Production of Biogas from Sugarcane Residues

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Abstract

Due to high production costs facing South African sugar manufacturing industries, production of sugar alone may not be profitable. For sugar manufacturing industries to be economically viable, a novel approach research on other value-added potential products is of paramount importance. The aim of this work was to conduct a feasibility study on biogas production from anaerobic digestion (AD) of sugarcane bagasse, molasses and leaves using cow dung as co-substrate.

Three sets of 12 independent batch laboratory experiments for each residue were carried out at temperature of 35°C and hydraulic retention time (HRT) of 14 days using 500 ml bottles as digesters. Design-Expert software was used for design of experiment, process optimisation and process modelling. One variable at a time (OVAT) and 2-Dimensional (2-D) graphical analysis methods were used to analyse the effects of cow dung to sugarcane residues (C:SR) feed ratio, media solution pH and digester's moisture content on biogas volume, methane yield and kinetic constants.

The results indicated that the effect of C:SR feed ratio, media solution pH and digester's moisture content on biogas volume, methane yield, biogas production potential, maximum biogas production rate and lag phase is mutually reliant between all variables, i.e., depended on conditions of other process variables. The optimum biogas volume generated by bagasse, sugarcane leaves, and molasses experiments were found to be 305.87 ml, 522.69 ml and 719.24 ml and respectively. The results showed that the optimum methane yield achieved by bagasse, sugarcane leaves, and molasses experiments were 28.75 ml/gVS, 87.18 ml/gVS, and 85.32 ml/gVS respectively.

The overall results showed that sugarcane bagasse, molasses and leaves can be potentially converted into biogas through AD process.

Dedication

To my dad

Preface

This project was conducted at Durban University of Technology. The experimental work started in April 2015 and was completed in December 2016 under supervision of Dr. Yusuf Isa.

Declaration

I, the undersigned **Sthembiso P Malunga (20250085)** declare that the work contained in this report was produced by me. All persons who assisted me to make this work successful have been acknowledged. Any work submitted from any other sources has been acknowledged.

This work has not been submitted before for any other degree or examination board at any university.

Place of Submission : Durban University of Technology
Name of the supervisor : Dr Yusuf Isa
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Signature of the Student : ______________________________

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Glossary

The following abbreviations or acronyms are used in this report:

ANOVA	Analysis of Variance
AD	Anaerobic Digestion
ATA	Anaerobic Toxicity Analysis
AcoD	Anaerobic co-digestion
Adeq Precision	Adequate Precision
Adj R-Squared	Adjusted R-Squared
BD	Biodegradability
BVS	Biodegradable Volatile Solids
BOD	Biological Oxygen Demand
BMP	Biochemical methane potential
COD	Chemical Oxygen Demand
C: N	Carbon to Nitrogen ratio
C:SR	Cow dung to Sugarcane Residues
C: SL	Cow dung to Sugarcane Leaves
C: M	Cow dung to Molasses
C: B	Cow dung to Bagasse
CV	Coefficient of variation
DOE	Design of experiment
GC	Gas Chromatograph
HRT	Hydraulic retention time
ISR	Inoculum to Substrate Ratio
TS	Total Solids
VS	Volatile Solids
VFA	Volatile Fatty Acid

STP	Standard Temperature and Pressure
ORP	Oxygen Reduction Potential
RVS	Refractory Volatile Solids
SL	Sugarcane leaves
SB	Sugarcane bagasse
SM	Sugarcane molasses
SASA	South African Sugar Association
SA	South Africa
SW	Sugarcane wastes
LCFA	Long-chain fatty acids
OLR	Organic loading rate
OVAT	One variable at time
Pred R-Squared	Predicted R-Squared
2-D	Two-Dimensional

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Terminology

Digester's moisture content	refers to the concentration of liquid content in the slurry charged in the reactors
Media solution	refers to water and salt solutions charged in the reactors
Biogas volume	Biogas volume is measured in (ml)
Methane yield	specific methane volume per mass of VS charged in the reactors(ml/gVS).
Feed ratio	refers to the actual mass of cow dung to the mass of residues charged into the reactors.

Chapter 1

INTRODUCTION

1.1. Introduction

Current global energy consumption is highly dependent on fossil fuels (Petrov, Bi and Lau 2017). These fossil fuels are not renewable, and their reserves are being depleted much faster than they are being formed Lackner (2010). Fossil fuels provide about 81 % of the world's commercial energy supply (McGlade and Ekins 2015). Utilization of fossil fuels has caused the increase in carbon dioxide (CO_2) concentration in the atmosphere (McGlade and Ekins 2015). Carbon dioxide emitted by combustion of fossil fuels had risen from 280 ppm from the beginning of industrial revolution to 385 ppm in 2010 and carbon dioxide in the atmosphere is rising by 2 ppm per year (Lackner 2010). About 60.0 g of CO_2 is released into atmosphere for every megajoule (MJ) of energy produced from fossil fuels (Lackner 2010). High concentration of CO_2 in atmosphere is likely to alter biology of oceans due to absorption of CO_2 by sea water and carbonic acid is produced which lowers the pH of sea water (Metz *et al.* 2005). Low water pH may interfere with the life of sea animals.

To accomplish low carbon dioxide emissions, biomass fuels can be considered as an alternative for fossils fuels. According to Egglestona *et al.* (2014) biomass fuels contribute about 10 % of primary energy requirements to meet the world energy demand. Although biomass fuels cannot replace fossil fuels, however, they can be used to supplement petrol, diesel and natural gas in combustion engines to produce electricity (Börjesson and Mattiasson 2008; Montoya, Olsen and Amell 2018). The availability of biomass source to produce biomass fuels at commercial scale is of great concern without disrupting food security because the farming of energy crops is likely to compete with food production for land, water and nutrients (Ertem, Neubauer and

Junne 2017; Lijó *et al.* 2017; Petrov, Bi and Lau 2017). To address food security issues, agricultural crops residues are commonly used as a source of biomass fuels (Cheng 2017).

1.2. Rationale

Sugarcane is a C4 plant, and has high rate of photosynthesis (Bezerra and Ragauskas 2016). It has been found that sugarcane has bio-conversion rate of 150 % to 200 % above the average plant (Gutiérrez *et al.* 2018). For this reason, sugarcane residues can potentially be converted to biomass fuels (Souza *et al.* 2018).

Sugar manufacturing industries produce mainly three types of residues, namely: bagasse, molasses and leaves (Holanda and Ramos 2016). Although there are many current industrial applications of these residues, but, a research for production of new value-added and environmentally friendly products is necessary to improve energy economy and profitability.

The aim of sugar manufacturing industries is to minimize wastes and improve profitability by producing high-value products from wastes residues generated (Bezerra and Ragauskas 2016). High-value products include, ethanol, furfural, furfuryl alcohol, biogas, electricity, etc. (Souza *et al.* 2018). Production of these products continue to play an important role in profitability and waste management (Illovo 2015). According to Illovo (2015) annual report 2012, production of these downstream products increased profitability by 24% in 2011/12 financial year compared to 2010/11 financial year.

South African Sugar Association SASA (2016) reported that sugarcane manufacturing industries produce about 75.0 % to 90.0 % of their energy requirements from bagasse. It has been found that high gaseous emissions (SO_2 CO and NO_X) emitted by bagasse fed boilers increase risk of respiratory tract infections, reduction of immune system and pulmonary diseases (Dotaniya *et al.* 2016). On other hand, biogas and ethanol fired boilers are more environmental friendly with no particulate emissions (Rabelo *et al.* 2011; Bezerra and Ragauskas 2016).

The trade-off on bagasse energy use (electricity production vs ethanol or biogas) economic and environmental assessments should be investigated. Biogas and ethanol are biomass fuels which can be derived from bagasse and sugarcane leaves and molasses (Agarwal 2014; Dotaniya *et al.* 2016; Salman 2018). They have been used as fuel for combustion engines to produce electricity to their high shock resistance (Jutakanoke *et al.* 2011; Souza *et al.* 2018). Ethanol is highly corrosive, as a result the use of ethanol as fuel for combustion engine is off limited use (Jigar *et al.* 2011). Biogas is corrosive too due to the presence of H_2S (Salas *et al.* 2012), however, the introduction of controlled amount of air during AD process can reduce H_2S concentration (Botheju and Bakkie 2010).

Biogas is a mixture of different gasses produced by breakdown of organic matter in the absence of oxygen in the process is called anaerobic digestion (Joshua *et al.* 2014; Kuusik *et al.* 2014; Kader *et al.* 2015; Lawal, Dzivama and Wasinda 2016; Bajpai 2017). Biogas is composed mainly of methane and carbon dioxide (Nijaguna 2002; Sajeena, Jose and Madhu 2014; Tengku *et al.* 2014) and can be upgraded into natural gas and other products (Makaruk, Miltner and Harasek 2010). Due to its high shock resistance, biogas can be upgraded into vehicle fuel standard (Persson, Jönsson and Wellinger 2006).

1.3. Objective of the study

The aim of this study was to assess feasibility of biogas production from sugarcane bagasse, leaves and molasses, using cow dung as co-substrate. Specific objectives of this work were:

- To determine the effect of process variables (cow dung to sugarcane biomass ratio, media solution pH and digester's moisture on biogas volume and methane yield.

- To develop mathematical models to predict the interactive effect between process variables and biogas volume or methane yield.
- To do a comparative analysis of sugarcane residues in term of biogas production.
- To model and determine kinetic constant values.

1.4. Thesis overview

Chapter 1: Highlights the introduction of the research work, and benefits of AD in biogas production and environmental impact of using fossil fuels.

Chapter 2: This chapter covers the relevant literature on biogas production process.

Chapter 3: This chapter discusses methodology used for design of experiments (DOE), initial characterization, handling and collection of substrates.

Chapter 4: Results of bagasse and cow dung experimental runs are presented and discussed. . Kinetics constant values results are presented and discussed

Chapter 5: Results of sugarcane leaves and cow dung experimental run are presented and discussed. Kinetics constant values results are presented and discussed.

Chapter 6: Results of molasses and cow dung experimental run are presented and discussed. Kinetics constant values results are presented and discussed

Chapter 7: The validation of experimental results are presented and discussed.

Chapter 8: Comparative analysis. Results of three sugarcane residues are compared.

Chapter 9: In this chapter, the thesis findings are discussed.

Chapter 2

LITERATURE REVIEW

2.1. Introduction

Production of biogas through AD process is the most attractive agricultural management technique due to its ability to control pollution; energy recovery and nutrient recycle simultaneously (Al Seadi *et al.* 2008; Agbor *et al.* 2011; Adekunle and Okolie 2015). Biogas is one of the most attractive form of biomass fuels because while it provides clean energy for the society, it also helps with waste reduction (Drożyner *et al.* 2013; Cheng 2017). Biogas is renewable as it is produced from biomass (Cheng 2017). Biomass is an organic matter that has stored energy through the process of photosynthesis (Drożyner *et al.* 2013). It exists in plants and may be transferred into animal through food chain (Petrov, Bi and Lau 2017).

Biogas typically refers to a mixture of different gasses produced by breakdown of organic matter in the absence of oxygen in the process is called anaerobic digestion (AD) (Joshua *et al.* 2014; Kuusik *et al.* 2014; Kader *et al.* 2015; Lawal, Dzivama and Wasinda 2016; Bajpai 2017). AD is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen (Sosnowski, Wieczorek and Ledakowicz 2003; Schnürer and Jarvis 2010).The main product of AD is biogas, which is composed mainly of methane and carbon dioxide (Nijaguna 2002; Sajeena, Jose and Madhu 2014; Tengku *et al.* 2014).

There are many factors which affects biogas production, namely: total solids (TS), volatile solids (VS), alkalinity, chemical oxygen demand (COD) and carbon to nitrogen ratio (C: N) inoculum concentration and others.

To improve the performance of AD process, two or more biomasses are digested simultaneously in a process called anaerobic co-digestion (AcoD) (Giuliano *et al.* 2013; Gashaw and Teshita 2014; Nordell *et al.* 2015; Shah *et al.* 2015). The aim of AcoD is to provide missing nutrients required. Laboratory tests must be performed to evaluate the suitability of each biomass to be used for in AcoD (Braun and Wellinger 2005; Tabatabae *et al.* 2011; Shin *et al.* 2013).

Biochemical methane potential (BMP) and anaerobic toxicity assays (ATA) are used to check for suitability of biomass for AD process (Gerardi 2003; Braun and Wellinger 2005; Schnürer and Jarvis 2010; Tabatabae *et al.* 2011; Shin *et al.* 2013).

2.2. Biochemical methane potential

Biochemical methane potential is used to measure anaerobic biodegradability of organic substance (Shin *et al.* 2013). It is mostly used to determine the concentration of organic matter in wastewater that can be anaerobically converted into methane (Samyuktha *et al.* 2015). BMP can be determined either theoretically or experimentally.

2.2.1. Experimental detrmination of BMP

Owen *et al.* (1979) developed a procedure to determine BMP experimentally, which is still being used by many researchers. Basic approach is to incubate small amount of biomass waste with inoculum and measure the amount of volume and concentration of methane in biogas generated. Tests are done by using test bottles in batch sequence. Media solution and substrates are mixed together in test bottles at optimal condition. Mixture is anaerobically digested until biogas production stops. At the end of the experiment biogas quality and quantity is measured.

2.2.2. Theoretical determination of BMP

Two methods are used to determine BMP theoretically, namely: stoichiometric reaction ratio and empirical gas laws.

2.2.2.1. Determination of BMP from stoichiometric ratio

Shi *et al.* (2016) used equation 2.1 to calculate theoretical BMP values based on stoichiometric ratio of empirical reaction. This method assumes that that all organic materials are converted only to CH_4 and CO_2 (Bajpai 2017).

$$BMP\,(ml\,CH_4\,/\,gVS) = \frac{(4a+b-2c-3d)\times 2.8}{12a+b+16c+14d} \text{ .... } 2.1$$

$$C_nH_aO_bN_c + \left(n-\frac{a}{4}-\frac{b}{2}+\frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2}+\frac{a}{8}+\frac{b}{4}+\frac{3c}{4}\right)CH_4 + \left(\frac{n}{2}+\frac{a}{8}+\frac{b}{4}+\frac{3c}{8}\right)CO_2 \text{ ......2.1.1}$$
$$+dNH_3$$

Where: $C_aH_bO_cN_d$ represents organic matter

a, b, c and d represent stoichiometric coefficients from reaction 2.1.1 (Bajpai 2017).

2.2.2.2. Determination of BMP from empirical gas law

Nielfa, Cano and Fdz-Polanco (2015) used equation 2.2 to calculate BMP from VS added.

$$\text{BMP(ml/g VS)} = \frac{n_{CH_4}\times RT}{P\times VS} \text{ ....2.2}$$

Where:

BMP represents theoretical biochemical methane potential, R represents universal gas constant (R = 0.082 atmL/mol K), T represents temperature of digester, P represent atmospheric pressure (1atm), VS (g) represents volatile solids of substrate and n_{CH_4} represents molecular amount of methane (mol) which can be calculated from equation 2.2.1.

$$n_{CH_4}(mol) = \frac{COD}{M_{CH_4}}\frac{COD(g)}{64(g\,/\,mol)} \text{ ....2.2.1}$$

COD and VS are the most common parameters which are used to describe the concentration of organic matter in substrates (Dioha *et al.* 2013; Tanimu *et al.* 2014). If the composition of organic material is known, it is possible to calculate between COD and VS content.

Angelidaki and Sanders (2004) used glucose complete oxidation to illustrate the relationship between COD and VS according to equation 2.2.2.

$$C_6H_{12}O_2(M=180)+6O_2(M=32)\rightarrow 6CO_2+6H_2O \text{ ....2.2.2}$$

Where: M represent molar masses of $C_6H_{12}O_2$ and O_2 are 180 g/ mol and 32 g/mol respectively.

According to Angelidaki and Sanders (2004) the ratio of COD-to-VS can be expressed according equation 2.2.3.

$$\frac{COD}{VS}=\frac{6\times M_{O_2}}{1\times M_{C_6H_{12}O_2}}=\frac{6\times 32}{180}=1.07g\,COD/g\,VS \text{ ....2.2.3}$$

Nielfa, Cano and Fdz-Polanco (2015) suggested that the value of COD/VS ratio is close to 1 for many organic substances because of their carbon oxidation state which is close to zero. It is therefore possible to estimate either COD or VS if one is known by using equation 2.2.3.

2.3. Anaerobic toxicity assays

Anaerobic toxicity assays measures substance's adverse effect on biogas production (Hansena *et al.* 2004; Moody 2010; Labatut, Angenent and Scott 2011). Easily digested substrate is added into the test bottles with media solution. Substrate at which ATA test is to be analysed is added gradually at different concentrations while measuring volume of biogas being released. If there is no change in the biogas production, then the substance is not toxic, should the biogas production rate decrease then the substance is toxic (Owen *et al.* 1979; Shin *et al.* 2013; Samyuktha *et al.* 2015; Hamilton 2016).

2.4. Anaerobic digestion process

There are four major stages of AD process namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gerardi 2003; Al Seadi *et al.* 2008; Schnürer and Jarvis 2010; Botheju and Bakkie 2011; Dobre, Nicolae and Matei 2014; Joshua *et al.* 2014). Figure 2.1 shows four basic steps of AD process.

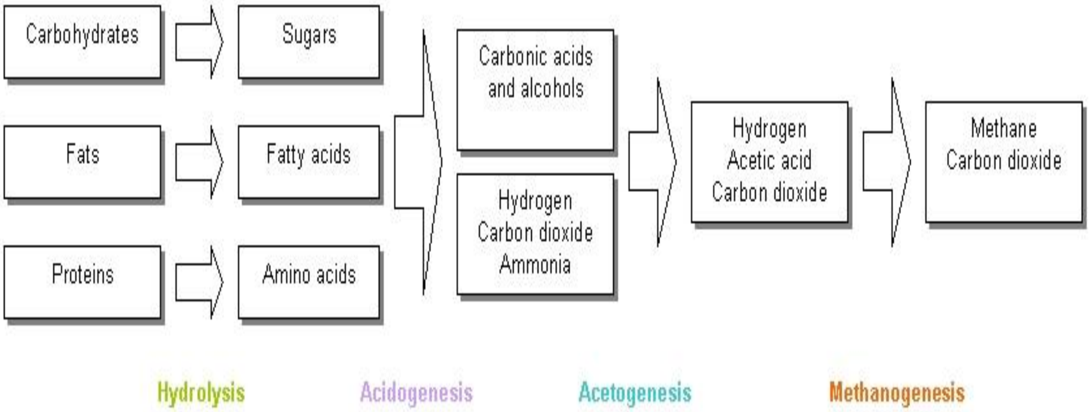

Figure 2.1: Summary of anaerobic digestion process (Thenabadu 2014)

2.4.1. Hydrolysis

Hydrolysis is the first stage of AD process where micro molecules such as carbohydrates, protein, lipids and polysaccharides are broken down into sugars, amino acid and fatty acid (Adekunle and Okolie 2015; Kader *et al.* 2015). According to Angelidaki and Sanders (2004) hydrolisis is a rate-limiting stage. The speed at which biogas is produced depends on hydrolysis. There are three anaerobes which are responsible for the processes on this stage, namely: bactericides, clostridia and facultative bacteria (Gerardi 2003; Schnürer and Jarvis 2010; Ali Shah *et al.* 2014).

To accomplish biodegradation, extracellular enzymes break down large molecules into smaller particles which can be consumed by microorganisms as source of energy and nutrition (Gerardi 2003). Acetic acid and hydrogen are produced in this stage but are used by the methanogens in the last stage of the AD process (Gerardi 2003; Schnürer and Jarvis 2010). Table 2.1 presents a list of some important hydrolytic enzymes which are responsible for hydrolysis stage (Merlin, Gopinath and Divya 2014). Equation 2.3 represents hydrolysis reaction where organic waste is broken into simple sugars (Bajpai 2017).

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2 \qquad 2.3$$

Table 2.1: List of some important hydrolytic enzymes, their substrates they act upon and products formed.

Enzymes	Substrates	Products
Proteinase	Protein	Amino acid
Cellulose	Cellulose	Cellobise and glucose
Hemicellulose	Hemicelluloses	Glucose, Xylose, Manose
Amylase	Starch	Glucose
Lipase	Fats	Fatty acid and glycerol
Pectinase	Pectine	Galoctose, arabinose and polygalacineatric acid

2.4.2. Acidogenesis

According to Gerardi (2003) acidogenisis is a biological reaction where simple monomers are converted into volatile fatty acids (VFA). In this stage, products of hydrolysis stage are transformed into propionic acid (CH_3CH_2COOH), butyric acid ($C_4H_8O_2$), acetic acid (CH_3COOH), formic acid ($HCOOH$), lactic acid ($CH_4H_6O_3$), ethanol (C_2H_5OH), methanol (CH_3OH), CO_2 and H_2 (Gerardi 2003; Joshua *et al.* 2014). Equations 2.4, 2.5 and 2.6 represent reactions where glucose is converted into ethanol, propionic acid and acetic acid respectively (Bajpai 2017).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad 2.4$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \qquad 2.5$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad 2.6$$

2.4.3. Acetogenesis

According to Al Seadi *et al.* (2008) acetogenesis is a biological reaction where VFA are converted into hydrogen, acetic acid and carbon monoxide. Equation 2.7 shows the transformation of propionic acid into acetic acid. Equations 2.8 and 2.9 represent glucose and ethanol conversion into acetate respectively (Bajpai 2017).

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad \text{.......} 2.7$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \text{.......} 2.8$$

$$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+ \quad \text{.......} 2.9$$

2.4.4. Methanogenesis

Methanogenesis is a biological reaction where acetates are converted into carbon dioxide and methane (Al Seadi *et al.* 2008; Adebayo, Jekayinfa and Linke 2014b). Equation 2.10 represents acetic acid being converted into methane and carbon dioxide. Equation 2.11 represents conversion of ethanol and CO_2 into methane and acetic acid. Equation 2.12 represents the conversion of CO_2 and hydrogen into methane and water (Bajpai 2017).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \text{.......} 2.10$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad \text{.......} 2.11$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \text{.......} 2.12$$

Biogas production is pH sensitive and requires pH of between 6.5 and 7.8 (Tabatabae *et al.* 2011; Ziemiński and Frąc 2012; Okonkwo, Aderemi and Okoli 2013; Dobre, Nicolae and Matei 2014).

2.4. Co-digestion

AcoD is defined as anaerobic treatment of mixture two or more different biomass types, with the aim of improving the efficiency of AD process by supplying missing nutrient (Aragaw, Andargie and Gessesse 2013; Giuliano *et al.* 2013; Kuusik *et al.* 2014; Nordell *et al.* 2015; Shah *et al.* 2015). Cow dung is commonly used as co-substrate for AD process. Cow dung is high in organic materials and rich in nutrients. Mixing cow dung with organic wastes has been successfully applied for biogas production by many researcher (Zhang *et al.* 2007; Kumar, Ou and Lin 2010; Aragaw, Andargie and Gessesse 2013; Giuliano *et al.* 2013; Gashaw and Teshita 2014; Nordell *et al.* 2015).

2.6. Biogas

Biogas is composed primarily of methane and carbon dioxide (Al Seadi *et al.* 2008). Its composition depends on substrates used and the conditions at which the AD process was conducted (Prakasha *et al.* 2015). Biogas varies from 50 to 78 % CH_4, 35 to 50 % CO_2, 2 to 4 % H_2O and 0 to 1 % H_2S (Nijaguna 2002; Al Seadi *et al.* 2008; Schnürer and Jarvis 2010; Sathish and Vivekanandan 2014; Prakasha *et al.* 2015).

2.7. Classification of substrates

Any material which when added into AD process becomes food for microbes and is called substrate (Achinas, Achinas and Euverink 2017). Properties of substrates have major impact on AD process stability, efficiency and quality of biogas and digestate produced (Krich *et al.* 2005). Wastewater treatment plant sludge, municipal wastes, industrial wastes, slaughterhouse wastes, animal manures and agricultural wastes are commonly used for AD process (Wagner *et al.* 2013). Substrates are classified in term of structure and composition , namely: carbohydrates, proteins, lipids and hemicelluloses (Esposito *et al.* 2012). Table 2.2 represents theoretical biogas composition from proteins, lipids and carbohydrates.

Table 2.2: Theoretical biogas composition

Substrate	Biogas formed composition(v/v) % (CH_4: CO_2)
Carbohydrate	50:50
Lipids	70:30
Protein	55:45

2.7.1. Carbohydrates

Carbohydrates are composed of monosaccharide, disaccharide, and polysaccharide. Monosaccharide cannot be broken down into sugars while disaccharides and polysaccharides can be broken down into sugars (Wagner *et al.* 2013; Adekunle and Okolie 2015). Carbohydrates consist of carbon, hydrogen, and oxygen and they are the main components of organic components from food wastes, agriculture-related factories, municipal solids wastes and household waste (Asif *et al.* 2011). Equation 2.13 represents conversion of carbohydrates ($C_nH_{n-2}O_{n-1}$) into equal amount of CH_4 and CO_2. Equation 2.13 indicates that carbohydrates yield 50% methane and 50% carbon dioxide (Krich *et al.* 2005).

$$C_nH_{n-2}O_{n-1} + nH_2O \rightarrow 0.5CH_4 + 0.5nCO_2 \quad \text{...2.13}$$

2.7.2. Lipids

Lipids are molecules that contain hydrocarbons and make up building block of the structure and function of living cells (Akpinar-Bayizit 2014). They are insoluble in water and release large amount of energy when they are metabolized (Akpinar-Bayizit 2014). They are naturally occurring molecules that include fats, waxes, sterols, diglycerides, phospholipids, glycerol triflate (Dowhan 1997).

Industries such as slaughterhouse, edible oil and fat refining, margarine, palm oil processing and meat packing industries produce effluent with high lipids (Li *et al.* 2002a). Hydrolysis of lipids results to long-chain fatty acids (LCFA) which may cause slow down biogas production. activity and decrease the concentration of adenosine which plays the important role in biochemical processes (Cirne *et al.* 2007). Lipids are degraded to VFA and LCFA and glycerol (Li *et al.* 2002b). Higher number of carbon and hydrogen atoms in lipids is the reason why high biogas production is achieved by lipids compared to proteins and carbohydrates.

Equation 2.14 represents conversion of lipids to methane and carbon dioxide where ratio of methane to carbon dioxide ratio is 70: 30 (Krich *et al.* 2005).

$$C_{54}H_{106}O_6 + 28H_2O \rightarrow 40CH_4 + 17CO_2 \qquad 2.14$$

2.7.3. Proteins

Proteins contain carbon, hydrogen, oxygen, nitrogen, and sulphur, and are composed of one or more chains of amino acids (Rajagopal, Mass and Singh 2013). Biomass that are high in protein are from meat processing factories, slaughterhouses, and animal manures (Rollon 2005). Wastes that are rich in protein contain high biological oxygen demand (BOD) but low carbon to nitrogen ratio (C: N)(Esposito *et al.* 2012). Wastes that have high protein contents are technically not feasible to use but theoretically have biogas potential (Al Seadi *et al.* 2008; Rainey 2009; Bélaich, Bruschi and Garcia 2012; Rajagopal, Mass and Singh 2013). This is due to high ammonia concentration released which has toxic effects on microbes. Equation 2.15 is the conversion of protein into methane, carbon dioxide, and ammonia.

$$C_{10}H_{20}O_6N_2 + 3H_2O \rightarrow 5.5CH_4 + 4.5CO_2 + 2NH_3 \qquad 2.15$$

Equation 2.15 shows that for every 55 % of methane produced, 45 % of carbon dioxide will be produced (Krich *et al.* 2005).

2.7.4. Lignocelluloses

Lignocelluloses are the most abundantly available raw material on earth (Mosier *et al.* 2005). They are composed of carbohydrates polymers i.e. cellulose, hemicelluloses, and lignin. Lignocelluloses material contains 40 to 50% cellulose, 25% hemicelluloses and 25% lignin (Kumar, Singh and Singh 2008; Jutakanoke *et al.* 2011).

Cellulose is composed of D- glucose, which is the most abundant organic polymer on earth and due to its polysaccharide structure, large amount of hydroxyl group exists along the cellulose backbone (Sun *et al.* 2016). Hemicellulose is a polysaccharide with a lower molecular weight than cellulose. It is formed from D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose,4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Sugars are linked together by β-1, 4- and sometimes by β-1,3-glycosidic bonds (Karp *et al.* 2013).

Hemicellulose is derived from several sugars and uronic acid. Xylan is the most abundant hemicelluloses (Agbor *et al.* 2011; Aboderheeba 2013). Lignin binds the different components of lignocelluloses biomass together and it is insoluble in water (Agbor *et al.* 2011; Aboderheeba 2013). Production of biogas and bio-fuels from these materials is complicated because polysaccharides are not easily biodegradable (Jutakanoke *et al.* 2011; Chandel *et al.* 2014). In order to process lignocelluloses, the polysaccharides must first be hydrolysed with acid or enzymes (Zheng *et al.* 2014).

2.8. Parameters affecting AD process

2.8.1. Overview

The efficiency of AD process depends on how well the optimum conditions of each factor are controlled. Table 2.3 shows some important optimum conditions of AD process (Christy, Gopinath and Divya 2014; Merlin, Gopinath and Divya 2014; Adekunle and Okolie 2015; Prakasha *et al.* 2015).

Table 2.3: Optimum values of some important parameters for AD process

Parameters	Hydrolysis/Acidogenisis	Methanogenisis
Temperature	25-35^oC	Mesophilic: 30-40^oC
		Thermophilic: 50-60^oC
pH	5.2-6.3	6.7-7.5
C: N ratio	10-45	20-30
Redox potential	+400-300mV	less than-250mV
C: N: P: S ratio	500:15:5:3	600:15:5:3

2.8.2. The effect of carbon to nitrogen ratio

Carbon and nitrogen must be in a correct proportion for optimum growth and activity of microbes (Siddiqui, Horan and Anaman 2011 ; Tanimu *et al.* 2014; Zhu 2007). Carbon is a source of energy and nitrogen is required for cell build-up (Tanimu *et al.* 2014). The optimum C: N ratio depends on the conditions at which the experiment was being conducted and substrates used (Siddiqui, Horan and Anaman 2011 ; Tanimu *et al.* 2014; Zhu 2007).

Zhu (2007) achieved the highest biogas production at C:N ratio of 25:1 where swine manure and rice hikes were co-digested at temperature of 35^oC for 21 days. Siddiqui, Horan and Anaman (2011) achieved the highest VS destruction of 93% at C: N ratio of 20:1 where industrial wastes was co-digested with swine manure at temperature of 37^oC and HRT of 10 days. Tanimu *et al.* (2014) achieved the highest VS removal efficiency of 85% at C: N ratio of 30:1, where different food wastes with different C: N ratio were anaerobically treated at mesophilic temperature conditions.

It has been found that some wastes lack either nitrogen or carbon when they are anaerobically digested into biogas production (Christy, Gopinath and Divya 2014; Merlin, Gopinath and Divya 2014; Adekunle and Okolie 2015; Prakasha *et al.* 2015). Table 2.4 represents C: N ratios for some materials which are commonly used as substrates for biogas production (Christy, Gopinath and Divya 2014; Merlin, Gopinath and Divya 2014; Adekunle and Okolie 2015;

Prakasha *et al.* 2015). Carbon content can be determined from its VS content according to empirical equation 2.16 (Jigar *et al.* 2011).

$$Carbon\,(\%) = \frac{\mathrm{VS}\,(\%)}{1.8} \quad \text{..2.16}$$

Nitrogen content can be determined by the Kjeldahl method. This method involves heating the substance with sulphuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulphate.

Table 2.4: C: N ratio of some material which are commonly used as substrates for biogas.

Material	C:N ratio
Cow dung	6-20:1
Chicken manure	3-10:1
Swine manure liquid	5:1
Straw	50-150:1
Grass	12-26:1
Potatoes	35-60:1
Sugar beet	55-46:1
Fruit and vegetables	7-35:1
Mixed food waste	15-32:1
Slaughterhouse waste	15-32:1

The effect of C:N ratio was investigated by Tanimu *et al.* (2014) using laboratory batch scale digesters. The experiments were conducted at constant pH (6.8) and temperature (35^{o} C) for 30 days using 1000 ml as digesters. Feedstock waste had an initial C: N ratio of 17 and was upgraded into feedstock 2 and feedstock 3 by adding more meat, fruits and vegetables. Table 2.5 represents C: N ratios of different feed stock at corresponding methane yield.

Table 2.5: Feedstock preparation for C/N by Tanimu et al. (2014)

Variable	Feedstock 1	Feedstock 2	Feedstock 3
Carbon (g)	23.99	44.55	62.30
Nitrogen(g)	1.45	1.73	2.02
C: N	16.50	22.75	30.84
Methane Yield (L/gVS)	0.32	0.45	0.68

Table 2.5 shows that the highest methane yield was achieved by feedstock 3 where C: N ratio had been adjusted to 30.84.

2.8.3. Temperature

Temperature is one of the most important factors that affects most of microbial activities (Pandey and Soupir 2011; Zhao 2011; Dobre, Nicolae and Matei 2014). Temperature influences the rate of most chemical or biological reactions (Sibiya, Muzenda and Tesfagiorgis 2014). Generally, reactions are faster at high temperatures. There are three temperature ranges at which anaerobic digesters can be operated, namely: psychrophilic, mesophilic and thermophilic (Al Seadi *et al.* 2008; Schnürer and Jarvis 2010; Botheju and Bakkie 2011; Sathish and Vivekanandan 2014; Barbazán 2015). Table 2.6 represents optimum temperature values for each temperature condition.

Table 2.6: Temperature conditions for AD process and its optimum value

Condition	Temperature range	Optimum Temperature	Source
Psychrophilic	<10ºC	-	(Nijaguna 2002)
Mesospheric	15-45ºC	35ºC	(Al Seadi *et al.* 2008)
Thermophilic	45 -65ºC	55ºC	(Schnürer and Jarvis 2010)

Arrhenius equation shown in equation 2.17 is used to quantify the effect of temperature in AD reactors.

$$K = K_o \times e^{\left(\frac{-E}{R-T_A}\right)} \quad \text{...} 2.17$$

Where: K = reaction rate

K_O = reaction rate constant

E = activation energy

R = universal gas constant (1.98 cal/molK)

T_A = absolute temperature (K)

ℓ = Euler number (2.71)

According to Barbazán (2015), the maximum growth rate increases with the increase in temperature until the new maximum growth rate is achieved. The change of maximum growth rate result in two processes: bacterial synthesis and bacterial decay. Net growth rate can be expressed as deference between two, according to equation 2.18.

$$K_{net} = K_1 \times e^{\left(\frac{-E_1}{R-T_A}\right)} - K_2 \times e^{\left(\frac{-E_2}{R-T_A}\right)} \quad \text{.........} 2.18$$

Where: K_{net} = net growth rate

K_1 = bacterial growth

K_2 = bacterial decay rate

The choice of temperature is critical to AD process. Temperature has strong influence on quality and quantity of biogas produced. Mesophilic bacteria can withstand high-temperature variation and large pH fluctuations (Ratkowsky *et al.* 1982; Nijaguna 2002; Gerardi 2003; Prakasha *et al.* 2015). Thermophilic bacteria are sensitive to pH variations (Pandey and Soupir 2011). The rate of biogas production is higher for thermophilic conduction than that of mesophilic condition. The advantage of operating biogas digester at thermophilic temperature conditions is the high rate of biogas production. This advantage is balanced by the cost associated with heating of the digester.

2.8.4. Moisture content

Water is essential for survival and movement of micro-organism and the optimum moisture content has to be maintained in the digester in order to achieve good biogas production (Lay, Li and Noike 1997). Some of researchers have reported the optimum value of digester's moisture content as 90% (Gerardi 2003; Zhang , Banks and Heaven 2012; Dobre, Nicolae and Matei 2014).

Excess water in a digester will lead to drop of biogas production per unit volume of feedstock and microorganisms are washed out in a continuous process (Schnürer and Jarvis 2010). Inadequate water leads to accumulation of acetic acids which inhibit the digestion process and hence production (Dobre, Nicolae and Matei 2014).

2.8.5. Availability of oxygen

Oxygen acts as an inhibitory and toxic agent in anaerobic digestion (Botheju and Bakkie 2010). Aerobic conversion of organic matter into CO_2 by aerobic respiration occurs if the digester has oxygen (Botheju and Bakkie 2010). Digester instability, slow start up, low methane yield occurs in the presence of oxygen (Kato, Field and Lettiga 1997). Hasengawa *et al.* (2009) observed that hydrolysis rate is higher under aerobic conditions than anaerobic conditions.

The presence of oxygen improves hydrolysis rate (Botheju and Bakkie 2011) but Kato, Field and Lettiga (1997) belived that methanogenesis has no tolerance of oxygen, therefore, AD process will not produce methane in the presence of oxygen. Botheju and Bakkie (2011) showed that the optimum amount of oxygen level in AD process may not be harmful to methane forming bacteria.

2.8.6. Ammonia toxicity

Ammonia is one of important compounds of AD process (Shi *et al.* 2016). It is formed from AD process of proteins compounds (Karlsson and Ejlertsson 2012). Ammonia may be present in the form of ammonium ion (NH_4+) or as gas (NH_3) and they are in equilibrium with each (Tabatabae *et al.* 2011). Table 2.7 represents ammonia nitrogen concentration and their effect on AD process (McCarty and McKinney 1961; McCarty 1964).

Table 2.7: Ammonia concentration and their effects on AD process

Ammonia nitrogen concentration (mg/L)	Effect on AD process
50-200	Beneficial
200-1000	No adverse effect
1500-3000	Inhibitory at higher pH values (7.4 -7.6)
Above 3000	Toxic

High ammonia concentration in a digester inhibits AD process due to accumulation of VFA (Shi *et al.* 2016). High ammonia concentration is common for AD of animal manures due to high ammonium nitrogen released compounds (Karlsson and Ejlertsson 2012). Concentration of free ammonia is directly proportional to temperature, therefore, there is a high risk of high free ammonia concentration at high temperatures (Shi *et al.* 2016).

2.8.7. pH

Optimal pH values for different stages of AD process are not the same. Table 2.8 represents optimum pH values for all stages of AD process. AD process performs well between pH range of 6.5 and 7.8 (Okonkwo, Aderemi and Okoli 2013; Kheireddine, Derbal and Bencheikh-Lehocine 2014). The optimum pH range for different kind of methanogenesis is presented by Table 2.9.

Table 2.8: Optimum pH values for different stage of AD process

Stage	Optimum pH	Source
Hydrolysis	4.0	Cameron and Gani (2011)
Acidogenisis	6.5	Nijaguna (2002)
Acetogenesis	6.0	(Christy, Gopinath and Divya 2014)
Methanogenesis	6.5-7.8	Tabatabae *et al.* (2011)

Function and characteristics, structure and chemical activity of enzymes are affected by pH as certain enzymes are only active at certain pH ranges.

Table 2.9: Optimum pH range for different kind of methanogenesis was presented by Tabatabae et al. (2011)

Genus	pH
Methanosphaera	6.8
Mathanothermus	6.5
Methanogeuim	7.0
Methanolacinia	6.6-7.2
Methanocrobium	6.1-6.9
Methanospirullium	7.0-7.5
Methanococcoides	7.0-7.5
Methanohalobium	6.5-7.5
Methanolobus	6.5-6.8
Methanothrix	7.1-7.8

Kheireddine, Derbal and Bencheikh-Lehocine (2014) studied the effect of initial pH on anaerobic digestion of dairy waste. AD process was conducted on a series of 570 ml batch scale reactors operated at initial pH of 4.0; 5.5; 7.0 and 9.5 at temperature of 55°C. 400 ml of cow dung were charged in each reactor. Results showed that the efficiency of COD removal for initial pH of 4.0; 5.5; 7.0 and 9.5 were 49.11; 63.75; 7; 90.8 and 79.64% respectively and corresponding with biogas volume of 163 ml, 100 ml, 2000 ml and 1500 ml. Results showed that initial pH of 7.0 achieved the highest biogas volume and the highest COD removal efficiency.

2.8.8. Alkalinity

Alkalinity is the ability of solution to neutralize acids. Alkalinity prevents rapid change of pH by buffering acidity created by acidogenesis process (Pereira, Campos and Motteran 2013). AD process is enhanced by the high alkalinity while low alkalinity causes accumulation of VFA (Adekunle and Okolie 2015). Alkalinity of a digester ranges from 2000 to 5000 g/L (Barbazán 2015). AD process charged with insufficient alkali compounds can be supplemented by certain chemicals to maintain the acceptable values of alkalinity.

Buffer solution consists of weak acid and it corresponding salts and can be explained by the equations 2.19; 2.20 and 2.21 (Barbazán 2015).

$$HA + H_2O \leftrightarrow H_3O^+ + A^- \quad \text{2.19}$$

$$K_A = \frac{\left[H_3O^+\right]\times\left[A^+\right]}{[HA]} \quad \text{2.20}$$

$$pH = pK_A + \log\frac{\left[A^-\right]}{[HA]} \quad \text{2.21}$$

Digester stability is enhanced by the high alkalinity and decrease in alkalinity causes accumulation of organic acids. Alkalinity of the digester is proportional to the composition of the substrates fed in the digester. Alkalinity of the digester should be between 2000 to 5000 g/L (Barbazán 2015**.** Should substrates have insufficient alkali compounds, certain chemicals are added to maintain the acceptable valve of the alkalinity. Table 2.10 represents the lists of chemicals which increase alkalinity of digester.

Table 2.10: Chemicals used to supplement for alkalinity in the digester (Tabatabae *et al.* 2011)

Chemical	Formula	Buffering Caution
Sodium bicarbonate	$NaHCO_3$	Na^+
Potassium bicarbonate	$KHCO_3$	K^+
Sodium carbonate	$NaCO_3$	Na^+
Potassium carbonate	K_2CO_3	K^+
Calcium carbonate	$CaCO_3$	Ca^{2+}
Calcium hydroxide	$Ca(OH)_2$	Ca^{2+}
Anhydrous ammonia	NH_3	NH^{4+}
Sodium nitrate	$NaNO_3$	Na^+

2.8.9. Chemical oxygen demand

Chemical oxygen demand is one of the most important properties that is used to determine the amount of organic matter in waste streams (Gerardi 2003; Bélaich, Bruschi and Garcia 2012). This oxygen is measured by using a strong chemical oxidizing agent in an acid medium.

Ghani (2009) conducted a laboratory test work to study the effect of COD on biogas production. Three set of experiments were performed on municipal waste leachate with different COD. Digester 1 was fed with COD of 3000 mg/L and digester 2 with 21000 mg/L of COD. The experiments were conducted at fixed temperature of 35 ^{o}C and pH of 7.3 at hydraulic retention time of 20 days. The performance was evaluated based on biogas production and COD removal efficiencies. Table 2.11 which represents experimental results shows that digester 1 achieved higher COD efficiency removal (46%) compared to digester 2 where efficiency of 33% was achieved. Digester 2 achieved higher biogas production (1.5 ml/ml leachate/day) compared to digester 1 where biogas production of 0.6 ml/ml leachate / day was achieved.

Table 2.11: Experimental results for the effect of COD on biogas production by Ghani (2009)

Digester	COD	% COD removal	Biogas production (ml/ml leachate/day)
Digester 1	2912.0	46.0	0.6
Digester 2	21056.0	33.0	1.5

This indicates that high biogas production may be achieved by high COD wastes, but the efficiency of AD process is reduced.

2.8.10. Volatile fatty acid

Volatile fatty acids (VFA) are produced from acidogenesis stage of AD process. There are four volatile fatty acids, namely: acetate, propionate, butyrate and lactate (Al Seadi *et al.* 2008; Al Seadi *et al.* 2013). Stability of anaerobic process is affected by the concentration of these VFA.

High concentration of VFA results in low biogas production (Aftab *et al.* 2014; Adekunle and Okolie 2015).

2.8.11. Volatile solids and organic loading rate

Volatile solid (VS) represents a difference between total solids and the ash content as obtained by complete combustion of substrates (Monnet 2003). Volatile solids have two components namely: biodegradable volatile solids (BVS) and refractory volatile solids (RVS) (Verma 2002). Waste with high BVS and low RVS is suitable for AD process, while waste that has low BVS and high RVS is not suitable substrate for AD process. Organic loading rate (OLR) is a measure of the capacity of anaerobic digester (Mähnert and Linke 2009). It is an important parameter for continuous operation (Mähnert and Linke 2009). It is expressed in kg COD or VS per m^3.

2.8.12. Hydraulic retention time

Hydraulic retention time is the length of time that the substrates take to remain in the digester during the AD process (Kim *et al.* 2006). The optimum HRT depends on the substrates used. There is a linear relationship between digester temperature and HRT. High temperature operated digesters result to a shorter HRT. Heo, Park and Kang (2004) reported that HRT is affected by waste composition, technology used, moisture contents of the AD process and other parameters.

2.8.13. Pre-treatment

Many researchers have proven that pre-treatment of substrates prior to anaerobic digestion is necessary to enhance biomass waste biodegradability, rate of digestion and quality of biogas generated (Agbor *et al.* 2011; Jutakanoke *et al.* 2011; Janke *et al.* 2015). According to Aboderheeba (2013) pre-treatment can reduce retention time from 25 days to approximately 7 days. Four methods of pre-treatment, i.e., physical treatment, chemical treatment, thermal hydrolysis and biological treatment are employed in AD process (Zheng *et al.* 2014). Physical pre-treatment involves disintegration methods like grinding and milling to reduce the particle size. Chemical pre-treatment uses acidic or alkaline solution (Zupančič and Grilc 2012; Sun *et al.* 2016).

2.8.14. Seeding

Seeding is the method used to start up the anaerobic digestion with previously digested material from another digestion plant. Alternatively, materials such as ruminant manure are often used to seed a new reactor, to increase the start-up time. There are also commercially available inoculums which can be used.

Rodriguez-Chaing and Dahl (2015) conducted the experiment to determine the effect of inoculums to substrate ratio (ISR). The optimum ISR of 0.8 was confirmed. The conclusion was that the lower the ratio the less gas produced. Pathak and Srivastavas (2007) concluded that 30 % of inoculum in the digester is optimal ISR. Dennis (2015) came into the similar conclusion that the increase in rumen fluid as inoculums for cow dung anaerobic digestion increases with the biogas production. Sunarso *et al.* (2012) showed that the increase in rumen fluid causes the biogas production to increase more than two times compare to manure without inoculum. Sunarso *et al.* (2012) concluded that the best results were obtained when the substrates to inoculums ratio was at 17.64 to 35.27. All the researcher come into the same conclusion that the more inoculum, the better the AD process, but there is space limitation when running BPM tests.

2.8.15. Particle size

Large particles in AD are responsible for low biogas production while smaller particles enhance biogas production rate (Sharma *et al.* 1988). Substrates size does not only increase the biogas production rate but also affects HRT (Mshandete *et al.* 2006).

Large particles slow down anaerobic process. Microbes will not be able to perform digestion function (Gashaw and Teshita 2014). Hydrolysis rate is related to amount of surface area available for digestion process (Schnürer and Jarvis 2010). Surface area is the key to hydrolysis process and the rate of anaerobic process depends on how fast the hydrolysis process is. Physical pre-treatment is required to speed up the hydrolysis process and thereby reducing the retention time (Karp *et al.* 2013). Reduction of retention time could increase hydrolysis rate and shortens digestion time. Figure 2.2 represents relationship between particle size and biogas yield.

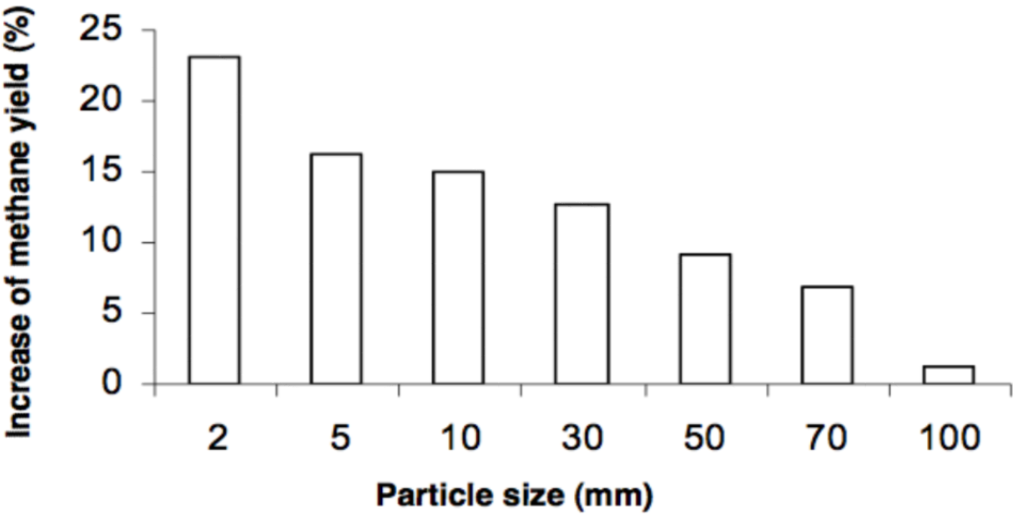

Figure 2.2: The effect of particle size on methane production (Schnürer and Jarvis 2010).

2.8.16. Nutrients concentration and other harmful materials

Required nutrients to enhance AD process are nitrogen, hydrogen, carbon and sulfur (Sorathia, Rathod and Sorathiya 2012). Addition of controlled amount of oxygen is very important to minimize the formation of H_2S and SO_2 (Schnürer and Jarvis 2010). Nitrogen and carbon are required for an optimum C: N ratio (Tanimu *et al.* 2014). Some compounds or chemicals hinder production of biogas when added into AD process. Presence of these materials at high concentration can lead to failure of AD process. Sorathia, Rathod and Sorathiya (2012) prepared a list of more common harmful materials and their maximum allowable concentration as shown in Table 2.12.

Table 2.12: Lists of some harmful substances and their maximum allowable concentrations

Material	Unit	Maximum concentration
Sulfate(SO_4^{-2})	ppm	5000
Sodium chloride	ppm	40 000
Copper (Cu)	mg/L	100
Chromium (Cr)	mg/L	200
Nickel (Ni)	mg/L	200-500
Cyanide (CN)	mg/L	25
ABS (detergent compound)	ppm	20-40
Ammonia (NH_3)	mg/L	1500 – 3000
Sodium(Na)	mg/L	3500-5500
Potassium (P)	mg/L	2500-4500
Calcium(Ca)	mg/L	2500-4500
Magnesium (Mg)	mg/L	1000-1500

2.9. Biogas potential from sugarcane residues

2.9.1. Sugarcane manufacturing industries

According to SASA (2016), South African sugar industry is ranked among the top 15 sugar producing countries in the world. It contributes about R12 billion in South African GDP (Illovo 2015) and has positive impact and catalyst to economic growth and development (SASA 2016).

Sugarcane is the one of the major agricultural crops found in KwaZulu-Natal and Mpumalanga provinces (Illovo 2015). Sugar manufacturing industries contribution to South African economy is enormous. According to SASA (2016), sugarcane growing comprises approximately 22500 sugarcane growers who are registered in KwaZulu-Natal and Mpumalanga provinces in South Africa which constitutes about R8 billion into direct income. Sugarcane industry contributes about 79 000 and 350000 of direct and indirect work opportunities respectively (SASA 2016). It estimated that about 2 % of South African population depends on sugar industry for living (SASA 2016).

Sugar manufacturing process generates moistly molasses, bagasse and trash or leaves. To increase profitability and waste management, these by-products or residues wastes are used to create value added products.

2.9.2. Bagasse

Bagasse is fibrous residue left after milling of cane sugarcane (Chandel *et al.* 2014). It is often used as a feed for boiler for steam generation. It is estimated that about 3000 kg of wet bagasse is produced when 10 000 kg of sugarcane is crushed (Rainey 2009). Moisture content of bagasse is between 40 to 50 % (Deepchand 2005; Chandel *et al.* 2014). Table 2.13 represents composition of sugarcane bagasse (Rainey 2009).

Caloric value of bagasse as fuel varies due to varying composition of sugarcane plant from which bagasse was extracted from (Deepchand 2005). Caloric value of bagasse can be calculated from Kumar, Kumar and Amit (2016)'s correlation as presented by equation 2.22

$$CV = 18260 - 207.63W - 182.6A - 31.14B \qquad \text{2.22}$$

Where: W represents moisture content of the bagasse in %

A represents ash content of the bagasse in %

B represents Brix content of the bagasse (%)

Bagasse fired boilers have low efficiency and gas flue particulate emissions are high between 8000 to 12 000 mg/Nm^3(Boshoff and Wh 1999). In South Africa a maximum limit is 200 mg/Nm^3 (Anon 1985). High gaseous emissions (SO_2 CO and NO_X) emitted by bagasse fed boilers are of great concern (Teixeira and Lora 2004). According to Chen *et al.* (2007) exposure of SO_2 CO and NO_X increase risk of respiratory tract infections, reduction of immune system and pulmonary diseases. Human exposure of SO_2 may lead to death (Saxena and Bhargava 2017). Exposure to carbon monoxide is associated with the increase risk of cardiopulmonary events including death (North *et al.* 2018). Mechanical collectors or cyclones, scrubbers, filters and electrostatic precipitators are used to reduce particulate emissions on bagasse fed boilers to reduce particulate emissions (Boshoff and Wh 1999).

Table 2.13: Typical fibre composition of bagasse

Compound	Composition (%w/w)
Cellulose	45-55
Hemicellulose	20-25
Lignin	1-4
Other	16-34

2.9.4. Molasses

Molasses is the mother liquor that remains from sugar industry after crystallization process (Osunkoya and Okwudinka 2011). It is commercially available, cost effective and contains high concentration of sugars and other valuable nutrients (Aftab *et al.* 2014). Molasses has many industrial applications such as ethanol fermentation and yeast fermentation. Due to its high COD, it is difficult to treat molasses anaerobically (Jiménez, Borja and Mart´ın 2004). Dilution of molasses as a pre-treatment method is necessary (Aftab *et al.* 2014). It is estimated that 1000 kg of sugarcane produces about 23 liters of molasses (Dotaniya *et al.* 2016). Molasses can be used a source of energy and it is generally regarded as a more available and cheaper source of carbohydrates. Table 2.14 represents molasses composition (Dotaniya *et al.* 2016).

Table 2.14: Composition of molasses composition

Constituents	Percentage (%w/w)
Sucrose	30-35
Glucose and fructose	10-25
Moisture	23-23.5
Ash	16-16.5
Calcium and potassium	4.8-5
Non-sugar compounds	2-3
Other mineral contents	1-2

2.9.5. Sugarcane leaves

Sugarcane leaves are made of fibre and parenchymatous tissue (Agarwal 2014). Fernandes *et al.* (2009) estimated that 1000 kg of sugarcane corresponds with about 250 kg of sugarcane leaves. They are composed of organic matter 25 %, organic carbon 14.5 % and the ash content of 3 % (Rainey 2009; Chandel *et al.* 2014). Composition depends on the region at which sugarcane was cultivated, crop variety and composition of the soil on which the sugarcane crop was planted (Jutakanoke *et al.* 2011). Table 2.15 represents typical sugarcane leaves composition.

Sugarcane leaves are burnt or left in the field to decompose due to high coast related to collection and transportation (Jutakanoke *et al.* 2011; Agarwal 2014; Chandel *et al.* 2014). According to Agarwal (2014) sugarcane leaves can potentially be converted into 1kWh/kg. Burning of sugarcane leaves has contributed to health related problems (Cancado, Saldiva and Pereira 2006). Production of fly ash when sugarcane leaves are burnt in the field has created severe damages on soil microbial diversity and raises environmental concerns (Coelho *et al.* 2008). Environmental and public health problems are likely to worsen in the future should sugarcane industries continue to burn residues for energy requirements (Cancado, Saldiva and Pereira 2006; Mazzoli-Rocha *et al.* 2008; Hiscox *et al.* 2015).

Table 2.15: Typical fibre composition of sugarcane leaves

Compound	Composition (%w/w)
Cellulose	33.3
Hemicellulose	18.1
Lignin	3.1
Other	45.5

Due to high lignin content in sugarcane leaves, they have limited industrial application. High lignin content is a barrier to access carbohydrates present to make value-added products (Chandel *et al.* 2014) . Removal of lignin can be achieved by using different pre-treatment processes available. Pre-treatment has been reported to increase accessibility to cellulose (Jutakanoke *et al.* 2011; Chandel *et al.* 2014; Zheng *et al.* 2014).

2.9.6. Justification of using cow dung as co-substrate

Cow dung is high in organic materials and rich in nutrients (Hamilton 2014). It is often used as an agricultural fertilizer and used to produce biogas (Adebayo, Jekayinfa and Linke 2014b). Mixing cow dung with organic wastes from industry and households has been successfully applied for biogas production (El-Mashad and Zhang 2010; Zhang *et al.* 2013; Adebayo, Jekayinfa and Linke 2014a; Maamri and Ammari 2014). Co-digestion of cow dung and other wastes play an important role in AD process and has an economical and has an environmental benefit as well (Zhang *et al.* 2013).

An attempt was done by Girija *et al.* (2013) to analyse microbiota of cow dung using 16rDNA approach. The following bacteria were identified: Bacteroidetes (38.3%), Firmicutes (29.8%), Proteobacteria (21.3%) and Verrucommicrobia (2%). These bacteria are strong degraders of the complex organic matter such as cellulose, lignocelluloses, xylose, chitin and xylem (Marten *et al.* 2009).

These findings justify the use cow dung as co substrate for AD process. Girija *et al.* (2013) further noticed that 65 % of clones identified belonged to anaerobic bacteria (Alastipes, Paludibacteria, Anaerovox and Akkermansia sp) and 35% of clones belonged to aerobic bacteria. Figure 2.3 shows microbials which were found in cow dung analysis.

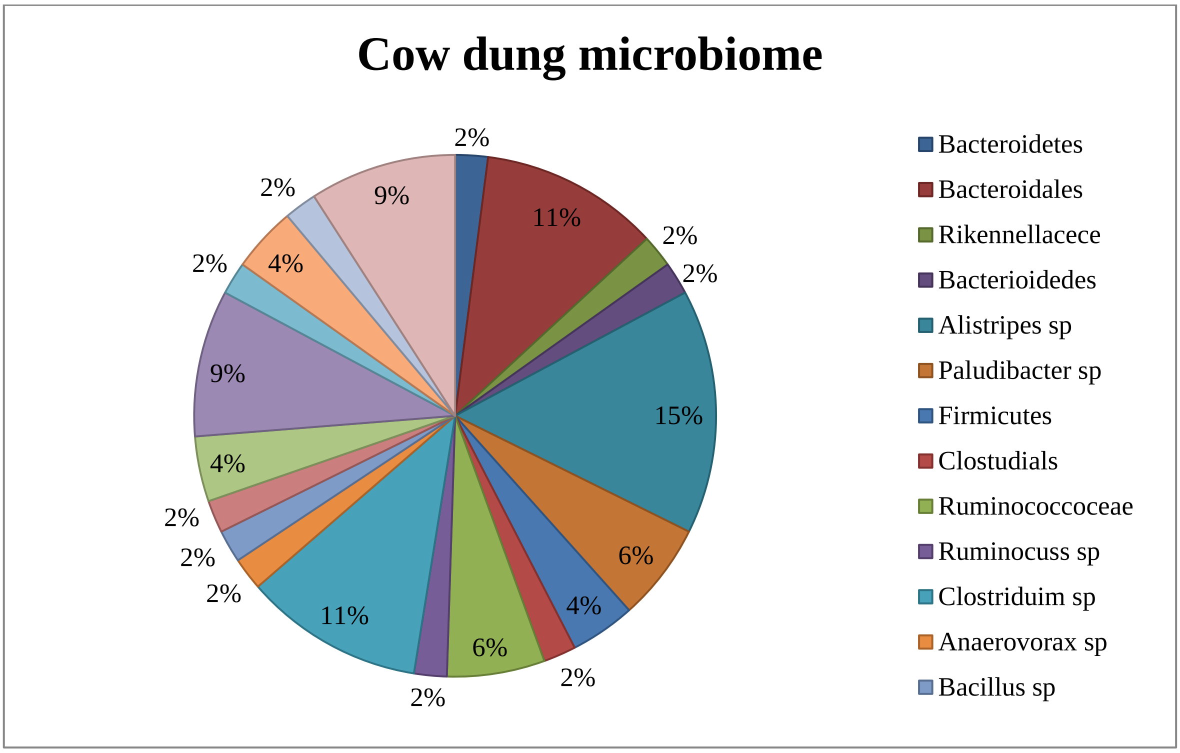

Figure 2.3: Cow dung microbiome by Girija et al. (2013)

2.10. Gomperzt function

Equations 2.10, 2.11 and 2.12 shows that methane and carbon dioxide are produced in methanogenesis stage of AD process, where , acetic acid, ethanol , hydrogen and CO_2 are being converted into methane, carbon dioxide and water by methanogenic bacteria (Bajpai 2017).

Zwietering *et al.* (1990) demonstrated that any bacteria growth obeys Gomperzt function. Methane production rate is proportional to methane forming bacteria growth rate and , therefore ,biogas production rate data can be applied to Gomperzt function (Ghatak and Mahanta 2017; Talha *et al.* 2018).

Gomperzt function is a 3-parameter mathematical model for time series, where growth is lowest at the start and lowest at the end. Gomperzt function is given by equation 2.23 (Tjørve and Tjørve 2017).

$$y(t) = ae^{-be^{-ct}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.23$$

Where: a is asymptote, b is the displacement along the x axis, c is the growth rate, e is the Euler's number and t is growth time.

Zwietering *et al.* (1990) modified Gomperzt function by substituting parameters (a, b and c) with A, U, and λ as he believed that parameters a, b and c do not have biological meaning. Modified Gomperzt equation is given by equation 2.24.

$$P = A\exp\left\{-\exp\left[\frac{Ue}{A}(\lambda - t) + 1\right]\right\} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.24$$

Where: P represents cumulative biogas volume (ml)

A represents biogas production potential (ml)

U represents maximum biogas production rate (ml/day)

λ represents lag phase (day)

t represents cumulative time for biogas production (day)

2.11. Related studies

Some important investigations on biogas production from sugarcane residues have been summarized in this section.

Mokobia *et al.* (2012) did a feasibility study on sugarcane leaves potential to produce biogas through AD process in laboratory scale batch digesters. The set up consisted of five 250 ml Buchner flask as digesters. 50 g of sugarcane leaves were charged in each flask with water volumes of 50.0 ml, 100.0 ml, 150.0 ml, 200.0 ml and 250.0 ml which correspond to sugarcane leaves-to-water ratio (g/ml) of 1:1, 1:2, 1:3, 1:4 and 1:5 respectively. Dilution ratio of 1:2 was found to be an optimum ratio where biogas volume of 239.4 ml was achieved. These results show that sugarcane leaves are potential source for biogas production. Although methane content was relatively low, but this study provides the basis for future research on biogas production from sugarcane leaves.

Janke *et al.* (2015) conducted comparative analysis on different sugarcane residues. Sugarcane residues investigated were, namely: vinasse, filter cake, bagasse, and straw. Characteristics which were analysed were, namely: VS, COD, macronutrients, trace elements and nutritional values. The overall objective of this study was to provide guidelines during the design of AD process when these sugarcane wastes are used as substrates. Results showed that bagasse and straw had higher average values of TS and VS (55 % and 96 % for bagasse, and 76.7 % and 86.3 % for straw) respectively. Biochemical methane potential (BMP) assays results revealed that methane yields varied considerably (199-326 ml/gVS) due to different characteristic of these wastes. Higher methane yield of 326 mL/gVS was achieved by bagasse compared to lower methane yield of 199 ml/gVS which was achieved by s sugarcane straws. The authors have successfully demonstrated that sugarcane residues have different characteristics which should be taken into consideration during the design of AD process. Furthermore, potential nutritional deficiencies were identified in these residues. Author concluded that pre-treatment, supplementation of nutrients or co- digestion of these wastes would improve biogas production

Ofomatah and Okoye (2013) studied the effect of pretreatment of bagasse during biogas production. Two set of experiments were conducted, namely: water soaked, and soda ash-soaked bagasse. 50 kg capacity bio-digesters were used. In both set of experiments, cow dung was used as co-substrate. Biomass wastes were digested for 35 days at mesophilic temperature. Ash/water: bagasse ratio was 1:3 in all reactors while bagasse-to-cow dung was 100: 0, 70:30, 50:50, 30:70 and 0:100. Results revealed that co-digestion of bagasse with cow dung did not improve biogas production instead it led to the steady state. Treatment of bagasse with ash resulted to 400% increase in biogas production due to breaking down of lignocelluloses present in bagasse.

Aftab *et al.* (2014) investigated the feasibility of sugarcane molasses to produce biogas. About 1800 ml of 10 times diluted molasses was anaerobically digested in 2 litres- digester at 37°C for 15 days. About 2 ml of sample was drawn daily for VS, TS and COD analysis. Biogas volume was measured daily by water displacement method. Results showed reduction in total solids from 11.24% to 7.74%, volatile solids from 9.88% to 6.62% and COD from 20.54 % to 5.42%. Total biogas produced by 1800 ml of 10 times diluted molasses produced 13.1 litres of biogas.

The effect of initial pH of solution on biogas production AD process efficiency using 400 ml of inoculum and nutrient solution was investigated by Kheireddine, Derbal and Bencheikh-Lehocine (2014). Laboratory batch reactors with working volume of 570 ml were used. Experiments were conducted at thermophilic conditions (55°C) for 50 days. Four pH conditions, 4; 5.5; 7 and 9.5 were investigated. The results showed that the efficiency of COD for the pH of 4; 5.5; 7 and 9.5 were 49.11; 63.75; 7; 90.8 and 79.64% respectively and the biogas produced were 163 ml, 100 ml, 2000 ml and 1500 ml for the pH of 4; 5.5; 7 and 9.5 respectively. The optimum pH was found to be 7.

Chapter 3

MATERIALS AND METHOD

3.1. Introduction

This is a quantitative investigation which was carried out at Durban of University of Technology (DUT) laboratory in 2016. Laboratory batch scale reactors were used to evaluate the interactive effects of C:SR feed ratio, media solution pH and digester's moisture content on biogas volume, methane yield and kinetic constants values.

The aim of this chapter is to detail methodology which was used to achieve goals and objectives of this research.

3.2. Materials

Cow dung, sugarcane leaves, bagasse and molasses which were used for this work were collected locally. They were dried, milled and sieved by using 350 µm sieve tray. The undersize particles were used for experiments. Waste water treatment plant sewage sludge which was used as inoculum was collected locally and was stored at 4 °C to avoid spontaneous fermentation.

3.3. Equipment

The following equipment were used:

- Gas chromatograph (GC)
    - GC was used to analyze the composition of biogas. Calibration is presented in section 3.7.2.
- Water bath
    - To maintain AD process at constant temperature.
- pH meter

    - pH meter was used to measure pH of media solution charged into digesters. pH meter specifications are presented in section 3.7.4.
- 13 × 500 ml Schott bottles
    - Used as digesters or reactors
- Mass balance
    - Mass balance was used for all weight measurement requirements. Section 3.7.5 details specifications of mass balance used.
- Glass tube 20mm ID, 750 mm long
    - Was used to measure the amount of biogas produced.
- Oven and furnace
    - Was used for TS and VS determination.
- Electric blender
    - This equipment was used to pulverize the bagasse and sugarcane leaves
- Measuring tape
    - It was used to measure water level displaced by biogas. Graduation of measuring tape is given in Section 3.7.3.
- Other accessories
    - Connecting tubes, volumetric flasks, rubber stopper caps, syringe for gas sampling, stop cock valves, clamps and 6mm glass tube.

3.4. Design of experiment

Design- Expert software offers designs that combine process factors and mixture components. In this work, Design-Expert's Combined (User-Defined) option was used for design of experiment and results analysis. Quadratic model was used for mixture components and linear models was used for process factors. Quadratic models are traditionally used for mixture components (Cornell 2011). Linear model for process factors was predicted as there were two levels (high and low) which were considered and it is permitted according to Montgomety (2001). Table 3.1 represents Design-Expert software input variables which were used for DOE. Table 3.2 represent design matrix with 12 points at which the experiments which be conducted. Mixture components were cow dung (X_1) and sugarcane biomass (X_2). Process factors were media solution pH (Z_1) and moisture content (Z_2).

Table 3.1: Input variables for design of experiment

Parameter	Variables	Levels	
		Low	High
Cow dung (g)	X_1	2.00	8.00
Sugarcane residue (g)	X_2	2.00	8.00
Media solution pH	Z_1	4.00	8.00
Digester moisture content (%)	Z_2	80.00	95.00

Table 3.2: Design matrix of DOE

Reactor	X_1 (g)	X_2 (g)	Z_1	Z_2 (%)
1	5.00	5.00	8.00	95.00
2	8.00	2.00	4.00	80.00
3	5.00	5.00	4.00	80.00
4	5.00	5.00	4.00	95.00
5	8.00	2.00	4.00	95.00
6	2.00	8.00	8.00	95.00
7	8.00	2.00	8.00	80.00
8	5.00	5.00	8.00	80.00
9	8.00	2.00	8.00	95.00
10	2.00	8.00	8.00	80.00
11	2.00	8.00	4.00	80.00
12	2.00	8.00	4.00	95.00

3.5. Experimental set up

Three sets of experiments were conducted, namely: bagasse and cow dung, sugarcane leaves and cow dung, and molasses and cow dung. Process conditions were adjusted as per Table 3.2. Experiments were conducted at mesophilic temperature condition (35°C) and hydraulic retention time of 14 days. Experimental set up is shown in Figure 3.1. Water displacement method was used to measure biogas volume generated. GC was used to analyse biogas composition at the end of each experiment. Reactors were charged with equal volume of inoculum. Based on the literature review, the optimum inoculation volume used was calculated to be 2 ml (Pathak and Srivastavas 2007; Sunarso *et al.* 2012).

Figure 3.1:Experimental set up for AD experiment.

3.6. Initial characterization of substrates

Standard procedure was used to determine moisture content, total solids and volatile solids of cow dung, molasses, sugarcane leaves and bagasse(Telliard 2001). Equations 3.1, 3.2 and 3.3 were used to calculate substrates's moisture content, total solids and volatile solids respectively.

$$X = \frac{M_1 - M_2}{M_1} \qquad 3.1$$

$$TS = 1 - (X) \qquad 3.2$$

$$VS = \frac{M_2 - M_3}{M_2} \qquad 3.3$$

Where: M_1 represents initial mass of substrates

M_2 represents dried sample mass after 24 hours at 105°C

M_3 represents mass of sample 3 hours at 550°C

X represents moisture content of substrates

TS represents total solids of substrates

VS represents volatile solids of substrates.

Detailed procedure and sample calculations are presented in Appendix C. Table 3.3 presents summary of initial characterisation results.

Table 3.3: Percentage fractions of initial characterization results of substrates

Substrates	Cow dung	Bagasse	Sugarcane leaves	Molasses
VS (w/w)	0.75	0.78	0.76	0.85
TS (w/w)	0.54	0.97	0.98	0.75
Moisture (w/w)	0.46	0.03	0.02	0.25

3.7. Analytical methods

Water displacement method was used to determine the volume of biogas generated. Measuring tape was used to measure the height of water displaced by biogas. Gas chromatography (GC) was used to analyse biogas composition. Standard pH meter was used to measure the pH of media solution. Mass of media solution and substrates were measured by using mass balance.

3.7.1. Water displacement method

Water displacement method is based on Greek philosopher Archimedes principle, which states that the amount by which water level rises when an object is submerged is equivalent to the volume displaced by the object. Biogas has lower density than water and is not soluble in water, hence it is possible to measure it by water displacement method. Figure 3.2 represents schematic diagram of water displacement method. Glass tube filled with water is inverted into reservoir of water. Gas from digester is transported by small tube into the inverted glass tube. As gas is being generated, it moves up into empty space of inverted tube and displaces water. Amount of water displaced is equal to biogas generated.

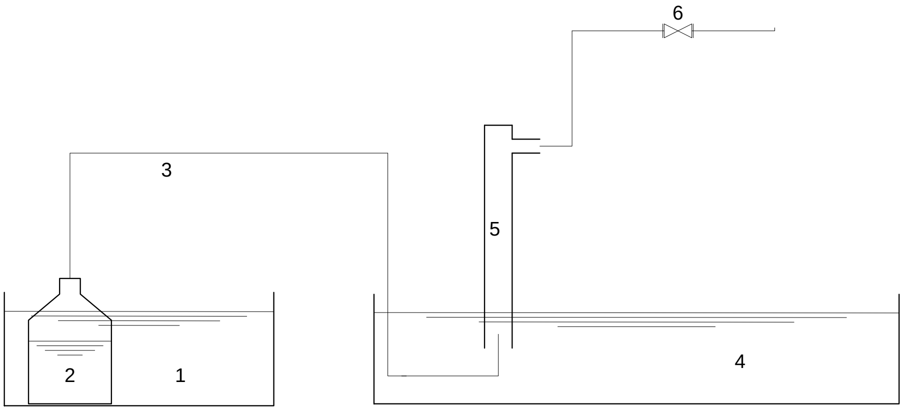

Figure 3.2: Schematic diagrams of anaerobic digestion: Water bath (1), 500ml Schott bottle (2), 6mm ID flexible tube (3), acrylic water basin (4), glass tube with the ID of 20mm with the opening at the bottom (5) and a clamp (6).

Cylinder volume formula was used to calculate volume of water displaced by biogas. Volume of cylinder can be calculated by using equation 3.4.

$$Y_i = \pi r^2 h \qquad \text{..........} 3.4$$

Where: r represents radius of the tube, in this work r equals to10 mm.

h represents height of water displaced (cm)

Y_i represent volume of water displaced (ml)

π is the constant which is approximated to 3.141

Sample calculations for water volume displaced are presented in Appendix C. Water volume displaced and corresponding amount of biogas volume generated for each run are presented in Tables A.1, A.2 and A.3.

3.7.2. Gas chromatography

Gas chromatography (GC) is used to analyse gasses that can vaporize without decomposition (Korytár *et al.* 2002). Gasses are heated into a temperature just above the boiling point. Time at which the gasses start to evaporate is recorded and compared to the time at which the standard sample evaporates.

In the current study, GC (Shimadzu-2014) equipped with a flame ionization detector and GS-Gaspro (30 m x 0.32 mm ID) packed column was used to analyse methane, carbon dioxide and hydrogen gasses. Nitrogen was used as the carrier gas. GC was set up as per the following conditions:

- Column oven: 80 ^{o}C
- Detector: 25 ^{o}C, 100 mA
- Filaments: 40 ^{o}C

Calibration was done by injecting high purity of methane, carbon dioxide and hydrogen gasses. Residence times for methane, carbon dioxide and hydrogen were 16, 21 and 14 minutes respectively. Sample of 100 micro litres was injected into GC. Different peaks were recorded against run time. Summary of the over results are presented in Tables A.4, A.5 and A 6.

3.7.3. Measuring tape

Daily water heights displaced by biogas were measured manually by using a standard measuring tape. Measuring tape was graduated with 1 mm, 0.5 mm and 10 mm lines, which implies that the accuracy of was approximately 0.01mm.

3.7.4. pH meter

High accuracy pH meter which was used to measure media solution pH of 4.00 and 8.00 had the following specifications:

- Accuracy: ± 0.01
- pH range: 0.00 to 14.00
- Temperature deviation: ± 0.05 ºC

3.7.5. Mass balance

Calibration certificate from external company with a good reputation indicated that the accuracy was 0.01 g. To confirm the accuracy of mass balance, objects with known mass were measured, it was found that the masses corresponded with value displayed by mass balance.

3.8. Data analysis

3.8.1. Total volatile solids

Table 3.3 was used to calculate the total VS charged into each reactor. Sample calculations are presented in Appendix C. Equation 3.5 was used to calculate total VS charged into each digester.

$$VS_{tot}(g) = TS_A VS_A M_A + TS_B VS_B M_B \quad \text{..} 3.5$$

Where: TS_A and TS_B represent total solids of substrates A and B respectively (%).

M_A and M_B represent initial mass of substrates A and B respectively (g).

VS_A and VS_B represent volatile solids of substrate A and B respectively (%).

3.8.2. Media solution

Media solution used for this work was prepared by dissolving a required amount of KH_2PO_4 and Na_2CO_3 into distilled water to obtain a media solution with pH of 10.44. Phosphoric acid was added into media solution to make two solutions with pH of 4.00 and 8.00 as required by experiment. Equation 3.6 was used to determine mass of media solution required to achieve required digester moisture as required. Derivation of equation 3.6 is presented in Appendix B. Sample calculations to determine mass of mass of media solution charged in each reactor is presented in Appendix C. The results media solution mass for each digestor is presented in Table A.10.

$$M_{sol} = \left(\frac{1-\varphi_{sl}}{\varphi_{sl}} \right) (TS_A + TS_B) - [X_A M_A + X_B M_B] \text{...} 3.6$$

Where: $\varphi_{sl} = 1 - Z_2$, represents solid content of a reactor. Z_2 represents digester's moisture content (%).

M_{sol} represents amount of media solution required (g)

M_A and M_B represent initial mass of substrates A and B respectively (g).

TS_A and TS_B represent total solids of substrates A and B respectively (g).

X_A and X_B represent moisture content of substrates A and B respectively (%).

3.8.3. Biogas volume

Total biogas volume represents the sum of daily biogas volumes generated in 14 days of experiment. Equation 3.7 was used to calculate the total biogas volume generated by one digester in 14 days.

$$Y_1 = \sum_{i=1}^{14} Y_i \text{...} 3.7$$

Where: Y_1 represents total biogas volume after 14 days (ml)

Y_i represent daily biogas volume generated by each reactor (ml)

Total biogas volumes result of cow dung -bagasse, cow dung-sugarcane leaves and cow dung-molasses experiments are presented in Tables 4.3, 5.3 and 6.3. respectively.

3.8.4. Methane yield

In this work, methane yield represents the efficiency of AD process relative to the mass of VS charged in each reactor. Equation 3.8 was used to calculate methane yield for each reactor.

$$Y_2 = \frac{Y_1}{VS_{tot}} \times n_{CH_4} \quad \text{...} 3.8$$

Where: Y_1 represents total of biogas volume (ml)

n_{CH_4} represents methane concentration as measured by GC

VS_{tot} represents total volatile solids charged into each reactor

Sample calculations of methane yield are presented in Appendix C. Methane yield results for each set of experiment: cow dung -bagasse, cow dung-sugarcane leaves and cow dung-molasses are presented in Tables 4.3, 5.3 and 6.3. respectively.

3.9. Statistical analysis

3.9.1. Modelling equation

Mixture components and process factors were represented by quadratic and linear models. Equations 3.8 and 3.9 represent quadratic model for mixture components and linear model for process factors respectively. Combined mixture-process model which is a product of the two models is presented by equation 3.10 (Anderson and Whitcomb 2000).

$$Y(X) = \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \text{..} 3.8$$

$$Y(Z) = \alpha_0 + \alpha_1 Z_1 + \alpha_2 Z_2 + \alpha_{12} Z_1 Z_2 \quad \text{...} 3.9$$

$$\begin{aligned}
Y(X,Z) &= \alpha_0\beta_1X_1 + \alpha_1Z_1\beta_1X_1 + \alpha_2Z_2\beta_1X_1 + \alpha_{12}Z_1Z_2\beta_1X_1 + \alpha_0\beta_2X_2 \\
&+\alpha_1Z_1\beta_2X_2 + \alpha_2Z_2\beta_2X_2 + \alpha_{12}Z_1Z_2\beta_2X_2 + \alpha_0\beta_{12}X_1X_2 + \alpha_1Z_1\beta_{12}X_1X_2 \\
&+\alpha_1Z_1\beta_{12}X_1X_2 + \alpha_{12}Z_1Z_2\beta_{12}X_1X_2 + \alpha_0\beta_{11}X_1^{\,2} + \alpha_1Z_1\beta_{11}X_1^{\,2} \qquad \text{......................3.10} \\
&+\alpha_2Z_2\beta_{11}X_1^{\,2} + \alpha_{12}Z_1Z_2\beta_{11}X_1^{\,2} + \alpha_0\beta_{22}X_2^{\,2} + \alpha_1Z_1\beta_{22}X_2^{\,2} \\
&+\alpha_2Z_2\beta_{22}X_2^{\,2} + \alpha_{12}Z_1Z_2\beta_{22}X_2^{\,2}
\end{aligned}$$

Where: Y represents methane yield (Y_2) or biogas volume(Y_1)

$\alpha_0, \alpha_1, \alpha_{12}, \beta_1, \beta_2, \beta_{11}, \beta_{12}$ *and* β_{22} represent model coefficient

X_1 and X_2 represent cow dung and sugarcane biomass respectively

Z_1 and Z_2 represent media solution pH and digester's moisture content respectively.

To simply equation 3.10, models terms with p values greater than 0.005 were eliminated by either backward or forward method. Terms with zero or close to zero co-efficient were eliminated.

3.9.2. Adequacy checks

Statistical test on whether the experimental data fit to the predicted model was conducted by using the analysis of variance (ANOVA). Coefficient of determination (R^2) was used to estimate relationship between predicted model and responses. F- Value was used to identify the model that best fit the experimental results. Calculated probability (p-value) was used to determine the statistical significance each term of model developed. Lack of fit test was used to determine whether the developed model was adequate to describe the data.

3.9.3. Optimization

Design-Expert's numerical optimization option was used for process optimasation. Optimization process uses developed statistical model (equation 3.10) to find optimum conditions to meet optimization goals. Solution with highest desirability was selected.

3.9.4. Kinetic constants determination

Kinetic constants values were determined for each reactor. MS word excel was used for determination of kinetic constant values. Simplified procedure used to determine kinetic constants values is presented in Appendix D. Results for kinetics constant values for each reactor are presented in Tables 4.1 ,5.1 and 6.1.

Chapter 4

RESULTS AND DISCUSSION: COWDUNG AND BAGASSE

4.1. Introduction

The aim of this chapter is to present results of cow dung and sugarcane bagasse co-digestion experiment. Mathematical models for biogas volume, methane yield and kinetic constant are presented. Analysis and discussions of the results including conclusions are also presented.

4.2. Kinetic constants

Figure 4.1 shows that at the beginning of AD process, biogas forming bacteria present in digesters became active and biogas production started and daily cumulative biogas volume started to rise. When biogas production rate reaches the maximum production, methane forming bacteria were acting on maximum amount of cow dung and bagasse. After 14 days, daily cumulative biogas volume began to drop. At day 14 did not mean that biogas production stopped but, it was assumed that data collected was sufficient to determine kinetic constant values with confidence.

Daily cumulative biogas volumes are presented in Table A.10. Daily cumulative biogas volumes were plotted against HRT as presented in Figure 4.1. Kinetic constants values are presented in Table 4.1.

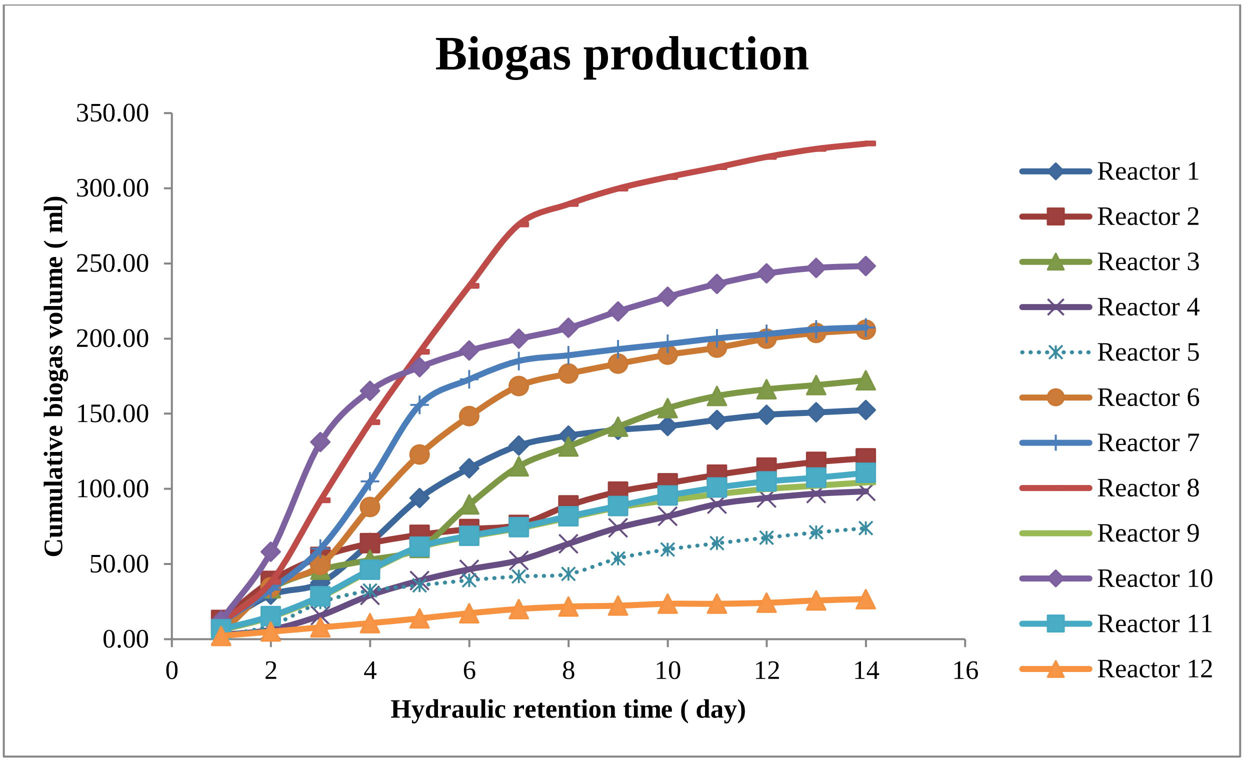

Figure 4.1: Daily cumulative biogas volume against HRT for cow dung and bagasse co-digestion

Table 4.1: Kinetic constants results for bagasse and cow dung co-digestion

Reactor	Cow dung (g)	Bagasse (g)	Media solution pH	Moisture content (%)	A (ml)	U (ml/day)	λ (day)
1	5.00	5.00	8.00	95.00	152.76	25.27	1.25
2	8.00	2.00	4.00	80.00	131.24	10.43	1.41
3	5.00	5.00	4.00	80.00	193.56	18.51	0.94
4	5.00	5.00	4.00	95.00	111.76	10.60	1.65
5	8.00	2.00	4.00	95.00	84.75	6.36	0.02
6	2.00	8.00	8.00	95.00	203.83	33.96	1.37
7	8.00	2.00	8.00	80.00	203.77	44.64	1.48
8	5.00	5.00	8.00	80.00	328.06	56.07	1.43
9	8.00	2.00	8.00	95.00	104.52	13.59	0.85
10	2.00	8.00	8.00	80.00	235.30	46.05	0.56
11	2.00	8.00	4.00	80.00	112.44	13.02	0.72
12	2.00	8.00	4.00	95.00	26.51	3.32	0.66

Table 4.1 shows that the highest biogas production potential of 328.06 ml and maximum biogas production rate of 56.07 ml/day were achieved by reactor 8. Lag phase is the time taken by a digester to produce first biogas , and is related to rate of hydrolysis (Nyoman and Seno 2010; Ghatak and Mahanta 2017). Higher lag phase of 1.65 day was achieved by reactor 4 which indicates that reactor 4 had slowest hydrolysis reaction. Lowest biogas production potential of 26.1 ml and maximum biogas production rate of 3.32 ml/day were achieved by reactor 12.

Table 4.1 shows that reactor 5 achieved the lowest lag phase of 0.02 day which indicates fast hydrolysis rate. Reactor 5 was charged with media solution with pH 4. The optimum pH for hydrolysis stage of AD process has been reported to be about 4.0 (Adekunle and Okolie 2015).

Detailed analysis for each process kinetic constant is presented in section 4.2.1.

4.2.1. Effect of process variables on kinetic constants

The effects of process variables on kinetic constants values were investigated by using OVAT approach. Comparison between two reactors' results which were operated at two fixed variables and one adjusted variable was performed.

4.2.1.1. Effect of cow dung-to-bagasse feed ratio

4.2.1.1.1. Analysis 1

Reactor 5 and 12 were charged with cow dung-to-bagasse (C: B) feed ratio of 8:2 and 2:8 respectively. Moisture content of digester and media solution pH were kept constant at 95.00 % and 4.00 respectively. Table 4.1 shows that reactor 5 achieved higher biogas production potential and maximum biogas production rate of 84.75 ml and 6.36 ml/day respectively compared to reactor 12 where lower biogas production potential and maximum biogas production rate of 26.51 ml and 3.32 ml/day were achieved respectively. Table 4.1 also reveals that reactor 12 achieved higher lag phase of 0.66 day compared to reactor 5 where lag phase of 0.02 day was attained.

It was observed that the reactor charged with high C: B feed ratio of 8:2 achieved higher biogas production potential and maximum biogas production rate compared to a reactor charged with low C: B feed ratio of 2:8. This indicates that high cow dung concentration in the feed favoured high biogas production potential and maximum biogas production rate values when reactors are operated at digester's moisture content 95.00 % and charged with media solution pH of 4.00.

Low lag phase value achieved by reactor 12 indicates that high concentration of cow dung in reactors improves biodegradability of AD process. This is the indication that hydrolysis rate of reactor 12 is faster than that of reactor 5. Higher lag phase value achieved by reactor 5 is the indication that high concentration of bagasse in a reactor may cause low start-up of AD process, therefore, the process takes longer to produce biogas.

4.2.1.1.2. Analysis 2

Reactors 6 and 9 were charged with C: B feed ratio of 2:8 and 8:2 respectively. Digester's moisture content and media solution pH were constant at 95.00 % and 8.00 respectively. Table 4.1 reveals that reactor 6 achieved higher kinetic constant values (A,.203.83 ml; U, 33.96 ml/day; λ, 1.37 day) compared to reactor 9 where lower biogas production potential, maximum biogas production rate and lag phase of 104.52 ml, 13.59 ml/day and 0.85 day were achieved.

This analysis reveals that reactor 6 which was charged with low feed C: B feed ratio (2:8) achieved higher kinetic constant values.

4.2.1.1.3. Analysis 3

Table 4.1 shows that reactors 7 and 10 were charged with C: B feed ratio of 8:2 and 2:8 respectively. Digester's moisture content and media solution pH were kept constant at 80.00 % and 8.00 respectively. Reactor 10 achieved higher maximum biogas production rate of 46.05 ml/day compared to reactor 7 where lower maximum biogas production rate of 44.64 ml/day was achieved. Higher biogas potential (235.30 ml) and lower lag phase (0.56 day) were achieved by reactor 10 compared to reactor 7 achieved where lower values of biogas production potential (203.83 ml) and lag phase (1.48 day).

4.2.1.1.4. Analysis 4

Reactors 2 and 11 were charged with C: B feed ratio of 8:2 and 2:8 respectively. Both reactors were charged with equal media solution pH and digester's moisture content of 4.00 and 80.00 % respectively. Table 4.1 reveals that reactor 2 achieved higher biogas potential of 131.24 ml compared to reactor 11 where lower biogas production potential of 112.44 ml was achieved. Reactor 11 achieved higher maximum biogas production of 13.02 ml/day at lower lag phase of 0.66 day compared to reactor 2 where lower maximum biogas production rate of 10.43 ml/day was achieved.

In this analysis, reactor charged with higher C: B feed ratio achieved higher biogas production potential but lower maximum production rate. Reactor charged with lower C: B feed ratio achieved higher maximum biogas production rate provided the reactors are operated at media solution pH and digester's moisture content of 4.00 and 80.00 % respectively.

4.2.1.1.5. Concluding comments

High biogas production potential and maximum biogas production rate values were achieved by reactors charged with high C: B feed ratio at digester's moisture content of 95.00 % and media solution with pH of 4.00.

High biogas production potential and maximum biogas production rate values were achieved by reactors which were charged with low C: B feed ratio when they were operated at 95.00 % digester's moisture content and media solution pH of 8.00.

The analysis indicated that the effect of C: B feed ratio on kinetic constants is depended on other process variables conditions and therefore cannot be generalised.

4.2.1.2. Effect of moisture content of the digester on kinetic constants

4.2.1.2.1. Analysis 1

Reactors 1 and 8 were charged with equal C: B feed ratio of 5:5, equal media solution with pH of 8.00 and operated at digester's moisture content of 95.00 % and 80.00 % respectively. Table 4.1 shows that higher kinetic constants values (A,328.06 ml; U, 56.07 ml/day; λ, 1.43 day) were achieved by reactor 8 compared to reactor 1, where lower kinetic constants values (A,152.76 ml; U, 25.27 ml/day; λ, 1.25 day) were achieved.

In this analysis, it was observed that low digester's moisture content operated reactor achieved higher kinetic constants values when charged with media solution with pH of 8.00 and with C: B feed ratio of 5:5.

4.2.1.2.2. Analysis 2

Reactors 11 and 12 were charged with equal C: B feed ratio of 2:8, media solution with pH of 4.00 and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Table 4.1 reveals that higher kinetic constants values (A, 112.44 ml; U, 13.02ml/day; λ, 0.72 day) were achieved by reactor 11 compared to reactor 12 where lower kinetic constants values were achieved (A, 26.51 ml; U, 3.32 ml/day; λ, 0.66 day).

This analysis reveals that a reactor operated at lower digester's moisture content achieved higher kinetic constants values when charged with media solution with pH of 4.00.

4.2.1.2.3. Analysis 3

Reactors 6 and 10 were charged with equal C: B feed ratio of 2:8, equal media solution pH of 8.00 and operated at different moisture content of 95.00 % and 80.00 % respectively as shown in Table 4.1. Table 4.1 shows that that higher biogas production potential (235.30 ml) and maximum biogas production rate (46.05 ml/day) were achieved by reactor 10 compared to reactor 6 results where lower biogas production potential of 203. 83 ml and maximum biogas production rate of 33.96 ml/day were achieved. Table 4.1 also reveals that reactor 6 attained higher lag phase value of 1.37 day compared to reactor 10 where lag phase of 0.56 day was achieved.

In this analysis, reactor which was operated at high digester's moisture content achieved higher lag phase indicates that at higher digester moisture content charged reactor (95.00 %) has low start up time. This may be related to inoculum dilution as more media solution is required to raise digester's moisture content from 80 % to 95 %.

4.2.1.2.4. Analysis 4

Reactors 3 and 4 were charged with equal C: B feed ratio of 5:5, equal media solution pH of 4.00 and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Table 4.1 reveals that higher biogas production potential and maximum biogas production rate (A,193.56 ml; U, 18.51 ml/day) were achieved by reactor 3, compared to reactor 4 where lower biogas production potential and maximum biogas production rate values (A,111.76 ml; U, 10.60 ml/day) were achieved by reactor 4. Reactor 3 and 4 achieved lag phases of 0.94 day and 1.65 day respectively.

4.2.1.2.5. Analysis 5

Reactors 2 and 5 were charged with equal media solution with pH of 4.00, C: B feed ratio of 8:2 and digester's moisture content of 80.00 % and 95.00 % respectively. Table 4.1 shows that reactor 2 which was operated at digester's moisture content of 80.00 % produced higher kinetic constant values (A, 131.24 ml; U,10.43 ml/day; λ, 1.41 day) compared to reactor 5 where lower kinetics constant values (A, 84.75 ml; U,6.36 ml/day; λ, 0.02 day) were achieved.

4.2.1.2.6. Concluding comments

Reactors which were operated at low digester's moisture content achieve higher biogas production potential and maximum biogas production rate compared to the reactors operated at high moisture content regardless of other process conditions at which they were operated.

Analyses show that lag phase values are not affected by digester's moisture content but depended on media solution pH at which reactors were operated.

4.2.1.3. Effect of media solution pH

4.2.1.3.1. Analysis 1

Reactors 5 and 9 were charged with media solution with pH of 4.00 and 8.00 respectively. Both reactors were charged with equal C: B feed ratio and digester's moisture content of 8:2 and 95.00 % respectively. Table 4.1 indicates that reactor 9 produced higher biogas production potential, higher maximum biogas production rate and lower lag phase values of 104.52 ml ,13.59 ml/day and 0.02 day respectively compared to reactor 5 results where lower biogas production potential, lower maximum biogas production rate and higher lag phase values of 84.75 ml, 6.36 ml/day and λ of 0.85 day were obtained. In this analysis, it was observed that higher kinetic constants values were obtained by a reactor which charged with media solution with pH of 8.00. Low lag phase value achieved by reactor 5 indicates that low media solution pH improves biodegradability when operated at 95.00 % digester's moisture content.

4.2.1.3.2. Analysis 2

Reactors 2 and 7 were charged with equal C: B feed ratio of 8:2, operated at equal digester's moisture content of 95.00 % and charged with media solution with pH 4.00 and 8.00 respectively as indicated in Table 4.1. Table 4.1 reveals that reactor 7 achieved higher kinetic constant values (A, 203.77 ml; U, 44.64 ml/day; λ, 1.48 day) compared to results of reactor 2 where lower biogas potential, maximum production rate and lag phase of 131.24 ml,10.43

ml/day and 1.41 day respectively were achieved. It was observed that a reactor which was charged with media solution with pH of 8.00 achieved higher kinetic constant values when operated at digester's moisture content of 95.00 %.

4.2.1.3.3. Analysis 3

Reactors 1 and 4 were charged with media solution with pH of 8.00 and 4.00 respectively as shown in Table 4.1. C: B feed ratio and digester's moisture constant were fixed at 5:5 and 95.00 % respectively. Reactor 1 achieved higher biogas production potential and higher maximum production rate (A, 152.76 ml and U, 25.27 ml/day) at lower lag phase of 1.25 day. Reactor 4 obtained lower biogas production potential and higher maximum production rate of 111.76 ml and 10.60 ml/day at higher lag phase of 1.65 day.

In this analysis, low media solution pH resulted to higher lag phase. Reactors charged with media solution with pH 8.00 obtained higher biogas production potential and maximum production rate when they were operated at 95.00 % digester's moisture content.

4.2.1.3.4 Analysis 4

Reactors 3 and 8 were charged with equal C: B feed ratio (5:5), operated at digester's moisture content of 80.00 % and charged with media solution with pH of 4.00 and 8.00 respectively as shown in Table 4.1. Higher kinetic constant values (A, 328.06 ml; U, 56.07 ml/day and λ,1.43) were achieved by reactor 8 compared to reactor 3 where lower values of kinetic constants (A, 193.56 ml; U, 18.51 ml/day and λ,0.94) were achieved. In this analysis, reactor charged with high media solution pH achieved higher values of kinetic constants.

4.2.1.3.5. Concluding comments

All analyses reveal that media solution with pH of 8.00 favours higher maximum production rate and biogas potential values.

Media solution with pH of 8.00 is close to optimum pH for biogas production. The optimum pH for biogas production is between 6.5 and 7.8 (Angelidaki and Sanders 2004; Christy, Gopinath and Divya 2014; Barbazán 2015).

Low lag phase values of reactors operated at low media solution pH (4.00) is related to hydrolysis stage of AD process. Lag phase is related to hydrolysis stage of AD process. The optimum pH for hydrolysis is close to pH of 4 (Kheireddine, Derbal and Bencheikh-Lehocine 2014; Sibiya, Muzenda and Tesfagiorgis 2014). This is the reason why reactors operated at 4.00 are likely to have a low start-up time.

4.2.2. Mathematical modeling of kinetic constant

Mathematical models for biogas production potential, maximum biogas production rate and lag phase are represented by equations 4.1, 4.2 and 4.3 respectively. ANOVA results are presented in Table 4.2.

Adjusted R-Squared (Adj R-Squared), Predicted R-Squared (Pred R-Squared) and Adequate Precision (Adeq Precision), coefficient of variation (CV), p-value and F values were used to check the significance of the models.

$$\begin{aligned}A = &-17.61X_1-57.08X_2+47.21X_1X_2+0.23X_1Z_2+4.66X_2Z_2\\&+0.38X_2Z_2-0.47X_1X_2Z_2\end{aligned} \quad \ldots\ldots\ldots\ldots\ldots4.1$$

$$\begin{aligned}U = &6.82X_1-5.65X_2+4.01X_1X_2-0.07X_1Z_2+1.15X_2Z_2+0.0029X_2Z_2\\&-0.039X_1X_2Z_2\end{aligned} \quad \ldots\ldots4.2$$

$$\lambda = -0.39X_1-0.038X_2+0.089X_1X_2+0.042X_1Z_2 \ldots\ldots\ldots\ldots\ldots4.3$$

Where: X_1 and X_2 represent weights (g) of cow dung and bagasse respectively

Z_1 is the pH of the media solution

Z_2 is the moisture content of the digester (%)

A is the biogas potential (ml)

U is the maximum biogas production rate (ml/day)

λ is the lag phase (day)

Table 4.2: ANOVA results of cow dung and bagasse co-digestions experiment for kinetic constants values models.

Model	R-Squared	Pred R-Squared	Adj R-Squared	F Value	p-value Prob > F	Adeq Precision	CV
A	0.9473	0.6899	0.8841	14.98	0.0047	12.780	17.36
U	0.8584	0.3224	0.6885	5.05	0.0481	6.468	41.78
Λ	0.6824	0.1550	0.5633	5.73	0.0216	6.974	6.974

4.2.2.1. Biogas production potential

Table 4.2 shows that biogas production potential (A) model as presented by equation 4.1 has F-value of 14.98 which implies that the model is significant. "Pred R-Squared" of 0.6899 is in reasonable agreement with the "Adj R-Squared" of 0.8841; "Adeq Precision" is greater than 4 which is the indication of an adequate signal.

4.2.2.2. Maximum biogas production rate

Table 4.2 indicate that maximum biogas production rate model presented by equation 4.2 has F-value of 5.05 which indicates the significance of the model. There is 4.81 % chance that F-value this large could occur due to noise. "Pred R-Squared" of 0.3224 is not as close to the "Adj R-Squared" of 0.6885. This may indicate a large block effect. "Adeq Precision of 6.468 indicates an adequate signal.

4.2.2.3. Lag phase

Lag phase (λ) model presented by equation 4.4 has F-value of 5.73 as indicated by Table 4.2. This implies that the model is significant. "Adeq Precision is 6.974 which indicates an adequate signal. This model can be used to navigate the design space.

4.3. Process modeling

Table 4.3 represents experimental results of cow dung and bagasse co-digestion. Cow dung and bagasse are represented by X_1 and X_2 respectively. Process variables are media solution pH (Z_1) and digester's moisture content (Z_2). Biogas volume and methane yield are represented by Y_1 and Y_2 respectively. Methane yield and biogas volume calculations are presented in Appendix C.

Table 4.3: Experimental results of biogas volume and methane yield for cow dung and bagasse co-digestion.

Reactor	X_1 (g)	X_2 (g)	Z_1	Z_2 (%)	Y_1 (ml)	Y_2 (ml/gVS)
1	5.00	5.00	8.00	95.00	152.43	13.65
2	8.00	2.00	4.00	80.00	120.37	10.64
3	5.00	5.00	4.00	80.00	172.23	19.57
4	5.00	5.00	4.00	95.00	98.37	8.64
5	8.00	2.00	4.00	95.00	73.86	7.77
6	2.00	8.00	8.00	95.00	205.86	15.3
7	8.00	2.00	8.00	80.00	207.43	24.44
8	5.00	5.00	8.00	80.00	329.69	29.52
9	8.00	2.00	8.00	95.00	104.34	9.44
10	2.00	8.00	8.00	80.00	248.29	19.54
11	2.00	8.00	4.00	80.00	110.63	6.45
12	2.00	8.00	4.00	95.00	26.71	1.63

Table 4.3 indicates that the highest biogas volume of 329.69 ml and methane yield of 29.52 ml/gVS were achieved from reactor 8, which was charged with C: B feed ratio of 5:5, media solution with pH of 8.00 and moisture content of 80.00 %.

The lowest biogas volume of 26.71 ml and methane yield of 1.63 ml/gVS were achieved in reactor 12 which was charged with C: B feed ratio of 2:8, media solution with pH of 4.00 and moisture content of 95.00 %. Table 4.3 also reveals that the best performing reactors were charged with C: B feed ratio of 5:5 compared to reactors which were charged with C: B feed ratio of 8:2 and 2:8 when they were operated at the similar conditions.

4.3.1. Biogas volume modelling

Biogas volume model is represented by equation 4.4. ANOVA results are presented in Table 4.4.

$$Y_1 = -9.9X_1 - 48.5X_2 + 42.30X_1X_2 + 0.145X_1Z_2 + 5.14X_2Z_1 + 0.28\,X_2Z_2 - 0.42X_1X_2Z_2 \quad \ldots\ldots\ldots\ldots 4.4$$

Where: X_1 and X_2 represent the mass of cow dung (g) and bagasse (g) respectively.

$Z_1$and Z_2 represent media solution pH and digester moisture content (%) respectively

Y_1 represents biogas volume (ml)

Table 4.4: ANOVA results of cow dung and bagasse co-digestion experiment biogas volume model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	72569.75	6	12094.96	14.19	0.0053
Linear Mixture	913.48	1	913.48	1.07	0.3479
X_1X_2	2823.12	1	2823.12	3.31	0.1284
X_1Z_2	57.02	1	57.02	0.067	0.8062
X_2Z_1	39371.95	1	39371.95	46.21	0.0010
X_2Z_2	202.65	1	202.65	0.24	0.6464
$Z_1X_2Z_2$	2133.55	1	2133.55	2.50	0.1744

R^2 =0.9445, Adjusted R^2=0.8780, Coefficients of variation (CV) =18.93, Adequate precision=12.039, Standard deviation =29.19, Pred R^2 =0.6966

Table 4.4 shows that biogas volume model has F-value of 14.19 which implies that the model is significant. P-value of 0.0053 implies that there is 0.53% chance that F-value could occur due to noise. Coefficient of determination (R^2) indicates how close the data fits the model. R^2 was found to be 0.9445, which implies goodness of fit. Pred R^2 of 0.6966 is in reasonable agreement with the "Adj R^2 of 0.8780. A difference of less than 0.2 is acceptable. Coefficient of variation (CV) is the measure of residual variation related to the size of the mean. High value of CV is the indication of the lower reliability of the experiment while lower CV means high reliability of the experiment. Table 4.4 shows that CV was found to be 18.93 %. Adequate precision measures the signal to noise ratio. Ratio greater than 4 is desirable. In this current work, adequate precision was found to be 12.03 which indicate an adequate signal. Therefore, this model can be used to navigate the design space.

4.3.2. Diagnostic checks for bagasse and cow dung biogas volume

After biogas volume model determination, it was necessary to inspect that all assumptions which were made were correct, because if they were incorrect, the model developed would be invalid and therefore would lead to faulty conclusions. This was achieved by performing diagnostic checks of the model. The following diagnostic checks were performed:

- Normal probability plot of studentised residual which is used to check normality of residual
- Studentised residual versus predicted values to check for constant errors.

According to Chambers *et al.* (1983) normal probability represents graphical technique for assessing whether data is normally distributed. The plot should be approximately linear. Predicted versus actual plot is used to check if experimental results are close to predicted values. For good of fit predicted vs actual plot should resemble straight line at 45 degrees.

Figure 4.2 represents normal probability plot. It can be observed that its shape is almost linear which is the indication that experimental data is normally distributed. Figure 4.3 represents predicted vs actual plot. Figure 4.3 is almost straight at 45 degrees which indicates that experimental results are close to predicted.

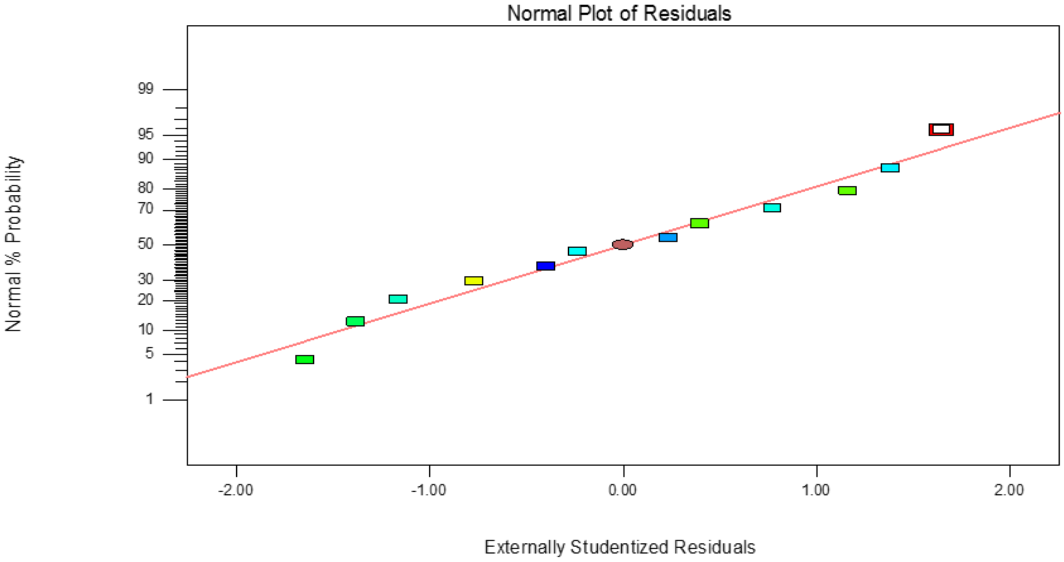

Figure 4.2: Normal probability vs studentized residual for bagasse and cow dung biogas volume model

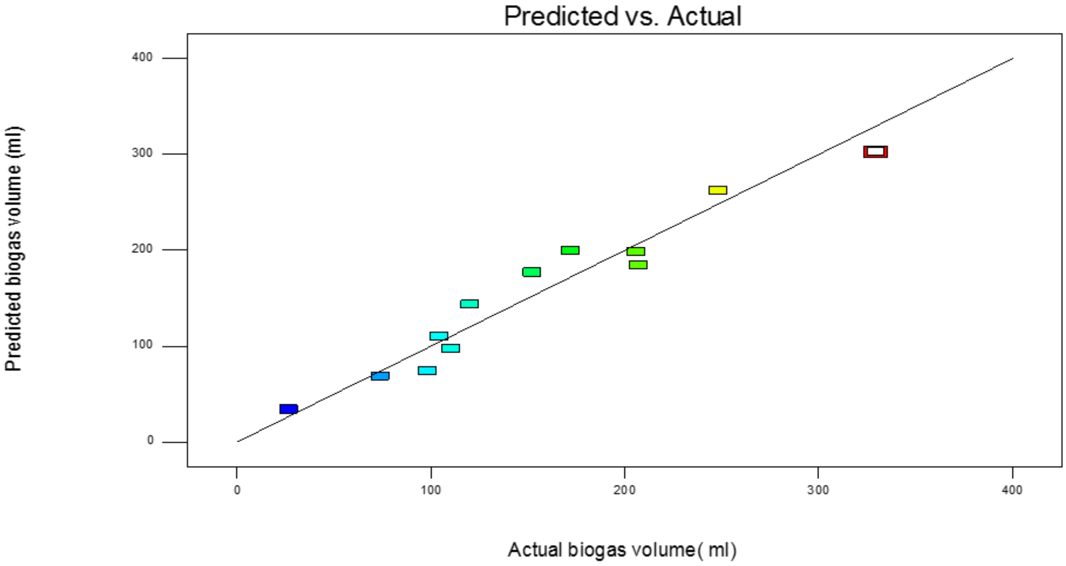

Figure 4.3: Normal probability vs studentized residual for bagasse and cow dung biogas volume model

4.3.3. Methane yield modelling

Methane yield model is represented by equation 4.5. ANOVA results are presented by Table 4.5.

$$Y_2 = -0.517X_1 - 7.78X_2 + 4.98\, X_1X_2 + 0.0097X_1Z_2 + 0.429\, X_2\, Z_1 + 0.059\, X_2Z_2 - 0.0494\, X_1X_2\, Z_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots 4.5$$

Where: X_1 and X_2 represent weights (g) of cow dung and bagasse respectively

Z_1 and Z_2 represent media solution pH and digester moisture content (%) respectively

Y_2 is the methane yield (ml/ g VS)

Table 4.5: ANOVA results for cow dung and bagasse co-digestion methane yield model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	660.06	6	110.01	8.76	0.0154
Linear Mixture	10.97	1	10.97	0.87	0.3929
X_1X_2	39.10	1	39.10	3.11	0.1380
X_1Z_2	0.25	1	0.25	0.020	0.8933
X_2Z_1	274.96	1	274.96	21.89	0.0054
X_2Z_2	9.19	1	9.19	0.73	0.4314
$X_1X_2Z_2$	29.64	1	29.64	2.36	0.1852
R^2 =0.9131, Adjusted R^2=0.8088, Coefficients of variation (VC) =25.53, Adequate precision=10.069, Standard deviation =3.54, Pred R-Squared =0.6236					

Table 4.5 indicates that methane yield model as presented by equation 4.5 has F-value of 8.76. P-value of 0.0154 indicates that there is 1.54 % chance that F-value this large could occur due to noise. "Pred R-Squared" of 0.6236 is in reasonable agreement with the "Adj R-Squared" of 0.8088; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this current work ratio of 10.069 indicates an adequate signal. This model can therefore be used to navigate the design space.

4.3.4. Diagnostic checks of bagasse and cow dung methane yield model

Normal probability plot shape of straight line as indicated by Figure 4.4 indicates that the data is normally distributed. Figure 4.5 represents predicted vs actual values plot which is a straight around 45 degrees. This is the indication of good of fit.

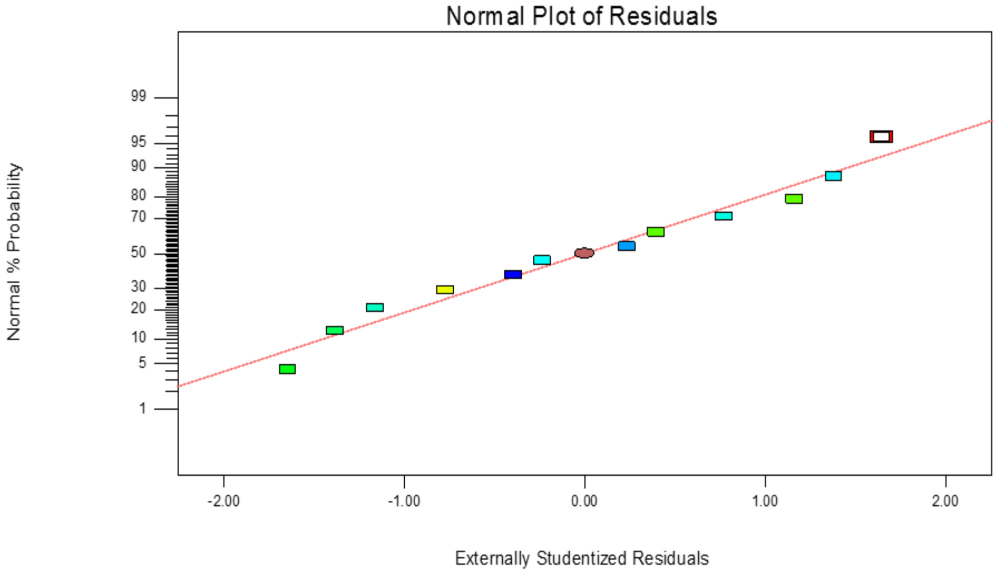

Figure 4.4: Normal probability plot of bagasse and cow dung model for methane yield

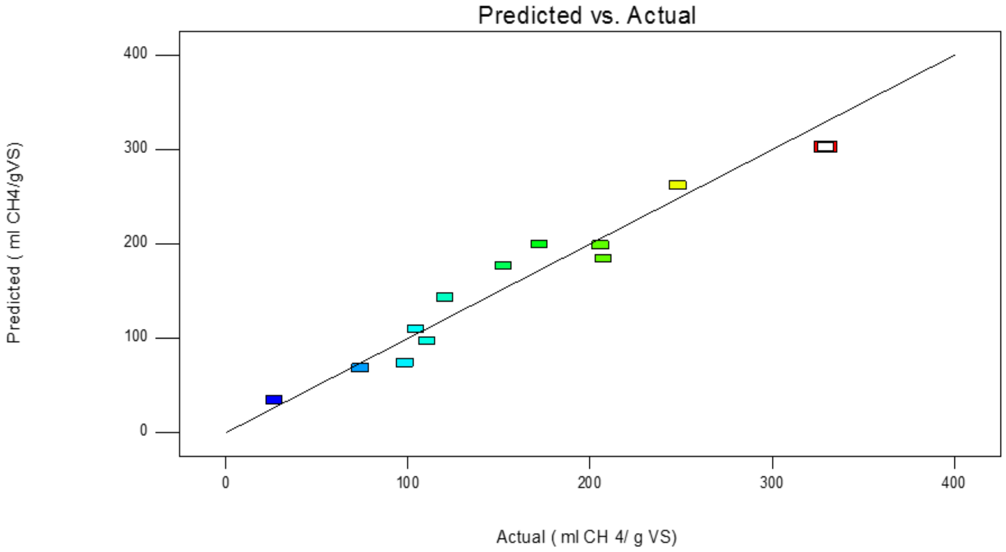

Figure 4.5: Predicted values versus the actual values of methane yield for cow dung ad bagasse

4.3.5. Effect of process parameters on biogas volume and methane yield

The effects of cow dung-to-bagasse ratio, media solution pH and digester's moisture content on biogas volume and methane were analyzed using OVAT and graphical analysis methods. Two-dimensional contour plots were used to analyze the effect biogas volume and methane yield. A contour plot provides 2-D view in which all points have same response are connected to produce contour lines of constant response. For the current work, value of one process variable is assigned at central position on Z-axis.

4.3.5.1. The effect of media solution pH on biogas volume and methane yield

4.3.5.1.1. Analysis 1

Reactors 5 and 9 were charged with equal C: B feed ratio of 8:2, digester's moisture content of 95.00 % and charged with media solution pH of 4.00 and 8.00 respectively. Table 4.3 shows that reactor 9 achieved higher biogas volume and methane yield of 104.34 ml and 9.44 ml/gVS respectively compared to reactor 5 where lower biogas volume of 73.86 ml and 7.77 ml/gVS were achieved.

In this analysis, it was observed that reactor charged with high media solution pH achieved higher biogas and methane yield values.

4.3.5.1.2. Analysis 2

Table 4.3 shows that reactors 2 and 7 were charged with equal C: B feed ratio of 8:2, digester's moisture content of 95.00 % and charged with media solution with pH of 4.00 and 8.00 respectively. Table 4.3 reveals that reactor 7 achieved higher biogas volume and methane yield of 207.43 ml and 24.44 ml/gVS respectively compared to reactor 2 where biogas volume and methane yield of 120.37 ml and 10.64 ml/gVS were achieved.

In this analysis, reactor which was charged with high pH (8.00) media solution achieved higher biogas volume and methane yield values.

4.3.5.1.3. Analysis 3

Reactors 3 and 8 were charged with equal C: B feed ratio (5:5), operated at moisture content of 80% and charged with media solutions pH of 4.00 and 8.00 respectively as shown in Table 4.3.

Higher values of biogas volume (329.69 ml) and methane yield (29.52 ml/gVS) were achieved by reactor 8 compared to reactor 3 where lower biogas volume and methane yield of 172.23 ml and 19.57 ml/gVS were achieved.

In this analysis, high media solution pH charged reactor attained higher biogas volume and methane yield values.

4.3.5.1.4. Analysis 4

Reactors 1 and 4 were charged with equal C: B feed ratio (5:5), operated digester's moisture content (95.00 %) and charged with media solution with pH of 8.00 and 4.00 respectively. Table 4.3 shows that reactor 1 achieved higher biogas volume and methane yield of 152.43 ml and 13.65 ml/gVS respectively. Reactor 4 achieved biogas volume of 98.37 ml and methane yield of 8.64 ml/gVS.

In this analysis, it can be observed that the reactor which was charged with media solution pH resulted to higher biogas and methane yield values.

4.3.5.1.5. Graphical analysis

The effect of C: B and digester's moisture content on biogas volume and methane yield at constant media solution pH was investigated. Contour lines in Figures 4.6 and 4.7 represent biogas volume and methane yield at constant media solution pH respectively.

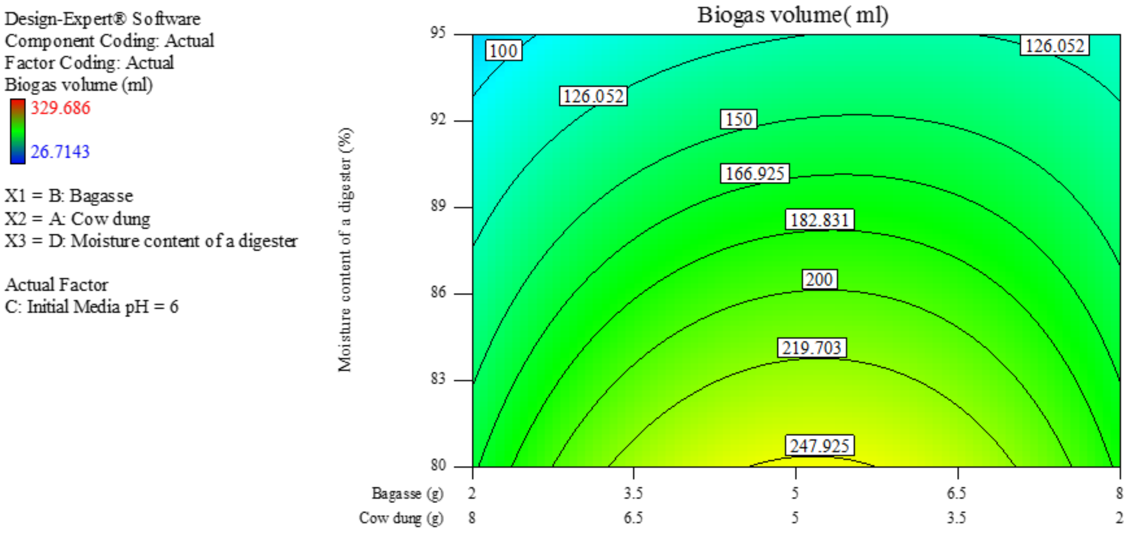

Figure 4.6: 2-D graphical representation of biogas volume model for cow dung and bagasse co-digestion at constant media solution pH

Figures 4.6 shows that biogas volume decreases with the increase of digester moisture content at constant C: B feed ratio. It can be observed that high biogas volume can be obtained at lower digester's moisture content. Figure 4.6 also shows that to maintain constant biogas volume digester's moisture content should be increased at low C: B feed ratio and be increased at high C: B feed ratio. At low C: B feed ratio the increase in cow dung concentration favors the increase in biogas volume. At constant C: B feed ratio, biogas volume decreases as digester's moisture content increases.

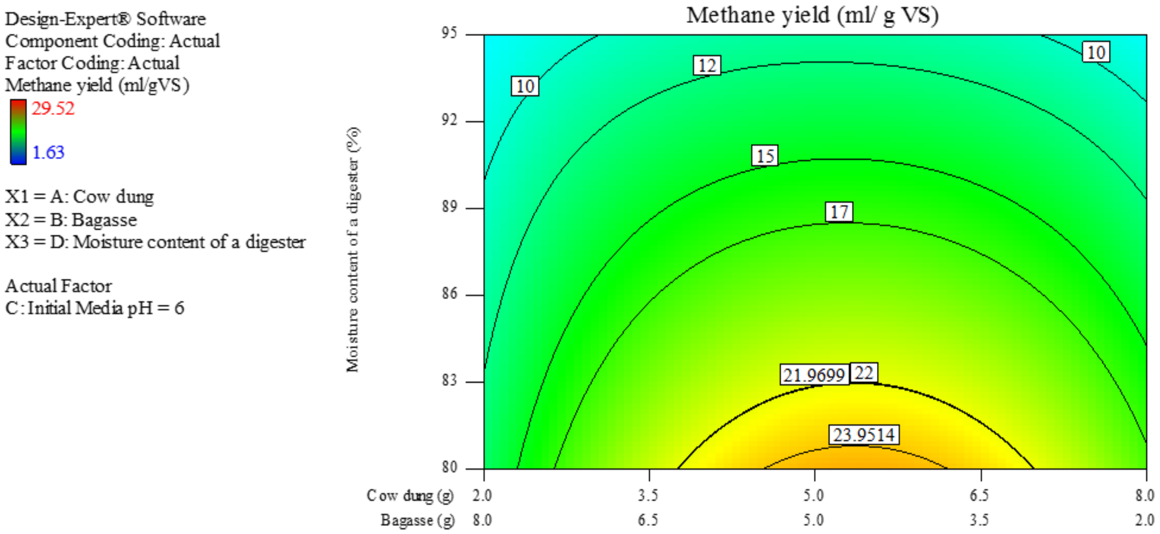

Figure 4.7: 2-D graphical representation of methane yield model for cow dung and bagasse co digestion at constant pH

Figure 4.7 indicates at constant C: B feed ratio methane yield decreases as digester's moisture content increases. The highest methane yield can be obtained at lower digester's moisture content at approximately C: B feed ratio between 3.5:6.5 and 6.5:3.5. At constant digester's moisture content methane yield increases as digester's moisture content increases at low C: B feed ratio, while at C: B feed ratio above 5:5, methane yield decreases as C: B feed ratio increases.

4.3.5.1.6. Concluding comments

Reactors charged with media solution with pH of 8.00 achieved higher biogas volume and methane yield regardless of conditions of other process variables. Media solution pH of 8.00 is close to the optimum pH. Optimal pH for biogas production is between 6.5 and 7.8 (Kheireddine, Derbal and Bencheikh-Lehocine 2014; Merlin, Gopinath and Divya 2014; Sibiya, Muzenda and Tesfagiorgis 2014; Adekunle and Okolie 2015; Prakasha *et al.* 2015).

Lower biogas volume and methane yield production in reactors charged with media solution at pH of 4.00 was observed. Low pH media solution produces high concentration of VFAs. According to Kheireddine, Derbal and Bencheikh-Lehocine (2014), pH below 6.5 produces high VFAs concentration which may cause AD process to slow down or fail.

Graphical analysis shows that both biogas volume and methane yield decrease as digester moisture content increases at constant C: B feed ratio. High biogas volume and methane yield are achieved at lower digester's moisture content.

4.3.5.2. Effect of digester's moisture content on biogas volume and methane yield

4.3.5.2.1. Analysis 1

Reactors 6 and 10 were charged with equal C: B feed ratio of 2:8, equal media solution with pH of 8 and operated at different moisture content of 95 % and 80 % respectively as indicated by Table 4.3. Table 4.3 shows that reactor 10 achieved higher biogas volume (248.29 ml) and methane yield 19.54 ml/gVS) compared to reactor 6 where biogas volume and methane yield of 205.86 ml and 15.30 ml/gVS were achieved respectively.

In this analysis high values of biogas volume and methane yield were achieved by reactor operated at lower moisture content of digester.

4.3.5.2.2. Analysis 2

Table 4.3 shows that reactor 3 achieved higher biogas volume and methane yield values of 172.23 ml and 19.57 ml/gVS compared to reactor 4 where biogas volume and methane yield of 98.37 ml and 8.64 ml/ gVS were obtained. Reactor 3 which was operated at lower digester's moisture of 80.00 % achieved higher biogas volume and methane yield compared to reactor 4 which was operated at 95.00 % digester's moisture content when charged with media solution with pH 4.00 and C: B feed ratio of 5:5.

It was observed that reactor operated at lower digester’s moisture content achieved higher values of biogas volume and methane yield.

4.3.5.2.3. Analysis 3

Reactors 1 and 8 were charged with equal C: B feed ratio (5:5), equal media solution with pH of 8.00 and operated at different digester’s moisture content of 95.00 % and 80.00 % respectively as presented in Table 4.3. Higher biogas volume and methane yield of 329.69 ml and 29.52 ml/gVS respectively were achieved by reactor 8 compared to reactor 1 where lower biogas volume and methane yield of 152.43 ml and 13.65 ml/gVS were achieved.

It was noticed that low digester’s moisture content operated reactor achieved higher biogas volume and methane yield.

4.3.5.2.4. Analysis 4

Reactors 11 and 12 were charged with equal C: B feed ratio of 2:8, media solution with pH of 4 and operated at digester’s moisture content of 80.00 % and 95.00 % respectively.

Table 4.3 reveals that higher biogas volume and methane yield of 110.63 ml and 6.59 ml/gVS respectively were achieved by reactor 11 compared to reactor 12 where lower biogas volume and methane yield values of 26.71 ml and 1.63 ml/gVS were achieved respectively.

This analysis reveals that a reactor operated at lower digester’s moisture content achieved higher biogas volume and methane yield when charged with media solution with pH of 4.00. Low cow dung concentration in a digester produces higher biogas when operated at 80.00 % moisture content. High concentration of bagasse in a reactor produces higher biogas at media solution of 4.00 and digester’s moisture content of 80.00 %.

4.3.5.2. 5. Analysis 5

Reactors 2 and 5 were charged with equal media solution with pH of 4.00, C: B feed ratio of 8:2 and digester’s moisture content of 80.00 % and 95.00 % respectively. Table 4.3 shows that reactor 2 which was operated at digester's moisture content of 80.00 % produced higher biogas volume (120.37 ml) and methane yield (10.64 ml/gVS) compared to reactor 5 where lower biogas volume (73.86 ml) and methane yield (7.77 ml/gVS) were achieved.

In this analysis, it was observed that low digester's moisture content charged reactors achieved higher biogas volume and methane yield. It was observed that reactor with 8.00 g of cow dung achieved higher biogas volume and methane yield at 80.00 % digester's moisture content.

4.3.5.2.6. Graphical analysis

Figures 4.8 and 4.9 represent 2-D contour graphical representation of biogas volume and methane yield models respectively at digester's moisture content fixed at central point of 87.5 %. Figure 4.8 indicates as media solution pH increases biogas volume increases at a constant C: B feed ratio. To maintain constant biogas volume as indicated by contour line, C: B feed ratio should be increased while media solution pH should be decreased at C: B feed ratio of about 2:8 to 5:5. At C: B feed ratio above 5:5, the increase in media solution pH together with the increase in C: B feed ratio is required to maintain constant biogas volume.

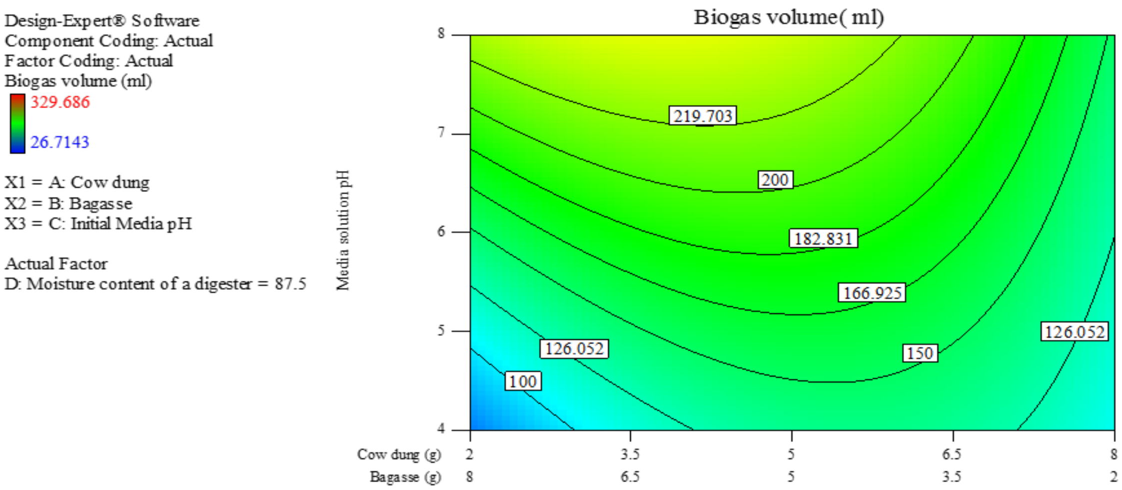

Figure 4.8: 2-D graphical representation of biogas volume model for cow dung and bagasse co-digestion at constant moisture content

Figure 4.9 indicates as media solution pH increases methane increases at a constant C: B feed ratio. To maintain constant methane yield as indicated by contour line, C: B feed ratio should be increased while media solution pH should be decreased at C: B feed ratio of about 2:8 to 5:5. At C: B feed ratio above 5:5, the increase in media solution pH together with the increase in C:B feed ratio is required to maintain constant methane yield. The linearity of methane yield in a region of C: B feed ratio of 3.5:6.5 indicates weak interaction between media solution pH and C: B feed ratio of methane yield.

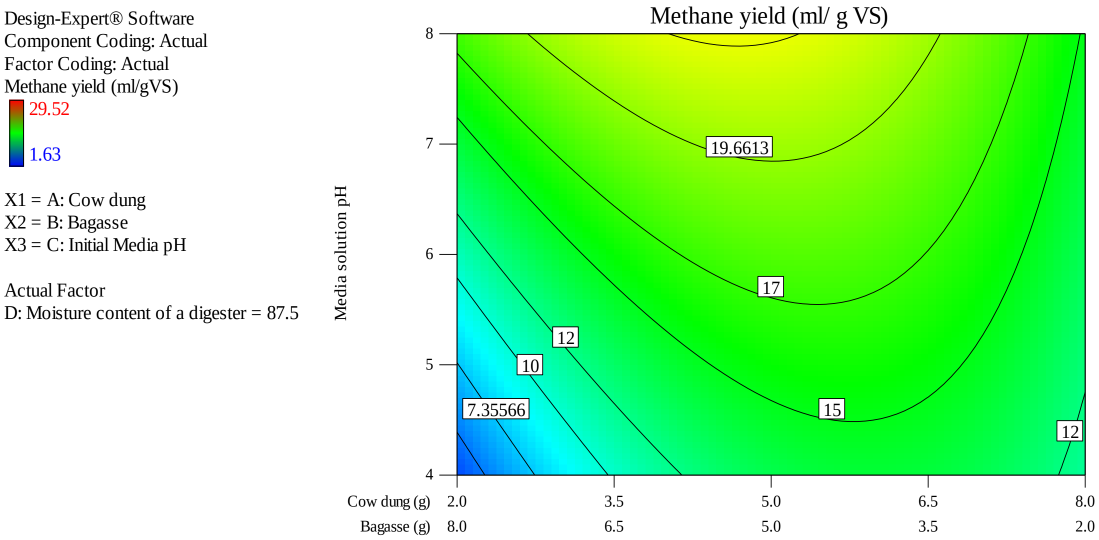

Figure 4.9: 2-D graphical representation of methane yield model for cow dung and bagasse co-digestion at constant moisture content

4.3.5.2.7. Concluding comments

The above analyses show that low digester's moisture content (80.00 %) charged reactors achieved high biogas production than reactors which were operated at high digester's moisture content regardless of conditions of other process variables.

High cow dung concentration in a reactor favoured high biogas production at media solution with pH of 4.00 and digester's moisture content of 80.00 %. High bagasse concentration in a reactor favoured high biogas production at media solution with pH of 4.00 and digester's moisture content of 80.00 %. At constant digester's moisture content and C: B feed ratio biogas volume and methane yield increase as media solution pH increases.

It was observed that to maintain constant biogas volume and methane yield, C: B feed ratio should be increased, and media solution pH should be decreased at higher C: B feed ratio.

4.3.5.3. The effect of cow dung-to-bagasse feed ratio on biogas volume and methane yield

4.3.5.3. 1.Analysis 1

Table 4.3 shows that higher biogas volume of 73.86 ml and methane yield of 7.77 ml/gVS were achieved by reactor 5 compared to reactor 12 where biogas volume and methane yield of 26.71 ml and 1.63 ml/ gVS were produced. Reactors 5 and 12 were operated at digester's moisture content (95.00 %) and media solution pH (4.00) and charged with C: B feed ratio of 8:2 and 2:8 respectively.

In this analysis, reactor charged with high C: B feed ratio achieved high biogas volume and methane yield values.

4.3.5.3.2. Analysis 2

Table 4.3 reveals that reactors 6 achieved higher biogas volume (205.68 ml) and methane yield (15.30 ml/gVS) compared to reactor 9 where biogas volume and methane yield of 104.34 ml and 9.44 ml/gVS were achieved. Reactors 6 and 9 were charged with C: B feed ratio of 2:8 and 8:2 respectively. Both reactors were operated at constant digester's moisture content and media solution with pH of 95% and 8.00 respectively.

It was noticed that reactor charged with low C: B feed ratio achieved high biogas volume and methane yield values.

4.3.5.3.3. Analysis 3

Reactors 2 and 11 were charged with equal media solution pH (4.00), operated at moisture content of 80.00 % and charged with C: B feed ratio of 8:2 and 2:8 respectively. Reactor 2 achieved higher biogas volume (120.37 ml) and methane yield (10.64 ml/gVS) compared to lower biogas volume (110.63 ml) and methane yield (6.45 ml/ gVS) achieved by reactor 11.

It was observed that reactor charged with high feed C: B feed ratio achieved high biogas volume and methane yield.

4.3.5.3.4. Analysis 4

Table 4.3 shows that reactors 7 and 10 were charged with C: B feed ratio of 8:2 and 2:8 respectively. Digester's moisture content and media solution pH were kept constant at 80.00 % and 8.00 respectively. Reactor 10 achieved higher biogas volume and methane yield compared to reactor 7 where lower biogas volume and methane yield were achieved.

In this analysis, it was noticed that high concentration of cow dung in the reactor achieved higher biogas production compared to reactors charged with 2.00 g of cow dung.

4.3.5.3.5. Graphical analysis

Figures 4.10 and 4.11 represent 2-D graphical representation of biogas volume and methane yield at central position of C: B feed ratio of 5:5. Both methane yield and biogas volume counter line are straight lines and parallel, which indicates that there a weak interaction between process variables on biogas volume and methane yield at C: B feed ratio of 5:5. Figures 4.10 and 4.11 reveal that to achieve the constant biogas volume and methane yield values at constant media solution pH, digester's moisture content should be increased. Both graphs indicate that as media solution pH increases, both biogas volume and methane yield increase.

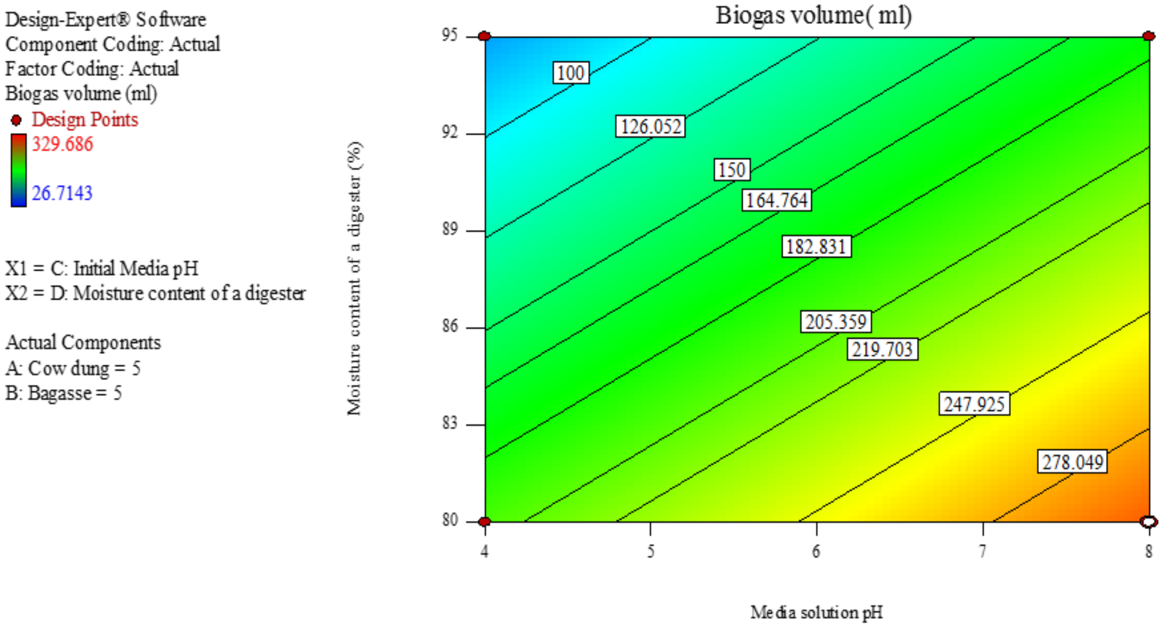

Figure 4.10: 2-D graphical representation of biogas volume model for cow dung and bagasse co digestion at cow dung-to-bagasse feed ratio of 5:5.

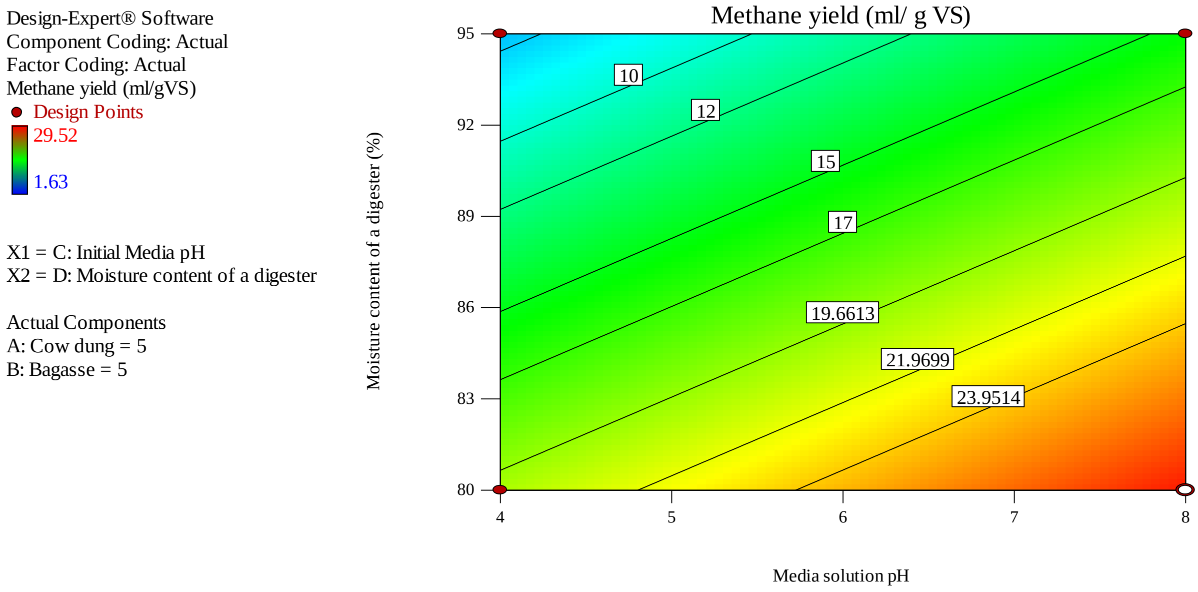

Figure 4. 11: 2-D graphical representation of methane yield model for cow dung and bagasse co-digestion at feed ratio of 5:5

4.3.5.3.6. Concluding comments

Reactors which were charged with high C: B feed ratio achieved higher biogas volume and methane yield when charged with media solution with pH of 4.00. Reactors charged with low C: B feed ratio achieved high biogas volume and methane yield when charged with media solution with pH of 8.00. Graphical analysis indicates that digester's moisture content should be increased to achieve constant biogas volume and methane yield values at constant media solution pH. In conclusion, the effect of C: B feed ratio on biogas volume and methane yield depends on media solution pH at which AD process was operated.

4.3.6. Optimization

Optimisation process was performed on single response and combined responses. This was done to have a better insight on how a single response alone compared to the combine responses. Design expert was used to perform optimisation of responses.

4.3.6.1. Optimization of biogas volume

Constraints used during optimisation process for biogas volume are presented in Table 4.6. Solutions found are presented in Table 4.7. Solution with highest desirability and highest biogas volume was selected as optimum value. The optimum biogas volume of 307.01 ml corresponds with media solution pH of 8.00, digester operated at 80.00 %, 4.27 g of cow dung and 5.73 g of bagasse.

Table 4.6: Constraints used during biogas volume optimization of cow dung and bagasse

Name	Goal	Limits	
		Lower	Upper
Cow dung (g)	minimize	2.00	8.00
Bagasse (g)	maximize	2.00	8.00
Media solution pH	is in range	4.00	8.00
Digester's moisture content (%)	is in range	80.00	95.00
Biogas volume (ml)	maximize	26.71	329.68

Table 4.7: Solutions found during biogas volume optimisation of cow dung and biogas digestion

Solution number	Cow dung (g)	Bagasse (g)	Media solution pH	Digester's moisture content (%)	Biogas volume (ml)	Desirability
1	4.27	5.73	8.00	80.00	307.10	0.925
2	4.14	5.86	8.00	80.00	306.96	0.925
3	4.45	5.55	8.00	80.00	306.82	0.925
4	4.25	5.75	8.00	80.15	305.87	0.921
5	4.10	5.90	8.00	80.27	304.70	0.918
6	4.15	5.85	8.00	80.33	304.32	0.916
7	5.00	5.00	8.00	80.00	302.40	0.910
8	4.46	5.54	7.81	80.00	301.35	0.906
9	3.23	6.77	8.00	80.00	297.64	0.894
10	2.00	8.00	8.00	80.00	261.76	0.776
11	2.68	7.32	8.00	86.38	247.77	0.730
12	6.07	3.93	4.54	80.00	208.64	0.600

4.3.6.2. Optimization of methane yield

Table 4.8 represents constraints used during optimisation process. Solutions are presented in Table 4.9. Solution with highest desirability (0.976) resulted to the highest methane yield (28.85 ml/gVS) was selected. Corresponding optimum process variables were 4.95 g cow dung, 5.05 g bagasse, media pH solution of 8 and 80 % moisture content of were selected.

Table 4.8: Constraints used during cow dung and bagasse methane yield optimisation process

Variable	Unit	Goal	Limits	
			Lower	Upper
Cow dung	g	is in range	2.00	8.00
Bagasse	g	is in range	2.00	8.00
Media solution pH		is in range	4.00	8.00
Digester's moisture content	%	is in range	80.00	95.00
Methane yield	ml/gVS	maximize	1.63	29.52

Table 4.9: Solutions for cow dung and bagasse methane yield optimisation process.

Solution Number	Cow dung (g)	Bagasse (g)	Media solution pH	Digester's moisture content (%)	Methane yield (ml.gVS)	Desirability
1	4.95	5.05	8.00	80.00	28.85	0.976
2	5.04	4.96	8.00	80.00	28.84	0.976
3	4.95	5.05	7.98	80.00	28.80	0.974
4	5.02	4.98	8.00	80.07	28.78	0.974
5	4.86	5.14	7.97	80.00	28.78	0.974
6	5.10	4.90	7.98	80.00	28.78	0.973
7	4.94	5.06	8.00	80.16	28.71	0.971
8	4.88	5.12	7.93	80.00	28.69	0.970
9	4.97	5.03	8.00	80.26	28.62	0.968
10	5.54	4.46	8.00	80.00	28.49	0.963

4.3.6.3. Combined optimization

Both biogas volume and methane yield were maximised as indicated in optimisation constrains in table 4.10. Optimisation solutions are presented in Table 4.11. Solution number 1 was selected as optimum conditions as indicated in Table 4.11. Digester operated at moisture content (80.00%), media solution pH (8.00), cow dung (4.64 g) and bagasse (5.36 g) are process conditions required to achieve optimum biogas volume (305.87 ml) and methane yield 28.75 ml/gVS.

Table 4.10: Constraints used for combined optimisation of biogas and methane yield for cow dung and bagasse digestion

Variable	Unit	Goal	Lower limit	Upper limit
Cow dung	g	is in range	2.00	8.00
Bagasse	g	is in range	2.00	8.00
Initial Media pH		is in range	4.00	8.00
Digester's moisture content	%	is in range	80.00	95.00
Biogas volume	ml	maximize	26.71	329.69
Methane yield	ml/gVS	maximize	1.63	29.52

Table 4.11: Solutions for combined optimisation process of biogas volume and methane yield for cow dung and bagasse co-digestion

Solution number	Cow dung (g)	Bagasse (g)	Media solution pH	Digester's moisture content %	Biogas volume (ml)	Methane yield (ml.gVS)	Desirability
1	4.64	5.36	8.00	80.00	305.87	28.75	0.947
2	4.53	5.47	8.00	80.00	306.51	28.66	0.946
3	4.81	5.19	8.00	80.00	304.53	28.83	0.946
4	4.43	5.57	8.00	80.00	306.88	28.56	0.945
5	4.67	5.33	8.00	80.06	305.23	28.71	0.945
6	4.64	5.36	7.98	80.00	305.32	28.70	0.945
7	4.95	5.05	8.00	80.00	302.98	28.85	0.943
8	4.78	5.22	7.96	80.00	303.83	28.74	0.943
9	4.65	5.35	8.00	80.21	304.08	28.57	0.940
10	4.98	5.02	7.92	80.00	300.61	28.68	0.936
11	4.36	5.64	8.00	80.31	304.50	28.22	0.935

4.4. Preliminary conclusions and findings

- Biogas production from cow dung and bagasse obeys Gomperzt law.
- High C: B feed ratio favored high biogas volume, methane yield and kinetics constant values when operated at media solution with pH of 4.00.
- Low C: B feed ratio favored high biogas volume, methane yield and kinetics constant values when operated at media solution with pH of 8.00.
- As media solution pH increase biogas production and methane yield increase. High cow dung concentration at any media solution pH favored the increase in both biogas volume and methane yield.

- The kinetics constants for cow dung and sugarcane leaves co digestion increases with the moisture content. The highest biogas potential, the highest maximum biogas production and lag phase rate observed in the reactors with high moisture contents. The reactors with the lower moisture content have a shorter lag phase.

- High production of biogas volume and methane were favorable at high media solution pH (8.00).
- The influence of C: B feed ratio and digester's moisture content on biogas production and kinetics constants values depended of the condition of other process variables.
- The optimum biogas volume for single optimization process was achieved at media solution with pH of 8.00, digester operated at 80.00 % moisture content, 4.27 g of cow dung and 5.73 g of bagasse.
- The optimum methane yield for single optimization process was found to be 28.85 ml/gVS at 4.95 g cow dung, 5.05 g bagasse, media solution with pH of 8 and digester's moisture content of 80%.
- Digester operated at moisture content (80.00%), media solution pH (8.00), cow dung (4.64g) and bagasse (5.36 g) are process conditions required to achieve optimum biogas volume of 305.87ml and methane yield of 28.75 ml/gVS.

Chapter 5

RESULTS AND DISCUSSION: COWDUNG AND SUGARCANE LEAVES

5.1. Introduction

The results of cow dung and sugarcane leaves co-digestion experiments are presented in this chapter. Mathematical models for biogas volume, methane yield and kinetic constants values are presented. The effects of cow dung, sugarcane leaves, media solution pH and moisture content of digester on methane yield, biogas volume and kinetic constants values were investigated and discussed.

5.2. Kinetic constants

AD process experiment was stopped at HRT of 14 days as it was showing signs of slowing down of daily biogas production. Cumulative biogas volume was plotted against HRT as shown in Figure 5.1. The experimental data was tested for the fitness in modified Gompertz equation. Kinetic constants values as they were determined by using MS Excel Solver are presented in Table 5.1.

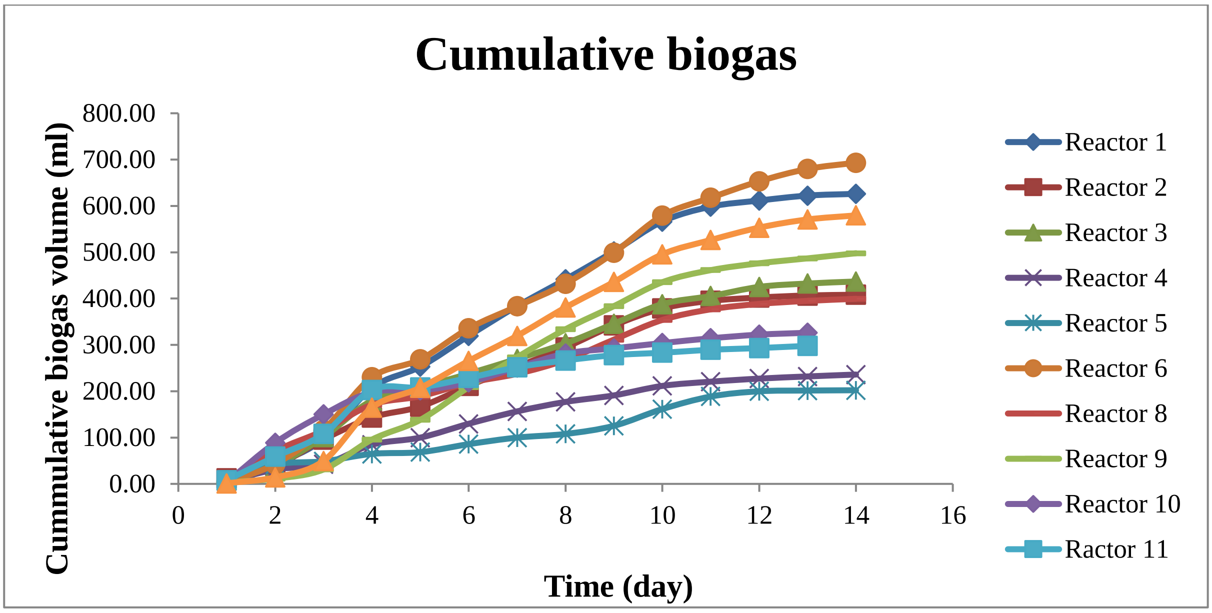

Figure 5.1: Cumulative daily biogas volume for cow dung and sugarcane leaves against HRT

Table 5.1: Kinetic constants values for cow dung and sugarcane leaves co-digestion experiment

Reactor	Cow dung (g)	Sugarcane leaves (g)	Media solution pH	Digester's moisture content (%)	A ml	U ml/day	Λ day
1	5.00	5.00	8.00	95.00	678.00	77.90	1.80
2	8.00	2.00	4.00	80.00	451.20	48.50	1.40
3	5.00	5.00	4.00	80.00	470.70	49.30	1.10
4	5.00	5.00	4.00	95.00	249.60	29.00	1.40
5	8.00	2.00	4.00	95.00	322.70	17.40	1.00
6	2.00	8.00	8.00	95.00	782.90	72.70	1.50
7	8.00	2.00	8.00	80.00	497.40	59.30	2.00
8	5.00	5.00	8.00	80.00	450.10	40.10	0.50
9	8.00	2.00	8.00	95.00	530.70	69.30	3.00
10	2.00	8.00	8.00	80.00	326.50	45.60	0.20
11	2.00	8.00	4.00	80.00	291.40	57.90	1.00
12	2.00	8.00	4.00	95.00	630.00	70.60	2.20

5.2.1. Effects of process variables on kinetic constants

Effect of process variables on kinetics constants was determined by using OVAT approach. In this section, results of two reactors operated at two fixed condition and one variable condition are compared. Conclusions are drawn based on the analysis.

5.2.1.1. Effects of digester's moisture content on kinetics constant

5.2.1.1.1. Analysis 1

Table 5.1 shows that reactor 9 achieved higher kinetics constants values (A,530.70 ml; U,69.30 ml/day and λ of 3.00) compared to reactor 7 where lower kinetic constants values (A,497.40 ml; U,59.30 ml/day and λ of 2.00) achieved. Reactors 7 and 9 were operated at digester's moisture content of 80.00 % and 95.00 % respectively. Reactors were both charged with equal C:SL feed ratio of 8:2 and media solution with pH of 8.00.

It was observed that reactor operated at lower digester's moisture content achieved higher values of kinetic constant.

5.2.1.1.2. Analysis 2

Reactors 11 and 12 were operated at digester's moisture content of 80.00 % and 95.00 % respectively as shown in Table 5.1. C:SL feed ratio and media solution pH were fixed at 2:8 and 4.00 respectively. Reactor 12 achieved higher kinetic constants values (A, 630.00 ml; U,70.60 ml/day and λ of 2.20 day) compared to lower kinetic constants values (A, 291.40 ml; U,57.90 ml/day and λ of 1.00 day) which were achieved by reactor 11.

It was observed that reactor operated at higher digester's moisture content achieved higher values of kinetic constants.

5.2.1.1.3. Analysis 3

Reactors 2 and 5 were charged with equal C:SL feed ratio of 8:2, media solution pH (4.00) and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Higher kinetic constants values were achieved by reactor 2 (A,451.20 ml; U,48.50 ml/day and λ of 1.40) compared to reactor 5 where lower kinetic constants values (A,322.70 ml; U,17.40 ml/day and λ of 1.00).

It was observed that reactor which was operated at low digester's moisture content achieved higher kinetic constants values.

5.2.1.1.4. Analysis 4

Reactors 6 and 10 were operated at digester's moisture content of 95.00 % and 80.00 % respectively. Media solution pH and C:SL were fixed at 8.00 and 8:2 respectively.

Table 5.1 reveals that higher kinetic constants values were achieved by reactor 6 (A, 782.90 ml, U,72.70 ml/day and λ of 1.50 day) compared to reactor 10 where lower biogas potential, maximum production rate and lag phase of 326.50, 45.60 ml/day and 0.20 day were achieved.

In this analysis, it was observed that reactor which was operated at 95.00 % digester's moisture content achieved higher kinetic constant values.

5.2.1.1.5. Analysis 5

Reactors 1 and 8 were charged with equal C: SL ratio of 5:5, equal media pH of 8.00 and operated at digester's moisture content of 95.00 % and 80.00 % respectively. Higher biogas production potential (678.00 ml), maximum biogas production rate (77.90 ml/day) and lag phase of 1.80 day were achieved by reactor 1 compared to biogas production potential of 450.10 ml, maximum biogas production rate of 40.1 ml/day and lag phase of 0.5 day which were achieved by reactor 8.

In this comparison, high digester's moisture content operated reactor achieved higher kinetic constants values.

5.2.1.1.6. Concluding comments

Results show that reactors operated at low digester moisture content (80.00 %) achieve higher kinetic constant values when charged with media solution with pH of 4.00 at higher C:SL feed ratio (8:2). Low digester's moisture content (80.00 %) operated reactors achieved higher kinetic constants values when charged with media solution with pH 4.00 and low C:SL feed ratio (2:8).

High digester's moisture content operated reactors achieved high kinetic constants values regardless of other process variables conditions. Above analyses show that there is interdependency between process variables on how each process variable influence kinetic constants values.

5.2.1.2. Effects of media solution pH on kinetic constant values

5.2.1.2. 1.Analysis 1

Reactors 1 and 4 were charged with media solution with pH of 8.00 and 4.00 respectively. Reactor 1 achieved higher kinetic constants values (A, 678.00 ml;77.90 ml/day and λ of 1.80

day) compared to reactor 4 where lower kinetic constants values (A, 249.60 ml; 29.00 ml/day and λ of 1.40 day) were achieved. Both reactors were operated at equal digester's moisture content and C:SL feed ratio of 95.00 % and 5:5 respectively.

In this analysis it was observed that reactor charged with media solution pH (8.00) achieved higher kinetic constant values.

5.2.1.2. 2.Analysis 2

Reactor 3 and 8 were charged with equal C:SL feed ratio of 5:5, operated at fixed digester's moisture content of 80.00 % and charged with media solution with pH of 4.00 and 8.00 respectively. Reactor 3 achieved higher kinetic constants values (A, 470.70 ml; 49.30 ml/day and λ of 1.10 day) compared to reactor 8 where lower kinetic constants values (A, 450.10 ml;40.10 ml/day and λ of 0.50 day) were achieved. In this comparison, high kinetic constant values were achieved by reactor charged with low media pH (4.00).

5.2.1.2. 3.Analysis 3

Table 5.1 shows that reactors 10 and 11 were charged with media solution with pH of 8.00 and 4.00 respectively. Digester's moisture content and C:SL feed ratio were fixed at 80 % and 2:8 respectively.

Higher biogas potential (326.60 ml), lower maximum biogas production rate (45.60 ml/day) and lower lag phase (0.20 day) were achieved by reactor 10 compared to reactor 11 where lower biogas potential (291.40 ml), higher maximum biogas production rate (57.90 ml/day) and higher lag phase of 1.00 day were achieved.

5.2.1.2. 4. Analysis 4

Table 5.1 show that digester's moisture content and C:SL feed ratio of reactors 2 and 7 were fixed at 95 % and 8: 2 respectively. Reactors 2 and 7 were charged with media solution with pH of 4 and 8 respectively. Reactor 7 achieved higher kinetic constants values (A, 497.40 ml;

59.30 ml/day and λ of 2.00 day) compared to reactor 2 where lower kinetic constant values (A, 451.20 ml; 48.50 ml/day and λ of 1.40 day).

In this analysis, the reactor charged with media solution with pH of 8.00 achieved higher kinetic constant values.

5.2.1.2.5. Concluding comments

Reactor charged with higher pH media solution achieved higher kinetic constants values when operated at digester's moisture content of 95.00 %.

Low pH media solution charged reactors achieved higher kinetic values when operated at 80% digester's moisture content.

5.2.1.3. Effects of cow dung-to- sugar cane leaves on kinetics constants values

5.2.1.3.1. Analysis 1

Reactors 6 and 9 were charged with equal media solution with pH of 8.00, operated at equal digester's moisture content of 95.00 % and charged with C: SL feed ratios of 2:8 and 8:2 respectively. Table 5.1 reveals that reactor 6 achieved higher biogas production potential, higher maximum biogas production rate and lower lag phase (A, 782.90 ml;72.70 ml/day and λ of 1.50 day) compared to lower biogas production potential, lower maximum biogas production rate and higher lag phase (A, 470.70 ml;69.30 ml/day and λ of 3.00 day) achieved by reactor 9.

5.2.1.3.1.2. Analysis 2

Table 5.1 shows that higher kinetic constants values (A, 630.00 ml;70.60 ml/day and λ of 2.20 day) were achieved by reactor 12 compared to lower kinetics constants values (A, 322.70 ml; 17.40 ml/day and λ of 1.00 day) achieved by reactor 5 when charged with media solution with pH of 4 and operated at digester's moisture content of 95.00 %. Reactors 5 and 12 were charged with C:SL feed ratio of 8:2 and 2:8 respectively.

5.2.1.3.1.3Analysis 3

Reactors 7 and 10 were charged with C:SL feed ratio of 8:2 and 2:8 respectively. Digester's moisture content and media solution pH were fixed at 80.00 % and 4.00 respectively. Higher kinetic constants values (A, 497.40 ml; 59.30 ml/day and λ of 2.00 day) were achieved by reactor 7 compared to reactor 10 results where lower kinetic constant values (A, 326.50 ml;45.60 ml/day and λ of 0.20 day) were achieved.

5.2.1.3.1.4. Reactor 4

Reactors 2 and 11 were charged with C:SL feed ratio of 8:2 and 2:8 respectively. Digester's moisture content and media solution pH were fixed at 80.00 % and 4.00 respectively.

Table 5.1 indicates that higher biogas production potential (451.20 ml), lower maximum biogas production rate (48.50 ml/day) and higher lag phase of λ of 1.4 day were achieved by reactor 2 compared to reactor 11 where lower biogas production potential (291.40 ml), high maximum production rate (57.90 ml/day) and lower lag phase of 1.00 day were achieved

5.2.1.3.1.5. Concluding comments

- It was observed that reactor charged with high C:SL achieved higher kinetics values.
- Reactor with low C:SL feed ratio achieved high kinetics constants when charged with media solution pH of 4.
- Reactors charged with high C:SL achieved lower lag phase when charged with media solution with pH of 8.00.

5.2.2. Mathematical modelling of kinetics constant

Mathematical models of biogas potential, maximum biogas production rate and lag phase are presented by equations 5.1, 5.2 and 5.3 respectively. ANOVA results presented in Table 5.2.

$$A = 164.86\, X_1 - 250.00\, X_2 + 4.23\, X_1 Z_1 - 1.69\, X_1 Z_2 + 2.85 X_2 Z_1 + 3.25 X_2 Z_2 \qquad \text{...........................5.1}$$

$$U = 61.24X_1 + 3.99X_2 - 7.62X_1Z_1 - 0.73X_1Z_2 - 2.65X_2Z_1 + 0.05X_2Z_2 + 0.10X_1Z_1Z_2 + 0.02X_2Z_1Z_2 \quad \ldots\ldots\ldots\ldots 5.2$$

$$\lambda = -0.08X_1 - 0.59X_2 + 0.05X_1Z_1 - 0.04X_2Z_1 + 0.011X_2Z_2 \quad \ldots\ldots\ldots\ldots 5.3$$

Table 5.2: ANOVA results of kinetic constant models for cow dung and sugarcane leaves co-digestion experiment

Model	R-Squared	Pred R-Squared	Adj R-Squared	F Value	p-value Prob > F	Adeq Precision	CV
A	0.7060	0.1379	0.4609	2.88	0.1150	5.478	25.38
U	0.9216	-0.0581	0.7845	6.72	0.0423	8.950	16.12
λ	0.8213	0.1600	0.6724	5.52	0.0302	7.893	30.59

5.2.2.1. Biogas production potential

Table 5.2 shows that biogas production potential model is significant due to F-value of 2.88. P-value of 0.1150 means that there are 11.50 % chances that this F-value could occur due to noise. “Adeq Precision” of 5.478 indicates an adequate signal.

5.2.2.2. Maximum biogas production rate

F-value of 6.72 as indicated in Table 5.2 implies that maximum biogas production rate model is significant. P-value of 0.0423 which implies that there are 4.23 % chances that F-value could be the results of noise. “Adeq Precision” is 8.950 which indicates an adequate signal.

5.2.2.3. Lag phase

Lag phase model is significant due to F-value of 5.52 as shown in Table 5.2."Adeq Precision" ratio of 7.893 indicates an adequate signal and can be used to navigate design space.

5.3. Process modelling

Table 5.3 represents results of biogas volume and methane yield results of cow dung and sugarcane leave co-digestion experiment. Cow dung and sugarcane leaves are represented by X_1 and X_2 respectively. Media solution pH and digester’s moisture content are represented by

Z_1 and Z_2 respectively. Biogas volume and methane yield are represented by Y_1 and Y_2 respectively.

Table 5.3: Experimental results for biogas volume and methane yield for cow dung and sugarcane leaves co digestion.

Reactor	X_1 (g)	X_2 (g)	Z_1	Z_2 (%)	Y_1 (ml)	Y_2 (ml/gVS)
1	5.00	5.00	8.00	95.00	626.06	50.09
2	8.00	2.00	4.00	80.00	408.26	49.20
3	5.00	5.00	4.00	80.00	436.86	26.60
4	5.00	5.00	4.00	95.00	236.03	28.74
5	8.00	2.00	4.00	95.00	174.77	20.32
6	2.00	8.00	8.00	95.00	693.31	26.63
7	8.00	2.00	8.00	80.00	462.00	53.73
8	5.00	5.00	8.00	80.00	399.46	18.76
9	8.00	2.00	8.00	95.00	497.83	93.68
10	2.00	8.00	8.00	80.00	326.86	23.18
11	2.00	8.00	4.00	80.00	299.51	21.24
12	2.00	8.00	4.00	95.00	579.54	58.22

The highest biogas volume of 693.31 ml was achieved by reactor 6 as shown in Table 5.3. The highest methane yield of 93.68 ml/gVS was achieved reactor 9. The lowest biogas volume and methane yield of 174.77 ml and 18.76 ml/gVS were achieved by reactors 5 and 8 respectively.

5.3.1. Biogas volume modelling

Biogas model is presented by equation 5.4 ANOVA results are presented in Table 5.4.

$$\begin{aligned} Y_1 &= +573.69X_1 - 201.05X_2 - 63.20X_1Z_1 - 6.62X_1Z_2 + 2.87X_2Z_2 \\ &+0.80X_1Z_1Z_2 \end{aligned} \quad \text{…………….....5.4}$$

Where: X_1 and X_2 present weights (g) of cow dung and sugarcane leaves respectively.

Z_1 and Z_2 present media solution pH and digester’s moisture content (%) respectively.

Y_1 represent biogas volume (ml)

Table 5.4: ANOVA results of cow dung and sugarcane leaves co-digestion experiment model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2.461E+005	5	49223.52	13.74	0.0031
Linear Mixture	15874.06	1	15874.06	4.43	0.0799
X_1Z_1	43358.28	1	43358.28	12.10	0.0132
X_1Z_2	86448.67	1	86448.67	24.13	0.0027
X_2Z_2	1.078E+005	1	1.078E+005	30.08	0.0015
$X_1Z_1Z_2$	53581.20	1	53581.20	14.96	0.0083
R^2 =0.9197, Adjusted R^2=0.8528, Coefficients of variation (VC) =13.97, Adequate precision=13.141, Standard deviation =59.85, Pred R-Squared =0.5269					

F-value of 13.74 as shown in Table 5.4 means that biogas volume model is significant. "Prob > F" of 0.0031 means that there is 0.31 % chance that F-value this large could occur due to noise. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable (Montgomety 2001). Table 5.4 indicates that "Adeq Precision" is 13.141 which indicates an adequate signal.

5.3.2. Diagnostic checks for biogas volume model

Normal probability and predicted versus actual plots were used to diagnostic checks. These checks to confirm validity of the model. Normal probability plot as indicated by Figure 5.2 shows that the model data is normally distributed. Predicted versus actual values plot as indicated by Figure 5.3 is linear around 45 degrees which indicate that the experimental results are close to predicted values.

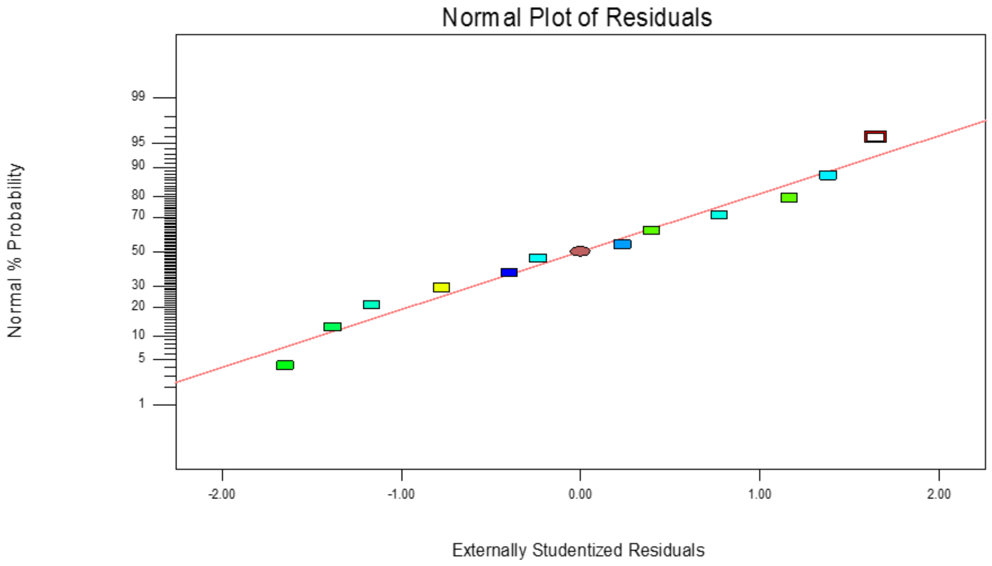

Figure 5.2: Normal probably plot for biogas volume model for sugarcane leaves and cow dung

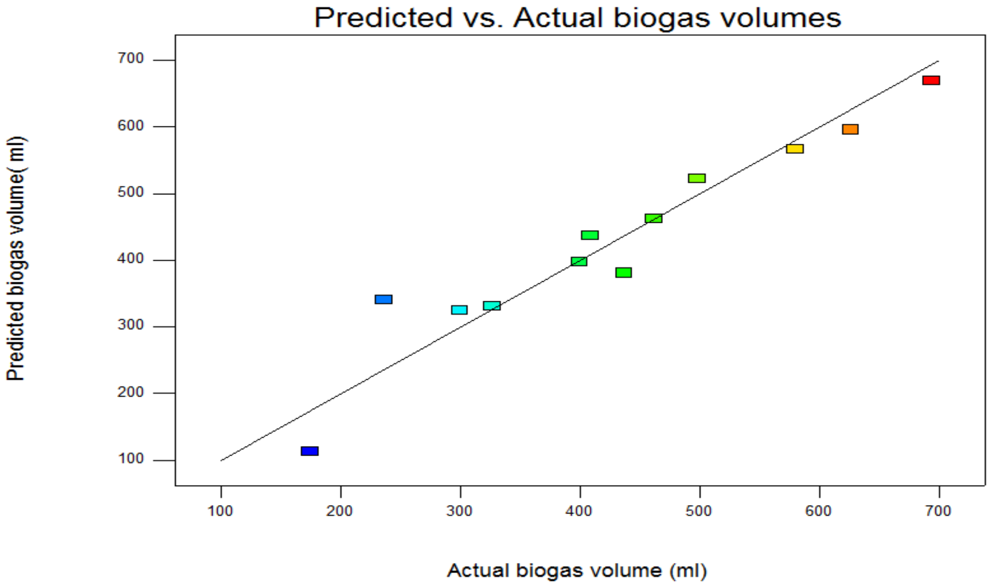

Figure 5.3: Actual versus predicted biogas volume for sugarcane leaves and cow dung model

5.3.3. Methane yield modelling

Methane yield results presented in Table 5.3 were used to create methane yield model for cow dung and sugarcane leaves co-digestion process. Reduced linear-2FI model as generated by

Design Expert is presented in equation 5.5. ANOVA results are presented in Table 5.5.

$$Y_2 = 58.75 X_1 - 13.30X_2 - 9.02X_1 Z_1 - 0.66X_1 Z_2 + 0.19X_1Z_2 + 0.12X_1 Z_1Z_2 \text{ ………..5.5}$$

Where: X_1 and X_2 are weights (g) of cow dung and sugar cane leaves respectively.

Z_1 and Z_2 represent media pH and digester moisture content (%) respectively.

Y_2 represent methane yield (ml/g.VS)

Table 5.5: ANOVA results of cow dung and sugarcane leaves co-digestion methane yield model

Source	Sum of Squares	df	mean Square	F-Value	p-value Prob > F
Model	4494.85	5	898.97	5.16	0.035
Linear Mixture	960.53	1	960.53	5.52	0.057
X_1Z_1	1432.96	1	1432.96	8.23	0.028
X_1Z_2	55.84	1	55.84	0.32	0.591
X_2Z_2	554.79	1	554.79	3.19	0.124
$X_1Z_1Z_2$	1391.95	1	1391.95	8.00	0.030

R-Squared =0.811, Adj R-Squared =0.654, Pred R-Squared= 0.060, Adeq Precision=7.77Std. Dev =13.19, C.V. %=33.65 PRESS=5207.85

Table 5.5 indicates that the model has F-value of 5.16 which implies that the model is significant. There is 3.50 % chance that this F-value could occur due to error. "Prob > F" less than 0.0500 of indicates that the model terms are significant. For this model X_1, X_2, X_1 X_2 and $X_1Z_1Z_2$ are significant. Adeq Precision of 7.47 indicates an equal signal. The model can be used to navigate the design space.

5.3.4. Diagnostic checks for methane yield model

Diagnostic checks for biogas volume model were conducted. Two plots, namely: normal probability and predicted values vs actuals values of biogas volume. These checks are critical as they confirm the validity of the model. Figure 5.4 represents normal probability plot against the studentised residual. The shape should be linear which translates into the good model. Figure 5.5 represents predicted versus actual values. Linear relationship along 45 degrees' line is the indication that experimental values are close to predicted values which further confirms the accuracy of the model.

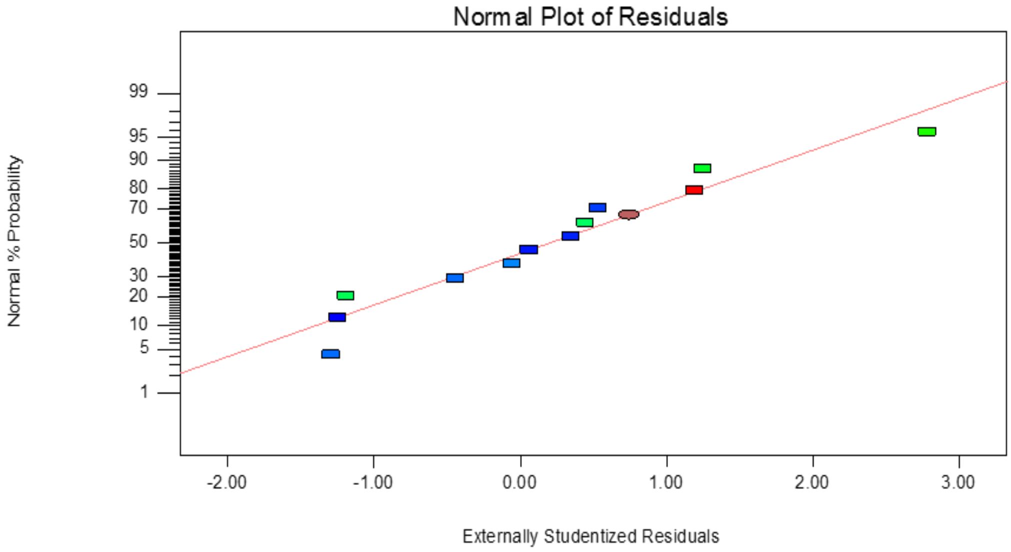

Figure 5.4: Normal probability plot of cow dung and sugarcane leaves co-digestion methane yield model

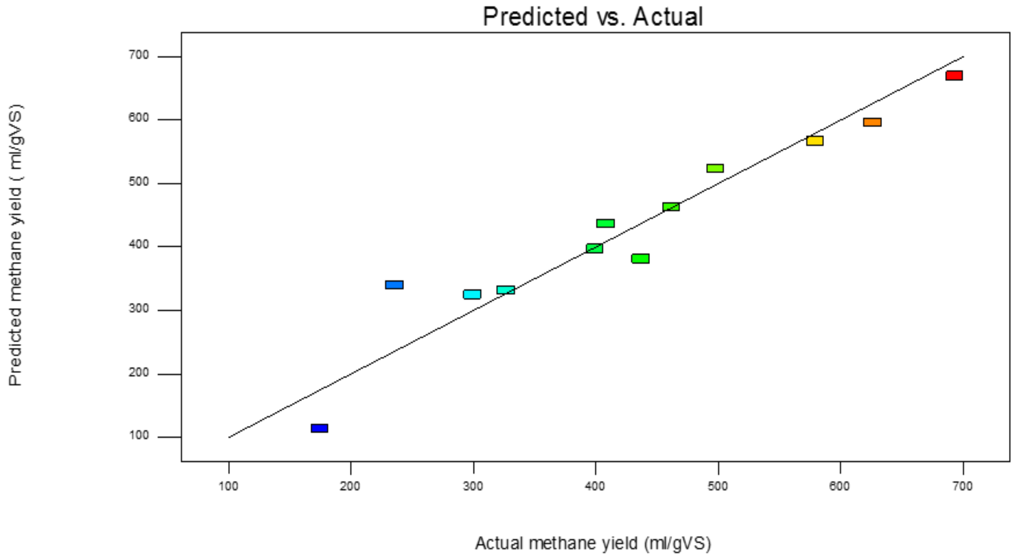

Figure 5.5: Actual vs predicted values plot for cow dung and sugarcane leaves methane yield model.

5.3.5. Effect of process parameters on biogas volume and methane yield

The effects of C: SL feed ratios, media solution pH and digester's moisture content on biogas volume and methane were investigated by using OVAT and 2-D graphical analysis methods.

5.3.5.1. Effect of digester's moisture content on biogas volume and methane yield

5.3.5.1.1. Analysis 1

Table 5.3 shows that reactor 1 achieved higher biogas volume (626.06 ml) and methane yield (50.09 ml/gVS) compared to reactor 8 where lower biogas volume and methane yield of 399.46 ml and 18.78 ml/gVS were produced. Reactors 1 and 8 were charged with equal C: SL of 5:5, equal media solution with pH of 8.00 and operated at digester's moisture content of 95.00 % and 80.00 % respectively.

It was observed that reactor operated at 95.00 % digester's moisture content attained higher biogas volume and methane yield.

5.3.5. 1.2. Analysis 2

Reactor 2 achieved higher biogas volume (408.26 ml) and methane yield (49.20 ml/gVS) compared to reactor 5 where biogas volume of 174.77 ml and methane yield and 20.32 ml/gVS were achieved. Reactors 2 and 5 were charged with equal media solution with pH of 4.00, C:SL feed ratio of 8:2 and operated at digester's moisture content of 80.00 % and 95.00 % respectively as indicated in Table 5.3. In this analysis, reactor which was operated at low digester's moisture content (80.00 %) achieved higher biogas volume and methane yield compared to a reactor operated at high digester's moisture content (95.00 %).

5.3.5. 1.3 Analysis 3

Reactors 11 and 12 were charged with equal C: SL feed ratio of 2:8, media solution with pH of 4.00 and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Table 5.3 reveals that higher biogas volume (579.54 ml) and methane yield (58.22 ml/ gVS) were achieved by reactor 12 compared to reactor 11 where lower biogas volume (299.51 ml) and methane yield (21.24 ml/gVS) were achieved.

This analysis shows that a reactor operated at high digester's moisture content achieved higher biogas production at media solution pH of 4.00 when charged with 2.00 g of cow dung and 8.00 g of leaves.

5.3.5. 1.4. Analysis 4

Reactors 6 and 10 were charged with equal C: SL feed ratio of 2:8, equal media solution pH of 8.00 and operated at different moisture content of 95.00 % and 80.00 % respectively as shown in Table 5.3. Table 5.3 reveals that higher biogas volume (497.83 ml) and methane yield (93.68 ml/g VS) were achieved by reactor 6 compared to reactor 10 where lower biogas volume (462.00 ml) and methane yield (53.73 ml/g VS) were achieved. In this analysis, reactor which was operated at high digester's moisture content achieved higher biogas production.

5.3.5. 1.5. Analysis 5

Reactors 3 and 4 were charged with equal C: SL feed ratio of 5:5, equal media solution pH of 4.00 and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Table 5.3 reveals that higher biogas volume of 436.83 ml and lower methane yield of 26.60 ml/ gVS were achieved by reactor 3 compared to reactor 4 where lower biogas volume of 263.03 ml and higher methane yield 28.74 ml/ gVS were achieved.

In this analysis, high biogas volume at lower methane yield was achieved by a reactor which was operated at 80.00 % digester's moisture content. This means high digester's moisture content charged digester produced biogas with high methane concentration.

5.3.5.1.6. Graphical analysis

Figures 5.6 and 5.7 represent 2D contour graphical representation of biogas volume and methane yields respectively. Digester's moisture content was fixed at central position of 87.5

% along Z axis. This was done to investigate the influence of combine effect of C: SL feed ratio and media solution pH on methane yield and biogas volume. Figures 5.6 and 5.7 represent an ideal system where digester's moisture content was fixed at central position. Contour lines represent biogas volume as shown in Figure 5.6. Figure 5.6 reveals that at constant C:SL feed ratio, biogas volume increases and at constant media solution pH biogas volume decreases. It was observed that as C:SL feed ratio increases contour lines are converging towards being linear and parallel which indicates that as C:SL increases the interaction between the process variables on biogas volume becomes weaker.

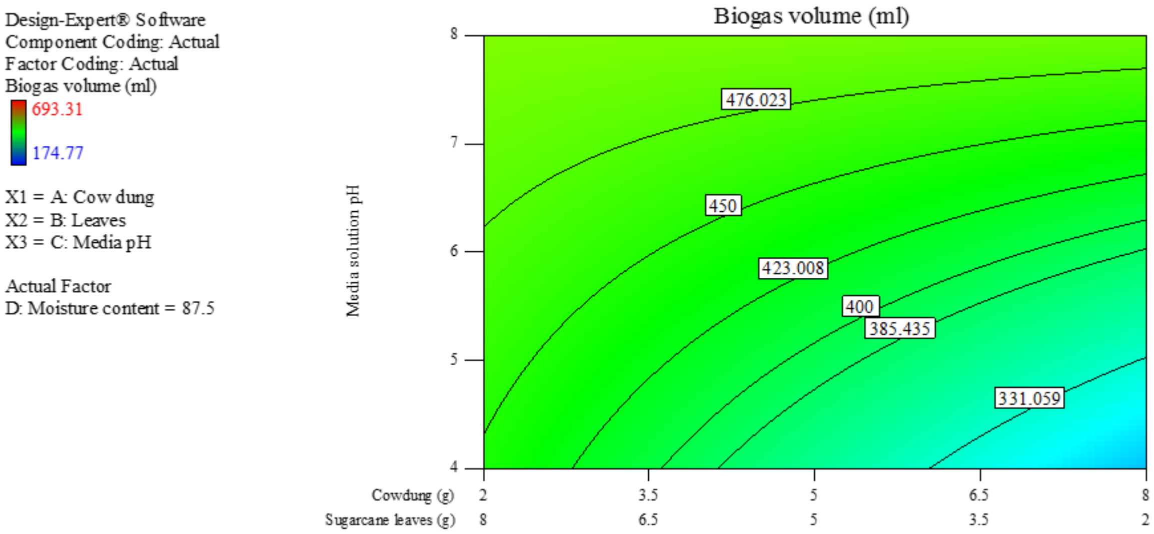

Figure 5.6: 2-D contour graphical representation of biogas volume models at constant moisture (87.5 %) content.

Contour lines represent methane yield in Figure 5.7. At constant media solution pH, methane yield increases with the increase in C:SL feed ratio as show in Figure 5.7. Figure shows that at constant C:SL methane yield increases with the increase of media solution pH. Figure 5.7 shows that constant methane yield can be attained by decreasing media solution pH while increasing C:SL feed ratio.

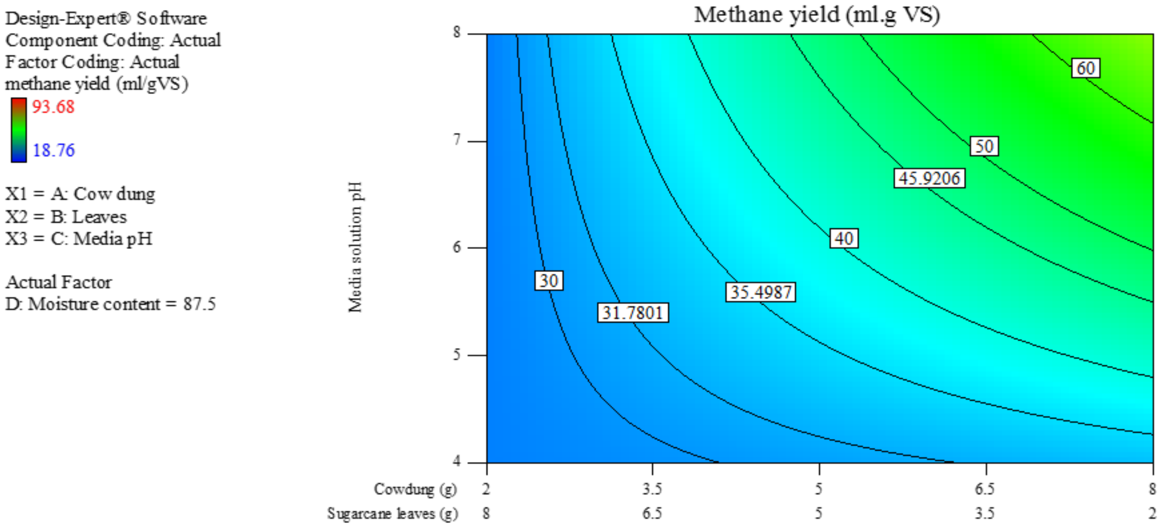

Figure 5.7: 2-D graphical representation of methane yield model at fixed moisture content.

In summary, Figure 5.7 indicates that as C: SL feed ratio and media solution pH increase, methane yield increases. It is also noted that the increase in C: SL ratio and decrease in media solution pH favours methane yield production.

5.3.5.1.7. Concluding comments

Reactors operated at low digester's moisture content (80.00 %) achieved higher biogas volume and methane yield compared to reactor which was operated at high digester's moisture content (95.00 %) when charged with low media solution pH.

High digester's moisture content resulted to high production of biogas volume and methane yield regardless of condition of other process variables.

Graphical analysis indicates that the increase of C: SL feed ratio and media solution pH increase favoured biogas volume and methane yield.

5.3.5.2. Effect of media solution pH on biogas volume and methane yield

5.3.5.2.1. Analysis 1

Table 5.3 shows that higher biogas volume (626.06 ml) and methane yield (50.09 ml/gVS) were achieved by reactor 1 compared to reactor 4 where lower biogas volume of 236.03 ml and methane yield of 28.74 ml/gVS were achieved.

Reactors 4 and 1 were charged with equal C:SL feed ratio (5:5), operated at equal digester's moisture content (95.00 %) and at media solution with pH of 4.00 and 8.00 respectively. In this analysis, reactor charged with media solution with high pH produced higher biogas volume and methane yield.

5.3.5.2.2. Analysis 2

Reactors 2 and 7 were charged with equal C: SL feed ratio of 8:2, operated at equal digester's moisture content of 80.00 % and charged with media solution at pH of 4.00 and 8.00 respectively. Table 5.3 shows that higher biogas volume of 462.00 ml and methane yield of 53.73 ml/gVS were achieved by reactor 7 compared to reactor 2 where lower biogas volume of 408.26 ml and methane yield of 49.20 ml/gVS were achieved. In this analysis, it is observed that reactor charged with media solution with high pH achieved higher biogas volume and methane yield.

5.3.5.2.3. Analysis 3

Reactors 5 and 9 were charged with media solution with pH of 4.00 and 8.00 respectively. Both reactors were charged with equal C: SL feed ratio and digester's moisture content of 8:2 and 95.00 % respectively. Table 5.3 indicates that reactor 9 achieved higher biogas volume (497.83 ml) and methane yield (93.68 ml/ gVS) compared to reactor 5 where lower biogas volume (174.77 ml) and methane yield (20.32 ml/ gVS) were achieved.

In this analysis, it was observed that higher biogas volume and methane yield values were achieved by reactor charged with media solution with pH of 8.00.

5.3.5.2.4. Analysis 4

Reactors 3 and 8 were charged with equal C: SL feed ratio (5:5), operated at digester's moisture content of 80.00 % and charged with media solution with pH of 4.00 and 8.00 respectively as shown in Table 5.3. Table 5.3 shows that lower biogas volume and methane yield of 399.48 ml and 18.78 ml/ gVS respectively were achieved by reactor 8 which was charged with media solution pH of 8.00 compared to reactor 3 where biogas volume and methane yield of 436.86 ml and 26.60 ml/gVS were achieved by reactor 3 which was charged with lower media solution with pH of 4.00.

In this analysis, it was observed that higher biogas production was achieved by media solution of 4.00 when reactor was charged with 5.00 g of cow dung and 5.00 of leaves.

5.3.5.2.5. Graphical analysis

Figures 5.8 and 5.9 are 2-D contour graphical representation of methane yield and biogas volume models for cow dung and sugarcane leaves co-digestion which were drawn at media solution pH set at central position of 6.00. Figure 5.8 reveals that at constant C:SL methane yield, methane yield increases with the increase of digester's moisture content. At constant digester moisture content, methane yield increases with the increase in C:SL feed ratio.

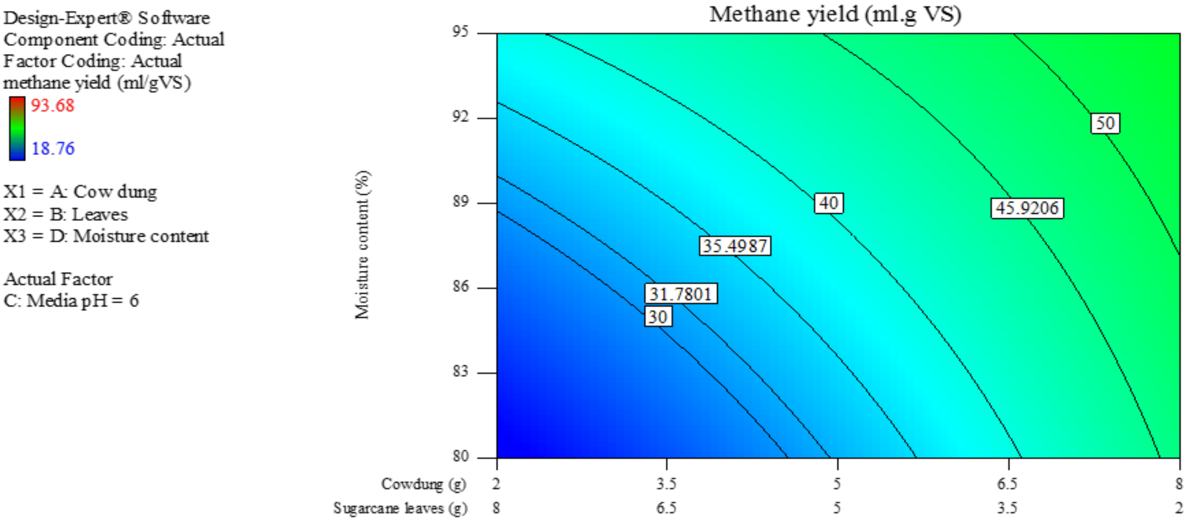

Figure 5.8: 2-D graphical representation of methane yield model at fixed media solution pH

In conclusion, Figure 5.8 shows that as C: SL feed ratio and digester's moisture content increases, methane yield increases.

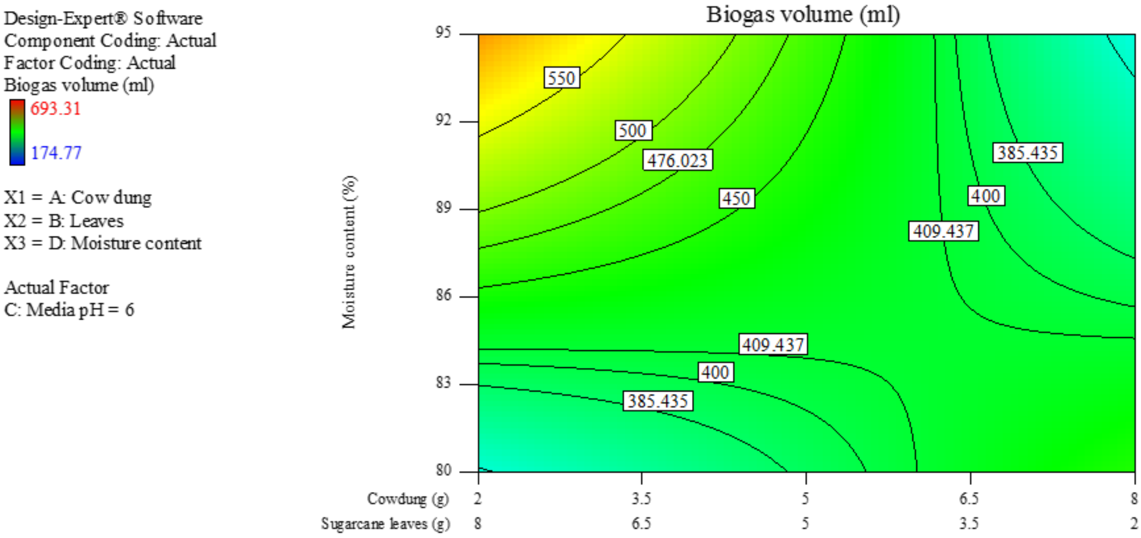

Figure 5.9: 2-D graphical representation of biogas volume model at fixed media solution pH

Figure 5.9 shows that high biogas volume values (450 to 550 ml) can be obtained at low C:SL feed ratio (2:8 to 5:5) and high digester's moisture content (> 86.00 %). Intermediate biogas values (409.437 ml) were obtained low and high S:SL feed ratio. For high biogas volume values region, biogas volume increases as digester's moisture content increases at constant S:SL feed ratio. Figure 5.9 also reveals that at C:SL feed ratio above 6.5:3.5, biogas volume decreases as C:SL feed ratio increases while at C:SL feed ratio below 6.5:3.5 biogas volume increases as C:SL feed ratio increases.

5.3.5.2.6. Concluding comments

Analyses 1 and 2 came into similar conclusions. This means that regardless of digester's moisture content at which the digesters were operated, reactor charged with media solution with higher pH will always favour higher biogas production.

Graphical analysis shows that at constant C:SL, methane yield increases as digester's moisture content increase.

5.3.5.3. Effect of C: SL feed ratios on biogas volume and methane yield

5.3.5.3.1. Analysis 1

Reactors 2 and 11 were charged with equal media solution pH (4.00), operated at equal digester's moisture content (80.00 %) and charged with C:SL feed ratio of 8:2 and 2:8 respectively. Table 5.3 shows that higher biogas volume (408.26 ml) and methane yield (49.20 ml/gVS) were achieved by reactor 2 compared to reactor 11 where lower biogas volume of 299.51 ml and methane yield of 21.24 ml/gVS were produced.

In this analysis, high C: SL feed ratio charged reactor achieved higher biogas volume and methane yield.

5.3.5.3.2. Analysis 2

Reactors 6 and 9 were charged equal media solution at pH of 8.00, operated at equal moisture content of 95.00 % and charged with C:SL feed ratio of 2:8 and 8:2 respectively. Table 5.3

reveals that reactor 6 produced higher biogas volume of 693.31 ml compared to reactor 9 where lower biogas volume of 497.83 ml was achieved.

It was observed that reactor 6 produced lower methane yield (26.63 ml/gVS) at higher biogas volume of 693.31 ml compared to reactor 9 where higher methane yield of 93.68 ml/gVS was achieved at lower biogas volume (497.83 ml). This indicates that reactor 9 achieved biogas with high concentration.

In this analysis, reactor charged with low C: SL feed ratio produced high biogas volume and low methane yield. Reactor charged with high C: SL feed ratio achieved lower biogas volume and higher methane yield. Table A.5 shows that biogas volume produced by reactor 6 had 26.00 % methane concentration while reactor 9 achieved 89.00 % methane concentration.

5.3.5.3.3. Analysis 3

Reactors 6 and 9 were charged with C: SL feed ratio of 2:8 and 8:2 respectively. Digester's moisture content and media solution pH were fixed at 95.00 % and 8.00 respectively. Table 5.3 reveals that reactor 9 achieved higher methane yield of 93.68 ml /gVS at 497.83 ml biogas volume compared to reactor 6 where lower methane yield of 26.63 ml/ gVS at biogas volume of 693.31 ml. This indicates that reactor charged with high cow dung concentration produce biogas with high methane concentration.

5.3.5.3.4. Analysis 4

Table 5.3 shows that reactors 7 and 10 were charged with C: SL feed ratio of 8:2 and 2:8 respectively. Digester's moisture content and media solution pH were kept constant at 80.00 % and 8.00 respectively. Reactor 7 achieved higher biogas volume and methane yield of 462.00 ml and 53.73 ml/gVS respectively compared to reactor 10 where lower methane yield (326.83 ml) and biogas volume (23.18 ml/gVS) were achieved.

In this analysis, reactor charged with higher C:SL feed ratio achieved higher biogas volume and methane yield.

5.3.5.3.5. Graphical analysis

Figures 5.10 and 5.11 are 2-D graphical representation of biogas volume and methane yield models respectively plotted at central position of C:SL feed ratio of 5:5. Figure 5.10 shows that at constant digester's moisture content biogas volume increases with the increase of media solution pH. At constant media solution pH, biogas volume increases as media solution pH increases.

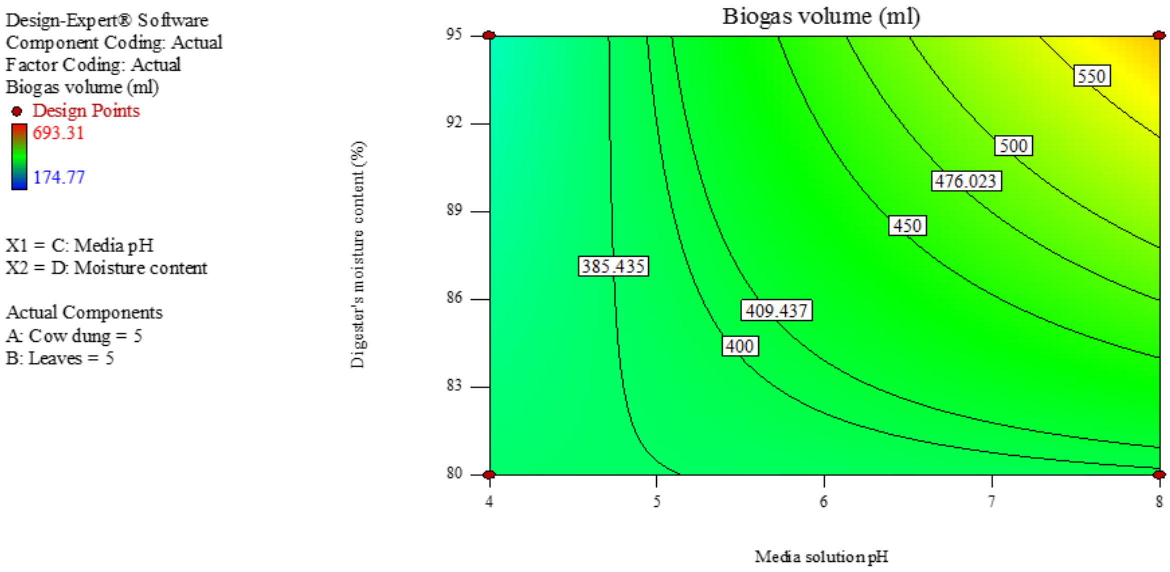

Figure 5.10: 2-D graphical representation of biogas volume model for cow dung and sugarcane leaves co-digestion at fixed C: SL feed ratio

Figure 5.11 shows that as moisture content increases, methane yield increases. The increase in media solution pH favours methane yield. Methane yield increases as digester's moisture content increases at constant media solution pH.

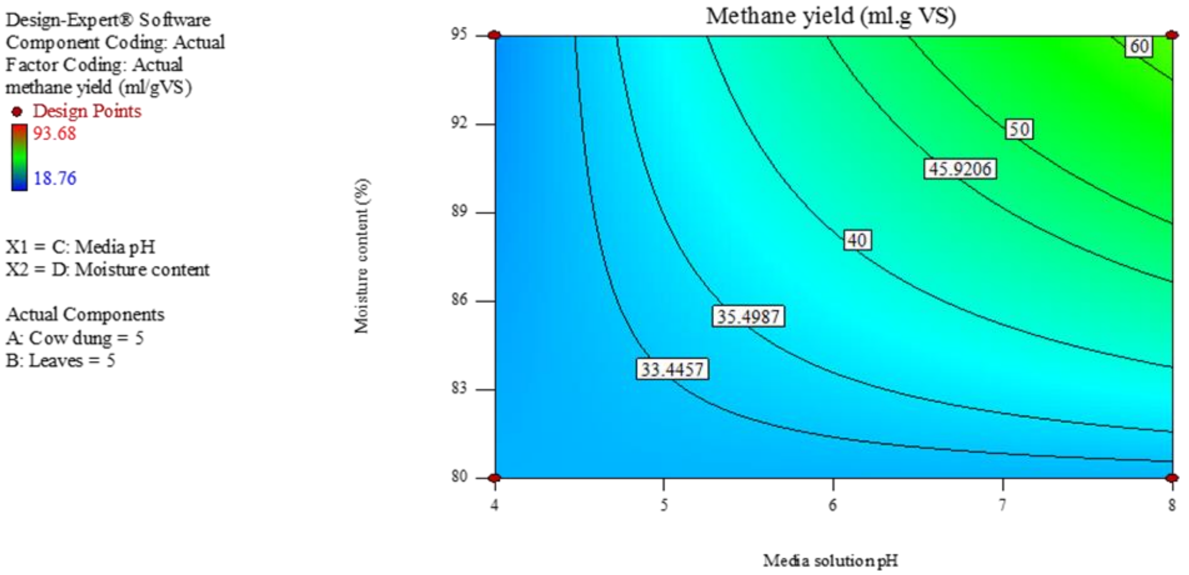

Figure 5.11: 2-D graphical representation of methane yield model for cow dung and sugarcane leaves co-digestion at fixed C: SL feed ratio

5.3.5.3.7. Concluding comments

High C: SL feed ratio charged reactor favours higher biogas volume and methane yield.

Graphical analysis reveals the increase in digester's moisture content and media solution pH favour biogas volume and methane yield.

5.3.6. Optimization

Constrained numerical optimisation method was used. Single and combined response optimisation approaches were used. Optimisation solution with the highest desirability value is recommended as optimum condition.

5.3.6.1. Biogas volume optimization

Table 5.6 represents constraints used for optimisation process. Table 5.7 represent solutions found during optimisation process. Optimum biogas volume of 669.19 ml was achieved at 2.00 g of cow dung, 8.00 g of sugarcane leaves, media solution pH of 8.00 and moisture content of 95.00 % as presented in Table 5.7.

Table 5.6: Constraints used for cow dung and sugarcane co-digestion biogas volume optimisation

Name	Units	Goal	Limits	
			Lower	Upper
Cow dung	g	is in range	2.00	8.00
Sugarcane leaves	g	is in range	2.00	8.00
Media solution pH		is in range	4.00	8.00
Digester’s moisture content	%	is in range	80.00	95.00
Biogas volume	ml	maximize	174.77	693.31

Table 5.7: Optimisation solutions for cow dung and sugarcane co-digestion biogas volume

Solutions number	Cow dung (g)	Sugarcane leaves (g)	Media solution pH	Digester’s moisture content (%)	Biogas volume (ml)	Desirability
1	2.00	8.00	8.00	95.00	669.19	0.953
2	2.00	8.00	8.00	94.92	667.45	0.950
3	2.00	8.00	7.92	95.00	667.02	0.949
4	2.00	8.00	8.00	94.76	663.80	0.943
5	2.00	8.00	8.00	94.62	660.63	0.937
6	4.70	5.30	8.00	95.00	603.23	0.826
7	4.87	5.13	8.00	95.00	599.21	0.819
8	7.74	2.26	8.00	95.00	528.94	0.683
9	8.00	2.00	8.00	95.00	522.68	0.671
10	7.22	2.78	8.00	89.67	507.53	0.642
11	8.00	2.00	8.00	80.93	466.34	0.562

5.3.6.2. Methane yield optimization

Table 5.8 represents goals of optimisation process. Optimisation solutions are presented in Table 5.9. Optimum methane yield was found to be 87.18 ml/gVS at 8 g of cow dung, 2 g of sugarcane leaves, and media solution pH of 8 and moisture content of 95% as shown in Table 5.9.

Table 5.8: Constrains used for methane yield optimisation of cow dung and sugarcane leaves co-digestion

Variable	Units	Goal	Limits	
			Lower	Upper
Cow dung	g	is in range	2.00	8.00
Sugarcane leaves	g	is in range	2.00	8.00
Media solution pH		is in range	4.00	8.00
Digester’s moisture content	%	is in range	80.00	95.00
Methane yield	ml/gVS	maximise	18.76	93.68

Table 5.9: Optimisation solutions of methane yield for cow dung and sugarcane leaves co-digestion

Solutions number	Cow dung (g)	Sugarcane leaves (g)	Media solution pH	Digester’s moisture content (%)	Methane yield (ml/gVS)	Desirability
1	8.00	2.00	8.00	95.00	87.18	0.913
2	8.00	2.00	8.00	94.83	86.73	0.907
3	7.74	2.26	8.00	94.92	84.88	0.883
4	8.00	2.00	8.00	94.01	84.52	0.878
5	8.00	2.00	8.00	93.59	83.40	0.863
6	6.62	3.38	8.00	95.00	76.12	0.766
7	8.00	2.00	4.47	80.00	46.56	0.371
8	2.00	8.00	4.00	95.00	38.99	0.270

5.3.6.3. Combined optimization

Table 5.10 represents constraints and goals of optimisation process. Table 5.11 represents optimisation solutions. Biogas volume of 522.69 ml and methane yield of 87.18 ml/gVS were selected as optimum values. These optimum values correspond with 8.00 g cow dung, 2.00 g sugarcane leaves, media solution pH of 8.00 and moisture content of 95.00 %.

Table 5.10: Optimisation constraints of biogas volume and methane yield for cow dung and sugarcane leaves co-digestion experiment.

Name	Units	Goal	Limits	
			Lower	Upper
Cow dung	g	is in range	2.00	8.00
Sugarcane leaves	g	is in range	2.00	8.00
Media solution pH		is in range	4.00	8.00
Digester's moisture content	%	is in range	80.00	95.00
Biogas volume	ml	maximize	174.77	693.31
Methane yield	ml/gVS	maximize	18.76	93.68

Table 5.11:Optimisation solutions for biogas volume and methane yield for cow dung and sugarcane leaves co-digestion.

Solutions number	Cow dung (g)	Sugarcane leaves (g)	Media solution pH	Moisture content (%)	Biogas volume (ml)	Methane yield (ml/gVS)	Desirability
1	8.00	2.00	8.00	95.00	522.69	87.18	0.783
2	8.00	2.00	8.00	94.94	522.43	87.01	0.782
3	8.00	2.00	7.98	95.00	520.19	86.77	0.778
4	7.60	2.40	8.00	95.00	532.49	83.95	0.775
5	5.01	4.99	8.00	93.23	572.19	59.54	0.646
6	2.03	7.97	8.00	95.00	668.41	39.25	0.510
7	2.35	7.65	6.40	95.00	612.59	40.21	0.492
8	2.13	7.87	6.14	95.00	615.12	39.37	0.483
9	8.00	2.00	4.63	80.00	441.02	46.58	0.437

5.4. Preliminary conclusions and findings

- Biogas production from cow dung and sugarcane leaves obeys Gomperzt law.
- Kinetics constant values increase with the increase of media pH, moisture content and C:SL feed ratio in all cases discussed.
- The increase in media solution pH favors maximum biogas production rate and biogas potential.
- High concentration of sugarcane leaves at low media solution pH results to higher biogas potential, maximum biogas potential and lower lag phase at moisture content of 80%.

- High digester’s moisture content (95 %) and high media solution pH (8) favors biogas volume and methane yield and low media solution pH (4).
- High moisture content and low media solution pH resulted to low biogas volume and methane yield.
- Reactor charged with low feed ratio produced high biogas volume with low methane concentration and high feed ratio resulted in low biogas volume with higher methane yield concentration.
- Optimum biogas volume and methane yield are 522.69 ml and 87.18 ml/gVS respectively. The optimum process conditions are: 8.00 g cow dung, 2.00 g sugarcane leaves, media solution pH of 8.00 and moisture content of 95.00%.

Chapter 6

RESULTS AND DISCUSSION: COWDUNG AND MOLASSES

6.1. Introduction

The aim of this chapter is to present results of cow dung and molasses co-digestion experiment. Mathematical models of biogas volume, methane yield and kinetic constants are analysed and presented.

OVAT and graphical analysis methods were used to investigate the effect of process variables on biogas volume, methane yield and kinetic constants values.

6.2. Kinetics constants

Molasses and cow dung co-digestion experiment was characterized by high production of biogas from first day of AD process and about 90 % of biogas produced was produced during the first 4 days of the AD process as indicated in Figure 6.1. At day 6, daily cumulative biogas produced was not increasing as shown in Figure 6.1, at this point it was assumed that there was no biogas production taking place.

The experimental data was tested for the fitness in the modified Gompertz equation. The kinetics constants were determined using the MS Excel Solver. Kinetic constants values are presented in Table 6.1

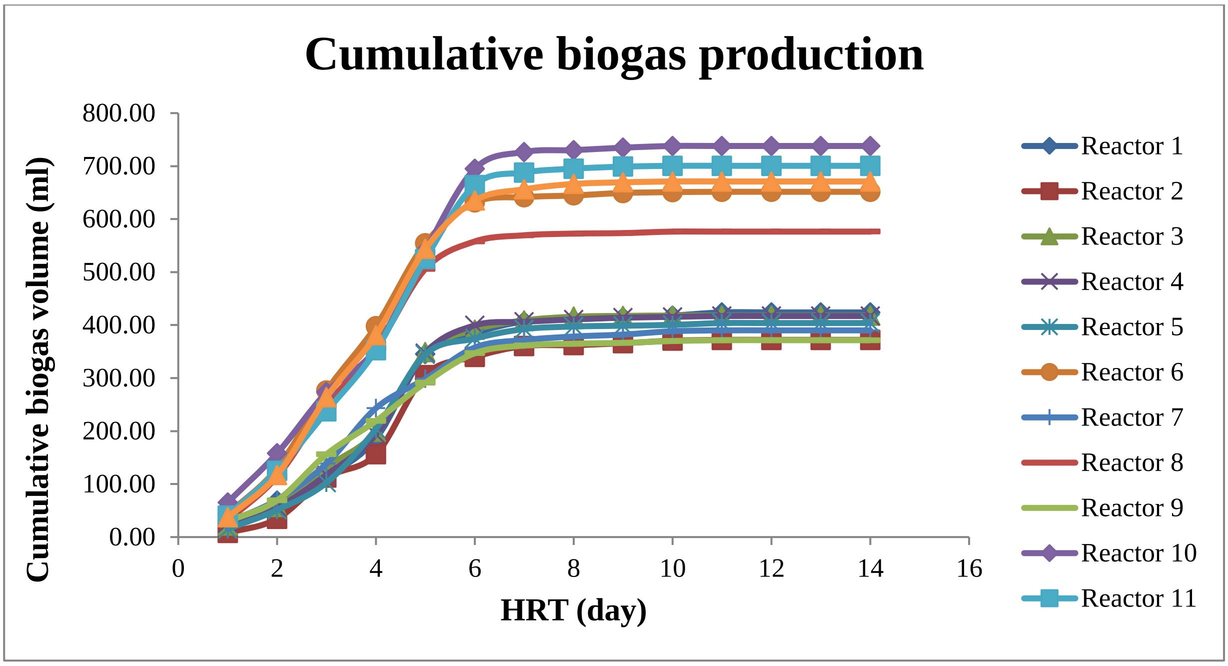

Figure 6.1: Daily cumulative biogas volume against HRT for cow dung and molasses anaerobic co-digestion.

Table 6.1: Results of kinetics constants values of cow dung and molasses anaerobic co-digestion experiment.

Reactor	Cow dung (g)	Molasses (g)	Media solution pH	Digester's moisture content (%)	A (ml)	U (ml/day)	λ (day)
1	5.00	5.00	8.00	95.00	428.1	109.64	1.88
2	8.00	2.00	4.00	80.00	375.35	104.85	2.07
3	5.00	5.00	4.00	80.00	424.32	115.17	1.87
4	5.00	5.00	4.00	95.00	422.21	121.16	2.03
5	8.00	2.00	4.00	95.00	406.35	125.2	2.13
6	2.00	8.00	8.00	95.00	659.88	170.76	1.34
7	8.00	2.00	8.00	80.00	392.95	93.74	1.37
8	5.00	5.00	8.00	80.00	583.27	157.56	1.39
9	8.00	2.00	8.00	95.00	375.95	87.93	1.24
10	2.00	8.00	8.00	80.00	756.96	159.00	1.25
11	2.00	8.00	4.00	80.00	714.92	168.11	1.55
12	2.00	8.00	4.00	95.00	680.62	170.02	1.45

The highest biogas potential of 756.96 ml was achieved by reactor 10 as indicated in Table 6.1.

Reactor 10 was charged with C: M feed ratio of 2:8, media solution pH of 8.00 and operated at digester’s moisture content of 80.00 %. Reactor 2 achieved the lowest biogas production potential of 375.35 ml charged with C: M feed ratio of 8:2, media solution pH of 4.00 and operated at digester’s moisture content of 80%.

Reactor 9 achieved the lowest maximum biogas production of 87.93 ml/day when charged with C: M feed ratio of 8:2, media solution pH (8.00) and operated at digester’s moisture content of 95.00 %.

The highest biogas maximum biogas production value of 170.76 ml/day was achieved by reactor 6 which was operated at C: M feed ratio of 2:8, media solution pH (8.00), operated at digester’s moisture content of 95.00 %.

6.2.1. The influence of process variables on kinetics constants

6.2.1.1. Effect of cow dung-to-molasses feed ratio on kinetics constants values

6.2.1.1.1. Analysis 1

Reactors 2 and 11 were charged with media solution with equal media solution pH of 4.00, operated at equal digester’s moisture content of 80.00 % and charged with cow dung-to-molasses (C: M) feed ratio of 8:2 and 2:8 respectively as presented in Table 6.1.

Table 6.1 shows that lower biogas production potential and lower maximum biogas production rate values (A, 375.35 ml; U, 104.85ml/day) were achieved by reactor 2 at higher lag phase of 2.07 day compared to reactor 11 where higher biogas production potential and biogas maximum biogas production rate values (A, 714.92 ml; U, 168.11 ml/day) were achieved at lower lag phase of 1.55 day. It was observed that the reactor which was charged with low C: M feed ratio achieved higher biogas production potential and maximum biogas production rate but low lag phase.

Biogas production potential and maximum biogas production rate are favoured by low C: M feed ratio when reactor was charged with media solution with pH of 4.00 and operated at 80.00 % digester's moisture content.

6.2.1.1.2. Analysis 2

Reactors 5 and 12 were charged with equal media solution with pH of 4.00, operated at equal digester's moisture content of 95.00 % and charged with C: M feed ratio of 8:2 and 2:8 respectively as indicated by Table 6.1. Higher biogas production potential of 680.62 ml, higher biogas maximum biogas production rate of 170.02 ml/day and lower lag phase of 1.45 day were achieved by reactor 12 compared to reactor 5 where lower biogas production potential (406.35 ml) and lower biogas maximum biogas production rate (125.2 ml/day) and higher lag phase of 2.07 day were attained.

It was observed that the reactor charged with low C:M feed ratio achieved high values of biogas production potential and maximum biogas potential but low log phase.

6.2.1.1.3. Analysis 3

Reactors 7 and 10 were charged with C:M feed ratio of 8:2 and 2:8 respectively. Both reactors were charged with media solution pH and digester's moisture content of 8.00 and 80.00 % respectively. Lower biogas production potential and maximum biogas production rate (A, 392.95 ml and U, 93.74 ml/day) were attained by reactor 7 at lag phase of 1.37 day. Higher biogas production potential and maximum biogas production rate values (A, 756.96 ml; U, 159.00 ml/day) were achieved by reactor 10 at lag phase of 1.25 day.

In this analysis low C:M feed ratio charged reactors achieved higher biogas potential and maximum biogas production rate but at low lag phase.

6.2.1.1.4. Analysis 4

Reactors 6 and 9 were charged C:M feed ratio of 2:8 and 8:2 respectively. Both reactors were charged with media solution pH and digester's moisture content of 8.00 and 95.00 % respectively. Higher kinetic constants values (A, 659.88 ml; U, 170.76 ml/day and λ of 1.34 day) were attained by reactor 6 compared to lower kinetics constant values (A, 375.95 ml; U, 87.93 ml/day and λ of 1.24 day) achieved by reactor 9.

In this analysis, reactor charged with low C:M feed ratio achieved higher kinetics constant values compared to reactors charged with higher C:M feed ratio.

6.2.1.1.5. Concluding comments

Low C:M feed ratio charged reactors achieved higher biogas production potential and maximum biogas production rate at low lag phase. Low C:M feed ratio translates to low cow dung and high molasses concentration. Table 6.1 shows that all reactors charged with low C: M feed ratio achieved higher biogas potential and maximum biogas production rate compared to reactors charged with high C:M feed ratio. This means that the introduction of cow dung in a molasses charged-reactors may hinder biogas production. Low lag phase achieved by reactors charged with low C:M feed ratio is because molasses was in the liquid phase and therefore the rate of hydrolysis reaction is faster.

6.2.1.2. Effect of digester's moisture content on kinetic constant values.

6.2.1.2.1. Analysis 1

Reactors 1 and 8 were charged with equal media solution at pH of 8.00, charged with equal C: M feed ratio of 5:5 and operated at digester's moisture content of 95.00% and 80.00 % respectively. Table 6.1 reveals that higher biogas production potential (583.27 ml) and higher biogas production maximum rate (157.56 ml/day) were achieved by reactor 8 at lag phase of

1.39 day. Reactor 1 achieved lower biogas production potential (428.10 ml) and lower biogas maximum production rate (109.64) ml/day at higher lag phase of 1.88 day.

It was observed that reactor operated at lower digester's moisture content achieved higher biogas production potential and maximum biogas production rate at constant C:M feed ratio (5:5) and media solution pH (8.00).

6.2.1.2.2. Analysis 2

Reactors 6 and 10 were charged with equal C: M feed ratio of 8:2, charged with equal media solution with pH of 8.00 and operated at digester's moisture content of 95.00 % and 80.00 % respectively. Table 6.1 reveals that higher biogas production potential and maximum biogas production rate (A,756.96 ml; U,159.00 ml/day) at low lag phase of λ,1.25 day were achieved by reactor 10 compared to reactor 6 where lower biogas potential and maximum biogas production rate values were achieved (A,659.88 ml; U, 170.76 ml/day) at higher lag phase of 1.34 day.

This analysis reveals that a reactor operated at low digester's moisture content achieved higher kinetic constants values compared to a reactor operated at high digester's moisture content.

6.2.1.2.3. Analysis 3

Reactors 11 and 12 were charged with equal C: M feed ratio of 2:8, charged with equal media solution pH of 4.00 and operated at moisture content of 80.00 % and 95.00 % respectively. Table 6.1 reveals that higher biogas production potential and lag phase (A, 714.92 ml; λ, 1.55 day) were achieved by reactor 11 at lower maximum biogas production potential of 168.11 ml/day compared to reactor 12 where lower biogas production potential and lag phase values (A, 680.62 ml; λ, 1.45 day) at higher maximum biogas production potential of 170.02 ml/day.

Low digester's moisture content favours higher kinetic constants values when reactors were charged with equal C: M feed ratio of 2:8 and charged with equal media solution pH of 4.00.

6.2.1.2.4. Concluding comments

Lower digester's moisture favours higher biogas potential and maximum biogas production rate regardless of other process variables conditions.

High digester's moisture content achieved lower lag phase. This means that biodegradability of substrates is improved when reactor is operated at high moisture content.

6.2.1.3. The effect of media solution pH on kinetic constant values.

6.2.1.3.1. Analysis 1

Reactors 5 and 9 were charged with media solution with pH of 4.00 and 8.00 respectively. Both reactors were operated at C:M feed ratio and digester's moisture content of 8:2 and 95% respectively. Higher kinetics constants values (A, 406.35 ml; U, 125.2 ml/day and λ of 2.13 day) were attained by reactor 5 compared to lower kinetics constant values (A, 375.95 ml; U, 87.93 ml/day and λ of 1.24 day) were achieved by reactor 9. High pH media solution favoured high kinetics constants values.

In this analysis, a reactor charged with low media solution pH (4.00) achieved higher kinetic constant values at C:M feed ratio of 8:2 and digester's moisture content of 95.00 %.

6.2.1.3.2. Analysis 2

Reactors 3 and 8 were charged with equal C: M feed ratio of 5:5, operated at digester's moisture content of 80% and charged with media solution with pH at 4 and 8 respectively as shown in Table 6.1. Higher biogas potential of 424.32 ml and higher maximum biogas production rate of 115.17 ml/day were achieved by reactor 8 at lag phase of 1.87 day. Reactor 3 achieve where lower biogas potential (583.27 ml), maximum biogas production rate (157.56 ml/day) and higher lag phase (1.39 day) were achieved as shown in Table 6.1.

6.2.1.3.3. Analysis 3

Reactors 1 and 4 were charged with equal C: M feed ratio of 5:5, operated at equal digester's moisture content of 95.00 % and charged media solution with pH of 8.00 and 4.00 respectively. Reactor 1 achieved higher biogas production potential (428.10 ml) and lower maximum biogas production rate (109.64 ml/day) at lag phase of 1.88 day compared to lower biogas production potential (422.21 ml) and higher maximum biogas production rate (121.16 ml/day) was achieved by reactor 4 at lag phase of 2.03 day as indicated in Table 6.1.

In this analysis, higher lag phase of 2.03 day was achieved by reactor 4 compared to reactor 1 where lower lag phase of 1.88 day.

6.2.1.3.4. Concluding comments

Media solution with high pH favoured high biogas potential values.

Maximum biogas production rate is favoured by reactors operated at low media solution pH.

Media solution with high pH favours low lag phase when reactor operated at digester's moisture content of 95%.

Reactors charged with media solution with low pH achieved higher lag phase at 95.00 % digester's moisture content.

Reactors charged with media solution with high pH achieved higher lag phase when operated at digester's moisture content of 80.00 %.

6.2.3. Mathematical modelling

Biogas potential, maximum biogas production rate and lag phase models are presented by equations 6.1, 6.2 and 6.3 respectively. Table 6.2 represents ANOVA results.

$$\begin{aligned} A = &-26.70X_1 + 96.5X_2 + 22.96X_1\ X2 + 0.79X_1Z_1 - 0.015X_2\ Z_2 \\ &-0.365X_1X_2Z_2 \end{aligned} \quad \ldots\ldots\ldots\ldots\ldots\ldots6.1$$

$$U = 12.13X_1 + 18.22\ X_2 - 0.52X_1Z_1 - 0.013X_1\ Z_2 + 0.31X_2Z_1 - 0.018X_2Z_2 \quad \text{.............................6.2}$$

$$\lambda = 0.65X_1 + 0.44X_2 - 0.19X_1X_2 - 0.022X_1Z_1 \quad \text{..6.3}$$

Where: X_1 and X_2 represent cow dung and molasses initial weight (g) respectively

Z_1 and Z_2 represent media pH and moisture content of the digester (%) respectively

A represents biogas potential (ml)

U represents maximum biogas production rate (ml/day)

λ represents lag phase (day)

Table 6.2: ANOVA results of kinetics constants models for molasses and cow dung co-digestion

Model	R-Squared	Pred R-Squared	Adj R-Squared	F Value	p-value Prob > F	Adeq Precision	CV
A	0.9400	0.6890	0.7622	18.96	0.0013	10.167	9.44
U	0.7900	0.3220	0.6153	4.52	0.0469	5.575	14.68
λ	0.9450	0.6680	0.8790	14.32	0.0052	9.690	6.97

6.2.3.1. Biogas production potential

Table 6.2 shows that biogas production potential model has F-values of 18.96, which implies that the model is significant. The "Pred R-Squared" of 0.7619 is in reasonable agreement with the "Adj R-Squared" of 0.8909. The difference is less than 0.2. Adeq Precision is greater than 4 which is the indication of an adequate signal.

6.2.3.2. Maximum biogas production rate

Table 6.2 shows that maximum biogas potential model has F-value of 4.52 which implies that the model is significant. P-value of 0.0469 means that there is 4.69% chance that F-value this large could occur due to noise. "Adeq Precision" measures signal to noise ratio. "Adeq Precision” ratio greater than 4 is desirable. The ratio of 5.58 indicates an adequate signal.

6.2.3.3. Lag phase

Model for lag phase which has F-value of 14.32, implies that the model is significant as shown by Table 6.2. "Adeq Precision "is 6.974 which indicates an adequate signal. "Prob > F" is 0.052 means that there is 0.52% chance that F-value of 14.32 could occur due to noise.

6.3. Process modelling

Table 6.3 represents results of biogas volume and methane yield results of cow dung and molasses co-digestion experiment. Cow dung and sugarcane leaves are represented by X_1 and X_2 respectively. Media solution pH and digester's moisture content are represented by Z_1 and Z_2 respectively.

Biogas volume and methane yield are represented by Y_1 and Y_2 respectively. The highest biogas volume and methane yield of 737.94 ml and 89.90 ml/gVS were achieved by reactor 10 charged with media solution pH of 8 and moisture content of 80 %, 2.00 g of cow dung and 8.00 g of molasses. The lowest biogas volume of 371.49 ml was produced by reactor 9 charged with 8.00 g of cow dung and 2.00 g of molasses, charged with media solution pH of 8.00 and operated at moisture content of 95.00 %.

Table 6.3: Experimental results of biogas volume and methane yield from cow dung and molasses co-digestion.

Reactor	X_1 (g)	X_1 (g)	Z_1	Z_2 (%)	Y_1 ml	Y_2 ml/gVS
1	5.00	5.00	8.00	95.00	423.97	69.95
2	8.00	2.00	4.00	80.00	372.11	64.28
3	5.00	5.00	4.00	80.00	418.00	36.09
4	5.00	5.00	4.00	95.00	417.06	50.41
5	8.00	2.00	4.00	95.00	403.86	46.51
6	2.00	8.00	8.00	95.00	651.51	73.86
7	8.00	2.00	8.00	80.00	390.03	62.20
8	5.00	5.00	8.00	80.00	576.40	87.36
9	8.00	2.00	8.00	95.00	371.49	47.72
10	2.00	8.00	8.00	80.00	737.94	89.90
11	2.00	8.00	4.00	80.00	700.54	59.27
12	2.00	8.00	4.00	95.00	671.00	60.17

6.3.1. Biogas model and methane yield

Biogas volume and methane yield models are represented by equations 6.3 and 6.4 respectively. ANOVA results for biogas volume and methane yield models are presented by Tables 6.4 and 6.5 respectively.

$$\begin{aligned} Y_1 &= -27.10X_1+86.67X_2+24.33X_1X_2+0.79X_1Z_2 \\ &\quad +0.07X_2Z_2-0.38X_1X_2Z_2 \end{aligned} \quad \ldots\ldots\ldots\ldots\ldots\ldots 6.4$$

$$Y_2=4.92X_1+1.83X_2+0.95X_2X_1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 6.5$$

Where: X_1 and X_2 represent cow dung and molasses weights (g) respectively.

Z_1 and Z_2 represent media solution pH and moisture content of digester (%)

Y_1 and Y_2 represent biogas volume (ml) and methane yield (ml/ g VS) respectively.

Table 6.4: ANOVA results of biogas volume model for cow dung and molasses co-digestion.

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F
Model	212823.24	5	42564.65	18.06	0.0015
Linear Mixture	187123.40	1	187123.40	79.39	0.0001
X_1X_2	932.83	1	932.83	0.40	0.5525
X_1Z_1	1678.20	1	1678.20	0.71	0.4311
X_2X_2	14.76	1	14.76	0.01	0.9395
$X_1X_2Z_2$	1733.51	1	1733.51	0.74	0.4241
R-Squared =0.9377, Adj R-Squared =0.8858, Pred R-Squared= 0.7507, Adeq Precision=9.851 Std. Dev =48.55, C.V. %=9.50 PRESS=56571.30					

Table 6.5: ANOVA results of methane yield model for cow dung and molasses co-digestion.

Source	Squares	df	Mean Square	F-Value	p-value Prob > F
Model	1831.25	2	915.63	7.78	0.0109
Linear Mixture	488.05	1	488.05	4.15	0.0721
X_2Z_1	1343.20	1	1343.20	11.42	0.0081
R-Squared =0.63367, Adj R-Squared =0.5522, Pred R-Squared= 0.3792, Adeq Precision=6.384 Std. Dev =10.85, C.V. %=17.40, PRESS=1793.123					

6.3.1.1. Biogas volume model

Table 6.4 indicates that biogas volume model has F-value of 18.06 which implies that the model is significant. "Prob > F" of 0.0015 indicates that that there is 0.15% chance that F-value this large could occur due to noise.

“Pred R-Squared" of 0.7507 is in reasonable agreement with the "Adj R-Squared" of 0.8858; i.e. difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable (Montgomety 2001). In this current work the value of Adeq Precision” ratio is 9.851 which indicates an adequate signal.

6.3.1.2. Methane yield model

Table 6.5 indicates that methane yield model has F-value of 7.78. This implies that the model is significant. P-value of 0.0109 indicates that there is 1.09 % chance that this F-value could occur due to noise.

Values of "Prob > F" less than 0.0500 indicates that all model terms are significant. Pred R-Squared" of 0.3795 is in reasonable agreement with "Adj R-Squared" of 0.5523; i.e. the difference is less than 0.2. "Adeq Precision" is 6.38 which indicates an adequate signal.

6.3.2. Diagnostic checks for biogas volume and methane yield models

Normal probability plots for biogas volume and methane yield are presented by Figures 6.2 and 6.3 respectively. Linear relationship of normal probability plots as indicated Figures 6.2 and 6.3 means that the experimental data are normally distributed around mean.

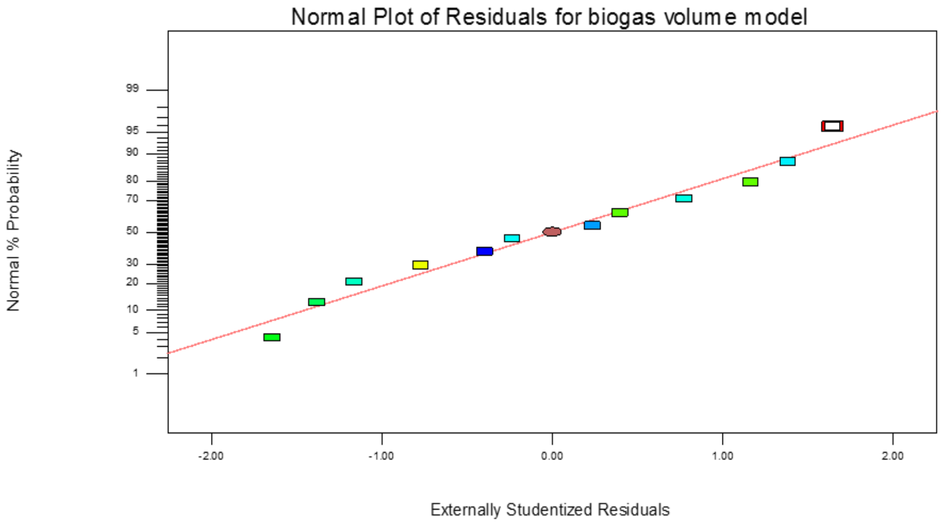

Figure 6.2: Normal probability plot of biogas volume model for cow dung and molasses co-digestion.

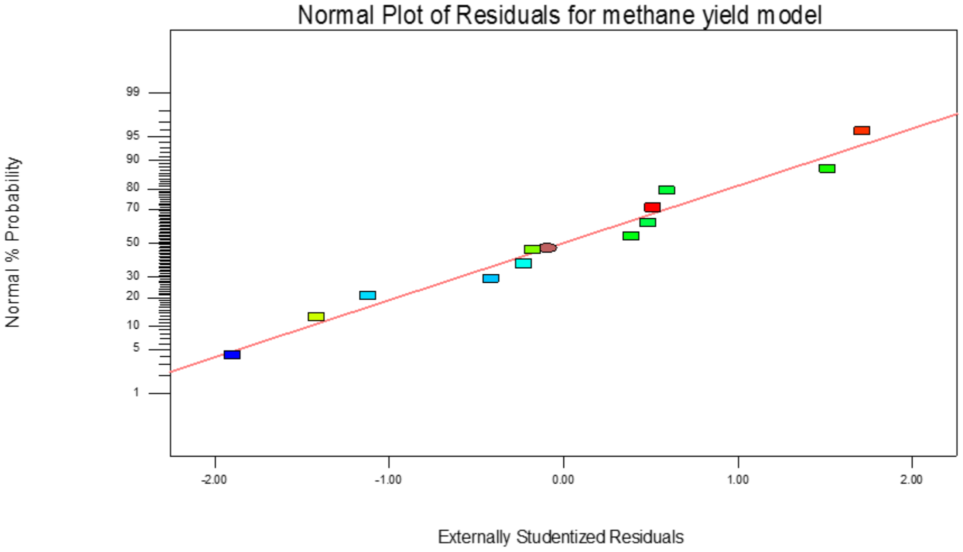

Figure 6.3: Normal probability plot of methane yield model for cow dung and molasses co-digestion.

Predicted vs actual values plot as shown in Figures 6.4 and 6.5 for biogas volume and methane yield are linear at about 45 degree's line which indicate that the experimental values are close to predicated values.

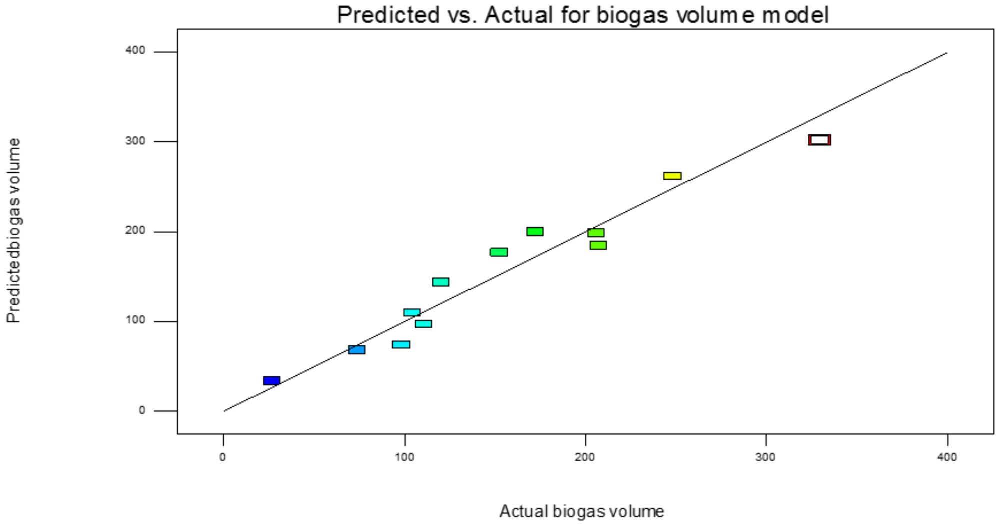

Figure 6.4: Actual vs predicted values for biogas volume model for cow dung and molasses co-digestion.

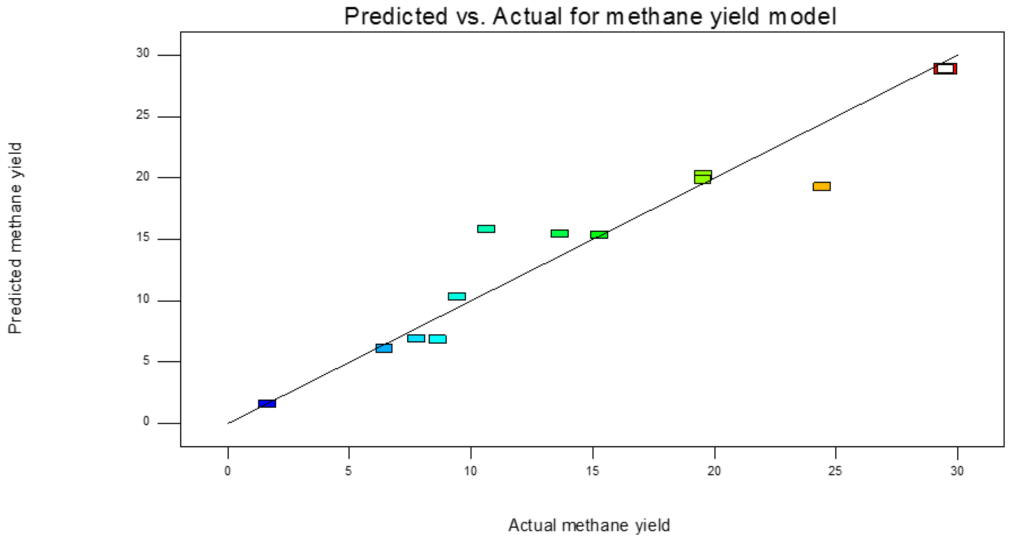

Figure 6.5: Actual vs predicted values for methane yield model for cow dung and molasses co-digestion.

6.3.3. Optimization

Biogas volume and methane yield were optimized simultaneously. Solution with highest desirability was selected as optimum condition. Table 6.6 presents the constraints which were used during optimization process. Table 6.7 represents solution found during optimization process. The optimum biogas volume and methane yield was found to be 719.24 ml and 85.32 ml/gVS respectively at moisture content of 80.00 %, media pH of 8.00, and 2.00 g of cow dung and 8.00 g of molasses as shown in solution number one.

Table 6.6: Optimisation constraints of combined optimisation for cow dung and molasses co-digestion

Name	Units	Goal	Limits	
			Lower	Upper
Cow dung	g	is in range	2.00	8.00
Molasses	g	is in range	2.00	8.00
Media solution pH		is in range	4.00	8.00
Digester’s moisture content	%	is in range	80.00	95.00
Biogas volume	ml	maximize	371.49	737.94
Methane yield	ml/gVS	maximize	36.09	89.90

Table 6.7: Optimisation solutions of biogas volume for cow dung and molasses co-digestion

Number	Cow dung (g)	Molasses (g)	Media solution pH	Digester's moisture content (%)	Biogas volume (ml)	Methane yield (ml/gVS)	Desirability
1	2.00	8.00	8.00	80.00	719.24	85.32	0.932
2	2.00	8.00	7.97	80.00	719.24	85.06	0.929
3	2.00	8.00	8.00	80.91	715.72	85.32	0.927
4	2.00	8.00	7.91	80.00	719.24	84.62	0.925
5	2.00	8.00	8.00	82.06	711.30	85.32	0.921
6	2.00	8.00	8.00	82.64	709.03	85.32	0.918
7	2.00	8.00	8.00	82.90	708.02	85.32	0.917
8	2.00	8.00	8.00	90.16	679.96	85.32	0.878
9	8.00	2.00	8.00	95.00	387.67	58.30	0.135

6.3.4. Effects of process variables on biogas volume and methane yield

6.3.4.1. The effect of cow dung to molasse feed ratio on biogas volume and methane yield

6.3.4.1.1. Analysis 1

Table 6.3 shows that reactors 7 and 10 were charged with equal media solution of pH of 8.00, operated at equal digester's moisture content of 80.00 % and charged with C:M feed ratio of 8:2 and 2:8 respectively. Table 6.3 reveals that higher biogas volume (737.94 ml) and methane yield (89.90 ml/gVS) were achieved by reactor 10 compare to reactor 7 where lower biogas volume (390.03 ml) and methane yield (62.20 ml/gVS) were attained.

6.3.4.1.2. Analysis 2

Reactors 5 and 12 were operated at equal digester's moisture content of 95.00 %, equal media solution with pH of 4.00 and charged with C:M feed ratio of 8:2 and 2:8 respectively. Table 6.3 reveals that reactor 12 produced higher biogas volume and methane yield of 671.00 ml and 60.17 ml/gVS respectively compared to reactor 5 where lower biogas volume (403.86 ml) and methane yield (46.51 ml/gVS) were achieved.

6.3.4.1.3. Analysis 3

Reactors 2 and 11 were charged with media solution with equal media solution pH of 4.00, operated at equal digester's moisture content of 80.00 % and charged with cow dung-to-molasses C: M feed ratio of 8:2 and 2:8 respectively as presented in Table 6.3. Table 6.3 reveals that reactor 11 achieved higher biogas volume (700.54 ml) and methane yield (59.27 ml /g VS) compared to reactor 2 where lower biogas and methane yield of 372.11 ml and 64.28 ml/ gVS were achieved.

6.3.4.1.4. Analysis 4

Reactors 6 and 9 were charged C:M feed ratio of 2:8 and 8:2 respectively. Both reactors were charged with media solution pH and digester's moisture content of 8.00 and 95.00 % respectively. Table 6.3 reveals that reactor 6, which was charged with lower C:M feed ratio achieved higher biogas volume and methane yield of 651.51 ml and 73.86 ml/gVS respectively compared to lower biogas volume and methane yield of 371.49 ml and 47.72 ml/gVS achieved by reactor 9.

6.3.4.1.5. Graphical analysis

Figures 6.6 and 6.7 are 2-D graphical representation of biogas volume and methane yield models plotted at C: M feed ratio fixed at central position of 5:5 in Z axis respectively.

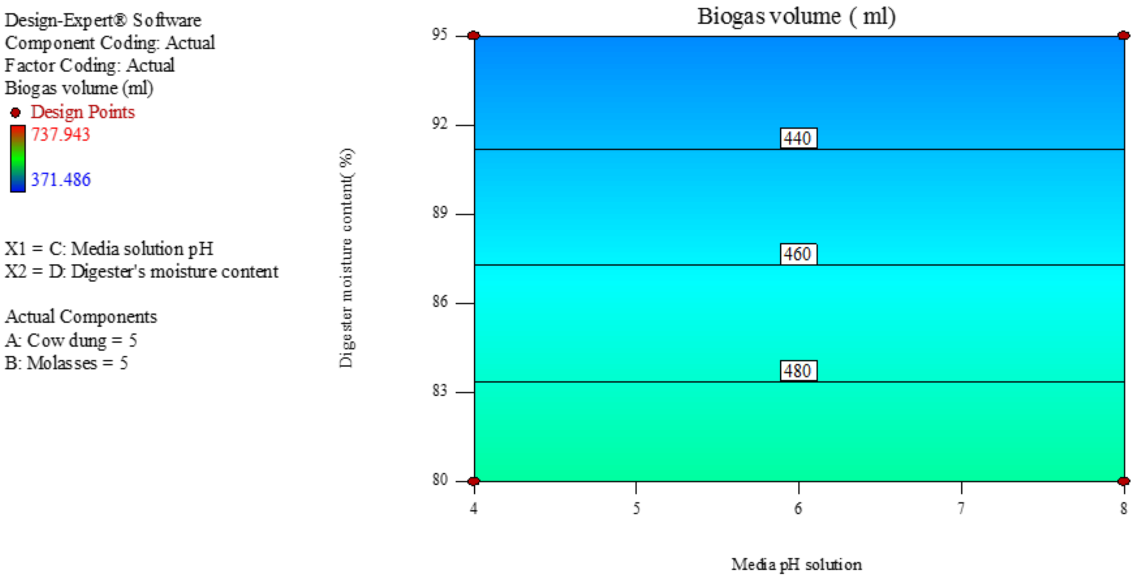

Figure 6.6: 2-D graphical representation of biogas model when C: M feed ratio is fixed at 5:5 for cow dung and molasses co-digestion.

Linearity of Figure 6.6 indicates weak interaction of media solution pH and digester’s moisture content on biogas volume at constant C:M feed ratio. Figure 6.6 also reveals that at constant media solution pH biogas volume decreases as digester’s moisture content increases. It can be observed that contour lines are parallel along x axis which indicates that biogas volume will not be affected by media solution pH if C:M feed ratio and digester’s moisture content remain the same.

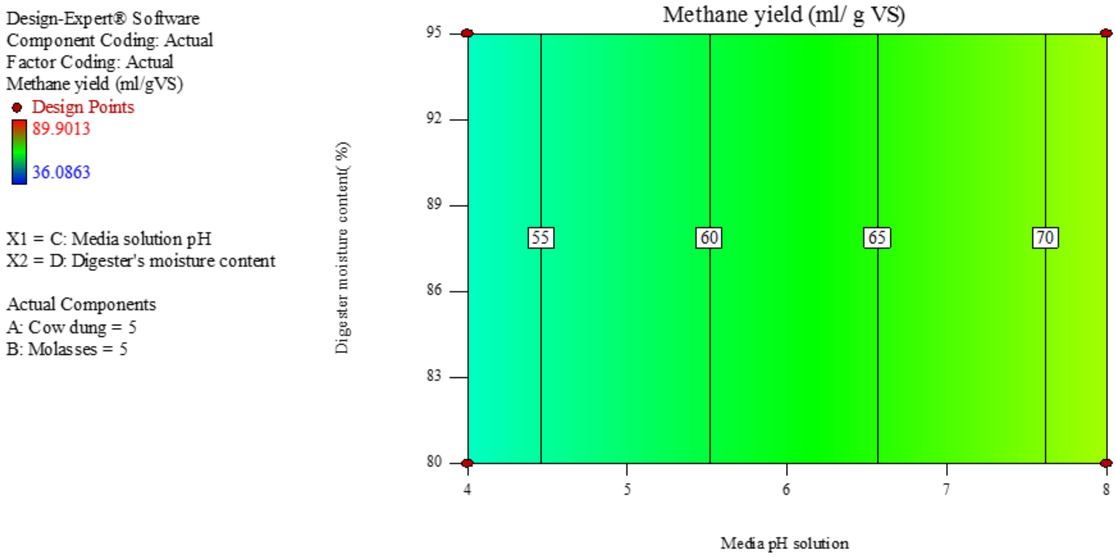

Figure 6.7: 2-D graphical representation of methane yield when C: M feed ratio is fixed at 5:5 for cow dung and molasses co-digestion.

Figure 6.7 shows that contour lines are linear and parallel along y-axis which is the indication that at constant media solution pH digester’s moisture content does not affect methane yield. Methane yield increases as media solution pH increases at constant digester’s moisture content.

6.3.4.1.6. Concluding comments

Low C:M feed ratio supports high biogas and methane yield production.

As media solution pH, decreases biogas volume and methane yield values decreases.

High C:M feed ratio has a negative effect on both biogas volume and methane yield.

Graphical analysis media solution pH and digester’s moisture content has a weak effect on methane and biogas yield effect.

6.3.4.2. Effect of media solution pH on biogas volume and methane yield

6.3.4.2.1. Analysis 1

Reactors 1 and 4 were charged with equal C: M feed ratio (5:5), operated at equal digester's moisture content (95 %) and charged with media solution with pH of 8.00 and 4.00 respectively. Table 6.3 shows that higher biogas volume 423.97ml and methane yield 69.95 ml/gVS were achieved by reactor 1 compare to reactor 4 where lower biogas volume and methane yield of 417.06 ml and 50.41 ml/gVS were produced respectively.

In this analysis, it is observed that reactor charged with high media solution pH produced higher biogas volume and methane yield. Media solution with pH of 8.00 favours both methane yield and biogas volume production when operated at 95% and charged with C:M feed ratio of 5:5.

6.3.4.2.2. Analysis 2

Higher biogas volume of 390.03 ml and lower methane yield of 62.20 ml/gVS were achieved by reactor 2 compared to lower biogas volume (372.11 ml) and higher methane yield (64.28 ml/gVS) achieved by reactor 7. Reactors 2 and 7 were charged with equal C:M feed ratio of 8:2, equal digester's moisture content of 80.00 % and media solution with pH of 4.00 and 8.00 respectively.

In this analysis, high media solution pH (8.00) resulted to higher biogas volume but lower methane yield. Media solution with low pH achieved lower biogas volume and higher methane yield.

6.3.4.2.3. Analysis 3

Reactors 6 and 12 were charged with equal C: M feed ratio (2:8), digester's moisture content (95 %) and charged with media solution with pH of 4 and 8 respectively.

Table 6.3 shows that higher biogas volume 671.00 ml and lower methane yield 60.17 ml/ g VS were achieved by reactor 12 compared to lower biogas volume 651.51 ml and methane yield 73.86 ml/gVS obtained by reactor 6.

6.3.4.2.4. Graphical analysis

Figure 6.8 represents 2-D graphical representation of biogas volume and methane yield models at constant media solution pH (6.00).

Figure 6.8 shows that as C:M feed ratio increases, biogas volume decreases. Biogas volume decreases as moisture content decreases. Figure 6.9 shows that as moisture content of digester decreases, methane yield increases. At constant digester's moisture content biogas volume decreases as C:M feed ratio increases.

Equation 6.4 below shows media solution pH and digester's moisture content do not affect methane yield as a result, plot for methane yield at constant media solution pH could not be drawn.

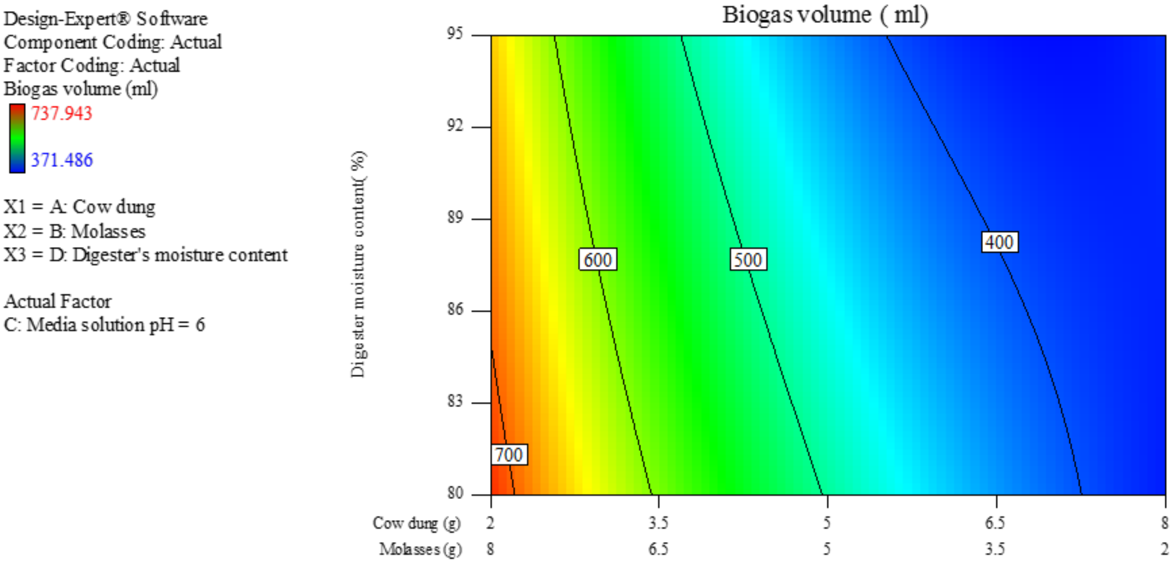

Figure 6.8: 2-D graphical representation of biogas volume at constant media solution pH for cow dung and molasses co-digestion.

6.3.4.2.5. Concluding comments

High pH media solution supports higher biogas volume and lower methane yield when operated at 80.00 % digester’s moisture content.

Reactors operated at 95.00 % digester’s moisture content supports higher methane yield when charged with media solution pH of 8.00.

Reactor operated at 80.00 % digester’s moisture content favours both methane yield and biogas volume regardless media solution pH.

6.3.4.3. Effect of digester’s moisture content on biogas volume and methane yield

6.3.4.3.1. Analysis 1

Higher biogas volume and methane yield of 737.94ml and 89.90ml/gVS were achieved by reactor 10 respectively compared to reactor 6 where lower biogas volume and methane yield of 651.51 ml and 73.86 ml/gVS were achieved.

Reactors 7 and 10 were charged with equal C:M feed ratio of 2:8, media solution with pH 8.00 and digester’s moisture contents of 95.00 % and 80.00 % respectively.

6.3.4.3.2. Analysis 2

Reactors 1 and 8 were charged with equal C: M feed ratio (5:5), equal media solution with pH of 8.00 and operated at digester’s moisture content of 95.00 % and 80.00 % respectively.

Table 6.3 shows that reactor 8 achieved higher biogas volume and methane yield of 576.40 ml and 87.36 ml/gVS respectively. Reactor 1 achieved lower biogas volume of 423.97 ml and methane yield of 69.95ml/gVS.

6.3.4.3.3. Analysis 3

Higher biogas volume (403.86 ml) and lower methane yield (46.51 ml/gVS) were achieved by reactor 5 compared to reactor 2 where lower biogas volume (372.11 ml) and higher methane yield (64.28 ml). were achieved. Reactor 2 and 5 were operated at moisture content of 80.00 % and 95.00 % respectively. Both reactors were charged with media solution with pH of 4.00 and C:M feed ratio of 8:2.

6.3.4.3.4. Analysis 4

Reactors 11 and 12 were charged with equal media solution with pH of 4.00, C:M feed ratio of 2:8 and operated at digester's moisture content of 80.00 % and 95.00 % respectively. Table 6.3 reveals that reactor 11 attained higher biogas volume (700.54 ml) and lower methane yield (59.27ml/gVS) compared to reactor 12 where lower biogas volume (671.00 ml) and higher methane yield (60.17 ml/gVS) were achieved.

6.3.4.3.5. Graphical analysis

Figures 6.9 and 6.10 are 2-D contour graphical representation of biogas volume and methane yield respectively plotted at digester's moisture content fixed at central position (87.50%). Figure 6.9 shows that contour lines are straight lines and parallel which indicates weak interaction of media solution pH and C:M feed ratio. Figure 6.9 shows that as C: M feed ratio decreases, biogas volume decreases. Figure 6.10 shows that as C: M feed ratio increases, methane yield decreases. The increase in media solution pH favours methane yield production. Figure 6.10 also shows that at constant C:M feed ratio methane yield increases as media solution pH increases.

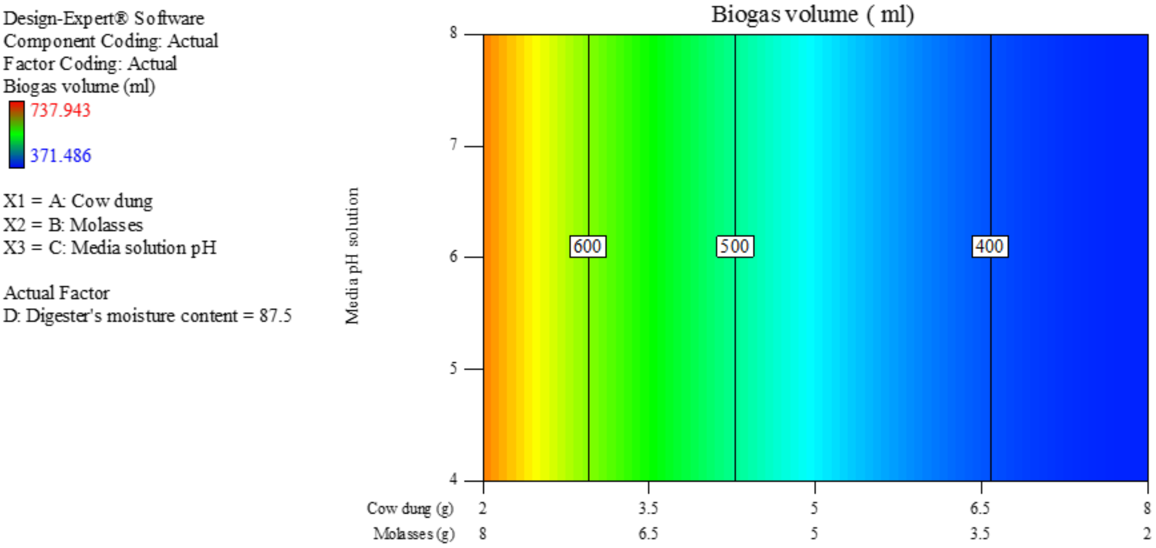

Figure 6.9: 2-D graphical representation of biogas volume at constant digester's moisture content for cow dung and molasses co-digestion.

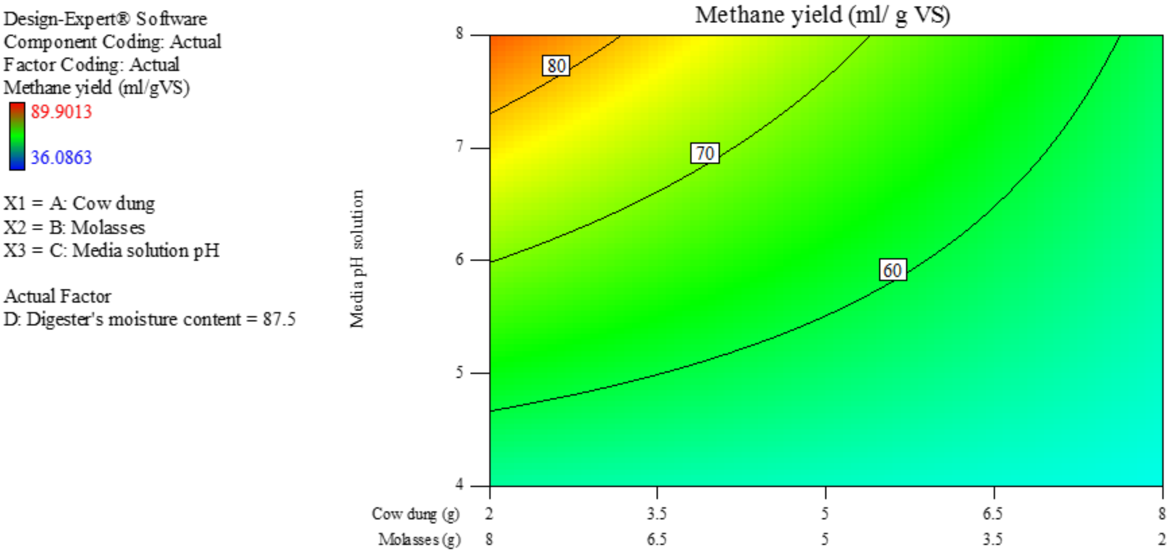

Figure 6.10: 2-D graphical representation of methane yield at constant digester's moisture content for cow dung and molasses co-digestion.

6.3.4.3.6. Concluding comments

High digester's moisture content supports achieved higher biogas volume and methane yield.

Reactor operated at lower digester's moisture content achieved higher biogas volume and methane yield when charged with C: M feed ratio of 5:5.

Reactor operated at digester's moisture content of 95 % produced higher biogas volume and lower methane yield when charged with media solution with pH of 8.00 while low media solution pH (4.00) produce high methane yield.

In conclusion, all reactors operated at moisture content of 80.00 % produced higher biogas volume and methane regardless of the convictions of other process variables.

6.4. Preliminary conclusions

Low C: M feed ratio charged reactors achieve high biogas potential and biogas maximum biogas values when charged with low pH (4.00) media solution and operated lower digester's moisture content of 80%.

Reactors changed with low media solution pH of 4 achieve low lag phase.

High digester's moisture content operated reactors produced higher biogas potential and maximum biogas production rate.

Media solution with pH charged reactors achieved high biogas potential and maximum biogas production rate and low lag phase when operated at similar conditions.

High concentration of molasses and low cow dung concentration lead to high biogas volume and methane at short lag phase.

The optimum biogas volume and methane yield are 719.24 ml and 85.32 ml/gVS respectively. The optimum conditions are digester's moisture content of 80.00%, media solution with pH of 8.00, 2.00 g of cow dung and 8.00g of molasses.

Chapter 7

MODELS VALIDATION

7.1. Background

Model validation is an exercise of demonstrating that the model is a reasonable representation of the actual process. A good model should produce system behaviour to achieve the objectives. There are two methods used to validate models, i.e., real system measurements and theoretical analysis.

Theoretical analysis is based on operational laws. This analysis checks if the model obeys certain theoretical laws. Should the model not obey theoretical laws, further investigations may be required. Real system measurement validation is the most reliable method where model-based results are compared to real system measurement. If there is large deviation, model can be calibrated by adjusting the model parameters. Real system measurement approach was used was used in the current study.

7.2. Models validation procedure

Biogas volume models for each sugarcane residues were validated. Nine experiments run were conducted within the same conditions used to develop the models. The results for each validation experiment are presented in Tables 7.1, 7.2 and 7.3.

Biogas volume for each residue was calculated from corresponding model. Calculated biogas volume was then compared with experimental values.

7.2.1. Cow dung and bagasse validation

Biogas volume model of cow dung and bagasse co-digestion experiment developed in chapter 4 is presented by equation 7.1. Experimental and calculated results presented in Table 7.1.

$$Y_1 = \text{-9.90X}_1\text{-48.5X}_2\text{+42.30X}_1\text{X}_2\text{+0.16X}_1\text{Z}_2\text{+5.14X}_2\text{Z}_1 \\ \text{+0.28X}_2\text{Z}_2\text{-0.42X}_1\text{X}_2\ \text{Z}_2 \qquad \text{……………………...7.1}$$

Where: X_1 and X_2 represent weight (g) of cow dung and bagasse

Z_1 and Z_2 represent pH media solution pH and moisture content of the digester (%)

Y_1 is the biogas volume (ml)

Table 7.1: Validation results of bagasse and cow dung co-digestion model

Reactor	CD-X_1 (g)	SB-X_2 (g)	pH-Z_1	Moisture Z_2 (%)	Calculated biogas volume (ml)	Experimental biogas volume (ml)	% Dev
1	4.00	6.00	8.00	80.00	978.17	529.69	45.85
2	6.00	4.00	4.00	95.00	927.29	448.7	51.61
3	1.00	9.00	6.00	95.00	225.88	173.6	23.15

Deviation between calculated and measured was found to be 45.85%, 51.61 % and 23.15 % for reactors 1, 2 and 3 respectively. Due to high deviation, the model was calibrated by suggesting an error term. New equation is presented by equation 7.2. Adjusting of co-efficient of model terms is also allowed.

$$Y_1 = \text{-9.90X}_1\text{-48.5X}_2\text{+42.30X}_1\text{X}_2\text{+0.16X}_1\text{Z}_2\text{+5.14X}_2\text{Z}_1 \\ \text{+0.28X}_2\text{Z}_2\text{-0.42X}_1\text{X}_2\text{Z}_2 + Error \qquad \text{……………..7.2}$$

7.2.2. Sugarcane leaves and cow dung

The model which was generated for biogas volume from sugarcane leaves and cow dung is presented by equation 7.3. Table 7.2 represents calculated and experimental values.

$$\text{Y}_2 = \text{58.75 X}_1\text{-13.30X}_2\text{-9.02X}_1\ \text{Z}_1\text{-0.66X}_1\ \text{Z}_2\text{+0.19X}_1\text{Z}_2 + \text{0.12X}_1\ \text{Z}_1\text{Z}_2 \quad \text{……………...7.3}$$

Where: X_1 and X_2 represent weight (g) of cow dung and bagasse

Z_1 and Z_2 represent pH media solution pH and moisture content of the digester (%)

Y_1 is the biogas volume (ml).

Table 7.2: Validation results for sugarcane leaves and cow dung model

Reactor	CD-X_1 (g)	SB-X_2 (g)	pH-Z_1	Moisture Z_2 (%)	Calculated biogas volume (ml)	Experimental biogas volume (ml)	% Dev
1	8.00	2.00	8.00	95.00	536.02	497.90	7.66
2	3.00	7.00	8.00	80.00	356.47	333.70	6.82
3	9.00	1.00	8.00	80.00	497.41	412.00	20.73

The calculated and measured biogas volume has a small deviation of about 11%.

7.2.3. Molasses and cow dung

Table 7.3 represents experimental and calculated values used to validate biogas volume for cow dung and molasses co-digestion experiment. Equation below represents biogas volume model for cow dung and molasses as was developed in chapter 6.

$$\begin{aligned} Y_1 &= -27.10X_1 + 86.67X_2 + 24.33X_1X_2 + 0.79X_1Z_2 \\ &+ 0.07X_2Z_2 - 0.38X_1X_2Z_2 \end{aligned} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.4$$

Where: X_1 and X_2 represent weight (g) of cow dung and bagasse

Z_1 and Z_2 represent pH media solution pH and moisture content of the digester (%)

Y_1 is the biogas volume (ml)

Table 7.3: Validation results for molasses and cow dung model

Reactor	X_1 (g)	X_2 (g)	Z_1	Z_2 (%)	Calculated Y_1 (ml)	Experimental Y_1(ml)	% dev
1	2.00	8.00	8.00	80.00	1138.84	755.00	33.70
2	3.00	7.00	8.00	80.00	1173.92	639.00	45.57
3	8.00	2.00	4.00	95.00	670.72	376.00	43.94

The average deviation between measured and calculated biogas volume was calculated to be 41.07 %.

7.2. Conclusions

Removal of insignificant terms during model development may have contributed to high deviations between calculated values and experimental values.

The resulted error terms could be determined experimentally for future study.

Chapter 8

COMPARATIVE ANALYSIS

8.1. Introduction

The aim of this work was to produce biogas from sugarcane residues. Results which were presented in chapters 4, 5 and 6 indicated that bagasse, molasses and sugarcane leaves have a potential to produce biogas. The question is now which of these three residues have ability to produce more biogas volume when equal amount of each residue is subjected to AD process to produce biogas at similar conditions.

Without looking at the economic side of AD process by ignoring costs related to handling, transportation and treatment methods of residues because these residues are not the same and therefore will require different methods of handling, treatment, etc. For technical point of view, the performance of each sugarcane residue was measured in terms of biogas or methane volume generated.

This chapter aims to assess the performance of bagasse, molasses and sugarcane leaves based on biogas volume or methane yield generated by each residue.

8.2. Biogas volume

Table 8.1 represents biogas volumes for each reactor in all three residues under study. Table 8.1 indicates that average biogas volumes for bagasse, molasses and leaves are 154.18 ml, 511.16 ml and 428.37 ml respectively. Based on these average values, it is clear that molasses and cow dung experiment performed better than other two residues, however, in some reactors sugarcane leaves and cow dung experiment individually achieved higher biogas volume than molasses and cow dung experiment.

Sugarcane leaves and cow dung experiment reactors 1,2,3,6 and 7 produced higher biogas volumes of 626.06 ml, 408.26 ml, 436.86 ml, 693.31 and 462.00 ml respectively, compared to molasses and cow dung experiment where lower total biogas volume of 423.97 ml, 372 ml, 418.00 ml, 651.51ml and 390.03 ml respectively.

Table 8.1: Total biogas volumes produced by a single reactor for each sugarcane residue

Reactor	**Biogas volume (ml)**		
	Cow dung and Molasses	Cow dung and Leaves	Cow dung and Bagasse
1	423.97	626.06	152.43
2	372.11	408.26	120.37
3	418.00	436.86	172.23
4	417.06	236.03	98.37
5	403.86	174.77	73.86
6	651.51	693.31	205.86
7	390.03	462.00	207.43
8	576.40	399.46	329.69
9	371.49	497.83	104.34
10	737.94	326.86	248.29
11	700.54	299.51	110.63
12	671.00	579.54	26.71
Average	511.16	428.37	154.18

8.3. Methane yield

Methane yield does not represent volume of methane produced but represents the efficiency of AD process for each reactor as a result the average values of methane yield were used. Table 8.2 represents methane yield for each reactor for each sugarcane residue.

Table 8.2 reveals that average methane yields of molasses, sugarcane leaves and bagasse experiments are 62.31 ml/gVS, 39.20 ml/gVS and 13.88 ml/gVS respectively. These results are evidence that molasses and cow dung experiment was better than other two residues.

Reactor 9 is the only reactor where sugarcane leaves and cow dung performed better than molasses experiments. Molasses and sugarcane leaves experiments in reactor 9 achieved 47.72 ml/ gVS below the average of 62.31 ml/ gVS and 93.68 ml/ gVS respectively.

Table 8.2: Results for methane yield for each reactor and each sugarcane residue.

Reactor	**Methane yield (ml/gVS)**		
	Cow dung and Molasses	Cow dun and Leaves	Cow dung and Bagasse
1	69.95	50.09	13.65
2	64.28	49.20	10.64
3	36.09	26.60	19.57
4	50.41	28.74	8.64
5	46.51	20.32	7.77
6	73.86	26.63	15.30
7	62.20	53.73	24.44
8	87.36	18.76	29.52
9	47.72	93.68	9.44
10	89.90	23.18	19.54
11	59.27	21.24	6.45
12	60.17	58.22	1.63
Average	**62.31**	**39.20**	**13.88**

8.5. Concluding comments

Molasses and cow dung experiment achieved the highest average biogas volume and more efficient as the highest average methane yield was achieved.

Chapter 9

CONCLUSIONS AND RECOMMENDATIONS

9.1. Introduction

This chapter presents the summary of preliminary conclusions and findings presented in chapters 4,5 and 6.

9.2. Thesis findings

- The following equations can be used to calculate methane yield, total biogas volume, total VS, daily biogas volume, and mass of media solution for two component mixtures.

$$Methane\ yield\left(ml/gVS\right)=\frac{Y_1}{VS_{tot}}\times n_{CH_4}$$

$$Total\ biogas\ volume\,(ml)=\sum_{i=1}^{14}\pi r^2 h_i$$

$$VS_{tot}\,(g)=TS_A VS_A M_A+TS_B VS_B M_B$$

$$Daily\ biogas\ volume\left(ml\right)=\pi r^2 h$$

$$Media\ solution\,(g)=\left(\frac{1-\varphi_{sl}}{\varphi_{sl}}\right)\left(TS_A+TS_B\right)-\left[X_A M_A+X_B M_B\right]$$

- Biogas can be produced from molasses, sugarcane leaves and bagasse.
- Molasses charged reactors achieve higher biogas volume and methane yield when compared to other reactor operated at the similar conditions.
- Reactors charged with low cow dung concentration perform better for molasses and cow dung experiment. Cow dung can be considered as an inhibitory substance for

molasses digestion. Anaerobic toxicity analysis should be conducted to determine the maximum amount of cow dung required to enhance biogas production.

- High media solution pH charged reactors achieve higher methane yield, biogas volume, and kinetic constants values.
- Digester's moisture content effect on methane yield, biogas volume and kinetic constants is dependent on media solution pH.
- The extent at which feed ratio influences methane yield, biogas volume and kinetics constant depend on sugarcane residue used.
- Optimum values of biogas volume and methane yield for molasses and cow dung is achieved at minimum cow dung concentration.
- Bagasse and cow dung achieved the highest biogas volume and methane yield at maximum cow dung concentration.

9.3. Future research

To develop an accurate method which could be used to estimate methane volume produced by a single substrate when subjected under anaerobic co-digestion process, i.e., to calculate an individual contribution of cow dung in cow dung and bagasse or to calculate bagasse contribution in a total biogas volume.

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Appendix

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1. Appendix A- Raw results

1.1. Daily water displaced

Height of water displaced by biogas for cow dung-bagasse, cow dung-leaves and cow dung-molasses co-digestion experiments are presented by Tables A.1, A.2, and A.3 respectively.

Table A.1:Daily heights of water displaced by biogas for cow dung and bagasse co-digestion experiment

	Height of water displaced by biogas (cm)											
Day	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	3.20	4.10	3.50	0.90	0.80	1.20	3.60	3.60	1.90	4.00	2.10	0.70
2	6.20	8.20	7.30	1.10	2.10	9.80	6.90	8.50	2.80	14.50	2.80	0.90
3	2.50	5.10	3.90	3.00	4.90	4.70	8.70	17.30	4.20	23.20	4.20	0.90
4	8.50	2.90	2.10	4.30	2.50	12.30	14.20	16.50	5.60	10.90	5.60	0.90
5	9.50	1.80	2.50	3.10	1.10	11.10	16.20	14.90	4.90	5.00	4.90	1.00
6	6.30	1.20	9.20	2.40	1.10	8.10	5.40	14.00	2.30	3.50	2.30	1.10
7	4.80	0.90	8.10	1.90	0.80	6.40	3.90	13.00	1.80	2.50	1.80	0.90
8	2.10	4.10	4.20	3.50	0.50	2.60	1.20	4.30	2.20	2.30	2.30	0.50
9	1.20	2.90	4.20	3.40	3.30	2.10	1.30	3.30	2.20	3.50	2.20	0.20
10	0.80	1.80	3.90	2.40	1.90	1.90	1.10	2.40	1.50	3.10	2.20	0.40
11	1.30	1.80	2.60	2.60	1.30	1.50	1.20	2.10	1.30	2.70	1.70	0.00
12	1.10	1.50	1.40	1.30	1.20	1.90	0.90	2.20	1.10	2.20	1.30	0.20
13	0.50	1.20	0.90	0.90	1.10	1.20	1.00	1.70	0.70	1.20	0.80	0.50
14	0.50	0.80	1.00	0.50	0.90	0.70	0.40	1.10	0.70	0.40	1.00	0.30

Table A.2: Daily heights of water displaced by biogas for cow dung and sugarcane leaves co-digestion experiment

Day	Heights of water displaced by biogas(cm)											
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	1.10	3.80	2.50	1.40	1.80	1.10	2.40	1.10	1.10	1.20	2.50	0.40
2	12.40	8.50	10.10	8.50	11.50	12.50	7.60	21.30	2.50	27.00	16.20	4.10
3	17.30	18.20	19.60	3.50	2.20	23.50	10.30	14.20	6.30	19.50	15.60	11.30
4	35.20	15.10	25.10	13.50	5.10	36.10	23.50	17.80	20.50	15.30	30.20	36.60
5	14.50	7.50	8.90	4.90	1.20	12.30	9.30	5.80	13.60	1.20	1.80	13.60
6	21.20	14.30	9.50	9.50	5.50	21.30	22.50	9.20	22.50	5.50	6.50	18.50
7	20.40	13.10	10.20	8.50	4.50	15.20	14.30	6.20	20.50	10.20	7.30	17.20
8	18.40	13.20	10.60	6.50	2.50	15.50	14.40	9.50	19.20	9.60	4.70	19.50
9	19.20	15.20	13.50	4.50	5.60	21.30	14.60	14.00	15.90	3.60	3.60	17.50
10	20.50	11.60	13.50	6.50	11.50	25.40	14.30	13.50	16.40	3.60	1.70	19.00
11	10.20	5.20	5.50	2.90	8.60	12.30	6.20	7.30	8.30	3.30	2.00	9.80
12	4.20	2.30	6.30	2.10	3.60	11.30	3.40	3.70	4.70	2.50	1.20	8.50
13	3.40	1.40	2.30	1.50	0.50	8.50	2.70	2.10	3.40	1.30	1.50	5.60
14	1.20	0.50	1.40	1.30	0.20	4.30	1.50	1.40	3.50	0.20	0.50	2.80

Table A.3: Daily height of water displaced by biogas for cow dung and sugarcane molasses co-digestion experiment

Day	Height of water displaced by biogas(cm)											
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	7.90	2.50	6.80	8.50	4.80	13.80	8.80	9.80	9.50	20.80	12.90	11.80
2	13.90	8.50	11.30	9.30	11.50	27.50	13.50	26.50	12.50	29.50	26.90	25.50
3	14.70	24.70	23.80	19.60	16.40	46.70	21.80	42.70	27.70	36.70	35.70	46.70
4	22.70	14.00	21.30	24.30	32.30	38.60	33.30	34.30	20.00	26.30	36.70	37.30
5	50.70	47.50	47.80	48.80	44.70	49.80	17.60	47.70	23.00	58.50	54.70	51.70
6	12.50	11.00	13.50	16.60	9.50	24.50	18.80	16.50	17.70	49.30	44.50	29.00
7	7.10	6.50	5.60	2.10	5.70	3.20	4.50	3.70	4.60	10.10	7.50	6.80
8	1.20	0.50	2.20	1.50	1.50	1.00	2.10	1.00	1.10	1.10	2.30	3.20
9	1.20	1.20	0.50	1.00	0.50	1.50	1.20	0.30	0.50	1.50	1.20	1.00
10	1.10	1.50	0.20	0.50	0.50	0.50	2.00	0.90	1.10	1.00	0.50	0.50
11	1.90	0.50	0.00	0.50	1.10	0.20	0.50	0.00	0.50	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

1.2. GC analysis

Tables A.4, A.5 A.6 represent overall biogas composition for bagasse-cow dung, leaves-cow dung and molasses-cow dung experiments respectively.

Table A.4: Overall biogas composition for cow dung and bagasse co-digestion experiment

Reactor	**Area (%)**		**Mass (g)**		**Composition (v/v)**	
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
1	74.89	25.10	119827.20	110475.20	0.52	0.48
2	66.87	33.12	107003.20	145741.20	0.42	0.58
3	84.34	15.47	134955.20	68090.00	0.66	0.34
4	73.08	24.77	116928.00	109005.60	0.51	0.48
5	70.84	25.74	113353.60	113291.20	0.50	0.50
6	75.01	25.89	120017.60	113920.40	0.51	0.49
7	76.84	21.75	122956.80	95730.80	0.56	0.44
8	74.98	25.01	119976.00	110066.00	0.52	0.48
9	67.45	31.54	107934.40	138780.40	0.43	0.56
10	76.64	23.35	122638.40	102740.00	0.54	0.46
11	64.79	35.15	103664.00	154699.60	0.40	0.60
12	60.61	29.38	96977.60	129311.60	0.42	0.58

Table A.5: Overall biogas composition for cow dung and sugarcane leaves co-digestion

Reactor	**Area (%)**		**Mass (g)**		**Composition (v/v)**	
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
1	64.23	27.38	102782.40	120476.40	0.46	0.54
2	78.91	21.08	126267.20	92765.20	0.57	0.42
3	65.70	43.29	105123.20	190511.20	0.35	0.64
4	86.83	13.14	138942.40	57851.20	0.70	0.29
5	77.25	22.79	123604.80	100298.00	0.55	0.44
6	35.69	35.69	57116.80	157062.40	0.26	0.73
7	77.47	22.52	123958.40	99114.40	0.55	0.44
8	51.06	48.39	81705.60	212933.60	0.27	0.72
9	95.62	4.37	153006.40	19232.40	0.89	0.11
10	71.21	28.21	113936.00	124124.00	0.48	0.52
11	71.39	28.60	114225.60	125870.80	0.48	0.52
12	71.40	12.85	114241.60	56566.40	0.68	0.33

Table A.6: Overall biogas composition for cow dung and molasses co-digestion

Reactor	**Area (%)**		**Mass (g)**		**Composition (v/v)**	
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
1	94.62	5.37	151395.20	23663.20	0.86	0.14
2	90.29	9.41	144470.40	41417.20	0.78	0.22
3	69.50	30.41	111200.00	133812.80	0.45	0.55
4	82.69	17.30	132313.60	76137.60	0.63	0.37
5	74.50	25.04	119200.00	110176.00	0.52	0.48
6	85.01	14.98	136017.60	65951.60	0.67	0.32
7	87.84	12.15	140556.80	53468.80	0.72	0.28
8	90.98	9.01	145576.00	39666.00	0.79	0.21
9	79.45	20.54	127134.40	90380.40	0.58	0.42
10	87.64	12.35	140238.40	54344.40	0.72	0.28
11	73.79	26.21	118064.00	115324.00	0.50	0.49
12	75.61	24.38	120977.60	107311.60	0.53	0.47

2. Appendix B: Derivation of water addition equation

Equation used to calculate the mass of media solution required for each reactor was derived from material balance. Mass fraction of the slurry represents solids present in the slurry. Slurry represent the mass of all solids and mass of all liquids in a mixture. Percentage solids of any slurry can be expressed by the equation A.1.

$$\text{Mass faction of the slurry}\,(\phi_{sl}) = \frac{\text{Mass of solids }(TS_s)}{\text{Mass of slurry }(M_{sl})} \text{ ....A.1}$$

By making M_{sl} the subject equation in equation A.1, then we have equation A.2

$$M_{sl} = \frac{TS_s}{\phi_{sl}} \text{ ....A.2}$$

TS$_s$ represents mass of dry solids. Total mass of the slurry is equal to total solids and mass of liquid as presented by equation A.3.

$$M_{sl} = M_L + TS_s \text{ .... A.3}$$

Where: M$_L$ represents mass of liquid in the slurry

By combining equations A.2 and A3, we get equation A.4

$$M_L + TS_s = \frac{TS_s}{\phi_{sl}} \text{ ....A.4}$$

By making M$_L$ the subject of equation in equation A.4, then equation A.4 can be re-arranged to equation A.5.

$$M_L = \left(\frac{1-\phi_{sl}}{\phi_{sl}}\right) \times TS_s \text{ ....A.5}$$

Since we are working with two components mixture, i.e., component A and B, therefore the total mass of mixture represent the sum of all components as expressed by equation A.6.

$$TS_s = TS_A + TS_B \text{ .... A.6}$$

By substituting equation A.6 into equation A.5, then we have equation A.7.

$$M_L = \left(\frac{1-\phi_{sl}}{\phi_{sl}}\right)(TS_A + TS_B) \text{ .... A.7}$$

Equation A.7 represents the total mass of liquid required to make required digester's moisture content when substrates are completely dry. In the current work, substrates were not dried therefore equation A.7 will not be accurate. Mass of water present in substrates due to moisture was compensated. Mass of water present in substrates can be calculated from equation A.8.

$$L_{AB} = X_A M_A + X_B M_B \text{ .... A.8}$$

Where: L_{AB} presents total mass of water present for components A and B respectively

X_A and X_B represent moisture content of components A and B respectively

M_A and M_B represent wet mass of components A and B respectively

Total water mass in a slurry is a sum of mass of water present. Equation A.9 represent mass of water required to make a slurry of required concentration.

$$L_F = L_T - L_{AB} \text{ .... A.9}$$

Where: L_F represents final mass of water. solution required making required to make are required slurry concentrations.

Equations A.7 and A.8 were substituted into equation A9 to get equation A.10.

$$M_{sol} = \left(\frac{1-\varphi_{sl}}{\varphi_{sl}}\right)(TS_A + TS_B) - [x_A M_A + x_B M_B] \text{ .... A10}$$

Unit for mass of media solution is in grams (g).

3. Appendix C: Sample calculations

3.1. VS, TS and moisture content of substrate

Sample calculations below demonstrate a procedure which was used to determine VS, TS and moisture content(X) of substrates.

Sample calculations of molasses

19.60 g of molasses sample (M_1) was placed in the oven set at 105°C for 24 hours. The final weight of the sample was recorded as M_2 and was found to be 14.75 g. Dried sample (M_2) was placed into the furnace operated at 550°C for 3 hours. After 3 hours, the weight of the sample was recorded as M_3 and was found to be 2.29 g.

Moisture content of molasses was calculated as per procedure below:

$$X = \frac{M_1 - M_2}{M_1} = \frac{19.60 - 14.75}{19.60} = 0.247$$

Therefore, moisture content of molasses was found to be 0.25 when rounded off into two decimal places.

Total solids (TS) of molasses was calculated from the following procedure:

$$TS = 1 - X = 1 - 0.247 = 0.753$$

The final value was also rounded off into two decimal places.

VS of molasses was calculated by substituting experimental values into equation 3.3 as following.

$$VS = \frac{M_2 - M_3}{M_2} = \frac{14.75 - 2.29}{14.75} = 0.85$$

VS, TS and moisture content of substrate were found to be 0.85, 0.75 and 0.25 respectively. Table A.7 represent the results for initial weights (M_1), weights after oven dried (M_2) and samples weight after being autoclaved (M_3) of all substrates.

Table A.7: Results of initial characterization of substrates

Description	Symbol	Cow dung	Sugarcane leaves	Bagasse	Molasses
Initial weight (g)	M_1	20.10	20.43	20.30	19.60
Weight after 24 hr in the oven (g)	M_2	10.85	20.00	19.60	14.75
Weight after 3 hours in the autoclave (g)	M_3	2.72	4.80	4.31	2.21
Moisture content of the substrate (w/w)	X	0.46	0.02	0.03	0.25
Total solids (w/w)	TS	0.54	0.98	0.97	0.75
Volatile solids (w/w)	VS	0.75	0.76	0.78	0.85

3.2. Total volatile solids fed in the digester

Reactor 1 for cow dung and bagasse co-digestion experiment was used to demonstrate the procedure which was used to calculate the total volatile solid (VS_{tot}) charged for each reactor.

Table 3.2 shows that reactor 1 was charged with 5.00 g of cow dung and 5.00 g of bagasse. Table A.7 shows that moisture content, TS and VS of cow dung are 0.46, 0.54 and 0.75 respectively. Bagasse has moisture content, TS and VS of 0.03 ,0.97 and 0.78 respectively.

Equation 3.5 was used to calculate total VS_{tot} as shown in a procedure below.

$$\begin{aligned} VS_{tot} &= TS_A M_A VS_A + TS_B VS_B M_B \\ &= 0.54 \times 0.75 \times 5 + 0.97 \times 0.78 \times 5 \\ &= 5.81 \text{ g} \end{aligned}$$

Similar approach was used to calculate total VS for all other reactors. Results of all reactors are presented in table A.8.

Table A.8: Total volatile solids charged for each reactor

Reactor	Total volatile solids charged per digester (g)		
	Cow dung and bagasse	Cow dung and leaves	Cow dung and molasses
1	5.81	5.75	5.21
2	4.75	4.73	4.52
3	5.81	5.75	5.21
4	5.81	5.75	5.21
5	4.75	4.73	4.52
6	6.86	6.77	5.91
7	4.75	4.73	4.52
8	5.81	5.75	5.21
9	4.75	4.73	4.52
10	6.86	6.77	5.91
11	6.86	6.77	5.91
12	6.86	6.77	5.91

3.4. Media solution fed in the digester

Equation A.10 was used to calculate required mass of media solution required to achieve digester's moisture content as indicated in table 3.2.

Reactor 1 for cow dung and bagasse co-digestion experiment was used to demonstrate the procedure which was used to calculate the mass of media solution charged in each reactor to achieve required digester's moisture content as indicated in table 3.2.

Table 3.2 shows that was reactor 1 was charged with 5.00 g of cow dung and 5.00 g of bagasse. Table A.7 shows that moisture content, TS and VS of cow dung are 0.46, 0.54 and 0.75 respectively. Bagasse has moisture content, TS and VS of 0.03 ,0.97 and 0.78 respectively. These values were substituted into equation A.10.

Table 3.2 reveals that reactor 1 was t operated at 95.00 % digester's moisture content, therefore digester's solids contents (ϕ_{sl}). The procedure is presented below:

$$
\begin{aligned}
M_{sol} &= \left(\frac{1-\varphi_{sl}}{\varphi_{sl}}\right)\left(TS_A + TS_B\right) - \left[x_A M_A + x_B M_B\right] \\
&= \left(\frac{1-0.05}{0.05}\right)\left(0.54\times 5.00 + 0.97\times 5.00\right) - \left[0.46\times 5.00 + 0.03\times 5.00\right] \\
&= 141.00g
\end{aligned}
$$

Therefore, mass of media solution of 141.00 g required to make slurry with 95% moisture content. Similar approach was used to calculate mass of media solution for all reactors. Table A.9 presents mass of media solution required to make slurry with required moisture content.

Table A.9: Mass of media solution charged per digester.

Reactor	**Mass of media solution charged (g)**		
	cow dung and bagasse	cow dung and leaves	cow dung and molasses
1	141.00	142.00	119.00
2	21.30	21.40	19.10
3	27.75	28.00	22.25
4	141.00	142.00	119.00
5	115.20	115.60	106.40
6	166.80	168.40	131.60
7	21.30	21.40	19.10
8	27.75	28.00	22.25
9	115.20	115.60	106.40
10	34.20	34.60	25.40
11	34.20	34.60	25.40
12	166.80	168.40	131.60

3.5. Biogas volume

3.5.1. Daily biogas volume

Reactor 1 (day 1) for cow dung and bagasse co-digestion experiment was used to demonstrate the procedure for biogas volume calculation. Table A.1 shows that height of water displaced by biogas on the first day (day 1) was measured to be 3.20 cm. Equation 3.4 was used to calculate the volume of biogas produced. The radius of tube used for biogas measurement was 1 cm. The value of Pi (π) was estimated to be 3.141.

$$
\begin{aligned}
Y_i &= \pi r^2 h \\
&= 3.141 \times (1)^2 \times 3.2 \\
&= 10.06\ ml
\end{aligned}
$$

Therefore, biogas volume generated by reactor 1 was found to be 10.06 ml. The similar approach was used to calculate biogas volumes generated by other reactors. Tables A.10, A.11 and A.12 represent daily biogas volumes for each reactor per day for 14 days of experiments.

Table A.10: Daily biogas volumes for cow dung and bagasse co-digestion experiment

Day	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R 11	R12
1	10.05	12.88	10.99	2.83	2.51	3.77	11.31	11.31	5.97	12.56	6.60	2.20
2	19.47	25.76	22.93	3.46	6.60	30.78	21.67	26.70	8.79	45.54	8.79	2.83
3	7.85	16.02	12.25	9.42	15.39	14.76	27.33	54.34	13.19	72.87	13.19	2.83
4	26.70	9.11	6.60	13.51	7.85	38.63	44.60	51.83	17.59	34.24	17.59	2.83
5	29.84	5.65	7.85	9.74	3.46	34.87	50.88	46.80	15.39	15.71	15.39	3.14
6	19.79	3.77	28.90	7.54	3.46	25.44	16.96	43.97	7.22	10.99	7.22	3.46
7	15.08	2.83	25.44	5.97	2.51	20.10	12.25	40.83	5.65	7.85	5.65	2.83
8	6.60	12.88	13.19	10.99	1.57	8.17	3.77	13.51	6.91	7.22	7.22	1.57
9	3.77	9.11	13.19	10.68	10.37	6.60	4.08	10.37	6.91	10.99	6.91	0.63
10	2.51	5.65	12.25	7.54	5.97	5.97	3.46	7.54	4.71	9.74	6.91	1.26
11	4.08	5.65	8.17	8.17	4.08	4.71	3.77	6.60	4.08	8.48	5.34	0.00
12	3.46	4.71	4.40	4.08	3.77	5.97	2.83	6.91	3.46	6.91	4.08	0.63
13	1.57	3.77	2.83	2.83	3.46	3.77	3.14	5.34	2.20	3.77	2.51	1.57
14	1.57	2.51	3.14	1.57	2.83	2.20	1.26	3.46	2.20	1.26	3.14	0.94

Table A.11: Daily biogas volume for cow dung and sugarcane leaves co-digestion experiment

Day	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	3.46	11.94	7.85	4.40	5.65	3.46	7.54	3.46	3.46	3.77	7.85	1.26
2	38.95	26.70	31.72	26.70	36.12	39.26	23.87	66.90	7.85	84.81	50.88	12.88
3	54.34	57.17	61.56	10.99	6.91	73.81	32.35	44.60	19.79	61.25	49.00	35.49
4	110.56	47.43	78.84	42.40	16.02	113.39	73.81	55.91	64.39	48.06	94.86	114.96
5	45.54	23.56	27.95	15.39	3.77	38.63	29.21	18.22	42.72	3.77	5.65	42.72
6	66.59	44.92	29.84	29.84	17.28	66.90	70.67	28.90	70.67	17.28	20.42	58.11
7	64.08	41.15	32.04	26.70	14.13	47.74	44.92	19.47	64.39	32.04	22.93	54.03
8	57.79	41.46	33.29	20.42	7.85	48.69	45.23	29.84	60.31	30.15	14.76	61.25
9	60.31	47.74	42.40	14.13	17.59	66.90	45.86	43.97	49.94	11.31	11.31	54.97
10	64.39	36.44	42.40	20.42	36.12	79.78	44.92	42.40	51.51	11.31	5.34	59.68
11	32.04	16.33	17.28	9.11	27.01	38.63	19.47	22.93	26.07	10.37	6.28	30.78
12	13.19	7.22	19.79	6.60	11.31	35.49	10.68	11.62	14.76	7.85	3.77	26.70
13	10.68	4.40	7.22	4.71	1.57	26.70	8.48	6.60	10.68	4.08	4.71	17.59
14	3.77	1.57	4.40	4.08	0.63	13.51	4.71	4.40	10.99	0.63	1.57	8.79

Table A.12: Daily biogas volume for cow dung and molasses co-digestion experiment

Day	R1	R 2	R3	R 4	R 5	R 6	R 7	R 8	R9	R10	R11	R12
1	24.81	7.85	21.36	26.70	15.08	43.35	27.64	30.78	29.84	65.33	40.52	37.06
2	43.66	26.70	35.49	29.21	36.12	86.38	42.40	83.24	39.26	92.66	84.49	80.10
3	46.17	77.58	74.76	61.56	51.51	146.68	68.47	134.12	87.01	115.27	112.13	146.68
4	71.30	43.97	66.90	76.33	101.45	121.24	104.60	107.74	62.82	82.61	115.27	117.16
5	159.25	149.20	150.14	153.28	140.40	156.42	55.28	149.83	72.24	183.75	171.81	162.39
6	39.26	34.55	42.40	52.14	29.84	76.95	59.05	51.83	55.60	154.85	139.77	91.09
7	22.30	20.42	17.59	6.60	17.90	10.05	14.13	11.62	14.45	31.72	23.56	21.36
8	3.77	1.57	6.91	4.71	4.71	3.14	6.60	3.14	3.46	3.46	7.22	10.05
9	3.77	3.77	1.57	3.14	1.57	4.71	3.77	0.94	1.57	4.71	3.77	3.14
10	3.46	4.71	0.63	1.57	1.57	1.57	6.28	2.83	3.46	3.14	1.57	1.57
11	5.97	1.57	0.00	1.57	3.46	0.63	1.57	0.00	1.57	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

3.5.2. Total biogas volume

Total biogas volume is the sum of daily biogas volumes generated in 14 days of experiment. Equation 3.7 was used to calculate the total biogas volume generated by one digester in 14 days. Cow dung and bagasse reactor 1 was used to demonstrate calculation procedure.

$$
\begin{aligned}
Y_1 &= \sum_{i=1}^{14} \pi r^2 h_i \\
&= 3.141 \times (1cm)^2 \times h_T(cm) \\
&= 152.39\, ml
\end{aligned}
$$

Where: h_T (total height of water displaced biogas) = 3.2 + 6.2 + 2.5 +8.5 + 9.5 + 6.3 + 4.8 + 2.1+ 1.2 + 0.8+1.3 +1.1+ 0.5 + 0.5

3.5.3. Daily cumulative biogas volume

MS excel spread sheet was used to calculate daily cumulative biogas volume from daily biogas. The results are presented in Tables A.13, A.14 and A.15.

Table A.13: Daily cumulative biogas volumes for cow dung and bagasse co-digestion

HRT (day)	Daily cumulative biogas volume (ml)											
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	10.1	12.9	11.0	2.8	2.5	3.8	11.3	11.3	6.0	12.6	6.6	2.2
2	29.5	38.7	33.9	6.3	9.1	34.6	33.0	38.0	14.8	58.1	15.4	5.0
3	37.4	54.7	46.2	15.7	24.5	49.3	60.3	92.4	28.0	131.1	28.6	7.9
4	64.1	63.8	52.8	29.2	32.4	88.0	105.0	144.3	45.6	165.3	46.2	10.7
5	94.0	69.5	60.7	39.0	35.8	122.9	155.9	191.1	61.0	181.0	61.6	13.8
6	113.8	73.2	89.6	46.5	39.3	148.3	172.9	235.1	68.2	192.0	68.8	17.3
7	128.9	76.1	115.0	52.5	41.8	168.5	185.1	275.9	73.9	199.9	74.5	20.1
8	135.5	88.9	128.2	63.5	43.4	176.6	188.9	289.5	80.8	207.1	81.7	21.7
9	139.2	98.1	141.4	74.2	53.7	183.2	193.0	299.8	87.7	218.1	88.6	22.3
10	141.7	103.7	153.7	81.7	59.7	189.2	196.4	307.4	92.4	227.9	95.5	23.6
11	145.8	109.4	161.9	89.9	63.8	193.9	200.2	314.0	96.5	236.3	100.9	23.6
12	149.3	114.1	166.3	94.0	67.6	199.9	203.0	320.9	99.9	243.3	105.0	24.2
13	150.9	117.9	169.1	96.8	71.0	203.7	206.2	326.2	102.1	247.0	107.5	25.8
14	152.4	120.4	172.2	98.4	73.9	205.9	207.4	329.7	104.3	248.3	110.6	26.7

Table A.14: Daily biogas volumes for cow dung and sugarcane leaves co-digestion

HRT (day)	Daily cumulative biogas volume (ml)											
	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9	R 10	R 11	R 12
1	3.5	11.9	7.9	4.4	5.7	3.5	7.5	3.5	3.5	3.8	7.9	1.3
2	42.4	38.7	39.6	31.1	41.8	42.7	31.4	70.4	11.3	88.6	58.8	14.1
3	96.8	95.9	101.2	42.1	48.7	116.6	63.8	115.0	31.1	149.9	107.8	49.7
4	207.4	143.3	180.1	84.5	64.7	230.1	137.7	171.0	95.5	198.0	202.7	164.7
5	253.0	166.9	208.1	99.9	68.5	268.7	166.9	189.2	138.3	201.8	208.4	207.4
6	319.6	211.8	237.9	129.8	85.8	335.7	237.6	218.1	209.0	219.1	228.8	265.6
7	383.7	253.0	270.0	156.5	99.9	383.4	282.5	237.6	273.4	251.1	251.7	319.6
8	441.6	294.5	303.3	176.9	107.8	432.1	327.8	267.5	333.8	281.3	266.5	380.9
9	501.9	342.3	345.7	191.1	125.4	499.1	373.7	311.5	383.7	292.6	277.8	435.9
10	566.3	378.7	388.1	211.5	161.5	578.9	418.6	353.9	435.3	303.9	283.2	495.6
11	598.4	395.1	405.4	220.6	188.6	617.6	438.1	376.8	461.4	314.3	289.5	526.4
12	611.6	402.3	425.2	227.2	199.9	653.1	448.8	388.5	476.1	322.1	293.2	553.1
13	622.3	406.7	432.5	231.9	201.5	679.8	457.3	395.1	486.8	326.2	297.9	570.7
14	626.1	408.3	436.9	236.0	202.1	693.3	462.0	399.5	497.8	326.9	299.5	579.5

Table A.15: Daily biogas volumes for cow dung and molasses co-digestion

HRT	Daily cumulative biogas volume (ml)											
(day)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
1	24.8	7.9	21.4	26.7	15.1	43.4	27.7	30.8	29.9	65.4	40.5	37.1
2	68.5	34.6	56.9	55.9	51.2	129.8	70.1	114.1	69.1	158.1	125.1	117.2
3	114.7	112.2	131.7	117.5	102.8	276.6	138.6	248.3	156.2	273.4	237.3	264.0
4	186.1	156.2	198.6	193.9	204.3	397.9	243.3	356.1	219.1	356.1	352.6	381.2
5	345.4	305.5	348.9	347.3	344.8	554.4	298.6	506.0	291.3	539.9	524.5	543.7
6	384.7	340.1	391.3	399.5	374.6	631.4	357.7	557.9	347.0	694.9	664.4	634.9
7	407.0	360.5	408.9	406.1	392.5	641.5	371.8	569.5	361.4	726.6	688.0	656.2
8	410.8	362.1	415.8	410.8	397.3	644.6	378.4	572.6	364.9	730.1	695.2	666.3
9	414.5	365.8	417.4	413.9	398.8	649.3	382.2	573.6	366.5	734.8	699.0	669.4
10	418.0	370.5	418.0	415.5	400.4	650.9	388.5	576.4	369.9	737.9	700.5	671.0
11	424.0	372.1	418.0	417.1	403.9	651.5	390.0	576.4	371.5	737.9	700.5	671.0
12	424.0	372.1	418.0	417.1	403.9	651.5	390.0	576.4	371.5	737.9	700.5	671.0
13	424.0	372.1	418.0	417.1	403.9	651.5	390.0	576.4	371.5	737.9	700.5	671.0
14	424.0	372.1	418.0	417.1	403.9	651.5	390.0	576.4	371.5	737.9	700.5	671.0

Legend: R represent reactor.

3.7. Methane yield

Reactor 1 for cow dung and bagasse co-digestion experiment was used to demonstrate the procedure which was used to calculate methane yield (Y_2). Equation 3.7 was used to calculate methane yield. Table 4.3 shows that biogas volume generated by reactor 1 for cow dung and bagasse was 152.43 ml. Methane concentration was found to be 0.52 as indicated in table A.4. Total VS of reactor 1 was found to be 5.81 g as indicated in table A.8. By substituting the above-mentioned values into equation 3.7, methane yield was calculated.

$$
\begin{aligned}
Y_2 &= \frac{Y_1}{VS_{tot}} \times n_{CH_4} \\
&= \frac{152.43}{5.81} \times 0.52 \\
&= 13.65\,\mathrm{ml/gVS}
\end{aligned}
$$

Therefore, methane yield was found to be 13.65 ml/gVS. Similar procedure was used to calculate Y_2 for all other reactors. Results for each reactor are presented in Tables 4.3, 5.3 and 6.3.

4. Appendix D: Kinetic constants determination

Reactor 1 for cow dung and bagasse co-digestion experiment was used to demonstrate the procedure which was used to determine kinetics constant values. Daily cumulative biogas volumes result as presented by table A.13 were used for sample calculations.

Daily cumulative biogas volume (Y) and calculated biogas volume (P) were plotted against HRT in the same chart as show in Figure A.1. Equation 2.24 was used to calculate the values of P by estimating the values for A, U, and λ.

Once normalized error between two curves was calculated, MS Excel Solver was used to minimise normalised error by adjusting the values of A, U, and λ. Table A.16 represents daily cumulative biogas volume, calculated biogas volume and estimated kinetics constants.

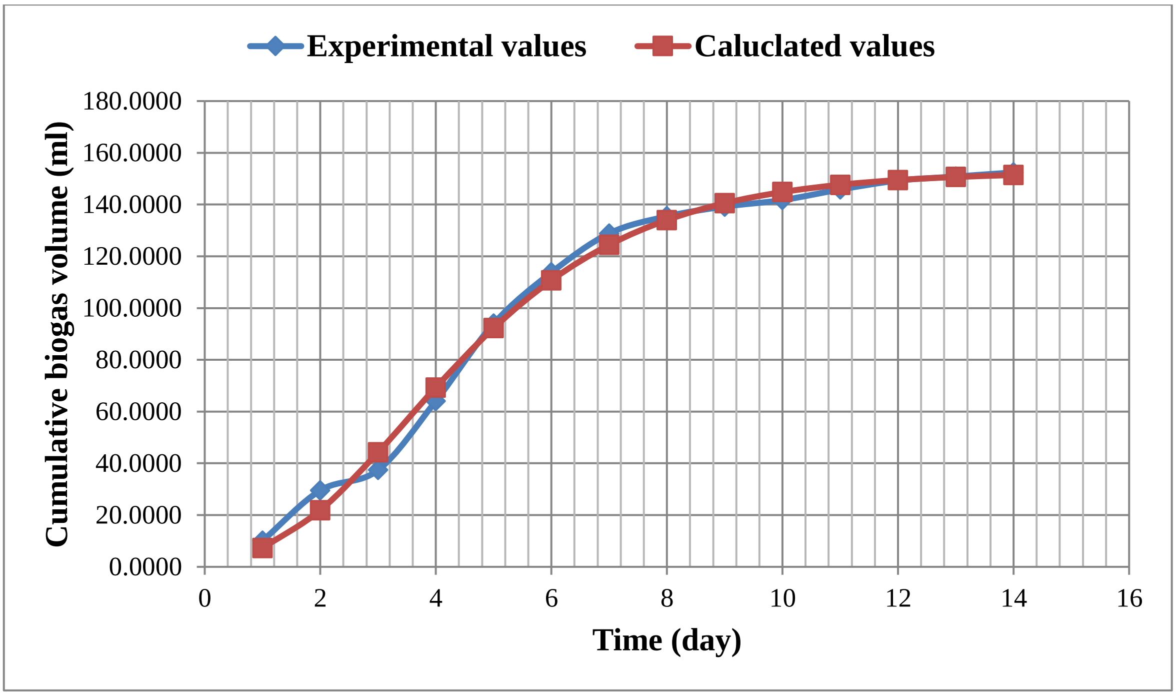

Figure A.1: Daily cumulative and calculated biogas volumes against HRT for cow dung and bagasse reactor 1.

Table A.106: Daily cumulative and calculated biogas volumes against HRT for cow dung and bagasse reactor 1.

Time (day)	**Cumulative biogas volume (ml)**					
	Experimental (Y)	Calculated (P)	$(Y_e\text{-}P)^2$			
1	10.06	7.27	7.74	Estimated values		
2	29.54	21.91	58.25	A	U	λ
3	37.40	44.26	47.15	152.75	25.27	1.25
4	64.10	69.32	27.15			
5	93.97	92.29	2.82			
6	113.77	110.76	9.03			
7	128.85	124.44	19.49			
8	135.45	134.03	2.03			
9	139.22	140.53	1.70			
10	141.74	144.84	9.61			
11	145.82	147.66	3.37			
12	149.28	149.48	0.05			
13	150.85	150.66	0.08			
14	152.42	151.41	1.03			
Sum normalized error			*189.45*			

Table A.16 shows that the values of A, U, and λ for cow dung and bagasse reactor 1 were found to be 152.76 ml, 25.27 ml/day and 1.25 day respectively. The results for other reactors are presented in tables 4.1, 5.1 and 6.1.

5.Appendix E: Commissioning

5.1. Background

The primary objective of commissioning is to have a well-functioning biogas experimental set up before an actual experiment is undertaken. Commissioning of the experimental set up involves careful planning and calibration of equipment. Operating errors and biogas leakages related problems were addressed. Cold and hot commissioning were done to ensure that the experimental results are accurate.

5.2. Cold commissioning

- Gas leaks tests were conducted by using by using bubble soap tests.
- The accuracy of water bath temperature sensor was checked and thermowells were cleaned.
- Volume measurement tests were conducted. This was achieved by introducing the known amount of air into the system. Volume of displaced by air was compared to the volume of air introduced.

5.3. Pre- commissioning

Before hot commissioning was conducted, it was necessary to check if experimental set up could handle the predicted biogas volume.

Pre- hot commissioning results reveals the following:

- 500 ml reactors were too small to handle total mass of substrates of 50.00 g for sugarcane leaves which was previously proposed. Total mass of substrates was dropped to 20.00 g.
- Total of 20.00 g of substrates produced high volume of biogas the set up could not handle. Total mass of substrates was dropped to 10.00 g.

5.4. Hot commissioning

Accuracy and integrity of any experimental results depend on how well hot commissioning was conducted. Hot commissioning helps the experimenter to familiarise him or herself with the equipment before the actual experiment is conducted.

To determine the accuracy of experimental results, two pre-experimental runs were conducted at similar conditions. The experiments were conducted on three sugarcane residues. Table A.17 represents hot commissioning results which were used to determine the accuracy of the actual experimental results.

Table A.17 reveals that the average percentage deviation (% dev) between two pre-experimental results was found to be 3.20 %, and therefore the actual experimental results as presented in tables 4.3, 5.3 and 6.3 were assumed to be close to the actual results.

Table A.17: Pre-experimental results

Reactor	**Residue**	**X_1(g)**	**X_2 (g)**	**Z_1**	**Z_2(%)**	**Biogas volume (ml)**		**% dev**
						Trail 1	Trail 2	
1	Bagasse	5.00	5.00	8.00	95.00	154.43	154.93	0.32
2	Bagasse	5.00	5.00	4.00	95.00	90.37	88.87	1.66
3	Bagasse	5.00	5.00	8.00	80.00	340.69	333.19	2.20
4	Leaves	5.00	5.00	8.00	95.00	628.06	670.56	6.77
5	Leaves	5.00	5.00	4.00	95.00	238.03	236.53	0.63
6	Leaves	5.00	5.00	8.00	80.00	401.46	422.46	5.23
7	Molasses	5.00	5.00	8.00	95.00	425.97	428.97	0.70
8	Molasses	5.00	5.00	4.00	95.00	444.06	428.06	3.60
9	Molasses	5.00	5.00	8.00	80.00	578.40	540.40	6.57