INVESTIGATING THE BIOGAS PRODUCTION POTENTIAL OF COFFEE CHERRY PROCESSING WASTES CO-DIGESTED WITH ANIMAL MANURE

MSc. THESIS

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HAWASSA UNIVERSITY, COLLEGE OF AGRICULTURE

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ADVISORS’ APPROVAL SHEET

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This is to certify that the thesis entitled “Biogas Production and Characterization from Coffee Cherries Processing Wastes” submitted in partial fulfillment of the requirement for the degree Master’s with specialization in bio-energy science and technology, the graduate program of the school of plant and horticultural sciences and has been carried out by WOLDE MATHEWOS DELKERO ID. No GPBESTR/0007/11, under our supervision. Therefore, we recommended that the student has fulfilled the requirements and hereby can summit the thesis to the School.

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We, the undersigned, members of the board of examiners of the final open defense by WOLDE MATHEWOS DELKERO have read and evaluated his thesis entitled "Biogas Production and Characterization from Coffee Cherries Processing Wastes" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirement for the degree of Master's science.

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DECLARATION

I hereby declare that this thesis is my own original work and has not been presented for a degree in any other universities, and all sources of materials used for this thesis have been properly acknowledged.

Name: ______________________ signature________________________

Place: College of Agriculture, Hawassa University, Hawassa Ethiopia

Date of submission: _______________________________

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ABBREVATIONS AND ACRONYMES

CH_3COOH	Acetic acid
APHA	American Public and Health Association
NH_3	Ammonia
ANOVA	Analysis of variance
BP	Biogas production
C: N	Carbon to Nitrogen Ratio
CO_2	Carbon dioxide
CV	Coefficient of variance
CH	Coffee husk
Cm	Centimeter
CP	Coffee pulp
CD	Cow dung
^0C	Degree Celsius
DFID	Department for International Development
ENEC	Ethiopian National Energy Committee
CH_4	Methane
FS	Fixed solid
G	gram
GHG	Greenhouse gases
HRT	Hydraulic retention time
I	Inoculum
IEA	International Energy Agency
Kg	Kilogram
LPG	Liquefied petroleum gas

Ml	milliliter
MC	Moisture content
M	Molarity
SNV	Netherland development organization
N_2	Nitrogen molecule
NGO	None governmental organization
OLR	Organic loading rate
PMC	Percentage of moisture content
PTS	Percentage of total solids
PVS	Percentage of volatile solids
P	Phosphorus
K	Potassium
SD	Standard deviation
SNNPR	Southern Nations, Nationalities and People's Region
TN	Total nitrogen
TS	Total solids
VS	Volatile solids

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ABSTRACT

As the human population is increasing from time to time, the energy demand also increasing in a similar fashion. To meet this energy demand, there is high dependence on traditional biomass and fossil fuel, which are major source of environmental degradation. On the other hand, agricultural and industrial wastes are becoming the sources of air and water pollution due to improper management in which otherwise can be used as a renewable energy sources. Biogas is a renewable alternative energy sources made from bio-wastes and has a huge potential for future energy security and environmental sustainability. In the present investigation a "batch feeding digester" was developed to evaluate the potential use of coffee cherries processing wastes: coffee husk, coffee pulp and their co-digestion with cow dung for biogas production at mesophilic conditions and to analyze the nutrient content of the bio-slurry to be used as a bio-fertilizer. In advance of biogas production, feedstock quality parameters such as moisture content, total solid, Volatile solid and carbon to nitrogen ratio was determined using standard methods in order to check biodegradability of substrate and balance of nutrients in the substrate. The biogas production was conducted using laboratory digesters having a capacity of five liter with working volume of four liter, operated for about 35 days in incubator at a temperature of 37^0*C. One way analysis of variance (ANOVA) was performed to compare variations among treatments. The result showed that the values of feedstock quality parameters were within the acceptable range for biogas production. The amount of biogas produced from 1:1 coffee pulp with cow dung, coffee pulp, 1:1 coffee husk with cow dung, cow dung and 1:1 of coffee husk with cow dung and coffee husk with was 372.33 ± 5.0, 349.88 ± 2.65, 320.07 ± 3.54, 291.43 ± 5.07 and 268.55 ± 4.16 ml/g VS, respectively. The result indicates that 1:1 ratio of coffee pulp with cow dung produced higher amount of biogas than coffee pulp alone. Significant differences were observed in biogas production potentials between mono-digested and co-digested treatments at* ($P<0.05$)*. The quality of biogas in terms of methane content was 60.0, 59.2, 58.4, 57.1, 52% respectively, for cow dung, coffee pulp with cow dung, coffee husk with cow dung, coffee pulp and coffee husk. This shows co-digestion improves biogas yield. Generally, the results encouraged the feasibility of the coffee husk, coffee pulp and their co-digestion with cow dung for biogas production in the study areas. Nitrogen, Phosphorous and potassium of bio-slurry were in the range of 1.8 - 2.25, 0.2-1.7 and 0.49-1.3% respectively. This showed that bio-slurry from all treatments had enough amounts of macronutrients that needed in bio- fertilizer.*

Keywords: *biogas, bio-slurry, coffee pulp, coffee husk, co-digestion.*

1. INTRODUCTION

1.1. Background and Justification

Energy is one of the essential needs of today’s society. As human population increase and their living standard improves, the demand for energy source also increasing globally. The situation calls for extensive utilization of fossil fuels for cooking, lighting, and industrial purposes (Florio *et al.*, 2019). The combustion of fossil fuel intern contributes for Greenhouse Gas (GHG) emission and the resultant global warming. One of the principal causes of this change is the atmospheric accumulation of carbon dioxide (CO_2) released during combustion of the fossil fuels (Wilkie, 2006).

Two billion people or about 40% of the world populations depend on solid biomass fuels including fuel wood, crop residues, charcoal and animal dung for cooking and heating (Cunningham *et al*., 2013). Of these, three-quarters (1.5 billion) people do not have an adequate and affordable supply of energy. Most of them are in the less developed countries, where they face a daily struggle to find enough fuel to warm their homes and cook their food. The problem is intensifying because rapidly growing populations in many developing countries create increasing demands for fire wood and charcoal from a diminishing supply (Cunningham *et al*., 2013).

Ethiopia is one of the least developed countries in sub Saharan Africa, suffers from a severe domestic energy problem. Among other things, the countries domestic energy problem can be manifested by relatively very low per capita energy consumption and the dominance of traditional biomass fuel use. Traditional biomass such as fuel wood, charcoal, dung, and crop residues has been serving a prime source of energy in the country. Thus fuel wood and agricultural residue accounts for about 92% energy sources in the country and have been used for cooking and heating (Abrham and Berta, 2015). In the year of 2013 total national consumption of wood was estimated to be 105.2 million tons per year of which charcoal accounts 5.7 million tons. The dependence on charcoal and fuel wood for fuel supply, especially for household consumption requires cutting of trees and bushes. It leads to deforestation which decreases the fertility of land by soil erosion and absorption of carbon dioxide from atmosphere. On the other hand, traditional use of biomass as energy is also harmful for the health of the people due to the smoke arising from burning of fuel wood and pollutes the environment (Susanne, 2013). In addition to health and pressure on forest, it increase burden on women and children in searching and collecting fuel wood. Women and children are usually liable for biomass fuel collection. The unbalanced share of burden of collecting and managing traditional fuels has resulted to women's disproportionate lack of access to education and income, and inability to escape from poverty (Dawit, 2012).

Now days, because of environmental concern, the world is shifting towards renewable energy source (Baeno-Moreno *et al*., 2019). Some of the most important benefits of using renewable energy are based on the organic composition, lack net CO_2 emission, the use of mainly locally available inexpensive resources and the fact that they are the best solutions covering directly the need of the local community. One of the sustainable and renewable energy technologies is biogas production from organic wastes (Popescu *et al.*, 2010).

Biogas system refers to the technology of digesting organic waste in anaerobic condition to produce combustible gas to generate clean renewable energy and bio-fertilizer with additional benefit of disposing agricultural residues, aquatic weeds, animal and human excrement and other organic matter (Grisel *et al*., 2014). Materials rich in lignocellulose, such as plant residues, represent the most promising renewable organic feedstock for biogas production, as their production does not compete for arable land (Sawatdeenarunat *et al.*, 2015; Chandra *et al*., 2012). The main components of biogas are carbon dioxide (30 - 50%) and methane (50-70%) which if released in uncombusted form is harmful to the environment (Bishir and Mbanefo, 2013).

A lot of work has been carried out on the conversion of biomass to biogas through anaerobic biodegradation using different substrates in various conditions. The feedstock ranges from plant wastes to animal wastes. Only a few reports were available on biogas production from coffee husks and coffee pulps at either laboratory scale or large scale.

Coffee is the second largest traded commodity in the world which generates large amounts of byproducts and residues during cherries processing. Coffee production is a livelihood for about 125 million people worldwide, mainly for developing countries (Bilhate *et al.*, 2018). Ethiopia is known to be the origin and gene pool for coffee Arabica (Labouisse *et al.*, 2008). Coffee is Ethiopia's prominent export commodity; and the income of more than one million households depends on coffee production. The production of coffee increases from time to time. For example annual coffee production in 2007 was 273,400 tones increased to 469,091 tons in 2016, while the cultivation area increased from 407,147 to 700,475 hectare (Bilhate *et al.,* 2018). The amount of coffee byproducts is directly related to coffee production. Ethiopian coffee is produced under forest, semi-forest, garden, and plantation production systems. Thus, about 95% of Ethiopia's coffee is produced by small holder farmers (Minten *et al.*, 2014).

Even though, coffee production is income for a millions people, coffee wastes and byproducts produced during coffee cherry processing constitute a source of severe contamination and pose serious environmental problems in coffee-producing countries (Yisehak, 2009). Therefore, the aim of present work was to investigate the potential use of coffee cherry processing waste as a feedstock for biogas production and testing the nutrient content of the by product, the slurry, to be used as organic fertilizer.

1.2. Statement of the Problem

Coffee is an important plant grown in different parts of Ethiopia by smallholder farmers mainly as a source of income for the family. It is the leading export crop and is the backbone of the countries as a source of foreign currency. Farmers grow coffee for its bean and it is common to see coffee pulp and husks discarded in coffee cherry processing areas, while potentially can be used as a source of energy and for maintenance for soil fertility. Kembata Tembaro zone in South Nation Nationalities Peoples Region (SNNPR) is an area in which farmer grow coffee as agricultural crop. According to Kembata Tembaro zone coffee, tea and spices directorate 2020 annual report, the annual average production of coffee cherry was 6032 ton per year. Kedida Gamela woreda is one of coffee producing woreda in the zone. At the same year in this woreda 1627.4 tone coffee cherry was processed in wet and dry method coffee processing industries. From wet coffee processing industry 43% of coffee pulp and in dry coffee processing 50% of coffee husk were produced.

In addition to processing the cherries by the farmers for household consumption, coffee cherry processors collect the coffee cherry from individual producers and process to prepare the bean mainly for export and to some extent for domestic market both of which release huge amount of coffee processing by product. It is known that coffee cherry processing for production of clean coffee bean has many environmentally unfriendly waste products such as coffee pulp, coffee husk and waste water from the production. Most of the time, coffee producers burn the coffee husk and others drain the coffee pulp and waste water to the nearby river or nearby open space. This improper way of removal of coffee pulp to rivers leads to water pollution, bad odors and concentration of toxic elements in soils. The activity results in decreased land productivity and increased use of chemicals for regeneration of land productivity. On other hand burning coffee husk emits greenhouse gases like CO_2 and nitrous oxide that cause global warming and major elements from plant like nitrogen (N), phosphorus

(P) and potassium (K) are also loss that plays a role in recycle of this element for next crop generation.

Generally, these creates socioeconomic and health related adverse impacts, many people and the farm animals are suffering from drinking this polluted water sources. On the other hands, farmers in the zone use traditional biomass, mainly fuel-wood as a source of energy for cooking, heating and lighting purposes. The situation may contribute environmental pollution through forest degradation and release of GHG that can be taken as a health threat. For these reason, it is important to change the situation by converting the coffee cherries processing waste products into renewable energy sources that benefit the community and the environment in coffee cherry processing areas. One option is utilization of these wastes as a feedstock for biogas production. The biogas produced can be used to fulfill household energy demands of the community in the study area and the by product, bio-slurry, can be applied to farm land as a fertilizer to improve the productivity.

1.3. Objective

1.3.1. General Objective

The general objective of the research was to evaluate the potential use of coffee cherries processing wastes and its co-digestion with cow dung for biogas production and analyze the plant nutrient content of the bio-slurry to be used as organic fertilizer.

1.3.2. Specific Objectives

The specific objectives of the present research were to:

- Characterize coffee pulp, coffee husk and its co-digestion with cow dung in terms of the moisture content (MC), total solid (TS), volatile solid (VS), carbon to nitrogen ratio (C: N) and power of hydrogen (pH).
- Determine the amount of biogas that can be produced using coffee pulp, coffee husk and its co-digestion with cow dung as a feedstock and analyze the quality of biogas.

- Analyze the plant nutrient content of bio-slurry to be used as organic fertilizer.

1.4. Research Questions

Based on the problems stated above the following questions were addressed.

1. Do the physiochemical characteristics of coffee husk, coffee pulp and their co-digestion with cow dung in terms of pH, MC, TS, VS, C: N suit for biogas production?
2. How much biogas is produced from coffee pulp, coffee husk and co-digestion of coffee husk and coffee pulp with cow dung?
3. Do the bio-slurry from coffee pulp and husk contain major plant nutrients to be used as bio-fertilizer?

1.5. Significance of the Study

Using coffee wastes for biogas production will have multipurpose advantages. It provides cheap energy source from bio-wastes that otherwise pollutes the environment. It contributes for reduction of forest degradation and maintenance of soil fertility. The finding of this research will serve as get way for development of technologies that help to convert waste products like coffee husk and coffee to alternative, clean and environmental friendly source of energy. The study will be awarded about the benefit of coffee processing waste which producers dispose and harm the environment. Apart from getting alternative source of energy for their domestic consumption, the by product in biogas production, the slurry, can be used as a bio fertilizer that can replace inorganic fertilizer and which improve the fertility of their farmlands. This might contribute to the efforts to reduce poverty as it decrease financial and economic costs expended on fuel and inorganic fertilizer. The study might also pave the way for the researcher to apply theoretical knowledge in to practice and to upgrade existing ability of doing the research. Policy makers, NGOs, Energy offices and respective universities may use the finding of this study to develop into large scale production.

2. LITRATURE REVIEW

2.1. Energy

Energy is a basic tool for sustainable development. Provision of sustainable energy in rural communities is vital for agricultural sustainability, food preparation, lighting, improving quality of health services and education. It has been realized that fossil energy causes greenhouse gas emissions that have adverse effects on the environment. Using fossil fuels causes the increase of CO_2 level in the atmosphere which is directly responsible for global warming. Therefore, it is an ongoing interest to find out a renewable and environmentally friendly source of energy for our industrial economies and consumer societies (Hossain *et al*., 2014).

There is still a very large gap in energy service provision between developed and developing countries. Developing countries use traditional biomass energy sources like, firewood, crop residues or animal wastes. These energy sources are relatively inefficient and hazardous for health. In other word, cooking with firewood, dung or crop residue is associated with a significantly higher health risks comparing to other forms of cooking, due to indoor air pollution (Abrham and Berta, 2015). On the other hand, alternative renewable sources such as solar, biofuel, hydro or wind energy are more efficient, less hazardous for health and more suitable, time and costs saving for its users (Hossain *et al*., 2014).

2.2. Biofuel

Biofuel is a fuel derived from biomass. Biomass is an organic matter from plant and animals sources. It includes mainly wood, agricultural crops and products, aquatic plants, forestry products, wastes and residues, and animal wastes (Mebrhatu, 2014). Biofuels have been promoted as part of the global energy blend to meet the climate change challenge. By the end of the 20th century, global commercial energy consumption was about 400 exa-joules (EJ) per year, with fossil fuels contributing about 85% of the total (Melillo *et al.,* 2014). Global energy demand projections indicate that energy demand could be in the range of 550-1000 EJ per year in 2050, depending on factors such as resource availability and policies (Clarke *et al*., 2007).

The deficiency and increasing prices of fossil fuel, together with fear about the environmental harm created by them, have resulted in increasing efforts to search for alternative energy sources. Presently, biofuel are considered as the most promising alternatives in energy generation that can compete with the fossil fuel and to reduce the world‘s dependence on fossil fuel. An increasing number of developing countries initiated biofuel production to meet domestic market and international demand. Reasons for searching biofuels production include, diversifying energy sources, ceasing dependence on imported fossil energy and reducing greenhouse gas emissions (Elbehri *et al*., 2009).

2.3. Types of Biofuel

Biogas, bioethanol and biodiesel are three major biofuels. Biogas is generated from anaerobic digestion of organic wastes. Bioethanol has a general transport fuel has been developed for over 30 years since it was a natural extension of brewing technology. Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats. It can be produced easily from common feedstock (vegetable oils, animal fats and recyclable cooking oils) by trans- esterification (Dainis *et al.*, 2014).

2.4. Biogas

Biogas is a clean burning gas produced by anaerobic digestion which is a biological process that occurs in the absence of oxygen and in the presence of anaerobic organisms at ambient pressures and temperatures of 30 - 70^{0}C (Rai, 2004).

2.4.1. Historical Background of Biogas Technology

Historical evidence indicates that anaerobic digestion process is one of the oldest technologies. The industrialization of anaerobic digestion began in 1859 with the first digestion plant in Bombay, India. Some reports indicate that anaerobic digestion has been exploited to produce biogas and fertilizer for hundreds, maybe thousands of years by the Chinese and Persians (Florian *et al.,* 2013).

Biogas technology was introduced to Ethiopia in October 12, 1966 Gregorian calendar. During that time the first batch type floating digester was constructed in Ambo collage of agriculture to generate energy required for the purpose of welding. The types of digester design introduced by government and nongovernment organizations were fixed dome or Chinese digester, floating drum or Indian digester and plastic plug (SNV, 2011).

2.4.2. Characteristics of Biogas

Biogas is flammable gas made of mixture of gases produced by methanogenic bacteria while acting upon biodegradable materials in an anaerobic condition. Biogas is about 20% lighter than air and has an ignition temperature in the range of 650^0C - 750^0C (Florian *et al.*, 2013). It is odorless after burning and colorless gas that burns with clear blue flame (Atkins *et al.*, 2008).

Biogas is different from other renewable energies because of its characteristics of using controlling and collecting organic wastes and at the same time producing fertilizer for use in agricultural irrigation. Biogas does not have any geographical limitations nor does it require advanced technology for producing energy, in addition to being very simple to use and apply (Jan, 2010). Moreover, domestic biogas could improve the livelihood situation of rural households regarding aspects of workload, health and sanitation (Getachew *et al*., 2006).

2.4.3. Biogas Composition

The composition of biogas mainly depends on feed material. Biogas is mainly composed of 50-70% methane (CH_4), 30-50% carbon dioxide (CO_2) and traces such as hydrogen sulfide (H_2S), nitrogen molecules (N_2), ammonia (NH_3), carbon monoxide (CO) and hydrogen gases (H_2) (Bishir and Mbanefo, 2013; Yitayal, 2011). The compositions of biogas have been presented in Table 1.

Table 1: Biogas composition (Bishir and Mbanefo, 2013; Yitayal, 2011).

No.	Substance	Percentage composition
1	Methane	50 – 70
2	Carbon dioxide	30 – 50
3	Nitrogen	0 – 5
4	Oxygen	0 – 2
5	Ammonia	0 – 1
6	Hydrogen sulfide	0 – 1
7	Hydrogen	5-10

2.4.4. Basic Principles of Biogas Production

Biogas is produced through the anaerobic digestion of decaying plant or animal matter. Biogas production occurs through the following steps, first is the decomposition or hydrolysis of the biodegradable material into molecules such as sugars. Next, these molecules are converted into acids. Lastly, the acids are converted into biogas. Anaerobic digesters use the bacteria's natural processes to capture and utilize the biogas in controlled environment (Fachagentur, 2010).

Biogas produced in anaerobic digesters is burned to generate clean and renewable energy. The main components of biogas are carbon dioxide 30 - 50% and methane 50 - 70%, which, if released in un-combusted form, is harmful to the environment as a particularly intoxicating greenhouse gas (Bishir and Mbanefo, 2013; Jan, 2010). By preventing the emission of methane with producing clean energy, anaerobic digesters make a twofold contribution to climate protection (Atkins *et al.*, 2008).

2.5. Potential of Coffee Residues for Biogas Production

Coffee is one of the world's most popular drink and important product. Globally million small producers depend on coffee for their living. The global coffee production per year on average accounts to 7 million metric tons. Ethiopia is the fifth country coffee producer in the world (Bilhate *et al.,* 2018). In Ethiopia, the main regional states involved in coffee production are Oromia, Southern Nations, Nationalities and People (SNNP), Sidama and Gambella (Bilhate *et al.*, 2018). As of 2014, there were 1026 wet and 696 dry milling stations in these regions. About 60% of the wet milling and 79% of dry milling stations were located in SNNP (including Sidama) and Oromia regions, respectively. The amount of coffee residue directly related to the production of coffee cherries processing. From wet coffee process the respective by-products are pulp (43%), mucilage (12%), and parchment (6.1%) on fresh weight basis of coffee cherries. Husk is the major single by-product of the dry method. It represents about 50% of the dry cherry (Bilhate *et al.*, 2018). The following Table 2 indicates wet and dry milling stations in Ethiopia.

Table 2: Wet and dry milling stations in Ethiopia (Bilhate *et al*., 2018)

Regional state	Wet milling	Dry milling	Grand total
Oromia	407	550	957
SNNPRS and Sidama	616	140	756
Gambella	3	6	9
Total	1026	696	1722

2.6. Anaerobic Digestion Stages

In a biogas process, large organic molecules (proteins, carbohydrates and lipids) are successively broken down into methane and carbon dioxide, a gas mixture called biogas. The presence of several different microbial communities is required for the biogas process to work anaerobically in order to form biogas. There are four key biological and chemical steps of anaerobic digestion process: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Jan, 2014).

2.6.1. Hydrolysis

Hydrolysis is the first stage of the organic waste decomposition process involving the breakdown of large organic polymer chains into smaller molecules such as simple sugars, amino acids and fatty acids. Saccharolytic and proteolytic micro-organisms break down sugars and proteins, respectively (Edison, 2014). As shown in the Table 3 different specialized bacteria produce a number of specific enzymes that catalyze the decomposition. The process is extracellular as it takes place outside the bacterial cell in the surrounding liquid (Weiland, 2009).

Table 3: Enzymes that help for substrate decomposition **(**Edison, 2014)

Enzyme	Substrate	Break down Products
Proteinase	Protein	Amino Acids
Cellulase	Cellulose	Glucose
Hemicellulase	Hemicellulose	Sugars (glucose, xylose, mannose)
Amylase	Starch	Glucose
Lipase	Fats	Fatty acids and glycerol
Pectinanse	Pectin	Sugars e.g. galactose, arabinose

2.6.2. Acidogenesis

It is a process where microorganisms of facultative and anaerobic group, collectively called as acid formers, hydrolyze and ferment compounds into acids and volatile solids. In some cases, these acids may be produced in such large quantities that the pH may be lowered to a level where all biological activity is seized. This initial acid phase of digestion may last about two weeks and during this period a large amount of carbon dioxide is given off (Ramaraj and Dussade, 2015).

2.6.3. Acetogenesis

The products formed during the acidogenesis stage are further broken down by various anaerobic oxidation reactions. The transition of the substrate from organic material to organic acids in the acid forming stages causes the pH of the system to drop. This is beneficial for the acidogenic and acetogenic bacteria that prefer a slightly acidic environment, with a pH of 4.5 to 5.5, and are less sensitive to changes in the incoming feed stream, but is problematic for the bacteria involved in the next stage of methanogenesis (Florian, 2013).

2.6.4. Methanogenesis

The final stage of anaerobic digestion is methane production stage, where methanogens produce methane from hydrogen, carbon dioxide and acetate as well intermediates products from hydrolysis, acidogenesis and acetogenesis by various methanogens active during this stage (Rai 2004).

Thus, $CH_3COOH \xrightarrow{\text{Methane forming bacteria}} CH_4 + CO2$

$$CO2 + 4H_2 \xrightarrow{\text{Methane forming bacteria}} CH_4 + H2O$$

Methanogens need a pH 6.5 to 8 to remain active and are therefore particularly sensitive to pH, presence of heavy metals and organic pollutants interference in the process (Liu and Withman, 2008). Because these organisms are important in the anaerobic oxidation, interference in the methanogenesis may have serious consequences for the entire process

(Edison, 2014). In other word if the pH is allowed to fall below 5, methanogenic bacteria cannot survive. Methanogenesis is the rate limiting process because methanogens have a much slower growth rate than acidogenesis. Therefore, the kinetics of the entire process can be described by the kinetics of methanogenesis (Ionel, 2010 and Rai, 2004).

However, anaerobic digestion can be considered to take place in these four stages, all processes occur simultaneously and synergistically, in as much as the first group has to perform its metabolic action before the next can take over, and so forth (Ionel, 2010). The following Figure 1shows the four Stages of anaerobic digestion diagram.

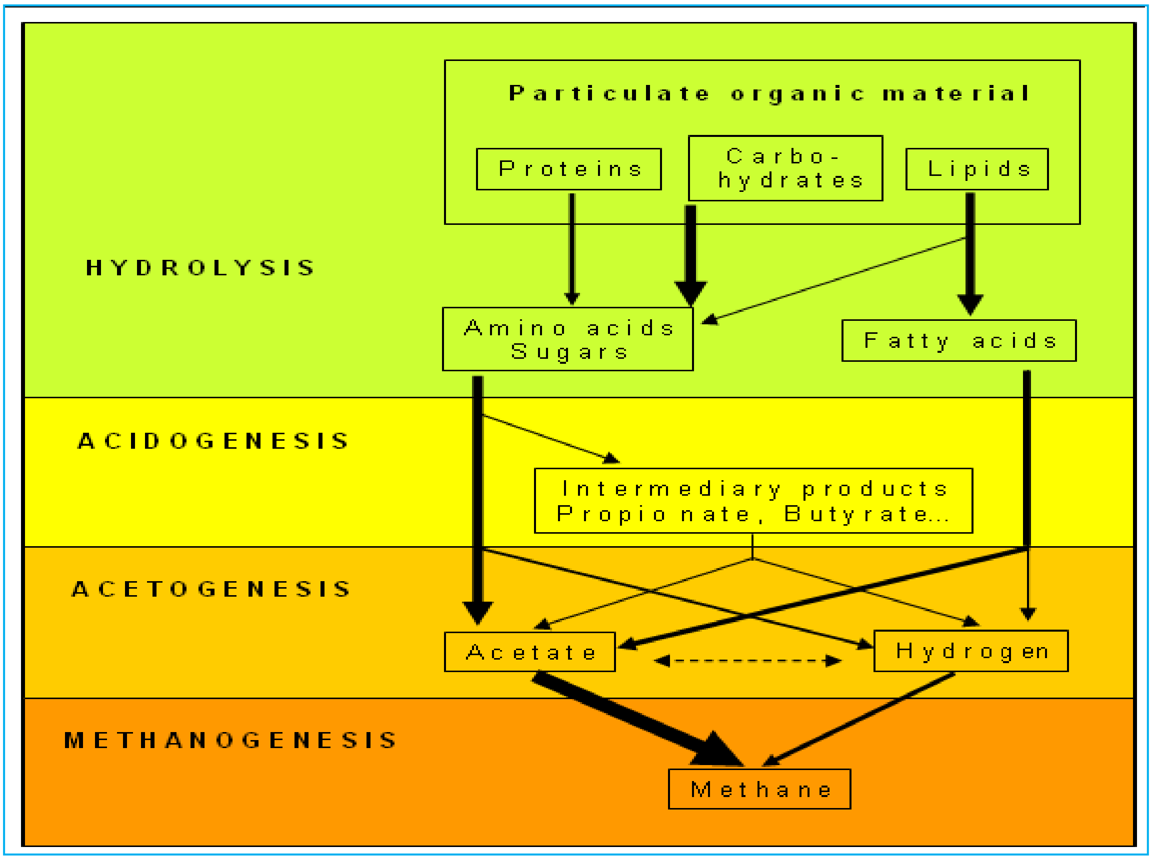

Figure 1: Stages of anaerobic digestion diagram (Suyog, 2012).

2.7. Factors Affecting Biogas Production

Environmental factors which influence biological reaction, such as, pH, temperature, nutrients and inhibitory concentrations are responsive to the external control in the anaerobic process. Any radical change in these factors can affect the biogas production (Whitman *et al.*, 2006)

2.7.1. pH

pH is one factor that affects digestion of substrates in reactors. Most microorganisms prefer a neutral pH range that is about PH 6.5 – 8.0 (Dioha, 2013). However, some organisms are active at both lower and higher pH values. There are several different organisms in the biogas process, and their pH requirements for optimal growth vary greatly. While fermenting, acid-producing microorganisms manage to live in relatively acidic conditions down to pH 5.0, most methane producers generally require neutral pH values to be active. Although most methane producers succeed best at neutral pH values, they remain active outside this pH-range (Whitman *et al.,* 2006). Methanogenic bacteria are very sensitive to pH and do not survive below a value of 5. If pH higher than 8.5 will start showing a toxic effect on methanogen population (Getachew *et al*., 2006; Jan, 2014).

2.7.2. Temperature

Temperature is the most critical process parameter that directly affects pathogens or bacteria, which are important to production of biogas. According to Meegoda *et al*., (2018), there are three temperature regions for anaerobic digestion: psychrophilic (< 25^0C), mesophilic (30-40^0C) and thermophilic (50-70^0C). The difference is at which temperature that the bacteria operate and metabolic rate of each type. In general mesophilic bacteria tolerate greater changes in their environment, including temperature. The stability of the mesophilic process makes it more popular in current anaerobic digestion facilities, although at the expense of longer retention times. However, thermophilic bacteria are sensitive to toxins and changes in the environment. Moreover, thermophilic bacteria need additional heating mechanisms such as insulation, circulation and solar heating system. Due to this, it is less attractive from an energetic point of view. Mesophilic bacteria are most active in the temperature range 30-40^0C and thermophilic bacteria in the range 50-70^0C (Mahanta *et al*., 2005; Nordberg, 2007).

2.7.3. Carbon to Nitrogen (C: N) Ratio

Generally, the carbon to nitrogen ratio is a major factor affecting the anaerobic process especially the methane yield and production rate. Therefore, the balance of carbon and nitrogen in a feed material is important. It is often suggested that an optimum C: N ratio is between 20:1 and 30:1. If the C: N ratio is very high, the nitrogen will be consumed rapidly by methanogens to meet their protein requirements and will no longer react on the left over carbon content of the material. As a result, gas production will be low (Rai, 2004).

On the other hand, if the C: N ratio is very low; nitrogen will be liberated and accumulated in the form of ammonia. NH_3 will increase the pH value of the content in the digester then it can inhibit the growth of the bacteria through NH_3 toxic concentration (Chaban *et al.,* 2006).

2.7.4. Particle Size

Particle size has some influence on gas production. The size of the feedstock should not be too large; if it is too large, it would result in the clogging of the digester and it would be difficult for microbes to carry out digestion. Smaller particles on the other hand would provide large surface area for adsorbing the substrate that would result in increased microbial activity and hence increased gas production. As suggested by Jan, (2010) out of five particle sizes 0.088, 0.40, 1.0, 6.0 and 30.0mm, maximum quantity of biogas was produced from raw materials of 0.088 and 0.40mm particle size. It suggested that a physical pretreatment of substrate such as grinding could significantly reduce the volume of digester required, without decreasing biogas production (Gollakota, 2004).

2.7.5. Total Solids and Volatile Solids

All feeding materials consist of solid matter and water. The solid consists of volatile solids and non-volatile solids or fixed solids. Volatile solids are the part of the total solids contents of the substrate that can be converted into biogas (Jan, 2010). When the percentage total solids (PTS) of substrate in an anaerobic continuous digestion process increases, there is a corresponding geometric increase in biogas production (Ionel, 2010).

The organic matter content is commonly determined from analyses of volatile solids. In the course of VS analysis, the total solids content of the material is determined first by removing all the water at 105^0C. Volatile solid is calculated as the amount of dry solids minus the amount of residual ash and is the part of the material that is biodegradable. This is sometimes referred to as loss on ignition, while the remaining ash residue is called residue after ignition (APHA, 1999).

2.7.6. Organic Loading Rate

The organic loading (OLT) rate is the quantity of organic matter fed per unit volume of the digester per unit time. High OLR and low sludge production are among the many advantages of anaerobic processes over other biological processes (Batstone *et al.*, 2002).

2.7.7. Hydraulic Retention Time (HRT)

Most anaerobic systems are designed to stay the waste for a fixed number of days. The number of days the materials stays in the tank is called the Hydraulic Retention Time (HRT). The HRT is important since it establishes the quantity of time available for bacterial growth and subsequent conversion of the organic material to gas. The retention time can only be accurately defined in batch type digester. Depending on the digester geometry and actual dilution rate, the effective retention time may vary widely for the individual substrate constituents and situations. Selection of a suitable retention time thus depends not only on the process temperature, but also on the type of substrate used (Jan, 2014). Shorter retention time is likely to face the risk of washout of active bacterial population while longer retention time requires a large volume of the digester. Normal retention period is between 30 and 45 days (Rai, 2004).

2.7.8. Pre-treatment of Feedstock

Plant biomass mainly consists of cellulose, hemicelluloses and lignin which is poorly degraded in anaerobic conditions, and the rate and extent of lignocelluloses utilization is severely limited due to the intense cross linking of cellulose with hemicelluloses and lignin as these materials form a scum and can easily clog the system. So, pre-treatment of the substrate in order to break the polymer chain so that increasing surface area and reduce lignin content is highly important (Sagagi *et al.*, 2009).

Treatments may be physical, biological or chemical and the most important physical pretreatment of biomass is particle size reduction leading to increase in available surface area and release of intracellular components (Grisel, 2014).

2.7.9. Inoculation

The use of a source high in anaerobic microbes to start up an anaerobic system is called inoculation. The quality and quantity of inoculums are critical to the performance and stability of bio-methanogenesis during startup or restart of an anaerobic digester. In manures and some wastes the microbes needed for digestion may be already present in the waste in small numbers, even though sufficient to act as inoculums, and will develop into a fully functional bacterial population if the right conditions are provided (Wilkie, 2008).

2.7.10. Co-digestion

Co-digestion is the simultaneous digestion of more than one type of biomass wastes in the same unit. Advantages of co-digestion include better digestibility, dilution of potentially toxic compounds, enhanced biogas production arising from availability of additional nutrients (Mshandete and Parawira, 2009). Lignocellulosic materials are characterized as carbon rich, poor in buffering capacity and in nutrients (Mata-Alvarez *et al.*, 2014). Mono-digestion of lignocellulosic materials often results in a slow process and low methane yield (Sawatdeenarunat *et al.*, 2015). This limitation can be overcome by using a co substrates, such as animal manure, can be used together with lignocellulosic biomass to supplement it with macro and micronutrients and buffering capacity (Mata-Alvarez *et al.*, 2014). For instance, co-digestion has been investigated for wheat straw with dairy and chicken manure (Wang *et al.*, 2012), rice straw with kitchen waste and pig manure (Ye *et al.*, 2013). These studies report higher methane yields approximately 200 - 400 mL/g VS compared with when using straw alone 120 - 200 mL/g VS, as a result of the higher energy content of the co-digestion materials and their complementary properties. But the high VS content of plant based materials can allow an increase in organic loading rate without reduce the hydraulic retention time and thus increase the volumetric biogas production (Moller *et al.*, 2004). Beside macronutrients, lignocellulosic materials such as agricultural residues are often low in trace elements such as iron, nickel, cobalt, selenium and tungsten, which are required for microbial enzyme activity (Demirel and Scherer, 2011).

2.7.11. Water

The production of biogas is ineffective if the substrates are too dilute or too concentrated in digester. With too little water, the activities of the micro-organisms will be affected and the quantity of biogas produced will be reduced. The dilution should be made to maintain an optimum total solid content. If the feed to the digester is too diluted, the solid particles will settle down into the digester and if it is too thick, the particles block the flow of gas formed at the lower part of digester. There is also higher risk of scum formation at the top of the slurry layer. In both cases, gas production will be less than optimal. Furthermore, most biogas digesters are designed for a total solids content of about 8%. A change of this ratio will have negative impact on functioning of the digestion process (Jan and Felix, 2010).

2.8. Benefits of Using Domestic Biogas

The biogas plant is mainly used for cooking, heating and lighting, therefore replacing energy sources such as fuel wood, dried dung, coal, or liquid petroleum gas (LPG), commonly used for these purposes in rural households (Hynek *et al*., 2018). In addition to the gas is valued for its use as a source of energy, the slurry uses for its fertilizing properties that mean it can be used as organic fertilizer. The advantages of using biogas for cooking is higher net efficiency, 5 times higher stove efficiency than traditional firewood stove (Jan, 2010). As indicated in Figure 2, in general biogas contributes three aspects of society that is economic, social and environmental, which are the pillars of sustainable development. These benefits can be direct or indirect and can occur on international, national, local, household, or individual levels of society (Gollakota, 2004).

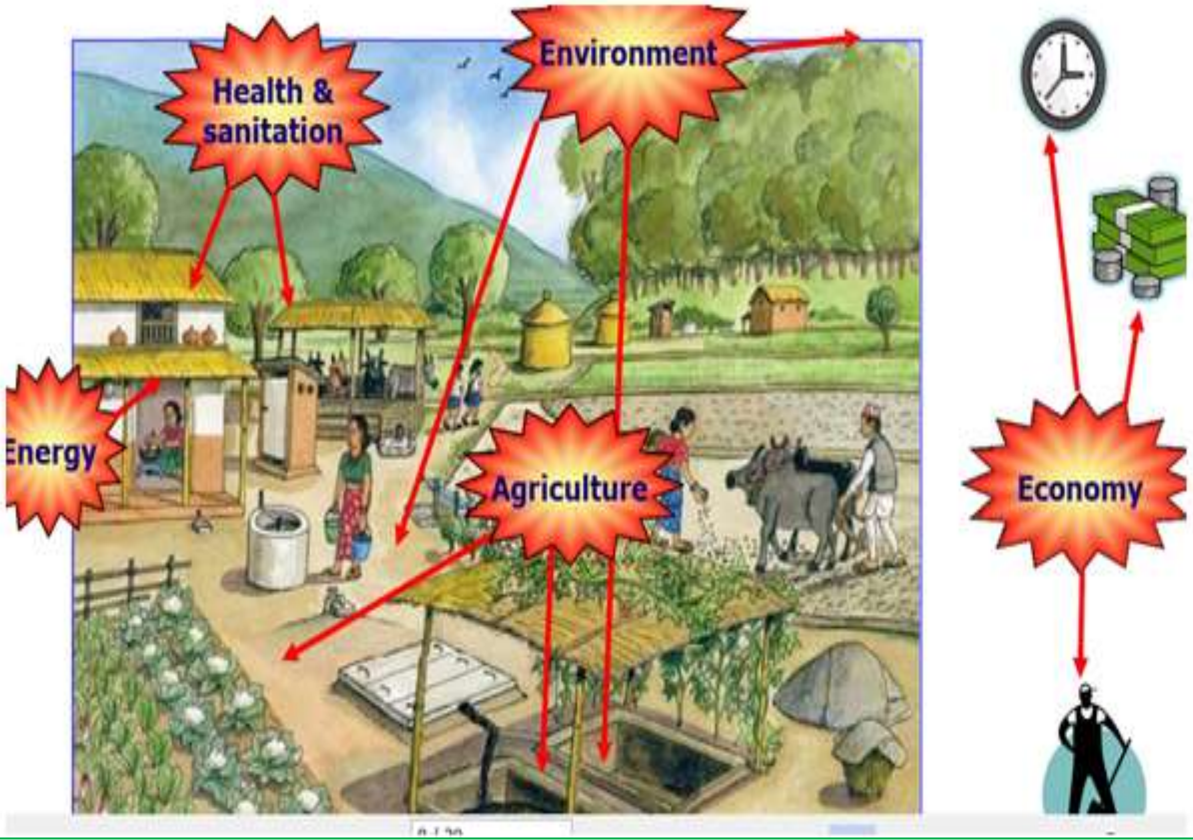

Figure 2: Benefits of biogas technology (Juliette, 2014)

2.8.1. Socio-economic Benefits

By investing in biogas plants, households could save time and energy, and have a supply of slurry that can be used as fertilizer in agricultural production. Biogas is widely accepted in Ethiopia as a cooking fuel and will mainly benefit women and children. 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 (Tereza, 2011).

A cost-benefit analysis of biogas plants yields positive net present values for households collecting their own energy sources. Even higher net present values are obtained for households purchasing all of their energy needs; these households stand to gain significantly from the financial benefits of energy cost savings with biogas technology. Additional economic impacts of biogas are to attract financial contribution from multi sector, revenues from saving electricity and fertilizer, revenues from CO_2 emission reduction, creation of sustainable employment opportunities for farmers and stimulus for regional economic development (Huong, 2010).

Additionally, Laramee and Daviss, (2013) states that the benefit of domestic bio-digesters helps especially to women and children, considering they are mostly responsible for cooking, fuel collection and agricultural activities.

2.8.2. Health Benefits

Since most households in developing countries are mostly depend on biomass from firewood for energy needs, deaths from acute respiratory infections as a result of indoor air pollution are extremely high (Mwirigi *et al*., 2014). Health related issues associated with biogas digesters include both clear benefits and potential risks. The main factors are associated with indoor air quality and pathogen distribution in the environment. Biogas offer several benefits by reducing odors and pathogens and providing renewable energy and fuel *(*Holm *et al.,* 2009*).* The health benefits of biogas are mainly related to a significant reduction of smoke and in-door air pollution compared to a traditional wood fire. Mostly women and children benefit from this as they spend lot of their time indoors cooking (Mwirigi *et al*., 2014).

2.8.3. Environmental Benefits

Biogas has positive environmental impacts. Biogas could potentially help to reduce global climate change. Biogas also reduces the about 60% emission of odorous substances during the storage and agricultural application and reduces hygienic sanitation and destruction of pathogen germs and disease causing agents. From a national perspective, the process of substituting biogas for firewood helped reduce the pressure on forests. This in turn has important implications for watershed management, slowing down deforestation and soil erosion. Treating residue in a biogas digester also reduces risks of contamination of soil and water (Grisel *et al*., 2014).

In addition, use of bio-slurry reduce the depletion of soil nutrients by providing organically rich nutrients resulting increased crop yield and hence reduces the pressure to expand cropland, displacement of mineral fertilizers, lowering their negative impact on the environment, increased recycling of organic matter and nutrients, Cost savings to farmers through enhanced use of own resources, reduced purchases of mineral fertilizer and higher nutrient efficiency, Potential for reduced air pollution from emissions of methane and ammonia form inorganic fertilizer (Lukehurst, 2012).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

A laboratory experiment was conducted at Hawassa University, Sidama region, southern Ethiopia. It is located at 270 km away from capital city of Addis Ababa. Geographically the area lies at $07^0$03'N and 038^o28'E, and at 1708 meter above sea level. The coffee husk and coffee pulp was collected from Kembata Tembaro zone, Kedida Gamela woreda. It lies between Longitude 37^0 51' East and 38^0 00' East and Latitude 7^0 11' North and 7^0 15' North with an elevation of 2122 meter above sea level. The area receives annual rainfall of 1200mm-1800mm (Hawassa meteorological center 2020).

3.2. Materials Required

The material and equipment required varies depending on the source of the biogas as well as the chemical analysis. The required material include: coffee husk, coffee pulp and cow dung which were used as feedstock for biogas production. Inoculant from active biogas plant is also required to start anaerobic digestion. For this specific study, the following equipment and chemicals were used to analyze physical, microbial and chemical characteristics of substrates.

- Concentrated and diluted Sulphuric acid (H_2SO_4), potassium sulphate (K_2SO_4), copper sulphate (CuSO4), potassium hydroxide (KOH), distilled water, methyl red indicator and bromocresol green indicator, boric acid and bromocresol green were used for nitrogen determination
- Citric acid and sodium chloride for making brine solution.
- Magnesium sulphate ($MgSO_4$), Phosphate, potassium nitrate (KNO_3) and asparagines, calcium chloride ($CaCl_2$), sodium chloride, ferric chloride agar, mannitol and bromothymol blue were used in microbial analysis.

- Acetic acid, calcium acetate and calcium lactate chemicals were used for determination of potassium and phosphorus during laboratory work.

Table 4: Equipment used during laboratory work

Equipment	Model/manufacturer	Application
Sampling bags	Not analyzed	For collecting inoculums
Plastic bottles	Aqua water plc.	As digesters
Polyethylene bags	Not analyzed	For storage of samples
pH meter	Hanna , Italy	For pH measurement
Electric oven	Germany	For MC,TS
Furnace	Germany, Type VMK3	For VS,FS
Plate dish	India	For TS, MC, VS
pestle and mortar	Not analyzed	For grinding
Sieve	Ethiopia/0.5mm/	For Sieving
Analytical balance	Kerns ALS(82-510g)	For Weight
Beakers/flasks/	Not analyzed	Multi -function
Measuring graduated cylinder	Roneyes life science	For measuring daily gas
Nitrogen digester	Not analyzed	For nitrogen determination
Kjeldhal flask	Not analyzed	For nitrogen determination
Distillation unit	Not analyzed	For nitrogen determination
Gas analyzer	GEOTECH, GA5000	For biogas composition
Microscope	Nicon ,SMZ 1500, USA	Microorganism observation
Incubator	Gerhardt, Wag TECH	Temperature control
Photometer	Wag TECH	For K
Spectrophotometer	Wag TECH	For P
Air bag	Medical/100ml	For gas collection
Connecting plastic tubes	Medical	For channeling of gas, brine
Petridishes	India, PD100G	For growth media
Slides	USA	For microscopic observation

3.3. Methods

In laboratory different analysis were carried out to characterize the samples and the biogas product.

3.3.1. Sample Collection and Preparation

About 20kg Coffee pulp was collected during pulping of the coffee cherries at Tefessa Gintamo coffee processing industry from Kembata Tembaro Zone, Kadida Gamella woreda and 20kg coffee husk was collected from Markos Abo dry coffee processing stations. The collected samples were spread on plastic sheet for 5 days on open sun for drying (Jatau *et al.*, 2001).

The dried samples were ground using pestle and mortar. The ground sample with particle size less than or equal to 0.5mm were separated and stored in black polyethylene bags, in a refrigerator at 4^0C until conducting laboratory analysis as recommended by (Wendland *et al*., 2006). This particle reduction need for microbes to carry out its digestion easily and provide large surface area for adsorbing the substrate that would increase microbial activity and hence increased biogas production.

Fresh cow dung was obtained from the dairy farm located at Hawassa university main campus. Approximately 15kg of cow dung was collected for this particular research purpose using random sampling method.

Inoculums were collected from near area of the effluent of an active biogas plant. Then physicochemical analysis of feed stock was done at Hawassa University Environmental Engineering laboratory, in Chemistry Department laboratory, Soil Science and Soil Microbiology laboratories.

3.3.2. Physicochemical Analysis of Substrates

Agricultural and industrial wastes contain large quantity of lignin, cellulose, hemicelluloses and carbon contents which is important source for biogas production. However, there are various parameters such as pH, moisture content, total solid, volatile solid content and fixed solid content which are among the main factors affecting the biogas production. Solids with high indigestible lignin concentration may not be suitable for microorganisms in biogas digester (Mihret *et al*., 2016). Therefore; total solids (TS), fixed solids (FS), and volatile solid (VS) analysis was conducted before the experiment using (APHA, 1999) standard methods. In addition to TS, FS, VS, carbon to nitrogen ratio (C: N); the bio-slurry macronutrients (N, P, K) were determined after digestion of process. The pH was measured initially and at the end of the experiment.

3.3.2.1. Moisture Content

Twenty gram of finely powdered air dried sample was weighed using analytical balance in a plate dish and heated in an electrical hot air oven at 105^0C for 4 hour and then taken out, cooled in desiccators and again weighed. The loss in weight is a measure of the moisture content of sample (APHA, 1999). It is expressed in percentage as indicated in Equation 1.

$$\%\mathrm{MC} = \frac{\mathrm{FSW} - \mathrm{DSW}}{\mathrm{FSW}} * 100\text{---} \quad (1)$$

Where, FSW = Fresh sample weight (air dried fuel sample)

DSW = Weight of the sample after oven dried

MC = Moisture content

3.3.2.2.Total Solids

For the determination of total solid, a clean evaporating plate dish was first dried in an oven adjusted at 105^0C for 1 hour, cooled in desiccators and weighed immediately before use. Then, 20g of each air dried sample was weighed using digital balance, and placed onto a pre-dried plate dish. Then, the dish was put inside an electric hot oven maintained at 105^0C. The plate dish was allowed to stay in the oven for 24 hours, and then taken out, cooled in desiccators and weighed as recommended by (APHA 2540 B, 1999). Then, the percentage of the TS was calculated using Equation 2 below:

$$\%TS = \frac{DSW}{FSW} \times 100 \text{ -- } (2)$$

Where: %TS = percentage of total solid

DSW = dry sample mass

FSW = fresh sample mass

3.3.2.3.Volatile Solids

The TS obtained from drying in electric oven was ignited at 550°C in furnace for four hours then taken out, cooled in desiccators and weighed to determine the volatile solid content. Then Equation 3 was employed to calculate the percentage of volatile solid content of the total solid (APHA, 1999).

$$\%\mathrm{VS} = \frac{\mathrm{DSW} - \mathrm{Ash}}{\mathrm{DSW}} \times 100 \text{ -- } (3)$$

Where: %VS = percentage volatile solid

DSW = dry sample weight

Ash = remaining mass after ignition which is called fixed solid

3.3.2.4. Organic Carbon Determination

There are different ways to determine organic carbon. For this particular research the organic carbon was determined using the volatile solid data and employing the Equation 4 (APHA, 1999).

$$\%\mathrm{OC} = \frac{MDS - \mathrm{Ash}}{1.8\, DSM} \times 100 \text{ -- } (4)$$

Where: %OC = Percentage of organic carbon

MDS = mass of dry sample

Ash = mass after ignition which is called fixed solid

3.3.2.5. Nitrogen Determination

The Kjeldahl procedure was employed to determine the total nitrogen content of the substrate. This method has three main steps. These are digestion, distillation and titration.

The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all organically bonded nitrogen in to ammonium ions (NH_4^+).

For current research work 2g of air dried fresh sample was measured using analytical balance and transferred to digestion tube with 15ml of concentrated sulfuric acid. Then 7g potassium sulphate to rise up the boiling point of the mixture and 3.5g of copper sulphate was added as a catalyst. Then it was heated at 370°C for three hours until the digest is black-white. Then cooled and 50ml water was added. The digested sample transferred to kjeldahl flask. Then 60ml of 50% Sodium hydroxide was added to change ammonium ion to ammonia in the digestate. Nitrogen was separated by distilling the ammonia and collecting the distillate in 20ml of boric acid solutions in receiving flask.

Then determination of nitrogen was done by titration, with a standard solution of 0.1N Sulphuric acid in the presence 20ml of bromocresol green and 4ml of methyl red color indicator in boric acid solutions. Titrate until the color change from green to pink.

Finally, the amount of nitrogen was calculated using Equation 5:

$$\%TN = \frac{(\text{ml titrant} - \text{ml black}) * \text{N of titrant} * 1.4007}{\text{sample weight}} \text{------------------------------} (5)$$

Where: %TN = Percentage of nitrogen,

N = Normal concentration (Normality)

Then Carbon to nitrogen ratio calculated by using Equation 6.

$$C{:}N = \frac{\%\ \text{carbon}}{\%\ \text{nitrogen}} \text{--} (6)$$

3.4. Microbial Analysis of Inoculum

To start up a new anaerobic process, it is important to use an inoculum of microorganisms to initiate the fermentation process. The common inoculum includes digested sludge, effluent or cow dung slurry from active biogas plant (Yadvika *et al.*, 2004).

Therefore, the inoculum was obtained from active biogas plant effluent found in Hawassa. Before adding inoculum to the digesters to start up anaerobic process, the type of microorganisms was identified. In order to identify the type of micro-organisms in inoculum, microbial growth media was prepared as: 1.0g of K_2HPO_4, 0.5g of KNO_3 and 0.5g asparagine was dissolved in distilled water and 0.2g magnesium sulphate, 0.1g calcium chloride, 0.1g sodium chloride and 0.002g ferric chloride was added. Then agar was added and dissolved, heated at 100^0C, and filtered. The Mannitol was added and cooled to 60^0C. pH was adjusted to 7.4 with adding bromothymol blue and autoclaved at 121^0C for 15 minutes. For serial dilution, 10ml of inoculum was added in 100ml distilled water in beaker and shacked. Then 9ml distilled water was added in 10 test tubes, then 1ml inoculum from beaker poured in to first tube, from 1st to 2nd, from 2nd to 3rd up to 10 test tubes. From each test tubes100 microliter was transferred to sterilized petridishes. Then prepared media poured in to petridish and cooled at 45^0C in incubator then put in top to down incubated at 28^0C for 2 days. Then the type of bacteria was examined by microscope as shown in Appendix Figure 7 (Thoronton, 1922).

3.5. Digester Setup and Configurations

Anaerobic digesters were constructed in batch-scale experiments. Biogas was produced out of the degradation of organic matter in 5 liter plastic bottle digester with 4 liter working volume and the remaining part used for gas holding. As shown Figure 3 three plastic bottles were arranged in such way that the first bottle contained substrate, the middle contained acidified brine solution and the last was used for collecting the brine solution that was ejected out from the second bottle by gas pressure. The acidified brine solution was prepared by dissolving sodium chloride in distilled water until a supersaturated solution was formed to prevent the dissolution of biogas in the water. Then three drop citric acid was added using a dropper to acidify the brine solution. The three plastic bottles were interconnected with a plastic tube having a diameter of 1cm. Thus, the biogas produced in digester would be driven from the first bottle to the second bottle that contained a brine solution so to displace a volume of the brine solution equivalent to the volume of biogas produced (Elijah, 2009). The lids of all digesters were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas. The temperature of all the digesters was maintained at 37°C (mesophilic condition) by keeping them in an incubator. The produced biogas measured twice a day using graduated cylinder.

Figure 3: Experimental set-up of anaerobic digestion

3.5.1. Digester Composition

3.5.1.1. Feed stocks

For the purpose of this study the amount of TS in each digester was fixed to be 320g taking the digesters volume in to consideration and the mass of dry samples of coffee pulp, coffee husk and fresh cow dung added to the 5 liters bottle digesters with working volume of 4 liters the remaining space was for biogas holding.

The amount of TS to be added in the digester obtained by using the following Equation 7 as recommended by (Elias, 2010; Yitayal, 2011). That is the TS in the slurry should be 8%.

$$ATS = 8\% * SL \text{ -- } (7)$$

Where, ATS = Total solid of sample in the digester

SL = Total slurry in the digester (4000g)

For determination of weight of fresh feedstock to be added in the in the digester should be obtained by using the following Equation 8:

$$\mathrm{WFS\ (g)} = \frac{\mathrm{ATS}}{\%\mathrm{TS}} \text{---} (8)$$

Where, WFS = Fresh sample weight added in digester

%TS = Percentage of total solid obtained after drying sample at oven at 105 ^{0}C

ATS = Amount of total solid in gram

3.5.1.2.Water Content

As reported by Elias, (2010) for better biogas production the water content of each sample should be adjusted to TS of 8% in the fermentation slurry. This is the basis for determination of the amount of water to be added for any given mass of total solid. Therefore, the feed stocks and inoculums were mixed with distilled water to get 8% TS solution in fermentation by using Equation 9.

$$\mathrm{ADW} = \left(\frac{\mathrm{ATS}-(8\% * \mathrm{DFS})}{8\%}\right) - \mathrm{inoculum} \text{ -------------------------------} (9)$$

Where, ADW = Amount of distilled water in ml

ATS = Amount of total solid in gram

DFS = Sun dried fresh sample in gram

3.5.1.3. Sample Proportions in the Digester

All treatments of coffee pulp, coffee husk, cow dung, ratio of coffee pulp to cow dung, coffee husk to cow dung and the amount of water that was added to get 8% TS slurry in fermentation. About 1% of inoculum from total volume of the fermentation slurry was added to each digester to serve as the starter culture (Neves *et al*., 2004). The digester contents are summarized in Table 5.

Table 5: Treatments and sample proportions in each digester

Trt	**PPCD %**	**PHCD %**	**FCD (g)**	**CD (g)**	**FP (g)**	**Pulp (g)**	**FH (g)**	**Husk (g)**	**I (ml)**	**WC (ml)**	**Tm**
T1	1:0	0:0	0	0	350	320	0	0	40	3610	4000
T2	0:0	1:0	0	0	0	0	345	320	40	3615	4000
T3	0:1	0:0	1600	320	0	0	0	0	40	2360	4000
T4	1:1	0:0	800	160	175	160	0	0	40	2985	4000
T5	0:0	1:1	800	160	0	0	172.5	160	40	2987.5	4000

Where, Trt = Treatment, PPCD = Proportion of cow dung with coffee pulp, PHCD = Proportion of Coffee husk with Cow dung, FD = Fresh cow dung, CD = Cow dung, FP = Fresh pulp, FH = Fresh husk, I = Inoculums, WC = Water content and Tm = Total mass

3.6. Determination of the Quantity of Biogas

The amount of gas produced was measured by using water displacement method (Tamirat, 2008). After the startup of the experiment, daily biogas yield of each digester was measured twice a day by using graduated circular cylinder until biogas production ceased. As indicated in Figure 3 biogas produced in digester would be driven from the first bottle to the second bottle that contained a brine solution. Thus, the displaced volume of the brine solution is equivalent to the volume of biogas produced.

The average daily biogas was calculated using Equation 10 that is cumulative biogas divided by retention time that is:

$$\mathrm{ADB} = \frac{\mathrm{CB}}{\mathrm{RT}} \text{--} (10)$$

Where, ADB = Average daily biogas production in ml

CB = Cumulative biogas production (the sum of daily biogas production) in ml

RT = Retention time (days of biogas production)

Biogas production in terms VS was calculated by using Equation 11:

$$\mathrm{BP}\left(\frac{\mathrm{ml}}{\mathrm{g}}\ \mathrm{VS}\right) = \frac{\mathrm{CB}}{\mathrm{AVS}} \text{--} (11)$$

Where, BP = Biogas production from total solids at the digester

CB = Cumulative biogas production

AVS = Amount of volatile solid in feedstock (g)

3.7. Determination of the Quality of Biogas Production

For determination of the quality of biogas another set up was conducted. In this case, each digester was filled with samples as described in above Table 5 all digesters sealed with super glue and deoxygenated gas bag of 100ml volume was used for gas collection (Figure 4). Each digester directly connected to the gas bags with plastic tubes having 1cm internal diameter and all treatments were replicated four times, closed and placed in incubator. The temperature was maintained at 37^0C (mesophilic condition). Then collected biogas in gas bag was taken by graduated syringe and was analyzed by using GEOTech gas analyzer. This gas analyzer helps to identify the percent composition of CH_4, CO_2, O_2, and H_2S in the biogas produced (Alemu, 2016).

Figure 4: Biogas collections for quality analysis

3.8. Determination of N,P, K of Bio-slurry

Bio-slurry is the byproduct of biogas. Bio-slurry used as organic fertilizer. It contains macronutrients nitrogen (N), phosphorus (P), potassium (K) and micronutrients. Therefore, after completion of gas production in digester, the amount of macronutrients: N, P and K values of the bio-slurry were analyzed. Kjeldhal method was used for the determination of nitrogen. For the determination of available potassium and phosphorus in bio-slurry (APHA 4500 P C 1999, APHA 3500 K B, 1999) protocol procedures were followed.

3.9. Experimental Design

The experimental design for biogas production was Completely Randomized Design (CRD) with five treatments and four replications. The treatments were T1= coffee pulp, T2 = coffee husk T3 =100 cow dung, T4=1:1 of cow dung with coffee pulp and T5= 1:1 of Cow dung with coffee husk.

3.10. Statical Analysis

All data were subjected to statistical analysis of variance using Statistical Analysis System software (SAS 9.0). One way analysis of variance (ANOVA) was performed to compare variation among treatments. List significance different test (LSD) at 0.05 probability level was used to separate mean of the treatments. Excel was used to generate histograms. Then the results were discussed and compared to other related works.

4. RESULTS AND DISCUSSIONS

4.1. Characterization of Feed Stocks

In order to determine the biodegradability and nutrient balance in coffee pulp and coffee husk: the total solid content, volatile solid content, organic carbon, total nitrogen and carbon to nitrogen ratio before inoculation and mixing were determined with four replications and their average values were summarized in Table 6.

Table 6: Characteristics of coffee pulp and coffee husk before mixing (mean ±SD)

Parameter	CP	CH	Recommended value	Reference
MC (%)	8.75 ± 0.06	7.25 ± 0.13	dried to 7-18	Oliveria *et al*.,2001; Rocha *et al.*, 2005; Barcelos *et al.*, 2001
TS (%)	91.25 ± 0.06	92.75 ± 0.13	91- 94	Mulugeta and Mebratu, 2017
VS (%)	88.00 ± 0.70	89.50 ± 1.00	70 – 90	Steffen *et al.*,2000
FS (%)	12.00 ± 0.70	10.50 ± 1.00	-	-
OC (%)	48.87 ± 0.25	49.72 ± 0.55	>40	El-Hendawy, 2003
N (%)	1.04 ± 0.03	1.27 ± 0.09	0.1- 2.4	Vander, 2004
C: N	47.01 ± 1.07	39.15 ± 2.78	20:1- 30:1	Dahlman and Forest, 2001
pH	5.72 ± 0.17	5.65 ± 0.10	6.5- 8.0	Whitman *et al.*, 2006; Anna and Asa , 2010; Dioha, 2013

Where, MC = Moisture content, CP = Coffee pulp, CH = Coffee husk, TS = Total solid, VS =Volatile solid, FS = Fixed solid, OC = Organic carbon, N = Nitrogen, C: N = Carbon to nitrogen ratio and pH = Power of hydrogen.

The total solid content, volatile solid content, organic carbon, total nitrogen and carbon to nitrogen ratio of cow dung before inoculation and mixing were determined with four replications and their average values were summarized in Table 7.

Table 7: Characteristics of cow dung before inoculation and mixing (mean ±SD)

Parameter	CD	Recommended value	Reference
MC (%)	80.00 ± 1.41	80 – 82	Rai, 2004
TS (%)	20.00 ± 1.41	18 – 20	Rai, 2004
VS (%)	79.25 ± 0.96	75 – 80	Steffen *et al.*, 2000
FS (%)	20.75 ± 0.96	20 – 25	Elias, 2010
OC (%)	44.02 ± 0.53	-	-
N (%)	2.20 ± 0.11	-	-
C: N	20.05 ± 1.00	20:1 - 30:1	Dahlman and Forest, 2001
pH	6.85 ± 0.05	6.5 - 8.0	Anna and Asa ,2010; Dioha, 2013; Whitman *et al.*, 2006.

Where, CD = Cow dung

4.1.1. Values of Total Solids and Volatile Solids Before Mixing

Substrate concentration can be determined in terms of volatile solids or total solids. Total solid is the measurement of dry matter as a percentage. As indicated in table 7 before mixing the substrates with water, inoculum and cow dung, the total solid content of coffee pulp was 91.25 ± 0.06%, which is in the range of total solid of coffee pulp 91- 94% reported by (Mulugeta and Mebratu, 2017). From total solid, the volatile solid content of coffee pulp was 88 ± 0.7%. Bilhate *et al*., (2018) reported nearly the same volatile solid result for coffee pulp (88.26%). These values indicate that large fraction of coffee pulp is biodegradable and thus it can serve as an important feedstock for biogas production.

The total solid content and the volatile solid content of the coffee husk were 92.75% ± 0.13 and 89.5 ± 1.0%, respectively. Wang (2013) reported nearly the same volatile solid for coffee husk (90.7%). This also indicates coffee husk is biodegradable and thus, can be used as feedstock for biogas production.

For cow dung the total solid was 20 ± 1.4% which is in range of 18 - 20% as reported by (Rai, 2004). From total solid content, the volatile solid content of cow dung was 79.25 ± 0.96%. This value is similar to volatile solid of cow dung 79.56% reported by (Yitayal *et al.*, 2017). This volatile solid value also indicates biodegradability of cow dung and can serve as feed stock for biogas production.

4.1.2. Values of Organic Carbon

The carbon content of coffee pulp, coffee husk and cow dung were 48.87%, 49.72% and 44.02% respectively. The carbon content of coffee husk agrees with 49.5% which is reported in previous study (Nguyen *et al.,* 2013). These values indicate that there is a high content of carbon in coffee pulp and coffee husk. It has been showed that the coffee husk and pulp rich in lignocellulosic material (Triolo, 2011). This high content of carbon in coffee husk and pulp are agrees as reported by (Mata-Alvarez *et al.*, 2014) that is lignocellulosic materials are characterized as carbon rich. Even though, this high carbon content makes the coffee husk a good feedstock for biogas production; concerns in coffee husk like inhibitors (tannin and caffeine) could inhibit the microbiological activities, which may affects the production of biogas yield (Hendriks, 2009). Therefore, such kind of limitation lignocellulosic substrates can be overcome by using a co-digestion with other substrates such as cow dung to supplement it with macro and micronutrients and buffering capacity (Mata-Alvarez *et al.*, 2014).

4.1.3. Values of Carbon to Nitrogen Ratio

Carbon to nitrogen ratio (C: N) of the feed stocks is another factor that affects the anaerobic digestion process. The C: N ratio expresses the relationship between the quantity of carbon and nitrogen present in organic materials. Materials with different C: N ratios differ widely in their yield of biogas. The bacteria use up carbon about 30 times faster than they use up nitrogen. Therefore, ideal C: N ratio for anaerobic bio-digestion is between 20:1 and 30:1 (Rai, 2004). If C: N ratio is much higher than this range, biogas production will be low, because the nitrogen content of the feed material will be consumed rapidly by methanogenic bacteria for meeting their protein requirements. On the other hand, if C: N ratio is very low, nitrogen will be liberated and will accumulate in the form of ammonia, which raises the pH value of the slurry in the digester (Saxon, 1998). Therefore, in this research C: N ratio was determined for each substrate.

The C: N of coffee pulp, coffee husk and cow dung in this experiment were 47.01:1, 39.15:1 and 20.05:1, respectively. C: N of cow dung agree with the value 20:1 to 30:1 as reported by (Dahlman and Forst, 2001; Yitayal *et al.,* 2017). However; C: N for coffee pulp and coffee husk in mono-digestion is much higher than recommended value for better biogas production. The previous research reported by Mihret *et al.,* (2016) also showed high amount of carbon to nitrogen ratio (93.49:1) for coffee husk. This indicates the need for further processing of feedstock for optimum biogas production and to make the ratio of C: N ratio between the ranges. One way is mixing coffee pulp and coffee husk with cow dung. There were also other organic feed stocks with high amount of carbon to nitrogen ratio. For example straw has 50 - 150 C: N ratio which needs mixing with other substrates such chicken manure that has low C: N ratio (Edison 2014).

4.1.4. Characteristics of Inoculum

It is important to use an inoculum of microorganisms to initiate the fermentation process. In other word, addition of manure slurry to the batch reactor as part of the starter improves the biogas production (Rojas *et al.* 2010). Therefore, the inoculum used in this study was manure slurry (effluent) collected from a well-functioning anaerobic digester in Hawassa city. Its physicochemical and microbial analysis was done. Morphological observation was done to isolate the type of microorganisms present in the culture of inoculum using compound microscope. The inoculum was cultured in agar nutrient media. Different colonies were formed in prepared agar media. From colonies, sample was taken by stirrer in order to differentiate a type of microorganisms by observing the morphology (shape) of bacteria using compound microscope. Different methanogenic bacteria were observed: such as methanobacterium, methanosarcina, methanospirillum and methanococcus. From those species, methanosarcina and methanospirillum were observed abundantly and played great roll in initiation of anaerobic digestion process.

In addition to microbiological analysis the physicochemical characteristics of the inoculums were analyzed and presented in Table 8. The relative smaller proportion of VS and TS was the indication of complete digestion of effluents except the presence of active microorganism population in the inoculum. Optimum pH was observed in inoculum. This optimum pH was important for methanogenic bacteria. In other word, methanogens need a pH 6.5 - 8 to remain active and are therefore this optimum pH makes the bacteria active for the anaerobic process.

Table 8: Physiochemical analysis of inoculum (mean ± SD)

Sr.no	Parameters	Result
1	MC %	95 ± 0.80
2	TS %	5 ± 0.80
3	VS %	5 ± 0.52
4	pH	7.8 ± 0.12

4.2. Characteristics of Feed Stocks Before Digestion (After mixing)

After mixing and inoculation the total solid, volatile solid content, organic carbon, total nitrogen and carbon to nitrogen ratio of coffee husk, coffee pulp, and co-digestion with cow dung were determined and their average values were summarized as following.

4.2.1. Moisture Content Values After Mixing

As indicated in Table 9 the mean moisture contents of coffee pulp, coffee husk, cow dung, 1:1 coffee pulp with cow dung and 1:1 coffee husk with cow dung were 92.00 ± 0.18%, 91.99 ± 0.05%, 92.01 ± 0.42%, 91.98 ± 0.96% and 91.97 ± 0.03%, respectively. This result showed that the moisture contents of all treatments were increased from the initial analysis done before inoculation and mixing. For instance, the mean moisture content of coffee pulp, coffee husk, and cow dung before mixing and inoculation was 8.75 ± 0.05%, 7.25 ± 0.12% and 80 ± 1.4%, respectively, but after mixing and inoculation the mean moisture contents of coffee pulp was 92.00 ± 0.18%, Coffee husk 91.99 ± 0.95% and cow dung was 92.01 ± 0.42%, respectively. Increasing in moisture content is due to the adjustment of total solids in the digester to 8% as per the recommendations of (Yitayal, 2011). Thus, the moisture content of all treatment was adjusted to appropriate moisture content for better biogas production and it was in the right range that is 65% - 95% (Demetriades, 2008).

Table 9: Moisture content after mixing

Treatment	MC (%)	Recommended value	Reference
CP	92.00 ± 0.18^{a}	65-95 %	Demetriades, 2008
CH	91.99 ± 0.05^{a}	65-95 %	Demetriades, 2008
CD	92.01 ± 0.42^{a}	65-95 %	Demetriades, 2008
CP:CD	91.98 ± 0.01^{a}	65-95 %	Demetriades, 2008
CD:CH	91.97 ± 0.03^{a}	65-95 %	Demetriades, 2008
CV	0.09	< 10%	Gomes, 2009
LSD	0.13		

*** means with the same letter are not significantly different at $p < 0.05$

Where, CV = Coefficient of variance, LSD = List significance difference, CP: CD = coffee pulp with cow dung, CH: CD = coffee husk with cow dung.

4.2.2. Values of Total Solids After Mixing

As indicated in Table 10 the percentage of all treatments of total solid adjusted to approximately 8%. This is because, if the value of TS concentration is too high in the digester, it inhibits the flow of gas formed at the lower part of digester, higher risk of scum formation at the top of the slurry layer, and the feed is not pumpable. Similarly, if the waste is too diluted, the digester is not fully utilized as more volume occupied by water with none substrate value. In both cases, gas production result will be less than optimal (Catarina, 2011). According to Igoni (2008), when percentage of total solids increases in the digester, the amount of water decreases, thus reducing the level of microbial activity, which then affects the amount of biogas, particularly at higher values of the TS%. Abbassi-Guendouz *et al.*, (2012) showed that the total methane production decreased with total solids contents increasing from 10% to 25% in batch anaerobic digestion under mesophilic conditions. Forster-Carneiro *et al*., (2008) also showed that the biogas and methane production decreased with total solids contents increasing from 20% to 30% in batch anaerobic digestion for food wastes. Therefore, as recommended by Yitayal, (2011) the total solid content of all treatments were adjusted to approximately 8% for better biogas production. Due to this arrangement none-significance difference were observed in total solids between all treatments at $p<0.05$.

Table 10: Total solid value after mixing and inoculation (mean ± SD)

Treatment	**TS (%)**	**Recommended value (%)**	**Reference**
CP	8.00 ± 0.18^a	8.0	Catarina, 2011
CH	8.01 ± 0.09^a	8.0	Elijah *et al*., 2009
CD	7.99 ± 0.42^a	8.0	Florian *et al*., 2013
CP:CD	8.02 ± 0.10^a	8.0	Ituen *et al.*, 2007
CH:CD	8.03 ± 0.02^a	8.0	Yitayal, 2011
CV	5.5	<10	Gomes, 2009
LSD	0.32		

*** means with the same letter are not significantly different at $p< 0.05$

4.2.3. Values of Volatile Solid After Mixing (mean ± SD)

As indicated in the Table 11, the percentage of volatile solids determined for coffee pulp, coffee husk, coffee pulp with cow dung, coffee husk with cow dung and cow dung were 89.50%, 89.75%, 86%, 85% and 80.3%, respectively. This showed there was slight change but none significance increase in volatile solids before mixing and after mixing. This is because there is no or very small quantity of organic matter in the inoculum. As described by Steffen *et al.,* (2000) the volatile solids content in total solids of all treatments were used in this experiment was in the range of 70 - 95 %.

Table 11: Volatile solid value after mixing and inoculation (mean ± SD)

Treatment	**VS (%)**	**Recommended value in %**	**Reference**
CP	89.50 ± 1.73^{a}	70-95	Steffen *et al*.,2000
CH	89.75 ± 0.96^{a}	70-95	Steffen *et al*.,2000
CP:CD	86.00 ± 1.83^{b}	70-95	Steffen *et al*.,2000
CH:CD	85.00 ± 1.82^{b}	70-95	Steffen *et al*.,2000
CD	80.30 ± 0.18^{c}	70-95	Steffen *et al*.,2000
CV	1.69	<10	Gomes, 2009
LSD	2.19		

*** Means with the same letter are not significantly different at (P<0.05)

4.2.4. Values of C:N

As indicated in Table 12, for the mixed treatments (1:1 ratio of coffee pulp with cow dung and 1:1 of coffee husk with cow dung) the C: N were 25.8% and 28.06% respectively, that is found in the possible ratio range set for carbon to nitrogen ratio of 20:1-30:1. Thus, for mixed substrates the balance of carbon and nitrogen is good for the microorganisms, so that mixed substrates could be used for anaerobic digestion to produce biogas. Therefore, mixing feedstock with high organic carbon and feed stocks with low organic carbon or high nitrogen content improve the nutrient balance. The C: N ratio of Cow dung was found in recommended range. However, C: N ratio of coffee husk and coffee pulp were higher than the range. This was due to the presence of high organic carbon in coffee husk and coffee pulp.

Table 12: Carbon to nitrogen value after mixing (mean ± SD)

Trt.	**OC (%)**	**N (%)**	**C: N**	**Recommended value**	**Reference**
CP	49.72 ± 0.97^{a}	1.04 ± 0.01^{d}	47.81 ± 0.91^{a}	20:1- 30:1	Rai, 2004
CH	49.86 ± 0.53^{a}	1.25 ± 0.06^{c}	39.93 ± 1.77^{b}	20:1- 30:1	Rai, 2004
CD	44.6 1 ± 0.10^{c}	2.20 ± 0.11^{a}	20.31 ± 1.02^{d}	20:1- 30:1	Rai, 2004
CP:CD	47.78 ± 1.02^{b}	1.85 ± 0.02^{b}	25.8 ± 0.64^{c}	20:1- 30:1	Rai, 2004
CH:CD	47.17 ± 0.94^{b}	1.70 ± 0.18^{b}	28.06 ± 3.12^{c}	20:1- 30:1	Rai, 2004
CV	1.70	6.24	5.48	<10	Gomes,2009
LSD	1.22	0.15	2.7		

*** means with the same letter are not significantly different at $p< 0.05$

4.2.5. Values of pH After Mixing and Inoculation (mean ± SD)

The pH value of the substrate in the anaerobic digester influences the growth of methanogenic microorganisms. Thus, the pH values of all substrates in the digester were recorded before digestion as shown in Table 13. As we can see from Table 6 before inoculation, the mean pH values of Coffee Pulp alone, Coffee husk alone and Cow dung alone were 5.72 ± 0.17, 5.65 ± 0.10 and 6.85 ± 0.05 respectively. However, after mixing the substrates in different proportions and inoculating, the mean pH of the all treatments (T1, T2, T3, T4 and T5) were 6.42 ± 0.15, 6.25 ± 0.13, 7.2 ± 0.12, 6.95 ± 0.13 and 7.0 ± 0.18 respectively.

Table 13: pH values after mixing and inoculation (mean ± SD)

Treatment	pH	Recommended value	Reference
CD	7.20 ± 0.12^{a}	6.0 - 8.0	Thy *et al*., 2003
CP:CD	6.95 ± 0.13^{b}	6.0 - 8.0	Thy *et al*., 2003
CH:CD	7.00 ± 0.18^{ab}	6.0 - 8.0	Thy *et al*., 2003
CP	6.42 ± 0.15^{c}	6.0 - 8.0	Thy *et al*., 2003
CH	6.25 ± 0.13^{c}	6.0 - 8.0	Thy *et al*., 2003
CV	2.11	<10	Gomes, 2009
LSD	0.21		

*** means with the same letter are not significantly different at $p < 0.05$

The result showed addition of distilled water, inoculation and co-digestion increases the pH value to the optimum range. One of the reasons for the increase in pH for inoculated treatments was addition of inoculum containing high ammonia concentration. In other word, as indicated in Table 8 the pH of inoculum was 7.8 which indicate the presence of high ammonia in the inoculum. Therefore, in addition to speeding up of the startup in the digestion process, the inoculums have good buffering capacity particularly for organic wastes (Girma *et al.*, 2004). The other reason was addition of distilled water; the pH value distilled water was 7.0 which also assisted for increasing the pH value of organic materials when mixed. The other reason was co-digestion; for example, fore co-digested treatments (T4 and T5) the pH values were 6.95 and 7.0 respectively. This was due to pH value of cow dung, in this particular research the average pH of cow dung was 6.8 which is near to neutral, when it was mixed with coffee pulp and coffee husk, the pH was increased for mixed treatments. Therefore, this result indicates the buffering capacity of co-digestion, which agrees with the idea of co-digestion of substrates such as cow dung, can be used together with lignocellulosic biomass to increase buffering capacity (Mata-Alvarez *et al*., 2014).

Thy *et al*., (2003) concluded the optimal pH for micro-organisms in anaerobic digestion is 6 - 8. However, according to Dioha, (2013) methane formation takes place within a relatively narrow pH interval of 6.5 - 8 for most methanogens. Therefore, in this investigation all treatments after mixing and inoculation were in the theoretical ranges of pH value set for better biogas production.

4.3. Amount of Biogas Production

4.3.1. Daily Biogas Production Trends

In this research biogas production was measured for about 35 days of digestion period until the gas production was ceased. The biogas production in batch condition is directly proportional to the specific growth rate of methanogenic bacteria in the bioreactor (Nopharatana *et al*., 2007). As indicated in Figure 5 at the beginning (day 1-3) the observed biogas production was very slow. This is due to the lag phase of microbial growth and adaptation of inoculums in the digester (Budiyono *et al*., 2010).

Then the observed daily biogas yield was shown progressive increasing trend from day 4 to 14 for each treatment as indicated in Figure 5. This showed the second phase called exponential phase. In the second phase there is an exponential production of biogas due to exponential increase of microorganisms and methanogenesis marks the final stage of anaerobic digestion, where accessible intermediates (small molecules) were consumed by methanogenic bacteria to produce methane (Ferry, 2010).

High peak point in biogas yield per day was seen in 14th day. Then later from day 15 to 34 progressive and regular decrease in biogas production was shown. This progressive and regular decline in biogas production was due to decline phase of microbial growth caused by the depletion of necessary nutrients in the substrate in the digester, the increase in ammonium concentration that resulted in an increased pH values to inhibit digestion and the gradual death of the methanogens (Castillo *et al.*, 1995; Hansen *et al.*, 1998). In day 35 all treatments showed zero biogas production except cow dung which showed zero biogas production at 31th day, this is due to low content of lignocellulosic content in cow dung (Li *et al*., 2011). Generally even though, the production of biogas it was low in days 1-3, the gas production starts from the second day. This early starting of biogas production confirmed that substrates didn't inhibit the microbial community activity in the inoculums during the setup of the experiment. The Figure 5 showed the progressive increase and decrease of daily biogas production in 35 days of biogas production.

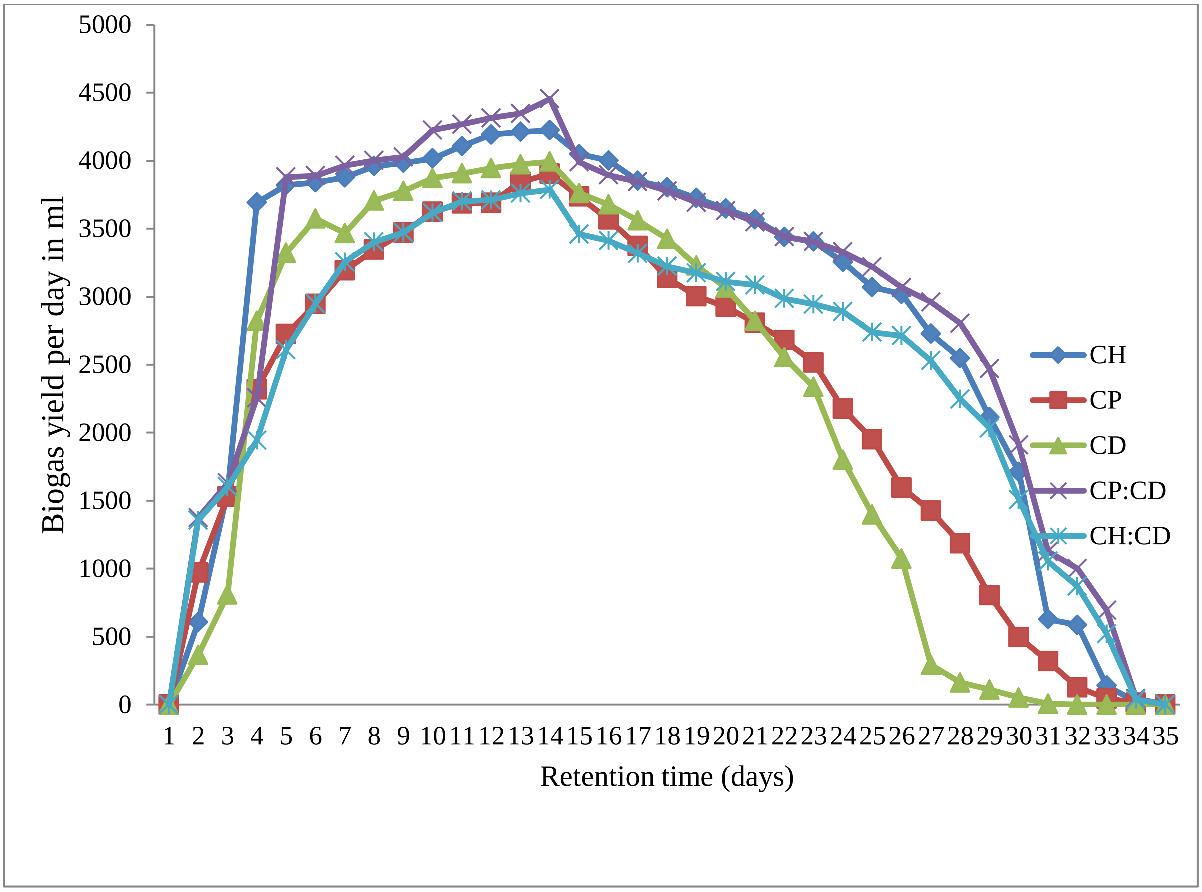

Figure 5: Daily biogas productions trends for each treatment.

4.3.2. Average Daily Biogas Rate

Average daily biogas production rate was determined by summing daily produced biogas (cumulative biogas) and dividing it with the retention time (Elijah, 2009). As indicated in Figure 6 average daily biogas production from coffee pulp (T1), coffee husk (T2), cow dung (T3), 1:1 ratio of coffee pulp with cow dung (T4) and 1:1 of coffee husk with cow dung (T5) was 2858.53, 2203.72, 2339.13, 2916.56 and 2487.88 ml/day respectively, from 320 grams of total solid for every 35 days. In all treatments average daily biogas rate showed significant difference at the $p<0.05$. High biogas production per day was observed from co-digestion of coffee pulp with cow dung and low daily average biogas production was recorded from coffee husk. This low production of coffee husk was due presence of tannins and caffeine inhibitors in coffee husk (Hendriks, 2009).

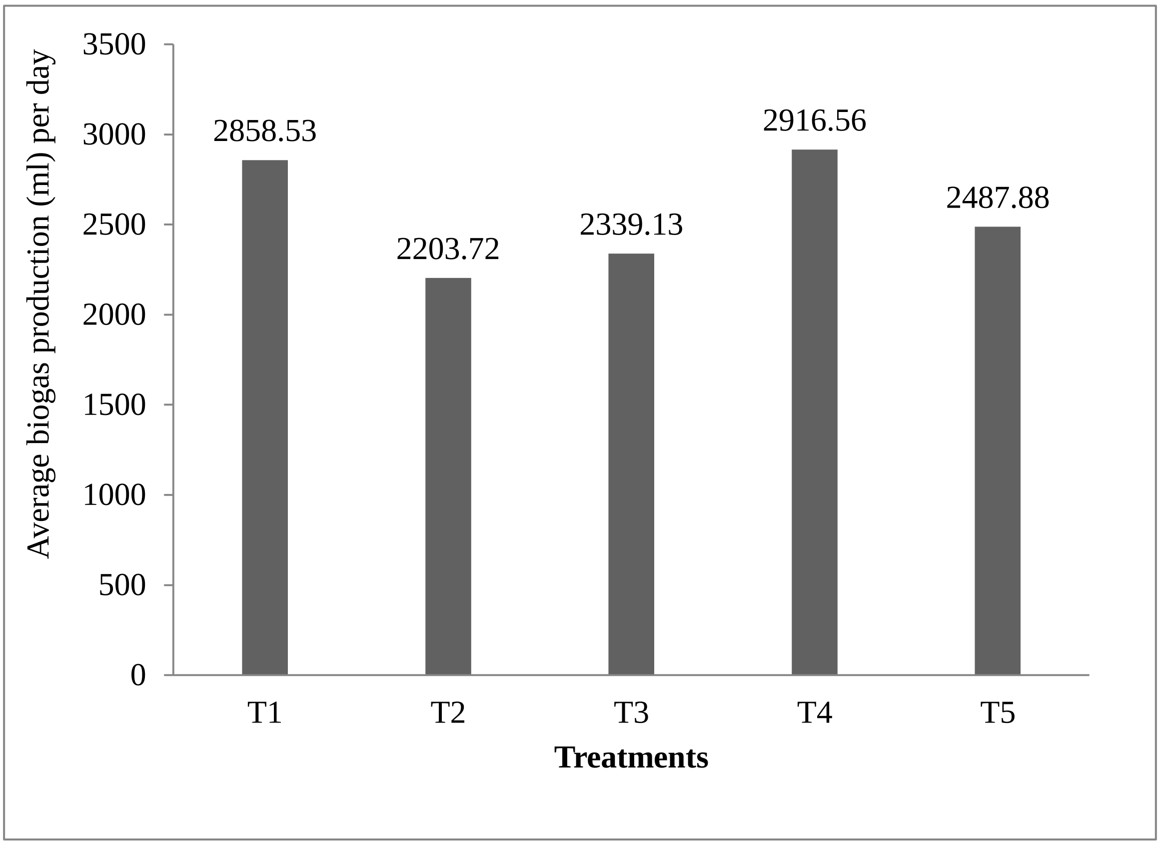

Figure 6: Average daily production of biogas in all treatments

4.3.3. Average Biogas Production in Terms of Volatile Solid

As shown in Table 14 in averages, 1:1coffee pulp with cow dung, coffee pulp, 1:1coffee husk with cow dung, cow dung, and coffee husk gave 372.33 ± 5.0, 349.88 ± 2.65, 320.07 ± 3.54, 291.43 ± 5.07 and 268.61 ± 4.16 ml/g VS of biogas respectively. In this case also high biogas was recorded in co-digestion types than sole substrates. That is comparatively mixture of cow dung to coffee pulp produced more biogas (372.33 ml/g VS) than cow dung alone (291.43ml/g VS) and coffee pulp alone (349.88 ml/g VS). Also mixture of cow dung to coffee husk produced more biogas yield (320.07 ml/g VS) than coffee husk (268.61ml/g VS) and cow dung alone (291.43ml/g VS). That means significance difference was observed between coffee pulp and 1:1coffee pulp with cow dung at ($P<0.05$). Also significance difference was observed between coffee husk and 1:1coffee husk with cow dung at ($p<0.05$). Therefore, it could be concluded that the higher production of biogas from the co-digestions due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (Tamirat, 2008).

The benefits of co-digesting plant materials with animal manure were first reported by Hills and Roberts (1981), by which it was found that manure could provide buffering capacity and a wide range of nutrients, while the added plant materials with high carbon content could improve the C: N ratio of the feedstock, thereby decreasing the risk of ammonia inhibition to the digestion process. So, this indicates that co-digestion of substrates maximizes biogas production which agrees with this current research result.

Even though, C: N ratio of coffee pulp was high, this not declined the production of biogas (Mihret *et al.*, 2016). Fresh coffee pulp alone was generated relatively the highest biogas production in comparison to cow dung. This is due to as indicated in Table 11 relatively there is high percentage of volatile solid content in the coffee pulp (89.5%) than cow dung (80.3%). High VS content of organic materials can produce high volumetric biogas than organic materials with low content of volatile solids (Moller *et al*., 2004). The other reason for low gas production from cow dung was due to low amount of cellulose, if pretreated, which has high energy content. On the other hand, coffee pulp has high amount of cellulose. Therefore, pretreated coffee pulp produces more biogas than cow dung (Grisel *et al*., 2013). Even if, the biogas yield of cow dung was lower than coffee pulp; cow dung is a common feedstock for production of biogas in Ethiopia. This is because in cow dung essential microorganisms were already presented, that used biogas production without adding inoculum. The other reason is availability of cow dung and absence of inhibitors in cow dung which may not need further pretreatment but coffee pulp needs pretreatment.

The minimum biogas production was recorded in digestion of coffee husk alone. This is due to coffee husk contains some amount of inhibitors like tannin and caffeine that inhibit the microbiological activities, which may affect the production of biogas yield (Hendriks, 2009). Conclusively, all observed treatments were between in the range 130 – 600ml/g of biogas production for different bio waste reported by (Bilhate *et al.,* 2018).

Table 14: Average biogas production from all in current research (ml/g .VS)

Sr.no	Treatments	Biogas yield ml/g .VS
1	CP:CD	372.33^{a}
2	CP	349.88^{b}
3	CH:CD	320.07^{c}
4	CD	291.43^{d}
5	CH	268.61^{e}
6	CV	1.34
7	LSD	6.3

*** means with the same letter are not significantly different at P<0.05

Coefficient of variance (CV) is Statical measure that helps to measure relative variability of a given data series. It is generally expressed in percentage. In this current investigation CV for biogas production was 1.34%. This low value was indication of high precision of the data. According to Gomes (2009), the higher CV indicates the greater level of dispersion around the mean. CV<10% is very good that is high precision, CV = 10-20% is good that is good precision, 20-30% is acceptable meaning low precision, and CV>30% is not acceptable (Gomes, 2009). Therefore, the current research CV values were in more acceptable ranges.

4.4. Quality of Biogas

Biogas is mainly composed of methane, carbon dioxide and low amount of other gases. The quality of biogas mainly depends on the amount of methane content. In other word, a good quality biogas has high percentage of methane. According to Liu, (2003) the methane ratio also depends on substrate composition, temperature and pH.

As indicated in the Table 15, the methane content of coffee pulp and coffee husk were 57.1% and 52% respectively. These results were nearly the same with previous research work on coffee husk (51.5%) and coffee pulp (56.8%) reported by (Bilihate *et al.*, 2018). The methane content of treatment cow dung was 60.02%. This observation was also similar to the previous research work on cow dung (60%) reported by (Sebola *et al.*, 2015). The methane content of coffee pulp with cow dung and coffee husk with cow dung were 59.2% and 58.4%. These shows next to cow dung; 1:1 of coffee pulp with cow dung and 1:1 coffee husk with cow dung were contained relatively high amount of methane. This might be associated with the synergetic effect of co-digestion on the quality of biogas produced. For instance, in different studies co-digestion has been investigated for different organic wastes: such as wheat straw with dairy and chicken manure Wang *et al*., (2018), rice straw with kitchen waste and pig manure (Ye *et al*., 2013). These studies report also showed higher methane yields (approximately 200 - 400 mL/g VS) compared with when using straw alone (120 - 200 mL/g

VS), as a consequence of the higher energy content of the co-digestion materials and their complementary properties.

The percentage composition of CO_2, O_2 and H_2S for coffee pulp from total volume of biogas were 42.3, 0.5, 0.08% and for coffee husk 47.1, 0.4, 0.08, respectively. The percentage composition of CO_2, O_2 and H_2S for cow dung was 38.5, 0.35 and 0.02 respectively. And also from total volume of biogas the percentage composition of CO_2, O_2 and H_2S for coffee pulp with cow dung were 40, 0.3 and 0.05, and also for 1:1coffee husk with cow dung 40.7, 0.24, 0.05, respectively.

Generally, all feed stocks results obtained in this finding were in agreement with theoretical range (50-70%) of methane content, (30-50%) carbon dioxide content, (0-2%) oxygen content, (0-1%) hydrogen sulfide content in biogas composition cited by (Yitayal, 2011). The amount of hydrogen sulfide present in biogas is usually should be below the toxic level of 0.08% (Volkmar, 2018). In current research work the obtained result of H_2S for all treatment was less than 0.08%. Therefore, the values were in acceptable range for H_2S in the biogas composition.

Table 15: Potential and quality of biogas for each treatment (Mean ± SD)

Sr. no	**Treatments**	**Biogas (ml/g VS)**	**CH_4 (%)**	**CO_2 (%)**	**O_2 (%)**	**H_2S (%)**
1	CP	349.88 ± 2.65	57.1	42.3	0.50	0.08
2	CH	268.61 ± 4.16	52.0	47.1	0.40	0.08
3	CD	291.43 ± 5.07	60.0	38.5	0.35	0.02
4	CP:CD	372.33 ± 5.08	59.2	40.0	0.30	0.05
5	CH:CD	320.07 ± 3.54	58.4	40.7	0.24	0.05

Where, CH_4 = Methane, CO_2 = Carbon dioxide, H_2S = Hydrogen sulfide and O_2 = Oxygen

4.5. Plant Nutrient Values of the Slurry

Bio-slurry is the byproduct of biogas. One advantage of anaerobic digestion is the use of the slurry as organic fertilizer. Different studies show that bio-slurry improves soil physical properties such as bulk density, hydraulic conductivity and moisture retention capacity (Yu *et al*, 2010). It is also inexpensive, does not pose any health hazard which is ecofriendly to the nature and produce better agricultural crops (Rabiul, 2016). Therefore, in this current research macronutrients and pH of the bio-slurry were analyzed in order to predict its use as plant nutrient.

4.5.1. Nutrient and pH Values of the Bio-slurry

The composition of bio-slurry depends upon several factors: the kind of feedstock (animal, human, or other feed stocks), water, and ages of the animals, types of feed and feeding rates (Devarenjan *et al.*, 2019). The macro-nutrients coffee pulp alone, coffee husk alone, cow dung alone, 1:1 ratio of coffee pulp with cow dung and 1:1 ratio of cow dung with coffee husk) were shown in Table 16.

The bio-slurry analysis result of present study showed that macronutrients such as: nitrogen, phosphorous and potassium in coffee pulp were 1.95%, 0.2%, and 1.25%, respectively. The result was with previous work reported by (vander vossen, 2004) that was 2% of nitrogen, 0.2% of phosphorus and 1.25% of potassium for coffee pulp bio-slurry.

The bio-slurry analysis result of nitrogen, phosphorous and potassium in coffee husk were 1.8%, 1.7%, and 1.3%, respectively. This result was in line with previous work reported by (Tekele, 2014); 1.7853% nitrogen, 1.75% phosphorus and 1.32% potassium for coffee husk bio-slurry. In current investigation in coffee husk bio-slurry, the amount of P and K were higher than other treatments. This was due to some times more nutrients from feed stocks retain in the digester because of the presence of inhibiting factors (Moller and Muller, 2012).

The observed nitrogen, phosphorous and potassium values for bio-slurry of cow dung were 2% nitrogen, 1.5% Phosphorous and 1.125% potassium. The amount of nutrients: nitrogen, phosphorus and potassium were in the range of previous report by (SNV, 2011); that was 1.1 - 2% for P and 0.89 - 1.2% for K. However, nitrogen content was lower than previous report cited by (Devarenjan *et al.*, 2019) which was 2.5% of Nitrogen for cow dung slurry.

The bio-slurry analysis result for 1:1 coffee pulp with cow dung of nitrogen, phosphorous and potassium were 2.1%, 0.21%, and 1.2%, respectively and for 1:1coffee husk with cow dung the bio-slurry analyzed result of nitrogen, phosphorous and potassium were 1.9%, 0.4%, and 1.0%, respectively.

Table 16: Values of macronutrients and pH in bio-slurry

Sr. no	Treatments	Composition of nutrients			pH
		% TN	P%	K %	
1	CP	1.95	0.2	1.25	7.3 ± 0.08
2	CH	1.8	1.7	1.3	7.3 ± 0.02
3	CD	2.25	0.22	0.49	7.7 ± 0.10
4	CP:CD	2.1	0.21	1.2	7.4 ± 0.70
5	CH:CD	1.9	0.4	1.0	7.5 ± 0.90
6	CV	1.1	1.6	2.4	1.07

Where, % N = percent of nitrogen, %P = percent of phosphorus, % K = percent of potassium

In current research work the amount of total nitrogen of all treatments was higher in bio-slurry than fresh feed stocks. This is because of the breakdown of organic matter during anaerobic digestion; organically bounded nutrients are mineralized in to directly available form. Most clearly, anaerobic digestion tends to increase the content of immediately available N in the form of ammonia-N (NH_4-N) (Moller and Muller, 2012).

Another factor for determining the slurry as bio-fertilizer is pH value. As indicated in Table 16, the pH of bio-slurry of cow dung, 1:1 coffee husk with cow dung, 1:1coffee pulp with cow dung, coffee pulp and coffee husk were determined and it was found that 7.7, 7.5, 7.4, 7.3 and 7.3 respectively. The values of treatments were in between the minimum and maximum accepted values of 7.3 and 9.0 respectively (Moller and Muller, 2012).

Conclusively, the analyzed results showed that bio-slurry of all observed treatments contains considerable amount of plant macronutrients and suitable pH value. Therefore, the bio-slurry of coffee husk, coffee pulp, cow dung and their co-digestions can be used as a good organic bio-fertilizer.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusions

Organic wastes especially agricultural and industrial wastes constitute an environmental pollutant, a threat to public health. However, these resources can be converted into useful products, like biogas which can contribute to alleviate the problems of rural and urban energy requirement. Therefore, the main aim of the present study was to produce biogas from coffee husk, coffee pulp and their co-digestion with cow dung and analyze the nitrogen, phosphorous and potassium value of the biogas by product, the bio-slurry to be used as organic fertilizer.

The biogas production potential of 1:1coffee pulp with cow dung, coffee pulp, 1:1coffee husk with cow dung, cow dung and coffee husk were 372.33 ± 5, 349.88 ± 2.65, 320.07 ± 3.54, 291.43 ± 5.07 and 268.61 ± 4.16 ml/g .VS, respectively. The result indicates that coffee pulp, coffee husk and their co-digestions could be used as a feedstock for biogas production at mesophilic temperature. The result also indicate 1:1 ratio of coffee pulp with cow dung produce high amount of biogas than coffee pulp alone, and coffee husk with cow dung produce high amount of biogas than coffee husk alone. On the other hand, next to cow dung the percentage composition of methane in biogas from co-digestion was higher than that of biogas from coffee husk and coffee pulp. The same is true for macronutrients of bio-slurry. Thus, it is revealed that mixing cow dung with coffee pulp and coffee husk enhances the biogas yield, its quality and plant nutrient values. In general, the analyzed biogas quality from all type of feed stock in the present study was in the ranges of theoretical standards. Also nitrogen, phosphorous and potassium value of bio-slurry of all treatments in this research encourages to be used as organic fertilizer. Therefore, coffee husk, coffee pulp and their co-digestion with cow dung can serve as alternative feedstock for biogas production and the bio-slurries can be used as organic fertilizer.

5.2. Recommendations

Upon the result of the present study, the following recommendations are drawn:

- In this investigation co-digestion of coffee husk and coffee pulp with cow dung was done 1:1ratio only. Therefore, the effect of co-digestion in different ratio should be studied.
- This investigation was done at mesophilic temperature (37^{0}C) only but it should be carried out at different temperatures like thermophilic conditions; and also using pre-treatments with different chemicals and physical agents.
- In this study investigation of macronutrients was done in laboratory level only. Further studies (field scale testing) required to understand the effect of bio-slurry on soil improvement and on yield of crops.
- For further investigation of bio-slurry quality, micro-nutrient content of coffee pulp, coffee husk, cow dung and their co-digestion should be studied.

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7. APPENDIX

Appendix Figure 1: Sample collection

Appendix Figure 2: Sample preparation (grinding and sieving)

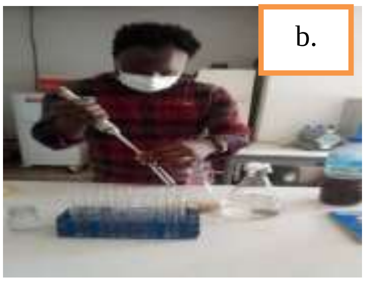
Appendix Figure 3: Analytical balance for measuring of samples

Appendix Figure 4: Physiochemical analyses of samples in oven and Furnaces

Appendix Figure 5: Measuring PH value for samples

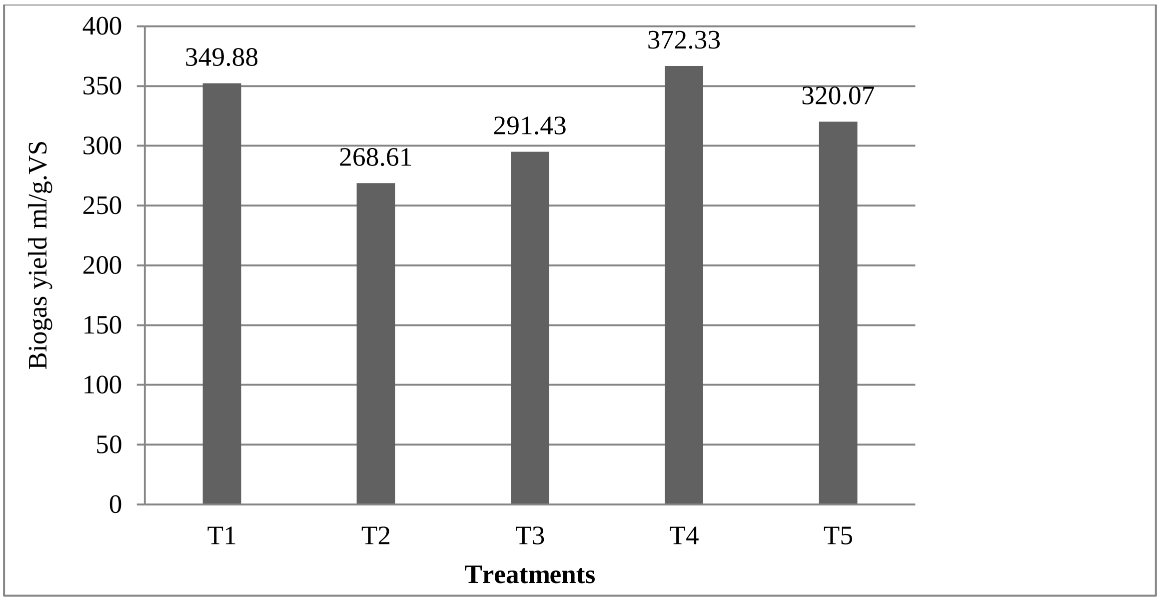
Appendix Figure 6: Kjeldahl methods (titration for nitrogen analysis)

a.



b.

Appendix Figure 7: Microbial analysis (a. inoculum collection, b. serial dillution, c. culturing inoculum and d. observation)



Appendix Figure 8: Average biogas yields ml/g VS for all treatments

Appendix Table 1: Raw data for percentage composition of feedstock's Parameters before mixing and adding water

Samples	Parameters								
	Rep	%MC	%TS	%VS	%FS	%C	%N	C:N	PH
Coffee pulp	1	8.8	91.2	89	11	48.6	1.02	47.65	5.7
	2	8.7	91.3	87.5	12.5	48.8	1.04	46.92	5.9
	3	8.8	91.2	88	12	49.2	1.08	45.55	5.8
	4	8.7	91.3	87.5	12.5	48.9	1.02	47.94	5.5
Coffee husk	1	7.4	92.6	89	11	49.44	1.4	35.3	5.6
	2	7.2	92.8	89	11	49.44	1.2	41.2	5.8
	3	7.3	92.7	89	11	49.44	1.2	41.2	5.6
	4	7.1	92.9	91	9	50.55	1.3	38.9	5.6
Cow dung	1	78	22	80	20	44.44	2.3	19.32	6.8
	2	79	21	80	20	44.44	2.1	21.16	6.9
	3	81	19	79	21	43.88	2.3	19.08	6.9
	4	78	22	78	22	43.33	2.1	20.63	6.8

Appendix Table 2: Data for characteristics of feed stocks after mixing and inoculation

		Parameters							
Trt	Rep	%MC	%TS	%VS	%FS	%C	%N	%C:N	PH
	1	91.9	8.1	88	12	48.88	1.03	47.46	6.3
	2	92.1	7.9	89	11	49.44	1.04	47.54	6.6
	3	91.8	8.2	89	11	49.44	1.05	47.1	6.5
T1	4	92.2	7.8	92	8	51.11	1.04	49.14	6.3
	1	91.95	8.05	89	11	49.44	1.3	38	6.1
	2	92.05	7.95	90	10	50	1.2	41.66	6.2
	3	91.96	8.04	89	11	49.44	1.2	41.2	6.4
T2	4	92	8.0	91	9	50.55	1.3	38.88	6.3
	1	92.04	7.96	80.4	19.6	44.66	2.3	19.41	7.1
	2	92.05	7.95	80.1	19.9	44.50	2.1	21.19	7.3
	3	91.98	8.02	80.5	19.5	44.72	2.3	19.44	7.1
T3	4	91.97	8.03	80.2	19.8	44.55	2.1	21.21	7.3
	1	91.97	8.03	87	13	48.33	1.87	25.84	6.9
	2	91.99	8.01	85	15	47.22	1.86	25.38	6.8
	3	91.98	8.02	88	12	48.88	1.83	26.71	7
T4	4	91.99	8.01	84	14	46.66	1.84	25.3	7.1
	1	91.99	8.01	87	13	48.33	1.5	32.22	6.8
	2	91.92	8.08	86	14	47.77	1.8	26.54	7.2
	3	92	8.0	84	16	46.67	1.9	24.67	7.1
T5	4	91.98	8.02	83	17	46.11	1.6	28.82	6.9

Appendix Table 3: ANOVA Table for moisture content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	0.03	0.08	0.11	< 0.97	0.09
Error	15	0.12	0.07			
Total	19	0.15				

Where, DF= degree of freedom, SS=sum square, MS= mean square and CV= coefficient of variance

Appendix Table 4: ANOVA Table for total solid content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	0.72	0.18	0.91	< 0.93	5.5
Error	15	2.99	0.2			
Total	19	3.72				

Where, DF= degree of freedom, SS=sum square, MS= mean square and CV= coefficient of variance

Appendix Table 5: ANOVA Table for volatile solid content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	238.96	59.74	28.4	< .0001	1.69
Error	15	31.85	2.12			
Total	19	270.81				

Where, DF= degree of freedom, SS=sum square, MS= mean square and CV= coefficient of variance

Appendix Table 6: ANOVA Table for fixed solid content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	239.32	58.83	33.43	< 0.0001	9.7
Error	15	26.85	1.79			
Total	19	266.17				

Where, DF= degree of freedom, SS=sum square, MS= mean square and CV= coefficient of variance

Appendix Table 73: ANOVA Table for carbon content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	73.73	18.43	28.15	< 0.0001	1.7
Error	15	9.82	0.65			
Total	19	83.55				

Where, DF= degree of freedom, SS=sum square, MS= mean square and CV= coefficient of variance

Appendix Table 84: ANOVA Table for nitrogen content after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	3.47	0.86	86.14	< 0.0001	6.24
Error	15	0.15	0.01			
Total	19	3.6				

Where, DF= degree of freedom, SS = sum square, MS = mean square and CV= coefficient of variance

Appendix Table 9: ANOVA Table for C: N ratio after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	2010	502.6	157.09	< 0.0001	5.48
Error	15	47	3.19			
Total	19	2058				

Where, DF= degree of freedom, SS= sum square, MS = mean square and CV = coefficient of variance

Appendix Table 10: ANOVA Table for pH after mixing

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	2.64	0.65	32	< 0.0001	2.11
Error	15	0.3	0.02			
Total	19	2.94				

Where, DF= degree of freedom, SS= sum square, MS = mean square and CV = coefficient of variance

Appendix Table 11: ANOVA Table for biogas yield ml/g VS production

Source of variation	DF	SS	MS	F-value	Pr. >F	CV
Treatment	4	26719	6679	360	< .0001	1.34
Error	15	277	18			
Total	19	26996				

Where, DF= degree of freedom, SS= sum square, MS = mean square and CV = coefficient of variance

Appendix Table 12: Laboratory result for total nitrogen of bio-slurry

Trt	V. blank	V. acid	Wt. of sample	N H2SO4	TN	TN average
T1R1	0.26	28.60	2	0.1	1.98	
T1R2	0.26	27.60	2	0.1	1.91	
T1R3	0.26	28.20	2	0.1	1.96	
T1R4	0.26	27.80	2	0.1	1.93	1.95
T2R1	0.26	26.00	2	0.1	1.80	
T2R2	0.26	26.50	2	0.1	1.84	
T2R3	0.26	25.70	2	0.1	1.78	
T2R4	0.26	25.80	2	0.1	1.79	1.80
T3R1	0.26	32.50	2	0.1	2.26	
T3R2	0.26	32.00	2	0.1	2.22	
T3R3	0.26	32.10	2	0.1	2.23	
T3R4	0.26	32.80	2	0.1	2.28	2.25
T4R1	0.26	30.50	2	0.1	2.12	
T4R2	0.26	31.00	2	0.1	2.15	
T4R3	0.26	29.70	2	0.1	2.06	
T4R4	0.26	30.00	2	0.1	2.08	2.10

T5R1	0.26	27.00	2	0.1	1.87	
T5R2	0.26	28.00	2	0.1	1.94	
T5R3	0.26	27.00	2	0.1	1.87	
T5R4	0.26	27.70	2	0.1	1.92	1.90

Where, TR indicates treatments and their replication