# Co-digestion of Cassava Biomass with Winery Waste for Biogas Production in South Africa

By

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## DECLARATION

I, **Unathi Liziwe Mkruqulwa**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology or the National Research Foundation of South Africa.

All intellectual concepts, theories, methodologies and material derivations and model developments used in this thesis and published in various scientific journals (except those of which the candidate is not the first author) were derived solely by the candidate and first author of the published manuscripts. Where appropriate, intellectual property of others was acknowledged by using appropriate references: The contribution of co-authors to conference papers and published manuscripts and supervisory capacity (Dr VI Okudoh) and co-supervisory capacity (Prof O Oyekola) are recognized.

**Signed** **Date**

## ABSTRACT

Renewable energy security for the future and better use of natural resources are key challenges that can be concurrently managed by a practical anaerobic co-digestion approach in the production of methane. For this study, co-digestion of cassava and winery waste was investigated for the production of biogas. Cassava biomass is a good substrate for biogas production due to its high carbohydrate yield per hectare (4.742 kg/carb) than most plants. Winery wastes constitute a lot of challenge in South Africa due to high amounts currently being dumped at landfills. Due to the chemical properties of the two substrates, it is envisaged that their co-digestion will produce more biogas than use of a single substrate.

Biomethane potential (BMP) tests were carried out in a batch, mesophilic (37 ˚C±0.5) reactor using cassava and winery waste singly and in combination at a ratio of 1:1 and ran for 30 days. Biogas optimization was also evaluated. The optimal conditions for methane production from anaerobic co-digestion of cassava biomass and winery solid waste using response surface methodology (RSM). The effects of temperature, pH and co-substrate ratios on the methane yield were explored. A central composite design technique was used to set-up the anaerobic co-digestion experiment was determined. Once the optimized values were established, biogas production from co-digestion of cassava biomass with winery waste was investigated using a single-stage 5 L mesophilic batch digester and the microbial dynamics inside the digester during co-digestion of cassava and winery waste in the single-stage 5 L mesophilic batch digester. The samples were collected on days 1, 15 and 30 of the anaerobic digestion period and DNA extracted from them while 16sRNA bacterial sequencing was performed.

The results for the BMP tests showed that cumulative methane yield for cassava, winery waste and in combination were 42, 21 and 38 $mLCH_4$ respectively. It was concluded that biogas production from anaerobic digestion was dependent on many factors such as pH, substrate properties and the ratio of different feedstocks used during co-digestion. The results from the optimization study were pH 7, temperature of 35 ˚C±0.5 and co-digestion ratio of 70:30 cassava to winery waste. The maximum methane yield of 346.28 $mLCH_4/gVS_{added}$ was predicted by the quadratic model at the optimal temperature of 35 $^{o}$C±0.5, pH of 7 and 70:30 ratio of cassava biomass to winery solid waste. Experimental results showed a close fit but higher methane yield (396 $mLCH_4/gVS_{added}$) than predicted values as indicated by the coefficient of determination ($R^2$) value of 0.9521. The response surface model proved successful in the optimization process of methane yield.

The single-stage 5L mesophilic batch digester with a co-substrate ratio of 70:30 cassava to winery waste produced a total of 819.54 mL/gVS biogas with a 62 % methane content.
The study of microbial community dynamics showed the presence of the bacteria that is responsible for each stage of anaerobic digestion. The study concluded that both winery waste and cassava substrates were favourable for biogas production and most underprivileged people in the rural areas with no access to electricity can produce & utilise it.

## DEDICATION

I wish to dedicate this thesis to my whole family who mean so much to me.

To my mom, Nontuthuzelo Mkruqulwa, the wisest teacher I have ever come across. Thank you for planting the seed. I am grateful for being able to finish what you started. Your unconditional love sustains me.

My daughter, Zintle Mkruqulwa, “those who hope in the LORD will renew their strength”; they will soar on wings like eagles; they will run and not grow weary, they will walk and not be faint” Isaiah 40:31. I am passing the ball to you. May you take it further?

To my dad, although he is no longer of this world, your teachings continue to regulate my life.

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## RESEARCH OUTPUTS

The following outputs are the contributions made by the candidate towards scientific knowledge during her master's candidacy:

1. Oral presentation at the 9th International Conference on Advances in Science, Engineering. Technology and Waste Management (ASETWM-17) organised by Eminent Association of Researchers in Engineering and Technology (EARET) under Eminent Association of Pioneers (EAP). Parys, South Africa. Title: Biomethane Potential from Co-digestion of Cassava and Winery Waste in South Africa.

2. Publication in Proceedings of the 9th International Conference on Advances in Science, Engineering, Technology and Waste Management (ASETWM-2017).

3. Abstract and oral presentation at the 7th International Conference on Engineering for Waste and Biomass Valorisation organised by WasteEng. Prague-Czech Republic. Title: Evaluation of co-digestion of winery solid waste with cassava biomass for optimal biogas production.

4. Manuscript submitted to Elsevier – Waste and Biomass Valorization journal. Title: Optimizing methane production from co-digestion of cassava biomass and winery waste.

## LAYOUT OF THESIS

The overall aim of this research is to produce and optimise the yield of biogas energy from winery waste in co-digestion with carbohydrate rich cassava in South Africa. This thesis is divided into the following chapters:

- **Chapter 1**: Introduction. This chapter provides the background information about cassava, winery waste and co-digestion. Furthermore, it provides a problem statement, hypothesis, objectives, the significance of and delineation of the study.
- **Chapter 2**: Literature review. In this chapter, biogas production, factors affecting biogas production, different digester operational configurations, cassava and winery waste characteristics and microbial community structure and dynamics in anaerobic digestion are discussed.
- **Chapter 3**: Materials and methods. This chapter lists materials, methods, and equipment used in this study to determine co-digestion of cassava biomass with winery waste.
- **Chapter 4**: This chapter comprises the results and discussion of the biomethane potential experiments.
- **Chapter 5:** This chapter comprises the results and discussion of the optimization experiments.
- **Chapter 6:** This chapter comprises the results and discussion of the scaled-up experiments.
- **Chapter 7:** This chapter comprises the results and discussion of the microbial community dynamics experiments.
- **Chapter 8**: This chapter presents the overall conclusions and also provides answers to research questions in Chapter 1. Recommendations for future research are also listed in this chapter.

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**Nomenclature**

| Symbol | Description | Units |
|---|---|---|
| Θ | Hydraulic Retention Time | d |
| V | Reactor Volume | $m^3$ |
| Q | Influent flow rate | $m^3/d$ |
| Θc | Solids Retention Time | d |
| X | Influent concentration | mg/L |
| $Q_w$ | Effluent flow rate | $m^3/d$ |
| $X_w$ | Effluent concentration | mg/L |

## CLARIFICATION OF BASIC TERMS AND CONCEPTS

| | |
|---|---|
| **Acetogenic bacteria** | Bacteria that generates hydrogen and anaerobically metabolize the alcohols and VFAs to form acetate, $H_2$, and $CO_2$ |
| **Acidogenic bacteria** | Bacteria that convert simple and soluble compounds like fatty acids, sugars and amino acids to $CO_2$, $H_2$, alcohol, and VFAs |
| **Anaerobic digester** | A closed storage tank producing biogas rich in methane |
| **Biogas** | A combustible gaseous fuel, rich in methane and also contains carbon dioxide and other trace elements that is produced through anaerobic digestion |
| **Biomethane Potential** | An indication of the biodegradability of a substrate and its potential to produce methane via anaerobic digestion. |
| **Cassava** | The tuberous crop with roots rich in starch used as food source in tropical countries. |
| **Co-digestion** | The simultaneous digestion of multiple substrates |
| **Hydrolysis** | A reaction involving the breaking of a bond in a molecule using water |
| **Methanogenesis** | The final stage of anaerobic digestion in which methanogenic bacteria form methane and carbon dioxide from acetate produced during acetogenesis |
| **Winery waste** | Waste produced from the winemaking process |

## GLOSSARY

| Abbreviation | Description |
|---|---|
| **AD** | Anaerobic digestion |
| **BMP** | Biomethane potential |
| **CD** | Cow dung |
| **C/N** | Carbon nitrogen ratio |
| **COD** | Chemical oxygen demand |
| **DO** | Dissolved oxygen |
| **GHG** | Greenhouse gas |
| **HRT** | Hydraulic retention time |
| **PCR** | Polymerase chain reaction |
| **PD** | Poultry droppings |
| **SD** | Swine dung |
| **TS** | Total solids |
| **VFA** | Volatile fatty acids |
| **VS** | Volatile solid |
| **WW** | Winery waste |
| **ZD** | Zebra droppings |

# CHAPTER 1

# INTRODUCTION

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# INTRODUCTION

## 1.1 Background

According to the United Nations, the African population is forecast to be around 2 billion people by the year 2050 (Ruppel & Althusmann, 2015). More energy will be required to meet the expanding demand posing a huge challenge to the African content in terms of energy security. In Southern Africa, a stable and abundant supply of energy is of vital importance in order to reduce poverty, stabilize democracy and economic growth. South Africa also faces an energy crisis with one of the major factors being the insignificant investment in the energy sector resulting in a backlog in infrastructure development for the past 20 years (Trollip et al., 2014). In 2005 Western Cape experienced an energy demand exceeding supply due to inadequate reserve margin which worsened in 2006 and 2007 resulting in the national load shedding implementation to maintain national electricity grid system integrity (Trollip et al., 2014).

About 95 % of electricity from South Africa is produced at local power stations but in order to meet the demand during peak supply, South Africa imports electricity mainly from the Cahora Bassa hydropower plant in Mozambique (Trollip et al., 2014). Alternative renewable energy sources are required to minimize imports to South Africa and dependency on non-renewable fossil fuels, reduce huge import budgets while meeting the rising energy demands (Hansupalak et al., 2015).

Conversion of agricultural biomass (e.g. cassava) and wastes (e.g. winery waste) into biogas is a good alternative which could solve some of these energy problems. Cassava plant thrives in drought conditions and requires low input of chemicals during cultivation. It also has small water footprint (21 $m^3$/GJ) and high carbohydrate yield per hectare (4.742 kg /carb) than most plants making it a desirable substrate for biogas production (Wang, 2002; Gerbens-Leenes et al., 2009; International Institute of Tropical Agriculture, 2015). Winery waste causes pollution if not disposed properly and should leaching of the waste occur, organic acids from winery waste cause a threat to soil and groundwater (Dillon, 2011). To reduce its organic load so as to prevent pollution, winery waste can be converted to a useful product such as biogas.

Biogas is a gaseous fuel, rich in methane (50-80 %) and produced through a biological route in an anaerobic digestion (AD) facility using a wide variety of substrate biomasses (Demuynck, 1984; Abdeshahian et al., 2016; Bundhoo et al., 2016) [**Table 1**].

**Table 1:** Comparison of biogas and natural gas (Kar & Sahu, 2012)

| Component | Nomenclature | Natural gas (%) | Biogas (%) |
|---|---|---|---|
| $CH_4$ | Methane | 85 | 50-80 |
| $CO_2$ | Carbon dioxide | 0.7 | 20-45 |
| $C_2H_6$ | Ethane | 2.85 | - |
| $C_3H_8$ | Propane | 0.37 | - |
| $C_4H_{10}$ | Butane | 0.14 | - |
| $N_2$ | Nitrogen | 14.32 | Trace |
| $O_2$ | Oxygen | <0.5 | Trace |
| $H_2S$ | Hydrogen sulphide | <0.5 | 0-1.5 |
| $NH_3$ | Ammonia | - | 0-0.45 |

Studies have shown that cassava tuber can be used for the production of biogas by anaerobic digestion and so can cassava peels (Wang, 2002; Ubalua, 2007; Gerbens-Leenes et al., 2009; Okudoh et al., 2014). In some countries like Nigeria, cassava is used as a food source and about 10 Million tonnes (Mt) of cassava are processed for garri, a staple food annually (Ubalua, 2007) which generates large quantities of waste. The cassava peels during cassava processing are considered as wastes and this makes up about 20-35 % of the cassava tuber (Ubalua, 2007). In most west African countries like Nigeria where cassava is used as a food source, waste and wastewater from cassava processing would likely be used than the actual cassava biomass. However, in South Africa, cassava is only used for animal feed and starch production therefore the use of cassava biomass for energy production will not have an impact on the fuel versus food debate. In addition to the AD process producing biogas, it also produces a by-product known as a digestate which is useful as a fertilizer (Okudoh et al., 2014). Cassava has high levels of nutrients (Calcium, Phosphorus, Nitrogen and Potassium) [**Table 2**] which contributes to its digestate being a good fertilizer (Okudoh et al., 2014).

During wine-making, large amounts of winery waste (1.3 - 1.5 kg winery waste per litre of wine in every batch produced) are generated and often its disposal becomes a problem to the wine industry (Lucas et al., 2010). If this winery waste is not disposed correctly, it can cause pollution. The use of winery waste for the production of biogas serves as an alternative waste disposal technique for the wine industry as it decreases pollution and its conversion to biogas

helps alleviate the energy crisis. Winery waste is high in nutrients (Potassium, Nitrogen and Calcium) which makes its digestate a good fertilizer (Lucas et al., 2010).

Co–digestion is the simultaneous digestion of multiple substrates. In economic terms, producing biogas from one agricultural substrate is not sustainable. Therefore, to increase the biogas yield, co-substrates with a high methane potential should be co-digested (Al Seadi et al., 2008, Riaño et al., 2011; Ziganshin et al., 2013; Fitamo et al., 2016). Another reason for exploring co-digestion of substrates is to balance the carbon nitrogen (C/N) ratio of the feedstock for anaerobic digestion (Zhang et al., 2016).

### 1.2 Hypothesis

- Co-digestion of cassava and winery waste with zebra droppings inoculum will give more biogas yield than a single substrate

### 1.3 Research questions

- How efficient is the co-digestion of cassava biomass with winery waste in biogas production?
- What are the optimal process conditions for biogas production from cassava biomass and winery waste for optimal biogas yield?
- What are the factors that affect the production of biogas from cassava and winey waste?
- Which microbial population are dominant during co-digestion of cassava and winery waste?

### 1.4 Research aim and objectives

The aim of this research is to produce and optimise the yield of biogas from winery waste in co-digestion with carbohydrate rich cassava using South Africa as a case study.

Objective 1:

To determine the potential and efficiency of co-digestion of cassava biomass with winery waste for the production of biogas using a batch digester

Work plan:

Fresh waste/cassava biomass was collected from plantation areas in Bizana, Eastern Cape. Also fresh WW was collected from Agricultural Research Council, Stellenbosch winery farm.

The physical and biochemical characteristics of fresh waste/cassava tubers were determined using modern analytical methods (APHA, 2000). Microbial inoculum (seed culture) was prepared by mixing fresh Zebra droppings (ZD) collected from a Stellenbosch farm game reserve. The anaerobic digestion process was performed using a commercial batch digester from Glass Chem (Pty) Ltd. The preliminary digestion was at mesophilic temperature (37˚C) for 20 days with a total volume of 5L.

Activity: 1.1
Planning stage: Literature Review. Sample and Material collection

Activity: 1.2
Determine the physical and chemical characteristics of winery waste and cassava biomass using analytical methods. The composition of cassava biomass and winery waste using standard methods was analysed viz. total solids, ash, fibre, nitrogen, sugars and trace elements

Activity: 1.3
Running experiments to determine potential and efficiency of the digestion process. Winery waste only. Cassava feedstock only. Co-digestion of both cassava feedstock and winery waste.

Activity: 1.4
Changes in biogas yield, gas composition, pH, alkalinity, volatile fatty acids, temperature, TS, volatile solids and organic content will be monitored.

Activity: 1.5
Data collection on the biogas yield using Biogas 5000 (Geospeed) gas counters. Analysing the slurry and the effluent using standard methods (APHA).

Objective 2:
To optimize the production of biogas using response surface method (RSM).

Work plan:
Optimizing total biogas yield using central composite design (CCD) and response surface methodology (RSM).

Activity: 2.1
Design of experiments using response surface method. Running all necessary experiments as outlined by RSM and optimising the production yield.

Activity: 2.2
Optimizing pH, Temperature, substrate ratio for biogas methane production.

Objective 3:
To investigate the potential production of biogas when the volumes are scaled-up using the optimized conditions.

Work plan:
The optimal conditions will be applied to produce biogas in the scaled batch digester, 5L working volume.

Activity: Design and installation of large scale (5 L) digester completed. Final application of optimal pH, temperature, alkalinity, VFAs, TS, VS and starch content of biogas production will be carried out.

Objective 4:
To investigate the microbial community structure and dynamics during co-digestion of cassava and winery waste.

Work plan:
Investigating the influence of the feedstock type on the microbial community involved during co-digestion of cassava biomass and winery waste

Activity:
Sequencing and fingerprinting of genes. Genomic Deoxyribonucleic Acid (DNA) samples will be polymerase chain reaction (PCR) amplified. Resulting amplicons will be purified, end repaired and illumina specific adapter sequence will be ligated to each amplicon.

**1.5 Significance of study**

Socially, the use of biogas will assist mostly people who are underprivileged especially in rural areas who do not have access to electricity. Biogas use also assists in terms of agriculture as the digestate can be used as fertilizer. Also, in the South African energy industry, biogas will

ensure energy security and shall therefore meet the rising energy demands. Environmentally, due to biogas being a renewable energy, it will reduce greenhouse gas (GHG) emissions which contribute to global warming. The reduction of the amount of winery waste organic load and depollution are also a viable additional motivation for building such plants.

### 1.6 Delineation of study

Digestate conditioning and its quality management will not be carried out. This will be carried out in future studies.

# CHAPTER 2

# LITERATURE REVIEW

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Cassava and its Characteristics

### 2.1.1 Cassava

Cassava (*Manihot esculenta*) is a root crop mostly grown in the tropics and is a major source of calories in developing countries of west Africa such as Ghana and Nigeria and ranks sixth in overall global crop production (Rosales-Soto et al., 2016). It is also known as yucca or manioc and is a long starchy root tube 5 cm in diameter and 20 cm long (Trade, 2016) [**Figure 1A**]. The height grows between 1 & 4 m (**Figure 1B**) and its root can grow up to 15 cm in diameter and 120 cm in length to weigh between 1 & 8 kg. It is the highest producer of carbohydrates among staple crops (Adelekan & Bamgboye, 2009) with its roots (if the cassava plant is 1-1.5 years old), having a starch content of between 18 & 32 % (**Table 2**) [Sirirote et al., 2010]. It grows well in counties with tropical climate but can also grow in temperate climates and is mostly grown by farmers in some developing countries (Adelekan & Bamgboye, 2009). It is an excellent source of carbohydrates compared to protein, fat and vitamins (**Table 2**) [Okudoh et al., 2014] . There are two types of cassava viz a viz bitter (*M. esculenta or utillisima*) and sweet (*M. dulcis*) cassava and the sweet is commonly grown due to its ability to produce greater yields (Okudoh et al., 2014). According to (Sirirote et al., 2010), the sweet type has a lower hydrocyanic acid content, and is therefore used for human consumption and the bitter type contains a high quantity of hydrocyanic acid, and is used mostly as animal feed and for industrial products.


**Figure 1: A,** Cassava tuber from Bizana, South Africa**; B,** Cassava plant (Zhang et al., 2014)

### 2.1.2 Cassava Composition

The physical and chemical composition of the cassava biomass are shown in **Table 2**.

**Table 2**: Cassava composition determined from fresh cassava and pre-treated cassava obtained from Bizana in Eastern Cape

| Composition | Unit | Dried cassava | Pre-treated (degraded) cassava |
|---|---|---|---|
| Moisture | % | 5.5 | 9.4 |
| Total Solids | % | 94.45 | 88.56 |
| Volatile solids | % | 98.2 | 97.24 |
| Protein | % | 2.25 | 2.5 |
| Total nitrogen | % | 0.36 | 0.4 |
| Total carbon | % | 45.6 | 44.2 |
| Ash | % | 1.7 | 2.5 |
| Calcium | % | 0.01 | 0.02 |
| Phosphorus | % | 0.05 | 0.11 |
| Iron | mg/kg | 1.15 | 2.08 |
| Sodium | mg/kg | 359.75 | 301.2 |
| Potassium | % | 0.26 | 0.5 |
| Cyanide | mg/kg | 0.88 | 2.08 |

All microorganisms involved in anaerobic digestion (AD) require building blocks in the form of salts to function e.g. sodium, potassium and chlorine. **Table 2** shows that cassava biomass already has some of these salts and that there is no need for them to be added to the process separately which makes cassava a good substrate for AD. Proteins, lipids, and carbohydrates are responsible for the methane production in anaerobic digestion (**Table 2**) [Goswami, 2016].

### 2.1.3 Cassava Production and Consumption

Cassava can be cultivated in harsh environmental conditions including areas with low or extreme rainfall or infertile, poor and sandy soil. It is easy to grow, recovers from pest damage and can be left in the ground from 7 months to 2 years after planting. It is also drought tolerant, can withstand climate variability and can grow on marginal soils (International Institute of Tropical Agriculture, 2015). It can withstand temperatures ranging from 18 to 25 ˚C and rainfall of 50 to 5000 mm annually. South Africa has an average temperature of 28 ˚C in summer, 464 mm rainfall annually making it a perfect country to grow cassava (International Institute of Tropical Agriculture, 2015). In South Africa, it is cultivated in Limpopo, Mpumalanga, Eastern Cape and northern KwaZulu-Natal mainly because of its protein content (1-2 %) which makes it suitable for animal feed and the starch content (85 %) which makes it suitable for starch extraction (Rosales-Soto et al., 2016; Veiga et al., 2016). Currently, 20 000 tons of cassava

starch are produced commercially (Department of Agriculture Forestry & Fisheries [DAFF], 2010).

## 2.2 Winery Waste and its Properties

### 2.2.1 Winery Waste

Winery waste (WW) is produced from the winemaking process. It is characterized by high biodegradable content making it a good substrate for AD for biogas production.

### 2.2.2 Winery Waste Composition

A typical WW contains: (i) wastewater (generated from cleaning operations) (ii) solid organic waste (grape marc, skins, pips, pomace etc.), (iii) GHGs ($CO_2$, volatile organic compounds, etc.) and (iv) packaging waste. The organic solid winery waste consists of grape pomace and filter waste (Dillon, 2011). Grape pomace consists of approximately 8 % seeds, 10 % stems, 25 % skins and 57 % pulp (Dillon, 2011).

The volumes and the load of pollution of WW vary due to the working period (i.e. vintage, racking, bottling), and the type of the wine produced (e.g. red, white, sparkling, etc.) (Iannone et al., 2016). The WW also consists of crude fibres, grape seeds, skin waste, marc, stalk and skin pulp, proteins, ethers and amino acids (Lucas et al., 2010). The major composition of winery waste is shown in **Table 3**. In similarity to cassava, winery waste is composed of lipids, soluble sugars and proteins that are responsible for the methane production in AD.

### 2.2.3 Winery Waste Production

For wine production to take place, water, energy, fertilizers and supplements (mainly organic) are needed, and the process itself produces different waste streams (Iannone et al., 2016). During the wine making process, grapes are crushed, destemmed and while the grape juice is fermented to produce wine, the remaining grape skin, lees, stalks, pomace, and seeds are generated and are referred to as winery solid wastes (Iannone et al., 2016). For each litre of wine produced, 0.7 – 14 L of wastewater is generated during cleaning operations (Da Ros et al., 2016). According to Dillon (2011), in South Africa, a company that processes winery waste in Wolseley and Worcester processed 20 000 tonnes of grape pomace in 2008, and 25 000 tonnes in 2009.

**Table 3**: Major components of Winery solid waste (Goswami, 2016)

| Composition | Unit | Winery waste |
|---|---|---|
| Moisture | % | 1.15 |
| Total Solids | % | 95.92 |
| Volatile solids | % | 83.86 |
| Protein | % | 11 |
| Total nitrogen | % | 1.76 |
| Total carbon | % | 50.40 |
| Ash | % | 15.95 |
| Calcium | % | 0.06 |
| Phosphorus | % | 0.16 |
| Iron | mg/kg | 28.05 |
| Sodium | mg/kg | 1191.9 |
| Potassium | % | 1.77 |
| Cyanide | mg/kg | 0.92 |

## 2.3 Biogas and its Production

### 2.3.1 Biogas

Biogas is a gaseous fuel produced through a biological route in an AD in which microorganisms break down biodegradable materials such as carbohydrates, proteins, and fats, into a mixture of methane ($CH_4$) and carbon dioxide ($CO_2$) and trace amounts of hydrogen ($H_2$), nitrogen ($N_2$), hydrogen sulphide ($H_2S$), and ammonia ($NH_3$), in the absence of dissolved oxygen (DO) (Ofoefule & Uzodinma, 2009). The heating value of 1 $m^3$ of biogas comprising 60 methane is estimated to be 21.5 MJ (Surendra et al., 2014).

### 2.3.2 Biogas Composition

The composition and quantity of the biogas are largely dependent on the feedstock used. Biogas composition is shown in **Table 4**. The main component of biogas, $CH_4$, constitutes about 50 – 80 % followed by $CO_2$ with 20 – 45 % (**Table 4**) (Anunputtikul & Rodtong, 2004) .

**Table 4**: Biogas composition (Anunputtikul & Rodtong, 2004)

| Gas | Concentration (%) |
|---|---|
| Methane ($CH_4$) | 50-80 |
| Carbon dioxide ($CO_2$) | 20-45 |
| Hydrogen sulphide ($H_2S$) | 50-5000ppm |
| Ammonia ($NH_3$) | 0-0.45 |
| Water ($H_2O$) | 0-10 |
| Nitrogen ($N_2$) | 0-5 |
| Oxygen ($O_2$) | 0-2 |
| Hydrogen ($H_2$) | 0-1 |

The theoretical biogas composition and yield can be calculated using the Busswell equation (**Equation 1**). According to Busswell equation, lipids yield more biogas (**Equation 1**). Also, the amount of volatile solids (VS) present in a substrate determines the organic matter of the substrate and the higher the VS, the higher the methane production (Goswami, 2016).

Busswell equation:

$$\boldsymbol{C_aH_bO_c + \left(a - {b}/{4} - {c}/{2}\right)H_2O \rightarrow \left({a}/{2} - {b}/{8} + {c}/{4}\right)CO_2 + ({a}/{2} + {b}/{8} - {c}/{4})CH_4} \quad (1)$$

For substrates containing proteins, nitrogen and sulphur, the Busswell equation is as follows:

$$\boldsymbol{C_aH_bO_cN_dS_e + [(4a - b - 2c + 3d + 2e)/4]\,H_2O \rightarrow [(4a - b + 2c + 3d + 2e)/8]\,CO_2 + [(4a + b - 2c - 3d - 2e/8)]CH_4 + dNH_3 + eH_2S} \quad (2)$$

**Equation 1** and **Equation 2** are applied to glucose, acetic acid, pyruvate and lipids and assume 100% conversion of substrate to biogas.

2.3.3 Microbiology and Stages of Biogas Production

The process of biogas production is a microbial process, which involves 4 steps, with each step using a particular bacterial community. The 4 steps involved in the conversion of biomass to biogas are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (**Figure 2**) [Intanoo et al., 2016; Bajpai, 2017].

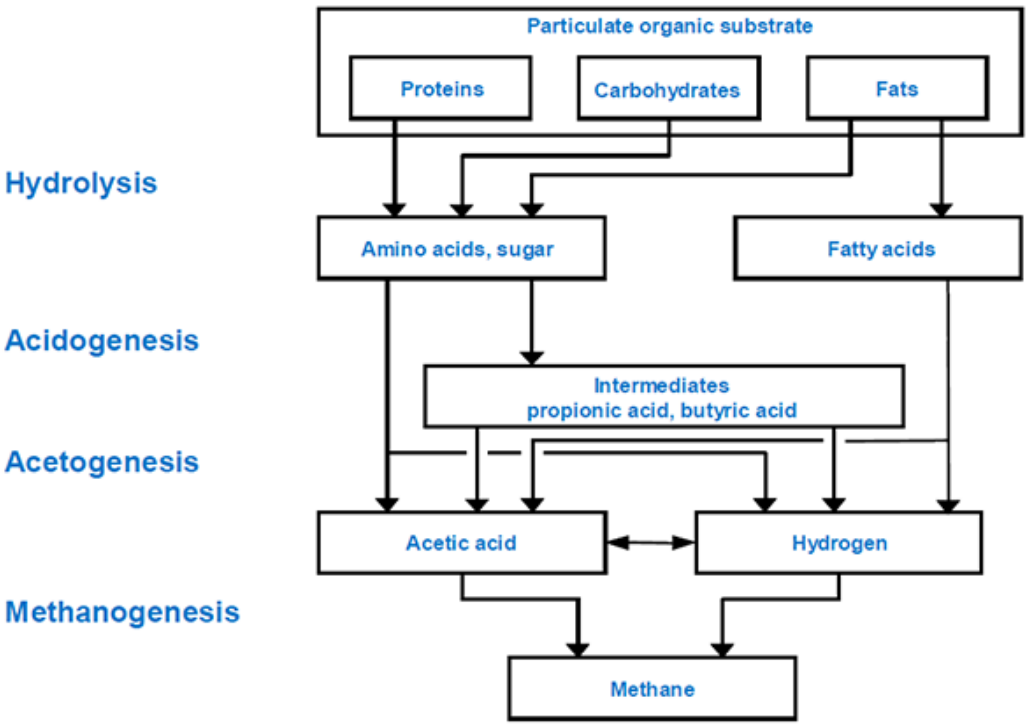


**Figure 2**: Flow chart showing stages/steps in the biogas production process (Bajpai, 2017)

During biogas production (**Figure 2**), complex high molecular weight carbohydrates, fats and/or proteins are hydrolysed into soluble polymers by means of the enzymatic action of hydrolytic bacteria and converted into organic acids, alcohols, $H_2$ and $CO_2$ (Demuynck et al., 1984). Volatile fatty acids (VFAs) and alcohols are then converted to acetic acid by acetogenic bacteria and finally methanogenic bacteria convert acetic acid formed during acetogenesis into $CO_2$ and $CH_4$ (Angelidaki et al., 2009). When the AD process is complete, the biomass is converted into biogas (mainly methane & carbon dioxide) with the resultant digestate discharged into vegetable gardens as fertiliser.

*Hydrolysis*

During hydrolysis, the molecule's bonds are broken by using water. This is the hydrolysis of biomass made up of materials such as carbohydrates, fats and proteins by hydrolytic bacteria to amino acids, fatty acids and simple sugars (Intanoo et al., 2016) [**Figure 2**].

*Acidogenesis*

This is the second step of anaerobic digestion following hydrolysis. In this step acidogenic bacteria break down the products obtained from the previous step (i.e. fatty acids, amino acids and sugars) to produce ammonia, $H_2$, $CO_2$, $H_2S$, shorter VFAs, carbonic acids, alcohols, as well as trace amounts of other by-products (Jorgensen, 2009; Intanoo et al., 2016). Even after acidogenesis has occurred the organic matter is still too large and cannot be used for methane production, so the biomass continues to the third stage of the process viz. acetogenesis.

*Acetogenesis*

In this step, acetogenic bacteria form acetate from carbon and energy sources. The acetogenic bacteria breaks down many of the products created in acidogenesis into acetic acid, $CO_2$ and $H_2$ (Jorgensen, 2009; Intanoo et al., 2016) [**Equations 3 - 5**]. Acetogenic bacteria break down the biomass to a point where it becomes utilizable by methanogenic bacteria for the production of methane.

According to Okudoh et al. (2014), the acetogenesis reactions are as follows:

$$2CH_3CH_2OH + 2H_2O \longrightarrow 2CH_3CO_2H + 4H_2 \quad (\Delta G^{\circ} \sim +19\ kj/mol) \quad (\mathbf{3})$$

$$4H_2 + CO_2 \longrightarrow 2H_2O + CH_4 \quad (\Delta G^{\circ} \sim -131\ kj/mol) \quad (\mathbf{4})$$

$$2CH_3CH_2OH + CO_2 \longrightarrow 2CH_3CO_2H + CH_4 \quad (\Delta G^{\circ} \sim -112\ kj/mol) \quad (\mathbf{5})$$

*Methanogenesis*

This is the fourth and last step of anaerobic digestion. Methanogenic bacteria produce methane from the products (mainly acetate) of acetogenesis as well as from some of the products formed during hydrolysis and acidogenesis (**Equations 6 - 7**). There are two ways to create methane in methanogenesis:

1. Hydrogenotrophic- carbon dioxide reacts with hydrogen to form methane and water.

2. Acetoclastic- acetate is converted to methane and carbon dioxide (Okudoh et al., 2014).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (\mathbf{6})$$

$$CH_3CO_2H \rightarrow CH_4 + CO_2 \quad (\mathbf{7})$$

According to Galand et al. (2005), in peatlands, the acetoclastic methanogenesis is the main pathway for producing methane than the hydrogenotrophic. Mach et al. (2015) also suggested that the acetoclastic methanogenesis is the most dominating pathway however for freshwater sediments and gut environments, the dominating methanogens are hydrogenotrophic.

### 2.4 Microbial Community Dynamics in Anaerobic Digestion

In order for biogas to be produced from an anaerobic digestion process, different species of microorganisms must be active and working together. These microorganisms need food in order to survive and grow. A substrate is used to feed the microorganisms which should contain a source of energy, vitamins and some trace elements (Schnürer & Jarvis, 2010). During anaerobic digestion, the microorganisms use the substrate to form new cells and the

metabolism yield different types of waste products. The waste products formed by a specific microorganism can be used as food by another (Schnürer & Jarvis, 2010). Different microorganisms use each other's waste products as substrates. Microbial waste products in a biogas process are fatty acids, carbon dioxide, and hydrogen (Intanoo et al., 2016).

The feedstock type has an influence on the microbial communities involved in anaerobic digestion (Ziganshin et al., 2013). The conversion of biomass to biogas in an anaerobic digestion is as a result of bacterial activities. Hydrolysis, acidogenesis and acetogenesis occur by distinct bacterial communities and methanogenesis is as a result of methanogenic bacteria. In a study of microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials by Ziganshin et al. (2013), several clostridia phylotypes were found in the anaerobic digesters with substrates containing chicken manure combined with cattle manure, cattle manure alone or in combination with maize straw or distillers grains. Under mesophilic conditions in a plant biomass fed anaerobic digester, *Clostridium spp.* are the most dominant bacterial class (Ziganshin et al., 2013).

Methanogenic archaea is responsible for the production of methane and carry the key enzyme of methanogenesis viz. co-enzyme B sulfoethylthiotransferase (Lebuhn et al., 2014). However, according to Schnürer & Jarvis (2010), there are only 2 groups of methanogens that are responsible for breaking down the acetate viz. *Methanosaeta* and *Methanosarcina*. A number of organisms such as *Methanobacterium, Methanococcus, Methanogenium* and *Methanobrevibacter* use hydrogen gas to form methane (Schnürer & Jarvis, 2010). All of these methanogens are part of a group of organisms called Archaea (Schnürer & Jarvis, 2010).

### 2.5 Biomethane Potential

Biomethane potential (BMP) is a test done with the intention to investigate the ultimate potential of an organic substrate in anaerobic digestion to produce methane (Angelidaki et al., 2009). This test assesses the biodegradability of substrates where microbiological, biochemical and physico-chemical aspects of the substrates are determined. They are conducted in batch, bench scales reactors and measure the amount of methane produced per gram of VS destroyed. The test can either be done on pure substrates or on a combination of substrates (co-digestion). The results obtained from BMP tests are important for validation and calibration of mathematical models (Esposito et al., 2012). According to Esposito et al. (2012), co-digestion of substrates resulted in higher biodegradability than a single substrate.

## 2.6 Bioreactor Technologies

Biogas production can be classified on the basis of mode (batch or continuous) and complexity (single or multi-stage) (Rowse, 2011).

### 2.6.1 Batch Systems

In a batch system, the influent feedstock is digested in a closed digester for a long time usually from 8 weeks to several months (Demuynck et al., 1984). It is advantageous due to its ease of operation and high removal efficiency of contaminants (Rowse, 2011). The problem with batch methane digesters is that the biogas production rate is very irregular (Demuynck et al., 1984). It is noticed to be high at the beginning of the digestion period and very low near its end. This system is very appropriate and popular for the biogas production of solid residues (Rowse, 2011).

### 2.6.2 Continuous Systems

This is an alternative to the batch digester and is often referred to as the completely mixed continuous system. Fresh influent feedstock biomass is added, and the digested mixed liquor is regularly removed, if not continuously (Rowse, 2011). If the conditions of the digester remain stationary, and the digester volume is kept constant then the biogas production rate will be more or less constant. An average production rate of 1 $m^3$ of biogas per $m^3$ working volume of digester per day can normally be obtained from a completely mixed continuous system (Demuynck et al., 1984). According to Demuynck et al. (1984), one of the disadvantages of the continuous system is that, for mechanical reasons, the concentration of the influent feedstock to be treated should not exceed 100 kg dry matter per $m^3$ of influent feedstock as it is found that at higher concentrations, the feedstock is no longer pumpable. In addition to that disadvantage, each time the methane digester is fed, the same quantity of digested mixed liquor comes out of the digester and carries away also part of the bacteria. The methanogenic bacteria have a low specific growth rate and to multiply and increase their number, they require about 2 to 5 days (Demuynck et al., 1984). As a result, the active biomass remains limited in a completely mixed continuous system and this limits the maximum possible biogas production rate (Rowse, 2011).

### 2.6.3 Single-stage

A single digester is used for this configuration and all the reactions (hydrolysis, acidogenesis, acetogenesis and methanogenesis) take place inside this digester (**Figure 3**). The pH of the tank needs constant monitoring as it can be reduced by the presence of acidogenic bacteria in the tank (Gulzow, 2010).

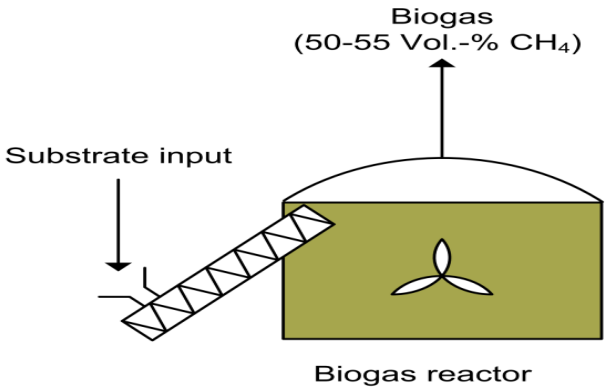


**Figure 3**: Layout of a single-stage digester (Xu et al., 2017)

2.6.4 Two-stage

For a two stage configuration, the first digester is for hydrolysis/acidogenesis and the other for acetogenesis/methanogenesis (**Figure 4**) (Aslanzadeh et al., 2013). Two reactors connected in series are used in a multi-stage configuration digester and separating the reactors into different stages assists in optimizing conditions auspicious to the growth of each group of organisms in each reactor (Aslanzadeh et al., 2013).

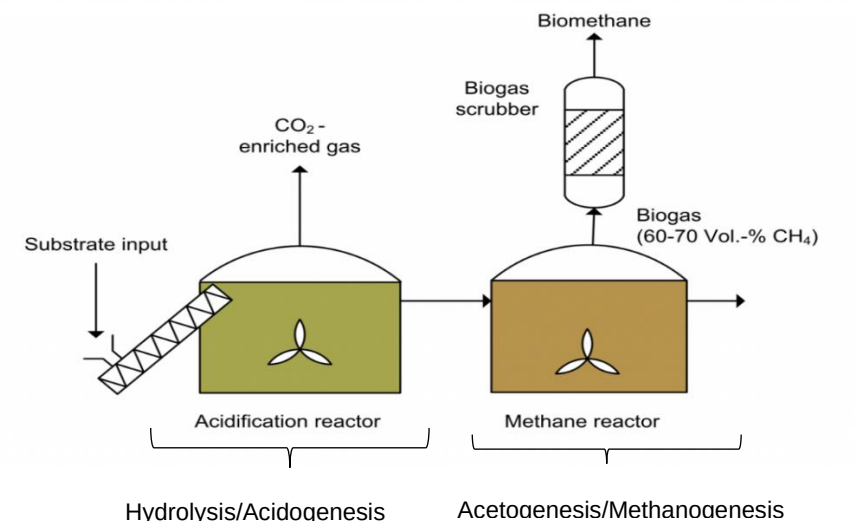


**Figure 4**: Layout of a two-stage digester (Xu et al., 2017)

### 2.7 Factors Affecting Biogas Production

The environmental conditions in which the reactions take place influences the yields and rates of the biogas production process. According to Dobre et al. (2014), there are two classes of factors that affect methane production viz. the physical and the chemical factors. The physical factors include temperature and mixing and the chemical factors include pH, the redox potential and the carbon to nitrogen (C/N) ratio. According to Kar & Sahu (2012), other factors affecting

methane production are the composition of the feedstock and the characteristics of the inoculum.

### 2.7.1 Temperature

The biogas production process is closely related to the temperature of the digester (Luo et al., 2010). There are various types of microorganisms viz. psychrophilic (~10 ˚C), mesophilic (~37 ˚C), thermophilic (~50 ˚C), and extremophilic/hyperthermophilic (>65 ˚C), therefore the temperature at which they grow and function also varies. The optimum temperature suitable for each type of microorganisms is closely related to the environment from which they originate (Schnürer & Jarvis, 2010). Microorganisms from human/animal intestines will likely grow best at 37 ˚C whereas those found in septic tanks best thrive at low temperatures (~10 ˚C).

The higher the temperature, the more biogas is produced (provided that the bacteria used are acclimatised to that climate) therefore at psychrophilic temperatures, biogas production rate is low. According to Schnürer & Jarvis (2010), temperatures normally used for biogas production are either mesophilic (37 ˚C) or thermophilic (55 ˚C). Another study conducted by Dobre et al. (2014) and Hobson et al. (1981) found that the greatest biogas production occurred when the digester temperature was in the mesophilic range of 32 to 40 °C. Most anaerobic digesters are operated at mesophilic conditions as it does not usually pay to run at psychrophilic conditions and only a small number of plants operate at thermophilic (above 55 ˚C) conditions.

### 2.7.2 Mixing

A methane digester requires a mixing device to increase contact between substrates and bacteria. There are several sophisticated mixing devices used for methane mixing but propellers or paddles are the most used. The effects of mixing the methane digester are not yet well known. Mixing is not compulsory for the methanogenic biology to take place (Demuynck et al., 1984). The main purpose of mixing is to achieve homogeneity inside the methane digester, to reduce scum formation and to also keep the anaerobic digester contents in suspension (Demuynck et al., 1984).

### 2.7.3 pH

Biogas production process requires an environment with neutral pH (i.e. pH 7) and when the value is below 6 or above 8, the process will be inhibited (Lay et al., 1997). According to Adelekan *&* Bamgboye (2009), a pH of between 7 and 8.5 is best for biogas production and normal gas production. The pH in the digester is a function of alkalinity, and takes into account, the concentration of VFA and the presence of bicarbonate ($HCO_3^-$). A rise in VFA concentration results in bicarbonate anions inadequacy and failure to maintain the desired pH level which therefore requires more bicarbonate (Lay et al., 1998).

### 2.7.4 Redox Potential

Anaerobic microorganisms in the AD process are very sensitive to the presence of oxygen. According to Kar & Sahu (2012), when organic compounds are broken down in the presence of oxygen, carbon dioxide will be produced but when the same compounds are broken down in the absence of oxygen, methane is produced. Kar & Sahu (2012), also noted that the presence of oxygen will inhibit the biogas production. It is therefore of utmost importance that the biogas digester be kept airtight.

### 2.7.5 Carbon/Nitrogen Ratio (C/N ratio)

The C/N ratio expresses the relationship between the mass of carbon and nitrogen present in organic materials (Adelekan & Bamgboye, 2009) and usually affects the biogas yield. The ideal C/N ratio for AD is between 20:1 and 30:1. At C/N ratio beyond this range, the nitrogen will be consumed rapidly by methanogenic bacteria thereby lowering the biogas production (Kar & Sahu, 2012; Adelekan & Bamgboye, 2009). Conversely, at lower C/N ratio, nitrogen will accumulate in the form of ammonia (Montingelli et al., 2015). Ammonia raises the pH value of the digested slurry. If the pH value is raised higher than 8.5, it will be toxic to the methanogenic bacteria in the digester and will reduce biogas production.

Adelekan & Bamgboye (2009), investigated biogas productivity of cassava peels, and the results showed that they have high organic carbon and low total nitrogen, and consequently a high C/N ratio. High C/N ratio results in the rapid consumption of nitrogen thereby not producing biogas appreciably (Kar & Sahu, 2012). However, the work points out that a material with high C/N ratio could be co-digested with another with a much lower C/N ratio to alleviate the ratio to an acceptable value of between 20 and 30.

### 2.7.6 Volatile Solids

Babaee & Shayegan (2011), described the volatile solids to be the part of the organic material solids which is biodegradable (i.e. can be digested by micro-organisms) while the rest of the solids is fixed and non-biodegradable. The concentration of the solids of the influent loaded into the digester affects the rate of biogas production (Rasi et al., 2011).

### 2.7.7 Organic Loading Rate

The organic loading rate is defined as the amount of volatile solids (fermentable solids) per unit of active biodigester volume per day (Kar & Sahu, 2012). The typical values of organic loading rates are reported as between 0.2 and 2 kgVS/$m^3$/day (Kar & Sahu, 2012), depending on the types of waste fed into the digester (Babaee & Shayegan, 2011). The type of waste

usually determines the level of biochemical activity that will occur in the digester (Babaee & Shayegan, 2011).

2.7.8 Hydraulic Retention Time

This is the average amount of time one reactor volume of actively digesting sludge stays within the reactor (Kim et al., 2006). Its numeric value is defined as:

$$\boldsymbol{\theta = \frac{V}{Q}} \quad \textbf{(8)}$$

Where ɵ, hydraulic retention time (d); V, volume of reactor ($m^3$) and Q, influent flow rate ($m^3$/d) HRT has a significant impact on biogas production. It must be long enough to retain the methanogens as these have long retention time compared to hydrolysis and acidogenesis bacteria (Shi et al., 2017). Ma et al. (2013) ran a sequential batch reactor treating a dilute waste stream which resulted in a failure when the HRT was less than 2 days due to the methanogenic microorganism's growth limits. Various studies have shown the optimum HRT to be about 20 days (Shi et al., 2017; Kaosol & Sohgrathok, 2012).

2.7.9 Solids Retention Time

Solids retention time is defined as "the mass of active biomass in the reactor divided by the mass of active biomass removed from the system each day" (Rittmann & McCarty, 2001). Its numeric value can be obtained from Equations 9 and 10 below:

$$\Theta_{\boldsymbol{c}} = \frac{\boldsymbol{active\ biomass}}{\boldsymbol{production\ rate\ of\ active\ biomass}} \quad \textbf{(9)}$$

$$\Theta_{\boldsymbol{c}} = \frac{\boldsymbol{V * X}}{\boldsymbol{Q_w * X_w}} \quad \textbf{(10)}$$

Where $\theta$c, Solids retention time (d); V, reactor volume ($m^3$); X, cell concentration in reactor (mg/L); Qw, flow rate out of reactor ($m^3$/d); Xw, cell concentration flowing out of the reactor (mg/L).

2.7.10 Volatile Fatty Acids (VFA)

VFAs are important intermediate products during AD therefore, its monitoring is of vital importance. They are a good parameter used to monitor biogas production from anaerobic digestion (Lützhøft et al., 2014) as they indicate the state and activity of microorganisms inside a digester. High VFA concentrations exceeding 13000 mg/L can be limiting for AD (Viéitez & Ghosh, 1999). VFA accumulation results in decreased pH which directly affects methanogenic

bacteria growth inside the digester and if left for long periods of time can result in acetogenic bacteria being formed. The most common VFAs in AD are acetic acid, butyric acid and propionic acid. Propionic acids and butyric acids are known to be methanogenic bacteria inhibitors hindering biogas production while acetic acid promotes methanogenesis resulting in biogas production (Nguyen, 2014). Acetic acid is directly linked to the anaerobic digestion end-product because during methanogenesis, acetic acid is converted to methane and $CO_2$. However according to (Dong-Jin et al., 2015) acetic and butyric acid should be the most predominant VFAs in anaerobic digestion. Some of the methods used to measure VFAs are gas chromatography, HPLC, titration method and so on (Lützhøft et al., 2014).

#### 2.7.11 Substrate/Inoculum Ratio (SI ratio)

Among other factors that ensure optimum biomethane conversion is the substrate/inoculum ratio. It is one of the primary drivers for a successful conversion of complex organic substances to methane. In order to complete the conversion process, provision of sufficient inoculum is required. The amount of inoculum added determines the yield and rate of methane produced. Eskicioglu & Ghorbani (2011) concluded that the yield and rate of methane is substrate and inoculum specific.

### 2.8 Biomass Pre-treatment Techniques Used in Anaerobic Digestion

The need for pre-treatment arises when the rate of breaking down of the substrate is slow thereby slowing biogas production. Slow breakdown of substrates could be caused by several factors including (i) toxic chemicals that inhibit the growth and activity of microorganisms, (ii) components prone to foaming or clumping and (iii) molecular structure not easily accessible to microorganisms (Bochmann & Montgomery, 2014).

There are various pre-treatment techniques used for energy crops. Some of these techniques include mechanical, ultrasonic, thermal, chemical and biological procedures. Some of these pre-treatment techniques are used in combinations. According to Okudoh et al. (2014), a possible combination for cassava pre-treatment is mechanical, biological and chemical pre-treatment technology.

#### 2.8.1 Mechanical

Milling, grinding and shredding are referred to as a mechanical pre-treatment technique for anaerobic digestion (Rodriguez, 2015). Mechanical pre-treatment grinds solid particles of the substrate so as to increase the substrate's surface area which enhances better contact between the substrate and bacteria (Ariunbaatar, 2014).

### 2.8.2 Ultrasonic

Ultrasonic pre-treatment technique uses waves to disrupt the substrate's cells in order to promote cavitation inside the cell (Rodriguez, 2015). This enhances the contact between the substrate and anaerobic bacteria (Zeynali et al., 2017). Rincon et al. (2014) investigated the effect of ultrasonic pre-treatment on two-phase olive mill solid waste (OMSW). The OMSW was ultrasonically pre-treated at a power of 200W and frequency of 24 kHz over a period of time. The maximum methane production rate was found to be 12 % higher than that obtained for untreated OMSW (Rincon et al., 2014). Wu-haan (2008) also evaluated ultrasonic pre-treatment on anaerobic digestion of biomass for methane production for corn-ethanol by-products. The results of the investigation showed that an increase in ultrasonic amplitude and treatment time resulted in an overall increase in methane produced (Wu-haan 2008).

### 2.8.3 Thermal

This pre-treatment technique utilises temperature to pre-treat the substrate to increase its solubilisation (Rodriguez, 2015). It also facilitates pathogen removal, improves dewatering performance and reduces viscosity of the digestate (Ariunbaatar, 2014). Gonzalez-Fernandez et al. (2012) investigated the thermal pre-treatment to improve methane production of *Scenedesmus* biomass. The experiments were performed at 70 and 90°C. The results showed the biomass pre-treated at 70 °C attained 22-24 % anaerobic biodegradability whereas the biomass pre-treated at 90 °C attained 48 % (Gonzalez-Fernandez et al., 2012).

### 2.8.4 Chemical

Acidic, alkali and oxidative pre-treatments solubilise polymers thereby favouring microbial degradation. It is used to achieve destruction of the organic compounds (Ariunbaatar, 2014). Acid pre-treatment involves addition of concentrated or dilute acids (Badiei et al., 2014). Strong acids can be sulphuric or hydrochloric acids. Alkaline chemicals include soaking the biomass in alkaline solutions such as calcium, potassium, sodium and ammonium hydroxide at a certain temperature for a certain amount of time (Badiei et al., 2014). Oxidative pre-treatment involves treating the biomass with oxidative agents such as ozone, hydrogen peroxide or oxygen (Badiei et al., 2014).

### 2.8.5 Biological

This pre-treatment technique is used to degrade the lignin and the hemicellulose in the biomass (Rodriguez, 2015). It can include both anaerobic and aerobic methods (Ariunbaatar, 2014). Aerobic treatment can be composting the substrate prior to AD. According to Rodriguez (2015), anaerobic pre-treatment occurs when hydrolysis and acidogenesis steps are separated from the final methane production step. Zhuo et al. (2018) investigated the use of bacteria for

improving the lignocellulose biorefinery process. The potential contribution of bacteria to lignocellulose pre-treatment was evaluated by physicochemical changes of corn stover with *Pandoraea* sp. B-6 bacterial strain before and after pre-treatment. The result obtained showed no difference in digestibility between the pre-treated and untreated corn stover (Zhuo et al., 2018). Hamidi (2006) investigated the effect of different biomass concentration on biological pre-treatment to chemical pulping process. Three different biomass concentrations (B1, B2 & B3) were used to biologically pre-treat banana stem waste. Once the stem wastes have been treated, they proceeded onto chemical pulping process. From this study, it was proven that the higher the biomass concentration, the higher the percentage of lignin degradation (Hamidi 2006). During the chemical pulping process, the pre-treated banana stem waste degraded the lignin faster than the unpretreated waste (Hamidi 2006). The biological pre-treatment helped reduce time and energy consumption in the chemical pulping process.

# CHAPTER 3

# MATERIALS AND METHODS

# CHAPTER 3

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Experiments were divided into four phases (Biomethane potential, Biogas Optimization, Up scaling using Single Stage 5 L Batch Digester and Microbial Community Structure & Dynamics Inside the 5 L Batch Digester) to achieve the aim and four objectives.

## 3.1 Sample Collection & Preparation

Fresh cassava (*Manihot esculenta* Crantz) [**Figure 5**] biomass was collected from plantation areas in Bizana, Eastern Cape in South Africa and stored in the refrigerator at 4 ˚C prior to utilisation. Some cassava samples were left to degrade under its natural flora (*Aspergillus niger* and *Rhizopus sp*.) to soften the tubers prior to digestion. Cassava was chopped into small pieces (1 $cm^3$) and oven dried for 48 hours and then milled into powder. Similarly, fresh winery waste was collected from a winery farm in Agricultural Research Council, Stellenbosch, South Africa. It was sun dried and milled into powder using a scientific RSA hammer mill SER no. 400 equipped with 2 mm sieve mesh (Okudoh et al., 2014).

**Figure 5**: Photo of cassava tuber from Bizana, Eastern Cape, South Africa

## 3.2 Analytical Methods

The collected cassava and winery waste were analysed for pH, total solids (TS), volatile solids (VS), ash content and moisture content in accordance with the standard methods (APHA, 2005). Total nitrogen, total organic carbon, calcium and phosphorus were also measured.

### 3.3 Microorganisms and Growth Medium

Fresh Zebra (*Equus quagga burchelli*) droppings (ZD) collected from a Stellenbosch farm game reserve were used as inoculum to start-up the experiment (Ellis & Smith, 2011). Some areas like Limpopo, KwaZulu-Natal and Western Cape have game farm reserves with zebras and the neighbouring rural areas can access these games reserves to collect the dung. For those areas that do not have zebras inoculum with similar characteristics can be used instead. The samples were collected in sterile plastic bags and stored in a refrigerator set at 4 ˚C prior to analysis. Before utilization, zebra dung was soaked in warm water and incubated at 37 ˚C for 7 days. It was then sieved and used as an inoculum for all the experiments (**Figure 6A & 6B**).


**Figure 6**: Preparation of inoculum: A, Zebra dung being sieved; B, sieved inoculum

### 3.4 Biogas Collection

The volume of the biogas produced was measured by downward displacement of water and collected by means of water-column. The gas was scrubbed using 1 M sodium hydroxide before collection. All gas volumes reported were corrected to STP (0 °C, 110.3 kPa) according to literature. The net biogas formed in each bottle with both substrate and inoculum was

subtracted from the gas formed from the bottle that has the inoculum only. This was done to account for the biogas formed from just the inoculum as determined from **Equation 11**.

$$Biogas\ produced(ml) = biogas\ from\ substrate(ml) - biogas\ from\ control(ml) \quad (\mathbf{11})$$

The generated methane volume was normalised against the VS using **Equation 12**

$$Cumulative methane\ yield(mLCH_4/gVS) = Net\ cumulative methane(mLCH_4)/Mass\ of\ VS\ added(g) \quad (\mathbf{12})$$

### 3.5 Experimental Set-up and Procedure

3.5.1 BMP Set-up and Procedure

The BMP test system for this study was conducted under reproducible and controlled conditions (**Figure 7**). Four experiments were conducted in triplicates i.e. twelve glass bottles with a volume of 500 ml each, were submerged in a water bath. The water bath was kept constant at 37 ˚C±0.5 throughout the duration of the experiments. After the bottles were filled up with the inoculum and substrates, pH was measured and when necessary was adjusted to pH 7 using 1 M sodium hydroxide (NaOH) or 32 % hydrochloric acid (HCl) solution prior to fermentation.

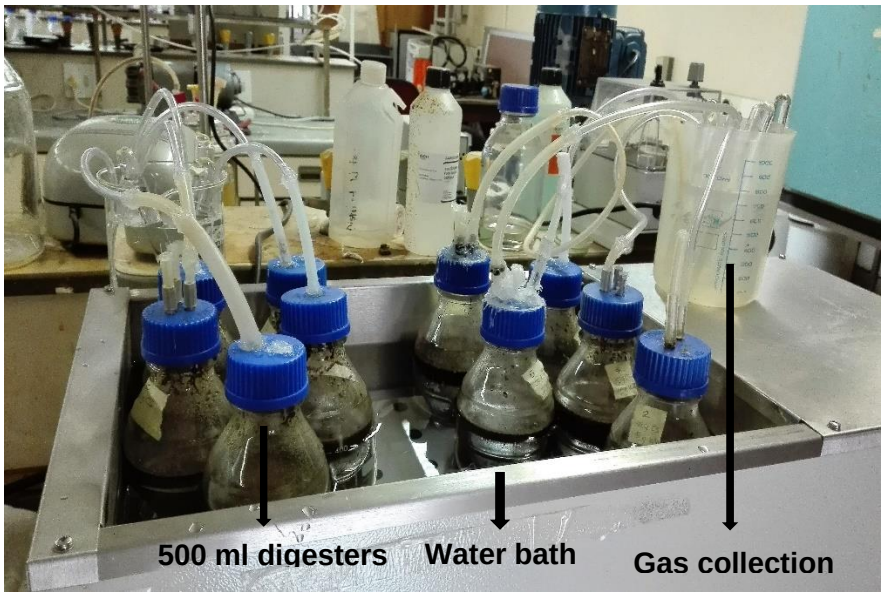


**Figure 7**: Picture showing BMP and optimization set-up

To rid the BMP test bottles of the oxygen and other gases, the contents of the bottle were bubbled with nitrogen gas for 3 minutes so as to remove all dissolved oxygen and the bottles were sealed immediately. The bottles were shaken manually twice a day. This was done to

achieve homogeneity inside the reactor, free trapped gases and to prevent scum accumulation. The 12 batch reactors were inoculated as shown in **Table 5**. 25 g of Zebra dung was filtered and measured to 250 ml for each experiment. Bottles 1-3 were each inoculated with 250 ml of zebra dung. This experiment with inoculum only is known as a control experiment and was run as a baseline for comparison. For bottles 4-6, each bottle was inoculated with 250 ml zebra dung and 25 ml cassava. Bottles 7-9 were each inoculated with 250 ml zebra dung and 25ml winery waste. Lastly, bottles 10-12 were inoculated with 250 ml zebra dung, 12.5 ml cassava and 12.5 ml winery waste. The last experiment was referred to as the co-digestion experiment. The experiments were terminated after 30 days.

**Table 5**: Bio Methane Potential Inoculation

| | Bottles 1-3 | Bottles 4-6 | Bottles 7-9 | Bottles 10-12 |
|---|---|---|---|---|
| Inoculum (ml) | 250 | 250 | 250 | 250 |
| Cassava (g) | 0 | 25 | 0 | 12.5 |
| Winery waste (g) | 0 | 0 | 25 | 12.5 |

Constant temperature was maintained by a water bath at set 37 ˚C±0.5 and checked by means of thermometers dipped in the bath. The pH was measured by a Crison basic 20 pH meter at room temperature before and after the experiments. Volatile fatty acid content was measured and quantified using HPLC before and after digestion.

### 3.5.2 Biogas Optimization Set-up and Procedure

#### *3.5.2.1 Experimental design and modelling*

Central composite design (CCD) was used for biogas optimization from co-digestion of cassava and winery waste. **Table 6** shows the chosen parameters for optimization which were pH ($X_1$), temperature ($X_2$) and co-digestion ratio ($X_3$).

**Table 6**: Factor levels showing real and coded values of the experimental plan

| Variable | Parameters | Level | | | | |
|---|---|---|---|---|---|---|
| | | -2 | -1 | 0 | 1 | 2 |
| $X_1$ | pH | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 |
| $X_2$ | Temperature | 25 | 30 | 35 | 40 | 45 |
| $X_3$ | Cassava: winery waste | 40:60 | 60:40 | 70:30 | 100:0 | 0:100 |

Each of the chosen variables was studied at five different levels on the CCD. The levels were allocated as -2, -1, 0, +1, and +2 respectively as shown. The output variable was the biogas yield (mLCH4/gVS).

*3.5.2.2 Regression modelling*

A second order polynomial was fitted to the model so as to determine the optimal point. The regression model was calculated by analysing the analysis of variance (ANOVA), p-and F-value. Co-efficient of determination, $R^2$ was used to express the adequacy of the model.

*3.5.2.3 Experimental procedure*

The optimization test system for this study was conducted under reproducible and controlled conditions. Twenty batch digestion tests experiments were conducted in 500 ml Duran Scott bottles which were submerged in a water bath. Cassava and winery waste were mixed at different ratios of 0:100, 40:60 & 70:30 and then incubated in a water bath at different temperatures (25, 35, and 45 ˚C ±0.5). The pH values (6.0 and 8.0) were adjusted to neutral (7.0) using 32 % hydrochloric acid (HCl) and 1 M sodium hydroxide (NaOH). Gas flow rate was measured daily by downward displacement of water. Each experiment lasted for 15 days and to rid the bottles of the dissolved oxygen and other gases, each bottle was flushed with nitrogen gas for 3 minutes. The bottles were shaken manually before reading each biogas flow rate. This was done to achieve homogeneity inside the reactor, free trapped gases and to prevent scum accumulation.

### 3.5.3. Up scaling Using Single-stage 5 L Mesophilic Batch Digester

The biogas production system for this study was conducted under reproducible and controlled conditions. A single-stage mesophilic batch digester with a volume of 5 L was used (**Figure 8**) to scale-up the experiment under optimized conditions. They were run at 35 ˚C±0.5, pH 7 and with a co-substrate ratio of cassava to winery waste (70:30). 250 g of zebra dung was

measured and mixed with water to make up 2.5 L. 175 g cassava and 75 g winery waste (70:30) was also measured and used. The pH was adjusted using 1 M sodium hydroxide (NaOH) or 32 % hydrochloric acid (HCl) solution to a final pH of 7 prior to fermentation. To rid the digester of dissolved oxygen and other gases, the contents of the bottle were bubbled with nitrogen gas for 6 minutes and thereafter sealed immediately. A 5 L biodigester (GlassChem Pty) with automatic pH, temperature and stirring content was used to carry out the experiment. Homogeneity inside the reactor was achieved by stirring at 180 rpm throughout the duration of the experiment. Constant temperature was achieved by using a heating mantle adjusted to the desired temperature of 35 ˚C±0.5 and was recorded daily. The pH was measured and recorded daily using a pH probe. Fermentation was carried out for 30 days while biogas production was measured daily. The gas was scrubbed using 1 M sodium hydroxide before collection. Biogas quantity was measured by downward displacement of water and the composition determined by using Geotech 5000 biogas analyser. The inoculum experiment served as the control.

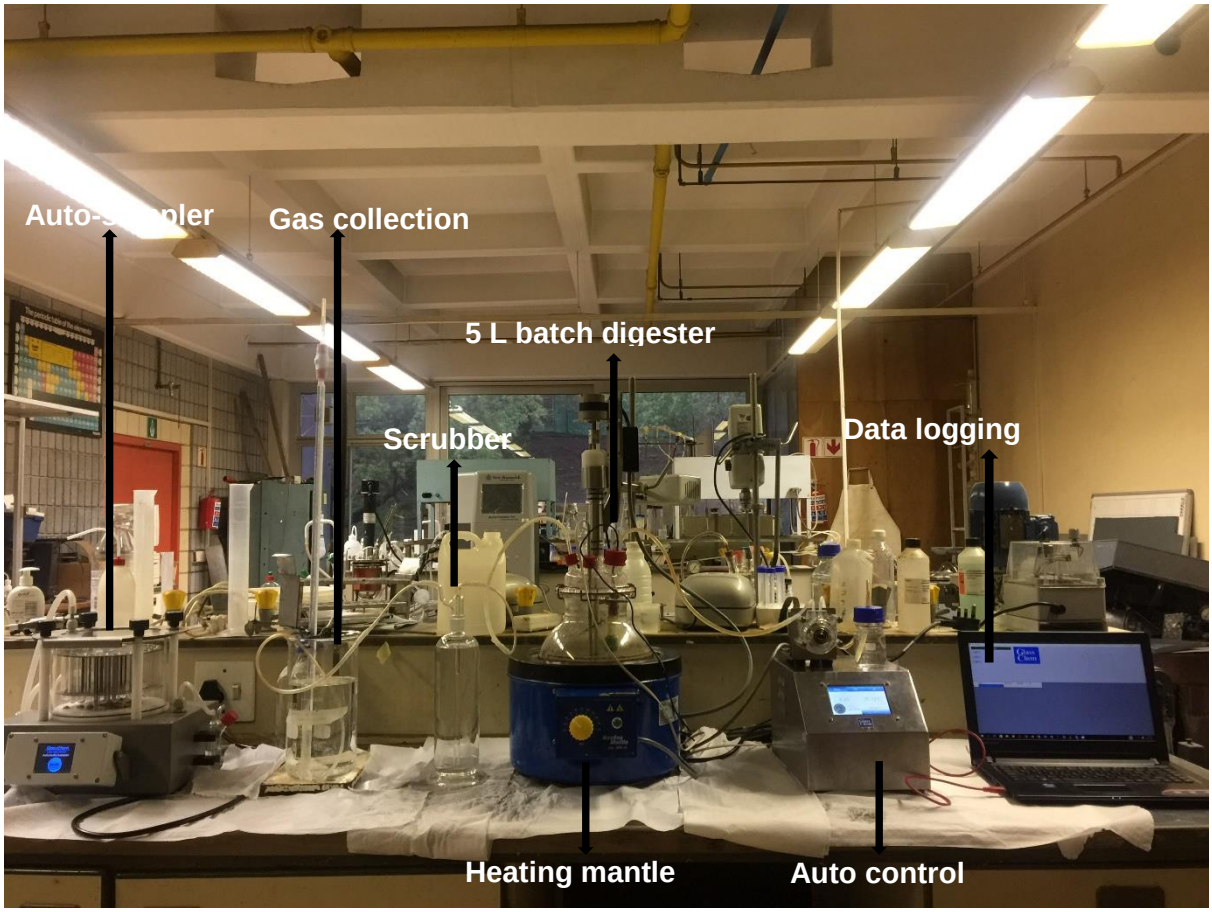


**Figure 8**: Single-stage 5 L mesophilic batch reactor used for up-scaling

### 3.5.4 Microbial Population Dynamics

Three samples were obtained from the single-stage 5 L mesophilic batch digester. Samples were taken on day 1, 15 and 30 of the digestion process using the auto-sampler, tested for pH and kept in the refrigerator at ±4 ˚C prior to analysis.

#### *3.5.4.1 DNA isolation*

DNA extraction from the samples was done using Powersoil DNA isolation kit. The samples were added to a bead beating tube for homogenization. Mechanical and chemical cell lysis occurred and the total genomic DNA was captured on a silica membrane. DNA was then washed and eluted from the membrane and was ready for PCR analysis and other downstream applications.

#### *3.5.4.2 PCR Amplification and bacterial gene sequencing*

Genomic DNA samples were PCR amplified using a universal primer pair (341F and 785R - targeting V3 and V4 of the 16S rRNA gene). Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon. Following quantification, the samples were individually indexed, and another purification step was performed. Amplicons were then sequenced on illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. 20 Mb of data (2x300 bp long paired end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

# CHAPTER 4

# BIOMETHANE POTENTIAL

## CHAPTER 4

## BIOMETHANE POTENTIAL OF CO-DIGESTION OF CASSAVA BIOMASS WITH WINERY WASTE FOR BIOGAS PRODUCTION

**4.1 Aim**: To determine the biomethane potential of co-digestion of cassava biomass with winery waste for the production of biogas using 500 ml batch digesters.

### 4.2 Objectives

The objectives for this part of the study were to:

- To determine the biomethane potential of zebra dung inoculum for the production of biogas using 3 x 500 ml batch digesters
- To determine the biomethane potential of cassava with zebra dung for the production of biogas using 3 x 500 ml batch digesters
- To determine the biomethane potential of winery waste with zebra dung inoculum for the production of biogas using 3 x 500 ml batch digesters
- To determine the biomethane potential of co-digestion of cassava and winery waste with zebra dung inoculum for the production of biogas using 3 x 500 ml batch digesters.

### 4.3 Introduction

For this part of the study, the biomethane potential of co-digestion of cassava biomass with winery waste for biogas production was evaluated. Biomethane potential (BMP) is a test done with the intention to investigate the ultimate biomethane potential of a substrate by anaerobic digestion (AD) (Angelidaki et al., 2009). This test assesses the biodegradability of substrates where microbiological, biochemical and physico-chemical aspects of the substrates are determined.

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***NB: Parts of this Chapter have been published in Proceedings of the 9th International Conference on Advances in Science, Engineering, Technology and Waste Management (ASETWM-2017).***

### 4.4 Materials and Methods

Fresh cassava & winery waste were mixed with zebra dung in a 500 ml batch digester on a ratio of 1:1 to test the biodegradability of the substrates (Refer to **Section 3**: Material & methods).

### 4.5 Results and Discussion

4.5.1 Substrate Characterisation

Cassava and winery wastes were characterised and the results showed major differences in some of the properties that were tested for. The physical and chemical characteristics of fresh cassava and winery waste are shown in **Table 7**. There are major differences between the protein contents (2.25 %) for fresh cassava and (11 %) for winery waste. The iron content was also found to be lower on cassava (1.15 %) than on winery waste (28.05). Sodium was found to be lower on cassava (359.75 mg/kg) compared to winery waste (1191.9 mg/kg). Both substrates (cassava and winery waste) have a high total solids content of 94.45 % and 95.92 % respectively and also a high volatile solids content of 98.20 % for cassava and 83.86 % for winery waste. A substrate with a high volatile solids amount is of vital importance for biogas production as this depicts the biodegradable amount in total solids. The nitrogen content (cassava - 0.36 % and winery waste - 0.4 %), calcium (cassava - 0.01 % and winery waste 0.06 %), potassium (cassava – 0.26 % and winery waste – 1.77 %), phosphorus (cassava- 0.05 % and winery waste – 0.16 %) and cyanide (cassava – 0.88 mg/kg and winery waste 0.92 mg/kg) showed very little difference.

**Table 7**: Physical & chemical characteristics of dried cassava and winery waste

| Characteristics | Unit | Dried cassava | Dried winery waste |
|---|---|---|---|
| Moisture content | % | 5.5 | 1.15 |
| Total solids | % | 94.45 | 95.92 |
| Volatile solids | % | 98.20 | 83.86 |
| Protein | % | 2.25 | 11 |
| Total nitrogen | % | 0.36 | 1.76 |
| Total carbon | % | 45.6 | 50.40 |
| Ash | % | 1.7 | 15.95 |
| Calcium | % | 0.01 | 0.06 |
| Phosphorus | % | 0.05 | 0.16 |
| Potassium | % | 0.26 | 1.77 |
| Iron | mg/kg | 1.15 | 28.05 |
| Sodium | mg/kg | 359.75 | 1191.9 |
| Cyanide | mg/kg | 0.88 | 0.92 |

The moisture content of the substrates was found to be low (5.5 % for cassava and 1.15 % for winery waste). Moisture content is important for anaerobic digestion. High moisture content results in more biogas yield whereas low moisture content yields low biogas (Devesa-Rey et al., 2011). Lancaster et al. (1982); Pandey et al. (2000) and Bayitse et al. (2015) reported the moisture content of cassava to be around 15 – 19 % for dry weight. Seenappa (2012) reported winery waste to have the ash content of 5 %, protein content of 11 %, calcium of 0.35 % and phosphorus content of 0.4 %.

The carbon/nitrogen (C/N) ratio of the two substrates was found to be high (127:1 for cassava and 29:1 for winery waste). The optimum carbon/nitrogen ratio is 20-30:1 for appreciable biogas production during anaerobic digestion. High C/N ratio indicates that the substrate is not good for anaerobic digestion and thus will not appreciably yield biogas (Ward et al., 2008). According to Ward et al. (2008), when one substrate has a high C/N ratio, it can be co-digested with a substrate that has low C/N ratio in order to balance the ratio and drop it to a value between 20-30:1. One of the reasons for co-digestion is to balance the C/N ratio of substrates. However, in the case of cassava and winery waste, both substrates have a high C/N ratio thereby causing low biogas yield when digested anaerobically. From the BMP results in **Table 8**, the biogas volume from cassava digestion is greater than the biogas volume from the co-digestion of cassava and winery waste. Addition of urea as a nitrogen source could be of vital importance in order to increase the nitrogen content of the digester thereby balancing the C/N ratio to 20-30:1 (Okudoh et al., 2014).

The protein content of cassava was found to be 2.25 % and 11 % for winery waste as shown in **Table 7**. During anaerobic digestion, carbohydrates and proteins are hydrolysed to soluble polymers by means of hydrolytic bacteria. High carbohydrate and protein contents result in high biogas yield with carbohydrates degrading more efficiently than protein (Yang et al., 2015). However according to Kovacs et al. (2015), protein content of the substrates should be kept minimal to avoid inhibition by ammonia. At high concentrations, free ammonia can inhibit biogas production during anaerobic digestion whereas at normal concentrations, it is an important nutrient for bacterial growth. Ammonia in the form of nitrogen which is generated by the deamination of amino acids can be used to monitor the degradation rate of the amino acids (Park et al., 2014).

4.5.2 Biomethane Potential

The biomethane potential was determined for all samples in triplicates and the average results are expressed in **Table 8**. Biogas production for cassava digestion started on day 4 of the digestion and dropped on day 22 whereas for winery waste, it started on day 4 of digestion and dropped on day 17. For co-digestion of both cassava and winery waste, it started from day 5 and dropped on day 20 (**Figure 9**) after which the biogas production was normalized using (**Equation 12**). The amount of methane in the biogas was found to be 62 % of the total biogas produced which was comparable to values obtained by Abdeshahian et al. (2016) [**Equation 11**]. The cumulative methane produced is shown in (**Figure 9**).

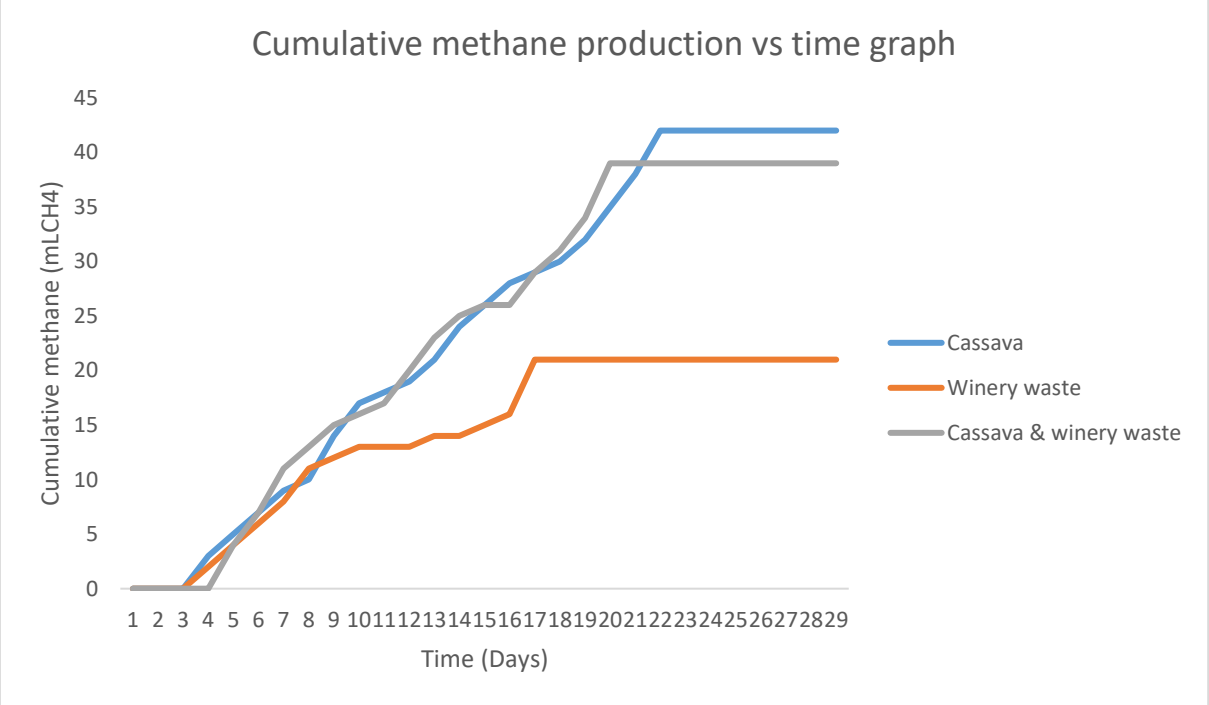


**Figure 9**: BMP test graph that shows the average methane yield

The results in **Table 8** show that cassava digestion produced more biogas than winery waste and was also found to have produced more biogas than the co-digestion of cassava and winery waste. This result was found to be contradictory to literature which states that co-digestion of substrates produces more biogas than a single substrate (Riano et al., 2011). This could be due to a number of factors which include the high C/N ratio of both substrates. This could be rectified by adding urea as a nitrogen source to adjust the C/N ratio to the optimum of 20-30:1. The other reason for less biogas production during co-digestion could be due to the cassava

to winery waste ratio. Winery waste has been found to have inhibiting factors for biogas production such as the presence of phenolics which inhibit biogas production (Kayembe et al., 2012). Lafka et al. (2007) studied winery waste phenolics using HPLC and found it to have major phenolics like gallic acid, catechin and epicatechin. Some of the other identified phenolics from winery waste were caffeic, syringic, vanillic, p-coumaric and o-coumaric acids (Lafka et al., 2007).

Another reason could be that, during co-digestion, the combination of the substrates increases the cyanide content of the digester making the environment more acidic. This acidity inhibits the anaerobic microbial activity such that they can't operate at their optimum best. This can also result in decreased biogas yield when the two substrates (e.g. cassava and winery waste) with high cyanide contents are co-digested compared to when a single substrate is used. Fresh cassava and winery waste were found to have 0.88 and 0.92 mg/kg (1 mg/kg~1 ppm) cyanide content respectively. However, Eze (2010) converted cassava waste from Gari processing industry to energy and biofertilizer and concluded that cyanide in cassava had no effect on the lack of biogas production if the amount of cyanide was less than 1 mg/kg. Another study found that during anaerobic digestion of cassava, the cyanide content of cassava was reduced concluding that the cyanide content does not have a negative impact on biogas production (Hassan & Nelson, 2012).

**Table 8**: Cumulative biogas produced from cassava, winery waste and co-digestion of both substrates

| Sample | Average cumulative methane ($mLCH_4$) | Average cumulative biogas (ml) | Cumulative methane (mLCH4/gVSadded) |
|---|---|---|---|
| Zebra dung + cassava | 42 | 67 | 1.62 |
| Zebra dung + winey waste | 21 | 33.8 | 0.9 |
| Zebra dung + cassava + winery waste | 38 | 61.3 | 1.58 |

4.5.3 Volatile fatty acids

The results of the VFA analysis obtained using an HPLC are shown in **Table 9**. The area and residence time of acetate, propionate and butyrate were determined for samples containing

zebra dung only, zebra dung + cassava, zebra dung + winery waste and zebra dung + cassava + winery waste.

Acetate, propionate and butyrate concentrations (**Table 9)** obtained at the beginning and on the last day of digestion during the BMP tests. The results show that during the digestion of zebra dung, there was no acetate at the beginning of the digestion process but was present at the end. This means that acetate was formed during AD. Propionate was found to be present at the beginning of digestion and was found to have increased at the end. Butyrate was not present both at the beginning and at the end of digestion. For cassava and zebra dung digestion, acetate was found at the beginning of digestion, increased significantly and persisted till the end. Butyrate was also present at the beginning of digestion but was not found at the end. For winery waste and zebra dung, acetate was present at the beginning, increased slightly during digestion and had a large increase towards the end. Propionate and butyrate were present at the beginning of the digestion but were not found at the end.

During co-digestion of the two substrates, acetate was present at the beginning, increased during digestion and was found to be more at the end. Propionate and butyrate were also present at the beginning of digestion and were not found at the end of the digestion period. According to Wijekoon et al. (2011), acetic and butyric acids are the most predominant VFA during anaerobic digestion. Acetic acid is necessary for anaerobic digestion as it is directly linked to methane and carbon dioxide formation. Gorris et al. (1989) found propionic acid to be completely degraded when acetic acid levels in the digester were low (less than 100mg/L) and that high acetic acid levels (more than 4700 mg/L) inside the digester blocked propionic acid degradation. This observation may be applicable to this experiment. For winery waste digestion and co-digestion of the two substrates, low acetate present in the digester resulted in propionate being completely degraded. According to Wijekoon et al. (2011), methanogenic bacteria has been found to be vulnerable to propionic acid concentration greater than 1.000~2.000 mg/L. Gourdon & Vermande (1987) also observed no inhibitory effect for propionate levels above 600 mg/L.

**Table 9**: VFA comparison before and after digestion

| Sample | VFA | Inlet concentration (mg/L) | Outlet concentration (mg/L) |
|---|---|---|---|
| Zebra dung only | Acetate | 0 | 136.62 |
| | Propionate | 144.11 | 149.2 |
| | Butyrate | 0 | 0 |
| Zebra dung + cassava | Acetate | 79.87 | 726.34 |
| | Propionate | 17,10 | 20.93 |
| | Butyrate | 56.73 | 0 |
| Zebra dung + winery waste | Acetate | 235.94 | 859.90 |
| | Propionate | 25.05 | 0 |
| | Butyrate | 52.91 | 0 |
| Zebra dung + winery waste + cassava | Acetate | 110.77 | 791.87 |
| | Propionate | 51.70 | 0 |
| | Butyrate | 154.02 | 0 |

# CHAPTER 5

# BIOGAS OPTIMIZATION

# CHAPTER 5

# OPTIMIZATION OF BIOGAS YIELD FROM CO-DIGESTION OF CASSAVA AND WINERY WASTE USING RESPONSE SURFACE METHOD

**5.1 Aim:** To optimize the production of biogas from cassava biomass and winery waste using response surface method (RSM).

## 5.2 Objectives

The objectives for this part of the study were to:

- Study the optimum substrate feeding ratio of cassava to winery waste for maximum biogas production, using response surface methodology;
- Study the optimum temperature for co-digestion of cassava biomass with winery waste for maximum biogas production, using response surface methodology;
- Study the optimum pH for co-digestion of cassava biomass with winery waste for maximum biogas production, using response surface methodology.

## 5.3 Introduction

During co-digestion, the substrate feeding ratio of one substrate to the other, pH and temperature are of vital importance. In this study, the optimum ratio of cassava to winery waste, temperature and pH were determined using response surface methodology.

## 5.4 Materials and methods

Design-Expert® Software version 6.0.8 was used to determine the optimal conditions for biogas production between cassava and winery waste. The chosen conditions were temperature, pH and co-digestion ratio of cassava and winery waste (Refer to **Section 3**: Materials & methods).

## 5.5 Results and Discussion

5.5.1 Response Surface Analysis Regression and Model Analytics

The experimental values obtained were subjected to response analysis so as to evaluate the relationship between the input variables namely pH ($X_1$), temperature ($X_2$) and co-digestion ratio ($X_3$).

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***NB: Parts of this Chapter have been submitted to Elsevier: Waste and Biomass Valorisation Journal for publication***

**Table 10**: Coded and real values of pH, different temperatures and different co-digestion ratios of substrate

| Run | Code values | | | Real values | | | Methane yield ($mLCH_4$) | Predicted values |
|---|---|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | $X_1$ | $X_2$ | $X_3$ | | |
| 1 | 0 | 0 | 0 | 7 | 35 | 70 | 353 | 346.28 |
| 2 | -2 | 2 | -2 | 6 | 45 | 40 | 121 | 105.12 |
| 3 | 0 | 0 | 0 | 7 | 35 | 70 | 346 | 346.28 |
| 4 | -2 | 0 | 0 | 6 | 35 | 70 | 237 | 283.33 |
| 5 | 2 | 0 | 0 | 8 | 35 | 70 | 285 | 295.33 |
| 6 | 8 | -2 | -2 | 8 | 25 | 40 | 100 | 93.72 |
| 7 | 0 | 0 | -2 | 7 | 35 | 40 | 189 | 235.33 |
| 8 | 0 | 0 | 0 | 7 | 35 | 70 | 358 | 346.28 |
| 9 | -2 | -2 | 1 | 6 | 25 | 100 | 101 | 94.72 |
| 10 | 0 | 0 | 0 | 7 | 35 | 70 | 378 | 346.28 |
| 11 | 2 | 2 | -2 | 8 | 45 | 40 | 122 | 114.12 |
| 12 | -2 | -2 | -2 | 6 | 25 | 40 | 115 | 98.72 |
| 13 | 0 | 0 | 0 | 7 | 35 | 70 | 396 | 346.28 |
| 14 | 0 | 2 | 0 | 7 | 45 | 70 | 250 | 279.53 |
| 15 | 2 | 2 | 1 | 8 | 45 | 100 | 136 | 138.12 |
| 16 | -2 | 2 | 1 | 6 | 45 | 100 | 117 | 109.12 |
| 17 | 0 | 0 | 0 | 7 | 35 | 70 | 360 | 346.28 |
| 18 | 2 | -2 | 1 | 8 | 25 | 100 | 108 | 109.72 |
| 19 | 0 | -2 | 0 | 7 | 25 | 70 | 235 | 262.13 |
| 20 | 0 | 0 | 1 | 7 | 35 | 100 | 235 | 245.33 |

The actual results were then fitted to a second order polynomial equation by means of multiple regression analysis. **Equation 13** was obtained based on mathematical regression models for biogas production and was fitted in term of coded factors:

$$396 + 6x_1 + 8.7\,x_2 + 5x_3 - 56.95x_1^2 - 75.45x_2^2 - 105.95x_3^2 + 3.50x_1x_2 + 5x_1x_3 + 2.00x_2x_3 \quad \textbf{(13)}$$

The adequacy and statistical significance of the model were ascertained on the results obtained by analysis of variance (ANOVA) as shown in **Table 11.**

**Table 11**: Analysis of variance (ANOVA) of the model for biogas production

| Source | Sum of Squares | DF | Mean Square | F value | Prob > F |
|---|---|---|---|---|---|
| Model | 2.125E+005 | | 23608.41 | 22.08 | < 0.0001 |
| A | 360.00 | 1 | 360.00 | 0.34 | 0.5745 |
| B | 756.90 | 1 | 756.90 | 0.71 | 0.4198 |
| C | 250.00 | 1 | 250.00 | 0.23 | 0.6391 |
| $A^2$ | 8920.51 | 1 | 8920.51 | 8.34 | 0.0161 |
| $B^2$ | 15656.82 | 1 | 15656.82 | 14.65 | 0.0033 |
| $C^2$ | 30872.51 | 1 | 30872.51 | 28.88 | 0.0003 |
| AB | 98.00 | 1 | 98.00 | 0.092 | 0.7683 |
| AC | 200.00 | 1 | 200.00 | 0.19 | 0.6745 |
| BC | 32.00 | 1 | 32.00 | 0.030 | 0.8661 |
| Residual | 10690.08 | 10 | 1069.01 | | |
| Lack of fit | 8981.25 | 5 | 1796.25 | 5.26 | 0.0463 |
| C.V. | 14.40 | | | | |
| PRESS | 45556.08 | | | | |
| R-squared | 0.9521 | | | | |
| Adj R- squared | 0.9090 | | | | |
| Pred R-Squared | 0.7959 | | | | |
| Adeq Precision | 10.924 | | | | |

The Model F-value of 22.08 implies the model is significant. There is only a 0.01 % chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 5.26 implies the lack of fit is significant. There is only a 4.63 % chance that a "Lack of Fit F-value" this large could occur due to noise. The p-value ($p<0.05$) of the quadratic regression model indicates that the model terms are significant. In the observed response values, the correlation coefficient, $R^2$= 0.9521, indicated that the sample variation of 95.21 % in the methane yield response could be explained by the model. A good statiscal model should have $R^2$ value in the range of 0.75-1 [21, 23]. The "Pred R-Squared" value of 0.7959 is in reasonable agreement with the "Adj R-Squared" value of 0.9090. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 10.924 indicates an adequate signal, meaning that this model can be used to navigate the design space. The percentage of coefficient of variation (CV %) is a measure of residual variation of the data relative to the size of the mean. Usually, the higher the CV value, the lower the reliability of experiment. Hence, a lower value of CV

(14.4 %) indicated a greater reliability of the experiment. The predicted residual sum of squares (PRESS) is a measure of how well the model fitted each point in the design. The smaller the PRESS statistics, the better would be the model fitting the data points. Here the value of PRESS was found to be 45556.08 meaning the model fitted the data points. **Table 11** also shows that the quadratic model terms ($A^2$, $B^2$, $C^2$) were sigificant ($p<0.05$). However the linear model terms (A, B, C) and the interactve terms (AB, AC, BC) were insignificant ($p>0.05$). Also the actual methane yield values were close to the predicted values (**Figure 10**).

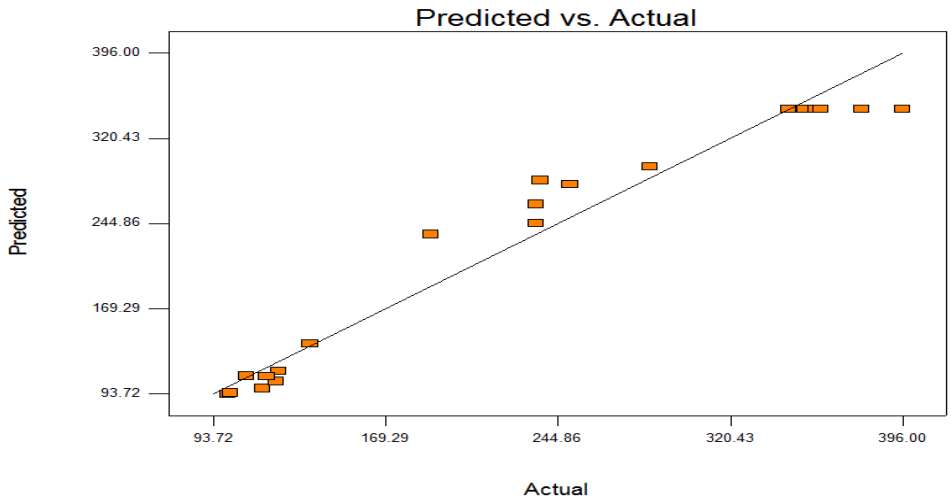



**Figure 10**: Actual vs. predicted biogas yield values

5.5.2 Response Surface Analysis and Interactions among Factors

*5.5.2.1 Relationship between pH and different temperature*

The relationship between pH and temperature was demonstrated using the three-dimensional response surface and the corresponding contour plot by keeping one variable at the central level of plot and contrasting the others inside the expected experimental range. Statistical difference was found to be non-significant ($P>0.05$) for interactive effect of pH and temperature but individual effects of these factors were significant on the digester performance. Carotenuto *et al.* (2016), found that at high temperature of 55 °C (thermophilic conditions) and high pH>8, the anaerobic digestion process still runs efficiently and even doubling the production rate of methane. The said authors found that for some animal manures like Buffalo, the digestion process can still run without any pH manipulation. In this study, the maximum methane yield (396 mL $CH_4/gVS_{added}$) was higher than the predicted maximum value (346.28 mL $CH4/gVS_{added}$) at a temperature of 35 $^oC\pm0.5$ and pH 7 (**Figure 11**).

It was also observed that the production of methane decreased when the temperature exceeded 35 $^oC\pm0.5$ during co-digestion of CB and WSW. When the temperature was at 25 $^oC\pm0.5$ there was a slight decrease in methane production.This could be due to the fact that

the chosen micro-organisms existed at about 35 °C which is the body temperature for zebras therefore they are most active at that temperature and when the temperature drops to 25 °C it causes a decrease in methane production. Methanogens have been observed to be very sensitive to extremes of temperature and pH [32] and have a very limited activity range. The optimum pH for methane production has been reported between 7 and 7.2 [Carotenuto et al., 2016; Demuynck et al., 1984].

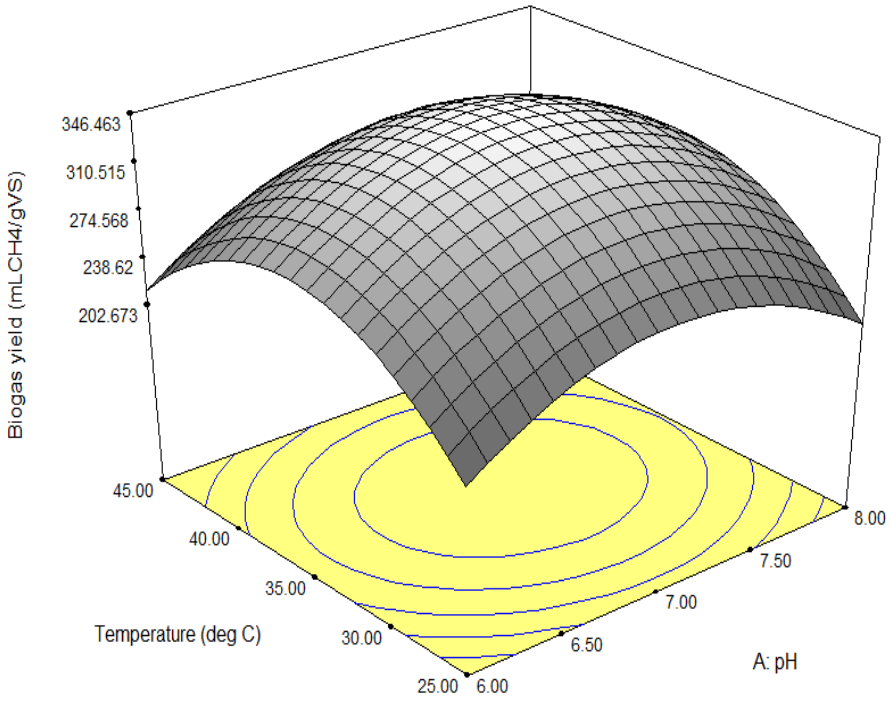



**Figure 11**: Response surface and contour lines for the effect of pH and temperature on specific methane production

*5.5.2.2 Relationship between the Effect of pH and Ratio of Substrate*

The interaction between pH and different ratios of cassava biomass and winery solid waste is shown in **Figure 12**. Both the pH and substrate ratios individually have significant impact **Equation 12** on methane production ($p<0.05$) but their interactive effect was statistically insignificant ($p>0.05$). In this study, methane production reached its peak at substrate ratio of 70 % CB and 30 % WSW (**Figure 12)**. Methane production was observed to decrease at 100 % WSW digestion and also when the quantity of WSW was more than that of cassava biomass. This could mean that WSW has inhibitory properties for methane production which could be attributed to a number of factors such as the presence of phenolics and the high carbon/nitrogen ratio when the two substrates were combined (DaRos et al., 2014).

The optimization of the pH in the digesters was selected to range from 6 to 8 (Henze 2002; Chanathaworn 2017). When the pH is less or greater than the optimum range (pH 7-8.5), the

methanogenic process gets inhibited thereby decreasing the rate of methane production (VanKessel et al., 1996; Adelekan & Bamgboye, 2009). One of the benefits of co-digestion is its buffering capacity in the digester during anaerobic digestion. A decrease in pH will lead to acidification and thriving acidogenic microorganisms due to the accumulation of volatile fatty acids (VFAs) in the early days of the anaerobic process and if not controlled can lead to digester failure (Ghaly 2000). In the present study, stable pH values at 7 favoured the production of maximum methane values at different yield combinations.

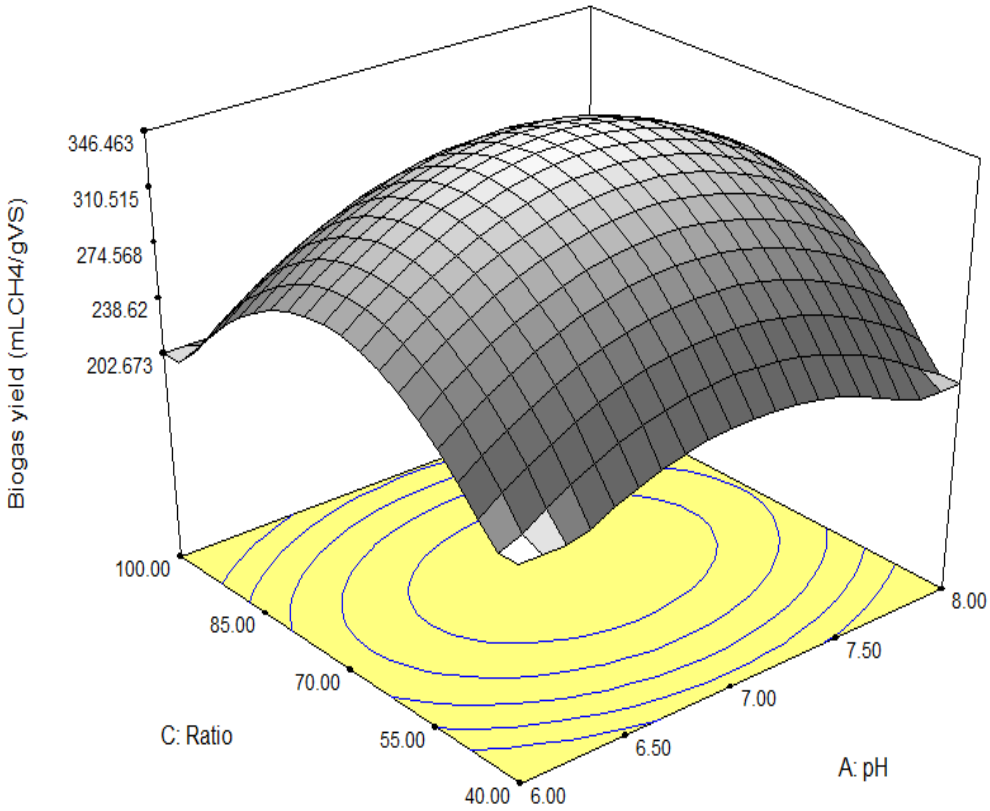



**Figure 12**: Response surface and contour lines for the effect of pH and substrate ratio on specific methane production

*5.5.2.3 Relationship between Effect of Ratio and Different Temperature*

The interactive effects of substrate ratios and different temperatures are shown in (**Figure 13)**. It was observed that an increase in the ratio of CB and temperature resulted in an increase in methane production and conversely the decrease in these two factors decreased methane production. The CB/WSW ratio individually affected methane production significantly ($p<0.05$) but its interactive effect with different temperatures was insignificant ($p>0.05$). The predicted maximum methane yield value (346.28 $mLCH_4/gVS_{added}$) of the substrate ratios and temperature are indicated by top of the surface (**Figure 13**) and was lower than the highest methane yield of 396 $mLCH_4/gVS$ recorded experimentally in this study at the ratio of 70:30

(70 % CB and 30 % WSW) and at 35 $^{o}$C±0.5 as shown in **Table 6.** This could be explained by the type of inoculum (zebra dung) used in this study where the methanogens are most active at the body temperature of 35 $^{o}$C.

The increase in temperature from mesophilic to thermophilic conditions promoted faster degradation of the substrates leading to ammonia inhibition according to a study carried out by Wang et al. (2014). According to the authors, this kind of ammonia inhibition could be avoided by balancing the C/N ratio of the mixed feedstock. The lowest methane production in the present study was at the ratio of 40:60 (40 % CB and 60 % WSW) at a temperature of 25 $^{o}$C±0.5. This result could be attributed to the fact that WSW degradation compound/s in itself is inhibitory to the methanogens due to the presence of phenolic compounds (DaRos et al., 2014) coupled with the low temperatures for methanogenic activity (Carotenuto et al., 2016; Lin *et* al., 2016). The combination of these two negative factors will ultimately lead to a lower methane yield.

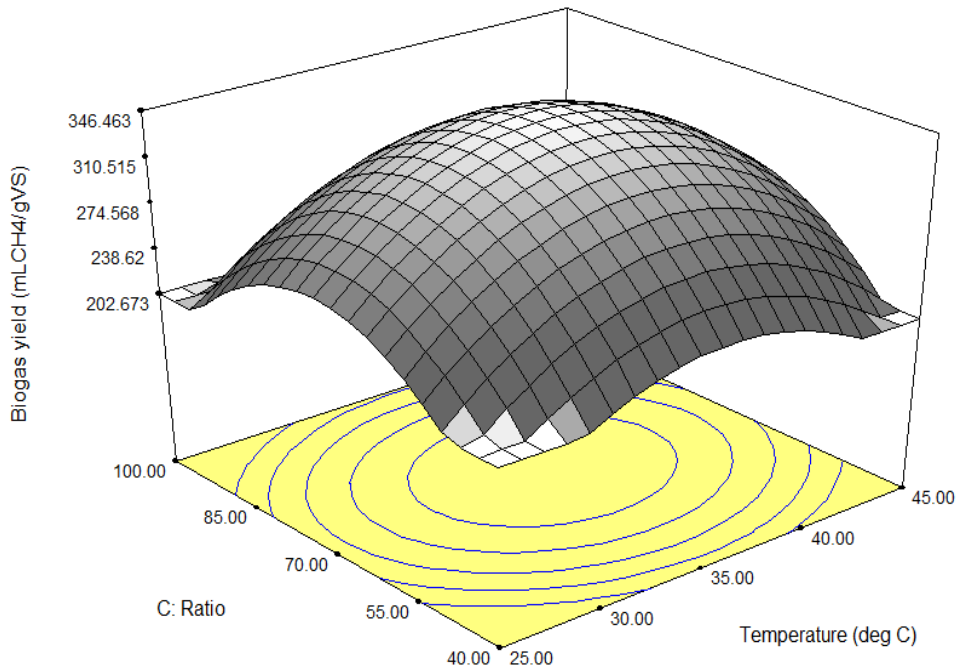



**Figure 13**: Response surface and contour lines for the effect of substrate ratios and temperature on specific methane production

5.5.3 Conditions for Optimum Response and Model Validation

The specific optimal conditions for methane production were determined using the second order polynomial model (**Equations 13**). The model predicted a maximum methane yield of 346.28 $mLCH_4/gVS_{added}$ for the optimal conditions. To ascertain the validity of the predicted

values, experiments were carried out at the optimal conditions of pH 7, temperature of 35 $^{o}$C±0.5 and 70/30 ratio of CB/WSW. The maximum methane production was recorded to be 396 mL$CH_4$/gVS. The obtained value is close but a bit higher than predicted. The co-digestion of two substrates has been reported to produce more methane than a single substrate (AlSeadi et al., 2013). Zhang et al. (2011) also reported a methane production of 259.46 mL/g$VS_{added}$ when CB residues and distillery waste water were co-digested using a batch thermophillic reactor. The optimum temperature for methane production from co-digestion of CB and WSW is 35 °C±0.5. It was observed that at 25 $^{o}$C and 45 $^{o}$C, methane production decreased, though there are various temperature conditions that micro-organisms exist in. Most anaerobic treatment plants around the world are operated at mesophilic conditions (Schnürer & Jarvis, 2010). This could be attributed to the sensitivity of methanogens to both low and high temperatures and hence the significant impact on the anaerobic digestion process (Lin et al., 2016; Tait et al., 2018).

Methane production presented several small peaks before production finally declined to very low levels. This could be related to the dynamic balance between the metabolism of acidogens and methanogens in the digester (Yan et al., 2015). The highly concentrated cassava biomass, at the early stages of digestion, provided sufficient organic acid for faster growth of the methanogens and resulted in an increased methane production. As the organic acid concentration becomes less, the methanogenic activity is reduced hence allowing acidogens to thrive. This causes a decrease in methane yield. This cycle of acidogenic and methanogenic activities continues until a decline in the final substrate concentration occurs as compared to the initial concentration.

### 5.6 Discussion

The results from **Table 10** show that the optimum temperature for biogas production from co-digestion of cassava and winery waste is 35 ˚C±0.5. It was noticed that at 25 $^{o}$C and 45 ˚C, biogas production decreased, though there are various temperature conditions that micro-organisms exist in. Most anaerobic treatment plants around the world are operated at mesophilic conditions (Schnürer & Jarvis, 2010). This could be attributed to high temperature sensitivity of micro-organisms to the thermophilic range.

Based on **Table 7**, it can be seen that optimum pH for biogas production of cassava and winery waste is pH 7. The reported optimum pH range for methanogenic bacteria is 7 – 7.2 (Demuynck et al., 1984). If the pH is less or greater than the optimum range (7 – 8.5), the methanogenic process gets inhibited thereby decreasing the rate of biogas production (Adelekan & Bamgboye, 2009). The analysis of variance in **Table 11** shows that the interactive effect of pH and ratio of cassava to winery waste were significant. The pH value is of vital importance in

anaerobic fermentation as it controls the activity of the methanogens (Okudoh et al., 2014). Based on the results presented in the current study, it can be seen that the optimum ratio for methane production between CB and WSW was 70 % CB and 30 % WSW. The high $R^2$ value of 0.991 showed that the RSM model was useful for methane yield prediction in agreement with Sathish & Vivekananda, (2016) and could be effectively used to predict methane production from agricultural food waste and animal manure (Yusof et al., 2014).

# CHAPTER 6

# UPSCALING USING A SINGLE-STAGE 5L MESOPHILIC BATCH DIGESTER

# CHAPTER 6

# INVESTIGATION OF THE POTENTIAL BIOGAS PRODUCTION WHEN VOLUME IS SCALED UP TO 5 L MESOPHILIC BATCH DIGESTER USING OPTIMAL CONDITIONS

**6.1 Aim**: To investigate the potential production of biogas when the volumes are scaled-up using the optimized conditions

## 6.2 Objective

- The optimal conditions will be applied to produce biogas in the single-stage 5 L mesophilic batch digester

## 6.3 Introduction

This chapter investigates biogas production from co-digestion of cassava biomass with winery waste using a single-stage 5 L mesophilic batch digester. The optimal conditions obtained from objective 2 will be used i.e pH 7, temperature of 35 ˚C±0.5 and co-digestion ratio of 70:30 cassava to winery waste.

## 6.4 Materials & Methods

A GlassChem® 5 L biodigester mesophilic batch digester was used to investigate biogas production using the optimized values obtained from chapter 5 (Refer to **Chapter 3**: Materials & methods).

## 6.5 Results and Discussion

A total of 837.54 ml biogas was produced when the single-stage 5 L mesophilic batch digester was added with a co-substrate ratio of 70:30 cassava to winery waste as shown on **Table 12**. Another experiment with 250 g of zebra dung mixed with water to make up 2.5 L was ran as a control for 30 days.18 ml of biogas was produced from this experiment which was subtracted from the total biogas produced from the co-digestion experiment giving a total biogas yield of 819.54 mL/gVS destroyed. The methane content of biogas was found to be 62 % resulting in 508.11 $mLCH_4$ produced and cumulative methane yield of 2.20 $mLCH_4/gVS_{added}$ calculated using **Equation 12**. On day 3, the pH of the digester started dropping from pH 7 to pH 6.1 (**Figure 14**) and this could be due to the production of volatile fatty acids. Due to large amounts of VFAs hindering methanogenic bacteria growth, the pH was increased to a neutral pH of 7

on day 4 by using a 1 M solution of sodium hydroxide (NaOH) which resulted in biogas production being observed on day 5. The decline in pH was also observed by Anunputtikul & Rodtong (2004) during the study of laboratory scale experiments for biogas production from cassava tubers using a 5L mesophilic batch digester. The maximum gas yield per day was observed as 35 mL$CH_4$ on days 6, 7, 9 &11 (**Figure 15**).This result was found to be comparable to the results obtained by Paepatung et al. (2009) which showed that cassava pulp produced 36.57 mL$CH_4$ per day from 120 ml mesophilic batch digesters. From day 12, biogas production started dropping. The temperature of the digester remained constant at 35 ˚C±0.5 throughout the duration of the experiment.

**Table 12:** Data showing the results of the co-digestion experiment using the single-stage 5 L mesophilic batch digester

| Days | Biogas yield (mL/day) | Methane yield (mL/day) | Temperature (˚C) | pH |
|---|---|---|---|---|
| **1** | 0 | 0 | 35 | 7 |
| **2** | 0 | 0 | 35 | 6.8 |
| **3** | 0 | 0 | 35 | 6.3 |
| **4** | 0 | 0 | 35 | 6.1 |
| **5** | 51,84 | 32 | 35 | 7.1 |
| **6** | 56,7 | 35 | 35 | 7 |
| **7** | 56,7 | 35 | 35 | 6.9 |
| **8** | 42,12 | 26 | 35 | 6.9 |
| **9** | 56,7 | 35 | 35 | 6.7 |
| **10** | 55,08 | 34 | 35 | 6.6 |
| **11** | 56,7 | 35 | 35 | 6.4 |
| **12** | 43,74 | 27 | 35 | 6.4 |
| **13** | 45,36 | 28 | 35 | 6.4 |
| **14** | 43,74 | 27 | 35 | 6.3 |
| **15** | 42,12 | 26 | 35 | 6.1 |
| **16** | 35,64 | 22 | 35 | 6 |
| **17** | 29,16 | 18 | 35 | 6 |
| **18** | 35,64 | 22 | 35 | 6 |
| **19** | 35,64 | 22 | 35 | 6 |
| **20** | 34,02 | 21 | 35 | 6 |
| **21** | 29,16 | 18 | 35 | 6.2 |
| **22** | 21,06 | 13 | 35 | 5.9 |

| | | | | |
|---|---|---|---|---|
| **23** | 17,82 | 11 | 35 | 5.7 |
| **24** | 12,96 | 8 | 35 | 5.7 |
| **25** | 9,72 | 6 | 35 | 5.7 |
| **26** | 6,48 | 4 | 35 | 5.4 |
| **27** | 4,86 | 3 | 35 | 5.4 |
| **28** | 4,86 | 3 | 35 | 5.3 |
| **29** | 4,86 | 3 | 35 | 5.2 |
| **30** | 4,86 | 3 | 35 | 5 |
| **Total** | **837.54** | **517 (62%)** | | |

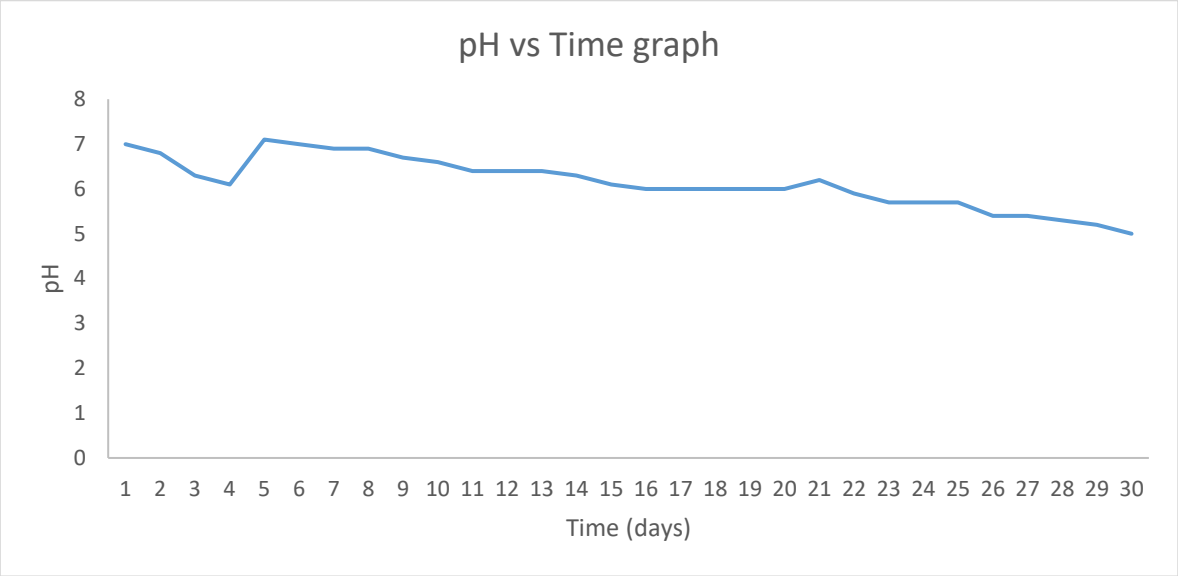



**Figure 14:** Graph showing the pH over time for the single-stage 5 L mesophilic batch digester experiment

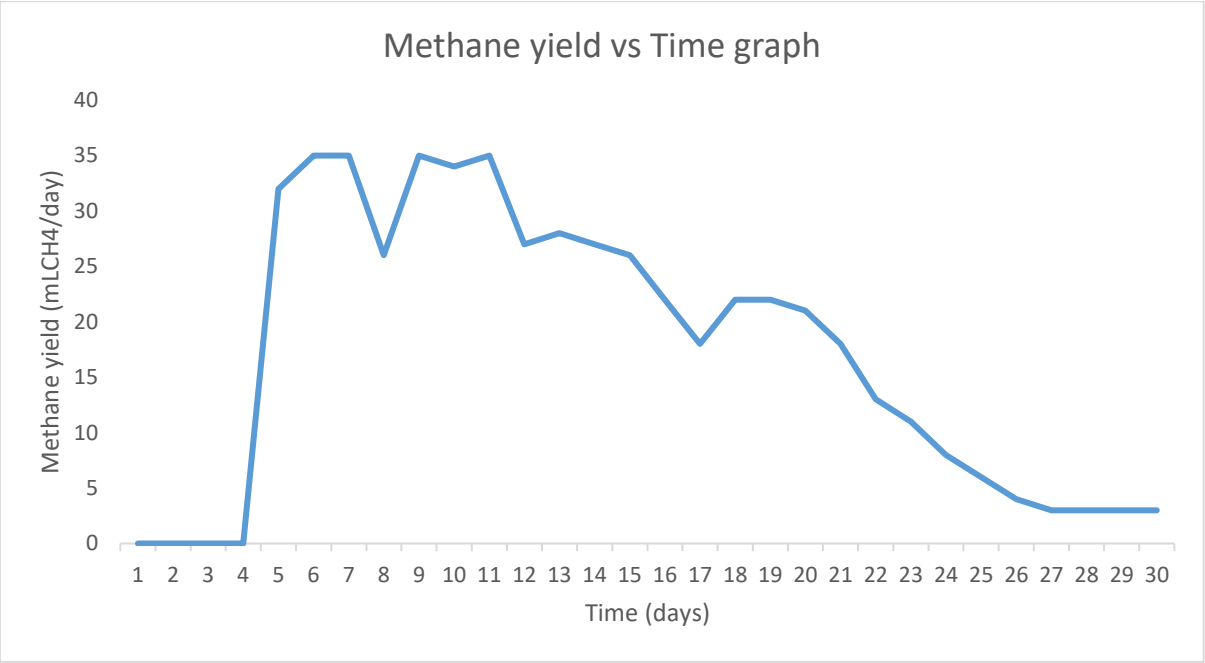



**Figure 15:** Graph showing the methane yield over time for the single-stage 5 L mesophilic batch digester experiment

# CHAPTER 7

# MICROBIAL COMMUNITY STRUCTURE & DYNAMICS

# CHAPTER 7

# INVESTIGATION OF THE MICROBIAL COMMUNITY STRUCTURE AND DYNAMICS DURING CO-DIGESTION OF CASSAVA AND WINERY WASTE FOR BIOGAS PRODUCTION

**7.1 Aim**: To investigate the microbial community structure and dynamics during co-digestion of cassava and winery waste for biogas production

## 7.2 Objectives

The objectives of this part of the study were to:

- DNA extraction from the three samples collected in the 5 L single-stage digester on day 1, 15 and 30 of the digestion process
- PCR amplification and bacterial gene sequencing of the three samples collected in the 5 L single-stage digester on day 1, 15 and 30 of the digestion process
- Assess changes in microbial community composition

## 7.3 Introduction

This chapter investigates the microbial dynamics inside the digester during co-digestion of cassava and winery waste in the single-stage 5 L mesophilic batch digester from Chapter 6. The samples were collected on days 1, 15 and 30 of the anaerobic digestion period and DNA extracted from them and 16s bacterial metagenomic analysis was performed.

## 7.4 Materials and methods: (Refer to Chapter 3: Materials & methods)

Genomic DNA samples were PCR amplified and the BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

## 7.5 Results and Discussion

### 7.5.1 16s Bacterial Metagenomics Analysis

The process of anaerobic digestion is achieved in four steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) by a group of micro-organisms called hydrolytic, acidogenic, and acetogenic as well as methanogenic bacteria. According to Bajpai (2017) species that are predominant in anaerobic digestion include *Clostridium, Peptococcus, Bifidobacterium, Desulfovibrio, Corynebacterium, Lactobacillus, Actinomyces, Staphylococcus, Streptococcus, Micrococcus, Bacillus, Pseudomonas, Selemonas, Veillonella, Sarcina, Desulfobacter, Desulfomonas* and *Escherichia coli* however they are largely dependent on the characteristics of the substrates.

From **Table 13** it was observed that archaeal communities were found to be much less than the bacterial communities. Bacterial communities were dominated by representatives of the phyla *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. The prevalence of *Bacteroidetes* and *Firmicutes* has been frequently reported for different AD reactors treating agricultural residues (Goux *et al*., 2015). Also the presence of *Desulfovibreo* is also of importance as it is a sulphate-reducing bacteria (Bajpai, 2017). *Desulfovibreo* was present on day 15 of the digestion period.

The read count from the results of the kingdom classification in **Table 13** for day 1 shows the presence of *Clostridium, Lactobacillus, Bacillus,* and *Pseudomonas*. On day 15, *Clostridium, Desulfovibrio, Lactobacillus, Bacillus* were present. Lastly on day 30, *Clostridium, Corynebacterium, Lactobacillus, Actinomyces, Micrococcus, Bacillus, Sarcina* were present.
The first step of anaerobic digestion known as hydrolysis is performed by hydrolytic bacteria, fungi and protists (Bajpai 2017). According to the results in **Table 13**, at the beginning of the digestion process there was a presence of bacteria & fungi to facilitate hydrolysis.

The second step of anaerobic digestion, acidogenesis, is performed by acidogenic bacteria namely *Lactobacillus* and *Propionibacterium sp.* (Bajpai 2017). Manyi-Loh *et al*. (2016) reported active acidogens to be of the family *Enterobacteriaceae.* These bacteria are found in human and animal intestines*. Aminobacterium*, *Psychrobacter*, *Anaerococcus*, *Bacteroides*, *Acetivibrio*, *Butyrivibrio*, *Halocella*, *Spirochaeta*, *Caldicellulosiruptor* and *Cellulomonas* (a facultative anaerobe of the phylum *Actinobacteria*) are also known to be anaerobic cellulose degrading bacteria (Manyi-Loh. *et al*., 2016). The results on **Table 14** shows the presence of *Actinobacteria* on day 1 of the digestion to be 1.74 % and a slight increase was observed on day 15 to 2.72 % and on day 30 there was a small amount (0.01 %) of this bacteria present in the digester.

Acetogenesis is performed by acetogenic bacteria namely *Acetobacter* sp. (Bajpai 2017). According to Manyi-Loh et al. (2016) *Syntrophobacter wolinii*, *Syntrophomonas wolfei* and *Smithella sp.* are responsible for acetogenesis.

Methanonegenesis is performed by methanogenic bacteria which belong to the domain *Archaea (*Manyi-Loh. *et al*., 2016*).* The results in **Table 13** show the presence of *Archaea* on day 1 of the digestion period and a slight increase on day 15 but there was no *Archaea* present on day 30.

From the kingdom classification in **Table 13**, it is observed that *Bacteria* is the most predominant followed by *Protozoa, Fungi, Plantae, Virus and Archaea*. Within the bacteria domain, *Proteobacteria* phylum was more abundant (**Table 14**).

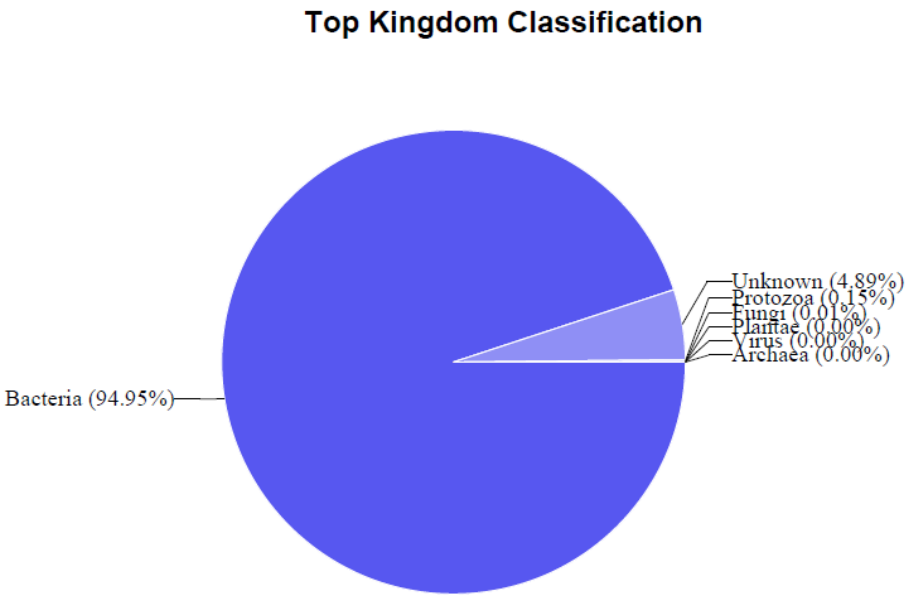
**Table 13**: Comparison of the kingdom classification from days 1, 15 and 30 respectively

| | **DAY 1** | | **DAY 15** | | **DAY 30** | |
|---|---|---|---|---|---|---|
| Kingdom | Read Count | % | Read Count | % | Read Count | % |
| Bacteria | 271553 | 94.95 | 312911 | 99.98 | 560942 | 99.84 |
| Unknown | 13982 | 4.89 | 8 | 0.0 | 871 | 0.16 |
| Protozoa | 435 | 0.15 | 49 | 0.0 | 18 | 0.00 |
| Fungi | 24 | 0.01 | 4 | 0.02 | 1 | 0.00 |
| Plantae | 3 | 0.00 | 2 | 0.00 | 8 | 0.00 |
| Virus | 1 | 0.00 | | | | |
| Archaea | 1 | 0.00 | 2 | 0.00 | | |

**Table 14**: Comparison of top phylum classification for days 1, 15 and 30

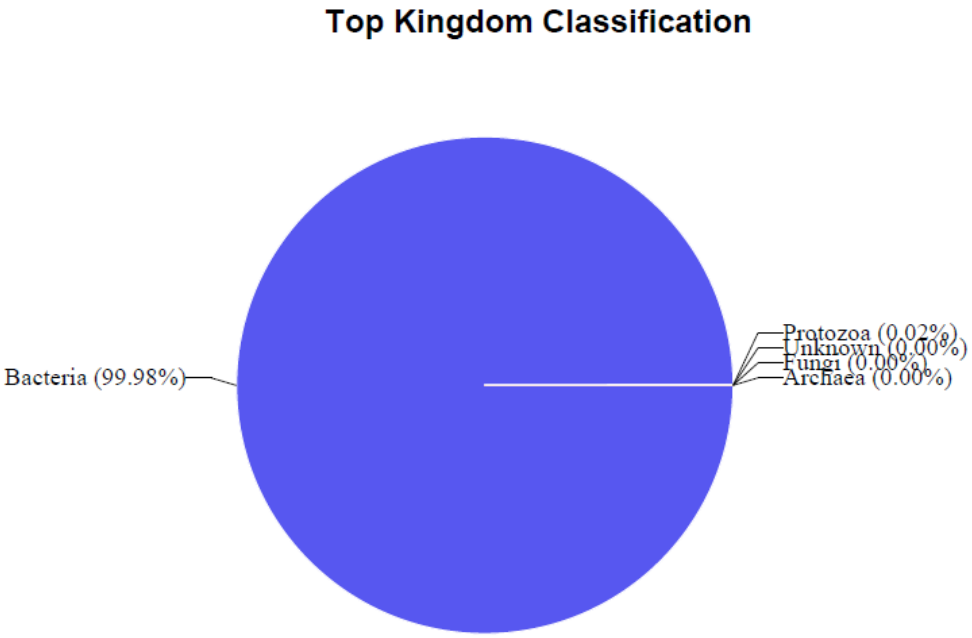
| | **DAY 1** | | **DAY 15** | | **DAY 30** | |
|---|---|---|---|---|---|---|
| Phyla Classification | Read Count | % | Read Count | % | Read Count | % |
| Proteobacteria | 162264 | 56.74 | 74748 | 23.88 | | |
| Unknown | 96227 | 33.65 | 54398 | 17.38 | 426353 | 75.89 |
| Firmicutes | 21535 | 7.53 | 174906 | 55.89 | 135386 | 24.10 |
| Actinobacteria | 4990 | 1.74 | 8506 | 2.72 | 55 | 0.01 |
| Chloroflexi | 399 | 0.14 | 133 | 0.04 | 2 | 0.00 |
| Ciliophora | 347 | 0.12 | 39 | 0.01 | 4 | 0.00 |
| Not assigned | 88 | 0.03 | 10 | 0.00 | 14 | 0.00 |
| Planctomycetes | 56 | 0.02 | 108 | 0.03 | 4 | 0.00 |
| Bacteroidetes | 50 | 0.02 | 49 | 0.02 | | |
| Ascomycota | 13 | 0.00 | 3 | 0.00 | 1 | 0.00 |
| Thermomicrobia | 7 | 0.00 | 37 | 0.01 | | |
| Zygomycota | 6 | 0.00 | | | | |
| Verrucomicrobia | 5 | 0.00 | 26 | 0.01 | | |
| Basidiomycota | 5 | 0.00 | 1 | 0.00 | | |
| Deferribacteres | 4 | 0.00 | | | | |
| Tracheophyta | 3 | 0.00 | | | 2 | 0.00 |
| Spirochaetes | | | 7 | 0.00 | | |
| Acidobacteria | | | 2 | 0.00 | | |
| Gemmatimonadetes | | | 1 | 0.00 | | |
| Proteobacteria | | | | | 13 | 0.00 |
| Bryophyta | | | | | 6 | 0.00 |

Day 1



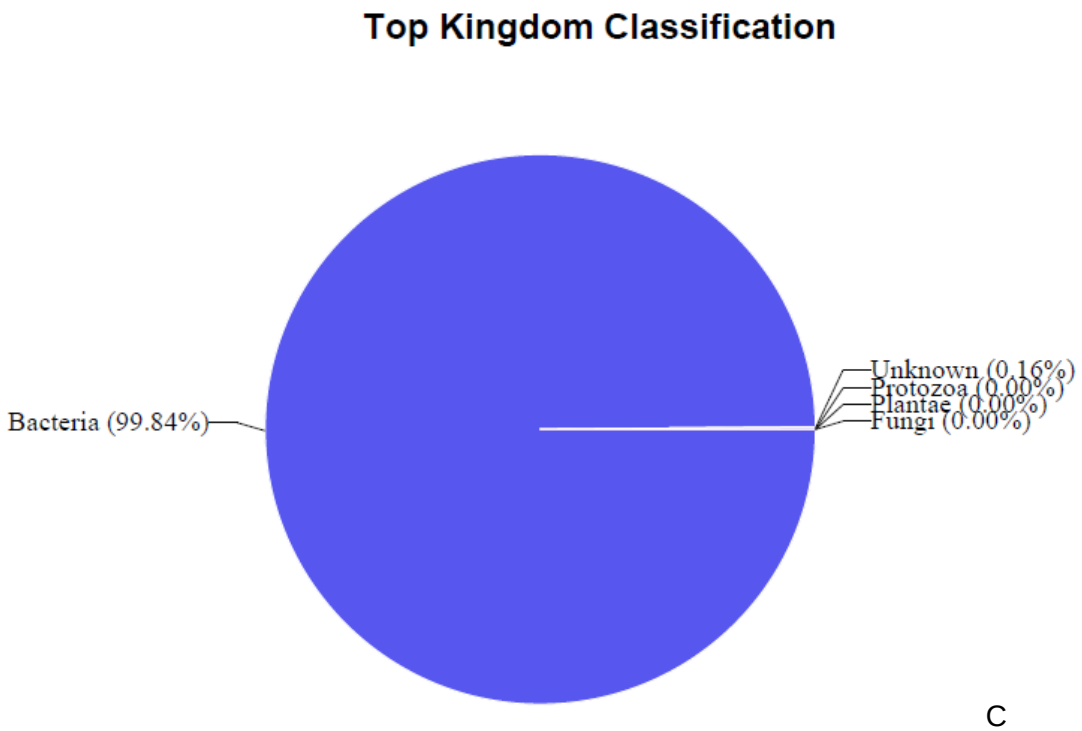

A

Day 15




B

Day 30




C

**Figure 16:** Top kingdom classification charts for microbial populations: **A**, Day 1; **B**, Day 15 and **C**, Day 30

# CHAPTER 8

# CONCLUSIONS

# AND

# RECOMMENDATIONS

# CHAPTER 8

# 7. CONCLUSIONS AND RECOMMENDATIONS - OVERVIEW

## 8.1 Conclusions

### 8.1.1 Bio-Methane Potential

The chemical composition of winery waste and cassava showed that both substrates were favourable for biogas production due to their high volatile solids and moisture content. Higher amounts of trace metals from both cassava and winey waste are optimal for anaerobic digestion. During BMP, the optimum temperature of 37 ˚C±05 showed great results for anaerobic digestion of the two substrates (cassava and winery waste). The obtained results from co-digestion of cassava and winery waste compared to the digestion of cassava alone were surprising. It was expected that the co-digestion of the two substrates would produce more biogas than a single substrate, however, the digestion of cassava (zebra dung + cassava) produced more biogas than the co-digestion of cassava and winery waste (zebra dung + cassava + winery waste).

### 8.1.2 Optimization

The ratio of cassava to winery waste proved to be the most significant factor during biogas production. Increasing the cassava quantity yielded more biogas but increasing the winery waste decreased biogas production. This decrease in biogas yield by winery waste could be due to the chemical properties of winery waste e.g the phenolics present in winery waste which hinder biogas production. The optimal conditions for biogas production were found to be pH 7, temperature of 35 ˚C±0.5 and cassava to winery waste ratio of 70:30.

The maximum response value for biogas production was 396 mL$CH_4$. From the results shown in **Table 10**, it is clear that the actual methane yield was close to the predicted methane yield. Co-efficient of determination, $R^2$, was found to be 0.9521 which shows that the model used can be used to predict methane production from co-digestion of cassava and winery waste. In conclusion, RSM can be a useful tool to predict methane production from co-digestion of cassava and winery waste for biogas production.

### 8.1.3 Single-stage 5 L Mesophilic Batch Digestion

The study of co-digestion of cassava with winery waste (70:30) using a single-stage 5 L mesophilic batch digester successfully produced 819.54 mL of biogas containing 62 %

methane. 1 g of volatile solids added produced 2.2 ml of methane (2.2 mL$CH_4$/gVS). These results showed that cassava and winery waste can be used in combination for the production of biogas provided the ratio, pH and temperature are monitored.

### 8.1.4 Microbial Community Dynamics

The study of microbial community dynamics showed the presence of the bacteria that is responsible for each stage of anaerobic digestion.

## 8.2 Recommendations

Recommendations for future studies are as follows:

- The C/N ratio was higher than normal and may have to be lowered by using urea as a nitrogen source
- Cultivation of cassava in South Africa especially in the rural areas as there is plenty of land where it can be cultivated for use in biogas production as well as food source thus creating jobs and alleviating poverty which will improve living standards
- The optimization study was found to be time consuming as each experiment has its own conditions therefore it would be recommended that some experiments be run simultaneously. The retention time could also be considered as a factor so as to determine how long each experiment took to reach completion. For this study all experiments were terminated after 15 days.
- Further studies on the microbial community dynamics will be required to capture the bacteria that is responsible for each stage of anaerobic digestion.
- No experiment on digestate was conducted in this study however, the potential to convert uncultivated lands to agricultural lands through digestate application could transform agriculture especially in rural areas. Vegetable gardens planted near an AD plant will bring economic potential to rural farmers.

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## APPENDICES

Raw Data for BMP Tests

**Table A.1:** Average biogas and methane yield per day for cassava only

| Days | Average Biogas yield per day (Cassava only) mLCH4 | Average Methane yield per day (Cassava only) mLCH4 |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 4,86 | 3 |
| 6 | 3,24 | 2 |
| 7 | 3,24 | 2 |
| 8 | 3,24 | 2 |
| 9 | 1,62 | 1 |
| 10 | 6,48 | 4 |
| 11 | 4,86 | 3 |
| 12 | 1,62 | 1 |
| 13 | 1,62 | 1 |
| 14 | 3,24 | 2 |
| 15 | 4,86 | 3 |
| 16 | 3,24 | 2 |
| 17 | 3,24 | 2 |
| 18 | 1,62 | 1 |
| 19 | 1,62 | 1 |
| 20 | 3,24 | 2 |
| 21 | 4,86 | 3 |
| 22 | 4,86 | 3 |
| 23 | 6,48 | 4 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| **Total** | **68,04** | **42** |

**Table A2**: Average biogas and methane yield per day for winery waste only

| Days | Average Biogas yield per day (WW) mLCH4 | Average Methane yield per day (WW) mLCH4 |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 3,24 | 2 |
| 5 | 3,24 | 2 |
| 6 | 3,24 | 2 |
| 7 | 3,24 | 2 |
| 8 | 4,86 | 3 |
| 9 | 1,62 | 1 |
| 10 | 1,62 | 1 |
| 11 | 0 | 0 |
| 12 | 0 | 0 |
| 13 | 1,62 | 1 |
| 14 | 0 | 0 |
| 15 | 1,62 | 1 |
| 16 | 1,62 | 1 |
| 17 | 8,1 | 5 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| **Total** | **34,02** | **21** |

Table A3: Average biogas and methane yield per day for co-digestion of cassava & winery waste

| Days | Average Biogas yield per day (CS+WW) mLCH4 | Average Methane yield per day (WW + CS) mLCH4 |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 6,48 | 4 |
| 6 | 4,86 | 3 |
| 7 | 6,48 | 4 |
| 8 | 3,24 | 2 |
| 9 | 3,24 | 2 |
| 10 | 1,62 | 1 |
| 11 | 1,62 | 1 |
| 12 | 4,86 | 3 |
| 13 | 4,86 | 3 |
| 14 | 3,24 | 2 |
| 15 | 1,62 | 1 |
| 16 | 0 | 0 |
| 17 | 4,86 | 3 |
| 18 | 3,24 | 2 |
| 19 | 4,86 | 3 |
| 20 | 6,48 | 4 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| **Total** | **61,56** | **38** |

**Figure A1**: Picture of a scale used for weighing samples

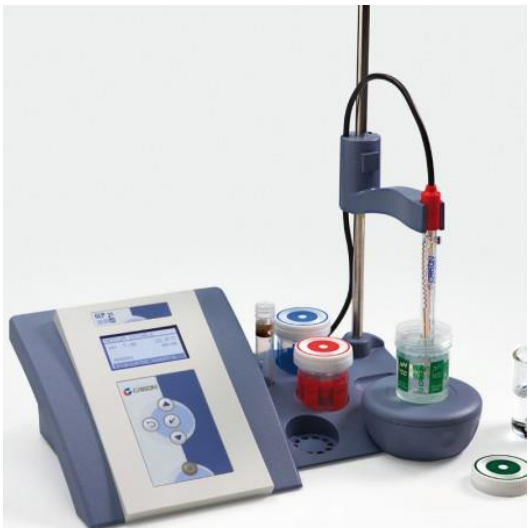

**Figure A2**: pH meter used to measure pH of samples

C Biogas Optimization Raw Data

**Table B1**: pH, Temperature, co-substrate ratio, methane yield per day and cumulative methane yield for the 20 reactors

| Bottle number | pH | Temp (Deg C) | Co-substrate ratio (CS;WW) | CS (g) | WW(g) | Methane yield per day (mLCH4) | | | | | | | | | | | | | | Cumulative Methane yield (mLCH4) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | |
| 1 | 6 | 45 | 40;60 | 20 | 30 | 0 | 0 | 20 | 40 | 20 | 11 | 9 | 9 | 6 | 6 | 0 | 0 | | 0 | 0 | 121 |
| 2 | 8 | 45 | 40;60 | 20 | 30 | 0 | 0 | 23 | 28 | 15 | 10 | 10 | 9 | 10 | 8 | 9 | 0 | 0 | 0 | 0 | 122 |
| 3 | 7 | 45 | 70;30 | 35 | 15 | 0 | 0 | 20 | 40 | 40 | 40 | 31 | 18 | 16 | 18 | 13 | 8 | 6 | 0 | 0 | 250 |
| 4 | 8 | 45 | 100;0 | 50 | 0 | 0 | 0 | 0 | 14 | 40 | 20 | 18 | 16 | 8 | 8 | 7 | 5 | 0 | 0 | 0 | 136 |
| 5 | 6 | 45 | 100;0 | 50 | 0 | 0 | 0 | 0 | 10 | 40 | 20 | 15 | 12 | 8 | 6 | 6 | 0 | 0 | 0 | 0 | 117 |
| 6 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 46 | 54 | 47 | 39 | 32 | 25 | 23 | 19 | 16 | 18 | 16 | 10 | 8 | 353 |
| 7 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 38 | 44 | 33 | 30 | 32 | 30 | 27 | 24 | 20 | 22 | 16 | 11 | 19 | 346 |
| 8 | 6 | 35 | 70;30 | 35 | 15 | 0 | 0 | 28 | 36 | 33 | 32 | 21 | 22 | 20 | 16 | 19,5 | 19 | 15,5 | 9 | 1 | 237 |
| 9 | 8 | 35 | 70;30 | 35 | 15 | 0 | 0 | 33 | 36 | 31 | 28 | 27,5 | 24 | 19 | 20 | 22 | 17 | 12 | 9 | 6,5 | 285 |
| 10 | 7 | 35 | 40;60 | 20 | 30 | 0 | 0 | 30 | 24 | 22 | 20 | 18 | 17 | 12 | 12 | 14 | 9 | 6 | 5 | 0 | 189 |
| 11 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 38 | 44,5 | 37 | 33 | 35,5 | 34,5 | 27,5 | 25 | 23 | 16 | 15 | 17 | 12 | 358 |
| 12 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 40 | 42 | 44 | 39 | 36,5 | 29 | 30 | 27 | 17,5 | 26 | 19 | 16 | 12 | 378 |
| 13 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 39 | 44 | 41,5 | 40 | 38 | 36,5 | 33 | 27 | 23,5 | 24 | 22,5 | 16 | 11 | 396 |
| 14 | 7 | 35 | 70;30 | 35 | 15 | 0 | 0 | 30,5 | 33 | 33,5 | 38 | 36,5 | 32 | 28 | 29 | 26 | 23,5 | 22 | 13 | 15 | 360 |
| 15 | 7 | 35 | 100;0 | 50 | 0 | 0 | 0 | 29,5 | 35 | 36 | 26 | 22,5 | 21,5 | 24,5 | 17 | 13,5 | 9,5 | 0 | 0 | 0 | 235 |
| 16 | 8 | 25 | 40;60 | 20 | 30 | 0 | 0 | 8 | 16 | 14 | 9 | 8 | 9 | 11 | 10 | 6 | 9 | 0 | 0 | 0 | 100 |
| 17 | 6 | 25 | 100;0 | 50 | 0 | 0 | 0 | 13 | 16 | 11 | 10,5 | 9 | 6 | 8 | 7,5 | 11 | 6 | 2 | 0 | 0 | 101 |
| 18 | 6 | 25 | 40;60 | 20 | 30 | 0 | 0 | 18 | 21 | 11 | 9 | 11 | 9 | 12 | 9 | 7 | 8 | 0 | 0 | 0 | 115 |
| 19 | 8 | 25 | 100;0 | 50 | 0 | 0 | 0 | 12,5 | 22,5 | 18 | 10,5 | 12 | 9,5 | 8 | 8,5 | 6,5 | 0 | 0 | 0 | 0 | 108 |
| 20 | 7 | 25 | 70;30 | 35 | 15 | 0 | 0 | 26 | 28 | 24 | 25 | 22,5 | 21 | 17,5 | 15 | 13 | 9 | 12 | 11 | 11 | 235 |