Biogas Production by Anaerobic Co - Digestion of Food Wastes with Goat Manure

By

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This is to certify that the thesis presented by Gidey Kiros, titled as "***Biogas Production by Anaerobic Co-Digestion of Food Wastes with Goat Manure"*** and submitted to the School of Bio and Chemical Engineering in partial fulfillment of the requirements for the award of degree of master of science in Environmental Engineering complies with the regulations of the university and meet the accepted standards with respect to originality and quality.

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Declaration

I declare that this thesis titled as "***Biogas Production by Anaerobic Co - Digestion of Food Wastes with Goat Manure***" is my own, original work done under the supervision of Mr. Teshome Worku (Ass. Prof.) at Addis Ababa University, AAIT in 2016/17 academic year for the partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering and that I have not previously submitted it entirely or in part for obtaining any qualification at any other university and all references used in this work have been properly cited and accredited.

Gidey Kiros ___________ ___________

Signature Date

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May God help us in all our journey

Table of Contents

List of Figures

List of Tables

Acronyms and Symbols

3R	Reduce, Reuse and Recycling
AD	Anaerobic Digestion
ANOVA	Analysis of variance
APHA	American Public Health Association
AWWA	American Water Works Association
BOD	Biochemical oxygen demand
BOD5	BOD after five days incubation period
BV	Biogas volume
CBO5	Carbonaceous BOD5
COD	Chemical oxygen demand
CRs	Crop residues
C/N	Carbon-to-nitrogen ratio
CSTRs	Continuously stirred tank reactors
CV %	Percentage of coefficient of variation
DOE	Design of experiment
FOG	Fat, Oil and Grease
FVW	Fruit and vegetable waste
FW	Food waste
FAO	Food and Agriculture Organization
GM	Goat manure
HRT	Hydraulic retention time
IIT	Indian Institute Technology
L/kg VS	Liter per VS in kilogram
L/LRd	Liters per loading rate in days
LCFA	Long chain fatty acid
LR	Loading rate
M	Molarity
MFW	Mixed food waste
MRs	Mixing ratios

MSW	Municipal solid waste
MY	Methane yield
N	Normality
NRB	Nitrate-reducing bacteria
OLR	Organic Loading Rate
OW	Organic waste
ppm	Parts per million
PS	Primary sludge
PVC	Polyvinyl chloride
R	Ratio
RNA	Ribosomal Nucleic Acid
SIK	Swedish Institute for Food and Biotechnology
SRB	Sulfate-reducing bacteria
SRT	Sludge retention time
TN	Total nitrogen
TS	Total solids
US EPA	United States Environmental Protection Agency
UV	Ultra violet
VFAs	Volatile fatty acids
VS	Volatile solid
VSS	Volatile suspended solids
WPCF	Water Pollution Control Facilities

Abstract

Due to the rapid population growth and unmanageable urbanization, one of the burning issues faced by the world today is management of all types of wastes. This study has been conducted on the production of biogas by anaerobic co-digestion of food wastes with goat manure. Food waste (FW), goat manure (GM) and their mixtures were anaerobically digested for 40 working days. The proximate composition analysis of the FW and GM had shown that, whereas the GM was higher in TKN value (2.1%), the MFW was higher in C/N ratio (34.0). During this study FW and GM were combined into five MFW to GM mixing ratios of 1:0, 1:1, 2:1, 1:2, and 0:1; and a total of 45 MRs (nine for each mixing system) were prepared in 1-liter plastic bottles. The pH of these MRs was adjusted to 6, 7, and 8 by adding 10 N NaOH. Three water baths were set to three temperature values of 30^o C, 35^o C and 40^o C in which the 45 plastic bottles of 1-liter capacity (15 plastic bottles in each water bath) as main digesters were immersed. The experimental AD of this study took 120 days (40 working days for each experimental setup) to produce biogas. The combination systems of the MFW and GM for co-digestion overcomes the imbalanced C/N ratio of the MFW. The efficiency of the mono-digestion of MFW only, GM only and co-digestion of the MFW with GM were studied and compared. Statistical design of the experiments and data analysis was investigated using the general factorial design. Statistical test had shown that the model "F values" for both the responses, the biogas volume and methane content in the biogas were statistically significant ($P<0.05$). The optimum MRs between the MFW and GM were found to be the 1:1 and 1:2 MRs at process parameters of 35^o C and initial pH of 7.0. The cumulative biogas generated at these conditions were found to be 11.4 and 17.3 liters respectively from the MRs of 1:1 and 1:2. Averagely, higher methane yield of 61.1% and 63.5% respectively was obtained by the co-digestion of MFW with GM at 35^o C and initial pH value of 7.0 from the MRs of 1:1 and 1:2. The results noted from this study showed that the anaerobic co-digestions of MFW with GM was better than mono-digestion of the single waste alone for biogas generation.

Keywords: Anaerobic co-digestion, biogas volume, digestion time, goat manure, methane yield, Mixed food waste.

1. Introduction

1.1 Background

One of the burning issues challenged by the planet nowadays is handling of all types of wastes and energy crisis. The rapid population growth and irrepressible urbanization has created serious problems of energy requirement and solid waste disposal in the environment, which resulted in environmental impact and global warming. Biological conversion of biomass to methane has received increasing attention in recent years. Issues pertaining to global warming and climate changes are receiving unprecedented attention among the scientific as well as political spheres both at national and international levels (Arthurl & Brew-Hammond , 2010).

Climate problems resulting from the green house effect, ozone depletion, etc. have all contributed to the recognition of the value of anaerobic digestion of organic wastes such as food wastes as an alternative renewable source of energy. Anaerobic digestion (AD) is a biological process that produces biogas from bio-degradable wastes by bacteria under poor or no oxygen conditions. Biogas generation and its utility as an alternative renewable source of energy is increasingly gaining attention, particularly among the developing countries (Abdulkareem, 2005). In the past two decades, AD has been applied as an effective technology for solving the energy shortage and environmental pollution problems of biotechnology industries and residential activities caused by heating and electricity generation (Madsen, Holm-Nielsen, & Esbensen, 2011; Song, *et al.*, 2012; Weiland, 2010). In addition to serving as an alternative energy source, biogas generation through AD process also enables us to do away with organic wastes whose accumulation in the environment would otherwise lead to numerous health related problems. The organic wastes mainly consist of household food wastes, leftover food stuffs, agricultural, human and animal excrements (Alemayehu, Solomon, & Chavan, 2014).

Current estimates put global food loss and waste between one third and one-half of all food produced. Loss and wastage occurs at all stages of the food supply chain or value chain. In low-income countries, most loss occurs during production, while in developed countries much food about 100 kilograms per person per year is wasted at the consumption stage. Food waste or food loss is food that is discarded or lost uneaten. The causes of food waste or loss are numerous, and occur at the stages of production, processing, retailing and consumption (Galanakis, 2015).

The waste management hierarchy suggests that reduce, reuse and recycling should always be given preference in a typical waste management system. However, these options cannot be applied uniformly for all kinds of wastes. For examples, organic waste is quite difficult to deal with using the conventional 3R strategy. Of the different types of organic wastes available, food waste holds the highest potential in terms of economic exploitation as it contains high amount of carbon and can be efficiently converted into biogas and organic fertilizer. There are numerous places which are the sources of large amounts of food waste and hence a proper food waste management strategy needs to be devised for them to make sure that either they are disposed of in a safe manner or utilized efficiently. These places include hotels, restaurants, residential societies, college or school office canteens, religious mass cooking places, airline caterers, food and meet processing industries and vegetable markets which generate organic waste of considerable quantum daily. Compared with the single digestion of feedstock such wastes, the co-digestion of food wastes and animal manures increases the rate of biogas production because of the greater balance between carbon and nitrogen (El-Mashad & Zhang, 2010) and improves AD efficiency (Guo, *et al*., 2014).

Food waste (FW) usually from residential, commercial establishments, institutional and industrial sources is generated at an ever-increasing rate every year with the rapid population growth and rising living standards in Ethiopia. It seems to be a good idea to reuse this favorable feedstock for energy recovery and municipal solid waste (MSW) reduction because FW contains high moisture and biodegradable organics and accounts for 40–50% of the weight of MSW. Various AD processes have been widely developed in many countries for the treatment of food waste. AD is the most attractive and cost-effective technology for treating sorted organic fraction of MSW such as food wastes (Forster-Carneiro, Pe´rez, & Romero, 2008).

Food wastes and animal manure have recently been used together to produce biogas by AD. Goat manure (GM) is an excellent raw material for AD because of its high total nitrogen content and fermentation stability (Wang, *et al*., 2006). High TN content is beneficial to co-digestion with food waste because it decreases the carbon-to-nitrogen (C/N) ratios of single food waste substrates to an optimum value. GM is also insensitive to acidification during anaerobic fermentation (Jain, Singh, & Tauro, 1981; Kanwar & Kalia, 1993). Hence, GM is an excellent raw material for anaerobic co-digestion process. Although various raw materials such as agricultural waste, animal manures, sewage sludge and food waste have been reported as potentially feasible for co-digestion (El-Mashad & Zhang, 2010; Creamer, *et al*., 2010; Luostarinen, Luste, & Sillanpa¨a¨ , 2009; Bouallagui, *et al*., 2009; Macias-Corral, *et al*., 2008), the suitable mixing ratios of multi-component substrates between GM and various organic wastes are largely unknown.

Biogas generation was introduced in Ethiopia in 1957/58 at Ambo Agricultural College, located 115 km west from Addis Ababa. Human excreta was used as the substrate in the production of the fuel (Mogues, 2009). In October 1962, the first biogas floating drum digester in Ethiopia was installed in the same college. This floating drum biogas system comprised of 7 m^3 digester which was charged with daily loading rate of about 100 liters of dung and water in a 1:1 ratio (Bilhat, 2009). The country side of Ethiopia, where about 85 % of the population be present in, suffers disproportionately from the problems of ever deteriorating qualities of traditional biomass fuels and their manifold adverse impacts, as well as inaccessibility of modern fuels. Thus, to address the problems of domestic energy and improve rural peoples' access to modern fuels, the government of Ethiopia has been undertaking various intervention measures. One of these measures is the development and distributions of domestic biogas. The present study aims at the production of biogas by anaerobic co-digestion of food waste with goat manure. The influence of different MFW to GM mixing ratios at three pH value levels and three temperature levels to produce biogas by anaerobic co-digestion was investigated and the best ratios were obtained by comparing the results. Furthermore, an optimum co-digestion condition of the MFW and GM for biogas production was proposed.

1.2 Statement of the problem

Due to the rapid population growth, and irrepressible urbanization one of the burning issues faced by the world today, Ethiopia in particular is handling of all types of wastes which resulted in global warming and environmental worsening. Unmanageable urbanization has resulted in discharge of too much and erratic food waste. Biogas technology is at its infant stage of expansion and distribution in many developing countries like Ethiopia. The main aspects monitoring its distribution include: strategies and organizations, financial limitation, grants, availability of inputs, awareness about the technology, consumers' considerations, and success stories about the technology. However, biogas technology is a multipurpose technology which assists in addressing economic, health, social and environmental problems at the same time. Thus, expansion and distribution of the technology reduces environmental impact and global warming as well as minimizes lack of energy and also improves peoples' status in the energy ladder. Energy recovery from municipal and agricultural wastes have gained importance due to two-fold reason: i). Waste volume reduction, ii). Energy recovery. The search for alternative renewable energy sources is needed not only for replacement of fossil fuels, but also meet environmental protection demands. Currently, worldwide food waste is a growing issue generated in significant quantities, and the disposal of it is controversial causing increased food prices and the resources required as well as in global warming and environmental impact. During this study food waste was anaerobically co-digested with goat manure to produce biogas.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to produce biogas by anaerobic co-digestion of food wastes with goat manure.

1.3.2 Specific objectives

The specific objectives of this study were:

- To characterize the composition of the food wastes and goat manure.
- To determine the optimal food waste and goat manure mixing ratio as well as C: N ratio of the food waste by anaerobic co-digestion of goat manure for high biogas production.
- To evaluate the effect of pH, Temperature and Hydraulic retention time on methane production from the food waste.
- To determine methane yield.

2. Literature Review

2.1 The concept of biogas production

Several studies have been carried out on AD process to determine the biogas production potential of solid organic wastes including food wastes. The following studies have been done by different researchers in different time and place about biogas production from waste substrates. It has been reported by Ahmad (2000) that the techniques of biogas production have been in existence since 1850s. Biogas production systems have several benefits, such as (a) eliminating greenhouse gas, (b) reduction of odor, (c) betterment of fertilizer, (d) production of heat and power. The decomposition of organic wastes under anaerobic conditions ultimately yields biogas as one of the by-products of the process. Potentially, all organic waste materials contain adequate quantities of the nutrients essential for the growth and metabolism of the anaerobic bacteria in biogas production (Khanal, 2008). Figure 2.1 shows the end products of organic waste decay.

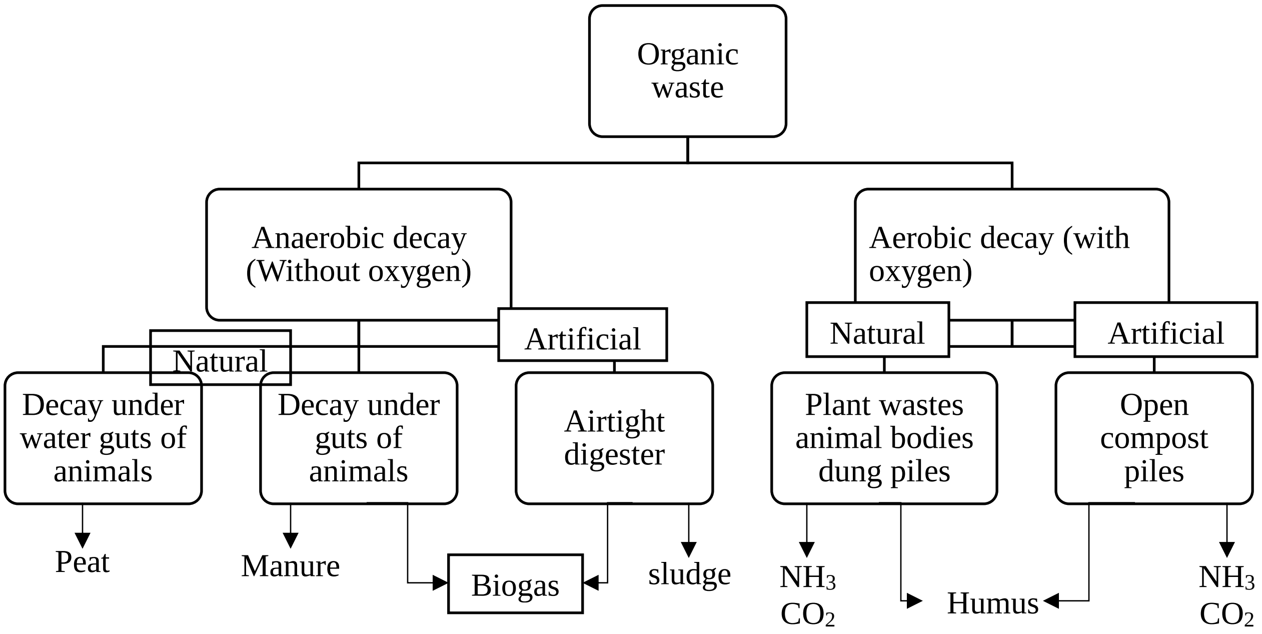


Figure 2. 1 End products of organic decay (Steadman, 1975)

Figure 2.1 indicates that biogas could be produced naturally from decay under water or the guts of animal, or artificially in an air tight digester. As a result, biogas has been described as a methane rich gas that is produced from the AD of organic materials in a digester (Itodo & Phillips, 2007); and biogas is a gas rich in methane which is produced by the fermentation of animal dung, human sewage or crop residues in an airtight container (General Environmental Multilingual Thesaurus, 2000). Biogas is a flammable gas produced by microbes when organic materials are fermented in a certain range of temperature, moisture content and acidities under airtight conditions. Biogas is a mixture of CH_4, CO_2, and small quantities of H_2S, N_2, H_2, CO, and several other hydrocarbon compounds (Table 2.1) (Ariane, 1979). The fact that methane (CH_4) is the major component of biogas, the properties of biogas could therefore be deduced from those of methane.

Table 2. 1 Typical biogas composition values (Deublin & Steinhauser, 2008)

Component	**Concentration (%Vol)**	**Origin**
Methane (CH_4)	50-75	Reduction of organic compounds
Carbon Dioxide (CO_2)	25-50	Reduction of organic compounds
Hydrogen Sulfide (H_2S)	0-3	Sulfate reduction by SRB
Nitrogen (N_2)	0-5	Nitrate reduction by NRB
Water (H_2O)	1-5	Water vapor due to heating
Ammonia (NH_3)	0-0.1	Reduction of nitrogen compounds

The conversion of complex organic matter to methane and carbon dioxide is possible only by the common action of at least four different groups of microorganisms. The essential microbial complex is comprised of hydrolytic bacteria, fermenting bacteria, acetogenic bacteria and methanogenic archaea. These groups of microorganisms have established syntrophic relationships where the later members of the food chain depend on the previous for their substrates, but they may also have significant influence on the earlier members in the chain by removing the metabolic products (Garcia, Patel, & Ollivier, 2000). Anaerobic digestion uses sequential breakdown of organic matter by syntrophic microbial communities to produce methane gas from organic substrates. A typical anaerobic bioenergy community contains over 300 different species of bacteria (Gerardi, 2003). Although AD can be considered to take place in four stages, all reactions occur simultaneously and are interdependent (Figure 2.2). The process by which these microbes break down organic substrates can be divided into four sequential stages each with their own process significance.

1. The first step of the anaerobic digestion process is **hydrolysis** in which complex insoluble polymers such as lipids, polysaccharides, and proteins or amino acids catalyzed by enzymes excreted from the hydrolytic and fermentative bacteria such as cellulase, protease and lipase are broken down into simpler constituents that are generally more soluble. End products of this reaction are soluble sugars, amino acids, glycerol and long chain carboxylic acids (Ralph & Dong, 2010). This step is essential because it enables the molecules to enter the bacterial cell wall. Hydrolysis is often considered the rate-limiting step because it involves the most complex series of syntrophic reactions and the largest molecule sizes (Werner, *et al*., 2011).

2. **Acidogenesis** is the next step and involves the conversion of the soluble monomers formed by hydrolysis into simpler alcohols and volatile fatty acids (VFAs). This stage is facilitated by microorganisms known as acid formers that transform the products of the hydrolysis into simple organic acids such as acetic, propionic and butyric acid as well as ethanol, carbon dioxide and hydrogen. Much of the VFAs produced during this step are short chain fatty acids (e.g. acetate, butanol, butyrate, ethanol, propanol) with acetate being the principal organic acid used as a substrate by the methane-forming organisms. Gases such as hydrogen and carbon dioxide released during this stage can be converted into acetate and/ or methane via certain strains of bacteria and thus are not inhibitory byproducts. Since many of the bacteria involved in hydrolysis are also involved in acidogenesis these two steps are often combined to form the term anaerobic fermentation (Ostrem & Themelis , 2004).

3. **Acetogenesis** occurs simultaneously to acidogenesis but by different syntrophic anaerobes. Here many of the acids and alcohols produced are degraded into acetate that can be used as a substrate by the methanogenic bacteria. The acetogenesis is completed through carbohydrate fermentation and results in acetate, CO_2 and H_2 compounds that can be utilized by methanogens. The presence of hydrogen is of critical importance in acetogenesis of compounds such as propionic and butyric acid. These reactions can only proceed if the concentration of H_2 is very low (Ralph & Dong, 2010). Thus, the presence of hydrogen scavenging bacteria is essential to ensure the thermodynamic feasibility of this reaction (Ostrem & Themelis , 2004).

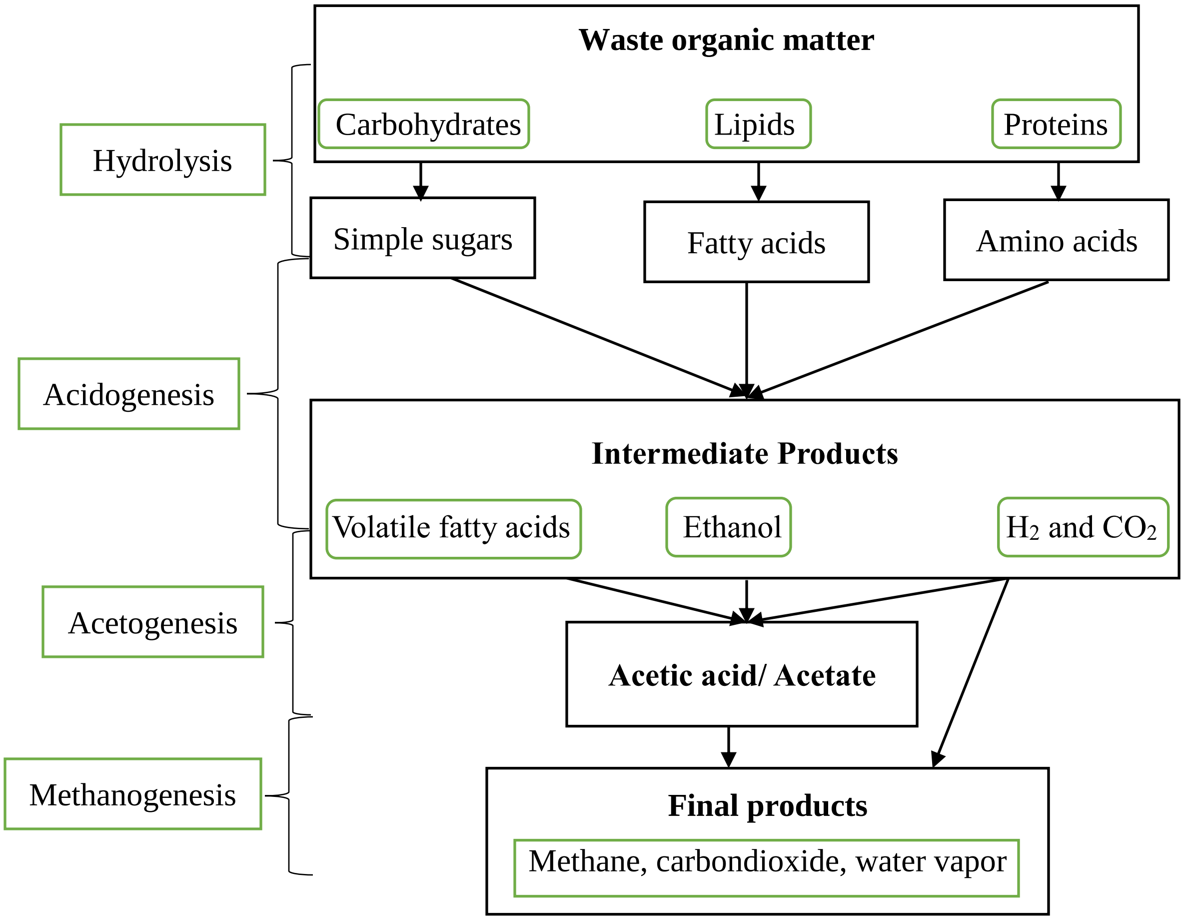


Figure 2. 2 Anaerobic digestion of organic matter

4. The last step in AD process is **methanogenesis**. The methane-forming organisms involved in this step are not classified as bacteria but as prokaryotic single celled organisms from the archaea kingdom. Their lack of membrane lipids, distinct RNA molecules, and peptidoglycan distinguishes them from being classified as bacteria (Khanal, 2008). There are three unique substrates utilization pathways for methane production to take place in the methanogenesis biochemical process (Table 2.2). Nearly 70% of the total methane gas is produced via the acetate reaction pathway (Gerardi, 2003).

Each group contains diverse microorganisms responsible for different metabolic tasks. Distinguishing characteristic of this anaerobic consortium is that different species of anaerobic microorganisms degrade one organic compound interactively, sharing energy and carbon sources from the compound (Schink, 1997). These organisms have developed specific kind of interdependent relationship called syntrophy, special kind of symbiotic cooperation of mutual dependence of the partner bacteria with respect to energy limitation where neither partner can exist without the other and together they exhibit a metabolic activity that neither one could accomplish on its own. Acid producing bacteria create an atmosphere with ideal parameters such as anaerobic conditions and compounds with a low molecular weight for methane producing bacteria. On the other hand, methane-producing microorganisms use the intermediates of the acid producing bacteria. Without consuming them, toxic conditions for the acid-producing microorganisms would develop (Suyog, 2010-2011). In this unique cooperation between two metabolically different types of microorganisms they depend on each other for degradation of a certain substrate for energetic reasons (Schink, 1997).

The syntrophic association between fatty acid oxidizing microbes, hydrogen-consuming methanogens, and acetate-consuming methanogens represents one syntrophic example in the methanogenic community. Fatty acids are converted by syntrophic oxidizers to acetate and hydrogen/CO_2, and these products are subsequently utilized by the two types of methanogens to form methane. Without this food chain, the degradation of fatty acids cannot occur unless coupled with the hydrogen and acetate consuming reactions because, the first step of the reaction is endergonic (Schink, 1997; Agdag & Sponza, 2007; Arsova, 2010). The reactions that occurs are as follows (Ralph & Dong, 2010; Forster-Carneiro, Pe´rez, & Romero, 2008):

Conversion of propionate to acetate:

$CH_3CH_2COO^- + 3H_2O \longleftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$; Free energy value: +76.1kJ (2.1)

Conversion of butyrate to acetate:

$CH_3(CH_2)_2COO^- + 2H_2O \longleftrightarrow 2CH_3COO^- + H^+ + 2H_2$; Free energy value: +48.3kJ (2.2)

Both reactions have unfavorable thermodynamics and unless in syntrophy with the hydrogen consuming bacteria (Equation 2.3) and methanogens (Equation 2.4 and 2.5) these organisms cannot exist. Particularly, hydrogen is the most important intermediate and the hydrogen-scavenging reaction makes the whole reaction energetically feasible.

Acetogenic reactions

$2HCO_3^- + 4H_2 + H^+ \longleftrightarrow CH_3COO^- + 4H_2O$; Free energy value: -104.6 kJ (2.3)

Methanogenic reactions

$CH_3COO^- + H_2O \longleftrightarrow CH_4 + HCO_3^-$; Free energy value: -31.0kJ (2.4)

$4H_2 + HCO_3^- + H^+ \longleftrightarrow CH_4 + 3H_2O$; Free energy value: -31.0kJ (2.5)

From this point of view, hydrogen consuming methanogens make an essential input to the production of methane and in driving the initial step of oxidation of the organic matter to be degraded by the heterotrophic microbes in AD. From the intermediates produced during AD; butyrate, propionate and acetate are the most important in addition to hydrogen. These substrates especially propionate and butyrate are also oxidized by the syntrophic association of fatty acid oxidizers and hydrogen-consuming methanogens (Bach-Knudsen, 1997).

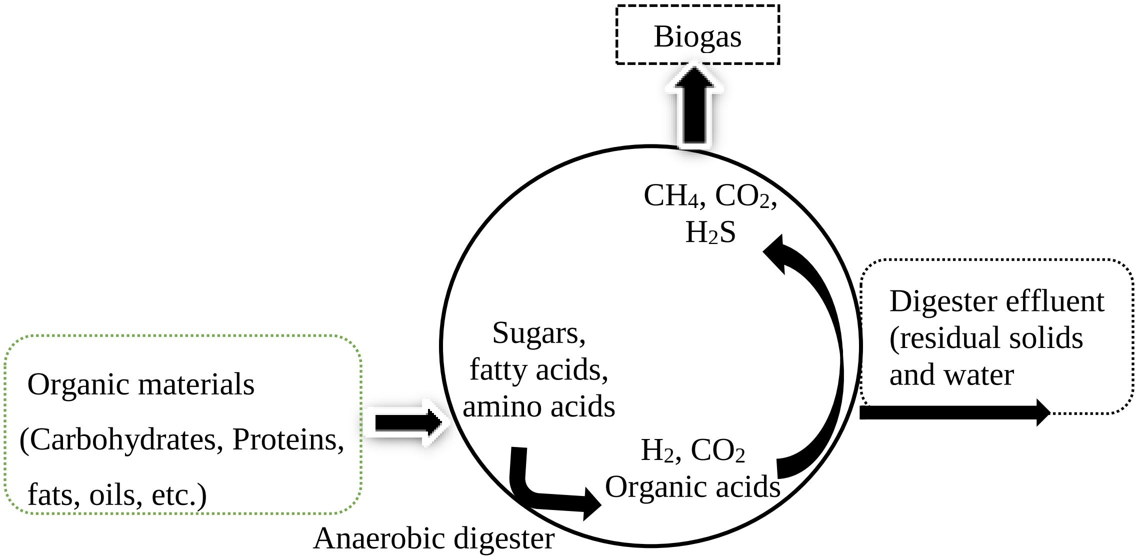


Figure 2. 3 Schematic diagram of organic waste conversion process in anaerobic digester

Table 2. 2 Substrate utilization pathways for methane production in AD environments

Type	Reaction Equation	Pathway utilization proportion
Acetate type	$CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3$ (2.6)	70%
Carbon dioxide type	$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$ (2.7)	30%
Methyl carbon type	$CH_3OH + H_2 \longrightarrow CH_4 + H_2O$ (2.8)	<1%

Although methane gas is the primary product of AD, other gases are produced, and gas composition varies widely based on feedstock characteristics as well as digester stability. Natural and anthropogenic sources account for 30 and 70 % respectively of the total methane released in the atmosphere every year. Major natural sources of methane are the wetlands and animal guts mainly insects and ruminants while, the main anthropogenic sources have been identified in the fossil fuel processing industries, rice fields and landfills. Biological activity has been identified to be the cause for more than 80% of the flux of the atmospheric methane (Ralph & Dong, 2010).

Humans have harnessed bio-methanogenesis for rapid and controlled decomposition of organic wastes and biomass feedstock to methane, carbon dioxide and stabilized residue. Since methane is a significant greenhouse gas AD has higher control over the methane production and contributes to lower the carbon footprint of the food waste management in the way that the fugitive emissions are lower than the emissions in the cases of the landfilling and aerobic composting (Levis, 2010).

Anaerobic digestion can be applied to a wide range of organic feedstocks including industrial, agricultural and municipal wastes. The types of feedstocks fed to an AD have a significant effect on the quantity and quality of biogas and solid effluent produced. For example, even in an optimally functioning digester system, agricultural wastes containing hemicellulosic and lignocellulosic material have low biodegradation efficiencies, and require effective pretreatment techniques to be a beneficial feedstock to digestion systems (Kumar, 2008). With no pretreatment, the biomass simply passes through the digestion system with little or no biodegradation. Comparatively, feedstocks high in fats and proteins are broken down within a few days while low molecular weight carbohydrates and alcohols biodegrade even more rapidly typically within a few hours (Kumar, 2008).

2.2 Anaerobic co-digestion advantages

Traditionally, AD was a single substrate and 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 simultaneous AD of multiple organic wastes used to increase methane production via the introduction of high-yielding readily digestible organic feedstocks in one digester is commonly referred to as anaerobic co-digestion. The key benefits of anaerobic co-digestion are improved nutrient balance, increased biogas production through the syntrophic effects of microorganisms, increased degradation of feedstock volatile solids and increased methane yield (El-Mashad & Zhang, 2010). However, care must be taken to avoid feedstocks that may inhibit the co-digestion process and thus reduce methane production.

Anaerobic co-digestion was reported to offer several benefits over digestion of separate materials such as increased cost efficiency, increased biodegradation of the treated materials as well as increased biogas production. The use of co-substrates usually improves the biogas yields from due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Mata-Alvarez, Mace, & Llabres, 2000). Various types of solid wastes streams such as sewage sludge (Heo, *et al*., 2003; Kim, Han, & Shin, 2003), cattle manure (Li, *et al*., 2009) and organic industrial waste has been used as co-substrate for anaerobic digestion of food waste.

Table 2.3 outlines a selection of full-scale and laboratory-scale co digestion studies that have been reported in the literature since 2000. The East Bay Municipal Utility District co-digestion process operates the nation’s largest co-digestion operation treating post-consumer food wastes along with municipal solid waste at a municipal wastewater treatment plant (US Environmental Protection Agency, 2010). From the sole standpoint of co-digestion and energy generation this project has shown great promise for the future of anaerobic co-digestion facility development in the US.

Table 2. 3 A selection of co-digestion studies utilizing using non-traditional OW since 2000

Co-digestates	Results	Reference
Post-consumer food waste and MSW	Waste and MSW Co-digestion of food waste increased biogas production by 35%	US EPA (2008)
FOG and MSW	Increased biogas production by 17 - 18% over control, but digestion of FOG only feasible up to 35% of total feed by volume	(Suto, *et al.*, 2006)
Grease trap waste and FOG	Increased methane yields 9 - 27% when 10 - 30% of grease trap feedstock was added	(Davidsson, *et al.*, 2008)
Yeast, food flavorings, restaurant, and brewery wastes	Notable synergism effects, increased biogas production by over 50%	(Zitomer & Adhikari, 2005)
FOG and MSW	Increased methane production yield by 37% at 64% FOG composition	(Wan, *et al.*, 2011)
Algal waste and paper sludge	Balanced C/N ratio using high-carbon paper waste with high-nitrogen algal sludge. Methane production rose 14% at 20-25:1 C/N ratio compared to algal sludge alone	(Yen & Brune, 2007)
Brewery waste, food waste MSW	Up to 70% increase in biogas compared to MSW alone	(Zitomer, *et al.*, 2006)
Fruit and vegetable waste, and MSW	Increase in biogas production by 27% compared to MSW alone	(Edelmann, Engell, & Gradnecker, 2000)

MSW = municipal solid waste. FOG = fat, oil and grease

Co-digestion can provide a better nutrient balance and therefore, better digester performance and higher biogas yields. Desai *et al.* (1994) reported the combination of whey and poultry manure had been found to be capable of maintaining the proper C/N ratio in the reactor. A highly-buffered system was obtained by co-digestion of solid slaughterhouse waste, manure, and FVW and the process worked well with gas yields of 0.81m^{3}kg^{-1}VS (Murto, Bjo¨ rnsson, & Mattiasson, 2004). Waste with poor fluid dynamics, aggregating wastes, particulate materials, floating wastes or materials with high disturbing or inhibiting components can be utilized more effectively as co-substrates when co-digest with well performing sewage sludge or liquid manure (Braun, 2002).

The addition of energy crops or silage as co-substrates allows for further increase in the biogas productivity of agricultural digesters (Braun, 2002). Animal manure usually contains high ammonia concentration that had an inhibitory effect on the glycolytic pathway. In co-digestion of plant material and manures, manures provide buffering capacity and a wide range of nutrients, while the addition of plant material with high carbon content balances the carbon to nitrogen (C/N) ratio of the feedstock, thereby decreasing the risk of ammonia inhibition (Lehtom¨aki, Huttunen, & Rintala, 2007). The following studies have also been done by different researchers in different time and place on anaerobic co-digestion of different substrates to determine the biogas production potential of these substrates like food wastes.

2.2.1 Anaerobic co-digestion of animal manure, energy crops and crop residues

Anaerobic co-digestion of grass silage, sugar beet tops and oat straw with cow manure was evaluated by (Lehtom¨aki, Huttunen, & Rintala, 2007) in semi-continuously fed laboratory continuously stirred tank reactors (CSTRs). It showed that it was feasible with up to 40% VS of crops in the feedstock. Compared with that in reactors fed with manure alone at a similar loading rate, volumetric methane production increased by 65, 58 and 16% in reactors fed with 30% VS of sugar beet tops, grass and straw respectively along with manure.

Gelegenis *et al.* (2007) examined a series of laboratory experiments in CSTRs at mesophilic conditions fed semi-continuously with various mixtures of diluted poultry manure and whey. Co-digestion of whey with manure was proved to be possible up to 50% input of whey by volume to the daily feed mixture without any need of chemical addition. However, specific biogas production (L/kg VS) remained roughly unchanged at the various whey fractions added in the feed mixture. The authors suggested that it is only due to the lower chemical oxygen demand (COD) of whey compared to that of manure. As whey fractions above 50% the reactor turned to be unstable as shown by the considerable decrease in pH and biogas production. Then, they scaled the experiments up to a continuously stirred pilot tank reactor and found biogas production increased from 1.5 to 2.2 L/LRd (almost 40%) for a hydraulic retention time of 18 days at 35^{o} C. Higher biogas production was due to the higher biodegradability of carbohydrates which is the main constituent of whey compared to lipids, the main constituent of manure and due to the improvement of C: N ratio balance (Lehtom¨aki, Huttunen, & Rintala, 2007) .

The possible use of potato tuber and its industrial by-products potato stillage and potato peels on farm-scale co-digestion with pig manure was also examined in a laboratory (Kaparaju & Jukka, 2005). The results showed that the potato tuber and its industrial by-products can be co-digested with pig manure at a loading rate of 2 kg VS m^{-3} day^{-1} in CSTR at 35° C.

2.2.2 Anaerobic co-digestion of animal manure and other organic waste

The potential of semi-continuous mesophilic AD for the treatment of solid slaughterhouse waste, fruit-vegetable wastes, and manure in a co-digestion process has also been experimentally evaluated and presented. They found that a combined treatment of different Bolivian waste types like manure (cattle and swine), solid slaughterhouse wastes (rumen, paunch content, and blood from cattle and swine), and FVW in a mesophilic co-digestion process gives the possibility of treating waste which cannot be successfully treated separately (Alvarez & Liden, 2007).

2.2.3 Anaerobic co-digestion of organic waste and municipal sludge

Results presented from the full-scale experiment on co-digestion of organic waste of domestic refuse (swill) with municipal sludge (Zupancic, Uranjek-Zevart, & Ros, 2007) had shown that anaerobic digestion is the solution to handling OW (swill) and above all it is very beneficial with little adverse impacts on the environment. They demonstrated that OW was virtually complete degraded and no increase in effluent VSS during the experiment as well as degradation efficiency increased from 71% to 81%. 80% increased biogas quantity was also observed.

Anaerobic co-digestion of sludge from grease traps and sewage sludge was also successfully performed both in laboratory batch tests and in continuous pilot-scale digestion tests (Davidsson, Lovstedt, la Cour Jansen, Gruvberger, & Aspegren, 2008). Single-substrate digestion of grease trap sludge showed high methane potentials in batch tests (845–928 ml/g VS_{in}) but could not reach stable methane production in the continuous digestion tests. Addition of grease trap sludge with digesting sewage sludge increases the methane potential and methane yield (amount of produced methane per added amount of VS).

The feasibility of the anaerobic co-digestion of a mixed industrial sludge with municipal solid wastes (MSW) was also investigated in three simulated anaerobic landfilling bioreactors. They concluded that anaerobic co-digestion of industrial sludge with MSW is a feasible process in the stabilization of the waste and in the treatment of leachate releases from the simulated anaerobic reactors. The supplementation of industrial sludge to MSW in simulated anaerobic bioreactors is a viable alternative for recovering high energy in the form of biogas with 72% methane content while at the same time improving the leachate quality (Agdag & Sponza, 2007).

The results obtained for the digestion of primary sludge (PS) and co-digestion of industrial sludge with fruit and vegetable fraction of MSW under mesophilic conditions has also been presented (Gomez, *et al*., 2006). The co-digestion of the fruit and vegetable fraction of MSW with PS produced more biogas than did the digestion of PS due to the higher concentration of VS contained in this feed. The parameters measuring the performance of the digestion process (specific gas production-SGP, and biogas yield) were more or less the same for the two kinds of feed evaluated. The application of a sudden increase in the organic load of the co-digestion systems led to higher gas production accompanied by a downgrading of the performance of the digesters even though the pH of the system was not affected (Gomez, *et al*., 2006).

The feasibility of the anaerobic co-digestion of five coffee wastes from the production of instant coffee substitutes and sewage sludge was assessed (Neves, Oliveira, & Alves, 2006). Methane yields in the range of 0.24–0.28 m^3/kg VS were obtained except for a barley-rich waste that achieved only 0.02 m^3 CH_4/kg VS. Four of the five wastes also presented a high reduction of TS (50–73%) and VS (75–80%), as well as 75–89% of the theoretical methane potential (350 l/kg COD removed).

The potential of mesophilic AD for the treatment of fats of different origin through co-digestion with the organic fraction of MSW was evaluated (Fernandez, Antoni, & Xavier, 2005). No important differences in the performance of the anaerobic co-digestion were observed when a fat from animal origin was suddenly changed by a fat of vegetable origin with a completely different long chain fatty acid (LCFA) profile. This may indicate that no important metabolic changes are implied in the degradation of different LCFAs with an acclimatized sludge. The authors concluded that the co-substrates may be variable in composition and thus, in expected inhibition.

2.3 Food wastes

2.3.1 Definitions

The international definition of solid waste includes municipal waste, industrial waste, mining and quarrying wastes, agricultural wastes and energy generation wastes (Beck & Parke, 2012). According to the definitions of the Food and Agriculture Organization (FAO) of the United Nations, food loss and food waste are different. There are five system boundaries in the food supply chains including agricultural production, postharvest handling and storage, processing, distribution and consumption (Figure 2.4) (FAO & SIK, 2011). Food loss refers to the decrease in edible food mass throughout the parts of the supply chain that specifically leads to edible food for human consumption. Food losses take place at production, postharvest handling and storage, and processing stages in the food supply chain (Buzby, *et al.*, 2011).

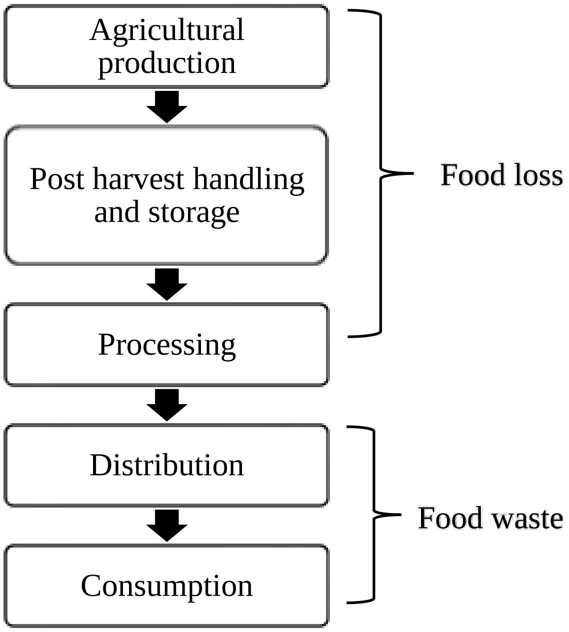


Figure 2. 4 Flowchart showing the FAO five system boundaries

Source: FAO & SIK (2011)

The unsustainable use of food resources has resulted in the generation of large amount of food waste in developing countries including Ethiopia. Presently, the worldwide MSW generation is about 2 billion tons per year. However, it is expected to rise to 3 billion tons by 2025. FVW production is also becoming a source of concern in municipal landfills because of its high biodegradability (Bouallagui, *et al.*, 2009). Food waste is the single largest category of MSW in Ethiopia. Biogas plant operators know well the advantages of adding fat residues or food wastes to their biogas plants. Food wastes collected from restaurants are highly desirable substrates for anaerobic digesters. These substrates are reported to yield 80% of the theoretical methane yields in 10 days of digestion time provided the various parameters affecting biogas generation are monitored properly (Neves, Oliveira, & Alves, 2006).

Food wastes are most of the time disposed of in landfills. The conversion of food wastes to energy is becoming a more viable practice due to the rapidly rising costs associated with energy supply and waste disposal and increasing public concerns with environmental quality degradation (Zhang, *et al*., 2007). Food waste has a potential for methane production depending on the type of food used. It can be digested rapidly making it a good source of material for AD. High calorie food wastes like bread, pasta and rice are easily degraded by fermentative bacteria, which produce large amount of organic acids which lowers reactor pH, inhibiting the methanogenic systems and limiting the generation of significant amount of methane (Dearman & Bentham, 2007). For instance, in Ethiopian higher institutions cafeteria wastes such as leftover of injera, which is made of teff, contains 15% protein, 3% fat and 82% complex carbohydrates and has high calorie content. The degradation of injera by fermentative bacteria produces large amounts of organic acids and lowers the reactor pH which in turn limits the generation of methane. Hence, co-digestion of these wastes with cow dung regulates the fluctuation of pH that would occur during digestion process (Alemayehu & Teshita, 2014).

Food wastes can be classified by whether they are avoidable. They include avoidable, possibly avoidable and unavoidable. The avoidable food waste is further divided into three groups according to the reasons for disposal. They comprise cooked, prepared or served too much, not used in time and others (Figure 2.5) (WRAP, 2009). Avoidable means the food was disposed simply because it is no longer wanted however, the food is still considered edible by the majority (Quested, *et al*., 2011). Possibly avoidable refers to the food in which some people eat but other does not and can be eaten means the food is not edible under normal situations. For instance, bones and apple cores are not edible (Parfitt, Barthel, & Macnaughton, 2010).

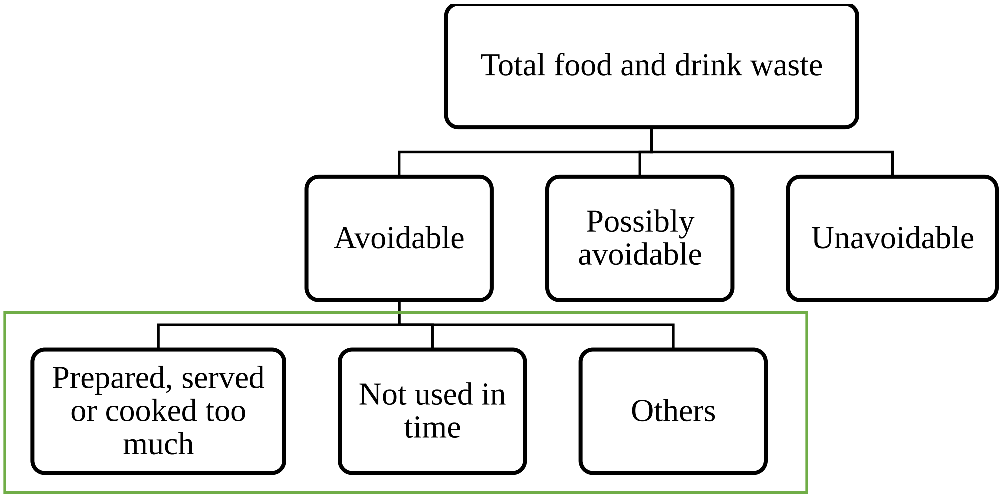


Figure 2. 5 Classification of food waste

2.3.2 Sources of food waste

The sources of food waste mainly originate from household, foodservice suppliers and food processing factories. The foodservice suppliers include supermarkets, wet markets, tuck shops, hotels, restaurants, meal box services and other catering outlets. Food distribution wastes food in improper food handling, packaging and transportation. Consumer food wastes are resulted from improper storage, wasteful food preparation processes, over-purchase behaviors and plate scraps (Griffin, Sobal, & Lyson, 2009). In the distribution stage, the wholesale and retail activities from food stores and supermarkets produce food wastes. The catering and beverages business like restaurants, bars and hotels create kitchen wastes. The hotel buffets produce leftovers by throwing away edible food for hygiene reasons. The improper household, foodservice supplier and customer behaviors are therefore the main causes of food waste in both distribution and consumption stages (FoE, 2013a).

2.3.3 The potential and limitation of food waste as substrate for AD

The US Environmental Protection Agency reports that food-based waste is the second largest category of MSW sent to landfills in the US, preceded only by paper. Food wastes account for approximately 14% of the total waste disposed of in landfills (US Environmental Protection Agency, 2010). The uncontrolled degradation of food wastes in landfills generates methane gas, which is considered a potent greenhouse gas, thus making it a substantial contributor to climate change (Alemayehu, Solomon, & Chavan, 2014). The US EPA has been at the forefront of diverting food wastes to AD facilities, whether they are privately owned or part of the public wastewater treatment system. They have listed multiple benefits for anaerobic co-digestion of food waste with other organic feedstocks like MSW, animal manure, etc. including economic benefits, climate change mitigation, and diversion opportunities. Diverting food waste and other organic wastes from landfills to anaerobic co-digestion facilities allows for the methane to be captured more efficiently and utilized beneficially while reducing the overall methane emissions. Furthermore, biogas produced at an anaerobic co-digestion facility is considered renewable, thus, there exists potential for greater greenhouse gas emissions reductions due to the energy offsets provided by an on-site energy generation system (Alastair, *et al*., 2008).

2.4 Goat manure as co-substrate for biogas generation

Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years. Goat manure, which is used as fertilizer in some countries can constitute a source of infection, since *Brucella* is eliminated in large numbers in urine and feces from infected animals. Human infection from accidental exposure to *Brucella* usually through inhalation in microbiology laboratories has been reported (Ensminger & Parker, 1986).

Goat manure is much drier than chicken manure, and it has a more balanced pH and less salt. The dry nature allows it to compost faster (Tessa , 2016). GM is an excellent raw material for anaerobic digestion because of its high total nitrogen content and fermentation stability. The TN contents of fresh GM (1.01%) is significantly higher than those of dairy manure (0.35%) and swine manure (0.24%). The high TN content is beneficial to co-digestion with CRs because it decreases the carbon-to-nitrogen (C/N) ratios of single CRs substrates (Wang, *et al*., 2006). GM is also insensitive to acidification during anaerobic fermentation (Jain, Singh, & Tauro , 1981; Kanwar & Kalia, 1993). Even though, different raw materials including food wastes have been stated as potentially viable for co-digestion (Creamer, *et al.*, 2010; Xie, *et al.*, 2011), the suitable mixing ratios between goat manure and food wastes are largely strange. In this study, the efficiency of biogas production by anaerobic co-digestion of FW with GM from five mixing ratios of the mixed food waste to GM have been investigated. By comparing the results, the best mixing ratios between the MFW and GM were obtained and an optimum co-digestion conditions were proposed.

Table 2. 4 Characteristics of common manures (Hess, 2016)

Manure source	**Characteristics**
Cattle	Cow manure is well-balanced, somewhat cool, and low in weed seeds, meaning that you don't need to be as careful in your composting process as with some other manures. Dairy manure is likely to be richer in nutrients than beef manure since milk cows are fed richer foods.
Poultry	Poultry manure is hot (high in nitrogen) and can burn plants if not properly composted. But on the plus side, the manure tends to be low in weed seeds. Poultry manure is also especially high in phosphorous, so you should limit applications if your soil phosphorous levels are already elevated.
Horses	Horse manure is hotter than cow manure, but not as hot as chicken manure. The amendment often contains weed seeds, making composting a must.
Pigs	Since pigs are somewhat carnivorous, their manure has more potential to transmit disease to humans. For optimal safety, use pig manure as you would human manure.
Sheep and Goats	Since sheep and goat pellets are relatively dry, they are easy to use in the garden. But the manure also tends to be relatively high in weed seeds and is somewhat hot that can burn plants from a gardening standpoint, so it's best to compost it before use.

2.5 Factors affecting biogas production

Several process parameters within an AD play key role in the physical environment and efficiency of waste digestion and biogas production potential. The microbial metabolism processes are dependent on many parameters so that for an optimum fermenting process, numerous parameters including pH value, temperature, solids concentration, HRT, VS and organic loading rate, inoculum, carbon–nitrogen ratio, toxicity, ammonium (NH_4), and water content need to consider maintaining for good treatment efficiency (Alastair, *et al*., 2008) and need to be controlled in the design and operation of AD reactors.

Many researchers evaluated the performance of an anaerobic system based on its methane production rate because, methanogenesis is regarded as the rate limiting step in anaerobic treatment of wastes. Anaerobic microorganisms especially methanogens are highly susceptible to changes in environmental conditions. Methanogens are highly vulnerable and extremely low growth rate so, an anaerobic treatment system requires careful maintenance and monitoring of the environmental conditions. A temperature change in the substrates or substrates concentration can lead to shutdown of gas production (Novaes, 1986). Some of these environmental conditions are shown in the Table 2.5 (Deublin & Steinhauser, 2008).

Table 2. 5 Environmental conditions and inhibitors in AD of wastes by methanogenic activity

Operation Parameters	**Inhibitors**
Hydrogen partial pressure	Oxygen (O_2)
Concentration of the microorganisms	Sulfur compounds
Type of substrate	Organic acids (fatty acids and amino acids)
Specific surface of material	Nitrate (NO_3^-), Ammonium (NH_4^+) ammonia
Cultivation, mixing and volume load	Heavy Metals
Temperature, Alkalinity and pH	Disinfectants, herbicides and insecticides
Organic Loading Rate (OLR)	Degree of decomposition of organic matter
Nutrients (C/N/P-ratio)	Foaming, Scum

2.5.1 Operating temperature

Anaerobic microorganisms are very sensitive to temperature. All metabolic processes in bacteria are brought about by enzymes. There is a temperature range within which these microbes thrive. When the temperature is not favorable, the enzymes may be denatured, which hampers their digestion process. In this regard, bacteria are classified according to their preferred temperature. It has a great effect on the growth, activity and survival of microorganisms. When operating at low temperature; chemical and biological reaction, and microorganisms' growth are slow down. The performance of AD system is greatly limited when the organic material degradation and the hydrolysis of suspended solids are at low temperature (El-Mashad & Zhang, 2010).

Under minimum growth temperature anaerobic microorganisms will even lose activity. When temperature rises, all the reactions in side microorganism like chemical, biological reaction and microorganisms' growth rate are peed up to the optimal range. The anaerobic degradation and treatment efficiency can achieve best results within the optimal range. But higher than the optimal temperature range nucleic acids, proteins and other cellular components will get irreversibly damaged and the system will shut down since the microorganisms lose activity (Luostarinen, Luste, & Sillanpa¨a¨ , 2009). It is interesting to note that AD in the natural environments occurred in a wide range of temperatures between 4º C (lake sediment) to 60º C (thermophilic digestion process); however, for the industrial practices, the temperature range is limited to 20-55º C. In the natural environments, the optimum temperature for the growth of methane forming archaea is 5-25º C for psychrophilic, 25-35º C for mesophilic, 45-60º C for thermophilic and >65º C for hyprethermophilic (Tchobanoglous & Burton, 1996).

It is generally understood that higher temperature could produce higher rate of reaction and thus promoting higher application of OLR without affecting the organic removal efficiency (Chae *et al.*, 2007; Poh & Chong, 2009). Although thermophilic condition could result in higher application of organic loading rates and better destruction of pathogens, at the same time it is more sensitive to toxicants and temperature control is more difficult (Gerardi, 2003). Furthermore, biomass washout that could lead to VFAs accumulation and methanogenesis inhibition could also occur if the thermophilic temperature could not be controlled (Poh & Chong, 2009). As a result, in tropical regions mesophilic temperatures are the preferred choice for anaerobic treatment of wastes (Yacob, *et al*., 2005).

2.5.2 Alkalinity and pH

The alkalinity and pH are related to each other and very promising to ensure a suitable environment for successful methanogenesis process. Alkalinity is produced as results of the hydroxides and carbonates of calcium, magnesium, sodium, potassium or ammonia and may also include borates, silicates and phosphates (Tchobanoglous & Burton, 1996). The alkalinity plays an important pH controlling role in the anaerobic treatment process by buffering the acidity derived from the acidogenesis process (Gerardi, 2003). Methane producing methanogens are known to be strongly affected by pH (Poh & Chong, 2009) and could only survive on a very narrow range of pH (Table 2.6) (Gerardi, 2003).

Table 2. 6 The optimum pH range for selected methanogens

Genus	**pH Range**	**Genus**	**pH Range**
Methanosphaera	6.8	*Methanococcoides*	6.5-7.5
Methanothermus	6.5	*Methanohalobium*	6.5-6.8
Methanogenium	7.0	*Methanolobus*	6.5-6.8
Methanolacinia	6.6-7.2	*Methanothrix*	7.1-7.8
Methanomicrobium	7.0-7.5	*Methanosaeta*	7.6
Methanosprillium	7.0-7.5		

The pH value of the reacting material is a pivotal factor in the AD of food waste. The importance of the pH is due to the fact that methanogenic bacteria are very sensitive to acidic conditions and their growth and methane production is inhibited in acidic environment. In batch reactors pH value is closer dependent of the retention time and loading rate. As such, the methanogenic activity will be severely affected once the optimum pH range is not met. The optimum growth conditions of *Methanosaeta Concilii* using a portable anaerobic micro tank was studied by (Steinhaus, *et al*., 2007). They reported that an optimum pH level of 7.6 revealing that even little variations on both sides of the optimum pH suppressed the growth of the methanogens. Several studies have also been reported on reactor failure or under performance simply due to pH reduction caused by accumulation of high VFAs in the anaerobic treatment system (Fabián & Gourdon, 1999; Poh & Chong, 2009).

In a study using synthetic wastewater in the thermophilic temperature it was found that at the pH of above 8.0, the methanogenesis was strongly inhibited and the value recorded for acetotrophic methanogenic test was zero (Viswanath, Sumithra Devi, & Nand, 1991). When investigating the role of pH in anaerobic degradation test it was found that the acidification led to the low performance of the anaerobic degradation, however the biodegradation was significantly increased once the wastewater when the pH was adjusted to above 6.5 (Fabián & Gourdon, 1999).

2.5.3 Composition of the food waste

The composition of food waste is variable depending on the time of the year, cultural habits, region etc. It is important to know the composition of the food waste in order to predict both the bio-methanization potential and the most efficient AD facility design. The bio-methanization potential of the waste depends on the concentration of the four main components: proteins, lipids, carbohydrates, and cellulose. This is due to the different bio-chemichal characteristics of these components (Neves, Oliveira, & Alves, 2006).

A large amount of research has been studied on biogas production from food wastes by AD process to improve biogas generation. The highest methane yields have systems with excess of lipids but with longest retention time. The methanization is fastest in systems with excess of proteins followed by the reactors with excess of cellulose and carbohydrate respectively. However, there are also inhibitory effects observed in the assays with excess of lipids and excess of proteins due to the VFA accumulation and ammonium nitrogen respectively. The lowest rates of the hydrolysis are the assays with an excess of lipids and cellulose, indicating that when these components are in excess, a slower hydrolysis is induced (El-Mashad & Zhang, 2010).

2.5.4 Organic loading rate

Loading rate is defined as the amount of raw materials fed per day per unit volume of digester capacity. OLR is a measure of the biological conversion capacity of the AD system. It is an important parameter that affects gas yield. Overloading of the system can result in low biogas yield. This happens due to accumulation of inhibiting substances such as fatty acids in the digester slurry (Arsova, 2010). If the digester is overfed, acids will accumulate, and methane production will be inhibited since micro-bacteria cannot survive in acidic situation. This is followed by a decrease in the main process parameters such as COD removal, specific methane production, and large number of suspended solids known as biomass washout in the effluent indicating that the reactor suffered a process imbalance and that biomass accumulated in the reactor (Converti, Ferraiolo, & Borghi, 1993; Fezzani & BenCheikh, 2007; Rincón, *et al*., 2008). This could be ascribed to an increase in the concentrations of the VFA with a consequent decrease in pH or to escalated levels of inhibitory or toxic compounds such as phenols, lignin and others (Tiwari, *et al*., 2006).

Similarly, if it is underfed, the gas production will also be low because of alkaline solution, which is also not a favorable condition for anaerobic bacteria (Teri, 1987). The effect of daily and alternate day loadings on biogas yield from raw dung was studied. According to (Moharao, 1974) a daily loading rate of 16 kg of volatile solids per m^3 of digester capacity produces 0.04-0.074 m^3 of gas per kg of raw dung fed. He further recommended loading rates for plants working on night soil ranging from 1.04 to 2.23 kg of volatile solids per m^3 of digester capacity. Higher loading rates are recommended only in cases where mean ambient temperature is high (Moharao, 1974).

Methanobacteriaceae and *Methanosaeta* were found the main methanogens in a laboratory scale up-flow anaerobic digester treating olive mill wastewater (Rizzi, *et al*., 2006). However, the authors also reported an interesting population shift by OLR variation. At lower OLR that was 6 kg COD/m^3d, hydrogenotrophic *Methanobacterium* predominated in the reactor but the number of cells/g sludge showed a 1000-fold decrease from 10^{11} to 10^8 when the OLR was increased to 10 kg COD/m^3-d. In contrast, phylotypes belonging to the acetoclastic *Methanosaeta* were not affected by OLR variation and at 10 kg COD/m^3-d, dominated in the biofilm (10^9 cells/g sludge) (Rizzi, *et al*., 2006).

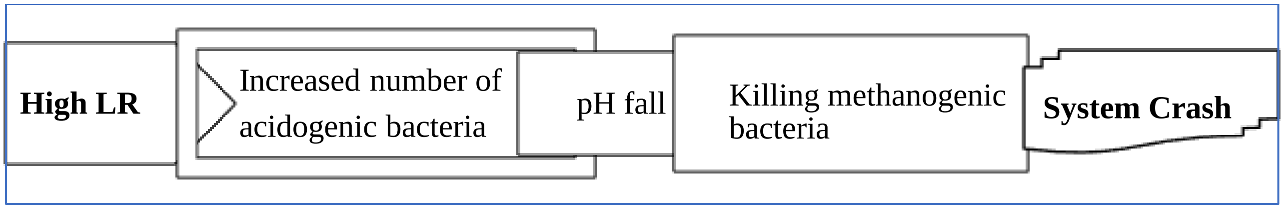


Figure 2. 6 Effect of the loading rate above the sustainable (Arsova, 2010)

The events that would occur in the case of overloading the system are shown in the Figure 2.6. It would cause proliferation of the acidogenic bacteria further decreasing the pH in the system and disturbing the population of the methanogenic bacteria. Loading rate can be calculated using the following equation:

$$\text{Loading rate (mg COD/m}^3\text{.day)} = \frac{\text{Organic matter (mg COD/m}^3\text{) x Flow rate (m}^3\text{/day)}}{\text{Operating volume (m}^3\text{)}} \qquad (2.9)$$

2.5.5 Hydraulic retention time

Retention time in an AD reactor refers to the time that feedstock stays in the digester. Hydraulic retention time (HRT) is the average period that a given quantity of input material remains in the digester to be acted upon by the methanogens. The retention time is calculated by dividing total volume of the digester by volume of input added daily. From the results of experiments on cattle-dung plant at IIT Guwahati, it was observed that the rate of gas generation is initially high and then gradually declines as the digestion approaches completion (Mahanta, *et al.*, 2004). The retention time in the AD reactors can be calculated using the following equation:

$$\text{Retention time (days)} = \frac{\text{Operating volume V (m}^3\text{)}}{\text{Flow rate Q (m}^3\text{/day)}} \qquad (2.10)$$

It is determined by the average time needed for decomposition of the organic material as measured by the COD and the BOD of the influent and the effluent material. The longer the substrate is kept under proper reaction conditions, the more complete its degradation will be. However, the rate of the reaction decreases with longer residence time, indicating that there is an optimal retention time that will achieve the benefits of digestion in a cost-effective way (Viswanath, Sumithra Devi, & Nand, 1991). The appropriate time depends on the type of feedstock, environmental conditions and intended use of the digested material (Ostrem & Themelis , 2004). Furthermore, retention time in the AD system depends on process temperature and total solid content. Mesophilic digesters have longer retention time (10-40 days) than thermophilic digesters. Also, the high solid content systems (dry processes) have longer retention time than low solid content systems (wet processes). Commonly used method for shortening the residence time in AD reactors is mixing the digester which is usually done by recirculation of the produced biogas back in the reactor.

2.5.6 Total solids

AD of food waste is considered as a standpoint way for its disposal. The total solids content of feedstocks affects the performances of AD process and the change of TS content will lead the change of microbial morphology in systems. The TS content of solid waste influences AD performance especially biogas and methane production efficiency (Pavan, Battistoni, & Mata-Alvarez, 2000). Previous studies have investigated on the role of TS content on AD performance in order to determine conditions for optimum gas production. In order to increase the efficiency of AD process, it is necessary to understand the role of the TS content on the behavior of the microbial communities involved in AD of organic matter from wet to dry technology. The effect of TS (5%, 10%, 15% and 20%) on the biogas production was investigated in AD reactors with mesophilic temperature condition and hydraulic retention time of 30 days. The experimental results had shown that the reactor with 10% of TS content yielded higher biogas compared with other reactors. The performances of mesophilic AD of food waste with different TS ranged from 5% to 20% were compared by different researchers and the microbial communities in reactors were investigated using 454 pyrosequencing technology. Pyrosequencing results revealed significant shifts in bacterial community with increasing TS contents. The proportion of phylum *Chloroflexi* decreased obviously with increasing TS contents while other functional bacteria showed increasing trend. *Methanosarcina* absolutely dominated in archaeal communities in three reactors and the relative abundance of this group showed increasing trend with increasing total solids contents (Jing *et al.*, 2014). It was also shown that the total methane production decreased with TS contents increasing from 10% to 25% in batch anaerobic digestion of cardboard under mesophilic conditions (Abbassi, *et al*., 2012). Forster-Carneiro *et al*.(2008), showed that the biogas and methane production decreased with the total solids contents increasing from 20% to 30% in dry batch anaerobic digestion of food waste.

2.5.7 Carbon/Nitrogen (C/N) Ratio

The C/N ratio represents the relationship between the amount of carbon and nitrogen contained in organic materials and it is an indicative measure of the nutrient balance that anaerobic organisms require for growth (Verma, 2002). The average C/N ratio of 20 – 25 has been stated as optimum for maximum yield of biogas and methane content by almost all workers referenced below. Analytical laboratory can be conducted to identify the carbon content, nitrogen content, and their individual C/N ratios each of which are useful for identifying codigestion feedstock blending ratios. These parameters can be optimized via co-substrate addition to optimize methane production (Wang, Xingang, & Gaihe, 2014). Achieving the optimum C/N ratio in the codigestion process ranging from 20-25:1 can be achieved with the methodical addition of high-carbon substrates and high-nitrogen substrates (Yen & Brune, 2007). Co-digestion of substrates has been preferred over mono-digestion due to several benefits associated with it. Mostly, specific methane production, ultimate methane production, methane production rate has been determined for evaluating the co-digestion process. Improvement in C/N ratio, higher bio-degradability, effective VS removal, eco-friendly sludge production has been regarded as merits of co-digestion process (Dioha, *et al*., 2013).

Materials with different C/N ratios differ widely in their yield of biogas. It is generally found that during AD microorganisms utilise carbon 25-30 times faster than nitrogen (Richards, *et al*., 1994). The C/N ratios in AD to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the feedstock vary between 10:1 and 30:1 (Richards, *et al.*, 1994) with an optimum, in most cases, C/N ratio between 15:1 and 25:1 (Yen & Brune, 2007). Excess amounts of some nutrients may however also become inhibitory to the AD process. Hence, a high C/N ratio will lead to a rapid consumption of nitrogen, in other words a reduced protein formation and a decline in the energy and structural metabolism of the anaerobes (Deublin & Steinhauser, 2008) resulting in lower substrate degradation efficiency and consequently in lower gas production rates (Zupancic , Uranjek-Zevart, & Ros, 2007). On the contrary, a low C/N ratio or too much nitrogen can cause ammonia to accummulate which would lead to pH value above 8.5 resulating in reduced anaerobic efficiency (Korres, *et al.*, 2013). Co-digestion will improve the characteristics of food waste including its content of micro and macro nutrients leading to a better C/N ratio.

Food waste has high carbon content used to generate high amount of biogas however, the single digestion of this substrate as a sole feedstock for biogas generation is not efficient due to its high carbon content and imbalanced C/N ratio. Goat manure has high total nitrogen content, fermentation stability, and is insensitive to acidic conditions during anaerobic digestion. The high nitrogen content of the goat manure is used to maintain and lower or balance the carbon to nitrogen ratio of food waste to optimal value for suitable biogas production and treatment efficiency. During this study, the production of biogas by anaerobic co-digestion of food wastes with goat manure was conducted. The influence of different MFW to GM mixing ratios at three pH value levels and three temperature levels to produce biogas by the anaerobic co-digestion was investigated and the best ratios were obtained by comparing the results. Furthermore, an optimum co-digestion condition of the MFW and GM for biogas production was proposed.

3. Materials and Methods

3.1 Materials

The study pointed at evaluating biogas production potential of food waste by anaerobic co-digestion with goat manure. Food wastes for the present study were collected from '*Amen Bahlawi Megbet'* and *'Piasa vegetable market'*. The food waste was composed of different fruit wastes and leftover foods such as injera, pasta, and macaroni mixed with different stews (Figure 3.1). To get the daily composition, the food waste was collected for five days and sorted separately in separated containers. Goat manure used during this study as co-digester was also collected from the local livestock farm near '*Qera*', *Beg- tera'*. Samples can degrade significantly during extended storage so that to prevent any organic decomposition thus avoid negative bias in the measurement of proximate content of the samples and to minimize odour during extended storage the wastes were stored in a fridge at 4^{o} C until analysis was begun (APHA, 1997).

Table 3. 1 Waste compositions used for the biogas production

Waste type	**Weight [g]**
Goat manure	12150
Food wastes	
Avocado peel	1735.71
Cabbage waste	1735.71
Carrot waste	1735.71
Potato peels	1735.71
Beetroot	1735.71
Mango peels	1735.71
Injera leftover mixed with stews	1735.71
Total	12150

Goat manure Mixed injera leftover Fruit peels waste

Figure 3. 1 Raw materials

The chemicals listed in appendix A used during this study were bought from the Atomic Educational Materials Supply PLC and Molary Trading P.L.C., Kirkos sub city, Addis Ababa.

The equipment used during this study are listed in table 3.2 and Appendix B.

Table 3. 2 List of different equipment used during the study

Equipment type	Purpose	Model and Manufacturer
Crucibles	Sampling for %TS, %VS, COD, BOD5 determination	
Fridge (set at 4° C)	Sample storage	
Electronic balance	Sample weight reading	Bosch wägesysteme GmbH, Germany
Oven	%TS determination	Memmert, Germany (100-800)
Muffle furnace	%VS determination	H.Jurgens and Co. Germany, Type VMK3
COD reactor	Digest samples for COD of samples determination	HANNA instruments Italy
BOD incubator	cBOD5 of samples determination	Lovibond® OxiDirect, Austria, TS 606/ 4-i
Photometer	Reading COD value of samples	HANNA instruments Italy
pH	Sample pH determination	Designed and manufactured in UK, JENWAY 3505

Fruit sparse mixer	Homogenization of food wastes	
Grinder	To grind goat manure to 1 mm	
GeoTech Biogas5000	Analysis of biogas composition (%CH_4, %CO_2, %O_2, H_2S (ppm))	GEOTech, GA5000
Graduated cylinder	Measuring volume of daily produced biogas and gas-displaced water collection	
Plastic bottles	Main digester, water container to be displaced by the gas produced	
Thermometer	Measure digester temperature	
Water bath	Minimizes temperature fluctuations within digesters	HWS24, temperature fluctuation +0.5°C
Plaster, UHU, Rubber stopper	Air tighten digester	

3.2 Methods

3.2.1 Analysis of proximate contents of sample

Many of the methods applied for the samples proximate composition determination listed in the present study were taken from the Standard Methods for Water and Wastewater Analysis (AWWA, APHA, & WPCF, 1992). The physical and chemical composition of the feedstocks were evaluated before digestion was started using standard procedures as outlined in APHA (1992). Parameters analyzed during this study includes total solids, fixed solids, volatile solids, total nitrogen content, pH, organic carbon content, C/N ratio, BOD and COD (Figure 3.2). The food wastes were separately weighed using weighing balance and ground by juicer then mixed together (Figure 3.4), whereas the goat manure used during this study as co-digester was milled to 1mm using grinder for analysis and feedstock preparation (Figure 3.3).

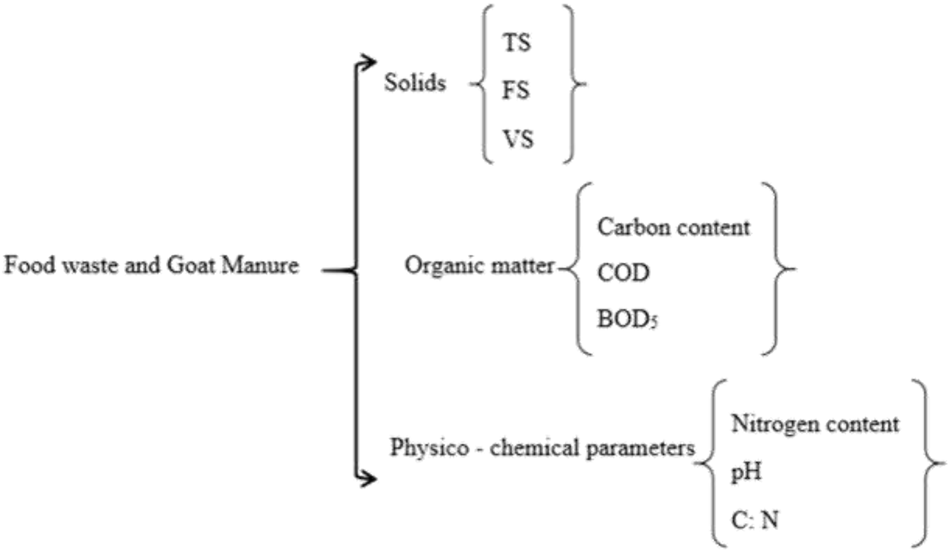


Figure 3. 2 Scheme of the characterization of food waste and goat manure

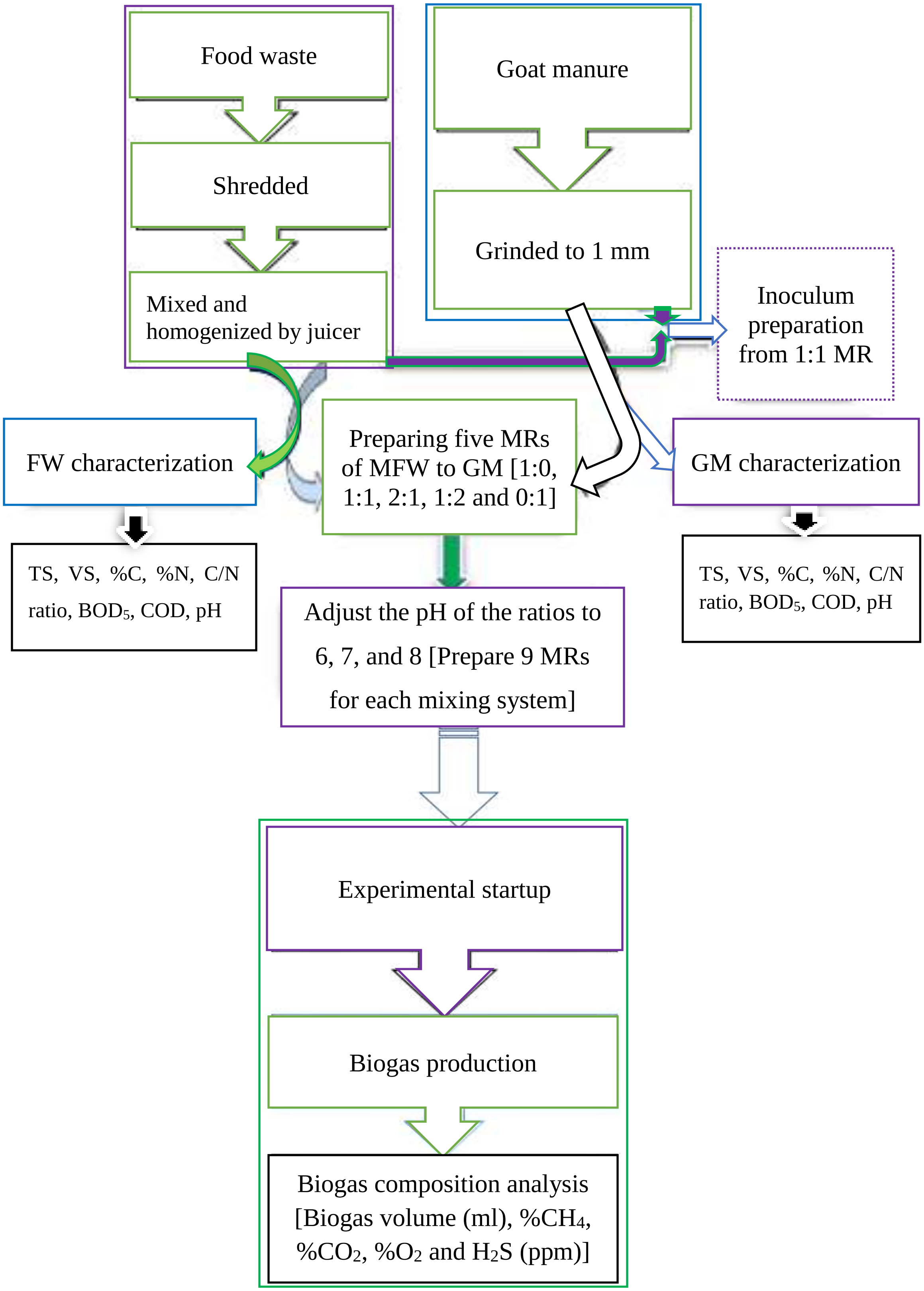


Figure 3. 3 Overall experimental work flow diagram

Figure 3. 4 Samples of different FW and GM ground for feedstock preparation

3.2.1.1 Determination of TS and VS

Devices used during this step were weighing balance, crucibles, drying cabinet or oven, and muffle furnace. For the TS content determination, food wastes were homogenized using juicer and goat manure was homogenized to 1 mm by using grinder. Eight clean empty crucibles which had previously been ignited in a muffle furnace at 550° C, cooled in a desiccator were weighed and recorded their corresponding weight (M_1). Samples of the homogenized wastes were filled in the crucibles separately, and the weight of the filled crucibles were once again weighed (M_2). The crucibles containing the samples were placed in an oven set to 105^{o} C for a period of 24 hours (Figure 3.5). After 24 hours, the samples were removed from the oven, weighed and then returned to the oven until constant weights were recorded (M_3) (APHA, 1997; Symons & Morey, 1941). The following formula was applied for the calculation of the TS content:

$$\%TS = \frac{M_3-M_1}{M_2-M_1} \times 100 \tag{3.1}$$

For the determinations of VS, the samples which were dried in the TS determination method were placed in a muffle furnace set at 550^{o} C and left there for 2 hours. The crucibles were then removed and were placed in an oven set at 105° C for 20 minutes to allow the dish to cool to around 105° C as placing a dish at 550° C in the plastic desiccator could have caused the plastic to crack. Once the crucibles were cooled they re-weighed (M_4) (APHA, 1997). The percentage of fixed (inorganic) solids in the samples was determined according to the following general formula:

$$\%FS = \frac{M_4-M_1}{M_2-M_1} x100 \tag{3.2}$$

The percentage of volatile solids in the samples was determined from the ash content of the samples using the following formula:

$$\%Ash = \frac{M_3-M_4}{M_2-M_1} x100 \tag{3.3}$$

$$\%VS = 100 - Ash\% \tag{3.4}$$

TS	Total Solid	%
VS	Volatile solids content	%TS
M_1	Mass of the empty crucible	(g)
M_2	Mass of the crucible after the sample was added	(g)
M_3	Mass of the crucible after drying at 105^{o} C	(g)
M_4	Mass of the crucible after burning at 550^{o} C	(g)

Figure 3. 5 TS and VS determination: drying oven (A) and maffle furnace (B)

3.2.1.2 pH determination

The pH of the samples was determined by placing 10 g of the homogenized samples in flasks containing 500 ml of distilled water. These were then stirred for 5 minutes and then let settled for 5 minutes and measured the pH using a pH meter. The pH of feedstocks for the digesters were adjusted to the required initial pH values 6, 7, and 8 to examine the effects of the initial pH on the biogas production and methane yield (Figure 3.6). The pH meter was dipped inside the glass bottles filled with the appropriate ratios of the wastes and adjusted to the required values by adding a 10 N NaOH solution to raise the pH to 6, 7 and 8.

Figure 3. 6 pH measurement

3.2.1.3 Carbon content

The carbon percentage of the samples was estimated based on the volatile solids content. VS are the components (largely carbon, oxygen, and nitrogen) which burn off an already dry sample in a laboratory furnace at 500-600°C, leaving only the ash (largely calcium, magnesium, phosphorus, potassium, and other mineral elements that do not oxidize). For most biological materials, the carbon content is between 45 to 60 percent of the volatile solids fraction. Assuming 56 percent (Adams, *et al*., 1951; Tom & Nancy, 1996; Badger, Bogue, & Stewart, 1979; Haug, 1993) the empirical formula used for the estimation of the % C was:

$$\% C =\cong \frac{56}{100} x VS \cong \frac{\% VS}{1.8} \tag{3.5}$$

3.2.1.4 COD determination

For the measurement of COD, samples of food wastes were homogenized using fruit sparse mixer prior to the test to improve accuracy and reproducibility. In the Palin test COD method, these samples were oxidized by digesting in a sealed reaction tube with sulphuric acid and potassium dichromate in the presence of silver sulphate as catalyst (Figure 3.7). The amount of dichromate reduced is proportional to the COD (AWWA, APHA, & WPCF, 1992).

Test Procedure

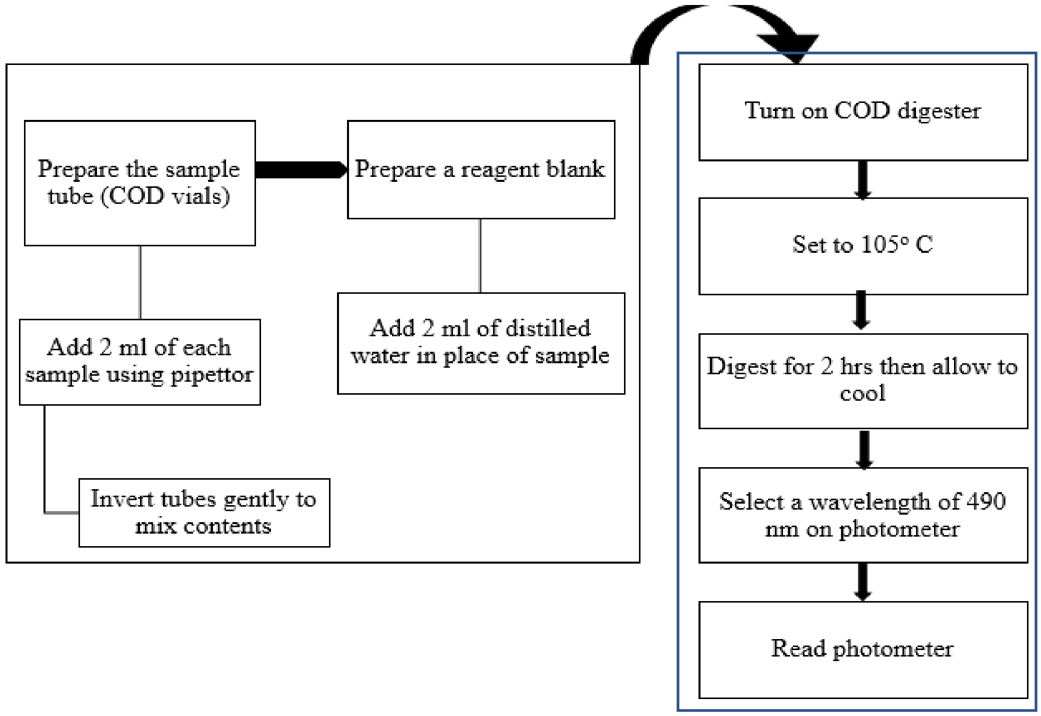


Figure 3. 7 COD test procedure flow diagram

From each sample, 2 ml volume of sample prepared by diluting 10 g of the samples in a flask containing 500 ml of distilled water (APHA, 1997) was added to a separated COD vials which had previously been premeasured reagents. A reagent blank was prepared by adding 2 ml of distilled water to the reagent tube then digested in the same manner as for the COD vials with the waste samples. The Nine filled COD vials one for the blank and the remaining eight for the samples were placed into a COD digester. The reactor was set to a digestion temperature of 150° C and 2 hours (APHA, 1997). Over the range of the test a color change from yellow (original vials color) (A) to blue (B) were produced (Figure 3.9). The color produced was indicative of the presence of COD (AWWA, APHA, & WPCF, 1992). The tubes were then cooled to room temperature. Once cooled, firstly the reagent blank was inserted into a photometer to set the instrument to zero absorbance before taking the samples reading (Jamie & Richard , 1996). The sample tubes were then inserted into the photometer and the readings were recorded. The results were expressed as milligrams of oxygen consumed per liter of sample.

Figure 3. 8 Crucibles containing samples for COD test

Figure 3. 9 Color change in COD vials

3.2.1.5 Determination of BOD_5

The sample volume is related to the expected BOD value. The OxiDirect® is designed to operate with different predicted BOD5 ranges and sample volumes, allowing BOD measurement up to 0 - 4000 mg/l without any dilution (Delzer & McKenzie, 2015). The optimum pH value for biochemical oxidation is between 6.5 and 7.5 (APHA, 1997).

To determine the 5-day carbonaceous biochemical oxygen demand ($CBOD_5$) 10 g of the samples in a flask containing 500 ml of distilled water were placed and mixed well, allowed to settle for five minutes. The pH values of the samples were then adjusted with 10 N NaOH to the acceptable range of 6.5-7.5. The samples were then filtered, and 56 ml of sample volume was measured using measuring cylinder and poured into the sample bottle. To inhibit nitrification, three drops of nitrification inhibitor B (Allyl Thiourea, or ATH) was added to each sample bottle. A clean magnetic stirring rod was added to each sample bottle and to absorb the CO_2 produced 4 drops of 45% potassium hydroxide solution was added to the seal gasket of the sample bottle. The seal gasket was inserted in the neck of the bottle. Finally, the BOD bottles were placed and incubated for five days at 20^o C (AWWA, APHA, & WPCF, 1992) and the readings were then taken after five days incubation period (Figure 3.10).

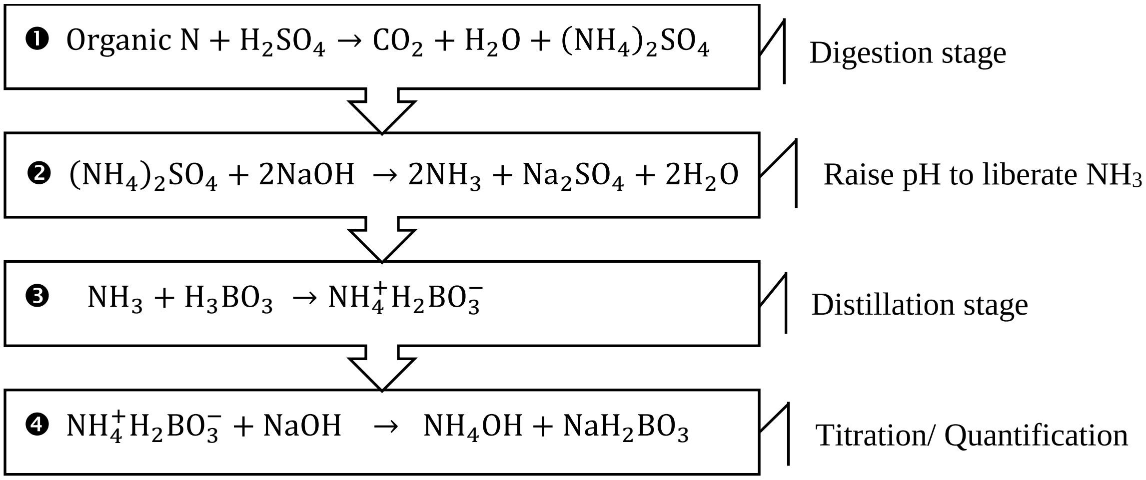
Figure 3. 10 Lovibond® OxiDirect BOD measuring system incubated with samples

3.2.1.6 Determination of Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) is used to determine the sum concentration of both organic nitrogen and ammonia nitrogen. The method involves a preliminary digestion to convert the organic nitrogen to ammonia, then distillation of the total ammonia into an acid absorbing solution and determination of the ammonia by titration method. The method consists of three basic steps: (1) **Digestion** of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia; (2) **Distillation** of the ammonia into a trapping solution; and (3) **Quantification** of the ammonia by titration with a standard solution. The method employed sulphuric acid as the oxidizing agent. A catalyst was added to hasten the oxidation of some of the more resistant organic substances (Figure 3.11). To determine the nitrogen content of the wastes all the food wastes were mixed together, and juicer was used to homogenize the mixture (Hiller, Plazin, & Slyke, 1948).

Test procedures

The digestion stage was accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The result was an ammonium sulfate solution (Figure 3.12). In this stage 5 gram of the homogenized MFW and ground GM were weighed and transferred to separate 500 ml Kjeldahl flasks. To each flask 150 ml distilled water was added. To liberate nitrogen as ammonium sulphate the organic matter in the waste samples was oxidized by adding 25 ml of concentrated sulphuric acid to each flask. The oxidation was kept rapidly at temperatures slightly above the boiling point of sulphuric acid (340 °C). To raise the boiling point of the digesting acid 20 g of potassium sulphate was added to each flask and to reduce bumping of the digestion mixture 1.5 g of alundum was added to each flask. To hasten the digestion process 2.5 g of copper sulphate as catalyst was also added to each flask.



❶ $\text{Organic N} + H_2SO_4 \rightarrow CO_2 + H_2O + (NH_4)_2SO_4$

❷ $(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

❸ $NH_3 + H_3BO_3 \rightarrow NH_4^+H_2BO_3^-$

❹ $NH_4^+H_2BO_3^- + NaOH \rightarrow NH_4OH + NaH_2BO_3$

Figure 3. 11 Process flow diagram of TKN steps

Figure 3. 12 Digestion stage: (A) for GM and (B) for MFW

Each flask was then heated slowly for about 2 hours with occasional swirling until a clear solution was obtained. After the digested mixture was changed to a whitish yellow color it was cooled. The mixture was then transferred into 200 ml volumetric flask and made up to 200 ml by adding distilled water (AWWA, APHA, & WPCF, 1992). The reaction involved during the digestion stage is:

$$\text{Organic N} + H_2SO_4 \rightarrow H_2O + (NH_4)_2SO_4 \qquad (3.6)$$

In the second stage of this method, 100 ml of the solution obtained by digestion was transferred to a Kjeldahl distillation assembly (Figure 3.13). To change the ammonium ions to ammonia the pH of the solution was raised by adding 100 ml of 40% NaOH until the solution becomes highly alkaline. To proceed the boiling during digestion very smoothly and with minimum danger of bumping by providing an evolution of fine bubbles of hydrogen gas (Bradstreet, 1940) 0.5 g of zinc dust was added to each flask. The Kjeldahl flask was attached to a water condenser then heated to boil off the NH_3 gas from the digest. To trap the distilled NH_3 in receiving solution the tip of the condenser was submerged in a flask of receiving boric acid solution. The reaction involved during this stage is,

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \qquad (3.7)$$

Boric acid was used as the receiving solution because the boric acid method has advantages over standard acid as a receiving solution: (1) prevents loss of ammonia by volatilization, (2) week acid to interfere with further titration of ammonium borate, (3) no need for back titration as in case of reaction with standard acid such as H_2SO_4 and HCl (Bradstreet, 1940). To prevent loss of ammonia during distillation, the receiving solution was kept on the ambient temperature that was below 29° C (APHA, AWWA, & WPCF, 1992). Distillation collecting distillate was carried out in volumetric flask containing 50 ml of 4 % boric acid and about 150 ml of distillate (total of 200 ml in flask) was collected. The boric acid captures the ammonia gas forming an ammonium-borate complex. As the ammonia collects the color of the receiving solution was changed to slight whitish. Three drops of phenolphthalein indicator were added to this distillate then finally this distillate was titrated with a standard solution of 0.1 N NaOH in a dropwise (Mohapatra, 2014; Verma, Khanna, & Kapila, 2010). The burette was turned off as soon as the color of the distillate was changed into pink color (Figure 3.14). The amount of the standard solution used from the burette was recorded. The reaction involved during this stage is:

$$NH_3 + H_3BO_3 \rightarrow NH_4^+H_2BO_3^- \tag{3.8}$$

$$NH_4H_2BO_3 + NaOH \rightarrow NH_4OH + NaH_2BO_3 \tag{3.9}$$

End point had been reached when the MFW and GM distillates were titrated with 52 and 75 ml of 0.1 M NaOH respectively (Figure 3.14). This means that all the ammonium-borate complex in the distillates were consumed by this much of the standard solution or 150 ml of the ammonium-borate complex were completely reacted with 52 and 75 ml of the standard solution 0.1 M NaOH. The total nitrogen content of the distillates as percentage of the original samples mass was calculated from the volume of NaOH used according to the following equations derived from the volumetric analysis method $C_1V_1 = C_2V_2$ and the detail calculation is computed in appendix D.

$$\%\ \text{Nitrogen} = \frac{(\text{ml standard solution}) \text{ x N of standard solution x } 1.4007}{\text{Weight of sample in grams}} \tag{3.10}$$

$$M_{NH4H2BO3} \text{ x } V_{NH4H2BO3} = M_{NaOH} \text{ x } V_{NaOH} \tag{3.11}$$

$$M_{NH4H2BO3} = \frac{M_{NaOH} \text{ x } V_{NaOH}}{V_{NH4H2BO3}} \tag{3.12}$$

(a) (b)

A: Kjeldal flask

C: Condenser

E: Heater

B: Stand- supports the setup mechanically

D: Receiving flask

Figure 3. 13 Distillation stage for GM (a) and for the MFW (b)

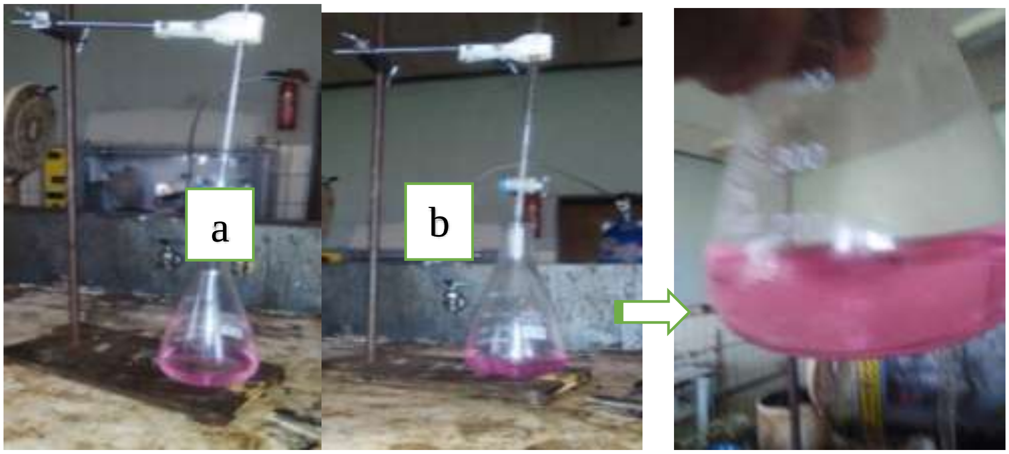


Figure 3. 14 Titration stage: for GM (a), for the MFW (b)

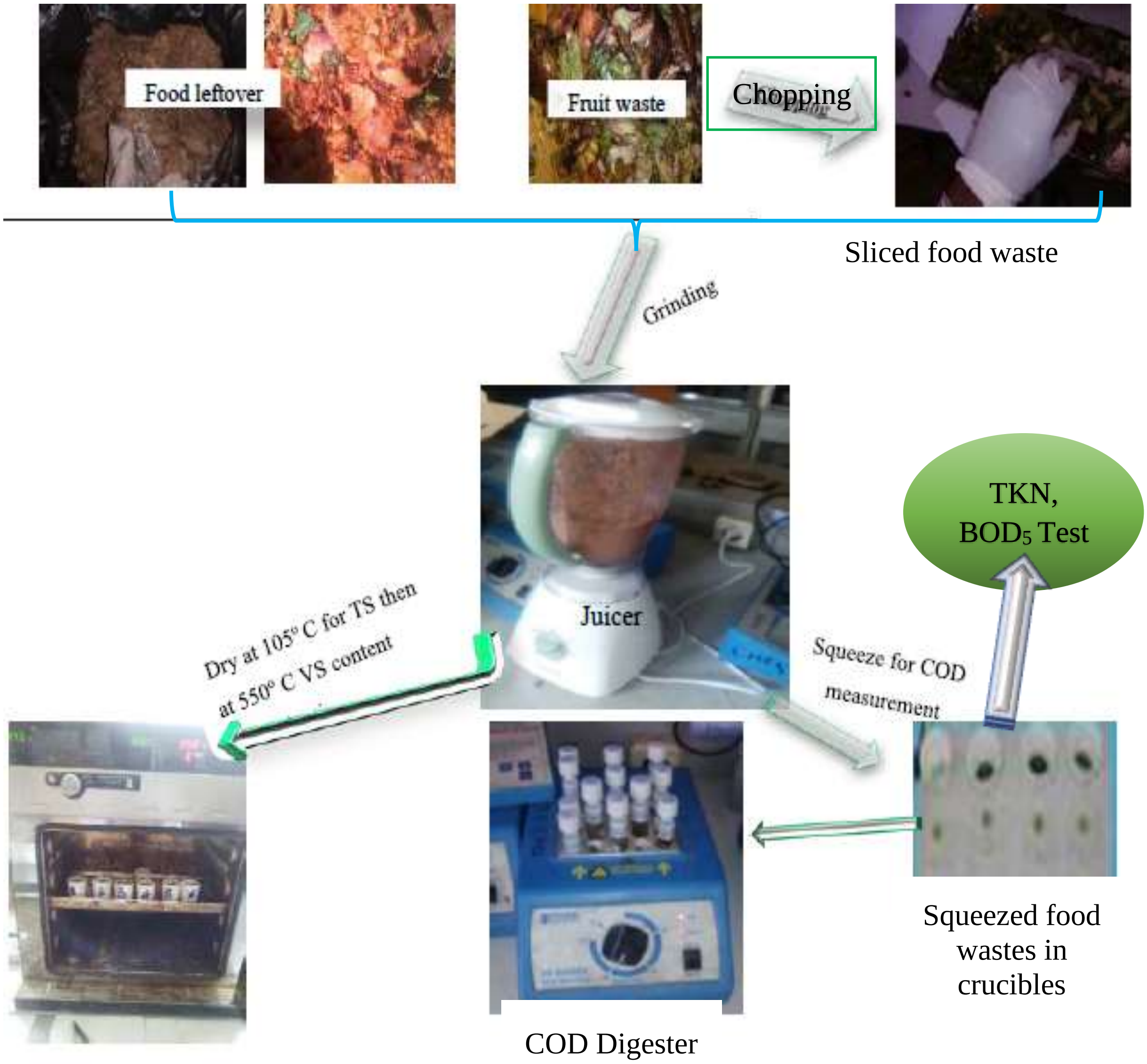


Figure 3. 15 Food wastes characterization and preparation for feedstock

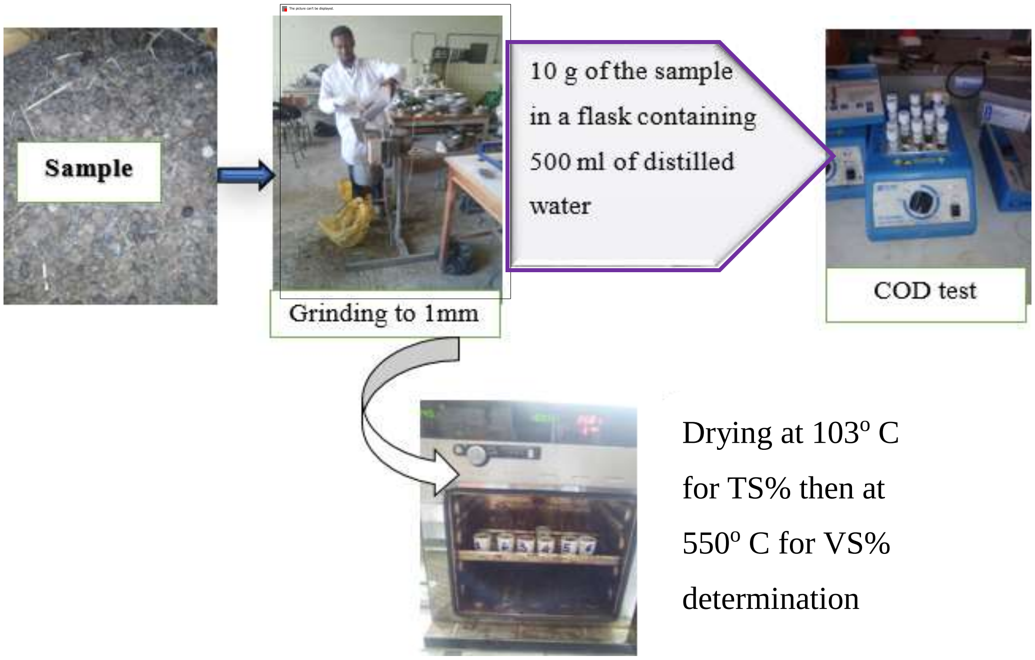


Figure 3. 16 Goat manure characterization and preparation as co-digester

3.2.2 Inoculum preparation

Digestion is a slow process and it takes at a minimum of three weeks for the microorganisms to adapt to a new condition when there is a change in substrate or temperature (Dieter & Angelika , 2010). To reduce the lag phase of the methanogenic activity for the substrates decomposition and to get a continuous biogas production an inoculum was used. The inoculum for this study was digested sludge prepared by acclimatization of the 1:1 ratio of MFW to GM in a bucket (Figure 3.17) after the wastes were reduced in size and homogenized. Before the experiment was started up a 13.5 kg of a 1:1 ratio of the MFW to GM was prepared and allowed to digest anaerobically in a bucket for fifteen days. Slurry of this digested was characterized for its proximate composition and fed into the digesters as methanogenic bacteria provider. As the microbes responsible for methane production of this slurry were adapted to both waste type this slurry was used as inoculum. This allows the microbial groups suitable for the food wastes and goat manure to able to habituate.

Figure 3. 17 Inoculum used for the study

3.2.3 Feedstocks preparation

Following the collection of the food wastes and goat manure, the FW were chopped manually using knife and homogenized using blender/juicer, and GM was ground to 1 mm size using grinder. The characteristics of these wastes were determined and prior to the commencement of the experiment the FW were thoroughly homogenized using a fruit sparse mixer to achieve minimal particulate size suitable for easy digestion and then mixed evenly with the co-digester GM. The mixtures used as feedstocks during this study were a combination of 1:0, 1:1, 2:1, 1:2, and 0:1 mixing ratios of MFW to GM. The digesters loaded with 1:0 (MFW only) and 0:1 (GM only) were used as control to determine the effect of MFW and GM co-digestion process. The fruit peels and leftover food stuffs portion of the mixed wastes fed into the digesters was consisted of wet weights of waste as indicated in Table 3.3. The TS content, VS, carbon content, nitrogen content and C/N ratio of the mixed wastes fed into the digesters were computed in Appendix D (Tom & Nancy, 1996; APHA, AWWA, & WPCF, 1992).

Table 3. 3 Waste composition and mixing ratio of feedstock for each digester

Mixing ratio*	**Sample Weight (g), FW to GM**	**% w/w**	**Volume of water added (L)**	**Volume of prepared sample fed into single digester (gm)**
1:0	540: 0	100% MFW	0.72	600
1:1	270:270	50%: 50%	3.16	600
2:1	360:180	33.33%: 66.67%	2.35	600
1:2	180:360	66.67%:33.33%	3.97	600
0:1	0:540	100% GM	1.04	600
Inoculum	150:150	50%:50%	1.86	200

Remark: * The mixing ratio used in this study was always mixed food waste to goat manure [MFW/GM].

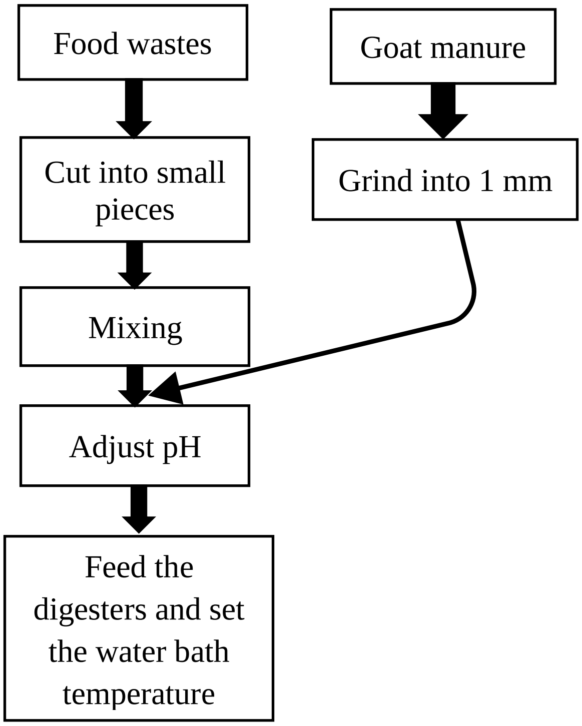


Figure 3. 18 Biogas production process for the co-digestion of food waste with goat manure

3.2.4 Experimental setup and startup

Anaerobic digesters were made from plastic bottles with capacity of 1 liter. The biodegradability and biogas yield of MFW co-digested with GM were determined at three different temperature levels and three different initial pH value levels by combining into five mixing ratios. In this study three experimental setups each having 15 experimental plastic bottles of 1 L capacity as main digesters filled with five MFW to GM mixing ratios (1:0, 1:1, 2:1, 1:2, and 0:1) that were adjusted their pH values to three different levels (6, 7, and 8) were carried out. To tolerate the temperature variation inside the digesters three water baths were used. In each water bath that were set their temperature to 30^{o} C, 35^{o} C and 40^{o} C, 15 plastic bottles filled with the samples of MFW and GM combined into the five different mixing ratios were immersed for 40 working days (Figure 3.20). The main digesters were connected by a gas delivery PVC tube to another 2 L plastic bottles filled with water to a marked area which were placed outside the water baths. These 2 L plastic bottles were also connected via PVC tube to a measuring cylinder or volumetric flasks for collection of the displaced water from the water filled 2 L plastic bottles. The 2 L plastic bottles were designed to have two openings; one inlet for the PVC tube coming from the main digester, which was designed to serve as gas outlet while the second served as gas-displaced water outlet to the measuring cylinder or volumetric flasks for daily produced biogas measurement. The gas produced in the main digesters chamber was collected in the water filled gas collection chambers by water displacement. The prepared digesters were manually shaken for one minute twice every two days (for working days) or three days (for weekend and holidays) days whenever after gas composition analysis was made.

The general representative for the experimental setup of the study is shown below (Figure 3.19). The working principle in the setup is: the AD process takes place in the 1 L plastic bottle digester (2), the temperature fluctuations inside the reactors were minimized by immersing the reactors in the water bath (1), the biogas produced (mixture of CH_4, CO_2, H_2S, O_2 and other trace components) from the 1 L digester was directed to the next 2 L plastic bottle filled with water (5) via the biogas PVC tube (4), this water filled 2 L plastic bottle was then directed via the PVC tube (6) to the measuring cylinder (7). Note that the biogas PVC tube was designed without touching the liquid (water) surface and the PVC tube for the gas-displaced water was immersed below the water surface to the bottom surface of the 2 L plastic bottle to prevent gas flow to the measuring cylinder.

The volume of biogas production from the reactors was recorded every two or three days by water displacement method. The volume of water displaced from the bottles was equivalent to the volume of gas generated. Gas composition was assessed by drawing periodical gas produced from the collecting plastic bottles (5) by connecting the PVC tube (6) to the gas sampling hose of the gas analyzer. In each time, a gas composition analysis was made, the 2 L plastic bottles were filled with water again to the marked area to know the succeeding new volume of gas produced. The experiments were run for 40 working days in continuous fermentation during which the following were carried out:

- Volume of biogas produced was noted every two or three days
- The time between the start of gas production and termination of the experiment
- Investigation of the gas composition to separate it to its different components

1. Water bath
2. Anaerobic digester (1 L)
3. Tight rubber septum
4. Biogas tube
5. Air tight biogas collection unit filled with water (2 L)
6. Tube for gas-displaced water
7. Measuring cylinder for displaced water collection

Figure 3. 19 General demonstrative for the experimental setup

Layout of the experimental setup at operating Temperature of 30° C

Layout of the experimental setup at operating Temperature of 35° C

Layout of the experimental setup at operating Temperature of 40° C

Figure 3. 20 Layout of the experimental setups at three different operating temperatures for different five mixing ratios and three pH values

3.2.5 Evaluation of digesters temperature

As stated previously, three water baths were used to uphold the temperature inside the digesters. Thermometer was used to determine the temperature received to the digesters from the heated water in the baths. The temperature within the digesters, on average, received the same amount of heat as the temperature of the heated water within the water baths. The temperature within reactors attained the same as the surrounding water temperature however, the organic matter within digesters is not in direct contact with the surrounding heated water in the water baths, this may be achieved due to the reaction within the reactors because of organic matter decomposition.

3.2.6 Biogas volume measurement and composition analysis

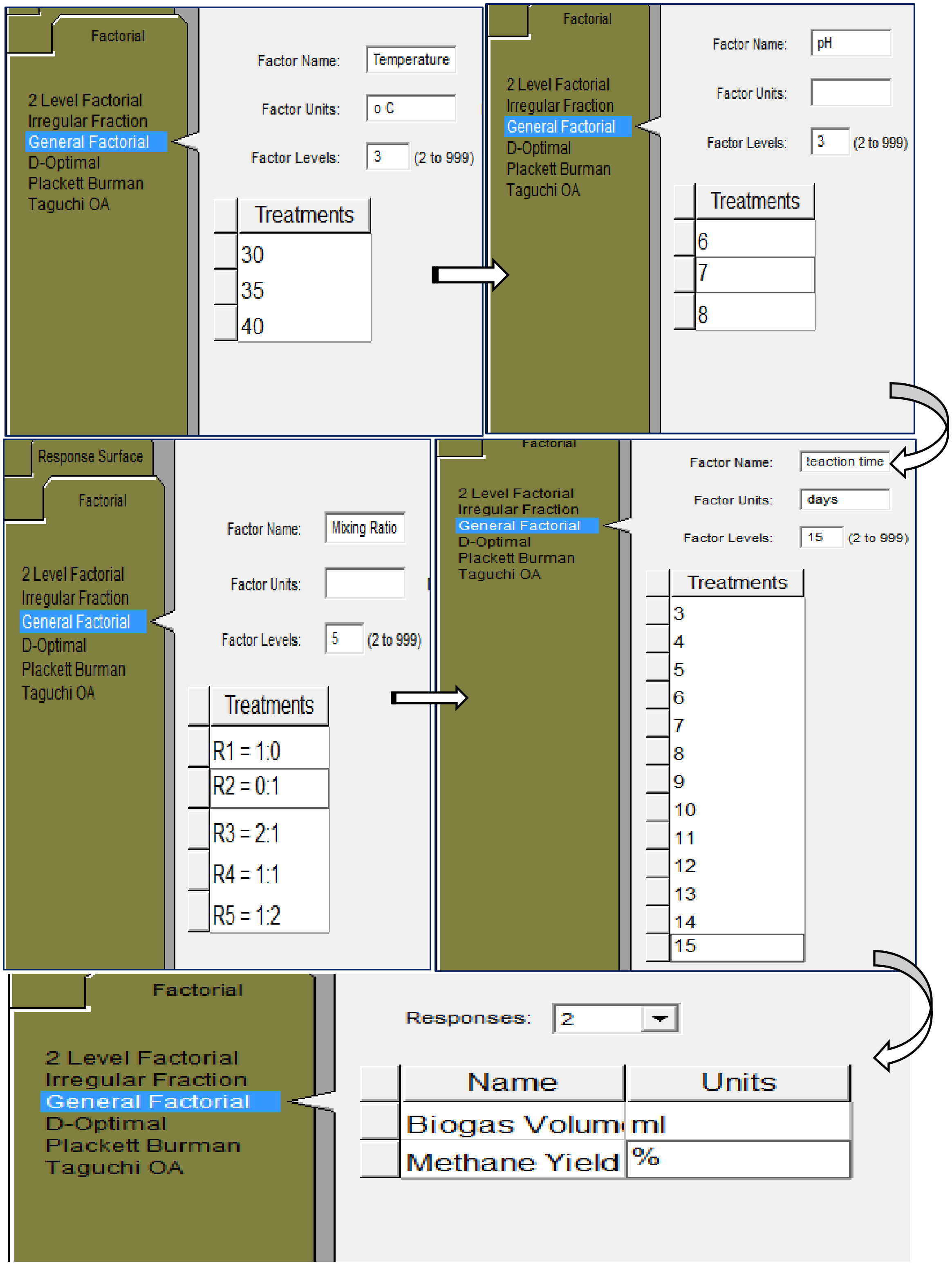
The experiment was lasted for about 120 days, 40 working days for each experimental setup, from February 10, 2017 to March 21, 2017 for the first-round experimental setup operated at 30^{o} C, from April 3, 2017 to May 12,2017 for the second round of the experimental setup that was operated at 35^{o} C and the next another 40 working days from May 19, 2017 to June 28, 2017 for the 40^{o} C experimental setup after which the experiment was terminated as the biogas production had become insignificant. Volume of biogas produced from each digester was measured by water displacement averagely once every two or three days interval. The produced biogas was characterized for its volume percentages of % CH_4, % CO_2, % O_2 and ppm H_2S composition on daily basis once every two or three days of interval using GeoTech biogas 5000 analyzer. The PVC tube from the gas collection chamber was connected to the gas sampling hose of the gas analyzer. The gas analyzer was switched on and waited until the display was ready. The built-in pump of the gas analyzer was turned on to draw out the gas from the 2 L plastic bottle into the analyzer. The results were recorded after the reading on the analyzer was stable. The biogas composition was displayed by the gas analyzer biogas 5000 as shown below in the figure 3.21.

Figure 3. 21 Biogas 5000 analyzer showing experimental results of biogas components

3.2.7 Software for design of experiment

To grasp the individual and interaction effects of the process parameters on biogas production and methane content Design expert version 6.0.8 and Microsoft excel 2016 were the two-software used during this study. Microsoft excel 2016 was used to see the progress formation of biogas volume and methane yield as well as the carbon dioxide fraction in the biogas produced with digestion time. Design Expert is a piece of software designed to help with the design and interpretation of multi-factor experiments. Design of experiment (DOE) is a well-accepted statistical technique able to design and optimize the experimental process that involves choosing the optimal experimental design and estimate the effect of the several variables independently and the interactions simultaneously. DOE is a systematic way of changing process inputs and analyzing the resulting process outputs in order to quantify the cause and effect relationship between them as well as the random variability of the process while using a minimum number of runs (Montgomery & Runger , 2002).

In the AD process the design expert software was used to help in design an experiment to see how the responses (biogas volume production and methane fraction in biogas) varies with changes in the process conditions such as the digestion temperature, initial pH, MR and digestion time. The effects of these independent variables on the two responses were analyzed using optimization techniques. To analyze whether a model results were significant, ANOVA was used and the interaction effect of the process variables on both biogas volume production and methane yield the general factorial design was employed. The procedure layout of the general factorial design followed during the study in analysis of the process parameters effect is presented below (Figure 3.22).



Factor Name: Temperature
Factor Units: o C
Factor Levels: 3 (2 to 999)

Treatments
30
35
40

Factor Name: pH
Factor Units:
Factor Levels: 3 (2 to 999)

Treatments
6
7
8

Factor Name: Mixing Ratio
Factor Units:
Factor Levels: 5 (2 to 999)

Treatments
R1 = 1:0
R2 = 0:1
R3 = 2:1
R4 = 1:1
R5 = 1:2

Factor Name: Reaction time
Factor Units: days
Factor Levels: 15 (2 to 999)

Treatments
3
4
5
6
7
8
9
10
11
12
13
14
15

Responses: 2

Name	Units
Biogas Volum	ml
Methane Yield	%

Figure 3. 22 Layout of procedures used General factorial design in Design Expert V 6.0.8

4. Results and Discussion

4.1 Proximate composition of samples and feedstocks

Table 4.1 shows the physicochemical characterization of the food wastes and goat manure used during this study. The TS, VS, COD and BOD values shows the amount of biodegradable matter available in the waste samples. The high values of these proximate content of the wastes indicate that there is large amount of digestible substances within the waste samples. As it can be seen from the table 4.1 goat manure, avocado peels, potato peels, injera leftover mixed with different types of stews and the mixture of the food wastes presents a high solids content. Upon mixing, the total solids content for the feedstocks fed into the digesters were adjusted to the optimal values. The total solids content for the mixing ratios of 1:0, 1:1, 2:1, 1:2 and 0:1 (MFW to GM) were found to be 11.40%, 49.72%, 36.95%, 62.50% and 88.04% respectively. These values are higher than the ideal value for anaerobic digestion of wastes. High solids digestion creates high concentrations of end products that inhibit anaerobic decomposition (Budiyono, Syaichurrozi, & Sumardiono, 2014).

The %TS, %VS and C/N ratio for the mixing ratios 1:1, 2:1 and 1:2 were calculated according to (Tom & Nancy, 1996) and were computed as shown in appendix D. For optimum biogas production, the total solid content of these mixed ratios was altered to an optimum value of TS content of 8.5% through simple dilution (Appendix D). The volume of water added to each mixing ratio to dilute the solid content and to achieve the ideal values of the TS of the feedstocks for suitable AD of the wastes was about 0.724, 3.159, 2.347, 3.970, 1.036 and 1.86 liters for the mixing ratios of 1:0, 1:1, 2:1, 1:2, 0:1 and inoculum preparation respectively (Table 3.3). The results of the analysis of the physicochemical parameters of the fresh substrates fed into the digesters after maintained to the required limits are shown below in table 4.2. To assess the effects of initial pH on biogas production and methane yield the pH values of the samples ranged from 4.48 to 7.87 (Table 4.1) were adjusted to 6 to 8 (Table 4.2) in the mixing ratios of 1:0, 1:1, 2:1, 1:2 and 0:1 that were fed into the digesters. Because the pH value of the samples was found to be about 5.45 upon mixing, this was done by adding 10 N NaOH to the mixed wastes to achieve to the required pH values of 6, 7 and 8 because optimal pH values of previous studies lay between 6 - 8 (Gerardi, 2003; Steinhaus, *et al.*, 2007).

Table 4. 1 Chemical characterization of wastes used in the study

Wastes type	**Parameters**							
	TS %	VS (%)	% C	%N	C/N	pH	COD mg/L	$CBOD_5$ gm O_2/L
Goat manure	88.04	93.78	52.10	2.10	26.77	7.87	2420	968
Avocado peel	13.39	88.21	49.00	-	-	6.35	1210	508.2
Cabbage waste	4.10	97.81	54.34	-	-	6.37	1430	550.6
Carrot waste	4.96	97.43	54.13	-	-	6.07	1470	536.7
Potato peels	18.77	82.87	46.04	-	-	5.60	1270	508
Beet	5.50	96.11	53.39	-	-	6.34	64	25.6
Mango peels	5.70	96.58	53.66	-	-	4.48	1420	528.2
Injera leftover mixed with different stews	27.37	70.92	39.40	-	-	6.80	420	168
Mixture of the food wastes	11.40	89.99	49.99	1.47	34.00	5.53	-	-

– Not determined

Table 4. 2 Proximate contents of waste feedstocks fed into digesters

Feed ratio [MFW: GM]	**%TS***	**C/N ratio**	**Influent pH**	**Digestion temperature**
1:0	8.5	34.00	6,7,8	30,35,40
1:1	8.5	25.57	6,7,8	30,35,40
2:1	8.5	26.22	6,7,8	30,35,40
1:2	8.5	25.21	6,7,8	30,35,40
0:1	8.5	26.76	6,7,8	30,35,40
Inoculum	8.5	Nd	7 [a]	-

* The value was adjusted through simple dilution to the ideal value of %TS for AD,

-- Not determined. a- pH of the inoculum was adjusted to the unbiased pH value 7.

As can be observed from table 4.1 above the nitrogen content of the wastes was found to be 1.47% and 2.10% and a corresponding C/N ratio of 34.00 and 26.77 for the mixed food wastes and goat manure respectively. The C/N ratio for the MFW was relatively found to be higher than that of GM and this was due to high carbon content relative to nitrogen content of the MFW than that of the GM. The C/N ratio of substrate in the range of 20–30 is considered to be optimum for anaerobic digestion of wastes (Li, Park, & Zhu, 2011; Ranalli, 2007). The low C/N ratio value of the GM, due to its high nitrogen content, was used to adjust the C/N ratio of the mixed food waste to 25.57, 26.22 and 25.21, to that of typically considered optimal value in the literatures for the waste digestion processes by mixing the MFW and GM in the ratios of 1:1, 2:1 and 1:2 respectively (Appendix D). These results recommend that the co-digestion of food wastes with goat manure could provide a more suitable C/N ratio and more well-adjusted nutrients for microorganisms' growth during co-digestion process thus, the mixing process might increase the biogas production.

4.2 Process parameters effect on biogas volume production

The rate of biogas volume produced for the biomass combination systems of the mixed food wastes and goat manure at three levels of temperatures 30^{o} C, 35^{o} C, 40° C and three initial pH level values 6.0, 7.0 and 8.0 were recorded on daily basis and is shown in the following graphs (Figure 4.1 -4.9). The daily recorded biogas volume produced, and its components fraction is shown in appendix E (Tables V-XIII). From the results of the co-digestion of MFW with GM and the graphs presented below it is possible to elucidate that maximum biogas were achieved by the anaerobic co-digestion of food wastes with goat manure with the mixing ratios of **R4 = 1:1** and **R5= 1:2** (food waste to goat manure) as the optimal mixing ratios at all the effective conditions. The total volume of biogas produced from the mixing ratios of **R1 = 1:0, R2 = 0:1, R3 = 2:1, R4 = 1:1** and **R5 = 1:2** is presented below in table 4.3.

Table 4. 3 Total volume of biogas produced from the different mixing systems

Temperature	**pH**	**Mixing ratio**				
		R_1 = 1:0	**R_2 = 0:1**	**R_3 = 2:1**	**R_4 = 1:1**	**R_5 = 1:2**
30^{o} c	6	2.5	3.1	4.6	7.7	14.47
	7	2.8	3.5	5.3	8.9	16.6
	8	2.6	3.2	4.8	8.1	15.1
35^{o}c	6	3.7	4.6	6.7	9.3	16.8
	7	**4.4**	**5.5**	**8.1**	**11.2**	**17.3**
	8	3.4	4.2	6.2	8.6	15.5
40^{o}c	6	2.8	3.51	4.9	7.3	12.5
	7	3.9	5.1	7.0	10.5	14.11
	8	3.0	3.7	5.6	6.8	12.8

From this experimental result, it was shown that the co-digestion of the mixed food waste with goat manure provided better biogas production than the mixed food waste (1:0) and goat manure (0:1) alone. The order of the mixing ratios in terms of the generated cumulative biogas volume can be arranged as R1 = 1:0 < R2 = 0:1< R3 = 2:1 < R4 = 1:1 < R5 = 1:2. From this it is clear to conclude that biogas yield improves by co-digestion of the mixed food wastes with goat manure and this increases with the amount of goat manure added as it is an excellent substrate for AD because of its high total nitrogen content and fermentation stability (Zhang, *et al*., 2007; Zhang, *et al*., 2013). This study has revealed that co-digestion of the mixed food wastes with goat manure was more useful than digesting each waste alone.

However, pH has an optimal range of 6.5 – 8.2 (Gerardi, 2003; Steinhaus, *et al*., 2007), for methanogenic activity to decompose organic matter to biogas; biogas produced at initial pH value of 7.0 was higher than at pH 6.0 and pH 8.0 at all input variables. From the daily produced biogas volume graphs presented in appendix E (Figures E2 – E10) it is revealed that all graphs show the same trends. Gas production was commenced during 7 days after the digesters operated and increased from the first day of digestion time to the fourth digestion time. This first increasing trend was possibly due to the presence of the methanogenic rich inoculum prepared from the 1:1 mixing ratio. The presence of this substrate helps the microbes to decompose easily degradable organic matter. The gas production was then shown a decreasing trend as can be shown from the middle part of the graphs (Figures E2 – E10). This drop stage in biogas volume production may also be due to the fact that the degradation of organic matter by methanogenic bacteria takes place in three phases. The pH value of the organic material is a key factor in the anaerobic digestion. The first phase consists of hydrolysis of the substrate into simple molecules such as fatty acids, simple sugars and alcohol causing a drop in pH of the substrate due to the accumulation of volatile fatty acids (Gerardi, 2003; Steinhaus *et al.*, 2007). This drop of the digesters pH caused to suppress the activity of methanogenic bacteria which results in the drop of the gas production.

The next stage of the digesters starting from the 10th digestion time (after 28 working days of digestion period) shows an increasing trend in biogas volume production. This degradation phase can be concluded as the conversion of products from the acid formation phase. This phase is indicated by a rise in pH (Forster-Carneiro, Pe´rez, & Romero, 2008) which resumed the methanogenic activity responsible for the gas formation. Based on the earlier studies mentioned above, the C/N ratio of substrate in range of 20−30:1 is considered optimum for anaerobic digestion of wastes (Li, Park, & Zhu, 2011; Ranalli, 2007). The C/N ratio for the mixed food waste was found to be 34 which is not in the recommended optimum range value in the literatures for wastes digestion. Thus, this sample containing 100% food waste had the least desirable value of carbon to nitrogen ratio which could be detrimental to the methanogenic activity. The deviation of the C/N ratio from the optimal range could influence the pH of the digesters system. The high in carbon content will give rise to more CO_2 formation and lowers the systems pH value, while high nitrogen content will increase production of ammonia gas that could increase the pH to the disadvantage of the microbes. During anaerobic fermentation microbes need a natural or mildly alkaline environment for efficient gas production (Augenstein, *et* al., 1997). Towards the end of the digestion period, there was a decrease in the gas volume production (Figures E2 – E10). Biogas production began to fall as the substrates available to the microbes to act on were being consumed and changed to methane.

The cumulative volume of biogas produced in milliliters are presented below (Figures 4.1-4.9). There was a gradual increase in the volume of biogas produced with the digestion time. From these it is clear to conclude that biogas production by anaerobic co-digestion was improved. The mixing ratio of the mixed food waste to goat manure with 1:2 ratio gave the highest cumulative gas of 17.34 liters followed by the 1:1 ratio 11.24 liters at 35^{o} C and initial pH value of 7.0. This may be due to the well-balanced C/N ratio in the1:1 and 1:2 ratio to the optimum ratio. The C/N ratio decreased i.e. balanced to an optimum value of C/N ratio from 34.0 to 25.21 with increasing the goat manure added (Table 4.2).

From the experimental results, it is also clear to conclude that highest biogas yield was achieved from all the digester systems operated at temperature 35^{o} C and pH value 7.0. However, the 40^{o} C operating temperature is in the mesophilic range, digesters operated at this temperature had shown that rapid gas production and rapid fall in gas production than the other two operating temperatures. This quick drop in gas production may be due to the quick accumulation of volatile fatty acids within this setup. In addition, digesters fed with GM only as a sole substrate and operated at this temperature had shown rapid drying. Temperature is a key factor influencing the system performance. It affects the physico-chemical properties of the organic matter in the digester, and the kinetics and thermodynamics of biological processes (Boe, 2006).

Even though, the TS content of all the digesters were maintained to the same optimal value of 8.5% (Appendix D), to the end of the digestion period digesters fed with GM as the sole substrate had shown rapid drying than the mixed food wastes and their co-digestion with goat manure. Digesters fed with the GM only were found to be hotter than the other digesters. From the TKN experimental analysis it has been shown that GM have high nitrogen content than the FW. Substrates with high nitrogen content increases the digester temperature in composting (Tom & Nancy, 1996; Hess, 2016). According to Le Châtelier principle the formation of ammonia from nitrogen and hydrogen substrate is based on an exothermic reaction. This reaction in AD occurs as the carbon content of the organic matter consumed with increasing digestion period and releases heat to the digesters which results an increase in the digester temperature. This rise in temperature causes killing microbes and the reduction of the moisture content of the substrates causing a decrease in organic matter conversion. Sequentially, this led to the reduction in biogas volume production and releasing the methane produced.

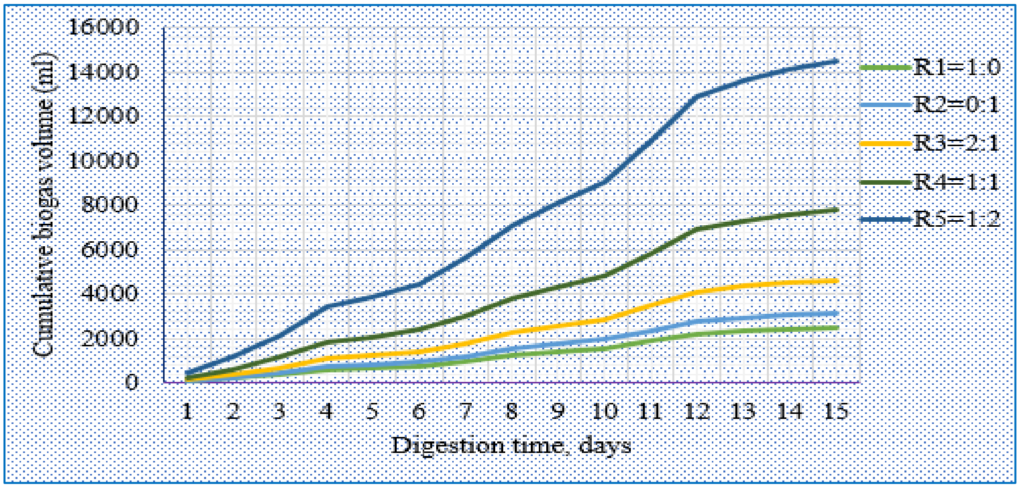


Figure 4. 1 Cumulative biogas volume at temperature 30° C and pH 6.0

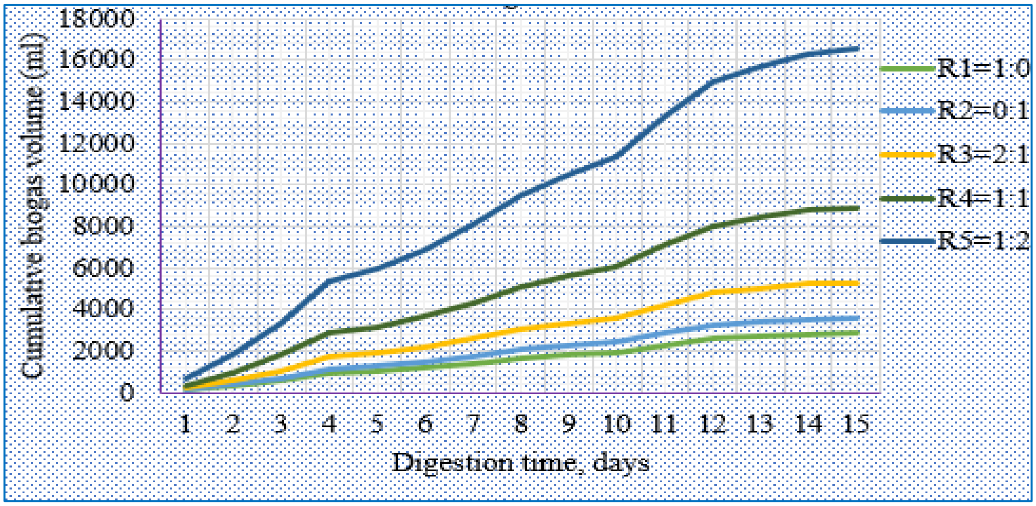


Figure 4. 2 Cumulative biogas volume at temperature 30° C and pH 7.0

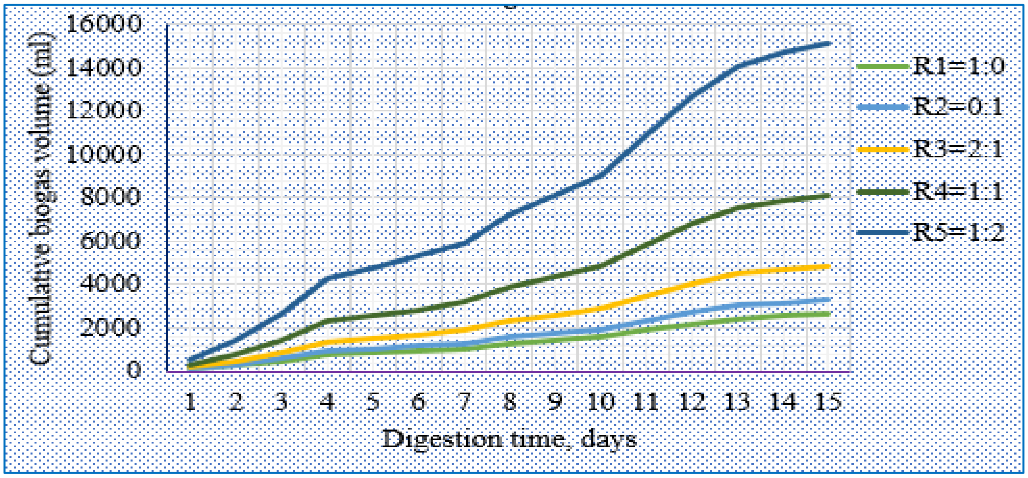


Figure 4. 3 Cumulative biogas volume at temperature 30° C and pH 8.0

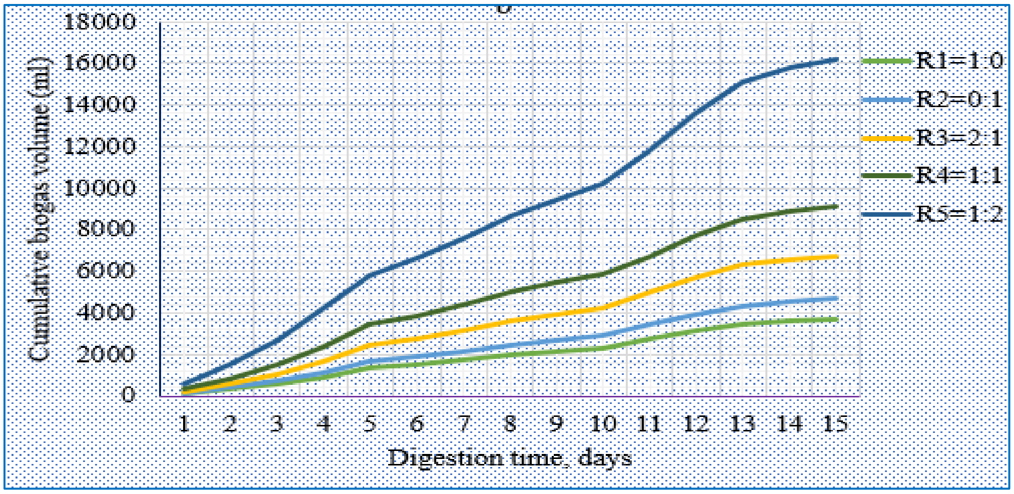


Figure 4. 4 Cumulative biogas volume at temperature 35° C and pH 6.0

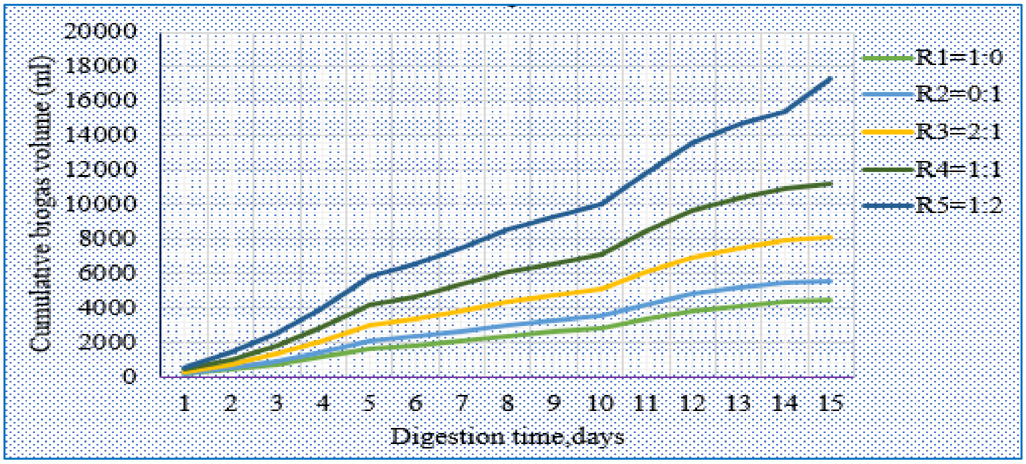


Figure 4. 5 Cumulative biogas volume at temperature 35° C and pH 7.0

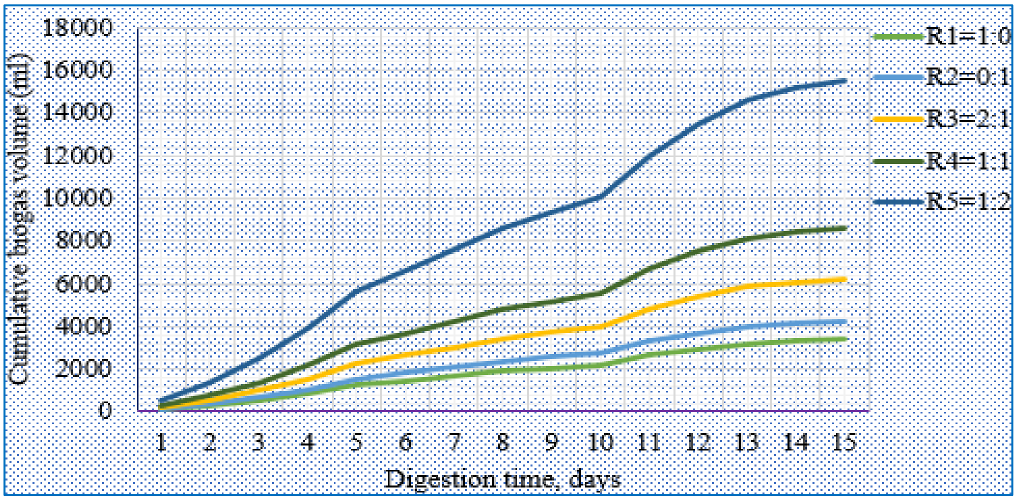


Figure 4. 6 Cumulative biogas volume at temperature 35° C and pH 8.0

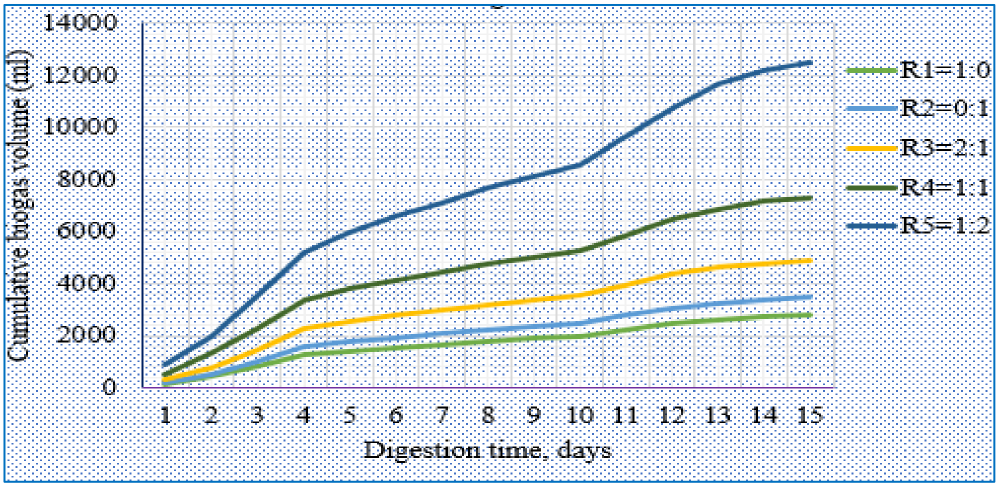


Figure 4. 7 Cumulative biogas volume at temperature 40° C and pH 6.0

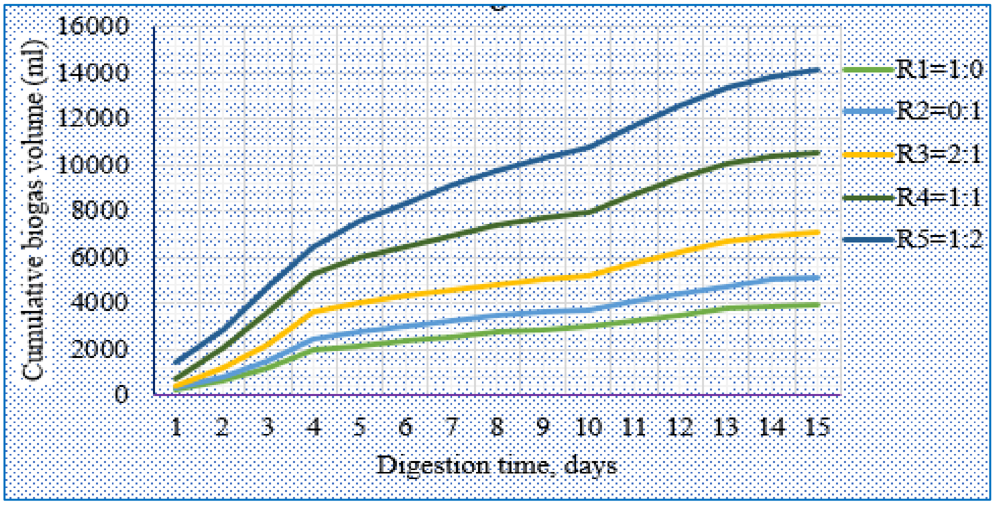


Figure 4. 8 Cumulative biogas volume at temperature 40° C and pH 7.0

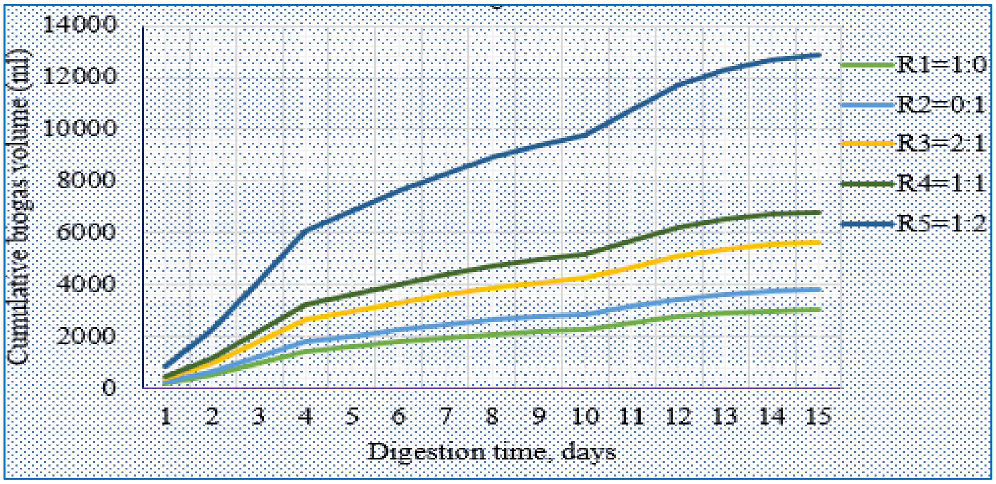


Figure 4. 9 Cumulative biogas volume at temperature 40° C and pH 8.0

4.3 Process parameters effect on CH_4 and CO_2 fractions in biogas

The average CH_4 fraction in the biogas produced for the mixing ratios of 1:0, 0:1, 2:1, 1:1 and 1:2 is presented below in the table 4.4. Other components detected in the biogas produced were carbon dioxide in % by volume as the second main component of the gas next to methane, oxygen in % by volume and hydrogen sulfide in ppm by volume.

Table 4. 4 Average methane fraction in the gas for the different mixing systems

Temperature	**pH**	**Mixing ratio**				
		R_1 = 1:0	**R_2 = 0:1**	**R_3 = 2:1**	**R_4 = 1:1**	**R_5 = 1:2**
30° c	6	47.6	49.1	51.9	54.3	56.2
	7	52.8	53.8	55.7	56.1	59.4
	8	48.5	50.7	53.5	56	58.1
35°c	6	50.7	54.9	60.8	55.6	57.5
	7	**51.7**	**53.8**	**56.9**	**61.4**	**63.5**
	8	50.9	55.4	59.6	61.9	63.1
40°c	6	47.6	48.1	50.9	53.3	55
	7	49.5	49.7	52.6	55	56.9
	8	48.9	51.9	54.7	57.2	59.2

The methane and Carbon dioxide fractions in the produced biogas are shown graphically below (Figures 4.10-4.18). Averagely, higher methane yield was obtained by the co-digestion of the mixed food wastes with goat manure at 35° C and initial pH value of 7.0 from the mixing ratios of 1:1 and 1:2 as the optimum MRs for methane yield. From the experimental results investigated and the graphs depicted below the concentration of methane in the produced biogas was increased with digestion time (Figures 4.10a-4.18a) and the concentration of carbon dioxide was decreased with digestion time (Figures 4.10b-4.18b).

The growth rate of methanogens can be significantly reduced when the pH value is less than 6.6 (Sandberg & Ahring, 1992). Reactors operated at initial pH of 6.0 were initially revealed higher CO_2 fraction than the digesters operated at initial pH values 7 and 8. The presence of higher organic material of the VS and COD of the waste samples causes the pH within the reactors that were operated initially at pH value of 6.0 to lower below this value. This drop in pH from the initial value showed that it passed through an acidic phase which concentrates VFAs. This concentrated VFAs lowers the pH to less than the initial value which resulted to rise the CO_2 concentration and in a decline the CH_4 content in the produced gas. However, the concentration of CO_2 was decreased with increasing the digestion time in all digesters. Previous studies on anaerobic digestion had shown that with increasing the digestion time, the pH value of the digester resumed to a stable state through the initial value until the end of the digestion time. The shapes of the graphs representing the CH_4 and CO_2 fractions in the biogas were nearly alike with the same digestion days (40 working digestion days) for all reactors. This signifies that the reactors maintain a stable pH with increasing the digestion time due to the consumption of VFAs and other earlier products by the methanogenic groups.

The methane content of the biogas produced in the reactors fed with mixed food wastes only (1:0) and goat manure only (0:1) was gradually increased but, smaller than the methane content in the reactors fed with mixed food wastes co-digested with goat manure with the 1:1 and 1:2 mixing ratios as the two optimum ratios for the digestion system from the digestion time day 1 (after 7 days from the start up to the evolution of gas was detected) to the digestion day 15 (after 40 working days) at all operating parameters. This signifies that the advantage of co-digestion of the mixed food waste with goat manure over the mono-digestion of food waste alone. The main components of the produced gas were CH_4 and CO_2. From the experimental results (Appendix E) and the graphs depicted below (Figures 4.10-4.18), it can be realized that when CH_4 fraction in the gas was high, CO_2 fraction was low. From the results, it is also possible to conclude that the CH_4% was better towards the end of the digestion days than earlier digestion time. Converti *et al.* (2009) suggested that there is more methanogenesis activity to the end of the digestion time resulting in higher methane content in the biogas. This was because the simple organic acids that were converted from the products of the hydrolysis by the acid formers were finished with increasing digestion time corresponding to the consumption of VFAs in the same time which in turn causes the decrease in the CO_2 fraction in the gas produced (Ostrem & Themelis , 2004).

For all the mixing ratios of the MFW and GM combination systems CH_4 fraction was improved with increasing the digestion period, however the fraction of the CO_2 in the biogas was declined in a similar manner. This may also be because biogas generation is related to the fraction of VS reduction. In addition, the two key pathways in CH_4 generation in AD are the transformation of H_2 and CO_2 to CH_4 and H_2O, and the conversion of acetate to CH_4 and CO_2 (George & Franklin, 1991) to the end of the digestion period.

Digesters operated at 35^o C and initial pH 7.0 had shown less CO_2 and more CH_4 fractions in the biogas formed to the end of the digestion time in comparison to the digesters operated at the other conditions. However, all the reactors regardless of the mixing ratios, had shown more CO_2 fractions in the produced gas in an early stage of the digestion time. Methanogenesis is the ultimate step of the anaerobic digestion process. In this step, CH_4 and CO_2 are formed by several methane forming microbes called methanogens. The most important substrates formed during anaerobic digestion for these microbes are CO_2, acetate and H_2 substrate (Liu & Whitman, 2008). The other substrates such as formate, methanol, methylamines and carbon monoxide can also be consumed by methanogens to produce methane in the last stage of the digestion time (Liu & Whitman, 2008). Acetate is the source of nearly 70% of the total methane gas produced via the acetate reaction pathway in AD (Gerardi, 2003). Therefore, it is expected that, higher CO_2 fraction will be detected in the initial stage of digestion time, and higher CH_4 will be obtained in the far ahead of the digestion time with the corresponding reduction in CO_2 fraction. Goat manure as co-digester overcomes the C/N ratio imbalances in the single digestion substrates of the MFW and improves the anaerobic digestion process (Zhang, *et al*., 2013). Methane content in the gas was well improved with the mixing ratio 1:1 next to the 1:2 in all reactors, and the 35^o C and initial pH 7 were found to be the optimum independent parameters for CH_4 production with an average optimum value of 61.1% and 63.5% respectively for the 1:1 and 1:2 combination systems.

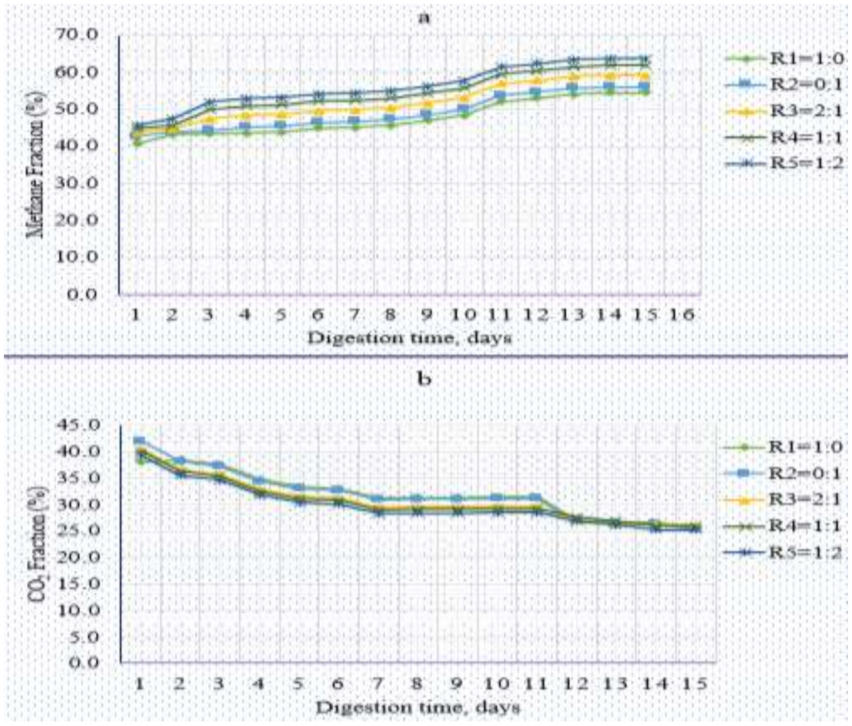


Figure 4. 10 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 30° C and pH 6.0

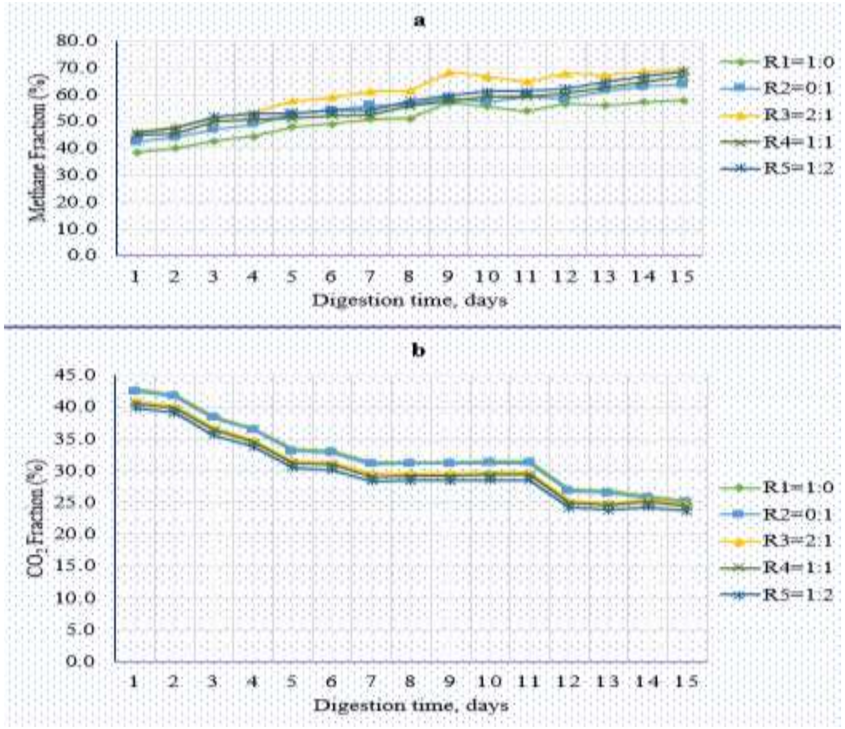


Figure 4. 15 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 35° C and pH 6.0

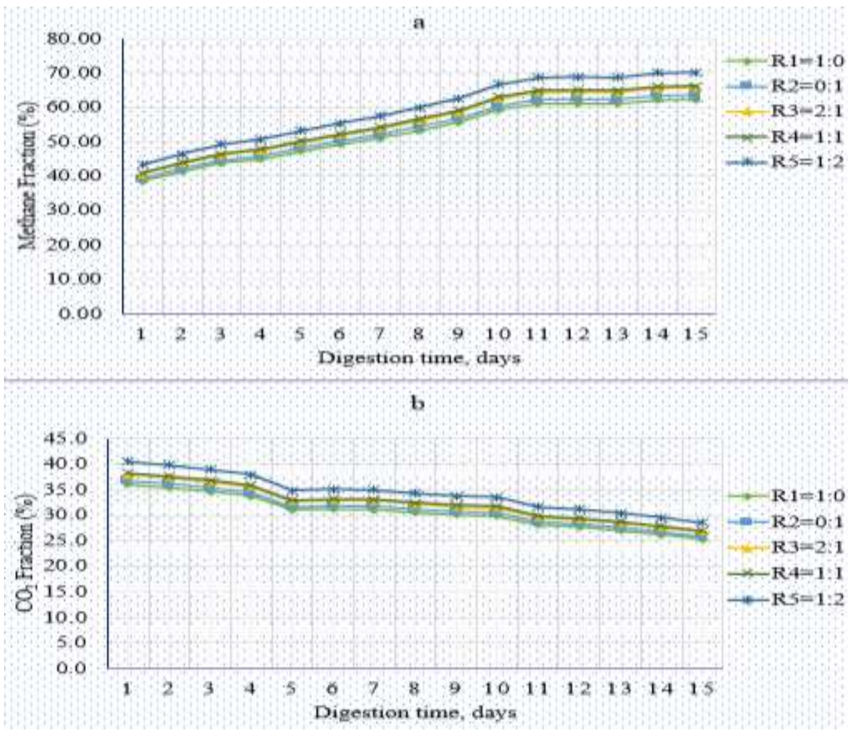


Figure 4. 11 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 30° C and pH 7.0

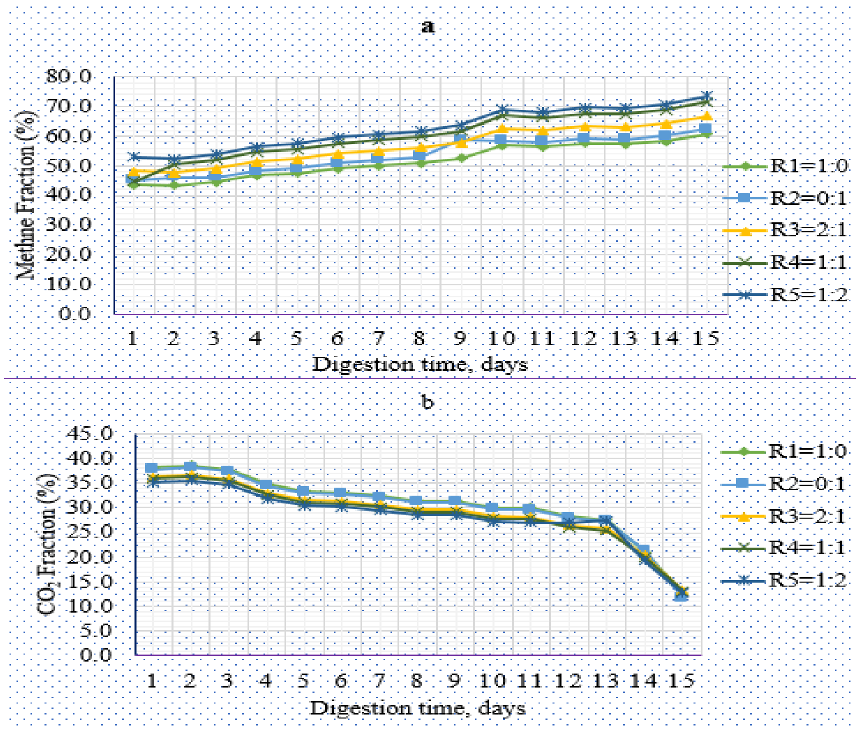


Figure 4. 16 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 35° C and pH 7.0

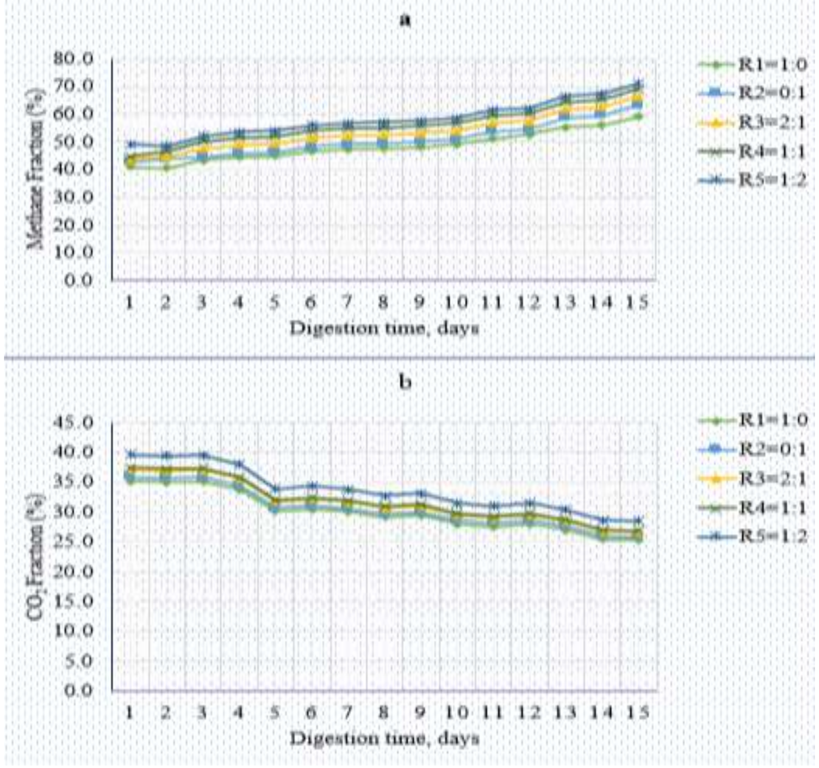


Figure 4. 12 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 30° C and pH 8.0

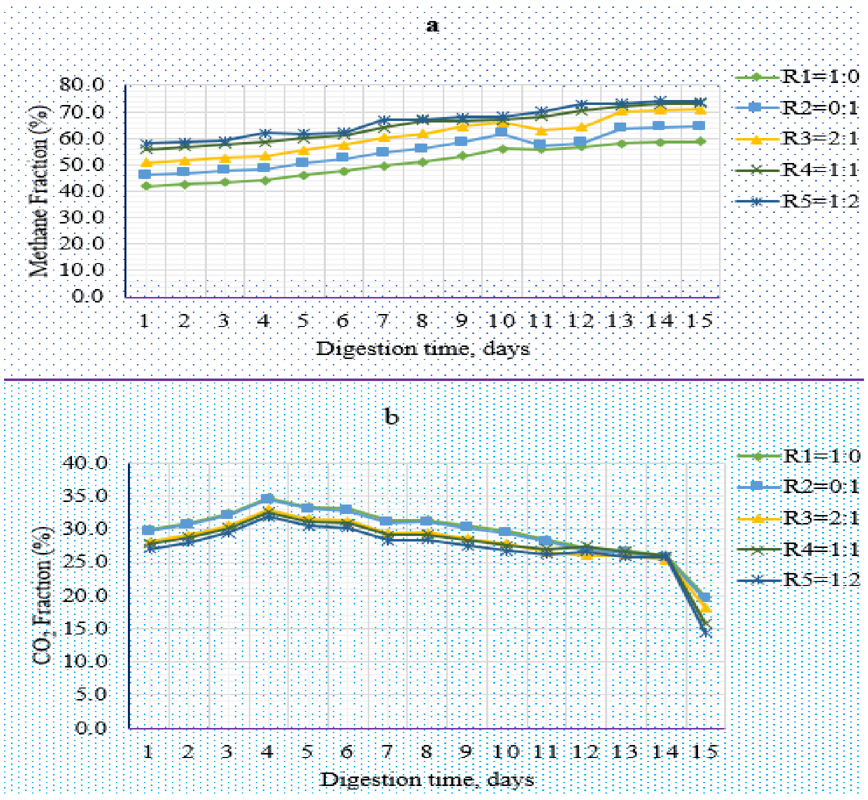


Figure 4. 17 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 35° C and pH 8.0

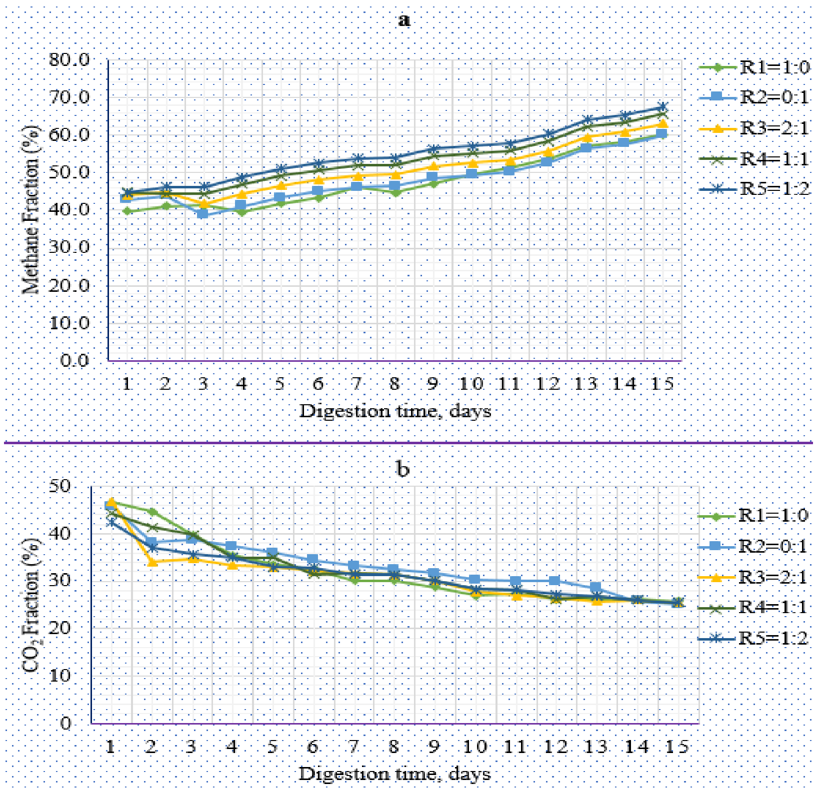


Figure 4. 13 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 40° C and pH 6.0

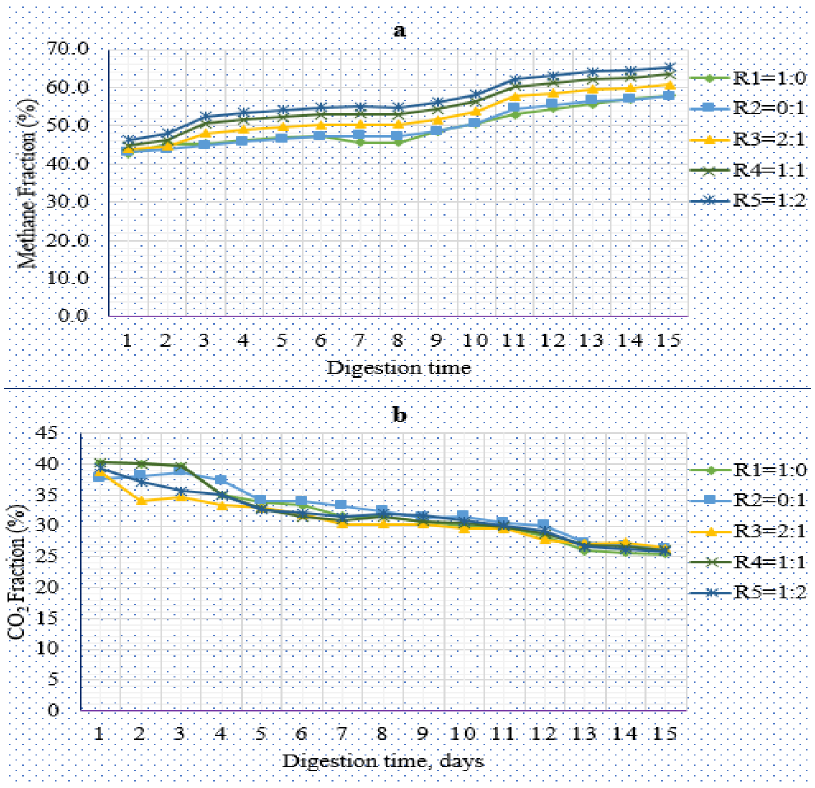


Figure 4. 14 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 40° C and pH 7.0

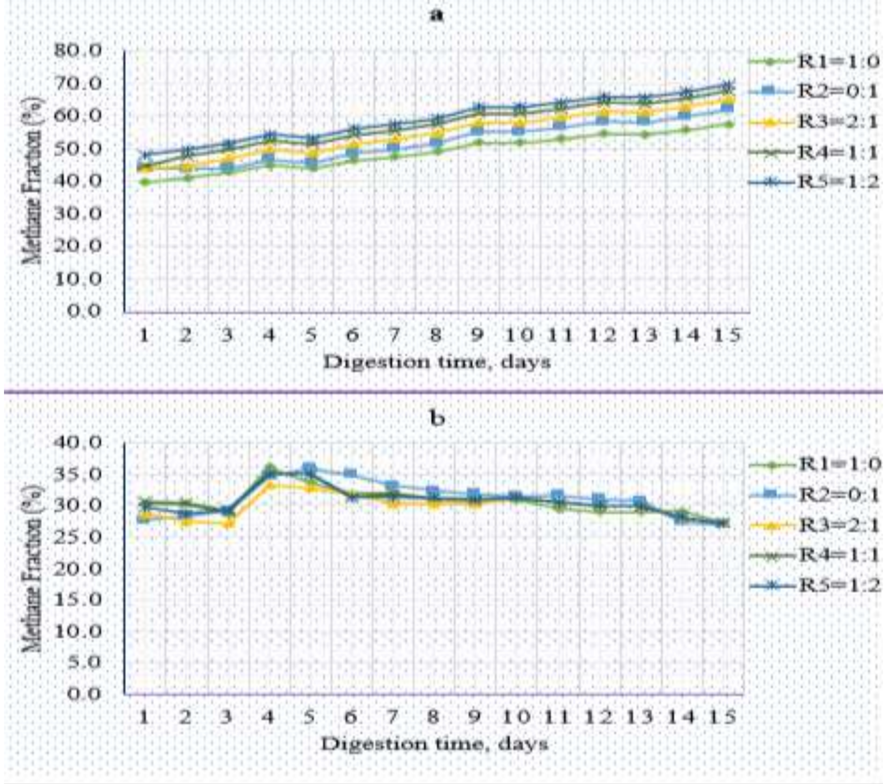


Figure 4. 18 CH_4 (a) and CO_2 (b) fraction in biogas at temperature 40° C and pH 8.0

4.4 The possibility of food waste co-digestion with goat manure

According to the experimental results presented in the previous, it can be concluded that the goat manure can be used as a co-substrate of the anaerobic digestion of the mixed food waste. Food waste mono-digestion for anaerobic digestion was found to be less effective for biogas production because of its imbalanced C/N ratio, and low pH due to the presence of fruit peel wastes which may have a detrimental effect to the methanogenic groups. The characteristics of the goat manure were appropriate for the anaerobic digestion due to the suitable ratio of C/N and high VS content. Co-digestion of the mixed food waste with the goat manure will result in better performance of removal efficiency and biogas yield as well as methane yield.

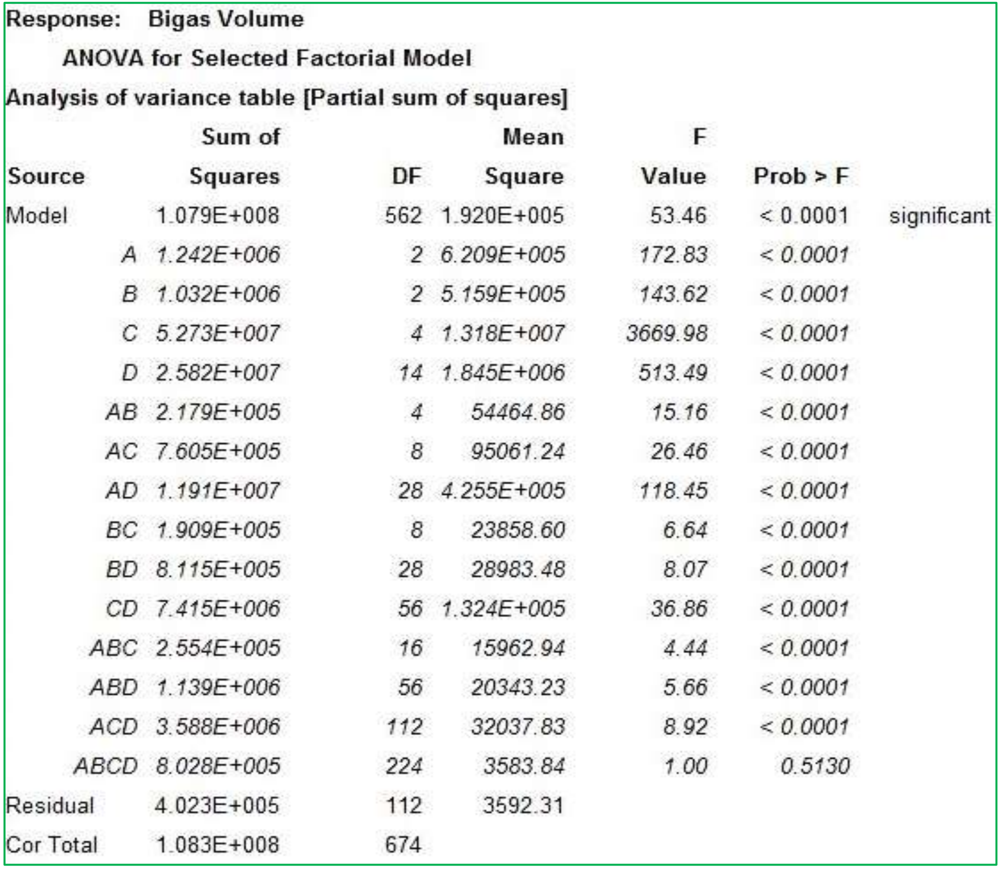
4.5 Statistical design of experiments and data analysis

4.5.1 Analysis of Variance (ANOVA)

Anaerobic digestion of wastes to produce biogas depends on large number of process variables. In this study four main factors i.e. temperature, initial pH, mixing ratio, and reaction time as independent variables were treated for investigation of their effects on biogas and methane yield. These four effective variables were considered at three levels for the digestion temperature, three levels for the initial pH, five mixing ratios and 15 levels for the digestion time. The interactive effect and optimal levels of these parameters for the biogas volume production and methane yield was simplified and studied using the general factorial design (design expert version 6.0.8). This program allows the user to have process variables that each has a different number of levels (Zinatizadeh, *et al*., 2007). The outcomes of this statistical approach are improvement in product yield, a reduction in process variability, a closer confirmation of the output response and a reduction in the experimental time and overall costs (Elibol, 2004; Montgomery & Runger , 2002).

Earlier studies have reported that the optimization of the process parameters as well as studied the individual and simultaneous effects of temperature, initial pH and retention time on biogas production and methane yield from palm oil mill effluent (Mohammadi, *et al*., 2016; Zinatizadeh, *et al*., 2007), acidic effluent coming from sugarcane juice hydrogen fermentation process (Reungsang, Pattra , & Sittijunda , 2012) and organic fraction of municipal solid waste (Beevi, Jose, & Madhu , 2014). However, the interactive effects of these factors on biogas and methane production from the mixed food wastes co-digested with goat manure have not so far been studied. In this study, the general factorial design was used for experimental modeling for screening the important factors, and investigating the nature of the relationship between the input factors and output data responses. To see the statistical significance of the models using ANOVA for the two responses (biogas volume and methane) the process variables were first treated as categorical factors. The ANOVA is necessary in determining the significance and adequacy of the model (Montgomery & Runger , 2002). The ANOVA results for the volume of biogas produced and methane yield is summarized in tables 4.5 and 4.7. The process variables were then treated as numerical factor to determine their optimal levels and their individual as well as interactive effects on biogas volume production and methane yield by surface plotting method (Figures 4. 21-4. 26).

Table 4. 5 Summary of ANOVA result for the volume of biogas produced



Response: Bigas Volume

ANOVA for Selected Factorial Model

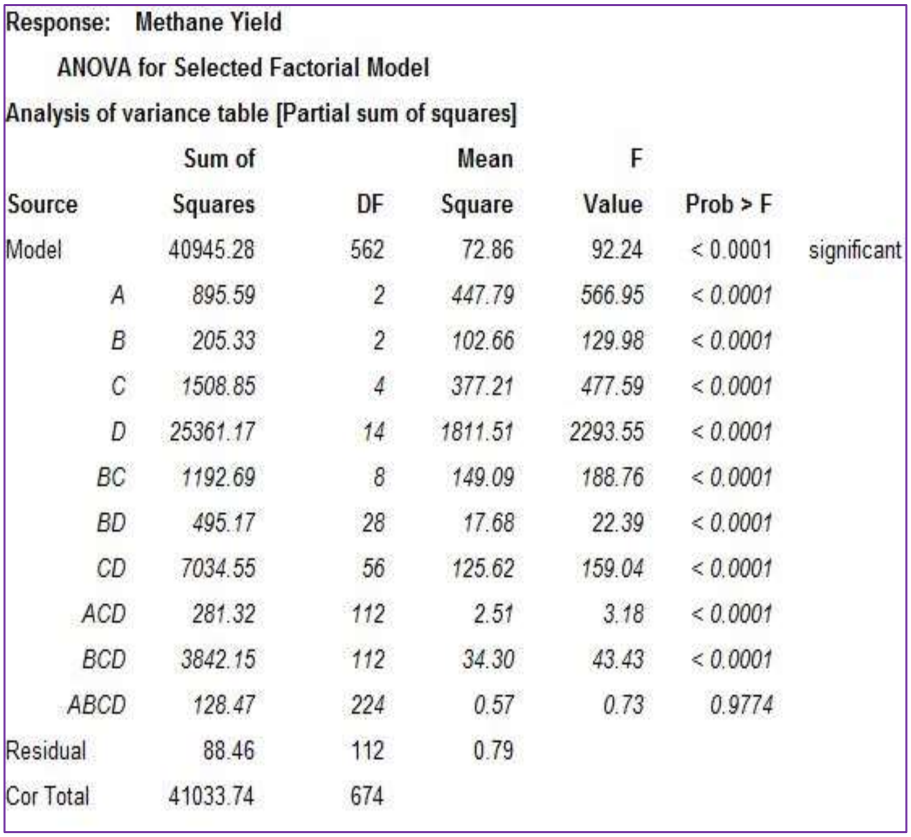
Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	1.079E+008	562	1.920E+005	53.46	< 0.0001	significant
A	1.242E+006	2	6.209E+005	172.83	< 0.0001	
B	1.032E+006	2	5.159E+005	143.62	< 0.0001	
C	5.273E+007	4	1.318E+007	3669.98	< 0.0001	
D	2.582E+007	14	1.845E+006	513.49	< 0.0001	
AB	2.179E+005	4	54464.86	15.16	< 0.0001	
AC	7.605E+005	8	95061.24	26.46	< 0.0001	
AD	1.191E+007	28	4.255E+005	118.45	< 0.0001	
BC	1.909E+005	8	23858.60	6.64	< 0.0001	
BD	8.115E+005	28	28983.48	8.07	< 0.0001	
CD	7.415E+006	56	1.324E+005	36.86	< 0.0001	
ABC	2.554E+005	16	15962.94	4.44	< 0.0001	
ABD	1.139E+006	56	20343.23	5.66	< 0.0001	
ACD	3.588E+006	112	32037.83	8.92	< 0.0001	
ABCD	8.028E+005	224	3583.84	1.00	0.5130	
Residual	4.023E+005	112	3592.31			
Cor Total	1.083E+008	674				

Table 4. 6 Model summary statistics for biogas volume produced

Std. Dev.	59.94	R-Squared	0.9963
Mean	492.00	Adj R-square	0.9776
C.V.	12.18	Pred R-square	0.8651
PRESS	1.461E+007	Adeq Precision	36.741

Table 4. 7 Summary of ANOVA result for the methane yield



Response: Methane Yield

ANOVA for Selected Factorial Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	40945.28	562	72.86	92.24	< 0.0001	significant
A	*895.59*	*2*	*447.79*	*566.95*	*< 0.0001*	
B	*205.33*	*2*	*102.66*	*129.98*	*< 0.0001*	
C	*1508.85*	*4*	*377.21*	*477.59*	*< 0.0001*	
D	*25361.17*	*14*	*1811.51*	*2293.55*	*< 0.0001*	
BC	*1192.69*	*8*	*149.09*	*188.76*	*< 0.0001*	
BD	*495.17*	*28*	*17.68*	*22.39*	*< 0.0001*	
CD	*7034.55*	*56*	*125.62*	*159.04*	*< 0.0001*	
ACD	*281.32*	*112*	*2.51*	*3.18*	*< 0.0001*	
BCD	*3842.15*	*112*	*34.30*	*43.43*	*< 0.0001*	
ABCD	*128.47*	*224*	*0.57*	*0.73*	*0.9774*	
Residual	88.46	112	0.79			
Cor Total	41033.74	674				

Table 4. 8 Model summary statistics for methane content in the biogas

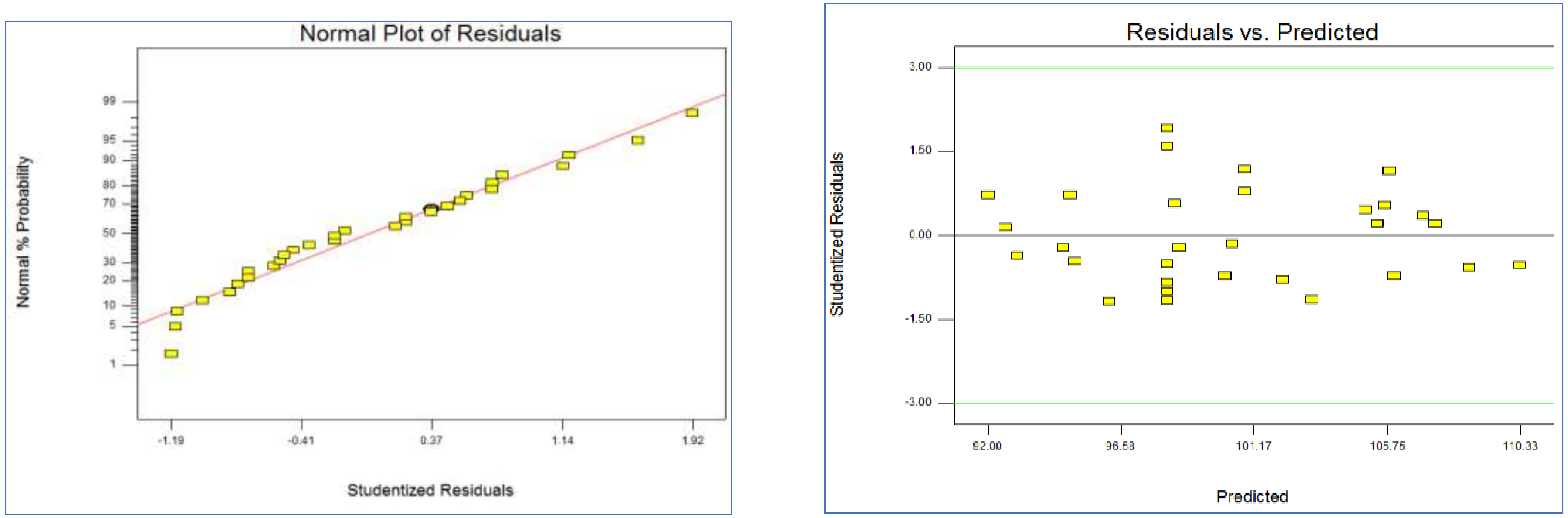
Std. Dev.	0.89	R-Squared	0.9978
Mean	54.60	Adj R-square	0.9870
C.V.	1.63	Pred R-square	0.9217
PRESS	3213.09	Adeq Precision	44.491

The independent variables coded as A, B, C and D are respectively referring to the process variables digestion temperature, initial pH, mixing ratio and retention time. The F-values 53.46 and 92.24 respectively for the volume of biogas produced and methane yield model implies that the two models are statistically significant ($P<0.05$). Effects with P-value less than 0.05 were better to indicate the consistency of a result (Montgomery & Runger , 2002). From the ANOVA result summarized above for the two model systems, there is only 0.01% chance that a " Model F-value" this large could occur due to noise. The values of " Prob > F" less than 0.05 in the tables 4.5 and 4.7 indicated that the model terms are statistically significant. In this case the individual model terms A, B, C, and D, and their interactions AB, AC, AD, BC, BD, CD, ABC, ABD, and ACD are statistically significant model terms of the produced biogas volume (Table 4.5). The individual model terms A, B, C, and D, and their interactions BC, BD, CD, ACD, CD, and BCD are also statistically significant model terms of the methane yield (Table 4.7).

For a good statistical model, the R^2 should be in the range of 0.75–1.0 which indicates a good fit of the model (Montgomery & Runger , 2002). The R-squared of 0.9963 and 0.9978 revealed that the models possibly will describe 99.63% and 99.78% of the variability in the biogas volume production and methane yield respectively (Tables 4.6 and 4.8). The adequate precision measures the signal to noise ratio. A value larger than 4 is required (Beevi, Jose, & Madhu , 2014; Montgomery & Runger , 2002). From the ANOVA results, a ratio of 36.741 and 44.491 were obtained for the biogas volume production and methane yield respectively (Tables 4.6 and 4.8) which shows an adequate signal. The adjusted R^2 0.9776 and 0.9870 respectively for the biogas volume production and methane yield were also very high, which indicated the higher significance of the models. The Pred R^2 values of 0.8651 and 0.9217 showed the reasonable agreement with the Adj R^2 values of 0.9776 and 0.9870 for the biogas volume and methane yield respectively. This indicated a good agreement between the observed and the predicted values. The percentage of coefficient of variation (CV %) is a measure of residual variation of the data relative to the size of the mean. Usually, the higher the value of CV, the lower is the reliability of experiment (Beevi, Jose, & Madhu , 2014; Montgomery & Runger , 2002). The low values of the CV 12.18% and 1.63% for the biogas volume and methane yield respectively confirmed a good precision and reliability of this experiment.

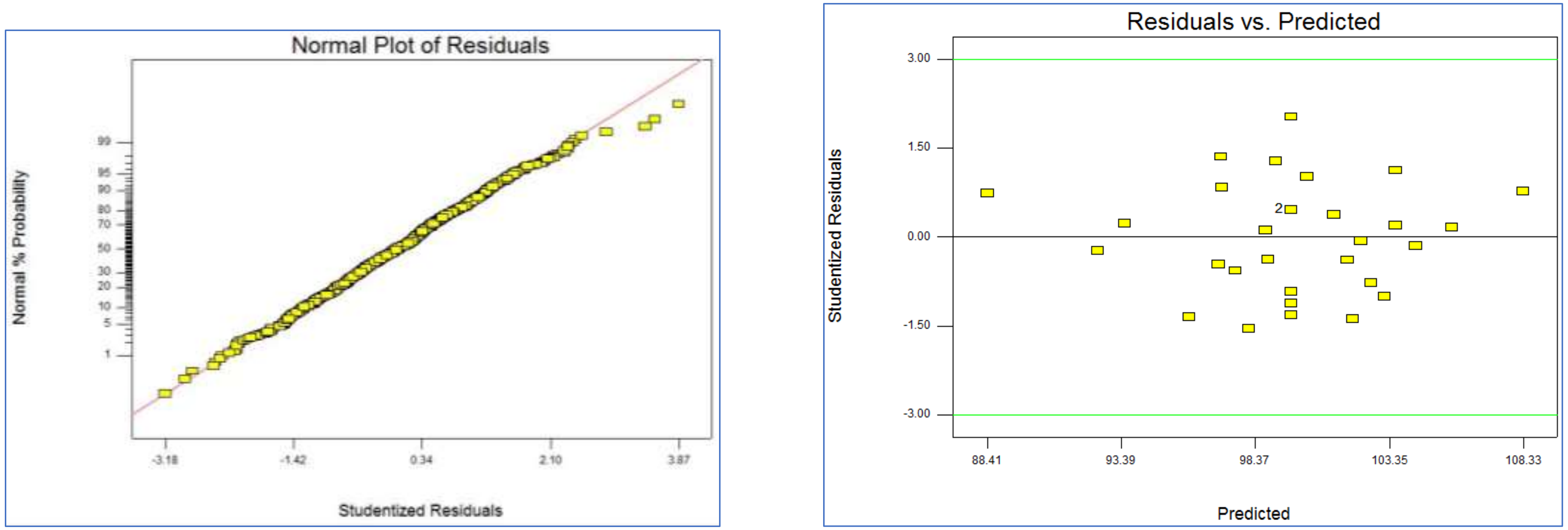
4.5.2 Optimization plots

Model graphs and diagnostics plots for the experimental data analysis was studied using the Stat-Ease software with Design Expert v.6.0.8 to study the individual and interactive effects of the process variables on volume of biogas production and methane yield, and to determine their optimal levels. The normal probability plots for the two responses is presented below (Figure 4.19a and 4.20a). The Applied statistics and probability for engineer textbook by Montgomery and Runger (2002) stated that a good normal probability plot should shows a linear straight line, as well stated that good residuals versus predicted response plot should be random scatter. An assessment on the normal probability plot for the volume of biogas produced and methane yield as shown in Figures 4.19a and 4.20a respectively revealed that the residuals generally fall on a straight-line concluding that the errors are distributed normally which indicated that the normality assumptions were valid for both the responses. The plots do not give any indication of serious problems. The residuals versus predicted response graphs for the volume of biogas produced and methane yield given in Figures 4.19b and 4.20b also revealed that they are randomly scattered. There is nothing uncommon in the residual plots.



(a) (b)

Figure 4. 19 Normal probability plot of residuals and Residuals versus predicted response plot for biogas volume produced data



(a) (b)

Figure 4. 20 Normal probability plot of residuals and Residuals versus predicted response plot for methane yield data

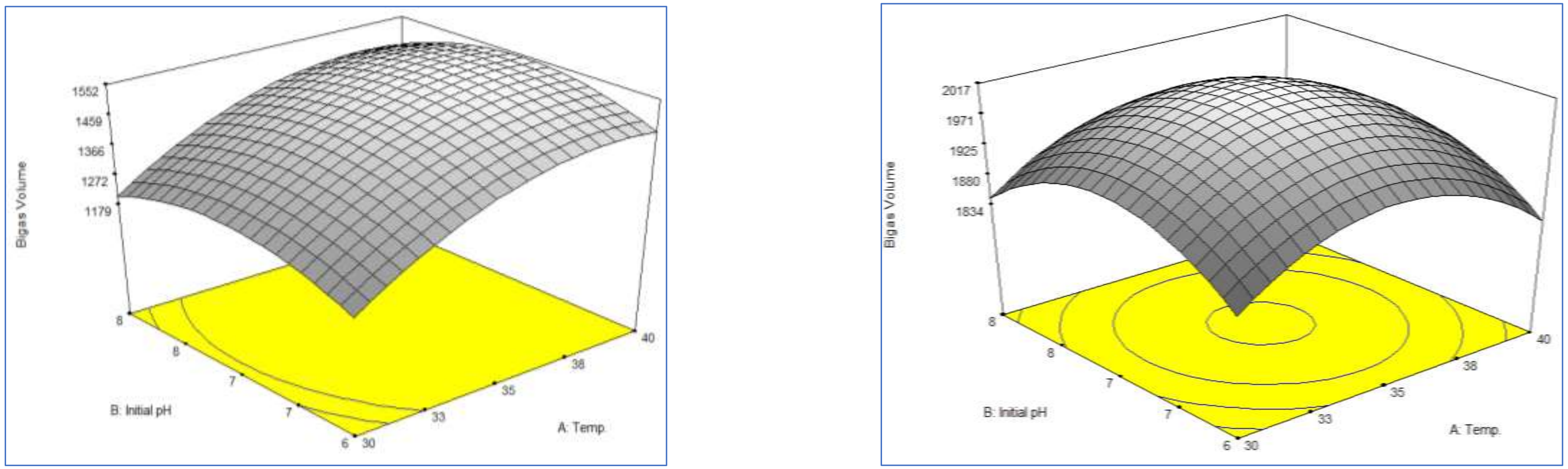
The simultaneous effects of the digestion temperature, initial pH and digestion time on biogas volume production and methane yield responses from the MFW anaerobically co-digested with GM is shown below (Figures 4.21-4.26). To simplify the work, the optimization plot for the two responses against the interactive effects of the process variables were limited only to the two optimal mixing ratios of 1:1 (a) and 1:2 (b).

The 3D surface plots indicated in Figure 4.21a and 4.21b for the MRs of 1:1 and 1:2 respectively showed that the initial pH of 7 and digestion temperature of up to 37.5^o C gave the maximum biogas production. When the initial pH and temperature were out of this condition the rate of biogas volume production decreased. These optimal values generated 1.54 and 1.94 liters biogas respectively for the 1:1 and 1:2. From the plots it is clear to conclude that the rate of biogas production increased with increasing initial pH from 6 up to initial pH of 7 and then production rate decreased with further increase in initial pH. The same trend was noticed in the effect of the digestion temperature on the rate of biogas production. Production rate increased as the operating temperature increased from 30^o C to 37.5^o C then the rate of production decreased beyond this value.

The surface plots in Figure 4.22 (simultaneous effects of digestion time and digestion temperature on biogas) showed that the biogas volume increased with reaction time up to a digestion period of 11.5 days (after about 31 working days) then the biogas volume diminishes beyond this working day to the end of the experiments. From these plots, it is also shown that biogas volume increased to a maximum point with digestion temperature up to 37.5^o C then after decreased with a further increase in the temperature. Even though higher temperature reduces the required digestion period, volume biogas production was decreased due to quick VFAs accumulation and rapid decomposition of organic substrate. The gradients of the volume of gas formation decreased with digestion time to the end of the experiments (from digestion period 31 working days ahead) because of the decrease of decomposable matter in the substrates. The biogas production also falls because of the destruction of bacterial enzyme by elevated temperature (Chae, *et al.*, 2007). The response surface plots shown in figure 4.23 shows the variation of biogas production with reaction time and initial pH. Both plots clearly show the increase in biogas volume with increasing the initial pH up to 7.5 then decreased with increasing the pH beyond this value which presents the effect of pH on the methanogenic bacteria (Poh & Chong, 2009). In addition, as stated in the previous paragraphs on the effect of digestion time on biogas volume, the volume of gas production increased with increasing reaction time then further increase in the reaction time increases in consumption of biodegradable substrate leading to decrease in biogas volume production.

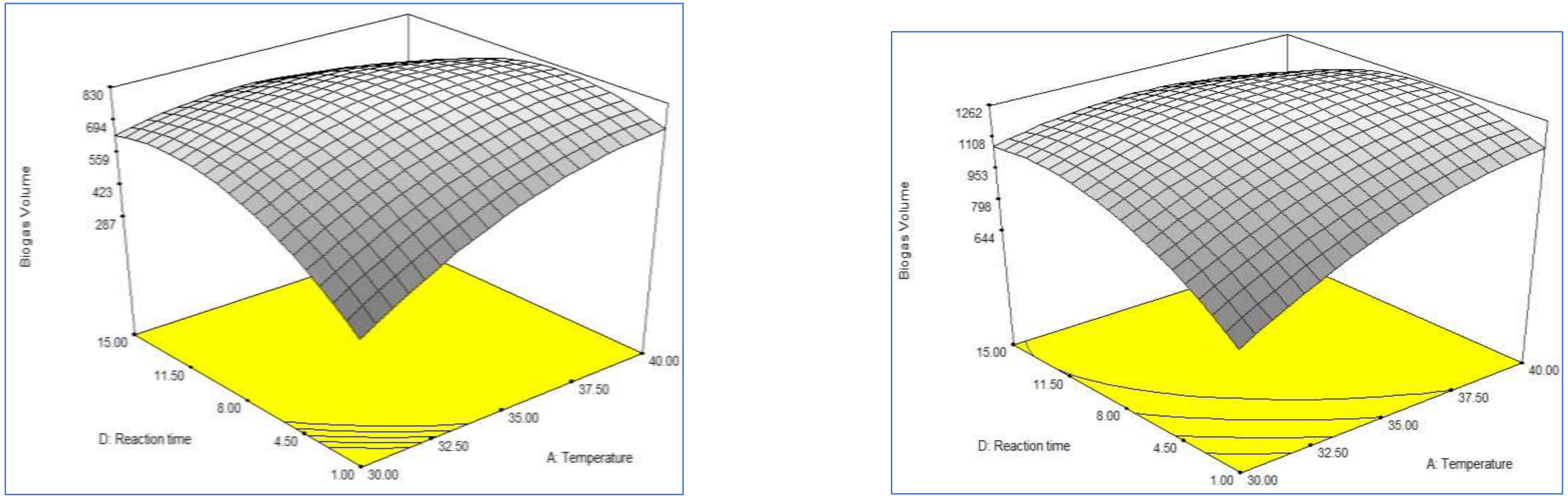
The response surface plots shown below (Figures 4.24-4.26) are representing the variation of methane yield from the optimal mixing ratios of 1:1 (Figures 4.24a-4.26a) and 1:2 (Figures 4.24b-4.26b) with the simultaneous effects of the process parameters digestion temperature and initial pH (Figure 4.24), reaction time and digestion temperature (Figure 4.25), and reaction time and initial pH (Figure 4.26). From the surface plots of figures 4.24 it was observed that the methane yield for both the mixing ratios increased with increasing the temperature from 30^{o} C to 35^{o} C then after decreased in methane content of the biogas produced with a further increase in this independent variable. An increase in methane content was also detected as the initial pH was increased from 6 to 7 (for the mixing ratio of 1:2) and from 6 to 7.5 (for the mixing ratio 1:1) in which an increasing beyond this value directed to the decrease in the response. The increasing range of the methane content for the 1:2 with the initial pH was up to 7.0 but for the 1:1 was up to 7.5 this may be due to the high nitrogen content which caused the rise in pH of the digesters fed with the 1:2 mixing ratio.

Response surface plots of Figures 4.25 shows the methane content variation in the biogas as a function of digester temperature and digestion time for the mixing ratios of 1:1 (Figure 4.25a) and 1:2 (Figure 4.25b). The methane content was increased with increased temperature from 30^{o} C to 35^{o} C for the 1:1 reactor and from 30^{o} C to 32.5^{o} C for the 1:2 reactor. The methane content for both the digesters was increased linearly with increasing digestion time. However, the presence of high nitrogen content in the digester fed with the goat manure only 0:1 and 1:2 mixing ratios increases the digester temperature the digester become too hot, killing the microorganisms (Tom & Nancy, 1996). Figures 4.26 shows the variation of the methane fraction in the biogas as a function of the digestion time and initial pH. The methane content increased with increasing all the digestion time as there are more methanogenic bacteria that can release the methane produced to the end of the digestion time (Converti, Ferraiolo, & Borghi, 1993) but decreased in a further increase in digestion time as a result of the consumption of subtrate to the end of the experiment.



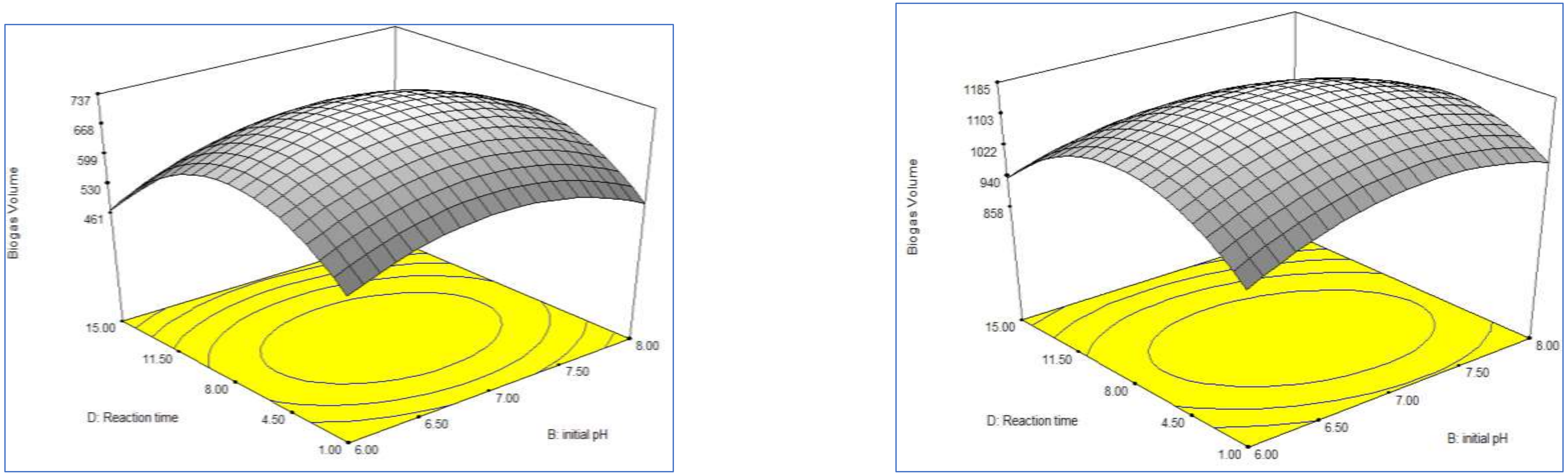
(a) (b)

Figure 4. 21 Surface plots showing the relationship between the experimental levels of temperature and initial pH on biogas volume production



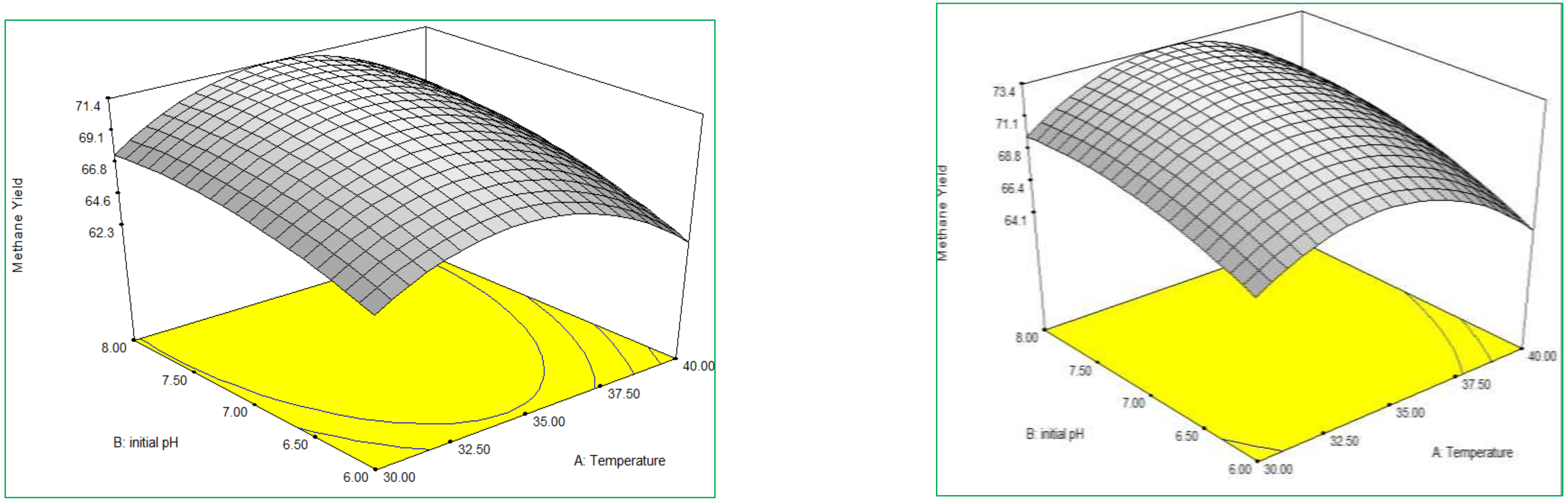
(a) (b)

Figure 4. 22 Surface plots showing simultaneous effect of digestion time and temperature on biogas volume



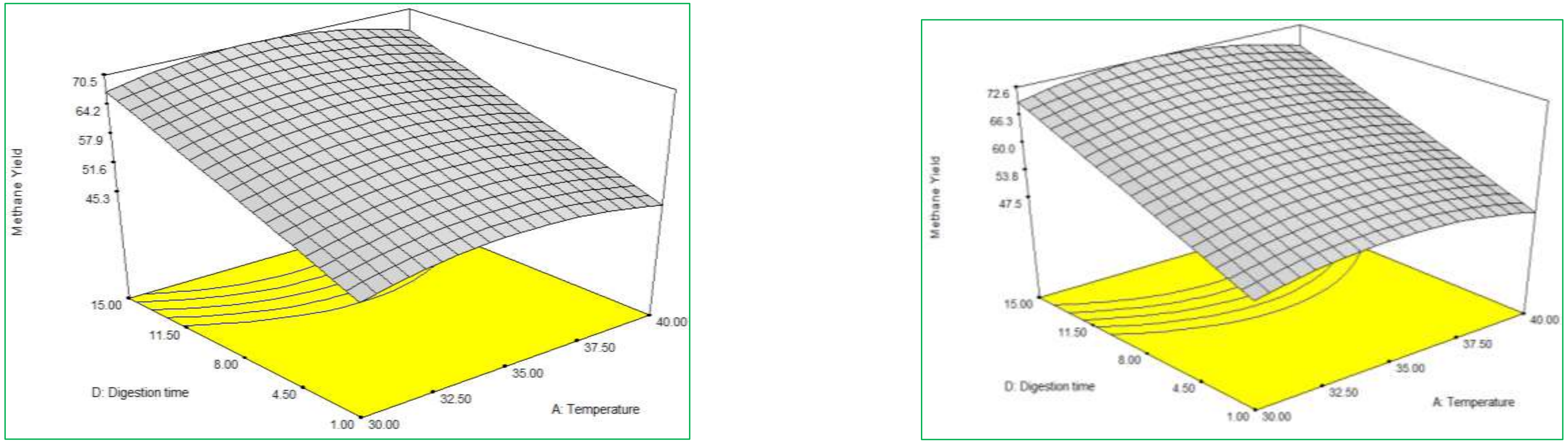
(a) (b)

Figure 4. 23 Surface plots showing the simultaneous effect of digestion time and initial pH on biogas volume



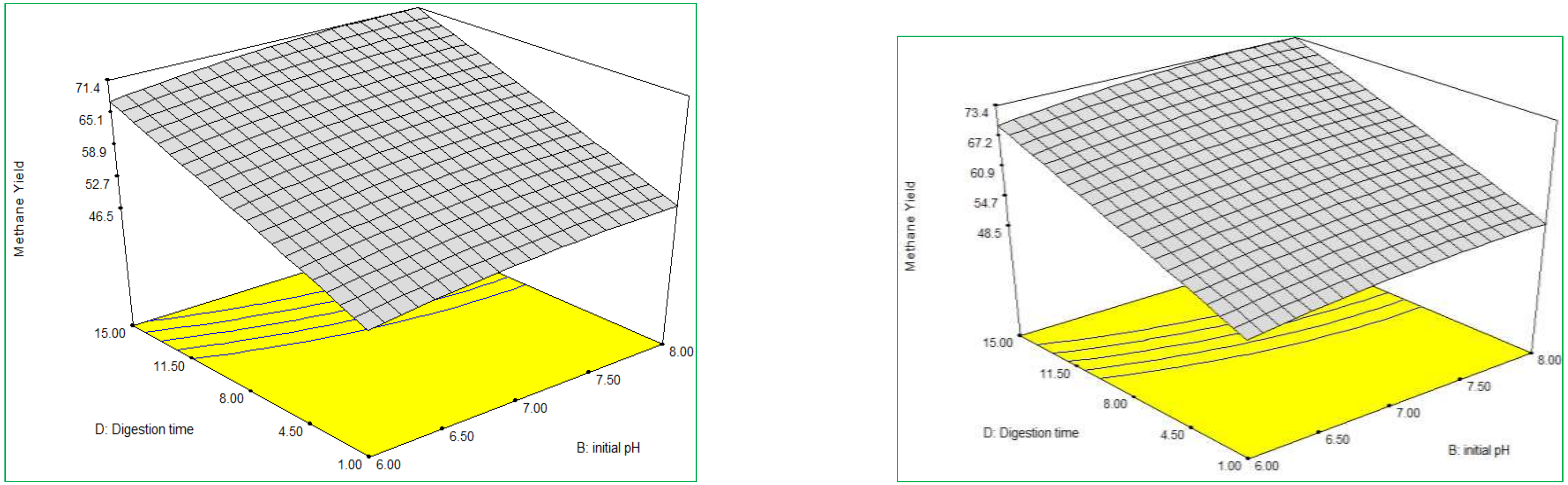
(a) (b)

Figure 4. 24 Surface plots showing the simultaneous effects of initial pH and temperature on methane yield



(a) (b)

Figure 4. 25 Surface plots showing the simultaneous effects of reaction time and temperature on methane yield



(a) (b)

Figure 4. 26 Surface plot showing the simultaneous effects of initial pH and reaction time on methane yield

5. Conclusions and Recommendations

5.1 Conclusion

This study dealt with the biogas production by anaerobic co-digestion of mixed food waste with goat manure. Even though food waste is easily biodegradable and has high VS, the mono-digestion of this substrate as a single feed stock for biogas production was not as efficient as co-digestion of it with goat manure. An inoculum prepared from the 1:1 mixing ratio after fifteen days digestion period was used to reduce the lag phase of methanogenic activity in degrading the substrates and to get a continuous biogas production.

The anaerobic co-digestion of food waste with goat manure for improving biogas generation overcomes the C/N ratio imbalances in mono-digestion of food waste or goat manure alone and boosts the process. Digesters fed with different five mixing ratios of the two substrates that were adjusted their initial pH values to three pH value levels 6, 7, and 8 were operated at three digestion temperature levels 30^o C, 35^o C and 40^o C for forty working days. The ratios of co-substrates of 540 g MFW with 0 g of GM, 0 g MFW with 540 g GM, 360 g MFW with 180 g GM, and 270 g MFW with 270 g GM, 180 g MFW with 360 g GM were termed as R1 = 1:0, R2 = 0:1, R3 = 2:1, R4 = 1:1, and R5 = 1:2. The potential of the anaerobic digester to generate biogas is closely related to the type of waste which is being used. From the experimental study noted and the data presented herein this document it is clear that all the physico-chemical parameters employed in this study were important for the improvements of biogas generation and methane yield at optimum process parameters. The type of waste and feedstock substrate used is very significant, the C/N ratio, the digestion period of the reactor, the mixing system, the initial pH and the reaction temperature are all very significant process conditions for improved biogas and methane yield. The results noted from this study also showed that the anaerobic co-digestions of mixed food waste with goat manure was better than mono-digestion of the single waste alone for biogas generation. The optimal mixing ratios were found to be the 1:1 and 1:2 (food waste to goat manure) at an initial pH of 7.0 and digestion temperature of 35^o C. These two combination systems were found to be well stable and balanced in C/N ratio for better functioning the methanogenic bacteria and were produced much more biogas than the other mixing ratios. The higher total carbon content of food waste in the 1:0 and rapid accumulation of VFAs in the 2:1 suppressed methanogenic growth and methanogenesis because of the lack of ammonium nitrogen and low pH. With increasing these acids pH of the digesters fed with these combination systems decreased leading to the inhibition of methanogenic activity. DOE software version 6.0.8 was used to examine the individual and simultaneous effects of the process parameters on the measured responses. The model summary statistics for the two measured responses were statistically good as they have the maximum pred R^2 and adj R^2.

5.2 Recommendations

From the experimental investigations conducted and the results obtained herein this study, it would be of greatest significance if the following suggested some future works would be taken into considerations፡-

- For efficient biogas production with high in methane content and good eco-friendly, MFW co-digested with GM could be used as substrate for anaerobic digestion process.
- Single substrate of MFW or GM alone as feedstock should not be used for biogas production. The use of MFW alone as substrate could lead to rapid high VFAs accumulation causing acidic effect of methanogenesis activity, and digester fed with GM alone as substrate could lead to rapid drying of digesters.
- Digestion systems with these substrates as feed stock should be preserved at mesophilic range, to keep working continue the possibility of using anaerobic digestion process to produce biogas from these co-substrates as alternative source of renewable energy.
- Food waste constitutes a considerable portion of MSW. Landfill volume in Ethiopia is becoming a threatened resource and is on the diminishing, with that will come severer waste management regulations, higher waste disposal prices, and the application of new waste diversion plans. FW generators could agree with local transportation organizations to send their generated wastes to anaerobic co-digestion facility.

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APPENDIX A

Chemicals

Table A 1 Chemicals used during this study

Chemicals	Code	Unit	Quantity
Distilled water	0261	Litter	5
Sodium hydroxide	0654	500 gm	3
Sodium thiosulphate	0477	500 gm	2
Phenolphthalein	1349	500 ml	2
Hydrochloric acid	0176	2.5 L	1
Aluminum oxide	174	500 gm	2
Potassium dichromate	994	500 gm	2
Ferrous Ammonium Sulphate	1145	500 gm	2
Potassium Sulphate	670	500 gm	1
Zink metal	12	500 gm	4
Boric acid	71	500 gm	3
Sodium Chloride	235	500 gm	2

Al_2O_3 Ethanol Distilled water FAS

COD vials Nitrification inhibitor Sulfuric acid Boric Acid

Figure A 1 Chemicals used

APPENDIX B

Equipment

Crucibles

COD tube tests

Weighing balance

COD digester

Desiccator

COD analyzer (photometer)

Drying oven

Maffle furnace

Figure B 1 COD test, %TS and VS Determination equipment

BOD Test

Table B 1 Recommended sample volumes for the 5-day biochemical oxygen demand test (Sawyer and McCarty, 1978).

Anticipated BOD range (mg O_2/L)	**Sample volume (ml)**	**Dosage ATH in drops**
0-40	428	10
0-80	360	10
0-200	244	5
0-400	157	5
0-800	94	3
0-2000	**56**	**3**
0-4000	21.7	1

AD construction materials

The following equipment were used for the construction of the experimental setups.

Plastic bottles

Hoses, Plaster, UHU

Figure B 2 List of main equipment for the AD construction

APPENDIX C

Reagents and Solutions Preparation

45% (w/v) Potassium hydroxide preparation

- 45 grams of KOH was dissolved in 100 ml distilled water

To make up 100 ml of **40% NaOH**,

$$40 \text{ gram x} \frac{100 \text{ ml}}{100 \text{ ml}} = 40 \text{ grams}$$

So, 40 grams placed in a beaker and added distilled water up to 100 ml.

4% (w/v) Boric acid

- 4 grams of H_3BO_3 dissolved in 100 ml distilled water
- To make 50 ml of 4% of H_3BO_3

$$4 \text{ gram x} \left(\frac{50 \text{ ml}}{100 \text{ ml}}\right) = 2 \text{ grams}$$

So, 2 g of H_3BO_3 placed in a beaker and added distilled water up to 50 ml.

0.1 N Sulphuric acid

- 0.1-gram equivalents of sulphuric acid per liter or 0.05 M H_2SO_4, since Normality (N) = Molarity (M) x n_{factor} , n_{factor} is usually the charge or valency, in this case it is 2.

This was prepared by dissolving 2.7 ml of 98% H_2SO_4 (molarity =18.4 M or 36.8 N) in 1 liter of deionized water. Density of concentrated H_2SO_4 is 1.84 gm/ml and molecular weight of 98.08 gm/mol as it is di acidic for normality it is 49 equivalent grams hence, $N = \frac{\left(\frac{98}{100}\right)(1000)(1.84)}{49} = 36.8\ N$;

Using the dilution method equation $N_1V_1 = N_2V_2$ to make 1 liter of 0.1 N H_2SO_4 from 36.8 N H_2SO_4; 1000 ml x 0.1 = 36.8 x V_2

- V_2 = 2.7 ml of 36.8 N H_2SO_4 dissolved in 1-liter distilled water.

10 N NaOH

N = MxZ, Z – number of OH^- for bases or number of hydrogen for acid

N = Mx1; Z = 1, N = M

10 N NaOH = 10 M NaOH

$$↳ 10 \text{ M} = 10 \text{ mol} \frac{\text{NaOH}}{1\text{L}}$$

$$= 10 * \frac{(22.9897 + 15.99 + 1.008)\text{g}}{\text{L}}$$

$$= 399.877 \approx 400 \text{ g/L}$$

Therefore, 400 grams of NaOH was dissolved in 1 liter of distilled water.

0.1 N NaOH = 0.1 M NaOH

» 0.1 M = 0.1 mol NaOH

= 0.1x (22.9897 + 15.99 + 1.008) g/L

= 39.99 g/L ≈ 40 g/L

40 grams of NaOH was dissolved in 1-liter distilled water.

APPENDIX D

Computation of the proximate content of the wastes

Table D 1 Proximate composition of wastes

Labeled Crucibles	Waste Type	M_1	M_2	M_3 [105° C]	M_4 550° C	%TS	%Ash	%VS	%FS
1	Avocado peel	35.50	74.50	40.72	36.12	13.39*	11.80	88.21	1.59
2	Cabbage waste	41.20	77.80	42.70	41.90	4.10	2.19	97.81	1.91
3	Carrot waste	36.10	69.20	37.74	36.89	4.96	2.57	97.43	2.39
4	Potato peel	41.70	66.10	46.28	42.10	18.77*	17.13	82.87	1.64
5	Beet waste	38.60	69.70	40.31	39.10	5.50	3.89	96.11	1.61
6	Mango peel	41.10	80.60	43.35	42.00	5.70	3.42	96.58	2.28
7	Other mixed food leftover wastes	41.50	55.37	47.84	44.71	27.37*	29.09	70.92	23.14
8	Goat manure	43.50	52.70	51.60	44.03	88.04*	6.22	93.78	5.76

Remarks: M_1 = weight of empty crucible, M_2= weight of raw waste + M_1, M_3 at 105° C = M_1+M_2 during drying at 105° C for %TS content, M_4 at 550° C = M_3 during drying at 105° C then at 550° C for % VS content, % TS = percent total solids content, % VS = percent volatile solids content. * Was adjusted to the require ideal TS % content for AD.

The values in the columns 7, 8 and 9 were calculated according to the equations 3.1, 3.2 and 3.3 respectively. For example, for Avocado peels the total solids content, volatile solids content and fixed solids content was calculated as follow:

$$\%TS = \frac{M_3 - M_1}{M_2 - M_1} \times 100 = \frac{40.72-35.5}{74.5-35.5} \times 100 = 13.39\%$$

$$\%Ash = \frac{M_3 - M_4}{M_2 - M_1} \times 100 = \frac{40.72-36.12}{74.5-35.5} \times 100 = 11.80\%$$

$$\%VS = 100 - \%Ash;\ 100 - 11.80 = 88.2$$

$$\%FS = \frac{M_4 - M_1}{M_2 - M_1} \times 100 = \frac{36.12-35.5}{74.5-35.5} \times 100 = 1.59\%$$

The total solids content, volatile solids, carbon content, nitrogen content and C/N ratio of the mixed food wastes as well as the mixing ratios fed into the digesters shown herein the table below were calculated using the equation below.

Table D 2 Characteristics of feedstocks fed into digesters of equal capacity

Characteristics	Food wastes to Goat manure ratio				
	1:0	**1:1**	**2:1**	**1:2**	**0:1**
% TS	8.5	8.5	8.5	8.5	8.5
% VS	89.99	91.89	91.25	92.52	93.78
% C	49.99	51.05	50.69	51.4	52.1
% N	1.47	1.79	1.68	1.89	2.10
C/N ratio	34.00	25.57	26.22	25.21	26.76

The %TS, %VS and C/N ratio for the feedstock fed into the digesters was prepared and calculated according to the following equations:

$$\%\mathrm{TS}_{\mathrm{FW}} = \frac{[\sum_{\mathrm{i}=1}^{7} \mathrm{Ww}_{\mathrm{i}} * \%\mathrm{TS}_{\mathrm{i}}]}{\sum_{\mathrm{i}=1}^{7}(\mathrm{Ww}_{\mathrm{i}})}$$

$W_{wi\,=}$ Wet weight of individual food waste (Table 3.1)

$\%TS_i$ = %TS of individual food waste (Appendix D Table 4.1)

$$\%\mathrm{TS}_{\mathrm{FW}} = 1735.71\ \mathrm{g} * \frac{13.39 + 4.1 + 4.96 + 18.77 + 5.498 + 5.70 + 27.37}{1735.71\ \mathrm{g}\ * 7}$$

$$= \frac{13.39 + 4.1 + 4.96 + 18.77 + 5.498 + 5.70 + 27.37}{7} = 11.398\ \%$$

The total solid content for the mixed food wastes is therefore,

$\mathrm{TS}_{\mathrm{FW}}(\mathrm{gm}) = \frac{11.398}{100} * 540 = \quad 61.549\ \mathrm{gm}$. To alter the solid content of this waste to the optimal value of solid content 8.5%; 8.5 gm = 100 ml → 61.549 gm ≈ 724 ml of water was added then 600 gm of this slurry was fed into the digesters with mixing ratio of 1:0.

- $\%\mathbf{TS}_{\mathbf{1:1}} = \frac{(\mathrm{FW}_{\mathrm{weignt}} * \%\mathrm{TS}_{\mathrm{FW}}) + (\mathrm{GM}_{\mathrm{weight}} * \%\mathrm{TS}_{\mathrm{GM}})}{\mathrm{FW}_{\mathrm{weight}} + \mathrm{GM}_{\mathrm{weight}}} = \frac{270 * 11.398 + 270 * 88.044}{270 + 270} = 49.721\%$

The total solid content for the mixed food wastes mixed with goat manure in the ratio 1:1 is therefore, $\mathrm{TS}_{1:1}(\mathrm{gm}) = 270 * \frac{11.398}{100} + 270 * \frac{88.04}{100} = 268.48\ \mathrm{gm}.$

☑ To alter the solid content of this mixed waste to the optimal value of solid content 8.5%; 8.5 gm = 100 ml → 268.48 gm ≈ 3158.59 $ml\ of\ water\ was\ added$ and the digesters with mixing ratio of 1: 1 were fed with 600 gm of this slurry.

- $\%\mathbf{TS_{2:1}} = \frac{(\mathrm{FW_{weignt}} * \%\mathrm{TS_{FW}}) + (\mathrm{GM_{weight}} * \%\mathrm{TS_{GM}})}{\mathrm{FW_{weight}} + \mathrm{GM_{weight}}} = \frac{360 * 11.398 + 180 * 88.044}{360 + 180} = 36.947\%$

  The total solid content for the mixed food wastes mixed with goat manure in the ratio 2:1 is

  $\mathrm{TS_{2:1}}(\mathrm{gm}) = 360 * \frac{11.398}{100} + 180 * \frac{88.04}{100} = 199.51\ gm$

☑ To alter the solid content of this mixed waste to the optimal value of solid content 8.5%

8.5 gm = 100 ml → 199.51 gm ≈ 2347.18 ml of water was added, and 600 gm of this slurry was fed into the digesters with mixing ratio of 2: 1.

- $\%\mathbf{TS_{1:2}} = \frac{(\mathrm{FW_{weignt}} * \%\mathrm{TS_{FW}}) + (\mathrm{GM_{weight}} * \%\mathrm{TS_{GM}})}{\mathrm{FW_{weight}} + \mathrm{GM_{weight}}} = \frac{180 * 11.398 + 360 * 88.044}{180 + 360} = 62.495\%$

  The total solid content for the mixed food wastes mixed with goat manure in the ratio 1:2 is

$$\mathrm{TS_{1:2}}\ (\mathrm{gm}) = 180 * \frac{11.398}{100} + 360 * \frac{88.04}{100} = 337.46\ \mathrm{gm}$$

☑ To alter the solid content of this mixed waste to the optimal value of solid content 8.5%

8.5 gm = 100 ml →337.46 gm ≈ 3970.12 ml of water was added, and 600 gm of this slurry was fed into the digesters with mixing raio of 1: 2.

- **$\%\mathrm{TS}_{0:1}$ = 88.044%**

  The total solid content for the goat manure only (1:0) is $\frac{88.04}{100} * 540 = 475.42$ gm

  →540 gm of the GM was diluted with1035.77 ml of water and the digesters with mixing ratio of 1: 0 were fed with 600 gm of this slurry.

$\mathrm{FW_{weight}}$ = Food waste weight (wet weight)

$\mathrm{GM_{weight}}$ = Goat manure weight (wet weight)

$\%\mathrm{TS_{FW}}$ = Total solids content of food waste

$\%\mathrm{TS_{GM}}$ = Total solids content of goat manure

Computation of nitrogen content of GM and MFW distillates

For the GM distillate

$M_{NH4H2BO3} = 0.1\ M \times \frac{75\ ml}{150\ ml} = 0.05\ M$

Moles of $M_{NH4H2BO3} = 0.05\ M \times 0.15\ L$

$= \underline{0.0075\ moles}$

❀ To determine weight of the ammonium-borate complex with molecular weights (N = 14.0067, H = 1.008, B =10.81, O = 15.999 g/mol) of 78.8617 g/mol:

Wt. $NH_4H_2BO_3$

$= 0.0075\ mol \times \frac{78.8617g\ NH_4H_2BO_3}{1\ mole\ NH_4H_2BO_3}$

$= 0.591\ g$

☑ By gravimetric analysis the amount of nitrogen present in the GM sample is:

Wt. N

$= 0.591\ g\ NH_4H_2BO_3 \times \frac{14.0067g\ N}{78.8617\ g\ NH_4H_2BO_3}$

$= 0.105\ g$

$\%\ N = \frac{0.105\ g}{5g} \times 100 = 2.10\%$

For the MFW distillate

$M_{NH4H2BO3} = 0.1\ M \times \frac{52\ ml}{150\ ml} = 0.035\ M$

Moles of $M_{NH4H2BO3} = 0.035\ M \times 0.15\ L$

$= \underline{0.0053\ moles}$

❀ To determine weight of the ammonium-borate complex:

Wt. $NH_4H_2BO_3$

$= 0.0053\ mol \times \frac{78.8617g\ NH_4H_2BO_3}{1\ mole\ NH_4H_2BO_3}$

$= 0.417\ g$

☑ By gravimetric analysis the amount of nitrogen present in the MFW sample is:

Wt. N

$= 0.417\ g\ NH_4H_2BO_3 \times \frac{14.0067g\ N}{78.8617\ g\ NH_4H_2BO_3}$

$= 0.074\ g$

$\%\ N = \frac{0.074\ g}{5g} \times 100 = 1.47\%$

The following formula was applied to calculate the C/N ratio for the feedstocks mixture:

$$\left(\frac{C}{N}\ \text{ratio}\right)_{\text{mixed foodwaste}} = \frac{\sum \text{weight of carbon in food wastes}}{\sum \text{weight of nitrogen in food waste}}$$

$$\left(\frac{C}{N}\ \text{ratio}\right)_{\text{mixture}} = \frac{\text{weight of carbon in food waste} + \text{weight of carbon in goat manure}}{\text{weight of nitrogen in food waste} + \text{weight of nitrogn in goat manure}}$$

$$= \frac{FW_{weight} * \%C_{FW} * (100 - \%M_{FW}) + GM_{weight} * \%C_{GM} * (100 - \%M_{GM})}{FW_{weight} * \%N_{FW} * (100 - \%TS_{FW}) + GM_{weight} * \%N_{GM} * (100 - \%TS_{GM})}$$

$$= \frac{FW_{weight} * \%C_{FW} * \%TS_{FW} + GM_{weight} * \%C_{GM} * \%TS_{GM}}{FW_{weight} * \%N_{FW} * \%TS_{FW} + GM_{weight} * \%N_{GM} * \%TS_{GM}}$$

Where, M_{FW} = Moisture content for the mixed food wastes = 100 - $\%TS_{FW}$ = 100 – 11.4 = 88.6%.

M_{GM} = Moisture contet for goat manure = 100 - $\%TS_{GM}$ = 100 – 88.04 = 11.96%

$$\left(\frac{\mathrm{C}}{\mathrm{N}}\right)_{1:1} = \frac{270 * 49.99 * (100 - 88.6) + 270 * 52.1 * (100 - 11.96)}{270 * 1.47 * (100 - 88.6) + 270 * 2.1 * (100 - 11.96)} = 25.57$$

$$\left(\frac{\mathrm{C}}{\mathrm{N}}\right)_{2:1} = \frac{360 * 49.99 * (100 - 88.6) + 180 * 52.1 * (100 - 11.96)}{360 * 1.47 * (100 - 88.6) + 180 * 2.1 * (100 - 11.96)} = 26.22$$

$$\left(\frac{\mathrm{C}}{\mathrm{N}}\right)_{1:2} = \frac{180 * 49.99 * (100 - 88.6) + 360 * 52.1 * (100 - 11.96)}{180 * 1.47 * (100 - 88.6) + 360 * 2.1 * (100 - 11.96)} = 25.21$$

- Volatile solids content for the feedstocks mixture were determined according to the following equation:

$$(\%\mathrm{VS})_{1:1} = \frac{\%\mathrm{VS}_{\mathrm{FW}} * \mathrm{FW}_{\mathrm{weight}} + \%\mathrm{VS}_{\mathrm{GM}} * \mathrm{GM}_{\mathrm{weight}}}{\mathrm{FW}_{\mathrm{weight}} + \mathrm{GM}_{\mathrm{weight}}}$$

$$\%\mathrm{VS}_{\mathrm{FW}} = 1735.71 * \frac{88.205+97.81+97.432+82.869+96.11+96.58+70.92}{7*1735.71} = 89.99\%$$

$$(\%\mathrm{VS})_{1:1} = \frac{89.99 * 270 + 93.78 * 270}{270 + 270} = 91.89$$

$$(\%\mathrm{VS})_{2:1} = \frac{89.99 * 360 + 93.78 * 180}{180 + 360} = 91.25$$

$$(\%\mathrm{VS})_{1:2} = \frac{89.99 * 180 + 93.78 * 360}{180 + 360} = 92.52$$

Where,

C_{FW} = Carbon (%) content of food waste

C_{GM} = Carbon (%) content of goat manure

N_{GM} = Nitrogen (%) content of goat manure

N_{FW} = Nitrogen (%) content of food waste

$\%TS_{GM}$ = Total solids (%) content of goat manure

$\%TS_{FW}$ = Total solids (%) content of food waste

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Figure D 1 Detail process of size reduction of food wastes

APPENDIX E

Daily produced biogas volume and its methane and carbondioxide content

Table E 1 Biogas volume, methane and carbon dioxide fractions at 30º C and pH 6.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed Food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	Feb 17, 2017	74	40.7	38.1	231	44.8	40.1	138	43.9	40.5	430	45.7	39.4	93	42.7	42.0
2	Feb 19, 2017	126	43.3	38.5	391	45.6	36.3	233	44.7	36.7	729	47.5	35.6	158	43.8	38.2
3	Feb 21, 2017	172	43.4	37.6	534	50.0	35.4	318	47.4	35.8	995	51.9	34.7	215	44.3	37.3
4	Feb 24, 2017	223	43.5	34.8	692	50.9	32.6	413	48.0	33.0	1291	52.8	31.9	279	45.2	34.5
5	Feb 26, 2017	68	43.8	33.4	211	51.2	31.2	126	48.9	31.6	393	53.1	30.5	85	45.5	33.1
6	Feb 28, 2017	105	44.9	33.1	327	52.2	30.9	195	49.6	31.3	609	54.1	30.2	132	46.5	32.8
7	Mar 3, 2017	200	45.0	31.3	622	52.4	29.1	371	49.8	29.5	1160	54.3	28.4	251	46.7	31.0
8	Mar 5, 2017	253	45.7	31.4	787	53.0	29.2	469	50.4	29.6	1467	54.9	28.5	317	47.3	31.1
9	Mar 7, 2017	170	48.3	31.4	529	54.3	29.2	315	51.7	29.6	986	56.2	28.5	213	48.6	31.1
10	Mar 10, 2017	163	52.1	31.5	507	55.7	29.3	302	53.1	29.7	945	57.6	28.6	204	50.0	31.2
11	Mar 12, 2017	331	53.0	31.5	1029	59.5	29.3	614	56.9	29.7	1545	61.4	28.0	415	53.8	31.2
12	Mar 14, 2017	335	53.6	27.1	1042	60.4	27.7	621	57.8	27.1	1635	62.3	27.0	420	54.7	26.8
13	Mar 17, 2017	125	54.4	26.8	398	61.4	27.0	232	58.8	26.3	725	63.0	26.3	157	55	26.5
14	Mar 19, 2017	95	54.6	26.7	295	61.8	26.0	176	59.2	26.3	551	63.7	25.3	119	56.1	26
15	Mar 21, 2017	57	54.1	25.7	177	61.8	26.0	106	59.2	24.3	330	63.7	25.3	71	56.1	25.4

Table E 2 Biogas volume, methane and carbon dioxide fractions at 30º C and pH 7.0

SN.	Gas sampling date [days]	Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]														
		1:0			1:1			2:1			1:2			0:1		
		Biogas [ml]	CH_4 [%]	CO_2 [%]	Biogas [ml]	CH_4 [%]	CO_2 [%]	Biogas [ml]	CH_4 [%]	CO_2 [%]	Biogas [ml]	CH_4 [%]	CO_2 [%]	Biogas [ml]	CH_4 [%]	CO_2 [%]
1	Feb 17, 2017	115	38.5	36.0	358	40.9	38.3	213	40.6	38.0	667	43.3	40.5	144	39.2	36.7
2	Feb 19, 2017	195	41.3	35.4	606	43.9	37.6	362	43.6	37.3	1131	46.5	39.8	244	42.1	36.1
3	Feb 21, 2017	266	43.7	34.6	827	46.5	36.8	493	46.1	36.5	1542	49.2	38.9	333	44.6	35.3
4	Feb 24, 2017	345	45.0	33.7	1073	47.8	35.8	640	47.5	35.6	1675	50.6	37.9	432	45.9	34.4
5	Feb 26, 2017	105	47.2	31.0	327	50.2	33.0	195	49.8	32.7	609	53.1	34.9	132	48.2	31.6
6	Feb 28, 2017	158	49.2	31.2	491	52.3	33.2	293	51.9	32.9	916	55.3	35.1	198	50.2	31.8
7	Mar 3, 2017	211	51.0	31.1	656	54.3	33.1	391	53.8	32.8	1223	57.4	35.0	264	52.3	31.7
8	Mar 5, 2017	244	53.2	30.5	759	56.6	32.4	453	56.1	32.2	1415	59.9	34.3	306	54.3	31.1
9	Mar 7, 2017	160	55.6	30.0	498	59.1	31.9	297	58.7	31.7	928	62.6	33.8	200	56.7	30.6
10	Mar 10, 2017	153	59.3	29.8	476	63.0	31.7	284	62.6	31.4	887	66.7	33.5	192	60.5	30.4
11	Mar 12, 2017	335	61.0	28.1	1042	64.8	29.9	621	64.4	29.6	1743	68.6	31.6	420	62.2	28.7
12	Mar 14, 2017	300	61.1	27.7	933	65.0	29.4	557	64.5	29.2	1739	68.8	31.2	376	62.3	28.3
13	Mar 17, 2017	124	61.0	27.0	386	64.8	28.7	230	64.4	28.5	719	68.6	30.4	155	62.2	27.5
14	Mar 19, 2017	105	62.1	26.2	327	66.1	27.9	195	65.6	27.6	610	69.9	29.5	132	63.4	26.7
15	Mar 21, 2017	45	62.3	25.3	140	66.2	26.1	83	65.7	26.7	261	70.1	28.5	56	63.5	25.8

Table E 3 Biogas volume, methane and carbon dioxide fractions at 30º C and pH 8.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	Feb 17, 2017	92	41.0	35.2	286	44.8	37.4	171	43.9	37.1	533	49.2	39.6	115	42.7	35.9
2	Feb 19, 2017	156	40.5	35.0	485	46.7	37.2	289	44.7	36.9	918	48.6	39.4	195	43.8	35.7
3	Feb 21, 2017	213	43.4	35.1	662	50.2	37.3	395	47.6	37.0	1220	52.1	39.5	267	44.4	35.8
4	Feb 24, 2017	276	44.6	33.7	858	51.6	35.8	512	49.0	35.6	1610	53.5	37.9	346	45.9	34.4
5	Feb 26, 2017	84	45.0	30.1	261	52.1	32.0	156	49.5	31.8	477	54.0	33.8	105	46.4	30.7
6	Feb 28, 2017	95	46.7	30.5	295	54.1	32.4	176	51.5	32.2	551	56.0	34.3	119	48.4	31.1
7	Mar 3, 2017	111	47.4	30.0	345	54.8	31.9	206	52.2	31.7	644	56.7	33.8	139	49.1	30.6
8	Mar 5, 2017	215	47.7	29.1	669	55.1	30.9	399	52.5	30.7	1247	57.0	32.7	268	49.0	29.7
9	Mar 7, 2017	165	48.0	29.4	513	55.7	31.3	306	53.1	31.0	957	57.6	33.1	207	50.0	30.0
10	Mar 10, 2017	153	49.2	28.0	475	56.6	29.8	284	54.0	29.5	887	58.5	31.5	192	50.9	28.6
11	Mar 12, 2017	325	51.2	27.5	1012	59.5	29.2	603	56.9	29.0	1615	61.4	30.9	407	53.8	28.1
12	Mar 14, 2017	304	52.7	28.0	945	60.1	29.8	564	57.5	29.5	1663	62.0	31.5	381	54.4	28.6
13	Mar 17, 2017	245	55.3	27.0	762	64.5	28.7	454	61.8	28.5	1421	66.4	30.4	307	58.7	27.5
14	Mar 19, 2017	103	56.0	25.4	320	65.3	27.0	191	62.7	26.8	597	67.2	28.6	129	59.6	25.9
15	Mar 21, 2017	75	59.1	25.3	233	69.0	26.9	139	66.4	26.1	435	70.9	28.5	94	63.3	25.8

Table E 4 Biogas volume, methane and carbon dioxide fractions at 35º C and pH 6.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	Apr 10, 2017	125	38.5	42.8	317	44.8	40.6	228	46.2	41.0	570	45.7	39.9	157	42.4	42.5
2	Apr 12, 2017	203	40.1	42.0	515	45.6	39.8	370	48.1	40.2	926	47.5	39.1	254	44.1	41.7
3	Apr 14, 2017	266	42.8	38.6	675	50.0	36.4	485	51.4	36.8	1215	51.9	35.7	333	47.1	38.3
4	Apr 17, 2017	336	44.5	36.7	852	50.9	34.5	613	53.4	34.9	1530	52.8	33.8	421	49.0	36.4
5	Apr 19, 2017	418	48.1	33.4	1060	51.2	31.2	763	57.7	31.6	1570	53.1	30.5	524	52.9	33.1
6	Apr 21, 2017	174	49.2	31.1	441	52.2	30.9	318	59.0	31.3	794	54.1	30.2	218	54.1	32.8
7	Apr 24, 2017	211	51.0	31.3	535	52.4	29.1	385	61.2	29.5	963	54.3	28.4	264	56.1	31.0
8	Apr 26, 2017	244	51.2	31.4	619	55.8	29.2	445	61.4	29.6	1113	57.7	28.5	306	56.3	31.1
9	Apr 28, 2017	167	57.1	31.4	424	57.7	29.2	305	68.5	29.6	762	59.6	28.5	209	58.7	31.1
10	May 1, 2017	171	55.6	31.5	434	59.4	29.3	312	66.7	29.7	780	61.3	28.6	214	57.2	31.2
11	May 3, 2017	438	54.0	31.5	865	59.5	29.3	799	64.8	29.7	1654	61.4	28.6	549	59.4	31.2
12	May 5, 2017	390	56.7	27.1	989	60.4	24.9	712	68.0	25.3	1779	62.3	24.2	489	58.3	26.8
13	May 8, 2017	315	57.2	26.8	775	62.8	24.6	575	67.2	25.0	1437	64.7	23.9	395	61.6	26.5
14	May 10, 2017	156	57.6	26.0	406	64.9	25.0	285	68.6	25.4	712	66.8	24.3	195	62.9	25.7
15	May 12, 2017	105	58.0	25.3	203	66.8	24.3	139	69.1	24.7	347	68.7	23.6	132	63.8	25.0

Table E 5 Biogas volume, methane and carbon dioxide fractions at 35^{o} C and pH 7.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	Apr 10, 2017	155	43.7	38.1	384	44.8	35.9	276	48.1	36.3	539	52.9	35.2	190	45.1	37.8
2	Apr 12, 2017	242	43.3	38.5	623	52.1	36.3	448	47.6	36.7	875	52.4	35.6	308	46.0	38.2
3	Apr 14, 2017	322	44.6	37.6	816	54.2	35.4	587	51.0	35.8	1146	56.1	34.7	403	46.0	37.3
4	Apr 17, 2017	407	46.7	34.8	1031	54.6	32.6	742	51.4	33.0	1448	56.5	31.9	509	48.3	34.5
5	Apr 19, 2017	506	47.5	33.4	1283	55.6	31.2	923	52.3	31.6	1802	57.5	30.5	634	49.1	33.1
6	Apr 21, 2017	211	49.2	33.1	534	57.6	30.9	384	54.1	31.3	750	59.8	30.2	264	51.0	32.8
7	Apr 24, 2017	255	50.1	32.4	647	60.8	30.2	466	55.1	30.6	910	62.7	29.5	320	52.0	32.1
8	Apr 26, 2017	295	51.0	31.4	749	60.0	29.2	539	56.1	29.6	1050	64.2	28.5	370	53.0	31.1
9	Apr 28, 2017	202	52.6	31.4	512	64.9	29.2	369	57.9	29.6	721	66.8	28.5	253	58.7	31.1
10	May 1, 2017	207	57.0	30.1	525	67.0	27.9	378	62.7	28.3	737	68.9	27.2	259	58.6	29.8
11	May 3, 2017	530	56.4	30.0	1544	66.3	27.8	967	62.0	28.2	1935	68.2	27.1	664	58.0	29.0
12	May 5, 2017	474	57.5	28.3	1202	68.1	26.1	865	63.3	26.5	1688	70.0	27.0	594	59.1	28.0
13	May 8, 2017	300	57.4	27.6	761	68.2	25.4	548	63.1	25.8	1069	70.1	27.4	376	59.0	27.3
14	May 10, 2017	218	58.0	21.1	553	70.7	21.0	398	64.2	20.4	777	72.6	19.3	273	60.0	21.4
15	May 12, 2017	110	60.8	12.6	279	71.6	13.1	201	66.8	13.3	392	73.5	12.7	138	62.4	11.8

Table E 6 Biogas volume, methane and carbon dioxide fractions at 35° C and pH 8.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	Apr 10, 2017	115	42.0	30.0	291	52.1	27.8	209	50.8	28.2	523	54.0	27.1	144	46.2	29.7
2	Apr 12, 2017	186	42.6	31.0	472	53.6	30.2	340	51..5	29.2	849	56.3	28.1	233	46.9	30.7
3	Apr 14, 2017	244	43.4	32.4	618	55.1	32.6	445	52.5	30.6	1112	56.0	29.5	306	47.7	32.1
4	Apr 17, 2017	308	44.0	34.8	781	56.0	31.2	562	53.2	33.0	1405	57.0	31.9	386	48.4	34.5
5	Apr 19, 2017	383	46.0	33.4	972	57.2	30.9	699	55.7	31.6	1748	58.6	30.5	480	50.6	33.1
6	Apr 21, 2017	210	47.5	33.1	533	61.3	29.1	383	57.5	31.3	958	61.2	30.2	263	52.3	32.8
7	Apr 24, 2017	215	49.7	31.3	545	62.0	29.2	392	60.1	29.5	981	62.1	28.4	269	54.7	31.0
8	Apr 26, 2017	234	51.0	31.4	593	63.4	28.3	427	61.7	29.6	1068	63.1	28.5	293	56.1	31.1
9	Apr 28, 2017	150	53.3	30.5	380	65.1	27.5	274	61.2	28.7	684	66.4	27.6	188	58.6	30.2
10	May 1, 2017	162	56.1	29.7	411	65.1	27.0	296	611.1	27.9	739	66.7	26.8	203	61.7	29.4
11	May 3, 2017	428	55.7	28.4	1085	66.0	27.4	781	64.0	26.6	1653	67.0	26.3	536	57.3	28.1
12	May 5, 2017	321	56.7	27.1	814	66.2	26.6	586	63.0	26.0	1464	68.5	26.7	402	58.5	26.8
13	May 8, 2017	254	58.0	26.8	644	67.0	25.9	464	65.2	26.0	1159	69.0	25.9	318	63.8	26.5
14	May 10, 2017	125	58.4	26.0	317	68.6	15.8	228	66.2	18.2	570	71.0	25.8	157	64.2	25.7
15	May 12, 2017	65	58.7	19.8	165	70.1	15.1	119	67.0	18.1	297	71.2	14.6	81	64.6	19.5

Table E 7 Biogas volume, methane and carbon dioxide fractions at 40^{o} C and pH 6.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	May 26, 2017	160	39.7	46.8	485	44.8	44.3	295	43.9	46.7	873	44.6	42.3	200	42.7	45.7
2	May 29, 2017	270	41.0	44.6	825	44.3	41.4	501	44.7	34.1	1111	46.2	37.1	338	43.8	38.1
3	May 31, 2017	369	41.2	39.7	970	44.6	39.7	652	41.7	34.7	1602	46.3	35.7	462	38.6	38.7
4	Jun 2, 2017	479	39.5	35.4	1086	46.9	35.0	829	44.3	33.3	1600	48.8	35.0	598	41.2	37.3
5	Jun 5, 2017	146	41.7	33.5	447	49.1	35.0	254	46.5	32.9	796	51.0	33.0	182	43.4	35.9
6	Jun 7, 2017	126	43.3	32.5	334	50.7	31.4	234	48.1	32.2	590	52.6	32.8	158	45.0	34.4
7	Jun 9, 2017	110	46.2	30.1	292	51.8	31.7	204	49.2	31.7	515	53.7	31.4	138	46.1	33.2
8	Jun 12, 2017	120	44.7	30.1	318	52.1	31.3	223	49.5	31.3	562	54.0	31.3	150	46.4	32.4
9	Jun 14, 2017	105	47.0	28.7	278	54.4	30.2	195	51.8	30.2	492	56.3	30.2	132	48.7	31.6
10	Jun 16, 2017	92	49.7	27.0	234	55.1	28.3	169	52.5	27.8	422	57.0	28.3	116	49.4	30.2
11	Jun 19, 2017	237	51.4	27.4	600	55.8	28.1	410	53.2	27.0	1080	57.7	28.1	296	50.1	30.0
12	Jun 21, 2017	264	53.6	26.1	596	58.3	26.2	407	55.7	26.4	1072	60.2	27.2	295	52.5	30.0
13	Jun 23, 2017	148	57.0	26.3	405	62.1	26.8	265	59.6	25.7	962	64.0	26.8	195	56.3	28.4
14	Jun 26, 2017	132	58.1	26.1	295	63.3	26.0	154	60.7	25.2	524	65.2	26.0	135	57.6	25.8
15	Jun 28, 2017	67	60.1	25.7	136	65.6	25.4	122	63.1	25.4	330	67.5	25.4	115	59.8	25.1

Table E 8 Biogas volume, methane and carbon dioxide fractions at 40º C and pH 7.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	May 26, 2017	247	42.7	40.3	768	44.8	40.3	447	43.9	38.7	1405	46.3	39.3	310	43.1	38.7
2	May 29, 2017	419	45.3	40.1	1279	46.2	41.1	769	44.7	34.1	1413	48.1	37.1	525	43.8	38.1
3	May 31, 2017	572	45.6	39.7	1345	50.6	39.5	1045	48.0	34.7	1685	52.5	35.7	716	44.9	38.7
4	Jun 2, 2017	725	46.2	35.0	1440	51.6	35.1	1348	49.1	33.3	1725	53.2	35.0	929	45.9	37.3
5	Jun 5, 2017	226	46.8	33.9	701	52.3	32.8	417	49.7	32.9	1149	54.4	32.6	284	46.6	34.1
6	Jun 7, 2017	200	47.4	33.3	504	52.9	31.4	291	50.3	31.9	791	54.7	32.1	247	47.2	34.0
7	Jun 9, 2017	190	45.7	31.5	479	53.1	30.9	276	50.5	30.3	752	55.1	31.4	234	47.4	33.2
8	Jun 12, 2017	165	45.6	31.5	416	53.0	31.4	240	50.8	30.3	653	54.9	32.0	203	47.3	32.4
9	Jun 14, 2017	125	48.6	30.6	315	54.3	30.7	182	51.7	30.3	495	56.3	31.6	154	48.6	31.3
10	Jun 16, 2017	111	50.5	30.1	283	56.4	30.4	204	53.8	29.5	510	58.3	30.8	140	50.7	31.5
11	Jun 19, 2017	271	52.9	29.7	726	60.3	29.9	523	57.2	29.5	926	62.2	30.1	359	54.6	30.5
12	Jun 21, 2017	264	54.5	28.4	720	61.2	28.7	519	58.6	27.7	873	63.1	29.3	355	55.6	30.1
13	Jun 23, 2017	255	55.6	26.0	647	62.2	26.8	465	59.6	27.1	809	64.5	26.6	320	57.8	27.2
14	Jun 26, 2017	112	57.0	25.6	285	62.6	26.6	215	60.0	27.3	425	65.3	26.2	240	57.9	26.8
15	Jun 28, 2017	92	57.9	25.4	150	63.3	26.0	111	60.8	26.4	291	66.1	25.9	123	58.1	26.3

Table E 9 Biogas volume, methane and carbon dioxide fractions at 40^{o} C and pH 8.0

SN.	**Gas sampling date [days]**	**Mixing Ratio [Mixed food waste to Goat manure, MFW:GM]**														
		1:0			**1:1**			**2:1**			**1:2**			**0:1**		
		Biogas [ml]	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**	**Biogas [ml]**	**CH_4 [%]**	**CO_2 [%]**
1	May 26, 2017	198	39.8	30.3	446	44.8	30.7	367	43.9	27.5	842	48.0	27.5	248	43.7	27.9
2	May 29, 2017	332	41.2	30.2	749	47.7	32.7	616	44.7	27.2	1415	49.6	27.5	416	43.8	28.4
3	May 31, 2017	447	42.7	28.7	1008	49.8	31.7	830	47.1	33.3	1510	51.7	29.8	560	44.0	29.1
4	Jun 2, 2017	457	44.9	36.1	1029	52.4	29.2	847	49.5	32.9	1643	54.3	28.4	572	46.7	34.7
5	Jun 5, 2017	181	44.0	33.7	407	51.3	35.1	335	48.7	31.9	769	53.2	29.5	226	45.6	35.9
6	Jun 7, 2017	173	46.3	32.0	389	54.1	35.0	321	51.5	30.3	736	56.0	36.1	217	48.4	34.9
7	Jun 9, 2017	160	47.5	32.1	360	55.6	31.1	297	52.9	30.3	681	57.5	35.7	200	49.8	33.2
8	Jun 12, 2017	148	49.0	31.1	333	57.4	31.4	275	54.8	30.3	630	59.3	31.9	185	51.6	32.4
9	Jun 14, 2017	105	51.8	30.8	236	60.8	31.0	195	58.1	30.3	447	62.7	31.8	132	55.0	31.9
10	Jun 16, 2017	88	51.8	31.0	198	60.8	31.2	163	58.1	31.3	375	64.1	31.6	110	55.1	31.5
11	Jun 19, 2017	231	53.0	29.5	521	62.2	31.2	429	59.6	30.6	985	65.9	30.1	290	56.5	31.7
12	Jun 21, 2017	235	54.5	29.0	529	64.0	30.1	436	61.4	30.1	1001	65.5	29.7	295	58.3	31.1
13	Jun 23, 2017	138	54.3	29.0	311	63.8	29.6	256	61.2	29.9	587	67.4	28.7	173	58.1	30.8
14	Jun 26, 2017	90	55.7	29.1	203	65.5	28.3	167	62.9	28.3	383	69.3	28.1	113	59.7	27.4
15	Jun 28, 2017	47	57.8	27.3	106	67.7	27.2	87	65.1	27.2	200	69.3	26.3	59	61.9	27.0

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Figure E 1 Experimental results of GA 5000 gas analyzer

Graphs of daily produced biogas volume

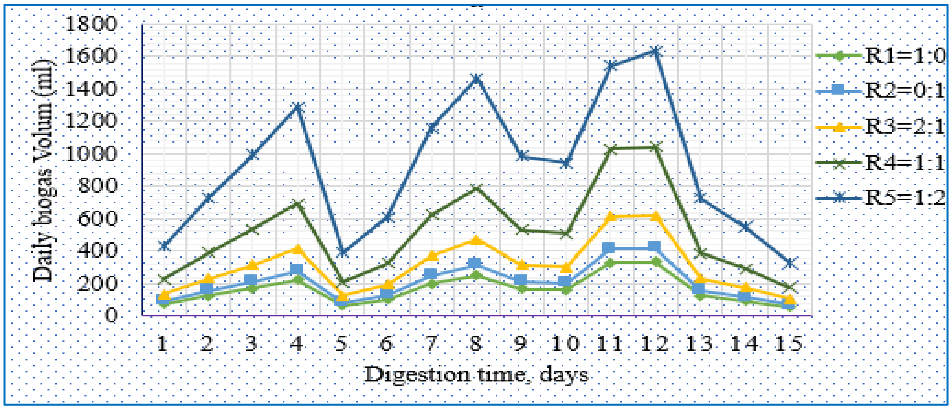

Figure E 2 Daily biogas volume at temperature 30° C and pH 6.0

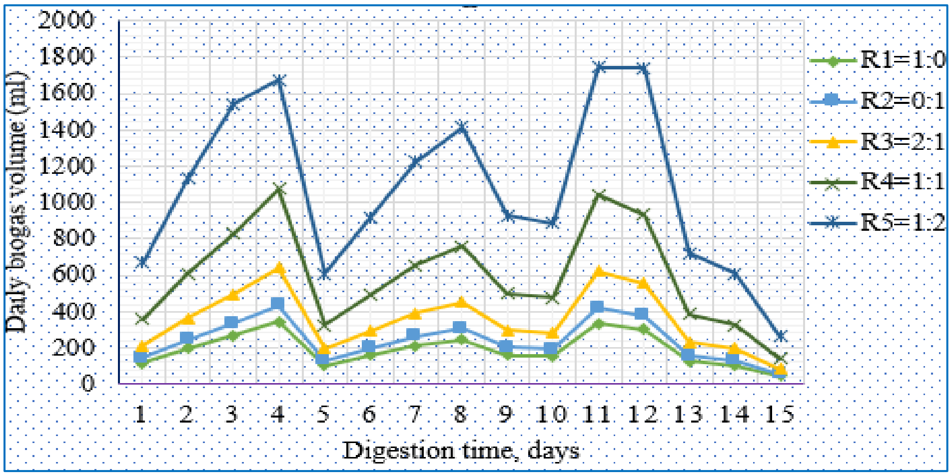

Figure E 3 Daily biogas volume at temperature 30° C and pH 7.0

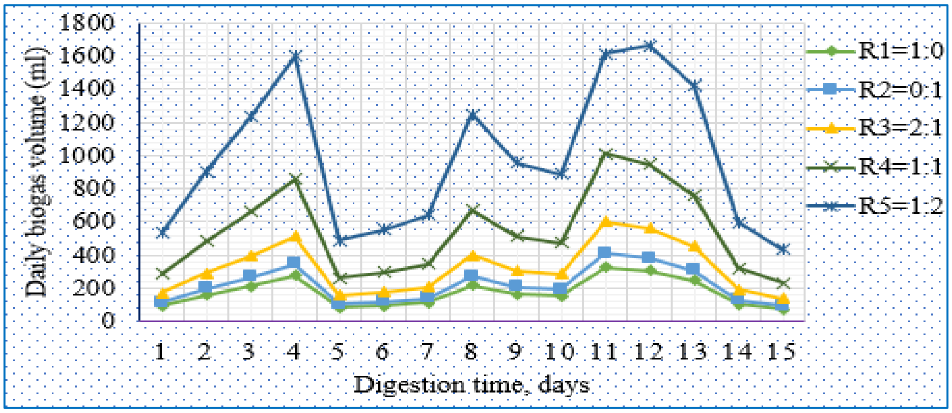

Figure E 4 Daily biogas volume at temperature 30° C and pH 8.0

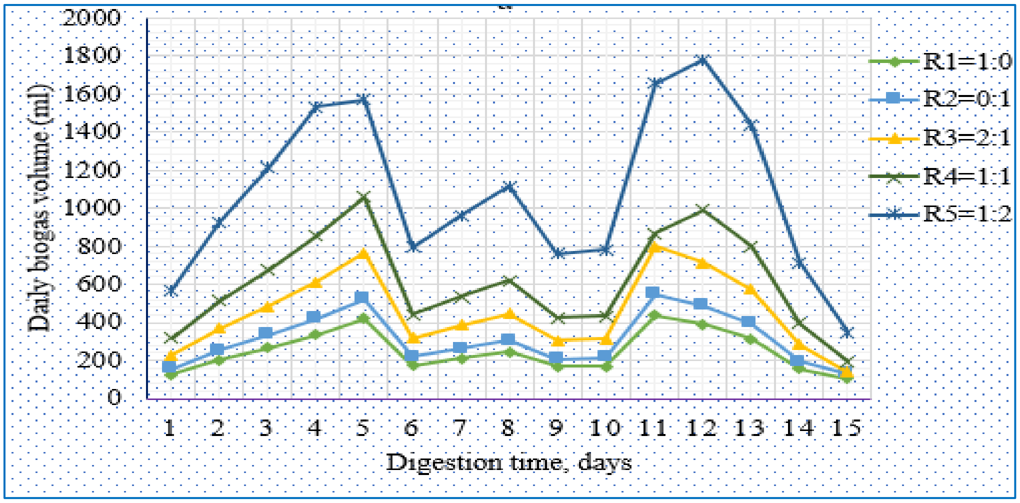

Figure E 5 Daily biogas volume at temperature 35° C and pH 6.0

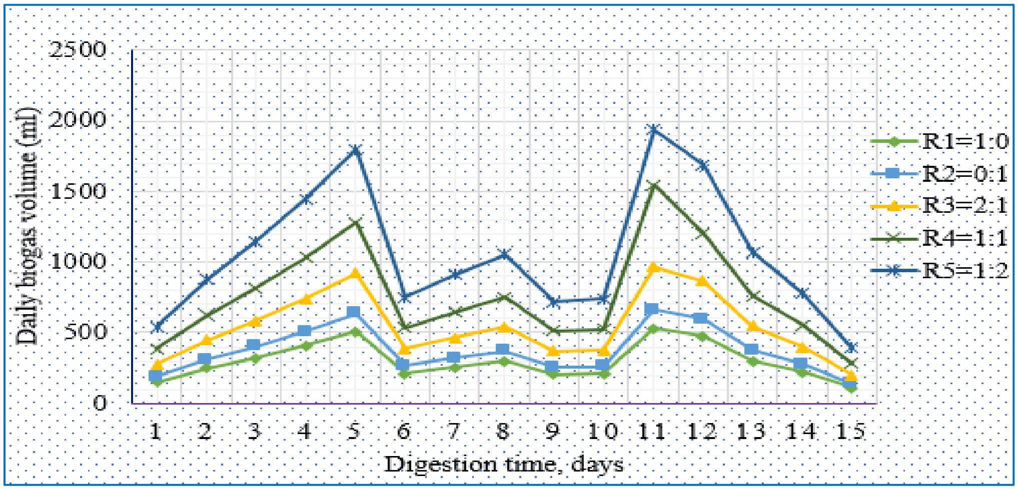

Figure E 6 Daily biogas volume at temperature 35° C and pH 7.0

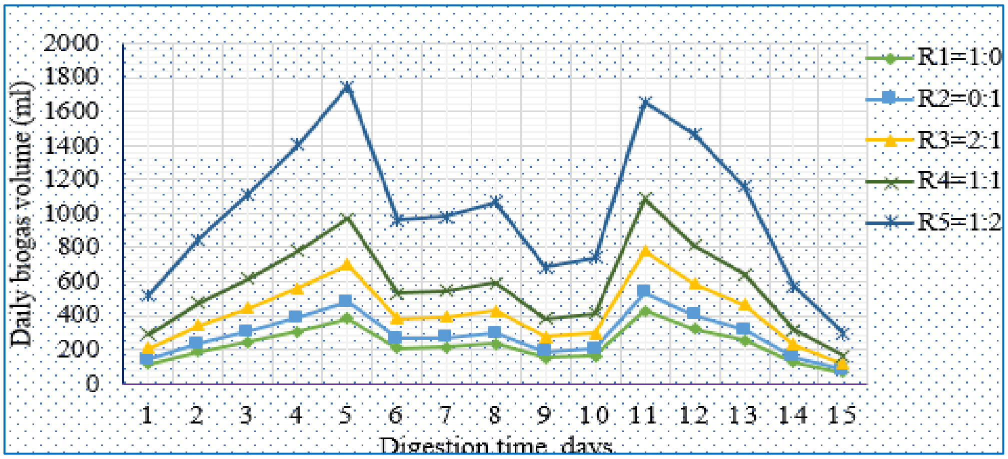

Figure E 7 Daily biogas volume at temperature 35° C and pH 8.0

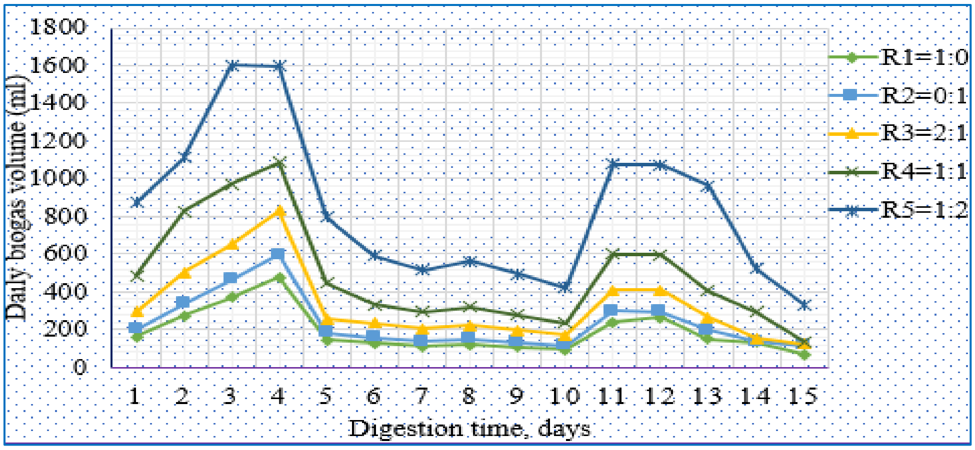

Figure E 8 Daily biogas volume at temperature 40° C and pH 6.0

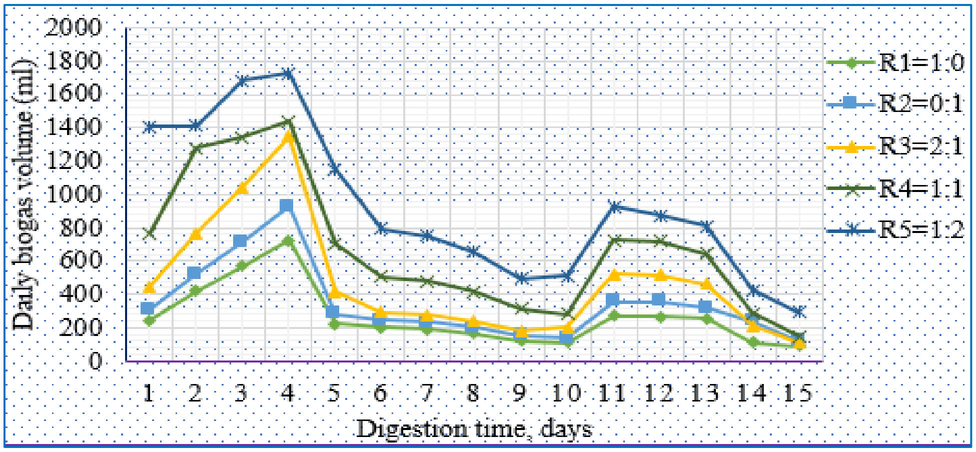

Figure E 9 Daily biogas volume at temperature 40° C and pH 7.0

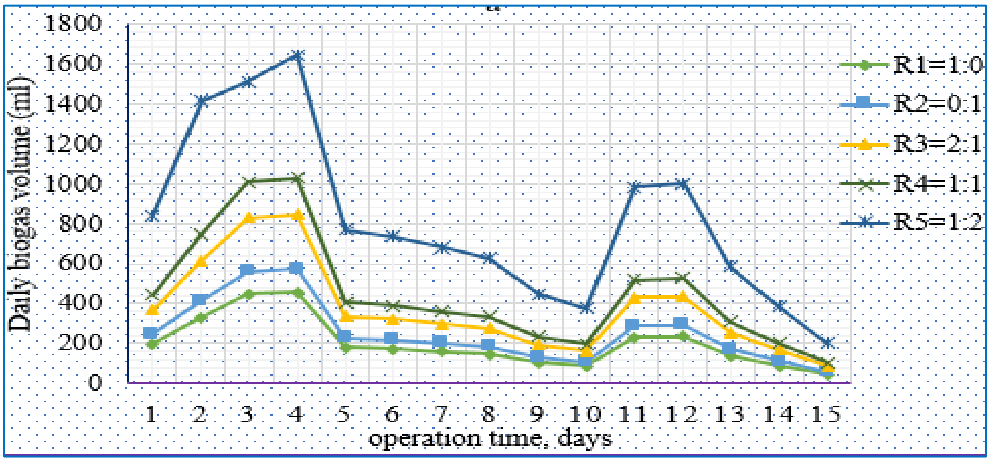

Figure E 10 Daily biogas volume at temperature 40° C and pH 8.0