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TITLE: BIOGAS PRODUCTION FROM CO-DIGESTION OF CATTLE MANURE AND FOOD WASTE

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DECLARATION

I, DAWIT ZERU BRHANE, hereby declare that this thesis represents my personal work, realized to the best of my knowledge. I also declare that all information, material and results from other works presented here, have been fully cited and referenced in accordance with the academic rules and ethics.

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ABSTRACT

There is a growing global demand of bioenergy production from animal manures and municipal solid wastes. In this study, the potential of cattle manure, food waste and their co-digestion for biogas production was investigated. The substrates were characterized for their physic-chemical properties, total solids (TS), volatile solids (VS) and C/N ratio and they were assessed in five combinations for their suitability to biogas production in 500 ml batch digesters. The five digesters of mixing ratios R_1=1:0, R_2=1:1, R_3=2:1, R_4=1:2 and R_5=0:1 were operated at three different temperature systems (35 ^{0}C, 40 ^{0}C and 45 ^{0}C) to investigate the effect of substrate level on the on biogas productivity and methane yield. Biogas production and its methane fraction was measured using airtight syringe sucking from the gas collecting plastic bags connected to the digesters, and gas analyzer regularly for 35 days. The amount of biogas obtained from the five mixing ratios R_1, R_2, R_3, R_4 and R_5 respectively is 1.97 L, 6.48 L, 3.96 L, 11.22 L and 2.52 L at 35 ^{0}C; 3.16 L, 8.65 L, 5.81 L, 13.25 L and 4.01 L at 40 ^{0}C; 2.55 L, 6.90 L, 4.67 L, 9.4 L and 3.56 L at 45 ^{0}C. Highest biogas productivity and methane yield was achieved from the AD system operated at 40 ^{0}C for all the respective digesters. The highest total biogas yields were attained from R_4 (33.34% food waste: cow manure 66.66%) at 40 ^{0}C as 13.25 L followed by R_4 (50 % cow dung: 50 % food waste) at 35 ^{0}C as 11.22 L and the lowest was from R1 (food waste alone) at 35 ^{0}C as 1.97 L. The average values of methane yield obtained from the mixing ratios 1:0, 1:1, 2:1, 1:2 and 0:1 were 57.76%, 55.76%, 55.25%, 58.63%, and 53.3% at 35 ^{0}C; 51.62%, 60.1%, 56.84%, 62.93%, and 53.26% at 40 ^{0}C; 49.43%, 54.78%, 52.28%, 56.76% and 49.63% at 45 ^{0}C. Comparatively, slightly higher methane yield was attained from the co-digestion of FW to CM in 1:2 and 1:1 ratios at 40 ^{0}C temperature. Statistical design of the experiments and data analysis was investigated using general factorial method of DOE. Statistical test revealed that the model "F values" for the two responses i.e. biogas productivity and methane yield in the biogas were statistically significant ($P<0.05$). This experimental finding showed that co-digestion of CM with FW improves the stability and productivity of anaerobic digestion system than mono-substrate digestion.

Keywords: *Anaerobic digestion, Co-digestion, Biogas, Methane yield, Mono-substrates, Cattle manure, Food waste.*

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ABREVIATIONS

AD	Anaerobic digestion
AcoD	Anaerobic co-digestion
CM	Cow manure
FW	Food waste
MSW	Municipal solid waste
FOG	Fat, Oil and Grease
TS	Total solid
VS	Volatile solid
LCFA	Long chain fatty acid
RT	Retention time
HRT	Hydrolic retention time
OLR	Organic loading rate
COD	Chemical oxygen demand
TKN	Total kjeldahl nitrogen
VFA	Volatile fatty acid
C/N	Carbon to nitrogen ratio
SRT	Solid retention time
CSTR	Continuously stirred tank reactor
DCD	Dry continuous digester
OFMSW	Organic fraction of municipal solid waste
BMP	Biomethane potential
IEA	International Energy Agency
FAO	Food and Agricultural Organization
APHA	American Public Health Association
ANOVA	Analysis of variance
DOE	Design of experiment

CHAPTER ONE: INTRODUCTION

1.1 Background

Climate change is unquestionably the most impending environmental concern that the world is facing today. Global temperature intensification will cause certain major effects on ecosystems, wildlife, food chains and ultimately human life. There is a common consent that global warming is due to the large scale anthropogenic release of greenhouse gases, which are primarily instigated by the generation of heat and power. Undeniably, still a large amount of the global energy demand is fulfilled by using conventional energy sources. According to the International Energy Agency (IEA), fossil fuels constituted up to 81% of the global primary energy supply in 2007 while renewable energy resources merely added 13%. Though much focus is being given to the technical and economic development of the application of renewable energy sources, fossil fuels will continue to be the leading energy sources worldwide, anticipated at 77% for the period 2007 – 2030. This reduction in the total share will be mainly compensated by the projected 2.5% annual energy demand growth until 2030 [1].

In most developing countries, mainly Asian and Sub-Sahara African countries, where the supply of energy is very low and expensive (on *per capita* and purchasing power basis, respectively), biogas technology has a far greater importance than it has to the developed countries. Hence, anaerobic digestion system in these countries has been mainly focused on energy production using biogas plants. Animal farming and waste manure generation is abundant in African countries; however, it is usually found highly dispersed unlike in developed countries [2]. Ethiopia is reported to be endowed with the highest livestock population in Africa. According to the 2016 data of the Food and Agricultural Organization statistics (FAOSTAT) the cattle population was estimated at about 59.5 million [3].Nowadays, massive amount of Food waste (FW) is dumped from households, commercial, institutional, and food processing factories on a daily basis alongside the rapid population growth and urbanization in Ethiopia. Its abundance is initiating researchers to bring an effective solution to treat the waste and create a resilient environment while produce alternative energy source. FW treatment which contains high moisture and biodegradable organics of the municipal solid waste is considered as a promising means of energy recovery and MSW reduction. Many countries have been extensively established AD systems for the

treatment of food waste. AD has become an attractive and economical system for treating sorted organic fraction of MSW (largely food wastes), and RE recovery [4].

Energy generation from anaerobic digestion system can be labeled as a matured technology, since it has been utilized already for many years and the study about the subject has been deep. Though the theme is progressive, there are still different problems that affect the optimal performance of its commercial application [5]. Biogas collection and utilization technologies have gradually mounted over the years, and energy recovery from biogas is developing into advanced waste to bioenergy technology [6]. By improving the system in different means; mixing different substrates or adjusting the carbon-nitrogen ratio of the feed, it is possible to enhance the production yield and the quality of the biogas [7]. Hence, its improvement adds a positive value in resource optimization, economic sustainability, high renewable energy mix, and therefore the means of its enhancement are worth of studying.

Biogas production is a biochemical process which occurs naturally from the decomposition of organic wastes or biomass residues in an airtight bio-digester system with the involvement of a group of natural metabolically active microorganisms[5]. Biogas is mainly methane (50–70%) and carbon dioxide (30–50%) with some trace gases. Taking into account the higher heating value (HHV) of methane to be 37.8 MJ/m^3 and no energy associated with the carbon dioxide (since it is the product of complete combustion), biogas has energy content in the range of 19 to 26 MJ/m^3 [8].

There are different types of biomass feedstocks such as animal manure, agricultural residues, food wastes, municipal solid wastes, etc. used for biogas production with different production potential both in quantities and qualities of the biogas. The choice of raw material to be fed to a specific type of anaerobic digester and its preliminary treatment has a major effect on the biogas yield [9]. Since the early years of twentieth century animal manure has been used as a major feedstock for biogas production in developing countries like India and China for rural energy supply [2].

In many cases, biogas feedstocks mainly cow manure is co-digested with various substrates in order to increase the organic content of the mixture and ensure optimum gas yield while stabilizing the digestion process and promoting gas production. Food waste is a better one for co-digestion because of its availability and suitable physicochemical characteristics. Comparatively, cow manure is an ideal base feedstock for co-digestion with other substrates

due to its good buffer capacity, which is significant for the stable performance of the anaerobic digester [10]. The total solid (TS) value of the feed (manure and food waste) should be below 2 – 12% for proper mixing and proper functionality of standard pumps which leads to efficient working of the bioreactor. From an economic perspective it is feasible when the raw materials are obtained from nearby places within a distance of 15 – 20 km [11].

This report is organized in chapters to present and discuss the work. Chapter 1 presented the overall introduction, objectives, and statement of the problem. Chapter 2 provides information about background, theory and principles of anaerobic digestion system. The detailed materials and methodology employed (experimental design and set up, analytical methods) are mentioned in Chapter 3. Result and discussion of experimental data presented in Chapter 4. Key conclusions and some potential gaps for future studies are discussed in Chapter 5.

1.2 Objective

1.2.1 General Objective

The main objective of this thesis is to improve biogas production system from co-digestion of different proportions of animal manure with food waste at three different temperature values (i.e. 35 ^{0}c, 40 ^{0}c and 45 ^{0}c).

1.2.2 Specific Objectives

The specific goals of this work are to:

- Characterize available biomass feedstock (i.e. cattle manure and food waste)**.**
- Identify mixing ratios that produce highest biogas volume and methane yield.
- Investigate the effect of temperature and digestion (retention) time on biogas volume and methane yield.

1.3 Statement of the Problem

Energy security is at the heart of the sustainable development goals which every nation is working strongly to achieving it. Nowadays, fossil fuels are the major source of energy in the world and small amount of the primary energy requirement is being met by renewable sources like solar, hydropower, geothermal and wind energy, biomass, municipal and agricultural wastes. Special emphasis has been given to biogas technology due to its two-fold reasons: I) environmental waste management and sanitation II) energy recovery. Due to the rapid population growth and urbanization, high energy demand and the need for waste management have become main challenges facing the world today which may lead to global warming and environmental pollution. Unstructured urbanization in developing countries has resulted in disorganized dissemination of large amount of municipal wastes and spoiled food waste.

Alternative renewable energy technologies development is required not merely for the substitution of conventional energy sources, but also assures environmental safety demands. Most developing countries including Africa have large number of livestock animals, which produce huge amount of manure, and considerable quantity of food waste of which significant amount of biogas can be generated. Apart from a few countries (like China, India, and Germany) which produce biogas from animal manure and food wastes, in most African countries wastes are not well managed and being dumped to the surrounding environment which are becoming the main source of pollution and contribute to global warming.

Nowadays, different African countries with the help of foreign organizations (mainly SNV Netherlands development organizations) are establishing household biogas production technology (from cattle manure) in rural areas to solve the problems of energy shortage and environmental sanitation. However, the production and methane yield of anaerobic digestion of manure is low because of the composition of the substrate (low organic content, C/N, NH_3 proportion). Though considerable amount of biogas is being generated from mono substrate (manure), the production can be improved by mixing different co-substrates with synergistic effect. In this study cattle manure and food wastes with different ratio are co-digested to observe the synergistic effect and identify the mixing ratio at which the system is optimized.

1.4 Research questions

1. What will be the effect of mixing different proportions of substrates (co-digestion) on biogas production and methane yield?
2. How do temperature and digestion time affect biogas production system?
3. How the AD system is affected by the interaction effect of mixing ratio, temperature and digestion time?

CHAPTER TWO: LITERATURE REVIEW

2.1 Principles of Biogas Production via Anaerobic Digestion

Anaerobic Digestion: is a biochemical process of organic waste degradation with the involvement of anaerobic microorganisms in an airtight system. With the commencement of both commercial and pilot anaerobic digestion plant designs in 1990s, biogas production from organic waste materials has gained global consideration. Various microbial groups break down organic wastes and results in the generation of biogas and additional energy-rich carbon-containing end products. A sequence of biochemical reactions such as hydrolysis, acidogenesis, acetogenesis and methanogenesis takes place in the process of anaerobic degradation [12].

Anaerobic digestion is applicable for various types of substrates including municipal, industrial and agricultural wastes, and plant residues. Moreover, this process is more advantageous than aerobic process because of its low energy intake for operation and a low biomass production and it is taken as a feasible technology in the management of organic waste while producing a renewable energy source [13, 14].

Anaerobic digestion system is environmentally friendly technology. Different researchers well-defined the benefits of this process to lessen environmental pollution in two main ways: the airtightness of the system avoid methane leakage into the environment, and combustion of the methane will emit carbon–neutral carbon dioxide (there is no net effect on atmospheric carbon dioxide and other greenhouse gases accumulation) [15]. However, anaerobic process incurs some difficulties such as long retention times and removal inefficiencies of organic compounds. The chemical conformation and structure of lignocellulosic resources slows down the rate of decomposition of solid organic waste materials. Hydrolysis of the complex organic matter to soluble compounds is taken as the rate-limiting stage of the series of biochemical processes for wastes which have high solid content. Hence, different physical, enzymatic and chemical pre-treatments are needed to enhance substrate solubility and speed up the biodegradation rate of solid organic waste [16].

Biochemical reactions

Methane production from biomass follows a general buswell equation [17]:

$$C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + nNH_3 + sH_2S + (c - x)\ CO_2$$

$$x = 1/8\ (4c + h - 2o - 3n - 2s)$$
$$y = 1/4\ (4c - h - 2o + 3n + 2s)$$

Different products are generated from the biodegradation of different food compositions as follows:

Carbohydrates: $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$

Fats: $C_{12}H_{24}O_6 + 3H_2O \rightarrow 4.5CO2 + 7.5CH_4$

Proteins: $C_{13}H_{25}O_7N_3S + 6H_2O \rightarrow 6.5CO2 + 6.5CH_4 + 3NH_3 + H_2S$

Anaerobic digestion is a complex process, which is classified into four stages of biochemical reactions, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Different specific groups of microorganisms are involved in each phases of the biological process, partly work in syntrophic interrelation and place various requirements on the environment[18].

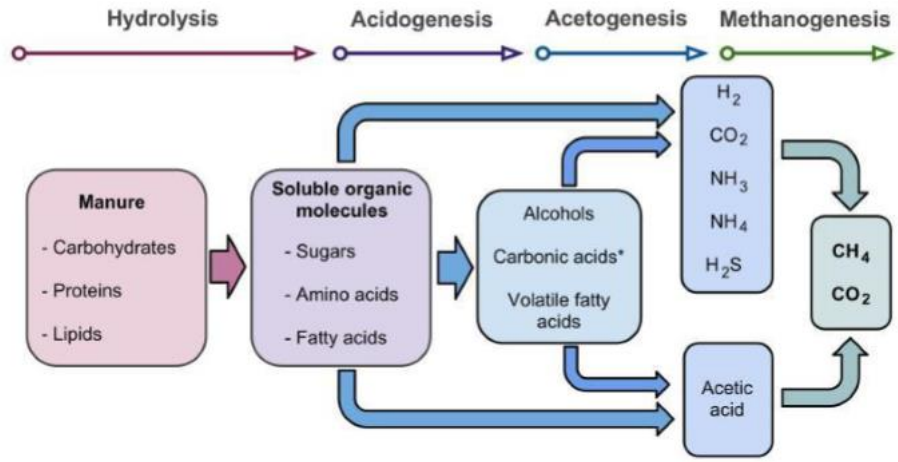


Figure 2.1: The anaerobic digestion pathway follows four major steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis[19].

1. Hydrolysis phase

In the first phase of the process, complex compounds, like carbohydrates, fats and proteins are degraded into their respective monomers by exoenzymes (hydrolase) of facultative and obligatorily anaerobic bacteria. The covalent bonds are split in a chemical reaction with water. Disintegration and hydrolysis of carbohydrates starts after some hours, the hydrolysis of proteins and fats within few days. Lignocellulose and lignin decomposition takes place slowly and incompletely. The facultative anaerobic microbes use the oxygen dissolved in the water and cause the low redox potential essential for obligatorily anaerobic bacteria [5].

Hydrolytic reactions takes place with the involvement of extracellular enzymes secreted from obligate or facultative anaerobic bacteria breakdown complex molecules in two phases. In the first stage a microbial colonization occurs where the hydrolytic bacteria shield the surface of solids. The bacteria on the surface release enzymes which degrade the macromolecules into their simpler compounds that can be used up by the hydrolytic bacteria and other group of bacteria as well. In the next stage, the particle surface will be decomposed by the bacteria at a constant depth per unit of time. The enzymes responsible for this reaction are: cellulose, cellobiase, xylanase and amylase for degrading carbohydrates into sugar monomers (monosaccharides), protease degrades proteins into amino acids and lipase decomposes lipids into LCFA and glycerol [20].

Hydrolysis rate is primarily influenced by organic material size, surface area, shape, biomass concentration, enzyme secretion and adsorption. It is generally reported that hydrolysis is the rate-determining step for digestion when the feedstock is in particulate form (e.g. animal manure, sewage sludge) whereas methanogenesis is the rate-limiting step for easily degradable feedstock [21].

2. Acidogenesis phase

The second phase of the biochemical reactions in AD process is acidogenesis (also termed fermentation) which is commonly described as an anaerobic acid-producing microbial process without an additional electron acceptor or donor [22]. The simple sugars and amino acids obtained from hydrolysis are digested to a various simpler molecules such as volatile fatty acids (VFA) including propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$) and acetic acid (CH_3COOH). However, the microbes oxidising LCFA are needed to use an external electron acceptor such as hydrogen ions or CO2 to generate H_2 or formate [23].

Simple sugar (e.g. glucose) degradation results in the emergence of diverse products such as VFA, ethanol and lactate with different yields of energy. The main pathway is determined by numerous factors such as substrate concentration, pH and dissolved hydrogen concentrations. For instance, lactic acid generation in a high level of organic loads becomes substantial. At pH value greater than 5 the production of VFA escalates to its maximum, while at lower pH (less than 5) ethanol production dominates. However, when the pH goes very low every process may come to an end [24].

Table 2.1: Glucose degradation into different products[25].

Products	Reaction
Acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$
Propionate + Acetate	$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$
Butyrate	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$
Lactate	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$
Ethanol	$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

3. Acetogenesis phase

The third stage of the biochemical reactions is termed as Acetogenesis. The products of acidogenesis serve as substrates for acetogenic bacteria. Acetogenic reactions are endergonic, of which the degradation of propionic acid requires $\Delta G\ f = +76.11\ kJ\ mol^{-1}$, and the degradation of ethanol entails $\Delta G\ f = +9.6\ kJ\ mol^{-1}$. In this phase, homoacetogenic bacteria continuously reduce exergonic H_2 and CO_2 to acetic acid [5].

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

Acetogenic bacteria are obligatory H_2 producers. The acetate formation by oxidation of LCFA (e.g., propionic or butyric acid) runs on its own and is thus thermodynamically feasible merely with very low hydrogen partial pressure. Acetogenic bacteria can acquire energy essential for their growth and survival only when the concentration of H_2 is very small. Therefore, acetogenic and methanogenic microbes must live and function symbiotically. Methanogenic bacteria function well only with higher hydrogen partial pressure. They continuously use up the products of the acetogenic phase from the substrate and maintain low hydrogen partial pressure suitable for the acetogenic bacteria. When the partial pressure of H_2 is low acetogenic microbes primarily produce CO_2, H_2 and acetate; when the partial pressure H_2 gets higher butyric, capronic, propionic, and valeric acids and ethanol are predominantly

formed. Of all these products, methanogenic organisms only process acetate, H_2 and CO_2 to produce methane[26].

The anaerobic transformation of alcohols and fatty acids occurs energetically at the expense of the methanogenics, where these get the substrates (H_2 , CO_2 , acetic acid) required for growth from the acetogenic bacteria in return. The acetogenic phase determines the rate of conversion in the final stage. The contribution of acetogenic microbes can be observed from the amount and yield of the biogas [22].

4. Methanogenesis phase

In this stage, fermentation products from the above series of reactions such as acetate, H_2 and CO_2 are converted to CH_4 and CO_2 by two groups of methanogenic archaea which are strict obligate anaerobes. Some other groups of methanogens are capable of growing on one-carbon compounds like methanol, formate, and methylamine. Mostly, methanogenic microbes are good at substrate utilization, as some of them are able to use only one substrate [27].

The archaea useful for the process of anaerobic digestion are generally classified into two main groups: namely, aceticlastic methanogens which degrade acetate into methane and carbon dioxide. The second group, called hydrogenotrophic methanogens utilize hydrogen as electron donor and CO_2 as electron acceptor to form methane. Almost all the methanogenic species are capable of producing methane from CO_2/H_2, while some species of methanogens are known for utilising acetate as a substrate. Nevertheless, from stoichiometric relations, most of the methane (around 70%) formed during anaerobic digestion comes from the acetate pathway. The hydrogen pathway is more energy yielding than the acetate pathway, and is usually not rate limiting. On the other hand, it plays a fundamental role in decreasing the hydrogen concentration of the system [28].

Furthermore, other than methanogenic reactions, the intermediate conversion between acetate and hydrogen catalysed by homoacetogenic bacteria plays a vital role in the methane production pathway as well. Based on the hydrogen concentration of the external environment, homoacetogens are able to either oxidize or synthesize acetate that permits for contention with various groups of microbes, including methanogens. As can be shown in the Table below, H_2 utilization by hydrogenotrophic methanogenesis is thermodynamically more favorable than homoacetogenesis ($\Delta G°'<0$). Concerning acetate degradation, aceticlastic methanogenesis is similarly more favourable than acetate oxidation. Hydrogenotrophic

methanogenes function well when the partial pressure of hydrogen is high, whereas aceticlastic methanogenesis is free from the effect of H_2 partial pressure. When the temperature increases (> 30°C) the acetate oxidation pathway turn out to be more favorable [29].

Table 2.2: Reactions related to methanogenesis (with standard temperatures)[27].

Type of reaction	Reaction	ΔG°' [kJ/mol]
Hydrogenotrophic methanogenesis	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-135
Aceticlastic methanogenesis	$CH_3COOH \rightarrow CH_4 + CO_2$	-31
Acetate oxidation	$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$	+104
Homoacetogenesis	$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$	-104

Hydrogenotrophic methanogenesis has been reported as a major controlling process in the whole scheme of anaerobic digestion. Its improper function will intensely disturb the syntrophic acetogenic bacteria and the entire fermentation process[30]. The accretion of reduced fermentation products in anaerobic digester is primarily because of poor elimination of hydrogen and acetate from the system due to different reasons. For instance, high amount of organic load in a digester raises hydrogen and VFA formation more than the capacity of methanogens leading to VFA buildup, or the declining in capacity of methanogens by toxic compounds and pH drop inhibition [29].

The hydrogen-utilizing methanogens are amid the rapidly growing microbes in the anaerobic digestion system as their average estimated doubling time is six hours, compared with 2.6 days for the slow-growing acetoclastic methanogens. Moreover, hydrogenotrophic methanogens have been observed less sensitive to environmental fluctuations than acetoclastic methanogens. Therefore, methanogenesis from acetate leads to be rate limiting in the anaerobic degradation of easily hydrolysable substrates [28].

2.2 Role of microorganisms in anaerobic digestion

The anaerobic digestion process can be facilitated by diverse group of microorganisms that transform complex organic molecules into their smaller molecules. An inoculum source is essential for the optimization of the waste/inoculum ratio. Sludge is frequently used as inoculum in waste treatment processes; though naturally selected strains or artificially mixed strains of microbes are also engaged. Moreover, cell aggregates in the form of flocs, granules,

biofilms, and mats, with dimensions specifically ranging from 0.1 to 100 mm might also be employed in the treatment system [31].

Various types of bacterial species have been stated as the main microbes involved in the anaerobic degradation process. Researchers stated that organic material is most possibly digested by heterotrophic microbes. A study reported that Clostridium species are among the commonly used ones in the anaerobic decomposition of organic wastes [32]. Nevertheless, it is very uncommon for a digestion system to depend merely on a single type of microorganism species and generally various groups of microbes are responsible for the anaerobic degradation process. According to researchers, group of microbes like actinomyces, Ralstonia, Thermomonospora and Shewanella are employed in the digestion of food waste into VFAs, and others like Methanosarcina and Methanobacterium/Methanobrevibacter predominantly involve in methane production. By means of denaturing gradient gel electrophoresis and DNA sequencing techniques, scientists observed hydrogenotrophic species (mainly, Methanobrevibacter sp., M. formicicum and Methanosarcina sp.) active in methane synthesis. A rise in methane content was also detected with the increase in the number of hydrogenotrophic species. Nevertheless, increased concentration of organic acids like acetic acid (>5000mg L^{-1}) and butyric acid (>3000mg L^{-1}) inside a fermenter has been observed to suppress microbial growth resulting in reduction of biogas production [33].

2.3 Biogas Compositions and Impurities

1. Methane and Carbon dioxide

Biogas is composed of predominantly methane and carbon dioxide. The methane contained in biogas provides energy feature to the gas. The type of feedstocks used for biogas production determines biogas productivity and methane yield. Table 2.3 summarizes biogas productivity and methane yield of some of the potential agricultural feedstocks.

Table 2.3: Biogas yields and methane content from agricultural feedstocks.([34],[17])

Feedstocks	%TS	%VS	RT (days)	Biogas yield (m^3/kg VS)	Methane content (%)
Cow slurry	5 - 12	75 - 85	20 - 30	0.2 – 0.3	55 - 75
Chicken slurry	10 - 30	70 – 80	>30	0.35 – 0.60	60 – 80
Garden wastes	60 - 70	90	8 - 30	0.2 – 0.5	NA
Whey	1 – 5	80 - 95	3 - 10	0.8 – 0.95	60 – 80
Fruit wastes	15 – 20	75	8 - 20	0.25 – 0.5	NA
Food remains	10	80	10 - 20	0.5 – 0.6	70 - 80

Carbon dioxide which is major component of biogas is not necessarily a contaminant; however it reduces the energy content of the biogas, lowering its heating value. Dissolving carbon dioxide from the gas mixture gives high fuel value to the digester gas. Carbon dioxide removal techniques are expensive and probably feasible merely if the biogas is to be upgraded to natural gas standard and sold commercially. The power generation machines designed particularly for alternative fuels are able to tolerate 30–50% of carbon dioxide in the gas. Hence, power generation using biogas doesn't require dissolving carbon dioxide from the gas mixture [35].

2. Moisture

Biogas generation from anaerobic digester is usually saturated with water vapour at the operating temperature. The moisture might condense and dissolve any hydrogen sulfide formed in the system which imparts corrosion to the piping system. Moisture can also hasten the corrosion of the sheaths in check and relief valves, gas meters, and regulators. The condensed water vapor mostly collects in the lower parts of piping, obstructing the flow of gas leading to pressure losses in the piping and high pressure in the digesters. Any obstruction in the gas line by condensed water vapour can be noticed by following the manometer pressure readings in the AD system. The manometer displays a zero pressure reading downstream of the blockage. Moisture content can be eliminated by cooling the gas using heat exchangers and removing the condensate [34].

3. Hydrogen Sulfide

Hydrogen sulfide is an exceedingly reactive element of biogas which produces sulfuric acid in presence of water vapour in AD system. The acid generated can decay gas pipes, gas storage tanks, and operation devices. H_2S predominantly affects many components of biogas handling system. Moreover, the inhalation of H_2S causes health problems and can be lethal at concentrations above 700 ppmv. After a short experience to the pungent rotten egg smell of the gas, the nose becomes dazed to odor, which leads to the riskily wrong conclusion that the exposure has lessened or disappeared. Thus, it is important to relay on a gas detector which can signify the least signal of the gas. The most common hydrogen sulfide scrubbing techniques include absorption using iron sponge, water scrubbing, dosing of iron salts to the feed, and biological oxidation [11].

4. Siloxanes

Siloxanes are impurities of emerging concern for facilities envisioning the exploitation of biogas as an energy source. Siloxanes are volatile organic compounds and are emitted as gas from AD process of municipal wastes. Oxidation of these organic complexes during utilization of the gas creates an abrasive solid which collects on moving parts or heat exchange faces leading to rapid deterioration and loss of heat transfer efficiency. Because of the high volatility nature of siloxanes at relatively low temperatures, the relationship between fermenter operating temperature and the amount of siloxane generation in the digester gas may be implied. There are explanations to consider that more siloxanes may be formed in the biogas where AD systems are heated for temperatures higher than mesophilic ranges. Particularly siloxanes are removed by applying graphite media filters or activated carbon or refrigerant dryers [34].

2.4 Factors Affecting Anaerobic Digestion

Anaerobic digestion system is extremely susceptible to fluctuations in environmental conditions. The microorganisms responsible for the different biochemical reactions (mainly methanogenic bacteria) are fragile when the operating conditions are not sustained at its optimum. Methanogens' high susceptibility and slow growth rate in an anaerobic handling system needs proper upkeep and follow up of the environmental conditions. Some of the major governing factors are temperatures (i.e. psychrophilic, mesophilic or thermophilic), pH (usually in the neutral range), nutrients and trace mineral concentration, toxicity, and optimum redox conditions.

2.4.1 Temperature

AD process, like other various biological systems, is highly temperature dependent. In an anaerobic system, methanogenesis takes place in three different optimum temperature ranges: psychrophilic, mesophilic, and thermophilic. Accordingly, the microorganisms are classified as psychrophiles, mesophiles, and thermophiles respectively. The anaerobic biochemical reaction rates commonly increase with rising temperature up to 60◦C [36]. AD system functions best with its highest efficiency at 5–15◦C for psycrophiles, 35–40◦C for mesophiles, and around 55◦C for thermophiles, with slower rates between these optima. Researchers stated that slower rates between these optima come from the incapability of the microbes to acclimatize the environment. Strong temperature dependence on the maximum substrate

consumption rates of microbes has been identified by many researchers [37]. Most industrial- and laboratory-scale anaerobic digesters function in the mesophilic temperature range.

Temperature is the most important factor on growth kinetics, such as growth yield, half-velocity constant, decay rate, and maximum specific growth rate. Therefore, the effect of temperature on methane generation rate is as a result of the aggregate effect of temperature on the growth kinetics. Thermophilic AD systems have an early methane generation at constant rate, irrespective of temperature fluctuations within the range of 50–70◦C. The rate is about 25–50% higher than mesophilic rate, depending on the nature of substrates [38]. Zinder (1984) reported that the hydraulic retention time (HRT) of thermophilic digestion system can be reduced in comparison to mesophilic processes due to faster growth of thermophilic, acid-consuming bacteria[39]. Besides, thermophilic condition creates pathogen free system. Thermophilic systems, however, have lower net yield (nearly 50% of mesophilic ones), thus bring about slow start-ups and susceptibility to loading variations, substrate changes, or toxicity.

Table 2.4: Mesophilic and thermophilic anaerobic digestion performance comparison (adapted from [40],[41])

Performance characteristics	**Mesophilic digestion**	**Thermophilic digestion**
Gas production rate	Contradictory reports	Contradictory reports
Pathogen reduction	Lower	Higher
Process stability	Higher	Lower (contradictory)
Energy requirement	Lower	Higher
Dewaterability	Contradictory reports	Contradictory reports
Methane content	Higher	Lower
Product/substrate inhibition	Lower	Higher
Odour	Lower	Higher

Generally, increasing temperature has a favorable effect on the metabolic rate of microbes and facilitates the digestion processes, but thermophilic process is hard to control and involves other extra energy to retain the constant temperature of the digester. Temperature fluctuation affects microbial growth and results in significant reduction in biogas production [38].

2.4.2 Alkalinity and pH value

Alkalinity and pH value of AD system has a major effect on the anaerobic treatment process. Apart from some microbes, majority group of microorganisms require and adapt neutral pH range for their growth. Various species of bacteria which take part in biogas production process prefer different optimum pH growth. The most suitable pH range to obtain maximum

biogas production in AD system is 6.8–7.2. Out of the four series biochemical reactions, methanogenic bacteria are most susceptible to pH changes and favor a pH value of approximately 7.0. Acidogenic microbes are relatively less subtle to pH and can tolerate in the range of 4.0–8.5. However, the optimum pH range for acidogenesis and hydrolysis is 5.5 to 6.5 [42].

The fact that microbes in AD system prefer different pH values, optimum pH ranges is taken as one main reason to classify some biodigesters into two-phase as acidogenic and methanogenic phases. It is also a critical factor as it affects the fraction of ionized and non-ionized forms (excessive hydrogen sulfide, fatty acids, and ammonia are toxic in their non-ionized forms). Generally, optimal alkalinity and pH value creates unruffled system for the digester's microorganisms. Mixing of various substrates can stabilize pH value by alleviating the extreme acidification condition. The fluctuation of pH value stimulated from mixing feedstocks during co-digestion is more stable and easier to uphold in optimal pH range in the degradation process in comparison to digestion of a single substrate [43].

Researchers reported that methanogenesis reaction was highly suppressed in the digestion of synthetic wastewater in the alkaline pH range (pH greater than 8) at thermophilic temperature and the value obtained for acetotrophic methanogenic test was zero. The effect of pH was studied in anaerobic degradation test and poor functioning of a digester and slow digestion was detected as a result of acidification of the system. Nevertheless, the rate of biodegradation was significantly increased as the pH value of the wastewater was set to above 6.5. Digesters involving manure have high feed bicarbonate buffering capacity and high amount of ammonia, that maintains the pH value in the range between 7.5 and 8.0, and the system can withstand slightly high amount of VFA before pH drop [29].

2.4.3 Type of Substrate

The different chemical composition of various substrates expresses the biochemical methane potential of feedstocks and helps to identify the proper substrates for anaerobic co-digestions. The substrates comprise various simple and complex organic materials which can be utilized in the AD process. According to their sources (animal manure and agricultural residues, municipal, food and industrial wastes), specific organic substances may dominate, though it is usually hard to identify the precise composition of the substrates. Chemical analysis of the substrates, while possible, delivers useful information for the expansion of the technological methods for substrate treatment and application in AcoD process. The biochemical

composition of different substrates helps to evaluate the biodegradability, bioaccessibility, and bioavailability of substrates.

Carbohydrate-rich organic feedstocks: Carbohydrates (generally sugars) are abundant substrates contained in most organic wastes with different proportions. Food waste (comprising waste from sugar factory, and fruit and vegetable processing) has high amount of simple sugars and disaccharides, which are simply degraded by methanogenic bacteria with the generation of volatile fatty acids (VFA). High concentration of simple sugars may result in rapid accumulation of VFA in the digester, lowered pH, and inhibition of methanogenesis. For stable functioning of anaerobic digesters, different substrates mainly of simple carbohydrates should be mixed with wastes of lower content of easily degradable organic matter [44].

Preliminary physical and biochemical treatment of complex carbohydrates plays a crucial role for efficient degradation process. Cellulose is the most abundant organic compound on earth and has high biogas production potential, regardless of its digestion difficulty. Starch is mainly found in most nutritional items such as pasta, wheat, rice, and potatoes. It contains straight or branched chains of glucose and is degraded comparatively easily in the biogas process. Utilization of agricultural residues without pre-treatment or mixing with other livestock wastes gives lower biogas yield as a result of high values of C/N ratio and lignin content. AcoD practice can also enhance the digestibility of cellulose and hemicelluloses, and the buffering effects of VFA and ammonia. Although lignocellulosic substances are sufficient resources, they are still challenging because of their complex pretreatment technique requirement and potential instability [45].

Protein rich organic substrates: similar to carbohydrates, proteins are found in almost all organic substrates. Wastes from pig and chicken manure, slaughterhouse, and end-products from ethanol industry (mainly stillage) are some of most common organic wastes with high protein content. Household wastewater and food waste which are good candidates for biogas also have protein, but in smaller amounts. Substrates of high protein contains high calorific value and generate a reasonably significant amount of methane in the biogas[46]. All amino acids in proteins contain a common amine functional group (−NH2). The concentration of ammonium and ammonia stays in equilibrium with each other to sustain the stability of the process which is highly determined by operational parameters (temperature and pH). Microbial decomposition of proteins discharges ammonium ions to the system resulting in the

inhibition of methanogenic bacteria. High ammonia (not ammonium) concentration brings about process instability, microbial inhibition and system failure at all. However, the problem can be solved by mixing suitable cosubstrates and fixing C/N ratio to its optimal value [47].

Fat rich materials: Significant amount of lipid containing wastes are generated from food processing factories, wastewater released from abattoirs, dairy products industry, edible oil industry, and olive oil mills. Due to its high biodegradability, fat rich organic materials are good candidates for the production of high biogas yield. Nevertheless, high amount of lipids bring different problems to the AD system, including blockage, microbial inhibition and adsorption to biomass (leading to mass transfer problems). Therefore, the concentration of long-chain fatty acids (LCFA) determines the proper functioning of AD system [46]. The breakdown of triglycerides releases LCFAs (more than 12 carbon atoms) and glycerol. Glycerol is easily transformed to biogas, whereas degradation of LCFAs is a more intricate process. High concentration of LCFAs may obstruct the operation of anaerobic microbes. Stearic and oleic acids concentration in the range of 0.2–0.5 gL−1 has a negative effect on methanogenesis phase [48]. Moreover, the detergent properties of LCFA group may create foaming in the digester, mainly at higher temperature. Its accumulation during co-digestion process can also cause inhibition of the process by forming toxic components. Co-digestion of carbohydrate-rich substrates with fat-rich resources (slowly degradable and rapidly degradable) results in nutrition balance, increase in stability, microbial enrichment, lower inhibitors accumulation, high efficiency of biogas production and methane yield [49].

2.4.4 Effect of C/N ratio

The C/N ratio of substrates signifies the relationship between the content of carbon and nitrogen present in feedstocks and is a good indicator for regulating AD systems[50]. High amount of C/N ratio shows quick nitrogen depletion by methanogens and results in lower gas generation. In contrast, organic materials of too low C/N value forms high amount of ammonia and leads to an increase in pH values, which is toxic to methanogenic bacteria. From different literatures the C/N ratios of co-digestions are significantly lower than mono-substrate materials, which indicate that mixing different substrates effectively reduces the C/N ratios of the feed. Researchers also reported that biogas production from mixture of different co-substrates is higher than the corresponding mono-substrate digestions [51].

Table 2.5: The values of C/N ratio for different substrates [52, 53].

Relatively lower C/N value materials		Relatively higher C/N value materials	
Feeds/substrates	C/N ratio	Feeds/substrates	C/N ratio
Cow dung	16 – 25	Rice straw	51 – 67
Poultry manure	5 – 15	Sugar beet/sugar foliage	35 – 40
Pig manure	6 – 14	Wheat straw	50 – 150
Sheep manure	30 – 33	Sugar cane bagasse	140 – 150
Kitchen waste	25 – 29	Corn stalks/straw	50 – 56
Fruits and vegetable waste	7 – 35	Sawdust	200 – 500
Food waste	3 – 17	Potatoes	35 – 60
Waste cereals	16 – 40	Algae	75 – 100
Grass/grass trimmings	12 – 16		
Goat manure	10 – 17		
Mixed food wastes	15 – 32		
Slaughterhouse waste	22 – 37		

Co-digestion of carbon-rich organic materials with nitrogen rich byproducts, such as kitchen wastes and animal manure helps maintain the process stability, the essential nutrients for the microbes and biogas production [52].The optimum value of carbon to nitrogen ratio (C/N) for stable AD system is in the range of 20 to 30, which is suitable to supply the required energy regardless of the other influences [54]. Substrates of optimal C/N value can maintain the possible nutritional requirement of the microbes. Hence, it is important to select substrates for co-digestion considering the effect of C/N value on the performance of the AcoD operation of biogas production. The C/N values of various organic materials are listed in Table 2.4. Nevertheless, it is uncertain to tell specifically the optimal ratio as the optimum C/N ratio might be influenced by numerous reasons like the type of substrate, chemical composition of the materials, and biodegradability. The deviation of the C/N ratio from its optimum value creates process instability, failure of the AD system and drop in biogas production [54].

2.4.5 Mixing and Retention Time

Internal mixing plays an important role in the efficient functioning of AD system. It helps proper mass transfer of organic materials for the active microbial biomass, avoids foaming by discharging gas bubbles generated in the medium and alleviates sedimentation of denser particulate materials. A digester does not require continuous agitation. Mixing intermittency and technique is determined by the type of agitator used, type of digester, and the TS value of the substrates; which might consume energy from 10 to 100 Wh/m^3 accordingly (Burton and Turner, 2003). To homogenize the organic material inside the digester, propellers can be employed if the substrate has low viscosity. To exclude the involvement of moving parts (agitator) within the digester, biogas recirculation through bottom of a digester or hydraulic

mixing by recycling of the digestate with a pump can be utilized to provide sufficient mixing [55].

Agitation system not merely affects the digestion process but is also costly to install, maintain, and operate. Hence, a good mixing system results in better productivity and cost. A moderate mixing rate is needed to present substrates to the microbes, but extreme agitation can lead to decreasing of biogas generation. Different researchers has stated that low mixing rate helps a reactor to better absorb shock loading disruption than did high speed mixing systems (Gomez et al., 2006), and minimizing mixing rate could enhance performance and also soothed a continuously-mixed unstable digester [56].

The solids retention time (SRT) refers to the time that solid materials are entrained in the reactor and is a critical element influencing reactor performance. SRT of an AD system depends on the type of substrate used; easily degradable substrates require shorter SRT and hardly degradable materials need longer SRT (Fig 2.2 left). The minimum SRT value can be estimated from the generation times of the microbes involved in the system. For SRT shorter than the generation time of the slowest microorganisms (commonly acetogens) a net washout of some microbes follows. Technical and economic factors determine the maximum SRT value since the ratio of additional gas yield to reactor volume drops at some point. Short retention time results in high gas generation rates (related to the reactor volume) as predominantly the easily degradable materials are digested. Concerning the total organic load, the shorter SRTs of the AD system the lower gas yields will be obtained (associated to the mass of volatile solids used). On the other hand, long SRTs increases gas yield and decreases gas generation rate (Fig 2.2 right) [57].

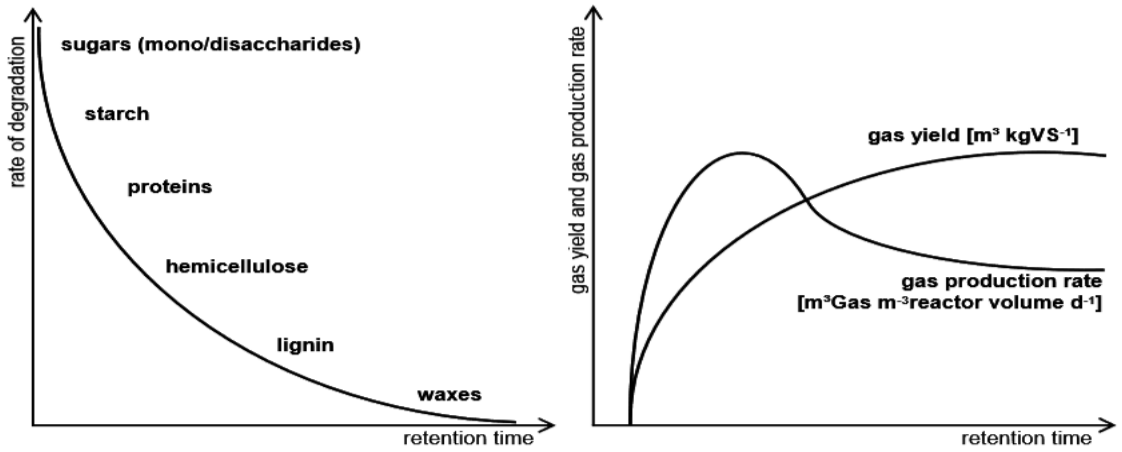


Figure 2.2: Rate of degradation of different substrates vs. retention time (left) and gas yield vs. retention time [57].

2.5 Anaerobic Co-digestion Technology

Traditionally AD technology was intended to provide a single purpose treatment and involved mono-substrate feedstocks. Nowadays, it has been understood that AD system, apart from waste management, can also serve as a significant energy source. The process of biogas generation from various organic wastes primarily depends on the composition of feedstocks that can be transformed into biogas, while their chemical conformations and biodegradability are among the main factors for the biogas and methane productions. Defining the extent of biodegradability, composition of the substrates, particle size and alkaline dosage helps to improve the biogas/methane production. Many research works have been done on the AD process of biogas production from various feedstocks as mono-substrates. On the other hand, direct use of substrates is difficult because of their nutritional imbalance, lack of the various key microorganisms and the effect of operational factors [58].

Co-digestion practice was suggested to alleviate the challenges of the mono-substrate system, like mixing agricultural residues with livestock droppings. Anaerobic co-digestion (AcoD) has been extensively applied to improve biogas yield of the digesters. Different studies have been published investigating AcoD of livestock manure with other different substrates to increase the biogas production rates. AcoD is a typical AD process of mixing two or more substrates which is a promising choice to solve the problems of mono-substrate digestion and expand the economic feasibility of AD plants as a result of higher biogas production. Co-digestion process results in better nutrient balance, higher biogas generation by the synergistic effects of microbes, enhanced degradation of substrate volatile solids and higher methane yield. However, care must be taken to avoid feedstocks that may inhibit the co-digestion process and thus reduce methane production [59].

Table 2.6: Co-digestion of selected feedstocks

Co-digestion	Result	Reference
FOG and MSW	methane yield increased by 37% at 64% FOG composition	[60]
Brewery waste, food waste MSW	Up to 70% increase in biogas compared to MSW alone	[61]
Fruit and vegetable waste, and MSW	Rise in biogas production by 27% compared to MSW alone	[62]
FW and MSW	Waste and MSW Co-digestion of food waste increased biogas production by 35%	[56]
Yeast, food flavorings, restaurant, and brewery wastes	significant synergism effects, increased biogas generation by over 50%	[63]
Grease trap waste and FOG	Increased methane yield 9 - 27% when 10 - 30% of grease trap feedstock was added	[64]

2.6 Configurations of Anaerobic Digesters

The design and operational mode of a digester is determined depending on the characteristics of available feedstocks. Main characteristics of substrates i.e. TS and VS values, density, biodegradability, particle size, and functional specific gravity. Digesters are classified as batch or continuous, wet or dry, and one-phase or multi-phase. Bioreactors can also be categorized mainly into mesophilic and thermophilic systems according to the operating temperature employed [65].

2.6.1 Dry batch digester

It is a type of reactor which involves a simple batch process (Fig. 2.3); and is commonly utilized for substrates of high TS value i.e. 30–40% [66]. When the substrate is fed into the digester at a specific time; gas generation starts, increases, peaks, drops and then stops. Once the operation comes to an end, half of the batch is ready for discharge and the remaining serves as inoculum for the next batch which results in effective RT. One of the advantages of batch reactors is its simplicity. Due to high solid content the system requires small thermal energy input. The processing needed is also low, resulting in small electrical parasitic demand. It is most convenient for treating wastes like OFMSW. The presence of inert contaminants doesn’t disturb the system; if, for instance, a piece of metal is in the substrate it

doesn’t intrude on any moving part. The system normally uses available construction and agricultural tools to introduce and discharge the substrate. Vertical garage door techniques are frequently used in these systems. Nonetheless, its main problem is that it may not increase the methane yield per unit of feed. For commercial developments, several batches are prepared and fed serially to attain relatively uniform generation of gas [65].

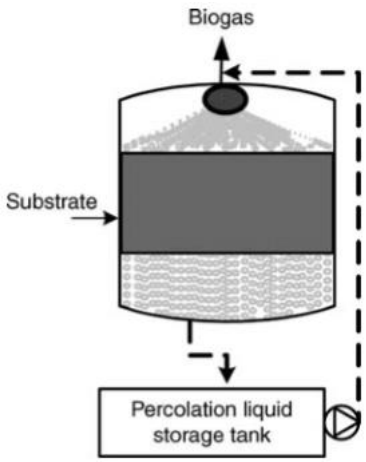


Figure 2.3: One-stage dry batch digester with sprinkling of liquor in a closed loop (adapted from [65]).

2.6.2 Continuously stirred tank reactors

It is also known as CSTRs. The digester facilitates a continuous wet process, involving substrates with TS value of 2 to 12%. CSTR is currently more applicable throughout the world in the treatment of sewage sludge, agricultural slurries and crops. Agitation system is the main design component of this process. Different methods can be used, for instance, in recycling biogas – paddle stirrers which revolve vertically in a spherical motion about a horizontal axis, paddles that rotate horizontally about a vertical axis and inclined paddle stirrers. Usually, the hydraulic retention time (HRT) of a CSTR is almost equal to solid retention time (SRT). Because of the slow growth rate of aceticlastic methanogenic bacteria and the syntropic feature of all trophic class of bacteria in the AD process, the system might fail due to washout when the generation time of the bacteria is lower than the retention time. An organic loading rate (OLR) is apt in between 1 to 4kgVS/m3 reactor/day (Murphy et al., 2011). The system works either as a single-stage or two stage system incorporating all microbial groups (acidogenic, acetogenic and methanogenic) in each reactor or stage (Fig. 2.4). Slurry from the second reactor recycles to the first vessel which helps to dilute the feedstock in addition to balancing the system [67]. In general, major part of the biogas is generated from the first vessel of the two step system. Thamsiriroj and Murphy (2010) obtained around 80% generation of gas from the first reactor in digesting grass silage.

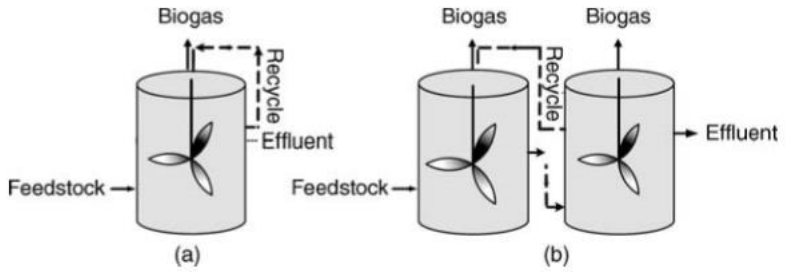


Figure 2.4: (a) One-step and (b) two-step CSTRs with liquid digestate recirculation (adapted from [35])

2.6.3 Dry continuous digester

Dry continuous digesters are like plug flow systems. In a PFS the feed is added at one point and flows inside the vessel as a plug without turbulence. Some effluent serving as inoculum is recycled and mixed with the fresh feed. Substrate degradation takes place alongside its flow through the digester of which substrate concentration gradient arises between the inflow and outflow ends of the reactor and, hypothetically, if the vessel is very long almost all the VS can be digested on getting the outlet. Therefore, in theory, the outlet stream should comprise lower concentrations of VFAs and yield higher removal efficiencies than from a totally mixed digester working at the same HRT. Nevertheless, friction from the walls, convection currents from heating systems and gas generation impart turbulence and mixing. DCD systems might be horizontal or vertical [68].

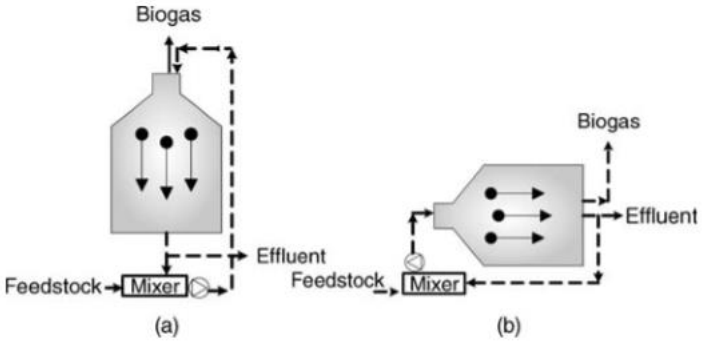


Figure 2.5: (a) Vertical and (b) horizontal dry continuous processes (adapted from [35]).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials and chemicals

Biogas production process involves different kinds of materials and chemicals for the proper functioning of the system. Biogas technology can be developed using easily available materials and chemicals. In this study, lab scale biogas production experiment was conducted to test the effect of co-digestion on the biogas yield. Cow manure and food waste were chosen as co-substrates due to their synergistic effect and abundance. The equipment and chemicals used in this study are listed in table 3.1 and 3.2 below.

Table 3.1: List of equipment used

Equipment type	Function
Crucibles	For sampling purpose
Oven	For TS measurement
Maffle furnace	For VS measurement
Fridge	For sample storage
Digital balance	For weighing purpose
Grinder and blender	For size reduction and homogenization
COD digester	Digest samples to measure COD content
Photometer	For analyzing COD reading
pH meter	To measure pH of sample
Air tight syringe	To measure the gas produced
500 ml Plastic flasks	Serves as Digesters
Hoses	For gas transportation
Plaster, UHU, Rubber stopper	For air tightness
GeoTech Biogas 5000	To analyze the gas compositions
Water bath	To maintain desired T^o
Thermometer	Temperature measurement

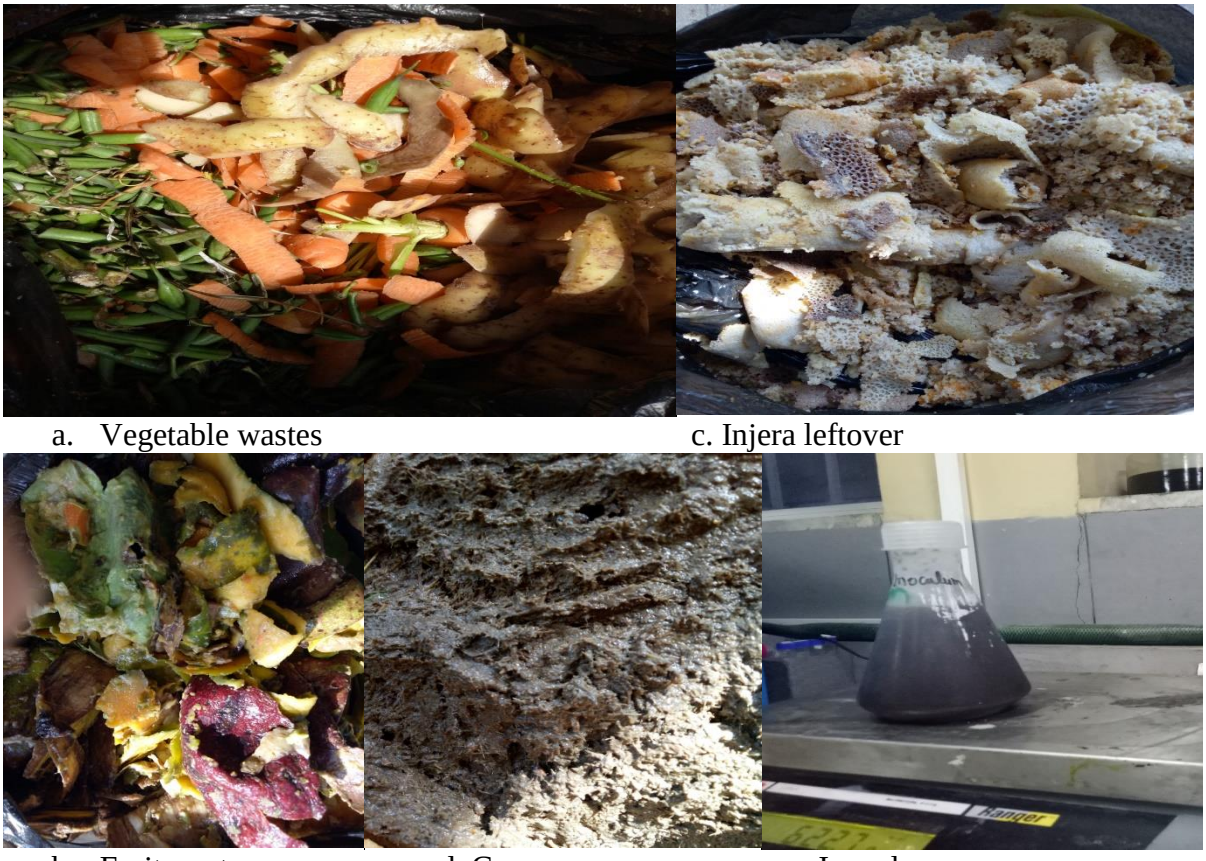
Table 3.2: List of chemicals used

Chemical name	Quantity
Sodium hydroxide	1kg
Phenolphthalein	1L
Sulpheric acid	2.5L
Distilled water	5L
Sodium chloride	1kg
Boric acid	1.5kg
Potassium sulphate	500gm
Sodium thiosulphate	1kg
Zinc metal	2kg
Aluminum oxide	1kg
Ferrous ammonium sulphate	1kg
Potassium dichromate	1kg

3.2 Methods

3.2.1 Sample collection and pretreatment

Food waste comprising of vegetable wastes (potato peels, cabbage and carrot wastes), fruits (banana, avocado, papaya, and mango peelings) and leftover injera was collected from local supermarket and restaurants in Addis Ababa and was transported to Addis Ababa University Institute of Technology research lab on 10th May, 2018. Dairy manure was collected from a nearby dairy farm in the city on the same day. The food waste was milled and blended using grinder and blender for homogenization. To remove the fibers from the manure, it was diluted with tap water and screened using a 250 mm sieve. The prepared food waste and dairy manure samples were kept in a refrigerator at 4 °C for later uses. Inoculum (spent manure) was obtained from local household biogas digester found in Addis Ababa and was used as the main seed.



a. Vegetable wastes c. Injera leftover

b. Fruit wastes d. Cow manure e. Inoculum

Figure 3.1: Feedstocks and inoculum collection

3.2.2 Sample characterization

For control and proper functioning of AD system feedstock has to be characterized prior to its introduction into a digester. Most of the methods employed for the proximate and ultimate analysis of the sample were taken from the standard methods for water and wastewater analysis [69]. The physico-chemical characteristics of the sample were evaluated as per the standard procedures outlined in APHA. Accordingly, TS, VS, C/N ratio, total nitrogen content, organic carbon content, pH, and COD of the sample were measured (Figure 3.2).

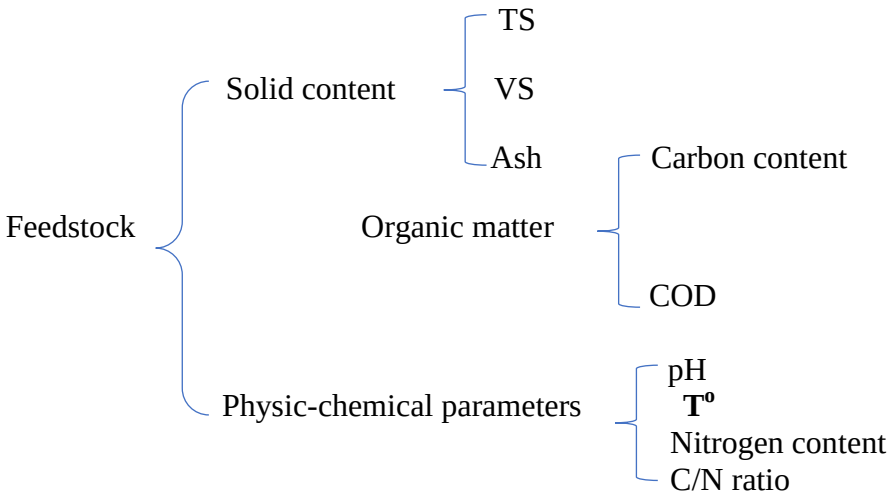


Figure 3.2: Scheme of feedstock characteristics

3.2.2.1 Determination of TS and VS

Total solid and volatile solid content of feedstock determines the biogas generation potential of an AD system. The materials used in the determination of TS and VS are; electronic balance, crucibles, desiccator, drying oven, and muffle furnace. For the determination of TS content, samples were taken 50gm from each collected raw material (i.e, fruit peelings, vegetable wastes, injera leftover and cow manure). Four clean dry crucibles were first weighed and recorded (M_1) prior to sample addition. The crucibles were then filled with the different sample types and weighed (M_2). The crucibles containing the samples were then put inside an oven set at 105 ^{o}C for a period of 24 hours. The crucibles were removed after 24 hours and weighed, and then placed back to the oven until constant weights were recorded (M3) [69]. The TS content was determined using the subsequent calculation:

%TS = $\frac{M_3 - M_1}{M_2 - M_1}$ x 100; where: M_1 is mass of the crucibles

M_2 is mass of sample filled crucibles

M_3 is mass of sample filled crucible after 24 hr in oven

Once the moisture content of the samples was removed, it was ready for further VS content determination. The crucibles filled with the samples were then placed inside a muffle furnace which was set at 550 ^{o}C for 4 hours. Once the ignition time is over, the crucibles were then removed and put in an oven set at 105 °C for 20 minutes to cool the crucibles to some extent as placing the hot crucibles might crack the plastic desiccator. Once the crucibles became comfortable for handling, their weights were then measured and recorded as (M_4) [69]. The percentage of volatile solids was then calculated by the simple expression as follows:

%VS = $\frac{M_4 - M_1}{M_2 - M_1}$ x 100 ; where: M_4 is mass of sample filled crucible after 4hrs in furnace

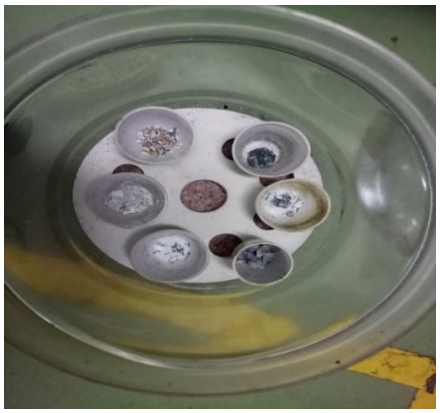
M1 is mass of empty crucible

M_2 is mass of sample filled crucibles

%Ash = 100 - %VS %Ash is percentage of ash content of the sample

a. Sample inside oven

c. Sample inside furnace



b. Sample inside desiccator

Figure 3.3: TS, VS and Ash content determination of sample

3.2.2.2 Carbon content determination

The organic content of a sample which majorly determines the BMP of a sample is mainly expressed by its carbon content. Different literatures state that the carbon content of most organic substrates is related to their volatile solid content. The main components of VS are largely carbon, oxygen, and nitrogen which completely burn in a muffle furnace at 500-600°C, leaving only ash particles (mainly calcium, phosphorus, potassium, magnesium, and other inert particulates which do not oxidize). For most organic materials, the percentage of carbon is in between 45 and 60 of the volatile solids fraction. From the Assumption of 56 % the following empirical formula was developed and used to estimate the carbon content [70]:

$$\% C = \frac{56}{100} (\%VS) = \%\frac{VS}{1.8}$$

3.2.2.3 Total Kjeldahl Nitrogen determination

Total Kjeldahl Nitrogen (TKN) is the most common method used to estimate the total concentration of both organic and inorganic nitrogen. The technique generally comprises three main processes; primarily digestion process, distillation, and titration. The three main sequential steps of the overall process are: (i) Digestion of the prepared sample in concentrated sulfuric acid with the involvement of a catalyst which converts organic nitrogen to ammonia; (ii) Distillation of the total ammonia into a trapping solution; and (3) Determination of the quantity of ammonia in the solution by titration with a standard solution. The digestion process was facilitated with the addition of copper sulphate as a catalyst. Two separate samples from food waste and cow manure were prepared and ready for the process.

The overall chemical process of TKN determination method is expressed as follows:

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

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

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

$$2NH_4H_2BO_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4 + 2H_3BO_3$$

Test procedure

Homogenized FW and CM samples (5gm each) were prepared and added into two different digestion flasks. 2 g of copper sulphate (as catalyst), and a little water to moisten the mixture were added and mixed gently. 25 ml of concentrated sulfuric acid was added and mixed by swirling. The flask was then allowed to boil for 3 hours or until the digest (which contains

ammonium ion) is white or pale yellow. 50 ml of concentrated sodium hydroxide solution was added to increase the pH of the solution and form ammonia gas. Distillation was used to transfer the volatile ammonia gas into an acidic trapping solution in the receiving flask. It was then cooled and 50 ml of distilled water was cautiously added and cooled again. The receiving acid solution containing ammonium ion was then transferred quantitatively into macro-kjeldahl flasks for titration. The titrant solution was then added drop by drop to the two macro kjeldahl flasks containing FW and CM samples until the end point was observed.

The following empirical expression is used to determine the total nitrogen content of any organic sample:

$$TKN\ (\%) = \frac{a - b}{S} \times N \times 0.014 \times 100 \times mcf$$

Where; a is ml of H_2SO_4 required for titration of sample

b is ml of H_2SO_4 required for titration of blank

S is air dry sample weight (5 g)

N is Normality of H_2SO_4 (0.1 N)

100 = volume (in ml) of the solution

0.014 = meq weight of nitrogen in g

mcf is moisture correction factor.

Figure 3.4: Digestion process of CM and FW in TKN technique

3.2.2.4 COD determination

Chemical oxygen demand or COD is a measure of the oxygen required from a strong chemical oxidant (most commonly potassium dichromate) for the degradation of an organic material. For COD determination of the sample, the Palin test COD method was employed samples were oxidized in a sealed reaction tube by a boiling mixture of potassium dichromate (in excess) and sulphuric acid in the presence of silver sulphate catalyst. After digestion, the amount of reduced $K_2Cr_2O_7$ is proportional to the COD value [71].

Test Procedure

Sample (10 g from each) was added to a flask and diluted by 500 ml of distilled water from which 2 ml volume of sample were added to COD vials containing premeasured reagents. A separate reagent blank was prepared by adding 2 ml of distilled water to the reagent tube and was ready for digestion with the sample containing COD vials which serves as a standard for the digestion of the samples. The three filled COD vials one for the blank and the other two for the samples (mixed FW and CM) were placed into a COD digester. The reactor was set to a digestion temperature of 150 ^{o}C and 2 hours according to the standard. During the process the colour of the samples were changed from yellow (A) to blue (B) which indicates there was consumption of oxygen (oxidation) as shown in Figure 3.5. The tubes were then cooled to ambient temperature. The vial containing the reagent blank was then inserted to a photometer to adjust the device to zero absorbance (reference reading). The COD vials containing samples were then introduced into the photometer and readings were recorded. The results were displayed as milligrams of oxygen used per liter of sample.

Figure 3.5: COD determination technique: sample preparation (A), COD reactor setup

3.2.2.5 pH determination

The acidity or alkalinity of the sample was measured by a 3505 pH meter. Sample solutions were prepared as of the desired proportions from the different substrates. The prepared samples were gently agitated and then allowed to settle for 2 to 5 minutes. Once the meter was properly calibrated, the probe of the pH meter was dipped into the different samples containing different substrate ratios and washed after measuring each sample using distilled water. Reading was then recorded after the pH meter comes to a stable pH value. The pH of all the samples was adjusted to be 7 to neglect the effect of pH variation on the AD system.

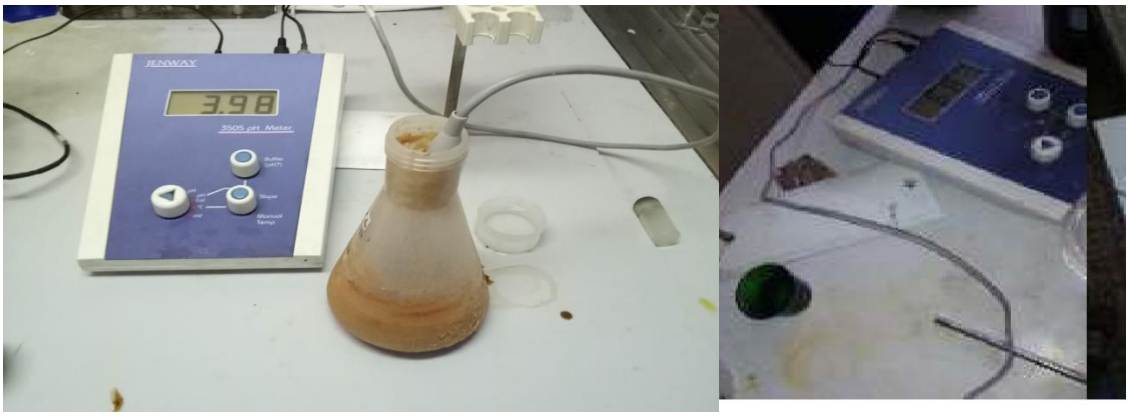


Figure 3.6: pH determinaiton

3.2.3 Feedstock preparation

Once the raw materials were collected, preliminary pretreatment processes were applied. The CM was screened to remove some inert particles from it and the FW, after sunlight drying, were chopped and milled using attrition mill and mixed well using blender. Their proximate and ultimate characteristics were then determined prior to sample preparation. According to the physic-chemical characteristics of the different wastes, feedstocks with different proportion of the substrates were then prepared. The ratios prepared during this study (indicating FW to CM) were R_1 = 1:0, R_2 = 1:1, R_3 = 2:1, R_4 = 1:2 and R_5 = 0:1. The two digesters containing mono substrate samples (R_1 and R_5) were prepared as controls for comparison with the other four digesters loaded with different ratios of co-substrates. Once the feedstocks were prepared 20% of inoculum were added to each digester as a starter and gently agitated prior to the startup of the AD operation. The computation of the proximate and ultimate analysis (TS, VS, TKN, %C, C/N) of the samples is computed in Appendix I.

Table 3.3: Prepared Feedstock ratios (FW to CM) fed to each digester

Mixing ratio	Weight of sample solution(g)	Inoculum added	Weight of sample fed into digester (g)
1:0	400:0	100	500
1:1	200:200	100	500
2:1	266:134	100	500
1:2	134:266	100	500
0:1	0:400	100	500

3.2.4 Experimental Set-up and Procedure

The experiments were done in a batch process using fifteen lab scale digesters, each with a working volume of 500 ml. All the digesters were connected to gas collecting plastic bags and tightened. The airtight gas measuring syringe, the digesters and the biogas collecting bags were all made up of glasses. The temperature of the system was set using water bath operating at three different desired temperature values (35 ^{0}c, 40 ^{0}c and 45 ^{0}c).

The feed to each digester (cattle manure and food waste mixture) were prepared as per the different ratios under investigation (1:0, 1:1, 2:1, 1:2, and 0:1) indicating FW to CM. Water was added to the prepared sample in each digester to obtain the desired TS value (12%) and the pH was adjusted to the optimum value (pH=7.5) by adding sodium hydroxide solution depending on the characteristics of the samples used. Active inoculum (20% of the sample), which was collected from local household digester, was added to each reactor. The slurry was then homogenized using electric blender. Three sets of batch AD systems, depending on the adjusted temperature values in the water baths (35 ^{0}c, 40 ^{0}c and 45 ^{0}c); containing five digesters each were established. The experiments were then allowed to run for 35 days until the generation of biogas becomes insignificant. All the fermenters were mixed by shaking the glass flasks by hand twice a day for one minute. The biogas was collected in the plastic bags for every three digestion days and was sucked by the airtight gas measuring syringe and pumped into the gas analyzer. The overall process of the experimental setup is represented in the figure 3.7 below.

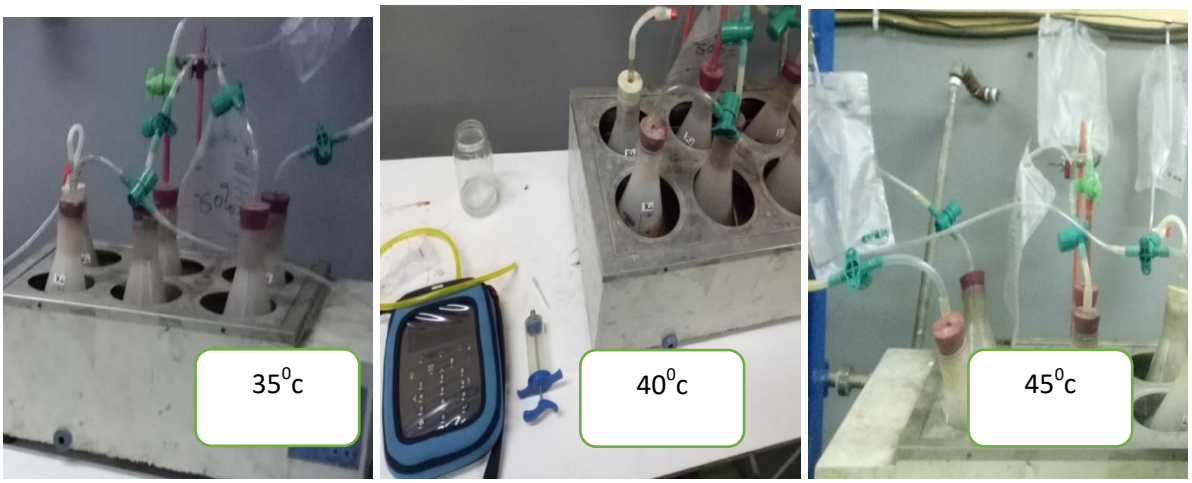


Figure 3.7: AD experimental setup

3.2.5 Biogas volume measurement and composition analysis

The experimental operation of the AD system was done in 35 consecutive days using three separate water bathes for the three different temperature systems (i.e. 35 ^{0}c, 40 ^{0}c and 45 ^{0}c) at a constant pH value. The experiment was ended after the generation of biogas was seen insignificant. The volume of the biogas generated (which was collected in plastic bags) was measured regularly using airtight syringe method once in every three days. The composition of the produced biogas was also characterized for its CH_4, CO_2, H_2S and other trace gases using GeoTech biogas 5000 analyzer by passing the gas from the syringe through the analyzer. Once the tip of the syringe is tightly fitted into the gas inlet of the analyzer, the analyzer was switched on and calibrated with the proper parameters. The built-in pump of the analyzer was then started to suck the gas into the analyzer. The results were then displayed on the screen of the analyzer and recorded. The figure below shows the Biogas analyzer GeoTech 5000 used in the measurement of the biogas composition.

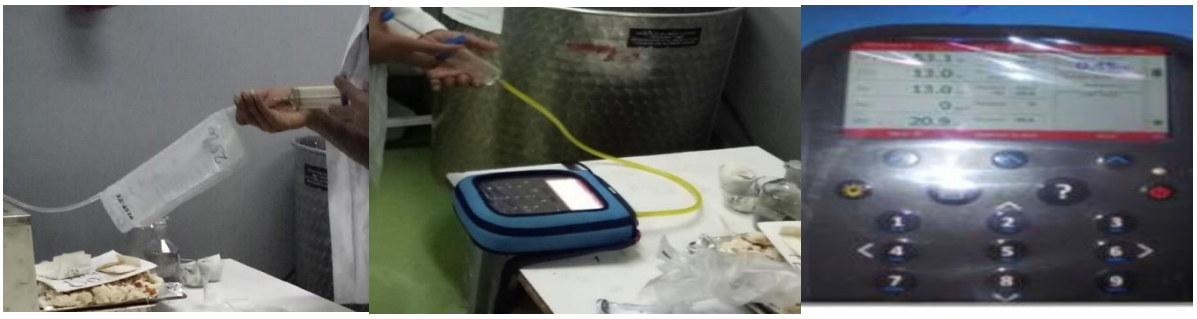


Figure 3.8: Biogas volume and composition measurement

3.2.6 Software for Experimental Design

AD system involving three factors with different levels were studied for the improvement of biogas production from co-digestion of Cow manure and Food waste, i.e. mixing ratio, temperature and digestion time. Design expert version 7.0.0 and Microsoft excel 2010 were employed to design the experimentation, analyze the data and illustrate the individual and interaction effects of the process parameters. The factor levels used in this study were chosen according to the practical functioning range for the anaerobic digestion of cattle manure and co-substrates. Microsoft excel 2010 was used to draw and observe the daily and cumulative biogas generation, and methane yield with respect to digestion period.

Design Expert is a piece of software developed to be used in designing and analyzing multi-factor experiments. Design of experiment (DOE) is most applicable statistical technique able to design and optimize experimental processes and provides the optimal design which estimates the effect of different process parameters and their simultaneous interaction effects. DOE is an efficient way of changing system inputs and analyzing the resulting process outputs so as to measure the cause and effect correlation as well as the arbitrary variability of the process while using minimum number of runs [72].

In this study, General factorial method of DOE was used in designing a lab scale AD system to see how the responses (i.e. biogas generation volume and methane yield) are affected by varying different process parameters such as the mixing ratio, operating temperature, digestion time. The effects of these independent variables and their interaction on the responses of the system were analyzed using optimization techniques. Statistical analysis method (ANOVA) was used to analyze whether the developed model and each model terms were significant and the interaction effect of all the process variables on both biogas production volume and methane yield. The scheme of the procedure followed in using general factorial to design the experiment is shown in figure 3.9 below.

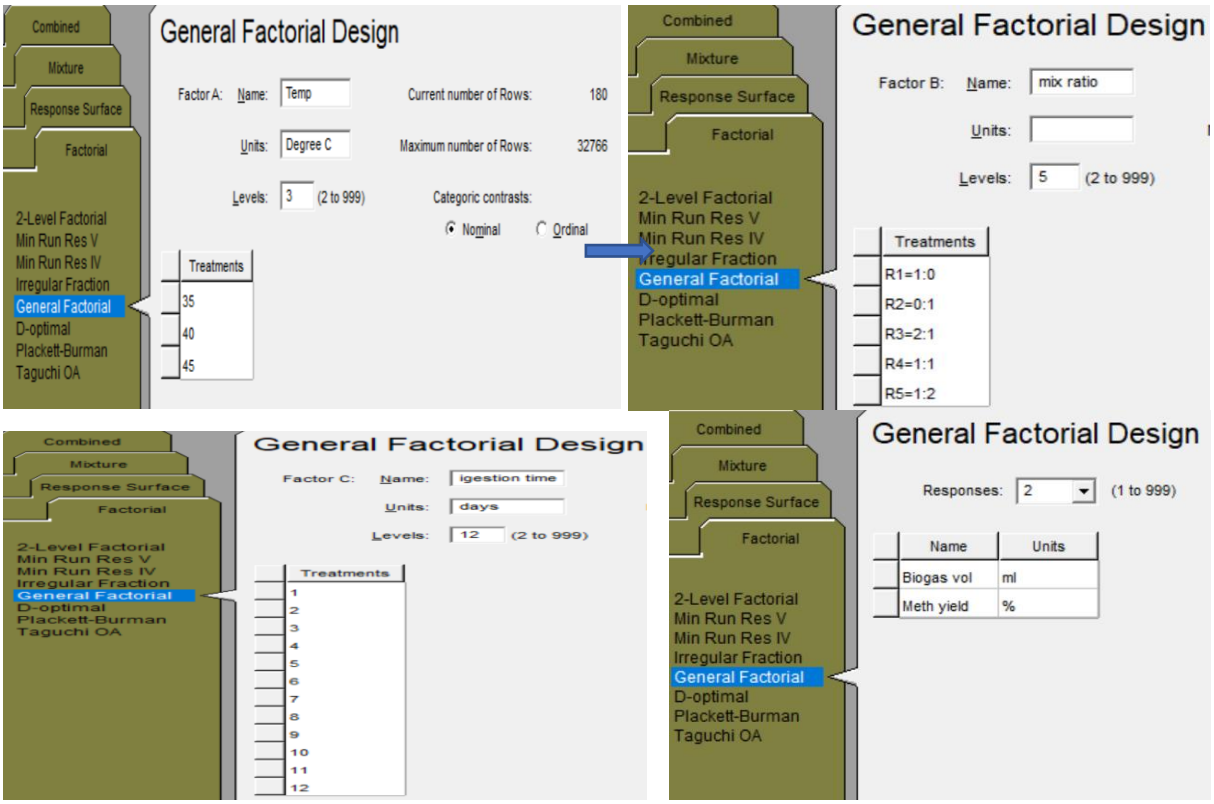


Figure 3.9: Scheme of the DOE employed using general factorial method

CHAPTER FOUR: RESULT AND DISCUSSION

4.1 Characteristics of Feedstocks

The cow manure and different food wastes were analyzed with respect to TS and VS content, carbon and nitrogen content, and COD values (Appendix I). The TS, VS, %C content, and COD values of the substrates indicate the amount of biodegradable organic materials available in the feedstocks. High values of these parameters show good BMP (Biochemical methane potential) of the substrates. There were some differences among the different substrates used. Mainly noticeable was the higher solid content (28.16%) of the injera leftover almost three times the values for fruit and vegetable peelings. Different studies reported that the optimal TS value for proper functioning of wet digestion is in the range from 7 to 12% [73]. The TS content of the feeds with different mixing ratios of FW to CM (1:0, 1:1, 2:1, 1:2 and 0:1) were found to be 15.96%, 17.51%, 16.99%, 18.03% and 19.06% respectively. These values are different from the optimal TS value for wet anaerobic digestion system. Hence, each feed in the digester were diluted with water and adjusted to the optimal TS content (12%). Higher solid content of feedstocks prevents proper mass transfer in the system and creates high concentrations of inhibitors which hinder anaerobic degradation [74]. The characteristics of the prepared feed in each digester are shown in table 4.1 below.

Table 4.1: Characteristics of prepared slurry fed to each digester

Analysis type	Prepared FW to CM ratios				
	1:0	1:1	2:1	1:2	0:1
%TS	15.96	17.51	16.99	18.03	19.06
%VS	87.39	85.16	85.91	84.42	82.93
%C	48.55	47.31	47.72	46.90	46.07
%N	1.61	1.71	1.67	1.74	1.8
C/N	30.16	27.67	28.58	26.95	25.60

The %TS, %VS, %C, %N and C/N for each feed to the digesters were computed according to the respective ratios as shown in Appendix I (table I2). For the proper functioning of the digesters the TS values of each digester were lowered to 12% by simple dilution. The amount of water added in preparing slurry of the feeds to each digester is computed in Appendix I. From different literatures a constant initial pH value of 7.5 which is reported as optimum was applied for all the digesters [75]. NaOH solution was used to adjust the lower pH values of samples in each digester.

The nitrogen content of the FW and CM was obtained to be 1.61% and 1.80% and a corresponding C/N ratio of 30.16 and 25 respectively. The C/N ratio of FW was comparatively higher than that of CM and is likely due to the higher organic content of the mixed food wastes. C/N ratio of 20 to 30 is reflected to be optimum for most anaerobic digestion systems [76]. The slightly higher value of FW is adjusted upon mixing with the lower value of CM. The C/N values for the 1:1, 1:2 and 2:1 was obtained to be 27.67, 26.95 and 28.58 respectively. These C/N values indicate that co-digestion could fulfill the optimum nutrient requirement of the microbes which results in improved biogas generation and methane yield.

4.2 Process Parameters Effect on Biogas Production and Methane Yield

The effect of mixing ratio, temperature and retention time on Biogas production from the AcoD of cow manure and food wastes were investigated. Biogas production from the three sets of AD systems (each containing five digesters of different mixing ratios) at three different temperatures (35 ^{0}C, 40 ^{0}C, 45 ^{0}C) and a constant initial pH (7.5) were recorded regularly once in three days for 35 days as shown in appendix II (Tables II-1 to II-3). The effect of mixing ratio and temperature on daily and cumulative biogas productivity is illustrated as shown in the curves below (Figures 4.1-4.3). As it is shown in the graphs biogas productivity from co-digestion of FW and CM was higher than mono substrate digestion under all operating conditions. Highest biogas production is obtained from the co-digestion of FW and CM in the mixing ratio of R=1:2. The cumulative biogas volume generated from all the digesters of different mixing ratios i.e. R_1 = 1:0, R_2 = 1:1, R_3 = 2:1, R_4 = 1:2 and R_5 = 0:1 (indicating FW to CM) was about 1.97, 6.48, 3.96, 11.22, 2.52 liters at a temperature of 35 ^{0}C and initial pH value of 7.5; 3.16, 8.65, 5.81, 13.25, 4.01 liters at a temperature of 40 ^{0}C and initial pH of 7.5; 2.55, 6.90, 4.67, 9.40, 3.56 liters at a temperature of 45 ^{0}C and initial pH 7.5 respectively.

From this experimental result, it was observed that co-digestion of FW and CM improved the yield of biogas than the digestion of the two feedstocks alone. The amount of cumulative biogas produced from the different mixing ratios is arranged as R_4=1:2 > R_2=1:1 > R_3=2:1 > R_5=0:1 > R_1=1:0; which indicates the enhancement of biogas productivity during co-digestion of cow manure and food wastes and productivity increases with the increase in the proportion of cow manure. Cow manure is considered as a good potential substrate due to its high nitrogen content and fermentation stability [77]. Hence, the study revealed that mixing cow

manure with food wastes creates a stable condition for the proper functioning of AD system which results in improved biogas yield.

From the graphs for daily biogas production (Figure 4.1a – 4.3a) it is observed that the trend of gas generation for the three AD systems is similar. Due to the addition of active inoculum gas generation was observed 3 days after the startup of the system. The rate of daily biogas generation was appeared to increase until the 3rd digestion period (i.e. the first 10 days) when active methanogen bacteria from the seed digest easily degradable organic materials. A decrease in gas generation was then observed in the meantime which reflects inhibition of methanogenic bacteria due to formation of intermediary compounds in the system. The gas generation trend is characterized by the effect of the three main stages of AD system on methanogenesis bacteria. The pH value of the system highly influences the methane producing microorganisms. Few days after the startup of the process hydrolysis of the complex organic materials to smaller molecules like simple sugars, amino acids, alcohols and fatty acids occurs and imparts a decrease in pH to the system. The drop in pH value impedes the activity of methanogenic microbes resulting in the decrease of biogas generation [78].

From the 8th to 10th digestion period (approximately in the twenties days of digestion), an increase in gas production was seen (figure 4.1a – 4.3b). During this digestion period it's easy to conclude that intermediate molecules from hydrolysis were converted to biogas. This phase is indicated by an increase in pH which facilitates the activity of methanogenic bacteria responsible for biogas generation[78]. Researchers stated that C/N ratio of feedstocks attributes to the alkalinity or acidity of AD systems and the optimal value is reported in the range of 20–30:1 [76]. The higher carbon contents of substrates in the digester (more CO_2 formation) the lower pH value of the system; whereas the higher nitrogen content resulting in increased ammonia formation the higher the pH value of the system. The C/N ratio of the FW (30.16) was slightly higher than the optimal range which resulted in the least biogas productivity. The deviation of the C/N ratio from the optimum range could influence the pH of the FW degradation system. Towards the completion of the digestion period, a decrease of gas generation was observed (Figures 4.1a - 4.3a). Gas production started to drop when the substrates available to the microorganisms to act on were already consumed up.

From the cumulative biogas production curves (Figures 4.1-4.3b), higher production was appeared from the digesters fed with co-substrates and a gradual increase in the volume of biogas generation with digestion time was observed. Thus, it is clear to conclude that AcoD of

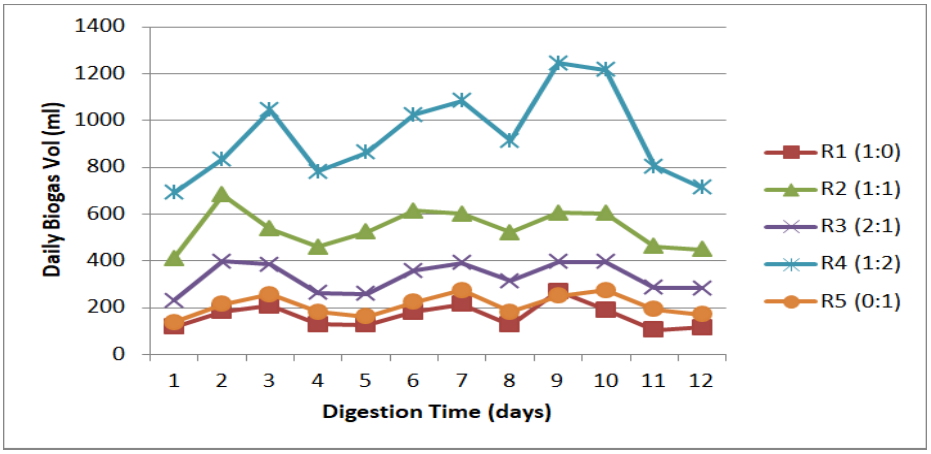
FW and CM could improve biogas productivity. Particularly, the highest biogas yield (13.25 L) was obtained from the co-digestion of FW to CM in 1:2 at 40 ^{0}C followed by 1:2 (11.22 L) at 35 ^{0}C. This might be as a result of an appropriate nutrient balance mainly C/N ratio in the 1:2 mixing ratio to the optimum value. The higher value of C/N ratio of FW was adjusted by adding CM (two-third in weight of FW) to 26.95 which could help for the best functioning of the AD system.

From the experimental results, the effect of temperature on biogas yield was observed. It is clearly seen that highest biogas production was attained from the AD setup operated at 40 ^{0}C (Figure 4.2). However, though rapid biogas production was observed in the AD system at 45 ^{0}C its cumulative biogas production was lower than the generation from the digesters in the other two temperature systems due to the rapid drop of production. The rapid fall of generation could be occurred as a result of rapid accumulation of VFA within the system. Thus, temperature can be considered as a major factor in the performance of an AD system. It affects the physico-chemical properties, kinetics and thermodynamics of the biochemical processes [29].

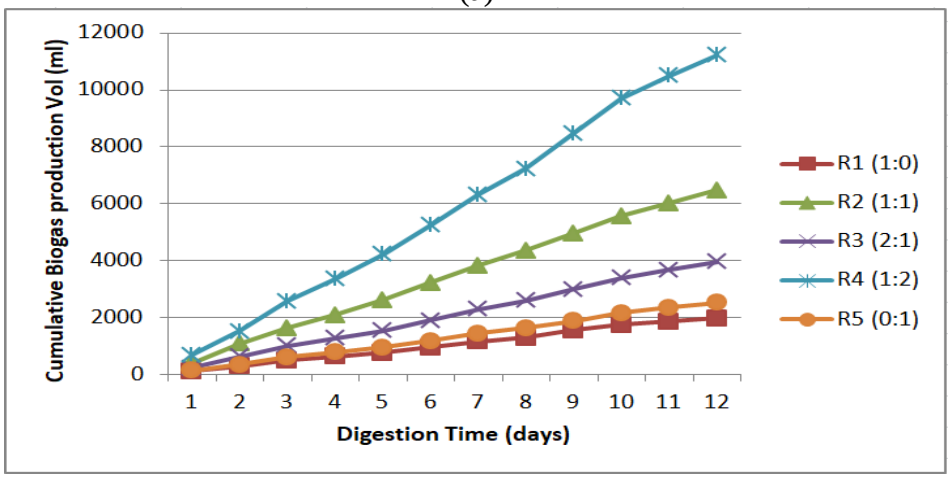
The effect of the parameters on methane yield is presented in the graphs shown below (Figures 4.1 - 4.3c). The methane fraction of the biogas produced varied from 40% to 70% in all the digesters with the minimum and maximum yields at the beginning and termination of the AD process respectively. The average values of methane yield obtained from the mixing ratios 1:0, 1:1, 2:1, 1:2 and 0:1 was 57.76%, 55.76%, 55.25%, 58.63%, and 53.3% at 35 ^{0}C; 51.62%, 60.1%, 56.84%, 62.93%, and 53.26% at 40 ^{0}C; 49.43%, 54.78%, 52.28%, 56.76% and 49.63% at 45 ^{0}C. Comparatively, slightly higher methane yield was attained from the co-digestion of FW to CM in 1:2 and 1:1 ratios at 40 ^{0}C temperature.

For all mixing ratios and operating temperatures methane yield of the biogas was increasing with retention time while CO_2 fraction was decreasing in a similar manner (Figure 4.7). This is supposed to be due to the fact that biogas production is related to the fraction of VS reduction. In addition, the two key pathways of methane formation in AD system namely the aceticlastic and hydrogenotrophic methanation which transform acetate, H_2 and CO_2 to methane takes place to the end of the digestion period [79]. In this stage, methane and carbon dioxide are generated by several methanogenic microbes. Other substrates such as methanol, formate, methylamines and carbon monoxide can also be digested by methanogens to produce methane in the late stage of the digestion period[80]. Acetate is the major source of the total

methane produced through the aceticlastic methanation reaction pathway [79]. Thus, it is expected to observe higher CO_2 fraction at the initial stage and higher CH_4 yield to the end of the digestion period.



(a)



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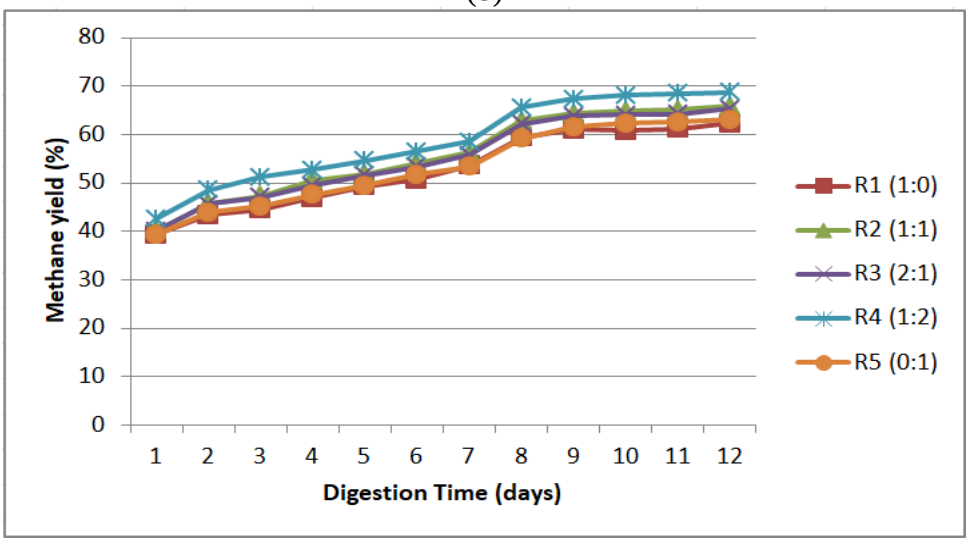
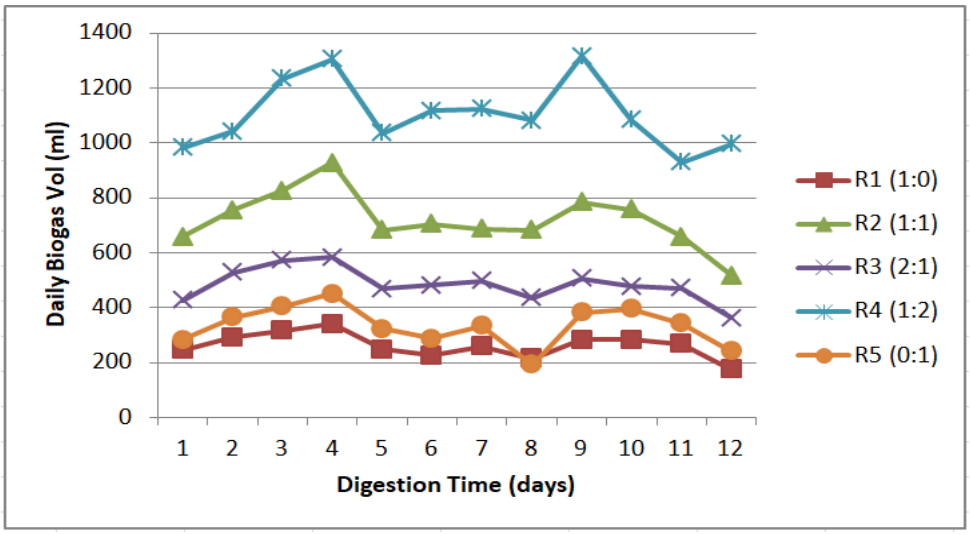
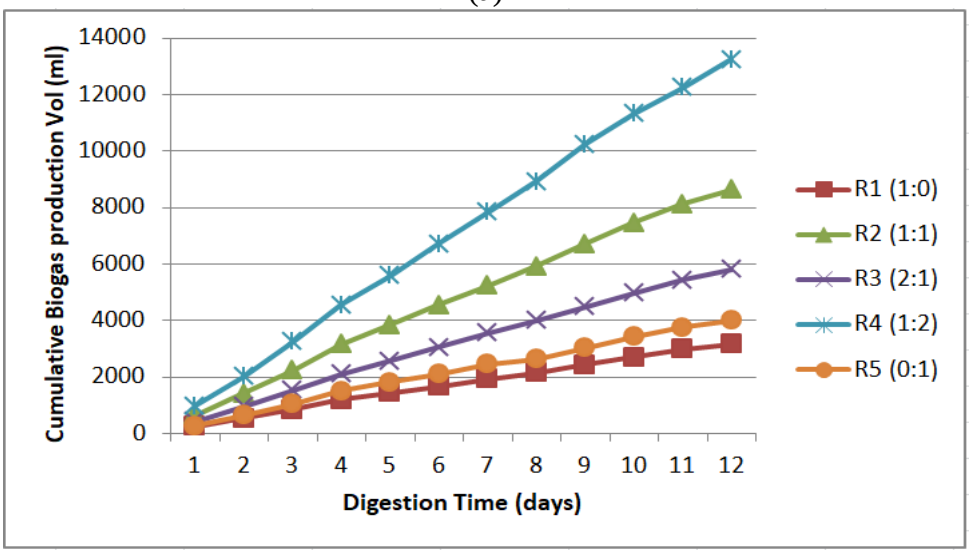


(c)

Fig 4.1: Daily (a), cumulative (b) biogas production and methane yield (c) vs. digestion time at 35^0c and pH=7.5



(a)



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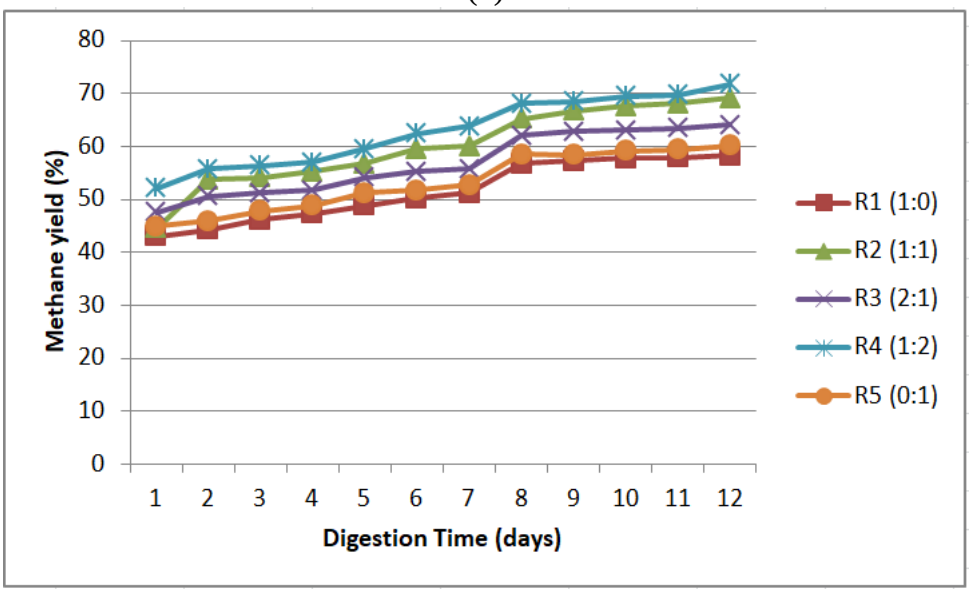
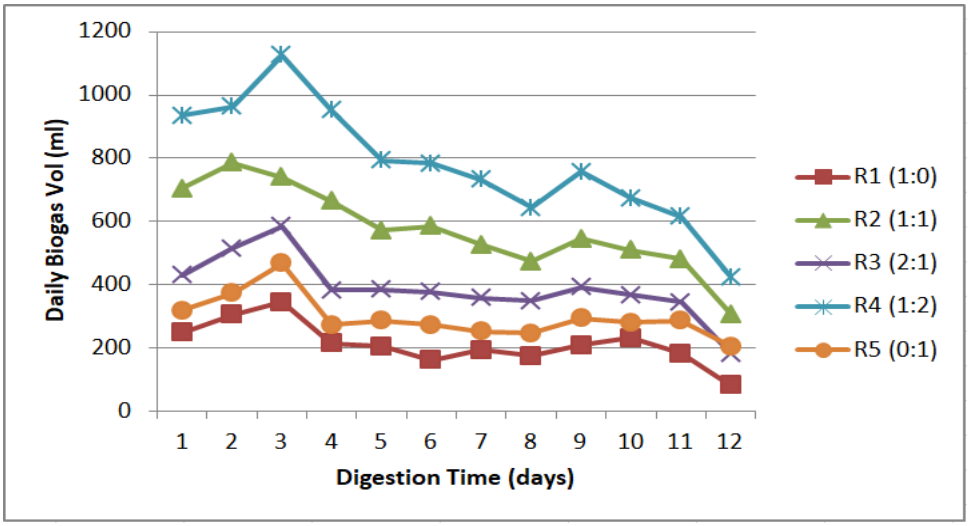
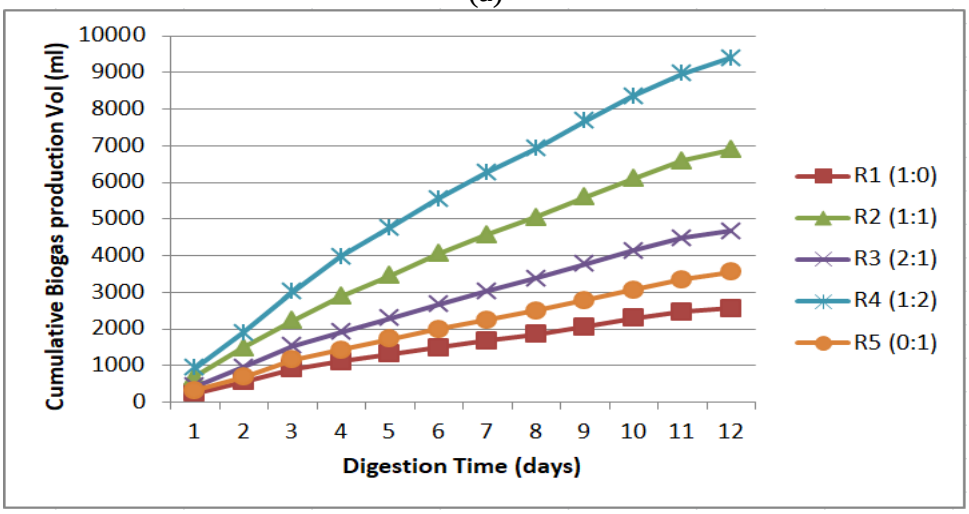


(c)

Fig4.2: Daily (a), cumulative (b) biogas production and methane yield (c) vs. digestion time at 40^0c and pH=7.5



(a)



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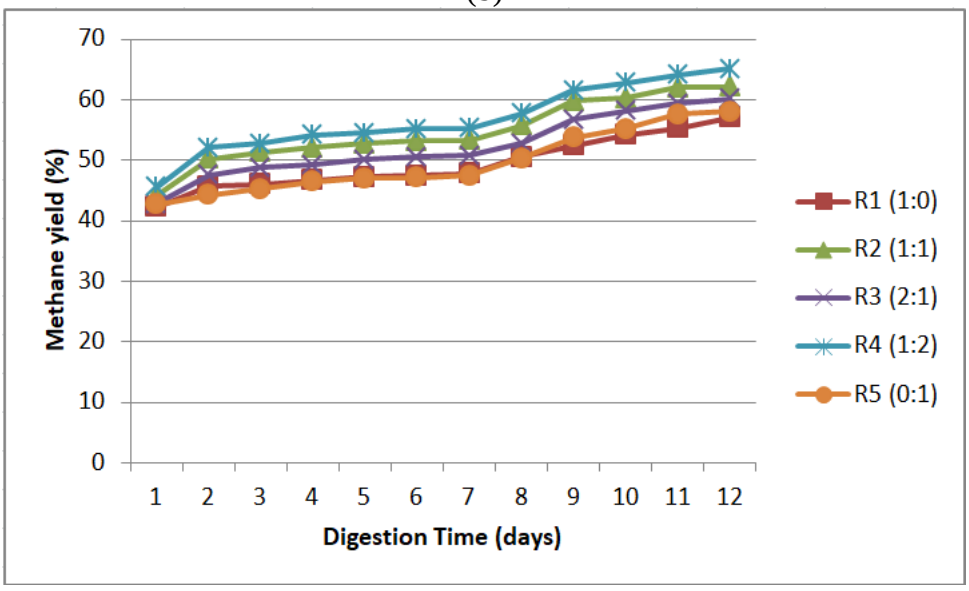

(c)

Fig 4.3: Daily (a), cumulative (b) biogas production and methane yield (c) vs. digestion time at 45^0c and pH=7.5

4.3 Statistical Design of Experiments and Data Analysis

4.3.1 Analysis of Variance (ANOVA)

Anaerobic digestion system depends on several process variables. In this study three main factors i.e. mixing ratio, temperature and retention time were treated for the investigation of their effects on biogas and methane yield. Different levels of process variables were considered i.e. three levels of digestion temperature, five mixing ratios and twelve levels for the digestion time. The interactive effect and optimal levels of these parameters for the biogas productivity and methane yield was simplified and studied using the general factorial method of design expert version 7.0.0 software tool. This tool allows the user to investigate the effects of process variables with different number of levels. The advantages of this statistical approach are product yield enhancement, reduction in process variability, confirmation of the output response and a reduction in the experimental time and overall costs [72].

Table 4.2: Model summary statistics for biogas volume produced

Std. Dev.	1.73	R-Squared	0.9336
Mean	21.18	Adj R-Squared	0.9266
C.V. %	8.18	Pred R-Squared	0.9172
PRESS	606.58	Adeq Precision	42.4381

Table 4.3: Model summary statistics for methane yield

Std. Dev.	1.78	R-Squared	0.9496
Mean	54.88	Adj R-Squared	0.9443
C.V. %	3.24	Pred R-Squared	0.9373
PRESS	637.32	Adeq Precision	57.2016

From the experimentally obtained data, mathematical models showing the effects of the different process parameters on the two responses namely biogas production and methane yield was developed (Equation 4.1 & 4.2). The models show individual and interactive effects of the three factors on biogas production and methane yield from the AcoD of food waste and cow manure. Analysis of variance (ANOVA) is the most common statistical method used in determining the significance and adequacy of the model and its terms [72]. From the model summary statistics quadratic model was suggested for both the responses. ANOVA was then used to analyze the effects of the categorical process variables on the two responses (biogas volume and methane yield) statistically. The statistical analysis summary (ANOVA) describing the significance of the model and each model terms is shown in tables 4.4 & 4.5 below. The process variables were then treated as numerical factors to obtain surface plot

showing simultaneous effects of temperature and digestion time on biogas volume and methane yield (Figures 4.6 and 4.7).

The mathematical model equation developed for the biogas production volume is:

$$Sqrt(Biogas\ vol) = +23.90 + 0.39 * A - 6.77 * B[1]4.61 * B[2] - 1.31 * B[3] + 3.40 * B[4] - 1.27 * C + 0.47 * AB[1] + 0.96 * AB[2] + 0.35 * AB[3] - 0.067 * AB[4] - 1.78 * AC + 0.023 * B[1]C + 0.58 * B[2]C + 0.041 * B[3]C - 0.54 * B[4]C - 3.10 * A^2 - 1.66 * C^2 \ldots\ldots\ldots Equation\ (4.1)$$

Where: A is temperature

B [B1, B2,…] is the different mixing ratios

C is digestion time

AB interaction between temperature and mixing ratio

AC interaction betweeen temperature and digestion time

BC interaction between mixing ratio and digestion time

Table 4.4: Summary of a square root transformed ANOVA for biogas volume produced

Response: Biogas vol						
Transform: Square root						
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Classical sum of squares - Type II]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6836.04	17	402.12	133.89	< 0.0001	significant
A-Temp	18.37	1	18.37	6.11	0.0144	
B-mix ratio	5999.84	4	1499.96	499.42	< 0.0001	
C-digestion time	114.12	1	114.12	38.00	< 0.0001	
AB	101.40	4	25.35	8.44	< 0.0001	
AC	149.29	1	149.29	49.71	< 0.0001	
BC	9.06	4	2.27	0.75	0.5565	
A^2	383.36	1	383.36	127.64	< 0.0001	
C^2	60.61	1	60.61	20.18	< 0.0001	
Residual	486.55	162	3.00			
Cor Total	7322.59	179				

The mathematical model equation developed for the biogas production volume is:

$$\text{Meth yield} = +57.35 - 1.29 * A - 3.62 * B[1] - 2.82 * B[2] - 0.091 * B[3] + 1.98 * B[4] + 9.97 * C - 0.37 * AB[1] - 0.54 * AB[2] - 0.20 * AB[3] + 0.77 * AB[4] - 2.53 * AC - 0.86 * B[1]C - 0.38 * B[2]C - 0.10 * B[3]C + 0.75 * B[4]C - 3.10 * A2 - 1.03 * C2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 4.2$$

Where: A is temperature

B [B1, B2,…] is the different mixing ratios

C is digestion time

AB interaction between temperature and mixing ratio

AC interaction betweeen temperature and digestion time

BC interaction between mixing ratio and digestion time

Table 4.5: Analysis of Variance summary for methane yield

Response: Methane yield						
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Classical sum of squares - Type II]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	9647.43	17	567.50	179.46	< 0.0001	significant
A-Temp	200.73	1	200.73	63.48	< 0.0001	
B-mix ratio	1642.05	4	410.51	129.82	< 0.0001	
C-digestion time	7041.72	1	7041.72	2226.79	< 0.0001	
AB	28.37	4	7.09	2.24	0.0667	
AC	301.58	1	301.58	95.37	< 0.0001	
BC	25.72	4	6.43	2.03	0.0921	
A^2	383.99	1	383.99	121.43	< 0.0001	
C^2	23.28	1	23.28	7.36	0.0074	
Residual	512.29	162	3.16			
Cor Total	10159.72	179				

The process parameters coded as A, B, and C implies temperature, mixing ratio and digestion time respectively. The F-values of the models for the two responses were 133.89 and 179.46 for biogas volume produced and methane yield respectively implying the significance of the models. Effects with P-value less than 0.05 were better to show the reliability of a model [72]. From the ANOVA summary (Tables 4.4 and 4.5) for the two models developed, 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 4.4 and 4.5 tells that the model terms are statistically significant. In this case, the individual model terms A, B, C; and their interactions AB and AC were statistically significant model terms of the produced biogas volume (Table 4.4). The

individual model terms A, B, C; and an interaction effect of AC model term were also statistically significant model terms of the methane yield (Table 4.5).

For a good statistical model, a value of R^2 in the range of 0.75 to 1.0 indicates a good fit of the model[72]. The R-squared values of 0.9336 and 0.9496 revealed that the models possibly describe 93.36% and 94.96% of the variability in the biogas volume production and methane yield respectively (Tables 4.2 and 4.3). The adjusted R^2 values of 0.9266 and 0.9443 respectively for the biogas volume production and methane yield were also high enough to show the significance of the models. The Pred R^2 values of 0.9172 and 0.9373 respectively for biogas volume produced and methane yield were in reasonable agreement (approximately within less than 0.2 variations) with the Adj R^2 values showing good fitting between the observed and predicted values. Adequate precision measures the signal to noise ratio and a value greater than 4 is required [72]. Adequate precision values of 42.4381 and 57.2016 were obtained for the biogas volume produced and methane yield respectively (Tables 4.2 and 4.3) showing an adequate signal of the responses. 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 lower the value of CV, the higher the reliability of experiment [72]. The low CV values of 8.18% and 3.24% respectively for the biogas volume produced and methane yield showed a good precision and reliability of the conducted experiment.

4.3.2 Model Diagnostic plots

The software (Design expert 7.0.0) also provided model graphs and diagnostic plots which illustrate the individual and interactive effects of the process variables on the two responses of the AD system. The normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line [72]. The normal probability plot of the two responses as shown below (Figure 4.4 c and 4.5 c) reflected that the residuals 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 plot of the residuals versus the ascending predicted response values tests the assumption of constant variance. The plot should be a random scatter [72]. The residuals versus predicted response graphs for the two responses (Figure 4.4 b and 4.5 b) revealed that the errors are randomly scattered. Predicted vs. actual plot helps detect a value, or group of values, that are not easily predicted by the model. The data points should be split evenly by the 45 degree line [72]. The graphs for biogas volume produced and methane yield (Figure 4.4 a, and 4.5 a) confirmed that the developed model could predict the experimental values.

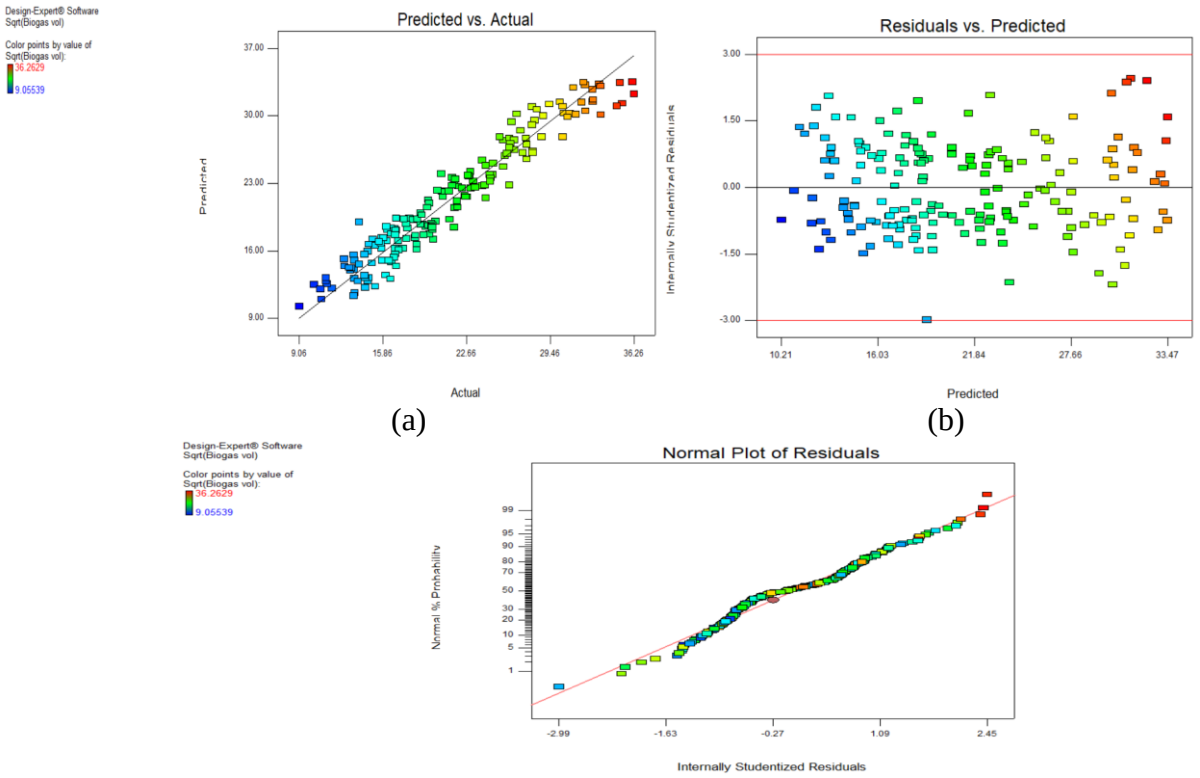

Figure 4.4: Predicted vs. actual (a), Residuals vs. predicted (b), and Normal plot of residuals (c) for biogas volume produced data

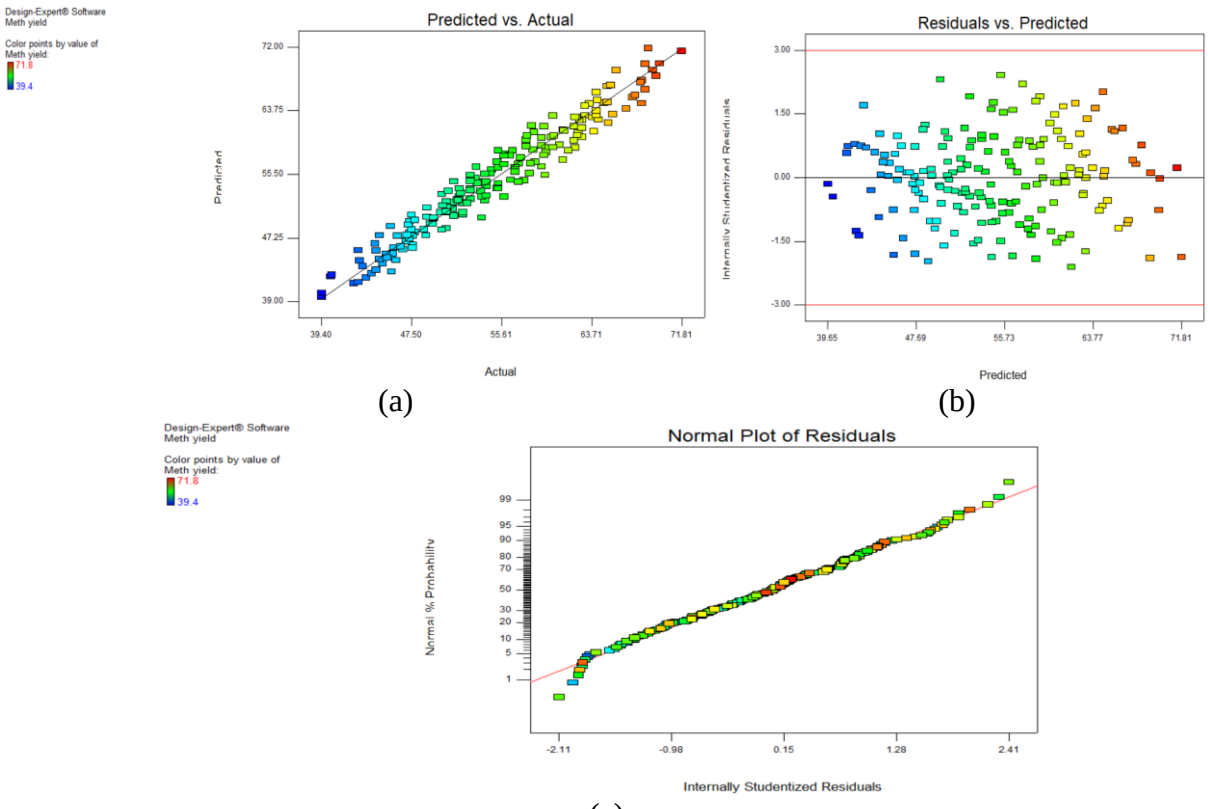

Figure 4.5: Predicted vs. actual (a), Residuals vs. predicted (b), and Normal plot of residuals (c) for methane yield data

The simultaneous effects of temperature and digestion time on biogas productivity and methane yield for the co-digestion of different proportions of FW and CM is presented as shown in the graphs below (Figure 4.6 and 4.7). Since mixing ratio was considered as a major and categorical factor, surface plots were plotted for each the mixing ratios showing the interaction effect of temperature and digestion time. Generally, co-digestion of the two feedstocks showed improved biogas productivity and methane yield than digestion of the individual substrates.

The surface plots in Figure 4.6 (interaction effects of temperature and digestion time on biogas productivity) revealed that rate of biogas generation increases with increasing retention time until a period of slow degradation occurs to the end of digestion period. From the plots, it is also observed that rate of biogas production increases with increasing temperature up until the optimum point (approximately 40 ^{0}C) and decreases with further increase in temperature. Even though higher temperature ranges increases the rate of biogas production, the cumulative production was decreased due to rapid VFAs accumulation and decomposition of organic substrates. The decrease of rate of gas production to the end of the digestion period is considered due to the reduction in the decomposable organic substrates.

The response surface plots displayed below (Figures 4.7) show the interactive effects of temperature and digestion time on methane yield for the five mixing ratios. From the plots it was observed that a slightly higher methane yield was obtained in the mixing ratios of 1:2 and 1:1. In general, methane yield was observed to increase with increasing digestion time and till some level in temperature.

The methane fraction of the biogas produced from the 1:0, 0:1, 2:1, 1:1 and 1:2 was seen to vary from 39 – 64%, 40 – 66%, 42 – 68%, 42 – 70%, and 45 – 73% respectively. Even though a slight increase in methane yield with increasing temperature was observed to the start of digestion time, an increase in temperature with increasing digestion time dropped the methane fraction. The overall increase in methane fraction with increasing digestion time is accounted to the presence of more methane producing bacteria to the end of the digestion time[81].

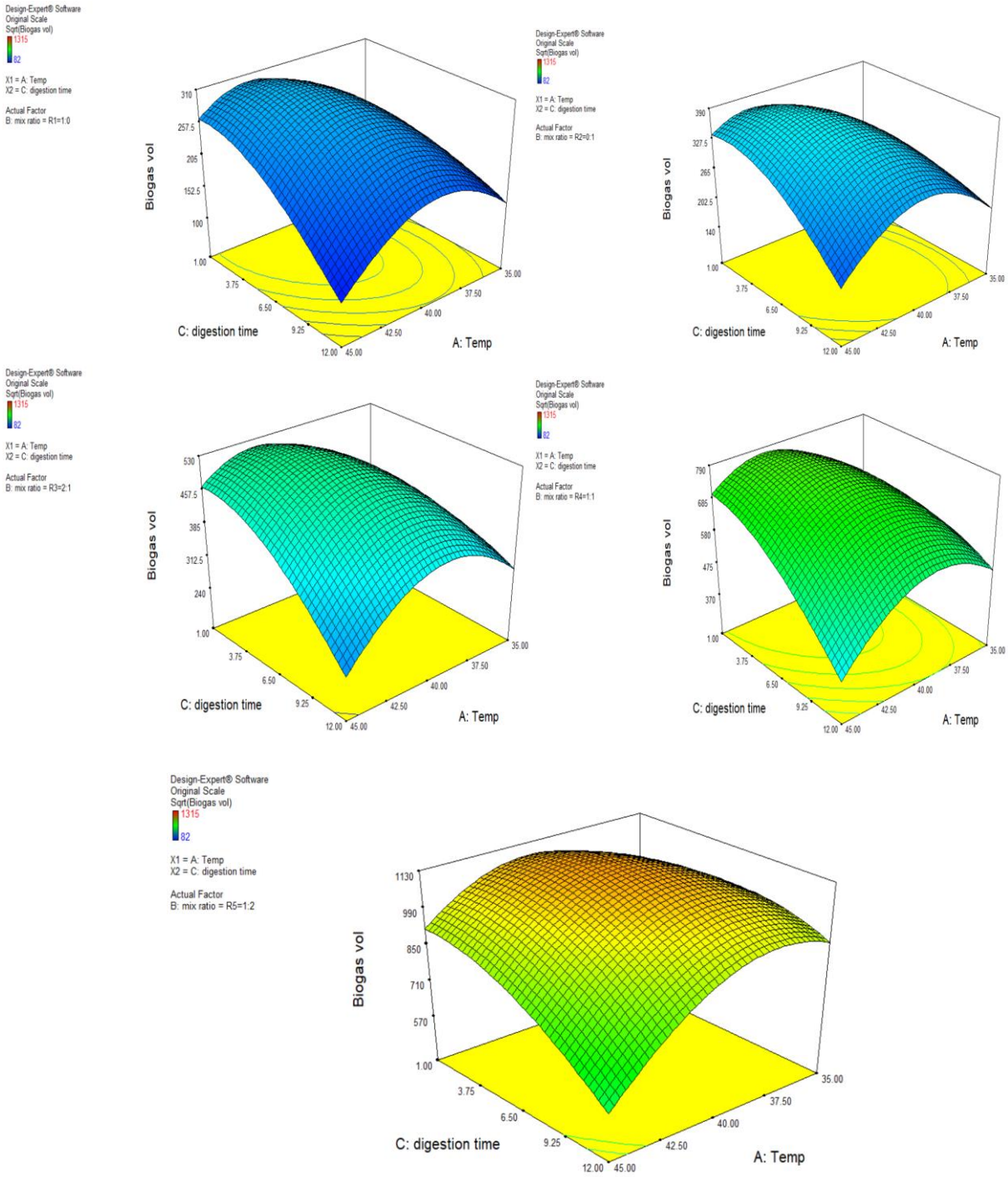

Figure 4.6: Surface plots showing simultaneous effects of temperature and digestion time on biogas volume for the five mixing ratios

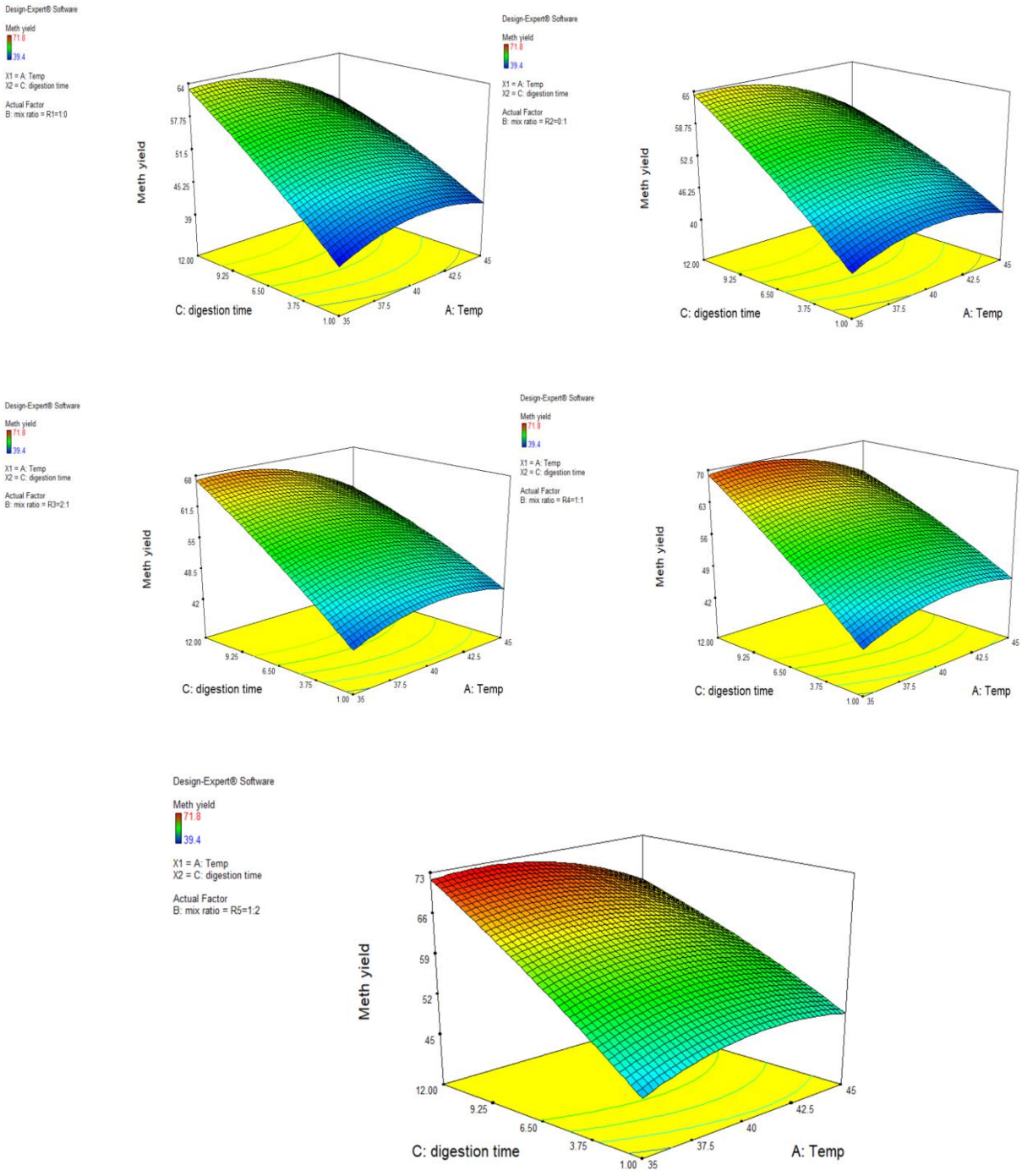

Figure 4.7: Surface plots showing simultaneous effects of temperature and digestion time on methane yield for the five mixing ratios

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Anaerobic digestion system is increasingly being recognized as the main technology for waste treatment, environmental protection, and energy generation in both developed and developing nations. There is a substantial quantity of decomposable organic waste with a great potential for biogas production. The effectiveness of AD system in energy production mainly depends on the substrate composition of the biodegradable organic waste and system temperature among the other factors. In this study, cow manure and food wastes were selected as co-substrates due to their potential and suitability for the AcoD system. The purpose of the present work was to improve biogas productivity and methane yield from the co-digestion of food waste and cow manure with different process parameters. The following conclusions have been drawn from the work illustrated in this paper.

Anaerobic co-digestion of the feedstocks is observed to be influenced by the composition of the substrates (i.e. food waste to cow manure proportion) in each digester. Higher biogas production and methane yield was achieved from the co-digestion of FW and CM compared to their mono-substrate digestion. Maximum biogas production and methane yield is obtained from the co-digestion of food waste and cow manure in the mixing ratios of 1:2 and 1:1 while all the other parameters were same for all the digesters. Thus, co-digestion is seen as a suitable means in the enhancement of biogas productivity by maintaining the required nutrient balance for the anaerobic digestion system.

In general, biogas production is highly affected by the type of potential feedstocks used and nutrient composition of the substrates. From this work, the effect of the three process parameters namely temperature, mixing ratio and retention time on biogas production and methane yield was observed. The type of potential feedstock, appropriate mixing ratio, C/N ratio, retention time and system temperature are all among the considerable factors for biogas production and methane yield enhancement. The study clearly showed that anaerobic co-digestion of food waste with cow manure provides better gas production and methane yield than mono-substrate digestion of the feedstocks separately. Highest productivity and methane yield was observed at 1:2 (indicating FW to CM ratio) mixing ratio and 40 ^{0}C temperature. The digester fed with co-substrates in 1:2 ratios was observed to have a balanced C/N ratio which stabilizes the digestion system for the better functioning of methanogenic microorganisms resulting in higher biogas generation. The effect of nutrient imbalance i.e. slightly higher and lower values of C/N content respectively in FW (1:0) and CM (0:1), and

rapid accumulation of VFAs in the digester fed with 2:1 mixing ratio showed a decrease in biogas production and methane yield by suppressing the activity of methanogenic bacteria possibly due to a decrease of ammonium nitrogen and low pH.

The statistical analysis of the experimental data results developed using the general factorial method of DOE software version 7.0.0 reflected the individual and interactive effects of the process parameters on the biogas productivity and methane yield. The significance of the developed mathematical models and each model terms was observed from the ANOVA results for both biogas volume and methane yield responses. The model summary statistics of the two responses showed a statistical suitability of the data as they have maximum pred R^2 and adj R^2.

5.2 RECOMMENDATION

Anaerobic co-digestion of potential feedstocks is an emerging R&D theme for the improvement of biogas production system. Mono-substrate digestion, mainly animal manure has been widely used as a feed in the AD system. However, the system has been showing problems of instability due to nutrient imbalance resulting in inefficient gas generation and methane yield. Co-substrate digestion of cow manure with different organic wastes has found to enhance the anaerobic digestion system, including co-digestion with food wastes described in this study. However, additional sorting and pretreatment techniques are required to remove inert materials found in food wastes which might damage digesters. It is also important to introduce proper mixing system for the better functioning of AD system. Moreover, similar optimization study is recommended to be done with a change in pH of the system. As a final point, further study on the co-digestion of cow manure with other available potential feedstocks at different operation parameters should be done for the optimization of anaerobic digestion system.

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APPENDIX I

Proximate analysis of feedstocks

Table I-1: Composition of substrates

Substrate type	Type of analysis									
	M1 (g)	M2 (g)	M3 (g)	M4 (g)	%TS	%VS	%C	%N	C/N	COD (mg/L)
Fruit peels	42.88	90.85	46.77	43.11	8.1	94.04	52.24	-	-	1370
Vegetable peels	44.25	100	50.94	44.7	11.62	91.67	50.92	-	-	940
Injera leftover	45.26	95.26	59.26	48.62	28.16	76.46	42.48	-	-	510
Mixed FW (the above three wastes)	-	-	-	-	15.96	87.39	48.55	1.61	30.15	1040.57
Cow manure	47.43	95.07	56.51	48.98	19.06	82.93	46.07	1.8	25.6	2350

The TS, VS, %C and %N values listed in the above table are computed as follows:

%TS = ((M3 – M1)/(M2 – M1)) * 100

%VS = ((M3 – M4)/(M3 – M1)) * 100

%C = %VS/1.8

%TKN = ((a – b)/S) * N * 0.014 * 100 * mcf

Where: M1 is mass of empty crucible

M2 is mass of crucible with sample

M3 is mass of crucible with sample after 24 hours in oven

M4 is mass of crucible with sample after 4 hours in furnace

a is ml of H_2SO_4 required for titration of sample

b is ml of H_2SO_4 required for titration of blank

S is weight of air dry sample (5g)

N is normality of H_2SO_4 (0.1 N)

100 is ml of the sample solutions

0.014 is meq weight of nitrogen in g

mcf is moisture correction factor

For example: For fruit peels; %TS = ((46.77 – 42.88)/(90.85 – 42.88)) * 100 = 8.1

For injera leftover; %TS = ((59.26 – 45.26)/(95.26 – 45.26)) * 100 = 28.16

For mixed FW; (8.1+11.62+28.16)/3 = 15.96

For cow manure; %TS = ((56.51 – 47.43)/(95.07 – 47.43)) * 100 = 19.06

For vegetable peels; %VS = ((50.94 – 44.78)/(50.94 – 44.25)) * 100 = 91.67

%C = 91.67/1.8 = 50.92

For cow manure; %VS = ((56.51 – 48.98)/(56.51 – 47.43)) * 100 = 82.93

%C = 82.93/1.8 = 46.07

For FW; %TKN = ((a – b)/S) * N * 0.014 * 100 * mcf

mcf = (100 - (100 – 15.96))/100 = 0.16

= ((36.14 – 0.2)/5) * 0.1 * 0.014 * 100 * 0.16 = 1.61

For CM; %TKN = ((a – b)/S) * N * 0.014 * 100 * mcf

mcf = (100 - (100 – 19.06))/100 = 0.19

= ((34.04 – 0.2)/5) * 0.1 * 0.014 * 100 * 0.19 = 1.8

The amount of water added to dilute the feeds in preparing slurry as per the respective mixing ratios is computed as follows:

For 1:0 slurry: TS content = $(\%TS_{FW}/100)*M_{FW}$ = (15.96/100)*400 = 63.84g

Since optimum %TS is 12%; 12g = 100ml;

63.84g = ?

Therefore, the volume of water added was 532ml.

For 1:1 slurry: TS content = (17.51/100)*400 = 70.04g

12g = 100ml; 70.04g = ?

The volume of water added was 584ml.

For 2:1 slurry: TS content = (16.99/100)*400 = 67.96g

12g = 100ml; 67.96g = ?

The volume of water added was 566ml.

For 1:2 slurry: TS content = (18.03/100)*400 = 72.12g

12g = 100ml; 72.12g = ?

The volume of water added was 601ml.

For 0:1 slurry: TS content = (19.06/100)*400 = 76.24g

12g = 100ml; 76.24g = ?

The volume of water added was 635ml.

The %TS and %VS values for the mixed FW were taken the average value of the three constituents.

APPENDIX II

Table II-1: Biogas volume and methane yield data for AD system at 35 ^{0}C and pH = 7.5

Biogas volume and methane yield data for AD sys at 35 oC and pH=7.5											
		Mixing ratio:Food waste to Cow manure									
		R1=1:0		R2=1:1		R3=2:1		R4=1:2		R5=0:1	
Sr no.	Gas sampl	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol	CH4 (%)
1	23-May	118	39.4	412	40.3	231	40.2	692	42.7	137	39.4
2	26-May	184	43.4	683	45.8	398	45.7	835	48.6	214	43.9
3	29-May	208	44.6	538	47.3	386	47.1	1045	51.2	258	45.2
4	1-Jun	128	47	461	50.6	263	49.5	783	52.8	182	47.6
5	4-Jun	126	49.4	524	51.9	260	51.6	863	54.7	162	49.5
6	7-Jun	183	50.8	614	54.1	358	53.4	1025	56.6	224	51.7
7	10-Jun	216	53.7	602	56.3	392	55.8	1084	58.5	274	53.4
8	13-Jun	126	59.5	521	62.8	315	62.2	914	65.6	183	59.2
9	16-Jun	271	61.1	605	64.3	396	63.9	1246	67.4	250	61.6
10	19-Jun	191	60.8	604	64.8	395	64.1	1216	68.2	274	62.4
11	22-Jun	105	61.2	462	65.1	285	64.2	805	68.5	194	62.6
12	24-Jun	116	62.3	450	65.9	284	65.4	714	68.8	172	63.1

Table II-2: Biogas volume and methane yield data for AD system at 40 ^{0}C and pH = 7.5

Biogas volume and methane yield data for AD sys at 40 oC and pH=7.5											
		Mixing ratio:Food waste to Cow manure									
		R1=1:0		R2=1:1		R3=2:1		R4=1:2		R5=0:1	
Sr no.	Gas sampl	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol	CH4 (%)
1	23-May	245	43.1	658	44.6	426	47.6	982	52.1	283	44.9
2	26-May	292	44.3	756	53.8	528	50.6	1042	55.8	365	45.9
3	29-May	316	46.2	827	54.1	572	51.2	1234	56.4	405	47.8
4	1-Jun	341	47.3	928	55.3	583	51.8	1305	57.1	452	48.9
5	4-Jun	248	48.9	683	56.8	468	54.2	1035	59.6	324	51.2
6	7-Jun	226	50.2	704	59.6	483	55.3	1118	62.5	287	51.8
7	10-Jun	260	51.3	687	60.1	497	55.8	1125	63.8	335	52.8
8	13-Jun	216	56.8	683	65.2	436	62.1	1082	68.2	194	58.6
9	16-Jun	284	57.3	785	66.8	506	62.8	1315	68.5	382	58.4
10	19-Jun	284	57.8	758	67.6	478	63.1	1085	69.5	396	59.2
11	22-Jun	267	57.9	658	68.1	471	63.5	929	69.8	342	59.4
12	24-Jun	176	58.3	518	69.2	362	64.1	995	71.8	241	60.2

Table II-3: Biogas volume and methane yield data for AD system at 45 ^{0}C and pH = 7.5

Biogas volume and methane yield data for AD sys at 45 oC and pH=7.5											
		Mixing ratio:Food waste to Cow manure									
		R1=1:0		R2=1:1		R3=2:1		R4=1:2		R5=0:1	
Sr no.	Gas sampl	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol (ml)	CH4 (%)	biogas vol	CH4 (%)
1	23-May	248	42.3	704	44.2	430	42.8	936	45.6	318	42.7
2	26-May	306	45.7	785	50.2	516	47.6	963	52.1	374	44.3
3	29-May	346	46.1	741	51.3	586	48.8	1126	52.8	468	45.3
4	1-Jun	214	46.7	664	52.1	382	49.2	952	54.2	273	46.5
5	4-Jun	206	47.3	572	52.8	385	50.1	794	54.6	286	47.1
6	7-Jun	161	47.5	586	53.2	378	50.6	783	55.2	273	47.2
7	10-Jun	194	47.8	527	53.3	358	50.8	732	55.3	253	47.5
8	13-Jun	174	50.6	473	55.8	348	52.8	643	57.8	246	50.3
9	16-Jun	208	52.5	546	59.8	392	56.8	758	61.6	294	53.8
10	19-Jun	232	54.2	510	60.3	368	58.2	674	62.8	281	55.2
11	22-Jun	182	55.3	481	62.1	346	59.4	615	64.1	287	57.6
12	24-Jun	82	57.1	308	62.2	182	60.2	424	65.1	206	58.1

APPENDIX III

Table III-1: Model summary statistics of biogas volume produced (Response 1)

Model Summary Statistics						
Source	Std. dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.6230	0.8375	0.8318	0.8236	1291.66	
2FI	2.3820	0.8729	0.8613	0.8450	1135.08	
Quadratic	1.7330	0.9336	0.9266	0.9172	606.58	Suggested
Cubic	1.6814	0.9432	0.9309	0.9125	640.66	Aliased

N.B: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Table III-2: Model summary statistics of methane yield (Response 2)

Model Summary Statistics						
Source	Std. dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.71	0.8745	0.8701	0.8640	1381.57	
2FI	2.37	0.9095	0.9012	0.8916	1101.26	
Quadratic	1.78	0.9496	0.9443	0.9373	637.32	Suggested
Cubic	1.43	0.9706	0.9641	0.9533	474.01	Aliased

N.B: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Research Grant Usage Description

Project duration	Tasks	Quantity	Estimated cost	
			In Birr and Dinar	**In USD**
	International flight (ALG-AA)	Return flight	100374 DZ	912.50
	Local flights (Algeria & Ethiopia)	-	5375 DZ 3027 ETB	160.97
	Softwares	one	12,420 ETB	460.00
	Internet and related fees	As per the requirement	3499 ETB	129.60
	Laboratory expenditure	Various types	30,331.25 ETB	1123.40
	Field trip accommodation	24 days	4800 ETB	177.78
	Finalizing and documentation	5 copies of thesis	4000 DZ	36.36
			Total	**3000.61**

NB: Conversion rate (1 USD = 27 ETB = 110 DZ)