Cape Peninsula University of Technology

APPLICATION OF IRON OXIDE NANOPARTICLES FOR BIOGAS YIELD OPTIMIZATION FROM WINERY SOLID WASTE AND SORGHUM STOVER

By

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DECLARATION

I, Carrelle Gabrielle Ossinga, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/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.

03/03/2021

Signed **Date**

ABSTRACT

Different methods and processes of optimizing the yield of biogas are currently being explored globally for better biomass management and renewable energy security. Winery solid waste is problematic in South Africa due to current disposal method to the environment and the way it is being handled. Similarly, a lot of waste is generated during sorghum harvesting; however, the stover represents a suitable feedstock for anaerobic digestion due to its high carbohydrate and protein content. Anaerobic digestion is one of the renewable energy technologies able to produce biogas from a variety of biomass sources. The addition of iron oxide nanoparticles (ION) has been touted to increase biogas production. Therefore, the aim of this study is to investigate the ability of ION to boost biogas yield via anaerobic digestion process from sorghum stover (SS) and winery solid waste (WSW). Biomethane potential tests were carried out at mesophilic conditions (37°C ± 0.5) in a batch reactor using SS and WSW singly and in combination at 1:1 ratio, in the absence and presence of ION. A 30-day retention time was used for all the tests. Biogas optimization was also carried out. The optimal conditions from three chosen factors viz., solid retention time (SRT), substrate ratio (SS/WSW) and concentration of iron oxide nanoparticles (ION) were investigated for biogas production using response surface methodology (RSM). The effect and relationship between these three factors on the biogas yield were also explored using CCD (central composite design) to determine the anaerobic co-digestion experiment. The upscaling experiment employed the use of optimal values in a 5L batch reactor. The results from the BMP tests for substrates with ION (wION) and without ION (w/oION) showed a cumulative methane yield of 9.5 $mLCH_4.gVS^{-1}$ WSW, 18.5 $mLCH_4.gVS^{-1}$ SS, and 44.6 $mLCH_4.gVS^{-1}$ substrate ratio for w/oION. Similarly, 36.3 $mLCH_4.gVS^{-1}$ WSW, 29.3 $mLCH_4.gVS^{-1}$ SS and 60 $mLCH_4.gVS^{-1}$ WSW+SS were obtained from wION. It was concluded that ION had a significant effect on biogas yield especially with WSW biomass where the increase was tripled. Results from the co-digestion experiment produced more biogas than single digestion. Optimization experiment using optimal conditions of 100 ppm for ION concentration, 80:20 substrate ratio and 25 days SRT produced maximum cumulative biogas yield of 51.9 $mLCH_4.gVS^{-1}$ which is higher than the RSM predicted value of 49.6 $mLCH_4.gVS^{-1}$ by the quadratic model. The RSM model proved successful in the optimization process with a determination coefficient (R^2) value of 0.9528. The upscaled experiment using a 5L batch reactor at mesophilic conditions with optimal values resulted in a cumulative biogas production of 522.97 $mLCH_4.gVS^{-1}$ with a methane content of 74%. The results of this study will affect the agro-industry as well as waste management practitioners.

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DEDICATION

I wish to dedicate this thesis to my family, particularly my mother.

To my mum, Victoire Flamine (MBA) who taught me the importance of hard work, effort, perseverance and faith. Thank you for the great model that you are in my life.

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 17th International Conference on Science, Engineering. Technology and Waste Management (SETWM-19) organized by Eminent Association of Researchers in Engineering and Technology (EARET), Johannesburg, South Africa. Title: Enhanced Biogas Production from winery Solid Waste through Application of iron Oxide Nanoparticles

2. Publication in Proceedings of the 17th International Conference on Science, Engineering, Technology and Waste Management (ASETWM-2017).
   Title: Enhanced Biogas Production from winery Solid Waste through Application of Iron Oxide Nanoparticles, serial number 44 page 241-245.

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CLARIFICATION OF BASIC TERMS AND CONCEPTS

- **AD:** Anaerobic digestion is a process which occurs when biodegradable organic matter in environments is broken down by microorganisms in the absence of oxygen to produce biogas. It is a natural activity that can be controlled in a way that allows the gases given off to be captured and made usable.

- **Batch process:** Existing process configuration in which a series of operations are carried out over a period of time on an identifiable reactor or digester.

- **Biogas:** A mixture of different gases obtained by anaerobic digestion

- **Catalyst:** A catalyst is an agent or compound that is added in a process to increase a gas production or speed up a chemical reaction. In this study iron oxide is used as a catalyst.

- **Co-digestion:** Co-digestion or in more explicit terms, the simultaneous digestion of two or more substrates is a method used to overcome the inconvenience of single digestion.

- **Continuous process:** Existing process digestion in which all operations occur at the same time and the substrate being used is not divided into detectable portions.

- **Inoculum:** Materials (essentially microorganisms) used to inoculate some other material or substance such as soil or compost.

- **Lignocellulose:** Lignin and cellulose work together to provide a structural function that is the origin of the stiffness and rigidity of the plants

- **Methanogenesis:** The last step of anaerobic digestion where methanogenic bacteria is converted into methane and carbon dioxide.

- **Mesophilic:** Mesophilic microorganisms are organisms that are optimally active at moderate temperatures, between 25°C and 40°C.

- **Nanoparticles:** Microscopic particles smaller than 100nm in diameter.

- **Thermophilic:** Thermophilic microorganisms are microorganisms with an optimum growth temperature of 50°C or more, a maximum up to 70°C and a minimum of 20°C.

GLOSSARY

Acronyms/Abbreviations	Definition/Explanation
AD	Anaerobic digestion
AGS	Anaerobic granular sludge
BMP	Biomethane potential
COD	Chemical oxygen demand
GHG	Greenhouse gas emission
ION	Iron oxide nanoparticles
LCFA	Long chain fatty acid
SEM	Scanning electron microscopy
SRT	Solid retention time
SS	Sorghum stover
WAS	Waste activated sludge
WSW	Winery solid waste
XRD	X-ray diffraction
ZD	Zebra dung

CHAPTER ONE
INTRODUCTION

1.1. Problem statement

The world is currently confronting challenges such as environmental pollution due to fossil fuel energy sources, an increase in organic waste production and in global energy demand due to rising human population, with approximately 88% of energy produced currently from fossil fuels (Pullen, 2015, Achinas et al., 2017). Australia recently experienced bush fires on an unprecedented scale in September 2019 due to climate change, with 6.3 million hectares burned (Ryan, 2020). South Africa has experienced a power shortage since 2007 due to breakdowns at power stations and also depletion of water and diesel resources (Hartleb, 2008). Many countries are dealing with waste management, waste reduction, and waste prevention and waste recycling which have become legislative and environmental issues. Traditional disposal methods (incineration, waste dumping and landfill) are offensive as they contribute to pollution and greenhouse gas emissions (GHG). Moreover, the methods represent a lost opportunity because little waste can be reused, recycled or have materials extracted from it. These materials represent proper feedstock for anaerobic digestion (Pullen, 2015).

For decades, production of biogas via the anaerobic digestion process has been an adequate solution to dealing with the need for energy and the lack of mineral oil resources. Constantly, researchers investigate methods to increase the biogas yield from biomass via this process through different combinations of substrates, catalysts, and operating factors. Africa and especially South Africa encompass a variety of feedstocks, which, used as biomass, represent an economic advantage. Considering the challenges listed, iron oxide nanoparticles is a catalyst which have shown the highest result in biogas increase among research on nanomaterials. Performing anaerobic digestion on sorghum stover and winery waste locally available with iron oxide may therefore result in tripled biogas yield that will favour a decrease in pollution and control of waste management (Pullen, 2015, Achinas et al., 2017).

Is iron oxide capable of furnishing satisfying results in anaerobic digestion by using dried substrates for increased biogas production? This could lead to improved waste management and energy security If the hypothesis proves true.

1.2. Background

For several decades, anaerobic digestion (AD) has been a well-studied technology to face the increase in energy demand and waste management. AD has been proven to be suitable not only because of its limited environmental impact, but also for its high potential for energy recovery (Ariunbaatar et al., 2014). AD is a convoluted biochemical process that converts complex organic materials into biogas, which is a needed renewable source of energy

(Ganzoury and Allam, 2015). Due to the biogas benefits, there have been several studies aiming to yield significant biogas yields (Jenicek et al., 2012). The biogas produced can be converted into heat and/or electricity production, upgraded to vehicle fuel or used as regenerative energy via injection of treated biogas into a natural gas grid (Angelidaki et al., 2009, Hoppe et al., 2009).

According to Michalska and Ledakowicz (2013), feedstocks and organics wastes used as biomass to yield a renewable energy source (biogas) must not conflict with sustainable development. Therefore, the choice of the substrate is a determining factor.

AD processes convert feedstock biomass into biogas. Sorghum is a substrate known to be versatile as different parts (stalks, sugar and leaves) produce methane after fermentation. The grain component is an excellent substitute for animal feed. Sorghum is also widely available due to its ability to grow in a wide range of temperatures. Remaining in the soil after the harvest, it possesses a good root system for the preservation of humus and soil nutrients. Additionally, optimization of growth and conversion of sorghum is possible due to a broad collection of hybrid strains. Sorghum stover has low lignin content adequate for high biodegradation (Jerger et al., 1987).

Winery waste used in a co-digestion anaerobic process with sorghum stover overcomes the inconvenience of single digestion and is considered more efficient. Wine production counts as a primary sector in the food processing industry, especially in South Africa which is a top producer with a share of 25 billion litre of the world market (Zacharof, 2017). Winery processes result in multiple types of waste (some of them listed as grape stalks, exhausted yeast, wine lees, grape marc and high loaded wastewater), producing 5 tons for each hectare/year of grape wine produced. The winery wastes are distributed throughout the year, although predominantly during the harvesting and production cycle. There are potential risks from some constituents if disposal isn't properly done. These solid wastes require expensive methods to process waste discharge. Therefore the need to reuse, recycle and recover energy from winery solid waste becomes apparent (Zacharof, 2017). Winery solid waste is characterised by high levels of chemical oxygen demand (COD), both particulate and soluble, as well as high biodegradability. (Da Ros et al., 2014).

Advances in nanotechnology have helped to improve biogas production via the development of processes using nanoparticles (Gonzalez-Estrella et al., 2013). Casals et al. (2014) confirmed the ability of iron oxide nanoparticles to lose or gain electrons, making it an ideal and versatile catalyst for boosting biogas production.

Several studies done on various additives to nanomaterial explored their effect on biogas yield. Researchers investigated different types of material, such as metal oxide, zero-valent metals, nano-ash and carbon-based materials. Various studies have shown that ZnO, CuO, Mn_2O_3

and Al_2O_3 have negative effects on biogas yield due to their toxicity, while TiO_2 and CeO_2 demonstrated mixed effects depending on their concentration. In addition, nano-iron oxide (Fe_3O_4) as well as metal nanoparticles encapsulated in porous SiO_2 showed positive effects and an increase in biogas yield. Various feedstocks used in these studies include anaerobic granular sludge (AGS), cattle manure, wastewater, waste activated sludge (WAS) and others. No studies have been made of dried substrates using iron oxide as catalyst (Ganzoury and Allam, 2015).

Considering the advantages of anaerobic digestion listed above, the choice of the substrate and pre-treatments are relevant to efficient biogas yields. This research will therefore pursue the importance of application of iron oxide nanoparticles to increase the biogas yield using affordable, available and efficient dried feedstock materials such as sorghum stover and winery waste in co-digestion.

1.3. Hypothesis and research questions

According to the above literature review, it is assumed that the utilization of iron oxide nanoparticles as a catalyst has the potential to enhance biogas and methane yield via anaerobic digestion using dried substrates.

In order to confirm or invalidate this hypothesis, the following research questions are posed:

- Is iron oxide capable of breaking the lignin-cellulose of sorghum stover?
- Is co-digestion better than single digestion in terms of yield?
- What are the best factors for a high yield of biogas?

1.4. Aim and objectives of the research

The main aim of this study is to investigate the ability of iron oxide nanoparticle to boost the biogas yield via anaerobic digestion process from sorghum stover and winery solid waste.

Specific objectives are as follows:

- To synthesize iron oxide nanoparticles and characterise them. Synthesis will be done by hydrothermal method and characterized by transmission electron microscopy (TEM) and X-ray diffraction (XRD) patterning.
- To evaluate biomethane potential using anaerobic co-digestion at mesophilic conditions with sorghum, winery waste and iron oxide nanoparticles. The following steps will be taken:
    i. Biomethane potential tests will be done for sorghum stover only and with iron oxide
    ii. Biomethane potential tests will be done for Winery solid waste alone and with iron oxide nanoparticle

iii. Biomethane potential tests will be done in co-digestion with sorghum stover and winery solid waste with and without iron oxide nanoparticle for a duration of 30 days.

- To find the optimal conditions for increased biogas production, biomethane tests will be done for three different factors: solid retention time, iron oxide nanoparticle concentration, and co-digestion ratio.

- To evaluate the biomethane potential of optimum conditions at an upscale level. Optimal values obtained from optimization will be applied in a 5L digester at mesophilic conditions to produce biogas.

1.5. Delineation of the research

The research study on iron oxide nanoparticles to boost yield biogas has the objective of determining and verifying their role in biogas production using sorghum stover and winery waste as feedstock. The effect of iron oxide has been verified only on wastewater treatment, but its ability to breakdown lignocellulose is not certain. Therefore, this study will investigate the potential of iron oxide nanoparticle only on two dried substrates.

1.6. Significance of the research

This research study will be relevant and benefit different areas like environmental engineering and scientific research. Environmental engineering, waste management and biogas production will be the principal domains served as the study represents a solution to dealing with waste, while responding to the worldwide need for biofuel. With regard to scientific research, the domain of nanotechnology will be enhanced, and the knowledge gained can be used in other areas in the future.

CHAPTER TWO
LITERATURE REVIEW

2.1 What is Biogas?

Biogas is a product of the anaerobic digestion process where methanogenic bacteria feeds off the input biodegradable substrate to give a mixture of different gases mostly composed of methane and carbon dioxide, with a small quantity of hydrogen and trace hydrogen sulphide. The decomposition of organic waste used as feedstock occurs in the absence of oxygen; for this reason the process responsible for producing this type of biofuel (biogas) is anaerobic digestion. Biogas is combustible due to its high methane content (50-75%) and produces a dark blue flame. It is a renewable energy source (Pullen, 2015).

Biogas is an energy source safe for the environment because it eases two main environmental problems consecutively. The first is worldwide contagious waste dumping on landfills that contributes to dangerous levels of methane released on a daily basis, and the secondly the dependence on fossil fuel energy to meet overall global energy demand (Pullen, 2015).

Biogas is a resource resulting from recycling materials via the biological conversion of organic waste into energy. Biogas generation recuperates waste materials that would otherwise cause landfill pollution; it avoids toxic chemical use in sewage treatment plants, and results in money, energy, and material saving by processing waste on-site. Methane gas contained in decomposing waste converts into carbon dioxide and has approximately 20 to 30 times the heat-trapping abilities of carbon dioxide. An illustration is that when a rotting loaf of bread converts into biogas, the environment surrounded by the loaf will be impacted roughly 10 times less than if it has left to rot in a landfill (Pullen, 2015).

Biogas production leads to another advantage. Nutrients existing in the organic waste are soluble in water due to the decomposition of organic substrate in a liquid environment and therefore form slurry rich in nutrients from this dissolution, normally used as fertilizer for plants. This resulting fertilizer is produced daily and consequently is a highly prolific by-product of anaerobic digestion (Pullen, 2015).

Biogas, renewable natural gas also named biomethane, can potentially be used not only for transportation as it is for biodiesel and bioethanol but also for heat and electricity generation (Nunez, 2019).

2.1.1. Biogas composition

According to Arthur et al. (2011), biogas yield depends on several factors listed as substrate composition, retention time, type of substrate, and biodigester conditions. The typical biogas

composition is represented in **Table 2.1** below. It is necessary to determinate the composition of biogas produced before usage as the presence of traces of hydrogen sulphide can be harmful, especially in internal combustion engines.

Table 2.1: Biogas composition (Ganzoury and Allam, 2015)

Compound	Yield (%)
Methane (CH_4)	50-75
Carbon dioxide (CO_2)	25-45
Water vapour (H_2O)	2-7
Nitrogen (N_2)	<2
Oxygen (O_2)	<2
Hydrogen sulphide (H_2S)	<1
Hydrogen (H_2)	<1
Ammonia (NH_3)	<1

2.2 Bioenergy

Bioenergy is renewable and carbon neutral because the carbon released during combustion is taken up during renovation of the biological resources, which happens over millions of years, thus making the resources continuously available (Paz, 2013).

There are three main types of bioenergy briefly described below and further elaborated from different feedstock shown in **Figure 2.1**.

2.2.1. Biodiesel

Biodiesel is a product of oils or fats using trans-esterification. It is produced from various substrates such as vegetable oils, soy, rapeseed, animal fats, mustard, flax, palm oil, hemp, field pennycress, and sunflower. Biodiesel with 100% purity contains less energy on volumetric basis than petroleum diesel. In other words, the higher the biodiesel percentage, the lower the energy content per gallon. Biodiesel is an operational solvent and washes dregs deposited by mineral diesel in engines. It efficiently washes the engine combustion chamber of carbon deposits, thereby improving maintenance efficiency (DGFS, 2005).

2.2.2. Bioethanol

Bioethanol is the most widely produced bioenergy. In the US, fuel is composed of 10% ethanol and 90% gasoline. The second largest producer of ethanol just behind US, Brazil, includes up to 27% of ethanol in its fuel. Fermentation of sugars produce bioethanol from substrates, such as sugar cane, wheat, corn, sugar beets and any sugar or starch that alcoholic drinks can be

prepared from. Ethanol may be commonly utilized in petrol engines to replace gasoline (DGFS, 2005).

2.2.3. Bio-oil

Oil and fat hydrogenation provide a diesel auxiliary. Diesel and hydrogenated oils can be mixed in all quantities. Hydrogenated oils have numerous benefits over biodiesel, which include their good performance at low temperatures, no storage constancy issues and no susceptibility to bacterial attack. It was estimated that 19 million tons of oil would be available from biomass by 2020 (DGFS, 2005).

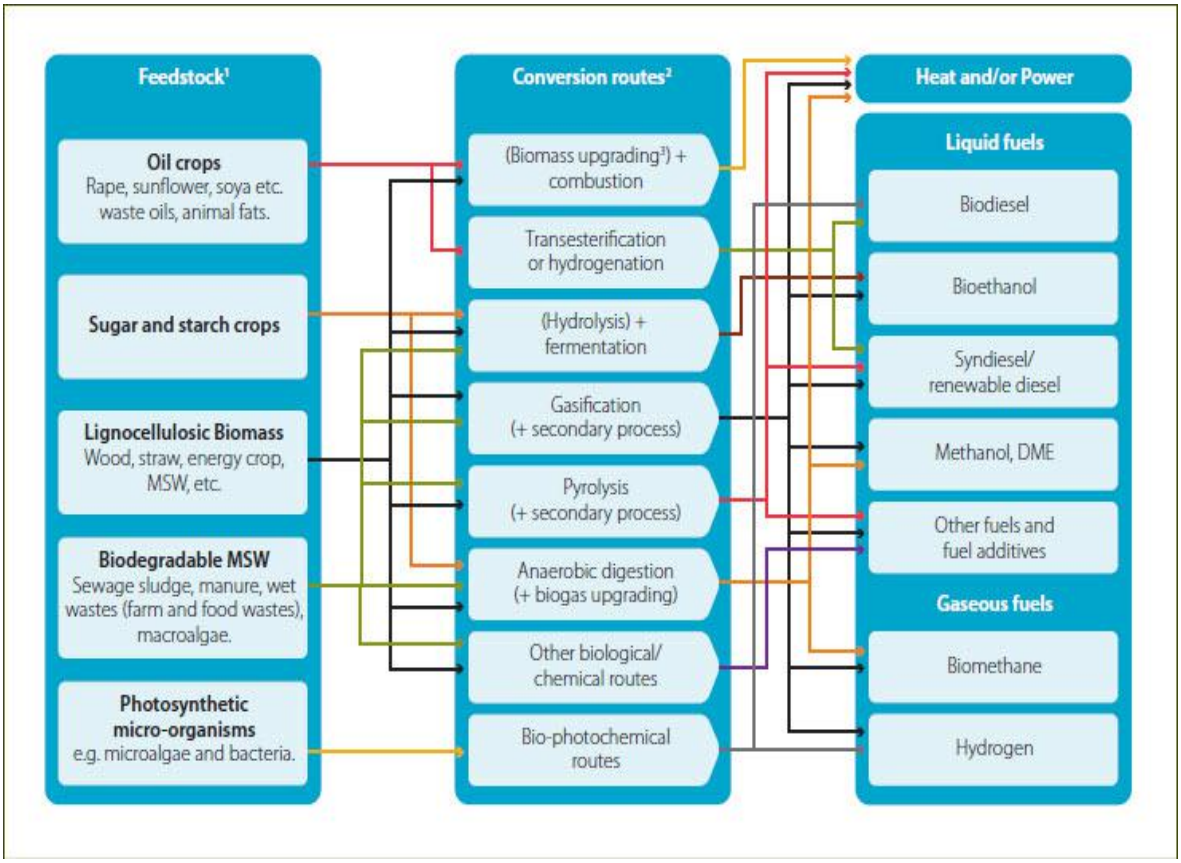

Figure 2.1: Feedstock conversion chain (Ausilio Bauen, 2009)

2.3 Biogas production techniques

According to Achinas et al. (2017), biogas is a multilateral renewable energy source produced via anaerobic digestion. This biogas production technique is an energy-efficient and environmentally friendly technology.

2.3.1. Anaerobic digestion

Anaerobic digestion is a series of biological processes that converts organic waste to energy (waste-to-energy). It occurs when biodegradable complex organic materials in environments

are broken down into simpler by microorganisms in oxygen-free environment for biogas production (Caruana and Olsen (2012).

There is diversity of substrate-like food scrapings, animal manure, wastewater, and sewage that can produce biogas via anaerobic digestion. **Figure 2.2** is a representation of biogas production from organic matter via anaerobic digestion (Achinas et al., 2017).

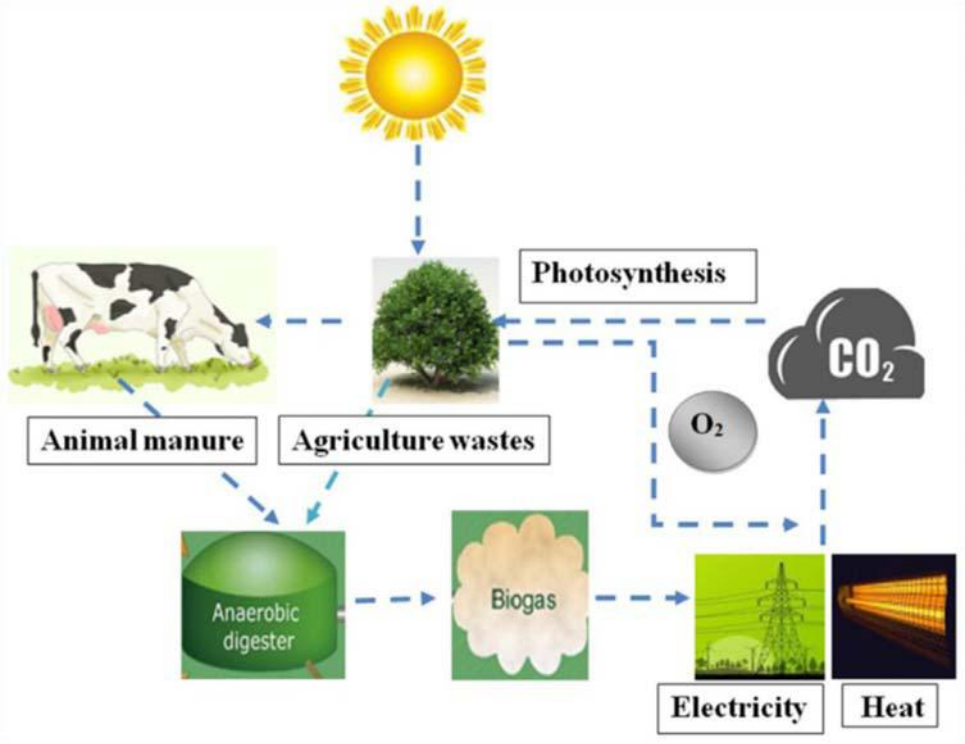

Figure 2.2: The CO_2 closed cycle in anaerobic digestion (Ganzoury and Allam, 2015)

2.3.1.1. Biochemical steps in anaerobic digestion

The AD process has four stages described below. Organic waste mixtures have a chemical composition of $(C_6H_{10}O_4)n$ on the equations represented in the different stages.

2.3.1.1.1. Hydrolysis

This is the first phase of AD which sees the breakdown of complex matter (carbohydrates, proteins, lipids, etc.) into sugars and amino acids. It is commonly a long-chain chemical compound, but hydrolysis breaks it down into single molecules with the aid of hydrolytic bacteria. Cellulase, protease and lipase are the enzymes which catalyze this process (hydrolysis). Enzymes are excreted by bacteria, resulting in fermentation and conversion of proteins to amino acids, lipids to long-chain fatty acids (LCFA), and polysaccharides finally to simple sugars. This assemblage of microbes is composed of a large group of potential bacteria able to flourish in the absence or presence of oxygen. The rate-limiting process for the complete digestion of materials with high-suspended solids (SS) over chemical oxygen

demand (COD) ratio is hydrolysis. It is usually due to the availability of reachable free surface area of the particles and the overall structure of the solid substrate. Lack of enzyme activity has no impact on rate-limiting process.

Moreover, the required reactor design depends on hydrolysis as at low temperature, it possibly will limit the overall process. The products of hydrolysis are the materials for acidogenic bacteria. **Equation (2.1)** shows a reaction of hydrolysis from the breakdown of organic waste into sugar where in the present case glucose is the substrate (Wang et al., 2010, Lohani and Havukainen, 2018).

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2O \qquad (2.1)$$

2.3.1.1.2. Acidogenesis

In the second phase, the fastest step in AD process, the distinct molecules of sugar and amino acids are broken down into ethanol and fatty acids by microorganisms under the action of acidogenic fermentative bacteria. Carbon dioxide, hydrogen sulphide and ammonia are also by-products resulting from this step. The composition of final products is dependent on the conditions of the reactor medium. In occurrence, acetate will remain the main final product if H_2-scavenging organisms such as methanogens efficiently remove H_2. However, if methanogenesis is delayed and H_2 accrues, more reduced products such as propionate and butyrate will be more potentially present. Hence, sewages of full or troubled anaerobic reactors contain often these more reduced intermediate products and turn out to be acidic. Besides the acidogenesis product, carbon dioxide, hydrogen and acetic acid will not be converted in the acetogenesis process and the methanogenic bacteria will use them directly in the final stage. **Equations (2.2)** and **(2.3)** are representative acidogenic reactions where glucose is converted into acetic acid and propionate, respectively (Wang et al., 2010, Lohani and Havukainen, 2018).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2OH + 2CO_2 + 4H_2 \qquad (\Delta G = -116.3kj/mol) \qquad (2.2)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \qquad (\Delta G = -36.5kj/mol) \qquad (2.3)$$

2.3.1.1.3. Acetogenesis

In the third phase, the ethanol and fatty acids obtained previously in the second phase are converted into carbon dioxide, hydrogen and acetic acid by acetogenic bacteria. There exist two categories of acetogenic bacteria known as hydrogen-producing acetogens and homo acetogens (Wang et al., 2010, Ersahin et al., 2011).

A low hydrogen partial pressure is required in order to avoid the inhibition of the metabolism of the microorganism during the acetogenesis reaction. This reaction is essential due to its ability to convert the products of acidogenesis which cannot be transformed directly into methane to methanogenic substrates. **Equations (2.4)** and **(2.5)** demonstrate the production

of acetic acid from butyrate and propionate by utilizing hydrogen-producing bacteria (Ersahin et al., 2011, Ganzoury and Allam, 2015).

$$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+ \quad (\Delta G = +19kj/mol) \tag{2.4}$$

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_{3^-} + 3H_2 \quad (\Delta G = -104.9kj/mol) \tag{2.5}$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (\Delta G = -112kj/mol) \tag{2.6}$$

Homoacetogenesis is the generation of acetic acid from dissolved H_2 and CO_2 by homo acetogens, as illustrated in **Equation 2.6** (Ersahin et al., 2011).

2.3.1.1.4. Methanogenesis

The last phase for biogas production sees the conversion of the remaining acetic acid and hydrogen into methane and extra carbon dioxide products via methanogenic bacteria. Acetoclastic and hydrogenotrophic methanogens are accountable for this transformation (Wang et al., 2010).

The conversion of acetic acid into methane is responsible for 70% of the methane production in AD as represented in **Equation (2.7)** This transformation is involved in acetoclastic process (Gallert and Winter, 2005, Ganzoury and Allam, 2015).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (\Delta G = -122.55kj/mol) \tag{2.7}$$

Alternatively, hydrogentrophic process involves the conversion of hydrogen and carbon dioxide into methane as represented in the **Equation (2.8)** below, and can be performed by almost all methanogens (Lackner et al., 2018).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (\Delta G = -135.6kj/mol) \tag{2.8}$$

In case of enough high concentrations of sulphate, methanogenic bacteria may compete with sulphate-reducing bacteria (Ersahin et al., 2011, Ganzoury and Allam, 2015). A graphic representation of the four biochemical stages involved in anaerobic digestion is shown in **Figure 2.3** below.

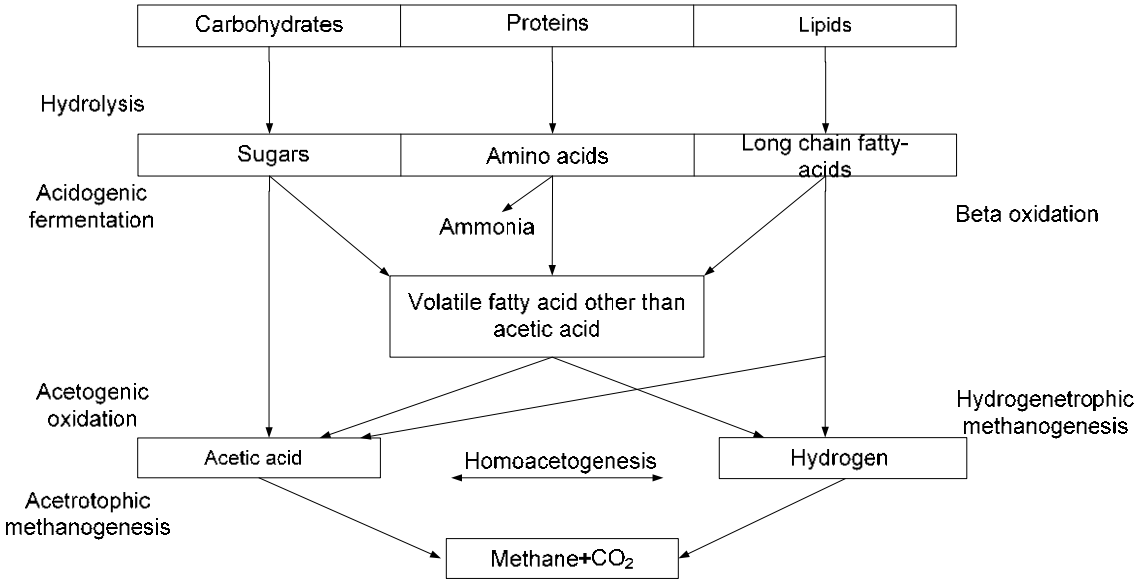

Figure 2.3: The stages of anaerobic digestion (Salminen and Rintala, 2002)

2.4. Parameters affecting AD

Biogas production activity is controlled by several factors such as pH, partial pressure, hydraulic retention time, C/N ratio, temperature, pre-treatment of feedstock, trace of metal and concentration of substrate (Schön, 2010). The most important parameters will be discussed in the following sections.

2.4.1. Temperature

Temperature affects anaerobic processes as in all biological processes. Anaerobic digestion can be worked in a large range of temperature and anaerobic microorganisms are commonly classified into three thermal groups, which are psychrophilic (under 20°C), mesophilic (between 20°C and 40°C) and thermophilic (45 to 70°C). Physical and chemical properties of the substrate may get disturbed depending on the anaerobic process temperature of the reactor. From this, the thermodynamic and kinetic reaction of the biological processes may also be affected. Increasing temperature has benefits as it shortens the reaction time and therefore the reactor hydraulic retention time (HRT), improves diffusivity and liquid-solid biomass separation. However, high temperatures are also responsible for an increase of the fraction of free ammonia, which inhibits the bacteria. The mesophilic process is the more common and suitable in current AD facilities due to its stability, but is realized at more extensive retention times (Schön, 2010, Singh and Kaushik, 2018).

2.4.2. pH

Three main types of bacteria are involved in the production of biogas: bacteria responsible for hydrolysis, fermentative bacteria, and methane-producing archaea bacteria. The fermentative

bacteria can be efficient in pH range from 4 to pH 8.5 with their optimum pH range situated between 5.0 and 6.0 whereas methanogenic archaea can be efficient in pH range from 5.5 to 8.5 with an ideal range of 6.5–8.0. ‘’Methane-producing’’ bacteria are responsible for the production of bicarbonate, which defuses the reduction of pH affected by acid-producing bacteria. The pH value of less than 5 .0 will kill methanogens. A pH value greater than 8 is lethal to most anaerobic organisms and consequently the inhibition of biological functions. High pH may be overcome by the addition of superior amount of feedstock (Singh and Kaushik, 2018).

2.4.3. Carbon/nitrogen ratio

There are indispensable elements for the growth of microorganisms present in the anaerobic digestion process. The synthesis of amino acid, proteins for example, needs one of the key nutrient elements, which is nitrogen. Nitrogen can convert a buffer compound into ammonia for the neutralization of the acidification process. Hence, it is a requirement for all feedstock to contain essential trace elements and nutrients for an effective anaerobic digestion (AD) process. A C/N/P ratio of 100:3:1 is appropriate for high methane yield. Deficiency of buffering capacity or lacking of nutrients for microorganism growth are consequences of significant deviation of C:N:P ratio (Lohani and Havukainen, 2018).

2.4.4. Solid retention time (SRT)

The SRT is an important factor that disturbs biochemical characteristics of organic materials. The SRT plays a role key in anaerobic digestion particularly for methanogens, which operate at low temperatures. SRT influences methanogenic activity. It should last long enough to provide the required methanogenic activity. The debut of methanogenesis is at SRT between 5 and 15 days at 25 °C and between 30 and 50 days at 15 °C although input substrate is a variable to it (Singh and Kaushik, 2018).

2.4.5. Organic loading rate

Organic loading rate controls the level of starvation of microorganisms in biological systems. The higher the ORL, the faster the microbial growth and the lower the ORL, the more starved the microorganisms. Conversely, with an ORL too high, microorganisms will not be able to consume all produced organic acids which will result an acidic state of the digester. Furthermore, high ORL may cause overloading with high quantities of organic material. Feedstock and reactor temperature are the principal factors determining OLR (Cioabla et al., 2012).

2.4.6. Effect of a particle size

The kinetics process of anaerobic digestion depends on the particle size of the feedstock (Lohani and Havukainen, 2018). According to a study by Kim et al. (2000) on the effects of

particle size, an increase in anaerobic thermophilic food waste digestion from 1.02 to 2.14 mm showed a decrease on the substrate utilization rate coefficient from 0.0033 to 0.0015 h^{-1}. This fact indicates that the smaller the particle size, the better the kinetic process and therefore the methane yield.

2.4.7. Inoculum to substrate ratio (I/S)

For optimum biodegradability of the solid waste and hence optimum biogas production, inoculum to substrate ratio is a fundamental parameter. Active anaerobic or animal inoculum is recommended to reduce digestion period and digestion volume. The ratio varies depending on the type of substrate used. (Raposo et al., 2006, Wang et al., 2010) investigated the influence of Inoculum/substrate ratio based on volatile solid of maize crops. The authors obtained similar results within a range of 3 to 1 (I/S) with methane production and no change with an inoculum/substrate ratio of 2. Raposo et al. (2009) further conclude that within a range of 3 to 0.8 I/S, anaerobic digestion is stable at mesophilic conditions. A maximum methane production was obtained by Boulanger et al. (2012) at an I/S ratio of 2.

2.5. Anaerobic digestion systems

In the AD process, batch and continuous digesters are the two existing configurations dependent on the feed materials being used, the structure of the digester and the operating conditions (Sio-iong et al., 2017).

2.5.1. Anaerobic digester configurations

2.5.1.1. Batch

During a batch process, the feed material is put into the digester when the process starts and sealed for the retention time. After breakdown, biogas is collected, and the digester partly emptied. The digester is not completely empty because of the inoculation of fresh organic material with bacteria from prior experiments.

2.5.1.2. Continuous

In contrast to batch processing, the continuous process involves an uninterrupted daily addition of substrates in phases to the digester. In this current situation, the final products are continuously removed thereby ending up in constant biogas production. One or multiple digesters in arrangement might be used (Sio-iong et al., 2017).

The biogas digester is selected taking in account dry and wet material content of the digester feedstock. Wet digestion has more wet material than dry digestion, with dry matter content less

than 15%; dry digestion has more dry material than wet with dry matter content above 15% (Sio-iong et al., 2017).

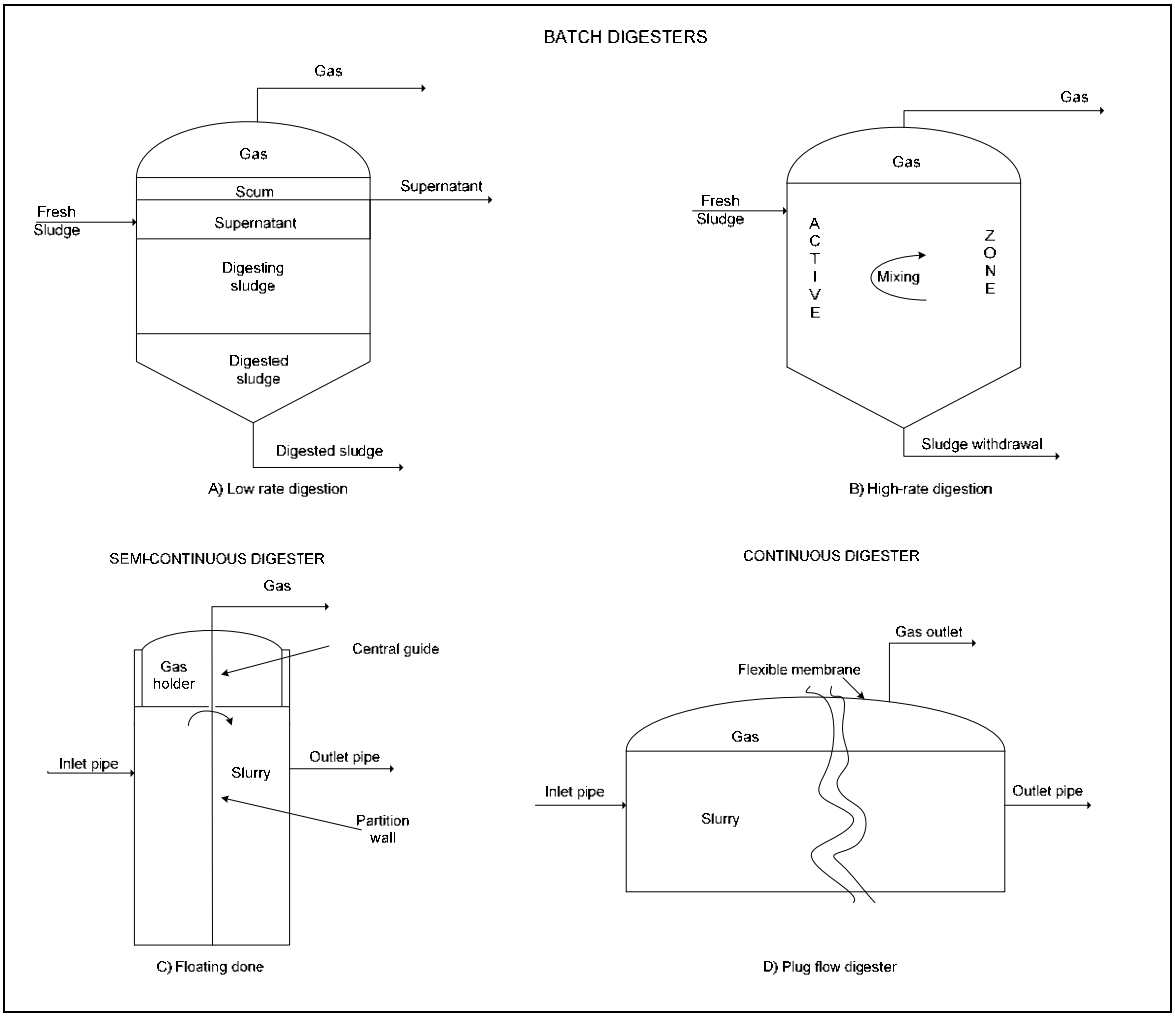

Figure 2.4: Different types of digesters: **A**, Low rate; **B**, high rate digestion (continuous); **C**, floating dome (semi continuous); **D,** plug flow (continuous). Adapted from (Marchaim, 1992, Abedeen, 2010)

According to the **Table 2.2** and **Figure 2.4** above and as stipulated by Eudald et al. (2014) the batch digester is a convenient configuration for sorghum stover and winery waste, both dried substrates.

Table 2.2: Comparison of various digesters types (Sio-iong et al., 2017)

Tech	Digester Type	Feedstock Type	HRT (days)	Biogas yield	Tech Level
Wet digestion	Covered lagoon	Manure	20-200	Poor	Low
	Plug flow	Manure	20-40	Poor	Low
	Completely mix	Liquid and solid	20-80	Good	Medium
	Fixed film	Liquid	1-20	Good	High
	UASB	Liquid	0.5-2	Good	High
Dry digestion	Batch	Agricultural and municipal feedstock	20-30	Good	Medium
	Vertical		20-40	Good	High
	Horizontal		20-40	Good	High

2.6. Feedstocks used for anaerobic digestion

Feedstock or substrate is the material that goes into the anaerobic plant digester to be broken down by microorganisms or bacteria and give off the biogas. It also means a material on or from which a microorganism lives or grows. It is crucial for an optimal and successful anaerobic digestion scheme. The reason is that different feedstocks have different energy yield rates and digest in different ways (Pullen (2015). In the case of this study, the feedstock is sorghum stover and winery waste as co-substrate.

2.6.1. Winery waste

Wine production is an important agricultural activity, with South Africa being the 7th largest producer worldwide. It is also a huge generator of discarded material, identified as winery waste. The main wastes produced by wine production industries are grape marc, grape stalks and wine lees (Devesa-Rey et al., 2011).

Grape marc is grape skin, pulp and seeds collected after the extraction of grape juice. Grape marc or pomace is characterized by high organic content, low nitrogen and phosphorus concentrations. It also possesses a large amount of carbohydrates and phenolic compounds. Wine lees are the remaining sludge collected after the fermentation of grape juice. Its characteristics are high organic material content, included by low pH and low electrical conductivity standards. The organic mixtures mostly involve ethanol, microbial biomass (mainly yeasts), phenolics and tartaric acid/tartrates (Eleutheria et al., 2016).

A short period of production connected with high organic content and unsteady configuration is a problem for winery waste beneficiation and treatment. Winery wastes are however being subjected to several treatment technologies. Wine lees are used to recuperate tartaric acid or ethanol, whereas grape marc is to produce lactic acid, polyphenols, ethanol, and soil fertilizer,

after composting. Moreover, and the crucial part, winery wastes may be utilized as substrate in AD for energy recovery (Eleutheria et al., 2016).

2.6.2. Sorghum stover

Sorghum constitutes a potential substrate with multiple utilities as the sugar, stalks, and leaves produce methane once fermented (Babu et al., 2015).

The content left in the field after harvesting is characterized as sorghum stover.Though it is comparable to grain sorghum, the stalks are juicy and have a high content of fermentable sugars (Babu et al., 2015).

According to Godin et al. (2016), lignin is considered as a phenyl-propane macromolecule originating from the cell walls of entire vascular plants, similar to sorghum stover. It is important to those plants due to its provision of structural and mechanical rigidity and protection from abiotic and biotic stresses. Lignin as well is responsible for the formation of a barrier adjoining the plants' polysaccharides: cellulose, hemicelluloses, and pectin, and hence it obstructs the enzymatic hydrolysis of plant cell wall polysaccharides in the rumen along with in bioconversion processes for biogas and biochemical production. In other words, the less the lignin content of the sorghum stover, the higher the biodegradation in anaerobic digestion process.

According to Godin et al. (2016) and Sattler et al. (2014), the reduction of lignin concentration into lignocellulose biomass advantages the increase of feed digestibility for reflective stirring and saccharification.

Musa et al. (2011) stated that sorghum stover has a lignin content of 11.8% which is relatively low but still adequate. The choice of the feedstock is justified in terms of food protection, waste management and their availability.

2.6.3. Co-digestion

According to Mata-Alvarez et al. (2014), co-digestion is the synchronized digestion of two or multiple substrates and is a method used to overcome the limitations of single digestion. Agro-industrial wastes and harvests are periodic feedstocks, which might reduce nitrogen. Co-digestion may improve the economic feasibility of the digester due to higher content of methane production.

2.7. Feedstock pre-treatment

Besides the activity of a catalyst for the boost of biogas yield production, pre-treatment methods correspondingly have their importance in the augmentation of anaerobic digestion. Chemical, thermal, mechanical and biological procedures are acknowledged and compared based on their efficiency, energy balance, environmental sustainability, operational and maintenance costs to evaluate more suitable pretreatments when necessary (Ariunbaatar et al., 2014).

Mechanical pretreatment consists of disintegrating and/or grinding solid particles of the substrate and therefore allow the release of cell compounds. A study by Carrère et al. (2010) reveals an improvement in the production of biogas of 24–140% in batch processes by sonication prior to the anaerobic digestion process. Thermal treatment, which leads to the disintegration of cell membranes is best for water sludge and lignocellulosic substrates. (Rafique et al., 2010) achieved a 78% rise in biogas production by pretreatment of lignocellulose substrates at 70 °C. Concerning chemical treatment, this method is appropriate to destroy organic compounds by means of strong acids, alkali or oxidant. However, this method has a disadvantage. Patil et al. (2011) found that alkaline pretreatment has a smaller outcome than mechanical pretreatment when they studied the influence of alkaline pretreatment of water hyacinth, which is known to have a lower lignin content than other plants. It therefore confirms that acidic and alkaline pretreatment are inefficient for feed materials with low lignin content. Biological pretreatment involves both aerobic and anaerobic methods along with the addition of specific enzymes. This pretreatment method is not that present in organic fraction of municipal solid waste but is employed in pulp and paper industries. Escamilla-Alvarado et al. (2012) examined the production of biogas with pre-treated thermophilic bacteria at 65°C and obtained a biogas yield range of 80-90%.

As a comparison of all these pretreatments and considering the substrates (sorghum stover and winery waste) used in this study, no pretreatment was needed. In fact, sorghum stover has low lignin content and therefore does not require pretreatment beside the use of the iron oxide nanoparticle catalyst, which can be an additive. This decision is furthermore advantageous economically and environmentally as only strictly necessary equipment will be used.

2.8. Nanotechnology in biogas production

Nanotechnology is a branch of science which allows scientists and researchers to work with units not bigger than micro level i.e. molecular and cellular levels(Rai and da Silva, 2016).

Based on recent studies, nanotechnology can display innovative properties, by altering the features of feed matter, for instance in the improvement of biogas production. Various nanomaterials like carbon, metal oxides, nano zero-valence, etc., represent real benefits to bioenergy production, particularly biogas production (Rai and da Silva, 2016).

Biogas is the resultant of anaerobic digestion process from organic, animal and/or human wastes. Organic waste types like agricultural, food, industrial and municipal wastes need carbon and nitrogen for their digestion process, meaning that the carbon: nitrogen (C/N) ratio is an important parameter for the digestion of any organic waste. Based on previous studies, the addition of metal ions in small quantities favours the activity of methanogenic bacteria. It acts as an additive which enhances the production of biogas (Rai and da Silva, 2016).

2.8.1. Iron oxide nanoparticles

Iron oxides are inorganic compounds which comprise iron and oxygen. As nanoparticles in nanoscale, they have shown potential for their use as catalytic materials, flocculants, and adsorbents, etc. (**Figure 2.5).** Currently, researchers like Casals et al. (2014), suggest that using iron oxide nanoparticles in AD might increase the degree of biogas production by over 200% (Sahu and K M, 2015).

2.8.2. Why iron oxide as catalyst?

According to Ganzoury and Allam (2015), nanomaterial additions have a certain effect on the rate of biogas production. They identified four categories of nanomaterial: metal oxides, zero-valent metals, nano-ash, and carbon-based material. The impact of these materials on biogas production were either negative or positive as shown in **Table 2.3**. Otero-González et al. (2014), investigated the effect of CuO nanoparticles on a long-time basis. The study demonstrated the inhibitive effect of CuO on methane production. The addition of CuO has been shown to decrease methane production by 15%. Similarly, the inhibitory effect of other nanoparticles and metals oxides has also been confirmed due to their toxicity on methane production (Mu and Chen, 2011, Gonzalez-Estrella et al., 2013) . The above-mentioned authors respectively researched ZnO nanoparticles and the effect of different metal oxides (Al_2O_3, CeO_2, CUO, Fe_2O_3, Mn2O3, TiO2, SiO2 and ZnO) on the AD of waste-activated sludge and anaerobic granular sludge respectively. They obtained a decrease in biogas production of 81% for the first experiment and within the range of 52% and 87% for the second experiment.

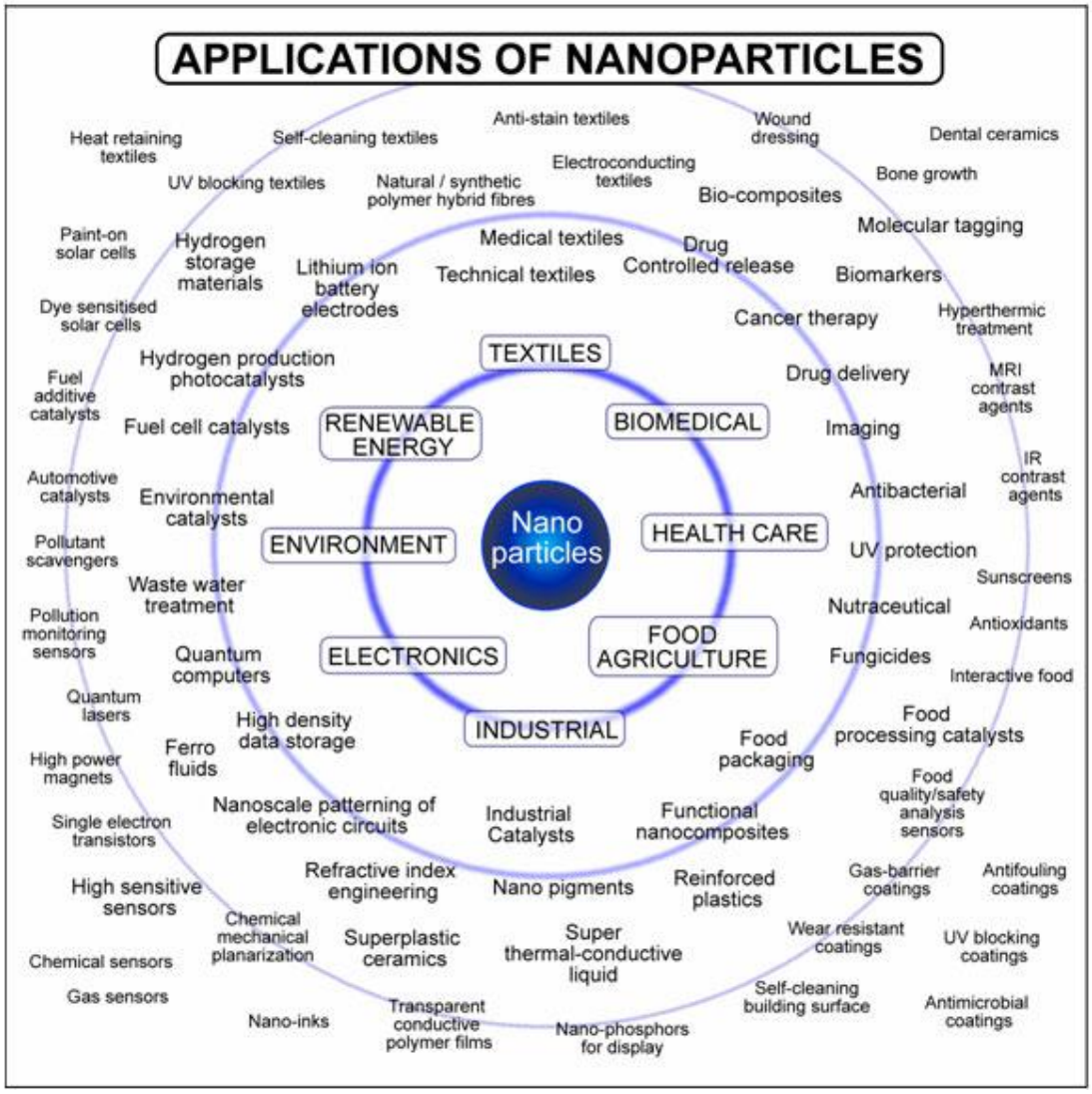

Figure 2.5: Major applications of nanoparticles (Tsuzuki, 2009)

In contrast, some nanomaterials helped to enhance the production of biogas. For instance, Casals et al. (2014) investigated the properties of iron oxide nanoparticles which supply living organisms without toxicity to the bacteria. In fact, the ability of iron oxide to lose or gain electrons makes it an effective additive if not used excessively. The study obtained a 180% increase in biogas production (234% increase in methane content) after 60 days retention time which is, according to the authors, the greatest amelioration to biogas production using nanoparticles to date. Similarly, A study by Su et al. (2013) on nano-zero valance iron on the AD of waste-activated sludge showed an increase in the biogas production of 30.4% and methane production of 40.4%, confirming that micro-valance electrons cause a significant increase in biogas production.

Some nanomaterials, however, show no effect on the production of biogas. García et al. (2012) conducted a 50-day study on the effects of Au and Ag NPs (20, 30 nm size) on waste residue

AD at moderate temperature (37 ±1 ºC) and thermophilic conditions (55 ±1 ºC). The results show that there is no significant difference in biogas production after adding 100 mg / l Au or 170 mg / l Ag NP. Also, Doolette et al. (2013) studied the effect of silver nanoparticles on anaerobic digestion of sludge. The methane production of AgNPs and Ag ions does not change. These results are consistent with a report by (García et al., 2012).

Nanomaterials seem to be potential additives during biogas production based on the studies described above. Therefore, the present study selected iron oxide nanoparticles as a suitable additive due to the presence of non-toxic Fe^{2+} and Fe^{3+} for co-digestion of sorghum stover and winery solid waste.

Table 2.3: Summary of different nanomaterials with their effect on biogas yield

Nanoparticles	Nanoparticle size	Feedstock	Temperature of AD	Incubation time (day)	Effect	Reference
CuO	37 nm	AGS	30	83	15% decrease in methane prod.	(Otero-González et al., 2014)
ZnO	<100 nm	WAS	35	105	81% decrease in methane prod.	(Mu and Chen, 2011)
Al_2O_3, CeO_2, CUO, Fe_2O_3, Mn_2O_3 ,SiO_2 and ZnO	10-30 nm	AGS	30	TMMP	53-75% decrease methane prod.	(Gonzalez-Estrella et al., 2013)
TiO_2	25 nm	AGS	30	TMMP	No effect	(Gonzalez-Estrella et al., 2013)
Fe_3O_4	7 nm	WWS	37	60	180% increase in biogas prod. 234% increase in methane prod.	(Casals et al., 2014)
Non-Zero valence iron	20 nm	WAS	37	17	Increase in biogas production by 30.4% and methane production 40.4%	(Su et al., 2013)

2.9. Synthesis methods of iron oxide catalyst

Several methods may be used to synthesize iron oxide nanoparticle such as hydrothermal, sol-gel, sono-chemical, and co-precipitation. A specific method is selected depending on what needs to be achieved using different criteria.

2.9.1. Hydrothermal method

Hydrothermal is one of the common methods used to create nanomaterials with different types of morphology. This procedure consists of mixing of the reactants in a container of water placed in an autoclave and set at high temperature and pressure conditions (Rao, Mukherjee et al. 2017).

Precise control of the temperature is a fundamental parameter to enable the synthesis of various nanomaterials. This method is convenient for the preparation of nanostructured materials with a large range of shapes as compared to other methods.

2.9.2. Microwave-assisted method

Conventional heating method are mostly used for solutions forming part of most chemical reactions. However, conventional heating procedures have some disadvantages such as high thermal gradient effect, slow reaction kinetics, and inconsistent and unwanted reaction conditions. For these reasons, microwave represents a good substitute to avoid the drawbacks of conventional heating processes (Rao et al., 2017).

Microwaves are basically electromagnetic energy which range between 300Mhz and 300Ghz. They interact with materials by way of two different mechanisms viz. dipole interaction and ionic conduction (Rao et al., 2017).

The microwave method has diverse advantages which makes it a reliable method to synthesize metallic nanostructures as well as metal oxides with a diversity of morphology. Some of these advantages include the fact that they are environmental friendly, clean, cheap and have brief reaction times for high production yield of desired constituents (Rao et al., 2017).

2.9.3. Polyol method

The polyol method is a promising synthetic way to synthetize multiple types of nanoparticles with numerous kinds of morphologies. It has been shown to be a versatile liquid-phase method which employs high boiling point and multivalent alcohols. Polyol alcohols are used as reducing agents and solvents, as well as for their capacity to control particle growth. Different types of polyols have been used in this process, e.g., ethylene glycol (EG), propylene glycol (PG), butylene glycol (BG), diethylene glycol (DG), triethylene glycol (TrEG), and tetraethylene glycol (TEG) amongst others. The polyol method allows the synthesis of nanoparticles in a

temperature range of 473-593K without high pressure and autoclaving. Other advantages of this method include the use of cheap metals since polyol alcohols have the properties of solubility and chelating ability (Rao et al., 2017).

2.9.4. Sono-chemical method

The sono-chemical method appears to be a recent versatile synthesis technique used in the biomedical industry. It consists of the utilization of high-intensity ultrasound, which requires conditions different from usual routes such as the hydrothermal method or wet chemical method. The conditions used in sono-chemical method are (a) speed of sound in liquid with range (1000–1500 ms−1); (b) ultrasonic wavelength between 100 μm and 10 cm; and (c) a frequency range of 20 kHz to 15 MHz Using this method assures a large diversity of metal and metal oxide nanoparticle morphologies (Rao et al., 2017).

2.10. Analytical and experimental methods of biogas production

Several methods are used to determine the quality and the quantity of biogas and methane production. Only the common ones which are the methods employed in this thesis will be described.

2.10.1. Biochemical methane potential test

Biochemical methane potential (BMP) is a crucial analytical technique to weigh the optimisation and implementation of the characterized anaerobic technologies. Nowadays, scientists rely on this method to define the maximum quantity of production of methane (Bo) of a definite feedstock. Additionally, BMP tests have the potential to determine the kinetic constant of the rate-limiting step, needed in anaerobic digesters to attain optimum operation and design. This batch assay is used to record the production of methane from matching a feedstock with an l anaerobic inoculum, from which only a small amount of methane is obtained (Holliger et al., 2016, Da Silva et al., 2018).

2.10.2. Liquid displacement gas measurement

Volumetric gas meters depend on the liquid displacement method in laboratories. These meters can easily be made with jars, plastic bottle or cylinder. Advantages of liquid displacement methods are the economic aspect, long-lasting period without maintenance and simplicity of construction. The goal is the collection and preservation of gases during liquid displacement. The gas measuring unit, known as gasometer, works on the principle of storage and does not give flowrate directly. Gas is collected with the aid of a glass container with a suitable liquid or barrier solution, which is displaced along with the collection of the gas (Parajuli, 2011).

Variation of room temperature and atmospheric pressure are factors affecting the accuracy of the gas measurement. Hence, it is necessary to convert the volume calculations from normal to standard. Conversions procedures are detailed below depending on the type of experiment. Recording the change in atmospheric pressure and temperature are necessary for conversion procedures. The gas pressure collected over the liquid solution and contained in the tube is the addition of biogas and vapor pressure. Therefore, the biogas pressure (Pbio) can be determined by deducting the vapor pressure of liquid (Pw) at the actual measurement of the temperature from the pressure of collected moist gas (P). **Equation (2.9)** is as follows:

$$P_{bio} = P - P_w \tag{2.9}$$

If the gas is collected over the liquid, static pressure occurs due to the level difference (P_{level}). **Equation (2.10)** therefore becomes:

$$P_{bio} = P - P_w - P_{level} \text{ or } \qquad P_{bio} = P - P_w + P_{level} \tag{2.10}$$

The volume of biogas produced can be converted from normal to standard conditions using combine gas law represented in **Equation (2.11)**:

$$V_o = V \times \frac{T_o}{T} \times \frac{P_{bio}}{P_o} \tag{2.11}$$

Where V is the actual volume of gas measured, V_o the gas volume at standard temperature and pressure conditions, P_o the standard pressure, T is the actual gas temperature at the time of measurement and T_o the standard pressure (Parajuli, 2011).

Calculation of vapor pressure Pw can be determined using the following modified equation from the Arden Buck equation:

$$P_w = 6.1121exp\left(\left(18.678 - \frac{T_C}{234.5}\right) \times \frac{T_C}{257.14 + T_c}\right) \tag{2.12}$$

Tc is the gas temperature in degree Celsius. Pw is the vapour pressure in hP (1hP= 0.1kPa).

There are commonly two types of gasometers, height and weight. In the height type, biogas can be inserted directly into the liquid column from the digester or by draining a gas bag. Gas volume is calculated from the change measurement in the barrier solution height. The weight type consists of weighing the displaced solution resultant of the gas volume from the reactant displacing the barrier solution into a container. **Figure 2.6** explains weight method by emptying a bag instead of connecting directly to the reactor (Parajuli, 2011).

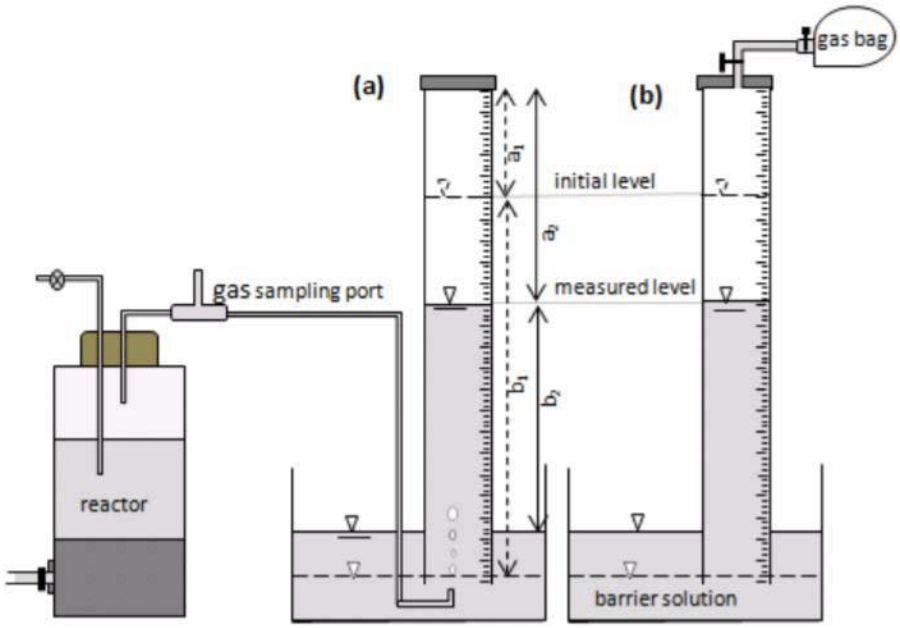

Figure 2.6: Measurement of gas: a) direct from a reactor using a cylinder meter; b) indirectly by collecting in a sample gas bag using a height meter

From height type, **Equation (2.13)** below shows gas volume calculation using a height meter:

$$V_o = \frac{T_o}{TP_o}\left[\left((P - P_w - \rho g b_1)Aa_1\right) - \left((P - P_w - \rho g b_2)Aha_2\right)\right] \qquad (2.13)$$

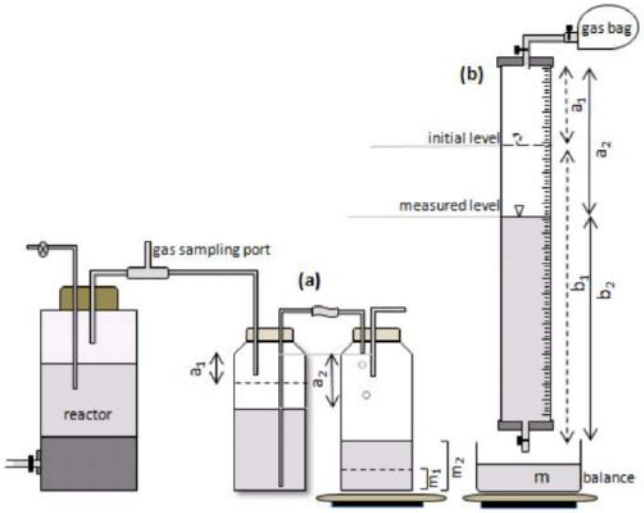

Figure 2.7: Measurement of gas: a) Direct from reactor using bottle meter; b) indirectly by collecting in a sample gas bag and using a height meter

From weight type, equations below show gas volume calculation by weighing displaced liquid in (1) a bottle meter or (2) a column meter:

$$(1)V_o = \frac{T_o(m_b - m_a)}{TP_o\rho}\left[P - P_w + \rho g\left(a_1 + a_2 + \frac{V_a}{A}\right)\right] \quad (2.14)$$

$$(2)V_o = \frac{T_o}{TP_o}\left\{\left[\left(P - P_w + \rho g\left(b_1 - \frac{m}{\rho A}\right)\right) \times A \times \left(a_1 + \frac{m}{\rho A}\right)\right] - [(P - P_w + \rho g b_1) \times A a_1]\right\} \quad (2.15)$$

Where a and b are height of gas and liquid, and m is the mass of the liquid recorded. Subscripts 1 and 2 are before/after measurement conditions, ρ is the liquid density, A represents cross sectional area and g acceleration due to gravity.

A consequent inconvenience of collection and measurement of biogas volume from liquid displacement is the inaccuracy caused by the biogas solubility through the barrier solution. There is a diversity of liquids which can be used as barrier solution: tap or carbonized water, acidified water, or oil. The gas solubility and diffusion depend on the type of liquid, temperature, gas pressure, liquid density, gas composition. Hence, it is inadequate to use the same correction factor for each time the gas is measured. Evaporations of barrier solution after a certain time are also a cause of inaccurate readings. A good technique of reducing gas solubility errors is to use the indirect liquid displaced method by emptying a gas bag instead of colleting directly from the reactor, as shown in **Figure 2.6** (Parajuli, 2011).

CHAPTER THREE
RESEARCH METHODOLOGY

This thesis embraces five types of experiment in two phases: 1) synthesis of iron oxide nanoparticles and characterization of the nanoparticle, and 2) biogas production process, characterization of the substrate, biomethane potential, optimization, and upscaling of the digester. This chapter describes the different materials used in each experiment, the experimental set-up, the sampling, and the analytical methods.

3.1. Substrates and preparation

3.1.1. Sorghum stover

Sorghum stover was purchased from ARC-Grains, Pretoria, South Africa. The substrate came half-dried (**Figure 3.1 A**) and half-wet in 5 kg quantities. It was dried in an oven at 60 °C for minimum of 4 hours. The procedure was repeated if the substrate was still wet until completely dried.

Sorghum was processed in a food processor and then ground in a POLYMIX® Lab Mill PX-MFC 90 D 230 V/ EU from Bench & Holm, Denmark which was set at 6000 x rpm. The substrate obtained was collected in sampling bag (**Figure 3.1 B**) conserved at room temperature.

Figure 3.1: A) Fresh sorghum and B) Sorghum powder

3.1.2. Winery solid waste

Fresh winery waste was collected from a winery farm at the Agricultural Research Council (ARC), Stellenbosch, South Africa. It was sun dried and milled into powder form (**Figure 3.2**) using a Hammer Mill SER No. 400 (Scientific®, SA) equipped with 2 mm sieve mesh (Mkruqulwa et al., 2019).

Figure 3.2: Pre-treated winery solid waste

3.1.3. Zebra dung

Fresh zebra dung (**Figure 3.3**) was collected from the Vredenheim Farm in Stellenbosch, South Africa. It was conserved in a foam bag with ice bricks during the trip back to the laboratory and stored in the fridge at 4°C prior to preparation.

Figure 3.3: Fresh zebra dung

3.2. Synthesis of iron oxide nanoparticle

Fe_3O_4 nanoparticles were obtained by hydrothermal synthesis. The following components were mixed in an autoclave: 1,98 g of iron II chloride; 8,08 g of iron III nitrate (non-anhydrous); 0,7 g of sodium dodecyl; 100 mL of water and 10 mL of ammonia. The aqueous solution was then heated in a water bath at 60 °C for 4 hours. The solution obtained was furthermore centrifuged for 5 min at 4000 x rpm. This step was repeated until the solution was clean. The wet powder (ppt) was put in a crucible and left to dry overnight. After that, it was reduced in powder form (**Figure 3.4**) with the aid of a molder and mortar.

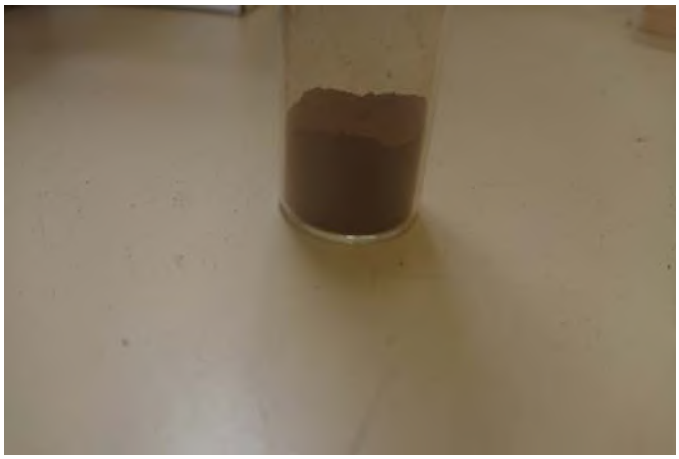

Figure 3.4: Iron oxide nanoparticles in powder form

3.3. Analytical methods

3.3.1. Characterization of iron oxide nanoparticles

X-ray diffraction and scanning electron microscopy were conducted on iron oxide nanoparticles at the Centre for Imaging and Analysis (UCT CIA) at the University of Cape Town, South Africa.

3.3.2. Biomass analysis

The substrates were analyzed for their total and volatile solids, ash and moisture content (experiment described below). Carbon, nitrogen, oxygen, calcium and phosphorus percentages were also determined via inductive couple plasma-atomic emission spectroscopy (ICP-AES) using Thermo ICap 6200 ICP-AES and elemental analysis using an Elementar Vario EL Cube Elemental Analyzer. Samples were prepared before ICP analysis with a microwave digester. Protein content was determined by applying a correction factor, 6.25 according to Mariotti et al. (2008), to the measured nitrogen content.

3.3.3. Characterization of substrate

Percentage volatile solid and total solids of the substrates are needed to produce biogas. The following procedure was done to determine this:

3.3.3.1. Drying

The weight of an empty crucible was recorded to four decimal places. 1g of sample was weighed. The total weight was recorded. The sample in the crucible was dried in the oven at +/- 105°C for a minimum of 4 hours. The weight of the dried sample was also recorded. The last step (drying) was repeated until there was no change in weight up to three decimal places.

3.3.3.2. Calcination

Once the weight of the dried sample was fixed it was calcined in a furnace at +/- 525°C for a minimum of three hours. The combined weight of the sample and crucible were recorded. It was repeated until there was no change in weight up to three decimal places.

Experiments were done in replicate.

3.3.3.3. Determination of % volatile and total solids

The average value of the replicate was used to determine the percentage volatile and total solids.

For the percentage total solid the following equation was used:

$$\text{TS (\%)} = \frac{\text{M(C+DS)} - \text{MC}}{MWS} \times 100\% \text{(Amano et al., 2017)} \quad (3.1)$$

for volatile solids and ash content:

$$\text{VS (\%)} = \frac{\text{M(C+DS)} - \text{M(C+A)}}{\text{MTS}} \times 100\% \text{(Amano et al., 2017)} \quad (3.2)$$

Or

$$\text{VS (\%)} = 100 - \left(\frac{\text{M(C+A)} - \text{MC}}{\text{MTS}} \times 100\% \right) \text{(Amano et al., 2017)} \quad (3.3)$$

And for moisture content:

$$\text{MC(\%)} = 100 - \left(\frac{\text{M(C+DS)} - \text{MC}}{MWS} \times 100\% \right) \text{(Amano et al., 2017)} \quad (3.4)$$

Where;

MC = mass of crucible used

$(C+DS)$ = mass of the dry sample plus crucible

MWS = mass of the wet sample

MTS = mass of the total solids

$(C+A)$ = mass of ash plus crucible

The procedure was used for the sorghum stover and the inoculum slurry. Characterization of winery waste and results were taken from research by Mkruqulwa et al. (2019).

3.4. Preparation of Inoculum

Inoculum, in this case zebra dung, was mixed in a 5 L plastic bottle with 1 L of pre-seed (previous inoculum prepared from another thesis already acclimatized with winery waste), 10 g of sorghum stover and winery waste each at once, to acclimatize. One litre (1L) of water was added to the mixture. The inoculum slurry was incubated at 37 ±0.5 °C in a bath of water for fourteen days. It was daily degassed. After that it was sieved and only the liquid was used as inoculum. More bottles were prepared when needed for further experiments.

3.5. Experimental technique

3.5.1. Biomethane potential set-up and procedure

Analytical BMP for this system was done in duplicate. Seven experiments were done in fourteen 500 mL Schott bottles with two connected screw cap (model GL 45 from Sigma-Aldrich) immersed in a water bath with integrated temperature control system (model TR5 and serial number F7571-0717 set at 37 ±0.5 °C.

Bottles were flushed with nitrogen before and after filling up with the substrate as well as the head space and the screw cap, for 1-2 minutes. A summary of the inoculation steps is shown in **Table 3.1** below. Bottles 1-2 had inoculum only (control); 3-4 had inoculum and WSW; 5-6 inoculum and SS; 7-8 SS/WSW at 50/50 ratio; 9-10 inoculum, WSW and ION;11-12 inoculum, SS and ION; and finally 13-14 had SS:WSW at 50/50 ratio with inoculum and ION. 100 mL of water was added for a total working volume of 400mL. The inoculum/substrate ratio was 2:1 in terms of volatile solids. Bottles were connected to scrubbing bottles with the aid of silicone tubes. Each scrubbing bottle was filled with water, sodium hydroxide 1M concentration and phenolphthalein as indicator. The scrubbing bottle were then connected to a plastic bottle filled up with acidified water (pH 1, H_2SO_4, 30mL) and 10g of table salt. The plastic bottle was used as a gasometer. Two holes were made in the plastic bottle cap, inlet and outlet entries, using silicone and vacuum grease to avoid entry of air. A second empty plastic bottle was connected to the gasometer to collect the water coming out of the first plastic bottle. Biomethane potential bottles were shaken twice daily to prevent scum and for homogenization inside the reactor.

Table 3.1: Biomethane potential inoculation

	Bottles 1-2	**Bottles 3-4**	**Bottles 5-6**	**Bottles 7-8**	**Bottles 9-10**	**Bottles 11-12**	**Bottles 13-14**
Inoculum (g.VS)	1.597	1.597	1.597	1.597	1.597	1.597	1.597
Sorghum stover (g.VS)	0	0.992	0	0.992	0	0.77	0.77
Winery waste (g.VS)	0	0	1.547	0	1.547	0.5	0.5
Iron oxide (ppm)	0	0	0	100	100		100
Water (mL)	100	100	100	100	100	100	100

Temperature was checked daily and maintained at 37°C +/-0.5 with the aid of a thermometer dipped in the bath and inside the bottle. The pH was measured at the beginning and the end of the run using a Crison® basic 20 pH meter at room temperature.

To maintain the water level in the bath water foam pieces were added. Despite that, water was added regularly to maintain the level. Sodium hydroxide (NaOH) or hydrochloric acid were used to correct the pH of the samples where needed. The experiment was run for 30 days.

3.5.2. Biogas collection and measurement

Biogas volume produced was collected and measured by displacement method using a water column as represented in the **Figure 3.5** below. Gas volumes recorded were converted to standard conditions as described in literature. The net biogas volume is the biogas produced by inoculum and substrate deducted from the biogas produced by the inoculum. Biogas volume is then normalized by dividing the volume produced by a gram of volatile solids ($g.VS^{-1}$). Qualitative analysis was done using a Geotech 5000 Biogas Analyzer (shown in **Figure 3.6**) to determine methane content.

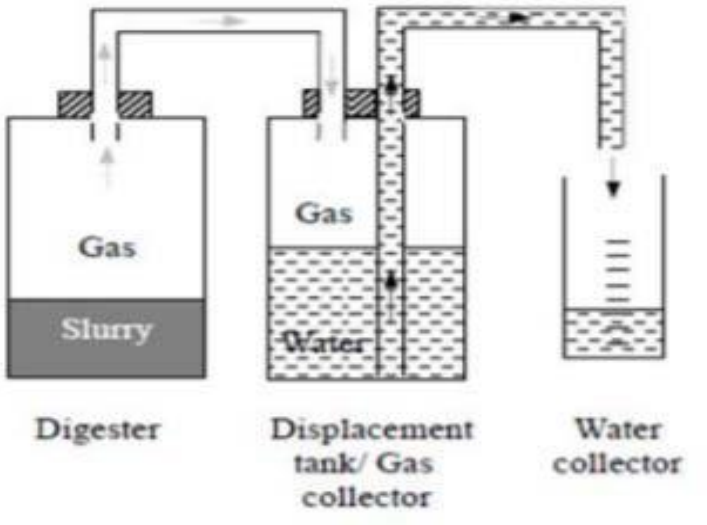

Figure 3.5: Displacement method for biogas production (Ojikutu, Abimbola and Osokoya, 2014)

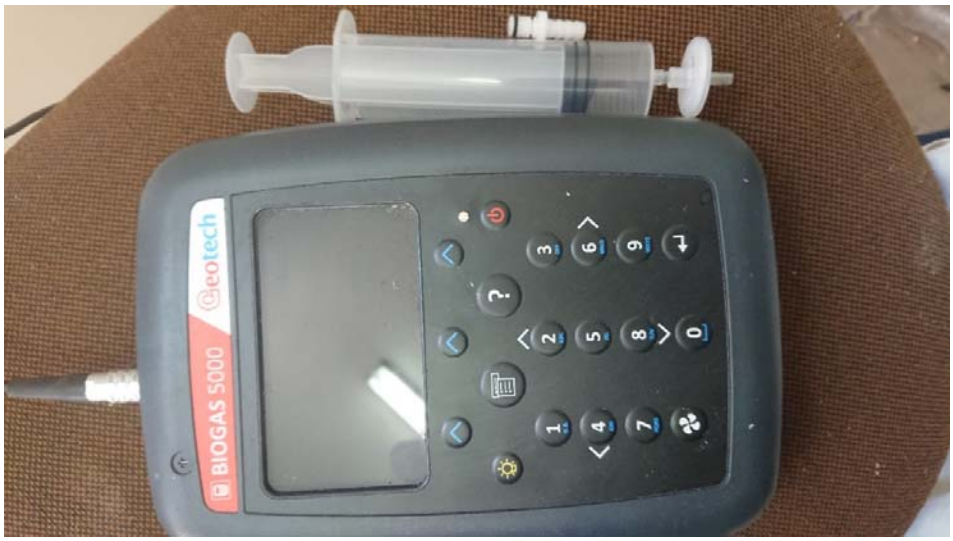

Figure 3.6: Biogas 5000 Geotech Analyser

3.6. Optimization experiment

3.6.1. Experimental design

Biogas optimization from winery solid waste and sorghum stover co-digestion through application of iron oxide nanoparticles was done by Central Composite Design in Design-Expert 11. Parameters chosen were solid retention time (10-25days), with concentration of ION (50-100ppm) and co-digestion ratio with respect to SS. **Table 3.2** below illustrates the different factors.

Table 3.2: Factors levels chosen from minimum to maximum values

Parameters	Units	Minimum	Maximum
SRT	Days	10	25
ION concentration	Ppm	50	100
Co-digestion ratio		20	80

Each factor was investigated at 5 levels: plus, and minus alpha (axial points), +1 and -1 (factorial points) and the center point. The response was the biogas production (mL).

A polynomial second order equation was fitted to the model to determine the optimal combination. The regression model was obtained through analysis of variance (ANOVA), p and F-values. R^2 and adequate precision measured the adequacy of the model.

3.6.2. Experimental procedure

Optimization BMP for this system was done in duplicate. 20 runs were done in 500mL Schott bottles with two connected screw caps (GL 45) immersed in water bath with an integrated temperature control system (model TR5 and serial number F7571-0717 set at 37 ±0.5°C.

Winery solid waste and sorghum stover were mixed at different ratios (0:100; 20:80; 50:50; 80:20 and 100:0) based on their volatile solids and in respect to SS (**Table 3.3**); iron oxide nanoparticles were added to the different mixtures at different concentrations of 33, 50, 75, 100 and 117 ppm based on the CCD results. Inoculum BMP was done for control. The composed samples were run for 5,10,18, 25 and 30 days. Biogas production was measured daily by displacement method of acidified and salted water. Biomethane potential bottles were flushed for 1-2 min before the experiment to get rid of other gases and shaken twice daily to prevent scum and for homogenization inside the reactor.

Table 3.3: Real ratios used during optimization experiment

Ratios SS:WSW		**Concentrations**	
Given	Equivalent	Given	Equivalent
0:100	$0g.VS^{-1}$	33	0.013g
20:80	0.16:0.64	50	0.02
50:50	0.4:0.4	75	0.03
80:20	0.64:0.16	100	0.04g
100:0	$0,8g.VS^{-1}$	117	0.047g

3.7. Upscaling experiment with 5L batch digester

Biogas yield from upscaling the system was done in duplicate. A 5L single stage mesophilic batch reactor (GlassChem Pty) (**Figure 3.7**) was used to conduct the upscaling of optimum conditions. Temperature and pH were controlled by integrated pH probe and heating mantle. A ratio substrate of 80:20 with respect to SS which corresponded to 1.237g of dried SS and 0.198 g of WSW were added to 0.4 g (100 ppm) of ION for a working volume of 3L for 25 days. The pH was adjusted to 7 with 1M sodium hydroxide (NaOH) or hydrochloric acid (HCl) when necessary before starting the experiment. Digester content was treated with 99.9% pure nitrogen gas for 2-3 minutes and sealed immediately after to get rid of dissolved gases in the mixture. Homogeneity inside the digester was assured by stirring at 200 x rpm throughout the experiment. Digestion was conducted for 25 days (optimum value) and biogas production was recorded daily. Clean gas obtained after scrubbing with 1M sodium hydroxide (NaOH) solution was collected daily. Collection was made by the upward displacement method via gasometer and qualitative analysis via Geotech 5000 Biogas Analyzer. Inoculum production was used as control.

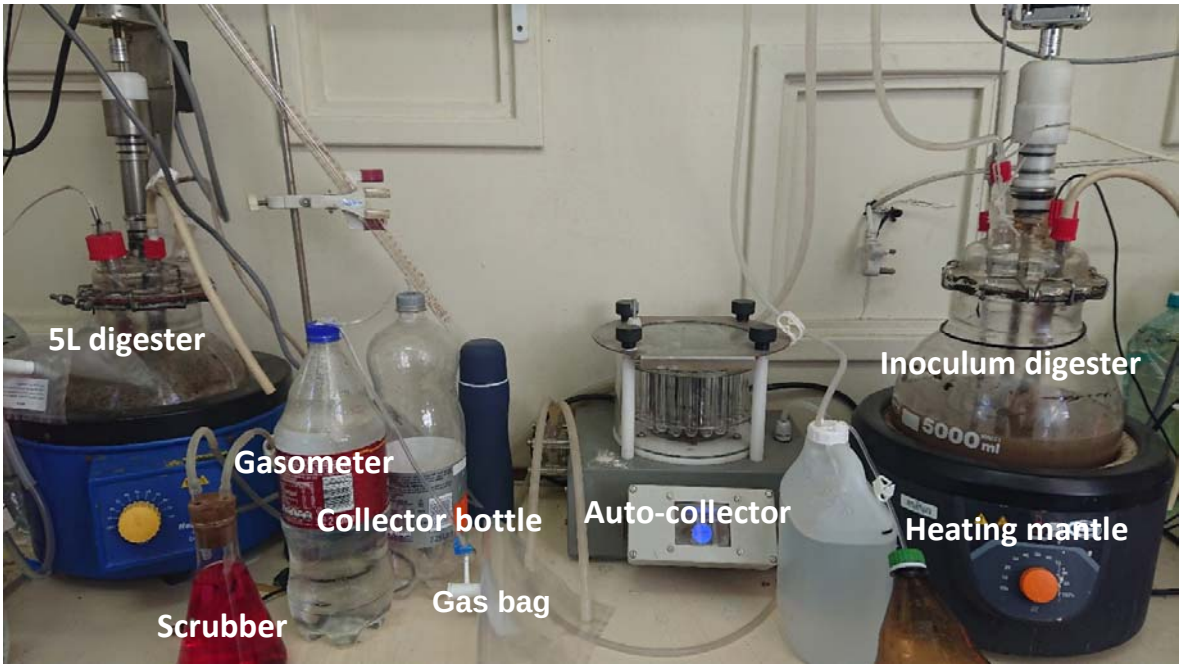

Figure 3.7: 5L single stage mesophilic digester set-up for upscaling

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. Biomethane potential from winery solid waste and sorghum stover via application of iron oxide nanoparticles

Biomethane potential through the application of iron oxide nanoparticles on winery solid waste and sorghum stover was investigated. The aim was to determine the effect of iron oxide nanoparticles on mono and co-digested dried substrates. Biomethane potential is a crucial technique which determines the maximum quantity of methane production of a defined feedstock.

4.1.1. Iron oxide nanoparticle characterization

The crystal structure, phase and size of an as-prepared sample of iron oxide nanoparticles was characterized used XRD and transmission electron microscopy. The XRD sample in **Figure 4.2** was compared with standard XRD patterns of Fe_3O_4 (JCPDS magnetite, maghemite-Q) represented in **Figure 4.1** to determine the nature of the prepared sample.

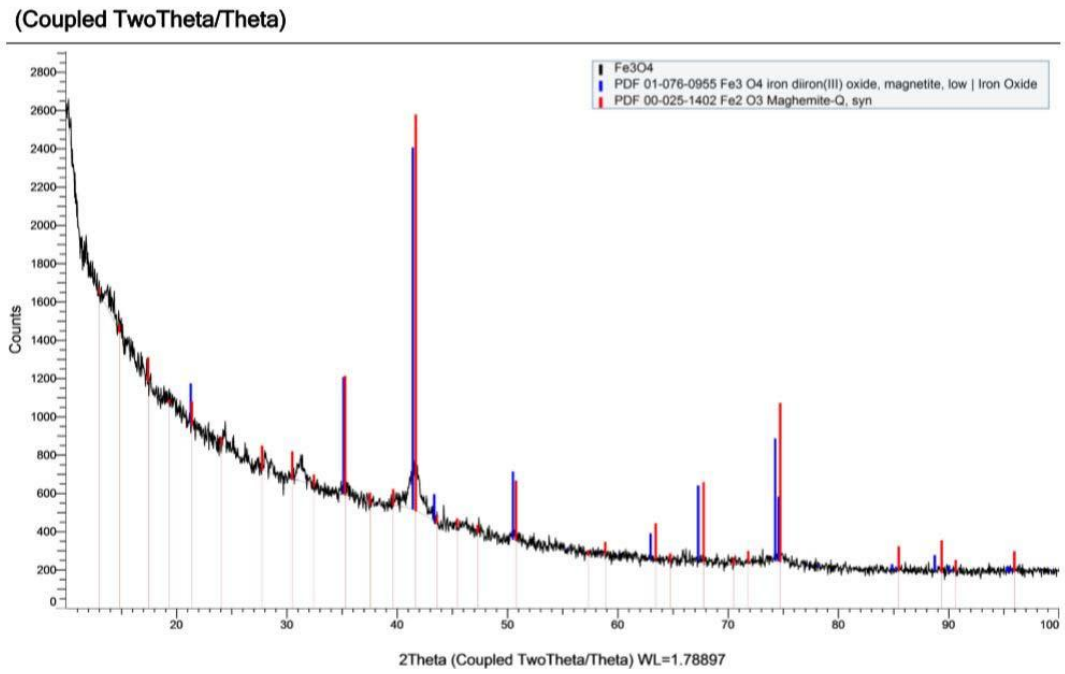

Figure 4.1: XRD patterns of Fe_2O_3 (red) and Fe_3O_4 (blue)

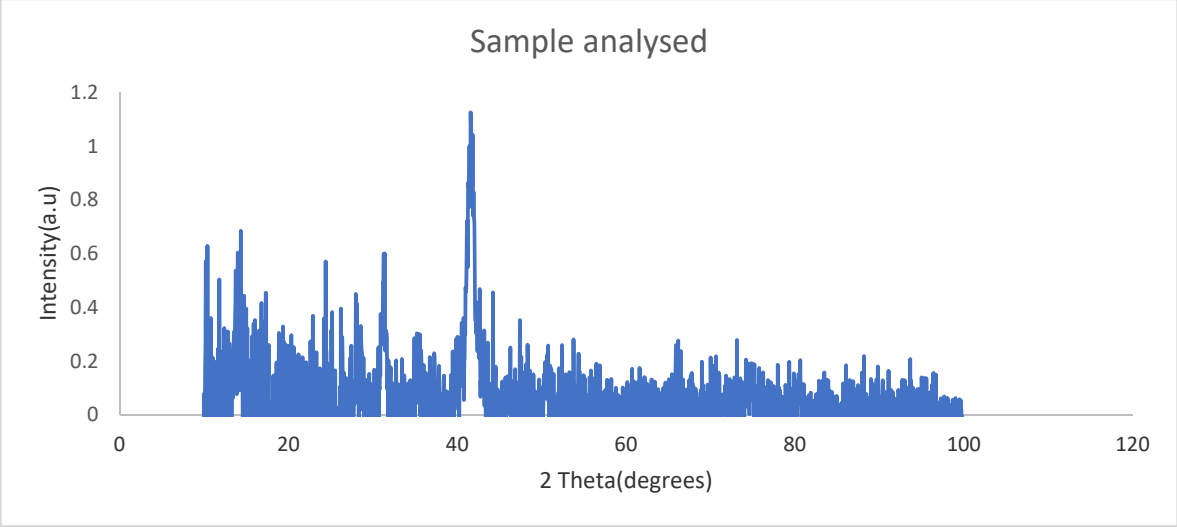

Figure 4.2: XRD pattern of the sample as prepared

The sites of the sample diffractions peaks are consistent with both Fe_3O_4 and Fe_2O_3 solid blue and red bars at 24, 35, 42 and 51 degrees. However, the intensity is not the same with peaks of Fe_3O_4 and Fe_2O_3 of lattice d 2.61. The first fact confirms the result of a mixture of Fe_3O_4 low magnetite and Fe_2O_3 maghemite-Q due to the similarities with both patterns. The second fact indicates the nature and size of the particles which are not ultra-fine and small. It could be said that the catalyst did not achieve an active state and a possible reason could be the low hydrothermal conditions. A mixture of iron (II) and (III) oxide is therefore available with both Fe^{2+} and Fe^{3+} oxidation states present. It is also important to underline the difficulty of distinguishing Fe_2O_3 and Fe_3O_4 nanoparticles because of their similar crystal structure (Iida et al., 2007). A rise of temperature could lead to a cubic spinel structure of Fe_3O_4, but could also interact with the size of the nanoparticles.

Transmission electron microscopic (TEM) images of the prepared sample were made, and results represented in **Figure 4.3** below, enlarged 2 and 3 times respectively. The size distribution of the nanoparticles was determined by using JAVA Image J software on the TEM images.

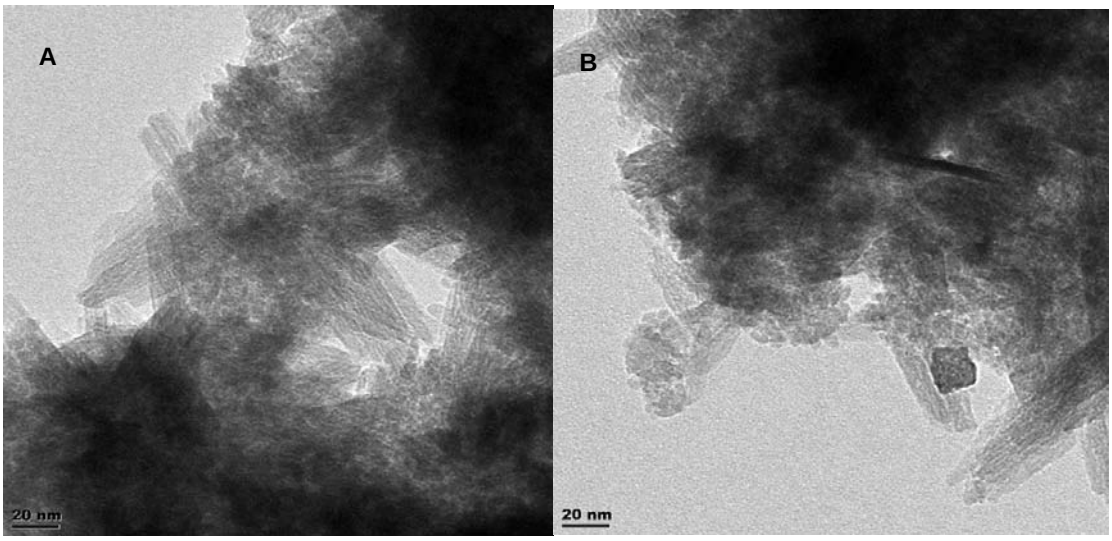

Figure 4.3: A, TEM images of iron oxide nanoparticles as synthesised; **B**, Enlargement of an area A both at a scale bar of 20nm

The iron (II) and (III) oxide nanoparticle mixture has an average diameter of 30nm and the aggregation in the micrograph is due the magnetite feature of the particles. The nanoparticles are composed of fine sub-crystallite and magnetite (Morsi and Hezma, 2019). The resulting size agrees with the results obtained by Hedayati et al. (2017) for the same method used. However, it is in contrast with Casals et al. (2014) who affirm that nanoparticles larger than 24 nm cause no alteration in biogas production. The effect of nanoparticles in this study could be explained by the compatibility between substrate microorganisms and iron and the state of iron nanoparticles not being pure Fe_3O_4.

4.1.2. Characterization of winery solid waste (WSW) and sorghum stover (SS)

Chemical composition and biodegradability are essential factors for biogas and methane production (Hagos et al., 2017).

The characterization of winery solid waste and sorghum stover shows differences represented in **Table 4.1** below. The two substrates had a high volatile solid content of 83.86% and 84.09% for WSW and SS respectively. However total solid content was higher for winery solid waste (95.92%) than sorghum stover (61.38%). Sorghum stover has a higher moisture content than winery solid waste, 38.62% compared to 1.15%. With regard to nitrogen, winery solid waste has a content (1.76%) twice that of sorghum stover (0.81%).

High volatile solid content shows the high ability of the feedstock for biodegradability and therefore biogas production rate. Nitrogen and carbon content were found to be respectively 1.76% and 50.40% for winery waste and 0.81% and 37.77% for sorghum stover. Hence it gives a carbon/ nitrogen ratio of 28.63 for the first substrate, which is acceptable. However, sorghum stover has been found with a carbon/nitrogen ratio of 46.63. For high methane yield a

carbon/nitrogen ratio between 20:1 - 30:1 is considered appropriate (Lohani and Havukainen, 2018). A proper ratio during the co-digestion of the two substrates would fix the inconvenient by adjusting the substrate proportions to bring it into the adequate range. Protein content of 11% and 5% were also found for winery solid waste and sorghum stover respectively with sorghum stover findings being similar to those of Harinarayana et al. (2005). Substrates rich in protein give a relatively high biogas yield and are rich in energy. However, ammonia contained in protein is toxic for methanogen bacteria at high concentration. Therefore an adequate amount of protein is required to provide enough nutrient without inhibiting methanogens (Hagos et al., 2017, Rabii et al., 2019). A 28.05 mg/kg iron content was discovered in winery solid waste compared to 636mg/kg in sorghum stover.

Trace metals are essential in anaerobic digestion as they stimulate methanogenic activity. Some metals (iron, cobalt, nickel, etc.) represent nutrient for methanogens (Rabii et al., 2019). Moisture content influence biogas production in anaerobic digestion. The higher the moisture content, the higher the biogas yield (Alnakeeb et al., 2017). Winery waste and sorghum stover moisture content were found to be respectively 1.15% and 38.62%. Phosphorus (0.16%), potassium (1.77%) and calcium (0.06%) content for winery solid waste were found to be similar to findings by Sousa et al. (2014), except for calcium which was higher. Phosphorus, potassium and calcium content of sorghum stover were 0.08%; 2.76% and 0.41% respectively, which is in accord with the findings of Pontieri et al. (2014).

Table 4.1: Physical and chemical properties of winery solid waste and sorghum stover

Characteristics	Units	Winery solid waste	Sorghum stover
Moisture	%	1.15	38.62
Volatile solids	%	83.86	84.09
Total solids	%	95.92	61.38
Total nitrogen	%	1.76	0.81
Ash content	%	15.950	15.91
Total carbon	%	50.40	37.77
Calcium	%	0.06	0.41
Potassium	%	1.77	2.76
Phosphorus	%	0.16	0.08
Protein	%	11.00	5.08
Iron	mg/kg	28.05	636
Sodium	mg/kg	1191.90	262
Cyanide	mg/kg	0.92	
C/N ratio		28.63	46.63

4.1.3. Biomethane potential tests

Biomethane potential tests were carried out to determine the maximum amount of methane produced with and without addition of iron oxide nanoparticles. The potential amount of biogas was determined first, represented in **Figure 4.4;** the potential amount of methane using BIOGAS 5000 (**refer to chapter 3**) is represented in **Figure 4.5.**

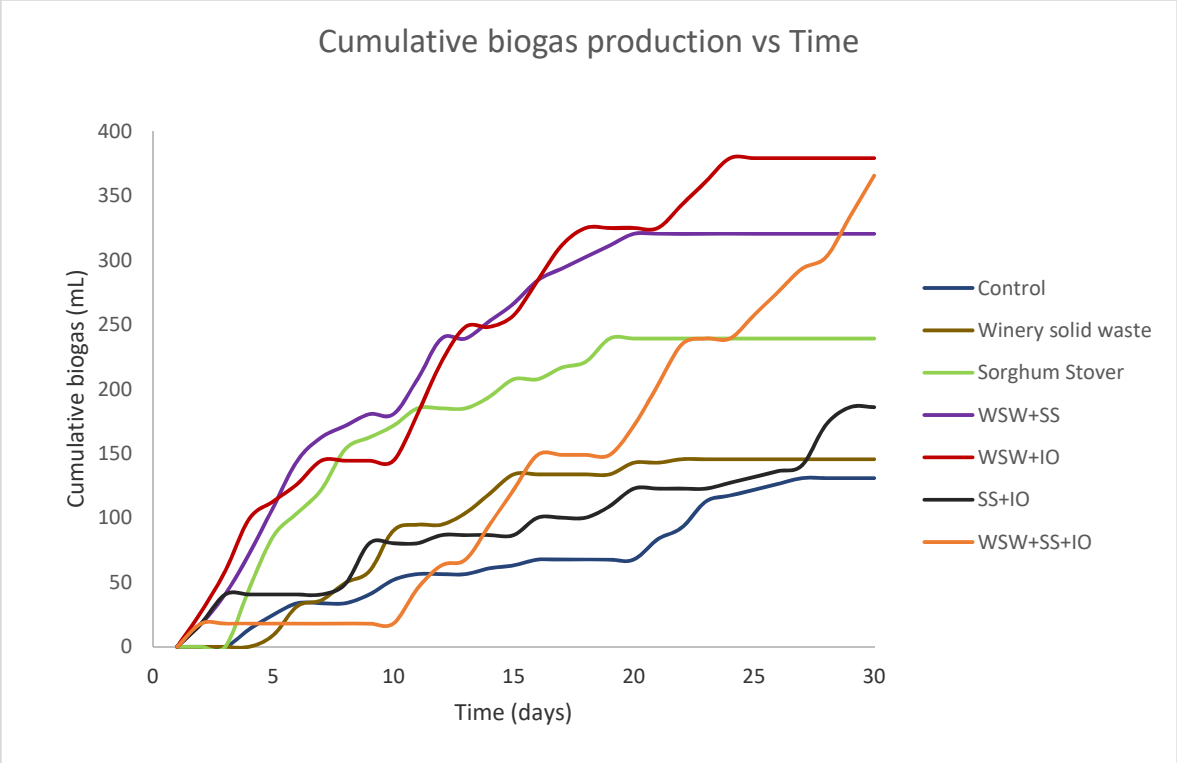

Figure 4.4: Biomethane potential test graph of biogas yield showing Inoculum (zebra dung only); winery solid waste; sorghum stover; co-digestion of WSW and SS at 50/50 ratio; winery solid waste with iron oxide nanoparticles; sorghum stover with iron oxide nanoparticles; and finally co-digestion substrate with ION

The maximum amount of biogas obtained with substrates after a period of 30 days is shown in **Figure 4.4**. Biogas produced by WSW with ION, co-digestion substrate without ION, and co-digestion substrates with ION and SS with ION all started at day 2. Their biogas production became constant respectively at day 24 and 20 for WSW with ION and co-digestion without ION, while SS with ION and co-digestion with ION were still increasing after day 30. Biogas produced by SS only started production at day 3 and became stable at day 18. WSW was the slowest biomass to start production practically at day 5 and stopped at day 20. The biogas volume produced by the control (inoculum) was below all other amounts of biogas produced by the substrates. It can be said that neither WSW nor SS inhibited the bacteria. It is important to notice that biogas production in the presence of ION was active before production in the

absence of nanoparticles. This could be explained by a threshold iron dose and results in the rapid increase of biogas production (Casals et al., 2014).

SS only produced 239 mL of biogas, WSW only produced 145 mL and co-digestion substrate 320mL. The co-digestion of substrate produced more biogas than sorghum stover and winery solid waste individually – respectively 81 mL and 175 mL more, which represents 34% and 120% increase of biogas compared to SS alone and WSW alone. This confirms the findings of Mata-Alvarez et al. (2014) which claims that co-digestion is superior to single digestion with more biomass.

SS with ION produced 186 mL of biogas compared with 239 mL of SS only. There is a 22% decrease which represents inhibition of the bacteria by the ION. As noted by Faisal et al. (2018), the conversion process by the interaction of nanoparticles is affected by inorganic contaminants obtained from inorganic material and molecular size. Nanomaterial could therefore not have an interaction with SS microorganisms. WSW with ION produced 380 mL of biogas which is 235 mL more than WSW alone and represents 162% increase in biogas production. Co-digestion substrates with ION produced 365 mL of biogas which represents an increase of 14%. This confirms the assertion of Casals et al. (2014) that iron oxide is a suitable additive to boost biogas production.

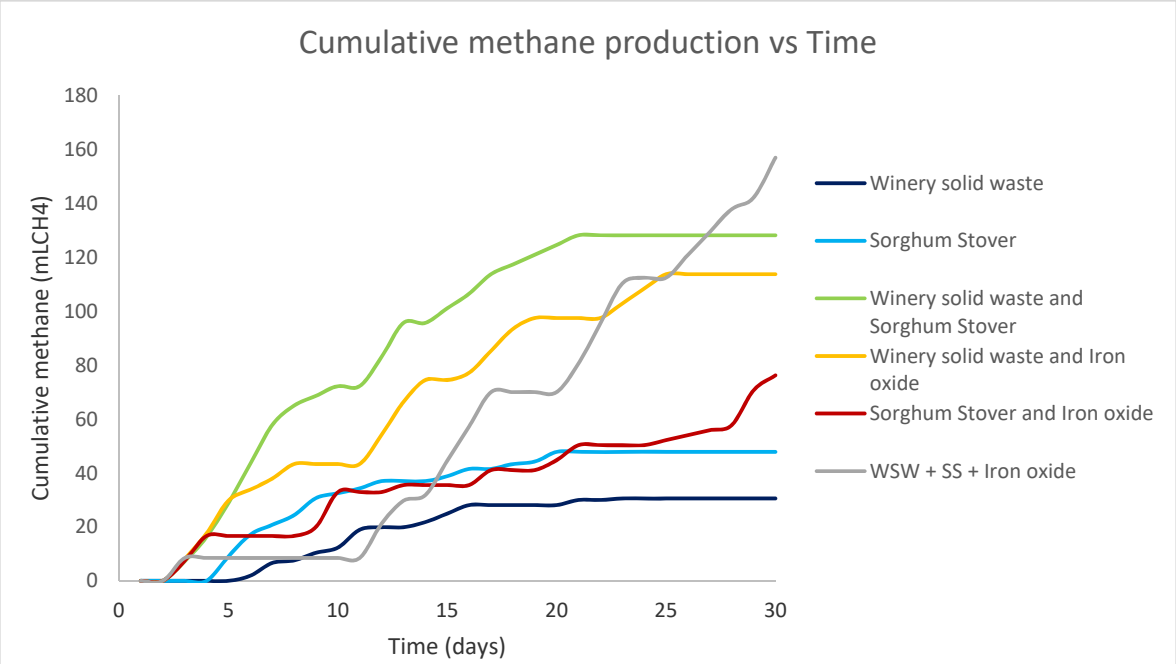

Figure 4.5: Biomethane potential test graph of methane yield

With regards to the quality of the biogas, the content of methane is the main goal. **Figure 4.5** shows the amount of cumulative methane obtained by the substrates with and without addition of iron oxide nanoparticles.

- WSW methane production went from 30 $mLCH_4$ to 114 $mLCH_4$. That represents an increase of 280% of methane yield. The methane percentage went from 21% without ION to 30% with ION.
- SS methane production went from 48 $mLCH_4$ to 76 $mLCH_4$. That represents a methane yield increase of 58%. SS without ION had 20% methane content and 41% methane content with addition of ION.
- Co-digestion substrate went from 128 $mLCH_4$ to 172 $mLCH_4$. There was a 34% increase in methane yield. Co-digestion went from 40% without ION to 47% methane content with ION.

Generally, there was an average increase of 51% in biogas production and 124% in methane production with the addition of iron oxide nanoparticles. These results fits with the statement that the non-toxic Fe3C ions contained in iron oxide nanoparticles enhanced the methane production by helping electron transport which improves the methane and hydrogen rate as well as stimulates bacterial growth production (Faisal et al., 2018).

Table 4.2: Summary of biogas, methane and normalized methane yield from different substrates and combinations

Sample	Average cumulative biogas (mL)	Average cumulative methane ($mLCH_4$)	Normalized cumulative methane ($mLCH_4.gVS_{added}^{-1}$)
ZD + WSW	145	30	9.54
ZD + SS	239	48	18.53
ZD + WSW + SS	320	128	44.64
ZD + WSW + ION	380	114	36.26
ZD + SS + ION	186	76	29.35
ZD + WSW + SS + ION	365	172	60

4.2. Optimization of biogas yield from co-digestion of winery solid waste and sorghum stover through application of iron oxide nanoparticles

Different factors affect the biogas production as mentioned in literature review. In this phase of experiment, optimum concentrations of iron oxide nanoparticle, co-digestion ratios and solid retention time were chosen and investigated to determine the best combination to exploit the maximum biogas using central composite design.

4.2.1. Response Surface Analysis Regression

The experimental data obtained were plotted into a response analysis and represented in **Table 4.3** to evaluate the relationship between the input variable (chosen factors) as solid retention time (A), concentration (B) and co-digestion ratio (C).

Table 4.3: Coded and actual values of iron concentration, solid retention time and co-digestion substrate ratios with their predicted and experimented results

Run Order	Solid retention time (days)		ION concentration (ppm)		Substrate ratio (SS:WSW)		Actual Value (mL)	Predicted Value (mL)
	Coded	Actual	Coded	Actual	Coded	Actual		
1	4.89	5	75	75	50	50	10.00	26.89
2	10	10	50	50	80	80	30.00	16.63
3	17.5	18	75	75	100.45	100	201.00	218.49
4	17.5	18	117.05	117	50	50	230.00	249.37
5	25	25	100	100	80	80	346.00	330.54
6	10	10	100	100	20	20	50.00	35.05
7	17.5	18	75	75	50	50	186.00	185.91
8	17.5	18	75	75	50	50	186.00	185.91
9	17.5	18	32.95	33	50	50	66.00	49.67
10	25	25	50	50	20	20	30.00	42.98
11	10	10	50	50	20	20	42.00	55.31
12	25	25	50	50	80	80	60.00	72.80
13	17.5	18	75	75	-0.45	0	39.00	24.55
14	17.5	18	75	75	50	50	186.00	185.91
15	17.5	18	75	75	50	50	186.00	185.91
16	10	10	100	100	80	80	251.00	235.87
17	17.5	18	75	75	50	50	186.00	185.91
18	25	25	100	100	20	20	50.00	61.22
19	17.5	18	75	75	50	50	186.00	185.91
20	30.11	30	75	75	50	50	110.00	96.15

Actual values obtained from the experiment were then fitted into a second order polynomial equation by means of multiple regression analysis. **Equation 4.1** is the resultant of mathematical regression models for biogas production and was in terms of coded factors:

$$= 186.04 + 22.93A + 61.72B + 60C + 5.63AB + 13.12AC + 55.88BC - 44.82A^2 - 13.71B^2 - 23.61C^2 \quad (4.1)$$

The accuracy and adequacy of the quadratic model were determined on the data obtained by analysis of variance (ANOVA) detailed in **Table 4.4** and **Table 4.5** below.

Table 4.4: Analysis of variance for the quadratic model for biogas production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.701E+05	9	18900.15	43.65	< 0.0001	significant
A-SRT	7181.83	1	7181.83	16.59	0.0022	
B-Conc. iron	52013.11	1	52013.11	120.13	< 0.0001	
C-Co-digestion ratio	49169.38	1	49169.38	113.56	< 0.0001	
AB	253.13	1	253.13	0.5846	0.4622	
AC	1378.13	1	1378.13	3.18	0.1047	
BC	24976.13	1	24976.13	57.69	< 0.0001	
A^2	28954.63	1	28954.63	66.88	< 0.0001	
B^2	2709.20	1	2709.20	6.26	0.0314	
C^2	8033.65	1	8033.65	18.56	0.0015	
Residual	4329.64	10	432.96			
Lack of Fit	4329.64	5	865.93			
Pure Error	0.0000	5	0.0000			
Cor Total	1.744E+05	19				

The model F-value of 43.65 indicates the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve it Eight out of ten terms were significant and therefore so was the model. The degree of freedom, five each for lack of fit and pure error ensure the valid lack of fit test. At least three for lack of fit and four for pure error is recommended.

Table 4.5: Fit summary for the biogas production

Std. Dev.	20.81	**R^2**	0.9752
Mean	129.95	**Adjusted R^2**	0.9528
C.V. %	16.01	**Predicted R^2**	0.8033
PRESS	34312	**Adeq Precision**	20.8852

In the results obtained, the coefficient of determination, R^2, was found to be 0.9528, which shows that 95.28% of the observed variation in the biogas yield response could be explained by the model. A high statistical measure of fit (R^2), ranging from 85% to 100% indicates that the stock performance moves relatively in line with the index. The larger R^2 is, the better the

regression model fits with actual values (van Ginkel, 2019). The predicted R-squared of 0.8033 is in reasonable agreement with the adjusted R-squared of 0.9528 as the difference is less than 0.2. An adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 20.885 indicates an adequate signal therefore this model can be used to navigate the design space. The coefficient of variation (CV) expresses the ratio of the standard deviation to the mean. The higher the percentage of CV is, the greater the level of dispersion around the mean. Hence, a relatively low percentage (16.01%) determines a more precise estimate. The predicted residual sum of square (PRESS) is a measure of the inconsistency between the data and the model estimation. The smaller the PRESS is, the better the model's predictive capability. The relatively low PRESS value of this study, found to be 34312, shows therefore that the model has a good predictive capability. **Figure 4.6** below reflects the proximity between the actual biogas volume values and the predicted ones.

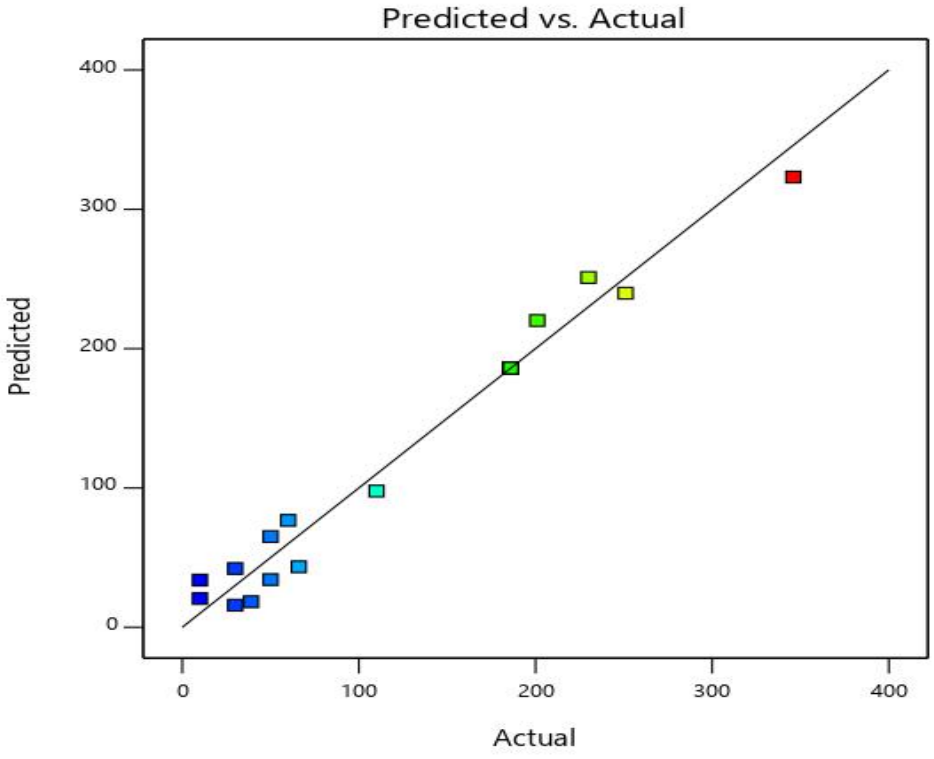

Figure 4.6: Model graph predicted vs actual values of biogas yield

4.2.2. Comparisons and interactions among factors

4.2.2.1. Relationship between iron concentration and solid retention time

A surface plot in 3D rendering was used to demonstrate the relationship between biogas yield (dependent variable), iron nanoparticle concentration, and solid retention time (both independent variables) and is represented in **Figure 4.7** below. The third factor was at a constant ratio of 80:20 with respect to sorghum stover. At this co-digestion concentration, a maximum value of 346 mL of biogas volume can be achieved at 25 days for an iron concentration of 100 ppm for a predicted value of 330.54 mL. Increasing the concentration of iron resulted in the increase of biogas volume until 100 ppm. Above this value (117 ppm) a decrease of biogas production was observed. This observation could be explained by the toxic effect of the nanoparticles on microorganisms when used in excess (Casals et al., 2014). The same result was observed when the solid retention time was increased. However, biogas volume decreased as the solid retention time exceeded 18 days, specifically at 30 days. This is in accordance with a study by Nges and Liu (2010) which states that the best operational SRT in terms of maximizing biogas production could be between 12 and 15 days at mesophilic conditions. ANOVA shows both SRT and NPs concentration have a significant effect on biogas volume, respectively P= 0.0022, F=16.59 and P< 0.0001, F=120.13. Both values of P are below 0.05. Iron oxide concentration contributed more to the biogas production based on the F values, which confirms the importance and ability of the nanoparticles in the change of biogas rate.

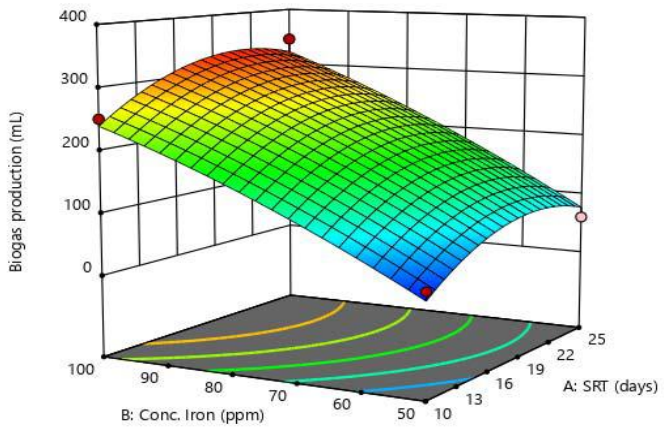

Figure 4.7: Three-dimensional response surface and contour lines of the effect of iron concentration and solid retention time on biogas production

4.2.2.2. Relationship between SRT and co-digestion

The interactive effect between the solid retention time and co-digestion substrates is shown in **Figure 4.8**. The darker region (in red) identifies the maximum amount of biogas predicted, 330.54mL at 25 days with a co-digestion substrate ratio of 80:20 with respect to sorghum stover. The design value of biogas volume obtained (346 mL) is above the predicted value characterized in the darker region (**Figure 4.8**) for the same parameters known at 25 days with a co-digestion substrate ratio of 80:20 with respect to SS as shown in **Table 4.3**. It could be due to the type of inoculum used (zebra dung) more active at mesophilic conditions (Mkruqulwa et al., 2019). With concentration of NPs being fixed at 100 ppm, biogas volume increased proportionally, with the increase of co-digestion substrate and SRT. ANOVA shows that both factors, SRT and co-digestion substrate have a significant effect on biogas yield. Both of their P-values are below 0.05. However, co-digestion substrate ($F=113.5$; $P< 0.0001$) has more impact on the biogas yield than SRT ($F=16.59$; $P=0.0022$). The co-digestion is directly related to the carbon/nitrogen ratio which when improved leads to a better microorganism activity. SS alone has a C/N of 46.63 while WSW has 28.63 (refer to **Table 4.1**), but the established mixture was found to be 22:1 which represents a suitable ratio to optimize biogas SRT and describe the average time spent by the microorganism in the digester, which may require another factor to be more efficient (Nges and Liu, 2010, Hallaji et al., 2019).

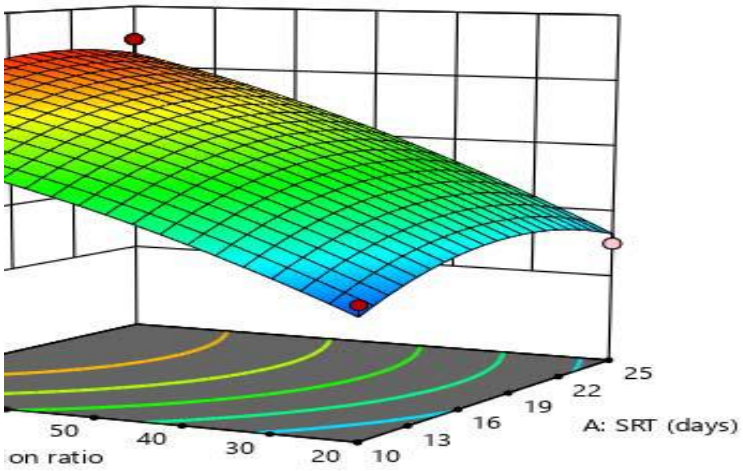

Figure 4.8: Three-dimensional response surface and contour lines of co-digestion substrate ratio and solid retention time (SRT) effect on biogas production

4.2.2.3. Relationship between concentration of iron oxide and co-digestion substrate

The interactive effect between concentration of iron oxide and co-digestion substrate is represented in **Figure 4.9** below. The curves in the response surface confirm the interaction effect of the model (Antony, 2014). SRT was fixed at 22 days close to the optimum value. An increase of NPs concentration and co-digestion substrate simultaneously increased the biogas volume. The predicted value of biogas volume (330.54 mL) characterized by the darker region was lower than the design value (346 mL) obtained in this study at 25 days with a co-digestion ratio substrate of 80:20 with respect to SS. It could be due to the type of inoculum used (zebra dung) which may be more active at mesophilic conditions (Mkruqulwa et al., 2019). ANOVA shows both ION concentration and co-digestion substrate have a significant effect on biogas volume, respectively P< 0.0001, F=120.13 and F=113.5; P< 0.0001. Both values of P are below 0.05. The factors have relatively the same degree of impact on the biogas volume. However NPs concentration is slightly higher (difference in f values).This could be explained by the fact that despite their nutritive effect on biogas, iron oxide nanoparticles have a toxic effect on microorganisms at high concentration (Casals et al., 2014).

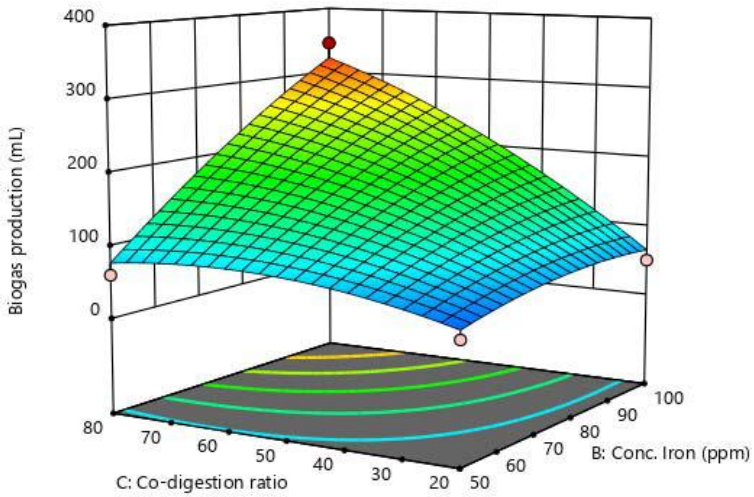

Figure 4.9: Three-dimensional response surface and contour lines of iron concentration and co-digestion substrate ratio effect on biogas production

4.2.3. Conditions for optimum response and model validation

The optimum conditions for optimal biogas production were predicted using the second order polynomial model (**Equation 4.1**). The model defined a maximum biogas yield of 330.54 mL for the optimal conditions. Biogas production shows optimum value of 346 mL in run 5 with experimental conditions of 100 ppm concentration of NPs, and 80:20 co-digestion ratio with respect to sorghum stover for a SRT of 25 days. The obtained value is slightly higher than the predicted value. According to Casals et al. (2014) the optimum concentration is accurate since iron oxide nanoparticles could be toxic if in excess. Lower concentration could also be effective but with a smaller amount of Fe^{2+} available to boost the microorganism activity, resulting in lower biogas and methane yield as observed in **Table 4.6**. Co-digestion ratio matches with the characteristics of the substrate; the phenolic content of the WSW is a possible criterion which could inhibit the biogas production, and the high C/N ratio of sorghum stover does not conform with high methane yield. The 80:20 ratio with respect to SS is therefore an alternative as 20% of WSW was used, reducing the inhibitory molecules (phenolic compounds) and the 80% of SS used balanced the C/N ratio to 22:1, which is a suitable ratio for high methane yield. It is shown in **Table 4.6** with the different values obtained at that ratio. Run 5 and 16 were among the higher values obtained with 346 mL and 251 mL respectively. According to Nges and Liu (2010), shortening of SRT leads to higher OLR, biogas and methane increase. From the average 30 days at mesophilic conditions to 25 days SRT, higher biogas and methane yield was observed from 110 mL and 19 mLCH_4 at run 20 to 346 mL and 148.7 mLCH_4 at run 5.

Table 4.6 also reveals the optimum conditions for the three chosen factors in this study. Analysis of variance (ANOVA) reveals that interactive effects between all factors were significant: SRT and concentration of iron, SRT and co-digestion ratio, and then concentration of iron and co-digestion ratio.

The optimal co-digestion ratio for biogas production from sorghum stover and winery solid waste adding iron oxide nanoparticles is 80:20 (SS: WSW). Average ratio (50:50) produced more biogas than WSW alone but less than SS alone. From 20% to 80% with respect to SS, biogas production increased. Sorghum stover alone produced a good amount of biogas but was still less effective than the mixture of substrates at adequate ratio. The 80:20 mixture encountered the limitation of each substrate individually, phenolic compounds for WSW and high C/N for SS.

Table 4.6: Coded and actual values of iron concentration, SRT and co-digestion substrate ratio with the practical results of biogas and methane yield

Run Order	Solid retention time (days)		ION concentration (ppm)		Substrate ratio (SS:WSW)		Biogas volume (mL)	Methane volume (mL CH_4)
	Coded	Actual	Coded	Actual	Coded	Actual		
1	4.89	5	75	75	50	50	10,00	1.3
2	10	10	50	50	80	80	30,00	3
3	17.5	18	75	75	100.454	100	201,00	26,13
4	17.5	18	117.045	117	50	50	230,00	71,3
5	25	25	100	100	80	80	346,00	148,78
6	10	10	100	100	20	20	50,00	20
7	17.5	18	75	75	50	50	186,00	33,48
8	17.5	18	75	75	50	50	186,00	33,48
9	17.5	18	32.95	33	50	50	66,00	21,78
10	25	25	50	50	20	20	30,00	15
11	10	10	50	50	20	20	42,00	21
12	25	25	50	50	80	80	60,00	6
13	17.5	18	75	75	-0.45	0	39,00	5,85
14	17.5	18	75	75	50	50	186,00	33,48
15	17.5	18	75	75	50	50	186,00	33,48
16	10	10	100	100	80	80	251,00	107,93
17	17.5	18	75	75	50	50	186,00	33,48
18	25	25	100	100	20	20	50,00	20
19	17.5	18	75	75	50	50	186,00	33,48
20	30.1134	30	75	75	50	50	110,00	19,8

The optimal concentration of ION for biogas production from SS and WSW through application of iron oxide nanoparticles is 100 ppm. Again, concentration of 50 ppm produced a relatively low amount of biogas compared to 75 ppm and 100 ppm. The 117 ppm concentration produced more biogas than 50 and 75 ppm, but less than 100 ppm. This could be explained by an excess of reactive iron ions and the consequently lethal effect on methane-producing microorganism (Casals et al., 2014). The 100 ppm concentration provided enough nutrients to the bacteria without inhibiting them.

The highest SRT (25 days) for biogas production was obtained from the mixture of SS, WSW and ION. The lowest amount of biogas obtained came from the lowest SRT of 5 days. As SRT increased from 10 to 25 days, biogas also increased, and higher biogas volumes were obtained between 18 and 25 days. At 30 days, normal SRT for mesophilic conditions, biogas yield was less than at 18 days under the same conditions. Shortening of SRT leads to an optimum use of the reactor without compromising volatile solid destruction efficiency. More biogas production is therefore obtained from 25 days than 30 days (Nges and Liu, 2010).

From the results obtained in this study, it was observed that the optimum ratio for methane production is 100 ppm of iron oxide concentration for a co-digestion ratio of 80:20 with respect to SS at 25 days. The high coefficient of determination (R^2) of 0.9528 confirms the utility of the response surface method model for biogas yield prediction and is relatable to the findings of Cu et al. (2015). It could therefore be used to predict methane production from biomass content of lipid, lignin and protein from manure and plant residues (Cu et al., 2015).

4.3. Upscaling using a single stage 5L mesophilic batch digester

Biogas production was evaluated at mesophilic conditions using a single stage 5L batch digester. Conditions were the same as optimum factors, 100 ppm of iron concentration for a co-digestion ratio substrate of 80:20 with respect to sorghum stover for 25 days. A co-experiment with 3 L of zebra dung was run simultaneously as control. **Table 4.7** below shows the biogas and methane yield obtained from the experiment. A total of 1760.54 mL biogas was obtained for 1302.8 $mLCH_4$ methane yield produced which represents a methane content of 74%. The 30 mL of biogas produced by the control was removed from the total volume to give 1730.54 mL of net biogas produced and 711.04 $mL.gVS^{-1}$. A methane yield of 1272.8 $mLCH_4$ was therefore produced for 522.97 $mLCH_4.gVS^{-1}$. Biogas production started on day 3, increased between day 9 and 20 but decreased at day 21 and remained constant afterwards till day 25 as can be observed in **Table 4.7**. Maximum temperature reached was 37.78 °C where biogas yield was the lowest. Good production of biogas was in the range of 36.60 - 37.02 °C as shown in **Table 4.7**. This could be explained by the sensitivity of the methanogens to change of temperature. Temperature influences the growth and metabolic activity of methanogens, therefore temperature regulation is important for high biogas production in methanogenic phase [**Figure 4.11**] (Wang et al., 2019). Also, the increase of temperature from 35°C to 37°C reduced the required time for the digestion process. Above 37°C the rate of biogas generation decreased. On the pH side, biogas was produced more between pH 7.2 and 7.6. Above a pH of 7.6, biogas production decreased. This is in accordance with Cioabla et al. (2012), who stated that the optimal pH range is 6.8 to 7.2 but the process can tolerate up to pH 8. The pH behavior of the optimum condition process shown in **Figure 4.10** is relatively linear starting with pH values at 7.63 and showing a small peak between 15 and 20 days. This indicates good behavior of the biomass mixture during anaerobic fermentation and has the advantage of a better control (Cioabla et al., 2012).

It is also important to mention that a better gas quality from 43% to 74% was obtained with an increase in reactor volume from 500mL Schott bottle to a 5 L single stage digester respectively. It constitutes therefore an increase of 72% of methane production. This can be explained by the larger amount of organic loading rate degraded in the 5 L digester.

Table 4.7: Observed daily data of optimum conditions for the Upscaling system

Day	Biogas Yield (mL)	Methane Yield (mL CH4)	Temperature(°C)	pH
1	0	0	37.01	7.63
2	0	0	37	7.6
3	0	0	37	7.6
4	72.19	53.42	36.93	7.58
5	62.26	46.07	37.78	7.23
6	62.26	46.07	37.77	7.23
7	62.26	46.07	36.73	7.31
8	61.36	45.41	36.63	7.23
9	90.23	66.77	36.65	7.29
10	90.23	66.77	36.67	7.4
11	90.23	66.77	36.68	7.25
12	90.23	66.77	37.26	7.26
13	90.23	66.77	36.75	7.3
14	94.75	70.11	36.75	7.31
15	90.24	66.77	36.99	7.32
16	90.24	66.77	36.75	7.48
17	90.24	66.77	36.73	7.66
18	90.24	66.77	36.86	7.52
19	108.28	80.13	36.75	7.6
20	81.21	60.09	37.02	7.77
21	76.70	56.76	36.75	7.75
22	66.77	49.41	37.01	7.75
23	66.77	49.41	37.01	7.81
24	66.77	49.41	37.02	7.73
25	66.77	49.41	37.03	7.86
TOTAL	1760.54	1302.8(74%)		

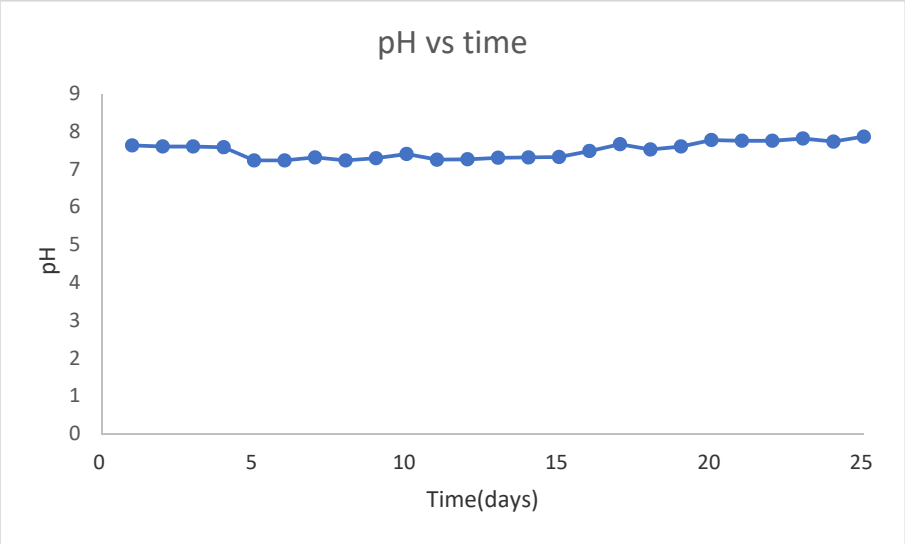

Figure 4.10: pH variation during anaerobic process

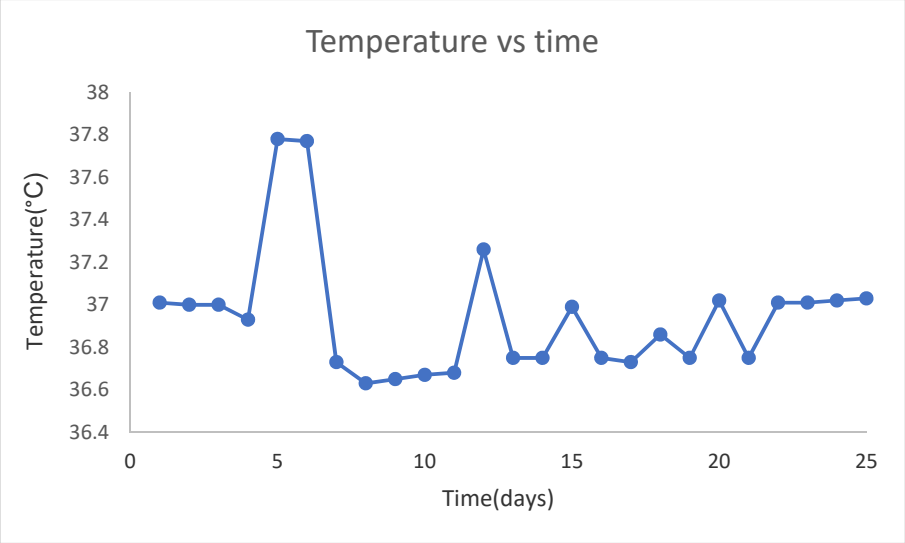

Figure 4.11: Temperature variation during anaerobic process

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

5.1.1. Characterization and biomethane potential

The chemical and physical properties of sorghum stover and winery solid waste show them as suitable substrates for biogas production due to their high total solids content, high volatile solids content and protein content. Higher concentration of trace metals for sorghum stover and winery solid waste are required for optimal anaerobic digestion. Iron oxide analysis showed the nanoparticles as a mixture of iron (II) and (III) oxides of 30 nm average size. The mixture contains the Fe^{2+} and Fe^{3+} ions which are nutritious for the methanogenic bacteria. During the BMP assay at optimal temperature of 37 °C ±0.5 for 30 days, sorghum stover and winery solid waste showed good results for anaerobic digestion amplified with the addition of iron oxide nanoparticles. The obtained results show an increase of biogas from individual substrates only to substrates with ION. The addition of the nanoparticles even tripled the biogas yield from winery solid waste. This confirms that WSW is more compatible to NPs than SS. Co-digestion of substrates also produced more biogas than the individual digestion of winery solid waste and sorghum stover.

5.1.2. Optimization

Among the three factors selected, iron concentration proved to have more influence during biogas production, followed slightly by co-digestion substrate ratio. SRT has less effect on the production of biogas. A right balance of iron oxide concentration produced more biogas while both excess and insufficient amounts produced less biogas. The decrease of biogas from excess iron concentration could be due to the toxic effect of Fe^{2+} at high concentration. Increasing the sorghum stover amount yielded more biogas but increasing the winery solid waste content decreased the biogas production. The decline of biogas yield for winery solid waste could be due to the chemical properties of WSW (i.e. phenolic compounds) which are inhibitory to the microorganisms. The optimum conditions for maximum biogas yield were 100 ppm of iron concentration, with sorghum stover to winery solid waste ratio of 80:20 at 25 days.

The maximum response value for biogas production obtained was 346 mL. The predicted value is close to the experimental value based on results obtained in **Table 4.3.** The coefficient of determination, R^2, found to be 0.9528, shows that the quadratic model used can predict the methane production from co-digestion of sorghum stover and winery solid waste with addition of iron oxide nanoparticles. In conclusion, RSM can be a valuable tool to determine methane production from co-digestion of winery solid waste and sorghum stover with the addition of iron oxide nanoparticles.

5.1.3. Upscaling the experiment to a 5L batch digester

The study of co-digestion of sorghum stover with winery solid waste (80:20) with the addition of 100 ppm iron oxide concentration using a single-stage batch 5 L digester at mesophilic conditions produced 1730.54 mL of biogas with a methane content of 74%. Methane content increased by 72% compared with the optimization experiment. These results showed that co-digestion of sorghum stover and winery solid waste with the addition of iron oxide can be used for biogas production at a larger scale provided the ratio and pH as well as temperature are kept constant.

5.2. Recommendations

The following further studies are recommended:

- A substitute for sorghum stover more compatible with iron nanoparticles to optimize biogas yield.
- An increase in the hydrothermal temperature for the synthesis of iron oxide nanoparticles to obtain pure Fe_3O_4. In fact, synthesis of nanoparticles can be done at different temperatures and the obtained nanomaterial tested in biogas production to determine if it is more effective. For this study, a size of 7 nm was planned, and 30 nm was reached.
- Further studies on the microbial community of the biomass can be done to determine the bacteria responsible at each stage of the anaerobic digestion

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APPENDICES

APPENDIX A: Raw data for BMP tests

Table A1: Average biogas and methane yield for sorghum stover only

Day	Average biogas yield per day from SS only(mL)	Average methane yield per day from SS only ($mLCH_4$)
1	0	0
2	0	0
3	0	0
4	45.11	9.02
5	40.60	8.12
6	18.04	3.61
7	18.05	3.61
8	31.58	6.32
9	9.027	1.80
10	9.02	1.80
11	13.53	2.71
12	0	0
13	0	0
14	9.02	1.80
15	13.53	2.71
16	0	0
17	9.02	1.80
18	4.51	0.90
19	18.04	3.61
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	**239.13**	**47.83**

Table A2: Average biogas and methane yield for winery solid waste only

Day	Average biogas yield per day from WSW only(mL)	Average methane yield per day from WSW only ($mLCH_4$)
1	0	0
2	0	0
3	0	0
4	0	0
5	9.02	1.89
6	22.55	4.73
7	4.51	0.95
8	13.53	2.84
9	9.02	1.89
10	31.58	6.63
11	4.51	0.95
12	0	0
13	9.02	1.89
14	15	3.15
15	15	3.15
16	0	0
17	0	0
18	0	0
19	0	0
20	9.02	1.89
21	0	0
22	2.71	0.57
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	**145.50**	**30.56**

Table A3: Average biogas and methane yield for co-digestion of sorghum stover and winery solid waste

Day	Average biogas yield per day for co-digestion of SS+WSW(mL)	Average biogas yield per day for co-digestion of SS+WSW($mLCH_4$)
1	0	0
2	18.05	7.22
3	22.56	9.02
4	31.58	12.63
5	36.09	14.43
6	36.09	14.43
7	18.04	7.21
8	9.02	3.60
9	9.02	3.61
10	0	0
11	27.07	10.83
12	31.58	12.63
13	0	0
14	13.53	5.41
15	13.53	5.41
16	18.05	7.22
17	9.02	3.61
18	9.02	3.61
19	9.02	3.61
20	9.02	3.61
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	**320.34**	**128.14**

Table A4: Average biogas and methane yield for sorghum stover in addition of iron oxide nanoparticles

Day	Average biogas yield per day for SS with ION (mL)	Average methane yield per day for SS with ION (mL CH_4)
1	0	0
2	18.05	7.40
3	22.56	9.25
4	0	0
5	0	0
6	0	0
7	0	0
8	8.12	3.33
9	31.58	12.95
10	0	0
11	0	0
12	6.31	2.59
13	0	0
14	0	0
15	0	0
16	13.53	5.55
17	0	0
18	0	0
19	9.02	3.70
20	13.53	5.55
21	0	0
22	0	0
23	0	0
24	4.51	1.85
25	4.51	1.85
26	4.51	1.85
27	4.51	1.85
28	31.58	12.95
29	13.53	5.55
30	0	0
Total	**185.89**	**76.21**

Table A5: Average biogas and methane yield for winery solid waste in addition of iron oxide nanoparticles

Day	Average biogas yield per day for WSW with ION (mL)	Average methane yield per day for WSW with ION (mL CH_4)
1	0	0
2	27.07	8.12
3	31.58	9.47
4	40.61	12.18
5	13.53	4.06
6	13.53	4.06
7	18.05	5.41
8	0	0
9	0	0
10	0	0
11	36.09	10.83
12	40.61	12.18
13	27.07	8.12
14	0	0
15	9.02	2.71
16	27.07	8.12
17	27.07	8.12
18	13.53	4.06
19	0	0
20	0	0
21	0	0
22	18.05	5.41
23	18.05	5.41
24	18.05	5.41
25	0	0
26	0	0
27	0	0
28	0	0
29	0	0
30	0	0
Total	**379**	**113.70**

Table A6: Average biogas and methane yield for co-digestion of sorghum stover and winery solid waste in addition of iron oxide nanoparticles

Day	Average biogas yield per day for co digestion(WSW+SS) with ION (mL)	Average methane yield per day for co digestion(WSW+SS) with ION (mL CH_4)
1	0	0
2	18.05	8.48
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	27.07	12.72
12	18.05	8.48
13	4.51	2.12
14	27.07	12.72
15	27.07	12.72
16	27.07	12.72
17	0	0
18	0	0
19	0	0
20	22.56	10.60
21	31.58	14.84
22	31.58	14.84
23	4.51	2.12
24	0	0
25	18.05	8.48
26	18.05	8.48
27	18.05	8.48
28	9.02	4.24
29	31.58	14.84
30	31.58	14.84
Total	**365.46**	**171.77**

APPENDIX B: Raw data for biogas optimization

Table B1: Biogas yield per day and cumulative biogas at different combination of iron concentration, Solid retention time and co-digestion substrate ratio

Run number	Iron conc.(ppm)	SRT(day)	Co-digestion ratio(SS:WW)	Biogas yield per day(mL)																																Cumulative biogas yield(mL)
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
1	75	5	50:50	0	0.45	2.26	6.77	0																										10
2	50	10	80:20	4.5	4.5	9.0	5.4	0.0	0.0	0.0	4.5	0.0	2.3																					30
3	75	18	100:0	0.0	4.5	9.5	24.8	0.0	17.1	23.0	27.1	15.8	31.6	6.8	0.0	9.0	13.5	9.0	9.0	0.0	0.0													201
4	117	18	50:50	0.0	0.0	13.5	22.6	18.0	15.8	15.8	22.6	29.3	3.6	9.0	13.5	12.6	3.6	18.0	13.5	9.0	9.0													230
5	100	25	80:20	45.1	15.8	18.0	27.1	45.1	18.0	21.2	27.1	27.1	6.8	6.8	9.0	11.3	0.0	0.0	0.0	0.0	20.3	13.5	0.0	0.0	13.5	13.5	0.0	6.3						346
6	100	10	20:80	0.0	0.0	0.0	0.0	15.8	0.0	13.5	13.5	2.3	4.5																					50
7	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
8	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
9	33	18	50:50	0.0	0.0	0.0	0.0	0.0	9.0	0.0	0.0	2.7	6.8	6.8	0.0	6.8	0.0	6.8	0.0	13.5	13.5													66
10	50	25	20:80	0.0	0.0	1.8	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.1	0.0	0.0	0.0						30
11	50	10	20:80	0.0	5.4	0.0	4.5	12.6	5.4	6.8	2.3	4.5	0.0																					42
12	50	25	80:20	0.0	0.0	2.3	2.3	1.8	3.6	0.0	0.0	4.5	15.8	4.5	6.8	0.9	0.0	3.6	0.0	2.3	3.6	0.0	4.1	1.8	2.3	0.0	0.0	0.0						60
13	75	18	0:100	0.0	0.0	13.5	0.0	0.0	0.9	4.5	0.0	0.0	0.0	9.0	11.3	0.0	0.0	0.0	0.0	0.0	0.0													39
14	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
15	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
16	100	10	80:20	5.0	40.6	45.1	20.3	24.8	24.8	20.3	15.8	22.6	31.6																					251
17	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
18	100	25	20:80	0.0	0.0	2.3	2.3	2.3	2.3	3.6	1.8	4.5	9.0	6.8	4.5	3.6	1.4	0.0	2.3	2.3	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0						50
19	75	18	50:50	0.0	9.0	1.8	0.0	29.3	31.6	9.0	4.5	15.8	9.0	15.8	4.5	9.0	8.1	13.5	6.8	9.0	9.0													186
20	75	30	50:50	0.9	0.0	0.0	3.6	0.0	0.9	1.8	9.0	2.3	0.0	13.5	4.5	5.4	2.3	4.5	4.5	0.0	9.0	6.8	6.8	9.0	4.5	4.5	1.8	9.5	4.5	0.0	0.0	0.0	0.0	110