Anaerobic Digestion of Different Feedstock Used at a Refurbished Gold Processing Mill for the Production of Biogas

By

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Declaration of Independent Work

I, Elly Mboneni (student number), hereby declare that the research submitted to the Central University of Technology, Free State, for the degree Master of Health Sciences in Environmental Health is my own independent work. The research project was conducted at the Central University of Technology, Free State under the supervision of Dr O de Smidt and co-supervised by Professor JFR Lues and Dr H Swanepoel.

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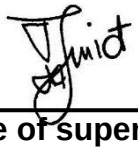

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DEDICATION

©To KHWATHISO NETSHIFHEHFE: Continue persisting and pushing forward. Be awesome and never settle for less. Preserve your uniqueness and always do it in the way that makes you happy… until…..EYEAMANTEU.

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Abstract

Anaerobic digestion is a technology that is commonly used to treat organic waste for biogas production. Biogas can be used to replace fossil fuel as a source of energy for electricity production. However, despite the benefits of anaerobic digestion, the degradation of organic material via this process is complicated and requires proper operational stability and applications to optimise methane (CH_4) production to promote its commercialisation.

This bioenergy project rehabilitated disused mine structures and land and aimed to produce biogas via anaerobic digestion of energy crops planted on contaminated soil. This study investigated and characterised the factors that needed understanding and had to be overcome for the proper start-up processes and operational procedures of a biogas digester. The study was divided into three parts: (a) A case study was conducted for which industrial digesters were commissioned. During Phase I, anaerobic digester 2 (AD2) was seeded with rumen solid contents (RSC) in ambient temperature. To improve AD2, municipal wastewater treatment sludge (MWWTS) was added as the second seed. AD2 was soon considered dormant and remedial methods were applied during Phase II. These methods included pH amendment by addition of lime and sodium hydroxide (NaOH), reducing the total solids (TS) by transferring the contents into an empty anaerobic digester (AD1), and the addition of inoculums obtained from an operational digester (DFOD) and prepared from long-standing cow manure (used as cow bedding) (LSM). During Phase III, process advancement was observed without any additional parameter adjustments. (b) Laboratory revitalisation of dormant digesters was performed in 5 L digesters. These trials were performed after Phase I to determine the methods that could be applied to remediate AD2.

Two methods for reviving the dormant digesters in batch laboratory tests were investigated, which included the addition of seeding material and increasing the retention time and temperature. The batch testing were divided into two trials: the first trial operated in psychrophilic and the second trial operated in mesophilic conditions (31.5±1.5ºC). (c) Biogas was produced from sugar beet roots (SBR), sugar beet leaves (SBL) and sorghum (SOR) used for phytoremediation of mine-

impacted land. The co-digestion potential of RSC with SBR and SOR at ratios of 25:75 and 50:50 was investigated. Rumen fluid was used as inoculum in both mono- and co-digestion tests and the trials were performed in mesophilic conditions.

The inoculation was declared a failure after Phase I because AD2 was overloaded. This resulted in a low CH_4 yield, high volatile fatty acids (VFA), unstable alkalinity, and a pH of 4. AD2 inoculation failed because RSC as an inoculum was inapt to provide consortia of facultative and anaerobic microorganisms and the method chosen for seeding was unsuitable. Moreover, the incorrect treatment of RSC as inoculant led to regular congestion of the system. To achieve successful inoculation of AD2, a reduction of TS and the addition of LSM were accomplished whilst AD1 was successfully seeded by mixing DFOD with AD2 digestate. AD1 and AD2 performances improved after the introduction of inoculums because the CH_4, VFA, pH and alkalinity in both digesters were at operational levels. During the laboratory revitalisation of dormant digesters, the digesters that operated in psychrophilic temperature took longer to produce biogas.

This process was characterised by low CH_4%, pH, gas production and higher VFA. The mesophilic digesters were characterised by lower VFA, higher CH_4% and gas production and a stable pH compared with the psychrophilic digesters. Temperature affected the quantity of VS (volatile solids) that were degraded because a higher VS removal was achieved in digesters operated in mesophilic temperature. The introduction of LSM and DFOD in the mesophilic digesters improved gas production and reduced the lag phase of microbial growth. During mono-digestion of RSC and energy crops, a profuse amount of biogas was produced from each feed: SBL produced a mean CH_4 concentration of ≈53% with a CH_4 yield of ≈282.60 $m^3\ t^{-1}$ VS. This was economical and thus offers an alternative to simply dumping this feedstock. RSC mono-digestion had a high CH_4 yield of 399.66±1.47 $m^3\ t^{-1}$ VS whilst co-digestion using RSC affected the biogas production positively, because the RSC:SOR (25:75) ratio mix had the highest CH_4 at 65% and the highest CH_4 yield of 515.45±4.91 $m^3\ t^{-1}$ VS.

It was concluded that a refurbished gold plant can be repurposed for biogas production, given that the correct inoculum is used and that proper process monitoring is practised. LSM and DFOD inoculums at different concentrations can be utilised to revitalise dormant digesters and are proficient in improving gas production, VS degradation, and reducing the lag phase of microbial growth of dormant digesters. This study proposes that the co-digestion of energy crops exposed to contaminated land with abattoir waste presents the possibility of rehabilitating soil and circumventing landfilling by organic waste, while at the same time producing bioenergy.

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Abbreviations

%	→ Percentage
$(NH_4)_2CO_3$	→ Ammonium carbonate
µm	→ Micrometre
a.m.	→ Ante meridiem
AD1	→ Anaerobic digester 1
AD2	→ Anaerobic digester 2
ANOVA	→ Analysis of variation
As	→ Arsenic
BOD	→ Biological oxygen demands
$CaCO_3$	→ Calcium carbonate
Cd	→ Cadmium
CH_3CH_2OH	→ Ethanol
CH_3COOH	→ Acetic acid
CH_3OH	→ Methanol
CH_4	→ Methane
CHP	→ Combined heat and power
CO	→ Carbon monoxide
Co	→ Cobalt
CO_2	→ Carbon dioxide
COD	→ Chemical oxygen demand
Cr	→ Chromium
CSTR	→ Continuous stirred tank reactors
CTC	→ Chlortetracycline
Cu	→ Copper
D1-D4	→ Digester 1, 2, 3 and 4
DFOD	→ Digestate from an operating digester
EU	→ European Union
FCM	→ Fresh cow manure
Fe	→ Iron
FOS	→ Volatile organic acids
g	→ Gram

GDI	→ Gas detection instrument
GDP	→ Gross domestic product
GHG	→ Greenhouse gas
GJ y^{-1}	→ Gigajoule per year
H_2	→ Hydrogen
H_2O	→ Water
H_2S	→ Hydrogen sulphide
H_2SO_4	→ Sulphuric acid
HCO_3^-	→ Bicarbonate
Hg	→ Mercury
$HgSO_4$	→ Mercury (II) sulphate
HRT	→ Hydraulic retention time
IDD	→ Industrial dormant digester
IDD-AD2	→ Industrial dormant digester AD2
Inoculum (0 L)	→ Digester loaded with no (zero) inoculum
$K_2Cr_2O_7$	→ Potassium dichromate (VI)
Kg m^{-3}	→ Kilogram per cubic metre
Kg mol^{-1}	→ Kilogram per mole
Kwh m^{-3}	→ Kilowatts hour per cubic meter
KWh	→ Kilowatt hour
L	→ Litre
LSM	→ Long standing manure
m^3	→ Cubic meter
m^3 d^{-1}	→ Cubic meter per day
m^3 h^{-1}	→ Cubic meter per hour
m^3 t^{-1} VS	→ Cubic meter per tonne volatile solids
mg	→ Milligram
MJ Kg^{-1}	→ Mega joules per kilogram
mL	→ Millilitre
mm	→ Millimetre
Mn	→ Manganese
mV	→ Millivolts
MW	→ Mega watts
MWWS	→ Municipality waste water sludge

MWWTS	→ Municipality wastewater treatment sludge
N_2O	→ Nitrous oxide
Na	→ Sodium
NaCl	→ Sodium chloride
NaCN	→Sodium cyanide
NH_4	→ Ammonia
NH_4^+	→ Ammonium
Ni	→ Nickel
O_2	→ Oxygen
ºC	→ Degree Celsius
OH	→ Hydroxide
OLR	→ Organic loading rate
ORP	→ Oxidation-reduction potential
OTC	→ Oxytetracycline
P	→ Phosphorus
p.m.	→ Post meridiem
Pb	→ Lead
pH	→ Potential of hydrogen
PO_4^{3-}	→ Phosphate
Pt	→ Platinum
RFI	→ Rumen fluid inoculum
RFI1	→ Rumen fluid inoculum from RSC1
RFI2	→ Rumen fluid inoculum from RSC2
RFI3	→ Mixture of RFI1 and RFI2 in a ratio of 1:1
RSC	→ Rumen solid content
RSC1	→ Rumen solid content from the skip on its way to disposal
RSC2	→ Rumen solid content from the skip after evisceration unit
Sb	→ Antimony
SBL	→ Sugar beet leaves
SBR	→ Sugar beet roots
SD	→ Standard deviation
SiO_2	→ Silicon dioxide
SOR	→ Sorghum
SRT	→ Solid retention time

TAC	→ Totales inorganic carbonate (German)
Ti	→ Titanium
TKN	→ Total Kjeldahl nitrogen
TS	→ Total solids
USA	→ United States of America
V	→ Vanadium
VFA	→ Volatile fatty acids
VOC	→ Volatile organic compounds
VS	→ Volatile solids
w/v	→ Weight/volume
Zn	→ Zinc
Zr	→ Zirconium

Chapter 1: Introduction

1.1 Background

In recent years the world has seen unique climatic changes and different indications of global warming, and the aggregation of toxic gases in the atmosphere has become a reality (Khanna & Zilberman, 2017; Blasing, 2016). Carbon dioxide (CO_2) is one of the most effective greenhouse gases (GHGs). This gas is accumulated by the use of fossil fuel to power machinery. However, fossil fuels are limited and are accompanied by a rapid reduction in the supply. The production of renewable energy using green methodologies and organic products is a significant way to ensure the world's future energy supplies with reduced detriment to the environment (Newman *et al.*, 2017; Mousdale, 2008). The search for these renewable energy sources has increased globally because the world has comprehended the impact of GHGs on the biosphere (Mielenz, 2009).

South Africa is the largest consumer of energy in Africa as it accounts for about 31% of the total energy consumption on this continent (Kolawole *et al.*, 2017; Sorrell *et al.*, 2010). This country contributes an approximate 1.4% of global CO_2 emissions and was responsible for 40% of Africa's CO_2 emissions in 2011 (Lin & Atsagli, 2017; Brown & Zhou, 2013). South Africa's electricity production is fossil fuel-based, as it is produced from coal. This results in intensive GHG emissions, and thus the electricity sector is a major emission contributor. Ninety per cent of the country's electricity is manufactured from coal, which has been the main provider of South Africa's primary energy since 2004. Currently, 23% of the world's primary energy comes from coal (Ahn *et al.*, 2017; Pegels, 2010; Franco & Diaz, 2009; Jeffrey, 2005). Even though South Africa supports the Kyoto treaty and other green energy initiatives, abandoning coal is not a decision that this country will make in the near future. It is therefore imperative that proposed alternatives for coal-sourced electricity production are well understood and supported by major consumers, such as the mining and agriculture sectors. It is also proposed that these alternative energy sources be phased in gradually.

In addition to high CO_2 emissions, South Africa also has waste management problems, particularly because waste is collected and disposed primarily at landfill

sites where methane (CH_4) gas is generated naturally by decomposition processes (Friedrich & Trois, 2016; Nahman *et al.*, 2012). Coal as a source of energy for electricity production can be replaced by CH_4 gas, which may be recovered from landfill sites where anaerobic fermentation occurs. Current literature indicate a landfill CH_4 emissions in South Africa to be between 0.2-0.4 Tg $year^{-1}$ (Bogner & Lee, 2005). Anaerobic fermentation of organic materials is a process that is applied with the aim of treating biodegradable wastes to break down and minimize landfill and to produce CH_4 which, in turn, can be used to produce heat or electricity (Whiting & Azapagic, 2014).

Many European countries are now producing biogas from energy crops which, when co-digested with organic waste material, results in an increase in the production rate and volume of biogas (Macias-Corral *et al.*, 2017). For anaerobic digestion of energy crops to be economical, such crops will have to entail the following: reduced energy usage for planting; reduced quantities of fertilizer and pesticides; limited negative environmental impact; and less energy in the pre-treatment of these crops. Moreover, the conversion of energy crops into biogas through anaerobic digestion has to result in a high specific CH_4 yield (Gourdet *et al.*, 2017; Mattioli *et al.*, 2017). Energy crops can also be used for soil amendments, such as the rehabilitation of contaminated soil prior to its use as feedstock for biogas production.

The mining sector has been key to the economic growth and development of South Africa, and this sector has had access to profuse mineral reserves of gold, platinum (Pt), titanium (Ti), chromium (Cr), manganese (Mn), vanadium (V), zirconium (Zr), phosphates (PO_4^{3-}), antimony (Sb), and coal. Between 1970 and 2000, the country produced more than 20% of the world's gold output, and it is acknowledged that the industrial revolution and economic development in this country were predominantly stimulated by the mining and agriculture sectors (Holliger *et al.*, 2016; Wickre *et al.*, 2004; Müezzinoğlu, 2003; Stilwell *et al.*, 2000). However, these sectors have also contributed substantially to the pollution of soil and water resources. For example, during gold mining processes electricity that is generated by fossil fuel is used abundantly, and a number of contaminants such as mercury (Hg), sodium cyanide (NaCN), and heavy metals are associated with recovering gold from its ore (Edinger *et al.*, 2008). Soil contamination due to mining activities is of great environmental concern, particularly because the main contaminants of soil worldwide are heavy metals such as cyanide, zinc, and lead (Lee *et al.*, 2014; Vamerali *et al.*, 2010).

Energy crops are utilised to extract contaminants from soil, water or other mediums via phytoextraction, which is an active, direct type of phytoremediation that is used to extract metals from soil (Vamerali *et al.*, 2010). Different energy crops such as maize and sorghum have thus been used to extract contaminants from soil (Meers *et al.*, 2010; Marchiol *et al.*, 2007; Dietz & Schnoor, 2001; Lombi *et al.*, 2001). Energy crops such as sugar beet and sorghum have also been demonstrated in the literature to have the capacity as feedstock for biogas production and they have been shown to have the potential to produce biogas (Ostovareh *et al.*, 2015; Brooks *et al.*, 2008; Demirel & Scherer, 2008; Parawira *et al.*, 2004). The cultivation of biomass on contaminated land projected for energy production purposes has been suggested as a potential solution for the production of valuable biomass while remediating the soil (Ruttens *et al.*, 2011). Biomass such as energy crops adds value to contaminated land because energy crops such as sugar beet have high land use efficiency and, if used as a substrate for bio-methanation, high CH_4 yields per hectare can be achieved. Moreover, the co-digestion of these energy crops with waste is economical (Jacobs *et al.*, 2017; Moeller *et al.*, 2015).

The degradation of organic materials is a complex process that relies on many different consortia of microorganisms. In a biological system such as an anaerobic digester, there are stages that involve various interactions of these microorganisms. The products that are of interest for end users in this process are the biogas and the fertilizers that are produced. The degradation of organic material transpires via the processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. If one of these processes is affected adversely, it can lead to process failure (Jain *et al.*, 2015). However, the start-up process and specifically the operation of large-scale anaerobic digesters can be fraught with complications. The success or failure of these systems depends on various factors such as the type of inoculum used (including its suitability, availability and cost), proper process monitoring, appropriate anaerobic conditions, suitable feedstock for continuous operation, homogenization methods, organic loading rate, hydraulic retention time, and biogas production rate (Cavinato *et al.*, 2017; Xu *et al.*, 2013; Lerm *et al.*, 2012; Sreekrishnan *et al.*, 2004; Weiland, 2003; Sonakya *et al.*, 2001; Hamdi, 1991; Koster & Lettinga, 1988).

1.2 Aim and Objectives

The aim of the study was to conduct a case study to document and critique the start-up process of an industrial scale anaerobic digester and to evaluate the efficacy of biogas production from waste that is co-digested with energy crops cultivated on mine-impacted land. The following objectives were devised to achieve this aim:

- Study the proper start-up foundation for an industrial anaerobic digestion process.
- Understand the factors that lead to industrial anaerobic digestion failure.
- Determine the chemical composition of different inoculums for anaerobic digesters.
- Determine the most suitable seeding material and methods to commission the start-up of a digester.
- Determine the influence of temperature on biogas production.
- Determine the chemical composition of energy crops cultivated in mine-impacted soil for use as feedstock.
- Determine the effect of rumen fluid inoculum (RFI) on the performances of an anaerobic digester aimed at biogas production from energy crops.
- Evaluate the aptness of the co-digestion of energy crops with rumen solid contents (RSC) for biogas production.
- Determine the most industrial relevant CH_4 concentration and yield from the different feedstock.

1.3 Chapter Layout

Chapter 2: Literature review. This chapter presents a discussion on current literature that was applicable to this study.

Chapter 3: Study structure, materials and methods. Anaerobic digester designs, timelines and analytical methods used for monitoring the anaerobic digestion process are described.

Chapter 4: Commissioning strategies for anaerobic energy crop digesters established at a gold mine: A case study. This chapter describes and analyses the start-up process of industrial anaerobic digesters fashioned from

recommissioned and refurbished gold processing equipment. Specific emphasis is placed on suitable equipment, organic loading rates, and timeous and accurate monitoring.

Chapter 5: Investigating methods for revitalising an industrial dormant digester (IDD). This chapter focuses on the different methods that were applied to revive an IDD. The parameters that are discussed include increasing the retention time, limiting agitation, changing operational temperatures, and the addition of a variety of seeding materials at different concentrations.

Chapter 6: Biogas production from rumen solid contents (RSC) and energy crops intended to remediate mine-impacted land. The use of sorghum, RSC, and sugar beet roots and leaves that had been cultivated on contaminated soil was investigated for the production of biogas by means of mono- and co-digestion trials.

Chapter 7: Concluding remarks. The main conclusions that were drawn based on the study findings and recommendations that the industry may employ are presented.

Chapter 2: Literature Review

2.1 Energy Security

Every country in the world needs energy sources to fuel economic growth because energy supply has a vital impact on social development. Energy derived from different energy sources is used for operating power plants, aeroplanes, automobiles and other machinery. There are many different ways of producing energy, but the most common ways of energy production pose environmental challenges, and this makes access to cheap energy sources (such as fossil fuels) essential for the functioning of the economy (Boleman *et al.*, 2011; Kerr, 2005). However, alternative methods of energy conservation are crucial, as all countries need a secure energy stream. When these streams are required to continue distributing energy, imported energy is reduced to allow independence of foreign energy sources. The second option is to increase the internal supply of energy, as this will give the country vast options, such as using alternative energy sources that address the need for renewable energy. Even though such measures are taken, the security of the fossil energy supply is usually threatened by political indifference, which, in turn, threatens the government structure of a country (Gupta & Verma, 2015; Moran & Russell, 2008; Bielecki, 2002).

To avoid the disintegration of governmental structures in South Africa, the first energy-efficient strategy for this country was published in 2005 with the intention of preparing the country for the development and implementation of efficient energy provision practices. The strategy aimed at lowering the cost of oil supplies or to achieve a null cost interaction. The strategy also focused on meeting the country's energy security supply goals by reducing the loads of energy that are imported from foreign soil. In this context, it must be acknowledged that it is inevitable that South Africa has to depend on other countries for crude oil because it lacks natural oil reserves. It is also understandable and practical that the country is conscious of the need for future supplies of energy (Winkler, 2005), whether renewable or non-renewable, as this will impact the performance of the country's economy.

2.2 Renewable and Non-Renewable Energy

Fossil fuels are regarded as non-renewable. These sources of energy were formed over millions of years from pressurised organic materials that were deprived of air at high temperature. Fossil fuels are used as a major fuel source for both transportation and the generation of electricity. However, the depletion rate of fossil sources compared with their rate of usage is not economical, and therefore fossil fuels are no longer suitable for long-term energy security strategies. Coal, crude oil, natural gases and nuclear power are some of the non-renewable energy sources that countries are dependent on (Shafiei & Salim, 2014; Curley, 2011; Pegels, 2010; Morris, 2006; Graham, 2005). Fossil fuels are limited resources, and the persistent use of these energy sources is fraught with complications such as emissions of GHGs. Therefore, to reduce the adverse environmental impacts associated with fossil fuels, scientists have devised ways to convert wind, solar, and geothermal biomass energy to generate power and heat. The energy generated by means of these resources is renewable (Salim *et al.*, 2014; Mohaibes & Heinonen-Tanski, 2004).

2.3 Energy Provision in South Africa

South Africa has one of the most energy-intensive economies in Africa, with the industrial sector using more than 37% of the total energy consumption in this country (Beidari *et al.*, 2017a). In this mineral rich country, extraction and processing of minerals are energy intensive processes. However, such processes have been regarded as a necessity because the contribution of the mining sector to the country's Gross Domestic Product (GDP) is substantial and thus it is argued that the portion of energy used by the industrial sector is proportional to the country’s industrial development. More recently, the industrial sector has shown a lesser use of energy compared to previous years, unlike the residential sector, which contributed 20% in 2000 to energy consumption. From 2007 to 2008, it consumed 25% of the energy resources, showing a high inclination in use, but currently the energy use of this sector has declined to only 23% (Beidari *et al.*, 2017b; Jørgensen, 2009).

After Egypt, South Africa is the second largest consumer of petroleum in Africa, and most of this country's demand for liquid fuels is met through crude oil imports, and thus the domestic prices of fuels are influenced by global crude oil prices (Khalid *et al.*, 2011; Ostrem *et al.*, 2004). In South Africa, coal is used in both the generation of electricity and the production of synthetic fuel, but the country has plans to move away from coal because burning coal has negatively affected the environment. For example, recent figures have indicated that the electricity sector contributes 66% to South Africa's CO_2 emissions, which has significant implications for the pollution of the environment. Against this background, it is noteworthy that the production of biogas through fermentation processes on a large scale will contribute to climate change moderation and will be a means of displacing fossil fuel based energy (Beidari *et al.*, 2017a; Shahbaz *et al.*, 2013; Jeffrey, 2005; Snyman & Botha, 1993).

2.4 Biogas

The result of the anaerobic fermentation of organic matter is a colourless biogas that may smell like rotten eggs. This gas is a mixture of methane (CH_4), CO_2, and other traces of gases such as hydrogen sulphide (H_2S), ammonia (NH_4), hydrogen (H_2), and nitrogen (N_2) (Makareviciene *et al.*, 2013; Rasi *et al.*, 2007). Biogas is produced via a natural process in landfills, by ruminant animals, in natural wetlands, and coal bed leakages, or through non-natural processes such as rice production, biomass controlled fermentation, and wastewater treatment processes (Bogner & Lee, 2005). The principal constituent of a biogas is CH_4; a high CH_4 concentration makes the biogas more attractive.

2.4.1 Characteristics of biogas

The feed material and techniques used in biogas production processes have a major impact on the composition of the biogas that is produced. The composition of a biogas ranges from 55-70% of CH_4, 30-45% of CO_2, 0-3% of N_2, 0-1% of H_2, and 0-1% of H_2S (Table 1) (Herout *et al.*, 2011; Rasi *et al.*, 2007; Weiland & Hassan, 2001). A biogas with 65% CH_4 concentration has a calorific value of 6 KWh, an ignition temperature of 700ºC, and a density of 1.2 kg m^{-3} (Salunkhe *et al.*, 2012; Rasi *et al.*, 2007). The lowest heating value (i.e., assuming that steam is produced) of CH_4 is 50 MJ kg^{-1}, which is higher than that of methanol, ethanol and petrol. Thus,

when biogas is produced for industrial application, a high CH_4 concentration and low impurities are prerequisites.

Table 1: General characteristics of biogas with slight modification from Rasi *et al* (2007)

Characteristics	Measurements
The composition of biogas	55-70% CH_4
	30-45% CO_2
	0-3% nitrogen
	0-1% H_2
	0-1% H_2S
Energy contents	6.0-6.5 KWh m^3
Explosion limits	6-12% biogas in air
Ignition temperature	650-750ºC
Critical pressure	75-89 bar
Critical temperature	-82.5ºC
Smell	Bad eggs
Molar mass	16.04 Kg mol^{-1}
Normal density	1.2 Kg m^{-3}

High protein feedstock tends to yield biogas that is constituted by high H_2S and other sulphide compounds (Wilber & Murray, 1990). Other impurities such as siloxanes, NH_4 and vast volatile organic compounds (VOC) have been reported to have compounded the biogas produced. A quartz-like structure (siloxanes and oxygen) results in silicon dioxide [SiO_2] when present in the biogas, and it remains on the surface of the operating machines and causes a decline in the flow levels of constituents. The results when this happens can be detrimental to the biogas plant as well as gas production rates (Rasi *et al.*, 2011).

2.4.2 Biogas application

What makes biogas an invaluable supplementary fuel is that it is a clean and renewable form of energy that is a good substitute for conventional sources of energy such as fossil fuels. Not only do fossil-based fuels have a toxic impact on the environment, but they are also depleting at a fast rate (Herz *et al.*, 2017; Hosseini & Wahid, 2014). From an environmental perspective, biogas has more advantages than fossil fuels and it is a more secure energy source. Just like fossil fuel energy, biogas is used for cooking, heating, cooling, and lighting – thus the entire spectrum

that is reliant on electricity. Moreover, the feedstock for the production of biogas is abundant because this gas can be produced from any organic material that is biodegradable and it can be obtained within a reasonable period of time from substrates such as organic waste and energy crops (Bond & Templeton, 2011; Weiland, 2010; Balsam & Ryan, 2006).

Biogas producers have different intentions for the end use of this gas, but most users will require that the biogas they use be produced in its purified form. The aim of purification is to remove any possible contaminants from the gas. CO_2, H_2S and water are some of the compounds regarded as impurities. The desired concentration of CH_4 in biogas will depend on the application that the gas is intended for (Weiland, 2010). Once the required quality of the gas has been reached, vast end-use applications are possible; thus if the purity required is high, then the energy use efficiency will also be high (Deng *et al.*, 2014). Many countries in the European Union (EU) inject biogas into the natural gas grid, which gives biogas many usage possibilities. Biogas is also used to replace petrol in the transport sector in countries such as Sweden, France, Switzerland and Denmark (Herout *et al.*, 2011; Raven & Gregersen, 2007). Most industrial applications of biogas are for steam production, and burning biogas in a boiler has become an established and reliable technology (Persson *et al.*, 2006). Moreover, micro-gas turbines such as combined heat and power (CHP) fuel engines are operated using biogas as a fuel, and these systems have become popular as they are characterized by low emissions of toxins (Matthews *et al.*, 2000). Another application of biogas is the production of other chemicals. This occurs because CH_4 as a feedstock necessitates the production of H_2, and the H_2 is used to power up fuel cells. This is an efficient method but it requires a pure CH_4 gas (Kumar *et al.*, 2013). Biogas is also used in most countries for the production of electricity to replace combustible feedstock such as coal. It is also used to replace poly-fuel and coal-generated electrical heating systems in mines.

In South Africa, biogas is predominantly used to fire boilers for industrial or commercial heating, but this is accompanied by high capital cost compared to coal-fired boilers (Bogner & Lee, 2005). In this country, biogas may be used to replace coal for the generation of electricity if the cost of using biogas is cheaper than that of coal. The profit of biogas projects will depend on the supplementary usage of the effluent, because, after the anaerobic fermentation process, the product that is of

economic attraction is a biogas with a ≥50% CH_4 concentration and a valuable digestate residue (Sewchurran & Davidson, 2017).

2.4.3 Biogas effluent

Biogas effluents, also known as digestate residues or slurry, have significant end-use applications in the agriculture sector, for example for the maintenance and improvement of soil fertility because the digestate is used as an organic fertilizer. Using these organic residues has many benefits for the environment because slurry is rich in nutrients (Pivato *et al.*, 2016; Møller *et al.*, 2009). Moreover, the chemical properties of anaerobic digestate such as ammonium (NH_4^+), total nitrogen ratios, total organic carbon contents, reduced biological oxygen demands (BOD), raised pH values, smaller carbon to nitrogen ratios, and reduced viscosities are features that ensure that digestate residues are valuable for further usage as either soil amendment agents or as fertilizers (Nkoa, 2014). The use of digestate residue to fertilise the soil reduces organic waste dumping in landfill sites and helps maintain soil nutrient levels. A further benefit is that microorganisms and pathogens that are harmful to crops are eliminated or inactivated by anaerobic fermentation. If the digestate is used around the human interface, it can be handled, stored, and applied with caution. Environmental and health risks can be identified and proper management protocols can be applied (Laitinen *et al.*, 2017; Bond & Templeton, 2011; Tambone *et al.*, 2010; Weiland, 2010; Holm-Nielsen *et al.*, 2009).

Digestate may meet the fertilizer needs of a farm and may be instrumental in optimising performance and ensure the economic sustainability of the agricultural business in question (Laitinen *et al.*, 2017; Nardin *et al.*, 2014). When energy crops (i.e., crops that are used for land remediation) are used as feedstock, the anaerobic digestate as a fertilizer is restricted by the presence of toxic heavy metals. The process of biogas and fertilizer production leads to direct environmental benefits such as replacing fossil fuels, as well as indirect environmental benefits such as enhanced land use and easier handling of organic waste (Börjesson & Berglund, 2007).

2.4.4 The environmental impact of biogas

Atmospheric changes due to an increase in the concentration of trace gases in the atmosphere have resulted in urban photochemical smog, acid deposition, as well as stratospheric and ozone depletion (Seinfeld & Pandis, 2016). On the other hand, the production of biogas from biomass has a positive impact on the environment. Various municipalities around the world produce vast quantities of organic waste material as total municipality waste. However, uncontrolled decomposition of organic waste results in the emission of CH_4 gas into the atmosphere, and CH_4 is more effective in trapping heat than CO_2. It is also a known fact that the concentration of GHGs, such as CO_2, CH_4 and nitrous oxide (N_2O), has increased in the last century (Ali *et al.*, 2016; Franchetti, 2013; Abdeshahian *et al.*, 2010) and that the concentration of GHGs in the atmosphere is rising at a rapid rate, leading to high climate changes. These changes have become a global dilemma because GHGs have accumulated globally over time.

Emissions by one country affect others, and international cooperation has been launched to mitigate GHG emissions on a global scale. It is evident that the total anthropogenic GHG emission has increased over the years, and annual GHG emissions grew by 2.2% from 2000-2010 compared to 1.3% from 1970-2000. The total anthropogenic GHG emissions from 2000-2010 were the highest in human history (Change, 2015). A study conducted in Zambia estimated that biogas production could result in reducing the direct use of biomass fuel by 36%, and that biogas production could result in 9.55 million tonnes of CO_2 emissions. Thus, 17.24 million tonnes of CO_2 emission could be avoided. It was further estimated that 10.38 million tonnes of nitrogen that are conserved could replace 13.23 million tonnes of biomass per annum (Shane & Gheewala, 2017).

Urbanization has increased and, because of population growth, it is expected to escalate over the next few years. This phenomenon has resulted in an increase in municipal waste, mainly food waste. Food waste is now discarded on a daily basis, and it is usually landfilled, incinerated, or composted with negative environmental consequences. Urban development is generally industrial based and is associated with a high usage of water. High quantities of wastewater and industrial waste are thus produced through industrial productivity. Wastewater is produced due to poor disposal of waste. Ocean dumping has become one of these practices and has been

banned in some countries where more appropriate treatment and disposal processes of waste have been introduced. Wastewater treatment and proper disposal of industrial and domestic waste are essential for environmental health and ecological sustainability. However, waste resulting from manufacturing processes is vast and varied, as it may include agricultural waste, food waste, and chemical waste (Tonon *et al.*, 2015; Brown & Li, 2013; Güngör-Demirci & Demirer, 2004; Clarke & Baldwin, 2002).

Fishery and agricultural waste is produced on a large scale globally, and such waste is a potential threat to the environment if uncontrolled disposal practices are applied. Agricultural waste such as fruit and vegetable waste from wholesale markets is known for its fragility, which makes landfill disposal of this type of waste a challenge (Pavi *et al.*, 2017; Tani *et al.*, 2016; Di Maria *et al.*, 2015; Scano *et al.*, 2014; Brown & Li, 2013; Kafle & Kim, 2013). Moreover, fish solid waste is reportedly difficult to dispose of appropriately, and the number of raw products converted into waste can be as high as 50% by weight (Ward & Løes, 2011).

However, organic wastes generated by the agricultural, industrial and fishery industries are good feedstock for biogas production because of their chemical composition. The disposal of these waste forms has become a problem worldwide but, fortunately, the use of these wastes as feedstock for biogas production is a growing field, particularly because this can help to alleviate environmental ruin caused by improper disposal of biodegradable waste (Pivato *et al.*, 2016; Jeihanipour *et al.*, 2013).

2.5 Biomass as Feedstock for Biogas Production

Biomass is composed of all the organic materials that are derived from plant materials and includes algae and animal waste (Demirbaş, 2001; Demirbas, 2000). Photosynthesis is a process that uses light, water, CO_2, and nutrients to convert light energy into chemical energy that is stored in biomass as carbohydrates. Through photosynthesis, a portion of radiation energy from the sun is used to fix more than 200 billion tonnes of carbon and CO_2 emitted to the environment, and CO_2 is recycled to be utilised again by plants to generate energy. Aquatic and terrestrial biomass has an energy content of more than 3000 billion GJ y^{-1}. Biomass has to be

grown and used in a sustainable manner for it to be carbon neutral (Govindjee, 2012; Reddy, 1994). Biomass can then be used to substitute fossil fuels for energy production, given that GHG emissions are reduced and also because biomass feedstock is renewable and abundant (Shanmugam *et al.*, 2017).

The energy stored in biomass is called biomass energy. Biomass can have many different applications when it has been converted into energy using different thermochemical and biotechnological processes (Fredriksson *et al.*, 2006). For example, biomass can be used directly as firewood that can result in a high net energy production. However, by directly burning biomass, different pollutants are produced that are harmful to the environment. When burnt biomass releases heat and CO_2 that was absorbed during photosynthesis, it means that using biomass energy does not add CO_2 to the environment. Biomass can also be used indirectly by alcohol fermentation of sugar crops or anaerobic fermentation to form biogas. Using biomass for biogas production is both energy efficient and beneficial to the environment, and this method of energy production is more promising compared to the direct burning of biomass (Gourdet *et al.*, 2017; Mckendry, 2002; Demirbaş, 2001). During ethanol production, the feedstock should always contain carbohydrates or simple sugars, but in biogas production, any form of biomass can be utilised. In an anaerobic process, biomass contents containing carbohydrates, fats and proteins are converted into simple derivatives that are converted into biogas. CH_4 production from biomass is more economical, has environmental benefits, and is a sustainable way of energy production compared to alcoholic fermentation (De Pérez, 2012; Baker & Keisler, 2011; Deublein & Steinhauser, 2011).

Biomass as feedstock for renewable energy production is a sensible choice because its feedstock is available in various forms such as organic waste, animal manure, energy crops, and crop residues (Hoogwijk *et al.*, 2003). For biomass to pass as a good substrate for biogas production, it should contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as the main components. The quality and quantity of the gas produced will depend on various factors. The following should be considered when choosing a substrate:

- Due to poor bioavailability, fats will require longer retention time compared to carbohydrates and proteins.

- When using potentially contaminated biomass such as municipal organic waste, pasteurization at high temperatures, or sterilization, is required (Kothari *et al.*, 2010; Braun *et al.*, 2008; Weiland, 2000).

Anaerobic digestion has been used for the treatment of animal manure and sewage sludge from aerobic wastewater treatment plants, but biomass such as leaves, food waste and municipal waste can be added and co-digested with manure to achieve a higher biogas yield. Co-digestion of waste with energy crops also improves gas production (Deublein & Steinhauser, 2011; Angelidaki & Ellegaard, 2003; Braun & Wellinger, 2003).

2.5.1 Energy crops

Energy crops are crops that are grown exclusively for energy production. Such crops have to be able to produce high gross energy potential and the net energy yield per hectare must be sufficient. The following are considered when selecting a crop of interest:

- CH_4 yield will be affected by the chemical composition of the crop; and
- The machinery for harvesting and methods of storing can affect the selection of a crop for CH_4 production (Amaducci *et al.*, 2016; Weiland, 2010).

Energy crops such as maize, sorghum, sugarcane, sugar beet, grass and different oil-bearing plants such as palms are convenient for bioenergy production. The profitability of energy crops for biogas is dependent on the price of agricultural raw materials and water consumption, and the rate of water consumption by a crop will affect the biofuel production cost (Jacobs *et al.*, 2016; Nuchdang *et al.*, 2015; Mahmood *et al.*, 2013; Zhang *et al.*, 2013).

Maize is a feedstock of choice for bioenergy production in countries such as the United States of America (USA) and Germany. Maize is favoured because of its high yield per hectare, its low production costs, and uncomplicated cultivation management. When sorghum is compared with maize, it has a high CH_4 yield (Jacobs *et al.*, 2016). Sorghum is less affected by drought because of its deep and dense roots, and the water use efficiency of this crop is higher compared to other crops (Amaducci *et al.*, 2016). However, sorghum has limitations such as sensitivity

to cold during its early stages of development, and this limitation has led to maize being favoured as a feedstock for bioenergy production in Europe and North America (Mahmood *et al.*, 2013; Schittenhelm, 2010).

Energy can also be extracted from crops such as sugarcane. This is done indirectly from waste produced after sugar production or directly from sugarcane cultivated as energy crops for bioethanol production. The bagasse and filter cake generated after the production of sugar or bioethanol can further be used to produce biogas to fully recover energy (Leite *et al.*, 2015). Sugar beet has a CH_4 potential yield that is close to that of silage maize. Sugar beet can also be used as a rotation crop with different other energy crops, and these crops are easy to digest because their dry matter consists mostly of sugar (Jacobs *et al.*, 2017; Moeller *et al.*, 2015). In Europe, grass has been cultivated for the production of CH_4 and grass as a co-substrate has shown higher biogas production rates than municipal solid waste. Palm oil, which is extracted for various end uses, is regarded as the most productive and economical product in the world. A large volume of effluent is produced during oil extraction from this by-product which is used as feedstock for biogas production via the four degradation steps of the anaerobic digestion process (Ohimain & Izah, 2017; Nizami *et al.*, 2009).

2.6 Anaerobic Fermentation

The biological treatment of organic materials can occur in the absence (anaerobic) or in the presence (aerobic) of oxygen. Aerobic fermentation is used in the treatment of industrial and municipal organic waste. During this process, the waste corresponding to $(CH_2O)n$ and the added oxygen corresponding to nO_2 degrades by aerobic respiration to end products nCO_2 and nH_2O (Mohaibes & Heinonen-Tanski, 2004). Anaerobic fermentation is the process of decomposition of complex organic matter such as a mixture of carbohydrates, lipids, and proteins by a microbial consortium in an oxygen-free environment. This process also occurs in nature in different environments such as watercourses, sediments, waterlogged soils and the mammalian gut (Boleman *et al.*, 2011). The growth rate of anaerobic microorganisms is considered to be lower than that of aerobic microorganisms. Typical reactions during anaerobic digestion are as follows (Jørgensen, 2009; Ostrem *et al.*, 2004):

- $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
  (Organic compound) → (Ethanol) + (Carbon dioxide)

- $2C_2H_5OH + CO_2 \rightarrow CH_4 + 2CH_3COOH$
  (Ethanol) + (Carbon dioxide) → (Methane) + (Acetic acid)

- $CH_3COOH \rightarrow CH_4 + CO_2$
  (Acetic acid) → (Methane) + (Carbon dioxide)

- $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
  (Carbon dioxide) + (Hydrogen) → (Methane) + (Water)

The anaerobic fermentation process occurs in different temperature ranges and differentiation is made between the psychrophilic (<25ºC), the mesophilic (≥25ºC) and the thermophilic temperature (>40ºC). The group of microorganisms involved in these processes are sensitive to temperature fluctuations (Kossman *et al.*, 1996; Noike *et al.*, 1985). The degradation of biological material under anaerobic fermentation occurs in four stages (Figure 1):

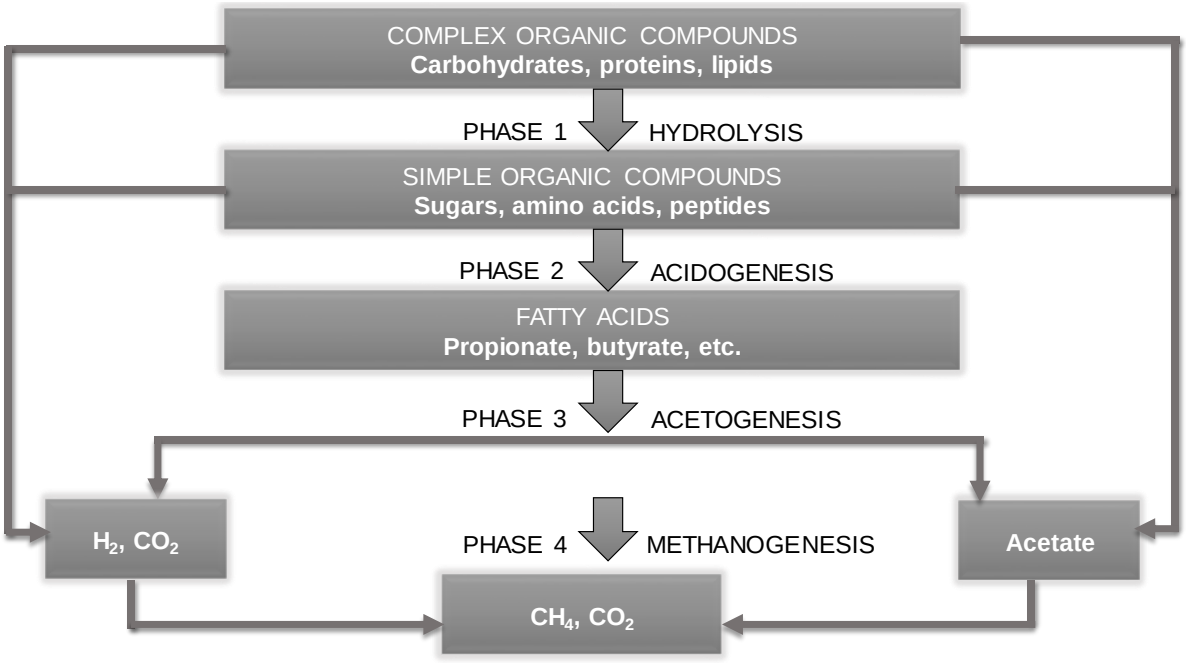

Figure 1: Diagram of the degradation steps in anaerobic digestion, adapted and modified from Speece (1996).

Complex organic materials are degraded to yield methane gas through four different phases, as is illustrated in Figure 1. A specific characteristic of CH_4 formation via the degradation of organic matter is its phasing, during which each of the steps accounts for a degradation of a different type of compound (Mata-Alvarez *et al.*, 2000; Speece, 1996; Noike *et al.*, 1985). The first phase of anaerobic digestion is hydrolysis, in which a specific group of enzymes is responsible for breaking down protein, carbohydrate, and fat polymers to smaller molecules (monomers) that will be utilised directly by anaerobic fermentation microorganisms. In the second phase (acidogenesis), the monomers caused by hydrolysis are used to form organic acids and alcohol. In the third step (acetogenesis), the microorganisms will use the organic acids and alcohol to form CO_2, H_2, and volatile fatty acids (VFA). The last stage is methanogenesis, which involves the production of CH_4 from CO_2, H_2 and VFA (Figure 1) (Li *et al.*, 2015).

2.6.1 Hydrolysis

During phase I (Figure 1), polymerised materials such as insoluble organic compounds are decomposed into soluble monomers and dimers. The formation of monosaccharides such as amino acids and fatty acids occurs during this phase. During hydrolysis, bacteria access extracellular enzymes from the group of hydrolases such as amylases, proteases, and lipases that are produced by appropriate strains of hydrolytic bacteria (Weiland, 2010). Hydrolysis of rigid but decomposable polymers such as cellulose, hemicellulose, and pectin is considered a stage that limits the rate of feedstock digestion. The rate of hydrolysis depends on parameters such as the size of particles, pH, enzyme production, diffusion, and adsorption of enzymes on the particles of materials subjected to the digestion process. All feedstock must undergo liquefaction by extracellular enzymes before being taken up by acidogenic bacteria (Rizwan *et al.*, 2015).

2.6.2 Acidogenesis

During this stage, anaerobic oxidizers utilise the soluble organic molecules from hydrolysis; these microorganisms are both obligate and facultative anaerobes. In a stable anaerobic digester, the main degradation pathway results in acetate, CO_2, and H_2. Acidogenesis may be a 2-directional process due to the effects of various

populations of microorganisms, implying that the process may be divided into hydrogenation and dehydrogenation. The basic pathway of transformation passes through acetate, CO_2 and H_2 (Figure 1), and other acidogenesis products play a trivial role. If the accumulation of electrons by compounds such as lactate, ethanol, propionate, butyrate, and higher VFA occurs, this is due to a microbial response to an increase in H_2 concentration in the solution. Among other products of acidogenesis, NH_4 and H_2S are responsible for the intense unpleasant smell during this phase of the process as acidifying microbes convert water-soluble chemical substances into fatty acids (Kumar *et al.*, 2013; Weiland, 2010; Jørgensen, 2009; Gerardi, 2003).

2.6.3 Acetogenesis

Fatty acids, aromatic fatty acids, and alcohols are intermediates formed during acidogenesis. These intermediates are not suitable to be utilized by the methanogenesis archaea. Proton reducing microbes and H_2 utilizers will oxidize the intermediates into acetate and H_2, which will be used by methanogenic archaea to form CH_4. Because of acetogenesis, H_2 is released, which exhibits toxic effects on the microorganisms that carry out this process (Ennouri *et al.*, 2016; Guo *et al.*, 2015; Kumar *et al.*, 2013).

2.6.4 Methanogenesis

Methanogenesis involves CH_4 production by methanogenic archaea. During this process, the CH_4 gas is produced from substrates (acetic acid, H_2, CO_2, formate, CH_3OH, methylamine, and dimethyl sulphide) which were produced during the acetogenesis phase. Despite the fact that only a few microbes are able to produce CH_4 from acetic acid, the majority of CH_4 arising in the digestion process results from acetic acid conversions by heterotrophic CH_4 bacteria. Only 30% of CH_4 produced in this process comes from CO_2 reduction caused by autotrophic CH_4 bacteria. Anaerobic degradation of organic matter to produce CH_4 relies on the complex interaction of different microorganisms, and the quantity and quality of biogas produced is affected by a number of different parameters (Kumar *et al.*, 2013; Weiland, 2010; Jørgensen, 2009; Gerardi, 2003).

2.7 Important Parameters Affecting Anaerobic Digestion and Gas Production

2.7.1 pH, alkalinity, and volatile fatty acid (VFA)

The pH value is a function of VFA concentration, bicarbonate (HCO_3^-) concentration, and alkalinity of the system as well as the fraction of CO_2 in the biogas. To keep the pH of a system from continuously changing, the relationship between the HCO_3^- and VFA concentrations requires adjustment on a regular basis (Liu *et al.*, 2008). In a bio-digester, pH fluctuations are common in different stages of the process, and within each phase of anaerobic digestion different groups of microorganism involved in each stage require different pH conditions for optimum growth. Anaerobic bacteria in the first phase (hydrolysis) and the second phase (acidogenesis) (Figure 2) of anaerobic fermentation of organic material prefer a more acidic environment. The acidogens are reported to perform best between pH 5.50 to pH 6.50 (Khalid *et al.*, 2011). Methanogens use organic acids as sustenance to produce CH_4, but they are intolerant to an acidic environment. It has been found that pH is a usual first pointer of process failure in different biological systems (Martin-Ryals, 2012; Weiland, 2010). The optimal value of pH for a digester differs depending on how the digestion process is carried out and on the feedstock of choice. The optimal range of pH for obtaining utmost biogas yields in an anaerobic digestion ranges from 6.50 to 7.50 (Arsova, 2010; Liu *et al.*, 2008).

When the process is balanced, the acidity in the reactor will be within range, and the buffer capacity in the reactor will be sufficient, thus alteration of pH will not be easy. Bio-digesters treating a slurry or high protein feedstock often have higher pH values due to a higher NH_4^+ content. NH_4 released in the sludge reacts with CO_2 and water, resulting in the production of ammonium carbonate ($(NH_4)_2CO_3$) that provides alkalinity to the system. Alkalinity is defined as an HCO_3^- to CO_2 balance that provides resistance to rapid change in acid or alkali concentrations. The optimum range of alkalinity has been reported to vary with bio-digesters (Montalvo *et al.*, 2017; Gerardi, 2003). Alkalinity is divided into partial and intermediate alkalinity, and a 2-point titration measurement that will determine the ratio of the two alkalinities is called the alkalinity ratio or a FOS/TAC ratio. A FOS/TAC (alkalinity ratio) value of below 0.30 indicates a stable process and a value above 0.80 indicates an unstable

process (Lili *et al.*, 2011). If organic materials in a bio-digester are digested quicker than the time, it takes methanogens to fully use the organic acids and CO_2, the dissolved CO_2 concentration in a gas will increase. More CO_2 will be dissolved as HCO_3^- in the liquid, resulting in a balanced pH (Patil *et al.*, 2011).

A pH of ≤6 is associated with process imbalance. A rapid formation of organic acids in a bio-digester and the existence of substances that will inhibit the methanogens' activities are two factors that will cause a decrease in alkalinity. Accumulation of VFA has the potential to decrease the pH of a bio-digester, and this happens if the first degradation phase runs too fast (Latif *et al.*, 2017; Zhang *et al.*, 2015a; Murto *et al.*, 2004). Acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, and enanthic acid are VFA to be expected in a digester. Propionic acid and butyric acid are high inhibitors of methanogens, whilst acetic acid is a predominant acid produced (Labatut & Gooch, 2014; Weiland, 2010).

2.7.2 Fermentation temperature

In many different biochemical processes, the high temperature that is attained is associated with an increased rate of reaction. In biogas digesters, different microorganisms adapt to different temperatures. Some microorganisms are more effective in psychrophilic conditions, whilst others prefer mesophilic or thermophilic conditions (Weiland, 2010). High temperature increases the reaction rate of a biochemical reaction, and the same applies to anaerobic digestion. Ideal substrates digested in a thermophilic environment degrade faster compared to ones in a mesophilic environment (Zupančič & Grilc, 2012; Madsen *et al.*, 2011; Levén *et al.*, 2007). Mesophilic digesters, unlike thermophilic digesters, do not need extra energy input for heating them. Digesters operated in psychrophilic conditions require a longer HRT, and these digesters are associated with low gas production when compared to mesophilic and thermophilic digesters.

2.7.3 Total solids (TS), volatile solids (VS) and ash

Total solids are the material residues left in the vessel after evaporation of a sample. It includes the total suspended solids presented as the total solids, while the weight loss on ignition is called volatile solids. Loss on ignition is not confined to organic matter. To characterize organic matter tests such as total organic carbon, BOD and

COD are applied (Greenberg Arnold & Clesceri Lenore, 1992). Ash concentration in a digester is used to measure the inorganics, while weight/volume (w/v) is used to classify the total solid content in a digester. TS and VS concentrations in a digester are expected to decrease as the retention time approaches, indicating that the microorganisms are utilizing the available organic content (Mudhoo, 2012). A digester with TS of ≤10% is categorized as a low solid anaerobic digester, the 10-20% range indicates medium solid, and high solid systems are ≥20%. High solid systems are also referred to as dry anaerobic digesters, where a high CH_4 yield is achievable with a 40-50% TS content (Abbassi-Guendouz *et al.*, 2012; Lehtomäki *et al.*, 2007). High solids digesters can reach a higher volumetric CH_4 production rate than low solids digesters at the same solid retention time (SRT) in mesophilic anaerobic reactors (Duan *et al.*, 2012).

2.7.4 Feeding and organic loading rate (OLR)

OLR is defined as the amount of VS or chemical oxygen demand (COD) fed to the system per unit volume per time. If a large amount of substrate is added to a digester, this affects the growth rate of the methanogens and the removal of the organic acids. The feedstock has to be fed to the digester at a rate adjusted to the growth rate of the microorganisms, balanced with the rate at which the microbes will utilize the organic acids. If the feed is to be changed, a gradual change is applied so that the microorganism can adapt to the new feed. When changing the feedstock, the energy content of the feed is an important factor and it can negatively affect the gas production rate. If feeding a digester is interrupted for hours or days, it can result in an unstable anaerobic digestion process (Martin-Ryals, 2012; Jørgensen, 2009).

2.7.5 Pre-treatment

Pre-treating feedstock by removing all non-biodegradable materials will increase the yield of an anaerobic digestion process. Small degradable particle size is the most desirable in a biogas plant, and different methods are applied to achieve a desirable and easy to degrade substrate. Pre-treatment methods such as the use of alkali chemicals, mechanical disintegration by high pressure, and thermochemical pre-treatment have been used to enhance the performance of the digesters and to explore digesters' full-scale applications (Montalvo *et al.*, 2017; Friedrich & Trois, 2016; Rodriguez *et al.*, 2015; Mata-Alvarez *et al.*, 2000).

2.7.6 Hydraulic retention time (HRT)

Methanogenic microorganisms have a long regeneration time but can double in numbers if the HRT is elongated. A minimum HRT of between 10 to 15 days is required for full growth of microbes and to avoid the washing out of CH_4 producing microbes from the reactor. Microbes found in the acidogenesis and acetogenesis phases have a short HRT of ≈2 days, and thus the risk of washing them out is minimal (Weiland, 2010; Dieter & Angelika, 2008).

2.7.7 Agitation

Agitation is done in order to connect the enzymes and microorganisms with the substrate. Both acetogenesis and methanogenesis microorganisms are required to be in close contact to achieve continuous degradation of organic materials. Thus, the mixing of a digester has to be adequate with moderate intensity and sufficient duration. Continuous stirred tank reactors (CSTR) are digesters that are most commonly used in wastewater and for farm application. The feedstock in these digesters has to be exposed to continuous agitation to avoid the formation of a surface crust (Tabatabaei *et al.*, 2011; Weiland, 2010; Kaparaju *et al.*, 2008).

2.7.8 Anaerobic digestion inhibitory substances

2.7.8.1 Hydrogen sulphide (H_2S)

H_2S is formed during the degradation of sulphur compounds. It is an inhibitory agent at a concentration as low as 50 mg L^{-1} (Märkl, 2005). H_2S has a precipitation effect on many metal ions; if present in a digester in high concentration, it will affect the bioavailability of trace elements, including iron (Fe). H_2S is also corrosive to digesters and pipelines that are made of copper (Cu), Fe and steel (Zhou *et al.*, 2016; Drosg, 2013; Kohl & Nielsen, 1997).

2.7.8.2 Heavy metal ions

Heavy metals become a problem if they are present in high concentrations (20-340 mg L^{-1}), but at low concentrations they are reported to be beneficial to the system. It is well known that they are present in high concentrations in municipal sewage waste. The heavy metals that have been identified to be of particular concern include

Cr, Fe, cobalt (Co), Cu, zinc (Zn), cadmium (Cd), and nickel (Ni) (Chen *et al.*, 2008). The relative sensitivity of acidogenesis and methanogens to heavy metals is as follows: Cu > Zn > Cr > Cd > Ni. If present in high concentrations, the heavy metals will disrupt the enzyme function and structure of microbes by binding with thiol and other groups of protein molecules (Mudhoo & Kumar, 2013; Li & Fang, 2007; Vallee & Ulmer, 1972).

2.7.8.3 Antibiotics and disinfectants

Antibiotics, pesticides and disinfectants in the feedstock have been reported to have a negative effect on the biodegradation activities and biogas formation rate in a digester. Inhibiting antibiotic concentrations have been reported in livestock manure but have been found to be of only minor significance in most cases. Antibiotics such as Oxytetracycline (OTC) are used in animal farming, but because the metabolism of OTC is poor, it is excreted in animal manure. OTC has been reported to have a negative effect on microbial activity. Antibiotics such as Aminoglycosides and chlortetracycline have an inhibitory effect on an anaerobic process (Drosg, 2013; Turker *et al.*, 2013). When thiamphenicol was added in an anaerobic digester, a significant difference in CH_4 production was found (Lallai *et al.*, 2002).

Pesticides sometimes occur in crops or harvest residues but the typical widespread use of pesticides is apparently not significant in the anaerobic digestion process. Disinfectant levels used for microbial and pathogen control are usually not higher than the recommended standard from where the feedstock is collected, because anaerobic digestion is inhibited by higher concentrations of disinfectants. However, high concentrations of heavy metals and acids are ostensive during mining processes. These metals include Cd, Pb (lead), As, Hg, and Cr (Ali *et al.*, 2013; Guo *et al.*, 2012; Chen *et al.*, 2008; Poels *et al.*, 1984).

Anaerobic digestion as a method for producing biogas has been used for the degradation of animal waste and other biomasses. There is an increasing interest in producing biogas from energy crops as a fuel for generating electricity. Clearly, seeding and operating a bio-digester is a complicated process that requires skill and knowledge.

Chapter 3: Study structure, materials and methods

3.1 Introduction

The bioenergy plant was established at a mine using recovered gold processing units for anaerobic digestion of energy crops. The crops considered as feedstock for biogas production were cultivated on contaminated land with the intention to decontaminate the land using the crops via phytoextraction. This chapter provides the details of the materials and methods used to accomplish the objectives of this research. The study was divided into 3 parts presented in chapter 4, 5 and 6 (Figure 2). During the unmonitored period between day 1-33, AD2 was added with an unknown amount of RSC this was done to understand the influence of the inoculum to the system. A case study involving the commissioning and start-up of the 1200 m^3 capacity anaerobic digesters is presented in chapter 4. The commissioning strategy was divided into 3 phases; during phase I, anaerobic digester 2 (AD2) was filled with RSC as inoculum between day 1 and day 97. Phase II continued from day 98-163 when AD2 was considered dormant, remedial applications such as pH amendment, TS% reduction and inoculum adjustment/variation were introduced. Phase III started from day 163-206 during which process advancement was observed without any additional parameter adjustments.

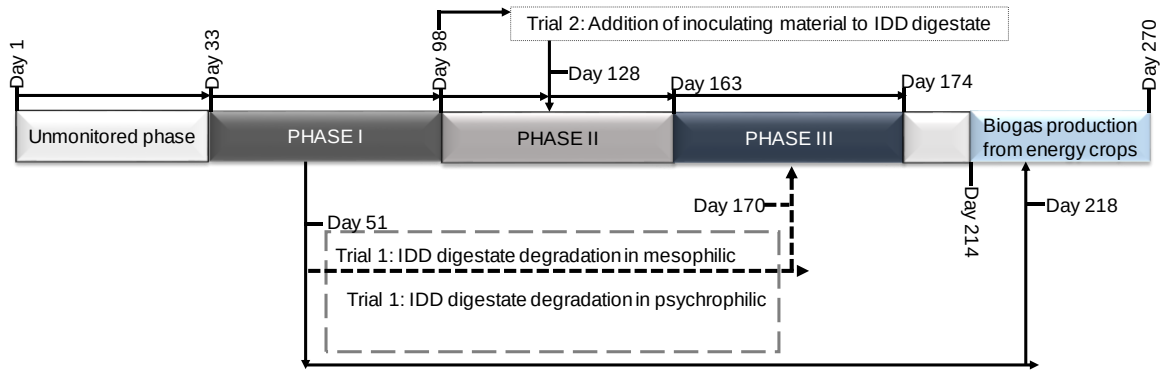

Figure 2: Illustration of the chronological process for data collection and presentation in Chapters 4, 5 and 6. During the commission of the industrial anaerobic digesters (AD1 and AD2) used in this study, the process was unmonitored from day 1 to day 33. AD1 and AD2 were commissioned in three phases from day 34 to day 163. On day 51, the first bulk of the digestate was collected from AD2 for Trial 1 and, on day 98, the second bulk of digestate was collected for Trial 2. During Phase II, 15% of the AD2 contents was transferred to AD1 and both were operated as separate entities in parallel. Laboratory trials for the production of biogas from energy crops and waste commenced on day 214.

The laboratory investigation included the operation of bench-scale anaerobic bio-digesters (2-5 L). The data are presented in Chapter 5 and Chapter 6. In Chapter 5, issues encountered when AD2 was considered dormant (referred to as industrial dormant digester [AD2-IDD]) were resolved by using digestate from AD2-IDD as digester slurry in a laboratory bench test. Two methods for reviving the AD2-IDD in batch laboratory tests were investigated:

- Trial 1: Increasing the retention time; and
- Trial 2: Addition of methanogens into the digesters to determine the effect of inoculum on AD2-IDD digestate.

Chapter 6 will discuss the production of CH_4 from bioenergy crops used for phytoremediation purposes in combination with rumen waste from a slaughterhouse from day 214 to day 270.

3.2 Design of the laboratory-based bio-digester

The experimental set-up was adopted and modified from Salam *et al.* (2015). The digesters (2 L or 5 L) containing slurry that had been sampled from AD2 and AD1 were connected to a plastic 5 L or 10 L container that was used as a gas collector, which was in turn connected to a 1 L measuring cylinder (Figure 3).

All the tanks used as digesters were made airtight and were tested for leaks before commencing with the experiments. The lids of each digester were sealed and were covered with extra protection layers. Plastic pipes were used to connect the digesters with the displacement tanks and the water outlet pipes. Scroll-in aluminium clamps were used to tighten the connections. Anaerobic digestion in this study was performed in batch type digesters. The airtight tanks were charged once with substrate/inoculums and/or pH adjustment chemicals, the tanks were then sealed, and fermentation was allowed to occur.

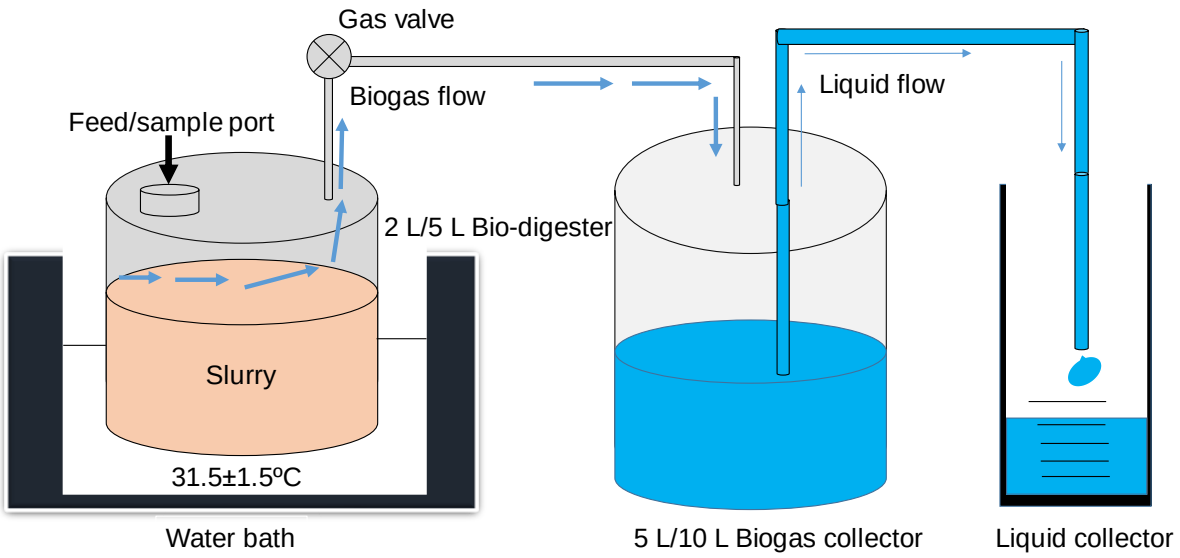

Figure 3: Schematic diagram of the bio-digester design for anaerobic digestion, the experimental set-up was adopted and modified from Salam et al. (2015). The experimental digesters (2 L or 5 L) were loaded with slurry (inoculum, feedstock or both) for the mesophilic trials. The digesters were immersed in a temperature controlled water bath filled with tap water. The digesters were fed through the sample/feed port that was also used for sampling the digestate whilst the gas was analysed via the gas valve. The 5 L or 10 L biogas collector was filled with brine solution and sealed and, when pressured, was used to measure the gas volume that had collected in a sealed measuring cylinder (liquid collector).

3.3 Analyses

3.3.1 Temperature, pH and oxidation reduction potential (ORP)

Temperature, pH and ORP were measured using the HI 8424 portable pH/ORP meter (Hanna Instruments). The temperature was also measured using a mercury in glass thermometer.

3.3.2 Volatile fatty acids (VFA)

The volatile organic compounds (VOC) representing the sum of organic acids were determined from the samples by a titration method adapted and modified from Nie *et al.* (2015), Schmidt *et al.* (2014) and Buchauer (1998). The samples were collected from a digester in a sample bottle. The VFA were analysed in the liquid phase of the samples after removal of the solids. The samples were filtered through a 2 mm sieve

to remove the solid contents. A filter paper was then used to remove the excess solids. The resultant fluid was filtered through a 0.45 µm membrane filter in syringe compatible casing. Filtered samples were collected in 50 mL titration beakers. An average Initial pH of the digester samples was recorded. The samples were titrated using 0.1 N sulphuric acid (H_2SO_4) in a pH range of 5.00 and 4.00 while mixed constantly on a magnetic stirrer.

3.3.3 Total alkalinity

The titration method that was applied was adopted from Lili *et al.* (2011) and Apha (1995). The samples collected were filtered through a sieve and then through a filter paper until all the solids were removed. Titration was performed at room temperature using 0.1 N H_2SO_4. During titration, all the titrant volumes were collected and the pH levels were recorded. The alkalinity and total alkalinity were determined using the following equations:

$$\text{Alkalinity mg } CaCO_3\ L^{-1} = \frac{A*N*50\ 000}{\text{mL Sample}} \qquad \text{(Equation 1)}$$

Where: A = mL standard acid, N = Normality of standard

$$\text{Alkalinity mg } CaCO_3\ L^{-1} = \frac{A*t*1000}{\text{mL Sample}} \qquad \text{(Equation 2)}$$

$$\text{Total alkalinity mg } CaCO_3\ L^{-1} = \frac{(2B-C)*N*50\ 000}{\text{mL Sample}} \qquad \text{(Equation 3)}$$

Where: B = mL titrant of the 1st recorded pH, C = total mL to read pH 0.3 unit lower, N = Normality of acid.

3.3.4 Solids content (TS, VS and ash)

The method used in this section was adopted from Xia *et al.* (2012), Eaton *et al.* (2005), Apha (1995), and Greenberg and Clesceri (1992). To determine TS and VS, a clean dish was heated to 105ºC for 1 hour. After the dish had been heated, it was stored and cooled until needed. The weight of the dish was measured every time before use. A sample was homogenized and evaporated to dryness in a drying oven. When transferring the samples, stirring was done using a magnetic stirrer. The dried samples were evaporated for 1 hour in an oven at 105ºC, left to cool in a desiccator,

and weighed. To determine VS, the residue from TS was ignited at 550ºC for 20 minutes (to a constant weight). TS was calculated according to Equation 4:

$$TS = \frac{m(dry)}{m(wet)} \times 100\% \quad \text{(Equation 4)}$$

VS was calculated according to Equation 5:

$$VS = \frac{m(dry) - m(ash)}{m(wet)} \times 100\% \quad \text{(Equation 5)}$$

Where: m (dry) = dry mass; m (wet) = wet mass; and m (ash) = mass remaining after ignition.

3.3.5 Gas production and composition

The volume of the biogas generated was measured by means of the liquid displacement method as adopted from Salam *et al.* (2015). The biogas was prevented from dissolving in water by adding brine solution. The brine solution was prepared by dissolving sodium chloride (NaCl) in water until saturation. The biogas produced in the bio-digester was delivered to the second chamber containing a brine solution (Figure 3). Because biogas is insoluble in brine, the pressure built up, forcing solution displacement. The displaced solution was measured to represent the amount of biogas produced. The volume of biogas produced after measurement was corrected for standard conditions of temperature and pressure at 20ºC and 10332.3 mm H_2O, respectively. Based on a combination of Boyle's Law and Lussac's Law, errors were taken into account. The concentration of CH_4 in the biogas was determined using a gas detection instrument (GDI) from Schauenburg Systems which uses infrared absorption approaches to determine the gas concentration. The GDI was equipped with a multi-gas detector and a methane-optimized sensor, and it was calibrated every day with 50% CH_4 calibration gas.

3.3.6 Ammonium-nitrogen

Drosg (2013) and Apha (1995) methods with slight modifications were used to determine the ammonium-nitrogen. The first volume of the sample was measured and recorded. Boric acid (2%) and indicator were mixed in an Erlenmeyer flask. After adding NaOH to the sample flask, distillation was performed allowing the ammonium

to be transferred to the Erlenmeyer flask. The Erlenmeyer flask was titrated with 0.1 N H_2SO_4 until the colour changed, and the calculation of ammonium-nitrogen concentration was performed afterwards.

3.3.7 Chemical oxygen demand (COD)

The sample was treated with COD of >50 mg O_2 L^{-1}. The sample was blended and pipetted into a 500 mL refluxing flask. After the addition of glass beads and mercury (II) sulphate ($HgSO_4$) into the flask, the contents was dissolved by adding H_2SO_4. Potassium dichromate (VI) ($K_2Cr_2O_7$) (0.04 M) solution was added into the flask, and it was then attached to a condenser and cold water was turned on. The condenser was covered to prevent foreign matter from entering the mixture. Refluxing was performed for 2 hours, and additional H_2SO_4 was added when necessary. Cooling and washing of the condenser were performed using distilled water. The reflux condenser was disconnected and the mixture was diluted to twice its volume with distilled water. After the solution had been cooled to room temperature, titration was performed using $K_2Cr_2O_7$ with FAS. A few drops (0.10-0.15 mL) of ferroin indicator were also added. The same volume of ferroin indicators was used for all titrations performed, and the colour changes were used as indicators for the endpoint of titration (Saady & Massé, 2013; Apha, 1995).

3.3.8 Total Kjeldahl nitrogen (TKN)

The required volume of sample was placed into the sample tube and NaOH was weighed and added into each sample tube. H_2SO_4 was added to the contents of each tube and digestion was performed. After digestion, the ammonia of the sample was distilled into a boric acid solution by steam distillation and it was then titrated with H_2SO_4. The method was verified by using glycine as the reference substance (Lindorfer *et al.*, 2008; Apha, 1995).

3.4 Statistical Analyses

All the data that were collected (as described in Chapter 5 and Chapter 6) were subjected to statistical analyses. To test the null hypothesis, the collected daily mean biogas yield data were subjected to analysis of variation (ANOVA). The t-statistic was used to compare the means of the daily gas obtained from different digesters operated at different settings and conditions. The confidence limit was set to 95%.

Chapter 4: Commissioning strategies for the anaerobic energy crops digesters established at a gold mine: A case study

4.1 Introduction

All industrial activities are dependent on energy, and currently the demand is satisfied by fossil fuels in most countries on the globe. In South Africa, most of the energy used for industrial purposes is met by coal (Sebitosi & Pillay, 2008). This country, like many other countries, desires to improve its industrial energy efficiency by limiting greenhouse gas emissions to a minimum in order to achieve sustainable and economical industrial development. Using biochemical processes allows the use of biomass to generate electricity and to provide process heat for industrial activities (Demirbaş, 2001). The electricity generated from coal is an undesirable commodity from an environmental perspective, but fossil fuel has and is still playing an enormous role in the mining sector. This sector has been key to the economic growth and development of South Africa (Ting *et al.*, 2015; Stilwell *et al.*, 2000).

During gold mining, fossil fuels are used in some metallurgical plants where different burners are used that are fitted with elution column heaters and kilns. The usual heaters use different non-renewable fuels and others are operated by electricity that is generated from coal. In addition to using fossil fuel during gold extraction, various other chemicals are associated with recovering gold from its ore. During gold extraction, high toxins are found in freshwater, which is a potential threat to both the ecosystem and humans (Edinger *et al.*, 2008; Wickre *et al.*, 2004; MüezzinogˇLu, 2003). Mine tailings are associated with different contaminators such as heavy metals, and thus different energy crops (sugar beet, sorghum, and maize) are required to extract contaminants from the soil (Meers *et al.*, 2010; Marchiol *et al.*, 2007; Dietz & Schnoor, 2001; Lombi *et al.*, 2001).

It was against this backdrop that this bioenergy project aimed to investigate the potential to substitute liquid fossil poly-fuel and fossil coal-derived electricity with renewable gas, with the ultimate aim of lowering the cost of energy production and providing an efficient way of energy use through faster heating in the kilns and

cleaner burning in the thermal oil heaters. It is thus envisaged that biogas will ultimately be produced from energy crops that are cultivated on mine impacted land that was contaminated during gold extraction. The project also aimed to encourage the control and reduction of energy costs, the reuse of existing infrastructure, and job creation. If a mine is to establish a biogas plant by utilizing energy crops to remediate contaminated land and to use it to cultivate feedstock for biogas production, the following are to be included in the basic layout of the plant. The plant will require a feedstock preparation station where sieving, sorting and pasteurization of feedstock occurs; fermentation tanks and a de-watering and composition station are required; and a gas conditioning station is a necessity (Raven & Gregersen, 2007; Hassan, 2003).

When starting a bio-digester for the first time, inoculation or seeding will be required. This process is necessary to provide the digester with proper microbial populations, and the microbes from the inoculating materials will require time to adjust to the substrate of interest (I Nyoman & Seno, 2010; Parawira *et al.*, 2005). After starting a digester, it is crucial to monitor the factors (H_2S, ammonia, heavy metals, antibiotics and disinfectants) that have a negative effect on the anaerobic fermentation process, and every biogas plant management should be responsible for developing its own process monitoring procedures. This chapter recorded the inoculation commission strategies and process monitoring protocols for the anaerobic energy crops digesters established at a gold mine as they occurred.

4.2 Materials and methods

4.2.1 Site description

The bioenergy plant is located at Welkom (coordinates: 27°58’59’’S; 26°43’15” E) in the Free State. Mining activities have been since the 1950s in this area (Phillips, 2013). However, several mines have shut when the mines became depleted, and the land that had been impacted and polluted with chemicals during mining became disused. The bioenergy project of focus in this study intended to structure a social enterprise that may eventually eliminate mine land negation by putting value back into that land. This was to be achieved by planting biomass that would remove contaminants from the soil produce renewable energy via anaerobic digestion. The

location of the biogas plant is situated close to the land that requires remediation and a reservoir containing municipal wastewater (Figure 4).

Figure 4: Geographical presentation of the bioenergy plant located at Welkom showing: (A) the wastewater reservoir; (B) the bioenergy farm; (C) the bioenergy plant; (D) the mine offices; and (E) the active mine tailing dam. Source: Google maps.

The location selected for this project was not burdened by environmental and hydrogeological constraints because it was outside protected areas. It allowed feedstock transportation at minimal cost; there was potential for further development on the surrounding land; and it was distant from the human residential area and animal dwellings in its vicinity.

4.2.2 Description of the bioenergy plant

The bioenergy plant that was constructed for this study was assembled in a plant that had once used for gold processing. This plant was scheduled to be demolished and the land rehabilitated. Thus, the redundant metallurgical plant was reserved to be used for bioenergy production. Several of the existing tanks were refurbished and modified for use as hydrolysis systems, bio-digesters and feedstock silos. The old steel refurbished Pachuca tanks that has once been used as gold processing units were used in this study as anaerobic fermenters. During the period of this investigation, the bioenergy plant was divided into a pre-treatment system (B); bio-digesters (C); a biogas conditioning area; (D) biogas storage tanks (E); and biogas end user (F) (Figure 5).

Figure 5: Visual representation of the process flow of the bio-energy plant. The feed/inoculum was delivered into the pre-treatment system (A), where it was mechanically treated (B). The booster pumps were responsible for pumping the feed/inoculum via a feed pipe into the digester (AD2, with a volume of 1200 m^3) (C). When gas was produced, it was directed to the gas buffer tanks after purification (D). Two buffer tanks (≈10 000 m^3 each) were used to store the gas.

The biogas production system was automated and the biogas components were connected to a computer network. The automated computer system was only used to control the valves, operate the homogeniser pump, and determine the digesters' temperature. The homogeniser pump was capable of pumping the slurry of ≤15% TS at a flow rate of 37 m^3 h^{-1}. The heat exchangers were not operational during the start-up phase, which means that no temperature regulation was possible. No foam and floating matter removal devices were installed; thus floating matters were inspected by looking through the inspection window. The feedstock/seeding materials were distributed through the pre-treatment system, where they were mechanically cut and homogenised using cutters. The resultant materials were passed through a sieving presser that reduced the size of the material to ≤2 mm. The materials were then pumped by the booster pumps into the digesters. The pre-treatment system was also used to separate stones and sand.

4.2.3 Commissioning strategy

The digesters were filled with tap water up to operational level (≈1100 m^3) to check the digesters for possible leakages. The pre-treatment system was inspected and tested before usage. Testing was initially performed using water, and later on small batches of rumen solid contents were added. The overflow pipes and pressure relief valves were also inspected and tested. The ethylene glycol was loaded to make sure that the relief valve's barometers had accurate readings. The plant was checked for proper system flows, the level control process, precise operation of all process

pumps, and an operational feedstock mixing system. All the machinery and devices that were to be operational during the inoculation process were inspected and tested before use, following all the mine standard operational procedures. The digesters were commissioned and inoculated between the end of spring and midsummer of 2017.

4.2.4 Inoculums

4.2.4.1 Rumen solids content (RSC)

The rumen solid waste that was used was initially intended for disposal by a slaughterhouse situated close to the bioenergy plant, but both the slaughterhouse and the local municipality favoured reprocessing it. RSC was selected as inoculum to start up AD2. It was delivered to the plant using waste containers called skip bins. These skip bins had a capacity of 6 m^3. RSC is usually a mixture of different cattle waste; similarly, the visual characteristics of the waste that was used showed that the major components were semi-digested fodder (cud), rumen skin, blood, fat and maize kernels. The RSC was characterized 3-4 times a week. Each time the samples were collected three times a day in batches of three and analysed in triplicate. The inoculum was measured for pH, ammonium concentration, total alkalinity, total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), ash, VS, VFA, and TS concentrations.

4.2.4.2 Municipality wastewater treatment sludge (MWWTS)

MWWTS was obtained from the municipality digesters that are used for the treatment of domestic sewage waste. MWWTS was characterized 2-3 times a week each time the samples were collected three times a day in batches of three and were determined in triplicate for each sample collected. The inoculum was measured for pH, TS, VS, and ash concentration.

4.2.4.3 Digestate from an operating digester (DFOD) and long-standing manure (LSM)

DFOD was collected from digesters operating at a mesophilic temperature. The digesters were using cow manure and industrial confectionary by-products for biogas production, and the inoculum was collected from a bioenergy plant situated ≈250 km away. The cattle manure that was on the floor of a shed (used as bedding for cattle)

for a period of ≥90 days was used to prepare LSM slurry. The manure was collected from a farm situated ≈30 km away from the bioenergy plant.

4.2.5 Inoculation procedure

RSC as a seeding material was intended to provide AD2 with appropriate microbes. The method followed to achieve a proper digester start-up comprised three phases that developed unintentionally during the process, as is represented in Figure 6 below. This unintentional division into three phases occurred because the first effort to seed the industrial digester failed, and subsequent efforts were subsequently made to achieve the desired outcome. After testing the digesters for leakages, AD2 was drained and the water was directed to the storage tanks. To inoculate AD2, tap water was used to deliver RSC into the digester, until half of the hydraulic operational level was reached (≈550 m^3).

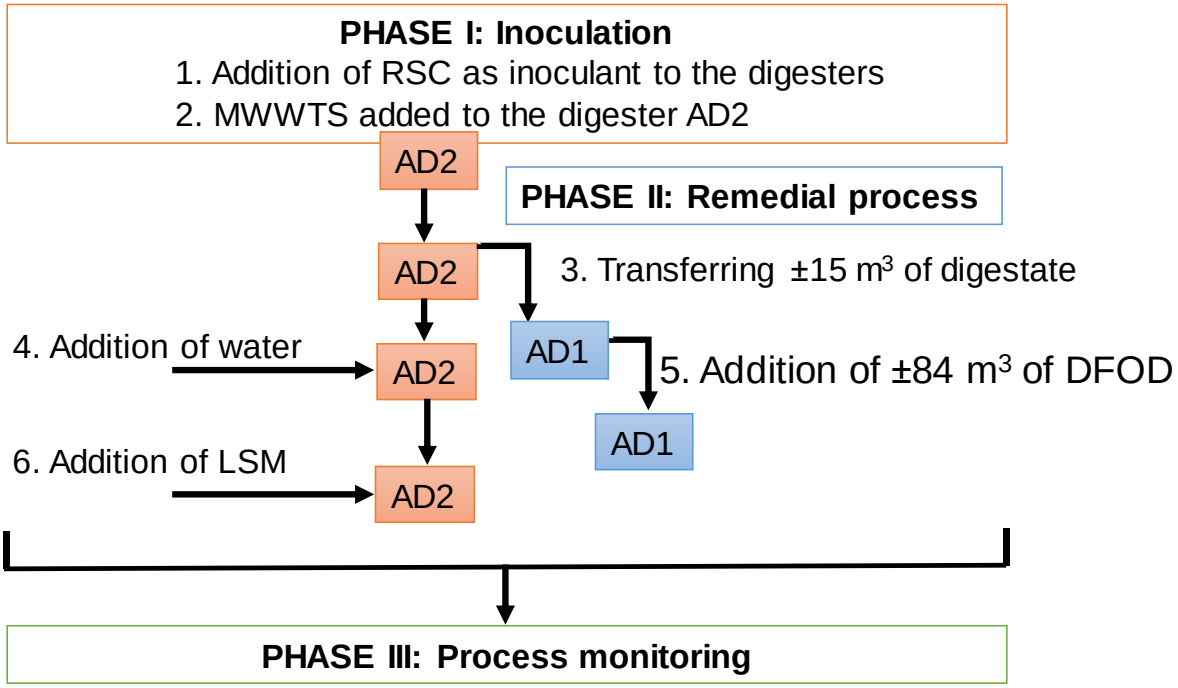

Figure 6: Steps followed to achieve the start-up of the industrial digesters: anaerobic digester 1 (AD1) and anaerobic digester 2 (AD2).

During Phase I, inoculation of AD2 with RSC and MWWTS failed, which resulted in the initiation of Phase II. Phase II involved different remedial applications, and AD1 was started by transferring the AD2 contents to it. The addition of inoculum from an operation digester (DFOD) and long-standing manure (LSM) in AD1 and AD2 achieved the seeding of both AD1 and AD2 separately. Phase III comprised process

monitoring when process advancement was observed without any additional parameter adjustments (Figure 6).

The introduction of RSC into the digester occurred via a pipeline that was connected to the mechanical pre-treatment system and directly to the digester, bypassing the hydrolysis tanks as (Figure 5). In earlier days of seeding, the RSC that was used was characterized by a large number of impurities. Manual sorting methods were employed to isolate the impurities, but this was primarily done in the pre-treatment system. The volumes of RSC added into the digester daily were measured using a weight scale at the slaughterhouse. MWWTS was used as the secondary inoculum to provide the anaerobes, and MWWTS was introduced on occasion on its own or concurrently with RSC. During Phase II (Figure 6), the seeding process was declared unsuccessful and remedial methods were initiated to resolve the dormant progression of AD2. This phase involved remedial applications such as pH amendment of AD2 by the addition of ≥15 tonnes of lime powder and ≥8 m^3 of 40% NaOH, and the TS concentration was reduced by transferring the contents of AD2 to an empty anaerobic digester 1 (AD1) and by adding water (≈250 m^3) to AD2. DFOD and LSM were added to AD1 and AD2 respectively in an effort to provide additional microbes. During Phase I, AD2 was agitated an average of 6-7 hours a day for 5 days a week. Agitation of AD2 was performed using slurry recirculation. As it was necessary to avoid the bottleneck effect, AD2 had a cone shape structure, and this facilitated the settlement of solids at the bottom. The exit opening from AD2 to the homogenising pump was situated at the bottom, and agitation prevented blockages. During Phase III, the remedial methods that had been applied were allowed to continue without any other parameter management. This phase was used to enhance the progress of the remedial methods and agitation was minimized to 2-3 hours a day for 5 days a week. The categorisation of the different phases was only done for clarity of this study.

4.2.6 Parameters that were measured

During Phases I to III, the following parameters: pH, ORP, temperature, CH_4, TS, VS, VFA, and alkalinity concentrations were determined using the methods described in Chapter 3.

4.3 Results and Discussion

Anaerobic digestion is a technology that is commonly used nowadays for treatment of organic waste for biogas production. The biogas can be used to replace coal as a source of energy for electricity generation. However, despite the many benefits of anaerobic digestion, the degradation of organic material via this process is complicated and requires proper operational stability and applications to optimize methane production to promote the commercialization of this process.

4.3.1 Selected seeding materials

Essential requirements of a good substrate include availability, affordable cost, ease of handling, the safety of operators, appropriate storage, the requirement for feeding, cleanliness, and the potential to contain a consortium of microbes (Suryawanshi *et al.*, 2013). These parameters are referred to in the argument for choosing rumen solid contents (RSC) as the inoculating material. The physical appearance of the seeding material included a large number of inorganic matter such as stones, sand and plastic clips. Organic materials that could be harmful to the instruments used included tails, bones, horns and other hard cow body parts. A manual sorting strategy was employed to remove the undesirable materials before adding the rest to the pre-treatment system, but the strategy proved to be unsuccessful. RSC was thus added directly into the digester via a pre-treatment system that was regularly blocked. The constituents of this seeding material thus regularly interfered with the booster pumps, feed pipes, the digester's outlet pipes, and the homogenising pump.

Before RSC was delivered to the plant from the slaughterhouse, it would have been discarded, and therefore it was supplied to the bioenergy plant free of charge. The slaughterhouse was capable of delivering more than 20 tonnes of RSC per day, which justified the decision to use this material. Moreover, the RSC required no storage facilities because it was mass-produced on daily basis from the slaughterhouse. During the early stages of seeding the AD2, the slaughterhouse was not informed of the importance of RSC cleanliness, and it had an obscene smell and was characterized by a mixture of much unwanted debris from the slaughterhouse. There was a possibility that the slaughterhouse was discarding disinfectant (used for cleaning their area of operation) into the skip bins. If this was so, the microbial population would have been affected negatively. No tests were done to determine

the availability of facultative anaerobes and methanogens, and thus the presence facultative anaerobes and methanogens in the RSC as the seeding material was questioned. In addition, the RSC was difficult and dangerous to handle by operators because it was characterized by a revolting appearance with an obscene smell and voluminous flies and maggots.

4.3.2 Characteristics of the selected inoculum

A low TS percentage characterising an inoculum is required to avoid overwhelming microorganisms with sustenance. This argument is supported by Yuan *et al.* (2014), who used a low TS percentage microbial consortium to enhance the anaerobic digestion of lignocellulose of municipal solid waste using a microbial pre-treatment method. The RSC used in this study had a high fibre content with a high TS concentration of 38.50±0.95% (Table 2). The high TS% of RSC was a perspective indicator that should have guided the bioenergy team to apply a well-understood dilution method for RSC in order to reduce the TS in the digester, because particle size reduction was essential. Palmowski and Müller (2000) indicate that, by reducing the size of organic waste, gas yield is improved and the retention time is reduced. The RSC that was loaded to AD2 was characterized by a higher average VFA concentration of 4972.00±240.00 mg L^{-1} (Table 2) that was delivered in different skip bins. Thus, the RSC with VFA concentration in excess of 5000 mg L^{-1} was determined.

When a feedstock for biogas production occupies a high quantity of VFA, the VFA concentration of the loaded digester is guaranteed to have a lower VFA concentration of ≥1000 mg L^{-1}. High VFA concentrations in a bio-digester are a cause of process imbalance (Drosg *et al.*, 2013; Ahring *et al.*, 1995). Yacob *et al.* (2006) established that a VFA of ≥2000 mg L^{-1} is passable for better gas production from digesting palm oil mill effluent in a 500 m^3 digester operated in mesophilic temperature. The high VFA and lower pH of RSC occurred because the RSC was probably decomposing, or other acid materials such as slaughterhouse cleaning chemicals had been added to the seeding material. However, higher VFA and the low pH of the RSC were good indicators for use and a helpful caution to condition the AD2 environment to favour a neutral pH.

Table 2: Chemical properties of rumen solid contents loaded to AD2 (Mean ±SD, n=12)

Parameters	Unit	Value
Ammonium (NH_4^+)	mg L^{-1}	210.00±0.23
Chemical oxygen demand (COD)	mg L^{-1}	46000.00±697.00
pH	pH units	5.17±0.35
Ash	%	38.11±0.98
Volatile solids (VS)	%	62.90±1.59
Total solids (TS)	%	38.50±0.95
Total alkalinity	mg L^{-1} $CaCO_3$	650.00±80.00
Total Kjeldahl Nitrogen (TKN)	mg L^{-1}	1341.00±58.00
Volatile fatty acids (VFA)	mg L^{-1}	4972.00±240.00

The average RSC fed into the digester contained more than 58% VS with a high amount of COD (Table 2). The VS, COD, TKN, TS and VS of the seeding material suggested that the RSC had veracious characteristics of a good feedstock for biogas production, but not those of a seeding agent. The RSC had been selected and used as a seeding material to reduce the lag time for the start-up of anaerobic digestion as inoculums derived from slaughterhouse waste had been used as a source of anaerobic microorganisms before (Sunarso *et al.*, 2010).

4.3.3 Phase I

4.3.3.1 Seeding process

AD2 experienced high VFA concentration during seeding and consequently had low pH. Low pH in a digester is capable of endangering the methanogens because these microorganisms thrive at neutral pH (Zhai *et al.*, 2015; Stams *et al.*, 2003). AD2 was intended to be operated as a low solid digester (≤10% TS), as Gupta and Verma (2015) had operated digesters loaded with agricultural wastes at the same TS%. However, in this study the inoculation predicaments of AD2 began with how the RSC as a seeding material had been prepared, and the approach that was used to equip the RSC as inoculum imposed a greater ratio potential of high levels of solids to the microorganism. When slaughterhouse wastes were used to provide facultative anaerobes and a methanogens population, they were prepared by diluting the slaughterhouse wastes with water and the slurry was then thoroughly filtered. This was done to acquire an inoculum dominated by microorganisms (Gu *et al.*, 2014; Wang *et al.*, 2014; Xu *et al.*, 2013). Córdoba *et al.* (2016) point out that most start-up

inoculums have the following in common: They are all potential carriers of microbes of interest, and fluid/slurry is commonly used. The RSC inoculum in this case study was unsuitable because it was thick and cluttered with large inorganic and organic matter. It was thus necessary to prepare the RSC to ensure a slurry or liquidised inoculum with a TS of 1-5%. Testing the RSC for potential microbes before use was also required in order to dismiss any uncertainty about the presence of the microbial population. This process was not novel, as Charles *et al.* (2009) determined the availability of methanogens in an organic fraction of municipal solid waste before using it as an inoculum. The RSC used in the current study was possibly exposed to competitive microorganisms and/or inhibiting chemicals considering how the rumen solids contents were handled at the slaughterhouse.

4.3.3.2 Solids concentration (TS% and VS%)

The liquid phase of the digester was analysed for TS and VS concentrations. The bioenergy plant aimed to determine TS and VS to assess RSC reduction and to reveal substrate utilization by microbes. The TS and VS concentrations in the digester were determined over a period of 65 days (Figure 7).

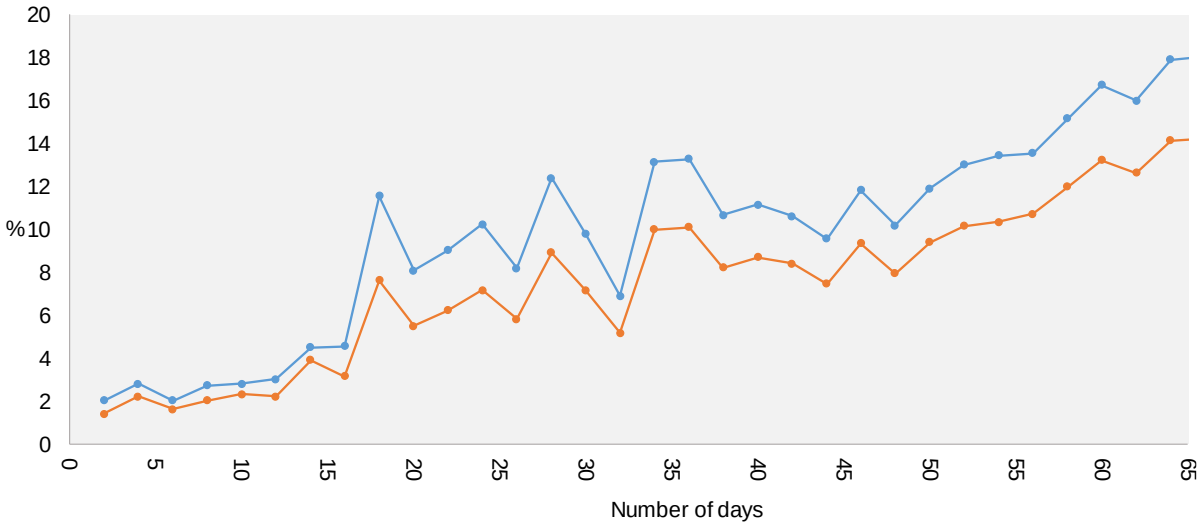

Figure 7: A graphical illustration of the daily concentration of TS% (●) and VS% (●) in AD2. Data are representative of 65 days monitored and commenced during phase I from day 33 to day 98. TS% and VS% fluctuated daily since the daily amounts of RSC introduced into the digester had different volumes and moisture content.

Given the quantity of RSC added per day, it is possible that the anaerobes in AD2 struggled to utilise the VS. The TS% in the digester was definitely not going to

decrease as expected. On day 1 after the RSC had been added to the content in AD2, the digester had a TS of 2%. The system experienced a blockage due to RSC loading and digester feeding had to be paused for three days. During this period, a reduction in TS and VS concentrations were expected, but no TS degradation occurred, as the TS determined on day 5 remained at ≥2%. The TS% of AD2 was between 2-4.5% from days 6-14. The TS% and VS% fluctuation between day 15 and day 50 occurred because of inconsistent mixing of the contents of the digesters and the erratic quantity of water that was used to prepare the RSC. On day 28, the TS was ≥12% and four days later it declined to ≥5%, but it increased to ≥13% on day 34. Upon observing that the TS was above the 10% target on day 46 (11.80%), tap water was poured into the digester. The addition of supplementary tap water diluted the TS to 10% on day 48. Although the TS% was now at the targeted value, operational volume, pH, methane concentration and VFA were still beyond the target range. It was acknowledged that the digester was performing below normal with low CH_4%, and it was presumed that the number of methanogens in the RSC was inadequate.

4.3.3.3 Effect of MWWTS on AD2 and CH_4 production

The second inoculum in the form of municipal wastewater treatment sludge (MWWTS) was introduced by combining it with water and RSC. The MWWTS analyses revealed a pH of 7.23±0.34 and TS of 2.20±0.08% (n=12). MWWTS was added to AD2 in small doses from day 35-38 (Figure 8).

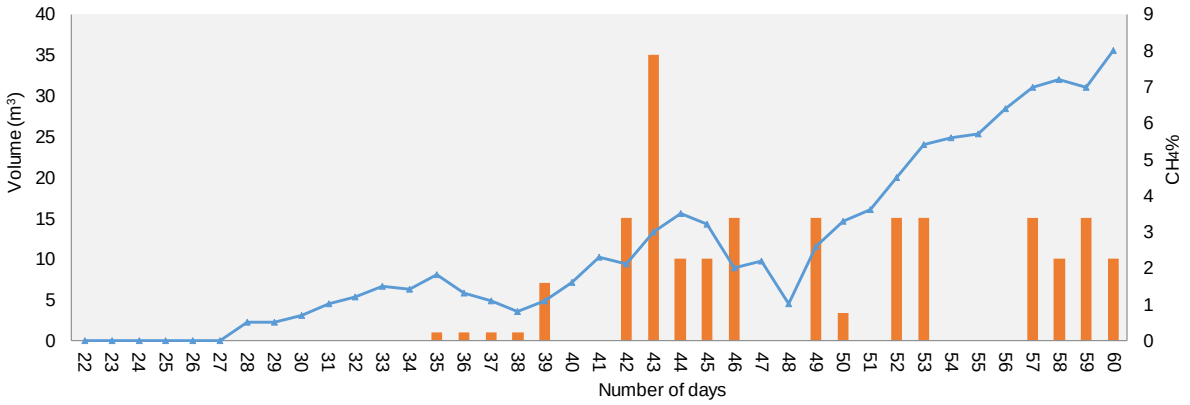

Figure 8: Graphical illustration of the quantities of municipal wastewater treatment sludge (MWWTS) (□ ▌) added (m^3) to AD2 and the daily CH_4 concentrations (%) produced in AD2 (▲). MWWTS was introduced as a secondary inoculum to AD2. The data are representative of 65 days of monitoring that commenced during Phase I on day 33 and continued to day 98.

The combination of MWWTS and RSC increased the TS concentration of the digester from ≥10% on day 49 to ≥18% on day 65. During the addition of MWWTS, the quantity of solids in the digestate was not taken into account when added in conjunction with the RSC. At one point, water was replaced by MWWTS to mix the RSC. This erroneousness assortment led to a costly high TS% in the digester, and the approach to increase microbial concentration using a combination of inoculums with high TS% needed rigorous examination before application. A low CH_4% of ≈0.5% was detected in AD2 from day 22-29, and it was apparent that the VS reduction and VFA utilisation were low. Total gas production is usually estimated to occur from the VS reduction over a certain retention time when operating a digester (Mudhoo, 2012). When inoculating AD2, the VS% increased with RSC loading and it reduced with TS% reduction, as was expected (Babel *et al.*, 2009). A notable VS reduction was not observed because the VS was not being utilized actively by the microbes. The AD2 environment did not allow microbial grow to establish, therefore low VS utilisation by the microbes was expected. The seeding material that was used had higher VFA readily available for usage by the available microbes for biogas production. If AD2 processes were balanced, the anaerobic microbes were going to utilize the abundant volatile acids first to produce methane.

The addition of MWWTS to AD2 had a positive effect on CH_4 production because, after the addition of this inoculum, a CH_4 concentration of 0.75% was detected on day 30 and 1.5% was achieved on day 33 (Figure 8). This encouraged the addition of MWWTS in increased quantities of 1 m^3 from day 34 to day 38. The trivial production of CH_4 in AD2 was attributed to the possibility that the newly introduced methanogens were able to utilize the abundant available organic acids (Figure 8). Farhat *et al.* (2018) were able to enhance biogas production when utilizing wastewater treatment sludge as inoculum. Babel *et al.* (2009) generated a high volume of biogas when sewage sludge was co-digested with brewery sludge at different mixing ratios. The quantity of MWWTS added (Figure 8) to AD2 was increased to 7 m^3 on day 39, and this had a positive effect on CH_4% because, on day 41, CH_4 increased to 2.3%. The quantity of MWWTS added to AD2 on day 43 was 35 m^3, which was followed by an increase in the CH_4 concentration of 3.5% on day 44. The CH_4% started declining from 3.5 to 1% from day 44 to day 46. A number of factors could possibly have affected the CH_4 content during this period. These include insufficient alkalinity, VFA accumulation, and ammonia inhibition that possibly resulted in the cessation of methane production. The addition of MWWTS

was paused for two days, and this decision had a positive result because the CH_4% increased from 1 to 2.6% from day 48-49. The positive influence of reducing agitation and discontinuing feeding was also reported by Lindmark *et al.* (2014) and Hamdi (1991). Between day 50 and day 65, an upward CH_4% increasing trend was observed, regardless of the quantity of MWWTS that was added (Figure 8). On day 60, the highest quantity of 8% CH_4 was observed, but there was no sign of significant VS reduction. Lu *et al.* (2008) demonstrated that this lag in VS reduction coincided with microbes that were still occupied with utilizing the residual VFA.

The delayed and lower CH_4 formation in AD2 could be attributed to numerous other factors, including oxygen concentration. This parameter was not measured during Phase I. The 1200 m^3 digester was not purged for oxygen because the expense of purchasing nitrogen gas for this purpose was considered too high. In retrospect, to have solved this, the addition of water to an operational level could have removed the oxygen, and this would have been preferable to starting the digester with a large volume of empty space. Gikas (2008) used water to effectively purge an industrial digester with an active volume of 10000 m^3 for inoculation. The high oxygen concentration in a digester is lethal to methanogens, which are sensitive to oxygen and easily perish if they come into contact with air (Koster & Lettinga, 1988). To avoid oxygen contamination, Mahdy *et al.* (2015) removed oxygen from digesters by purging the headspace with helium before digesting sludge and microalgae biomass.

CH_4 formation on the day 30 was a good indicator that the methanogens were now available in the digester. It is argued that the addition of fresh seeding material possibly provided these microorganisms. When attempting to inflate a gasbag with a volume capacity of 500 mL from the roof of AD2, no gas was collected in the gasbag even when the CH_4 concentration was higher (day 52 to day 60; Figure 8). The gas that was produced in AD2 was possibly settling in the headspace and was not enough to outflow, because biogas comprises of CH_4 that is lighter and CO_2 that is heavier than air, and therefore the biogas was expected to rise slowly. The feeding regime and VS reduction clearly showed that the digester was being overfed because of continuous feeding which resulted in organic overload (Lerm *et al.*, 2012).

4.3.3.3 pH, alkalinity and volatile fatty acids (VFA)

It was expected that the pH, alkalinity and VFA concentrations would be balanced to ensure a stable digester. However, AD2 experienced pH fluctuation (Figure 9) because of high VFA accumulation during the inoculating stage and poor buffer capacity (alkalinity). Fluctuations in pH have a negative impact on anaerobes and methanogens (Macias-Corral *et al.*, 2008). Figure 9 shows that a high accumulation of VFA occurred which caused pH lowering in AD2, and this possibly affected methanogen growth (Zhang *et al.*, 2013).

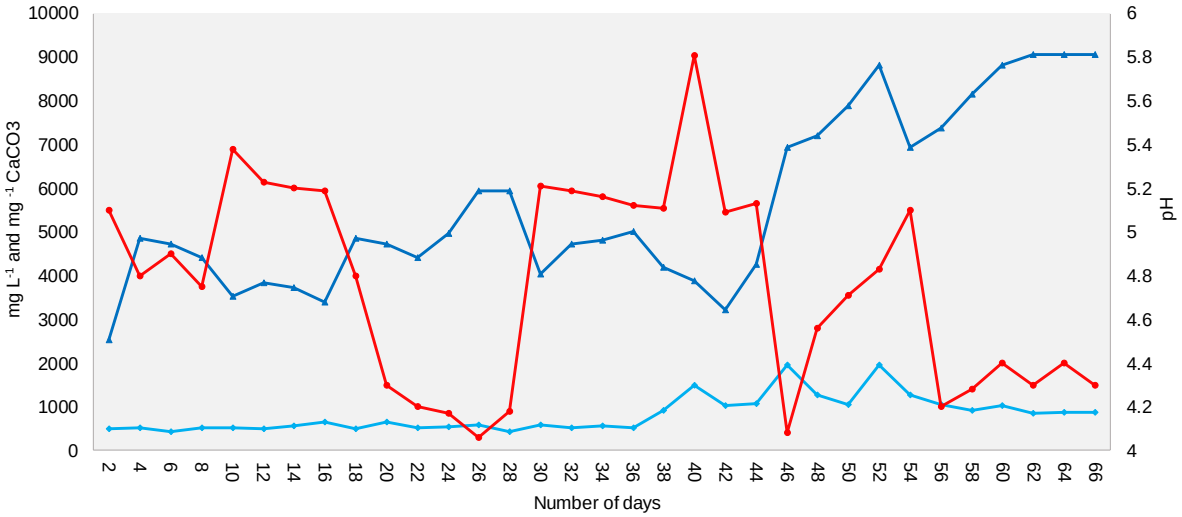

Figure 9: pH (●), VFA (▲) and alkalinity (♦) variations during seeding of AD2. The data are representative of 65 days of monitoring that commenced during Phase I on day 33 and continued to day 98.

The anaerobic digestion process is divided into different phases as the microorganisms involved have different pH preferences. The ones that are responsible for VFA formation prefer a lower pH of ≤5.00 whereas methanogens prefer a pH of 6.80 to 8.00 (Boe *et al.*, 2010). AD2 was fed with high VFA content and low pH inoculum (Table 2) and no pH adjustments were employed in the digester. This affected the pH of AD2 during the inoculation period to be on an average of 4.70±0.13. The highest pH reached during Phase I was 5.61±0.21.

To maintain a stable pH, a high level of alkalinity (≥1000 mg L^{-1}) is required. Higher alkalinity is equal to greater buffer capacity, which promotes stable pH (Liu *et al.*, 2008). RSC is substance rich with nitrogen, protein, and amino acids, and alkalinity is generated by the degradation of nitrogenous substances (Jiang *et al.*, 2013). A

stabilized alkalinity is between 1000-15000 mg L^{-1} $CaCO_3$, and values of ≤1000 mg L^{-1} $CaCO_3$ are indicative of digester instability (Li *et al.*, 2018; Martín-González *et al.*, 2013). Thus, an eventual increase in AD2 alkalinity was expected based on the ammonia and TKN values determined for the RSC (Table 2), and the low initial alkalinity went unnoticed. A constant alkalinity of 485.46±30.16 mg L^{-1} $CaCO_3$ was observed from days 1-36, which was well below the recommended value. The alkalinity was able to reach a maximum value of 1959 mg L^{-1} $CaCO_3$ during Phase I. At the end of this phase, the alkalinity value was again at an instability range of 860 mg L^{-1} $CaCO_3$ (Figure 9). Manser *et al.* (2015), Grimberg *et al.* (2015), and Subramanian and Pagilla (2014) indicate that unstable alkalinity is attributed to overloading, temperature fluctuations, and a lower microbial concentration in the digester.

The daily concentration of VFA in AD2 always exceeded 1000 mg L^{-1}, and between days 44 and 52 an exponential increase in VFA concentration was observed (Figure 9). The methanogens were not given enough time to multiply, the RSC and MWWTS materials overwhelmed the digester, the organic acid formation was favoured, and the methanogens quantity decreased. An ideal RSC inoculum is expected to generate VFA ranging between 1000-4000 mg L^{-1} because it possesses materials that are hard to digest (Martín-González *et al.*, 2013). However, the VFA concentration was at a maximum of 9054±203 mg L^{-1} on day 65, which indicated that AD2 was unstable considering that a total VFA of a stable system is <1000 mg L^{-1} and of an unstable digester it is >4000 mg L^{-1} (Drosg, 2013). A VFA concentration of 2500-3500 mg L^{-1} is permissible, but only if accompanied by elevated total alkalinity concentrations (13000-15000 mg L^{-1} $CaCO_3$) (Ahmed *et al.*, 2016; Drosg, 2013). A decrease of VFA concentration occurred on day 54, but there was no reflection of this occurrence in the CH_4% that was produced. It may be surmised that VFA was accumulated due to the overloading of the system. Thus, if the RSC loading was passive or ceased, a low VFA accumulation was to be favoured if a supplementary retention time was permitted (Zhang & Jahng, 2010).

4.3.3.4 Temperature

The temperature of the digester was not regulated, and thus the digester was affected by the temperature of the environment, and fluctuations occurred (Figure 10). The daily temperature was recorded in the morning, at midday and in the

afternoon. The AD2 temperature difference was calculated for morning-midday, midday-afternoon, morning, and afternoon.

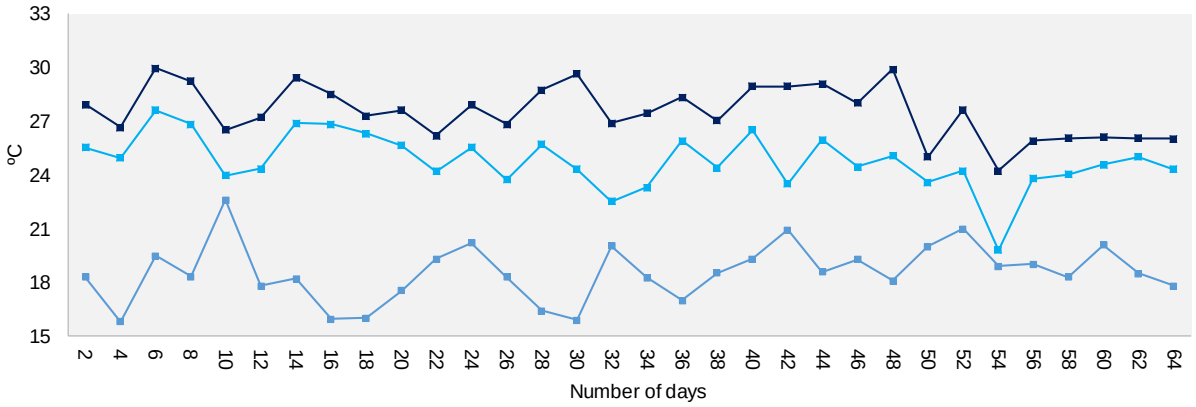

Figure 10: Graphical illustration of digester (AD2) temperature variations in the morning (■), at midday (■), and in the afternoon (■). The data that are presented are representative of 65 days of monitoring that commenced during Phase I on day 33 and continued to day 98. The temperature of the digester was not regulated and therefore the contents was affected by environmental temperature fluctuations.

The rate of temperature increase is dependent on individual plants' conditions, and rates between 0.15-0.45ºC day^{-1} have been reported at different plants operating between mesophilic and thermophilic temperatures (Lindorfer *et al.*, 2008). A temperature that is below 25ºC is referred to as psychrophilic in this study as is suggested by Shitophyta (2016).

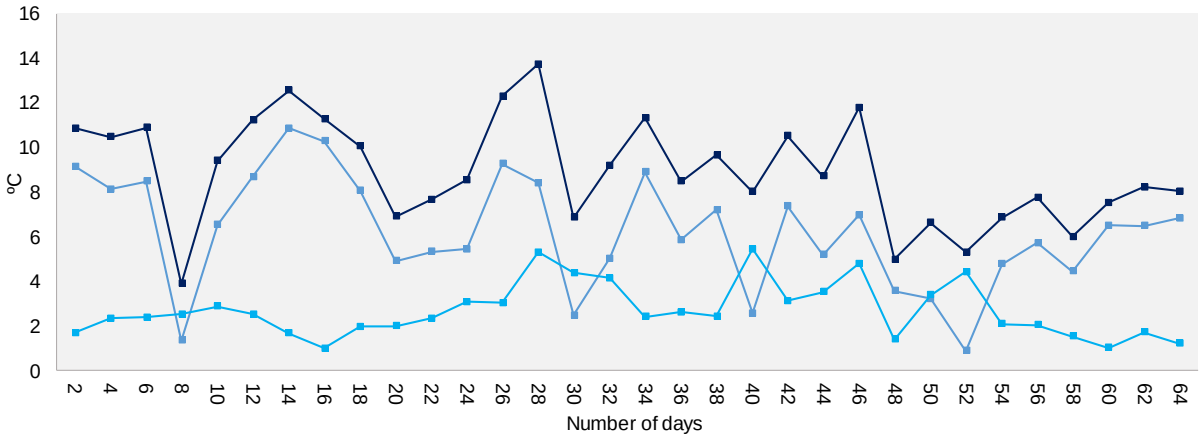

Figure 11: Digester (AD2) temperature fluctuation differences between morning-midday (■), midday-afternoon (■), and morning-afternoon (■). The data are representative of 65 days of monitoring that commenced during Phase I on day 33 and continued to day 98.

Average temperature readings of 18.51±0.32ºC were observed in the morning, an average temperature of 25.00±0.70ºC was observed at midday, and an average of 27.30±0.94ºC was observed in the afternoon. The temperatures were recorded three times a day for a period of 65 days. The atmospheric temperature increased as summer advanced, but the morning temperature of the digester decreased to a minimum temperature of 15.00±0.30ºC (Figure 11). The digester operated in low temperatures in the morning, and higher temperatures at midday and in the afternoons. This fluctuation predicament happened every day.

When the RSC was observed, the physical appearance displayed high dominance of cellulosic materials such as forage and maize kernels. These materials are resistant to microbial and enzyme digestion at lower temperatures. Saady and Massé (2013) describe a low microbial activity and biogas production rate from lignocellulosic biomass when digested at a low temperature, and this was also observed in the case of AD2. The reason why AD2 was operated at ambient temperature was guided by the delayed installation of heat exchangers, as low temperatures contributed to the unsuccessfulness of the inoculation process in AD2. Wei *et al.* (2014) report that high solid content and low temperature are not always terminal when operating a digester. For example, when barley straw was co-digested with animal manure in psychrophilic temperature with TS of 20%, a profuse volume of gas was observed. The success of the latter study relied on lengthened retention time. In the current study, when AD2 was inoculated, attention was focussed only on continuous loading of the RSC with the expectation of CH_4 production. TS and VFA concentrations of AD2 were also expected to be naturally balanced without intervention.

The temperature fluctuations between morning and midday were measured and found to be on average 3.05±0.40ºC day^{-1}. During the 18^{th} day of operation, there was only a 1.01±0.30ºC difference between morning and midday temperatures. On day 30, a 5.00±0.62ºC high difference in temperature was observed (Figure 11). The temperature fluctuations between morning and afternoon were high, as a daily average of 9.07±0.43ºC was measured. During the 28^{th} and 29^{th} days, temperature fluctuations of 12.10±0.31ºC and 13.70±0.2ºC were observed. Temperature fluctuations are a major contributor to high total VFA accumulation and, if VFA is accumulated, reducing feeding rate is beneficial because temperature sensitivity increases with load rate. A period of more than a week is also required for microbes to adapt to the new temperature. Process failure can occur if temperature changes

are in excess of 1ºC day^{-1}, thus temperature fluctuations of more than 0.6ºC day^{-1} are avoided in high temperature operated digesters (Nandi *et al.*, 2017; Appels *et al.*, 2008; Choorit & Wisarnwan, 2007). Psychrophilic digesters' temperature changes per day are expected not to be more than 2-3ºC (Kossman *et al.*, 1996; Noike *et al.*, 1985). Because this was not the case, the anaerobes and methanogens in AD2 were surely going to find it hard to adapt. Lindorfer *et al.* (2008) point out that temperature fluctuations affect the general performance of digesters and have negative economic consequences for the plant, and this is what was observed during AD2 inoculation.

4.3.3.5 Agitation

The purpose of agitation is to enhance the proliferation of microorganisms, to ensure substrate contact and distribution, and to ensure uniform pH and temperature (Nandi *et al.*, 2017; Brehmer *et al.*, 2012). During AD2 seeding, a crust formed at the top and agitation was applied to debilitate the crust. During inoculation, when the digester was not agitated (i.e., at weekends), a high CH_4 concentration was achieved. This also occurred when the mixing pump was not functional. The frequency of AD2 agitation had an influence on CH_4 production, thus moderate mixing has been reported to be positive because low and high mixing have been shown to have a negative effect on the methanogens (Ohimain & Izah, 2017). At the end of Phase I, AD2 had acquired unexpected results. In this period, the pH of the digester was fluctuating between 3.90 and 4.43. Moreover, the CH_4 dejected to a low concentration of ≤3% and TS% was not decreasing because the addition of the RSC was subsequently done on a daily basis.

4.3.4 Phase II: Remediation of the digester

Phase II was necessitated after it was concluded that the AD2 inoculation strategy had not resulted in CH_4 production. Remediation methods were investigated and Phase II was initiated. During this period the CH_4 concentration plummeted to ≤1% and the pH was between 3.50 and 4.00. The VFA concentration was ≥5000 mg L^{-1} and the alkalinity level was between 500 mg L^{-1} and 800 mg L^{-1} $CaCO_3$. TS concentration was ≥18% and the total weight of the RSC that was added to the digester was estimated at >180 tonnes. AD2 was also filled to ≈50% of total volume. To accomplish digester remediation, it was proposed that the pH should be improved by adding alkaline solutions, TS% would be reduced by reducing the digester's

contents, RSC feeding was to cease, and methanogens were to be added in the form of digestate from an operational digester (DFOD) and/or cow manure.

4.3.4.1 pH control

pH is a measurement of H^+ activity in an aqueous solution. Because of the simplicity to determine pH, it is the most used parameter for anaerobic digestion monitoring. Normal pH values for anaerobic digestion processes are in the range of 6.50-7.50, but different values are found in stable anaerobic digestion processes because pH is the result of the presence of several compounds such as high concentration of VFA, NH_4-N and high solids (Zhai *et al.*, 2015).

a) Addition of lime

The order of buffering capacity of different alkaline chemicals from high to low is CO_3^{2-}> HCO_3^-> OH− (Chen *et al.*, 2015). The addition of $CaCO_3$ has been used to achieve a stable pH after the added VFA has been digested at increased organic loading rates (Melamane *et al.*, 2007). It was thus, decided to add lime powder to AD2 in an attempt to adjust the pH from acidic conditions to basic acceptable pH conditions. A titration method that was applied to the small-scale laboratory digester indicated that a large volume (≈64 tonnes) of lime was required to change the pH by 0.5. When lime is added in a bio-digester, it is undigested and will remain in the digester until flushed out. After the addition of ≈15 tonnes of lime to AD2, no significant digester pH change was observed. Adjusting the pH from acidic to basic conditions by the addition of lime can create an environment favouring methanogenic microorganisms without keeping pace with the equilibrium between the acetogenic and methanogenic population, and this has a detrimental effect similar to overfeeding (Chen *et al.*, 2015).

b) Addition of 40% NaOH

Loading rumen content concurrent with NaOH led to an exothermic reaction that instigated system heating. This caused the pump's sensors to read a false overheating signal throughout the system and the booster pumps and the cutter were automatically switched off. The addition of NaOH increased the pH by ≈0.80, but the temperature upsurge caused by this chemical paused all operational activities for two days. NaOH is used to solubilize and to improve the biodegradability of solid feed. When a high concentration of NaOH is used to pre-treat feed, high COD has been reported to solubilize (Penaud *et al.*, 1999). Rafieenia

et al. (2018) and Strik *et al.* (2006) utilized NaOH to control the pH of the digesters that they used. Cationic elements such as sodium (Na) are released due to the addition of NaOH for pH adjustment, and the inhibition effect of Na was reported to be 11.5 g Na^+ L^{-1}, corresponding to 2% NaOH (Cao *et al.*, 2017; Chen *et al.*, 2008). A concentration of 40% NaOH is high to affect anaerobic microbes negatively, but fortunately a slight quantity was utilized. A sample was collected from the digester an hour after NaOH had been added and before any agitation. The sampled feedstock appeared liquefied and was hot. Therefore, if proper dilution of NaOH had been applied, system impairment could have been avoided. NaOH also posed some danger to the operators because they were not equipped to work with such a hazardous chemical.

4.3.4.2 Addition of methanogens and TS% reduction

If VFA is amassed in a digester due to overloading, this can be corrected by reseeding and suspending the feeding of the digester or by adding an alkaline in requisite quantities. It is possible to increase gas yield and reduce the retention period by the addition of inoculum (Sreekrishnan *et al.*, 2004). Laboratory digesters have been revived by removing half of the contents and replacing it with fresh methanogenic sludge (Melamane *et al.*, 2007). The method for TS% reduction that was applied in the current study involved the transfer of the AD2 contents to AD1. AD2 was then diluted with ≈250 m^3 water until the TS concentration was ≤8%.

4.3.4.3 Addition of inoculums into AD1 and AD2

A volume of ≈84 m^3 digestate from an operational digester (DFOD) was added to AD1. The DFOD was characterized by TS concentration of 2.10±0.11% and a pH of 7.28±0.93 (n=3). The dilution of AD2 with water (TS% reduction) had no impact on gas production or on the VFA, alkalinity and pH of the digester. During this stage, RSC feeding was stopped and the MWWTS was also stopped because it was too expensive. DFOD was not acquired for AD2, because it was retrieved from a distant area and was also expensive. AD2 was subsequently inoculated by using cow dung. Unfortunately, fresh cow dung was expensive and the only material that was available locally at a cheaper price was dry and old cow manure. This cow manure, referred to as long-standing manure (LSM), was the most readily obtainable and affordable type of organic material to stimulate a large industrial scale digester. LSM was characterized by high sand content determined in the laboratory as ash% (inorganics) constituting more than 40% of the TS. The LSM slurry was added to

constitute ≈30% of the total AD2 contents. This digester was characterized by a high sand content that was responsible for damaging the pump blades and causing additional system blockages.

4.3.5 Phase III: process monitoring

Phase III was performed for 43 days from day 163 to day 206 (Figure 2). The day on which process monitoring started was assigned as day 1 and the last day as day 43. This assignment is represented by Figure 12 and Figure 13. After applying the remedial methods described above, the digesters were monitored and no attempt was made to manipulate any digester parameters. During this period, the digesters were treated as a batch digester and analysed for significant parameters. The digesters were not fed to allow the growth of the anaerobic bacteria, and the VFA, alkalinity, pH, and temperature were monitored to make sure that the slurry was not dropping into an acidic state. These parameters were monitored 2-3 times a week and CH_4% was determined every second day (Figure 12 and Figure 13).

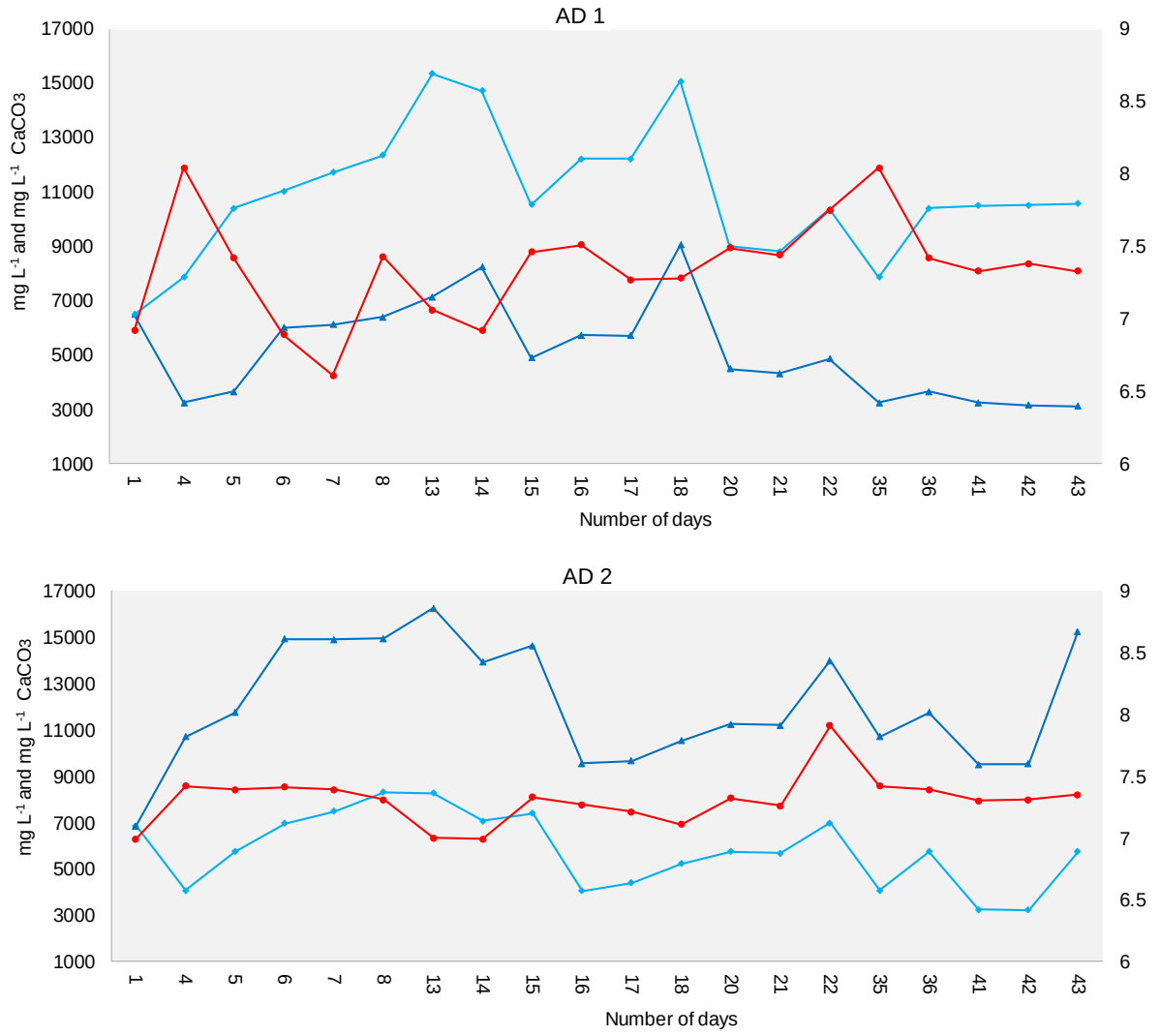

Figure 12: pH (●), VFA (▲) (mg L^{-1}), and alkalinity (♦) (mg L^{-1} $CaCO_3$) variation of AD1 and AD2 during Phase III. The data are representative of 43 days of monitoring that commenced in Phase III on day 163 and continued to day 206. AD1 contained ≈15 m^3 of AD2 digestate and 84 m^3 of DFOD. AD2 contained ≈ 490 m^3 of digestate and ≈210 m^3 of LSM slurry.

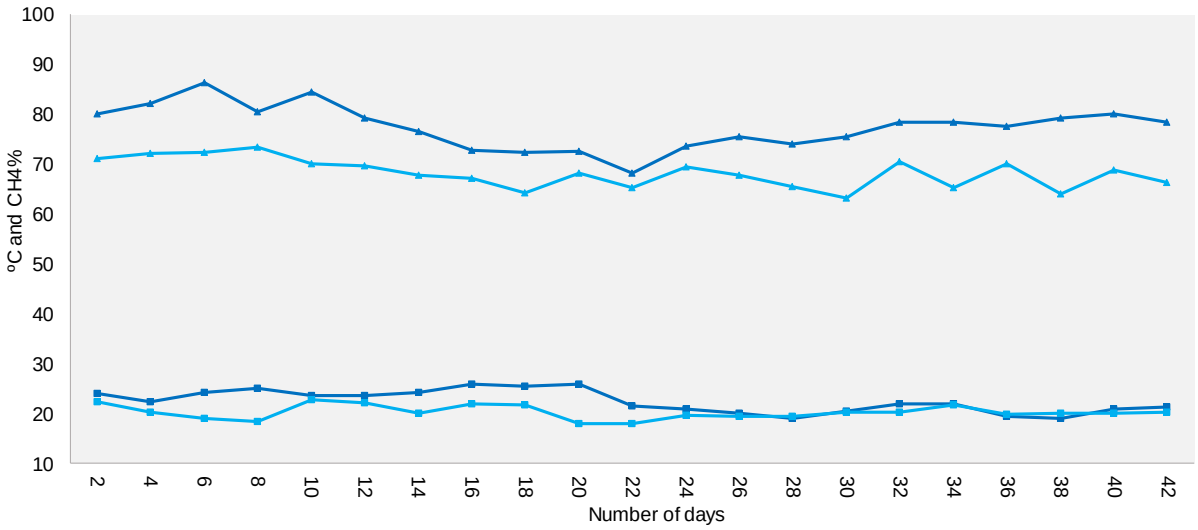

Figure 13: Graphical illustration of the CH_4 concentration (%) of AD1 (▲) and AD2 (▲) and the daily temperature variations (ºC) of AD1 (■) and AD2 (■) during Phase III. The data presented in Figure 13 are representative of 43 days of monitoring which commenced in Phase III on day 163 and continued to day 206. AD1 contained ≈15 m^3 of AD2 digestate and 84 m^3 of DFOD. AD2 contained ≈490 m^3 of digestate and ≈210 m^3 of LSM slurry.

During this phase, the total retention time in both AD1 and AD2 was sufficient for the growth of the methanogens. The agitation rate in both AD1 and AD2 was reduced to only three times a day for 45 minutes for five days a week. This change in agitation impacted the stability of the digesters positively. Anaerobic digestion is a complex process that requires strict anaerobic conditions with an ORP of -200 mV (Appels *et al.*, 2008). The ORP was measured every second day during Phase III, but the presented values were measured on days 41, 42 and 43 for both AD1 and AD2. The ORP values were -155, -151, -204 for AD1 and -138, -122, -156 mV for AD2. This indicated that the digesters were anaerobic and were approaching an anaerobic steady state of -300 mV (Demirel & Scherer, 2008).

4.3.5.1 AD1 and AD2 analysis

During Phase III, agitation of AD1 and AD2 was reduced to 2-3 hours per week because it had become evident that excessive agitation affected the digesters' performances negatively. After the digesters had been loaded with DFOD and LSM separately, a balanced and self-inoculated system that maintained an optimal acidogenic-methanogenic microbial ratio was achieved. A consistent production of biogas was developed (Figure 12), particularly as DFOD and LSM as microbial cultures appeared to have affected the digesters positively. On the first day of Phase

III, AD1 had a pH of 6.92 whereas AD2 had a pH of 6.99. The VFA was 6500 and 6850 mg L^{-1} for AD1 and AD2 respectively. This was an indication that both the digesters' pH was corrected by the addition of both inoculums, but the VFA was still high. However, because feeding of the digesters had been stopped, it created an opportunity for the methanogenic population to utilise the VFA that was available. The pH of AD1 increased to 8.04 on day 4 with a reduction of VFA to 3245 mg L^{-1}, attaining an alkalinity of 4625 mg L^{-1} $CaCO_3$. On the same day, AD2 had a higher alkalinity of 6625 mg L^{-1} $CaCO_3$ that was reflected by a pH of 7.42 and a VFA concentration of 4075 mg L^{-1}, indicating that the microbial activities in these digesters were high. Both digesters were now favouring acetogenesis and methanogenesis.

The high pH in AD1 on this day was possibly due to the high accumulation of ammonia, which is toxic at a pH of above 7 (Ward *et al.*, 2008). On day 7, the pH levels of AD1 and AD2 were 6.61 and 7.39 respectively, with an increase in VFA accumulated of 6125 and 7478 mg L^{-1} respectively. AD2 had a stronger buffering capacity because the pH did not lower when higher VFA accumulated on that day. The pH fluctuation of AD1 was possibly from the acetogenic organisms that had produced more acetate and the number of methanogens available to utilise it was reduced. On day 7, the alkalinity of AD2 was 7425 and 5600 mg L^{-1} $CaCO_3$ for AD1, which suggests that the buffering capacity of AD1 was more affected by the production of fatty acids than that of AD2. On day 13, the pH of AD2 had reduced to 7.00 because the VFA had accumulated to 8254 mg L^{-1}, and AD1 had a pH of 7.06 with a higher alkalinity of 8200 mg L^{-1} $CaCO_3$, indicating that even when VFA had accumulated to 7145 mg L^{-1}, the digesters maintained a stable pH. On day 15, the pH of AD1 had fluctuated to 7.46 with a high reduction of VFA, which resulted in 4905 mg L^{-1}.

A reduction of VFA was also experienced in AD2, because the VFA measure 7385 mg L^{-1} and the pH was 7.33. On day 18, a high level of VFA of 9055 mg L^{-1} was accumulated in AD1, and the pH was 7.28. On days 22, 35, 36, 41, 42 and 43, AD1 had a VFA level of 4865, 3245, 3660, 3245, 3132 and 3123 mg L^{-1} respectively, accompanied by an alkalinity of 5500, 4625, 6750, 7250,7365, and 7450 mg L^{-1} $CaCO_3$ respectively. It was clear that between day 22 and day 43, VFA were being utilized and the alkalinity was increasing, which indicated process balance that was also reflected by the CH_4% of 68.2 on day 22. A respective 78.45%, 77.56%, 79.20%, 80.06% and 78.35% CH_4 content was attained on days 35, 36, 41, 42, and

43 (Figure 13). CH_4 production in AD1 averaged at 75.80±4.98%, and these measurements were confirmed by four different GDIs. The digester was operating between 19-26ºC (Figure 13). It seemed that the inoculum-feed ratio had been well modified to maintain a constant pH, increase CH_4%, and reduced VFA accumulation. Thus higher buffering capability was created (Gunaseelan, 1995).

On day 22, AD2 had a pH of 7.91 with an increase in VFA accumulation of 6980 mg L^{-1}. On day 35, the VFA and pH levels had reduced to 4075 mg L^{-1} and a pH of 7.42, while the pH was 7.39 on day 36. The pH was constant on days 41and 42, and on day 43 it had increased to 7.35. The VFA in AD2 fluctuated from 5735 mg L^{-1} on day 36, to 3245, 3222 and 5735 mg L^{-1} on days 41, 42 and 43 respectively, whilst the alkalinity was 6000, 6250, 6311 and 9500 mg L^{-1} $CaCO_3$ on days 36, 41, 42 and 43 respectively. The CH_4% was 65.35 on day 22, and 65.2%, 69.96%, 68.87% and 66.3% on days 36, 41, 42 and 43 respectively (Figure 13). AD2 was characterized by a high pH of ≥7.30 because degradation of nitrogenous matter was taking place. This occurred mostly due to the presence of protein and urea because this digester consisted of large volumes of slaughterhouse waste and LSM. During the earlier days, the VFA that had accumulated were higher, probably because of high ammonia production (Angelidaki & Ellegaard, 2003), but from days 36 to 43 the VFA accumulation had reduced because methanogenesis was favoured. The microbial communities were adapted to psychrophilic conditions because the digester was operating between 18-23ºC. CH_4 production in AD2 averaged at 67.86±3.46% over the batch operation period of 43 days (Figure 13).

The co-digestion of both AD1 and AD2 with DFOD and LSM separately possibly adjusted the carbon-nitrogen (C: N) ratio. Because protein-rich materials have a lower C/N ratio, the co-digestion allowed proper microbial growth that led to process stability. AD2 and AD1 had a steady-state rate of methane production and accumulation of VFA, indicating high microbial growth and suggesting that the systems were not inhibited. DFOD and LSM improved the fermentation of RSC and the physicochemical parameters that allowed better-quality CH_4% of AD1 and AD2. When a digester is overloaded with organic materials, consistent low pH values and poor CH_4 production are the consequences. It was evident that the RSC that was used was perilous to the system, as it resulted in clogging of pumps and pipelines. It was thus clear that the RSC as a single seeding substance would not have started up AD2 during the retention time as had been intended in Phase I.

4.4 Conclusion

The initial unsuccessfulness of AD2 seeding was due to the unsuitable method that had been chosen to inoculate the digester and the use of RSC as an inoculum because it was unsuitable for providing consortia of facultative and anaerobic microorganisms. It soon became evident that the parameters that were monitored often were important, but that they were not able to provide early warning of process instability during inoculation. This study established that DFOD and LSM can possibly be used to revitalise industrial dormant digesters although the addition of LSM will introduce sand and stones in a digester. Regardless of the quantities of total solids present in a dormant digester, if proper system requirements are achieved such as proper seeding material, correct process monitoring, precise hydraulic retention time, impeccable agitation, proper feeding rates, the system can then balance naturally and high-quality CH_4 can be produced. This study also provided a pragmatic view that the old steel refurbished Pachuca tanks that had once been used as gold processing units can be repurposed for biogas production, given that the correct inoculum is used and proper process monitoring is applied.

Chapter 5: An investigation of methods for revitalising an industrial dormant digester (IDD)

5.1 Introduction

Anaerobic digestion has been used to treat high organic waste from multiple industries such as distilleries, paper factories, slaughterhouses, cheese factories and olive oil mills (Maragkaki *et al.*, 2017; Rajeshwari *et al.*, 2000). Degradation of organic matter to produce methane (CH_4) via anaerobic digestion occurs in four phases. The growth of microorganisms in different phases of anaerobic degradation of the substrate has a specific chronology and order, and each consortium has to adapt to breaking down a specific substrate, as the product formed by one group serves as a subsequent substrate for the next (Jørgensen, 2009; Ostrem *et al.*, 2004; Mata-Alvarez *et al.*, 2000; Noike *et al.*, 1985). All these different consortia need to be active and symbiotic for the formation of biogas, and an uninterrupted collaboration of this microorganism is required in order to achieve high biogas production (Gerardi, 2003). When the anaerobic process becomes unbalanced, the effect is unfavourable for biogas generation as it leads to low gas production, low CH_4 concentrations, and high volatile fatty acids (VFA) production (Chen *et al.*, 2008).

To start up a new anaerobic process, it is important to use a proper inoculum of microorganisms. Seeding material will help establish an anaerobic microbial flora as the lag phase will be eliminated and this will result in increased biogas production and good gas quality (Sreekrishnan *et al.*, 2004). Commonly used seeding materials include digested sludge from a running bio-digester, wastewater treatment sludge, rotting manure from a pit, cow manure slurry, and rumen fluid. Rumen fluids are a better inoculum than rumen solids because rumen solids provide a diminutive number of microorganisms (Gulhane *et al.*, 2017; Franke-Whittle *et al.*, 2014; Gu *et al.*, 2014; Wang *et al.*, 2014; Xu *et al.*, 2013). Many different species of bacteria are found in the anaerobic process and they have different needs and functions (Rizwan *et al.*, 2015; Kumar *et al.*, 2013). Important factors to consider when operating a successful digester include the type of feedstock used, environmental conditions, and the presence of potential toxins (Drosg, 2013; Suryawanshi *et al.*, 2013). If an

appropriate substrate is selected and supplied, optimum performance of the microbial population can be achieved. The more varied the composition of the organic material, the more components are available for growth, and thus the greater the diversity of organisms that can grow (Schnurer & Jarvis, 2010).

Different anaerobic digestion parameters such as temperature, pH, and VFA can negatively affect the dynamics of microbial consortia. Operational defects such as overfeeding and improper monitoring procedures will also have a negative effect on microorganisms (Cavinato *et al.*, 2017; Divya *et al.*, 2015). Typical causes of organic overload and acidification are because of incorrectly measured inputs or increased mixing which lead to the inclusion of unreacted materials in the digestion process (Jiang *et al.*, 2012). An organic overload occurs when the quantity of organic matter fed into the bio-digester exceeds the total degradation capacity of the microbes to produce biogas (Roubík *et al.*, 2017; Massart *et al.*, 2006). Additional factors that can lead to an inert digester are: an unexpected change in pH, an increase in organic matter concentrations, an alteration of the loading rate, or the introduction of toxic compounds into the system (Tian *et al.*, 2015).

To revive an adversely affected digester, techniques such as decreasing the loading rate of feed, increasing hydraulic retention time (HRT), retention of feeding, and the use of nutrients have been employed (Sonakya *et al.*, 2001; Callander & Barford, 1983). When a digester's pH is out of range, it is indicative of an anaerobic process dormant. The digester can be revived by removing half of the content and replacing it with fresh methanogenic sludge (Melamane *et al.*, 2007). During Phase II of the current study that was described in the previous chapter, affirmation of AD2 as dormant occurred when the pH, VFA, CH_4 yield and biogas volume were below the acceptable ranges for biogas production. The research process and findings that are presented in this chapter reflect on an investigation into variations in hydraulic retention time (HRT), temperature, and inoculum type in a laboratory scale digester to determine possible solutions for resolving challenges experienced with industrial size dormant digesters (as was the case with IDD-AD2 in this study).

5.2 Materials and Methods

Samples of IDD-AD2 digestate were collected on day 34, and these bulk samples were used in Trial 1. The second sample that was collected from AD2 on day 98 was used for Trial 2 (Figure 2).

5.2.1 Trial 1: IDD-AD2 digestate degradation in psychrophilic and mesophilic conditions

IDD-AD2 digestate degradation was conducted in both mesophilic and ambient temperatures. Trials were performed in 5 L digesters that were designed in the style as depicted in Figure 3. Trial 1 was conducted in nine batch anaerobic digesters as depicted in Figure 14.

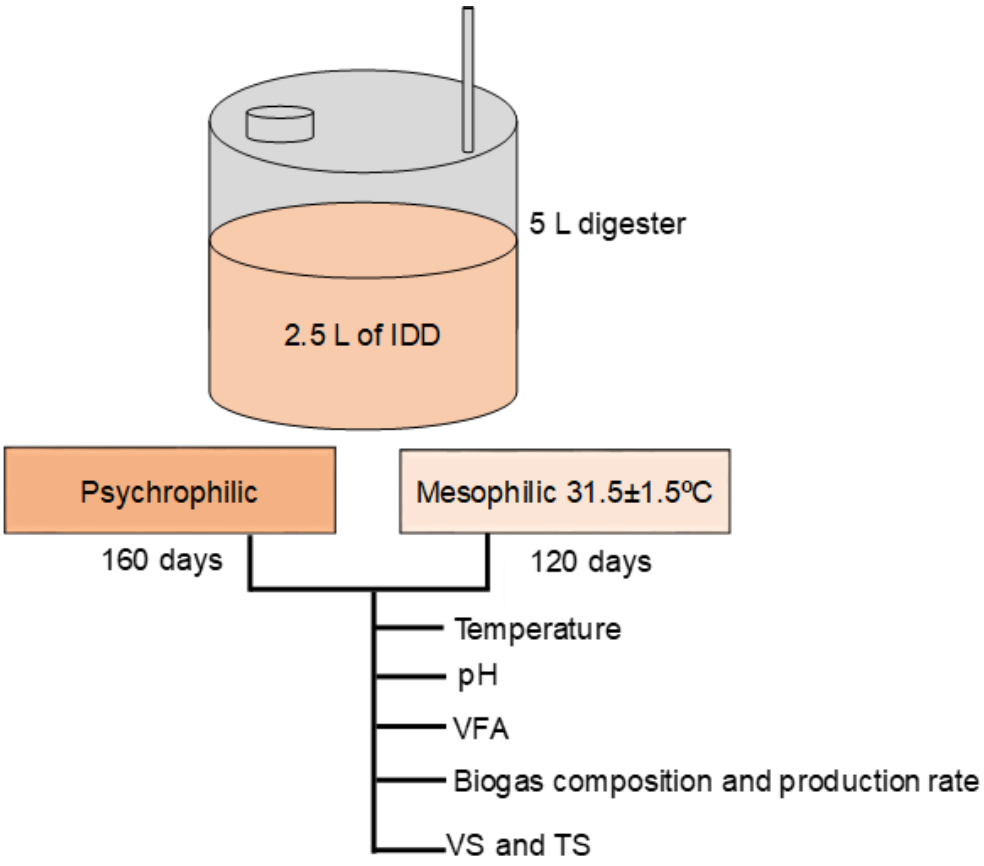

Figure 14: Schematic illustration of the batch digester set-up and the test parameters. Six digesters were operated in psychrophilic conditions for 160 days and three in a mesophilic condition (31.5±1.5ºC) for 120 days. The conditions that are important operational parameters for the anaerobic digestion of digestate slurry were determined.

The batch test experiments were divided into two groups. The trials that were operated in psychrophilic/ambient temperature utilised six digesters. Three digesters were operated in a mesophilic condition (i.e., the controlled temperature was maintained at 31.5±1.5ºC) (Figure 14). The mesophilic digesters are presented as D5-D7 and the psychrophilic digesters as D1-D4. A volume of 2.5 L of the digestate was added to each 5 L digester, and the digesters were sealed for 120 days for mesophilic trials and for 160 days for psychrophilic trials. The digestate material from IDD-AD2 was loaded without any pre-treatment, and the TS content of the digesters was maintained at ≥18%. VS and TS reduction were used as indicators of digestion advancement.

To investigate process developments, the following parameters were also measured: pH, temperature, retention time, VFA, amount of gas produced, and CH_4 concentration. The contents of the trial bio-digesters were mixed by vigorous manual shaking three times a day for ±15 seconds (i.e., in the morning, at midday, and in the late afternoon every day). The method of mixing was not a reflection of how the IDD-AD2 was mixed, as in this phase of the study, mixing was intended to be reduced. The pH was determined every second day, and the TS and VS were determined before and after anaerobic digestion. The level of the VFA was determined at selected times while the temperature was determined daily. The parameters were determined using the same methods and materials as described in Chapter 3.

5.2.2 Inoculum comparison of the trials

To achieve the goal for Trial 2, the seeding materials of interest were investigated and selected. The experimental procedure that was applied in this section of the study was intended to determine the best inoculum feedstock source that could be used for the revitalization of IDD-AD2. Depending on availability and accessibility, the inoculums were prepared from rumen solid contents (RSC) that had been obtained from a slaughterhouse, municipal wastewater treatment sludge (MWWTS), digestate from an operating digester (DFOD), and long-standing manure (LSM) that had been used as cow bedding. Ample literature is available on fresh cow manure (FCM) as a seeding material, and therefore the inoculum prepared from FCM was compared with the other selected seeding materials (Haryanto *et al.*, 2018; Ukpai *et al.*, 2015; Abubakar & Ismail, 2012).

5.2.2.1 Source of inoculum materials and preparation

a) Rumen fluid inoculum (RFI) preparation

The RFI was prepared from rumen solid contents (RSC) which had been collected from a nearby slaughterhouse. The RSC included all the waste from the slaughterhouse and was collected in a skip bin meant for disposal. This RSC is referred to as RSC1. The RSC that was collected just after the evisceration unit included only organic waste such as skin, bones and other organics and this is referred to as RSC2. Rumen fluid inoculum 1 (RFI1) was prepared from RSC1 and the inoculum that was prepared from RSC2 is referred to as rumen fluid inoculum 2 (RFI2). RFI1 and RFI2 were prepared by mixing 5 kg of RSC1/2 feedstock with 25 L of water. This was done in two separate 50 L containers. To prepare RFI1 and RFI2, a sample was collected from the respective containers and the samples were primed by passing RSC:water mixture through sieves of different sizes (1250, 500,.12.5, 2 mm) . The mixture was filtered using filter paper and a 1 µm cartridge filter was then used to achieve a high microbes to solid ratio. RFI3 was prepared by mixing RFI1 and RFI2 in a ratio of 1:1.

b) Municipality wastewater treatment sludge (MWWTS) and inoculum obtained from the operational plant (DFOD)

MWWTS was obtained from government digesters used to treat domestic sewage waste that is operated in mesophilic temperature. DFOD was obtained from an operational plant that treats cow manure and industrial confectionary by-products in mesophilic temperature. DFOD and MWWTS were both used without any modification and were both purchased from the sources.

c) LSM inoculum preparation

LSM was selected because it is cheap, abundantly available, and had been found to be the most feasible feedstock available for the bioenergy project because only LSM and rumen fluid inoculum (RFI) were assessable free of charge. Cattle manure that had been on the floor in a shed where it had been used as bedding for cattle for a period of more than 90 days was collected. LSM was collected from the same farm where the FCM was collected and was stored in an open 20 L container for one or two day(s) before use. The LSM slurry was prepared by adding tap water in a ratio of 5:1 (water:LSM), and supplementary water was used to create an LSM slurry with high moisture content. To prepare the LSM inoculum, the slurry was thoroughly

filtered by using a number of sieves of different sizes (1250, 500, 12.5, 2 mm). The slurry prepared from this feedstock was characterized by high inorganics.

d) FCM inoculum preparation

FCM was collected from a local farm where it was harvested minutes after excretion. It was used as soon as possible after collection. The FCM inoculum was prepared by mixing fresh cow dung with tap water in a ratio of 1:1. The prepared slurry was filtered using a 2 mm sieve to remove excess solids.

5.2.2.2 Experimental procedure

The compositions of the various inoculums were determined. Inoculum comparisons were performed in 2 L digesters designed in the same manner as depicted in Figure 3, except that a 5 L gas collector was used instead of a 10 L one. The different digesters were all filled with 1.5 L of inoculum and they were thoroughly sealed. Each laboratory reactor digestion trial was performed in duplicate at a temperature of 31.5±1.5ºC. The inoculums were tested for accumulative and daily biogas production for a period of 30 days and the readings were recorded.

5.2.3 Trial 2: Addition of inoculating material (methanogen addition) to IDD-AD2 digestate

The effect of inoculums on digester revival or enhancement was investigated. All the digesters were filled with 2.5 L of IDD-AD2 digestate, which was followed by the addition of 0.75 L, 1 L and 1.25 L of the selected inoculums respectively (Figure 15).

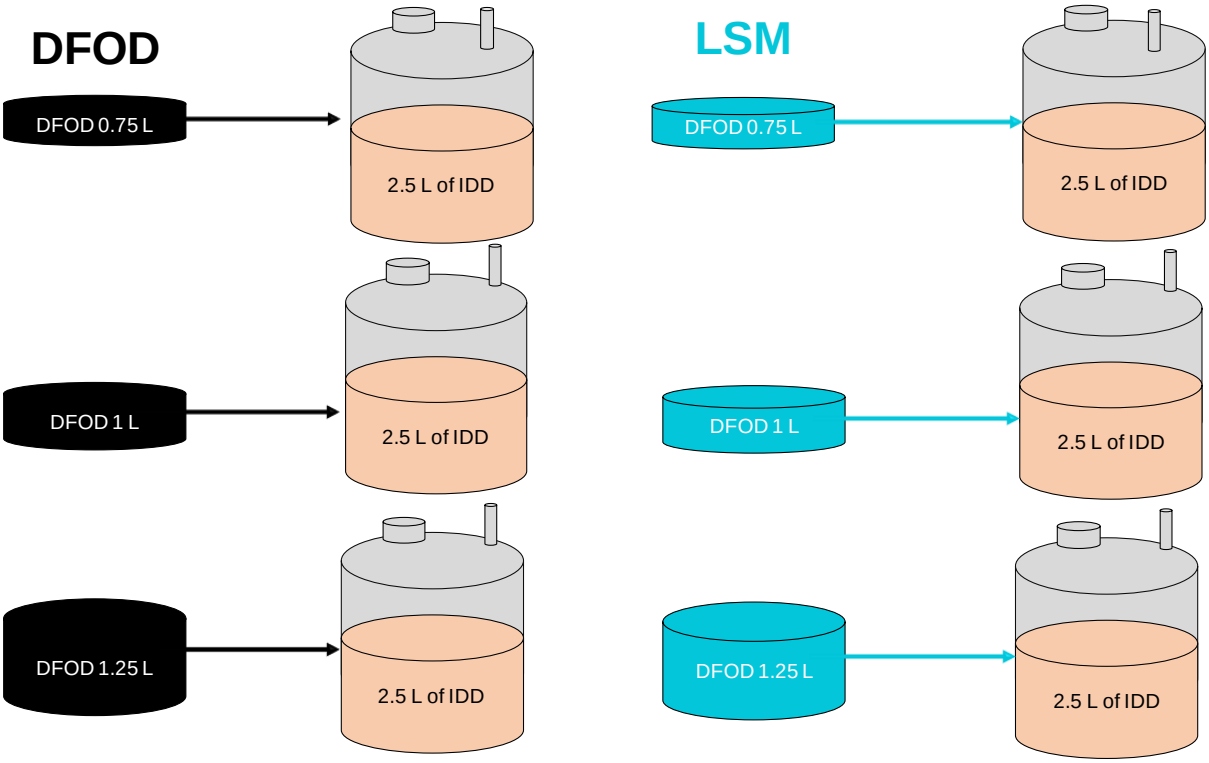

Figure 15: Schematic illustration of Trial 2 experimental design. Digesters loaded with 2.5 L of industrial dormant digestate were loaded with different inoculum volumes of 0.75 L, 1 L and 1.25 L of LSM and DFOD respectively. The digesters were allowed to digest in a mesophilic condition (31.5±1.5ºC) for a period of 30 days.

Each LSM and DFOD experiment involved nine different digesters. The tests were performed as illustrated in Figure 15. These digesters were operated in a temperature of 31.5±1.5ºC in triplicate. The digesters were sealed for 30 days and the parameters that were determined were pH, temperature, retention time, TS and VS reduction, VFA, gas production, and CH_4 concentration. The methods and materials described in Chapter 3 were used.

5.2.4 Calculations

To determine the rate of biogas production, the quantity of VS in the digester was determined on day 1 and again after 30 days of anaerobic digestion. After measuring the total volume of biogas that had accumulated, the rate of biogas production was determined using the following equation:

$$\text{Biogas production rate} = \frac{\text{Total gas accumulated (mL)}}{\text{Quantity of VS degraded (mg)}} \qquad \text{Equation 6}$$

5.3 Results and Discussion

The trials were conducted to investigate the digestibility of unpasteurized rumen solid contents (RSC). The duration at which a significant quantity of RSC was digested and the associated amount of gas produced were of particular interest. It was deemed imperative that the study should provide an insight into whether RSC can be considered an appropriate inoculum to start up an industrial digester.

5.3.1 Characteristics of the digestate

5.3.1.1 pH before anaerobic digestion

The psychrophilic digesters are identified as D1-D4 and the mesophilic digesters are identified D5-D7. Digestate obtained from the industrial dormant digester AD2 (IDD-AD2) was digested without pH adjustment. The pH levels of D1-D4 and D5-D7 were determined before starting the trials, and the established average pH of all the digesters was 4.63±0.05 before D5-D7 were exposed to heating. When cow rumen and slaughterhouse waste were used as substrates, a pH of 6-7 was determined (Newman *et al.*, 2017). The low pH of the digestate in the digesters indicated that the digesters were to progress below the optimum pH range. The pH levels of D1-D4 and D5-D7 were projected to balance themselves to the desired pH and to attain a buffering capacity without the addition of any chemicals. The ideal pH for an anaerobic digestion is 6.80-7.20 (Weiland, 2010), whereas Mshandete *et al.* (2006) indicate that a pH of 6.50-8.50 is the optimum range. However, in the trial the average pH level of D1-D4 and D5-D7 was lower because of a higher VFA concentration in the IDD-AD2 digestate. The high VFA concentration of the digestate was because the digestate that as used was in the process of digesting when collected and was thus already semi-digested. Stabnikova *et al.* (2008) operated successful digesters using leachate with a VFA concentration of 9450.00 mg L^{-1} and a pH of 5.00.

5.3.1.2 Solids concentration

The digestate that was used to start up the digesters were characterized by a TS concentration of ≈18.20%, a VS% of ≈79.00, a pH of 4.63±0.05, and VFA of ≥8050 mg L^{-1}. The moisture content of the digesters before starting them up was low, as

substrates with a high TS content are difficult to mix, which decreases the degree of digestion (Dai *et al.*, 2013). A high TS% results in inadequate mixing. Abbassi-Guendouz *et al.* (2012) experienced low gas production in digesters with TS of ≥10% and mixing in these digesters was important for gas production. A digestate with a higher TS of ≈18% was expected to have a lower VS destruction and a lower CH_4 production rate because thick sludge is considered an important operational factor that affects digester performances (Karim *et al.*, 2005).

5.3.2 Remediation of dormant digesters in psychrophilic and mesophilic conditions

Anaerobic digestion that takes place at temperatures of below ≤25ºC are referred to as psychrophilic, whereas mesophilic conditions operate at a temperature of ≥25ºC (Shitophyta, 2016). Two out of six psychrophilic digesters were unsuccessful, as they were characterized by non-detection of CH_4 and no pressure developed in these two lifeless digesters to force liquid displacement. Because no microbial testing was performed, the digesters were considered unsuccessful via a cessation change of VS, VFA, and the pH, and thus a good environment that allows microbial grow was not established in these two digesters. When determining different parameters in the mesophilic digesters, close results were obtained as the digesters performed similarly. In the psychrophilic digesters, there was a slight difference in the performances of the digesters. For example, the individual psychrophilic digesters required different start-up times. Biogas production, CH_4 concentrations, VFA, alkalinity, pH levels and temperature were used as indicators of process development in digesters D1-D4 and D5-D7.

5.3.2.1 pH levels and VFA in the digesters

The mesophilic and psychrophilic digesters were operated in the batch/single stage, and all the biochemical reactions occurred in a single fermenter. The anaerobic microbes involved in these reactors were varied. The optimal pH levels also varied as they were either alkaline or acidic and the physiological needs were also unique. Single-stage digesters have been reported to always perform below the optimal level, resulting in either less biogas production or less CH_4 content (Song *et al.*, 2004). The cause of delayed biogas production observed in both mesophilic and psychrophilic digesters was the unregulated pH regime that was employed during

this study, which led to an average operational pH of 5.89±0.12 in D1, 6.20±0.51 in D2, 6.56±0.29 in D3, and 5.78±0.33 in D4 (Figure 16).

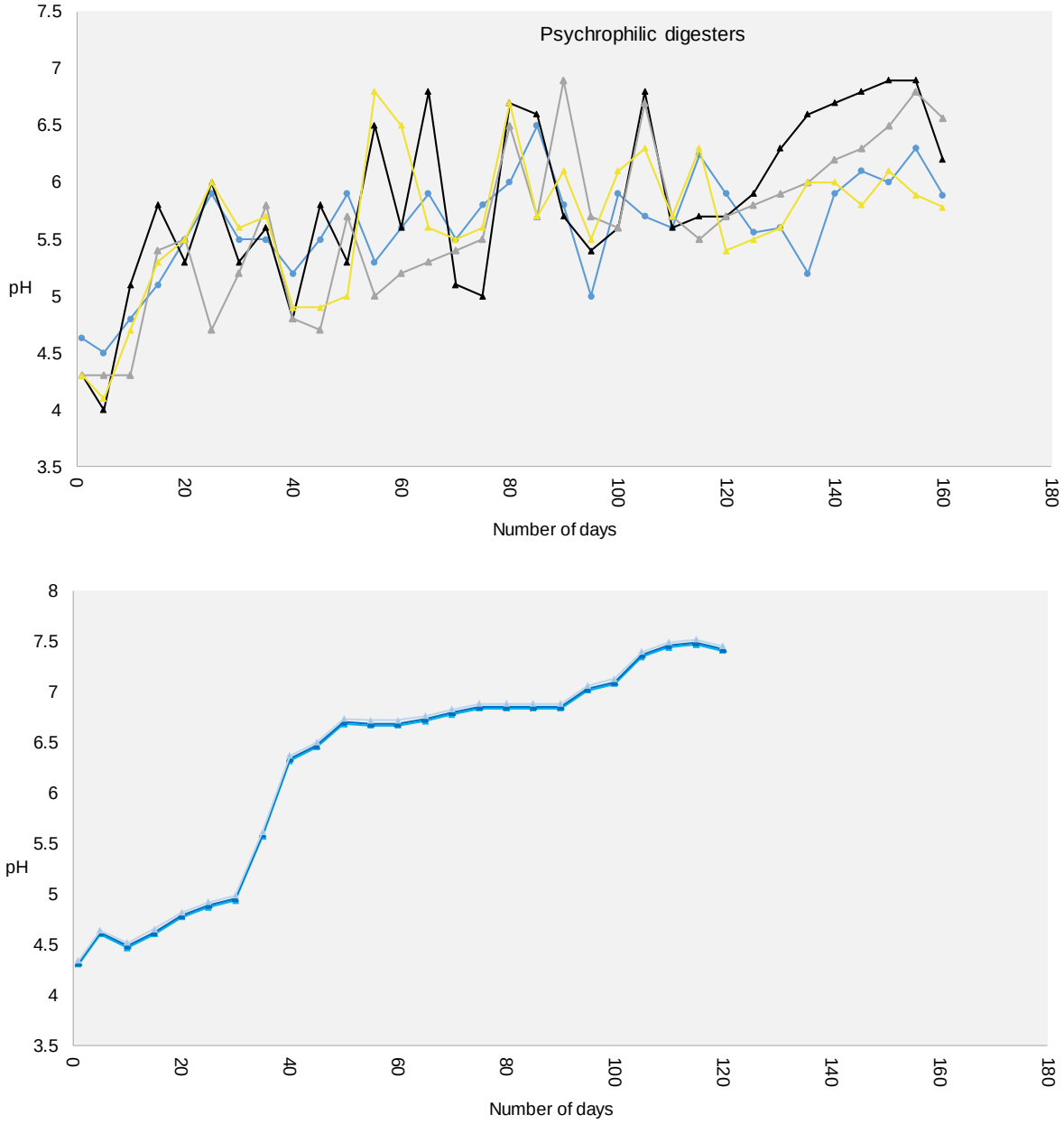

Figure 16: The pH in the psychrophilic digesters (D1 (▲), D2 (▲), D3 (▲) and D4 (▲) and in the mesophilic digesters D5 (▲), D6 (▲), and D7 (▲).The data are representative of 160 days of monitoring that commenced in Trial 1 on day 51 and continued to day 218.

The pH of the two unsuccessful digesters was ≈4, and pH fluctuation was observed in the psychrophilic digesters. The pH values of the psychrophilic digesters were below the recommended operational values of 6.5-7.0 (Weiland, 2010). The pH for D1 was 4.5 while it was 4.0 for D2, 4.1 for D3, and 4.3 for D4 on day 5. The pH levels of these digesters were low due to a high presence of VFA that were not yet

utilized by the microbes (Lu *et al.*, 2008). The pH of D1 fluctuated between 5.2 and 5.9 from day 25-65. During the same period, D2 had a pH of between 4.8 and 6.8, D3 had a pH of 4.8-6.8, and D4 had a pH of 4.7-6.5. The pH of D1 was between 5 and 5.6 from day 66 to day134, and on day 155, it fluctuated to a pH of 6.3, which went down to 5.89 on the last day. The pH of D2 fluctuated to a lower value of ≈5 on day 75, but 5 days later it was 6.7. From days 95-115 it fluctuated between 5.4 and 6.8. A gradual increase in pH was noticed from day 116 to day 155. D3 had a pH fluctuation of 5.5-6.7 from day 55 to day 115, and the pH was between 5.4-5.8 from day 120 to day 160. D4 had a lower pH of between 4.7 and 6.9 from day 25 to day 90, and from day 115 the pH increased slowly from 5.5 to 6.5 while it was 6.8 on day 160. The increase in pH between day 116 to day 155 and day 115 to day 160 for D3 and D4 respectively was due to VFA utilisation and alkalinity development in the digesters (Jiang *et al.*, 2013; Hejnfelt & Angelidaki, 2009).

The general pH fluctuation in digesters D1-D4 was caused by unstable reactions occurring in the digesters. Also, the hydrolysis, acedogenesis and methanogesis steps were not balanced (Mata-Alvarez *et al.*, 2000; Speece, 1996). Because the pH levels in these digesters were not regulated, VFA accumulation regularly occurred causing a drop in pH that affected the methanogens negatively. Ward *et al.* (2008) reported a reduction in the growth rate of methanogens at pH ≤6.60. The VFA concentrations in the psychrophilic digesters ranged between 4000-4833 mg L^{-1} on day 160. Drosg (2013) and Siegert and Banks (2005) indicate that a VFA level of ≥4000 mg L^{-1} is toxic to the digester and that the digester will be in between states (i.e., stable or unstable), whilst VFA levels of >4000 mg L^{-1} have a negative effect on microbes and will result in low gas production or digester demise. Bouallagui *et al.* (2004) suggest that reduction in VFA production indicates that acetate and propionate production is decreasing. If the methanogens were utilizing some of the VFA available and the concentration of VFA remained above the recommended value, the digesters were then in favour of the hydrolysis step. The mean pH of the psychrophilic digesters in this study was observed to be at low operational value with high fluctuations due to the low buffering capacity of the RSC.

The average concentration of VFA in mesophilic digesters was above 5000 mg L^{-1} for the first 35 days, which was caused by a low mean pH of between 4.30±0.58 (Figure 17). The increase of VFA concentration is attributed to low utilization of VS

and VFA provided by the semi-digested IDD-AD2 digestate. From day 40 to day 80, the pH of the mesophilic digesters increased from an average value of 6.31±0.25 to 6.80±0.21 (Figure 18). The VFA concentration was at an average of 5432.00±60.3 mg L^{-1}. The highest VFA accumulated of 6031±30.5 mg L^{-1} was on day 47 (Figure 17). There was a significant reduction of VFA concentration from day 59 to day 120, which was attributed to a high pH value of 7.43±0.60. On day 105, the pH was 7.30±0.07, and on day 120 the pH=7.40±0.11 and the VFA concentration was 2800.00±262.00 mg L^{-1}.

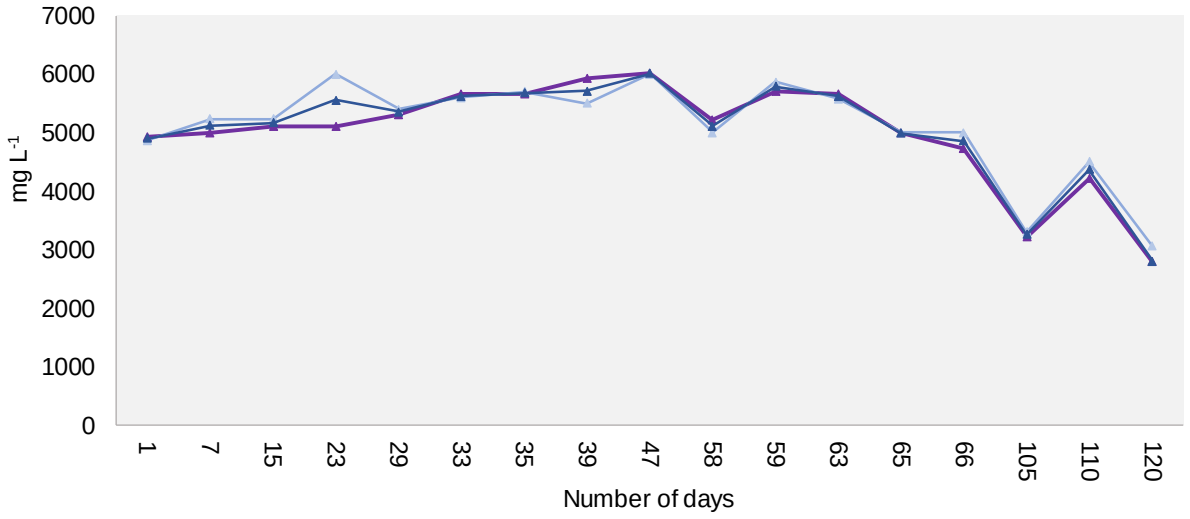

Figure 17: VFA variations during operation of D5 (▲), D6 (▲), and D7 (▲). The data are representative of 160 days of monitoring that commenced in Trial 1 on day 51 and continued to day 218.

Strik *et al.* (2006) revealed the same behaviour in the mesophilic digesters they used when pig waste was co-digested with maize. D5-D7 digesters operated at an expected pH during the last two weeks of operation. Similar pH conditions were reported by Bougrier *et al.* (2018) and Salminen and Rintala (2002) when they used brewery and slaughterhouse wastes as feed respectively. RSC has a high ammonia concentration potential, and thus the formation of a high ammonia concentration in the current trials possibly occurred during the degradation of the RSC, which caused elevated pH levels (Chen *et al.*, 2008). The ammonia that was produced when heat was applied possibly favoured the buffering of the digesters. Increasing temperatures in D5-D7 balanced the pH and lowered the retention time required for gas production, while the VFA accumulation was also lowered when compared to the psychrophilic digesters.

5.3.2.2 Gas production

a) Gas quality

CH_4 was detected earlier in the mesophilic digesters compared to the psychrophilic digesters. Low microbial activity was possibly the cause of low gas production during the earlier days of the trial because low temperature affects the cellular structure and function of bacteria (D'amico *et al.*, 2006). In the mesophilic digesters, a CH_4% of 7.3±1.9 was noted on day 5. The gas quality in the mesophilic digesters showed a CH_4 concentration improvement on day 30, which was between 10-20%. The CH_4% of these digesters was high compared to that of the psychrophilic digesters during the same period. After 120 days of operation, the average CH_4% in the mesophilic digesters was 45±3%. The mesophilic digesters (D5-D7) had a poor average CH_4 concentration compared with similar waste mentioned in the literature. For example, Newman *et al.* (2017) achieved a 60% CH_4 concentration in ≈60 days of operating a digester in mesophilic temperature using a mixture of organic slaughterhouse waste as feed. Hejnfelt and Angelidaki (2009) obtained a high CH_4 concentration of 69-75% from similar organic waste in less than the degradation period that was achieved for D5-D7 in the current trials.

It was noted that the CH_4 concentrations in the digesters were different during the initial stages under psychrophilic conditions because D1 had a 2% CH_4 on day 30, D2 had a 2.5% on day 40, and D3 had a 1% and D4 had a 0.9% on day 35. During days 30-40, low-pressure gas was produced that failed to displace the liquid in the measuring cylinder. A higher CH_4 content was expected in D1-D4 because Connaughton *et al.* (2006) reported a CH_4 concentration of ≥50% in less than 40 days of operating digesters in psychrophilic temperature using slaughterhouse waste. After 160 days of operating D1-D4, the mean CH_4 concentration was only 27.4±3.6%. When comparing D1-D4 with other digesters operated in low temperatures using lignocellulose feed and other similar types of feed, Saady and Massé (2013), Dhaked *et al.* (2010) and Hill *et al.* (2001) noted that the microbial activities in D1-D4 were low and that the CH_4 yield was poor. Trzcinski and Stuckey (2010) argue that psychrophilic temperatures can decrease the number and activities of microorganisms.

b) Weekly biogas production

To condense the data that were collected, the gas volume collected over a week (for the purposes of this study a week was five days) was converted to a standardized volume per day, added, and presented in this section of the study as weekly biogas production. The method to represent cumulative biogas production was adapted from Comino *et al.* (2010) and Macias-Corral *et al.* (2008). The trial digesters that operated under low temperature were expected to have an extended start-up time. Digesters D1-D4 experienced a sluggish start-up of gas production, but Saady and Massé (2013) reported a shorter lag phase of fewer than five days for the psychrophilic digesters in their study. Rapid digester start-up periods at low temperatures were also reported by Bouallagui *et al.* (2004) and Kashyap *et al.* (2003). Connaughton *et al.* (2006) also observed a rapid start-up period in psychrophilic digesters when brewery wastes were used as feed, and these digesters had higher rates of COD removal and a higher biogas volume when compared to D1-D4. It was speculated that a longer trial period was required for the development of psychrophiles in D1-D4 (Abubakar & Ismail, 2012). When the weekly and accumulative biogas production of all psychrophilic digesters were compared, no statistically significant difference was determined. This was also the case for the mesophilic digesters because the p-values were >0.05. The reason for the delayed gas production in both the mesophilic and psychrophilic digesters might have been the extended lag phase of microbial growth because the contents were adjusting to the new environment (Cirne *et al.*, 2007; Angelidaki & Ahring, 1992). The high organic solid content to microorganism ratio possibly caused the extended lag phase (Najafpour *et al.*, 2006) because both the mesophilic and psychrophilic digesters had abundant nourishment with a high proportion of organic solid to the microorganism, which possibly overwhelmed the limited microbes that were available. Abubakar and Ismail (2012) suggest that the biogas production rate in batch conditions is directly equal to the specific growth of methanogens. This implies that the volume of gas produced during this study was a reflection of the quantity of microbes that was available.

Neither the mesophilic not the psychrophilic digester was purged of oxygen. Oxygen abided in the digesters during the early stages, and so the aerobic microorganisms were exposed to. After the aerobic bacteria had used up the oxygen, the acid-forming bacteria became active and gas production began. In D1-D4, a small volume

of biogas was noticed during the earlier days (Figure 18). Gas production in the psychrophilic digesters started earlier in D1 than in the other three digesters.

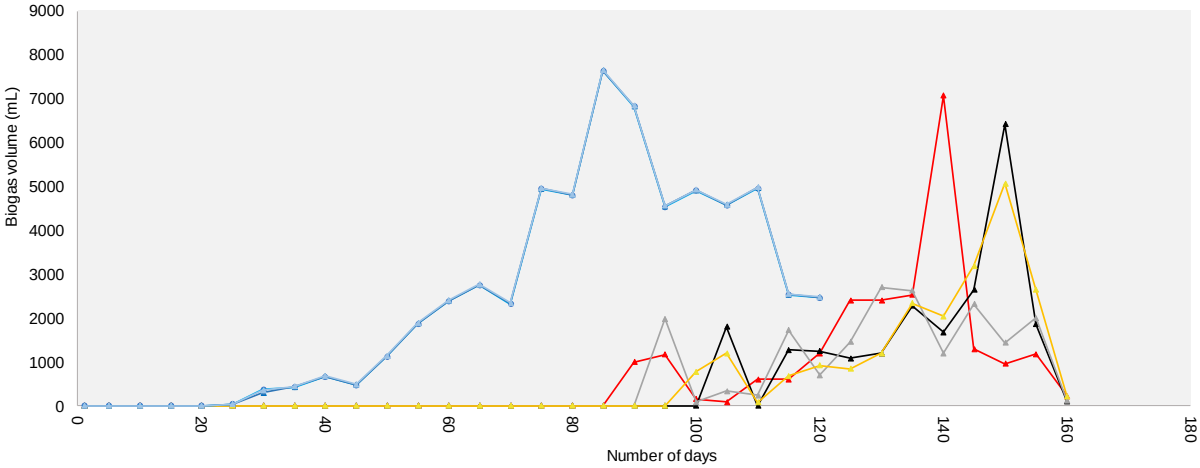

Figure 18: Weekly biogas production in the mesophilic digesters D5 (▲), D6 (▲), and D7 (▲) and the psychrophilic digesters D1 (▲), D2 (▲), D3 (▲) and D4 (▲). The mesophilic digesters were operated for 120 days and the psychrophilic digesters were operated for 160 days. The volumes of gas that were produced were collected and recorded every five days. These volumes are expressed as the volume of gas produced per week. The data are representative of 160 days of monitoring that commenced in Trial 1 on day 51 and continued to day 218.

A noticeable volume of biogas that was produced in the psychrophilic digesters occurred after 85 days after commencement. This occurred because the microorganisms that had been inactive had now adapted to the new environment and thus they became active and gas was produced (Grimberg *et al.*, 2015). From day 86 to day 90, the biogas volume for D1 was 996 mL. In D2 it was 83 mL, in D3 it was 1980 mL, and in D4 it was 72 mL (Figure 18). The biogas production was thus more varied in D1-D4 compared to what occurred in D5-D7. It may be argued that if the psychrophilic digesters were representative of IDD-AD2, then AD2 required a longer time to start producing biogas. Thus when or if feeding was stopped, there was a 33.33% chance that AD2 was going to fail. Wei *et al.* (2014) needed a long solid retention time of ≥80 days for the digesters to start producing biogas at low temperature and low air pressure conditions when sorghum was used as feed, demonstrating that digesters containing high fibre content require a longer period of time to start producing gas at low temperatures. The most noticeable gas production that occurred in D5-D7 started on day 25, a small volume of 40.00±9.28 mL was collected. Appels *et al.* (2008) point out that higher temperature increases the

solubility of organic compounds and enhances the biological and chemical reaction rates occurring in them. Thus the heat applied to D5-D7 was able to activate the microbial population that was available in the digestate. This was the case because earlier gas production occurred in these digesters than in the psychrophilic digesters. The occurrence of high microbial activity in the mesophilic digesters was indicated by the high weekly gas production that was measured in these digesters (Figure 18). Yadvika *et al.* (2004) also found that, when operating digesters in mesophilic and thermophilic conditions, gas production was improved, but Kim *et al.* (2002) elaborated that slower growing methanogens in a single stage digester were affected more by a higher temperature. They argued that this was due to an unbalanced reaction rate between acidogens and methanogens.

The current study found that D1 produced the highest weekly gas production rate of 7068 mL, while D2=6420 mL, D3=2700 mL, and D4=5070 mL. The differences in the weekly production rates were attributed to inconsistent agitation resulting in improper homogenization that, in turn, affected the digesters' performance. The highest gas volume achieved in the mesophilic digesters occurred between days 81-85, when the gas volume amounted to 7632.03±432.00 mL. A lower gas production in D1-D4 was produced during the last weeks of operation, while the same lower biogas production was observed in D5-D7 as a volume of 2460.20±60.19 mL was produced (Figure 18). This low production was probably due to a lack of sufficient microbes to utilize the VFA or lignocellulosic constituents available. D1-D4 showed a high biogas fluctuation, which indicated process instability caused by higher TS% and VFA accumulation. Astals *et al.* (2012), Bouallagui *et al.* (2003) and Kim *et al.* (2002) demonstrated that high TS% resulted in lowered pH due to VFA accumulation. They argue that if a lack of sufficient microbes to utilize the VFA exists, gas production will cease, resulting in process instability. Gas production in D5-D7 showed a stable increase from day 25 to day 40, while a small decrease was observed from day 46 to day 50. From day 51 to 90, an exponential increase was observed until the highest weekly gas production was reached on day 81.

Low biogas production was observed in D1-D4 (days 96-100, 101-105, and 111-115) as well as in D5-D7 (days 120-125, 45-50, 90-95, and 110-120). This was attributed to a number of factors such as unbalanced nutrient to microorganism ratio, unstable pH value, low microbial activities, and the presence of certain quantities of components in the digestate such as cellulose and lignin that are relatively difficult to

degrade and require longer retention times for hydrolysis (Zhai *et al.*, 2015; Zhang *et al.*, 2015b; El-Mashad & Zhang, 2010; Taherzadeh & Karimi, 2008; Schofield *et al.*, 1994). It was also observed that, in both D1-D4 (days 91-95, 106-110, 126-135, 136-140, and 140-145) and D5-D7 (days 25-40, 50-80, and 95-100) higher weekly biogas production occurred (Figure 18). The escalation of biogas production in each of these digesters in these periods probably occurred due to an increase in microorganisms that occurred concurrently with the development of favourable conditions in the digesters (Weiland, 2010). The mesophilic digesters started producing gas earlier than the psychrophilic digesters and a higher weekly gas production was observed in D5-D7 than in D1-D4. This was due to the higher temperature that was applied to D5-D7 which had an impact on gas production. Bouallagui *et al.* (2004) observed a similar behaviour of slow gas production in digesters operated in psychrophilic conditions, where it took ≥80 days for digesters to start producing a significant amount of biogas volume.

c) Cumulative biogas

The conversion of VS to biogas in an anaerobic digester is controlled by the degradation time that is assigned, as increasing the retention time does not always make a significant contribution to the increased destruction of VS (Mudhoo, 2012). Even when the degradation time was increased in the current study, the D1-D4 psychrophilic digesters produced an uneconomically low gas volume (Figure 19).

The total volumes of biogas that accumulated in D1-D4 were varied: D1=22856 mL, D2=21574 mL, D3=18923 mL, and D4=21190 mL. The differences in the accumulated gas volumes in the four psychrophilic digesters occurred because the organic carbons that were contained in these digesters did not equally degrade and were thus not equally converted to biogas during a similar retention time as the volume of biogas produced is related to the quantity of VS that is degraded. It is also most likely that the methanogens were utilizing the available VFA (Lu *et al.*, 2008). Moreover, the nutrient compositions and microbial populations were probably not similarly balanced. The gas accumulations in all four the digesters showed a low production of biogas in general. According to Alvarez and Lidén (2009), the gas they

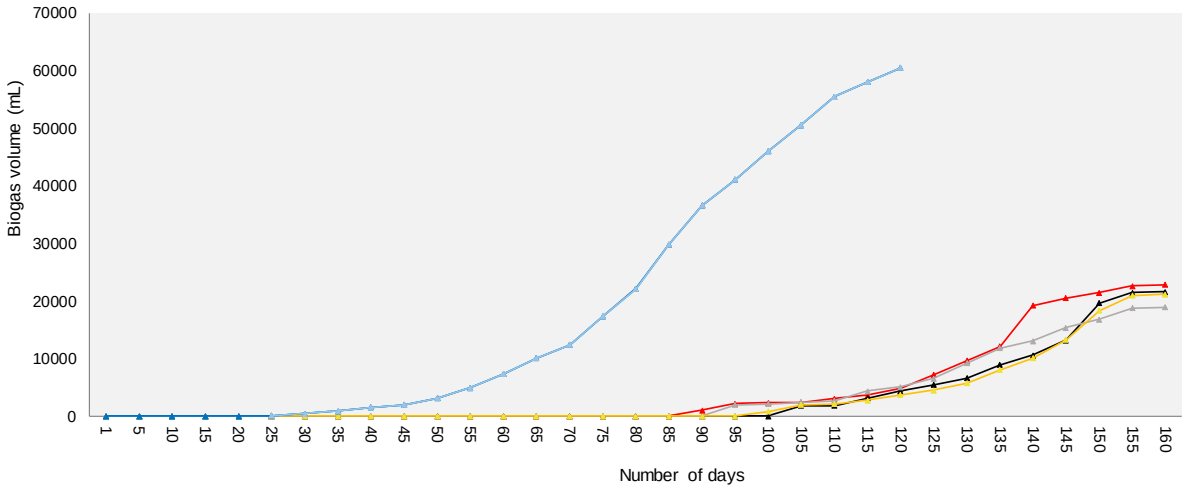

Figure 19: Cumulative biogas production in the psychrophilic digesters D1 (▲), D2 (▲), D3 (▲) and D4 (▲) and the mesophilic digesters D5 (▲), D6 (▲), and D7 (▲). The cumulative gas volumes represent the total volume of gas that accumulated in a week for 160 days in the psychrophilic and for 120 days in the mesophilic digesters. The data are representative of 160 days of monitoring that commenced in Trial 1 on day 51 and continued to day 218.

obtained when cow waste was digested at low temperature was higher compared to the volume that was obtained in D1-D4. Also, even when animal excretions were used as feedstock at low temperature, the accumulated production of biogas after 80 days was high (Ferrer *et al.*, 2009).

The mesophilic digesters (D5-D7) acquired a total biogas volume of 60534±122.98 mL after 120 days of operation (Figure 19). These digesters produced ≈16000 mL more biogas than the psychrophilic digesters on day 55, and the general biogas accumulation in the mesophilic digesters was more than that of the psychrophilic digesters. The observation that mesophilic digesters are capable of producing more biogas than psychrophilic digesters is consistent with data reported by Connaughton *et al.* (2006) and El-Mashad *et al.* (2004). High temperature improves the anaerobic biodegradation of complex organic matter because the microbes in high-temperature digesters are capable of efficient use of the substrate. When the digesters were operated in thermophilic and mesophilic conditions, high biogas yield was achieved by Vindis *et al.* (2009) and Gavala *et al.* (2003). However, the biogas that was produced from the digestion of rumen waste under mesophilic conditions in the current study was comparatively low compared to the production obtained by Kafle and Kim (2013), Hejnfelt and Angelidaki (2009), and Murto *et al.* (2004), who used similar feeds. The IDD-AD2 digestate was a volumous mixture of cud, some meaty

substances, fats and traces of blood. Digesters with mixed substrates tend to exhibit a tendency to increase biogas production. This was true when rice straw was co-digested with kitchen waste and pig manure by Ye *et al*. (2013). Panichnumsin *et al.* (2010) observed the same positive effect of co-digestion, which unfortunately was false during the operation of the D1-D4 and D5-D7 digesters in the current study. Moreover, the gas yields of D1-D4 and D5-D7 were disappointing in terms of CH_4 production, but when temperature elevation occurred it did revitalize the IDD-AD2 digestate to a certain extent to produce more gas than before. The digesters' microbial population activities were apparently rejuvenated, but high TS% and VFA accumulation, low pH, low biogas production and CH_4% characterized these digesters.

5.3.2.3 Impact of temperature on the digesters

The four psychrophilic digesters were operated at uncontrolled ambient temperatures but the temperature differences were not substantial. In these conditions, it was observed that the rate of the anaerobic digestion of sludge and CH_4 production were proportional to the digesters (D1-D4) temperatures that ranged from 17.65±0.33 to 25.90±1.63ºC. Murto *et al.* (2004) operated their digesters at a similar temperature range to produce biogas from mixed solid wastes. The temperature fluctuations in the psychrophilic digesters were not high when compared to the temperature fluctuations in industrial anaerobic digester 2 (AD2) (Chapter 4). In the experimental design, the temperature fluctuations in D1-D2 ranged from 2.00±0.02ºC between morning and midday, at 4.30±1.03ºC between midday and afternoon, and at 5.60±.0.01ºC between morning and afternoon. Lindorfer *et al.* (2008) reported that temperature fluctuations were detrimental to their digesters and that a close monitoring method was necessary. Lettinga *et al.* (2001) attained an improved growth and enrichment of methanogens and acetogens in the psychrophilic digesters they used. Hagos *et al.* (2017) found that microorganisms grew more optimally at mesophilic and thermophilic temperature ranges compared to psychrophilic digesters, and that high temperature had a positive effect on the metabolic rate of microorganisms and accelerated the degradation processes in their study.

5.3.2.4 Biodegradation of organic compounds

The TS and VS were determined on day 1 before the digesters were sealed and again on day 160 for the psychrophilic and on day 120 for the mesophilic digesters.

The methanogens produced biogas by degradation and conversion of VS. At the start-up of the laboratory digesters, a volume of ≈36000 mg of total solids was determined in the 5 L digesters. The TS and VS that were determined after 160 days of psychrophilic digestion showed a slight reduction, meaning that the biogas that had been produced was mostly produced from the VFA that were already available in the digestate. The lower degradation rate of feed indicated that the methanogens were only slightly active during the longer retention time. The gas production rate of D1-D4 was 2.30±0.14 mL mg^{-1} VS which occurred due to 9165.70±346.30 mg removal of VS, and the gas production rate for D5-D7 was 2.36±0.28 mL mg^{-1} VS from the biodegradation of 25675.71±884.00 mg (72.62±8.30%) of the initial VS. Increased temperature remarkably improved VS degradation. The lower degradation of VS in D1-D4 was attributed to lower pH and temperature levels. The VS reduction per mg in relation to the biogas production and yield in the mesophilic digester were closely related to each other, unlike what occurred in psychrophilic conditions. This was possibly the effect of temperature in balancing the different anaerobic digestion processes in the digester, and resulted from the activated microorganisms involved. It is undeniable that temperature is one of the most important factors that impact the digestion of solid organic waste (Gaby *et al.*, 2017; Bouallagui *et al.*, 2004; Gavala *et al.*, 2003; Veeken & Hamelers, 1999). Ortner *et al.* (2014) obtained different results as a high CH_4 production was achieved at low temperature by using suitable solid slaughterhouse waste. They observed a high degradation rate with low formation of ammonia toxicity.

The organic contents in the mesophilic and psychrophilic digesters were similar as they both contained a higher percentage of organic contents. Given the degradation time, the mesophilic digesters delivered a better gas production rate than the psychrophilic digesters. Increasing degradation time and operating digesters in psychrophilic conditions took longer to revitalize them, and the digesters' characteristics were fluctuating and lower pH levels, high VFA, low CH_4 concentrations and low gas production were observed. When the temperature of the dormant digesters was increased, the pH of the digesters was able to balance and the accumulation of VFA was lower compared to the psychrophilic digesters.

5.3.3 Characterization and comparison of inoculums

5.3.3.1 Characteristics of the inoculums

The intention of inoculum preparation was to ingest a high concentration of microbes into the digesters loaded with IDD-AD2 digestate that was operated in mesophilic temperature conditions. The implementation of this trial was to compare the cumulative and daily biogas production using different inoculums. A concentrated and active inoculum source is important to reduce digestion time and to improve digester efficacy (Holliger *et al.*, 2016; Salminen & Rintala, 2002). Rumen fluid, cow dung and sludge from operating digesters had been used before to start up digesters (Rafieenia *et al.*, 2018; Córdoba *et al.*, 2016; Nuchdang *et al.*, 2015; Lopes *et al.*, 2004). The inoculums prepared were characterized by TS maintained below 4% in order to approximate municipal wastewater treatment sludge (MWWTS) and digestate from an operating digester (DFOD) (Table 3).

Table 3: Characteristics of the inoculums selected for the upbringing of IDD-AD2 digestate

Parameters	FCM	LSM	RFI1	RFI2	MWWTS	DFOD
TS%	3.28±0.16	2.63±0.09	1.23±0.10	1.25±0.11	2.25±0.11	3.29±0.16
VS%	2.31±0.13	1.52±0.02	0.93±0.01	0.71±0.01	1.61±0.02	2.06±0.03
VS% of TS	73.81±0.61	55.86±0.06	65.42±0.08	66.23±0.02	65.03±0.27	63.13±0.02
pH	7.41±0.12	7.85±0.03	5.23±0.06	5.29±0.05	7.01±0.56	7.21±0.02

The inoculums that were prepared from fresh cow manure (FCM), MWWTS and DFOD had a characteristic pH close to operational level of between 6.5 and 7.5 (Boe *et al.*, 2010; Ward *et al.*, 2008) (Table 3). Rumen fluid inoculum 3 (RFI3) was prepared by mixing RFI1 and RFI2. Both RFI1 and RFI2 had a lower pH and VS%. Characterization of RFI3 was not performed, but LSM and DFOD were characterized by lower VS% when compared to FCM (Table 3). LSM had lower VS% of TS and higher ash%, and the inoculum consisted of high sand contents during preparation.

5.3.3.2 Biogas production

The operation of the digesters containing inoculums occurred in mesophilic conditions (31.50±1.50ºC) and there was no degradation test of the substrate in this trial. The gas quality produced during LSM digestion was promising compared to the gas quality in RFI1, RFI2 and RFI3. After 30 days of running the LSM and FCM digesters, mean CH_4 concentrations of 50.6±0.2% and 59.1±0.2% were determined

respectively. The same range of CH_4 reading was obtained when cow manure was used as feedstock for anaerobic digestion (Abubakar & Ismail, 2012; Ashekuzzaman & Poulsen, 2011). When comparing the cumulative biogas trend of the inoculums (Figure 20), the digesters showed the same upward trend; however, as was expected, FCM showed the highest gas production.

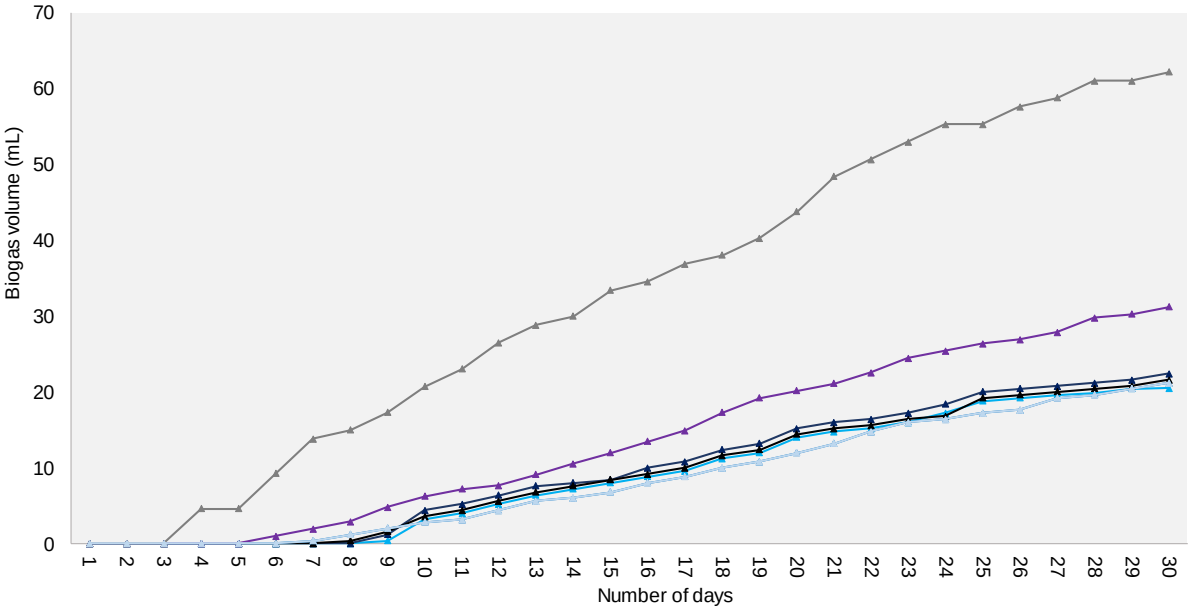

Figure 20: Cumulative biogas of different inoculums: DFOD (▲), MWWTS (▲), LSM (▲), FCM (▲), RF3 (▲), RFI2 (▲) and RFI1 (▲).The operation of inoculum digesters was in mesophilic temperature, operated in duplicate and the cumulated gas present the amount of gas collected every day. Data are representative of 30 days monitored and commenced during trial 2 from day 98 to day 128.

RFI3 produced a larger volume of biogas compared to RFI1 and RFI2. The increase in biogas production occurred because of high organic content available in the mixture, because RFI1 and RFI2 had higher TS%. The TS% in all the inoculums used was lower than 4, and this is reflected by the low gas volume produced and also because of the small quantities of inoculums used. To avoid large differences in biogas volume production, the duplicate experiments were homogenised in the same manner. The biogas trends (Figure 20) showed that more biogas was accumulated daily, which implies that more microorganisms were available to utilize the available substrates (Suryawanshi *et al.*, 2013; Mudhoo, 2012; Demirel & Scherer, 2008). Moreover, the lag phases were expanded, probably because the acedogens were favoured during the earlier days of the experiment. The biogas accumulated was ongoing after 30 days, indicating that degradation of the small material was still occurring. LSM produced gas earlier than DFOD. These results were not expected

because DFOD was in the process of digesting when collected. Thus the microbes possibly adapted faster in the new environment than expected (Grimberg *et al.*, 2015).

The total gas levels produced in 30 days for MWWTS, DFOD, RFI3, FCM, RFI2, LSM and RFI were 20.70±3.23, 23.98±0.74, 24.00±0.03, 62.90±1.51, 22.60±2.03, 26.90±0.54 and 23.11±1.00 mL respectively. The combination of RFI1 and RFI2 improved the gas production from the rumen fluid inoculum per day. I Nyoman and Seno (2010) obtained a higher biogas production rate and efficiency from the rumen fluid inoculum compared with the cow manure inoculum. The digesters that produced more gas at a faster rate were considered the ones with the highest numbers of microorganism (Abu-Bakr & Ismail, 2012). The order of inoculum with the highest number of microbes to the lowest is presented as follows:

FCM>LSM>RFI3>DFOD>RFI1>RFI2>MWWTS.

FCM and LSM had higher gas production rates per day on average and more gas volumes were attained in 30 days. Based on availability and expense, LSM and DFOD were chosen over FCM to be used to revitalize the industrial and laboratory digesters. LSM performed well because methanogens are capable of surviving in dry cattle dung stored for prolonged periods of time, often more than 24 months (Esposito *et al.*, 2012). Avoiding RFI3 as inoculum to revitalize the digesters was because these digesters were already overwhelmed with rumen solid contents.

5.3.4 Addition of inoculum into IDD digestate

5.3.4.1 pH

The mesophilic digesters (D5-D7) from Trial 1 that operated without the addition of inoculums are referred to as inoculum (0 L) in this section. Inoculum (0 L) had a mean VFA concentration of 4000 mg L^{-1} for the first 35 days with a low average pH of 4.30±0.58. The addition of LSM/DFOD 0.75 L, 1 L and 1.25 L had a positive effect on the pH of the dormant digesters that contained IDD-AD2 digestate. The pH levels of the digesters after addition of the inoculums were 5.61±0.20, 6.23±0.10, and 6.54±0.31 for the digesters loaded with LSM 0.75 L, 1 L and 1.25 L respectively. These levels suggest that the inoculum probably provided a high buffering capability. After 30 days of operation, the mean pH levels for digesters with LSM 0.75 L, 1 L

and 1.25 L were 7.89±0.11, 7.60±0.25 and 7.52±0.30 respectively. The mean operational pH levels of the digesters loaded with DFOD 0.75 L, 1 L, and 1.25 L were 6.80±0.39, 7.10±0.03 and 7.30±0.13 respectively. A high production of ammonia that was released during digestion probably caused these high pH levels in the LSM digesters. Ammonia (NH_3) and ammonium (NH_4^+) are accumulated from breaking cow manure and slaughterhouse waste, and they can inhibit anaerobic digestion processes (Turker *et al.*, 2013). The pH levels of the digesters loaded with DFOD and LSM were in the same range as those of the digesters operated by Esposito *et al.* (2012) and Buendía *et al.* (2009) when slaughterhouse waste was used as feed. DFOD and LSM were able to balance the pH of dormant digesters, with LSM 0.75 L and DFOD 1.25 L being the most effective.

5.3.4.2 Biogas production

a) Gas quality

Inoculum (0 L) digesters showed a low mean CH_4% between day 1 and day 5, whilst during the same period digesters LSM 0.75 L, 1 L and 1.25 L had a CH_4 concentration of ≈10%, ≈17%, and ≈8% respectively. Digesters DFOD 0.75 L, 1 L and 1.25 L had a CH_4% production of ≈12%, ≈7%, and ≈11% respectively during the same period. Inoculum (0 L) had a 10-20% CH_4 concentration on day 30, and during the same period digesters LSM 0.75 L, 1 L and 1.25 L showed a 62±0.4%, 65.8±0.4% and 68.1±0.9% mean CH_4 respectively. Digesters DFOD 0.75 L, 1 L and 1.25 L had mean CH_4 concentrations of 58.2±0.5%, 55.3±0.1%, and 59±1.8% respectively during the same period. LSM 1.25 L and DFOD 1.25 L provided microbes that adapted quicker to the new environment and thus produced high-quality biogas because the number of active microbes available impacted the volume of biogas produced. It appeared that the volume of inoculum that was introduced into the digesters affected the relationship between the microbial species' adaptation and the CH_4 contents. Similar CH_4% results were reported when different mixtures of abattoir waste were digested using cow manure as inoculum (Hejnfelt & Angelidaki, 2009). The addition of both LSM and DFOD caused an improvement of CH_4 content, and different LSM concentrations improved gas quality than different DFOD concentrations. This implies that, when attempting to revitalize dormant digesters in mesophilic temperature, using LSM and DFOD as inoculums will improve CH_4 quality.

b) Daily biogas production

The digesters that were revitalized by adding DFOD 0.75 L, 1 L and 1.25 L produced an average of 50.30±0.70 mL, 134.61±1.92 mL and 191.40±2.71 mL per day respectively. The maximum gas volume obtained in digesters DFOD 0.75 L, 1 L and 1.25 L in a day was 158.21±11.97 mL on day 27, 391.20±20.22 mL on day 20, and 652.47±60 mL on day 8 respectively (Figure 21).

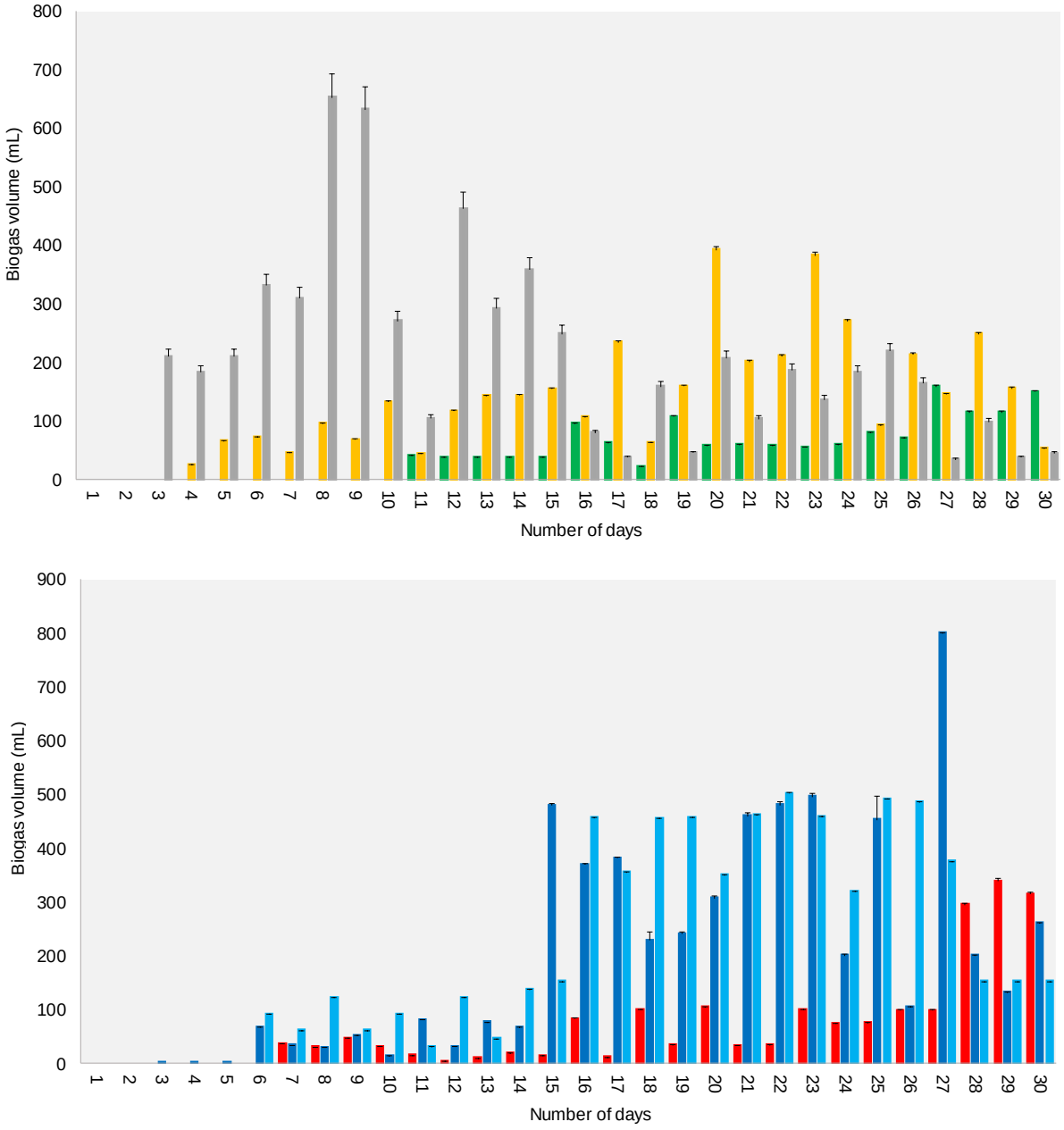

Figure 21: Daily average biogas yield from IDD digestate co-digested with DFOD inoculum of 0.75 L (■), 1 L (■) and 1.25 L (■), and LSM inoculums of 0.75 L (■), 1 L (■) and 1.25 L (■) .The graphs above indicate the volumes of gas produced every day by each inoculum concentration individually, plotted as averages and standard deviations presented as error bars. The data are representative of 30 days of monitoring that commenced in Trial 2 on day 98 and continued to day 128.

LSM 1.25 L produced a higher volume of biogas per day whilst LSM 0.75 L produced the lowest. Digesters LSM 0.75 L, 1 L and 1.25 L produced averages of 65.78±0.72 mL, 200.87±72.00 mL and 217.58±72.14 mL per day respectively. The average volume of biogas produced per day was higher in the LSM digesters. The high daily biogas production in the LSM digesters was an indication that the microorganisms had probably acclimated to the substrate quicker because of the active inoculum used (Suksong *et al.*, 2017). LSM 1 L on day 27 and DFOD 1.25 L on day 8 produced the highest biogas volumes in a day (Figure 21). When digesting rumen solid waste, Aragaw and Gessesse (2013) noted low gas production after 55 days of operation, which was due to the accumulation of high levels of ammonia. After the addition of LSM to dormant digesters, the production of biogas occurred earlier compared to inoculum (0 L). The addition of 0.75 L of LSM reduced the lag phase of the mesophilic digesters up to 6 days, compared to 25 days for inoculum (0 L). A further reduction in the lag phase occurred in the LSM 1 L digesters. In the DFOD digesters, gas production started earlier compared to both inoculum (0 L) and the ones supplemented with LSM.

The addition of 1 L of DFOD promoted an earlier production of biogas on day 3, while the DFOD digesters with 1.25 L started producing on day 2. The availability of readily biodegradable organic matter in DFOD and the presence of a high content of the methanogens contributed to the earlier gas production. The DFOD inoculum was from an active digester, and thus the microorganisms from this inoculum were expected to acclimate faster, which was a peculiar contradiction to what was observed when DFOD was digested on its own (Figure 20). The contradiction could be explained by the presence of the microbes in DFOD that probably required a suitable substance for quicker activation. DFOD instigated a rapid start-up of the digesters and had a good buffering capacity. The highest concentration of DFOD (1.25 L) caused the highest volume of gas to be produced per day when compared to other DFOD concentrations. The digesters that had been loaded with the highest quantity of LSM concentration (1.25 L) produced the highest average volume of biogas per day when compared to all the other digesters with LSM and DFOD concentrations.

c) Cumulated biogas

On day 30, inoculum (0 L) produced 578.30±66.90 mL whilst digesters LSM 0.75 L, 1 L and 1.25 L produced 1973.40±20.61 mL, 6025.95±93.44 mL and 6527.40±52.63 mL respectively (Figure 22). The digesters that were supplemented with DFOD 0.75 L, 1 L and 1.25 L accumulated 1509.75±23.92 mL, 3976.83±66.31 mL and 5742.00±8.97 mL of biogas respectively in 30 days (Figure 22). This means that the higher the volume of inoculum that was added the more biogas was produced. Both LSM and DFOD with the highest volumes produced the highest biogas volumes. Forster-Carneiro *et al.* (2008) state that the volume of inoculums used during seeding is proportional to the biogas that is produced.

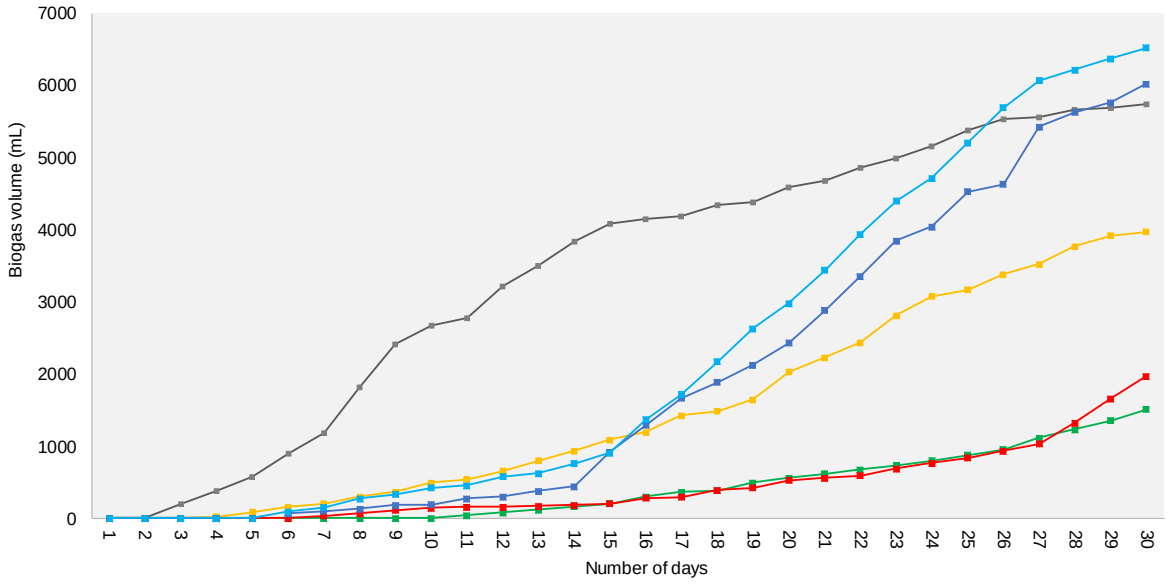

Figure 22: Cumulative biogas production from the digestion of IDD digestate co-digested with DFOD inoculums of 0.75 L (■), 1 L (■) and 1.25 L (■), and LSM inoculums of 0.75 L (■), 1 L (■) and 1.25 L (■).The data in Figure 22 are representative of 30 days of monitoring that commenced in Trial 2 on day 98 and continued to day 128.

The different volumes of LSM resulted in more biogas accumulation than in the dormant digesters that had been loaded with different volumes of DFOD. LSM 1.25 L produced the highest gas volume. Rabah *et al.* (2010) were able to produce ≥2000 mL of biogas in two weeks, and thus demonstrated that more gas was produced from only 200 g of slaughterhouse solid waste. Chen *et al.* (2008) state that the one factor that is a major contributor to digesters' toxicity is ammonia that forms when slaughterhouse waste is digested. Process instability due to ammonia often results in VFA accumulation, which leads to a decrease in pH. Cuetos *et al.* (2010) failed to

produce a high volume of biogas from slaughterhouse waste when co-digested with municipality waste, but the biogas volume that they obtained was higher than what was obtained in the current study, because of the lower retention time in their study.

d) Gas improvement after the addition of various quantities of inoculum

Wang *et al.* (2014) and Xu *et al.* (2013) indicate that a constructive effect of inoculum reflects a stable performance of digesters. This was corroborated by the findings of this study as the digesters that had been loaded with higher volumes of LSM had a stable performance. The digesters loaded with LSM of 0.75 L, 1 L and 1.25 L achieved ≈54%, ≈83% and ≈84% gas improvement when compared to inoculum (0 L) for the first 30 days of operation respectively. Thus increasing the concentration of LSM from 0.75 L to 1 L improved gas production by ≈51% and an improvement of ≈54% of gas production occurred when LSM was increased from 1 L to 1.25 L. The improvement in gas production in the digesters loaded with LSM probably occurred because a large number of microbes was now available to utilize the feed, as indicated by Schofield *et al.* (1994). This implies that the more LSM added the more methanogens were added, resulting in the improvement of gas production. However, a threshold exists as continuing to add LSM does not ensure biogas production improvement beyond a certain point.

In the digesters that were loaded with 0.75 L, 1 L and 1.25 L DFOD gas production was improved with inoculum (0 L) by ≈45%, ≈75% and ≈82%, respectively. There was a gas production improvement of ≈45% from DFOD 0.75 L to 1 L. This was also true when the DFOD volume was increased from 1 L to 1.25 L, because an ≈18% improvement in gas production was achieved. The addition of 0.75 L of DFOD to the digestate increased the total volume of gas that accumulated more than when 1 L and 1.25 L of DFOD were added. Thus higher quantities of inoculums provided higher TS, which increased the viscosity and created an environment that inhibited the microorganisms from fully utilizing the VS and organic acids that were available. The highest DFOD added to the IDD-AD2 digestate (1.25 L) had a lower effect on the total gas accumulated than when 0.75 L of LSM was added. When both inoculums were used to develop laboratory dormant digesters containing IDD-AD2 digestate, a better total gas production was achieved when 1 L of LSM was added to the digestate. The total gas production was improved in the LSM digesters than in the DFOD digesters because all the different digesters that were loaded with

different volumes of LSM produced better total gas volumes than what was produced by the digesters loaded with DFOD. It was therefore confirmed that ultimate gas yields, as well as the daily volumes of biogas produced, are dependent on the inoculum: thus large inoculation volumes ensure high microbial activity, low risk for overloading, and low risk for inhibition (Sawatdeenarunat *et al.*, 2015). The statistical analyses that were performed revealed that increasing the addition of inoculum from 0.75 L to 1 L and 1.25 L of DFOD and LSM had a significant effect for the production of higher volumes of biogas. The digesters that were loaded with LSM revealed a close relationship between the quantities of LSM added and the biogas produced.

5.3.4.3 Organic biodegradability

To determine the biodegradability of IDD-AD2 digestate effectively, the TS and VS of LSM and DFOD inoculums were determined and the VS after digestion was determined by subtracting the weight of the inoculums. VS reduction per mg in inoculum (0 L) was ≈72.62%, which resulted in a 2.36±0.28 mL mg^{-1} VS biogas production rate after 120 days. Banks and Wang (1999) achieved a 50% feed degradation when mixed abattoir wastes were anaerobically digested in 30 days. The VS reduction rates in the digesters with LSM 0.75 L, 1 L and 1.25 L were 58.03±0.24%, 59.00±0.01%, 59.16±0.44% respectively, which resulted in gas production rates of 0.93±0.01, 0.28±0.04 and 0.30±0.00 mL mg^{-1} VS respectively in 30 days of operation. The digesters that were loaded with DFOD 0.75 L, 1 L and 1.25 L had 77.41±0.91%, 73.29±0.13% and 72.61±0.55% VS reduction rates on day 30, with gas production rates of 0.05±0.02, 0.15±0.03 and 0.23±0.06 mL mg^{-1} VS for DFOD 0.75 L, 1 L and 1.25 L respectively. Thus, the addition of LSM and DFOD to dormant digesters better improved the total biogas that accumulated with high VS reduction compared with the rates that were achieved with inoculum (0 L) digesters. It appeared that LSM provided the dormant digesters with a population of active microbes and that the effect of these microbes on VS reduction was lower compared to DFOD microbes. This assertion is based on the volume of biogas produced per VS reduction after the selected period. The high VS reduction in the DFOD digesters may be attributed to the positive synergetic effect of DFOD with RSC in providing balanced nutrients, increased buffering capacity, and a decreased effect of toxic compounds. In DFOD, the lowest quantity (0.75 L) that was added to the IDD-AD2 digestate was responsible for high VS reduction, but resulted in the lowest gas production rate. The effect of the slight DFOD that was required to destruct large VS

was because DFOD had a low TS of 3.29±0.16% and a small quantity of VS of 2.06±0.03, with probably a greater microbial population to solid ratio. In this context, Gu *et al.* (2014) argue that the source of an inoculum will affect the digestion results depending on the feedstock that is added.

The LSM feed had been exposed to heat and was impacted by environmental conditions while in the shed form where it was collected. It thus had fewer nutrients and high ash% and TS% and thus negatively affected microbial population. Conversely, the microbial population in DFOD was expected to be hyperactive because this inoculum had been taken directly from an operational digester. VS reduction in digesters loaded with LSM and DFOD suggested that the higher LSM volume added promoted higher RSC digestion, whilst the lowest DFOD volume added promoted higher RSC digestion. However, the same cannot be said for the total volume of biogas that was accumulated. LSM and DFOD inoculums were advantageous because they increased the VS degradation rate, enhanced biogas production, shortened the starting time, and made the digestion process more stable than in the inoculum (0 L) digesters that contained only RSC.

5.4 Conclusion

Increasing both temperature and retention time was required to revitalize dormant digesters because in these conditions a high CH_4 concentration, digester stability and large gas volumes were obtained. The temperature increase also affected the degradation of volatile solids positively. Attempting to revitalize digesters without elevating the temperature was possible, but a longer retention time was required and this was deemed uneconomical. LSM and DFOD inoculums were plausible for utilization in dormant digester revitalization as these inoculums were found to be proficient in improving gas production, volatile solids degradation, and reducing the lag phase of microbial growth in dormant digesters. The most significant finding of this investigation was that the use of LSM to revive digesters in mesophilic temperature positively affected the performance of the digesters compared to the use of DFOD. However, DFOD may be more economical than LSM because a smaller quantity was used to revive the dormant digesters as the use of DFOD resulted in high CH_4 content and high volumes of biogas. LSM is unfortunately characterized by high quantities of inorganics. The use of DFOD to revive a digester

was also found effective in maintaining the TS% to a minimum. The use of DFOD and LSM as inoculums to revive an unresponsive, dormant industrial digester was promising and considered economically beneficial because by using either, it may not be necessary to empty an anaerobic digester of its contents, as was the case with the 1200 m^3 anaerobic digester (AD2).

Chapter 6: Biogas production from rumen solid contents (RSC) and energy crops intended to remediate mine-impacted land

6.1 Introduction

The mining sector in South Africa contributes significantly to the GDP and is one of the major job creators in this country. However, mining operations' excessive soil contamination that is caused by the use of various chemicals is inevitable. These contaminants can be extracted from the soil using crops to rehabilitate mine tailings (Aderholt *et al.*, 2017; Bahadur *et al.*, 2017; Lee *et al.*, 2014; Ali *et al.*, 2013), and these crops can in turn be used for energy production. Using crops to extract contaminants can help rehabilitate the mine tailings, phytoremediation is a process in which plants are used to degrade or immobilize contaminants from soil or water. This process uses relatively cheap technology which is solar-driven and performed in situ (Gupta *et al.*, 2013; Salt *et al.*, 1998; Raskin, 1996). Pollutants such as benzene, toluene, ethylbenzene, xylenes, lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) can be removed by phytoremediation using biomass (Karimi, 2013; Perry *et al.*, 2012; Angelova *et al.*, 2011; Raskin, 1996). Phytotransformation, phytoextraction, phytostabilisation, phytovolatilization and rhizofiltration are different types of phytoremediation (Gupta *et al.*, 2013). Phytoextraction uses green plants to uptake pollutants from the environment, and the success of this process is dependent on the yield of biomass and efficient transfer of metals from plant root to shoot (Attinti *et al.*, 2017; Bravo *et al.*, 2017; Shtangeeva *et al.*, 2017; Gupta *et al.*, 2013).

The crops that are used in these processes are generally referred to as 'energy crops'. Such crops can be cultivated for phytoremediation and they are used for biogas production. However, because these crops absorbed contaminants, they pose a threat and may disrupt the anaerobic digestion process and it has been demonstrated that the toxic effect of heavy metals disrupts enzyme function and the structure of microbes (Mudhoo & Kumar, 2013; Willscher *et al.*, 2013).

Sorghum in particular has been used for phytoremediation to reduce soil contamination, and high absorption rates of contaminants from soil by this crop have been reported. Sorghum is thus widely cultivated for remedial purposes and is used

quite extensively for biogas production (Gomes, 2012). Sugar beet, sorghum and sugar beet waste pulp have all been demonstrated as feedstock for biogas production (Ostovareh *et al.*, 2015; Brooks *et al.*, 2008; Demirel & Scherer, 2008; Parawira *et al.*, 2004).

Meat and other animal products that are generated at slaughterhouses during meat production are used and wastes such as skins, fats and bones are particularly targeted (Cuetos *et al.*, 2008). Masses of slaughterhouse wastes are generated yearly, making disposal an important part of solid waste management (Xia *et al.*, 2012; Cuetos *et al.*, 2008). The method of disposal of this waste is important to the environment and to both animals and human health and should be conducted in a manner that avoids diseases outbreaks. Slaughterhouse waste is an ideal substrate for anaerobic digestion, but is regarded as a difficult substrate to use because of its high protein and lipid content. The inhibition of anaerobic digestion of slaughterhouse waste is usually caused by high ammonia concentrations, which are produced during the degradation of proteins (Afazeli *et al.*, 2014; Xia *et al.*, 2012; Cuetos *et al.*, 2008). However, the treatment of slaughterhouse waste via anaerobic digestion has been found to be promising because it combines energy production and waste treatment, and thus the use of slaughterhouse waste to produce biogas has become a common practice in some regions (Alvarez & Liden, 2008).

Energy crops are usually co-digested with waste materials because co-digestion uses all the nutrients in waste and balances the microbial population by enabling a stable anaerobic fermentation process with a balanced pH. Co-digestion of energy crops with waste has the potential to enhance gas yields and general anaerobic digestion performances. Solid slaughterhouse waste has been co-digested with different biomass substances and this has been proved to enhance biogas production (Alvarez & Liden, 2008; Amon *et al.*, 2007).

This chapter presents the methods and findings based on an investigation to determine the production of biogas from sorghum (SOR), sugar beet roots (SBR) and sugar beet leaves (SBL) that had been exposed to mine-contaminated soil. The use of rumen solid contents (RSC) as an additional feedstock for co-digestion with energy crops for biogas production was also investigated.

6.2 Materials and Methods

Sorghum and sugar beet were selected as energy crops to be used as feedstock in the bioenergy project. This project initially intended to use only sugar beet roots to produce biogas, and thus the leaves were to be discarded at the farm or used as soil nourishment. Unfortunately, the agricultural land available for cultivation of energy crops was not sufficient to produce enough feedstock to meet the projected demand of the digesters. Because the bioenergy farm was situated close to one of the major slaughterhouses in the Free State, the bioenergy plant was earmarked for the incorporation of slaughterhouse waste as an additional feedstock for biogas production in the future.

6.2.1 Sample and inoculum preparation

6.2.1.1 Inoculum preparation

Rumen fluid inoculum 3 (RFI3) was prepared by mixing rumen fluid inoculum 1 and 2 (RFI1 and RFI2) in a 1:1 ratio. RFI1 and RFI2 were prepared using materials and methods as were described in Chapter 5 (5.2.2.1 a).

6.2.1.2 Feedstock preparation and collection

a) Rumen solid contents (RSC)

RSC was collected from a slaughterhouse that was situated close to the bioenergy plant. After collecting the RSC, it was dried on a plastic surface using direct sunlight for a period of 1-2 days. A manual sorting of the RSC was undertaken to remove unwanted materials such as stones, bones, and plastics. When it was consdered sufficiently ‘cleaned’, the RSC was shredded to particle sizes of ≈2 mm before loaded into a digester.

b) Sugar beet leaves (SBL) and sorghum (SOR)

SBL were collected from the farm and ensiled using the method adopted by the farm. This involved ensiling the green harvest by covering it with plastic bags until required. The same method was used to ensile SOR, which was also collected from the farm. When required, the SBL and SOR were shredded to a size of ≈2 mm before usage.

c) Sugar beet roots (SBR)

The SBR that were used were collected from the bio-plant silos. The particle size of the SBR in the silos was ≈8 mm. When the SBR compound was required, it was shredded to a size of ≈2 mm before use. The SBR compound that was used in this study was stored in a silo for about two days.

6.2.2 Experimental set-up

No chemical pre-treatment was applied to the feedstock in this particular investigation. All the experiments were performed in 2 L reactors designed according to the same approach as described in Figure 3. In this investigation, 5 L collection containers were used in which the gas could be generated. These digesters operated between 31.50±1.50ºC. The temperature was kept constant by placing the digesters in a temperature controlled unit. The working volume of the digesters was kept at ≈1.7 L and this headspace was allowed to avoid over foaming. The SBL, SOR, SBR and RSC were studied for mono and co-digestion using rumen fluid inoculum (RFI3). All the experiments were operated in batch mode and the characteristics of the substrates and inoculum were analysed. Each mono- and co-digestion experiment was operated in triplicate, as illustrated in Figure 23.

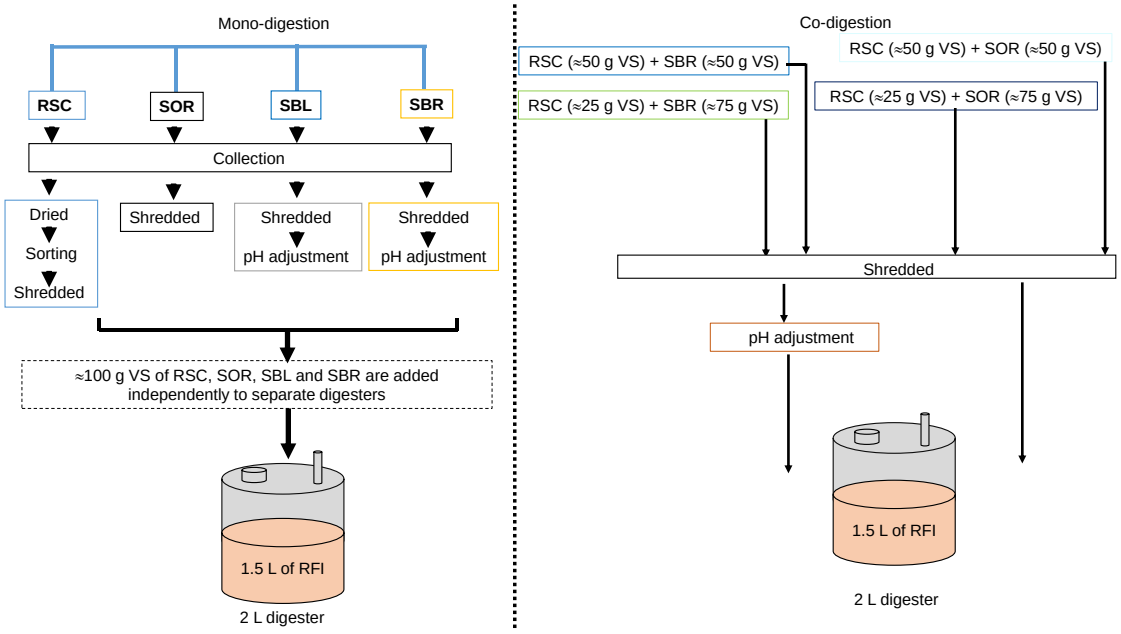

Figure 23: Representation of mono- and co-digestion experiments of energy crops and rumen solid contents. The mono-digestion that is illustrated above involved the digestion of 100 g of VS of each feedstock in triplicate. Co-digestion was conducted with ≈50 or ≈25 g VS of RSC in ratios of 25:75 and a 50:50 (RSC:SBR and RSC:SOR) respectively.

6.2.2.1 Mono-digestion

The separate digesters were filled with a total of ≈100 g VS of feedstock (SBL, SOR, SBR and RSC). The digesters were operated using rumen fluid inoculum (RFI3) in all the triplicate batch experiments. The digesters involving SBL and SBR mono-digestion required pH adjustment, which was performed using a few drops of a 10% NaOH solution only on the first day (Figure 23).

6.2.2.2 Co-digestion

The ratios selected for co-digestion of energy crops with RSC avoided the potential replacement of energy crops with RSC. Ratios of 1:3 and 1:1 were selected as optimum mixtures as these ratios were suitable to avoid viscosity problems. The feedstock mixtures were prepared by blending ≈50 or ≈25 g VS of RSC in 25:75 and a 50:50 (RSC:SBR and RSC:SOR) mix ratios respectively. All the co-digestion experiments involving SBR were adjusted for pH using 10% NaOH; this was done only on the first day. The contents of all the digesters were mixed by shaking the digesters manually 2-3 times a day for ±15 seconds to improve homogeneity. The CH_4 content and biogas volume of the digesters were determined every day, while the pH was determined at 3-5 day intervals. The VFA levels were determined before and after digestion.

6.3 Results and Discussion

6.3.1 Feedstock characteristics

Feedstock composition affects the quality of the biogas produced (Heidarzadeh Vazifehkhoran *et al.*, 2016). When comparing the results for SOR used in this study with those of other studies, the VS% of the TS content in this study was lower (Table 4). The low VS% of SOR could probably be attributed to the loss of organic solids during ensiling (Mahnert *et al.*, 2005). It has been established that the transformation of organic matter into gases can occur if improper storage is applied (Heidarzadeh Vazifehkhoran *et al.*, 2016). Moreover, using SBL as feedstock for biogas production is perceived as more suitable than SBR because SBR are usually associated with sand (Cheesman, 2004). This was also true for this study as sand was collected with

the roots during the harvesting process of the sugar beet that was used in the experiments.

Table 4: Characteristics of raw SBL, SOR, SBR, RSC and rumen fluid inoculum (Mean ±SD, n=4).

Parameters	SBL	SOR	SBR	RSC	RFI3
pH	3.40±0.03	6.40±0.30	3.20±0.01	4.70±0.61	7.03±0.03
TS (%)	21.64±0.26	20.00±2.12	20.90±0.33	17.16±0.84	1.20±0.50
VS (%)	19.12±0.15	14.10±0.11	20.30±0.40	14.59±0.44	1.03±0.59
VS % of TS	88.35±1.95	70.51±10.70	97.31±0.91	85.03±15.03	85.83±9.46

The pH of the RSC was far off from the preferred operational value (Table 4). Boe *et al.* (2010) and Murto *et al.* (2004) also reported a low pH of 5.90 for raw rumen solid waste. The pH levels of raw SBL and SBR were also lower than the operational value (Boe *et al.*, 2010), and these pH levels were lower than the level for SOR (6.40±0.30) (Table 4). The same low pH ranges of 3.27-3.50 for sugar beet leaves and 3.30 for sugar beet roots were determined before the commencement of the experiments (Ahmed *et al.*, 2016; Demirel & Scherer, 2008; Weiland, 2003). When Demirel and Scherer (2008) utilized sugar beet for mono-digestion, buffering agents were added regularly in order to keep the pH of the reactor stable. The digesters in this study were not regulated for pH because an understanding of whether the digesters could produce economical CH_4 yields when no pH adjustment chemicals are used.

6.3.2 Effects of the inoculum on the digester

The pH of sorghum in the digester was low (5.97±0.30) when compared to the raw feed (6.40±0.30), probably because the sorghum had been exposed to the air before digestion and it had possibly been digested aerobically to produce acids (Sanderson *et al.*, 1997; Egg *et al.*, 1993). This effect was noted for most of the raw feed (Table 4) because all the pH levels varied in the prepared feedstock loaded to the digesters (Table 5). The digesters in this study were operated with rumen fluid inoculum 3 (RFI3). The inoculum was added to all the digesters before the operation. The pH of RFI3 was ≥7 with a TS% of 1.20±0.50. The average pH of the SBR digesters was 3.62±0.05 before addition of the inoculum, and after addition of the inoculum the pH of SBR was elevated to 5.04±1.53, which was still far from the operational value (Fang *et al.*, 2011; Boe *et al.*, 2010) (Table 5). The pH of RSC was also low when loaded to the digester, but it was elevated by the addition of rumen fluid.

Table 5: pH levels before and after addition of the inoculum (RFI3) (Mean ±SD, n=3)

Digesters	Average pH (Before addition of RFI3)	Average pH (After addition of RFI3)	pH adjustment with 10% NaOH
SBR	3.62±0.05	5.04±1.53	7.03±0.06
SOR	5.97±0.30	7.02±0.58	ND
RSC	4.95±0.08	5.21±0.41	ND
SBL	5.24±0.14	5.88±0.33	7.01±0.03
RSC:SOR (25:75)	4.68±0.96	6.90±0.36	ND
RSC:SBR (25:75)	4.96±0.32	5.33±1.02	7.00±0.01
RSC:SBR (50:50)	3.82±0.09	5.12±0.24	7.14±0.10
RSC:SOR (50:50)	5.93±0.03	6.32±0.51	ND

*ND = not determined

The pH was elevated in most digesters, although not all reached optimum operational values. The low pH levels determined in the RSC:SBR (25:75) and RSC:SBR (50:50) digesters indicated that the rumen fluid in these digesters possibly had a low buffering capacity (Jiang *et al.*, 2013; Liu *et al.*, 2008; Ward *et al.*, 2008). In the digesters loaded with RSC:SOR (50:50) and RSC:SOR (25:75), RFI3 was able to increase the pH to close to the operational value (Boe *et al.*, 2008). The same elevation actions by an inoculum were observed by Neves *et al.* (2004), because acidification of the digesters loaded with kitchen waste was avoided by using a granular inoculum. No alkalinity was added to the digesters loaded with only RSC, SOR, and RSC:SOR (25:75 and 50:50) ratio mixtures, and thus the only expected source of alkalinity was the inoculum. In the mono-digestion trials, it was assumed that the nitrogen, macronutrients, and micronutrients were provided by the inoculum (Gulhane *et al.*, 2017; Wang *et al.*, 2014; Sreekrishnan *et al.*, 2004).

6.3.3 The pH and VFA levels in the mono- and co-digestion digesters

Sugar beet consists mainly of simple sugars, which are broken down first and rapidly, when compared to fibrous materials. When a rapid reduction of these sugars occurs, it causes the pH to decrease in the digesters (Demirel & Scherer, 2008). The accumulation of acids due to this reaction was possibly what was experienced in all the digesters that were loaded with SBR (Table 7). The mono-digestion of the SBL and SBR had the highest pH fluctuations (Figure 23), signifying that regular digester buffering was required. The average pH of the SBL digesters was ≥7 during operating, whilst that of the SBR was generally ≤7 (Figure 23).

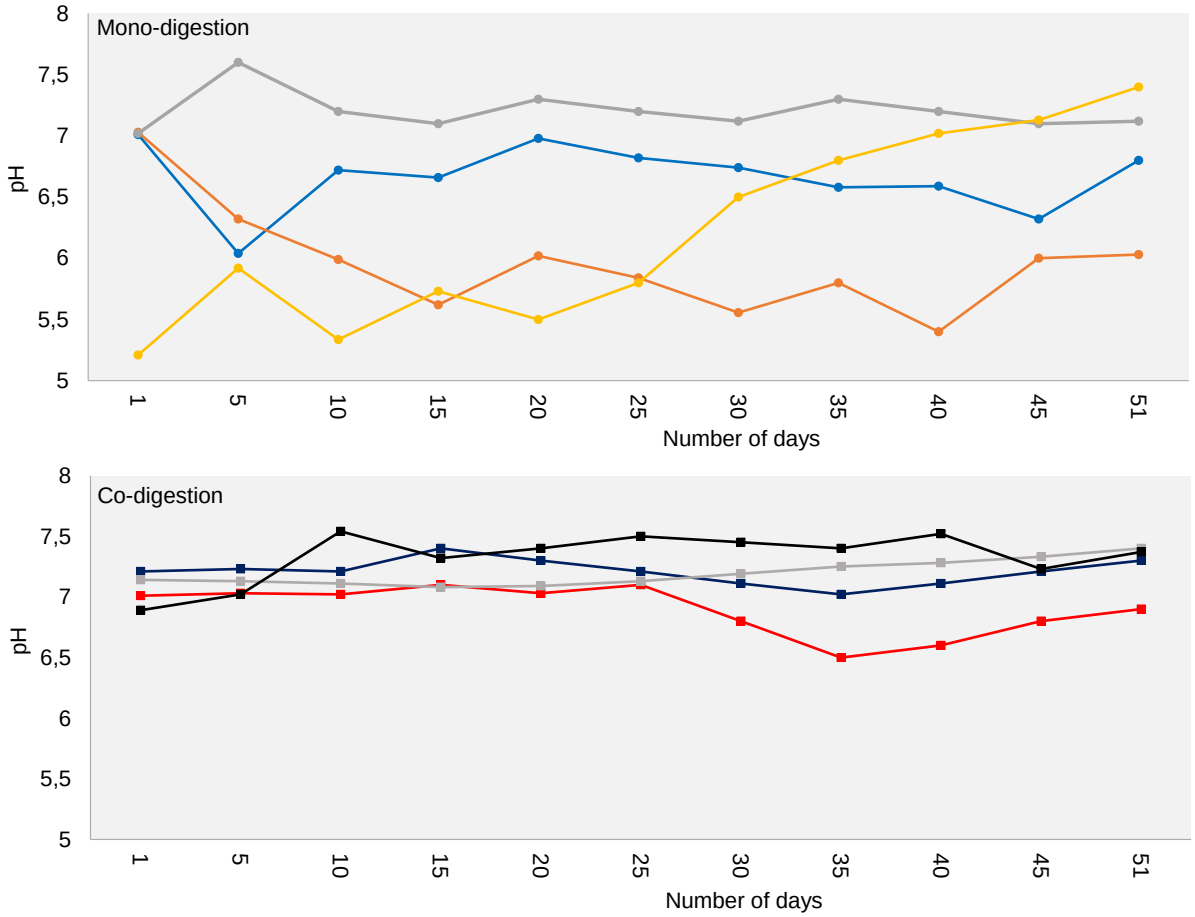

Figure 24: pH variations of mono-digestion of SBR (●), SOR (●), RSC (●) and SBL (●) and co-digestion of RSC:SOR (25:75) (■), RSC:SBR (25:75) (■), RSC:SOR (50:50) (■) and RSC:SBR (50:50) (■) ratio mixtures. The data above are representative of 51 days of monitoring that commenced on day 214 and continued to day 270.

The pH levels of SBL and SBR after digestion were 6.80±0.04 and 5.80±2.31 respectively. Demirel and Scherer (2008) determined the same pH range after digestion of whole sugar beets. When SBR and SBL were co-digested with RSC, all the digesters operated at a pH level of ≥6, indicating that RFI3 was possibly the major provider of alkalinity in those digesters (Gunaseelan, 1995). The pH in the RSC mono-digesters was lower during the earlier days of operation, likely so because the microbial consortium was still adapting, or VFA had accumulated (Suksong *et al.*, 2017; Chen *et al.*, 2008). On day 20, the pH started escalating from 6.50±0.64 to 7.11±3.60 on day 51. From day 10 to day 51, the digesters loaded with sorghum operated within a pH range of 7.1-7.3. It was noticed that five days after the alkaline solution had been added to the SBR and SBL digesters, they experienced a decrease in pH, but during co-digestion of SBR with RSC this occurrence was

absent, possibly because of the fibre and alkalinity provided by the RSC. The RSC:SBR (50:50) ratio mixtures experienced a pH decline between day 26 and day 27, and this decline continued until day 35. This was probably the period when the digesters accumulated a higher quantity of VFA and, after day 36, the microbes were starting to utilize the VFA (Liu *et al.*, 2008). The pH of the RSC:SOR (50:50) ratio mixture was generally high, and from day 15 to day 51 the digester operated between a pH of 7.23-7.52. On day 10, some of the digesters had a pH of 7.54. This was close to the toxic value of 8 (Macias-Corral *et al.*, 2008) and occurred possibly because of the ammonia that must have accumulated due to the degradation of the RSC (Ward *et al.*, 2008).

Table 6: Volatile fatty acids and pH of mono- and co-digestion before and after anaerobic digestion (Mean ±SD, n=3)

Digesters	VFA before (mg L^{-1})	VFA after (mg L^{-1})	Mean pH (after digestion)
SBL	1200±132.21	2378.68±12.10`	6.80±0.04
SBR	2043.60±59.20	3317.52±85.96	5.80±2.31
SOR	522.45±20.81	1105.15±82.53	7.02±1.02
RSC	1037.22±55.76	1267.33±58.34	7.11±3.60
RSC:SBR (25:75)	575.44±34.04	2656.72±66.01	6.8±1.03
RSC:SOR (25:75)	1077.44±97.00	1749.12±77.23	7.31±4.21
RSC:SBR (50:50)	1849.86±69.84	2842.69±103.81	6.10±9.03
RSC:SOR (50:50)	501.65±80.80	575.44±34.04	7.20±0.17

VFA is one of the most important parameters in a digestion process because it has a direct correlation with the digesters' performances. The SBR mono-digesters started at 2043.60±59.20 mg L^{-1} VFA, which increased to 3317.52±85.96 mg L^{-1} after 51 days (Table 7). The pH of the SBR and SBL digesters was adjusted with a few drops of NaOH solution in the beginning in an attempt to balance the digestion process. These digesters operated between unstable and stable conditions that were reflected by pH fluctuation. The SOR digesters were characterized by a low VFA concentration of 575.44±34.04 mg L^{-1} before digestion, but after digestion the VFA concentration was 1105.15±82.53 mg L^{-1}, which suggests that the digesters were at a stable state on day 51 (Drosg, 2013). This was also supported by the average pH of ≥7 during operation. The low VFA accumulation from this feedstock was in agreement with that of other studies. For example, Antonopoulou *et al.* (2008) detected a pH of ≥7 and VFA of ≤1500 mg L^{-1} after anaerobic digestion of sorghum. The RSC digesters had a VFA level of above 1037.22±55.76 mg L^{-1} on day 1, and after 51 days the VFA concentration was 1267.33±58.34 mg L^{-1} with a neutral pH of 7.11 (Table 8). In the beginning, the mix ratios of RSC:SBR (25:75) and RSC:SOR

(25:75) had low VFA concentrations of 575.44±34.04 mg L^{-1} and 1077.44±97.00 mg L^{-1} respectively. VFA accumulations of 2656.72±66.01 mg L^{-1} and 1749.12±77.23 mg L^{-1} occurred in both RSC:SBR and RSC:SOR in the 25:75 ratio respectively, but the VFA levels that accumulated were still far from the toxic value of 3500 mg L^{-1} (Drosg, 2013). A VFA level of 2842.69±103.81 mg L^{-1} was observed in the digester containing the RSC:SBR (50:50) ratio mixture, and the accumulation of VFA was comparable to what occurred when sugar beet and solid abattoir waste were digested, even when the pH was regulated to normal using different pH regulation regimes (Alkaya & Demirer, 2011). All the digesters that were operated during these tests had a VFA level of <3500 mg L^{-1}, which suggests that RFI3 provided good alkalinity, attesting to the fact that RFI3 was good inoculum as it was able to provide a necessary number of methanogens to act on the VFA that were produced (Sreekrishnan *et al.*, 2004).

6.3.4 Gas production

6.3.4.1 Mono-digestion

a) Daily biogas production

During this study, biogas production levels and CH_4 concentrations were measured on a daily basis. Gas production started on day 7 in the digesters loaded with SBR, producing 360.90±44.30 mL. The SBL digesters started producing on day 8 with an initial gas production of 120.08±23.01 mL. The adaptation of the microbes to the SBR was probably rapid (Suksong *et al.*, 2017; Grimberg *et al.*, 2015). The digesters loaded with SOR started producing gas on day 6, producing 218.40±12.89 mL. Initial gas production in the digesters loaded with RSC started on day 10 at a volume of 50±2.00 mL. In digesters loaded with SOR and RFI3 possibly caused a quick start-up of the digestion process by providing a good buffering capacity that helped to keep the pH of the digesters within the operational range (Gulhane *et al.*, 2017; Wang *et al.*, 2014). The delayed and low biogas production with RSC was probably because the microorganisms did not have any easily degradable nutrients available (Manser *et al.*, 2015). The initial gas produced was the highest for SBR and SOR. SBR produced the highest biogas in the earlier days because biogas yield depends on the composition of the substrate, and sugar beet after storage consists mostly of simple sugar and alcohols that can be converted into biogas easily and quickly (Sauthoff *et al.*, 2016). The gas production per day during the mono-digestion tests differed, probably because of the different quantities of nutrients and the number of

microbes available to utilize the available substrates that were in turn dependent on the feedstock in the digesters (Suryawanshi *et al.*, 2013; Demirel & Scherer, 2008). Microbes in the SBR and SBL digesters were expected to adapt faster because the pH had been adjusted to operational value (Table 6), but VFA accumulation possibly inhibited microbial activities (Suksong *et al.*, 2017; Zhai *et al.*, 2015).

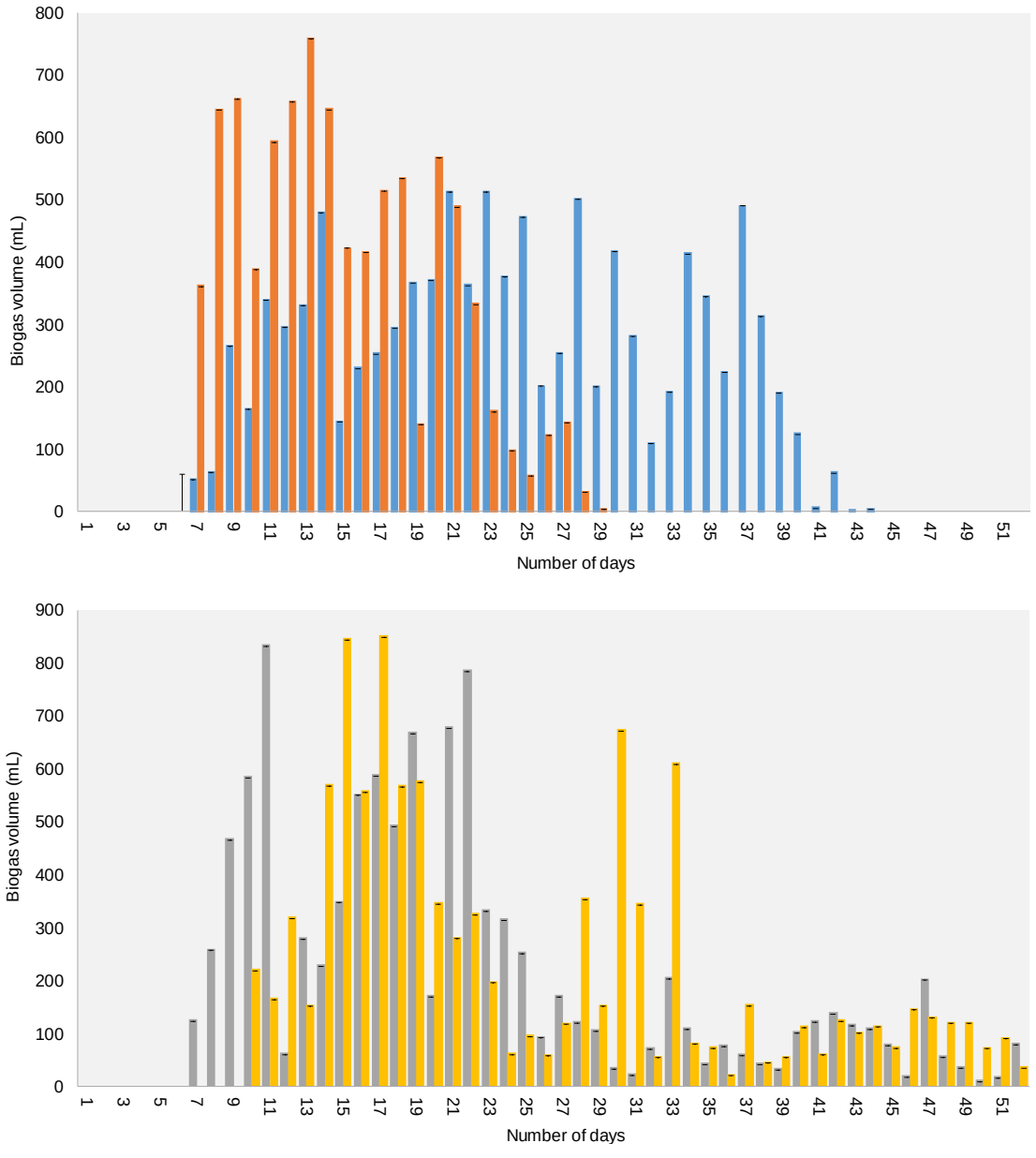

Figure 25: Daily biogas production from mono-digestion of SBR ( ), SBL ( ), SOR ( ) and RSC ( ) in batch digesters. The graphs indicate the volumes of gas produced every day by each feedstock individually, plotted as averages and standard deviations presented as error bars. The data are representative of 51 days of monitoring that commenced on day 214 and continued to day 270.

The digesters containing different feedstock were characterized by different daily biogas production (Figure 24). Daily gas production of the SBL digesters ranged from 195.90±10.56 mL, 167.80±4.44 mL, 196.00±8.00 mL and 194.70±0.34 mL per day with biogas achieved for the SBR, SOR and RSC digesters respectively. The digesters containing SBR probably experienced low daily gas production because, after the extended digestion time, easily degradable compounds were exhausted. The highest daily gas production levels in the SBL digesters were 412.00±43.00 mL on day 23 and 757.30±21.80 mL on day 13. For SOR, 731.60±17.43 mL was measured on day 11 and the RSC digesters reached a maximum daily gas production of 748.40±78.00 mL on day 17 (Figure 23). The digesters with SOR and RSC continued to produce biogas, albeit much less, until day 51. The SBL digesters stopped producing gas from day 42 to day 45, while the SBR digesters stopped producing gas on day 29 to day 31. Initially, it was thought that these digesters had stopped because the VS that were available were fully degraded; however, these digesters were characterized by high VFA concentrations which caused the exhaustion of the microbes, which then hindered gas production (Zhai *et al.*, 2015).

b) Cumulative biogas

Because the digesters contained different feedstock, they were characterized by various cumulative biogas yields. The biogas that accumulated from the mono-feeds was elaborated by different curve trends, but these digesters showed the same behaviour from day 1 of operation to day 5 because there was no biogas production (Figure 25). The microbes were in the process of adaptation during this period, and it is possible that they needed time to acclimatize to the substrate due to slow activated inoculum (Grimberg *et al.*, 2015). The graph developments of SOR, RSC and SBL were again equivalent from day 47 to day 51, and these digesters were probably facing the same low aggregated gas volume and low daily gas production during this timeline (Figure 25). The low gas production during this period was because RSC and SOR, which have a high cellulose content, were most probably available as a substrate for anaerobes. It is possible that, in the SBL digesters, insufficient buffering capacity occurred. Moreover, the inoculum caused unstable pH levels that resulted in high VFA accumulation, which subsequently caused a reduction in methanogenic activities (Zhai *et al.*, 2015).

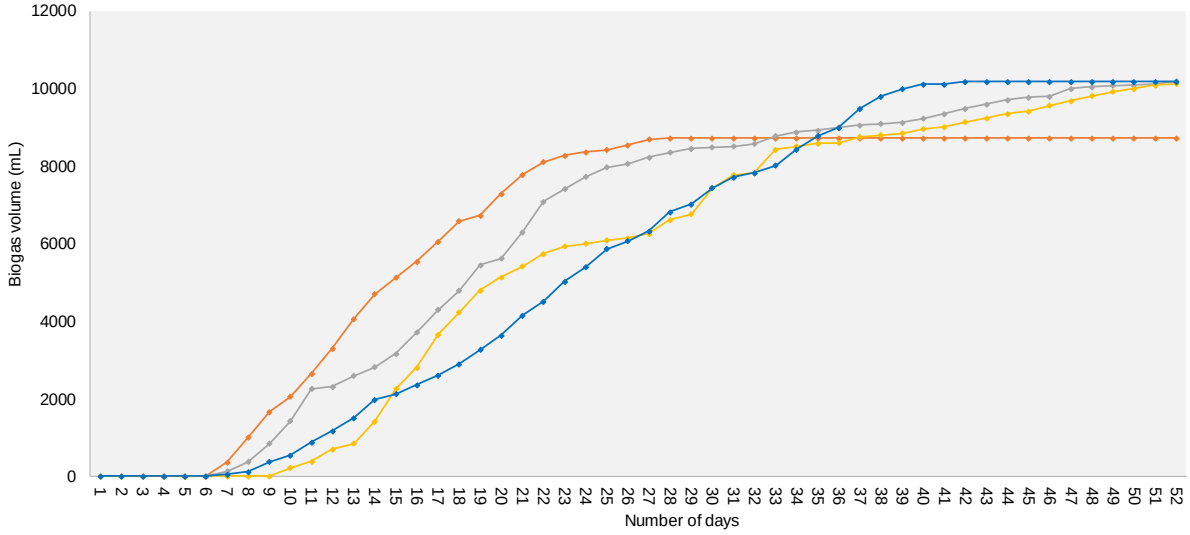

Figure 26: Cumulative biogas from mono-digestion of SBR (◆), SOR (◆), RSC (◆) and SBL (◆) in batch tests. Data are representative of 51 days monitored and commenced from day 214 to day 270.

The total biogas that accumulated from highest to the lowest levels occurred in the following: SOR>SBL>RSC>SBR. The total biogas volumes that were produced in the SBL, SBR, SOR and RSC digesters were 10185.80±15.28 mL, 8723.00±160.30 mL, 10190.00±81.40 mL, and 10123.01±69.30 mL in this order respectively. SBR produced the lowest total biogas volumes which were similar to the findings by Subramanian and Pagilla (2014) and Demirel and Scherer (2008) who declared that sugar beet was a poor substrate, but that SBL was better as it produced higher biogas volumes. The lower gas production volumes during the digestion of SBR may probably be attributed to captivated contaminants from the soil, because when maize was used for phytoremediation of contaminated soil, the biogas produced from this feed was reduced by half when compared to fresh maize, and CH_4 concentration was also affected (Šotnar *et al.*, 2014). Thus captivated metals from the soil affect digesters negatively, but because no metal analyses were performed in this investigation, it may only be surmised that that metals were captivated via phytoextraction, as was demonstrated by Attinti *et al.* (2017) who used grass to captivate contaminants. When primed well (as it was done in this study), RSC may be a good feedstock for biogas production as it produced gas volumes that were in the same range as the SBL and SOR tests and higher gas volumes than the SBR tests. These findings were in agreement with those of Cuetos *et al.* (2008) who, after digestion of mixtures of solid slaughterhouse waste, attained high gas production and eliminated more than 90% COD. Sugar beet leaves are generally not considered for energy yield because they are used in fields for soil fertility purposes (Jacobs *et al.*, 2017). The same was true for the SBL used in this study. If high biogas volume

was the target, SBL as a feedstock for biogas production is more suitable than SBR and RSC. This suggests that, if sugar beet is chosen as feed for biogas production, using it as a whole plant will be more beneficial because the differences in pH, TS% and composition of the feed of SBR and SBL had a noteworthy impact on the performances of the digesters (Schnurer & Jarvis, 2010).

6.3.4.2 Co-digestion

a) Daily gas production

The progression of the daily gas production with the co-digestion of RSC:SBR and RSC:SOR (25:75 and 50:50 ratio mixtures) is presented in Figure 26. The first biogas volume that was collected for RSC:SOR (25:75) was on day 7, for RSC:SBR (25:75) it was on day 6, and for the 50:50 ratio of RSC:SBR and RSC: SOR ratios it was also on day 7.

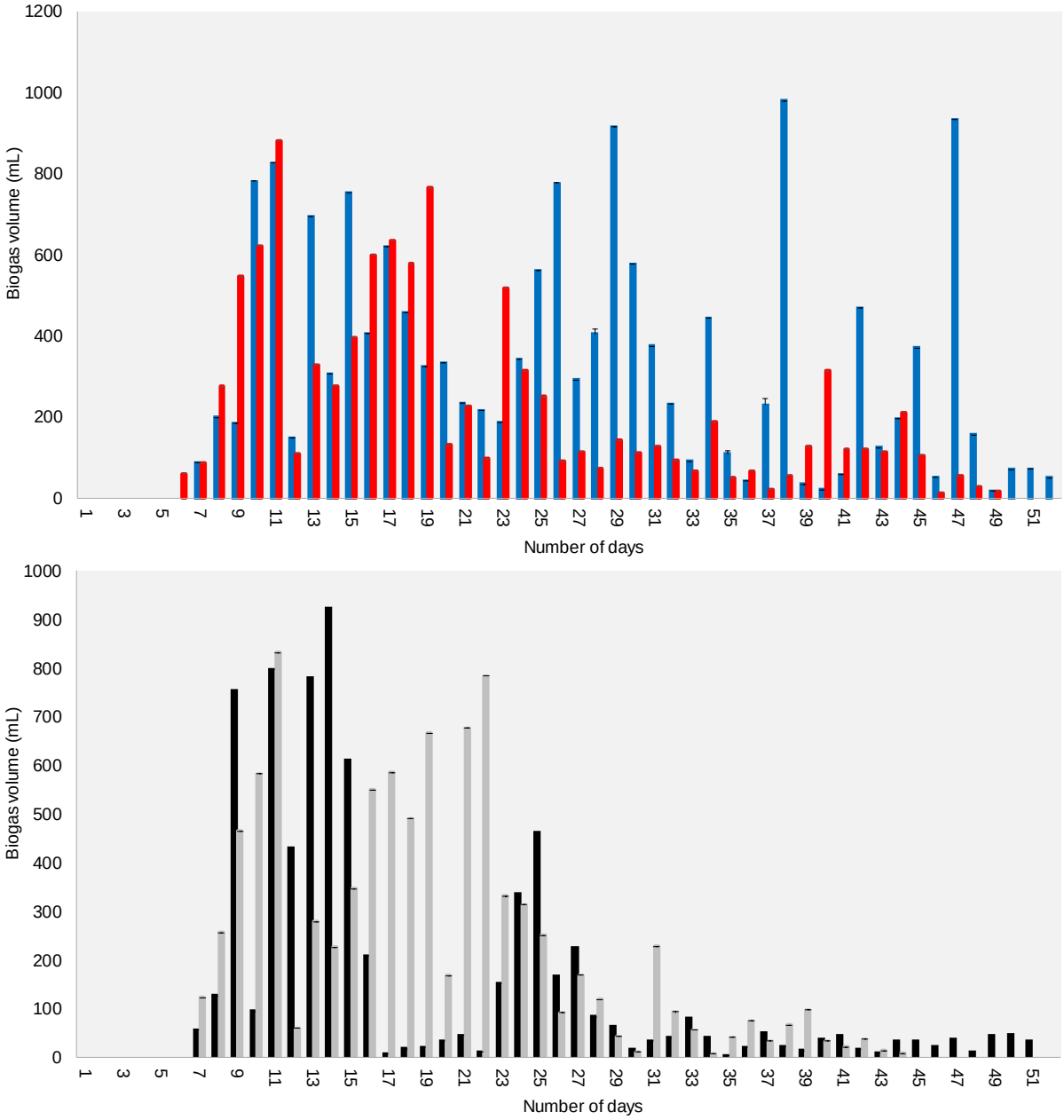

Figure 27: Daily biogas production with ratio mixtures RSC:SBR (25:75) ( ), RSC:SOR (25:75) ( ), RSC:SBR (50:50) ( ) and RSC:SOR (50 :50) ( ). The graphs indicate the volumes of gas that were produced every day by each feedstock individually. The findings are plotted as averages and standard deviations presented as error bars. The data are representative of 51 days of monitoring that commenced on day 214 and continued to day 270.

The digesters loaded with RSC:SOR (25:75) had a daily average gas production of 302.20±100.45 mL and they achieved a maximum daily biogas production of 979.90±76.00 mL on day 38. Digesters RSC:SOR (50:50) produced a maximum of 831.60 ±55.20 mL on day 11, but gas was generated until day 44 at an average rate of 180.53±45.20 mL per day (Figure 26). During mono-digestion, SOR started producing biogas earlier than RSC and was also the highest biogas producer per

day, followed by SBL. RSC:SOR (25:75) started producing biogas earlier, but this was because the digesters were possibly using substrates provided by SOR. The higher average daily gas production during the earlier days occurred possibly because balanced C/N and fibre contents were provided by both feedstocks (Suksong *et al.*, 2017; Lehtomäki *et al.*, 2008). When the quantity of RSC was increased and that of SOR was reduced in the RSC:SOR (50:50) ratio, there was a reduction in the average volume of biogas produced per day as well as in the TS of TS%. Moreover, fraction fibre, buffering capacity and nutrients were possibly affected by the change in the feed composition. Both the RSC:SOR and RSC:SBR (50:50) ratios had a rapid initial gas production, but the gas production started declining from day 34. This might have been due to a shorter lag phase growth that instigated a quick degradation of readily biodegradable organic matter in the substrates (Demirel & Scherer, 2008; Parawira *et al.*, 2004). The low biogas production from day 34 indicates a reduction in methanogenic activities, which may be attributed to various factors (Subramanian & Pagilla, 2014; Zhang *et al.*, 2013).

Digesters with a RSC:SBR (25:75) ratio mixture produced a daily average biogas of 194.30±34.90 mL, but the maximum average gas production reached in a day was 979.90±23.33 mL on day 11. Digesters with an RSC:SBR (50:50) mix reached their maximum average daily gas volume on day 14, equating to 922.33±16.00 mL, and the daily average gas that was produced was 138.15±41.65 mL per day (Figure 26). The RSC:SBR (50:50) mixture had a lower average daily production rate compared to each individual feedstock. Digesters that are loaded with high fibre contents usually experience low solid degradability (Zhang & Jahng, 2010). During the earlier days of production at both 25:75 and 50:50 ratios of RSC:SBR, SBR was possibly the major provider of substrates of simple sugars that were converted directly to CH_4. Parawira *et al.* (2004) reported the same quick degradation of sugar beet during the earlier days of operation. All the 50:50 ratio mixtures selected had a lower daily biogas production when compared to RSC, SBL and SOR in mono-fermentation. The mixture of RSC:SOR (25:75) had the highest biogas production per day with the highest gas production in one day when compared to the other mixtures. RSC:SBR/SOR (25:75) ratio mixtures were both better options than the 50:50 ratio mixtures with regards to daily gas production. The RSC:SBR (25:75) ratio proved to be a good biogas producer per day because the digesters contained a larger number of microbes acting on the VS available per day (Mudhoo, 2012). This

implies that, if biogas is intended for daily use, this ratio mixture can potentially deliver a higher biogas yield per day than the other ratios.

b) Cumulative biogas

The digesters containing the RSC:SOR (25:75) ratio mixtures had a good increase of accumulated gas (Figure 27). This mixture possibly resulted in a balanced VS% and nutrients, which resulted in a large volume of biogas after co-digesting the two feeds. The maximum volume of gas that accumulated in these digesters was 15716.90±149.90 mL, which was higher than with the mono-digestion of RSC and SOR. A horizontal curve movement was virtually absent in the cumulated biogas of the RSC:SOR (25:75) ratio mixture (Figure 27) and this is attributed to the continuous production of biogas. High biogas production from this ratio mixture was possibly influenced by the balanced fibre and higher buffering capacities provided by the well-balanced contents in these digesters (Suksong *et al.*, 2017; Gunaseelan, 1995). The RSC:SOR (25:75) digesters produced ≈50% of the total gas produced on day 25. While, the RSC:SOR (50:50) ratio mixture accumulated a low biogas of 9207.00±101.33 mL and ≈87% of the total gas produced in 25 days, which suggests that the feed in these digesters were being utilized effectively by the microbes and that this affected gas production positively during the earlier days (Hejnfelt & Angelidaki, 2009; Angelidaki & Ellegaard, 2003). The low biogas that accumulated due to the RSC:SOR (50:50) ratio mixture was probably due to the fact that the most available materials for microbes were lignocellulosic because the mixture contained a higher fibre content (Manser *et al.*, 2015). It is most likely that the lignocellulose materials in these two type of feedstocks were degrading at the same slow rate. Motte *et al.* (2013) elaborated that, when digesting high solid (≥15%) digesters, optimizing TS content and particle size needs to occur to achieve process stability.

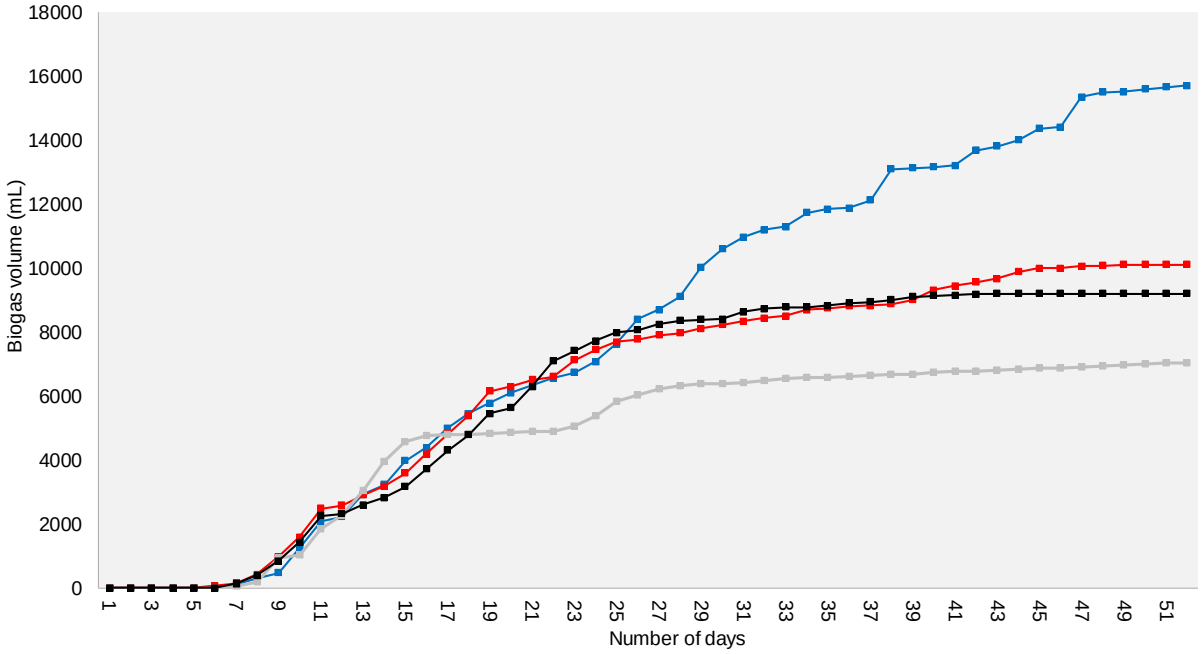

Figure 28: Cumulative biogas from RSC:SOR (25:75) (■), RSC:SBR (25:75) (■), RSC:SOR (50:50) (■) and RSC:SBR (50:50) ratio mixtures (■). The data that are presented in the graphs above are representative of 51 days of monitoring that commenced on day 214 and continued to day 270.

The RSC:SBR (25:75) digesters accumulated a maximum of 10102.93±12.00 mL of biogas, which was less than what was collected in the RSC mono-digestion test, but higher than the mono-digestion of SBR. These digesters started having a horizontal motion on the curve during the last few days (Figure 27), which was possibly due to the fact that the available substrates (alcohols and simple sugars) that were mostly supplied by SBR were exhausted (Demirel & Scherer, 2008; Parawira *et al.*, 2005). The RSC:SBR (25:75) digesters produced ≈76% of the total gas produced on day 25 via positive synergic effect. Aboudi *et al.* (2016) noted the same synergic effect when sugar beet roots were co-digested with cow manure, because the biogas yield was improved. This was also noticed when sugar beet was co-digested with grass silage, which improved gas production and also resulted in high CH_4 yields (Ahmed *et al.*, 2016; Umetsu *et al.*, 2006). The RSC:SBR (50:50) digesters produced a low total biogas of 7045.67±74.00 mL and ≈83% of it had been produced by day 25. These digesters were possibly utilizing the simple sugars mostly provided by SBR. This was evident by the horizontal movement of the curve that occurred after day 25, which presented a low or no gas production (Figure 27). The microbes provided by RFI3 possibly had a poor adaptability to lignocellulose digestion because, when approaching the last days of monitoring, the gas production levels declined. Digesters RSC:SBR (50:50) produced the lowest biogas after 51 days when

compared to all the mono- and co-digestion trials. RSC:energy crops (50:50) ratio mixtures performed poorly because of high VS% which increased the total organic load of the digesters, thus resulting in a longer retention time needed for the digesters to reach maximum performance (Farhat *et al.*, 2018; Babel *et al.*, 2009).

The low or no gas accumulation that is presented in Figure 26 and Figure 27 as follows: Day 9 to day 10, day 15 to day 23, and day 25 to day 51 for the RSC:SBR (50:50) ratio mixture. Day 11 to day 13, day 21 to day 22, and day 43 to day 51 for RSC:SBR (25:75) ratio mixture. Day 11 to day 13, day 19 to day 21, day 26 to day 29, and day 31 to day 51 for the RSC:SOR (50:50) ratio mixture. The cessation for the RSC:SOR (25:75) ratio mixture on day 11to day 13, day 33 to day 36, day 38 to day 41, and day 42 to day 45 probably occurred because the microbial activities had ceased during these periods. This cessation is generally more noticeable in digesters loaded with SBR. In these digesters, carbon was possibly degraded easily and quickly, favouring VFA formation without keeping pace with the utilization of the acids by methanogens and, in turn, probably inhibited microbial activities (Roubík *et al.*, 2017; Chen *et al.*, 2015; Massart *et al.*, 2006). The slow degradation of complex materials possibly occurred in digesters such as RSC:SOR (50:50) because they were loaded with high fibre content. The low biogas yield of RSC:SOR (50:50) was possibly an indication that methane production was inhibited (Lu *et al.*, 2008; Ward *et al.*, 2008). In the other digesters, the carbon to nitrogen was probably not available for use by microbes (Ward *et al.*, 2008). Mixing RSC and SOR in a 25:75 ratio produced the highest average volume of gas over 51 days. When using SBR and SOR to co-digest with RSC, the gas production of SBR and SOR was sustained and prolonged. The mono-digestion resulted in a quick biogas production that ended earlier (Figure 26 and Figure 27). Thus, if biogas volume is the target, a mixture of RSC and SOR in a 25:75 ratio seems economical because the total volume of gas produced by this mixture was more compared to all the other mono- and co-digestions.

6.3.5 Biogas composition and yield

During the mono-digestion experiments, SBL produced the lowest mean CH_4 concentration of ≈53%; with the lowest CH_4 yield of 282.60±0.46 m^3 t^{-1} VS. The biogas yield for SBR was 361.38±44.12 m^3 t^{-1} VS with a CH_4% of ≈58% (Table 6).

The CH_4 content of the biogas that was produced from SBR and SBL was in agreement with the findings of other studies (Demirel & Scherer, 2008). When sugar beet silage was used for biogas production, a CH_4 content of ≈53% was attained. Moeller *et al.* (2015) reported a CH_4 of between 68-72%, whilst Ahmed *et al.* (2016) and Umetsu *et al.* (2006) reported a 57-59% CH_4 concentration from sugar beet roots. Esposito *et al.* (2012) projected a 750-800 $m^3\ t^{-1}$ VS potential yield of SBL and 730-770 $m^3\ t^{-1}$ VS for SBR. Moeller *et al.* (2015) reported a high CH_4 yield for SBL whilst Nordberg and Edström (2003) determined a CH_4 potential yield of forage beets plus leaves to be 456 $m^3\ t^{-1}$ VS.

Table 7: Biogas composition and yield for mono- and co-digestion experiments (Mean±SD, n=50).

Digesters	Mean CH_4 (%)	Total biogas produced (mL)	CH_4 yield ($m^3\ t^{-1}$ VS)
SBL	53	10185.80±15.28	282.60
SBR	58	8723.00±160.30	361.38
SOR	64	10190.00±81.40	326.08
RSC	57	10123.00±69.30	399.66
RSC: SBR (25:75)	65	10102.93±12.00	450.09
RSC: SOR (25:75)	61	15716.90±149.90	515.45
RSC: SOR (50:50)	61	9207.00±101.33	272.63
RSC: SBR (50:50)	50	7045.67±74.00	167.75

SOR digesters produced a mean CH_4 concentration of ≈64%, which was in agreement with the findings of other studies. Klimiuk *et al.* (2010) and Antonopoulou *et al.* (2008) produced biogas with a 58-64% CH_4 concentration from sorghum. SOR had a CH_4 yield of 326.08±2.60 $m^3\ t^{-1}$ VS which was lower than the estimated potential of 520-620 $m^3\ t^{-1}$ VS (Esposito *et al.*, 2012). The RSC digesters produced a mean CH_4 concentration of ≈57% and a CH_4 yield of 399.66±1.47 $m^3\ t^{-1}$ VS (Table 6). The CH_4 content was lower than and the CH_4 yield was comparable to other studies, because Hejnfelt and Angelidaki (2009) produced ≈70% CH_4 content from mixed slaughterhouse waste while they also reported a CH_4 yield of 225-619 $m^3\ t^{-1}$ VS. Nordberg and Edström (2003) reported a potential yield of 225 $m^3\ t^{-1}$ VS from mixed animal by-products. The CH_4 yield is an important economic factor in an anaerobic digester if the CH_4 yield is the target. The current study thus argues that RSC is the best feedstock to use because it produced the highest CH_4 yield when compared to other mono-digestions. Moreover, the findings of this study pertaining to CH_4 yields using energy crops did not differ significantly from reports in the literature for energy crops that had not been exposed to contamination.

The RSC:SBR (25:75) mix had the highest average gas quality of ≈65% in comparisons of both mono- and co-digesters, which was an improvement when compared to CH_4% of mono-digestion of each individual feedstock. Thus, co-digestion can improve the biogas yield. Parawira *et al.* (2004) improved the gas production of mono-digestion of SBL by 6-31% by co-digesting SBL with potato waste. In the current study, a mixture of RSC and SOR at a ratio of 25:75 produced the highest CH_4 yield of 515.45±4.91 $m^3\ t^{-1}$ VS, whilst the RSC:SBR (50:50) ratio mixture produced the lowest yield when compared to all the different ratio mixtures and mono-digestions (Table 6). Digesters with 25:75 ratio mixtures of RSC:SBR and RSC:SOR both produced high CH_4 yields when compared to the other ratio-mix experiments. These ratios had the highest CH_4 concentrations, implying a high caloric value which is economical and desirable. Synergistic increases in CH_4 yields were also found when sugar beet was co-digested with crops such as maize and grass silage (Ahmed *et al.*, 2016). The impact of RSC on the biogas production using energy crops was encouraging. Using RSC to co-digest with energy crops could help reduce the space required for arable land. When RSC was prepared well, it proved to be a good feedstock with the potential to produce higher CH_4 yields.

6.4 Conclusion

This study demonstrated that bioenergy production from crops used for phytoremediation of mine-impacted land compared well with that of energy crops reported in the literature that were not exposed to contamination. Sugar beet leaves produced a mean CH_4 concentration of ≈53% and a CH_4 yield of ≈282.60 $m^3\ t^{-1}$ VS. These levels are economical and thus the use of sugar beet leaves for biogas generation offers an alternative to simply dumping this feedstock. RSC produced a total biogas volume of ≈10171 mL with a CH_4% of ≈57% and a CH_4 yield of ≈399 $m^3\ t^{-1}$ VS, which are levels that suggest that RSC is a good feedstock for biogas production. Moreover, the production of biogas from RSC co-digested with energy crops produced higher CH_4 yields and biogas volumes, especially when an RSC:SOR (25:75) ratio mixture was used. This study thus argues that the co-digestion of energy crops that were exposed to contaminated land with abattoir waste has the potential to rehabilitate soil. This will circumvent landfilling using organic waste while it will, at the same time, produce bioenergy efficiently.

Chapter 7: Concluding Remarks

Anaerobic digestion is a method with the potential capability of reducing landfill and GHG emissions while concurrently producing bioenergy. Starting a digester can be a complex process that is fraught with complications, such as selecting a proper seeding method and materials, creating appropriate anaerobic conditions in an industrial digester, choosing a proper method of loading a seed/feedstock, selecting a proper feedstock, and a good feeding rate. The bioenergy project that was undertaken intended to explore the production of biogas from energy crops to start up an industrial digester (AD2) with RSC as a seeding material.

The seeding of AD2 (an industrial size digester that had been used at a gold mine) was studied to determine the most effective start-up methodology and to explore factors that could lead to digester failure. During seeding of AD2, a vast number of technical problems were experienced. To seed AD2 successfully, the following were required: proper seeding materials, correct process monitoring, proper retention time, and impeccable agitation rates and intervals. When the optimal levels of these processes had been determined, the system balanced naturally and high-quality CH_4 was produced. It was clear that the old steel refurbished Pachuca tanks that had once been used as gold processing units could be repurposed for biogas production, given that the correct inoculum was used and proper process monitoring was applied. When AD2 experienced instability, changes were applied promptly and conservatively, if analytic feedback of laboratory results was the guide for the changes applied on AD2 during seeding it would have benefited digester-seeding process.

During the rehabilitation of AD2, some of the rehabilitation methods that were applied were probably correct, but more time to exhibit process progression was necessary. When resolving digester instability, it is advisable to avoid implementing numerous solutions simultaneously while the process is still in its early stages. The study found that when alkaline solutions were added to adjust the pH of AD2, positive results were achieved. However, efficient methods to adjust the pH of industrial digesters to favour the environment for microbial growth in all process phases require more investigation.

Also, a proper process monitoring regime needs to be investigated further as it was evident that the parameters that were used to monitor AD2 were important, although they were often not able to provide early warning of process instability during inoculation.

Operational stability of an anaerobic digestion process is vital for commercialization. A number of factors caused the digester's failure or instability, and therefore understanding these factors is vital when operating a digester. When starting a bioenergy plant that will be operating digesters of industrial size, the management team needs to ensure that team members are knowledgeable about both systems engineering and biotechnological applications, and that ample time is dedicated to that single project. This study provides a general and pragmatic view of these processes, urging that proper operation of an industrial digester requires representative, accurate, timeous and continuous monitoring of the parameters that will positively influence the microbial communities for biogas generation. It is cautioned that haphazard, incorrect and continuously inapt data generation and interpretation can lead to process failure. If the data that are generated are not interpreted on a continuous basis, process stability will be disadvantaged, and preventative or corrective actions will not be taken appropriately. Real-time dependable measurements of all parameters are thus vital.

Laboratory analyses of important parameters associated with industrial digesters can provide vital data. It is important to make sure that the sampling process is executed properly and that the samples are sent to the laboratory on time. This may require strategic initiatives such as establishing a standardized sampling protocol, a sampling schedule, an on-site laboratory, and instating dedicated courier services.

Low levels of pH, CH_4% and alkalinity and a high TS% and VFA indicated that AD2 had become dormant after 98 days of operation. Laboratory trials to rehabilitate the industrial digester demonstrated that increasing the temperature and the retention time would achieve high CH_4 production. When temperature elevation was impracticable, digesters in psychrophilic conditions were revived nonetheless, but the revival period was extended. When LSM and DFOD were used to revive laboratory dormant digesters, the biogas production was improved and the lag phase was reduced. These results were better compared with those of digesters that were operated in mesophilic conditions without the addition of any inoculum. Adapting the

LSM as the inoculum of choice in the large-scale digester was proficient in improving gas production, volatile solids degradation, and in reducing the lag phase of microbial growth. It was also determined that using DFOD to revitalize AD2 was an economical choice. The study also proposes that when RSC is used as a feedstock/inoculum of choice, the ammonia concentration should be analysed regularly to stem its effects because ammonia was often implicated during this study as a potential inhibitor of anaerobic digestion. During these trials, it was also recognized that analysing the microbial population is essential. Further trials to determine the optimum temperature for digester revitalization will benefit the improvement of biogas generation in association with other methods intended to reduce the organic retention time and increase the biodegradability, whilst still operating at a temperature that is energy conserving. Thus further investigations of inexpensive methods to insulate digesters to avoid temperature fluctuations in psychrophilic conditions will benefit industrial digesters that need to operate a low temperature.

When determining CH_4 yields from energy crops and RSC via mono- and co-digestion, it was established that bioenergy production from crops used for phytoremediation of mine-impacted land compared well with that of earlier studies using energy crops that had not been exposed to contamination. Rumen fluid inoculum was used for digesting energy crops, and it uplifted the pH of feedstock that had low pH levels. The results that were obtained for this phase of the study indicated that energy crops that had possibly been contaminated produced high CH_4 yields and biogas volumes. This indicated a potentially economical process as no pH was adjusted, but it is possible that this process will be different in continuously fed digesters. When energy crops were co-digested with RSC, an RSC:SOR (25:75) ratio mixture produced the highest biogas volume and CH_4 yield.

The trials that were conducted in this study were performed in batches, thus the hydraulic retention time and organic loading rate analyses will be important when operating digesters in continuous feed trials. The results that were obtained for the batch tests need to be supported by additional tests for microbial analyses and for determining ammonia, COD, and heavy metal (retention and accumulation) concentrations, as well as VFA accumulation during digestion. Ratio mixtures such as RSC:SBR (25:75) and RSC:SOR (50:50) produced sufficient levels of CH_4%, but

less than RSC:SOR (25:75) ratio mixtures. A further investigation into different ratio mixtures will enrich high biogas production.

During the operation of the digesters in these trials, potential contamination by captivated metals was conceivable. However, the potential impact of heavy metal content on the digesters was not measured, thus determining heavy metal concentrations in the digestate in future trials is essential. Moreover, to conclusively understand that the energy crops captivated the contaminants, proper laboratory experiments utilizing relevant analysis procedures are necessary. The investigation of a proper method to ensile and store energy crops to attain VS is important as the storage method that was applied affected VS% of the energy crops. If energy crops are selected as feedstock for biogas production, it is important to select ones that can be cultivated locally. If the co-digestion of energy crops and RSC is favoured, it is essential to procure proper machinery to prepare the feed so that it is convivial for an expensive pre-treatment system.

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