EVALUATION OF BIOGAS PRODUCTION POTENTIAL FROM ANAEROBIC CO-DIGESTION OF KHAT (*Catha edulis*) AND FOOD WASTE WITH COW DUNG IN JIMMA TOWN, SOUTHWEST ETHIOPIA

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RESEARCH PAPER SUBMITTED TO DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY, PUBLIC HEALTH FACULTY, INSTITUTE OF HEALTH SCIENCES, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCES AND TECHNOLOGY

OCTOBER, 2017

JIMMA, ETHIOPIA

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Declaration

I, the undersigned, declare that this research paper is my original work and has not been presented for the award of a degree in any university and all the sources of materials used for this thesis have been properly acknowledged.

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Abstract

Biogas is produced by bacteria through the bio-degradation of organic material under anaerobic conditions. The aim of this study was to evaluate biogas production potential from anaerobic co-digestion of khat (*Catha edulis*) and food waste with cow dung. Khat waste, food waste, and cow dung were used for this study. Khat waste samples were collected from khat markets of Jimma town, whereas; food and cow dung were collected from Jimma University College of Agriculture and Veterinary Medicine. Ten treatments with different mixing proportions were conducted from khat waste, food waste, and cow dung. These all treatments were evaluated for their potential of gas production. The gas produced was measured by water displacement method until it stopped to produce any more gas. The CH_4 content in the biogas was determined by allowing the gas to pass through a CO_2 absorption solution. The physico-chemical parameters were determined in a laboratory-scale bench digester for a total period of 41 days at a mean temperature of 22.5 ± 3 °C monitored by Styrofoam. The mean, standard error of the mean for average biogas yield and methane percentage of the triplications of the ten treatments, and correlation statistics at 5 and 1 % significant level, respectively were carried out using SPSS software. The results in this study indicated that the mean biogas produced in ascending order was T3 (5526 mL), T4 (5855 mL), T9 (6003 mL), T8 (6020 mL), T6 (6218 mL), T7 (6375 mL), T10 (6423 mL), T1 (6823 mL), T6 (7310 mL) and T5 (8565 mL) per 100g fresh mass sample. T5 produced the highest biogas yield. This may be because of its optimum C/N ratio (25:1) and synergistic effects of organic matter in terms of provision of nutrient for the growth of methanogenic bacteria. This study helps to improve energy shortage and is a very promising way to overcome the problem of energy demand and waste treatment of our country (Ethiopia).

Keywords: Anaerobic digestion, Biogas, Cow dung, Injera, Khat

Acknowledgement

From the very beginning, I would like to thank the Almighty God for providing me the staying power and survival to carry out my thesis work. I express my deepest appreciation to my advisors Mr. Dessalegn Dadi and Mr. Wondwossen Birke for their constructive comments, support and sound advice in all aspects of my research paper. I am really appreciative their objective and tolerant attitude, as well as creating very pleasant working conditions. My gratitude also goes to Jimma University, Department of Environmental Health Sciences and Technology staffs and laboratory technicians for allowing me the chance to conduct this thesis work. Lastly but not the least, my great thank from the bottom of my heart goes to my wife Tigist Olana and my son Friwak Mekonnen, who helped me in all what I need and supported me to be successful in my study.

Acronyms /abbreviations

AD – Anaerobic Digestion

ANOVA – Analysis of Variance

ASTM – American Society of Testing and Materials

CD – Cow Dung

CSA – Central Statistical Agency

FW – Food Waste

KW – Khat Waste

MC – Moisture Content

OLR – Organic Loading Rate

SPSS – Statistical Package for Social Science

SRT – Solids Retention Time

TS – Total Solid

USDA –United States Department of Agriculture

VS – Volatile Solid

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CHAPTER ONE: INTRODUCTION

1. 1. Background of the study

Biogas is an alternative and renewable energy source produced through anaerobic digestion of organic matter whereby the organic matter is converted into a combustible biogas rich in methane and a liquid effluent. Biogas consists of 55 to 80% methane and 20 to 45% carbon dioxide, small amounts of other gases such as ammonia (NH_3), hydrogen sulfide (H_2S), and water vapor. The rate of biogas production varies with different conditions and parameters like temperature, stirring; feed concentration, catalyst concentration and pH. Biogas is produced using solid wastes containing organic matters (Dhanalakshmi and Ramanujam, 2012).

There are many resources ready to serve the mankind on demand. Out of the many renewable options, wind, solar and biomass energies are considered as the major sources. Biogas technology is a very attractive way to utilize biomass sources for fulfilling partial energy requirements. Biogas system can provide multiple benefits to the users and aid to protect the environment. Biogas systems produce biogas through anaerobic digestion of organic materials (Deepanraj *et al.*, 2014). The outlook of turning organic wastes into biogas through a low-cost process has certainly increased the interest around this technology and has required several studies aimed to develop methods that could improve the performance as well as the efficiency of this process (Esposito *et al*, 2012).

Solid waste management is a critical issue all over the world. The problem is more sever in developing countries especially, fast growing towns and cities of developing countries (Venkateswarlu *et al*., 2015). The estimated quantity of Municipal Solid Waste generated worldwide is between 1.7–1.9 billion metric tons making cities a threat to the environment. It is also expected to increase to approximately 2.2 billion metric tonnes per year by 2025 while, in sub-Saharan Africa is approximately 62 million tonnes per year (US EPA, 2002). Millions of tons of wastes are generated each year from agricultural, municipal, and industrial sources. For example, in 2001, a total of 229.2 million tons of solid waste was produced in the United States (US EPA, 2002).

Inadequate management of solid waste in most cities in the tropical areas leads to pollution of the atmosphere, soil, and ground water due to GHG emissions and toxic leachate (Sharholy *et al.*, 2008;

Horaginaman *et al*., 2011). Rapid growth of population and uncontrolled urbanization has created serious problems of energy requirement and solid waste disposal (Hailu, 2010).

In today's fast-growing world, the rate of energy consumption is rising at unexpected rates with each passing day. Ethiopia has also part of this global trend particularly over the last decade. To meet its growing energy requirements, the country has been investing hugely in developing its hydroelectric power generating capacity from water source like Gilgel Gibe dam, Abay River dam, Fincha dam and the like. Besides, the country also relies substantially on the fuel it imports to meet its energy demand. Development of renewable and sustainable energy source is the best solution to the country's energy demands (Deressa *et al.*, 2015). The raise of solid waste generation, continually rising petroleum cost and the fast addition of GHGs into the atmosphere is a global problem that has to be solved soon. Waste materials can have an economic benefit if managed accordingly in addition to protection of the environment from pollution (Moghadam & Mokhtarani, 2009).

Using fossil fuels alone as primary energy source results global climate change, environmental degradation and human health problems. Moreover, the recent rise in oil and natural gas prices may derive the current economy toward alternative energy sources such as biogas (Sunarso *et al*., 2012). Ethiopia produces plenty of solid wastes (Hailu, 2010). A variety of agricultural, industrial and domestic wastes can be digested by microorganisms anaerobically. The process can tackle both energy recovery and pollution control (Muzenda, 2014).

One of the biomass wastes, which have the potential to supplement SW is khat waste (CSA, 2002). In Ethiopia, khat is being grown for sale not only in its traditional areas in Hararghe but in Jimma, Shashemene, Sidamo, Kembata, Gurage, and even as far as Debre Libanos, Gojam and Tigray. Total land in khat in 2004/05 was over 120 thousands hectares and was higher by 8% from the preceding years (CSA, 2002).

1.2. Statement of the Problem

As predicted by Aremu and Agarry (2013) the world will have 9 to 10 billion people in the year 2040 which generate waste and must be provided with energy and materials. This rapid growth of population and uncontrolled urbanization cause energy crisis, generate millions of tons of wastes, shortage of final waste disposal and increase environmental and public health problems. The recent rise in oil and natural gas prices and problems connected with using fossil fuels are also the other problems of our globe.

The most prominent issues in Ethiopia's domestic energy sector include; heavy reliance on biomass fuels, by tradition relatively high domestic energy consumption, low level of renewable energy and/or energy efficiency technology, energy demand in most areas significantly exceeds the supply. As a result_ fuel wood is over-harvested in many areas, contributing to deforestation of already ecologically sensitive areas; fuel wood and charcoal have been and are rapidly becoming more expensive; households and large institutions cope by substituting fuel wood with dung cake and agricultural residue (Getachew *et al.*, 2006).

The need for ultimate source of energy is a vital issue of the present day in Ethiopia. This is because of the international energy crises that follows the extraordinary price growth of hydrocarbon fuel in the international market and environmental issues that are found in Ethiopia where people are highly dependent on burning of bio fuels (i.e. firewood, charcoal and cow dung) for house hold purposes. Although the country has abundant energy resources, its potential is not yet well developed due to lack of capacity and investment. As a result, all of the needed fossil fuel and gas is imported from abroad, and its fuel wood resource exploited unsustainably, which led to deforestation and hence land degradation in the country. The proper design and implementation of biogas technologies can boost socio-economic development and address environmental concerns. Based on their implementation in the past, these technologies have not always been successful in rural Ethiopia (Gerardi, 2003).

Numerous studies have been conducted by several researchers in order to produce biogas from biomass using anaerobic digestion. Indeed, biogas production from different organic materials like cow dung with rice husk (Iyagba *et al.,* 2009), cattle manure with rumen fluid inoculums (Budiyono *et al*., 2010), cow dung with poultry litter (Animut, 2013), Corn-Cob with Waste Paper (Aremu and Agarry, 2013), cattle slurry with maize stalk (Adebayo *et al.*, 2014), fruit and vegetable wastes mixed with different wastes (Deressa *et al*., 2015) and others were performed. However, no research has been conducted in the production of biogas from the mixture of khat *(Catha edulis)* and food waste co-digesting with cow dung. To fill this gap, biogas production potential of khat and food waste co-digested with cow dung was conducted in this study.

1.3. Significance of the study

One requirement for sustainable development is the availability of adequate energy services for satisfying basic needs, improving social welfare, and achieving economic development. Dependence on oil imported from foreign countries affect the national energy securities and energy security of global economies has become one of the most challenging problem that needs to be resolved as the fossil sources are fast diminishing and irreplaceable. The alarming energy demand and consumption rate of the present global status is currently exponentially exceeding the rate of local supply sources, becoming an issue of concern.

Consequently, the challenge of energy for sustainable development will require uninterrupted effort on the part of international organizations, national governments, the energy community, civil society, the private sector and individuals. Turning solid biomass to liquid and gas by efficient and greener technologies is essential in order to minimize environmental pollution, land degradation and social welfare. Thus, the new concept for treating khat and injera left over with cow dung anaerobically to produce biogas- a clean renewable alternative energy with many applications is more realistic option.

According to this study, biogas plants are successfully utilized to displace woody fuels and dung in our country (Ethiopia). As a result of this, the output of this research may help the community for many purposes. The main benefits are: production of energy for lighting, heat, electricity, improved sanitation (reduction of pathogens, worm eggs and flies), reduction of workload (less firewood collecting), environmental benefits (fertilizers substitution, less greenhouse gas emission) and create job opportunity for those who will engage in biogas production. This study also helps the decision makers (EPA and Policy formulating bodies) in obtaining waste disposal option, saving the environment and budgets spend for non-renewable energy sources.

CHAPTER TWO: LITERATURE REVIEW

2.1. Biogas Technology

Biogas originates in the process of bio-degradation of organic material under anaerobic conditions from archebacteria. The natural generation of biogas is an important part of the biogeochemical carbon cycle. Methanogens (archebacteria) are the last link in a chain of microorganisms which degrade organic material and return the decomposition products to the environment as a source of renewable energy (Rai, 2010).

Biogas systems use anaerobic digestion to recycle organic waste, turning it into biogas, for energy (the gas), and valuable soil products (liquid and solids), using a natural, biological process. After simple processing, biogas is a renewable substitute for natural gas, and the digested materials, the liquid and solids can be turned into a wide variety of useful soil products, similar or identical to peat moss, pellets and finished compost. Organic wastes are manure from dairies, sludge filtered from wastewater, municipal solid waste, food waste, yard clippings, crop residues and more (Benjamin, 2004; USDA, 2014).

2.2. Co-digestion

Co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics, being one of the main advantages of the anaerobic technology (Fernandez *et al*, 2005). Recent works on co-digestion have been showed that there is synergism effect among the co-digested substrates. For instance, the work of Sosnowski *et al.*, (2003) showed that optimization of the carbon to nitrogen ratio was found when municipal wastes and sewage sludge are co-digested. The improvement of the buffer capacity is also reported as a positive effect in the co-digestion process by Mshandete *et al*., (2004).

Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. Traditionally, anaerobic digestion was a single substrate, single purpose treatment. Recently, it has been realized that AD as such became more stable when the variety of substrates applied at the same time is increased. The most common situation is when a major amount of a main basic substrate is mixed and digested together with minor amounts of a single, or a variety of additional substrate

(Braun, 2002). The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Mata-Alvarez et al., 2000).

2.3. Anaerobic Digestion

Anaerobic digestion is a process of controlled decomposition of biodegradable materials under managed conditions where free oxygen is absent, at temperatures suitable for naturally occurring mesophilic or thermophilic anaerobic archaea species, that convert the inputs to biogas (Fantozzi and Buratti, 2009).

2.4. Stages of anaerobic digestion

Anaerobic digestion is a complex process consisting of a mixed biological system in which organic materials such as carbohydrates, lipids, and proteins are utilized by microorganisms in their normal metabolic activities. It occurs in a basic biological and chemical step of anaerobic digestion as a result of the activity of a variety of microorganisms (Rai, 2010).

2.4.1. Hydrolysis

The first stage in the anaerobic digestion process is hydrolysis. In this stage, the complex organic polymers are converted into simple soluble molecules. The lipids (fats) are converted into fatty acids, carbohydrates (polysaccharides) into simple sugars (monosaccharides) and proteins into amino acids (Fantozzi and Buratti, 2009). Hydrolysis stage is carried out by different groups of facultative or obligate fermentative bacteria through excreting extracellular enzymes (Jingquan, 2002). Lipases convert lipids to long-chain fatty acids, proteases converts proteins to amino acids and the polysaccharides such as cellulose, starch and pectin are hydrolyzed to monosaccharides by cellulases, amylases and pectinases (Fricke *et al.*, 2007).

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{Hydrolysis}} n\,(C_6H_{12}O_6)$$

2.4.2. Acidogenesis

In the second stage, the soluble compounds produced through hydrolysis are converted into volatile fatty acids (C_1-C_5), H_2, CO_2, ethanol and some organic nitrogen and sulfur compounds (Khoiyangbam *et al.*, 2011). The acids produced in this stage are acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$) and valeric acid ($CH_3CH_2CH_2CH_2COOH$) (Moghadam and Mokhtarani, 2009). The acetic acid formed in this stage is directly taken to last stage and the other products are taken to third stage for further degradation by acetogens.

$$n(C_6H_{12}O_6) \xrightarrow{\text{Acidogenesis}} n(CH_3COOH)$$

2.4.3. Acetogenesis

Third stage of anaerobic digestion process is acetogenesis. In this stage, the volatile fatty acids having more than two carbon atoms (from acidogenesis stage) are converted into acetic acids, hydrogen and carbon dioxide with the help of acetogens (Khanal, 2008).

$$n(C_6H_{12}O_6) \xrightarrow{\text{Acetogenesis}} n\,(CH_3COOH)$$

2.4.4. Methanogenesis

In the last stage, the methanogenic bacteria (methogens) produce methane by consuming acetic acid, hydrogen and some carbon dioxide. Around 66 % of methane is formed from acetic acids by means of acetate decarboxylation and remaining 34% of methane is formed from carbon dioxide reduction (Nayono, 2009).

$$CH_3COOH \xrightarrow{\text{Methane forming bacteria}} CH_4 + CO_2$$

$$CO_2 + 4H_2 \xrightarrow{\text{Reduction}} CH_4 + 2H_2O$$

Also, CO_2 can be hydrolyzed to carbonic acid and methane

$$CO_2 + H_2O \xrightarrow{\text{Hydrolysis}} H_2CO_3$$

$$4H_2 + H_2CO_3 \xrightarrow{\text{Reduction}} CH_4 + 3H_2O$$

2.5. Parameters affecting anaerobic digestion process

There are many factors that affect the performance of anaerobic digestion. Some of the factors which are having major influence in anaerobic digestion are elaborated below.

2.5.1. Temperature

Temperature is the most important parameter to be considered in anaerobic digestion. Different species of methanogens function optimally in three different temperature ranges: 45–60°C thermophilic, 20–45°C mesophilic and below 20°C psychrophilic. The rate of biogas production increases with an increase in temperature. In biogas digestion process, only mesophilic and thermophilic temperature ranges are considered important because anaerobic digestion reaction essentially stops below 10°C. The bacteria available for digestion process are sensitive to temperature fluctuation, so, it is necessary to maintain a constant temperature. Thermophilic bacteria are more efficient in terms of retention time, loading rate and gas yield, but they need higher heat input and are also sensitive to temperature fluctuations and environmental variables than mesophilic (Fantozzi and Buratti, 2009).

2.5.2. Solid to water content

Water and raw material should be added together to generate slurry with required consistency. Production of biogas is inefficient if the slurry is too dilute or too thick. The optimum solid concentration may vary from 7- 25% depending on the type of raw material used (Abbasi *et al.*, 2013). Sewage waste contains very low solid content and so optimum level can be achieved by adding solid matters like crop residues, weed plants etc.

2.5.3. pH

The optimum pH value of the anaerobic digester is at 6.7 to 7.59 (Deublein and Steinhauser, 2008). The pH value will not be constant throughout the process. The volatile fatty acids production rate is much higher than the methane production rate, resulting in pH value below the optimum range and can inhibit methanogens, because they are very sensitive to acid conditions (Fantozzi and Buratti, 2009). Reduction in pH can be controlled by the addition of chemicals such as sodium carbonate, sodium bicarbonate, gaseous ammonia, ammonium hydroxide, lime, potassium and sodium hydroxide (Khanal, 2008).

2.5.4. Retention period

The time period for which the organic material remains inside the digester for biogas generation is known as retention period. The retention period depends on the type of feedstock and the temperature used (Rai, 2010). Solids retention time (SRT) and hydraulic retention time (HRT) are the two significant retention times in anaerobic digestion process. SRT refers the time that bacteria (solids) remain inside the digester. HRT is commonly used to denote substrate retention time. It is the time spent by the input slurry, inside the digester from the instant of its entry to its exit (Abbasi *et al*., 2012).

2.5.5. Organic loading rate

Organic loading rate (OLR) is an important parameter which affects the biogas production in anaerobic digestion, particularly when the digestion takes place in continuous flow mode (Abbasi *et al*., 2012). OLR is a measure of biological conversion capacity of the anaerobic digestion system. It can be expressed as the amount of raw material (kg of volatile solids) fed to the digester per unit volume per day (Fantozzi and Buratti, 2009). Overloading easily affects the digestion process due to accumulation of acids. The optimum loading rate is in between 0.5 kg and 2 kg of total volatile solids per unit volume of the digester per day which can be chosen based on type of raw material, retention time and the process temperature. Higher loading rates are recommended only in cases where mean ambient temperature is high (Khoiyangbam *et al*., 2011).

2.5.6. Carbon/ Nitrogen ratio

The relationship between the amount of carbon and nitrogen present in the raw materials is represented by the C/N ratio (Shefali, 2002). The carbon-nitrogen (C/N) ratio is one of the important factors in the production of biogas. The elements of carbon (in the carbohydrates) and nitrogen (in the form of proteins, ammonia nitrates) are the major food for anaerobic bacteria (Rai, 2010).

The consumption of carbon by bacteria is 30 times faster than the nitrogen consumption. Therefore, for optimum rate, the availability of carbon in the substrate should be 20- 30 times higher than nitrogen (i.e. C/N ratio between 20 and 30). If the C/N ratio is high, then rapid consumption of nitrogen by methogens takes place and results in lower bio-gas production. Lower C/N ratio leads to ammonia accumulation and pH values exceeding 8.5 which are toxic to methogens (Gizachew, 2011). To maintain the optimum C/N ratio in the digester, substrates of high C/N ratio can be co-digested with lower C/N ratio substrates. The effect of C/N ratio of various feeds on biogas production showed that C/N ratio of 26:1 gives maximum biogas yield compared to others (Fricke *et al.* , 2005).

2.5.7. Mixing/agitation

Mixing or agitation is required in the digester to maintain homogeneity and process stability (Kaparaju *et al.*, 2008). Mixing helps to combine the fresh incoming material with microorganisms and prevents from thermal stratification and scum formation in the digester. Mixing maintains uniformity in substrate concentration, temperature and other environmental factors. Also, it prevents solid deposition at the bottom of the digester (Karim *et al*., 2005). Mixing can be done either by using mechanical stirrers or by recirculation of the digester slurry using centrifugal pumps (Nayono, 2009).

2.5.8. Pretreatments

Pretreatment could be done by various techniques like mechanical, thermal, chemical, biological pretreatment (Fang *et al.*, 2011). Mechanical pretreatments such as milling and ultrasonic pretreatments are used in anaerobic digestion process. Milling is used to reduce the particle size of the feedstock before taken into the digester. Particle size is one of the important parameter which plays significant role in the biogas production. Smaller particle size leads to increase the substrate utilization because smaller particle size provides increased microbial activity (Nayono, 2009). Biogas production can be increased when the organic and inorganic compounds in the feedstock are partially solubilized during thermal pretreatment (Bougrier *et al*., 2008; Mudhoo, 2012).

During thermal pretreatment, the organic and inorganic compounds in the feedstock are partially solubilized before hydrolysis which reduces digester volume and increases the biogas production (Mudhoo, 2012). Chemical pretreatment technique includes acid, alkaline, oxidation and ozonolysis pretreatment. Application of these chemical pretreatments results in higher solublization and biodegradation of cellulose, hemicelluloses and lignin, which are the main components of biomass (Fang *et al*., 2011).

In anaerobic digestion process, increase in biogas production and higher calorific value of the gas can be achieved by applying pretreatments to substrate (Yadvika *et al.*, 2004; Fang *et al.*, 2011). Pretreatment breaks down the complex structure of organic compounds into simpler molecules and make them more susceptible to microbial degradation (Yadvika *et al*., 2004). According to Yadvika *et al*., (2004), out of five particle sizes (0.088, 0.40, 1.0, 6.0 and 30.0 cm), maximum quantity of biogas was produced from raw materials of 0.088 and 0.40 mm particle size.

2.5.9. Toxicity effects

Mineral ions, heavy metals and detergents are some toxic materials that inhibit the normal growth of microorganisms in the digester. Small quantity of mineral ions (e.g., sodium, potassium, calcium, magnesium, ammonium and sulphur) also stimulates the growth of bacteria, while very high concentration of these ions leads to toxic effects. For example, presence of NH_4^+ from 50 to 200 mg/L stimulates the growth of anaerobic microbes, whereas, concentrations above1500 mg/L produces toxicity. Similarly, heavy metals such as copper, nickel, chromium, zinc, lead etc., in small quantities are essential for the growth of bacteria but their higher concentrations has toxic effects. Detergents including soap, antibiotics, organic solvents etc. also inhibit the activity of methane producing bacteria and hence addition of these substances in the digester should be avoided (Martı´nez *et al.*, 2002).

2.5.10. Seeding

To start up a new anaerobic process, it is critical to use inoculums of microorganisms to commence the fermentation process. The common seeding materials include digested sludge from a running biogas plant or material from swage (Martı´nez, 2002). Sunarso *et al.*, (2012) states that inoculums caused biogas production rate and efficiency increase more than two times in compare to substrate without inoculums. It was reported that 30 % inoculums concentration is most suitable for anaerobic treatment (Pathak and Srivastava, 2007).

CHAPTER THREE: OBJECTIVES OF THE STUDY

3. 1. General Objective

The general objective of this study was to evaluate the biogas production potential from anaerobic co-digestion of khat (*Catha edulis*) and food waste with cow dung in Jimma town, Southwest Ethiopia

3.2. Specific objectives

- To characterize the physico-chemical parameters (total solid, volatile solid, pH, C/N ratio, temperature, organic carbon, and moisture content) of the substrate AD
- To find out the best biomass mix for gas production
- To determine methane content of the biogas

CHAPTER FOUR: METHODS AND MATERIALS

4.1. Study Area and Period

The study was conducted at laboratory of Jimma University College of Agriculture and Veterinary Medicine, Ethiopia, which is located 350 km south-west of Addis Ababa (capital of Ethiopia). It's geographical coordinates are approximately 7° 41′ N latitude and 36° 50′ E longitude. Jimma is found in an area of average altitude of about 1,780 m above sea level. The mean annual maximum temperature is 30 °C, and the mean annual minimum temperature is 14 °C (FDRE, 1998). Data collection and experiments was conducted during June and July months of 2017. In general, the study period was 41 days.

4.2. Sample collection

Sample was collected by primary collection system, collection of waste from the source of generation. Waste was picked up by hand and collected in clear plastic by wearing glove and the collected waste was transported to the study site. Three different types of wastes were considered for this study: khat left over, food wastes/left over and cow dung. Khat left over samples were collected from khat markets of Jimma town where khat was regularly chewed (Merkato and Becho Bore khat market in this study) and the required quantity of food left over (injera left over) was collected from Jimma University student cafeterias, whereas; cow dung samples were collected from dairy farm of Jimma University College of Agriculture and Veterinary Medicine. To avoid contamination, all samples were collected before having contact with any other type of waste.

4.3. Sample preparation

The co-digestion experiments were conducted in bench mode in 2L plastic digester with 3 different substrates (khat left over, food left over and cow dung) prepared in ten different mix ratios. The substrates were prepared separately and labeled as treatment (T) 1 to 10 made in the following proportion. The proportion of mixed waste was modified based on the study conducted by Iyagba *et al.* (2009) in Nigeria and Adebayo *et al.* (2014) in Germany. Accordingly;

T1 = [100 % KW] (Khat Waste only)

T2 = [75 % KW] + [25 % FW] (3:1)

T3 = [75 % KW] + [12.5 % FW] + [12.5 % CD] (6:1:1)

T4 = [50 % KW] + [50 % FW] (1:1)

T5 = [50 % KW] + [25 % FW] + [25 % CD] (2:1:1)

T6 = [37.5 % KW] + [37.5 % FW] + [25 % CD] (3:3:2)

T7 = [25 % KW] + [75 % FW] (1:3)

T8 = [25 % KW] + [50 % FW] + [25 % CD] (1:2:1)

T9 = [12.5 % KW] + [75 % FW] + [12.5 % CD] (1:6:1)

T10 = [100 % FW] (Food Waste only)

As suggested by Balsam (2002), Rai (2004), Muryanto *et al*. (2006), and Budiyono *et al.* (2010) substrates were mixed with appropriate amount of tab water and inoculums to achieve the recommended 7-9 % total solids content in the fermentation slurry for better biogas production. The amount of total solids used in this study was 100 g considering the total volume of the digester (Tadesse, 2014). The total amount of liquid (tab water and inoculums) added to the digester was then determined by the following formula.

$$Y = \frac{mTS - 8\ \%\ X}{8\ \%}$$

Where, mTS = mass of total solids (100g)

X = mass of fresh substrate

Y = mass of fluid

It was reported that 30 % inoculums concentration is most suitable for anaerobic treatment (Pathak and Srivastava, 2007). For this study, the anaerobic sludge was collected from the anaerobic reactor at Jimma Degitu Hotel and 375 mL for one digester with triplicate seed sludge was added to start up the anaerobic digestion. Then, by fixing the amount of inoculums (375 mL in this study considering the total volume of the substrate) that was added finally to facilitate digestion, the amount of tab water added was then determined using the following formula;

Z= Y-375

Where; Z = amount of tab water

Y = total amount of liquid

Treatments were randomly arranged in the laboratory and done in triplicates. Initial pH values were also maintained within the pH range of 6.7 to 7.59 for optimal biogas production (Yadvika *et al*., 2004). The proportions of the substrates added in the digesters (total amount of liquid and fresh sample) with three replicates were arranged based on the above formula in Table 1 below assuming 1g is equivalent to 1mL.

Table 1: The main contents of each digester

Treatments	X (g)				Y (mL)			(X+Y) (mL)
	KW	FW	CD	Total (X)	Water (Z)	Inoculums	Total (Y)	
T1	333.3	0.0	0.0	333.3	541.7	375	916.7	1250
T2	250.0	71.4	0.0	321.4	553.6	375	928.6	1250
T3	250.0	35.7	70.3	356.0	519.0	375	894.0	1250
T4	166.7	142.8	0.0	309.5	565.5	375	940.5	1250
T5	166.7	71.4	140.6	378.7	496.3	375	871.3	1250
T6	125.0	107.2	140.6	372.8	502.2	375	877.2	1250
T7	83.3	214.3	0.0	297.6	577.4	375	952.4	1250
T8	83.3	142.9	140.6	366.8	508.2	375	883.2	1250
T9	41.7	214.3	70.3	326.3	548.7	375	923.7	1250
T10	285.7	0.0	0.0	285.7	589.3	375	964.3	1250

4.4. General procedures and Setup of the experiment

Anaerobic digesters were generally constructed in bench-scale experiments where biogas was produced out of the degradation of organic matter in 2L plastic digester with appropriate working volume. One tube was taken through the stopper which acts as an outlet for the gas and closed airtight. Thirty bottles were taken for the experiment with triplications. The lids of all digesters were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas. After measuring the initial pH values of all the digesters, the pH values was arranged in the range of 6.7 to 7.59 by adding buffer solution to obtain optimum pH value as recommended by Deublein and Steinhauser, (2008)

(Table 2). The amount of air initially present in the empty bottle was sacked by syringe after substrate feeding and sealing the digester to maintain anaerobic digestion as shown in Figure 1 below.

Figure 1: Sacking air by syringe to maintain anaerobic digestion

The temperature fluctuation of all the digesters were controlled at ±3 ^{0}C using Styrofoam having 3cm thickness to control maximum temperature fluctuation. The amount of gas produced was measured by downward water displacement method using 90 % NaCl solution (Yetilmezsoy *et al.*, 2008) as illustrated in Figure 2B below. The daily gas production was measured and recorded for all treatments until the gas production stops.

Figure 2: A: Triplicate arrangement of digester, B: Determination of biogas by water displacement method

4.5. Measured parameters

4.5.1 Total Solids (TS)

The total solid content of substrates were determined using the standard method as reported by (Sluiter *et a.l*, 2008). 10 g of freshly collected samples of each of khat waste, food waste and cow dung were weighed using electrical balance, and placed inside an electric hot air-oven maintained at 105 °C using a crucible (see Figure 3 below).

Figure 3: Drying samples using hot air oven

The crucible was allowed to stay in the oven for 24 hours, then taken out, cooled in a desiccator and weighed. Then the percentage of the TS was calculated as:

$$\% \, TS = \frac{M \, dry}{M \, fresh} \times 100$$

Where: M_{fresh} = fresh mass and

M_{dry} = dry mass after heating at 105°C for 24 hrs using a hot air oven

4.5.2. Volatile Solids (VS)

Volatile solid content of the feedstock is one of the factors in the biogas production (Macias-Corral *et al.*, 2008). Volatile content of the raw material was determined by drying the samples at 550 ^{0}c furnace for three hours. Then, the mass of ash was weighed to determine VS content of the substrate (see Figure 4 below).

Figure 4: A. Drying samples using furnace, B. Remaining mass after ignition

The following formula was employed to calculate the percentage of volatile solids content of the TS.

$$\% VS = \frac{M\,dry - M\,ash}{M\,dry} \times 100$$

Where, M dry = dry mass and

M ash = remaining mass after ignition at 550 ^{0}c furnace for 3hrs.

4.5.3. Moisture content (MC)

Moisture content is the amount of water evaporated from the sample. To determine the percentage of moisture content in the samples, 10 g of each fresh substrate was dried in an oven at 105 °C for 24 hours and reweighed. Then the moisture content in percent was determined by using the following formula based on American Society of Testing and Materials (ASTM, 2001).

$$\% MC = \frac{Wi - Wf}{Wf} \times 100$$

Where; MC = moisture content

Wi = initial weight of sample in grams,

Wf = final weight of sample after drying at 105 °C in grams.

4.5.4. Organic carbon (OC)

The total organic carbon content was obtained from percentage of volatile solids data using an empirical equation as reported by Barrington *et al.* (2002).

$$\% OC = \frac{\% VS}{1.8}$$ Where, VS - Volatile solid, OC - Organic carbon

4.5.5. Determination of total nitrogen (crude protein)

The Kjeldahl procedure was employed to analyze the total nitrogen content of the feed stocks (Gerardi, 2003). 0.3g dried samples of each of samples (khat, food and cow dung) were placed on a digestion tube with 15 mL of concentrated sulfuric acid (Figure 5). 7 g of each potassium sulphate and copper were then added. The digestion tube was placed into a digestion block where it is heated at 370 °C for 4hrs. Sodium hydroxide was then added to change ammonium ion to ammonia in the digestate, and the nitrogen was separated by distilling the ammonia and collecting the distillate in 0.1 N sulfuric acid solutions.

Figure 5: Digestion tube filled with sample

Determination of the amount of nitrogen on the condensate flask was done by titration of the ammonia with a standard solution of 0.1N sodium hydroxide in the presence of methyl red indicator in 0.1 N sulfuric acid solutions. Finally, the amount of nitrogen present was calculated using the formula:

$$\% \text{ N} = \frac{[1.4007 \text{ x} (\text{Va}-\text{Vb}) \text{ x N}]}{\text{W}}$$

Where, Va: volume of acid used for sample titration

Vb: volume of acid used for the blank

N: Normality of acid

W: sample weight in grams

1.4007: conversion factor mill equivalent weight of nitrogen and N percent

$$\% \text{ CP} = \% \text{ N} \times \text{F}$$

Where, CP= Crude Protein

F = conversion factor (F =6.25 for all forages)

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

$$\text{Carbon to nitrogen ratio} = \frac{\% \text{ carbon}}{\% \text{ nitrogen}}$$

4.5.6. Substrate pretreatment

Particle size is one of the important parameter which plays significant role in the biogas production. In AD process, the substrate (khat and food left over) were pretreated to increase biogas production and calorific value of the gas as reported by Yadvika *et al.*(2004) and Fang *et al.*(2011) (Figure 7). Pretreatment breaks down the complex structure of organic compounds into simpler molecules and make them more susceptible to microbial degradation (Yadvika *et al.*, 2004). Pretreatment was done by mechanical pretreatment using grinder (Fang *et al.*, 2011). Grinder was used to reduce the particle size of the feedstock before taken into the digester. The size of the sample solid waste to be used in the experiment was reduced manually to approximately 0.5– 0.75 cm and placed into ten laboratory-scale anaerobic bench digesters set up for this experiment. Smaller particle size leads to increase the substrate utilization because smaller particle size provides increased microbial activity (Nayono, 2009).

Khat waste food waste cow dung

Figure 6: Collected wastes for the study

Khat waste Food waste Cow dung

Figure 7: Pretreated wastes ready for the study

4.6. Determination of biogas and methane yield

The gas produced was measured by water displacement method (Yetilmezsoy *et al.*, 2008) (Fig.2). The total biogas from each treatment was measured until it stopped to produce any more gas. The biogas produced was collected to gas bag by syringe to separate CH_4 from biogas (Figure 8).

Figure 8: Collecting biogas for methane determination

Then, the methane content in the biogas was determined by allowing the gas to pass through a CO_2 absorption solution. Then, the CO_2 in the biogas was removed by CO_2 absorption reactor illustrated in Figure 9 below and the remaining biogas was measured (almost CH_4). CO_2 absorption solution was prepared from 3M of 1L NaOH solution, 4mL of 99.5% ethanol, 1mL of water and 20 mg of phenolphthalein. Finally, the methane quality was determined (Shealy & Nuber, 2007). It was reported that the major proportion (i.e., 73 %) of biogas was methane while other gases constitute a minor fraction of the total (Barik *et al.*, 2013).

Figure 9: CO2 Absorption column

4.7. Data Analysis

Fishers Least Significant Difference was used to investigate statistical significance between the different treatments, whereas paired samples T-test was used to investigate statistical significance within a treatment. The mean, standard error of the mean for average biogas yield and methane percentage of the ten treatments, and correlation statistics at 5 and 1 % significant level, respectively were carried out using SPSS software. In addition, the biogas yield and the percentage of methane yield of all treatments were compared in line graph and bar chart to show the optimum gas production.

CHAPTER 5: RESULTS AND DISCUSSION

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

The Physico-chemical characteristics of khat waste, food waste and cow dung mixed in different proportions were determined before and after AD and the results are discussed below.

Table 2: Comparison of pH, percentage of total solid and moisture content between before and after AD of the various substrates (values were mean ± SE, n = 3)

Treatments	pH			Total solid (%)		Moisture content (%)	
	Initial	Adjusted	Final	Initial	Final	Initial	Final
T1	6.73 ± 1.00	6.73 ± 1.00	7.95 ± 0.10	30.00 ± 1.20	16.44 ± 0.00	70.00 ± 2.10	83.56 ± 1.00
T2	4.34 ± 0.10	7.23 ± 0.12	7.86 ± 0.11	31.25 ± 2.00	13.32 ± 0.10	68.75 ± 0.50	76.68 ± 0.13
T3	5.85 ± 0.12	7.30 ± 0.11	7.98 ± 0.05	29.10 ± 1.00	16.76 ± 1.00	70.90 ± 1.03	83.24 ± 0.06
T4	4.03 ± 0.09	6.74 ± 0.01	8.00 ± 0.03	32.50 ± 0.50	19.60 ± 0.23	67.50 ± 1.26	80.40 ± 0.50
T5	5.50 ± 0.02	7.12 ± 0.06	8.03 ± 0.01	28.19 ± 1.10	12.68 ± 1.00	71.81 ± 0.05	87.32 ± 2.36
T6	5.40 ± 0.00	6.86 ± 0.06	7.66 ± 0.12	28.82 ± 2.20	20.16 ± 0.05	71.19 ± 1.19	79.84 ± 2.00
T7	3.85 ± 0.15	6.95 ± 0.03	7.79 ± 0.09	33.75 ± 2.00	16.72 ± 0.10	70.00 ± 0.25	83.28 ± 1.50
T8	4.73 ± 0.06	7.00 ± 0.00	7.93 ± 0.05	29.44 ± 0.30	14.52 ± 0.09	69.31 ± 1.22	75.48 ± 1.30
T9	4.40 ± 0.00	7.10 ± 0.09	7.81 ± 0.10	32.22 ± 0.42	14.48 ± 2.00	67.78 ± 2.33	76.52 ± 2.06
T10	3.95 ± 0.16	7.10 ± 0.10	8.14 ± 0.00	35.00 ± 0.11	22.40 ± 1.00	65.00 ± 0.09	77.60 ± 0.07

Each of these results are described and discussed below.

5.1.1 pH

Experimental analysis showed that the pH of 100 % KW substrate before anaerobic digestion was 6.73 ± 1.00, whereas that of 100 % FW was 3.95 ± 0.16 (Table 2). The pH value of T1 (100 % KW) was not adjusted because it was between the optimum pH ranges of biogas production as indicated by Deublein and Steinhauser (2008), while the pH of the other treatments was not in the recommended range which requires adjustment (Table 2). The optimum pH value of the anaerobic digester is at 6.7

to 7.59 (Deublein and Steinhauser, 2008). The pH value of T2 to T10 was below 6.7 when measured initially. Reduction in pH for T2 to T10 was controlled by addition of sodium hydroxide as a buffer solution (Khanal, 2008). The pH was adjusted to 6.7 to 7.59 by adding buffer system in the digester for substrate mixtures diverging from normal pH before start of digestion in this experiment as reported by Teodorita *et al.*, (2008). Comparison of the initial, adjusted and final pH of the AD substrates was shown in Figure 10 below.

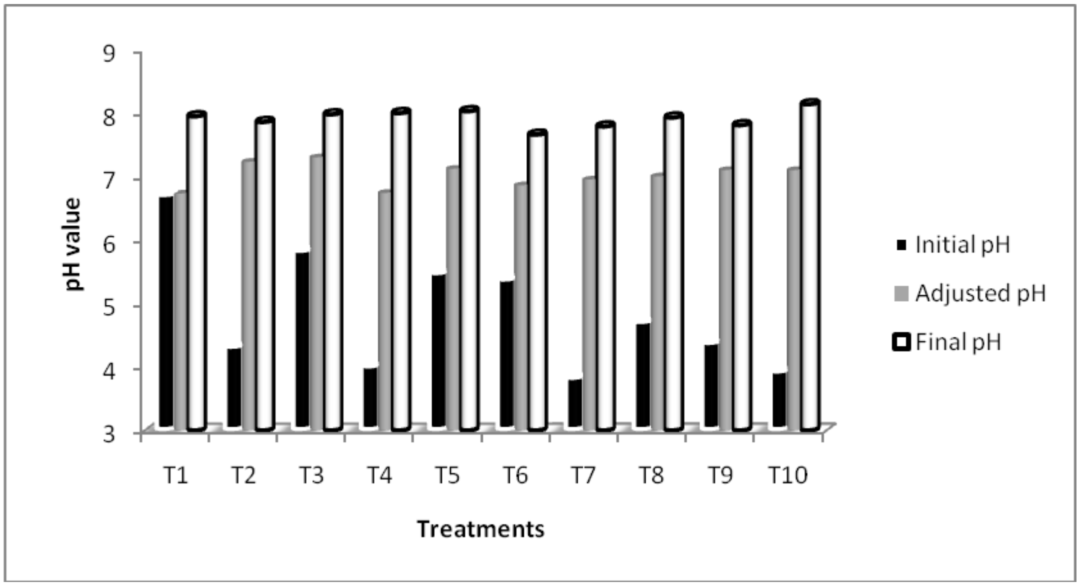

Figure 10: Comparison of initial, adjusted and final pH of the AD substrates

Significant differences were seen in pH values within treatments before and after anaerobic digestion (Paired samples T-test, $P<0.05$). The pH of the treatment was initially low, which might be due to the formation of acids by acidogenic bacteria. However, it was increasing as the time of incubation increased. This may be due to digestion of volatile acid and nitrogen compounds through methanogenic bacteria (Mahanta *et al*., 2004). Generally, the pH was between 6 and 8 (Table 2), which was suitable for most methanogenic bacteria to function since most of the methanogens grow best at the pH range of neutral environment (Deublein and Steinhauser 2008). Increase in pH value of the substrates after AD of all treatment may be attributed due to production of alkali compounds, such as ammonium ions during the degradation of organic compounds in the digester (Gerardi, 2003).

5.1.2. Total solid

The initial percentage of total solid (% TS) content of the feedstock was between 28.19 and 35 %, with the highest value for T10 and lowest value for T5 (Table 2). According to Balsam (2002), Muryanto *et* *al*. (2006), and Budiyono *et al.* (2010), the optimum solid content obtained for biogas production is in the range 7–9 %. Furthermore, Sadaka and Engler, (2003) reported that the higher solid content in feed decrease the cumulative volume of biogas production. The initial percentage of total solid content of the feedstock did not agree the recommended range of 7-9 %. Substrates were mixed with appropriate amount of tab water and inoculums to achieve the recommended 7-9 % total solids content in the fermentation slurry for better biogas production. The percentage of total solid and organic carbon of different feed stocks before and after anaerobic digestion was illustrated in Figure 11 below.

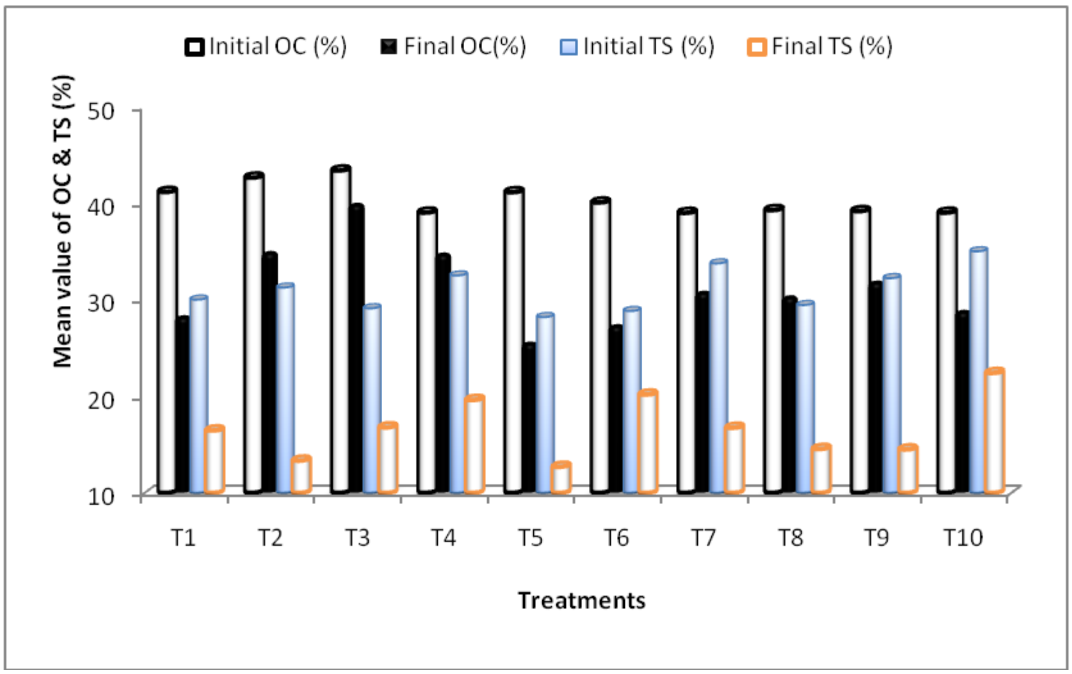

Figure 11: Comparison of the percentage of total solid (TS) and organic carbon (OC) of different feed stocks before and after anaerobic digestion

5.1.3. Volatile solid (VS)

The VS content of all the treatments was in the range of 70.16 to 78.05 % (Table 3). This VS was in agreement with the recommended value for biogas production (70–95 %) reported by Steffen *et al.*, (2000). Potential of gas production can usually be estimated from the VS loading of the digester and the percentage of VS reduction through digestion. The percentage of volatile solid and organic carbon between before and after AD of the various substrates was compared in Table 3 below.

Table 3: Comparison of % organic carbon and % volatile solid between before and after

Treatments	Volatile solid (%)		Organic carbon (%)	
	Initial	Final	Initial	Final
T1	74.07 ± 1.12	50.53 ± 1.00	41.15 ± 0.00	28.07 ± 2.02
T2	76.73 ± 2.09	62.51 ± 1.53	42.63 ± 0.62	34.73 ± 1.05
T3	78.05 ± 1.10	71.50 ± 2.03	43.36 ± 0.11	39.72 ± 0.07
T4	70.22 ± 0.05	62.23 ± 0.87	39.01 ± 0.27	34.57 ± 1.36
T5	74.02 ± 1.03	45.61 ± 0.16	41.12 ± 1.72	25.34 ± 0.17
T6	72.09 ± 0.04	48.87 ± 1.76	40.05 ± 2.30	27.15 ± 2.24
T7	70.16 ± 0.62	55.10 ± 2.00	38.98 ± 1.08	30.61 ± 1.18
T8	70.67 ± 2.08	54.25 ± 1.69	39.27 ± 2.00	30.14 ± 1.90
T9	70.45 ± 1.00	56.99 ± 2.04	39.16 ± 1.43	31.66 ± 2.33
T10	70.20 ± 0.06	51.53 ± 0.05	39.00 ± 1.62	28.63 ± 1.21

Significant differences were observed between digesters in % VS both before and after AD. After AD, VS of all substrate types were significantly decreased, but more decrease was observed in T5 and T6 than in other substrates. This suggests that mixing and optimum C/N ratio can enhance degradation and biogas production. Removal of VS after AD implies its conversion to biogas. Volatile solids destruction was a good parameter for evaluating the efficiency of anaerobic digestion (Abuabaker and Ismail, 2012).

5.1.4. Moisture content

Experimental results showed that there is significant difference in moisture content values within treatments before and after anaerobic digestion (Paired samples T-test, $P < 0.05$). The mean moisture content of partially dried substrates before anaerobic digestion was 70.00 ± 2.10, 68.75 ± 0.50, 70.90 ± 1.03, 67.50 ± 1.26, 71.81 ± 0.05, 71.19 ± 1.19, 70.00 ± 0.25, 69.31 ± 1.22, 67.78 ± 2.33 and 65.00 ± 0.09 % for T1 to T10 respectively (Table 2). It has been reported that the highest methane production rates occur at 60 to 80 % of humidity (Khalid *et al.*, 2011). The initial moisture contents did agree with the recommended range. But after anaerobic digestion the mean moisture content of T1 to T10 were

83.56 ± 1.00, 76.68 ± 0.13, 83.24 ± 0.06, 80.40 ± 0.50, 87.32 ± 2.36, 79.84 ± 2.00, 83.28 ± 1.50, 75.48 ± 1.30, 76.52 ± 2.06 and 77.60 ± 0.07 % respectively (Table 2), showed increased moisture content (water) than before. This was due to added tab water to adjust moisture contents in the digester. The percentage of volatile solid and moisture content of different feed stocks between before and after anaerobic digestion were compared in Figure12 below.

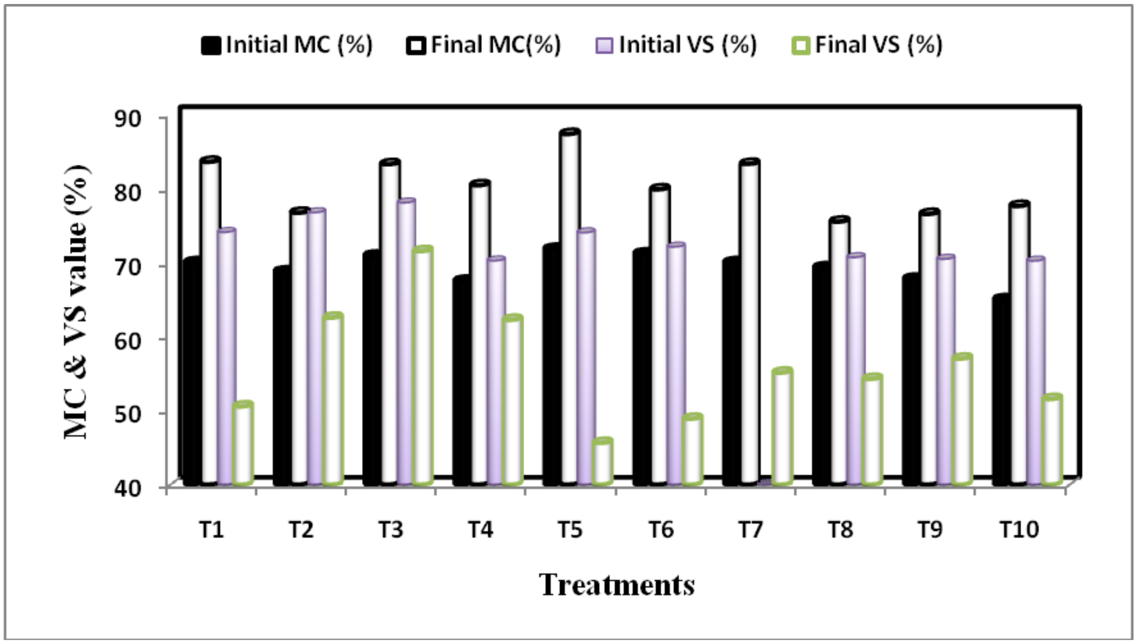

Figure 12: Comparison of the percentage moisture content (MC) and volatile solid (VS) of different feed stocks before and after anaerobic digestion

5.1.5. Organic carbon

The percent degradation of organic carbon for T5 was higher than all (from 41.12 ± 1.72 to 25.34 ± 0.17, i.e., 61.62 % reduction) (Table 3). The results also revealed that there were differences in percentage organic carbon in all mix ratios between before and after AD (paired samples-T-test, $P<0.05$). Comparison of initial and final % C showed that % C significantly decreased AD in all substrate types. Organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or biogas production (Gerardi, 2003). Therefore, the decrease in C reflects the degradation process during anaerobic digestion (Devlin *et al*., 2011). More degradation of organic carbon was observed in mixed substrates. This suggests that mixing can enhance degradation and biogas production.

5.1.6. Carbon to Nitrogen ratio

Too much increase or decrease in the carbon/nitrogen ratio affects biogas production (Gerardi, 2003; Yadvika *et al*., 2004; Deublein and Steinhauser 2008). The carbon/nitrogen ratio of the feed stocks affected the anaerobic digestion process. As a result, methane yield and its production rates were highly influenced by the balance of carbon and nitrogen in the feeding material. The C/N ratios of T1 to T10 were 26.04 ± 0.50, 27.33 ± 1.00, 28.53 ± 0.10, 23.34 ± 1.12, 25.07 ± 1.07, 24.27 ± 0.21, 22.79 ± 0.15, 21.82 ± 0.17, 21.17 ± 0.10 and 23.08 ± 1.00 % respectively (Table 4). The Carbon to Nitrogen ratio and ash content of the substrates before anaerobic digestion was illustrated in Table 4 below.

Table 4: Carbon to Nitrogen ratio and ash content of substrates before anaerobic digestion

Treatment	Initial % of C	Initial % of N	Initial % of TN	C:N ratio	Initial % of Ash
T1	41.15 ± 0.00	1.58 ± 0.00	9.88 ± 0.70	26.04 ± 0.50	4.2 ± 0.00
T2	42.63 ± 0.62	1.56 ± 0.01	9.75 ± 0.0.40	27.33 ± 1.00	1.8 ± 0.01
T3	43.36 ± 0.11	1.52 ± 0.00	9.50 ± 0.10	28.53 ± 0.10	4.6 ± 0.00
T4	39.01 ± 0.27	1.67 ± 0.03	10.44 ± 0.14	23.34 ± 1.12	2.0 ± 0.03
T5	41.12 ± 1.72	1.64 ± 0.04	10.25 ± 0.03	25.07 ± 1,07	2.2 ± 0.02
T6	40.05 ± 2.30	1.65 ± 0.07	10.31 ± 0.20	24.27 ± 0.21	2.3 ± 0.00
T7	38.98 ± 1.08	1.71 ± 0.00	10.69 ± 0.45	22.79 ± 0.15	2.1 ± 0.10
T8	39.27 ± 2.00	1.80 ± 0.08	11.25 ± 0.60	21.82 ± 0.17	1.2 ± 0.12
T9	39.16 ± 1.43	1.85 ± 0.00	11.56 ± 0.16	21.17 ± 0.10	1.9 ± 0.11
T10	39.00 ± 1.62	1.69 ± 0.01	10.56 ± 0.11	23.08 ± 1.00	1.4 ± 0.10

The C:N contents of all substrates were measured to be between 21.17 ± 0.10 and 28.53 ± 0.10 % (Table 4) which agrees with the value of (20:1 to 30:1) reported by (Arogo *et al.*, 2009). This indicates that khat waste could serve as a substrate for biogas production even without mixing it with food waste and cow dung. For the mixture treatments, the ratio was still in the range of 21:1 to 30:1. Thus, in all substrates the ratio of C: N is good for bacteria so that their combination or each alone could be used for anaerobic digestion to produce biogas.

5.1.7. Temperature

Both the mean temperature and the temperature fluctuations adversely affect the performance of a biogas digester (Fantozzi and Buratti, 2009). The day time temperature of the room where digestion took place was measured three times a day: at morning (7:00 AM), noon (1:00 PM) and dusk (7:00 PM) and the result was shown in Figure 13 below.

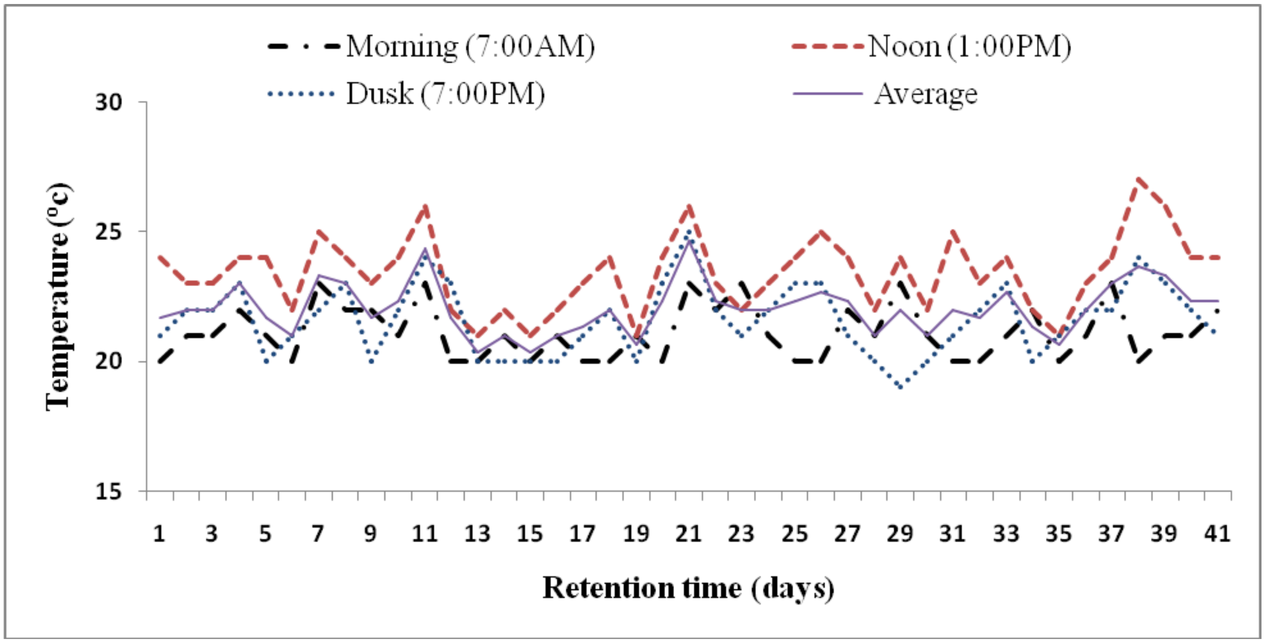

Figure 13: Daily room temperature where digestion took place

Temperature is an important factor in the biogas production. Most of the acid-forming microorganisms grow under mesophilic conditions; however, for methanogens, a higher temperature is favorable Deublein and Steinhauser (2008). Microorganisms exhibit optimal growth and metabolic rates within a well-defined range of temperatures (20–35 °C) (El-Mashad *et al.*, 2004). It is found that the minimum and maximum day time average temperatures of the study room were 20 and 24.67 °C respectively. The observed temperature of the study area was also within the range of what is considered to be suitable for digestion (Fantozzi and Buratti, 2009).

Temperature fluctuations in the thermophilic range should be no more than ± 1°C. In the mesophilic range, the microorganisms are less sensitive; therefore, fluctuations of ± 3°C can be tolerated (Deublein and Steinhauser 2008). However, it was observed that there was a maximum fluctuation of temperature (± 4.67°C) during this experiment (Figure 13), which was beyond the tolerance range for the mesophilic microorganisms (Ahn and Forster, 2002; Deublein and Steinhauser 2008). This fluctuation was minimized by using thick covering of the digesters 3cm thickness styrofoam which could bring the digesters' temperature fluctuation to less than ±3°C as recommended by NRCS, (2005).

5.2. Biogas production

The mean biogas produced in ascending order was indicated that T3 (5526 mL), T4 (5855 mL), T9 (6003 mL), T8 (6020 mL), T6 (6218 mL), T7 (6375 mL), T10 (6423 mL), T1 (6823 mL), T6 (7310 mL) and T5 (8565 mL) per 100g fresh mass sample. Gas production was noticed from day one of the experiment in all substrate types. However, the amount of biogas measured varied with substrate type; highest for T6 and lowest for T7 at day 1 (Table 5). Average values for biogas, methane and percentage of methane production for each treatment were summarized in Table 5 below.

Table 5: Triplicate average values for biogas, methane and percentage of methane production for each treatments

Treatments	Biogas produced (in days)	Mean biogas Yield (m^3)	CH_4 Yield (m^3)	%CH_4 Yield
T1	39	6.82×10^{-3}	3.67×10^{-3}	53.80
T2	36	6.22×10^{-3}	3.39×10^{-3}	54.52
T3	35	5.53×10^{-3}	2.89×10^{-3}	52.20
T4	35	5.86×10^{-3}	3.06×10^{-3}	52.28
T5	41	8.57×10^{-3}	5.17×10^{-3}	60.36
T6	37	7.31×10^{-3}	4.28×10^{-3}	58.58
T7	35	6.38×10^{-3}	3.56×10^{-3}	55.89
T8	35	6.02×10^{-3}	3.24×10^{-3}	53.74
T9	35	6.00×10^{-3}	3.28×10^{-3}	54.59
T10	35	6.42×10^{-3}	3.72×10^{-3}	57.86

The fact that gas production occurred on the first day of the experiment suggests the existence of microbes in the added inoculums to act on readily degradable materials of the substrates (Kamthunzi, 2008; Animut, 2013). Triplicate daily mean values for biogas production of each treatment in AD were shown in Figure 14 below.

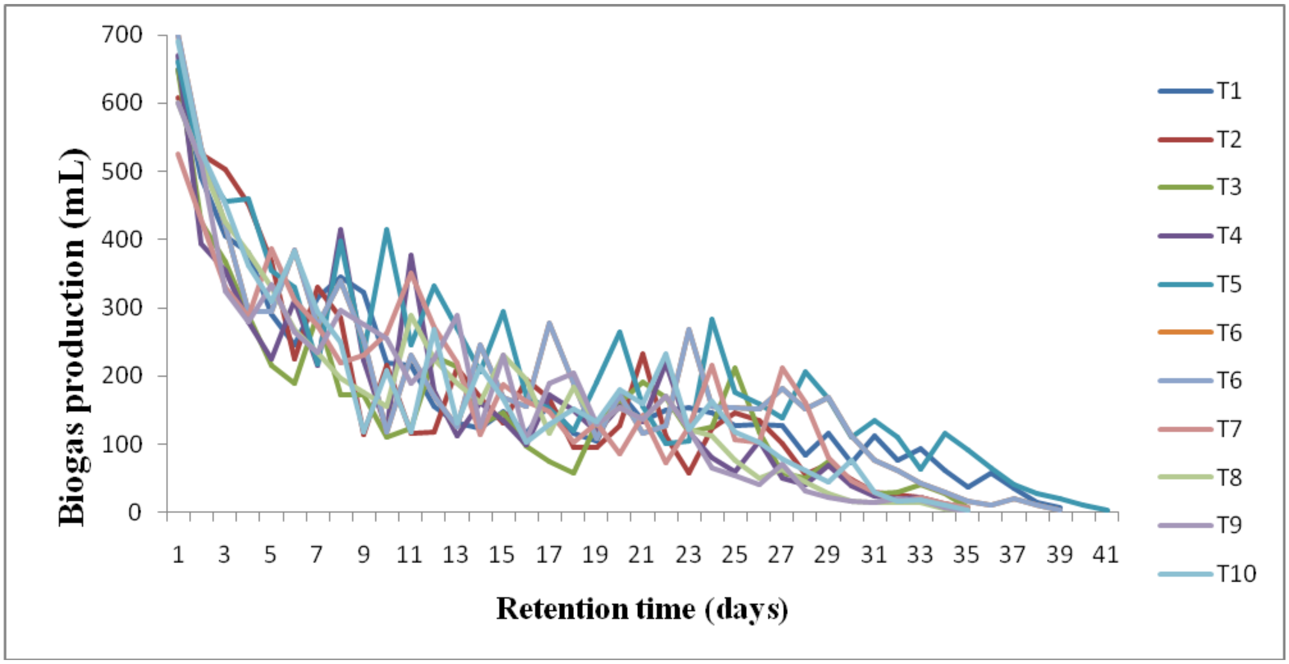

Figure 14: Daily mean biogas yield of different substrate proportion

Biogas production showed fluctuating decline after the first day of measurement and eventually reached 0 mL after fifth week of the experiment (Figure 14). The decline of biogas production may be due to depletion of readily decomposable substrate and accumulation of toxic wastes due to increasing microbial population in reverse case of depleting nutrients (decline phase of microbial) in the digester (Ahn *et al.*, 2009). For optimal performance of the microbes, the pH within the digester should be kept in the range of 6.7 to 7.59 (Deublein and Steinhauser, 2008). The results in this study indicated that the final pH values of all the digesters were above 7.66. This indicates that there is high accumulation of ammonia. The pH value below or above 6.7 to 7.59 may restrain the process in the digester since micro-organisms and their enzymes are sensitive to pH deviation (Yadvika *et al.*, 2004). There was a significant difference between the substrates in an overall biogas yield ($p < 0.05$).

All substrate types resulted in significantly higher cumulative biogas yield with the highest cumulative biogas production (8565 mL) observed in T5 and lowest (5526.67 mL) for T3. The higher gas production from T5 could be due to good C/N ratio, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (Macias-Corral *et al.*, 2008; Li *et al.*, 2009; Abayneh, 2013). Triplicate cumulative values for biogas production of each treatment in AD were shown in Figure 15 below.

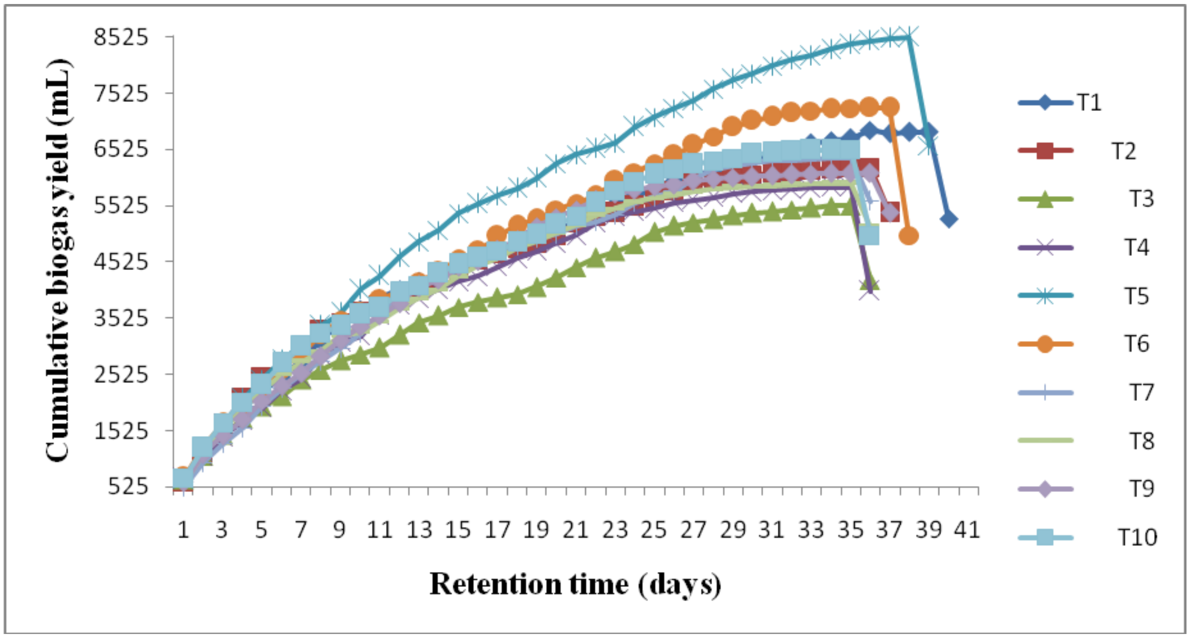

Figure 15: Triplicate cumulative values for biogas production of each treatment

The total methane content of T1 to T10 was 53.80, 54.52, 52.20, 52.28, 60.36, 58.58, 55.89, 53.74, 54.59 and 57.86 % per 100g fresh sample, respectively (Table 5), which is again within the recommended value range (50 to 75 %) (EEMBPM, 2002; Jemmett, 2006; Dominik, 2007). T5 had highest methane while T3 had the lowest methane content (Fig. 10), and this might be due to its good C: N ratio (25:1) for T5 than T3 (Hartmann *et al*., 2004) (Table 4). Triplicate means values of methane production of the AD were shown in Figure 16 below.

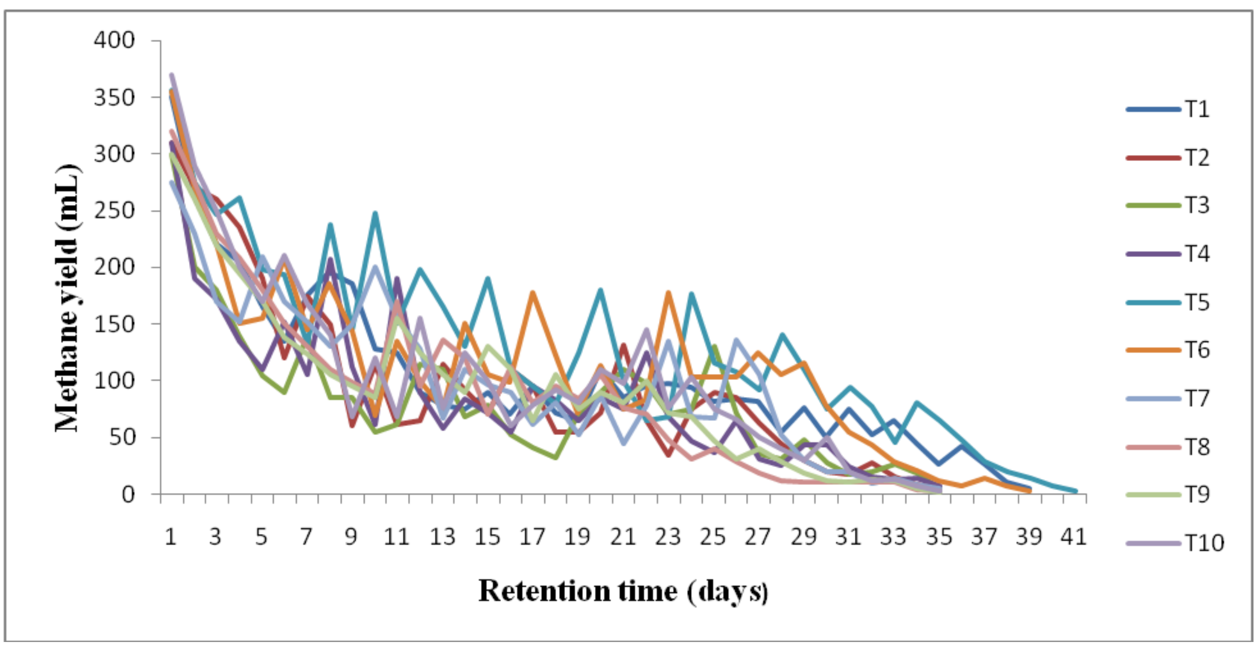

Figure 16: Triplicate means values of methane production

A statistically significant positive correlation between retention time and methane production was also observed with Pearson correlation (P < 0.01) (Appendix 18). Thus, the quality of methane increased as the retention time increased and finally ceases as biogas yield stops. The reason could be the existence of more and more methanogenic bacteria conversion of acidic substances including CO_2 to CH_4 (Nayono, 2009). Triplicate means values of percentage of methane production of the AD were shown in Figure 17 below.

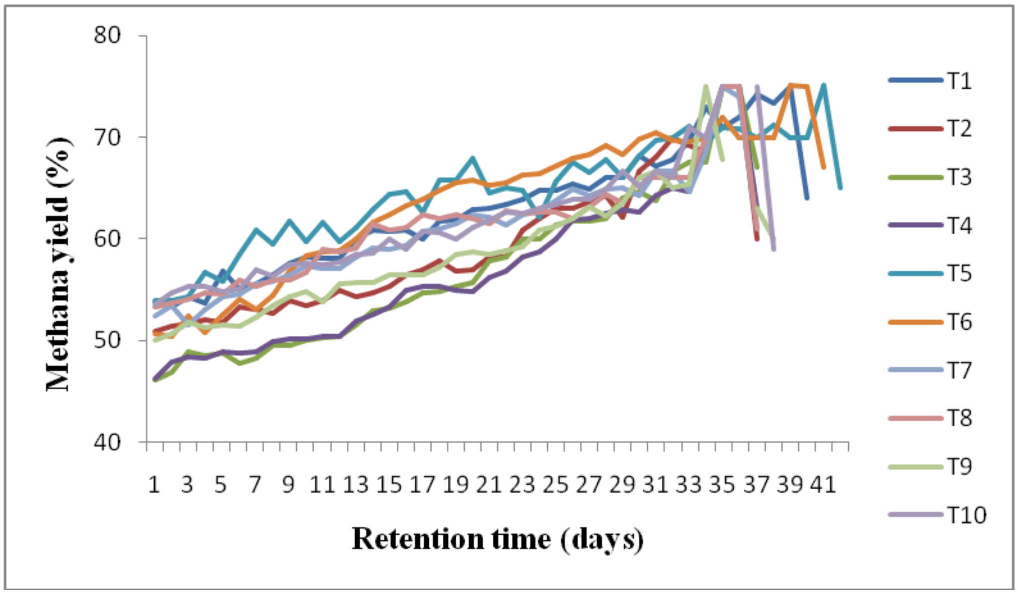

Figure 17: Triplicate means values of percentage of methane production

Statistically significant differences were seen in the yield of CH_4 within T5, T6, and T10 treatments of anaerobic digestion (Paired samples T-test, $P < 0.05$). The percentage of methane produced in descending order was indicated that T5 (60.36 %), T6 (58.58 %), T10 (57.86 %), T7 (55.89 %), T9 (54.59 %), T2 (54.52 %), T1 (53.80 %), T8 (53.74 %), T4 (52.28 %) and T3 (52.20 %). The overall amount of biogas produced by T5 was found to be higher than others (Fig. 12). This could be because of sufficient supply of nutrient for digesting bacteria from different feedstock and application of good C/N ratio (25:1) (Table 4) that enhances fast degradation of the organic waste (Fricke *et al.*, 2007). The total biogas, methane and its overall percentage of methane production of all feedstocks in anaerobic digestion was summarized in Figure 18 below.

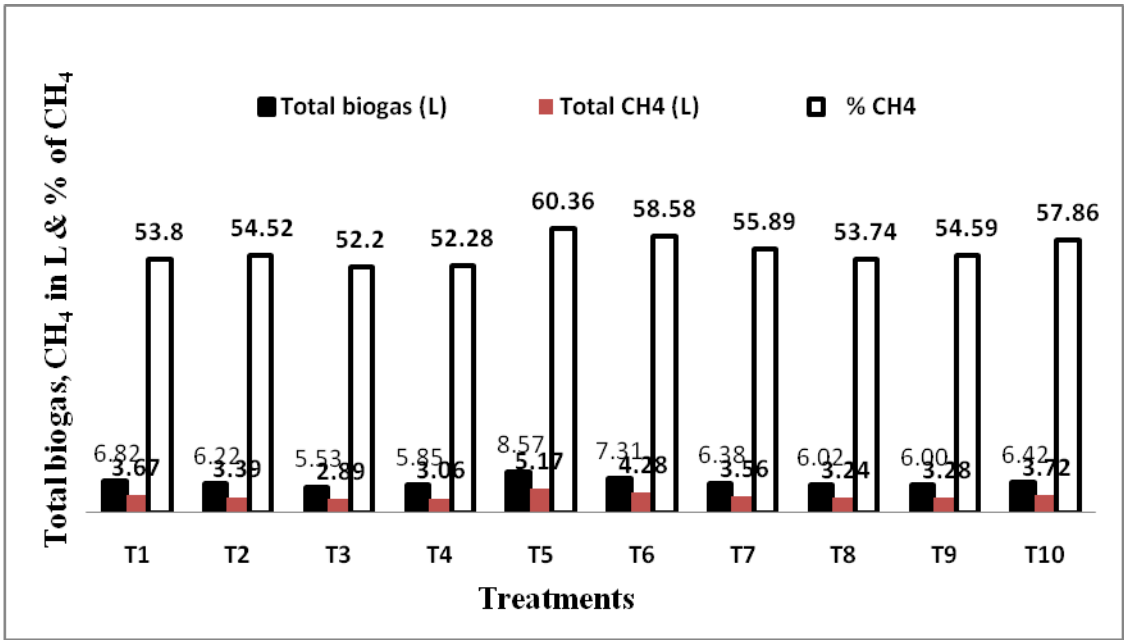

Figure 18: The total biogas, methane and its overall percentage of methane production of different feed stocks in anaerobic digestion

Descriptive assessment of fuel wood consumption data reveals about 81.5 and 93 % of the households used firewood and charcoal, respectively, mainly for food preparation. On average, monthly per-capita firewood consumption and monthly per-capita charcoal consumption were 10 kg each (Bereket *et al.*, 2002). The caloric value of charcoal is 10.7kWh/kg, while firewood has a caloric value of 5.5kWh/kg. On the basis of this, the total caloric value of charcoal and firewood used in Jimma town as a source of energy was found to be 205×10^6 kWh/year (1,284 kWh/cap/year) and $110x10^6$ kWh/year (660 kWh/cap/year), respectively. The total waste generated daily in Jimma town was ca. 88,000 kg, and the average per capita generation rate was 0.55 ± 0.17 kg/capita/day (Getahun *et al.*, 2012).

One dairy cow produces 27kg of manure each day, which amounts to 10,000kgs or ten tons of fresh manure per year**.** On a farm with 1,000 cows this totals 10,000 tons of fresh manure per year. In an anaerobic digestion process this can produce enormous amount of one million kilowatt hours (kWh) of electricity per year. So, one single cow produces manure worth 2.7kWh of electricity every day. It only takes 2.4kWh to power a 100 watts light bulb all day long (FAO, 2011).

The total energy demand of Jimma town was 1,944 kWh/ cap/year. The results in this study indicated that the total methane yield potential using 40 kg of khat and food waste substrate was measured to be

1607 and 1901 kWh/cap/year which covers 82.7 % and 97.8 % of the total energy demand of the area respectively. Thus, this technology will able to provide a clean fuel from renewable feedstock and help to improve energy shortage (Mshandete and Parawira, 2009; Richard *et al*., 2011).

5.2.3. Pilot study for combustibility of the gas produced

Pilot study for the combustibility test was performed for the biogas produced in 0.5L plastic digester by connecting the small hose of plastic tube to Bunsen burner. The hose was connected to the Bunsen burner. The biogas in each of the bottle was found to light the burner and produced light. The diagram for this test was shown in Figure 19 below.

Figure 19: Pilot study for combustibility of the gas produced

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study reflects that, selective and appropriate amount of co-digestion should be used to improve the biological and nutritive environment in the digester for microbes increasing biogas production. Biogas can be produced from co-digestion of KW, FW and CD. More degradation of organic carbon was observed in mixed substrates. The results in this study indicated that the mean biogas produced in ascending order was T3 (5526 mL), T4 (5855 mL), T9 (6003 mL), T8 (6020 mL), T6 (6218 mL), T7 (6375 mL), T10 (6423 mL), T1 (6823 mL), T6 (7310 mL) and T5 (8565 mL) per 100g fresh sample. This suggests that mixing can enhance degradation and biogas production. Mixing KW and FW with CD, especially in 2:1:1 ratio would optimize gas yield, its quality and provision of nutrient for growth of methanogenic bacteria. Substrate of 25:1 C/N ratio is optimum for anaerobic digestion process. Long retention time during anaerobic digestion increases methane quality of biogas. KW, FW and CD could be used as a substrate for biogas production at a temperature range of 20.33 to 24.67 °C around the digester environment. The temperature fluctuation can be monitored at ±3 °C using Styrofoam. Methane content of biogas can be separated using CO_2 absorption solution. Temperature of Jimma town was suitable for anaerobic digestion even during rainy seasons. This technology has remarkable application in the future for sustainability of environment (treatment of wastes) and production of energy as an extra benefit. Production of biogas and methane from solid waste in Jimma town could be important for energy production, keeping the environment clean, and minimizing the effects of climate change by cleaner green energy production and reducing the output of greenhouse gases. Everyone can apply this technology at house hold level since this technology is easily operated and carried out from locally available materials.

6.2. Recommendations

- The experiment was conducted during rainy season. Hence, further study should be investigated by mixing KW, FW and CD especially in 2:1:1 ratio to evaluate effect of seasonal variation on production of gas during dry season.

- Further study is necessary to look gas production under incubated mesophilic temperature because maximum gas production was observed at maximum temperature.

- Digester containing low TS (T5) produced highest gas yield. Thus, further study is supposed to be carried out by feeding lower TS because higher organic content in the digester feed may decrease the cumulative volume of gas production.

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8. Appendices

Appendix Table A1: Daily mean biogas yields from co-digestion ± SE (mL) (n = 3)

Days	Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	650 ± 5.0	608 ± 2.9	650 ± 0.0	670 ± 0.0	660 ± 10.0	700 ± 0.0	525 ± 5.0	600 ± 0.0	600 ± 0.0	690 ± 10.0
2	491 ± 7.6	525 ± 5.0	426 ± 5.8	393 ± 5.8	510 ± 0.0	535 ± 5.0	430 ± 10.0	513 ± 5.8	513 ± 5.8	530 ± 10.0
3	405 ± 5.0	503 ± 5.8	368 ± 2.9	355 ± 5.0	455 ± 5.0	420 ± 10.0	330 ± 10.0	425 ± 5.0	325 ± 5.0	451 ± 7.6
4	381 ± 7.6	451 ± 2.9	288 ± 2.9	280 ± 5.0	460 ± 0.0	295 ± 5.0	286 ± 2.9	380 ± 0.0	280 ± 0.0	361 ± 2.9
5	290 ± 8.7	366 ± 7.6	215 ± 5.0	225 ± 5.0	355 ± 5.0	295 ± 5.0	386 ± 5.8	330 ± 10.0	333 ± 5.8	310 ± 10.0
6	245 ± 5.0	225 ± 5.0	188 ± 7.6	311 ± 2.9	330±10.0	385 ± 5.0	311 ± 2.9	268 ± 10.4	265 ± 5.0	381± 7.6
7	315 ± 5.0	330 ± 5.0	290 ± 0.0	215 ± 5.0	216 ±7.6	273 ± 10.4	273 ± 7.6	235± 5.0	235 ± 5.0	295 ± 5.0
8	345 ± 5.0	285 ± 5.0	171 ± 7.6	415 ± 5.0	398 ± 2.9	340 ± 0.0	218 ± 7.6	196 ± 2.9	296 ± 2.9	248 ± 2.9
9	321 ± 2.9	113 ± 2.9	171 ± 5.8	223 ± 5.8	235 ± 5.0	253 ± 5.8	230 ± 5.0	175 ± 5.0	275 ± 5.0	118 ± 5.8
10	220 ± 0.0	215 ± 5.0	110 ± 0.0	121 ± 7.6	415 ± 8.7	118 ± 2.9	261 ± 7.6	155 ± 5.0	255 ± 5.0	208 ± 2.9
11	215 ± 5.0	115 ± 0.0	123 ± 7.6	376 ± 2.9	245 ± 5.0	230 ± 0.0	350 ± 10.0	288 ± 7.6	188.3±7.6	118 ± 2.9
12	155 ± 5.0	118 ± 2.9	228 ± 10.4	178 ± 7.6	331 ± 10.4	170 ± 10.0	271 ± 2.9	225 ± 5.0	225 ± 5.0	268 ± 5.8
13	130 ± 10.0	212 ± 2.9	213 ± 2.9	111 ± 7.6	270 ± 10.0	125 ± 5.0	220 ± 0.0	188 ± 2.9	288 ± 2.9	130 ± 0.0
14	123 ± 2.9	168 ± 5.8	128 ± 2.9	160 ± 10.0	206 ± 5.8	245 ± 5.0	113 ± 2.9	161 ± 2.9	125 ± 5.0	213 ± 2.9
15	148 ± 2.9	130 ± 10.0	146 ± 2.9	135 ± 5.0	295 ± 5.0	168 ± 7.6	186 ± 7.6	230 ± 10.0	230 ± 10.0	166 ± 2.9
16	115 ± 5.0	195 ± 5.0	96 ± 5.8	98 ± 7.6	170 ± 10.0	155 ± 5.0	163 ± 2.9	195 ± 5.0	106 ± 5.8	101± 2.9
17	158 ± 2.9	166 ± 5.8	75 ± 5.0	171 ± 7.6	151 ± 7.6	278 ± 2.9	148 ± 7.6	115 ± 5.0	188 ± 7.6	128 ± 2.9
18	115 ± 0.0	95 ± 5.0	58 ± 7.6	150 ± 5.0	120 ± 10.0	188 ± 7.6	101 ± 2.9	183 ± 5.8	203 ± 5.8	151 ± 2.9
19	105 ± 5.0	95 ± 5.0	130 ± 0.0	118 ± 2.9	190 ± 5.0	108 ± 7.6	131± 2.8	128 ± 2.9	128 ± 2.9	133 ± 5.9
20	175 ± 5.0	126 ± 2.9	161± 5.8	155 ± 5.0	265 ± 5.0	171 ± 2.9	85 ± 8.7	153 ± 5.8	153 ± 5.8	180 ± 5.0
21	135 ± 5.0	231 ± 2.9	190 ± 5.0	133 ± 2.9	155 ± 5.0	115 ± 5.0	141 ± 2.9	136 ± 7.6	136 ± 7.6	158 ± 2.9
22	150 ± 0.0	111 ± 2.9	168 ± 7.6	220 ± 5.0	100 ± 0.0	126 ± 5.8	73 ± 2.9	170 ± 5.0	170 ± 5.0	231 ± 2.9
23	153 ± 10.4	58 ± 7.6	116 ± 7.6	116 ± 2.9	105 ± 5.0	268 ± 2.9	125 ± 5.0	121 ± 2.9	121 ± 2.9	121 ± 2.9
24	145 ± 5.0	123 ± 2.9	125 ± 5.0	80 ± 10.0	283 ± 7.6	155 ± 5.0	215 ± 0.0	111 ± 2.9	65 ± 5.0	163 ± 5.8
25	126 ± 2.9	145 ± 5.0	211 ± 7.6	60 ± 5.0	175 ± 5.0	153 ± 5.0	106 ± 5..8	76 ± 7.6	53 ± 2.9	118 ± 5.8

26	128 ± 7.6	135 ± 0.0	116 ± 2.9	105 ± 5.0	158 ± 7.6	151 ± 2.9	103 ± 7.6	50 ± 0.0	40 ± 0.0	103 ± 7.6
27	126 ± 2.9	100 ± 10.0	58 ± 11.5	50 ± 5.0	138 ± 2.9	182 ± 2.9	211 ± 2.9	63 ± 2.9	70 ± 5.0	78 ± 5.8
28	83 ± 5.8	55 ± 5.0	50 ± 5.0	40 ± 5.0	206 ± 7.6	151 ± 10.4	161 ± 5.8	45 ± 5.0	31 ± 2.9	61 ± 2.9
29	115 ± 5.0	70 ± 5.0	75 ± 5.0	68 ± 7.6	166 ± 7.6	168 ± 5.7	80 ± 5.0	28 ± 5.8	21 ± 5.8	45 ± 5.0
30	73 ± 2.9	48 ± 7.6	43 ± 5.8	38 ± 7.6	110 ± 5.0	111 ± 2.9	46 ± 5. 8	16 ± 2.9	16 ± 7.6	76 ± 5.8
31	111 ± 2.9	30 ± 0.0	26 ± 5.8	23 ± 5.7	110 ± 5.0	111 ± 2.9	30 ± 0.0	15 ± 5	15 ± 5.0	30 ± 0.0
32	76 ± 10.4	25 ± 5.0	30 ± 10.0	20 ± 5.0	110 ± 0	61 ± 5.8	15 ± 5.0	15 ± 8.6	20 ± 0.0	16 ± 5.8
33	93 ± 2.9	21 ± 2.9	40 ± 5.0	21 ± 5.8	63 ± 2.9	41 ± 2.9	21 ± 5.8	15±10	18 ± 5.8	18 ± 5.8
34	61 ± 2.9	11 ± 2.9	26 ± 7.6	10 ± 5.0	115 ± 10	30 ± 10.0	11 ± 2.9	5 ± 5.0	6 ± 2.9	10 ± 0.0
35	36 ± 5.8	6 ± 2.9	6 ± 2.8	3 ± 2.9	91 ± 2.9	16 ± 5.8	6 ± 2.9	3 ± 2.9	1 ± 2.9	3 ± 2.9
36	58 ± 7.6	0 ± 0.0	0 ± 0.0	0 ± 0.0	65 ± 5.0	10 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
37	35 ± 5.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	40 ± 10.0	20 ± 5.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
38	15 ± 5.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	26 ± 5.8	10 ± 5.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
39	6 ± 2.9	0 ± 0.0	0 ± 0.0	0 ± 0.0	20 ± 5.0	3 ± 2.9	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
40	0 ± 0.0	0 ± 0	0 ± 0.0	0 ± 0.0	10 ± 5.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
41	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
42	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Total	6823 ± 2.9	6218 ± 36.2	5526.7 ± 22.5	5855 ± 65.0	8565 ± 74.7	7310 ± 43.3	6375 ± 27.8	6020 ± 13.2	6003 ± 5.8	6423 ± 42.5

Appendix Table A2: Daily biogas production (mL) from the combination of chat and food waste in the ratio 3:1 (T2)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	605	610	610	608.3	19	95	90	100	95.0
2	520	530	525	525.0	20	125	130	125	126.7
3	510	500	500	503.3	21	230	235	230	231.7
4	450	450	455	451.7	22	110	115	110	111.7
5	375	365	360	366.7	23	65	60	50	58.3
6	230	220	225	225.0	24	120	125	125	123.3
7	335	325	330	330.0	25	150	140	145	145.0
8	290	285	280	285.0	26	135	135	135	135.0
9	115	115	110	113.3	27	90	110	100	100.0
10	210	220	215	215.0	28	55	50	60	55.0
11	115	115	115	115.0	29	65	75	70	70.0
12	120	115	120	118.3	30	40	55	50	48.3
13	210	215	210	211.7	31	30	30	30	30.0
14	165	175	165	168.3	32	25	20	30	25.0
15	120	140	130	130.0	33	20	25	20	21.7
16	195	200	190	195.0	34	15	10	10	11.7
17	160	170	170	166.7	35	10	5	5	6.7
18	90	100	95	95.0	Total	6195	6260	6200	6218.3

Appendix Table A3: Daily biogas production (mL) from the combination of chat, food and cow dung biomass in the ratio 6:1:1 (T3)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	650	650	650	650.0	19	130	130	130	130.0
2	430	430	420	426.7	20	165	155	165	161.7
3	365	370	370	368.3	21	190	195	185	190.0
4	290	285	290	288.3	22	175	160	170	168.3
5	210	220	215	215.0	23	110	125	115	116.7
6	180	195	190	188.3	24	130	120	125	125.0
7	290	290	290	290.0	25	205	210	220	211.7
8	180	165	170	171.7	26	120	115	115	116.7
9	175	165	175	171.7	27	65	65	45	58.3
10	110	110	110	110.0	28	50	55	45	50.0
11	130	115	125	123.3	29	75	70	80	75.0
12	240	220	225	228.3	30	40	50	40	43.3
13	215	210	215	213.3	31	30	30	20	26.7
14	130	125	130	128.3	32	20	40	30	30.0
15	150	145	145	146.7	33	40	45	35	40.0
16	90	100	100	96.7	34	25	35	20	26.7
17	70	75	80	75.0	35	10	5	5	6.7
18	65	50	60	58.3	Total	5550	5525	5505	5526.7

Appendix Table A4: Daily biogas production (mL) from the combination of chat and food biomass in the ratio 1:1 (T4)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	670	670	670	670.0	19	120	115	120	118.3
2	400	390	390	393.3	20	155	150	160	155.0
3	360	355	350	355.0	21	130	135	135	133.3
4	285	275	280	280.0	22	220	225	215	220.0
5	220	230	225	225.0	23	115	120	115	116.7
6	315	310	310	311.7	24	80	70	90	80.0
7	210	220	215	215.0	25	65	55	60	60.0
8	420	410	415	415.0	26	110	100	105	105.0
9	230	220	220	223.3	27	55	45	50	50.0
10	130	115	120	121.7	28	40	35	45	40.0
11	375	380	375	376.7	29	75	60	70	68.3
12	170	180	185	178.3	30	45	40	30	38.3
13	120	105	110	111.7	31	30	20	20	23.3
14	170	150	160	160.0	32	20	15	25	20.0
15	140	130	135	135.0	33	25	25	15	21.7
16	90	100	105	98.3	34	10	15	5	10.0
17	170	165	180	171.7	35	5	5	0	3.3
18	145	155	150	150.0	Total	5920	5790	5855	5855.0

Appendix Table A5: Daily biogas production (mL) from the combination of chat, food and cow dung biomass in the ratio 2: 1:1 (T5)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	650	670	660	660	22	100	100	100	100
2	510	510	510	510	23	110	105	100	105
3	460	450	455	455	24	290	275	285	283
4	460	460	460	460	25	180	170	175	175
5	350	355	360	355	26	165	150	160	158
6	320	340	330	330	27	140	135	140	138
7	225	210	215	216	28	205	200	215	206
8	395	400	400	398	29	160	175	165	166
9	230	240	235	235	30	105	110	115	110
10	410	425	410	415	31	135	130	140	135
11	240	250	245	245	32	110	110	110	110
12	335	340	320	331	33	65	60	65	63
13	260	280	270	270	34	115	105	125	115
14	200	210	210	206	35	90	95	90	91
15	300	290	295	295	36	70	60	65	65
16	180	160	170	170	37	50	30	40	40
17	160	145	150	151	38	30	20	30	26
18	130	110	120	120	39	25	15	20	20
19	190	195	185	190	40	10	5	15	10
20	260	265	270	265	41	5	0	5	3
21	150	155	160	155	Total	8620	8480	8595	8565

Appendix Table A6: Daily biogas production (mL) from the combination of chat, food and cow dung biomass in the ratio 3: 3:2 (T6)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	700	700	700	700	21	120	110	115	115
2	530	540	535	535	22	130	120	130	126
3	410	430	420	420	23	270	265	270	268
4	300	290	295	295	24	160	150	155	155
5	290	300	295	295	25	125	115	120	120
6	380	385	390	385	26	155	150	150	151
7	270	285	265	273	27	180	185	180	181
8	340	340	340	340	28	160	140	155	151
9	250	260	250	253	29	175	165	165	168
10	120	115	120	118	30	110	115	110	111
11	230	230	230	230	31	85	70	75	76
12	160	180	170	170	32	65	65	55	61
13	130	120	125	125	33	40	45	40	41
14	250	245	240	245	34	30	40	20	30
15	170	160	175	168	35	20	20	10	16
16	160	150	155	155	36	10	10	10	10
17	280	275	280	278	37	20	15	25	20
18	195	180	190	188	38	10	5	15	10
19	115	110	100	108	39	5	0	5	3
20	170	170	175	171	Total	7335	7335	7260	7310

Appendix Table A7: Daily biogas production (mL) from the combination of food and chat biomass in the ratio 3:1 (T7)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	525	520	530	525.0	19	130	135	130	131.7
2	420	430	440	430.0	20	95	80	80	85.0
3	340	320	330	330.0	21	140	145	140	141.7
4	290	285	285	286.7	22	75	70	75	73.3
5	390	380	390	386.7	23	120	130	125	125.0
6	310	315	310	311.7	24	215	215	215	215.0
7	280	275	265	273.3	25	100	110	110	106.7
8	210	220	225	218.3	26	110	95	105	103.3
9	235	225	230	230.0	27	215	210	210	211.7
10	270	255	260	261.7	28	165	165	155	161.7
11	360	340	350	350.0	29	80	75	85	80.0
12	270	275	270	271.7	30	40	50	50	46.7
13	220	220	220	220.0	31	30	30	30	30.0
14	115	110	115	113.3	32	15	10	20	15.0
15	185	180	195	186.7	33	25	25	15	21.7
16	160	165	165	163.3	34	10	15	10	11.7
17	150	155	140	148.3	35	5	10	5	6.7
18	100	105	100	101.7	Total	6400	6345	6380	6375.0

Appendix Table A8: Daily biogas production (mL) from the combination of chat, food and cow dung biomass in the ratio 1: 2:1 (T8)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	600	600	600	600	19	130	125	130	128
2	520	510	510	513	20	150	150	160	153
3	430	420	425	425	21	135	130	145	136
4	380	380	380	380	22	170	165	175	170
5	330	340	320	330	23	120	125	120	121
6	265	260	280	268	24	110	115	110	111
7	230	240	235	235	25	70	85	75	76
8	195	200	195	196	26	50	50	50	50
9	175	180	170	175	27	65	65	60	63
10	155	160	150	155	28	40	45	50	45
11	280	295	290	288	29	35	25	25	28
12	230	225	220	225	30	20	15	15	16
13	190	185	190	188	31	15	20	10	15
14	160	160	165	161	32	10	10	25	15
15	240	220	230	230	33	15	25	5	15
16	200	190	195	195	34	5	10	0	5
17	120	110	115	115	35	5	5	0	3
18	180	190	180	183	Total	6025	6030	6005	6020

Appendix Table A9: Daily biogas production (mL) from the combination of food, chat and cow dung biomass in the ratio 6: 1:1 (T9)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	600	600	600	600	19	130	125	130	128
2	520	510	510	513	20	150	150	160	153
3	330	320	325	325	21	135	130	145	136
4	280	280	280	280	22	170	165	175	170
5	330	340	330	333	23	120	125	120	121
6	265	260	270	265	24	60	65	70	65
7	230	240	235	235	25	50	55	55	53
8	295	300	295	296	26	40	40	40	40
9	275	280	270	275	27	75	65	70	70
10	255	260	250	255	28	30	35	30	31
11	180	195	190	188	29	25	15	25	21
12	230	225	220	225	30	10	25	15	16
13	290	285	290	288	31	15	20	10	15
14	130	120	125	125	32	20	20	20	20
15	240	220	230	230	33	15	25	15	18
16	100	110	110	106	34	5	10	5	6
17	190	180	195	188	35	0	5	0	1
18	210	200	200	203	Total	6000	6000	6010	6003

Appendix Table A10: Daily biogas production (mL) from the combination of food biomass only (T10)

Days	Rep.1	Rep.2	Rep.3	Average	Days	Rep.1	Rep.2	Rep.3	Average
1	690	680	700	690.0	19	130	140	130	133.3
2	540	530	520	530.0	20	185	180	175	180.0
3	460	450	445	451.7	21	155	160	160	158.3
4	365	360	360	361.7	22	235	230	230	231.7
5	300	320	310	310.0	23	120	125	120	121.7
6	375	390	380	381.7	24	160	170	160	163.3
7	300	295	290	295.0	25	125	115	115	118.3
8	250	245	250	248.3	26	110	105	95	103.3
9	125	115	115	118.3	27	85	75	75	78.3
10	210	205	210	208.3	28	65	60	60	61.7
11	120	115	120	118.3	29	40	45	50	45.0
12	265	275	265	268.3	30	70	80	80	76.7
13	130	130	130	130.0	31	30	30	30	30.0
14	210	215	215	213.3	32	20	20	10	16.7
15	170	165	165	166.7	33	25	15	15	18.3
16	100	105	100	101.7	34	10	10	10	10.0
17	125	130	130	128.3	35	5	5	0	3.3
18	150	150	155	151.7	Total	6455	6440	6375	6423.3

Appendix Table A11: Daily temperature (^{o}c) of the room where digestion took place

Days	Morning (7:00AM)	Noon (1:00PM)	Dusk (7:00 PM)	Average	Days	Morning (7:00AM)	Noon (1:00PM)	Dusk (7:00PM)	Average
1	20	24	21	21.67	22	22	23	22	22.33
2	21	23	22	22.00	23	23	22	21	22.00
3	21	23	22	22.00	24	21	23	22	22.00
4	22	24	23	23.00	25	20	24	23	22.33
5	21	24	20	21.67	26	20	25	23	22.67
6	20	22	21	21.00	27	22	24	21	22.33
7	23	25	22	23.33	28	21	22	20	21.00
8	22	24	23	23.00	29	23	24	19	22.00
9	22	23	20	21.67	30	21	22	20	21.00
10	21	24	22	22.33	31	20	25	21	22.00
11	23	26	24	24.33	32	20	23	22	21.67
12	20	22	23	21.67	33	21	24	23	22.67
13	20	21	20	20.33	34	22	22	20	21.33
14	21	22	20	21.00	35	20	21	21	20.67
15	20	21	20	20.33	36	21	23	22	22.00
16	21	22	20	21.00	37	23	24	22	23.00
17	20	23	21	21.33	38	20	27	24	23.67
18	20	24	22	22.00	39	21	26	23	23.33
19	21	21	20	20.67	40	21	24	22	22.33
20	20	24	23	22.33	41	22	24	21	22.33
21	23	26	25	24.67					

Appendix Table A12: Triplicate Cumulative values for biogas production of each treatments

Days	T1	T2	T3	T 4	T5	T6	T7	T8	T9	T10
1	650.00	608.33	650.00	670.00	660.00	700.00	525.00	600.00	600.00	690.00
2	1141.67	1133.33	1076.67	1063.33	1170.00	1235.00	955.00	1113.33	1113.33	1220.00
3	1546.67	1636.66	1445.00	1418.33	1625.00	1655.00	1285.00	1538.33	1438.33	1671.67
4	1928.34	2088.33	1733.33	1698.33	2085.00	1950.00	1571.67	1918.33	1718.33	2033.34
5	2218.34	2455.00	1948.33	1923.33	2440.00	2245.00	1958.34	2248.33	2051.66	2343.34
6	2464.34	2680.00	2136.66	2235.00	2770.00	2630.00	2270.00	2516.66	2316.66	2725.00
7	2779.34	3010.00	2426.66	2450.00	2986.67	2903.33	2543.34	2751.66	2551.66	3020.00
8	3124.34	3295.00	2598.33	2865.00	3385.00	3243.33	2761.64	2948.36	2848.36	3268.34
9	3446.00	3406.33	2770.00	3088.33	3620.00	3496.66	2991.64	3223.36	3123.36	3386.67
10	3666.00	3621.33	2880.00	3210.00	4035.00	3615.00	3253.31	3278.36	3378.36	3595.00
11	3881.00	3736.33	3003.33	3586.67	4280.00	3845.00	3603.31	3466.69	3566.69	3713.33
12	4036.00	3854.66	3231.66	3765.00	4611.67	4015.00	3874.98	3691.69	3791.67	3981.66
13	4166.00	4066.36	3445.00	3876.67	4881.67	4140.00	4094.98	3880.00	4080.00	4111.66
14	4289.34	4234.69	3573.32	4036.67	5088.34	4385.00	4208.31	4041.72	4205.00	4325.00
15	4437.67	4364.69	3720.00	4171.67	5383.34	4553.00	4394.98	4271.72	4435.00	4491.69
16	4552.67	4559.69	3816.66	4270.00	5553.34	4708.00	4558.31	4466.72	4541.72	4593.39
17	4711.00	4676.36	3891.66	4441.67	5705.01	4986.62	4706.64	4581.72	4730.05	4721.72
18	4826.00	4771.36	3950.00	4591.67	5825.00	5174.95	4808.34	4765.00	4933.38	4873.39
19	4931.00	4866.36	4080.00	4710.00	6015.00	5283.28	4940.04	4893.38	5161.71	5006.72
20	5106.00	4993.03	4241.66	4865.00	6280.00	5454.95	5025.04	5046.71	5315.04	5186.72
21	5241.00	5224.67	4431.66	4998.33	6435.00	5569.95	5166.71	5183.41	5451.74	5345.00
23	5391.00	5336.37	4600.00	5218.33	6535.00	5696.62	5240.04	5353.41	5621.74	5576.60
24	5544.33	5394.70	4716.66	5335.00	6640.00	5964.95	5365.04	5475.11	5743.44	5798.30
25	5689.33	5518..03	4841.66	5415.00	6923.34	6119.95	5580.04	5586.81	5808.44	5961.60
26	5816.00	5663.03	5053.33	5475.00	7098.34	6273.28	5686.67	5663.48	5861.77	6079.90

27	5944.33	5798.03	5170.00	5580.00	7256.67	6424.95	5790.04	5713.48	5901.77	6183.20
28	6071.00	5898.03	5228.33	5030.00	7395.00	6606.62	6001.74	5776.81	5971.77	6261.53
29	6154.33	5953.03	5278.33	5670.00	7601.67	6758.30	6163.41	5821.81	6003.44	6323.20
30	6269.33	6023.03	5353.33	5738.33	7768.34	6926.62	6243.41	5850.14	6025.11	6368.20
31	6342.63	6071.36	5396.66	5776.66	7878.34	7038.30	6290.08	5866.81	6041.78	6444.87
32	6454.33	6101.36	5423.33	5800.00	8013.34	7109.96	6320.08	5881.81	6056.78	6474.87
33	6530.97	6126.36	5453.33	5820.00	8123.34	7171.63	6335.08	5896.81	6076.78	6491.54
34	6624.30	6166.36	5493.33	5841.66	8186.67	7213.30	6356.75	5911.81	6095.11	6509.87
35	6685.97	6188.03	5520.00	5851.66	8301.67	7243.30	6368.42	5916.81	6101.78	6519.87
36	6722.64	6199.70	5526.67	5855.00	8393.34	7259.97	6375.09	5920.14	6101.78	6523.20
37	6870.97	6206.37	_	_	8458.34	7269.97	_	_	6103.45	_
38	6815.97	_	_	_	8498.34	7273.30	_	_	_	_
39	6830.97	_	_	_	8525.00	_	_	_	_	_
40	6837.64	_	_	_	8545.00	_	_	_	_	_
41	_	_	_	_	8555.00	_	_	_	_	_
42	_	_	_	_	8558.34	_	_	_	_	_

Appendix Table A13: Triplicate average values for amount of biogas, methane and percentage of methane production of digester 1 & 2

Treatment-1				Treatment-2			
Days	Biogas (mL)	Methane (mL)	Methane (%)	Days	Biogas (mL)	Methane (mL)	Methane (%)
1	650.0	350	53.85	1	608.3	310	50.96
2	491.7	262	53.36	2	525.0	270	51.42
3	405.0	220	54.32	3	503.3	260	51.66
4	381.7	205	53.71	4	451.7	235	52.03
5	290.0	165	56.89	5	366.7	190	51.82
6	245.0	135	55.10	6	225.0	120	53.33
7	315.0	175	55.56	7	330.0	175	53.03
8	345.0	195	56.52	8	285.0	150	52.63
9	321.7	185	57.62	9	113.3	60	53.89
10	220.0	128	58.18	10	215.0	115	53.48
11	215.0	125	58.14	11	115.0	62	53.91
12	155.0	90	58.06	12	118.3	65	54.93
13	130.0	78	60.00	13	211.7	115	54.33
14	123.3	75	60.81	14	168.3	92	54.65
15	148.3	90	60.68	15	130.0	72	55.38
16	115.0	70	60.87	16	195.0	110	56.41
17	158.3	95	60.00	17	166.7	95	56.99
18	115.0	71	61.74	18	95.0	55	57.89
19	105.0	65	61.90	19	95.0	55	56.84
20	175.0	110	62.85	20	126.7	72	56.97
21	135.0	85	62.96	21	231.7	132	58.20
23	150.0	95	63.33	23	111.7	65	58.28
24	153.3	98	63.91	24	58.3	34	60.81
25	145.0	94	64.82	25	123.3	75	62.00
26	126.7	82	64.75	26	145.0	90	62.96
27	128.3	84	65.45	27	135.0	85	63.00
28	126.7	82	64.90	28	100.0	63	63.63
29	83.3	55	66.00	29	55.0	45	64.28
30	115.0	76	66.08	30	70.0	30	62.07
31	73.3	50	68.18	31	48.3	20	66.67
32	111.7	75	67.16	32	30.0	17	68.00
33	76.7	52	67.82	33	25.0	28	70.00
34	93.3	65	69.65	34	21.7	15	69.22
35	61.7	45	72.97	35	11.7	8	68.55
36	36.7	26	70.90	36	6.7	5	74.96
37	58.3	42	72.00				
38	35.0	26	74.28				
39	15.0	11	73.33				
40	6.7	5	74.96				

Appendix Table A14: Triplicate average values for amount of biogas, methane and percentage of methane production of digester 3 & 4

Treatment-3				Treatment-4			
Days	Biogas (mL)	Methane (mL)	Methane (%)	Days	Biogas (mL)	Methane (mL)	Methane (%)
1	650.0	300	46.15	1	670.0	310	46.26
2	426.7	200	46.87	2	393.3	190	47.93
3	368.3	180	48.86	3	355.0	172	48..45
4	288.3	140	48.55	4	280.0	135	48.21
5	215.0	105	48.83	5	225.0	110	48.89
6	188.3	90	47.78	6	311.7	152	48.77
7	290.0	140	48.28	7	215.0	105	48.84
8	171.7	85	49.51	8	415.0	207	49.87
9	171.7	85	49.51	9	223.3	112	50.15
10	110.0	55	50.00	10	121.7	61	50.14
11	123.3	62	50.27	11	376.7	190	50.44
12	228.3	115	50.36	12	178.3	90	50.46
13	213.3	110	51.56	13	111.7	58	51.94
14	128.3	68	52.99	14	160.0	84	52.50
15	146.7	78	53.18	15	135.0	72	53.33
16	96.67	52	53.79	16	98.33	54	54.92
17	75.0	41	54.67	17	171.7	95	55.34
18	58.33	32	54.86	18	150.0	83	55.33
19	130.0	72	55.38	19	118.3	65	54.93
20	161.7	90	55.66	20	155.0	85	54.84
21	190.0	110	57.89	21	133.3	75	56.25
22	168.3	98	58.22	22	220.0	125	56.82
23	116.7	70	60.00	23	116.7	68	58.28
24	125.0	75	60.00	24	80.0	47	58.75
25	211.7	130	61.41	25	60.0	36	60.00
26	116.7	72	61.71	26	105.0	65	61.90
27	58.33	36	61.72	27	50.0	31	62.00
28	50.0	31	62.00	28	40.0	25	62.50
29	75.0	48	64.00	29	68.3	43	62.93
30	43.33	28	64.62	30	38.3	43	62.61
31	26.67	17	63.74	31	23.3	24	64.29
32	30.0	20	66.67	32	20.0	15	65.00
33	40.0	27	67.50	33	21.7	13	64.61
34	26.7	18	67.49	34	10.0	14	70.00
35	6.7	5	74.96	35	3.3	7	75.00

Appendix Table A15: Triplicate average values for amount of biogas, methane and percentage of methane production of digester 5 & 6

Treatment-5				**Treatment-6**			
Days	Biogas (mL)	Methane (mL)	Methane (%)	Days	Biogas (mL)	Methane (mL)	Methane (%)
1	660.00	356	53.94	1	700.00	355	50.71
2	510.00	275	53.92	2	535.00	270	50.47
3	455.00	247	54.28	3	420.00	220	52.38
4	460.00	261	56.74	4	295.00	150	50.84
5	355.00	198	55.77	5	295.00	155	52.54
6	330.00	193	58.48	6	385.00	208	54.03
7	216.67	132	60.92	7	273.30	145	53.05
8	398.33	237	59.49	8	340.00	185	54.41
9	235.00	145	61.70	9	253.30	144	56.85
10	415.00	248	59.76	10	118.30	69	58.33
11	245.00	151	61.63	11	230.00	135	58.69
12	331.67	198	59.70	12	170.00	100	58.82
13	270.00	165	61.11	13	125.00	75	60.00
14	206.67	130	62.90	14	245.00	151	61.63
15	295.00	190	64.40	15	168.30	105	62.38
16	170.00	110	64.70	16	155.00	98	63.22
17	151.67	95	62.64	17	278.30	178	63.95
18	120.00	79	65.83	18	188.30	122	64.78
19	190.00	125	65.78	19	108.30	71	65.54
20	265.00	180	67.92	20	171.70	113	65.81
21	155.00	100	64.51	21	115.00	75	65.22
22	100.00	65	65.00	22	126.67	83	65.52
23	105.00	68	64.76	23	268.33	178	66.34
24	283.33	176	62.12	24	155.00	103	66.45
25	175.00	115	65.71	25	153.33	103	67.17
26	158.33	107	67.58	26	151.67	103	67.91
27	138.33	92	66.51	27	181.67	124	68.25
28	206.67	140	67.74	28	151.67	105	69.23
29	166.67	110	66.00	29	168.33	115	68.32
30	110.00	75	68.18	30	111.67	78	69.85
31	135.00	94	69.63	31	76.67	54	70.43
32	110.00	77	70.00	32	61.67	43	69.73
33	63.33	45	71.05	33	41.67	29	69.59
34	115.00	80	69.56	34	30.00	21	70.00
35	91.67	65	70.91	35	16.67	12	71.98
36	65.00	46	70.77	36	10.00	7	70.00
37	40.00	28	70.00	37	20.00	14	70.00
38	26.67	19	71.24	38	10.00	7	70.00
39	20.00	14	70.00	39	3.33	2.5	75.07
40	10.00	7	70.00				
41	3.33	2.5	75.07				

Appendix Table A16: Triplicate average values for amount of biogas, methane and percentage of methane production of digester 7 & 8

Treatment-7				**Treatment-8**			
Days	Biogas (mL)	Methane (mL)	Methane (%)	Days	Biogas (mL)	Methane (mL)	Methane (%)
1	525.0	275	52.38	1	600.0	320	53.33
2	430.0	230	53.48	2	513.3	275	53.72
3	330.0	170	51.51	3	425.0	230	54.12
4	286.7	152	53.02	4	380.0	208	54.73
5	386.7	210	54.31	5	330.0	180	54.55
6	311.7	170	54.54	6	268.3	150	55.97
7	273.3	152	55.61	7	235.0	130	55.32
8	218.3	130	55.88	8	196.7	110	55.93
9	230.0	150	56.52	9	175.0	98	56.00
10	261.7	200	57.32	10	155.0	88	56.77
11	350.0	155	57.14	11	288.3	170	58.96
12	271.7	128	57.05	12	225.0	95	58.76
13	220.0	67	58.18	13	188.3	136	59.13
14	113.3	110	59.12	14	161.7	120	61.53
15	186.7	97	58.93	15	230.0	70	60.87
16	163.3	90	59.38	16	195.0	112	61.09
17	148.3	62	60.68	17	115.0	80	62.34
18	101.7	81	60.98	18	183.3	95	61.96
19	131.7	53	61.51	19	128.3	84	62.34
20	85.0	88	62.35	20	153.3	105	61.96
21	141.7	45	62.12	21	136.7	76	61.46
22	73.3	78	61.36	22	170.0	70	62.76
23	125.0	135	62.40	23	121.7	48	62.46
24	215.0	68	62.79	24	111.7	31	62.68
25	106.7	67	63.75	25	76.67	40	62.61
26	103.3	136	64.84	26	50.0	29	62.00
27	211.7	105	64.25	27	63.3	18	63.16
28	161.7	52	64.95	28	45.0	11	64.44
29	80.0	30	65.00	29	28.3	10	63.54
30	46.7	20	64.28	30	16.7	10	65.98
31	30.0	20	66.67	31	15.0	10	66.00
32	15.0	10	66.67	32	15.0	10	66.00
33	21.7	14	64.61	33	15.0	10	66.00
34	11.7	8	68.55	34	5.0	3.5	70.00
35	6.7	5	74.96	35	3.3	2.5	75.00

Appendix Table A17: Triplicate average values for amount of biogas, methane and percentage of methane production of digester 9 & 10

Treatment-9				Treatment-10			
Days	Biogas (mL)	Methane (mL)	Methane (%)	Days	Biogas (mL)	Methane (mL)	Methane (%)
1	600.0	300	50.00	1	690.0	370	53.62
2	513.3	260	50.65	2	530.0	290	54.71
3	325.0	220	51.76	3	451.7	250	55.35
4	280.0	195	51.32	4	361.7	200	55.30
5	333.3	170	51.51	5	310.0	170	54.84
6	265.0	138	51.43	6	381.7	210	55.02
7	235.0	123	52.34	7	295.0	168	56.95
8	296.7	105	53.38	8	248.3	140	56.38
9	275.0	95	54.28	9	118.3	68	57.47
10	255.0	85	54.84	10	208.3	120	57.60
11	188.3	155	53.76	11	118.3	68	57.47
12	225.0	125	55.56	12	268.3	155	57.76
13	288.3	105	55.75	13	130	76	58.46
14	125.0	90	55.67	14	213.3	125	58.59
15	230.0	130	56.52	15	166.7	100	60.00
16	106.7	110	56.41	16	101.7	60	59.00
17	188.3	65	56.52	17	128.3	78	60.78
18	203.3	105	57.27	18	151.7	92	60.65
19	128.3	75	58.44	19	133.3	80	60.00
20	153.3	90	58.70	20	180	110	61.11
21	136.7	80	58.53	21	158.3	98	61.89
22	170.0	100	58.82	22	231.7	145	62.58
23	121.7	72	59.17	23	121.7	76	62.46
24	65.0	68	60.89	24	163.3	103	63.06
25	53.3	47	61.30	25	118.3	75	63.38
26	40.0	31	62.00	26	103.3	66	63.87
27	70.0	40	63.16	27	78.33	50	63.83
28	31.7	28	62.22	28	61.67	40	64.86
29	21.67	18	63.54	29	45.00	30	66.67
30	16.67	11	65.98	30	76.67	50	65.21
31	15.00	10	66.67	31	30.00	20	66.67
32	20.00	13	65.00	32	16.67	11	65.98
33	18.33	12	65.46	33	18.33	13	70.98
34	6.67	5	74.96	34	10.00	7	70.00
35	1.67	1.3	77.84	35	3.33	2.5	75.00

Appendix 18. Correlation of percentage of methane with retention time

Correlations

		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
T1	Pearson Correlation	1	.371*	.383*	.381*	.031	.988**	.360*	.356*	.373*	.358*
	Sig. (2-tailed)		.017	.013	.014	.848	.000	.021	.022	.016	.021
	N	41	41	41	41	41	41	41	41	41	41
T2	Pearson Correlation	.371*	1	.997**	.998**	-.303	.430**	.995**	.994**	.997**	.996**
	Sig. (2-tailed)	.017		.000	.000	.054	.005	.000	.000	.000	.000
	N	41	41	41	41	41	41	41	41	41	41
T3	Pearson Correlation	.383*	.997**	1	.999**	-.257	.446**	.992**	.989**	.996**	.993**
	Sig. (2-tailed)	.013	.000		.000	.105	.003	.000	.000	.000	.000
	N	41	41	41	41	41	41	41	41	41	41
T4	Pearson Correlation	.381*	.998**	.999**	1	-.268	.442**	.994**	.991**	.998**	.994**
	Sig. (2-tailed)	.014	.000	.000		.091	.004	.000	.000	.000	.000
	N	41	41	41	41	41	41	41	41	41	41
T5	Pearson Correlation	.031	-.303	-.257	-.268	1	.082	-.339*	-.355*	-.301	-.351*
	Sig. (2-tailed)	.848	.054	.105	.091		.609	.030	.023	.056	.024
	N	41	41	41	41	41	41	41	41	41	41
T6	Pearson Correlation	.988**	.430**	.446**	.442**	.082	1	.421**	.415**	.430**	.415**
	Sig. (2-tailed)	.000	.005	.003	.004	.609		.006	.007	.005	.007
	N	41	41	41	41	41	41	41	41	41	41
T7	Pearson Correlation	.360*	.995**	.992**	.994**	-.339*	.421**	1	.999**	.996**	.998**
	Sig. (2-tailed)	.021	.000	.000	.000	.030	.006		.000	.000	.000
	N	41	41	41	41	41	41	41	41	41	41
T8	Pearson Correlation	.356*	.994**	.989**	.991**	-.355*	.415**	.999**	1	.995**	.998**
	Sig. (2-tailed)	.022	.000	.000	.000	.023	.007	.000		.000	.000
	N	41	41	41	41	41	41	41	41	41	41
T9	Pearson Correlation	.373*	.997**	.996**	.998**	-.301	.430**	.996**	.995**	1	.996**
	Sig. (2-tailed)	.016	.000	.000	.000	.056	.005	.000	.000		.000
	N	41	41	41	41	41	41	41	41	41	41
T10	Pearson Correlation	.358*	.996**	.993**	.994**	-.351*	.415**	.998**	.998**	.996**	1
	Sig. (2-tailed)	.021	.000	.000	.000	.024	.007	.000	.000	.000	
	N	41	41	41	41	41	41	41	41	41	41

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 19: Descriptive and ANOVA outputs

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
T1	Between Groups	6154.141	38	161.951	.115	.999
	Within Groups	2813.449	2	1406.724		
	Total	8967.589	40			
T2	Between Groups	19029.680	38	500.781	914.502	.001
	Within Groups	1.095	2	.548		
	Total	19030.775	40			
T3	Between Groups	18004.336	38	473.798	161.089	.006
	Within Groups	5.882	2	2.941		
	Total	18010.218	40			
T4	Between Groups	17748.038	38	467.054	93.920	.011
	Within Groups	9.946	2	4.973		
	Total	17757.984	40			
T5	Between Groups	1120.585	38	29.489	4.718	.190
	Within Groups	12.500	2	6.250		
	Total	1133.085	40			
T6	Between Groups	6636.763	38	174.652	.124	.999
	Within Groups	2827.432	2	1413.716		
	Total	9464.195	40			
T7	Between Groups	19650.523	38	517.119	381.012	.003
	Within Groups	2.714	2	1.357		
	Total	19653.237	40			
T8	Between Groups	19824.954	38	521.709	384.394	.003
	Within Groups	2.714	2	1.357		
	Total	19827.668	40			
T9	Between Groups	19030.050	38	500.791	2.773E3	.000
	Within Groups	.361	2	.181		
	Total	19030.411	40			
T10	Between Groups	19967.706	38	525.466	1.367E3	.001
	Within Groups	.769	2	.384		
	Total	19968.474	40			

Appendix 20: multiple comparisons of the substrate

One-Sample Test

	Test Value					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
T1	25.592	40	.000	59.84415	55.1181	64.5702
T2	14.715	40	.000	50.12634	43.2416	57.0111
T3	14.492	40	.000	48.02415	41.3265	54.7218
T4	14.546	40	.000	47.86537	41.2148	54.5159
T5	77.699	40	.000	64.58415	62.9042	66.2641
T6	25.073	40	.000	60.23146	55.3763	65.0866
T7	14.916	40	.000	51.63634	44.6399	58.6328
T8	14.965	40	.000	52.03585	45.0084	59.0633
T9	14.690	40	.000	50.04024	43.1555	56.9249
T10	14.941	40	.000	52.13415	45.0818	59.1865