OPTIMIZATION OF BIOGAS YIELD THROUGH CO-DIGESTION OF SUGARCANE VINASSE AND PRE-TREATED MAIZE STALKS

BY:

KIPLAGAT MOHAMED KIBET

THESIS SUBMITTED TO SCHOOL OF ENGINEERING, DEPARTMENT OF MECHANICAL & PRODUCTION ENGINEERING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN ENERGY STUDIES.

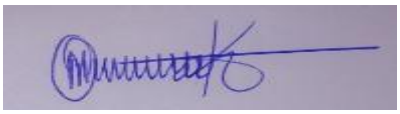
MOI UNIVERSITY

SEPTEMBER, 2021.

DECLARATION

Declaration by the student:

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without prior written permission of the author and/or Moi University.

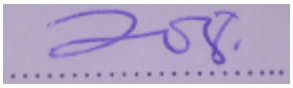
Signature:  Date: 23/09/2021

Kiplagat, Kibet Mohamed

TEC/PGMP/14/18

Declaration by supervisors:

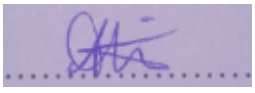
We declare that this thesis has been submitted for examination with our approval as university supervisors.

Signature:  Date: 23/09/2021

Prof. Zachary Otara Siagi

Mechanical & Production Engineering

Moi University, Eldoret - Kenya

Signature:  Date: 23/09/2021

Dr. Cleophas Achisa Mecha

Department of Chemical &Process Engineering

Moi University, Eldoret - Kenya

DEDICATION

I dedicate this work to my parents Mr. and Mrs. Kiplagat Indany, my wife Eudiah Sang, my son Ryan Kigen and to all my brothers and sisters; Rashid, Hassan, Sophie and Betsy.

ACKNOWLEDGEMENT

I would like to extend thanks to the following people and institutions, who so generously contributed to the work presented in this thesis. For without them this work would not have been completed.

I. **ACE-II PTRE center** for fully funding the program to completion.

II. My Supervisors, **Prof. Zachary Otara Siagi** and **Dr. Cleophas Achisa Mecha** for their valuable supervision throughout the course of this research. I thank them wholeheartedly.

III. Similar, profound gratitude goes to **ACE II PTRE** and **Moi University staff** for their support during my time in the institution.

IV. My colleges Alex, Ruto, Brain, Malanda, Mwape and Zeddy

V. Finally, but by no means least and above all the **Almighty God** for His grace, strength, sustenance and above all, his faithfulness and love.

ABSTRACT

Sugarcane vinasse contains toxins which pose serious challenges to the environment if not disposed properly. Previous studies have shown that anaerobic digestion (AD) of vinasse is a viable option for producing biogas and organic load reduction, however, biogas yield from anaerobic digestion (AD) is as low as 16%. The main objective for this study was to co-digest vinasse and pre-treated maize stalks (maize residues are abundant in most farms as a result of it being the major crop grown by most farmers) in order to find the optimum biogas yield. Specifically, the study aimed at characterizing the substrates; optimize operating conditions for biogas production and to characterize the gas and the resultant sludge (digestate). The vinasse was collected from Muhoroni Sugar Company and the maize stalks from maize farms in Uasin-Gishu county-Kenya. To characterize the substrates, the pH, moisture content, chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC) and nitrogen content were determined based on standard methods. The experiment design was done based on response surface methodology specifically Box-Benhken experiment design was used to optimize the biogas production by determining the experimental design matrix. The pH was varied from 6.5 to 7.5, temperature from 35^0C to 40^0C and ratio of substrates (maize stalks ratio to vinasse) from 25% to 75%.The biogas was analyzed using gas chromatography on flame ionization detector (FID) and the digestate potential hydrogen (pH), TS, TSS, TDS and COD were determined based on standard methods. The study established that the pH, moisture content, COD,TS, TSS, TDS, TOC and nitrogen content for vinasse were 4.34, 93.91%, 71.28gO/l, 7.05%, 6.04%, 1.01%, 2.23g/l and 2.7g/l respectively. The pH, moisture content, TS, TSS, TDS, TOC and nitrogen content for maize stalks were 7.52, 9.52%, 91.50%, 90.12%, 7.38%,

49.51g/kg and 1.28g/kg respectively. Consequently, the optimum yield was 3.99ml/ml of substrate at pH, temperature and substrate ratio conditions of 7.0, 37.5^{o}C and 50% respectively. The study further found that biogas yield from co-digestion was 3.99ml/vol. of substrate while digesting the substrates (Vinasse and maize stalks) independently yielded 1.32ml/ml of substrate and 1.54ml/ml of substrate respectively. Co–digestion bioreactors operating at 40^{o}C yielded shorter start-up times of 4 days while the ones operating at 37.5^{o}C and 35^{o}C were 7 and 10 days respectively. Analysis of the co-digestion product indicated that the average methane yield was 61.91%. In conclusion, the study established that the characteristics of the substrates and the optimum conditions made favorable conditions for multiplication of bacteria; in addition it also inferred that co-digestion enhances efficiency of the digester and subsequently more biogas yield is attained. The study recommends pH adjustment during different stages of methanogenesis for higher biogas yield and also it puts forward a room for co-digestion of vinasse with more other substrates.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATION

AD	Anaerobic digestion
APHA	American Public Health Association
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
COMESA	Common Market for Eastern and Southern African
EAC	East African Community
KALRO	Kenya Agriculture & livestock Research Organization
KEBS	Kenya Bureau of Standards
KPH	Potassium Hydrogen Phthalate
pH	Potential hydrogen
TDS	Total dissolved solids
TS	Total solids
TSS	Total suspended solids
UNDP	United Nations Development Program
CEC	Cation exchange capacity

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

High oil prices, increasing population, industrialization, and the decline in energy security have all led to an increase in global interest in biofuels. However, despite this growth, the global market for biofuels is still in its infancy. The future global potential for biofuel production is also difficult to estimate, due to a number of factors including the limits of natural resources and the need for food security above biofuel use. However, studies on biomass availability have concluded that by 2050, the possible contribution of biomass to global energy supply could vary from 100 EJ/year to 400 EJ/year, which represents 21% to 85% of the world‘s current total energy consumption, estimated at 470 EJ. Although biofuels are only a fraction of total biomass, biofuels still have the potential to play a significant role in meeting future global energy demand, if developed through appropriate channels (Bailis *et al.*, 2015). Many developing countries are faced with the dilemma of finding alternative energy sources with reduced environmental impact. Wind energy, hydro, solar and biofuels energy sources are potential solution. According to Earley et al. (2009), the ethanol and biodiesel production increased from about 4.8 billion gallons in 2000 to about 21 billion gallons in 2008, in the world. The goal of replacing fossil fuels with biofuels has resulted in high production of ethanol (Gil, 2011).

Ethanol is a prominent biofuel in the world because of its advantages; however, there is a challenge in disposing vinasse, which is the effluent from the distillation columns of ethanol industries (Wilkie *et al.,* 2000). It is said that for each liter of ethanol produced, between 0.8 and 3.0 liters of vinasse are obtained (Asocaña, 2011). Vinasse has dark color and low pH (Iqbal S., 2016). Concentration of total solid and chemical oxygen

demand (COD) value are very high. The pH condition of vinasse is 3.25-4.97 whilethe total solid (TS) value in vinasse is 63,000-79,000 mg/L (Iqbal S., 2016). Whereas, Budiyono *et al., 2014* reported that vinasse contains COD content of 299,250 mg/L. Wilkie *et al.,* 2000 further stated that vinasse is an organic liquid residue comprising of about 93% water, 5% organic matter mainly unfermented sugars and other carbohydrates and about 2% of inorganic dissolved solids. Vinasse contains many kinds of organic compounds such as acetic acids, lactic acids, glycerol, phenols, polyphenols and melanoidins (Budiyono *et al.*, 2014).

These vinasse characteristics pose serious challenges to the environment in disposal as supported and stated by the following authors; Vinasse contains abundant organic materials and has strongly acidic. It's COD and BOD content are very high (Iqbal S., 2016). If vinasse is discharged directly in to the rivers without treatment, water biota will be death. Dissolved oxygen in the rivers is used by oxidation bacteria to degrade COD and BOD. Hence, the availability of dissolved oxygen is running out, so water biota cannot breath and finally death (Summardiono *et al.,* 2013). A strongly acidic characteristic of vinasse causes remobilization of heavy metal in soil (Kafle *et al.,* 2012). The dark color in vinasse is not good for environment. Environment will be dirty and unsightly. Besides that, it also can hamper penetration of sun light in to the rivers, so water plant in the riverbed cannot do photosynthesis (Iqbal S., 2016). Soluble salts in vinasse can cause soil salinity and sodicity. Hence, soil structure become poor, not fertility. Vlyssides *et al.,* 1997 stated that high concentration of P and N nutrients cause eutrophication in water bodies. The temperature of fresh vinasse that is out from distillation unit is 65 – 105oC. If vinasse is disposed to bodies water, not cooled before, temperature of bodies water can increase. It can disturb the fish activity (Siles *et al.,* 2011).

Attempts have been made to establish ways to disposing vinasse with minimum negative effect on the environment. In Brazil, vinasse is applied directly to soil because of its organic matter and nutrient content; potassium, nitrogen and phosphorus which makes it a good organic fertilizer for sugarcane farms (Ferraz *et al.,* 2015).From an economic perspective, the soil application of vinasse represents the simplest and cheapest solution. However, continuous application of vinasse to the soil results in soil and groundwater contamination, leaching and salinization and seed germination inhibition (Ferraz *et al.,* 2015). Dávila *et al.,* (2009) evaluated the electro-flotation/oxidation process for vinasse, obtaining reductions in COD by 58%. Similarly, Yusuf (2007) achieved a 90% reduction in total organic carbon in vinasse through electro-coagulation with the use of a supporting electrolyte and the gradual addition of hydrogen peroxide. Goncalves (2006) performed research for the treatment of the vinasse by utilizing coagulation and flocculation. The study evaluated several variables influencing COD removal which was used to develop a model. The model demonstrated that the COD removal varied as a function of the pH and mixing. The best results were achieved when calcium oxide and ferrous sulfate were used, with the pH values of 12.4 the removal efficiencies were 52 and 44%, respectively. The study established that resulting sludge could be used as a fertilizer because it was rich in nutrient content. The major challenge in utilizing sludge as organic fertilizer from vinasse is the high pH of 12.4which cause soil pollution. According to Satyawali et al. (2007), anaerobic treatment is the most attractive primary treatment of vinasse due to the BOD and COD removal being over 80%, and energy recovery in the form of biogas. Ribas (2006) stated that the anaerobic reactors are a promising alternative because they accomplish a high rate of organic load removal and produce biogas. Iqbal S. (2016) in his review further supported and concluded that it is more effective

to degrade organic materials through anaerobic digestion than aerobic treatment. However, the value of COD removal is not maximum because of the presence of phenolic compounds in vinasse. He further stated that anaerobic digestion is a viable option for sugarcane vinasse processing and enables energy recovery as biogas production. To further support and minimize these challenges, the current study will co-digest vinasse with maize stalks.

Co–digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. The most common situation is when a major amount of a main basic substrate (such as manure or sewage sludge) is mixed and digested together with minor amounts of a single, or a variety of additional substrates. Better digestibility, enhanced biogas production/methane yield arising from availability of additional nutrients, improved buffer capacity with stable performance as well as a more efficient utilization of equipment and cost sharing have been highlighted as advantages of co-digestion (Mshandete & Parawira, 2009). Researchers have shown that co-digestion has resulted in improved methane yield by as much as 60% compared to that obtained from single substrates of as low as 16% (Babel, Sae-Tang & Pecharaply 2009). A wide variety of substrates, animal and plant wastes, as well as industrial wastes such as carbonated soft drink sludge and brewery wastes have been used for biogas production (Iyagba, Mangibo & Mohammad (2009).

Studies have established that maize stalks could be used as raw material for anaerobic fermentation (Adebayo *et al.,* 2014; Zhong *et al.,* 2011 and Bruni, 2010). A pretreatment process is required to decompose cellulose to reduce the volume of material and increase production of biogas (Antognoni *et al.,* 2013). In Uasin-Gishu county maize stalks are abundant since they are left in the farms and even some times

burned. The current study will co-digest vinasse and maize stalks to result in increased biogas yield.

1.2 Problem Statement

In Kenya, Mumias Sugar Company had intended to burn their vinasse as fuel in addition with wood chips in a vinasse-fired boiler to generate steam. However, the project failed due to inappropriate vinasse combustion technology and currently the company stores vinasse in a large dam. These challenges called for alternative disposal methods of such liquid wastes.

The study therefore sought to enhance biogas production through co-digestion of sugarcane vinasse and pre-treated maize residues. The process was intended to produce energy, reduce the COD and increase the pH of the resultant effluents; hence the by-product can be disposed into the environment as a soil amendment material.

The study sought to enhance biogas production through co-digestion of sugarcane vinasse and pre-treated maize stalks. This consequently reduced the COD of vinasse which can be disposed and/or used as fertilizer.

1.3 Objectives

1.3.1 Main objective

To optimize biogas yield through co-digestion of sugarcane vinasse and pre-treated maize stalks.

1.3.2 Specific objectives

To be able to accomplish this, the research was guided by the following specific objectives.

i. To determine the characteristics of substrates such as: pH, Moisture content, COD, TS, TSS, Nitrogen, Carbon and C/N ratio
ii. To evaluate the effect of pH, Temperature and Maize stalk ratio on

optimization of biogas yield.

iii. To characterize biogas and the resulting sludge.

1.4 Justification

Vinasse characteristics; usually an acidic compost (pH: 3.5–5), dark brown slurry, with a high organic content (COD: 50–150 g /L), and an unpleasant odor to humans (España-Gamboa *et al*. 2013) pose very serious challenges to the environment as stated by various studies. Summardiono et al. (2013) stated that if vinasse is disposed to the rivers without treatment, water biota will be death. This is because the dissolved oxygen in the rivers is used by oxidation bacteria to degrade COD and BOD. Hence, the availability of dissolved oxygen is running out, so water biota cannot breath and finally death. Strongly acidic of pH vinasse is not good for environment since it causes remobilization of heavy metal in soil (Kafle *et al.*, 2012). The dark color also can hamper penetration of sun light in to the rivers, so water plant in the riverbed cannot do photosynthesis (Iqbal S., 2016). Soluble salts in vinasse can cause soil salinity and sodicity. Hence, soil structure become poor, not fertility (Kafle *et al.*, 2012). Ferraz *et al.*, (2015) further supported that application of vinasse to the soil results in soil and groundwater contamination, leaching and salinization and seed germination inhibition.

1.5 Scope of the Study

This study focused on determining the most suitable pH, Temperature and substrate ratio for optimum production of biogas. The experiment design was done based on response surface methodology (Box-Benhken experiment design) to investigate the three factors (pH, Temperature and substrate ratio) under three levels. The characteristics of the substrates were determined using standard methods. Biogas

sample composition was determined and analyzed using gas chromatography on flame ionization detector (FID).

1.6 Organization of the Thesis

This thesis describes the optimization of biogas yield from co-digestion. The organization of this thesis is a follows. Chapter 1 introduces the concept in detail. It describes the background, problem statement, justification and aims of the study. Chapter 2 is focusing on reviewing available information about biogas technology, Vinasse characteristics, problems associated with disposal of vinasse and proposed ways of vinasse treatment. It also focuses on why we chose maize stalks as a substrate for co-digestion. Chapter 3 presents materials and methods used in the study. It lists the materials and describes the methods used. It also presents the experiment design and experiment setups. Chapter 4 contains results, analysis and discussions. On this chapter the effects of Temperature, pH and Substrate ratio are shown, interpretation and discussion of the experimental results are also presented. Chapter 5 contains the conclusions and recommendations of the study. It focuses on the findings of how pH, Temperature and Substrate ratio affects the biogas yield and ways of improving the yield. It also gives the summary of the study and directions for future work. The Appendix contains the descriptions of all methodologies used in the study.

CHAPTER TWO

LITERATURE REVIEW

This chapter presents a review of the literature relevant to this study. On this chapter a review of existing knowledge and gaps in biogas production from agricultural waste will be analyzed. The information presented in this section is as follows. Background related to biogas, anaerobic digestion, factors affecting biogas production, composition and characteristics of biogas, anaerobic co-digestion-Vinasse and maize stalks, pretreatments methods and summary.

2.1 Background of Biogas

Biogas typically refers to a mixture of gases produced by the breakdown of organic matter in the absence of oxygen, usually consisting of certain quantities of methane and other constituents. Biogas can be produced from raw materials such as agricultural waste, manure, municipal waste, plant material, sewage, green waste or food waste. According to Mclnerrney and Bryant (1981), the production of biogas involves the breaking down of complex polymers to soluble products by enzymes produced by fermentative bacteria which ferment substrate to short-chain fatty acids, hydrogen and carbon dioxide. The objective of this research was to optimize the production of biogas from Vinasse and maize stalks waste.

2.2 Biogas Production

Biogas production by anaerobic digestion is a process involving four main stages, namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis, stages that take place under the action of several species of bacteria *(*Gould, 2014). In the first stage, hydrolysis, fermentation bacteria convert the insoluble organic matter (cellulose) into sugars, amino acids and fatty acids.

During these step microorganisms of type *Clostridia*, *Micrococci*, *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Selenomonas*and*Streptococcus*are acting on the substrates *(Cirne, Lehtomaki, Bjornsson, Blackall*, 2007).

Acidogenesis is the fastest stage in the process of anaerobic conversion of complex organic matter, also known as acid fermentation stage. From this stage will result organic acids (acetic acid, propionic acid and butyric acid), fatty acids with short chain, alcohols, H_2, respectively CO_2 (Kalyuzhnyi, Veeken, Hamelers, 2000). In the third stage, acetogenic bacteria convert fatty volatile cids and alcohols into hydrogen (H_2), carbon dioxide (CO_2) and acetic acid, which represent the substrate for the last stage of the process, methanogenesis (Yi, Jia, Fuqing, Yebo, 2014)

In the last stage of anaerobic digestion, methanogenesis, are involved methanogenic bacteria which are very sensitive to changes of environmental factors, such as the pH and temperature. Under these conditions, the methanogenic bacteria are considered to be the limiting factor for the speed of the anaerobic digestion process (Chen, Cheng, Creamer, 2008). During this stage, microorganisms convert the previously formed hydrogen and acetic acid into methane (CH_4) and carbon dioxide (CO_2).

The following flow chart summarizes the different stages of the anaerobic digestion.

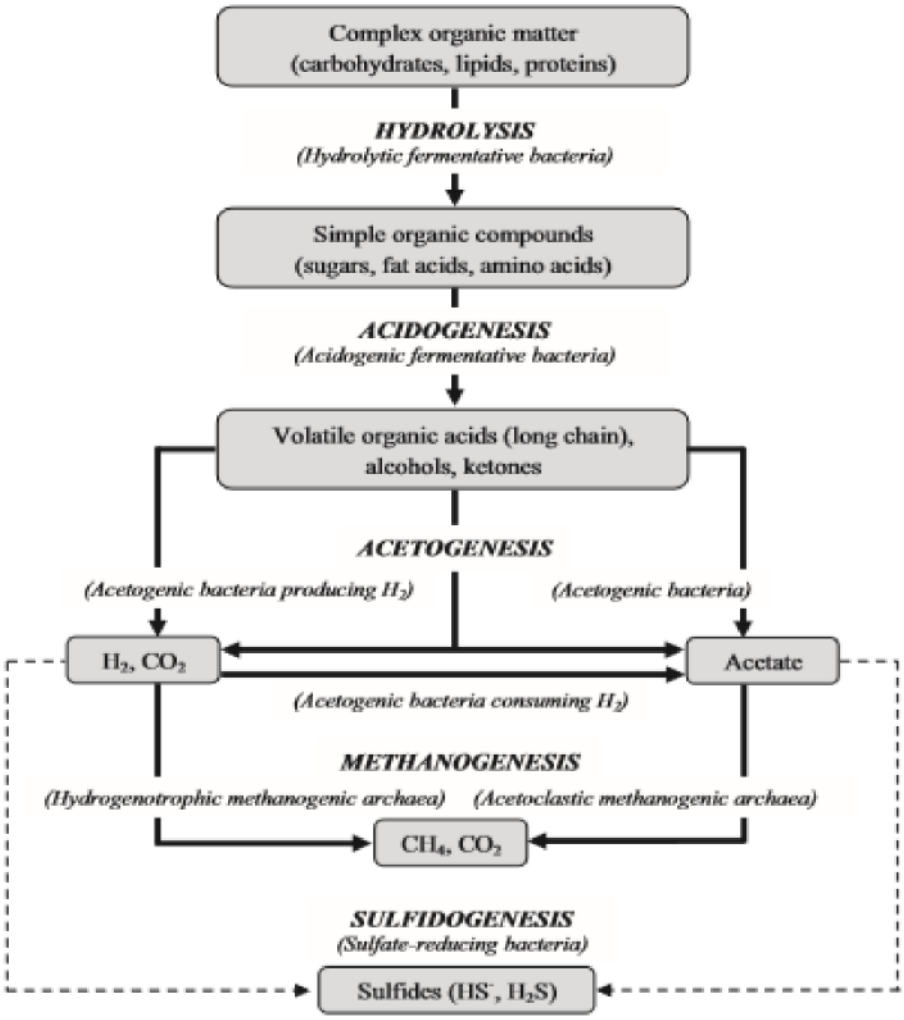


Figure 2.1 Scheme of the anaerobic digestion of complex organic matter, depicting the steps and microbial populations involved, (Moraes *et al.*, 2015)

The composition of biogas obtained in the process of anaerobic digestion varies depending on the used feedstock. Generally, the biogas has two major compounds, methane (CH_4) and carbon dioxide (CO_2), but it can also contains traces of impurities, such as hydrogen sulphide (H_2S), nitrogen (N_2) and ammonia (NH_3) (Da Costa Gomez, 2013).

It has to be noted that the methane yield varies significantly among different substrates based on their chemical composition in Figure 2.2 (Kougias *et al.*, 2018)**.** The theoretical methane yields of typical substances suitable for anaerobic digestion are presented in Figure 2.3

Category	Substrate	Methane yield[a] (mL-CH_4/g VS)	Reference
Livestock manure	Cattle manure	242–399	[2–5]
	Mink manure	239–428	[2,6]
	Pig manure	313–322	[3,7,8]
	Poultry manure	107–438	[2,8–10]
Agricultural wastes	Barley	322–335	[3]
	Corn silage	270–298	[5,11]
	Fruit & Vegetable waste	153–342	[8,12]
	Meadow grass	282–388	[2,3,7]
	Palm Oil Mill Effluents	378–503	[12]
	Rice straw	279–280	[8,13]
	Ryegrass	140–360	[3,11]
	Switchgrass	122–246	[5,8]
	Wheat	245–319	[3,8]
Oil/LCFA	Rapeseed oil	704±13	[14]
	Oleic acid	837±0.3	[14]
Household/ Municipal/ Industrial wastes	Kitchen waste	541–683	[8,15]
	Organic fraction of municipal solid waste	300–570	[16,17]
	Solid cattle slaughterhouse wastes	561–657	[16]
	Sewage sludge	249–274	[18,19]
Macroalgae	*Laminaria digitata*	359±5	[20]
	Saccharina latissima	285±19	[20]

Figure 2.2 Methane yields of various organic residues. (Kougias *et al.*, 2018)

Compounds	COD/VS (g/g)	CH_4 yield[a] (mL-CH_4/g VS)	CH_4 yield[a] (mL-CH_4/gCOD)	CH_4 content[a] (%)
Carbohydrate $(C_6H_{10}O_5)_n$	1.19	417	350	50
Protein[b] $C_5H_7NO_2$	1.42	497	350	50
Lipids $C_{57}H_{104}O_6$	2.90	1015	350	70
Ethanol	2.09	732	350	75
Acetate	1.07	375	350	50
Propionate	1.51	529	350	58
Iso-butyrate/Butyrate	1.82	637	350	63
Iso-valerate/Valerate	2.04	714	350	65

Notes: a) Methane yields are calculated at standard temperature and pressure conditions, i.e. 0°C and 1 atm. It is assumed that all the organic matter is converted to methane and carbon dioxide. b) Nitrogen is converted to ammonia (NH_3 or NH_4^+)

Figure 2.3: Theoretical Methane yields of typical compounds. (Kougias *et al.*, 2018)

Factors affecting biogas production are discussed below;

2.2.1 pH value

The optimum biogas production is achieved when the pH value of input mixture in the digester is between 6 and 7. The pH in a biogas digester is also a function of the retention time. In the initial period of fermentation, as large amounts of organic acids are produced by acid forming bacteria, the pH inside the digester can decrease to below 5. This inhibits or even stops the digestion or fermentation process.

Methanogenic bacteria are very sensitive to pH and do not thrive below a value of 6.5. Later, as the digestion process continues, concentration of NH_4 increases due to digestion of nitrogen which can increase the pH value to above 8. When the methane production level is stabilized, the pH range remains buffered between values of 7.2 to 8.2 (Maishanu *et al.*, 1990*).*

2.2.2 Temperature

The methanogens are inactive in extreme high and low temperatures. The optimum temperature is 35^{o}C. When the ambient temperature goes down to 10^{o}C, gas production virtually stops. Satisfactory gas production takes place in the mesophilic range, between 25^{o}C to 30^{o}C. Proper insulation of digester helps to increase gas production in the cold season. When the ambient temperature is 30^{o}C or less, the average temperature within the dome remains about 4^{o}C above the ambient temperature (Lund, Andersen and Torry-Smith, 1996).

2.2.3 Loading rate

Loading rate is the amount of raw materials fed per unit volume of digester capacity per day. In Nepalese conditions, about 6 kg of dung per m^3 volume of digester is recommended in case of a cow dung plant. If the plant is overfed, acids will accumulate and methane production will be inhibited. Similarly, if the plant is underfed, the gas production will also be low (BSP, 1992).

2.2.4 Retention time

Retention time (also known as detention time) is the average period that a given quantity of input remains in the digester to be acted upon by the methanogens. In a cow dung plant, the retention time is calculated by dividing the total volume of the digester by the volume of inputs added daily. Considering the climatic conditions of Nepal, a retention time of 50 to 60 days seems desirable. Thus, a digester should have

a volume of 50 to 60 times the slurry added daily. But for a night soil biogas digester, a longer retention time (70-80 days) is needed so that the pathogens present in human excrete are destroyed. The retention time is also dependent on the temperature and up to 35^{o}C, the higher the temperature, the lower the retention time (Lagrange, 1979).

2.2.5 Toxicity/ inhibitors

Mineral ions, heavy metals and the detergents are some of the toxic materials that inhibit the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g. sodium, potassium, calcium, magnesium, ammonium and sulphur) also stimulates the growth of bacteria, while very heavy concentration of these ions will have toxic effect (BRTC 1989). For example, presence of NH_4 from 50 to 200 mg/l stimulates the growth of microbes, whereas its concentration above 1,500 mg/l produces toxicity (Kougias *et al*., 2017 and Fotidis *et al.,* 2014). Similarly, heavy metals such as copper, nickel, chromium, zinc, lead, etc. in small quantities are essential for the growth of bacteria but their higher concentration has toxic effects (Fotidis *et al.,* 2014). Likewise, detergents including soap, antibiotics, organic solvents, etc. inhibit the activities of methane producing bacteria and addition of these substances in the digester should be avoided (Labatut *et al.,* 2014)

Although there is a long list of the substances that produce toxicity on bacterial growth, the inhibiting levels of some of the major ones are given in Table 2.1.

Table 2.1: Toxic level of various inhibitors; (The Biogas Technology in China, BRTC, China (1989))

Inhibitors	Inhibiting Concentration
Sulphate (SO_4--	5,000 ppm
Sodium Chloride or Common Salt (NaCl)	40,000 ppm
Nitrate (Calculated as N)	0.05 mg/ml
Copper (Cu++)	100 mg/l
Chromium (Cr+++)	200 mg/l
Nickel (Ni+++)	200 – 500 mg/l
Sodium (Na+)	3,500 -5,500 mg/l
Potassium (K+)	2,500 – 4,500 mg/l
Calcium (Ca++)	2,500 – 4,500 mg/l
Magnesium (Mg++)	1,000 – 1,500 mg/l
Manganese (Mn++)	Above 1,500 mg/l

2.3Bio-digesters

The Bio-digester is a physical structure, commonly known as the biogas plant. Since various chemical and microbiological reactions take place in the Bio-digester, it is also known as bio-reactor or anaerobic reactor. The main function of this structure is to provide anaerobic condition within it. As a chamber, it should be air and water tight. It can be made of various construction materials and in different shape and size. Construction of this structure forms a major part of the investment cost. Some of the commonly used designs are discussed below.

2.3.1 Floating drum digester

In this design, the digester chamber is made of brick masonry in cement mortar. A mild steel drum is placed on top of the digester to collect the biogas produced from the digester. Thus, there are two separate structures for gas production and collection. With the introduction of fixed dome Chinese model plant, the floating drum plants became obsolete because of comparatively high investment and maintenance cost along with other design weaknesses. In Nepal, KVIC design plants have not been constructed since 1986.

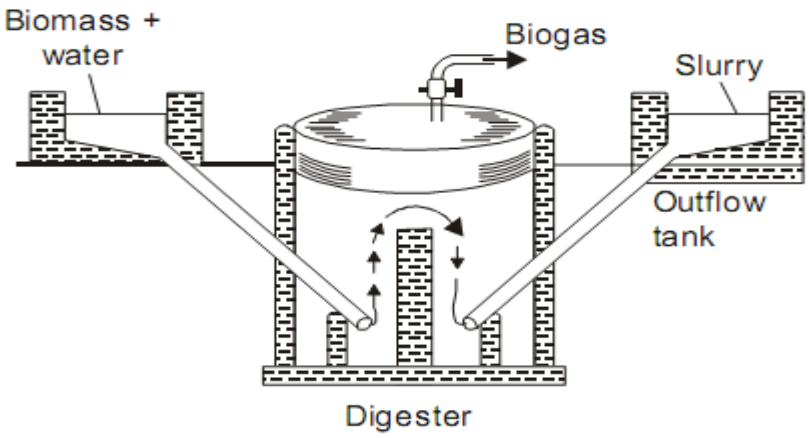


Figure 2.4: Floating drum digester (Source: Raja et al., 2006)

2.3.2 Fixed dome digester

Fixed dome Chinese model biogas plant (also called drumless digester) was built in China as early as 1936. It consists of an underground brick masonry compartment (fermentation chamber) with a dome on the top for gas storage. In this design, the fermentation chamber and gas holder are combined as one unit. This design eliminates the use of costlier mild steel gas holder which is susceptible to corrosion. The life of fixed dome type plant is longer (from 20 to 50 years) compared to KVIC plant. Based on the principles of fixed dome model from China, Gobar Gas and Agricultural Equipment Development Company (GGC) of Nepal have developed a

design and have been popularizing it since the last 17 years. The concrete dome is the main characteristic of GGC design.

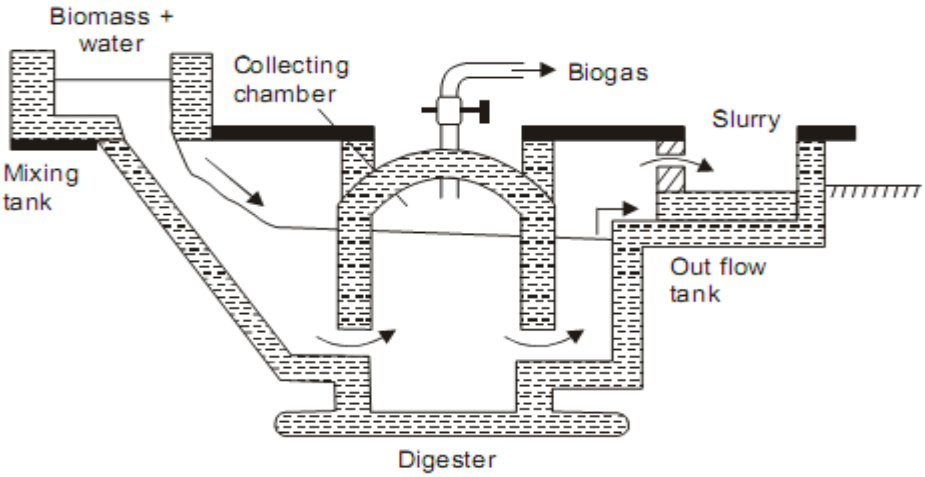


Figure 2.5: Fixed dome digester (Source: Raja et al., 2006)

2.3.3Deenbandhu model

In an effort to further bring down the investment cost, Deenbandhu model was put forth in 1984 by the Action for Food Production (AFPRO), New Delhi. In India, this model proved 30 percent cheaper than Janata Model (also developed in India) which is the first fixed dome plant based on Chinese technology. It also proved to be about 45 percent cheaper than a KVIC plant of comparable size. Deenbandhu plants are made entirely of brick masonry work with a spherical shaped gas holder at the top and a concave bottom. The South Asian Partnership/Nepal (SAP/N), an INGO working in Nepal, has introduced Deenbandhu model plants in Bardiya district of Nepal. About 100 plants were constructed by SAP/N in the villages of Bardiya district in 1994. Preliminary studies carried out by BSP did not find any significant difference in the investment costs of GGC and the Deenbandhu design plants. Recently, Environmental Protection and Social Development Association (EPA), a NGO, has constructed modified Deenbandhu design plants in Bardiya district which is also approved by Biogas Support Programme (BSP). In addition to above designs developed

particularly for household use in developing countries, there are other designs suitable for adoption in other specific conditions. Though they are not of much relevance to present conditions in Nepal, they could prove useful in the future. These designs are briefly described below for reference.

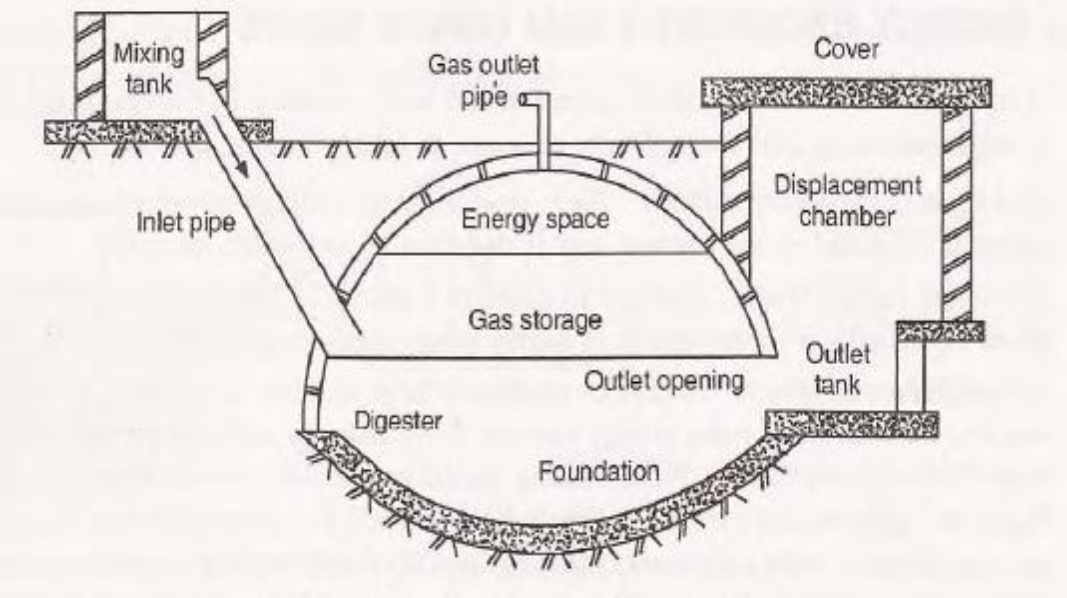


Figure 2.6: Deenbandhu model (Source: Raja et al., 2006)

2.3.4Bag digester

This design was developed in 1960s in Taiwan. It consists of a long cylinder made of PVC or red mud plastic. The bag digester was developed to solve the problems experienced with brick and metal digesters. A PVC bag digester was also tested in Nepal by GGC at Butwal from April to June 1986. The study concluded that the plastic bag Bio-digester could be successful only if PVC bag is easily available, pressure inside the digester is increased and welding facilities are easily available (Biogas Newsletter, No. 23, 1986). Such conditions are difficult to meet in most of the rural areas in developing countries.

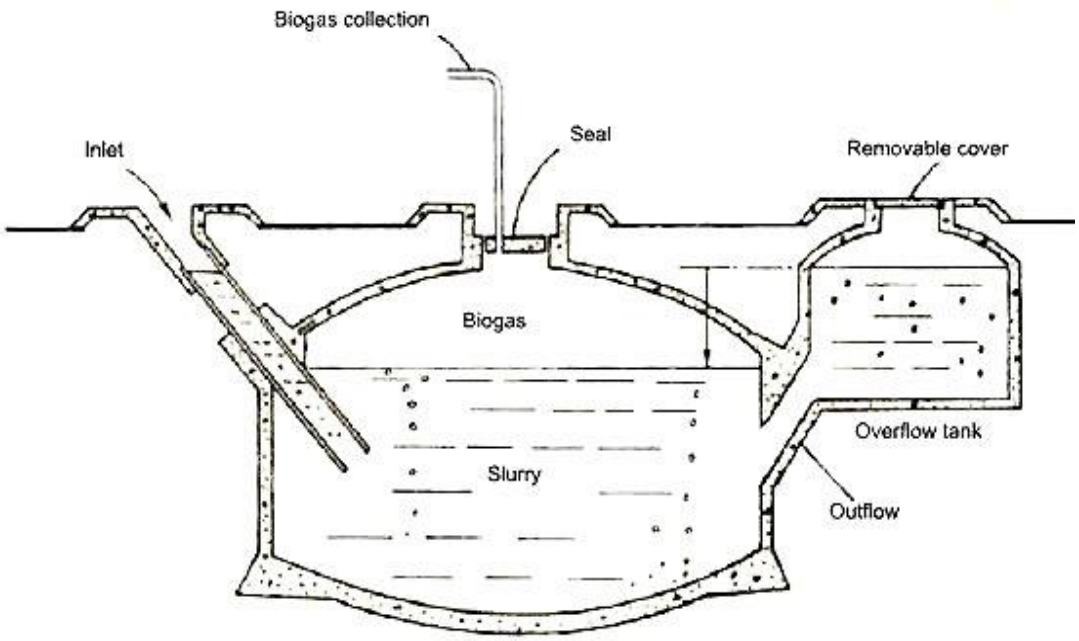


Figure 2.7: Bag digester (Source: Biogas Newsletter, No. 23, 1986)

2.3.5Plug flow digester

The plug flow digester is similar to the bag digester. It consists of a trench (trench length has to be considerably greater than the width and depth) lined with concrete or an impermeable membrane. The reactor is covered with either a flexible cover gas holder anchored to the ground, concrete or galvanized iron (GI) top. The first documented use of this type of design was in South Africa in 1957.

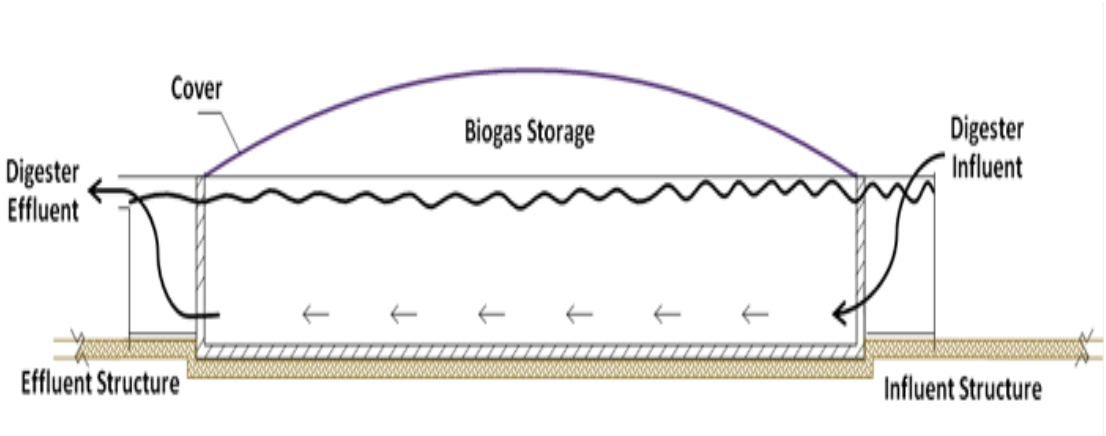


Figure 2.8: Plug flow digester (Source: Bio-energy Systems Report, 2004)

2.3.6 Anaerobic filter

This type of digester was developed in the 1950's to use relatively dilute and soluble waste water with low level of suspended solids. It is one of the earliest and simplest types of design developed to reduce the reactor volume. It consists of a column filled with a packing medium. A great variety of non-biodegradable materials have been used as packing media for anaerobic filter reactors such as stones, plastic, coral, mussel shells, reeds, and bamboo rings. The methane forming bacteria form a film on the large surface of the packing medium and are not carried out of the digester with the effluent. For this reason, these reactors are also known as "fixed film" or "retained film" digesters (Bio-energy Systems Report, 2004).

2.3.7 Up flow anaerobic sludge blanket

This UASB design was developed in 1980 in the Netherlands. It is similar to the anaerobic filter in that it involves a high concentration of immobilized bacteria in the reactor. However, the UASB reactors contain no packing medium; instead, the methane forming bacteria are concentrated in the dense granules of sludge blanket which covers the lower part of the reactor. The feed liquid enters from the bottom of the reactor and biogas is produced while liquid flows up through the sludge blanket. Many full-scale UASB plants are in operation in Europe using waste water from sugar beet processing and other dilute wastes that contain mainly soluble carbohydrates (Bio-energy Systems Report, 2004). Such reactor has not been experimented in Nepal. There are also other designs of anaerobic reactors which are of less interest in the context of Nepal due to their limited utility. Reduction in investment cost using alternative construction materials has been one of the main driving forces in the development of new designs. In an effort to achieve this objective, use of bamboo, plastics and other such cheap construction materials have also been tried with varying

degree of success (Cortsen *et al.*, 1995; Beteta, 1995). However, all such reported success stories are yet to take the form of implementation program in a mass scale.

2.4 Factors Influencing the Selection of a Particular Design or Biogas Model

The main factors that influence the selection of a particular design or model of a biogas plant are as follows:

i. **Economic**. An ideal plant should be as low-cost as possible (in terms of the production cost per unit volume of biogas) both to the user as well as to the society. At present, with subsidy, the cost of a plant to the society is higher than to an individual user.

ii. **Simple design**. The design should be simple not only for construction but also for operation and maintenance (O&M). This is an important consideration especially in a country like Nepal where the rate of literacy is low and the availability of skilled human resource is scarce.

iii. **Utilization of local materials**. Use of easily available local materials should be emphasized in the construction of a biogas plant. This is an important consideration, particularly in the context of Nepal where transportation system is not yet adequately developed.

iv. **Durability**. Construction of a biogas plant requires certain degree of specialized skill which may not be easily available. A plant of short life could also be cost effective but such a plant may not be reconstructed once its useful life ends. Especially in situation where people are yet to be motivated for the adoption of this technology and the necessary skill and materials are not readily available, it is necessary to construct plants that are more durable although this may require a higher initial investment.

v. **Suitable for the type of inputs**. The design should be compatible with the type of inputs that would be used. If plant materials such as rice straw, maize straw or similar agricultural wastes are to be used, then the batch feeding design or discontinuous system should be used instead of a design for continuous or semi-continuous feeding.

vi. **Frequency of using inputs and outputs**. Selection of a particular design and size of its various components also depend on how frequently the user can feed the system and utilize the gas.

2.5 Co-digestion

Co-digestion or co-fermentation is an efficient process in terms of energy that can improve the performance of anaerobic digestion by adding a secondary substrate which is rich in nutrients lacking from the initial feedstock (Parawira *et al.*, 1823)

According to *Mata-Alvarez et al*., 2011, the digestion of various feed stock in the same digester can establish a positive sinergy, enabling the microbial growth. *H.M. El-Mashad* and *R. Zhang* 2010 tested the efficiency of co-digestion process for a substrate consisting of cow manure and food waste, compared to separate digestion of the two substrates. They reported that biogas production obtained from food waste was of 657 L/kgVS after 30 days of digestion, while from fine, coarse and non-sieved fraction of cow manure were obtained 436, 404, respectively 366 L/kgVS. In terms of co-digestion of the two substrates, two mixtures were formed with the following composition: 32% food waste and 68% cow manure, the first mixture, respectively 48% food waste and 52% cow manure for the second mixture. After a period of 30 days of digestion, biogas production was of 455 L/kgVS for the first mixture and 531 L/kgVS for the second mixture. It was found that cow manure in co-digestion with food waste favors the increase of biogas production.

Another study of co-digestion was conducted by Zhang T. *et al.,* 2013, which followed the production of biogas from goat manure and several types of crop residues (wheat straws, corn stalks and rice straws) in different proportions of mixture. The results showed that the process of anaerobic co-digestion of goat manure with corn stalks or rice straws were effective, improving significantly the production of biogas. Thus, feedstock used in the following proportions, goat manure (GM)/corn stalks (CS) (30:70), GM/CS (70:30), GM/rice straw (RS) (30:70) and GM/RS (50:50) produced, after 55 days of digestion, 14840, 16023, 15608, respectively 15698 mL of biogas.

K. Bulkowska *et al., 2012* developed a study on the co-digestion process of energy crops of *Zea mays L.* and *Miscanthus sacchariflorus*, with pig manure in the following percentages: 0%, 7.5%, 12.5% and 25%. The authors concluded that pig manure favors biogas production and the content of methane, unlike the anaerobic digestion of individual energy crops.

Mirela *et al., 2017*, conducted co-digestion of animal manure and maize silage, the results showed that the use of maize in co-digestion process shows a positive effect on the yield and concentration of resulted methane. Adebayo *et al., 2014* determined the biogas production and methane concentration for cow slurry and maize stalk at mesophilic temperature (37^{o}C). They reported that higher biogas and methane yield was recorded at substrate with higher ratio of maize stalks.

Gimaiye *et al.,* 2019 co-digested avocado fruit peel and animal manure with the aim of optimizing biogas yield. The study showed that avocado peal waste is a potential substrate for biogas production and co-digestion improves efficiency of biogas production:- the substrate containing the animal manure were found to produce biogas with shorter time than the avocado fruit peal alone.

The advantages of the co-digestion process can be summarized as follows:

- Increases loading of readily biodegradable matter depending on the chemical composition of the used substrates (Thong *et al., 2012* and Borowski *et al.*, 2014).
- Improves buffer capacity of the influent mixture maintaining the pH levels within the range for methanogenesis (Zhang *et al., 2013*).
- Provides better nutrient balance, especially for improving the C/N ratio (Tsapekos *et al.*, 2017).
- Dilutes inhibitory compounds avoiding deterioration of the anaerobic digestion process (Tsapekos *et al.,* 2015).
- Leads to higher volumetric methane production (Sondergaard *et al.,* 2015).
- Promotes synergistic effects leading to advance biodegradation (Kougias *et al., 2018*).
- Contributes in solving problems related to the digesters' stirring or pumping, especially while processing solid wastes (Angelidaki *et al.,* 2003).
- Improves the economics of biogas plants (Koupaie *et al.,* 2014).

2.6 Sugarcane Vinasse

Vinasse is the effluent from the distillation columns of ethanol industries. Vinasse is also known as mosto, stillage, thin stillage, distillery wastewater, distillery spent wash, and distillery slops. The production of ethanol from biomass, whether from sugar crops (sugar beets, sugar cane, molasses, etc.), starch crops (corn, wheat, rice, cassava, etc.), dairy products (whey) or cellulosic materials (crop residues, herbaceous energy crops, bagasse, wood, or municipal solid waste), results in the production of vinasse (Wilkie *et al.,* 2000). The composition of vinasse is mostly water (93%) and solids (7%). It has a sharp smell and dark and light brown color. Its acidic pH (pH

3.5-5) and highly corrosive nature make this effluent approximately 100 times more polluting than domestic sewage (Freire and Cortez, 2000; Ribeiro *et al.,* 2007). All of these characteristics make vinasse an environmentally worrisome effluent as highlighted below.

Vinasse is a rich source of salts and organic contents, ranging from 24,000 to 80,000 mg L^{-1} of minerals and 4000 to 64,000 mg L^{-1} of organic matter. It has high contents of potassium, calcium, magnesium, sulfate, total phosphorus, and nitrogen, and significant concentrations of cumulative heavy metals such as copper (2.2-37.8 mg kg^{-1}), lead (0.5-8.8 mg kg^{-1}), zinc (2.7-47.7 mg kg^{-1}), and other metals that are detected, such as As, Cd, and Hg. All of these can contaminate soil, groundwater, sediments, and superficial water (Chen and Cho, 1993; Fuess and Garcia, 2014).

Therefore, vinasse can be used as a fertilizer owing to its nutrient content, mainly calcium and potassium, and its high organic material content and dissolved solids (Prasad *et al.,* 2008), which could be toxic or contaminating under certain conditions. Thus, vinasse can pollute soil and groundwater if disposed of in the environment. The content of dissolved organic matter in vinasse is high, varying from 10,973 to 14,801 mg L^{-1} of dissolved organic carbon (Christofoletti *et al.,* 2013). This high organic content may cause problems in groundwater through an increase in nutrient supply (Espan˜a- Gamboa *et al.,* 2011).

Vinasse with a high concentration of soluble solids is obtained when sugarcane, sugar beet, sweet sorghum, grape, and agaves are used. The characteristics of vinasse depend mainly on the raw material used for bioethanol production. In the case of sugarcane, this includes molasses, the substrate used in the fermentation process, the type and efficiency of fermentation and distillation, and the variety and maturation of the sugarcane. From these, 9-14 L of vinasse can be obtained per liter of alcohol

produced (Espan˜a-Gamboa *et al.,* 2011). This residue has high organic matter with elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which vary between 6-25 and 15-65 g L^{-1} O_2, respectively (Freire and Cortez, 2000; Ribeiro *et al.,* 2007). Its COD is high, ranging from 30 to 40 g/L (Polack *et al.*, 1981), which is associated with the putrefaction process that takes place as soon as it is discharged, releasing foul gases that make its environment unbearable. Typically, an ethanol distillery produces 12 liters of vinasse per liter of ethanol. Its solids content vary between 2 and 7%, if derived from sugarcane juice (Cortez and Pérez, 1997).

These waste waters also contain phytotoxic, antibacterial, and recalcitrant compounds such as phenols, polyphenols, and heavy metals, which have negative effects on microorganisms and plants at disposal sites.

Highly colored compounds are also found in vinasse. This characteristic leads to reduced sunlight penetration in rivers and lakes, thereby reducing photosynthetic activity and dissolved oxygen concentrations and causing hazardous conditions for aquatic life (Prasad *et al.,* 2008).

With regard to the concentration of solids in vinasse (Irisarri, 2006), this can vary according to the degree of dilution. Therefore, for diluted, semiconcentrated, concentrated, and solid vinasses, their amounts of total solids are 8%-10%, 20%-30%, 55%-60%, and 99%-99.9%, respectively. The composition of concentrated vinasse at 60 ^{o}Brix is 60%-65% dry matter, 16%-20% ashes, 4%-8% crude protein, 35%-42% carbohydrates, 5% sugars, and 4% potassium (Pe´rez and Garrido, 2006).

In general, diluted vinasse contains 377.0 g kg^{-1} dry matter of organic carbon and 380.1 g kg^{-1} dry matter of total carbon, whereas concentrated vinasse is composed of 359.8 and 363.9 g/kg dry matter of organic and total carbon, respectively (Parnaudeaua *et al.,* 2008). Concentrated vinasse is used as an alternative to overcome

some of the problems encountered when diluted vinasse has to be handled. The use of concentrated vinasse as a fertilizer avoids contamination of water bodies and facilitates its transport over longer distances, resulting in a lower-cost alternative. In addition, water from diluted vinasse is an important resource which can be used for different applications in the sugarcane industry, as well as to irrigate crops.

The increase in sugarcane production and processing to obtain important products such as sugar and ethanol has the negative aspect of increasing industrial residues as well. Several physicochemical characteristics of vinasse have been studied (Espan˜a-Gamboa *et al.*, 2011; Pe´rez and Garrido, 2006; Robertiello, 1982; Scull *et al.*, 2012). Vinasse characteristics such as density, viscosity, electric conductivity, boiling point, specific heat capacity, and heat of combustion have average values of 1.031 kg m^{-3}, 1.38 cP, 16.4 dS m^{-1}, 100.25^{o}C, 0.934 cal g $^{-1o}C^{-1}$, and 3.39 cal g^{-1}, respectively (Pe´rez and Garrido, 2006). The low pH (average of 4.3), electric conductivity, and chemical elements present in sugarcane vinasse may cause changes in the chemical and physicochemical properties of soils and watercourses, with frequent discharges over a long time, and may also cause adverse effects on agricultural soils and biota in general. In view of this, studies and green methods need to be developed aimed at sugarcane vinasse recycling and proper disposal.

2.7 Environmental concerns about vinasse

Environmental concerns regarding vinasse have been the focus of research and social feedback regarding regulating its disposal and applications, due to the volume of vinasse produced and its characteristics. Therefore, regulatory frameworks and laws worldwide have been developed over the years. In United States and Brazil, the first and second major producers of ethanol and consequently the highest generators of this byproduct the following detailed frameworks have evolved.

In the United States, the Resource Conservation and Recovery Act regulated by the Environmental Protection Agency (EPA) deals with the disposal of hazardous wastes, in particular in Parts 260 to 273.

Although many countries permit the use of vinasse in fertirrigation, the Renewable Fuel Standard Program (RFS2) Regulatory Impact Analysis (EPA EPA-420-R-10-006, 2010) states that this practice is not currently allowed in the United States. Nevertheless there are no specific restrictions on the use of vinasse as a fertilizer in the US Code of Federal Regulations. On the other hand, environmental legislation forbids the inappropriate disposal of vinasse into rivers, lakes, the ocean, and soil, owing to the high nutrient loading and the potential for polluting waterways (EPA EPA-420-R-10-006, 2010).

Even though in Brazil the disposal of vinasse in watercourses has been forbidden since 1978(Portaria MINTER no. 323, from November 29, 1978), no Federal Act has been applied regarding the disposal of this residue. On the other hand, a Law Project has been filed which establishes rules for vinasse storage, transport, and soil application as a byproduct of the sugarcane industry (PL 5182/2013). Despite this, the National Water Resources Policy (Act No. 9.433/1997) and the National Solid Waste Policy (Act No. 12.305/2010) requires the Brazilian states to establish their own laws and regulatory frameworks based on the national policies.

In this way, Sa˜o Paulo state, the largest Brazilian producer of ethanol and therefore of vinasse, has atechnical normative which defines the criteria and procedures for the storage, transportation, and application of vinasse in agricultural soil as well as the control of groundwater sampling monitoring(CETESB P 4231/2006). This normative reports all federal and state acts to be observed regarding storage, transportation, and application of vinasse.

Despite the regulatory frameworks and the potential of vinasse as a contaminant, the disposal of this residue in watercourses and soils has been the most common practice over the past 30 years. In the early 1980s, different destinations for vinasse were applied worldwide. In Brazil, vinasse used to be discharged into an adjacent waterway or land area and used for fertirrigation; in Australia, this residue was released in marine outfall, carried out to a conventional sewage or lagoon treatment, and or used in anaerobic digestion. On the other hand, in Japan, vinasse was incinerated to an ash to be used as fertilizer, and in the United States it was evaporated to be employed as animal and aquaculture feed (Willington and Marten, 1982).

Most recently, vinasse has been disposed of in sacrifice zones, which consist of ponds or swales (Marchi *et al.,* 2006), soaked with untreated vinasse, and thus became unusable for any other purpose (Corazza, 2006). Besides this procedure, other actions such as transportation and even storage for cooling are potential causes of soil and water contamination.

It has been possible to identify the infiltration of vinasse from conducting channels using porous cup probes and groundwater reservoirs by monitoring their pH, electrical conductivity, BOD, and complete nitrogen series: N-Kjeldahl, ammoniacal-N, NO_3^- and NO_2^- (Ludovice, 1997). Fig. 2.8 illustrates the main forms of the introduction of vinasse into water bodies.

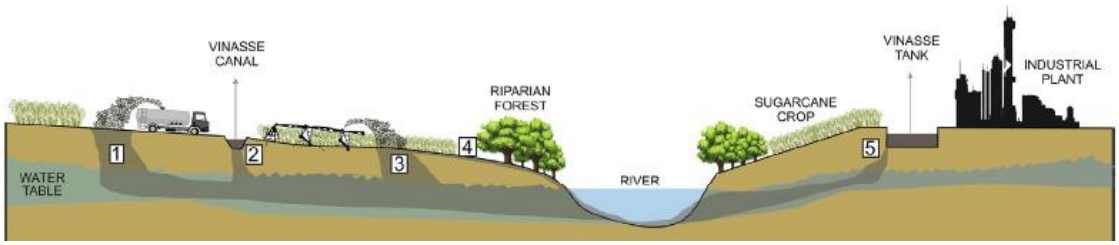


Figure 2.9: Main forms of introduction of Vinasse into water bodies: (1) and (3) Percolation from fertirrigation; (2) Percolation from a vinasse canal spill; (4) Runoff over soil; (5) Percolation from a vinasse tank. (Carrilho *et al.,* 2017)

Besides contamination resulting from storage, vinasse has been used in fertirrigation with direct application to the soil acting as a fertilizer owing to its high concentration of essential macronutrients and micronutrients. This procedure has as its main advantages economic agricultural inputs and clean water for irrigation (BNDES and CGEE, 2008; Christofoletti *et al.*, 2013; Fuess and Garcia, 2014; Macedo, 2005; Pimentel *et al.*, 2007; Sheehan and Greenfield, 1980; Smeets *et al.*, 2008; Willington and Marten, 1982).

Nevertheless, these practices may have adverse effects both for the environment and for agriculture. The first impacts of the disposal of vinasse on soil are those of the microbiota that comprise the vinasse, leading to development of fungi and bacteria that work in the mineralization and immobilization of nitrogen and its nitrification, denitrification, and biological fixation, as well as microorganisms that participate in biogeochemical cycles of other chemical elements (Giachini and Ferraz, 2009).

In the first 10 days after vinasse application, the pH experiences a considerable reduction and then rises abruptly, reaching values greater than 7. This is a result of the action of microorganisms, favoring the development of bacteria responsible for the decomposition of the vinasse, which returns the soil to its original pH value before the application of vinasse (Rossetto, 1987; Silva and Ribeiro, 1998).

When the organic matter of vinasse is incorporated into the soil, colonization by fungi takes place, converting it into humus and neutralizing soil acidity (Silva *et al.*, 2007). In this way, the process of microbiological decomposition of vinasse affects the physical and chemical characteristics of the soil.

With respect to the chemical contamination of soils, there are reports of increased concentrations of macronutrients such as K and P and metals such as Na, Fe, Zn, Mn, and C (Barros, 2010). The organic matter present in the vinasse gives the soil a lot of

negative charge, which hinders the leaching of cations and increases the cation exchange capacity (CEC) (Mutton *et al.,* 2014). In addition, the continued use of vinasse for fertirrigation can promote the saturation of cations in the soil (mainly K^+), resulting in, problems for groundwater (owing to the leaching of components), which may be contaminated, even with heavy metals.

Depending on the soil characteristics, the vinasse can affect its resistivity. The resistivity measurement of clay soils contaminated by vinasse has low resistivity values (between 10 and 90 U m), probably because of the influence of salts present in this effluent associated with water retention in the soil. These values are compared with those caused by contamination from manure, which is also conductive (Cruz *et al.*, 2008).

Moreover, direct and continuous application of vinasse in watercourses and soil can lead to serious environmental problems (Fuess and Garcia, 2014; Mohana *et al.,* 2009; Pant and Adholeya, 2007). The contamination of groundwater will depend on factors such as the soil composition and permeability/compression, which influence the velocity of the contaminant's transit into the aquifer; the shallowness of the water table; the relief of the region; rainfall and/or irrigation; soil pH; the amount of soil organic matter; and the composition of vinasse (Bedient *et al.,* 2000; Saracino and Phipps, 2002).

Fertirrigation areas in Brazil, monitored for 15 years, denote the maintenance of soil fertility and show an increase of NO_3^- in groundwater (Cruz *et al.,* 1992). In addition, pH is reported as being the main factor of fish toxicity when vinasse is disposed of in watercourses, including the development of liver disease in tilapia (Botelho et al., 2012; Ferreira et al., 2011; Marinho et al., 2014).

In Pernambuco, Brazil, the contamination of groundwater and of the Ipojuca River by a vinasse storage lagoon was indicated by changes in electrical conductivity, BOD, COD, concentrations of total dissolved solids, Cl^-, K^+, Ca^{2+}, and Mg^{2+} (Rolim *et al.*, 2013).

Some contaminants introduced into the environment by vinasse are of concern to human and animal health. As an example, NO_3^- ions are one of the main components of vinasse (around150-1600 mg L^{-1}) and are soluble in water and not promptly adsorbed in the soil, which contributes to their transport to groundwater. The EPA's Safe Drinking Water Interim Health Standard established 10 mg L^{-1} of NO_3^- for nitrogen, which equals 45 mg L^{-1} of NO_3^- (Saracino and Phipps, 2002).

The contamination by NO_3^- of water for human consumption can lead to serious health problems, and the intake of NO_3^- by children causes a drop in oxygen levels in the blood, leading to a potential death.

The authors also found the contamination of surface and groundwater by phosphate and nitrate, which has impacts on human and animal health and promoting interference in the development of plants and environmental eutrophication (Meurer *et al.,* 2000; Stevenson, 1986).

The impacts of practicing fertirrigation may affect other components of the environment. For instance, a study of the emission of greenhouse gases, especially N_2O, CH_4, and CO_2 from stored vinasse and from its application to soil, created an increase of 15% in greenhouse gas emissions in the agricultural phase of the ethanol production process. From the emitted gases, 99.8% was CH_4, and when applied to the soil, vinasse potentiated N_2O emissions and increasedCO_2 emissions in 46 and 31 kg/hectare in areas of burned and raw sugarcane, respectively (Oliveira *et al.*, 2015).

The disposal of vinasse can also cause population explosions of insects. An example is the Stomoxyscalcitrans outbreaks, a hematophagous fly that attacks animals and humans and can transmit diseases in sugarcane-producing regions of Brazil (Correa *et al.*, 2013). The influence of different sugarcane subproducts, bagasse, straw, straws with vinasse, and filter cake, on increasing this bug population was smaller on straw with vinasse than that observed for flies on the filter cake. Furthermore, the large area covered by filter cake would be potentially capable of producing around 24 million S. calcitrans/month in only 12.23% of the cultivated area where vinasse was applied monthly.

Table 2.2 shows the main reported environmental impacts promoted by using fertirrigation vinasse in soil.

Table 2.2: Environmental Impacts of Vinasse Use in Fertirrigation (Carrilho *et al.*, 2017)

✓ Commitment of soil structure and water bodies owing to high acidity and concentration of sulfates with consequent reduction in crop yields
✓ Soil salinization
✓ Increased salt concentrations in groundwater
✓ Increase in soil instability
✓ Overload of organic substances in soil, which may lead to clogging of pores of the soil and reduction in microbial activity caused by to low concentrations of dissolved oxygen, generating unpleasant odors as result of consumption of organic matter by anaerobic digestion
✓ Hyper-soil fertilization that can lead to the destabilization of soil structure and eutrophication of water bodies
✓ Contamination by abundant ions in the composition of vinasse such as Cl^-

and NO_3^- and heavy metals such as copper, lead, and zinc

- ✓ Permanent acidification of water bodies
- ✓ Interference in process of photosynthesis by aquatic plants and microalgae owing to high color and turbidity of vinasse
- ✓ Inhibition of seed germination with consequent reduction in crop yields
- ✓ Increase in soil resistivity
- ✓ Moderate increase in population of S. calcitrans (fly that attacks animals)
- ✓ Gas emissions that cause the greenhouse effect

Nevertheless, a number of studies have been conducted with the objective of finding an optimal solution, regarding the difficulties associated with the large volumes of vinasse. As a result some alternatives have been proposed,

In Australia, Brazil and other countries have been applying untreated vinasse to fertilize sugarcane fields for many years. However, using vinasse for fertilizer generates water quality problems, due to its high COD, low pH, and high concentrations of various constituents (Turner *et al.*, 2002).

Goncalves (2006) performed research for the treatment of the vinasse by utilizing coagulation and flocculation and the factorial planning technique. The study evaluated several variables, specially, the COD removal. The COD removal efficiencies were 52% and 44% respectively. The study established that resulting sludge could be used as a fertilizer because it was rich in nutrient content. The major challenge in utilizing sludge as organic fertilizer from vinasse is the high pH of 12.4 which cause soil pollution. The resulting sludge could be used as a fertilizer because it was rich in

nutrient content. Fernández *et al.,* (2001) used activated carbon and natural zeolite as support materials in their research for vinasse treatment in an anaerobic fluidized bed reactor. The study achieved an organic loading rate of 10 kg COD/m^3 day, with COD removal above 70%. The methane production was 2 l/d.

According to Satyawali *et al.* (2007) anaerobic digestion is the most attractive primary treatment due to the BOD removal rate being over 80%, in addition to the energy recovery in the form of biogas. Dávila *et al.,* (2009) evaluated the electro-flotation/oxidation process for vinasse, obtaining reductions in COD by 58%. Similarly, Yusuf (2007) achieved a 90% reduction in total organic carbon in vinasse through electro-coagulation with the use of a supporting electrolyte and the gradual addition of hydrogen peroxide

Ribas (2006) has stated that the anaerobic reactors have shown to be a promising alternative because they accomplish a high rate of organic load removal and produce biogas. Wilkie *et al.,* (2000) advocated the advantages of the anaerobic digestion because of its effective reduction of the organic load and because it produces biogas.

2.8 Maize Stalks

Maize is a cereal crop that is grown widely throughout the country and generally consumed by Kenyans than any other grains (IITA, 2009). It can be eaten after cooking or smoking and can also be converted into animal feeds. The wastes of maize which are left behind after harvest include the husks, chaff, stalks and the leaves (Oseni and Ekperigin, 2007). Chaffs, cobs and stalks are among the prominent wastes associated with maize. These disposed maize wastes have some positive and negative effects on the environment. In Kenya, maize is abundantly produced and valued as the stable food for Kenyan households (IITA, 2009).

Lignocellulose is the main component of plants and it is made mainly of cellulose, hemicellulose and lignin. The carbohydrate fraction of lignocellulose (cellulose and hemicellulose) is called holocellulose. Cellulose is the main component of lignocellulose. Cellulose is a polymer made of cellobiose units (two β-1, 4-glycosidic bound glucose molecules) rotated by 180 degrees with respect to the neighbor molecules. Cellobiose units form chains with number of units between 100 and 14000. These chains are grouped into water-insoluble aggregates (elementary fibrils) that present crystalline regions and less ordered amorphous regions. Elementary fibrils are organized in microfibrils, which are embedded into a matrix of hemicellulose and lignin (Klemm *et al.,* 2005).

Hemicelluloses are not chemically homogeneous. Hemicellulose is composed mainly by pentose sugars and the basic structure is formed by 1,4-bound xylose units with different side chains. Other carbohydrates forming hemicellulose are arabinan, mannan, galactan, glucan. The degree of polymerization of hemicellulose is between 70 and 200. Depending on the plant species, hemicellulose is acetylated to different degrees: some of the hydroxyl (-OH) groups at C_2 and C_3 of the xylose units are replaced by O-acetyl groups (Sassner *et al.,* 2008).

Lignins are phenolic polymers with complex three-dimensional structure. The monomeric unit is composed by an aromatic nucleus with an aliphatic chain, $(C_6\text{-}C_3)^n$. Lignin polymers are formed by syringyl, guaiacyl and p-hydroxyphenyl units that are chains formed from sinapyl alcohol, coniferyl alcohol and pcoumaryl alcohol, respectively. The composition of lignin changes depending on the plant species and age. Guaiacyl lignin is the most abundant compound in lignins from conifers, syringyl and p-hydroxyphenyl in dicotyledons angiosperms, while lignins from

monocotyledons angiosperms (grasses and herbaceous crops) contain the three components in similar proportions (Widsten & Kandelbauer, 2008).

Anaerobic digestion of these wastes will not only produce biogas and a residue organic waste that has superior nutrient qualities over the usual organic fertilizer, it also minimizes environmental pollution as it reduces greenhouse emission (Voss, 2007). The use of low-cost feedstock is crucial to obtain cost-effective biotechnologies for biogas production (Rabelo, 2009). In biogas plants digesting agricultural residues (lignocellulose), the low digestibility of the substrate causes a loss of methane production and limits the overall efficiency of the process (Jin et al., 2009). Agricultural residues such as straw and maize stalks are among the low-cost feedstock's, but they are relatively recalcitrant to anaerobic digestion and need treatment to be efficiently degraded in biogas processes (Demirbas, 2008).

The quality of the substrate can be further improved with treatments prior to anaerobic digestion. Treatments on lignocellulosic materials are made to facilitate the hydrolysis of the sugars (cellulose and hemicellulose). However, being successful in improving the methane yield of the substrate is not enough for a treatment to be considered optimal for biogas production. The degradation or loss of organic matter and the formation of inhibitors have to be avoided. Some treatments are capital-intensive, while other treatments are too slow or have high energy requirements. The choice of the treatments for industrial applications has to take into account technological and environmental factors such as energy balance, recycling of chemicals and downstream processes (Liu & Zhang, 2008).

2.9 Pre-treatment Methods

Pre-treatment of substrates can increase biogas production and volatile solids and solubilisation of substrates which make it more accessible to enzymes (Tanaka *et al.*,

1997). They are particularly useful in the digestion of lingo-cellulosic materials as they contain high cellulose or lignin level. Pre-treatment can disrupt these recalcitrant polymers chemically, thermally or physically. The addition of pre-treatment can enhance the biogas rate or reduce the time of start-up, however, the additional cost must be considered to be balanced against resultant improvements in efficiency (Alastair J. Ward 2008).

Lignocelluloses are tough materials which have complex and rigid structures resistant to mechanical stress and enzymatic attack, insoluble in water. Water molecules cannot enter the lignocellulosic fiber because of the combination of accessible surface area, presence of lignin andcrystallinity of cellulose. The fibres are protected and strengthened by lignin which is inhibiting to the action of enzymes (Saulnier *et al.*, 1995). Furthermore, the crystalline structure of cellulose decreases the availability of surface contact to enzymes (Hendriks, 2009).

1. It is hard to identify the most suitable pre-treatment for all types of lignocellulosic materials (Hann-Hägerdal *et al.*, 2006). The effective pre-treatment should have three qualities: Increase the porosity of the substrate which makes the carbohydrates more accessible for enzymes,
2. Preserving the different fractions without losing or degrading organic matters
3. Limiting the formation of inhibitors.

Furthermore, the pre-treatment should take economic issues into consideration. Each pre-treatment has advantages and drawbacks. The optimal operation depends on the characteristics of the materials. The main purpose of pre-treatment for biogas production is to increase the accessibility to the hemicelluloses content of the lignocellulosic material (Hendriks, 2009)

Several pre-treatment techniques have been studied intensively prior to both biogas and ethanol production from lignocellulosic substrates with respect to facilitating the biological degradation. Each of the treatment methods has its advantages and drawbacks. These pre-treatment methods can be divided into mechanical, thermal, chemical as well as, biological treatments or a combination of these techniques as it is shown in Table 2.3.

Table 2.3: Pre-treatment Techniques (Muller, 2000)

Technique	Subdivision
Mechanical	Milling
Thermal	Steam explosion
	Thermal hydrolysis
Chemical	Acid hydrolysis
	Alkaline pre-treatment
	Ionic liquids pre-treatment
Biological	Fungi
Co-digestion	Two or more substrates

I. Thermal pre-treatment

It has been known for many years that a thermal pre-treatment can improve the degradability of sludge. While the carbohydrates and the lipids of the sludge are easily degradable, the proteins are protected from the enzymatic hydrolysis by the cell wall. Heat applied during thermal treatment destroys the chemical bonds of the cell wall and membrane, thus makes the proteins accessible for biological degradation. Maximum biodegradability, in percentage, meaning the maximum percentage of substrate COD that is converted to methane, was calculated so that biodegradability can serve as an indicator for measuring the biogas production (El-Mashad *et al.*, 2004). Thermal pre-treatment has been studied using a wide range of temperatures ranging from 60 to 270 º C. In practice, the optimum temperature is in range of 160-

180ºC and treatment times from 30 to 60 minutes pressure associated to these temperatures may vary from 600 to 2500 kPa (Weemaes & Zeeman, 1998). Various experiments and research of thermal pre-treatment have been done to proclaim this conclusion.(Li & Noike, 1992) showed that optimum temperature in terms of 33% volatile suspended solids degradation increased and 100% methane production was at 170ºC and contact time was 60min. No further improvement for longer contact times (Gavala *et al.*, 2008) who concluded that temperature and duration of the optimum pre-treatment depend on the nature of the sludge: the greater the proportion of difficult hydrolyzing biological sludge substrates, higher the intensity of pre-treatment needed. Research was done to compare the thermal pre-treatment performance of waste activated sludge collected from urban wastewater plants with untreated sludge samples under (130 º C, pH=10, 150 º C and 170 º C during 30min) conditions (Bougrier *et al.*, 2006). The results indicated that there was positive effect on solubilisation rates and methanization when thermal pre-treatment was added. Particularly, the 170 º C treatment led to comparable results in anaerobic digestion performance increase: about 80% improvement in removal of matter and in biogas yield. (Haug and co-workers, 2002) worked on thermal treatment at lower temperatures in order to improve dewater ability as well as digestibility and at the same time avoid the problems that occurred with higher temperature thermal pre-treatment. They showed that the largest effect on digestibility was for activated sludge was at 175ºC. This temperature was about the limit for digestibility before digestion was inhibited (presumably because of the formation of inhibitory and/or refractory compounds).

At 175 º C, digestion of the thermally pre-treated sludge resulted in an increase of 60-70% in methane production over not pre-treated sludge. Higher temperatures led to decreased gas production.

In general, thermal pre-treatment of waste activated sludge can considerably increase methane production for mesophilic anaerobic digestion with temperatures between 25- 45ºC and to a lesser extent for thermophilic anaerobic digestion with temperatures above 45ºC, for that thermophilic digestion is already more efficient at volatile suspended solids reduction and methane production as compared with mesophilic digestion, hence reduces benefits of pre-treatment.

Most works have shown that excessively high temperatures (higher than 170-190º C) lead to decreased sludge biodegradability in spite of achieving high solubilisation efficiencies. Indeed, in some cases, there is formation of toxic, refractory compounds during pre-treatment which is a major drawback (Delgenes *et al.,* 2002). In previous works, the effect of thermal treatment on biogas production is summarized in Table 2.4

Table 2.4: The effect of thermal treatment on biogas production *(Source: Delgenes et al., 2002)*

Sludge	Treatment condition	AD conditions	Results
Activated sludge (Haug *et al.*,1978)	175^{O}C 30 min	CSTR HRT: 15 days 35^{O}C	Increase of CH4 production (+82%)
Activated sludge (Stuckey & McCarty,1978)	175^{O}C 60 min	Batch 25 days 35^{0}C	Increase of CH4 production (+42%)

Activated sludge (Y.Y & Noike, 1992)	$175^{O}C$ 60 min	CSTR HRT: 5 days $35^{O}C$	Increased of gas production (100%)
Activated sludge (Fjord side, 2001)	$180^{O}C$	WWTP 45000 PE CSTR HRT: 15 days	Increased of biogas production (+80%)
Digested mixed sludge (Dohanyos et al, 2004)	$170^{O}C$ 60 min 0.8 MPa	Batch 20 days	Increased of gas production (+49%)
Activated sludge (Valo et al, 2004)	$170^{O}C$ 60 min	Batch 24 days $35^{0}C$	Increased of gas production (+45%)
	$170^{O}C$ 60 min	CSTR HRT:20 days $35^{0}C$	Increase production of CH_4 (+81%)
	$170^{O}C$ 30 min	Batch 24 days $35^{0}C$	Increase production of CH_4 (+78%)
Activated sludge (Bougrier et al., 2008)	$170^{O}C$ 30 min 7 bar	CSTR HRT:20 days	Increase production of CH_4 (+51%)
	$170^{O}C$ 30 min 7 bar	Batch	Increase production of CH_4 (+50%)

170^{O}C 30 min 7 bar	Continuous HRT: 12 days	Increase production of biogas (+40 -50%)

II. Chemical pre-treatment

Chemical pre-treatment is also an efficient and cost-effective method to hydrolyze the cell wall and membrane and thus increase solubility of the organic matter contained within the cells. According to different principles, chemical methods can be divided to acid and alkaline (thermal) hydrolysis, oxidation.

The most frequent studies oxidative methods are ozonation and peroxidation. Acid and alkaline hydrolysis will be introduced in the thermo-chemical pre-treatment part.

III. Ozonation

Ozone (O_3) is a strong cell-lytic agent, which can kill the microorganisms in activated sludge and further oxidize the organic substances released from the cells (Cui & Jahng, 2004; Saktaywin *et al.*, 2005). Of the techniques to disintegrate sludge, ozonation of sludge is one of the effective ways and yields the highest degree of disintegration (Muller, 2000). Following ozonation, the characteristics of the sludge are greatly changes. The flocs are broken down into fine, dispersed particles. Floc integration and solubilisation generates a large number of micro-particles dispersed in the supernatant in addition to soluble organic substances (Libing *et al.*, 2009). The sludge biodegradation is affected by ozone dose. Several researchers have investigated the impact of ozone dose on sludge biodegradation.

Ozonation treatment has two counteracting effects: degradation of molecules and cell structures that are non-degradable for methanogenic bacteria will increase

biogas production; oxidation of organic molecules that are degradable for methanogenic bacteria will decrease biogas production (Levlin, 2010). It was found that around 60% of soluble COD generated due to ozonation was biodegradable at the early stage of ozonation, while the remaining soluble organic matter was refractory (Saktaywin *et al.*, 2005).When the ozone dose was 0.1 g O3/g TSS, the biodegradation was about 2-3 times greater compared with raw sludge in both aerobic and anaerobic conditions for 5 days. (Yeom *et al.*, 2002)

The biogas production increased with 80% at ozone treatment with 0.1 g O3/g COD, the effect was not pronounced at higher ozone concentration (Weemaes *et al.*, 2000). the previous works are summarized in Table 2.5.

Table 2.5: The effect of ozonation pre-treatment on biogas production *(Source: Weemaes et al., 2000)*

Sludge	Treatment conditions	AD conditions	Results
Mixed sludge (Paul *et al.*, 2005)	0.1g O_3 g^{-1} COD	Batch 30 days 33^0C	Increase of CH_4 biogas production(+100%),respectively
Activated sludge (Bougrier *et al.*,2007)	0.15g O_3 g^{-1} TS	Batch 18 days 35^0C	Increase of biogas production (+145%)
Activated sludge (Valo *et al.*,2004)	H_2O_2: 150 mmol.L^{-1} $FeSO_4$: 5mmol.L^{-1} 90^0C,60 min	Batch 24 days 35^0C	Increase of biogas production (+16%)

Due to its well-known potential and performance, sludge ozonation is used in combination with activated sludge process in wastewater treatment plant. A review of

studies concerning the combination of ozonation with activated sludge process has been recently proposed. The schematic process is as shown in figure2.10 (Chu *et al.*, 2009).

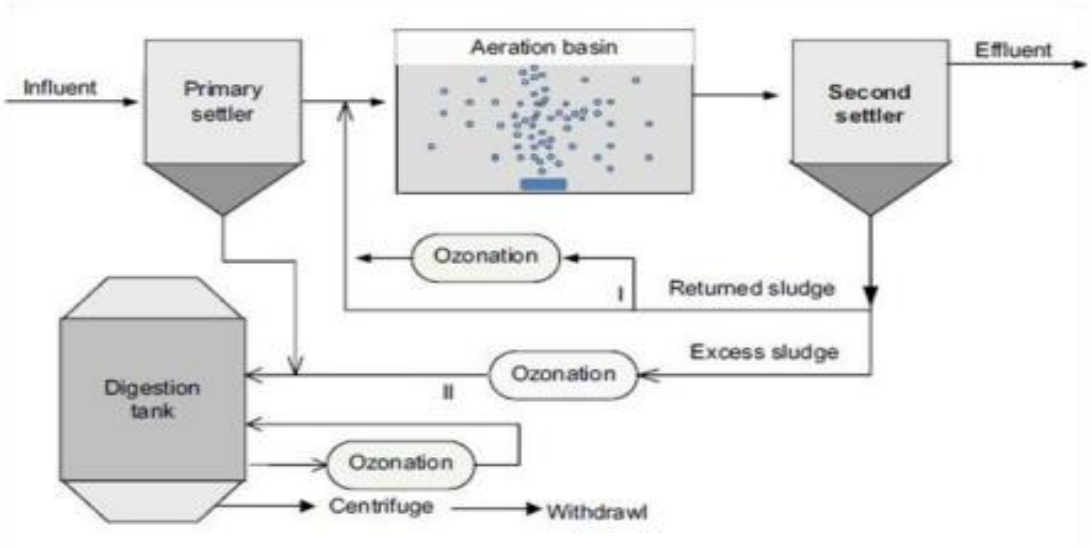


Figure 2.10: Ozonation process (Source: Chu et al., 2009)

Ozonation can be introduced to the returned activated sludge line (Route I) or to the sludge digestion line (Route II). For Route I, the ozonation aims to reduce excess sludge production by promoting cryptic growth.

IV. Thermo chemical pre-treatment

Alkali treatment is normally combined with thermal treatment; it‘s so called thermo chemical treatment. There is no consensus on the efficiency of alkali agents. The efficacy of alkali agents in order for sludge solubilisation was Sodium Hydroxide (NaOH)>Potassium Hydroxide (KOH)> Magnesium Hydroxide ($Mg(OH)_2$) and Calcium Hydroxide ($Ca(OH)_2$), (J. Kim *et al.*, 2003). Separately it was demonstrated that pre-treatment with KOH was more efficient than using NaOH (Penaud *et al.*, 1999). With regard to the effect of thermo chemical pre-treatment (addition of alkali) on solubilisation and biodegradability, different studies give contradictory results. (Haug *et al.*, 1978) determined a decrease in biodegradability of

60%, while (Penaud et al. 2000) observed no effect on the biodegradability. (Tanaka *et al.,* 1997) showed that thermo chemical pre-treatment led to significant increase in biodegradability, which could reach 230%. It's noted that thermo chemical pre-treatment gives the best results in the biogas production compared with thermal, chemical, ultrasonic methods under the same conditions.

V. Mechanical Pre-treatment

Mechanical pre-treatment plays an important role because it favours solubilisation of particulate matters in liquid phase. In general, the most often used techniques in mechanical pre-treatment are ultrasonication, grinding and high pressure homogenization. By these methods, the aim is to increase the degradability of organic matters by disrupting the flocs and/or lysing the bacterial cells. The principles and applications of the methods above will be introduced hereafter.

i. Ultrasonic pre-treatment

Ultrasonication is a promising and effective mechanical pre-treatment method to enhance the biodegradability of the sludge. This technology has several inherent merits like efficient sludge disintegration (>95%), improvement in biodegradability, improved bio solids quality, increase in methane percentage in biogas, no chemical addition, less retention time, sludge reduction and energy recovery (1kW) of ultrasound energy generates 7 kW of electrical energy including losses (Pilli *et al.,* 2010). Ultrasonication enhances the sludge digestibility by disrupting the physical, chemical and biological properties of the sludge. As mentioned above, hydrolysis is the rate-limiting step in anaerobic digestion process. Ultrasonication accelerates the hydrolysis reactions by disrupting cells. The bacterial cells are disunited by pressure waves and cavitations generated from an ultrasonic generator leading to elution of intracellular organic substances (Takatani *et al.,* 1981).

In addition, some soluble particulate organic matter may even be transformed into a soluble state under the cavitational explosion of transient bubbles. The disruption of sludge particles derived from sonication treatment would enhance subsequent acidogenesis, acetogenesis and methanogenesis reactions, which would in turn lead to an improvement in methane generation and reduction of sludge volume (Kuan *et al.,* 2007).

ii. Particle size reduction

One predominant technique is the wet milling, which is more of a grinding method. Wet milling uses small beads to rupture cell walls, the size of the beads used are thus critical for maximal sludge disintegration (Baier & Schmidheiny, 1997). Of several milling devices, the ball mill using small diameter (0.2-0.25 mm) balls has the best performance (Allan & Talat, 2007). The use on an agitator ball mill was studied. Sludge was pressed through a cylindrical or conical space by an agitator including shear-stresses high enough to break the bacterial cell walls (Kunz *et al.*, 1994).

iii. High pressure homogenizer

One of the most frequently used methods for large-scale operation is high pressure homogenization, compressing the sludge to 60 MPa (Harrison, 1991). The compressed suspension is then depressurized through a valve and projected at high speed against an impaction ring. The cells are hereby subjected to turbulence, cavitation and shear stresses, resulting in cell disintegration (Lise *et al.*, 2008). Some studies reporting the effects of high pressure homogenizer on biogas production are also summarized in Table 2.6.

Table 2.6: Effect of Particle size reduction on biogas production(Source: Lise *et al.,* 2008)

Sludge	Treatment conditions	AD conditions	Results
Activated sludge (SRT:7 days) (Baier & Schmidheiny, 1997)	Balls diameter:0.25mm Balls velocity:10m/s 9 min, 60^0C	Batch 21 days 37^0C	Increase of biogas production (+10%)
Anaerobic digested sludge (Baier & Schmidheiny, 1997)	Balls diameter:0.25mm Balls velocity:10m/s 9 min, 60^0C	Batch 21 days 37^0C	Increase of biogas production (+62%)
Activated sludge (Choi *et al.,* 1997)	Plate collision ΔP: 30 bar	Batch 26 days 35^0C	Increase of VS removal (+43%)
Activated sludge (Engelhart *et al.,* 1999)	Homogenizer ΔP:300 bar (750 kJ/kg TS)	CSTR HRT: 10-15 days 35^0C	Increase of CH4 production (+60%)
	Homogenizer ΔP: 600 bar	CSTR HRT: 20 days 36^0C	Increase of biogas production (+18%)

2.10 Summary

On this chapter a review of existing knowledge and gaps in biogas production have been enumerated. Information about biogas technology from anaerobic digestion, biogas digesters, factors affecting biogas production, composition of biogas to anaerobic co-digestion have been showed. Vinasse characteristics, challenges facing its disposal and proposed alternatives for treatment have been reviewed. Existing literature on maize characteristics, potential substrate for biogas production and ways of treatment have also been highlighted.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Collection of Raw Materials

The test substrates consisted of maize stalks and sugarcane vinasse. Maize stalks was collected from the farms in Uasin-Gishu County (Indany Kipsum farm) during harvesting season and dried. The maize stalks samples were milled and dried at ambient conditions to average equilibrium moisture content of 10% (±1.5).

The Vinasse was obtained from Muhoroni Sugar Company, ethanol plant, collected in to 20 litres plastic container and transported to the laboratory.

Table 3.1: Materials, reagents and apparatus

Raw materials	**Reagents**	**Equipment/Apparatus**	
			Model
Maize stalks Vinasse	Sodium hydroxide Calcium hydroxide Hydrochloric acid Distilled water $(NH_4)_2SO_4$ Copper sulphate Potassium dichromate solution ($K_2Cr_2O_7$) Silver sulfate (Ag_2SO_4) $HgSO_4$ Iron sulfate heptahydrate Potassium sulphate	Weighing balance – Digital Milling machine Digital pH meter Thermobalance Beaker Clamps Measuring cylinders Thermometer Conical Flask Gas delivery tubes Water bath Gas chromatograph Kjeldahl apparatus & condenser UV-Vis Spectrometer Moisture analyzer Photometer COD reactor	Ohaus-Scout Pro Alfa Machines HANNA (HI98128) HANNA Agilent(3962138D-Variant) Beckman DU640 AGRIPRO605 HANNA(HI 83300) HANNA(HI839800)

3.2 Characterization of Substrates

3.2.1 Determination of pH of the substrates

The pH of vinasse was measured using a Hanna pH-meter directly. The maize stalks were sun-dried for 15 days, and then it was milled and sieved to obtain the different substrate particle size; 0.5 mm, 1 mm, 2 mm (Elely *et al.*, 2018). 5g of crushed (1mm) maize stalks was placed in a glass test tube, 150ml distilled water was added and stirred, after 3 hours the pH readings were taken. The samples were made in three replicates.

3.2.2 Determination Moisture content

The moisture content of the substrates was measured using moisture analyzers. The moisture content for vinasse was determine using MAX50 moisture analyzer while for maize stalks AGRIPRO 6095 moisture meter was used. The procedure was done in three replicates

3.2.3 Determination of nitrogen

Nitrogen content for the substrates was determined based on Persulfate standard method – 4500-N_{org} D (LAMNDA 900). The method determines total nitrogen content by oxidation of all nitrogenous compounds to nitrate. Alkaline oxidation at 100 to 110^{o}C converts organic and inorganic nitrogen to nitrates. The total nitrogen is determined by analyzing the nitrate in the digestate. The procedure is shown in the appendix IV.

3.2.4 Determination of carbon content

The carbon content for the substrates was determined from Kenya Agricultural & Livestock's Research Organization (KALRO), Soil laboratory, Nairobi centre. A total organic (TOC) analyzer was used to analyse the carbon samples. The samples were

analyzed based on 5310- Total organic carbon standard method for examination of water and waste water. The results are attached in the appendix.

3.2.5 Determination of C/N ratio

The C/N ratio was determined by dividing the total organic carbon content by the total nitrogen content, according to Xiaojiao *et al.,* 2014. The carbon content and the nitrogen content of the respective substrates (VN & MS) were determined based on the standard methods highlighted in III and IV above. The equation used to determine the ratio was as follows;

$$C/N = \frac{W1\ C1}{W1x\ N1} \quad \text{, for each substrate} \qquad \text{(i)}$$

$$C/N = \frac{(W1xC)+(W2xC2)}{(W1xN1)+(W2xN2)} \quad \text{, for the mixture} \qquad \text{(ii)}$$

Where W_1, W_2 and W_3 were the TS weight in a single substrate in the mixture, C_1, C_2 and C_3 were the organic carbon content (g kg^{-1}VS) in each substrate and N_1, N_2 and N_3 were the nitrogen content (g kg^{-1}VS) in each substrate.

3.2.6 Determination of COD

COD was determined based on closed reflux, Colorimetric standard method-5220D. The standard and substrate samples were treated in Standard potassium dichromate solution (K_2Cr_2O) and Sulfuric acid reagent, the reduction of dichromate absorbance was measured using UVs spectrometer. The standard samples were prepared using potassium hydrogen phthalate with COD equivalence. The concentration of the samples was obtained from the standard curve. The COD was calculated using:

$$\text{COD as g/L} = \frac{(B-A)\ x\ M}{1000}$$

Where:

A = mg/L meter reading for the sample

B = mg/L meter reading for blank

M = Dilution Factor for the sample

3.2.7 Determination of Total Solids (TS), Total suspended solids (TSS) and Total dissolved solids (TDS)

TS, TSS and TDS for the substrates was determine based on standard methods (2540B, 2540C & 2540D) for the examination of water and waste water by Eaton *et al.*, (1995). For Vinasse it was well mixed and evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105^{o}C while maize stalks were dried directly. The decrease in weight over that of the empty dish represents the solids. The standard operating procedures are on the appendix. The relationship between TS, TSS and TDS is given by the following expression;

TS = TSS + TDS

3.3 Experiment Execution

3.3.1 Pretreatment of maize stalks

Milled maize stalks were pretreated using sodium hydroxide (NaOH) according to Song *et al.*, 2014. The crushed maize stalks samples were mixed with sodium hydroxide and then heated in water bathe for 3 hours at 100^{o}C. Neutralization was done using HCL to reduce the pH. The pretreatment was done for removal of lignin and degradation of hemicelluloses (Song *et al.*, 2014). The procedure is at Appendix III.

3.3.2 Design of experiment

A response surface design was used to define the experiment matrix. Box Benhken is a good design over the other designs for response surface methodology because it permits; estimation of parameters of the quadratic model, building of sequential

designs, detection of lack of fit and the design is economical since it has fewer runs required for optimization The effect of three factors; pH(X_1), substrate ratio(X_2), and Temperature(X_3) was studied on three response levels using response surface methodology. The software package design expert was used to determine the experiments design matrix and its statistical analysis.The temperature, pH and substrate ratio was investigated.

pH range: 6.5-7.5, $X_1(pH) = \frac{(pH-7)}{0.5}$ (3.1)

Substrate ratio 1:100 $X_2(SR) = \frac{(SR-50)}{25}$ (3.2)

Temperature (T) range**:** 35-40^oC, $X_3(T) = \frac{(T-35)}{2.5}$ (3.3)

A total of 15 runs were carried out based on the Box Behnken experiment design, Montgomery D., (2013). The ranges for the various operational parameters were selected based on information from research work on anaerobic digestion experiments for other substrates in mesophilic range (Girmaye *et al.*, 2019).

Digestion pH, X_1

The pH, X1 of the substrates was regulated by addition of either 1 molar sulfuric acid or 1 molar sodium hydrogen carbonate in the substrate for feeding the reactors (Girmaye *et al.* 2019). The pH was maintained at 6.5, 7.0 and 7.5 as shown in the design experiment.

Substrate ratio SR, X_2

The substrate ratio will range from 25%-75% where; (Maize: Vinasse) 25-75:75-25 the values were obtained from research on optimized co-digestion using different substrates (Neelam *et al.,* 2019).

Digestion temperature (^oC), X_3

Natural digestion temperatures values 35^{o}C, 37.5^{o}C and 40^{o}C was controlled using calibrated thermostatic water baths (Girmaye *et al.,* 2019). Temperature is controlled by immersing the digesters in a water bath. A record of daily temperatures was kept. Temperature adjustment is to be done on an hourly basis. A record of temperature at 4 hour intervals is kept for the period of operation.

3.3.3 Experiment design combinations

Box Benhken experimental design was used for this experiment, where three factors at three levels were investigated. A total of 15 runs were conducted. Each run had three factor level combinations. Three center-runs make up a total of 15 required runs in the Box-Behnken design as shown in literature review, Montgomery D., (2013). Another four runs were run as control experiments.

The biogas production was quantified based on the liquid displacement method. The displaced water was measured to represent the amount of biogas produced (Budyono *et al.,* 2014). Biogas volumes were measured daily starting from next day of inoculation for about 25 days (Girmaye *et al.,* 2019). The accumulated biogas yield was calculated using equation below:

$$\text{Accumulated Biogas Yield } = \sum_{n=1}^{n} Vn$$

V(mL) is the volume of biogas per day and n is the number of the days analyzed.

Table 3.2 shows the various level combinations for each run of the Box-Behnken experimental design.

RSM design was also used to determine the optimum conditions for the research, from the response surface contour we were able to identify the optimum points of the three variables (pH, Temperature and mixing ratio) and also use the model of the response surface to predict results.

Table 3.2: Natural and coded design variables

Run	Natural variables			Coded variables			
	pH	SR (Ration of Maize)(%)	T (^oC)	x_1	x_2	x_3	Biogas Yield,R1 (ml)
1	6.5	50	40	-1	0	1	
2	6.5	50	35	-1	0	-1	
3	7.0	75	35	0	1	-1	
4	7.0	25	40	0	-1	1	
5	7.0	50	37.5	0	0	0	
6	7.5	25	37.5	1	-1	0	
7	6.5	75	37.5	-1	1	0	
8	7.0	50	37.5	0	0	0	
9	7.0	75	35	0	1	-1	
10	7.0	50	37.5	0	0	0	
11	7.0	25	35	0	-1	-1	
12	7.5	50	35	1	0	-1	
13	7.5	75	37.5	1	1	0	
14	6.5	25	37.5	-1	-1	0	
15	7.5	50	40	1	0	1	

Three conical flasks were arranged in a way that the first flask contained substrate; the middle contained water and the last for collecting water that was expelled out of the second container (Girmaye and Ebsa, 2019). All the flasks were connected with plastic pipes having a diameter of 5mm. The pipe connecting the first bottle to the second was fitted above the substrate in the first bottle to the top of the second bottle to help gas collection in to second as shown in Figure 3.1 and 3.2 Thus, the biogas produced was driven from the first flask to the second flask that contains water so as to displace a volume of the water equivalent to the volume of biogas produced. The cocks of all digesters were sealed tightly using clear silicon clue in order to control the entry of oxygen and loss of biogas as indicated in Figure 3.2 below. Shaking of digesters was done manually on daily basis to ensure contact between the substrate molecules and microbial cells.

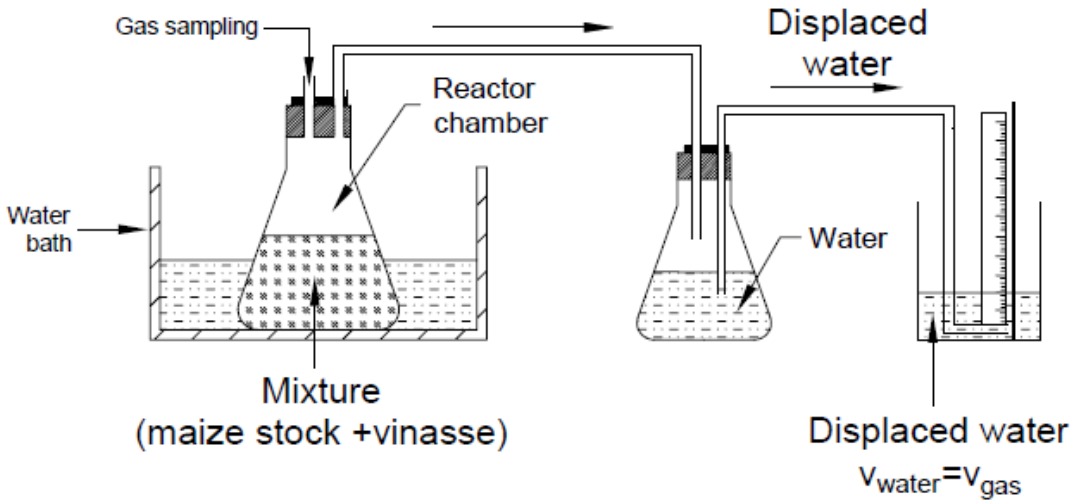


Figure 3.1: Schematic setup

Figure 3.2: Experiment setup

3.4 Characterization of Biogas and Resultant Sludge

A gas chromatograph was used to determine components in the biogas. The characteristics of the sludge (pH, COD, TSS and TDS) were determined based on the standard methods described below.

3.4.1 Determination of biogas composition

The sample for biogas composition determination was drawn from the digester experiment runs using a syringe; analysis was done using Agilent gas chromatography (MRC/GC/3962138D-Variant) on flame ionization detector (FID) as described by Chamarthi S. *et al.*, (2013). The temperature for column chamber, inlet chamber and detector were 150^{o}C, 200^{o}C and 250^{o}C, respectively. High purity nitrogen was used for carrier gas in this study, and the flow rate for nitrogen was 2.0 ml/min. The split ratio of gas sample in inlet chamber was 20:1, which is used to control the amount of biogas flew into column, and prevent the unconventional peak, such as flat peak, trailing peak. The procedure is clearly outlined in the appendix VI.

3.4.2 pH for sludge

The pH of the sludge was measured using a Hanna pH-meter directly. The sludge was well mixed and put into three 50mL beakers and the pH reading taken. The Final pH was obtained from calculating the mean of the three samples.

3.4.3 Determination of COD for sludge

COD for the sludge was determined based on closed reflux, Colorimetric standard method (1995). The sludge samples were treated in Standard potassium dichromate solution (K_2Cr_2O) and Sulfuric acid reagent, the reduction of dichromate absorbance was measured using UVs spectrometer. The standard samples were prepared using

potassium hydrogen phthalate. The concentration of the sludge was obtained from the standard curve.

3.4.4 Determination of TSS and TDS

TS for the sludge were determined based on standard methods (2540B and 2540C) for the examination of water and waste water by Eaton *et al.,* (1995). The well mixed sludge is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105^{o}C. The decrease in weight over that of the empty dish represents the solids.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characteristics of the substrates

4.1.1 pH

The pH of maize stalks and vinasse were obtain from the pH meter. The procedure was done in triplicates and their average as shown in Table 4.1

Table 4.1 pH of the substrates

Item	Sample/Run	Volume(ml)	Average pH
Vinasse	3	30	7.53
Maize stalks	3	30	4.34

4.1.2 The Moisture

The moisture content for vinasse was determine using MAX50 moisture analyzer while for maize stalks AGRIPRO 6095 moisture meter was used. The results were recorded in Table 4.2

Table 4.2: Vinasse Moisture content

Item	Sample/Run	Mass(grams)	Moisture content (%)
Vinasse	3	20	93.912
Maize stalks	3	5	9.520

4.1.3 Total Solids (TS)

Gravimetric standard method was used to determine the TS of the substrates. Vinasse samples stirred to homogenize before the samples were taken. The procedure was done in triplicates and recorded in Table 4.3

Table 4.3: Total solids

Substrate	Samples run	Average Weight (grams)		
		W_0	W_1	W_2
Vinasse	3	180.7	211.9	182.9
Maize stalks	3	180.7	182.7	182.53

The percent total solid was calculated using standard relations,

$$\% \text{ Total solids} = \frac{W2-W0}{W1-W0} \times 100$$

(a) % Total solids of maize stalks = $\frac{182.53-180.7}{182.7-180.7} x\ 100 = 91.5\%$

(b) %Total solids of Vinasse = $\frac{182.9-180.7}{211.9-180.7} x\ 100 = 7.05\%$

4.1.4 Total suspended solids (TSS) for substrates

TSS of the substrates was determined by filtering a known mass of sample using a 2 micron filter paper. The Filter paper residue is rinsed with distilled water before drying.

5g of crushed maize stalks was placed in a glass test tube, 150ml distilled water was added, stirred, and left for 4 hours before determining TSS. The samples were made in replicates and the results recorded in Table 4.4

Table 4.4 Total suspended solids (TSS)

Substrate	Samples run	Average Weight (grams)		
		W_0	W_1	W_2
Vinasse	3	1.2	41.2	3.616
Maize stalks	3	1.2	6.2	5.706

The percent total suspended solids was calculated using standard relations below,

$$\% \text{ Total suspended solids} = \frac{W2-W0}{W1-W0} x100$$

Where: W_1 = Mass of Sample + W_0

$$\% \text{ TSS for Vinasse} = \frac{3.616-1.2}{41.2-1.2} x100 = 6.04\%$$

% TSS for Maize stalks = $\frac{5.706-1.2}{6.2-1.2} x\ 100$ = 90.12% Note that; Average values where used

4.1.5 Total dissolved solids (TDS)

Total dissolved solids were determined by subtracting total suspended solids from total solids using the relation: TS = TSS + TDS. TSS and TS values were obtained from Table 4.4 and 4.5

% TDS for Vinasse = TS – TSS = 7.05 – 6.04 = 1.01%
% TDS for Maize stalks = TS – TSS = 91.50 – 90.12 = 1.38%

4.1.6 COD determination

COD for vinasse was determined based on closed reflux, Calorimetric standard procedure outline in chapter 3. The standard curve was plot and the absorbance of the samples read from UV-Vis photo-spectrometer.

Table 4.5: Calibration curve

Sample Number	KHP1	KHP2	KHP3	KHP4	KHP5	KHP6
Strength in mg/L	500	250	125	62.5	31.25	15.625
Absorbance	0.067	0.051	0.01	0.009	0.008	0.004

Using the results above a standard curve was plotted in Appendix I,

Sample absorbance were read from the UV-Vis photo-spectrometer and recorded in the table 4.6 below, there absorbance were read from the standard curve on appendix I.

Table 4.6: COD sample data

Samples run	**ml of sample**	**A mg/l**	**B mg/l**	**M (Average)**
3	2	0	330	216

COD as g/L = $\frac{(330-0)x\ 216}{1000}$ = 71.28 g/l

4.1.7 Determination of nitrogen content

A standard curve was constructed by plotting standard sample absorbance due to NO_3-against strength/concentration. The table 4.7 below shows the results for absorbance against strength for the standard samples

Using the sample absorbance, sample concentration was directly obtained from the standards curve. And the results tabulated in the appendix.

Table 4.7: Nitrogen content for substrates

Substrate	Number of runs	Average Nitrogen content
Maize stalks	3	12.13
Vinasse	3	2.7

Characteristics of components of the substrates (maize stalks and Vinasse) determined based on standard procedures outlined in chapter 3 were summarized in table 4.8 below.

Table 4.8: Composition of various components of Maize stalks and Vinasse

Parameters	Maize stalks	Vinasse
pH	7.52	4.34
Moisture %	9.52	93.91
COD g/l	**	71.28
Total solids (%)	91.50	7.05
Total suspended solids %	90.12	6.04
Total dissolved solids %	7.38	1.01
Carbon content (TOC)	49.51g/kg	2.23g/l
Nitrogen content	1.28g/kg	0.27g/l
C/N ratio	38.68	8.25

Key: Number of repetition (n) =3, except for pH and TOC which has n = 1. **Not applicable.

The composition of the substrates posted in the table above is similar to the values reported in the literature. Maize stalks characteristics: moisture content, TS, TSS and TDS which are important components for anaerobic digestion were similar to those posted by Carlos *et al*., 2014. Similarly vinasse composition was within the range posted by Maria *et al., 2017* and Bruno *et al.,* 2015. Anaerobic co-digestion, for the production of biogas and digestate, can be a powerful technology to obtain a gaseous fuel and simultaneously obtain also a fertilizer. To optimize the process, it was necessary to characterize in detail the substrate before and during the process. Gas analysis was performed to determine the heating value and also the contaminants inside it. All these measurements were standardized and are discussed in this chapter to provide information to the study and researchers in the field of biogas.

4.2 Biogas yield

Anaerobic digestion of vinasse wastes co-digested with pre-treated maize stalks at temperature range of 35^0C – 40^0C, at different ratios and at pH range of 6.5 – 7.5 for 25 days. The results were recorded in Table 4.9.

Table 4.9: Biogas Yield

Run	**Natural variables**			
	pH	Substrate ratio (Ratio of Maize stalks)(%)	Temperature (^{o}C)	Biogas Yield,R1 (ml)
1	6.5	50	40	410
2	6.5	50	35	519
3	7.0	75	35	550
4	7.0	25	40	403
5	7.0	50	37.5	998
6	7.5	25	37.5	412
7	6.5	75	37.5	626
8	7.0	50	37.5	869
9	7.0	75	40	795
10	7.0	50	37.5	933

11	7.0	25	35	772
12	7.5	50	35	687
13	7.5	75	37.5	782
14	6.5	25	37.5	454
15	7.5	50	40	546
16	7.0	100% VS	37.5	329
17	7.0	100% MS	37.5	385
18	7.5	100% VS	37.5	132
19	5.5	100% VS	37.5	228

Key: VS – vinasse, MS – Maize stalks

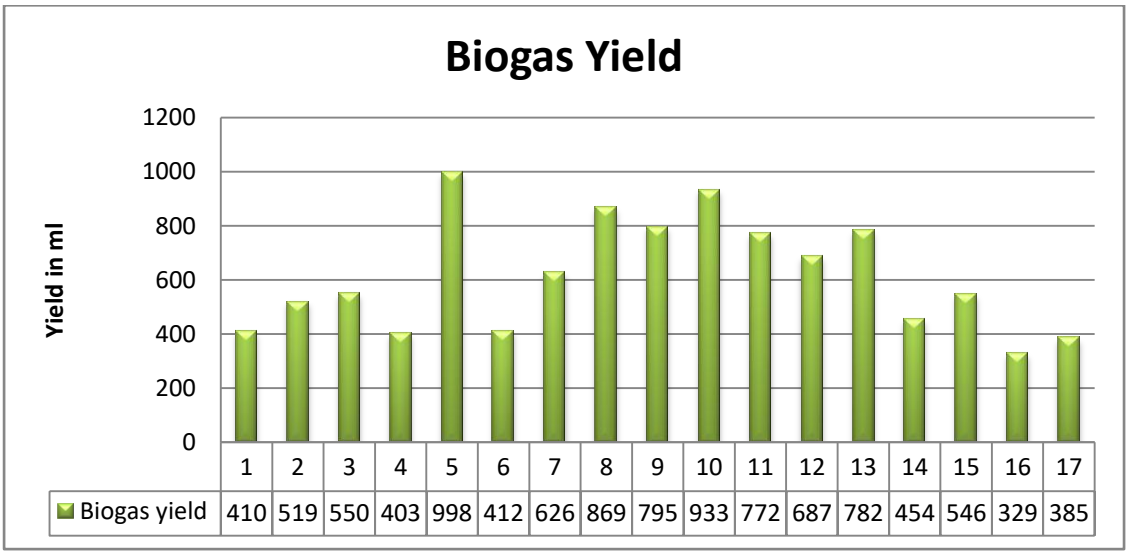


Figure 4.1: Daily biogas yield

Table 4.9 and figure 4.1 shows the cumulative daily biogas production during anaerobic digestion (AD), during the 25 day monitoring period. According to the cumulative daily trend of biogas yield during the experimental development, the treatments that were done through co-digestion (50 % Vinasse and 50 % Maize stalks), had a significant difference regarding to those treatments that were done through one substrate. The treatments that had the lowest methane production were 100 % Vinasse and 100 % maize stalks; this evidenced that it is not convenient to work with one consortium alone; this is due to antagonistic action of mixed population of microorganisms that promote a better microbial growth during the anaerobic digestion (Quintero *et al.*, 2012). Digestion of more feed stocks in same digester establishes a positive synergy, habitat for cells and more substrates

(hemicellulose and cellulose) that enable the microbial growth and significantly promoting production of biogas. In this research the biogas yield from co-digestion (vinasse and maize stalks) increased compared to biogas production from vinasse alone; these results are similar with results reported by Budiyono *et al.*, 2014.

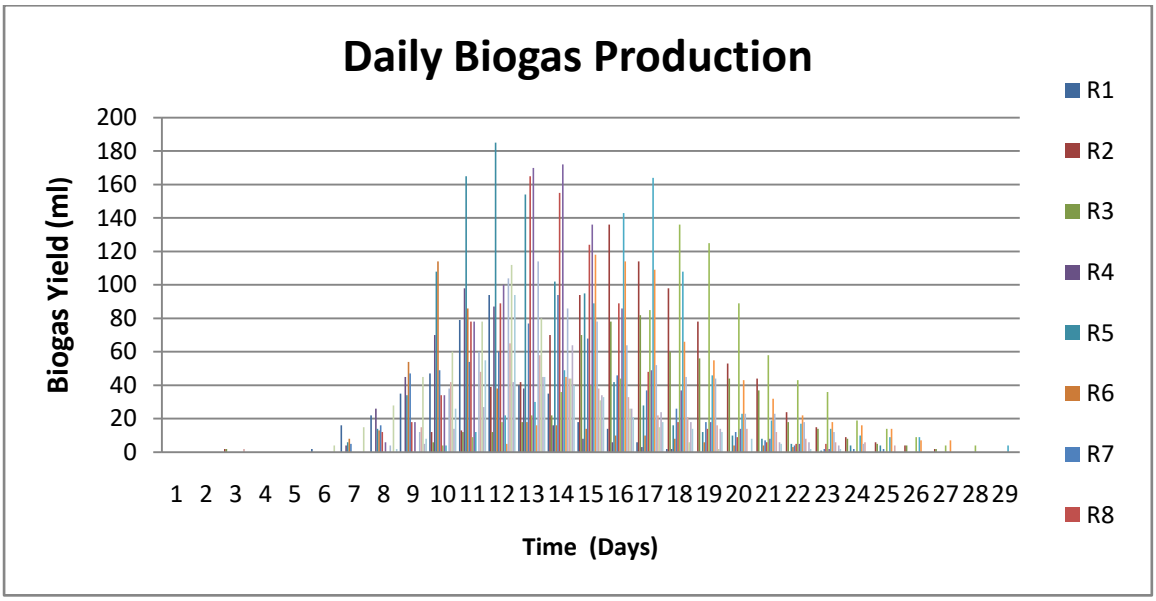


Figure 4.2 Daily biogas productions

Maximum production of biogas was produced by run 5 (50% VN 50% MS, pH - 7.5 and at 37.5^{0}C - T) followed by runs 10 and 8 respectively operating at the same conditions as run 5. All the runs followed a normal curve trend; increase in biogas yield to an optimum yield where it starts to decline gradually. The results also showed that the three factors: substrate ratio, pH and Temperature to a big extend affect the biogas production, this is in agreement with the previous studies by Girmaye *et al.*, 2019 and Yaldiz *et al.*, 2014. These results were also analyzed using design expert showing the optimum run 5, figure 4.12 shows the 3D contours.

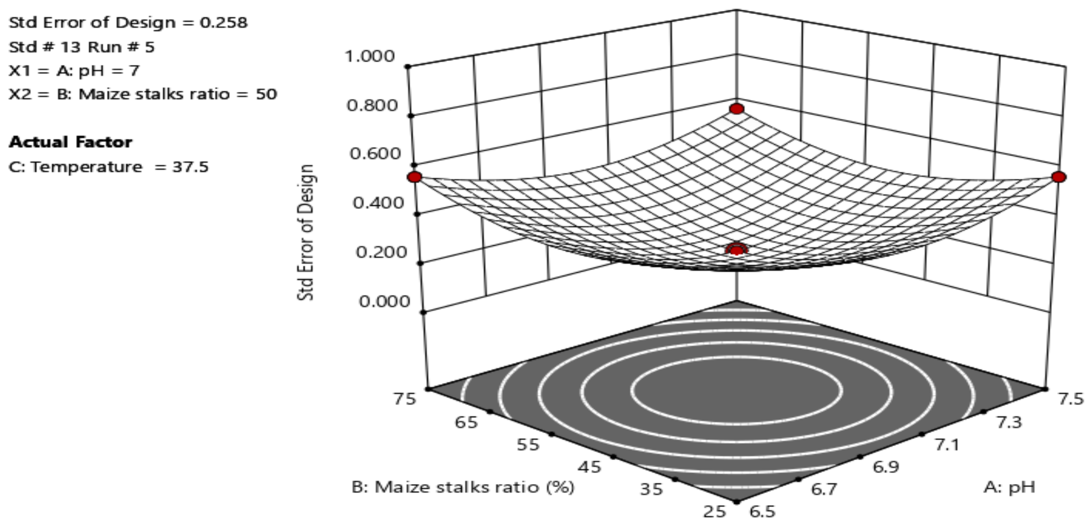


Figure 4.3: 3D surface contours

4.3 Effect of Temperature, pH and Maize stalks ratio on biogas production

Three factors; Temperature, pH and Maize stalks ratio were investigated at three levels. A total of 17 runs were run for a period of time. Run 1-15 had different combinations of the factor levels while run 16, 17, 18 and 19 were control experiments.

The model that predicts the biogas yield in terms of coded factors is given by the following equation. Yield,

$$Y_{Biogas\ yield\ ml} = 933.33 + 52.25X_1 + 89X_2 + 46.75X_3 + 49.5X_1X_2 - 8X_1X_3 + 153.5X_2X_3 - 227.17{X_1}^2 - 137.67{X_2}^2 - 165.67{X_3}^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. \textit{(vi)}$$

Where X_1 refers to the pH, X_2 refers to the substrate ratio, and X_3 refers to the reaction temperature inside the water bath. The second-order polynomial vi above was applied to determine the relationship between variables and responses and regression coefficients were calculated. From the RSM model's sum of squares obtained from the Design Expert Software demonstrated that the second-order polynomial regression was suitable to explain the relationship between input variables and output

(responses). The equation above showed that the significance of factors affecting biogas yield obtained from RSM were in the following order:

$$X_2X_3, X_2,\ X_1,\ X_1X_2, X_3,\ X_1X_3, X_2^2,\ X_1^2\ and\ X_3^2.$$

This equation was then used to plot response surface and contours of biogas yield. The relationship between yield and the three factors are shown in figures 4.3. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 4.10 ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.240E+05	9	58217.77	9.06	0.0129	significant
X_1-pH	21840.50	1	21840.50	3.40	0.1245	
X_2-sub ratio	63368.00	1	63368.00	9.87	0.0256	
X_3-temp	17484.50	1	17484.50	2.72	0.1599	
X_1X_2	9801.00	1	9801.00	1.53	0.2716	
X_1X_3	256.00	1	256.00	0.0399	0.8496	
X_2X_3	94249.00	1	94249.00	14.67	0.0122	
X_1^2	1.905E+05	1	1.905E+05	29.66	0.0028	
X_2^2	69977.03	1	69977.03	10.89	0.0215	
X_3^2	1.013E+05	1	1.013E+05	15.78	0.0106	
Residual	32115.67	5	6423.13			
Lack of Fit	23795.00	3	7931.67	1.91	0.3622	not significant
Pure Error	8320.67	2	4160.33			
Cor Total	5.561E+05	14				

The **Model F-value** of 9.06 implies the model is significant. There is only a 1.29% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case: X_2, $X_1 X_2$, ${X_1}^2$, ${X_2}^2$, ${X_3}^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.91 implies the Lack of Fit is not significant relative to the pure error. There is a 36.22% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good, the model fits and can be used to navigate the design space.

Table 4.11 Fit Statistics

Std. Dev.	80.14	**R^2**	0.9422
Mean	650.40	**Adjusted R^2**	0.8383
C.V. %	12.32	**Predicted R^2**	0.2817
		Adeq. Precision	9.0557

The **Predicted R^2** of 0.2817 is not as close to the **Adjusted R^2** of 0.8383 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq. Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.056 indicates an adequate signal. This model can be used to navigate the design space.

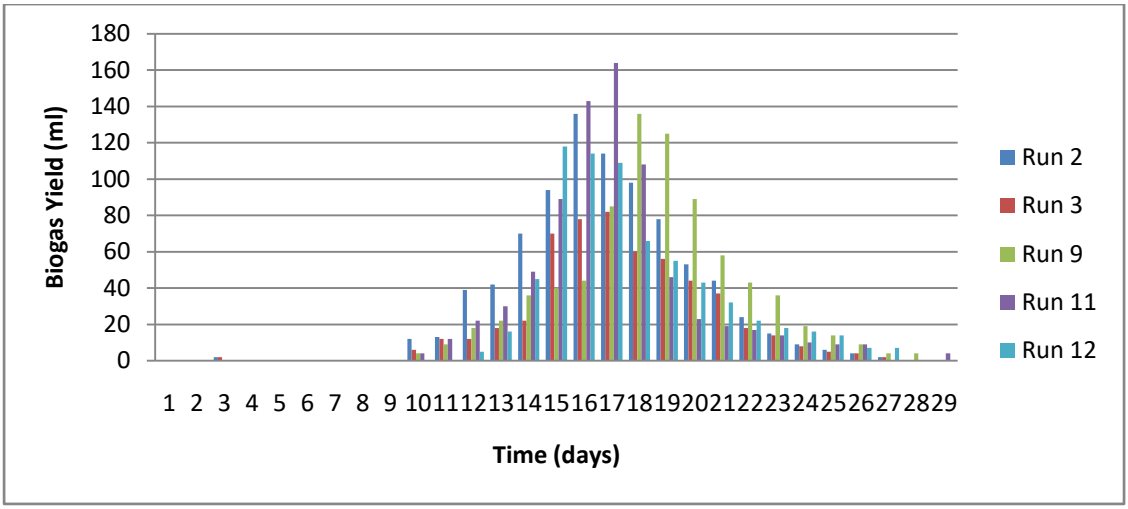
4.3.1 Temperature

The table 4.10 below shows the operating temperature of the water bath for the 17 runs used in the experiment.

Table 4.12: Operating temperatures for the runs

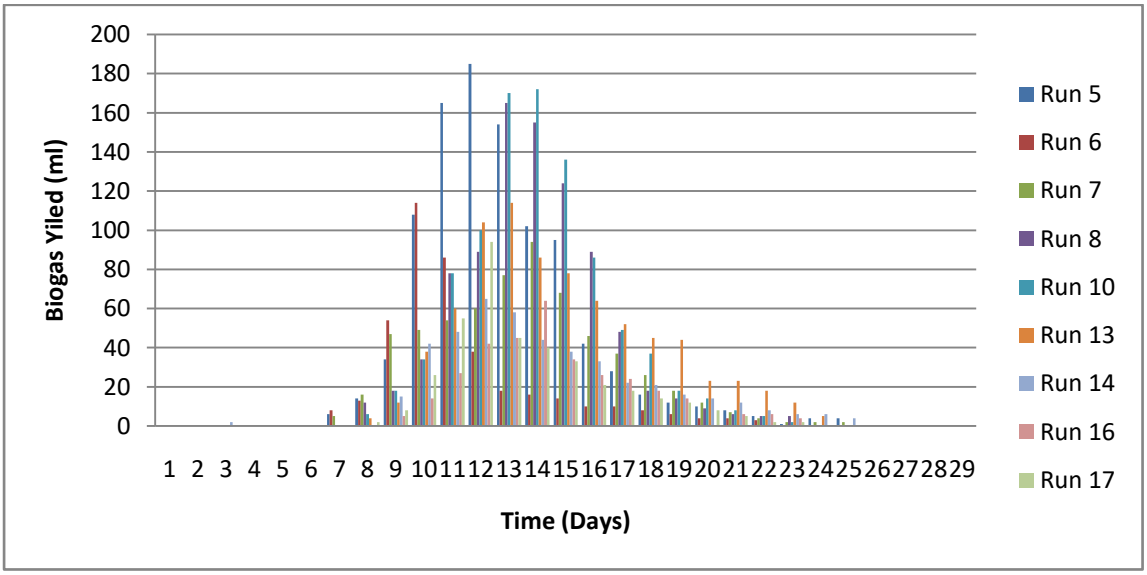
Run	Water bath Temperature (^{o}C)
2,3,9,11 and 12	35
5,6,7,8,10,13,14,16,17, 18 and 19	37.5
1,4 and 15	40

For these different levels of temperature the following graphs 4.1, 4.2 and 4.3 illustrates the behavior of the biogas yield per day over the period experiment.



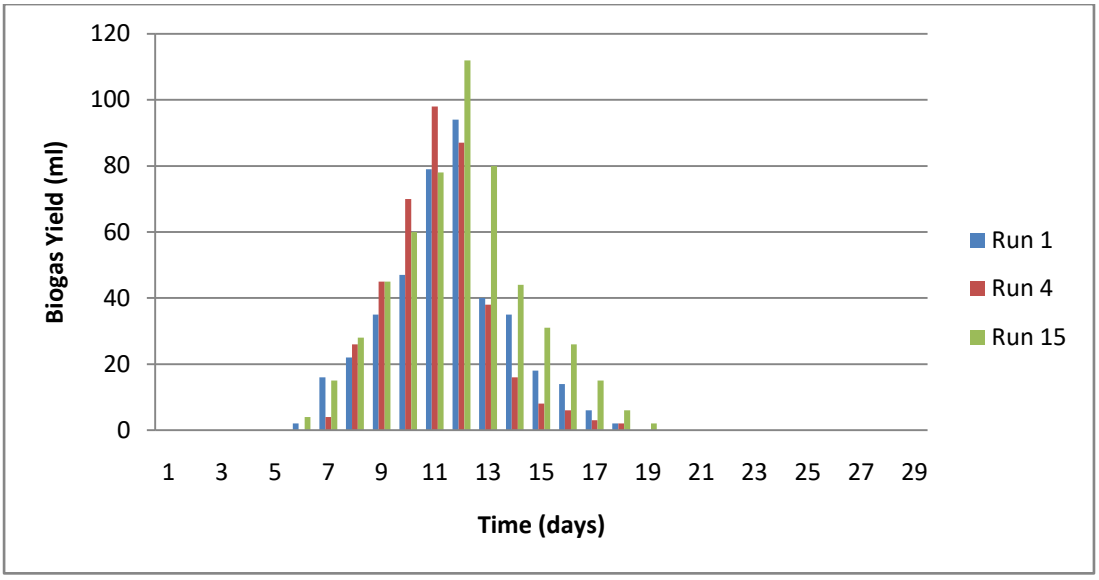
***Experimental conditions: Run 2** – 50% VS + 50% MS, pH of 6.5, **Run 3** - 25% VS + 75% MS, pH of 7.0, **Run 25**% VS + 75% MS, pH of 7.0, **Run 11- 75**% VS + 25% MS, pH of 7.0, Run **12- 50**% VS + 50% MS, pH of 7.5*

Figure 4.4: Daily biogas yields for runs operating on water bath at 35^0C



***Experimental conditions: Run 5** – 50% VS + 50% MS, pH of 7.0, **Run 6**- 75% VS + 25% MS, pH of 7.0, **Run 7-75**% VS + 25% MS, pH of 6.5, **Run 8- 50**% VS + 50% MS, pH of 7.0, Run **10- 50**% VS + 50% MS, pH of 7.0, **Run** 13– 25% VS + 75% MS, pH of 7.5, **Run 14** - 75% VS + 25% MS, pH of 76.5, **Run 16-** 100% VS, pH of 7.0, **Run 100**% MS, pH of 7.0*

Figure 4.5: Daily biogas yields for runs operating on water bath at 37.5^0C



***Experimental conditions: Run 1** – 50% VS + 50% MS, pH of 6.5, **Run 4** - 75% VS + 25% MS, pH of 7.0, **Run 15-50**% VS + 50% MS, pH of 7.5.*

Figure 4.6: Daily biogas yields for runs operating on water bath at 40^0C

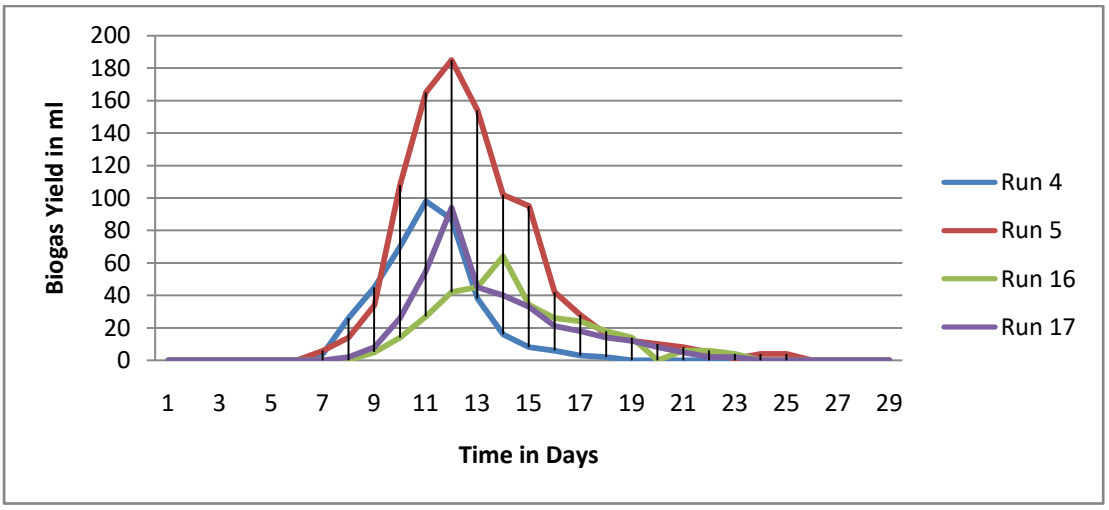
Runs' operating on water bath at 35^0C; run 2, 3,9,11 and 12 produced biogas after 8th – 9th day, there was a smooth rise in the production and prolonged for 15 days. Run 5, 6, 7, 8, 10, 13, 14, 16 and 17 produced within 6^{th} – 8^{th} day, moderate rise in production and followed by the same moderate drop. Run 1, 4 and 15 were run at 40^0C water bath; they produced within 3^{rd} -4^{th} day followed by sharp increase and finally a sharp decrease.

The results showed that, 37.5^0C is the optimum temperature for biogas production from co-digestion of vinasse and maize stalks. Previous research also showed that optimum temperature for biogas production is at mesophilic range of temperature (30-40^0C) (Vindis et. al, 2009). The results further indicated that anaerobic digestion is much sensitive to temperature change. At higher temperature the retention time tend to reduce since high temperature speeds the degradation of the substrates, also the bacteria concern for the degradation are affected by change in temperature which was noted by Ganiyu, 2005. The same is also strongly supported by Hutma, 2003 who

revealed that increase in temperature cause increase in inhibition of free ammonia (NH_3).

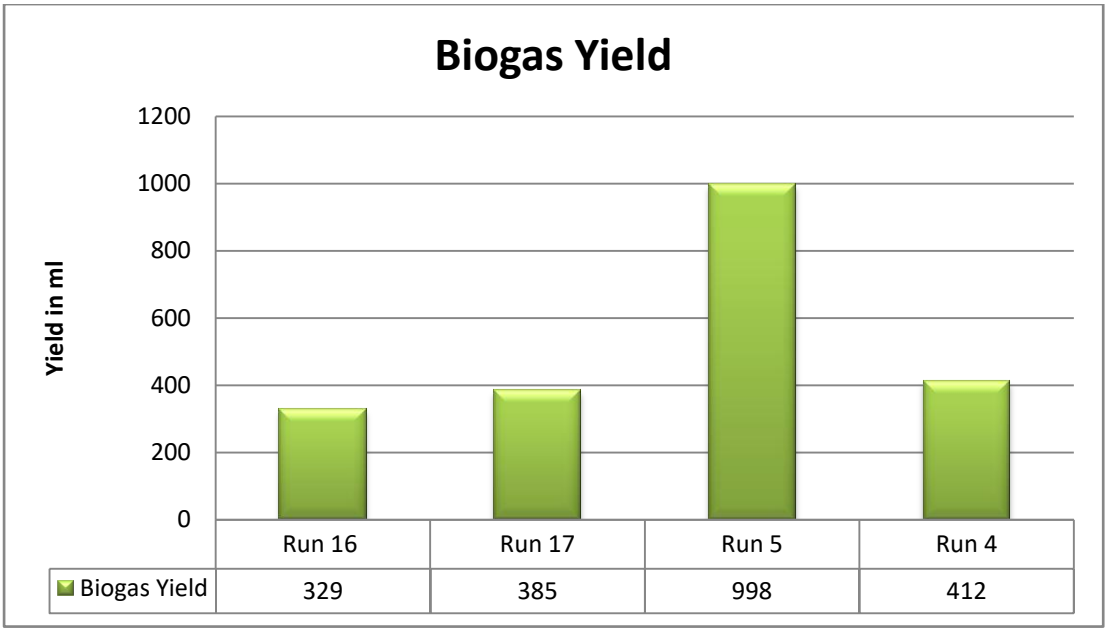
4.4 Effects of substrate ratio - Co-digestion

The study further investigated the relationship between substrate ratio and biogas yield; the column chart below shows the effect of substrates on biogas yield. The yield for Runs 4,5,16 and 17 were compared based on the fact that run 16 and 17 were run at 100% vinasse and 100% Maize stalks respectively while runs 4 and 5 were the minimum and optimum runs respectively. The results from this investigation informed the study the benefits of digesting more substrates since more substrates resulted to high yield of biogas and improved the quality of the digested.



***Key: Run 4** – 75% VS + 25% MS, pH of 7.0 and Temperature of 37.5^{0}C, **Run 5** - 50% VS + 50% MS, pH of 7.0 and Temperature of 37.5^{0}C, **Run 16** - 100% VS, pH of 7.0 and Temperature of 37.5^{0}C, **Run 17** - 100% MS, pH of 7.0 and Temperature of 37.5^{0}C*

Figure 4.7: Daily biogas productions



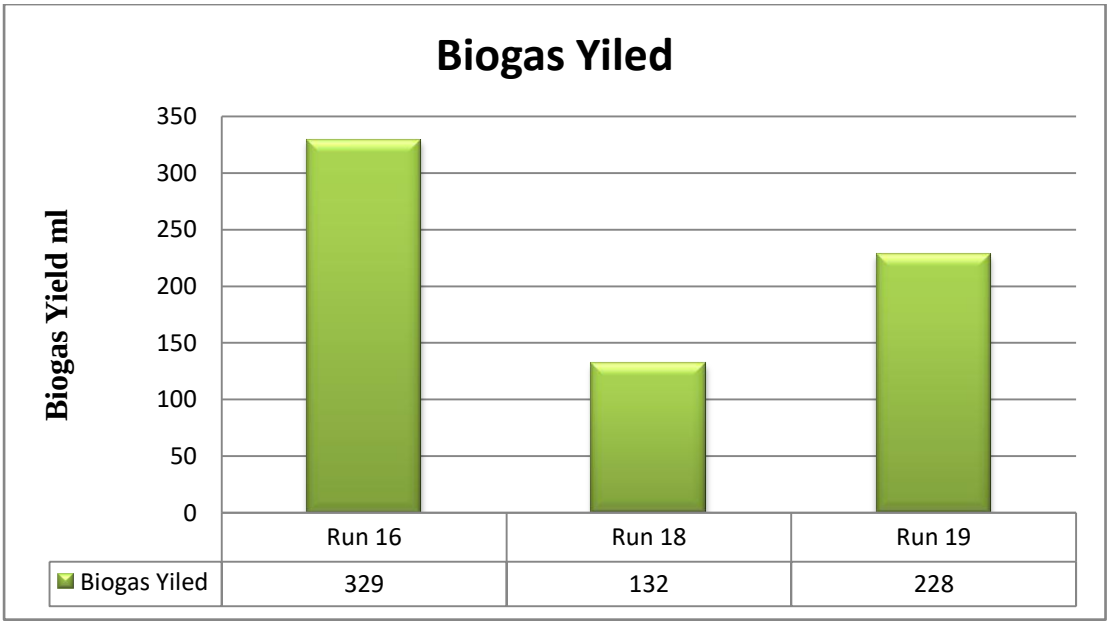
Key: Run 4 *– 75% VS + 25% MS, pH of 7.0 and Temperature of 37.5^{0}C,* ***Run 5*** *- 50% VS + 50% MS, pH of 7.0 and Temperature of 37.5^{0}C,* ***Run 16*** *- 100% VS, pH of 7.0 and Temperature of 37.5^{0}C,* ***Run 17*** *- 100% MS, pH of 7.0 and Temperature of 37.5^{0}C*

Figure 4.8: Biogas productions

The results show that biogas yields increases with increase in the substrate ratio. This is supported by Ogunwande, 2012.

4.5 Effects of pH

Three setups were run to investigate the effect of pH on biogas yield. The chart below shows the results.



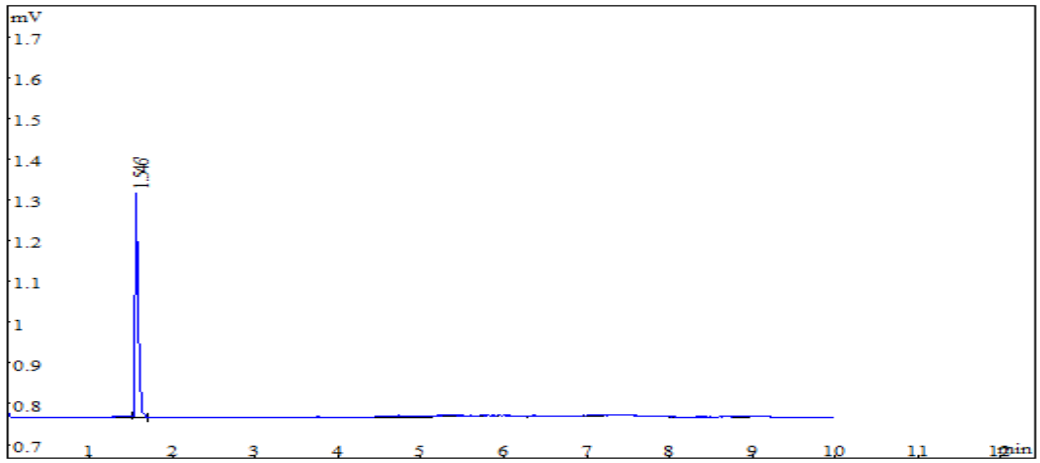
***Key: Run 16** - 100% VS, pH of 7.0 and Temperature of 37.5^{0}C, **Run 18** - 100% VS, pH of 7.5 and Temperature of 37.5^{0}C, **Run 19** - 100% VS, pH of 5.0 and Temperature of 37.5^{0}C,*

Run 16 showed the highest cumulative biogas yield at pH of 7. At pH of 7.5 (Run 18) the yield is relatively higher but reduces sharply at pH of 5.0 (Run 19). This suggests that pH 7.0 resulted in biogas yield followed by 7.5 and 5.0. This is supported by Dioha (2013) who stated that bacteria causing degradation are highly sensitive to both high and low pH and grow better in pH range of 6.5-8.0.The same is further noted by Ogunwande (2012) who stated that at very low pH/ high alkaline leads to the disintegrative of microbial granules and subsequently failure of the digestion process. The measured high yield of biogas may be due to the higher initial pH that promoted the hydrogenotrophic methanogenesis during which the CO_2 and H_2 are converted into CH_4 and H_20 (Schink, B. 1997). From this investigation the results informed the study on importance of pH before digestion on biogas yield.

4.6 Biogas composition

The composition of the biogas was analyzed using a gas chromatograph. The samples were taken from the optimum run number 5 while the standard gas was prepared in

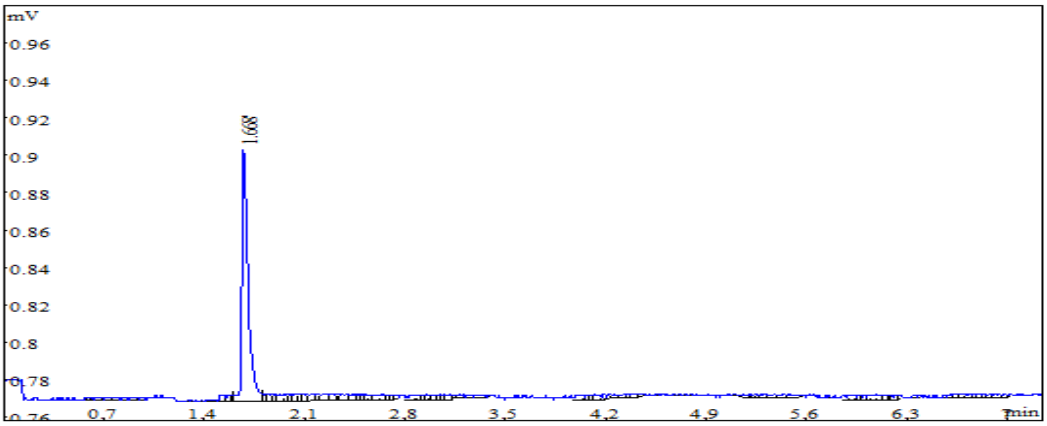
the lab. Chromatogram of the 2µl standard gas is shown in figure 4.6 below. The chromatogram showed one peak representing methane. The retention time of methane peak was 1.546 min at column temperature, inlet chamber and detector chamber temperature of 150^{o}C, 200^{o}C and 250^{o}C respectively.



Rank	Time	Name	Area%	Area
1	1.546		100	1262
Total			100	1262

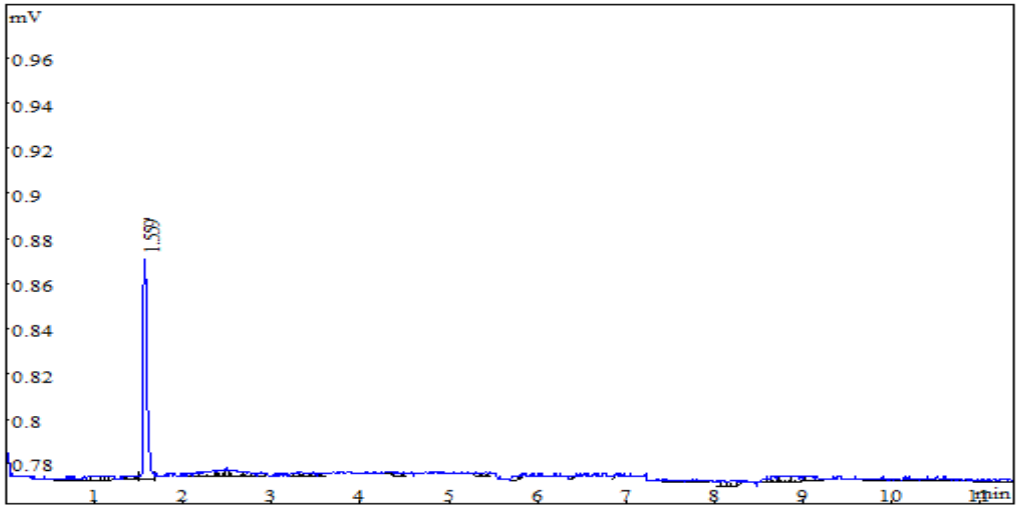
Figure 4.9: Chromatogram of the standard gas

To estimate the methane present in the samples, the same amount as of the standard gas was injected to the GC and the column temperature, inlet chamber and detector chamber temperature were 150^{0}C, 20^{0}0C and 250^{0}C respectively. Three samples where run on the gas chromatography and there chromatograms shown in figures 4.10, 4.11 and 4.12 below.



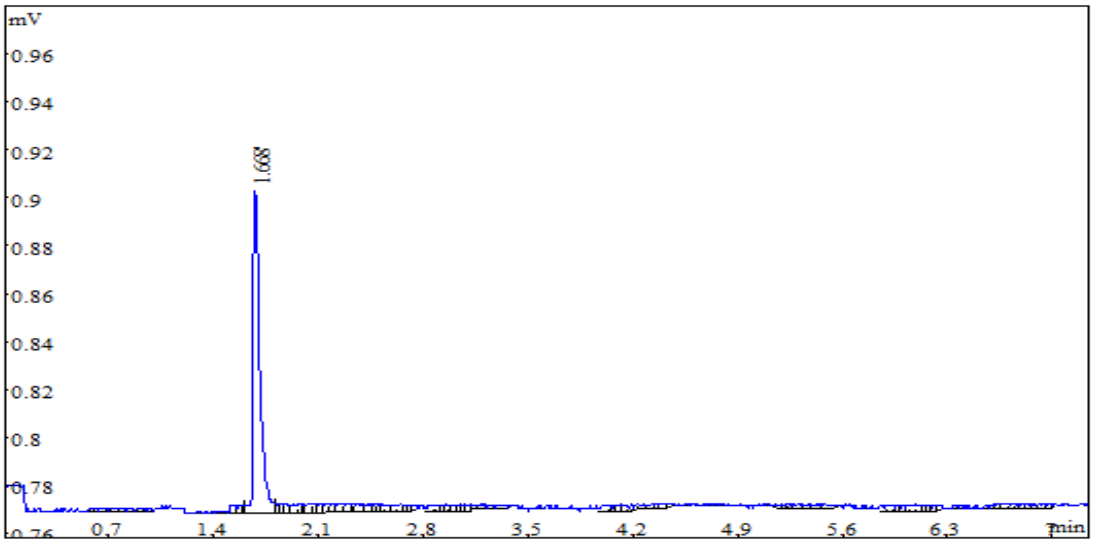
Rank	Time	Name	Area%	Area
1	1.668		100	880
Total			100	880

Figure 4.10: Chromatogram of sample gas 1



Rank	Time	Name	Area%	Area
1	1.559		100	681
Total			100	681

Figure 4.11: Chromatogram of the sample gas 2



Rank	Time	Name	Area%	Area
1	1.668		100	783
Total			100	783

Figure 4.12: Chromatogram of the sample gas 3

Table 4.11 below shows the area of the peaks for the standard gas and the sample gases

Table 4.13 Peak area for the injected gases

Samples	Peak Area
Standard gas	1262
Sample gas 1	783
Sample gas 2	681
Sample gas 3	880

From the area measurements the methane concentration in each sample was calculated as a percentage of the total area of standard sample. The percent methane in samples was calculated using the relations,

% methane $=\frac{A}{B} x100\%$

Where; A – Area of the peak for 2µl sample gas

B – Area of the peak for the 2µl standard gas

Note: Standard gas produced was assumed to be 100% Methane

The results of sample concentration from the above calculations were recorded in Table 4.12. The table showed the percentage of methane in the three samples of gas analyzed.

Table 4.14: Percentage Methane in the sample gas

Sample Gas	Peak Area	% $NH_4 = \frac{peak\ area}{1262} x100\%$
1	783	62.04
2	681	53.96
3	880	69.73
Average		**61.91**

4.7 Sludge composition

The pH, COD, TSS and TDS of the resultant sludge were determined based on standard procedures in Chapter 3. The table 4.13 below shows composition of the sludge.

Table 4.15: Composition of sludge

Parameters	Sludge
pH	6.7
COD in g/L	42.27
TS (%)	35.65
TSS (%)	34.88
TDS (%)	0.77

From the above results and the results from substrate characterization the organic removal rate were 59.3%.

Organic removal rate = $\left(\frac{A-B}{A}\right)$ * 100%

Where:-

A - COD before digestion

B - COD after digestion.

The characterization of sludge was very important process as it informed the study on viability of the digested to be used as a fertilizer. The organic removal rate showed the extent of digestion and the efficiency of the digester and or the process. Gas analysis was performed to determine the heating value and also the impurities in it. All these properties were characterized to provide information to the study and researchers.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Review of the Research Objectives

The main objective that guided this study was to optimize biogas yield through co-digestion of sugarcane vinasse and pre-treated maize stalks. To be able to accomplish this, the research was guided by the following specific objectives.

i. To determine the characteristics of substrates such as: pH, Moisture content, COD, TS, TSS, Nitrogen, Carbon and C/N ratio
ii. To evaluate the effect of pH, Temperature and Maize stalk ratio on optimization of biogas yield.
iii. To characterize biogas and the resulting sludge.

5.2 Key Findings as per the Objectives

Based on the results obtained, the study found that the characteristics for the substrates were favorable for anaerobic co-digestion. It also found that anaerobic co–digestion bioreactors with temperature and substrate ratio conditions of 35^{o}C and 50%:50% respectively yielded more and start-up times shorter than 22 days. It is also inferred that the optimum pH for co-digestion of vinasse and maize stalks was 7.0.

5.3 Conclusions

In conclusion, this study found that:

1. The optimum pH, temperature and maize stalks ratio for biogas production from co-digestion of vinasse and pre-treated maize stalks were 7.0, 37.5^{o}C and 50% respectively, these optimum conditions made favorable conditions for multiplication of bacteria.

2. Co–digestion bioreactors operating at 40^{o}C yielded shorter start-up times than the ones operating at 37.5^{o}C and 35^{o}C respectively this is because the higher the temperature the faster there is degradation process).
3. In addition the study also inferred that co-digestion enhances efficiency of the digester and subsequently more biogas yield is attained.
4. The removal rate of TS and COD further supports and strengthens the reported results.

5.4 Research contribution to the theory and practice

The research studied the Optimization of biogas production through co-digestion of sugarcane vinasse and pre-treated maize stalks. Research on optimization of biogas production from organic waste specifically vinasse and maize stalks is of much relevance in the energy and agricultural sectors. The thesis has therefore attempted to contribute to theoretical knowledge and practice.

5.5 Recommendations

Since there was no control over crucial parameters such as particle size, pH adjustment during different stages of methanogenesis and mixing (there was no continuous stirring throughout the digestion process). The study therefore recommends controls over some parameters to be done to improve the methane yield. Particle size variation, pH adjustment and stirring for higher biogas yield

5.6 Future Research

The study also puts forward a room for co-digestion of vinasse with more other substrates. Nonetheless, the biogas production from these residues allows an adequate treatment of the same, mitigating pollution problems in places adjacent to the growing

areas. Finally the study suggested that further analysis be conducted to evaluate the effects of input materials on the characteristics of biogas.

REFERENCES

Adebayo, A. O., Jekayinfa, S. O., & Linke, B. (2014). Anaerobic co-digestion of cattle slurry with maize stalk at mesophilic temperature. *American Journal of Engineering Research (AJER)*, *3*(1), 80-88.

Al Seadi, T., Rutz, D., Prassl, H., Köttner, M., & Finsterwalder, T. (2008) *Biogas Handbook;* University of Southern Denmark: Funen, Denmark.

Anthony M. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Journal of Renewable Energy* 31: 23 85–2392.

Antognoni, S., Ragazzi, M., Rada, E. C., Plank, R., Aichinger, P., Kuprian, M., & Ebner, C. (2013). Potential effects of mechanical pre-treatments on methane yield from solid waste anaerobically digested. *International Journal of Environmental Bioremediation & Biodegradation*, *1*(1), 20-25.

Anunputtikul, W. (2004). Biogas production from cassava tubers. *[Disertation] Suranaree University of Technology.*

Bailis, R., Drigo, R., Ghilardi, A., & Masera, O. (2015). The carbon footprint of traditional woodfuels. *Nature Climate Change*, *5*(3), 266-272.

Bruni, E. (2010). *Improved anaerobic digestion of energy crops and agricultural residues*. Technical University of Denmark (DTU).

Budiyono, B., & Sumardiono, S. (2014). Effect of total solid content to biogas production rate from vinasse. *International Journal of Engineering*, *27*(2), 177-184.

Budiyono, B., Syaichurrozi, I., & Sumardiono, S. (2013). Biogas production from bioethanol waste: the effect of pH and urea addition to biogas production rate. *Waste Technology*, *1*(1), 1-5.

Budiyono, Widiasa I. N., Johari S., Sunarso. (2010b). The Kinetic of Biogas Production rate from Cattle Manure in Batch Mode. *International Journal of Chemical and Biological Engineering*

Budiyono, Widiasa I. N., Johari S., Sunarso.(2010a). Increasing Biogas Production Rate from Cattle Manure Using Rumen Fluid as Inoculums. *International Journal of Basic & Applied Sciences.*

D., Adholeya, A., 2007. Biological approaches for treatment of distillery wastewater: a review. *Bioresource Technology* 98, 2321-2334.

Earley, J. H., Bourne, R. A., Watson, M. J., & Poliakoff, M. (2015). Continuous catalytic upgrading of ethanol to n-butanol and> C 4 products over Cu/CeO 2 catalysts in supercritical CO 2. *Green Chemistry*, *17*(5), 3018-3025.

Eaton, D.W., Clesceri, L.S., Greenberg, A.E., & Franson, M.A.H. (1995). *Standard methods for examination of water and wastewater,* American public Health Association, Washington DC.

Ezekoye, V. A., & Okeke, C. E. (2006). Design, construction, and performance evaluation of plastic biodigester and the storage of biogas. *The Pacific Journal of Science and Technology*, *7*(2), 176-184.

Fernandes, T. A. R., da Silveira, W. B., Passos, F. M. L., & Zucchi, T. D. (2014). Laccases from Actinobacteria--what we have and what to expect. *Advances in Microbiology*, *4*(6), 285.

Girmaye, K., & Ebsa, K. (2019). *Optimization of Biogas Production from Avocado Fruit Peel Wastes Co-digestion with Animal Manure Collected from Juice Vending House in Gimbi Town, Ethiopia*. Fermentation Technology

Kayhanian, M., & Rich, D. (1995). Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements. *Biomass and bioenergy*, *8*(6), 433-444.

Lauterböck, B., Nikolausz, M., Lv, Z., Baumgartner, M., Liebhard, G., & Fuchs, W. (2014). Improvement of anaerobic digestion performance by continuous nitrogen removal with a membrane contactor treating a substrate rich in ammonia and sulfide. *Bioresource technology*, *158*, 209-216.

L.T., Garcia, M.L., 2014. Implications of stillage land disposal: a critical review on the impacts of fertirrigation. *Journal of Environmental Management* 145, 210-229;

Lv, Z., Hu, M., Harms, H., Richnow, H. H., Liebetrau, J., & Nikolausz, M. (2014). Stable isotope composition of biogas allows early warning of complete process failure as a result of ammonia inhibition in anaerobic digesters. *Bioresource technology*, *167*, 251-259.

McCarty, P. L. (1964). Anaerobic waste treatment fundamentals. *Public works*, *95*(9), 107-112

Moraes, B. S., Zaiat, M., & Bonomi, A. (2015). Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives. *Renewable and Sustainable energy reviews*, *44*, 888-903.

Mohana, S., Acharya, B.K., Madamwar, D., 2009. Distillery spent wash: treatment technologies and potential applications. *Journal of Hazardous Materials* 163, 12-25; Pant,

Mshandete, A., Björnsson, L., Kivaisi, A. K., Rubindamayugi, M. S., & Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renewable energy*, *31*(14), 2385-2392.

Ofoefule, A. U., Uzodinma, E. O., & Anyanwu, C. N. (2010). Studies on the effect of anaerobic Digestion on the microbial flora of Animal wastes: 2. Digestion and modelling of process parameters. *Trends in Applied Sciences Research*, *5*(1), 39-47.

Palmowski L., & Müller J. (1999). Influence of the size reduction of organic waste on anaerobic digestion. *International symposium on anaerobic digestion of solid waste. Barcelona,* 137–144.

Quintero, M., Castro, L., Ortiz, C., Guzmán, C., & Escalnte, H. (2012). Enhancement of starting up anaerobic digestion of lignocellulosic substrate: fique's bagasse as an example, *Bioresource Technology*, **108** (2012), 8–13.

Ramos, I., Peña, M., & Fdz-Polanco, M. (2014). Where does the removal of H2S from biogas occur in microaerobic reactors?. *Bioresource Technology*, *166*, 151-157.

Raskin, L., Rittmann, B. E., & Stahl, D. A. (1996). Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms. *Applied and Environmental Microbiology*, *62*(10), 3847-3857.

Rohstoffe eV, F. N. (2012). Guide to Biogas; From Production to Use. *Gülzow-Prüzen, Germany*, 75.

Scherer, P., Neumann, L., Demirel, B., Schmidt, O., & Unbehauen, M. (2009). Long term fermentation studies about the nutritional requirements for biogasification of fodder beet silage as mono-substrate. *biomass and bioenergy*, *33*(5), 873-881.

Schmidt, T., Nelles, M., Scholwin, F., & Pröter, J. (2014). Trace element supplementation in the biogas production from wheat stillage–Optimization of metal dosing. *Bioresource technology*, *168*, 80-85Siles, J. A., García-García, I., Martín, A., & Martín, M. A. (2011). Integrated ozonation and biomethanization treatments of vinasse derived from ethanol manufacturing. *Journal of Hazardous Materials*, *188*(1-3), 247-253.

Somayaji, D., & Khanna, S. (1994). Biomethanation of rice and wheat straw. *World journal of microbiology & biotechnology*, *10*(5), 521-523.

Song, Z., Liu, X., Yan, Z., Yuan, Y., & Liao, Y. (2014). Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PloS one*, *9*(4), e93801.

Tang, Y. Q., Fujimura, Y., Shigematsu, T., Morimura, S., & Kida, K. (2007). Anaerobic treatment performance and microbial population of thermophilic upflow anaerobic filter reactor treating awamori distillery wastewater. *Journal of Bioscience and Bioengineering*, *104*(4), 281-287.

Wang, X., Lu, X., Li, F., & Yang, G. (2014). Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. *PloS one*, *9*(5), e97265.

Wilkie, A. C. (2008, January). Biomethane from biomass, biowaste, and biofuels. In *Bioenergy* (pp. 195-205). American Society of Microbiology.

Wilkie, A. C., Riedesel, K. J., & Owens, J. M. (2000). Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass and Bioenergy*, *19*(2), 63-102.

Yavuz, Y. (2007). EC and EF processes for the treatment of alcohol distillery wastewater. *Separation and purification technology*, *53*(1), 135-140.

Zhong, W., Zhang, Z., Luo, Y., Sun, S., Qiao, W., & Xiao, M. (2011). Effect of biological pretreatments in enhancing corn straw biogas production. *Bioresource technology*, *102*(24), 11177-11182.

APPENDICES

Appendix I: Preparation of reagents for COD determination

Standard potassium dichromate solution, 0.0167M: Dissolved 4.913g K_2Cr_2O, previously dried at 103^0C for 2 hour in 500ml distilled water, 167ml conc H_2SO_4 and 33.3g HgSO4. Dissolve, cool to room temperature and diluted to 1000ml.

Sulfuric acid reagent: Ag_2SO_4, reagent was added to conc H_2SO_4 at the rate of 5.5g Ag_2SO_4 /kg H_2SO_4

Ferroin indicator solution: 1.485g 1,10-phenan-throline monohydrate and 695 mg $FeSO_4.7H_2O$ was dissolved with distilled water and diluted to 100ml.

Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.10M: 39.2g $Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in distilled water. 20ml conc H_2SO_4 was added, cooled and diluted to 1000ml

$$\text{Molarity of FAS} = \frac{Volume\ \ 0.016MK2Cr2O\ solution\ \ titrated\ ,ml}{Volume\ \ FSA\ used\ \ in\ titration\ ,ml} = x\ 0.10$$

Mercuric sulfate, HgSO4, crystals or powder

Potassium hydrogen phthalate (KHP) standard: Potassium hydrogen phthalate ($HOOCC_6H_4COOK$) was lightly crushed and dried to constant weight at 120^0C. Dissolved 425 mg in distilled water and dilute to 1000ml.

Procedure

Treatment of sample- measure a suitable volume of sample and reagents into tube/ampule as indicated in the table

Measurement of dichromate reduction- invert cooled samples, blanks and standards several times and allow solids to settle before measuring absorbance. Read absorbance and compare to calibration curve. Use optically matched culture tubes or ampules for greater sensitivity

Preparation of calibration curves- prepare at least 5 standards from potassium hydrogen phthalate solution with COD equivalence from 20-900 mg O_2/L. Make up to volume with distilled water

Table 6.1: Sample and reagent quantities for various digestion vessels

Digestion Vessel	Sample ml	Digestion Solution (ml)	Sulfuric acid Reagent (ml)	Total Final Volume (ml)
Culture tubes:				
16 X 100 mm	2.5	1.5	3.5	7.5
20 X 150 mm	5.0	3.0	7.0	15.0
25 X 150 mm	10.0	6.0	14.0	30.0
Standard 10-mL Ampoules	2.5	1.5	3.5	7.5

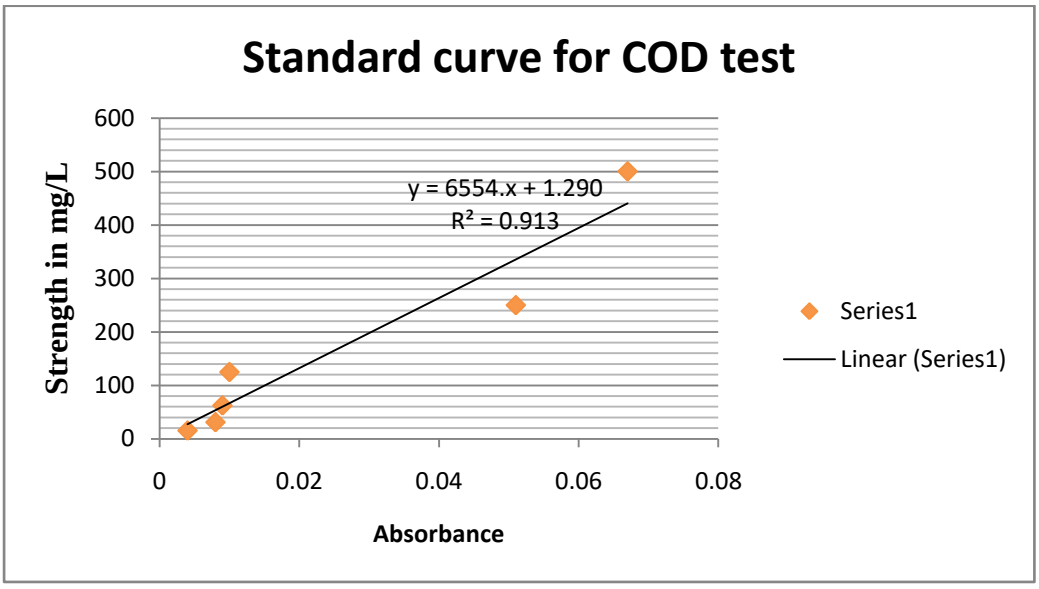


Figure 6.1: Standard curve for COD test

COD as g/L = $\frac{(B-A)x\ M}{1000}$

Where:

A = mg/L meter reading for the sample

B = mg/L meter reading for blank

M = Dilution Factor for the sample

Appendix II: Gravimetric procedures for determination of TS, TSS and TDS

Samples of maize stalks and vinasse were prepared in triplicates. Vinasse samples stirred to homogenize before the samples were taken. The procedure below was used to determine the TS for the substrates.

TS Procedure - Vinasse

I. Empty crucible weight was taken to the nearest 0.01g. Recorded as W_0
II. 20ml of vinasse sample placed on the prepared evaporating crucible.
III. The sample placed in the crucible and weighted, recorded as W_1.
IV. The samples were dried to 103^0C to 105^0C for 30 minutes.
V. Cooled to room temperature and weighed.
VI. The residue heated for 30 minutes, cooled and weighed.
VII. Heating, cooling and weighing procedure was repeated until there was no weight change and the final weight was recorded as W_2.

The above procedure was done in triplicate

Maize stalks

I. Empty crucible weight was taken to the nearest 0.01g. Recorded as W_0
II. 5g sample placed on the prepared evaporating dish.
III. The sample placed in the crucible and weighted, recorded as W_1.
IV. The sample was dried to 103^0C - 105^0C for 10 minutes.
V. Cooled to room temperature and weighed.
VI. The residue heated for 10 minutes, cooled and weighed.
VII. Heating, cooling and weighing procedure was repeated until there was no weight change and the final weight was recorded as W_2.
VIII. The above procedure was done in triplicate

Determination of total suspended solids (TSS)

TSS in vinasse was determined by filtering a known mass of sample using a 2 micron filter paper. The Filter paper residue is rinsed with distilled water before drying. The procedure used was as follows: The procedure was done in triplicates.

1. The mass of 2 micron filter paper was determined in grams. Recorded W_0

2. A known mass of Vinasse sample was filtered. (Mass recorded as W_1 = Mass of vinasse + W_0)
3. The residue on the filter paper was rinse with distilled water
4. The filter paper was dried at 103^0C till there was no mass change.
5. Cooled to room temperature and the mass were noted.W_2
6. Change in mass represented weight for total suspended solids

Total dissolved solids (TDS)

Total dissolved solids were determined by subtracting total suspended solids from total solids using the formula: TS = TSS + TDS

Appendix III: Maize Stalks Pretreatment Procedure

I. Mixture of Maize stalks and NaOH with a ratio of (0.25-1.5% w/v) was prepared

II. The mixture was then placed in a hot water bathe for 3 hours at $100^{O}C$ and stirring was done at an hour interval.

III. The pre-treated mixture was removed from the hot water bathe and cooled.

IV. The pH was measured

V. Neutralization was done using 2M HCL to reduce the pH

VI. The sample was then dried at 55^{o} C.

Appendix IV: Persulfate procedure for determination of Nitrogen content (LAMNDA 900)

Procedure

a) *Calibration curve:* Prepare NO_3 – calibration standard in the range of 0 to 29 mg NO_3 – N/L by diluting to 100 ml the following volumes of intermediate nitrate solution: o, 1.0, 2.0, 4.0,…….29.0 ml. Treat standards in the same manner as samples.

b) *Digestion:* To culture tubes add 10 mL sample or standard or a portion diluted to 10mL.

Sample concentration

Table 6.2: Sample concentration

	Sample runs	Absorbance	Conc.(A) standard curve reading	B = A*(Dilution factor)	Nitrogen content % = (14/62)*B*100
Maize stalks	1	5.37857	0.05402856	0.5402856	12.2
	2	5.54464	0.05535712	0.5535712	12.5
	3	4.935714	0.050485712	0.50485712	11.4
Vinasse	1	0.112	0.011896	0.11896	2.7
	2	0.134	0.012072	0.12072	2.7
	3	0.101	0.011808	0.11808	2.7

Appendix V: Absorbance for standard samples

Sample Number	1	2	3	4	5
Absorbance	0.067	0.051	0.01	0.009	0.008
Strength in mg/l	500	250	125	62.5	31.25

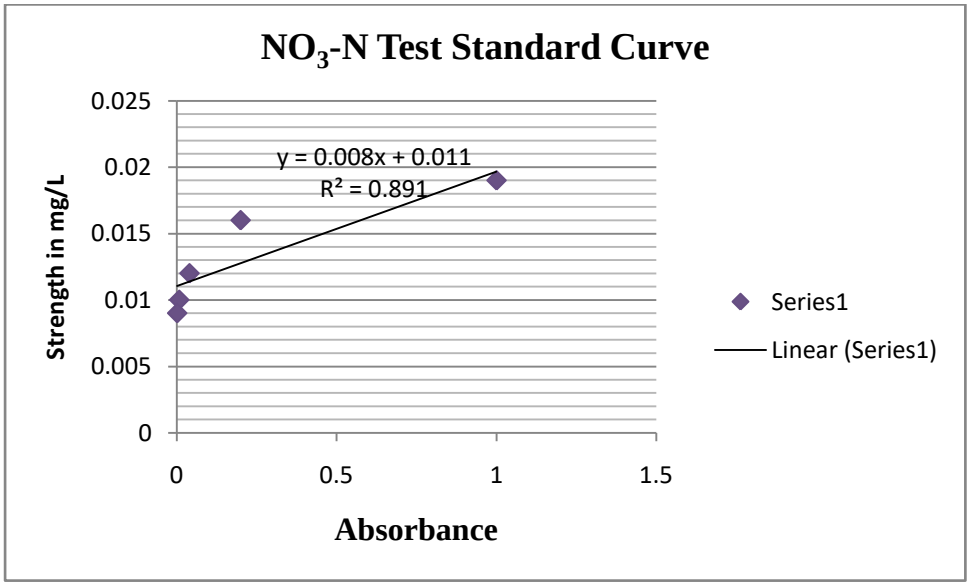


Figure 6.2: NO_3-N Test Standard Curve

Appendix VI: Procedure for Biogas Characterization

1. A sample will be introduced into a heated small chamber via syringe through a septum. The heat (internal oven at $60^{O}C$) facilitates volatilization of the sample and sample matrix.
2. The carrier gas then either sweeps the entirety (split mode) or a portion (split mode) of the sample into the column. In split mode, a part of the sample/carrier gas mixture in the injection chamber is exhausted through the split vent. Split injection is preferred when working with samples with high analyte concentration ($>$ 0.1%) whereas splitless injection is best suited for trace analysis with low amounts of analyte ($<$0.01%). In split less mode the split valve opens after a pre-set amount of time to purge heavier elements that could otherwise contaminate the system. This pre-set time can equal the total run time to effectively keep the purge closed.
3. An electronic detector is used to monitor the outlet stream from the column; thus the time at which each component reaches the outlet and the amount of that component can be determined and displayed by the monitor.

Preparation of a standard methane gas

The standard methane gas used for quantification of the methane present in the gas samples was prepared in the lab as described below.

1. A mixture of sodium acetate and soda lime was heated.
2. Powdered sodium acetate is mixed with four times the amount of soda lime. As the contents are heated, methane gas is produced. It is collected by the downward displacement of water.

$$CH_3COONa + NaOH \xrightarrow[\text{Heat}]{CaO} CH4 + Na_2CO_3$$

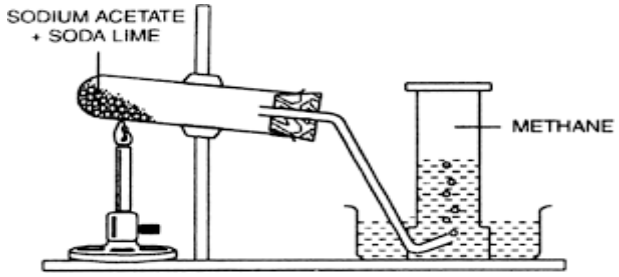


Figure 6.3: Standard methane gas preparation schematic setup

For this experiment methane produced was collected directly on a balloon to prevent it from contamination with water vapour.

Figure6.4: Standard methane gas preparation experiment setup