ANAEROBIC DIGESTION OF DAIRY MANURE WASTEWATER, FOOD AND FRUIT WASTE, A SUSTAINABLE SOURCE OF BIO-ENERGY AND WASTE MANAGEMENT

By

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The potential for anaerobic co-digestion (AD) organic waste streams such as dairy manure waste water, fruit and food waste is a well-established process. A pilot study, utilizing a new constructed 40.248 m^3 plug-flow, three chamber anaerobic reactor was conducted to determine the effects of temperature, electrical conductivity (EC), pH, organic loading rate (OLR) over a 20 day hydraulic retention time (HRT) on biogas yield, waste volume reduction, water soluble minerals, reduction of pathogenic microorganisms (*Escherichia coli O157:H7*), phytotoxcity and anaerobic microbial population dynamics.

The reactor was equipped with a build-in temperature sensor which allowed for the internal temperature to be recorded from the circulation of water via an 8L biogas geyser. The reactor was also fitted with three ports connected to the three individual chambers, from which samples for EC and pH were lifted for analysis. Dairy manure wastewater had a total soluble (TSS) concentration (2.59 g/l) which was considerably lower than the anticipated 10% TSS. This meant the reactor had an OLR of 2.59 kg/$m^3_{reactor}$/day (Total suspended solids) and 1.97 kg/$m^3_{reactor}$/day volatile suspended solids (VSS). To maintain an organic retention time (ORT) equivalent to HRT, co-digestion of fruit and food waste was than calculated based on the TSS and VSS content of dairy manure wastewater.

The results validated that the reactor's temperature plays an important role in the production of biogas and reduction of pathogenic microorganisms of the digestate. Due to the low temperatures during the trial period, no viable biogas yields were detected using the current water displacement system and thus no biogas quality analysis were conducted.

Temperature data confirmed that at temperatures below 20 °C, there was no linear relationship between the salinity indicator, EC and temperature. In addition, these low psychrophilic temperatures at a HRT of 20 days had no comparable impact on the reduction of pathogenic microorganisms. However, a 97% reduction in TSS entering the reactor was observed in all digestates. Water soluble minerals entering the reactor through feedstock were comparable to the soluble mineral concentrations the digestate. Although, the digestates contained high

concentrations of mineral nutrients such as ammonium which were above the legal limit of waste water to be discharged in the environment.

At concentrations below 75% none of the digestates showed significant phytotoxicity effects quantified with tomato seed germination. While, the data also seem to suggest that OLR had a noticeable influence on microbial population dynamics. Overfeeding the reactor induced an instant decreased on pH and also increased the microbial population species that colonize the different chambers. Under current sub-operating conditions compounded by low OLR, the AD reactor was not competitive enough to replace the current composting operation.

OPSOMMING

Die potensiaal vir anaërobiese mee-vertering (AD) van organiese afvalstrome soos suiwelmis afvalwater, vrugte- en voedselafval is 'n goed gevestigde proses. Vir ‘nloodsstudie, is gebruik gemaak van 'n nuut geboude 40. 248 m^3 propvloei, drie-kamer anaërobe reaktor om die uitwerking van temperatuur, elektriese geleiding (EG), pH en organiese laai tempo (OLR) oor 'n 20 dag hidrouliese retensietyd (HVT) op biogas opbrengs, afvalstroom volume vermindering, water oplosbare minerale konsentraises, die vermindering van patogene mikro-organismes soos (*Escherichia coli* O157: H7), fitotoksisiteit en mikrobiese anaërobiese bevolking dinamika te bestudeer.

Die reaktor is toegerus met 'n inhuis temperatuur sensor wat dit moontlik gemaak het om die interne reaktor se temperatuur te monitorword sirkulasie van water deur 'n 8 L biogas verhitter. Die reaktor is ook toegerus met drie poorte wat gekoppel is aan die drie individuele kamers, waaruit monsters vir EG en pH ingesamel kon wordvir ontleding. Suiwelmis afvalwater het 'n totale oplosbare wastetof (TOVS) konsentrasie getoon wat aansienlik laer is as die verwagte 10% TOVS. Dus het die reaktor het 'n OLR van 2. 59 kg / $m^3_{reactor}$ / dag (TOVS) en 1. 97 kg / $m^3_{reactor}$ / dag vlugtig gesuspendeerde vastestowwe (VSS) getoon. Om 'n organiese behoud tyd (ORT) gelykstaande aan HVT te bereid, is mee-vertering van vrugte- en voedselafval geïmplimenteer gebaseer op die TSS en VSS inhoud van suiwelprodukte mis afvalwater.

Die resultate bevestig dat die temperatuur van die reaktor 'n belangrike rol in die produksie van biogas en die vermindering van patogene mikro-organismes uit die afvloeiwater speel. As gevolg van die lae temperature, is geen lewensvatbare biogas opbrengs waargeneem in die die huidige water-verplasing-stelsel nie en dus is geen biogas kwantifisering gedoen nie.

Temperatuur data het wel bevestig dat by temperature onder 20 ° Cr geen lineêre verhouding tussen die soutgehalte aanwyser EG en temperatuur bestaan nie. Verder het hierdie lae temperature op 'n HVT van 20 dae geen vergelykbare impak op die vermindering van patogene mikro-organismes gemaak nie. 'n Vermindering van 97% in TSS in die reaktor is wel waargeneem in alle afvloeiwater. Water oplosbare minerale in die invoer van die reaktor was vergelykbaar met die in die afvloeiwaer. Alhoewel, die afvloeiwater hoër konsentrasies

van minerale soos ammonium bevat het wat die wettige perk oorskry het fir die storting van afvalwater in die omgewing.

Konsentrasies onder 75 % van al die afvloeiwater het geen beduidende fitotoksisiteit effekte op tamatie saad ontkieming getoon nie. Die data daarop dat OLR 'n merkbare invloed op die mikrobiese bevolkingsdinamika het. Oorvoeding die reaktor het 'n direkte effek getoon op pH en ook die toename in mikrobiese spesies bevolking wat die verskillende kamers koloniseer. Onder die huidige sub-optimale omstandighede, was die AD reaktor was nie mededingend genoeg om huidige stelsel van kompostering te vervang nie.

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Dedications

I dedicate this thesis to my mother, Loise Kafula for her invaluable support and love she showered me with during the memorable and challenging moments of my academic journey and to God the Almighty for the wisdom, protection, guidance and many blessing he has given to me.

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List of Abbreviations

AD: Anaerobic digestion.

EC: Electrical conductivity

OLR: Organic loading rate.

ORT: Organic retention time.

HRT: Hydraulic retention time.

TSS: Total suspended solids.

VSS: Volatile suspended solids.

C: N: Carbon to Nitrogen ratio.

DM: Dairy manure wastewater.

DF: Dairy manure wastewater and fruit waste.

DFF: Dairy manure wastewater, fruit and food waste.

DFd: Dairy manure wastewater and food waste.

CHAPTER 1

General Introduction

1. Background to the research question

The increase in the global human population, economic growth, emergence of new markets and social mobility has resulted in an increased demand for plant and livestock products and services. To keep up with the current demand, agricultural systems have responded by intensifying production. This response, however, resulted in increased pressure on the environment which serves as both source and sink for raw materials and waste (Hooda *et al*. 2000).

Frosch (1996) noted that inefficient practices of resource exploitation often result in a generation of huge quantities of waste, posing significant risks to human health and the environment alike. In addition, it becomes evident that externalities emanating from waste pollution and contamination are not only confined to the sphere of public health and environmental concerns, but that this extends into negative effects on the economy. This argument draws support from Turan *et al*. (2009) who noted that indecorous solid waste management practices have serious health implications on the environment and public health such as pollution of water sources, liberation of unpleasant odours, pest infiltrations and gas explosions.

It becomes evidently clear that waste management affects not only the environment, but is intertwined with economic and social concerns which makes it a rather complex problem. To remedy and possibly reverse some of these complex waste management problems, waste management authorities have been timelessly exploring waste management alternatives which include options such as, incineration, composting and recycling (Oteng-Ababio *et al*., 2013). However, some of these alternatives have several drawbacks such as not being economically feasible (Kinobe *et al*. 2015) or exacerbating current public health and environmental concerns (Lee *et al*., 2004; Vidanaarachchi *et al*., 2006).

However, these alternatives are likely only being taken under consideration under where there is sufficient capital and capable human resources to deal with adequate volumes in order to justify investment. When constrained with limited capital and human resources, it is imperative to quantify waste volumes during the decision making process before a transition from conventional to an alternative waste management option is made. Quantifying waste volumes is essential as this enables the flow information for waste management authorities or stakeholders to allow investors to make informed decisions (Oteng-Ababio *et al*., 2013).

Several authors (Gay *et al*. 1993; King & Murphy, 1996; Purdy & Sabugal, 1999; Oteng-Ababio *et al*. 2013) have tested and demonstrated several methodologies of quantifying municipal solid waste volumes. However, literature on the quantification of waste streams in agriculture such as fruit waste, dairy manure and effluent, remains limited to the author's knowledge. This is in spite of agriculture being the primary producer of organic biomass such as food found in municipal solid waste.

2. Introduction

According to Avaci *et al*. (2013) biomass waste in the form of plant and livestock residues such as crop leftovers and manures are some of the largest sources of available bio-energy sources in both rural and agro-industrial areas. These types of waste are renewable and subsequently inevitable as they intertwined with food production systems. Although, organic matter and nutrients contained in waste produced at the farm level are readily available and have a higher chance of been recycled back into the system, the probability greatly diminishes as the distance from the farm increases.

According to the Food and Agriculture Organization (2013) it is estimated that 40% of food produced for human consumption is either lost or wasted worldwide. Food losses represent a subset of the wholesome-edible food that is not consumed at either retail or consumer level, while food waste represent a subset of food loss that has the potential to be recovered for human consumption (Hodges *et al*., 2011). This FAO estimation on global food waste continues to be cited by many contemporary authors today, although with contradictions. Authors such as Parfitt *et al.* (2010) argued that food waste can be difficult to estimate and scrutinize as it is heavily reliant on limited databases gathered from across food value chains.

In most instances, food waste is quantified at retail level, where output from agricultural systems becomes inputs aimed for human consumption (Parfitt *et al.*, 2010). Parfitt *et al.* (2010) further continues to state that several definitions of food waste exist, largely influenced by the complexities of the food value chain.

In 1981 the FAO defined food waste to be, wholesome edible material produced for human consumption that is spoiled, discarded or consumed by pest. This implied that food waste is any fraction or wholesome food items that are not consumed, but lost from food value chain making it similar to the definition by Hodges *et al*. (2011) on food losses. However, in 2013, the FAO rearticulated its definition of food waste to include food losses or reduction in weight or nutritional value of food destined for human consumption that is left to spoil or discarded, while still fit for human consumption.

On the contrary, Stuart (2009) defined food waste to be any edible material that is intentionally fed to livestock or a by-product from food processing, whereas Evans (2011) defined food waste to be a consequence of how material is disposed through a trajectory that connects it to a waste stream and that surplus production cannot necessarily be classified as waste although it is treated as waste. Although Evans (2011) is not simplistic about food waste, the author’s argument that surplus production does not fall under the decree of waste fails to justify that surplus production was initially produced and destined for human consumption. Whereas, Stuart’s definition remains open to critique especially, when large quantities of commodity crops such as grain and soya that can be consumed by humans tends to be produced to feed livestock and thus these are not wasted but, converted from one form into another. This is despite challenges that these food items fed to livestock might not be efficiently converted to food products and that some are converted to inevitable products such as manures and spoiled feed.

Despite, there being several comparisons and contrasts from numerous authors, food waste and food losses all represent a wastage and loss of resources and must be managed in such a way that some of these are recovered. However, the absence of efficient waste management alternatives especially in developing economies has been attributed as the leading cause why scores of organic waste end up in landfills, burned or buried. Heaped and pressed-down, organic waste in landfills decays anaerobically releasing detrimental greenhouse gases such as methane (CH_4) and carbon dioxide (CO_2).

In addition to the CH_4 and CO_2 produced from food waste sources, (Steinfeld *et al*. 2006) noted that livestock induced land-use practices generate about 2.4 billion tons of CO_2 per annum. According to Garnett (2009) greenhouse gas emission from anthropogenic sources such as the livestock farming will continue to be one of the prominent threats to the environment as long as global consumption of meat and dairy products continues to rise.

These predicaments are further exacerbated by economic growth and increasing global population, increase in disposable incomes especially in emerging economies coupled by the diversification of diets from plant proteins to nutrient dense foods such as meat and dairy. According to Moss *et al.* (2000) there is a correlation between the increasing human population and concentration of greenhouse gases such as methane in the atmosphere, based on that approximately 70% of methane emissions is proposed to emanate from anthropogenic sources. In addition, Schellnhuber *et al.* (2006) highlighted that greenhouse gas emissions are responsible for the pronounced 2 °C rise in atmospheric temperatures, which is far above pre-industrial levels and that such changes are expected to expose us to extreme weather and climate changes.

Organic waste

For the purpose of this study, the author defines organic waste to be waste generated as consequence of a system feedback loop from consumers through value chain back to producers. These will include generation of organic waste during production (food losses), post-harvest during grading and classification, spoilage during storage, processing right through the value chain until the end of the product life cycle when it is discarded by the end user (food waste).

This definition of organic waste is adapted from Parfitt *et al.* (2010), who argued that organic waste streams in agriculture can occur at the farm gate during harvesting and threshing to drying, storage, processing (primary-secondary processing), product evaluation (quality control), packaging, marketing, post-consumer and when the product is finally discarded and disposed. This can also include by-products such as manure from dairy production systems and fruit losses before harvesting and pomaces from the production of value added products such as beverages.

Organic waste at the farm gate such as fruit and vegetable waste can be generated as a consequence of attaching a cosmetic value to food through grading and classification system that only allow fruit or vegetable possessing a certain shape, colour and size to enter the food supply chain.

In spite of this, food waste is not the only kind of waste that finds its origins at the farm gate. Nutrient rich livestock manures, post-farm gate protein-rich waste as that from abattoirs and retailers. These types of waste are inevitable and are linked to agriculture through a feedback loop from consumers back to producers.

Organic waste from agricultural systems presents an ever increasing challenge to farm waste management as the volumes can be overwhelming. These waste volumes need to be handled in a way that is economically viable, environmentally friendly and with minimal impact on society. A number of potential waste management strategies exist in the form of aerobic and anaerobic treatment. However, the absence of effective anaerobic waste management alternatives at some junctions are the leading causes why scores of food waste end up in landfills, incinerated, buried or worse, dumped into the areas where it becomes a bio-safety hazard whilst reducing the aesthetic value of the environment.

The premise of integrated waste management is for organic waste such as manure, food and fruit waste not to have an end to their life-cycles but, for these waste to be utilised as a resource base in order to re-capture opportunities that could have been lost from the food production system. These include stabilization of waste so that it can be returned safely back to the environment. Recovery of energy, water and use of waste as a compost medium and help create farmland and increase food production though adding nutrients while reducing on the need for ever increasing cost of synthetic fertilizers.

Increasingly, there has been increasing interest in anaerobic digestion especially with regard to the use of reactors as an alternative waste management strategy. Despite anaerobic digestion being regarded as a more sustainable alternative to organic waste management. A vast majority of studies have been conducted from the laboratory bench and thus it is important to evaluate this alternative at a semi-commercial scale in order to facilitate and build confidence for a wider adoption of the technology, especially at farm level and agro-industries that produce vast quantities of organic semi-solid and liquid waste.

3. Problem statement

Anaerobic digestion of organic waste streams, dairy manure wastewater, fruit and food waste at a semi commercial scale is not yet an established efficient and sustainable waste management alternative for the production of biogas, waste volume reduction and that the digestate can be suitable compost medium. In order to evaluate the efficiency, economic feasibility, mitigation of environmental and social concerns of anaerobic digestion away from the laboratory bench, it is imperative to evaluate the potential of different organic waste streams with regard to biogas production, organic waste volume reduction and reduction of pathogenic microorganisms such as *Escherichia coli* O157: H7 (*E. coli*) in digested waste (digestate) before it is utilized as an alternative source of fertilizer. In addition, it is just as important to analyse if the digestate to be used as fertilizer has any phytotoxic effects on plant growth. In addition, it remains imperative to evaluate the effects of anaerobic digestion on water soluble minerals present in both feedstock and digestate in order to avoid environmental pollution and contamination. Because anaerobic microorganism have been correlated to biogas production, it will also be essential to monitor microbial population dynamics during the digestion process in order to elucidate the volatile acid accumulation, pH changes, nutritional composition, toxicity, which are factors that can lead to anaerobic digestion process failure.

4. Research question

Is anaerobic digestion of organic waste streams, dairy manure wastewater, fruit and food waste at a semi-commercial scale an efficient and sustainable waste management alternative for the production of biogas, waste volume reduction and can the digestate be a suitable fertilizer?

5. Research aim

The aim of this research is to:

Reassure prospective stakeholders that anaerobic digestion technology could be sustainable alternative organic waste management treatment strategy for livestock manures and waste waters, food and fruit waste. This includes the generation of renewable bio-energy, organic liquid fertilizer while mitigating environmental and social concerns associated with conventional waste management practices such as landfilling, open-dumping, burying, incineration and composting.

6. Research objectives

Quantify dairy and fruit waste volumes and evaluate current waste management practices at Stellenbosch University Welgevallen experimental farm per annum.

1. Determine the efficiency and sustainability of an anaerobic reactor to process dairy manure at Welgevallen experimental farm.
2. Determine efficiency of co-digesting dairy manure waste water with food and fruit waste substrates on production of methane and reduction of waste volume.
3. Determine the economic sustainability of an anaerobic reactor to process organic waste streams at Welgevallen experimental farm.

7. Significance of the research

The study could potentially add to investor confidence in the bio-energy sector especially for aspiring independent power producers, but also contributes to the sustainable management of organic waste streams to address economic, environmental and social concerns induced by conventional waste management such as landfilling, burying, incineration and dumping waste in the environment.

Many would come to an agreement that anaerobic digestion (reactors) is not a novel technology. Literature shows that anaerobic digestion technology has been broadly investigated and demonstrated (El-Mashad & Zhang, 2010). Although there are numerous demonstrations of small-scale and commercial anaerobic reactors worldwide, the majority of studies have been limited to reactors on laboratory benches. While, economic feasibility studies on the technology in South Africa have been limited to survey methods with emphasis on small scale reactors at a household level in rural South Africa.

In addition, literature is scant on the economic feasibility of semi-commercial reactors in South Africa especially under the Mediterranean climate of the Western Cape. The Western Cape is home to the largest dairy cattle population in the country and thus has a potential to cause environmental concerns such a pollution. In addition, waste from the fruit industry have the potential to act as repositories for diseases and parasites if not treated adequately.

The commercialization and use of reactors may thus offer the agro-industry the opportunity to effectively manage waste volumes in a more sustainable way through the recovery of energy and subsequent production of organic fertilizer. In addition, the industry helps mitigate environmental and social concerns and also boosts local economies through the sale of carbon credits and saving of energy and fertilizer overhead costs.

Finally, there is limited literature on the fate of water soluble minerals, phytotoxicity, and conflicting literature on the ability of anaerobic reactor to eliminate pathogenic microorganism after the anaerobic digestion process. Including the combination of dairy manure, food and fruit waste in one feedstock for anaerobic digestion.

8. Scope and limitations

Although, thermophilic anaerobic digesters have been demonstrated to yield several advantages such as efficiency in handling higher organic solid waste and pathogenic microorganism destruction, its use has been limited due to higher energy requirements and poor process stability as a result of increased bacterial decay which can be double that of mesophilic digestion (Kim *et al*., 2002).

The study was limited to intermittently fed, non-stirred, three-phase, plug-flow anaerobic digester with a hydraulic retention period of 20 days. The reactor was not be heated up to 35°C during the initial start-up, but will be operated under current environmental conditions until such a time period when it produces enough biogas (methane) to heat it up to the ideal mesophilic operating temperature.

The study consisted of four treatments namely (1). Dairy manure wastewater (control), (2) Dairy manure wastewater and fruit waste (Golden Delicious apples), (3) Dairy manure wastewater, fruit and food waste, (4) Dairy manure wastewater and food waste. Food waste was obtained from catering services of the Stellenbosch University student residences and fruit waste from the University farm orchards. Fruit waste was harvested from the Stellenbosch University farm as part of a study by researcher(s) in the Department of Horticultural Sciences.

Despite, the possibility that there might be a significant concentration of pathogenic microorganisms in the organic waste streams such as *Salmonella*, *Escherichia coli* O157: H7 will be the only pathogen of interest that will be monitored if it survives or is destroyed during the anaerobic digestion process and excludes any other potential pathogens.

Because, volatile fatty acids (VFA's) have been reported to cause significant reductions in the pH level of an anaerobic digester (Dinopoulou, 1988), which can have an influenced on anaerobic microbial populations. The study was also limited to obtaining pH measurements and not that of VFA's. In addition, by monitoring anaerobic microorganism population dynamics, it will be possible to explain the influence of pH and ultimately VFA's on the efficiency of the anaerobic digester to treat organic waste.

Regarding water soluble minerals, the mineral compounds of interest will be Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) , Na (Sodium), Manganese (Mn), Iron (Fe), Zinc (Zn), Boron (B) and Copper (Cu). The study will then try to elucidate which minerals have the potential to be toxic, leach, utilized by plants and if there are sufficient quantities to satisfy the plants mineral requirements or should they be supplemented or diluted.

According to Westerman & Bicudo (2005) it is not easy to determine environmental cost and benefits of an alternative waste management. In addition, it is also not easy to quantify economic cost and benefits to society as a result of switching to an alternative waste management option. Despite, having several methods of evaluating the economic feasibility of a farm investment, most require validated data collected over a period of time in order to make a meaningful conclusion.

To evaluate the economic feasibility of an anaerobic reactor investment to manage waste at Welgevallen experimental farm, we are left with three options, (a) compiling a long-term capital budget, (b) a break-even budget and (c) a partial budget. Although a long-term capital budget will be the most ideal, it would require an extrapolation of several factors and variables under consideration, whereas the lack of clear streams of income and cost savings will make it equally difficult to compile a break-even budget. This makes the partial budget the most suitable option as we can use readily available information and data that is relevant and of interest to the study.

9. Definition of key terminology

Mesophilic bacteria are defined as microorganisms that are capable of thriving under temperatures between (30-40 °C), whereas thermophilic refers to microorganisms that thrive within the temperature range of (50-60 °C), while psychrophilic refers to those microorganisms that are able to thrive within environmental temperatures of (0-20 °C).

Feedstock is defined as raw materials that serves as input fed into a process to produce an output and can thus be either renewable or non-renewable. However, for the purpose of this study, feedstock will be biomass materials that are either of plant or animal origin in the form of manure, food and fruit waste.

10. Chapter overview

Chapter two is a literature review, whilst chapter three will be the quantification of organic waste volumes and evaluation of current waste management practices at one of the Stellenbosch University, Welgevallen experimental farm. This study will then conclude with chapter four, which will research findings of biogas yield and composition, waste volume reduction, pathogenic microorganism reduction, soluble water nutrient, phytotoxicity and economic feasibility investing in an anaerobic reactor. Recommendations and suggestions for prospective research will also be provided.

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CHAPTER 2

Literature review

2.1. Introduction

Anaerobic digestion (AD) can be defined as a number of biological processes, under anaerobic conditions i.e. without oxygen, by which biodegradable material is broken down to yield a source of bio-energy in the form of biogas, which is chiefly composed of methane (CH_4) and carbon dioxide (CO_2). Gerbens-Leenes *et al*. (2009) defined bio-energy, as energy that is derived from biomass sources. Bio-energy is thus regarded as fuel that has the potential to succeed fossil fuels once they become exhausted and, at the same time, help mitigate adverse effects of harmful greenhouse gas emissions (Faaij, 2006).

Therefore, biogas from AD, can be utilised as a sustainable source of bio-energy. This occurs when methane (CH_4), hydrogen (H) and carbon monoxide (CO) are combusted or oxidized with oxygen. This process releases energy, which allows biogas to be used as a fuel for either heating or cooking. Alternatively, biogas can be used in a gas engine for the combine production of heat and electricity.

Biomass (biodegradable material) is defined to be 'matter of organic origin' by the Food and Agriculture Organization (2006), whereas Gerbens-Leenes *et al*. (2009) adds that biomass is any matter that can be derived from the biosphere and can be from plant or animal origin. Blok (2006) noted that organic matter remains the most important biosphere source of renewable energy that can be converted into various forms of fuels such as biogas, ethanol and charcoal. For AD, biodegradable sources can include organic waste such as livestock manure (Nielsen & Angelidaki, 2008), fruit waste (Lastella *et al*, 2002), food processing waste (Sigge & Britz, 2007) etc.

In addition, biogas production, AD has the capacity to reduce organic waste volumes and pathogenic microorganisms present in the source organic material/ feedstock. Without adequate waste management practices, large quantities of biomass waste have the potential to become a public health hazard and pollute the environment (Salminen *et al*., 2001). At a time period when the world is faced with the dilemma of maintaining environmental integrity, there is an on-going demand to replace conventional industrial practices with those that produce fewer to no waste products (Khan *et al*., 2015). AD thus remains one plausible alternative to achieve this goal.

Lastly, AD converts organic nitrogen (N) to ammonia which can be a valuable source of nitrogen for plants if converted to nitrate after/during application (Wang, 1991; Tiquia *et al*., 1996). Before the advent of waste management systems, household waste which vastly contained organic matter, was important source of plant nutrients (Dalemo *et al*., 1997). However, this practice decreased as the filtration of inorganic material at household levels increased and organic fertilisers at the farm level were out-competed by synthetic fertilizer. As a result, organic waste management moved from a resource-base to the disposal of undesirable material (Dalemo *et al*., 1997) resulting in applying primarily chemical/inorganic fertilizers to crops.

Marchaim *et al*. (1991) noted that agro-industries produce large quantities of biomass waste rich in nutrients that can be used as renewable resource base in the production of bio-energy fuels and organic soil augmenters. Otterpohl *et al*. (1997) pointed out that organic waste obtained from agricultural systems should be reverted back to provide nourishment and revitalize these soils. To ensure that the effluent (liquid and solid waste streams) resulting from the anaerobic digestion process is a suitable fertilizer replacement to mitigate environmental concerns, it is imperative to evaluate the AD success in reducing microbial pathogenic pressure in the original waste source. It is also essential to understand the influence of the anaerobic process on water soluble minerals.

Current unsustainable biomass waste management practices are increasingly being challenged to investigate alternative, more sustainable waste management options (Dalemo *et al*. 1997). As subsequently result, AD technologies are gaining more attention with regard to a sustainable biomass waste management alternative especially for agricultural waste with high levels of moisture. The long term sustainability of this alternative is largely dependent on the supply of raw materials (Khan *et al*., 2015). Models and methods to identify and quantify accessible waste volumes streams are thus critical to consider before investing in any bio-energy infrastructure to ensure economic feasibility and a sustainable option.

This includes gaining a realistic perspective of the availability and composition of waste volumes and evaluating existing waste management practices to ensure the optimal economic value extraction from waste. AD systems can treat biomass waste with considerably high moisture concentrations such as wastewaters to produce bio-energy and has the potential to eliminate certain pathogenic microorganism occurring in organic waste resources. In addition, the digestate from AD can be considered as an alternative source of organic fertilizer with potential to command a market value.

2.2. Aspects in AD

2.2.1. Biogas as source of bio-energy

Concerns around future energy supplies are increasingly drawing attention to renewable energy sources such as biomass (Avaci *et al.,* 2013). Bagliani *et al.* (2010) noted that this is largely motivated by the determination to de-couple the world's heavy dependence on non-renewable fossil fuels such as oil, gas and coal, versus the profound need to reduce the alarming rate of atmospheric greenhouse gas emissions (Havlik *et al.,* 2011). These concerns are some of the primary reasons behind the shifting to carbon neutral fuels and help stretch the lifespan of fossil fuels (De Vries *et al*., 2007).

Literature referring to renewable energy sources such as biomass only started receiving additional attention after the first oil crisis of the 1970's (Jacobsson & Johnson, 2000; Balat *et al*., 2009), but interest subsided with the discovery of vast oil reserves in the 1980's (Appels *et al.*, 2011). Present day pursuits are enhanced by innovative technological developments such as thermochemical, physiochemical and biochemical technologies, which allow for the competitive exploitation of biomass to produce bio-energy. In addition, other sources of renewable energy include hydroelectric, photovoltaic, and wind technology (Liserre *et al.,* 2010), which represents 19 % of the world's energy production share.

Nonetheless, Appels *et al*. (2011) highlighted that biomass is likely to become an increasingly significant source of renewable energy, particularly because power generation can be guaranteed, unlike other sources which are intermittent. Hydroelectric energy generation requires a minimal volume of water flowing from a certain height, while photovoltaic energy production requires the sun to be shining and wind turbines, require mapping out areas with significant wind flow velocities. Traditionally, the construction of large hydroelectric power generation dams have never been without social, economic and environmental ramifications such as the relocation and resettlement of displaced local inhabitants (Fearnside, 1999; Tilt *et al*., 2009), flooding of vast upstream areas of significantly fertile land, disruption of downstream rural economies and greenhouse gas emissions that is often overlooked (Fearnside, 2002). Additional concerns of hydroelectric power include disruption and destruction of natural habitats of aquatic and plant biodiversity. This entails that these alternative sources of renewable can only be entirely exploited in areas where social, environmental and economic conditions are favourable (Bagliani *et al*., 2010) whereas, bio-energy technologies like AD can easily be exploited without any resistance.

2.2.2. Sourcing of biomass waste

In principle, AD can utilize any degradable biomass to produce biogas. However, for efficient performance, the utilization of organic matter with inherently high lignin concentrations are not ideal, unless adequately pre-treated (Faaij, 2006). The diverse chemical and physical properties of most biomass waste make certain resources only suitable to be converted with alternative conversion technologies. Buffiere *et al.* (2008) noted that, in recent years, the use of biomass waste in anaerobic digestion treatments has been increasing at an annual rate of 25% for waste streams such as sewage, livestock manures and crop residues. These are ideal biomass resource bases as they are renewable and do not compete with food production for high quality agricultural land, rendering biomass waste an ideal source of renewable energy.

Nonetheless, as the quest for renewable bio-energy sources continues to grow, a new stream of cultivated biomass energy crops emerged for the production of bio-diesel and ethanol. These new streams of energy crops compete with food production, while costs associated with producing one unit of bio-energy from cultivated biomass far exceeds the cost of producing the biomass mass rather than using biomass waste (Faaij, 2006). According to Hill *et al*. (2006), ethanol and biodiesel from cultivated energy crops yields 25% and 93% net energy invested respectively, while they produce 12% and 41% less greenhouse gases relative to fossil fuels. However Gerbens-Leenes *et al*. (2009) argued that cultivated biomass energy resources have a significantly higher water footprint in comparison to biomass waste, reducing its appeal as a sustainable, renewable source of bio-energy.

The major cultivated energy crops for the production of ethanol and bio-diesel predominantly remain corn, sugar cane (Solomon *et al.* 2007), rape seed (canola), sorghum, wheat, sugar beet (Demirbas, 2009), soybean (Hill *et al*. 2006), palm oil and jatropha (Tiwari *et al*., 2007). However, most of these are important staple crops while crops such as corn and wheat have the ability to significantly influence global food prices. Despite this, the search for alternative renewable energy sources does not seem to be overwhelming enough to dissuade several economies from cultivating energy crops.

Havlik *et al.* (2011) noted that in support of bio-energy production, many developed countries such as the United States of America, Brazil, China, India and European countries have introduced policies to support and regulate use and production of fuels from cultivated crops as a measure to lower the dependency on non-renewable fossil fuels. Although there has been resistance against the use of food sources such as grain in the production of biofuels (Solomon *et al.,* 2007), rendering support for the commercialization and diffusion of more sustainable renewable energy in many countries is lacking (Suurs & Hekkert, 2009).

According to Callaghan *et al.* (2002), the utilization of biomass feedstocks for the production of bio-energy is the most likely option to gain commercial interest provided that it is economically sustainable in the long-run. Common barriers to adoption are mainly technical economic feasibilities such as high cost of investment, system maintenance and bio-energy conversions (de Souza *et al.,* 2013). Nevertheless, Nonhebel (2005) projected that in the near future, biomass will likely supply most of renewable energy, but concluded tentatively that biomass alone will not be able to meet the world’s energy requirements.

In 2002, renewable energy sources alone contributed 14%- 20% of the world's energy mix (Madlener & Stagl, 2005; Akella *et al*., 2009; Apples, 2011) predicted that this will be reduced to 13% over the next nine years. The energy mix of renewable energy sources remains relatively dominated by readily biomass energy sources such as wood used for heating and cooking especially in developing countries such as those in sub Saharan Africa. While, advanced technologies such as hydropower, wind, photovoltaic, and geothermal that have a potential of becoming sustainable energy sources only contributed 2% (Akella *et al*. 2009) of the energy mix.

2.2.3. Quantification of biomass waste volumes

During the conception process of developing alternative waste management systems, it is imperative to quantify readily available waste streams (Bandara *et al*., 2007). Various waste quantification models and methodologies like economic input-out analysis, solid-waste-sorting sampling methods were developed which enable us to understand the concept of waste management. However, literature on the quantification of biomass waste at the primary producer level remains limited. Nonetheless, unambiguous quantification of waste volumes will aid improve management in this economic sector with regards to the magnitude of the problem as well as assist with the selection of sustainable alternatives for waste reduction on site.

Nonetheless, Parfitt *et al*. (2010) argued that quantification of waste streams still remains fixated on ancient methods of quantification such as collecting waste samples before weighing them. Although much more accurate, this is a tedious practice and where models and data were available, this process could be reduced by a rapid and accurate estimation of waste volumes.

Quantification of waste streams through modelling received supplementary consideration during times of increased public concerns with regard to conventional waste management practices such as landfills coupled with renewed interest for waste material (Gay *et al*., 1993). However, Gay *et al*. (1993) quantification of organic waste streams such as municipal waste, can be costly and inconsistent, with large standard deviations with more than 25%, confirming the need for more accurate models.

Purdy & Sabugal (1999) utilized a solid-waste-sorting sampling method to quantify waste volumes by estimating the organic waste composition of solid waste in the city of Davao, Mindanao, Philippines. The study involved collecting composite solid waste samples from randomly selected refuse trucks, before organics were sorted out and weighed. This data was then used to estimate the total amount of organic waste produced by the city by determining the weight of each truck offloading at the landfill.

Gay *et al*. (1993) applied methodology defined as the Economic Input/ Output analysis (EIO). The method is based on a philosophy that volumes of sale in one sector are the outputs of another. This included estimation of waste volumes and composition by converting sales data of durable and non-durable goods at regional level into estimates of solid waste generation. The authors argued that this is an improved method to quantifying waste such as that of municipal areas, a process that is time consuming and yet costly which is often manifested by the small sample sizes.

Bandara *et al*. (2007); King & Murphy (1996) utilized the survey sampling theory to estimate the average amount of waste produced from a single residential unit in the Broward Country Florida, Atlanta. This approach involved a random pre-selection of refuse trucks from which solid waste samples were obtained. These sample weights were recorded, whereby the total weight was divided by the number of households each truck covered per collection route. Their purpose was to estimate the volume of waste generated per household in order to obtain a ratio of waste per household. This waste ratio was than extrapolated to provide an estimate of the amount of waste generated per household on an annual basis.

2.2.4. Availability and composition of biomass waste

The composition of biomass waste vary depending on the source (e.g. kitchen waste versus sludge) as well as the final destination of the waste (landfill versus sewers). Furthermore availability of biomass waste for further reuse will depend on the aim of re-use or recycling (composting, biogas, etc.), the required volumes, type of waste (solid or liquid) and sustainability of the source in relation to the processing plant. These factors will contribute towards the efficiency of the processing plant/unit and therefore need to be investigated and quantified accurately before considering re-use/recycling of the biomass.

In the USA, food waste constitutes the largest waste stream component of solid waste quantified by weight products (Zhang *et al*., 2007). This includes waste from households, commercial establishments such as schools, restaurants, hotels and agro-processing industries (Zhang *et al*., 2007). Moisture contents of these food waste streams can vary between 74-90% while, total and volatile solid can range from 80-97% whereas, carbon to nitrogen ratios can be 14.7-36.4 (Zhang *et al*., 2007).

As a result of relatively uncertainty with regard to the availability, physical and chemical properties of organic waste streams during anaerobic digestion process, it is very difficult to compare studies utilizing diverse waste streams (Hallenbeck, 2009). Variables such as time and area in which the waste was collected can significantly influence the physical and chemical properties of biomass waste streams (Mata-Alvarez *et al*., 1992; Alibardi & Cossu, 2015), depending on living standard, lifestyle choices, cultural diversity, and individual's attitudes towards waste, which can be influenced by government policy or social pressure.

Nonetheless, biomass waste streams will remain an important alternative resource base for the sustainable production of bio-energy and organic fertiliser. These include waste streams that originate from both livestock and crop productions systems that can be categorized as either primary, secondary or tertiary waste (Faaij, 2006). According to Faaij (2006) primary waste streams are those generated during the production food crops and forestry products principally at farm level whereas, secondary waste are generated when value is added such as during processing of consumer products while, tertiary waste only become available after value has been extracted from a product and may include organic fractions found in municipal solid waste and sewages

2.2.5. Physical and chemical composition of biomass waste

Physical and chemical properties of biomass waste are two important dynamics that can influence the type of technology required and economic benefits that can be derived from a bio-energy conversion alternative (Rapport *et al*., 2008). Organic waste streams with total solids in excess of 15% are regarded as waste with a high load of total solids. These waste streams have a tendency to produce higher concentrations of ammonia that can result in the AD process becoming unstable (Duan *et al*., 2012). In addition, the importance of total solids cannot be underestimated as it can significantly influence the organic loading rate and, ultimately the hydraulic retention time of an anaerobic digester (Krupp *et al*., 2005).

An ideal carbon to nitrogen ratio is important for optimal performance of the AD process. Although the ideal carbon to nitrogen ratio (C: N) is often debated in literature, 20:1-30:1 remains the widely acceptable range (Yen & Brune, 2007). While, several authors reported that fruit and vegetable waste inherently have a high C: N ratio and moisture levels that can exceed 60-80% (Lastella *et al*., 2002; Faaij, 2006), others found fruit and vegetable waste to have a low carbon to nitrogen ratio, coupled with a low cellulose content, rendering it a challenge for commercial use and important to quantify the source when interpreting the results (Lin *et al*., 2011; Khan *et al*. 2015).

Food waste on the other hand can contain high salt concentrations and heavy metals levels that can present a challenge to waste management. Appels *et al*. (2011) highlighted that municipal solid waste contain high levels of heavy metals that can affect the efficiency of AD. Recognising that a significant large proportion of municipal solid waste is food, the probability of contamination with heavy metals in food waste increases with an increase in the food component. Food waste can furthermore contain high levels of sodium chloride (NaCl) used to add flavour or preserve food. Salt can increase osmotic pressure and dehydrate anaerobic microorganisms (Yerkes *et al*. 1997), whereby the efficiency of the AD processing is reduced.

Mendez *et al*. (1995) reported that high concentrations of sodium ions (Na^+) can interfere with the metabolisms of anaerobic microorganism. In order to obtain organic waste of the highest quality, pre-sorting of the waste is essential. However, this can significantly increase operational costs, whereas the presence of water soluble minerals such as NaCl makes it nearly impossible to separate such minerals from waste such as food.

Mata-Alvarez (2003) showed that organic waste streams with high moisture concentrations are inclined to decompose rapidly under anaerobic conditions. Waste streams that decompose rapidly under anaerobic conditions yielded higher volumes of biogas (Lin *et al*., 2011). One major drawback of rapidly decomposing biomass such as fruit waste is that AD is inclined to be overpowered by hydrolysis, which cascades into overwhelming methanogenesis - leading to instability of the entire digestion process (Lin *et al*., 2011; Arhoun *et al*., 2013). Organic waste streams such as fruit waste yielded high levels of volatile fatty acids during AD, which resulted in lowering pH levels and inhibiting the activity of methanogenic microorganisms (Mata-Alvarez *et al*., 1992).

2.3. Existing organic waste management practices

Solid waste management operations have evolved over time, from practices of merely collecting and dumping waste indiscriminately in landfills, to sustainable alternatives, material recovery/re-cycling and composting. These paradigm shifts are not only as influenced by environmental consciousness but, also due to the recognition of social and economic gains that can be obtained from alternative waste management strategies (Colón *et al*., 2015). These are further supported by numerous life cycle analyses conducted to evaluate the environmental effects on alternative waste management propositions (Finnveden, 1999; Clift *et al*., 2000; Lundie & Peters, 2005; Cleary, 2009; Cherubini *et al*., 2009; Laurent *et al*., 2014).

According to Laurent *et al*. (2014) the need for waste management practices that integrate environmental sustainability cannot be overemphasized. Furthermore there is increasing evidence that waste management systems that disregard social concerns are often destined for failure (Joos *et al*., 1999). Yet, despite sufficient knowledge on waste management alternatives, the ultimate sustainable waste management practice still seem elusive to mankind without being open to scrutiny.

2.3.1. Landfills

In China, nearly 90.5% of their solid waste streams are still send to landfills (Lin *et al*., 2011). In most instances, these waste contain large concentrations of up to 60% biodegradable matter, because of the food component. Landfilling food waste present an environmental hazard, because when food decays under anaerobic conditions, greenhouse gases such as methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) are released, while valuable nutrients originating from agriculture, are trapped without further use.

In order to mitigate this, European Union (EU) member states agreed in 1999 to reduce the amount of biodegradable biomass waste ending up in landfills by up to 65% before July 2016 (Murto *et al.,* 2004). Thereafter Sweden introduced a policy to ensure that no bio-degradable waste ends up in landfills from 2005. This included introducing a tariff for every ton of waste that ends up in a landfill (Murto *et al.,* 2004).

2.3.2. Composting

Several waste management alternatives have been developed to manage organic biomass waste - all with varying degrees of social, economic and environmental impacts. Waste management alternatives to divert biomass waste from landfills include composting (Lee *et al.* 2004; Lou *et al.* 2013) and AD have been suggested. Despite, composting being regarded as a sustainable waste management alternative to landfills, the process too releases greenhouse gases into the atmosphere (Kuroda *et al*., 1996; Sommer & Moller, 2000). After conducting a life cycle analysis from several of composting sites, Edelmann *et al*. (1999) reported higher methane emissions than they had previously assumed. In the present environment, when there is a worldwide consensus to reduce greenhouse gas (GHG) emissions through the Kyoto protocol (1997), conventional composting does not offer an entirely sustainable alternative with respect to addressing these environmental concerns (Callaghan *et al*., 2002).

According to Boucher *et al*. (2009), Hydroxide (OH^-) remains one of the natural sinks for methane that escapes into the atmosphere. Methane reacts with (OH^-), to produce carbon dioxide, methanol and formaldehyde, which are all intermediate products and thus not every molecule of oxidized methane results into the production of another greenhouse gas in the form of carbon dioxide. However, unlike carbon dioxide, methane can have the capacity to be exploited as a source of renewable energy.

2.3.3. Anaerobic digestion (AD) and biogas production

Appels *et al.* (2011) defined AD as a biochemical process by which anaerobic microorganisms break down biomass within an aqueous environment to produce bio-energy, while (Lastella *et al*., 2002) defined AD as a process by which biodegradable waste is broken down in the absence of oxygen and that it is a complex process requiring specific environmental conditions and microbial organisms.

The definition by Avaci *et al*. (2013) included the biomas reduction in more detail to include simple compounds such as methane (CH_4), carbon dioxide (CO_2), water vapour (H_2O) and may include contaminants such as hydrogen sulphide and ammonia. It is however Shin *et al*. (2010) who provided a more comprehensive definition by defining AD as a complex, multi-phase biochemical process through which organic matter undergo digestion in a cascade of series mediated by micro-organisms to produce biogas which is chiefly composed of methane and carbon dioxide. The inherent ability of anaerobic microorganisms to breakdown high moisture organic matter and produce a source of renewable energy as biogas led to the utilization of anaerobic digestion as an alternative organic waste management strategy (Lin *et al*., 2011).

AD has several benefits with regard to waste management, some of which are the ability to stabilise (Talbot *et al*. 2008) and reduce waste volumes (Möller *et al*., 2008). In addition, anaerobic digestion produces a renewable carbon neutral source of bio-energy commonly referred to as bio-methane that can be utilized to generate electricity (Avaci *et al*. 2013), heating or combined heating and electricity generation. However, anaerobic digestion process is not the only waste management alternative available for the production of methane.

Methane can also be produced from biomass via a process of gasification (Chynoweth *et al*., 2001). Gasification, which is a partial oxidation thermochemical process, yields a bio-energy source referred to as syngas containing methane, carbon monoxide, hydrogen, carbon dioxide and various contaminants in varying proportions (Bridgwater, 1994; Bridgwater, 2003). Similar to AD, the process occurs over a number of sequential steps namely: drying for the evaporation of moisture, followed by pyrolysis which yields gas, solid char and vaporised tars or oils and finally the partial oxidation of the solid chars, pyrolysis of both tars and gases (Bridgwater, 2003).

Gasification technologies are either autothermal or allothermal – with the allothermal process requiring an external heat sources, whereas autothermal provides partial combustion of the heating energy required (Karellas *et al*., 2008). Gasification of biomass requires temperatures ranging from 350-600 °C for low-temperature catalytic gasification and temperature of 500-750 °C and above for high-temperature water gasification (Matsumura *et al*., 2005) – making it a process with very high initial thermal inputs compared to AD.

In addition, for economic considerations, gasification is limited to feedstock with less than 50% moisture content such as wood, or to cost-effective process that can mechanically dehydrate biomass (Chynoweth *et al*., 2001). This implies that for feedstocks such as dairy manure waste waters, food waste and fruit with less than 15% total solids, gasification will require a pre-treatment process in order to lower moisture concentrations to feasible levels. Agricultural waste such as slurries and fruit waste will thus have to undergo drying before high temperature required for gasification can be achieved (Chynoweth *et al*., 2001). Because of this constraint, AD remains a more competitive alternative to gasification per unit volume of energy for feedstock with high moisture concentrations than that with low concentrations.

Furthermore, stabilized biomass solids a by-product of AD, commonly referred to as digestate, is rich in plant available ammonia (Vavilin *et al*., 2008) and can thus be utilized as a liquid fertilizer or compost medium if nutrition levels are suitable. However, if not stable enough to as liquid fertilizer or compost medium, it can further undergo aerobic treatment by incineration with very minimal generation of pollutants (Appels *et al.*, 2011) to produce mineral ash, which can be applied to the soil.

AD thus offers an encouraging alternative to organic waste management, as it not only offers the opportunity for waste volume reduction, but also mitigates the quantity of greenhouse gas emissions into the atmosphere (Lou *et al*., 2013). Disadvantages of AD include carbon dioxide in biogas which reduces the gas calorific value (Ryckebosch *et al*. 2011) and contaminants such as hydrogen sulphide which are corrosive (Bond & Templeton, 2011). The gas will thus need to be purified, resulting in additional costs, to remove the carbon dioxide and contaminants in order to obtain bio-methane with a higher calorific value (Ryckebosch *et al*., 2011).

2.4. Factors influences AD efficiency

Several factors are critical to the efficient performance of the AD process including: i) temperature, ii) pH (Dinopoulou, 1988), iii) organic loading rate, iv) hydraulic retention time (Molnar & Bartha, 1988), v) nutrient availability (Chandler *et al*. 1980; Angelidaki & Sanders, 2004) and vi) design of AD.

2.4.1. Temperature

Temperature plays an important role in the AD process as it significantly influences the activity of the microbial population responsible for breaking down organic matter. Anaerobic bacteria can thrive under varying temperature conditions as long as it does not exceed the upper limit where bacterial growth rate is exceeded by decay (Saleh & Mahmood, 2004). The bacteria can thus be classified as psychrophilic (0-20°C), mesophilic (20-42°C) and thermophilic (42-75°C). Although, anaerobic bacteria can survive under psychrophilic condition, such conditions are not conducive to effectively break down biomass and produce biogas.

2.4.2. PH

PH (or degree of acidification) influences the anaerobic process by affecting the extent to which AD affluent will acidify from the accumulation of volatile fatty acids (VFAs) (Dinopoulou, 1988). Acidogens, responsible for the breakdown of organic matter in the first phase of AD prefer an acidic environment of 6.2, while methanogens thrive in the pH range of 6.8-7.2 for optimal performance (Mudrack & Kunst, 1986). However, for the efficient performance of the AD, it is imperative that the pH be maintained between methanogenic limits to prevent acidogenic bacteria from dominating the process which can result in the accumulation of unwanted VFAs (Saleh & Mahmood, 2004). Saleh & Mahmood (2004) emphasised the importance of ensuring that the contents of the AD provide sufficient buffering capacity to prevent excessive accumulation of VFAs accumulation.

2.4.3. Organic loading rate

The organic loading rate (OLR) refers to the volume of feedstock fed into the anaerobic digester on daily basis. The OLR is of particular significance, because it directly affects the hydraulic retention time (HRT). If the hydraulic retention time is too long, excessive feeding of the anaerobic digester results in the disruption of microbial populations and subsequent washout of organic matter, which can lead to the failure of the AD process. Chynoweth *et al*. (2001) noted that, mesophilic anaerobic digesters operated at a temperature of 35 °C corresponded to a hydraulic retention time of 20-30 days.

2.4.4. Nutrition availability

Nutrient availability is an essential contributor towards the efficiency of AD. The vigour of microbial organisms inside the reactor is greatly dependent on the availability of easily biodegradable carbohydrates (Chandler *et al*. 1980), protein and lipids (Angelidaki & Sanders, 2004). Carbohydrates such as cellulose and lignin are not easily degraded by hydrolytic fermentative bacteria (Dong *et al*., 2009). Although, nutrients in feedstock such as dairy manures and fruit waste is considered organic, inorganic particles such as sand may present a challenge to the AD process by occupying reactor space and subsequently increasing the HRT.

To enhance the digestibility of feedstock, pre-treatment of waste streams with high contents of cellulose and lignin compounds has been utilised in order to increase accessible surface area of the feedstock to anaerobic microbes (Kratky & Jirout, 2011). Pre-treatment can include i) mechanical maceration (Kratky & Jirout, 2011), ii) heating, iii) use of fermentative fungi and bacteria, iv) enzymes and v) chemical treatment. Pre-treatment can be costly and thus, for economic viability, it is imperative to consider an option that is suitable for local needs. Furthermore pre-treatment to reduce particle size can consume about 33% of the total energy demand.

2.4.5. AD reactor types

According to Bond & Templeton (2011), different types of reactor designs exist, ranging from low-rate, simple Chinese fixed dome digesters and Indian floating drum and balloon digesters to stirred and heated reactors in temperate regions of the developed world. Chynoweth *et al*. (2001) reported that conventional designs are fast being replaced with new innovative reactors to accommodate a vast diversity in organic waste streams.

Stirred or leach bed batch reactors are effective for treating feedstocks with high total solid contents (> 10%), whereas settling reactors are more suitable for feedstocks such as sewages and slurries (5-10% total solids) (Chynoweth *et al*., 2001). Under similar conditions, AD has the capacity to reduce organic waste volumes by as much as 60%, with yields of 0.24 m3 methane per kilogram of volatile solids added (Chynoweth *et al*., 2001). Nonetheless, the two most common types of AD are the semi-continuous and batch reactors (Rao & Singh, 2004). Despite this, Rao & Singh (2004) added that batch reactors are simple, which makes them easy to maintain and operate, while requiring less capital.

2.5. Conversion of biomass to biogas

The AD process is largely mediated by a cascade of reaction sequences through which organic matter is broken down to yield methane, carbon dioxide, hydrogen oxide (Talbot *et al.*, 2008). These sequential reactions are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gujer & Zehnder, 1983; Charles *et al*., 2009; Shin *et al*., 2010).

During hydrolysis, fermentative bacteria break down organic solids into liquid state (Angelidaki & Sanders, 2004). Complex molecules of carbohydrate (polysaccharides) are depolymerized by enzymatic actions into soluble polymer sugars such as glucose and fructose. While, proteins are broken down to amino acids and fats, into fatty acids and glycerol. To avoid process failure at the hydrolysis phase, acetogenic bacteria digest and breakdown products from hydrolysis into organic acids such as volatile fatty acids and acetate (Siegrist *et al*., 2002). Finally, during methanogenesis, *Methanogen archaea* utilizes the acetate (Siegrist *et al*. 2002), formate and hydrogen to produce methane and carbon dioxide.

According to Hori *et al*. (2006), methanogenesis (last phase of AD) is referred to as the rate-limiting phase, because *Methanogenic archaea* have an inherent slow growth rate and are more sensitive to environmental changes such as temperature, pH and chemicals in feedstocks (Hori *et al*., 2006). In contradiction, Bougrier *et al*. (2007) reported that the rate-limiting phase is the first phase of hydrolysis, for the treatment of activated sludge. Similar findings were also reported by Salminen *et al*. (2000) who found that AD of solid poultry slaughter waste was limited by hydrolysis as a result of high concentration of propionate, also confirming findings by Broughton *et al*. (1998) who treated sheep tallow. Despite, AD being a dynamic process influenced by the interactions of microbiological, biochemical and physiochemical elements, the stability of the AD process is dependent on the critical balance between the symbiotic growths of principal microbial populations (Angelidaki *et al*., 2009).

2.6. Anaerobic bacteria population dynamics

The biomass volume of methanogens in an AD can be correlated to methane yield (Morris *et al*. 2010 *cited by* Appels *et al.*, 2011). This statement is contested by Hori *et al*. (2006) who argued that the population of anaerobic *Marchaea* and *Acidogenic bacteria* had no direct relationship with biogas production rate. This is despite the hypothesis that if anaerobic microbes are responsible for the digestion of organic matter and liberation of methane and carbon dioxide, population dynamics of these microorganisms will have a significant influence on biogas production and organic waste reduction rate.

Despite their significance, knowledge on anaerobic microbial population dynamics remains relatively poor (Klocke *et al*., 2007). Additionally, microbial populations can contain thousands of interdependent species, many of which cannot be cultured under standard laboratory conditions, and the complexity of these microbial populations can be the leading reason why there is a deficiency of basic knowledge on anaerobic digestion systems (Apples *et al*., 2011).

Biochemical and physiochemical aspects such as pH, volatile fatty acids (VFAs) and ammonia concentration all affect the contribution of microorganisms to processing efficiency (Pind *et al*., 2003). VFAs concentration can also be an excellent indicator of the metabolic status of the AD process (Fernández *et al*., 2005) Nonetheless, a comprehensive understanding of anaerobic microbial population shifts is imperative to gain a perspective of the stability of the anaerobic process (Hori *et al*., 2006).

Anaerobic microorganisms are categorized into four classes, namely hydrolytic fermentative bacteria, acidogenic, acetogenic and methanogens (Delbes *et al*., 2000; Chouari *et al*., 2005). Although, AD microbes enjoy a syntrophic co-existence within mixed microbial culture, each class has different nutritional requirements, pH, growth kinetics and tolerance for specific environmental conditions (Demirer & Chen, 2005). Hydrolytic and acidogenic microorganisms are heavy feeders with similar growth kinetics and ability to thrive under similar environmental conditions such as pH and temperature (Demirer & Chen, 2005). Whereas, acetogenic and methanogenic microorganisms are relatively slow feeders, have slower grow kinetics and also thrive under similar pH and environmental conditions. Acidogenic bacteria are primarily responsible for digesting and breaking down solid organic matter into liquid and gas form to yield organic acids such as acetate, butyric, propionic, lactic, alcohols and ketones. *Methanogenic archaea* convert these organic acids into biogas and carbon dioxide (Cooney *et al*., 2007).

In order to avoid process failure and optimize biogas production, the two consortia of acidogenic and methanogenic microorganisms are separated between two or more anaerobic digestion reactors (Shin *et al*., 2010; Yang *et al*., 2003). Acidogenic bacteria are allowed to thrive at a lower pH and short hydraulic retention time of 1-2 days, whereas methanogenic bacteria function best at a neutral pH with a longer hydraulic retention time of 10-20 days (Demirel & Yenigun, 2002; Blonskaja *et al*., 2003). These aspects necessitate an increase of the understanding of microbial population shifts in the AD in order to avoid process failures and optimize the overall performance of the process (Lee *et al.,* 2008; Talbot *et al.,* 2008). Limited literature on the monitoring of anaerobic microorganism populations' confines the optimum used the AD process to break down solid organic waste efficiently (Ueno *et al*., 2007; Shin *et al*., 2010).

There also seems to be limited literature on how microbial population shifts could affect biogas yield (Hori *et al*. 2006) with regard to large scale reactors utilizing dairy cow manure, fruit and food waste, taking into consideration that fruit and food waste can vary greatly in composition. Further research into AD population shifts to elucidate the working mechanisms of the AD process addition was proposed by Appels *et al.* (2011) as well as Talbot *et al.* (2008) who noted that changes may occur in population shifts without apparent changes in reactor performance.

2.7. Biogas from mono-digestion and co-digestion

An AD system can either be operated as mono, with only one type of feedstock, or co-digesters by combining several feedstocks (Demirel & Scherer, 2008). In addition, the choice of operating a mono or co-digester will be influenced by factors such as the availability and composition of organic waste streams. Mono- or co-digestion of slurries and maize silage, sugar beet and fermented green forages are increasingly becoming of importance for the production of biogas in Germany (Klocke *et al*., 2007).

Biogas produced during the AD process is mainly composed of methane (36 -75%), carbon dioxide (15-60%) and small concentrations of contaminants such as water vapour and traces of H_2S hydrogen sulphide (Avaci *et al*., 2013; de Souza *et al*., 2013) However, the concentration of each value is influenced by the origin of the gas source and their composition (Avaci *et al*., 2013). Thus, different feedstocks have the potential to yield biogas with explicit properties Qiao *et al*. (2011). Methane, the energy carrier of biogas, has a calorific value of 21-24 MJ/m^3 (Dimpl, 2010), nearly an equivalent to 6 kWh/m^3 (Bond & Templeton, 2011). However, de Souza *et al*. (2013) reported a calorific value of 8 500 kcal-12 000 kcal m^{-3} that is influenced by the quality of the gas.

The most widely used method to determine the quality of biogas is gas chromatography. Mata-Alvarez *et al.* (1992) analysed biogas composition using a Shimandzu GC-9A gas chromatograph with a porapak q column, 0.125 inch diameter and 3 meters (m) long. The oven temperature was maintained at 37°C and thermal conductivity temperature of 100°C.

Cooney *et al*. (2007) used an Agilent Technologies 6890 model gas chromatographer with two gas columns maintained at 30°C.

With regard to sampling to quantify biogas volumes, de Sousa *et al*. (2013) collected 4 samples for analysis daily and analysed the biogas methane composition using Drager X-am 7000. Cooney *et al*. (2007) and Lin *et al*. (2011) used gas calibrated high precision gas meters connected to the head space of the reactor. Rao & Singh (2004) recorded biogas yield at a fixed time each day with the water displacement method. To validate their measurements, the water was prepared per specification of the standard methods (APHA, 1989). ElMashad & Zhang (2010) recorded the daily head space pressure with a pressure gauge (model 3150, Wal, Germany) in order to determine the volume of biogas. To validate the results, the biogas in the headspace was released under water and the head space pressure was recorded again to be used as the initial measurement for the next recording. Recorded pressure measurements were then converted into biogas volumes using the following model

$$V_{biogas} = \frac{P \times V_{head} \times C}{R \times T}$$

Where:

V_{biogas} = daily biogas volume (L)

P = absolute pressure difference (mba)

V_{head} = volume of head space (L)

C = molar volume (22.42 L mol^{-1})

R = universal gas constant (83.14 Lmbar K^{-1} mol^{-1})

T= absolute temperature (K)

2.7.1. Mono-digestion

Because of economic drawbacks, several critics discourage the solitary utilization of feedstocks such as bovine manure in reactors, citing low biogas yields. Co-digesting alongside other feedstocks such as food, fruit and vegetable waste produced higher yields (Callaghan *et al.,* 2002; El-Mashad & Zhang, 2010). On the contrary Klocke *et al*. (2007) reported that mono-digestion of fodder beetroot had a higher biogas yield when operated at a hydraulic retention time of more than 106 days.

Despite, mono-digestion of food waste yielding more methane, food waste have a tendency to degrade rapidly, releasing ammonia faster than feedstocks such as fruit and vegetables (Lin *et al*., 2011). High ammonia concentrations often emanate from organic waste with high concentration of N and have been causing process failures as ammonia poisons anaerobic microbes (Fricke *et al*., 2007). In addition, mono-digestion of waste such as fruit and vegetable also have a tendency to destabilize the AD process rendering it inefficient as a result of low C: N ratio of 15-17, rather than the 22-35 recommended in literature (Lin *et al*., 2011).

However, Habiba *et al*. (2009) reported high C: N ratios (36±1.2) for fruit and vegetable waste and highlighted that nitrogen (i.e. amino acids) are essential for the growth vigour of anaerobic microorganism, which is a pre-requisite for cell growth and reproduction. A low C:N ratio implies that the feedstock contain high levels of nitrogen mainly in the form of protein, which makes it ideal for co-digesting with complementary feedstock in order to stabilize and increase the efficiency of the AD process (Bouallagui *et al*., 2009; Habiba *et al*., 2009, Neves *et al*., 2009).

2.7.2. Co-digestion

Anaerobic co-digestion involves the addition of different waste streams to a single reactor, each with its own physical and chemical properties (Habiba *et al.*, 2009). According to El-Mashad & Zhang (2010), co-digestion of two or more feedstocks improves the nutrient balance and enhances the growth vigour of anaerobic microorganisms. In addition, co-digestion also has economic benefits in that it enables the dilution of waste streams with high concentrations of toxic compounds such as salts to be treated successfully (Lo *et al*., 2010; Sosnowski *et al*., 2003). Co-digestion also has additional benefits compared to mono-digestion such as higher volatile solid degradation efficiencies with yields such as 88% obtained for 30:70 activated sludge: fruit and vegetable waste (Habiba *et al*., 2009).

Co-digestion of cow manure together with supplementary waste streams has been considerably evaluated by several authors. El-Mashad & Zhang (2010) experimented with cow manure alongside food waste, while Callaghan *et al*. (2002) evaluated co-digestion of cow manure and fruit waste, whereas Alvarez & Liden (2008) evaluated co-digestion of slaughter house waste, manure and fruit and vegetable waste. Nonetheless there is limited literature with emphasis on evaluating co-digestion of cow manure, food and waste in the winter rainfall Mediterranean climate of the Western Cape, South Africa. Cow manure, which is notably rich in minerals such as nitrogen (N) and phosphorus (P), compliments waste streams such as fruit and vegetable waste with low levels of N and P rendering it an ideal combination for anaerobic co-digestion (Callaghan *et al.*, 2002; Ward *et al*., 2008).

Although, co-digestion of organic waste streams is becoming increasingly popular (Appels *et al*. 2011), the digestion mechanism is not adequately understood because of the high complexity of the process, especially with regard to the dynamics of microbial population during digestion. In addition, it is also not understood how the addition of diversified waste streams to a stable reactor will impact the performance of the AD process (Callaghan *et al*., 2002).

El-Mashad & Zhang (2010) evaluated co-digestion of manure and food waste compositions of different particle sizes utilizing an AD system, with a HRT of 30 days, at mesophilic temperature of 35°C. This laboratory bench study, had two treatments of fine and coarse fractions and was inoculated with 10 ml of inoculum obtained from another bench reactor. The first treatment comprised a composition of 68%:32% manure: food waste, while the second had, 52%:48%. According to Salminen *et al.* (2001) the addition to a newly operating anaerobic reactor not only introduces anaerobic microorganisms, but accounted for a high total solids (TS) of 54% and volatile solids (VS) 52% reduction compared to treatments that received no inoculum (37% and 35% respectively).

The researchers also highlighted that the higher biogas yield from the coarse fractions compared to the unscreened manure could also have been influenced by presence of spillage of maize silage and grain. However, it is also important to note that for co-digestion, the addition of feedstocks such as fruit and food waste which come with varying degrees of textures will necessitate pre-treatment of the organic fractions in order to achieve a homogeneous texture.

In the food waste experiment, only 79.1% of the entire biogas yield was recorded at 20 days. This was possibly due to accumulation of intermediates, which inhibited methanogen bacteria during the first 5 days, confirming results of Wang *et al*. (1997). Average methane was 53.7% with a standard deviation of 14.8%, while recorded VS reduction to be 82%. During the co-digestion experiment, the two treatments of manure and food waste had a biogas yield of 90.3% and 95.1% after 20 days of AD and achieved a reduction of 64% VS.

2.8. The efficiency of AD to reduce pathogenic micro-organisms

The presence of zoonotic pathogens in organic waste streams such as manures from agricultural production systems present a public health hazard often as a result of air, water and soil contamination (Côté *et al*., 2006). Despite, the possible presence of pathogenic microorganism in untreated livestock waste, such as manures, these continue to be widely used as organic crop fertilizer.

2.8.1. Escherichia coli

One of the most important pathogenic microorganism is enterohemorrhagic *Escherichia coli* (*E.coli*) O157:H7. Despite, a large majority of *E.coli* strains being regarded as harmless, the O157:H7 strain has been documented to cause severe enterohemorrhagic enteritis, kidney failure and death among children and the elderly (Rangel *et al*., 2005). Although most E.*coli* poisoning in humans have been documented from the consumption of contaminated beef, recent outbreaks have been linked to the consumption of contaminated leafy green vegetables crops fertilized with water contaminated by ruminant manure (Hilborn *et al*., 1999; Horby *et al*., 2003; Callaway *et al*., 2008). The application of manure as fertilizer presents a public health and environmental risk as pathogens have been found to survive in the soil after long periods of time -increasing the chances of these pathogens finding its way into water sources via runoffs (Avery *et al*., 2004).

2.8.2. Reduction of *E.coli* concentrations in digestate

One of the challenges of AD lies in the inability to utilize raw solid and liquid waste streams directly as a suitable soil amender (Abdullahi *et al*. 2008) due to potential phytotoxicity (McLachlan *et al*. 2002). The effluent will thus need further post-treatment such as composting or dilution, which can increase costs associated with the AD process in a closed systems approach. Another concern is that the effluent may also become a safety hazard (Sahlsröm, 2003) if inoculum for pathogenic bacteria such as E.*coli* are not significantly destroyed during the AD process. However, Saunders *et al*. (2012) noted that AD of organic waste streams under mesophilic conditions has the potential to reduce the chances of applying pathogenic organisms onto the land.

Nicholson *et al*. (2005) reported that storage of waste such as manure can help reduce concentration of pathogenic microorganism, but does not eliminate them completely. On the contrary Avery *et al*. (2004) highlighted that storage can eliminate 90% of pathogenic microbes from manures within 38 days, however complete elimination requires 4-5 months, or more. *Escherichia coli* has been documented to survive in water for 35 days (Rosen *et al*. 2000), while the pathogen has also been documented to survive for 200 days in soil and 300 days in slurries (Environmental Protection Agency) as *cited by* Saunders *et al*. (2012).

Despite, AD being regarded as a cost-effective method of eliminating pathogenic microorganisms such as *E.coli*, Côté *et al*. (2006) noted that there is limited knowledge on the subject matter with no literature references about the elimination of *E.coli* from a two phase reactor. According to Côté *et al*. (2006) temperature and hydraulic retention time are two influential factors important in determining the extent of pathogenic microorganisms' elimination during the anaerobic digestion process. In addition, Crane & Moore (1986) argued that moisture, extreme changes in pH and solar radiation are also critical factors that influence the decline in pathogenic microbe populations.

Bendixen (1994) reported a significant reduction of pathogens in organic waste under thermophilic conditions, whereas mesophilic conditions had no effect on pathogens. Contrary to this, Kumar *et al*. (1999) reported a fast elimination of zoonotic pathogens such as *E.coli* and *Salmonella* at mesophilic conditions of 35 °C for up to 20 and 10 days, respectively from cattle slurries, while Côté *et al*. (2006) reported total elimination of *E.coli* O157: H7 under mesophilic conditions for a laboratory bench scale digester maintained at a temperature of 20°C for 20 days with pig slurry. Juris *et al*. (1996) also reported similar findings with a complete elimination of *E.coli* EC5 for pig slurry at a higher mesophilic temperature range of 35-37 °C at 18 days in a larger digester of 800 litres.

2.8.3. Detection and enumeration of *E.coli* colony forming units (cfu/ml)

The common method for detecting and quantifying *E.coli* O157:H7 is the incubation of samples at 35°C on sorbitol MacConkey agar for 48 hours (Armstrong *et al*., 1996). Unlike 93% of human *E. coli* isolation methodologies, the sorbitol MacConkey agar plate method exploits the fact that *E.coli* O157:H7 does not ferment the sorbitol rapidly (March & Ratnam, 1986) which makes counting of bacterial coliforms a more reliable process. In addition to the sorbitol MacConkey test, an alternative method of detecting and quantifying *E.coli* O157:H7 is to grow the bacteria quicker on non-sorbitol selective enriched broth media overnight. Counting of the bacteria can thus be done within 72 hours at room temperature of 25 °C.

2.9. Water soluble minerals in anaerobic digestate

Water soluble minerals such as nitrogen compounds, phosphorus and potassium are of particular economic interest to agriculture. However, losses of these mineral compounds through seepage, leaching or run-off in agricultural systems has been the centre of attention for environmental and public health concerns (Hooda *et al*., 2000).

In both livestock and plant production systems, losses of soluble mineral compounds are of particular interest as these minerals can cause eutrophication of water ecosystems, thereby affecting water quality and rendering it unfit for agricultural use, human consumption or supporting of aquatic life. It is thus important that during the process of considering alternative waste management practices such as anaerobic digestion, these concerns are not overlooked, but addressed.

Ciavatta *et al*. (1993), observed that composting, an aerobic process, has the ability to reduce the solubility of heavy metals as the organic matter becomes stabilized. In addition, Tiquia *et al*. (1996) observed a decline in water extractable metals Copper (Cu) and Zinc (Zn) from compost, after composting. The authors attributed this to the formation of mineral complexes with chelating organic compounds, making them biologically unavailable for plants to absorb as they become water insoluble.

Möller & Stinner (2010) observed that undigested manure contained water soluble concentrations of 45% to 70% before anaerobic digestion. These concentrations were significantly reduced to 25% and 45% respectively after anaerobic digestion. On the contrary, Gooch *et al*. (2007) *cited* by Möller & Stinner (2010) found a higher concentration of phosphorus in anaerobically digested manure fractions than in the undigested manure. Möller & Stinner (2010) concluded that AD had no influence on phosphorus uptake by plants and this was confirmed by Loria & Sawyer (2005) who performed laboratory bench experiments.

Topper *et al*. (2006) noted that the total mass of mineral nutrients such as phosphorus and potassium remain relatively constant, although some of the phosphorus can be converted to soluble form during anaerobic digestion. This elucidates why Möller & Stinner (2010) observed a higher concentration of phosphorus after anaerobic digestion. However, the method of analysis could have also been a determining factor in this contradiction. It is expected that the mineral composition of the solution will remain relatively constant before and after AD. The effect of thermal energy and pH variation during the process of AD can change the active state of certain mineral elements e.g. N_2 which may occur as NO_3^- or NH_4^+.

2.10. Phytotoxicity

AD effluent could be utilized as organic fertilizer to supply essential plant nutrients for plant growth, by separating liquid and solid fractions of the effluent (Liedl *et al*., 2006). However, elevated concentrations of salt could influence nutrient availability and nutrient uptake (Grattan & Grieve, 1999) by posing a phytotoxicity risk. Zucconi *et al*. (1981) defined phytotoxicity as the toxic effects of a compound to cause short or long-term damage to a growing plant. It is thus important to conduct phytotoxicity before digester effluent can be applied as fertilizer to growing plants.

2.10.1. Phytotoxicity tests

Even though a widely accepted germination index does not seem to exist in current literature (Komilis & Tziouvaras, 2009), a germination index greater than 70% is accepted as non-phytotoxic (McLachlan *et al*., 2002). Cheung *et al*. (1989) showed that the sensitivity of plants to phytotoxcity tests is dependent on the quantity of nutrient reserves available to a germinating seed and that larger seeds such as those of cereals and legumes are less prone to phytotoxicity due to their larger nutrient reserves.

The use of vascular organisms for phytotoxcity tests as alternative to germination tests has been reported by (Khoufi *et al*., 2009; Young *et al*., 2012). As the presence of various complex chemicals makes it difficult to pin-point which factor is causing the symptoms (Tam & Tiquia, 1994), the use of vascular plants for phytotoxcity analysis instead of seed germination tests has been proposed ad it will enable detection of the presence of biological toxins in effluent waste (Walsh *et al*., 1991). It also enables researchers to assess possible adverse effects that potential soil augmenters will have on germination and seedling development during the critical first days of growth (Lewis, 1995).

Organic fractions from AD have the potential to be used as source of compost based fertiliser and thus close the organic waste and nutrient recycling for plants (Zhang *et al.,* 2007). Phytotoxcity test can be used to avoid environmental risks associated with the application of organic soil amenders back onto farmland (Tiquia *et al*., 1996). According to Mata-Alvarez *et al*. (2000), effluent from AD is not suitable for the direct application on farm land due to the high moisture contents and notably a high concentration of potentially phytotoxic volatile fatty acids. It is thus recommended that the effluent undergoes aerobic treatment such as composting in order to obtain a high-quality complete product (Poggi-Varaldo *et al*., 1999). However, one of the major drawbacks of this treatment is the loss of nitrogen through volatilisation which can greatly reduce its economic value.

2.10.2. The role of mineral nutrient elements in phytotoxicity

During the AD process, ammonia is produced and proteins broken down into amino acid (Tiquia *et al*., 1996; Vavilin *et al*., 2008). Despite ammonia becoming readily available for plants to take up, it has the potential to be phytotoxic (Tiquia *et al*., 1996). Similarly, intermediate compounds of AD such as organic acids, also have the potential to be phytotoxic, as they have the potential of speeding up microbial activity in the soil (Marambe *et al.*, 1993).

Initially, nitrogen in undigested manure enters the reactor either as ammonium or organic nitrogen in the form of protein (Topper *et al*., 2006). The formation of ammonium occurs at such a fast rate, that 95% is produced during the first 12 hours and can be present in the manure before it is collected. During AD, ammonium stays intact with additional ammonium being released with the digestion of protein into simple molecules such as amino acids. When this ammonium is incorporated into the soil, it undergoes rapid transformation by microorganisms into nitrite and eventually into nitrate which is readily absorbed by the plants (Topper *et al*., 2006).

Hansen *et al*. (1998) noted that total ammonia concentration, pH and temperature have a profound effect on free ammonia present in a reactor. AD becomes more sensitive to increments in pH (Koster, 1986), while free ammonia increases with an increase in temperature (Angelidaki & Ahring, 1994). Another factor impacting on phototoxic symptoms in plants is the concentration of mineral salts in digestate that can influence the availability of nutrients, competitive uptake and transport in plants (Grattan & Grieve, 1999).

2.10.3. Indicator plants for phytotoxicity

Young *et al*. (2012) evaluated phytotoxcity from effluent of an anaerobic reactor fed with cereal residue on *Lactuca sativa* (Lettuce) seeds and observed that concentration of mineral salts, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were the primary causes of phytotoxcity after AD. Abdullahi *et al*. (2008) evaluated the influence of phytotoxcity on the germination index of radish (*Raphanus sativus*) seeds to establish the effect of anaerobic and aerobic post-treatment on quality and stability of the organic fraction of municipal solid waste as a soil amendment. In this bench study, the AD was operated at thermophilic conditions of 55 °C for 15 days before phytotoxcity tests were conducted. The radish seeds were sown directly onto the organic fractions. Water extracts from 50 gram samples were obtained and diluted with 100 ml of distilled water at 15 °C. The samples were shaken for 6 hours before centrifuging at 8000 revolutions per minute (rpm) for 20 minutes at 20°C. Seeds were sown onto filter paper in petri dishes and moistened with 5ml water extracts. These were then incubated at 25 °C in a dark area for 72 hours to obtain the germination index. Abdullahi *et al*. (2008) observed that seed germination index was relatively higher with increasing dilutions, which indicated that phytotoxicity decreased with a reduction in biological organic matter in dilutions.

2.11. Economic feasibility of an anaerobic reactor

Although, renewable energy sources have several environmental and social advantages over fossil fuel energy, adoption and implementation of such technologies remains largely constrainted by technical economic factors such as the competitiveness against cheaper fossil energy when it reaches the final consumer. The initial cost of investing in renewable energy technology such as bioenergy can be significantly high. Nevertheless, there are several alternative processes capable of utilizing organic waste streams for the production of renewable bioenergy. These are namely, thermochemical (Chynoweth *et al*. 2001); physiochemical and biochemical technologies (Appels *et al*., 2011). However, these technologies all differ with regard to their primary aim and market requirements for direct heat or steam and thus differ in their utilization of capital, economies of scale, energy conversion ratio, energy transport and environmental impact of the conversion process (Chynoweth *et al*., 2001).

Faaij (2006) noted that, to improve the economic feasibility of an AD plant, the plant should be located at the point at which biomass waste is produced. The vast majority of biomass waste streams tends to be bulky, high in moisture and of low economic value per unit volume, which makes transporting these waste over long distances a significantly costly exercise.

Avaci *et al*. (2013) evaluated the economic feasibility of electricity generation utilizing swine culture originated in Brazil. They reported that small scale anaerobic reactors generating electricity from methane at costs of US$ 0.12 kWh^{-1} were not economically feasible. Gwavuya *et al*. (2012) evaluated the economic feasibility of a generator providing 40 kWh at US$ 99, 31 MWh^{-1} from swine slurry biogas in rural Ethiopia and concluded that this option can be economically feasible if the demand and price of energy increases. Because of high total capital cost, it is of vital importance to maintain operational cost at minimal levels to improve the economic benefits from small scale reactors.

Laramee & Davis (2013) investigated the economic feasibility of small scale anaerobic digesters domestic households in Arusha, Tanzania. A cross-sectional research design method was implemented to collect data through interviews to access the economic benefits among households with/without an anaerobic reactor. Variables under consideration included household energy consumption, energy bills, greenhouse gas emissions, farm incomes and the use of synthetic fertilizers. Laramee & Davis (2013) used the net present value (NPV) and payback period to quantify the feasibility and considered the capital investment to build a reactor, opportunity cost of energy fuels (wood), time value expanded to procure such fuels and cost of manure management. Benefits that were too complex to objectively quantify such as, increments in family income, improvement of family health and nutrition were excluded in the study. Economic cost and benefits were discounted at current domestic loan interest rates. Laramee & Davis (2013) concluded that investment for reactor is economically feasible for 6 $m^2$and 30 m^2under these circumstances, with initial total capital outlay and installation being the largest expenditures association with investing in a household reactor.

Smith *et al*. (2014) evaluated the financial and economic feasibility of AD systems as an alternative source of thermal energy for rural households in Okhombe, KwaZulu-Natal, South Africa. This was a desktop study, followed up with a survey to obtain qualitative data from randomly selected population sample of 135 households. The data generated was then used to evaluate the economic, social and environmental costs and benefits of households if they were to invest in small pre-fabricated anaerobic reactors. The authors included the opportunity cost of time saved as a result of using biogas instead of going out to search for fire per annum. Savings on energy and opportunity cost of time were calculated on the basis of the local minimum wage received by an unskilled labourer as proposed by (Austin & Blignaut, 2008). While, variables such as increase in farm revenue, health and nutrition improvements were excluded from the model. Nonetheless, the authors concluded that a reactor (pre-fabricated reactor with a capacity to be fed 20 kg and 20 litres of water daily from a local energy company retailed at ZAR 22 743.00 is not a financially feasible investment for a rural household. However, it would become an economically feasible investment when the worth of a human life, value of a statistical life (VOSL = US$ 9.1 million) estimated by United States of America Environmental Protection Agency in 2011 is factored into the analysis.

Avaci *et al*. (2013) used a different approach to determine the economic feasibility of two semi commercial scale reactors using swine slurry from a commercial with hog farming operation. The reactors had a capacity of 29.2 m^3 and 7.3 m^3 respectively, and a reasonably conservative hydraulic retention period of 30 and 20 days respectively. Biogas produced from the units was used to generate electricity with a 50 m^3 $hour^{-1}$motor generator operated for 10, 16 and 20 hours day^{-1}. The financial and economic feasibilities were than examined considering sale of carbon or no carbon credits. In this study, both financial and economic feasibility analysis were based on commercial value of the reactor which included excavation and installation, cost and energy generated from the motor generator. The following parameters were used: These included, Liquid Present Value (LPV or NPV), IRR and payback period.

A sensitivity analysis of selling carbon credits was also factored into the investment analysis. Carbon credits trading at USD 14.50 and an equivalence of methane to carbon dioxide ratio of 21 units, the sequestration of one ton methane easily traded for US$ 304.50. The authors noted that carbon credits sales significantly reduced the payback period from 27.5, 18, 14 years to 8.5, 6 and 6 years respectively when the motor generators is operated for 10, 16 and 20 hours day^{-1}, respectively.

However, just like Avaci *et al*. (2013); Laramee & Davis, (2013); Smith *et al*. (2014) also concluded that investing in an anaerobic reactor is not financially feasible but, remains an economic feasible option with the sale of carbon credits. One major limitation in published studies with regard to economic feasibility of anaerobic reactors as an alternative and sustainable waste management practice, is that the majority of studies is based on extrapolation of results from bench scale reactors (Cooney *et al*., 2007; Lin *et al*., 2011;

Callaghan *et al*., 2002; El-Mashad & Zhang 2010) and no reference is made to semi-commercial anaerobic reactors.

2.12. Conclusion

The ever increasing world human population is inevitably causing an increase in agricultural systems organic waste volumes. Waste volumes such as slurries and others with high moisture contents have limited economic treatment options that can effectively reduce waste volumes within a reasonable period time (Chynoweth *et al*., 2001; Mata-Alvarez, 2003). It has become evident that AD is a suitable waste management alternative for onsite waste management with high moisture contents (Faaij, 2006). AD has the ability to stabilize and reduce waste volumes, to produce a renewable source of carbon neutral energy in the form of biogas and, when operated optimally, to reduce pathogen populations enabling utilization of solid and liquid waste streams for agricultural use (Zhang *et al*., 2007; Ward *et al*., 2008; Gerbens-Leenes *et al*., 2009; Saunders *et al*., 2012).

However, there are contradictions as to whether AD can reduce pathogenic microbes such as E. *coli* consistently at the mesophilic temperature of 35°C (Kumar *et al*. 1999) and how possible phytotoxicity may impact on further use of waste stream products as fertilizers in agriculture. This includes limited references on the effect of AD on water soluble minerals such as nitrogen compounds, potassium, phosphorus and sodium chloride.

Quantification of the efficiency of AD for reduction of waste volumes of different waste streams has also not been addressed sufficiently as most literature focuses on the production of biogas under these conditions.

Utilization of the liquid and solid waste streams after AD seems to be limited to fertilization of agricultural crops and application of compost-like media to improve soil fertility. Often these results were not presented to the full potential of application or mentioning possible pitfalls in utilizing these products under field conditions. There seems to be a huge potential for future research in this field. Also the aspect of further mining of these two waste streams seems to be underestimated – as mentioned in a recent local publication (Khan *et al*., 2015).

Lastly, most of the studies referred to results obtained from bench reactors performed under laboratory conditions and extrapolations were used for commercial application. Thus, even though AD is no new concept and using reactors for organic waste management has been widely reported on – certain aspects still require more attention and quantification under semi-commercial conditions. These are the aspects that will be addressed in the proposed study.

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CHAPTER 3

Quantifying dairy and fruit waste volumes and evaluating current waste management practices at Stellenbosch University Welgevallen experimental farm per annum

3.1. Introduction

The increase in global human population and demand for products such as meat, dairy, fruit, vegetables and fibre has led to the intensification of agricultural production systems as a feedback mechanism to meet these demands. As a consequence of increased production, one of the systems responses that persists is an increase in the volumes of organic waste production. These waste volumes present an ever increasing challenge to waste management amid social, economic and environmental concerns. Typical organic waste management practices at farm level have been land application, landfilling, burying, constructed wetlands and composting.

To develop an alternative waste management, it is important to quantify the waste generated and the composition of the waste streams (Bandara *et al*., 2007). Likewise, it is also equally imperative to understand how the current waste management strategies address, social, economic and environmental concerns. According to Bandara *et al*. (2007) various models and methodologies have been used to gain an in-depth understanding of waste generation.

Authors such as Purdy & Sabugal (1999) conducted field studies to estimate the quantity of waste and its composition in the city of Davao, Mindanao, Philippines. This included collected waste samples from randomly selected refuse trucks, sorted into various categories of waste and weight. This data was then used to estimate the total amount of waste in the city by weighing to determine the load each truck carried.

While, authors such as such as Gay *et al*. (1993) used a methodology referred to as the Economic Input/ Output analysis (EIO). This method is based on a philosophy that volumes of sale in one sector are the outputs of another. This included estimated waste volumes and composition by converting sales data for a region into estimates of solid waste generation.

Authors such as King & Murphy (1996), utilized the survey sampling theory to estimate the average amount of waste produced from a single residential unit in a municipality. Their study entailed determining the volume of waste a truck loaded. This load was then divided by the amount of residences each truck covered in order to determine the volume of waste each household generated.

The objective of this study, which included a survey, was to gain an understanding to quantify the scale and magnitude of biomass waste volumes including past waste management strategies at Welgevallen experimental farm. However, the outcome of the study is not aimed at replacing existing waste management strategies, but to complement and ensure sustainable waste management at this Stellenbosch University farm. This also included gaining perspective of current biomass waste management strategies and how this can be used to regain opportunities lost from Horticulture, viticulture and dairy production waste.

3.2. Problem statement

The importance of quantitative data during the decision making process of an alternative waste management strategy cannot be over emphasized. Resources are scares and have competing multiple uses. Data availability and synthesis also become imperative to consider entry and exit barriers of different waste management strategies. In order to come up with sustainable waste management strategies it is imperative that stakeholders have sufficient information on which to base investment decision making. This includes the type of infrastructure, equipment, logistics, human resources, society and its impacts of the environment.

Dairy, viticulture and horticulture production are part of several experimental and production systems of economic significance at the Stellenbosch University, Welgevallen experimental farm. Despite their economic significance, these three production systems are responsible for an estimated 1 027 tons of biomass waste annually. These waste streams especially dairy waste present an ever increasing challenge management on the farm. This is necessitated by the need to reduce the chances of health hazards to humans, reduce water, air and soil contamination, while reducing the possibilities of waste acting as repositories of diseases for both crops and livestock.

Dairy waste constitutes nearly 97% of this biomass whereas the other 3% is from horticulture and viticulture production. Seasonal horticulture production includes fruit such as citrus, apples, plums, pears, peaches. Both production systems posed and ever increasing challenge with regard to waste management. Nonetheless, dairy waste have always been the focus of attention owing to the farm's proximity to an urban landscape and an important natural resource capital, the Eerste River.

The river flows from the Jonkershoek Nature reserve, through Stellenbosch before it continues to reach sea at Macasar. People in the vicinity of the river utilize it for recreational purposes while, farmers utilize its water to irrigate cash crops. On numerous occasions, dairy manure waste would find its way into this river especially during the rainy season which is not only a health hazard, but also an environmental concern. As a precaution, the Stellenbosch Municipality timely collects water samples flowing off the farm through the water canal for microbial analysis in order to monitor zoonotic pathogen concentration of Escherichia *coli* O157:H7 in the waste. However, concerns over N and P transport into the river cannot be ruled out.

Despite, waste presenting farm management with day-to-day challenges, it is equally important to understand how the role of the farm has changed as a system with regard to changes in waste management strategies over the past couple of years. Inclusive of this, is how these strategies affect society through a system of complex tangible and intangible relationships. Especially those directly and indirectly affected by the activities of the farm. Society demands to have food and beverages such as milk, fruit and wine.

General public privileged enough to live near farms like Welgevallen want to relish in the sight of dairy cows peacefully grazing in the meadows. However, the presence of flies that are part of the dairy production system. The same individuals may envy the aesthetic value provided for vineyards and fruit orchards especially when they are in full bloom, but are concerned with chemical sprays that guard against plant pest and diseases.

3.3. Justification

Potential environmental and public health hazards emanating from organic farm waste are not only limited to the boundaries of the farm. Agricultural landscapes as systems are connected to other systems through complex structures and thus pollution of the air, water and soil ecosystems with waste originating from farms can have far reaching concerns beyond the farm gate. Despite these predicaments, quantification of farm waste volumes remained relatively side-lined by research as literature is generally concentrated on developing models of quantifying organic waste at municipal and district levels.

The significance of quantitative data is of particular interest to agricultural waste management as it can help producers make informed decisions before converting to an alternative waste management strategy. Resources are increasingly becoming scares and to a certain extend have multiple competing uses. Data accessibility and synthesis thus become imperative in order to advance investment confidence before capital expenditure for new waste management alternatives are made.

3.4. Legislation on Agricultural waste management

The policy on integrated waste management was introduced by the South African government, Department of Environmental Affairs and Tourism (1998) to ensure development that is economically, environmentally and socially sustainable. The policy placed strong emphasis pertaining to curbing water, air and land pollution from all economic and social activities. In addition, the National Waste Management Strategy (DEA, 2011) approved by cabinet set to implement a strategy to promote minimization, reuse, recycling and recovery of wastewater streams.

Welgevallen experimental farm as an entity falls under the obligation to comply with the set policy guidelines, because of its capacity to produce waste that must be managed to avoid possible detrimental impacts on health and environmental pollution. The biggest consideration with regard to Welgevallen is pollution that can enrich and at the same time increase pathogenic microbial population of the Eerste River. In addition, guidelines from the South African Department of Water Affairs (DWAF, 1996) and World Health Organization (WHO, 1989) stipulated that water containing less than 1000 *Escherichia coli* per 100 millilitre (ml) or (10 colony forming units/ml) is indeed safe for the irrigation of agricultural fresh produce.

3.5. Horticulture waste management

Citrus and deciduous fruit production occurs on 6 hectares of the farm of the Welgevallen experimental farm. Despite utilizing 5% of the total farm size, these enterprises remain integral components of the farming operations. This mainly attributed to the fact that the horticulture production enterprises are managed in accordance to global Good Agricultural Practice (Global G. A. P) principles, which enables the farm to not only produce fruit for both domestic and export markets.

Van Kerwel (2014, W. Van Kerwel, personal communication, Stellenbosch University, Welgevallen experimental farm manager) noted that the economic sustainability of the farm is an integral component of the farm's operations. It remains in the farm's interest to prevent and minimise post-harvest fruit losses and ensure that most of the produce reaches the pack house. Average fruit waste volumes can be as much as 10 tons of citrus, 3 tons of apples, 5 tons of pears, 1 ton of Nectarines, 3 tons of plums, but it can be more. Overall, the horticulture enterprise produces approximately 27 tons of fruit waste on an annual basis and are calculated by weighing the fruit in 0.45 ton containers. Fruit waste quantities at Welgevallen experimental farm are presented in Table 1.

Van Kerwel (2014) noted that pre-harvest losses are largely as a result of sunburn, which is progressively becoming a common occurrence among apple cultivars. This may be attributed to elemental conditions such as, unexpected heat waves that are becoming increasingly more prevalent with each growing season. While, additional fruit losses are direct result of damaged or fruit getting blown off the trees by the wind before they are harvested. In addition, post-harvest waste is largely composed of fruit that does not meet the classification standards and are sorted out at the farm before the fruit is send off to a pack house. Fruit losses on the farm thus vary with each growing season influenced by several factors such as weather, pest and diseases and classification standards.

In the course of the preceding decade, fruit waste on the farm commanded no commercial value, was a liability and had to be disposed in the most cost-effective way. In addition, the farm faced no real constraint regarding fruit waste management and as a result fruit waste was simply buried on designated areas on the farm. The costs of such a waste management system were nonetheless labour, excavation of burial pits and transport of waste to the pits. This practice was successful, since it prevented putrefying fruit from emitting unpleasant odours, attracting pests and acting as inoculum repositories of pathogenic plant diseases. Furthermore, the practice not only disposed of waste volumes, but decomposing fruit ultimately added nutrients and soil organic matter helping to improve soil health and fertility in these burial sites.

Currently, the practice of burying fruit no longer remains a viable option to farm operations. A lack of suitable fruit burial sites poses an ever increasing constraint to this practice. Simultaneously, the Faculty of Agricultural Science's adopted a 'Green initiative' policy targeted at investigating alternative approaches to managing different streams of organic waste. At the moment all waste volumes from horticulture and viticulture generated on the farm are directed towards a windrow composting operation.

Irrigated agriculture remains the largest user of fresh water in the country and at a time period when the country has been declared a water stressed nation, agricultural production cannot afford to pollute water sources such as rivers from which its livelihoods are derived. Inputs such as water, energy and other natural capital resources needed for production are largely sourced from the environment whereas, inevitable waste products from production are returned to the environment. The environment thus has multiple economic uses by acting as a source and a sink. The challenge is striking a balance between production efficiencies and how waste is managed to avoid environmental degradation.

Consequently, consumers are increasingly becoming aware about the effect of unsustainable production systems such as the effects of intensified agricultural production and how uncontrolled waste management practices affects societies and the environment in which these farms are located. Despite, aerobic waste management options such as composting being regarded as environmentally sustainable, opportunities to compliment and yet extract the greatest possible economic value from biomass waste could be of great benefit to aerobic waste management alternatives such as composting.

3.6. Dairy waste management

At any given time, the farm has an average herd of 100 lactating Holstein Friesian cows. Plans to expand the current herd to 200 lactating cows are in the near future. This will be done to optimize the full production capacity of the newly renovated and improved milking parlour. Despite the ambition, several constraints remain eminent. One such constraint is the availability of sufficient grazing pastures during rainy winter seasons when pastures become heavily waterlogged making grazing nearly impossible (Plate 1). When this occurs, the prospect of manure waste water flowing into water canals and reaching the Eerste River is amplified (Plate 2).

Waterlogged pastures further pose serious animal health concerns while providing a conducive breeding habitat for flies to thrive (Plate 3). Flies not only become a nuisance to grazing cows but, also to communities on the periphery of the farm. To overcome these challenges, manure produced on the farm before 2012, were hauled off the farm to a central location where the public would collect it for personal use. At that moment in time, disposing dairy waste outside the farm gate was out of necessity, because the alternative of applying it onto farm pastures could have easily exceeded the ability of the soil to receive it as a fertilizer.

According to Coetzee (2014, personal communication, Stellenbosch University, Welgevallen experimental farm dairy manager) a plausible solution for the dairy is to accommodate most if not all lactating cows under feedlot conditions during the rainy winter seasons. This will not only safeguard the cows against the elements such as weather, but afford a conducive environment for the cows to become more productive. At the same time, such an undertaking will permit better control and management of manure, which will be flushed-out and pumped to the reactor or contained in a constructed wetland, which is the current alternative to manure management.

Unlike, fruit waste that is seasonal, dairy manure waste water remains reasonably constant throughout the year subject to the number of cows that are on the farm at a particular time. An estimate by the dairy manager, indicates that the dairy produces in excess of 1 000 metric tons of manure waste water per annum. This waste is divided between two streams which are milking parlour and that from the feedlot. However, the milking parlour waste represents only a mere 10% of the estimated 1000 metric tons of waste water from the two streams. An estimated comparison of dairy manure wastewater and fruit waste is presented in Table 1.

Manure waste at the feedlot is flushed-out on a daily basis. The process uses 19 m^3 of water flowing down the sloped feedlot into a collecting pit before some of it enters the constructed wetland without any pre-treatment. Waste from the milking parlour is primarily composed of manure, whereas that from the feedlot can be a wide-ranging combination of manure, sand and fibre rich Lucerne used as bedding material for the cows.

The presence of inorganic particles such as sand and high cellulose fibrous matter in the feedlot manure will thus pose challenge for anaerobic digestion than that from the milking parlour. Sand does not degrade, it settles down at the bottom of the anaerobic reactor and has the potential to increase HRT, flushing out essential anaerobic microorganisms, which ca lead to possible process failure. Under such instances, the digester will have to be cleaned often in order to remove the sand particles.

While, manure waste water from the feedlot is flushed into a constructed wetland, waste from the milking parlour is transported twice on a weekly basis to the composting site. However, during events of heavy rainfall, the wetlands capacity to contain manure waste waters is compromised to prevent it from flowing into water canals.

This prompted the Stellenbosch Municipality to timely collect water samples flowing of the farm in order to monitor the presence of indicator pathogenic micro-organisms in water flowing off the farm into the river. One of the water quality indicator pathogen of particular interest to the municipality is *Escherichia coli* (*E. coli*) serotype O157:H7 of which ruminants are natural carriers of these pathogenic bacteria. The bacteria lives undetected in the digestive tract of ruminants and can spread to humans either through direct or indirect contact (Callaway *et al*., 2008).

According to Unc & Goss (2004) the changes in environmental conditions for the pathogen to survive outside the digestive tract of the ruminant are often considered unfavourable. On the contrary, *E.coli O157:H7* has been documented to survive for extended periods of time (Maule, 2000) and is influenced by how the manure or slurry is managed (Nicholson *et al*., 2000).

Research suggests that populations of total *E. coli* O157:H7 shed in faeces is exacerbated by feeding ruminants such as cattle grain based diets (Callaway *et al*., 2009). On the contrary, Van Baale *et al*. (2004) argued that cattle fed forage based diets had higher faecal *E.coli O157:H7* concentrations as much as by two fold for longer periods of time than those fed grain diets. The probability of *E.coli O157:H7* shedding by the dairy cows on Welgevallen experimental farm thus increases as a result of feeding a diet containing of nearly 55% grain in proportions of 30% maize, 7% barley, 8% oats and 10% wheat. The diet also include protein sources such as 7% soya and 2% blood meal.

Constructed wetlands are a common waste management practice on farms to target excess nutrients from livestock sludge (Sooknah & Wilke, 2004). Knight *et al*. (2000) noted that offer a low-cost and low-maintainace alternative and that interest in the utilization of constructed wetlands has been increasing to intercept waste waters before it leaves the farm either via surface runoff or groundwater infiltration. According to Cronk (1996) by intercepting waste waters constructed wetlands prevent excessive nutrient run-offs, whereas Danalewich *et al.* (1998) added that they help prevent eutrophication of downstream receiving water sources such as lakes and slow moving rivers.

Sooknah & Wilke (2004) noted that constructed wetlands can also be exploited for the production of high nutritive value floating aquatic plants to remove nutrients from waste water. The plants can then be harvested and fed to livestock such as fresh water fish, poultry or pigs. In addition, these plants can be fed into an anaerobic digester or composted alongside dairy manures. The only drawback is that some floating aquatic species such as hyacinth have the potential to become invasive in the absence of biological control agents.

Knight *et al* (2000) noted that wetlands have the ability to reduce faecal coliforms in dairy manures by an average efficiency of 92% and little effect on the reduction of salts. However, this statement is open to criticism as it could be expected that plants would be able to absorb excess soluble mineral salts present in the waste water. Faecal coliforms are reduced due to exposure to sunlight, predation, increased competition for resources and toxins (Cronk, 1996).

According to Knight *et al* (2000) it is a common practice that constructed wetlands will have some form of pre-treatment facility such as a settling basin or anaerobic lagoon to stabilize raw organic waste. Although a constructed wetland is an attractive waste management alternative of organic waste with high moisture contents, they are inefficient without pre-treatment (Cronk, 1996). However, there is limited data on the performance of these pre-treatment facilities, although, it is estimated that they have the ability to remove 50-75% of total biochemical oxygen demand (BOD) and total suspended solids (TSS) in waste water before it is put through a wetland (Knight *et al.,* 2000).

The constructed wetland at Welgevallen experimental farm has no pre-treatment facility such as an aerobic or anaerobic lagoon (Plate 4). In order to remove sediments from pre-treatment facilities, the waste is often agitated in order to obtain a homogenous mixture that is than sprayed onto agricultural land via powerful irrigation pumps. The spraying creates small particles that drift away with the wind and has been attributed to health problems such as asthma. This technique also results in the release of odours to people living in the immediate surroundings.

3.7. Windrow composting for waste management

In an effort to reform waste management, in the year 2012, a windrow composting operation was introduced as part of the 'Green initiative' by the Faculty of Agricultural sciences at Stellenbosch University. Today, manure once a liability and destined for dumping is transported on a weekly basis to the composting site located on the premises.

McDoughall *et al*. (2008) although aerobic waste management alternatives such as composting have the advantage of being cost efficient, the alternative does not offer the capacity for energy recovery. In addition, composting operations are energy and water intensive and require significantly larger portions of land. This also includes the likelihood of water soluble minerals seeping through the soil and results in the compaction of the soil in the long-run. Composting is also greatly influenced by the weather, such that it takes on average 8 weeks for compost to mature in the summer and even more during the cold and rainy winter months.

On the contrary Mata-Alvarez (2000) argued that anaerobic digestion is the most cost effective alternative among biological treatments due to its high energy recovery and its limited environmental impacts. In addition, Westerman & Bicudo (2005) noted that on farm composting operations allows for reduction in volume, which leads to a concentration of nutrients in compost, reduces odour, kills pathogens, adds an economic value to organic waste while stabilizing the organic waste for cost effective transportation.

Since the inception of the 'Green initiative', dairy manure particularly that from the milking parlour has been composted alongside fruit and waste before it is applied back onto farmland or University's common grounds. According to Cronk (1996) dairy waste particularly that from the milking parlour has high biochemical oxygen demand due to the presence of butter fat, milk sugars and contaminants such as detergents used in cleaning and disinfection of dairy infrastructure and equipment.

In comparison to disposing waste off the farm, the composting operation has a much a lower carbon footprint and less tractor hours as a result of reduce millage. Transport is hired at a going rate of R 380.00/hour which translates to R 760.00/week spend on transporting waste to the compost site. At two trips per week, this would amount to nearly as much as R 39 520.00 expended on transport on an annual basis.

3.8. Opportunities and challenges

Despite waste presenting farm management with day-to-day challenges, it is vitally imperative to understand how the role of the farm has changed as a system with regard to changes in waste management strategies over the past couple of years. How these changes affect society through a system of complex tangible and intangible relationships. Especially, individuals who are directly and indirectly affected by the management decisions made on the farm.

Several members of public residing in urban societies privileged enough to live on the periphery of farms like Welgevallen have the opportunity to view wild birds and dairy cows peacefully grazing in the meadows. However, the presences of insects such as flies that form part of the dairy production system are not always an occurrence society takes pleasure in. The same applies for the free ecosystem services such as the aesthetic value provided for by vineyards and fruit orchards in bloom. However, this too comes at a cost one of which is the timely chemical sprays in the same vineyards and orchards to guard against plant pest and diseases which also forms part of the production system.

It is evident that horticulture, viticulture and dairy waste management at Welgevallen experimental farm is not only largely constrained by environmental and economic concerns, but, by social concerns too. It is thus imperative that, plausible solutions to alternative waste management strategies do not add to existing economic, environmental and social concerns but try a mitigate them were possible.

Thus far, windrow composting has been successfully utilized as an alternative biomass waste management strategy at the farm especially when to overcome challenges which were associated with ancient waste management regimes of burying and disposing waste off the farm. However, this waste management strategy is geared more towards research although revenue a minimal amount of revenue is generated from this undertaking. Although composting is a well-established organic waste management practice to stabilize waste Alibardi & Cossu (2015) point out that some of the drawbacks are in energy consumptions such as fossil fuels and problems in the compost market. Although composting of organic waste continues to grow in popularity, an alternative waste treatment option such as anaerobic digestion has several advantages such as production of energy and reduction in the cost of aeration (Chynoweth *et al*., 2001).

Fruit waste persists to be the feedstock of choice during composting operations due to ease of handling, transportation, high organic matter and low levels of heavy metals (Yang *et al.,* 1998). Composting thus allows safer application of manure nutrients onto agricultural land. Compost improves soil organic matter essential for the production of crops; however, the quality of compost is difficult to standardize and as such particular composts yield superior results than others (Bailey & Lazarovits, 2003).

Despite this drawback, compost remains a comparatively sustainable solution to applying raw manure by reducing the risk associated with vast loss and seepage of nutrients such as nitrates and phosphorus into surface or ground water sources leading to contamination (Yun *et al*., 2000). Whereas, Smith *et al*. (2001) argued that composting just like other organic waste management practices such as land filling and constructed wetlands have been documented to release organic compounds of carbon, nitrogen and phosphorus.

Bernal *et al*. (2009) noted that composting involves a partial mineralisation of substrates which results in the liberation of carbon throughout the process, which compensates for the stabilization of the remaining organic biomass. Organic carbon losses can be as high as 67% in cattle manure. Nonetheless, composting remains an environmental cautious practice than the application of raw manures onto farmland, which often results in the rapid decomposition of the organic matter by soil microorganisms leading to the production of intermediate metabolites (Lee *et al.,* 2004).

3.9. Alternative waste management options

Alternative waste management options at Welgevallen experimental farm are not only constrained by environment, economic and social concerns, but have to align with the green initiative as per directive of the Faculty of Agricultural Sciences of 2012. Nonetheless, plausible alternative options will either be aerobic or anaerobic options. According to Avaci *et al.* (2013) aerobic waste treatment such as incineration have been researched and demonstrated in European countries for the management of chicken bedding whereas, high moisture waste such as swine slurry would undergo anaerobic digestion.

Incineration has the ability to rapidly reduce waste volumes whilst, ensuring the destruction of pathogenic microorganism at the same time. The process however, requires thermal energy and emits harmful greenhouse gases such as carbon dioxide into the atmosphere. In addition raw dairy, viticulture and horticulture waste at Welgevallen experimental are likely to contain high levels of moisture making incineration nearly impossible without pre-treatment. Appels *et al*. (2011) noted that incineration only becomes a viable option when the moisture content of biomass is below 60%. In addition, incineration can only be used as an alternative practice if there are no other viable options to manage waste and if relevant authorities sanction the practice.

Aerobic alternatives such as landfilling have even larger environmental concerns to it such as displacement of plant nutrients that could have been returned to the point of production, loss of potential soil organic matter which ultimately translates into loss of valuable farmland. This is mainly attributed to the fact that nutrients get trapped and are lost forever in landfills and while undergoing the process of anaerobic decomposition, they release harmful greenhouse gases such as Carbon Dioxide and Methane.

One major drawback of landfills emanates from the production leachate that can contain concentrated toxic chemicals, which likely contributes to contamination of surface and ground water sources. Although modern landfills are now constructed with a permeability liner, leakage is greatly dependent on the permeability (Narayana, 2009) or failure of the liner. In addition, as the human population continues to grow, land ownership per capita declines. This causes an increase an increase in land prices which ultimately includes the opportunity cost of landfilling space (Huhtala, 1997).

3.10. Anaerobic digestion

Windrow composting and the constructed wetland at Welgevallen experimental farm would greatly benefit from pre-treatment facilities to supplement waste management. The efficiency of the wetland to avoid contamination and pollution of water sources is compromised as a result of no pre-treatment. One major trade-off of the wetland is that water from the dairy slurry seeps through the wetland and is lost from the system and not recycled whereas, composting time could be greatly reduced. Björklund *et al.* (1999) highlighted that composting on a large scale can increase environmental concerns when compared to anaerobic digestion.

In addition, composting requires more land, while the process is largely influenced by the weather such that windrow compost takes about 8 weeks to mature during the warmer summer months and even longer during the cold rainy winter period. Although composting requires more labour than anaerobic digestions over the long-run, one major drawback of large commercial operations is the need for larger quantities of water and energy needed during aeration intervals.

In order to effectively contribute to the “green initiative” movement, a promising alternative to possible biomass waste management strategies at Welgevallen would be controlled anaerobic digestion through the utilization of a reactor. A reactor would provide a viable pre-treatment alternative to the current waste management practices. Mata-Alvarez (2000) argued that controlled anaerobic digestion can be utilized effectively to treat high moisture waste with dry matter contents of less than 40%.

Dairy manure, food and fruit waste can represent an important source of carbohydrates, proteins and fats in varying proportions. This makes these materials important and yet interesting feedstocks for the production of bio-energy as they are readily available waste and renewable. Utilizing fruit waste as a resource signifies a promising opportunity to reduce environmental concerns as biogas production is recognised as carbon neutral source of renewable energy (Ward *et al.,* 2008).

Controlled anaerobic digestion reduces odour emissions (Smet *et al.* 1999), reduces uncontrolled emissions of methane from manure during storage (Apples *et al.* 2011) and also produces a stabilized compost based organic fertilizer (digestate), which that can be further processed into composted or directly applied back to farmland (Mata-Alvarez, 2000; Alvarez & Liden, 2008). The anaerobic process also increases the nitrogen availability to the plant which in return also increases the reactor substrates fertilization efficiency (Avaci *et al.,* 2013).

Anaerobic digestion thus affords organic waste management an ability to regain opportunities that could have been lost by conventional waste management strategies such as burying, landfilling and incineration. Apart from generating renewable bio-energy, which can either be utilized for heating, generate electricity of combined heat and electricity generation. In addition, the farm will help mitigate greenhouse gas emissions, most notably methane and carbon dioxide, which will give the farm an ability to trade in carbon credits. The incentive for carbon credits thus has the potential to make anaerobic digestion a very attractive option (Chynoweth *et al*., 2001).

Apart from the generation of bio-energy and carbon credits, the digestate from the reactor can be utilized to amend soil contingent on its phytotoxic properties or it be further processed into compost. Despite controlled anaerobic digestion been regarded as waste management alternative that has the potential to address economic, environmental and social concerns better than most waste management alternatives, it remains too early to make assumptions that the anaerobic digestion technology is the appropriate waste management alternative for sustainable management of agricultural organic waste.

3.11. Discussion

Although models and methodologies of quantifying organic waste such as food waste in municipal areas, literature on models and methods of estimating organic waste at farm level remain limited. Salminen & Rintala (2002) also echoed the same sediment by stating that there is limited literature available for the quantification and characterisation of agro-industry solid by-products such as waste from poultry slaughter houses. Nonetheless, Gay *et al*. (1993) model of Economic Input/Output analysis (EIO) can be adapted to estimate agricultural waste production at the farm level. The model has several advantages in the sense that it can be cost effective with the potential of obtaining data that does not have significantly large standard deviations.

Weiss (2004) estimated that manure production can be estimated by quantifying milk production and that on average 2. 2 kilogram (Kg) of manure is produced for every kilogram (kg) of milk produced by a Holstein Friesian dairy cow. Weiss (2004) noted that this can be influenced by the choice of ingredients and nutrient composition of the animal diet. However, Weiss (2004) observed a significant association between dry matter intake and manure production contrary to substantial variations between milk yield and manure production, which implied that increased milk yields do not necessarily equate to increased manure production.

Although, there are methods that can be adapted to estimate organic waste such as dairy, it is increasingly difficult to adapt existing models for estimating fruit losses. Hodges *et al*. (2011) noted that estimating fruit waste can be difficult and unreliable, however, two main approaches have been considered that includes timely measuring the amount of fruit losses or use questionnaires by asking those who have experienced fruit losses.

Hodges *et al*. (2011) further added that although several studies have been conducted to estimate post-harvest losses in the United States of America (USA), none have been able to estimate total loses either on the farm or during processing, whereas in developing countries such studies are relatively unknown. According to Muth *et al*. (2007) there is also a limited pool of peer-reviewed literature on food waste studies in the USA.

3.12. Conclusion

The introduction of the 'Green initiative' by the Faculty of Agricultural Science in 2012 has seen a tremendous shift in biomass waste management practices at Welgevallen. This transformation, driven towards more sustainable biomass waste management strategies led to the reduction in the volume of waste been buried or transported off the farm. The shift towards composting has allowed the farm to production to return essential soil organic matter and nutrients that could have been lost or buried in several locations on the farm.

In order to address this subject, there is a need to investigate alternatives to the current practices of composting and wetland. One of the promising solutions is the use of an anaerobic reactor. This is a carbon neutral waste management strategy that would not only cut down on the farm's carbon footprint by reducing greenhouse gas emissions but, affords an opportunity to convert one of these gases into a valuable renewable energy source.

Although, constructed wetlands have multiple uses, the wetland at Welgevallen experimental farm without pre-treatment facilities remains a liability to waste management, which can be off-set by the inclusion of an anaerobic reactor in the dairy waste management mix.

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Table 1: Fruit waste quantities at Welgevallen experimental farm.

Fruit type	Weight (tons)	Time of availability
Apples	3	February - April
Pears	5	January - March
Citrus	10	March - April
Nectarines	1	November
Plums	3	January - March
Grape pomace	5	February
Total weight	**27**	

Table 2: Estimated dairy manure wastewater and fruit waste produced at Welgevallen experimental farm

Organic waste	Tons	Percentage
Cow manure	1000	97%
Fruit waste	27	3%
Total	**1027**	

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Plate 1: Dairy cows on waterlogged pastures at Welgevallen experimental farm

Plate 2: Water canal on the farm that connects to the Eerste River

Plate 3: Dairy cows, ankle deep in a waterlogged paddock at Welgevallen experimental farm

Plate 4: A constructed wetland to manage dairy manure wastewater at Welgevallen experimental farm

Chapter 4

Determining the efficiency and sustainability of an anaerobic reactor to process dairy manure wastewater at Welgevallen experimental farm

4.1. Background

According to the Department of Agriculture and Forestry (DAFF) (2013), South Africa's dairy industry produces an estimated 0.5% of global dairy production. Dairying occurs in all 9 of the country's provinces, of which the three largest producers are the Western Cape, Eastern Cape and KwaZulu Natal.

According to DAFF (2013), the South African dairy industry has over 2 474 milk producers, directly employs 60 000 employees and over 40 000 indirect jobs across the value chain, while producing milk and dairy products for both local and export markets. With such a large footprint, the significance of the dairy industry to the South African economy can thus not be underestimated. The DAFF (2013) report highlighted that the national dairy cow herd size increased by 99% over the past decade, while milk production increased by 17% over the same period. With a nearly 100% increase in the national dairy herd size, it will be not be unfounded to estimate a similar increase in dairy manure wastewaters over the same period.

The Water Research Commission (1989), as cited by (Strydom *et al*. 1997), estimated that the South African dairy industry had a water footprint of 4.5 million m^3 of which 75-95% is often discharged as effluent wastewater. According to Molobela & Sinha (2011), 52% of fresh water allocation in South Africa is primarily used for agricultural purposes, while 10% is allocated for human use. This is of particular concern especially because South Africa is classified at a semi-arid country that receives on average 160-330 mm of rain per annum. Strydom *et al*. (1997) further noted that the bulk of dairy manure wastewater is either directed into municipal sewage systems or alternatively sprayed onto pastures as readily available liquid fertilizer.

Directing dairy manure wastewater into municipal sewage systems places additional complications on already stressed municipal sewage systems. While, spraying dairy manure wastewater as liquid fertilizer has been implicated in causing public health concerns for communities living in the vicinity of concentrated animal feeding operations (Greger & Koneswaran, 2010). In addition, customary manure storage systems such as constructed wetlands release greenhouse gases into the atmosphere, while these also pose contamination and pollution of surface and groundwater sources (Greger & Koneswaran, 2010)

Thus, evaluating sustainable waste management alternatives to moderate the impact of increasing dairy herds in future is justified and important, both for the agricultural sector and the environment.

4.2. Introduction

Consumers are becoming increasingly conscious of the externalities emanating from the production systems that produce products and services they consume (de Boer *et al*., 2006). This awareness takes cognisance of the fact that these products not only have the potential to affect their wellbeing, but also that of societies in close proximity of the production units and consequently, the environment. As a response, modern day consumers are progressively starting to demand products from production systems that are not only economically sustainable but, also from production systems that comprehend environmentally and socially sustainability (Vermeir & Verbeke, 2008).To satisfy consumer demands, various entities along the supply value chain are increasing promoting strategies such as fair trade in an effort to create alternative sustainable production systems that take environmental and social concerns into perspective (Murray & Raynolds, 2000).

Intensive dairy production systems have a relatively significant impact on the environment in which they operate, as they produce large quantities of dairy manure wastewater. If not managed well, these wastewater have the potential to cause serious and detrimental environmental and social problems (Greger & Koneswaran, 2010). The externalities of waste management remain one of the major constraints to successful intensification of agricultural systems such as dairying. Although an array of innovative alternatives pertaining to the management of waste have been research and demonstrated, anaerobic digestion (AD) remains one of the most sustainable options provided that the economic returns are favourable (Callaghan *et al*., 2002). In addition, AD of waste such as dairy manure wastewater is an established waste management alternative in some parts of the world. However, most studies regarding AD refer to fundamental research performed on small scale laboratory models (Strydom *et al*., 1997; Callaghan *et al*., 2002; Murto *et al*., 2004; Alvarez & Liden, 2008).

AD closely mimics the digestion of feed material through the digestive system of ruminants such as dairy cows, which makes dairy manure a suitable AD feedstock due to the presence of essential anaerobic microorganisms that are critical to the overall efficiency of an anaerobic reactor (Bond & Templeton, 2011). These are excreted alongside faeces. Therefore, when using fresh dairy manure as feedstock, sufficient anaerobic microbial inoculum is available for AD and it is not necessary to add inoculum.

Existing literature indicates that temperature remains one of the most important variables that has a direct influence on the AD process (Ahring, 1992). To afford a conducive microbial environment and optimize the production of biogas anaerobic reactors are designed and operated with a mesophilic (20-42°C) or thermophilic (42-75°C) temperature range (De la Rubia *et al*., 2005). In addition, anaerobic reactors can also be operated at psychrophilic temperature (0-20°C) which will result in a longer HRT and lower yields of biogas. However, thermophilic conditions have been observed to be more effective at speeding up the AD process by reducing the HRT, increasing methane yield and improving the efficiency of reducing pathogenic microorganisms in the digestate. The only major drawback it that reactors operated at thermophilic temperatures have been reported to be more prone to process failures, due to a higher accumulation of toxic ammonia (Angelidaki & Ahring, 1994). Ammonia permeates through the cell membranes of microorganisms, resulting in proton imbalances rendering microbes to become inactive (Kroeker *et al*., 1979).

In addition to temperature, parameters such as EC remain important to meet the requirements of the National Water Act 36 of 1998. EC which is dependent on temperature is an indicator of how well a substance can conduct electricity. According to Hayashi (2004) the relationship between EC and temperature is linear, such that 1ºC in temperature would result in a 2%

increase in EC. Under environmental conditions were temperature is increasing, the movement of concentrated ions under an electrostatic potential also increases which subsequently leads to an increase in EC of a substance. On the contrary Millero (2001) as cited in (Hayashi, 2004) noted that the EC-temperature relationship of substances such as natural water sources can be generally nonlinear, although this can be relatively small for waters within an environment temperature range of 0-30 ºC. EC observations at different temperatures thus remains critical to adjusting EC values to, which correspond to a standard temperature (25 °C) for meaningful interpretation (Hayashi, 2004).

EC thus makes it possible to gain a perspective of the amount of dissolved salts in a solution (µS/cm). In agriculture and in particular for the integrated use of anaerobic digestate as a fertilizer source, EC can be used to serve as a guideline for the application of digestate onto farmland. According to NRCS (1999) water with EC values of 2000 µS/cm or less have been found to be suitable for irrigation whereas, it has been found to be toxic above 4000 µS/cm.

In addition to temperature and EC, pH also plays a significant role such that the ideal pH for optimal microbial growth and biogas production falls within a narrow spectrum of 6.8-7.2 (Ward *et al*., 2008). This range caters for methanogens whose ideal pH range remains around 7.0, while that of acid producing bacteria (hydrolytic and acidogenic microorganisms) is around 5.5-6.5 (Kim *et al*., 2003).

Another important factor to consider in order to avoid process failure is OLR. Calculation of the organic loading rate (OLR) and estimation of the digestibility of feedstock depends on dry matter (DM) and volatile solid (VS) content of feedstock. Due to the high moisture content of dairy manure wastewater, DM is determined by analysing total suspended solids (TSS), which subsequently also includes determining volatile suspended solids (VSS). Because, microbes break down and digest VS, it is also important to determine the carbon to nitrogen ratio (C: N) of feedstock into order to ensure that the microbial populations do not suffer from nutritional shocks and acclimatise well to the reactor. Anaerobic microbes have been documented to utilize 25 to 30 times the equivalent of carbon compared to nitrogen (Sreekrishnan *et al*., 2004). Imbalances such as feedstock with low C: N ratio are likely to result in the production of toxic concentrations of ammonia and an accumulation of volatile fatty acids (VFA's) that can cause process failure.

Livestock waste such as manure contain important quantities of Nitrogen (N), Phosphorus (P) and Potassium (K) and other water soluble minerals that can be used to complement benefits derived from the use of fertiliser (Hooda *et al*., 2000). Despite this importance, the increase loss of these water soluble nutrients from agricultural systems either through leaching or runoff can serious implications on public and environmental health. Minerals such as nitrogen and phosphorus have been found to cause eutrophication of fresh water sources (Sharpley & Menzel, 1987) subsequently leading to the deterioration of water quality (Sharp & Withers, 1994).Therefore for sustainability concerns, it is vital to evaluate the effects of AD on water soluble minerals in order to facilitate the attachment of an economic value to the digestate as an alternative liquid fertilizer.

Nitrogen and Ca can affect the buffering capacity of a reactor. Nitrogen in dairy manure wastewater enters the reactor as either organic nitrogen or ammonium. During AD, organic N is mineralized to produce ammonia (NH_3) and ammonium (NH_4) with the major percentage being ammonium nitrogen (Fricke *et al*., 2007). Ammonium concentrations of up to 1000 mg/l remains critical at maintaining sufficient buffering capacity and promoting microbial growth (Fricke *et al*., 2007). However, high ammonium concentrations above 3000 mg/l caused detrimental effects to the AD process through the inhibition of methanogenesis (McCarty & McKinney, 1961).

Apart from calcium and nitrogen, digestate contains other essential minerals that can be useful in supplementing essential macro and micro nutrients needed for plant growth and productivity. Macro-minerals/ nutrients includes minerals such as (N), phosphorus (P) and potassium (K). In addition, mineral nutrients such as calcium (Ca) and Magnesium (Mg) remain equally important. Whereas, minerals such as boron (B), zinc (Z), iron (Fe), Copper (Cu), Manganese (Mn) and Magnesium (Mg) are considered important micronutrients. However, before digestates can be used as fertilizer, it is essential to evaluate the phytotoxic effects of this possible fertilizer.

Livestock manures can contain high ammonia concentrations that can inhibit anaerobic digestion by inhibiting optimal microbial growth (Hansen *et al*., 1998). However, high ammonia concentrations can also be beneficial especially when co-digestion alongside feedstock such as food and fruit waste that have low nitrogen levels. Higher energy feedstocks, those with lower C: N ratios (of the order 18 and lower) will be fed to a maximum of 2.5 $kg/m^3_{reactor}/day$ (Volatile suspended solids) in order to avoid poisoning of the anaerobic bacteria with ammonia because of the high nitrogen levels. In composting experiments, Sanchez-Monedero *et al*. (2001) observed that high ammonium concentrations during the first week of composting can be responsible for the low microbial biomass. In addition to ammonium, free ammonia at concentrations at 150 g/l in the reactor has been observed to cause growth inhibition of anaerobic microbes (Gallert *et al*., 1998).

To quantify microbial diversity, bacterial cells which contain DNA must first be extracted from the effluent. After DNA is detected via the gel electrophoresis technique, the DNA is than amplified with specific primers via the Polymerase Chain Reaction (PCR) process which targets a highly conserved gene 16s rRNA (Shin *et al*. 2010) before microbial species diversity are quantified with the Automated Ribosomal Intergenic Spacer Analysis (ARISA). ARISA does not identify species, but quantifies genetic diversity through a generation of frequency peaks on a graph, with each peak representing a distinct microbial species. However, high species diversity does not imply beneficial microorganisms and that these will have a positive influence on biogas yield, but that biogas yield will be influenced by a high microbial biomass yield of keystone hydrolytic, acetogenic, acidogenic and methanogen species.

4.3. Problem statement

AD efficiency as a sustainable alternative for dairy manure wastewater in a semi-commercial, plug flow AD has not yet been established with reference to the production of biogas, pathogenic microorganism elimination, waste volume reduction and the potential of digestate as compost medium and/or possible fertigation source.

4.4. Objective

The aim of this study was to quantify these parameters in a semi-commercial, plug flow AD on Welgevallen experimental farm according to the requirements of the National Water Act (NWA) (Act 36 of 1998) with reference to the guidelines in Table3.

4.5. Material and methods

4.5.1. System design

The current study utilized a recently constructed three chamber (Fig 25), plug-flow anaerobic reactor with a volumetric capacity of 40.248m^3 on Welgevallen experimental farm, Stellenbosch University. The design of the chamber is added as annexure 1. The theory behind plug flow-reactors is that feedstock will move coherently through the bio-digester without mixing the content in the axial directions (Ward *et al*., 2008). However, this theoretical hypothesis can be validated by hydrodynamic studies using easily detectable tracers such as Lithium chloride (Olivet *et al*., 2005) fed-into the bio-digester alongside feedstock and monitoring the time interval when Lithium is detected in the digestate, (Ward *et al*., 2008) which can also be interpreted at the HRT.

This reactor only started operating 2 months before the first trial commenced due to logistical problems. It has a daily volumetric loading rate of 2.1m^2 of dairy manure wastewater, supplied as two deliveries of 50% each. Dairy manure waste (feedstock) was flushed from the dairy cow feedlots into a sump and then pumped automatically with a Wilo JDSK 20 pump (3 min) via a 75mm delivery line, to the reactor. The pump was set to deliver feedstock daily around 08:30 and 18:30. The supply of 2.1 m^3 volumetric loading rate was heavily dependent on the reliable manual cleaning of the feedlots by dairy employees. A pump control meter located in the reactor’s control room records volumetric loading rate and validates the time the pump is active on a daily basis. The feedlot accommodated 50 dairy cows per day.

This experiment had an OLR of 2.59 kg/m$^3_{reactor}$/day (Total suspended solids) or 1.97 kg/m$^3_{reactor}$/day (Volatile suspended solids) which is considered relatively conservative which makes it fairly easy for anaerobic microorganisms to digest. Livestock manures can contain high ammonia concentrations that can inhibit anaerobic digestion by inhibiting optimal microbial growth (Hansen *et al*., 1998). However, high ammonia concentrations can also be beneficial especially when co-digestion alongside feedstock such as food and fruit waste that have low nitrogen levels. Higher energy feedstocks, those with lower C: N ratios (of the order 18 and lower) will be fed to a maximum of 2.5 $kg/m^3_{reactor}/day$ (Volatile suspended solids) in order to avoid poisoning of the anaerobic bacteria with ammonia because of the high nitrogen levels. Ammonia levels can become toxic to anaerobic bacteria above 4000mg/L depending on the acclimatisation the bacteria (Angelidaki & Ahring, 1994).

4.5.2. Treatments:

This was a mono-digestion of dairy manure wastewater, which served as a control experiment. The experiment commenced in July 2015 with a daily volumetric feeding rate of 2.1m^3 of feedstock which corresponded to a HRT of 20 days.

4.5.3. Experimental variables

Variables that were measured as well as the frequency thereof, are summarised in Table 4. For analyses of liquid samples, a volume of 500 ml was sampled at each occasion. Sampling occurred at the same time of the day in the morning between 08-09h00. The reactor is equipped with three sampling ports, connecting to the individual chambers. Samples for EC, pH and microbial community diversity analysis were lifted from each chamber using a 3 phase electric motor pump into plastic containers labelled accordingly.

4.5.4. Temperature and Electrical conductivity (EC)

Unlike other start-up reactors that receive heating from an external source during initial start-up, no additional heating was provided during the start-up phase as the design made provision for using methane as main energy source for the system to qualify as energy self-sustainable. The reactor was thus designed to produce sufficient quantities of biogas to be available for a gas geyser to heat the unit via hot water recirculating system connected to all three chambers under ideal conditions

Temperature and EC observations were recorded daily after dairy manure wastewater was pumped into the digester at 08:30. Temperature was recorded (between 09h00-10h00) automatically from the reactor control panel as heated water circulated in a network of pipes between the installed geyser and the reactor. Before temperature was recorded, the heated water circulation pump was switched from automatic circulation to manual for 45 minutes. EC measurements were recorded within 30 minutes after sampling (insulated container to preserve temperature) in a laboratory on the University campus.

4.5.5. pH measurements

Digestate sub-samples were lifted from each per chamber, using a single-phase induction motor pump into 500ml containers which were labelled accordingly. These samples were taken to a laboratory on the campus within 30 minutes after sampling where pH measurements were recorded using a Hanna H1 991300 pH/EC/Total dissolved solids (TDS) and temperature meter from Hanna instruments. The pH meter was calibrated on a weekly basis with standard solutions 7.01 and 4.01. EC, with a standard solution value of 1413 µS/cm, was also determined.PH observations were recorded for each of the three reactor chambers.

4.5.6. Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

Samples for quantification of the C:N ratios were analysed by a commercial laboratory (Bemlab (Pty) Ltd, Strand) and results are presented in figure 8.Total suspended solids (TSS) and volatile suspended solids (VSS) were analysed by glass filter technique (APHA, 1998) summarised in annexure 2.

TSS and VSS were than calculated accordingly to the following formulas (APHA, 1998) and summarised in Table 5:

$$\text{Total suspended solids (g/L)} = \frac{(B - A) \times 1000}{\text{Sample volume(ml)}}$$

$$\text{Volatile suspended solids (g/L)} = \frac{(B - C) \times 1000}{\text{Sample volume(ml)}}$$

Where:

A = Weight of filter disc and porcelain dish after step 1.

B = Weight of filter disc and porcelain dish after step 2.

C = Weight of filter disc and porcelain dish after step 3.

4.5.7. *Escherichia coli* O157: H7

To ensure that the AD digestate complied with the requirements of the National Water act 36 of 1998, the potential efficiency of the reactor to reduce concentrations of pathogenic *E*.*coli* over a HRT of 20 days had to be quantified. A sample of dairy manure wastewater was collected at the inlet of the reactor to determine initial *E.coli* concentrations on day 1 (10 July 2015). A second sample of the digestate was collected on day 20 (30 July 2015) and stored at 4 °C to prevent further growth of pathogenic bacteria until analyses are possible. Analyses were performed using the standard pour plate technique (APHA, 1971). The protocol is summarised as annexure 3.

4.5.8. Water soluble minerals

A sample of feedstock collected at the inlet on day 1 (10 July 2015) and another, of the digestate collected on day 20 (30 July 2015) at the outlet of the reactor, were sent to commercial laboratory (Bemlab Pty Ltd, Strand) for analysis. To obtain a representative composite sample for analysis, the sample was drawn at the inlet of the reactor during the second minute of the 3 minutes when the pump delivered feedstock into the reactor in the morning. The digestate sample was collected from inside the discharge pump chamber of the reactor after 20 days (30 July 2015).

4.5.9. Phytotoxicity

Phytotoxicity of the digestate was evaluated by quantifying germination percentage of seeds according to literature (Mitelut & Popa, 2011). Tomato seeds were selected because of their relatively small size and small quantities of mineral nutrient reserves, rendering them susceptible to most phytotoxicity symptoms, excluding ammonia, Cu and Zn (Tiquia *et al*. (1996).

Germination rate was determined to assess if digestate produced from the reactor over a HRT period of 20 days has any phytotoxic effects on relative germination percentage of tomato seeds. Tomato seeds (HEINZ 1370) were selected to estimate percentage germination between 7-14 days. The seeds were purchased from a local retailer in Stellenbosch and sown in petri dishes lined with sterile towel paper. The experiment was a completely randomised design (CRD) with 5 treatments and three replications. The data was subjected to the statistical analysis system software (SAS) 9.3 for a one way Anova.

Treatments comprised of a control (distilled water), and DM digestate concentrations of 25%, 50%, 75% and 100%.Treatments comprise 5 ml to moisten the towel tissue in which 10 tomato seeds were sown. Petri dishes were then incubated at room temperature inside a dark area for 72 hours to obtain the seed germination rate (Table 7). As this was only performed at the end of the all experiments, digestates were collected from the reactor outlet sump and stored at 4 °C for 60 days (DM manure wastewater), 40 days (DM wastewater and apple waste), 20 days (DM wastewater, apple and food waste) and 5 days (DM wastewater and food waste).

4.5.10. Anaerobic microorganism population diversity

Quantification of the anaerobic microbial population diversity was performed to over 20 days to indicate microbial dynamics during the process. All analyses were performed in the Department of Microbiology, Stellenbosch University. Samples from each of the three reactor chambers were transported to the laboratory in plastic containers within 30-60 minutes. Samples collected on a Saturdays or Sundays were kept in at 4 °C and analysed on the following Monday. To obtained bacterial cells of the waste water, samples were centrifuged at 10 000 revolutions per minute (rpm) for 15 minutes at 4 °C whereby solids (colloids) including bacterial cells settled to the bottom while the clear liquid was discarded. The samples were now ready for DNA extraction performed according to the Zymo spin test kit manufacturer’s (Zymo Research, Orange, California) protocol in annexure 4.

Polymerase Chain Reaction (PCR)

Although, sampling for DNA extraction was done every third day during each treatment. Only samples extracted on day 1, 4, 7, 10, 13, 16 and 19 were used for PCR and ARISA, due to time constraints. The standard protocol utilized for PCR analysis can be found in annexure 5.

4.6. Results and Discussion

4.6.1. Temperature and Electrical conductivity

The experiment commenced during the rainy winter season of the Western Cape. Without additional heating, the reactor's temperature during this treatment fluctuated between 10-14 ºC, which was lower than the 35 °C needed by anaerobic microorganisms to perform optimally (Fig 1). Nonetheless, psychrophilic conditions have been observed not to have a significant impact on hydrolytic bacteria (Ward *et al*., 2008), but the viability of methane producing methanogens is severely compromised resulting in low biogas production, which was validated by this study.

In addition, to temperature, the EC of the reactor during this period increased over time (Fig 1), taking into consideration that some data could not be collected on days 2, 3, 5, 6, 7, 8, 9 and 10 due to technical constraints. However, a sharp decline in EC was observed on day 14 in chambers 2 and 3. Although, an increase in EC can be an indication of water pollution from water sources like rivers and dams, in the agricultural context, an increase in EC can be a potential indicator of increased concentrations of water soluble nutrients such as nitrates (Sanchez-Monedero *et al*., 2001). Sanchez-Monedero *et al*. (2001) observed a significant correlation between EC and nitrate concentrations during aerobic composting of organic waste when ammonium was converted to nitrate due to a liberation of hydrogen ions. Similar observations were reported by Sharma (2003) in composting trials, who noted that an increase in EC can be related to the digestion and breakdown of organic matter which leads to the liberation of water soluble mineral salts such as phosphate, potassium and ammonium.

4.6.2. pH

During the start-up phase, the trends pH in both three chambers seems to be considerably unstable. Despite this instability, pH remained within the optimal microbial growth range especially during the last 6 days of the experiment (Fig 2). The instability could have been a result of VFA's production by fast growing hydrolytic and Acidogenic bacteria which exceeded the ability of slower growing acid consuming acetogens and methanogens to produce biogas from acid which in turns yields alkalinity and avoid pH decline (Kim *et al*., 2002).Alternatively, it could also be partly due to the fact that the reactor has only been active for a relatively short time. Ward *et al*. (2008) noted that acid producing bacteria have the ability to thrive at low psychrophilic temperatures, which helps elucidate the progressive decline in pH over time. This may explain the decline in chambers 2 and 3 between days 15 to 18.

4.6.3. Instantaneous biogas production (quantity) and composition

Biogas yields are dependent on the optimal temperature, pH, OLR and HRT. Despite, the pH rate being within the optimal range of 6.8-7.2 for the duration of the experiment, temperatures in the reactor were too low for optimum microbial functioning and hence low or undetectable biogas yields resulted. This confirms reports from Lettinga *et al*. (2001) that the solubility of gaseous compounds such as methane, carbon dioxide, hydrogen sulphide and hydrogen increases as the temperature drops below 20 °C. Thus, these compounds will be higher in the effluent of reactors operated at low psychrophilic temperatures than those operated at high mesophilic or thermophilic temperatures (Lettinga *et al*., 2001).

4.6.4. Total Suspended Solids (TSS) and Volatile Suspended Solids

Forster-Carneiro *et al*. (2008) noted conventional reactors require feedstock with less than 10% TS whereas, modern systems can accommodate feedstocks with more than 20% TS. Considering that our DM wastewater contained a mean TSS content of 2.59 g/L (Table 5) and volumetric loading rate of 2.1m^3 of feedstock per day, the OLR was 5.44 kg of which 4.13 kg is VSS. This is an equivalent of 0.259% TSS, which is considerably lower than the conservative 10% TSS that conventional reactors require. The low TSS can be explained by the large quantities of water that can exceed 19 m^3 (380 L/ cow) used to flush manure from the feedlots daily which diluted the DM concentration in the inflow. Adapting Weiss (2004) thesis that 2.2 kg of manure is produced for every 1 kg of milk, the average Holstein Friesian dairy cow with yield of 20 kg milk would produce 44 kg of manure. The entire herd of 50 cows in the feedlots would produce 2.2 m^3 of manure per day, which would be an equivalent of 11.57 % in the manure wastewater where 19 m^3 of flush water is used daily.

TSS reduction of 80.70% and 75.51% VSS in dairy manure wastewater and digestate was observed (Table 6). Despite this noticeable reduction in TSS, the reactor yielded little to no biogas. This high reduction in TSS and VSS could not have possibly resulted from anaerobic digestion of solids by anaerobic microorganisms. A possible explanation could be that the solids have settled at the bottom of the reactor to form sediments as observed when the unit had to be cleaned out to repair a major leakage during the initial phase.

4.6.5. Escherichia coli

Colony forming units per millilitre (cfu/ml^{-1}) increased from log 4.75 cfu/ml^{-1} in dairy manure wastewater to log 4.82 cfu/ml^{-1} dairy manure wastewater digestate (Figure 3). This outcome was expected due to the low reactor temperatures recorded during this time period. Kim *et al*. (2002) argued that reactors operated under mesophilic digestion or lower are not capable of producing pathogen free digestates and that these can only be attained in reactors operated at higher temperatures such as thermophilic reactors. In order to comply with the National Water act 36 of 1998, the digestate produced will thus require additional treatment such as chlorination before it can be used as an alternative source of liquid fertilizer or discharge into natural water ecosystems such as the Eerste River

4.6.6. Water soluble minerals and nutrients

Compared to DM wastewater with a C: N ratio of 2.4:1, the digestate showed a higher C: N content with ratio of 1:1 (Figure 4). Despite, the low psychrophilic temperatures observed, a decline in carbon and increase in nitrogen would represents microbial activity, which could have been responsible for the decline in pH mentioned earlier. This draws support from Wang *et al*. (2014) who observed that C: N ratio had a significant influence on pH especially in reactors operated at mesophilic temperatures.

Turning to water soluble minerals and other derivative N compounds (Figures 5 and 6), there were no differences between soluble mineral nutrients concentrations in the diary manure wastewater feedstock compared to the digestate. These findings draw support from Möller & Müller (2012) who noted that several authors did not find any statistically significant evidence to proof a reduction nor an increase in digestates for minerals such as K, Fe, Mg and Na. However, Marcato *et al*. (2008) found significant losses for minerals such as Ca and Mg which tend to crystallize into phosphates and carbonates. Consequently, calcium concentrations declined from 257.7 mg/l before to 156.98 mg/l after digestion, which signifies a 40% reduction and concurs with findings from a previous study (Marcato *et al*. 2008).

In addition, calcium concentration between 250-400 mg/l have been observed Parkin & Owen (1986) to moderately inhibit anaerobic digestion. While, Jackson-Moss *et al*. (1989); Ahn *et al*. (2006); argued that concentrations of 500 - 700 mg/l can cause anaerobic microbial inhibition. According to Parkin & Owen (1986) it is therefore possible that initial Ca concentrations at 257.7 mg/l could have contributed towards moderately inhibition of AD.

The reduction of approximately 40% in Ca concentration during AD can be partly attributed to the importance of Ca also in the growth of anaerobic microbes. Ca concentrations between 100-120 mg/l has been shown to be essential for the growth of anaerobic microbes as it stimulates the formation of biofilms and granules that are colonized by anaerobic bacteria (Huang & Pinder, 1995). On the other hand, large quantities of Ca can precipitate into calcium carbonate that can cause the reactor to loose buffering capacity (Van Langerak *et al*., 1998).

In addition to Ca, N derivatives can also affect the buffering capacity of the reactor. Although, the concentration of ammonium was substantially lower in the digestate than the reported 1000 mg/l ammonium concentration essential for maintaining sufficient buffering capacity and promoting microbial growth Fricke *et al*. (2007).

Although, the specific nutrients or soluble minerals liable for displaying phytotoxicity are not exclusively known, Tiquia *et al*. (1996) highlighted that several micro minerals such as Cu, Zn (figure 9) and nitrogen compounds like NH_4^+-N are some of the most important phytotoxic chemicals. In addition, organic acids present in digestates have been observed to display phytotoxicity by causing a surge in soil microbial populations, causing microbes to exhaust essential soil O_2 available to plants (Marambe *et al*., 1993). Salminen *et al*. (2001) observed that several chemicals present in digestates such as palmitic acid also displayed a negative phytotoxic correlation towards root growth in Chinese cabbage, whereas medium fatty acids and chemical oxygen demand (COD) displayed the same phytotoxic effects on the root growth of ryegrass.

4.6.7. Phytotoxicity

The tomato seed germination results was subjected to R commander data analysis software, a significant difference observed for the treatments (p = 0.0361) indicated that either one or more of the dairy manure wastewater digestate concentrations does have phytotoxicity effects on the germination of tomato seeds (Table 7). To establish which digestate concentration had a phytotoxic effect on seed germination, a pairwise comparison test of means was conducted (Table 8). The 100% of dairy manure wastewater digestate yielded a relative germination mean of 76.7%, which according to Sánchez *et al*. (2008), would indicate phytotoxicity. This result suggested that dairy manure wastewater digestate will have to be diluted before it can be utilized as an alternative liquid fertilizer source. This effect is possibly due to a combination of various mineral nutrients as specific nutrients or soluble minerals liable for displaying phytotoxicity are unknown Tiquia *et al*. (1996)

4.6.8. Anaerobic microorganism population dynamics

Plate 5 illustrates extracted DNA from the reactors effluent, whereas (Plate 6) illustrates DNA amplification after PCR. These two processes were prerequisites for DNA sequencing in order to quantify the anaerobic microbial population dynamics via ARISA. The analysed ARISA data is presented in Table 9 and Figure 7.

The ARISA data was subjected to a gene map software analysis tool to assess the anaerobic microbial population diversity present in the reactor. Gene map generates a series of peaks to depict the detection of a particular microbial species. Microbial diversity in the reactor decreased over time. This trend in community diversity was expected and validates some degree of selection between aerobic and anaerobic microorganisms by the reactor in the absence of O_2. Feedstock pumped into the reactor in the afternoon were exposed to an aerobic conditions for approximately 10 hours on a daily basis before it was flushed-out of the feedlot. During this period, various aerobic and anaerobic microbial communities could have colonize the manure, although aerobic microbes would have had a comparative advantage to thrive as the anaerobic bacteria are likely to initiate starvation responses (Roslev & King, 1995) in order to survive outside the rumen environment. Therefore, the microbe population entering the reactor in the second flush may have comprised of a higher aerobic component when the feedstock entered the reactor, making it more difficult for aerobic microbes to survive and compete for nutrients in an anaerobic environment. Under such conditions, aerobic microbes find it difficult to survive and eventually die. This resulted in only keystone anaerobic microbial communities thriving and hence the decline in species diversity confirming observations from

Goux *et al*. (2015) that, with increasing organic loading rates, distinct microbial communities begun to dominate the reactor.

Assuming that the 1st chamber would be colonized by fast growing hydrolytic bacteria and the 3rd chamber by slow growing methanogens, the 2nd chamber would either be colonized by acidogenic or acetogenic bacteria. The microbial diversity in chamber 2 decreased from 28 species on day 1, to 17 on day 13, before increasing to 30 species (Table 9 and Figure 9). This could be attributed to direct competition between microbial communities trying to establish themselves in the reactor.

4.7. Conclusion

Anaerobic digestion as a sustainable alternative for dairy manure wastewater management was quantified for different parameters at psychrophilic temperatures using a semi-commercial, 3 chamber, and plug flow-anaerobic reactor. The first treatment validated that temperature remains an important variable in the AD process, particularly for the reduction of colony forming units in the digestate. The data also seem to suggest that, temperature does not appear to have influenced the EC of effluent under low psychrophilic temperatures. Throughout the experiment, EC of the dairy manure remained around 3000µS/cm, which is much higher than the legally excepted 2 000 µS/cm limit set using wastewater for irrigation purposes or discharge into the environment with low discharging volumes of 50 m^3/day. This also exceeds the limit of < 700 µS/cm that will be relevant should we dispose of all 2000 L we pump daily.

pH remained relatively unstable during the first 20 day period, but this is common in most common start-up reactors where microbial populations are still trying to acclimatise and establish themselves in the new environment. However, pH range was still conducive for the functioning of most of the microorganisms and this was not the primary reason for the lack of biogas.

The high colony forming units of log 4.82 cfu/ml^{-1} exceeded the allowable 1 cfu/ml^{-1} for human safety and confirmed inefficiency of AD under our conditions. The digestate will thus need to undergo further treatment to ensure that it does not pose public health hazards.

AD was efficient regarding waste volume management with regard to changes in TSS and VSS if the reduction were not primarily due to solids settling at the bottom of the reactor. This we could not monitor in the present situation. A major drawback of solids settling at the bottom of the plug flow-reactor is that this could lead to a reduced HRT and possible washout of slow growing methanogenic microorganisms, resulting in low biogas production and trapping water soluble minerals. Using a plug flow-AD did not compromise the nutrient mineral concentrations of the feedstock as reflected in the digestate.

The nitrogen concentrations in the digestate was relatively low compared to optimal nutritional needs of a cash crop such as lettuce, which require 120 mg/l to 240 mg/l of nitrogen (Cuppett *et al*., 1999). The digestate will thus need to undergo further pre-treatment such as denitrification of NH_4^+N to NO_3 in order for the solution to be viable as fertiliser. Care has to be taken regarding the EC for this type of application of the digestate. The relatively low manifestation of the digestate's phytotoxicity effects on tomato seed germination is positive, but will require further studies on other indicator plant species to ensure suitability as possible fertiliser source, should enrichment be possible for certain nutrients.

The assessment of microbial community diversity within the anaerobic reactor elucidated the systems dynamics of microbial population. The data provided vital evidence that, within a recently defined environmental space, there will always competition between microbial communities to colonize environments of which the keystone adaptive species live on to thrive, while other communities cease to exist.

Goux *et al*. (2015) noted that the efficient performance of an anaerobic reactor is largely dependent on dynamic microbial community structures. This study could have benefitted not only from quantifying microbial community diversity, but also from sequencing and identification of important anaerobic microbial species. Such an analyses could have also indicated more specifically how the failure of optimum temperature and low DM feedstock inflow compromised optimum AD. The study thus serves as a foundation on which more research can add to the scientific pool of knowledge through the identification of keystone microbial species in plug flow anaerobic reactors. This can also include the development of microbial inoculum for reactors, which can be used to optimize the efficiency of plug flow reactors for bio-energy production and waste management.

Even though the digestate is relatively diluted due to the low DM loading, the digestate was still not suitable for either direct fertigation or dumping in the river. To comply with the National Water Act 36 of 1998 and avoid environmental pollution or contamination, the present digestate will still have to be treated either by chlorination or the AD operational management improved to function optimally, before it can be used as liquid fertilizer, irrigation of discharged into the environment such as water ecosystem.

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List of tables

Table 3: South Africa's waste water specifications for land irrigation (Republic of South Africa, 2013).

Variable	Limit 50 m³/day	Limit 500 m³/day	Limit 2 000 m³/day
pH	6.0-9.0	6.0-9.0	5.5-9.5
Electrical conductivity (EC)	<2000 µS/cm	<2000 µS/cm	<700 µS/cm
Suspended solids	–	–	< 25 mg/l
Chemical Oxygen Demand (COD)	< 5000 mg/l after removal of algae	< 400 mg/l after removal of algae	< 75 mg/l
Ammonia nitrogen	–	–	< 3 mg/l
Nitrate/ Nitrite	–	–	< 15 mg/l
Phosphorus	–	–	< 10 mg/l
Faecal coliforms	< 1000/100ml	< 1000/100ml	< 1000/100ml

Table 4: Experimental variables under consideration during the treatment.

Parameter	Unit	Frequency	Comment
Temperature	°C	Daily	
Electrical conductivity	µS/cm	Daily	Each Chamber
pH	Units	Daily	Each Chamber
Instantaneous gas production	mbar/hour	Daily	
Biogas composition	%	Every 3rd day	
Total suspended solids	%	Twice	Inlet & Outlet
Volatile suspended solids	%	Twice	Inlet & Outlet
Biogas composition	%	Every 3rd day	
Escherichia coli O157:H7	cfu/ml	Twice	Inlet and Outlet
Microbial population diversity	N/A	Every 3rd day	
Water soluble minerals	mg/L	Twice	Inlet & Outlet
Phytotoxicity	Germination %	Once	Digestate

Table 5: TSS and VSS in dairy manure wastewater (Feedstock) on day 1 (10 July 2015).

			Filtrate	**5ml**		
Replicate	Weight A (mg)	Weight B (mg)	Final (mg)	TSS (g/L)	VSS (g/L)	VSS%
1	38.81	38.83	38.82	2.74	2.06	75%
2	36.10	36.12	36.11	2.50	1.94	78%
3	40.23	40.24	40.23	2.52	1.88	75%
			Mean	2.59	1.96	

Table 6: TSS and VSS in dairy manure wastewater digestate on day 20 (30 July 2015)

			Filtrate	**20 ml**		
Replicate	Weight A (mg)	Weight B (mg)	Final (mg)	TSS (g/L)	VSS (g/L)	VSS %
1	39.72	39.72	39.72	0.43	0.41	96%
2	38.82	38.83	38.82	0.55	0.53	96%
3	43.34	43.35	43.34	0.53	0.51	97%
			Mean	0.50	0.48	

Table 7: Summary of means and multiple comparison test on the phytotoxicity effects of dairy manure digestate on tomato seed germination after 72 hours

Treatment	**Mean Germination**	**Least significance difference**
0%	9.67	b
25%	9.33	ab
50%	9.00	ab
75%	9.33	ab
100%	7.67	a
P -value	0.0361	

Table 8: Anaerobic microbial population diversity in dairy manure wastewater effluent.

Day	Chamber	Species richness
1	1	25
1	2	28
1	3	28
4	1	31
4	2	27
4	3	30
13	1	20
13	2	17
13	3	22
19	1	25
19	2	30
19	3	25

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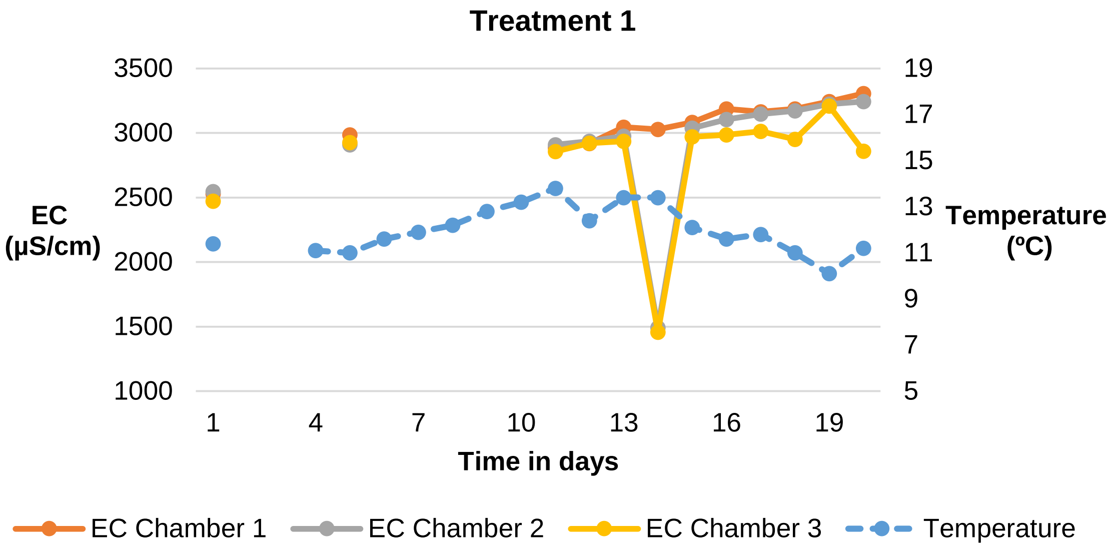

Figure 1: Temperature and electrical conductivity of the reactor digesting dairy manure wastewater over a HRT of 20 days from 10-30 July 2015.

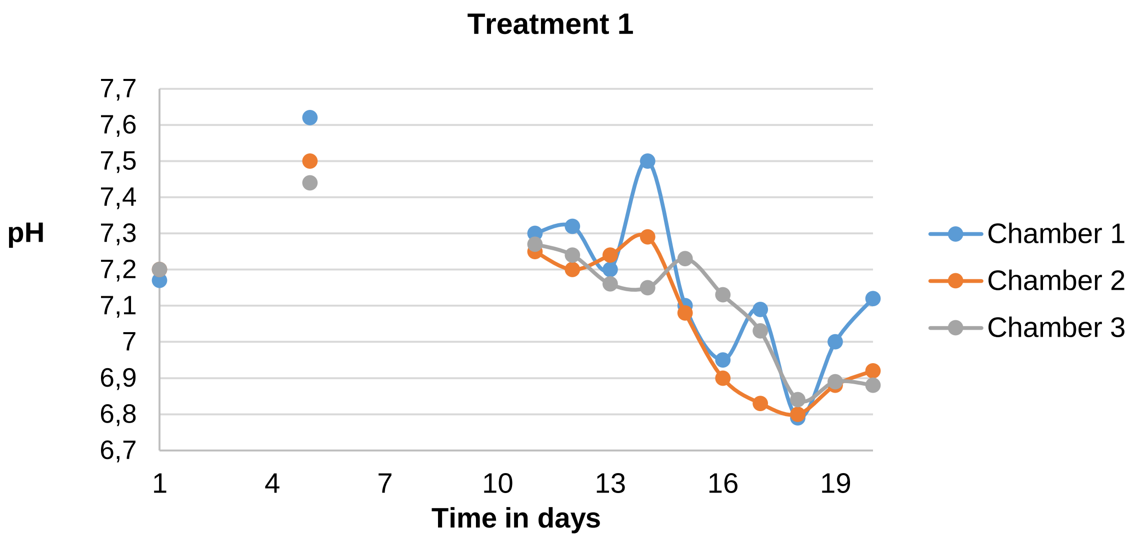

Figure 2: pH values of the reactor digesting dairy manure wastewater over a HRT of 20 days from 10-30 July 2015.

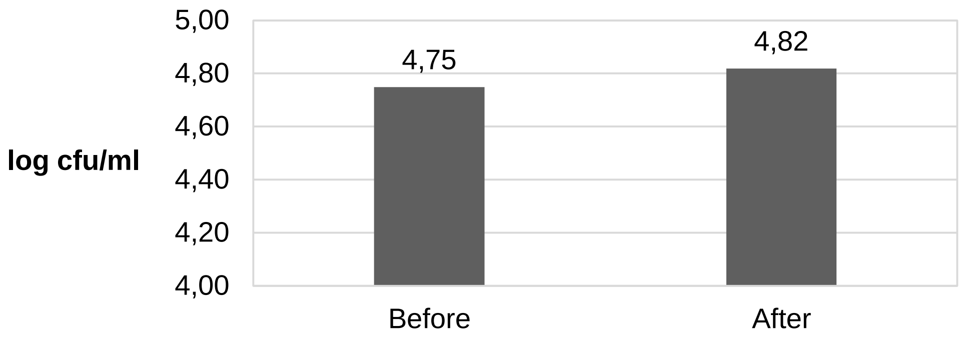

Figure 3: *E.coli* counts in dairy manure wastewater feedstock and digestate after a HRT of 20 days.

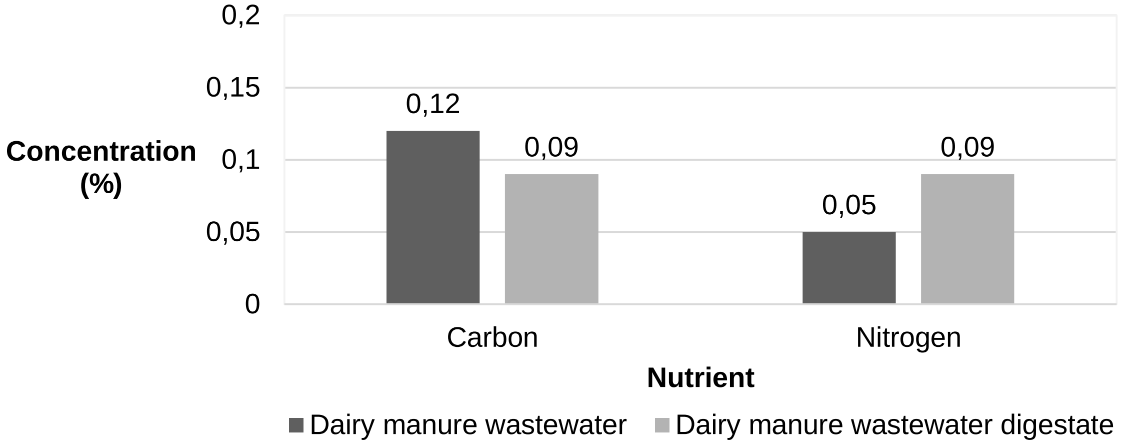

Figure 4: Carbon: Nitrogen content of DM and DM digestate.

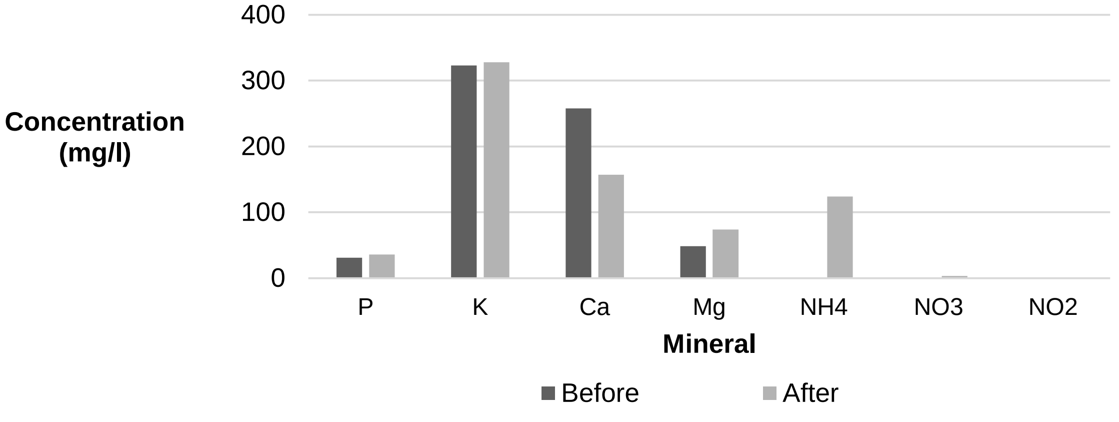

Figure 5: Water soluble macro minerals and nitrogen compounds in dairy manure wastewater and digestate.

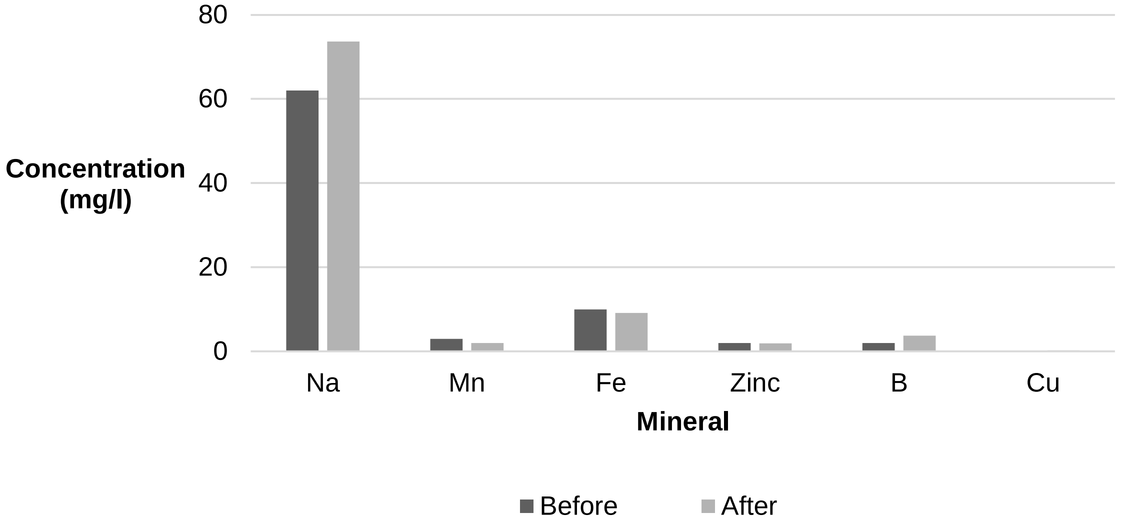

Figure 6: Water soluble micro minerals in dairy manure wastewater and digestate.

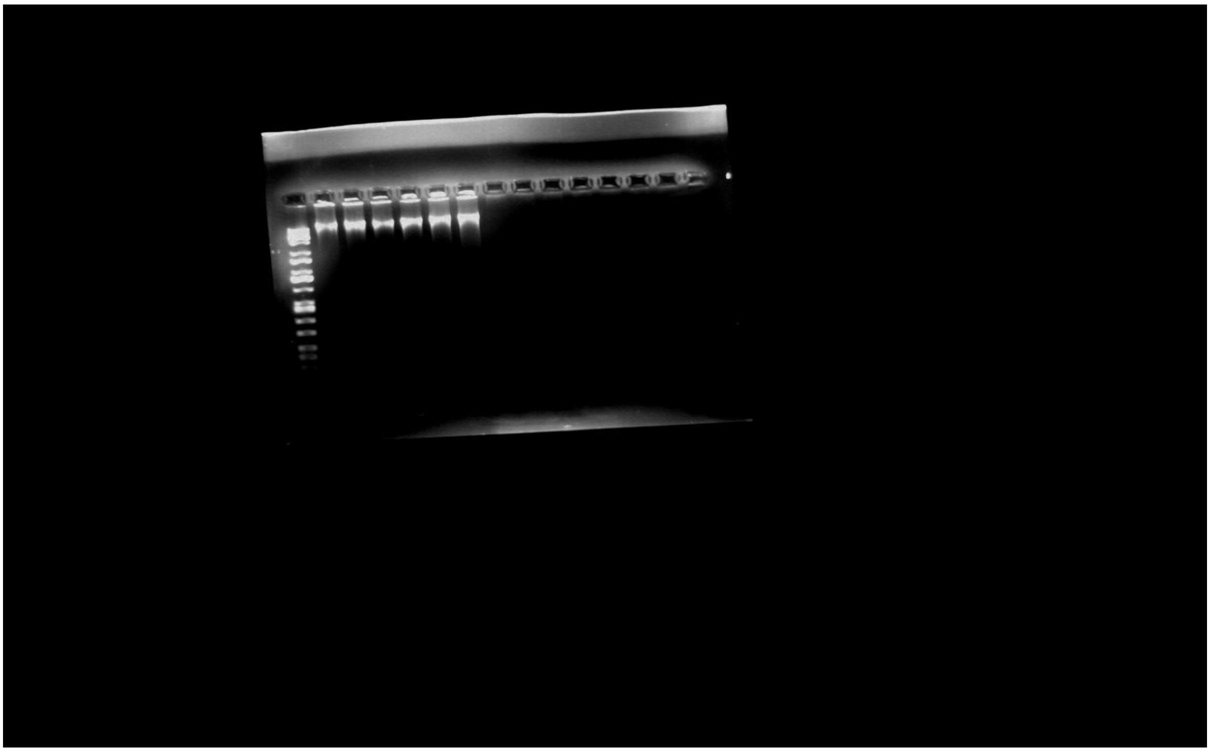

Plate 5: Visualization of microbial DNA after gel electrophoresis.

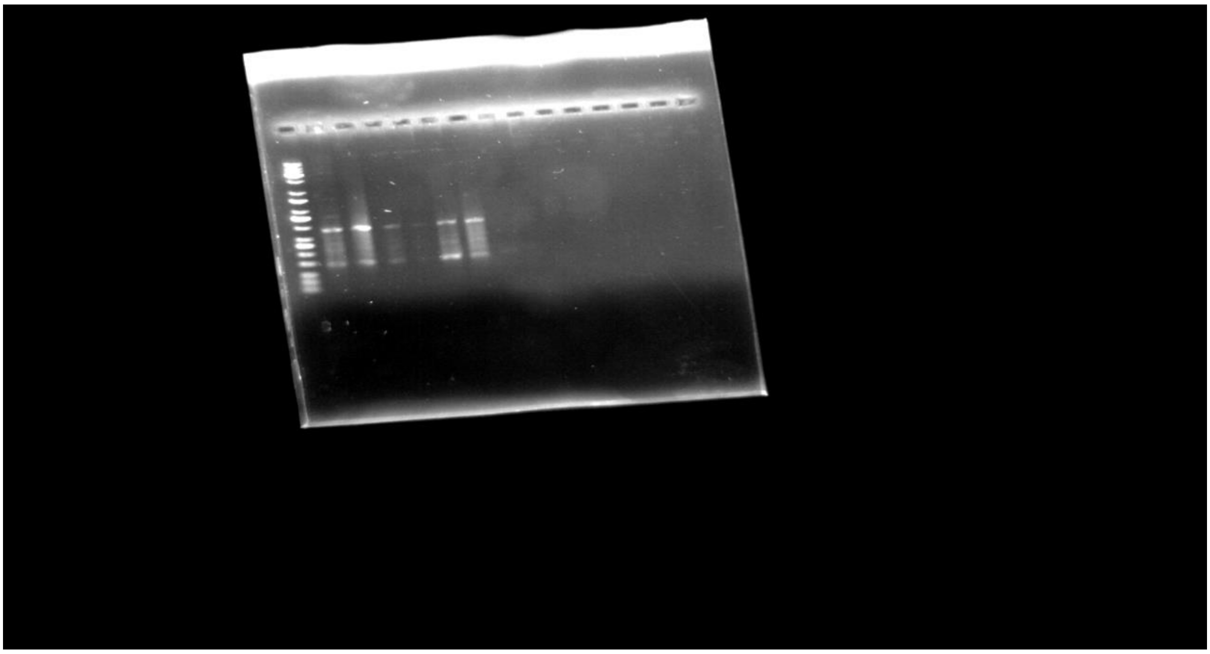

Plate 6: Visualization of microbial DNA amplification after PCR

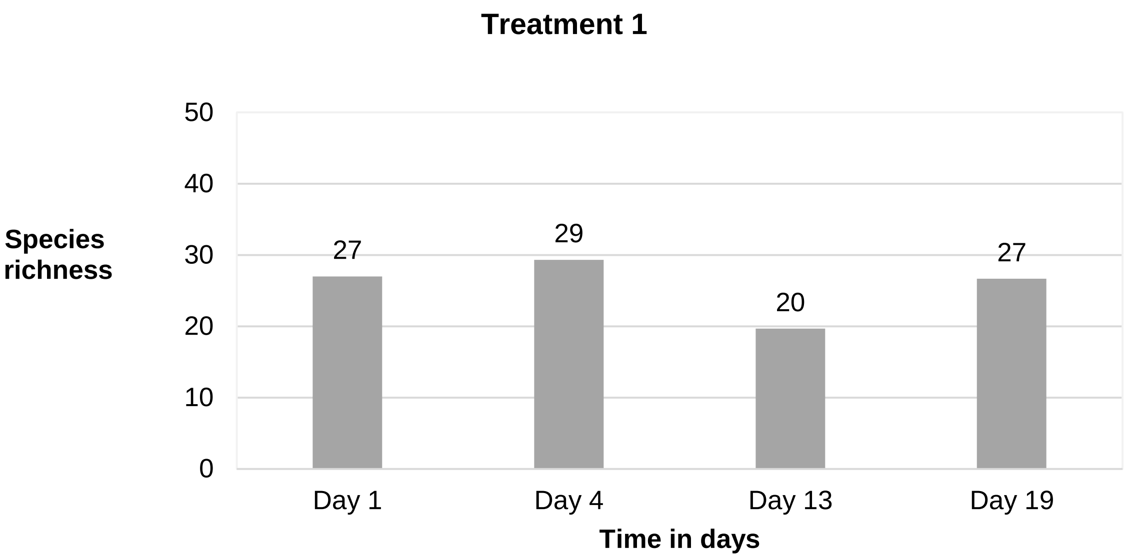

Figure 7: Mean microbial community diversity in reactor treating dairy manure waste water over HRT of 20 days.

Chapter 5

Determining the efficiency and sustainability of an anaerobic reactor to co-digest dairy manure wastewater, fruit and food waste at Welgevallen experimental farm

5.1. Introduction

South Africa ranks as one of the largest producers of citrus in the world. The country exports 85% of its fruit by value, making the fruit industry one of most export orientated (Ross, 2007). Although, the bulk of locally produced fruit is earmarked for the export markets, unpredicted weather conditions, pest and diseases have been implicated in influencing the total volume of production that end up on export market shelves. Oelofse & Nahman (2013) noted that damaged fruit, often of low economic value, become of particular concern and may have contributed approximately 57% of the total food waste composition. This is particular environmental concern especially when the country's carbon footprint from food waste alone is estimated at 4.14 tons of Carbon dioxide (CO_2) per ton, making fruit a significant contributor to greenhouse gas emissions (Oelofse & Nahman, 2013). Although waste, such as fruit and food waste, can be composted and used as fertilizer, vast volumes end up in landfills where it undergoes anaerobic digestion releasing gasses such as methane (CH_4) and CO_2 into the atmosphere.

Conventional waste management practices such as landfilling are increasingly becoming constrained by economic factors such as, scarcity of land near areas of waste production. To overcome some of these constraints and lower the environmental impact of organic waste streams such as food, the South African government through the Department of Environmental Affairs (DEA) has been contemplating phasing-out the practice of municipal landfills in favour of more sustainable waste management alternatives such as recycling, composting and anaerobic digestions (AD) for the production of bio-energy and composting (DEA, 2011).

According to Oelofse & Nahman (2013), South Africa produces an estimated 9.04 million tons of food waste per annum, 89% of which originates from local production. This is comparable to de Lange & Nahman (2015) who estimated food waste in South Africa to be 12.6 million tons. With such large quantities, food waste make investment in alternative waste management technologies such as AD more attractive and ensure that the nutrient cycling system remains closed. In addition, this would also reduce producer's dependence on commercial fertilizer and lower the input costs of food production if the digestate can be used as an alternative source of fertiliser (Yiridoe *et al*., 2009).

Regardless of these characterization and statistics, data on fruit waste in South Africa remains relatively limited. As a result, the majority of studies on the utilization of fruit waste for the production of bio-energy has been limited to waste from agro-processing industries.

5.2. Fruit waste

Attempts by authors such as Parfit *et al*. (2010) to quantify food waste quantities were chiefly driven by the need to assess the social implications of waste in relation to global malnutrition. Waste such as fruit that originate from agricultural production systems add further economic, environmental and social concerns to agriculture which are not easy to quantify. This predicament is further compounded by the relative difficulty to obtain reliable data or models to estimate fruit waste losses at the farm level in order to help inform decision making on sustainable waste management strategies.

The long-term sustainability of anaerobic reactors is largely dependent on the availability and accessibility of biomass as feedstock. However unlike dairy and food waste, adaptation of models and methodologies to quantify deciduous, citrus and sub-tropical fruit waste volumes at the farm gate remain elusive. Fruit waste volumes are influenced by a complexity of factors which are some of the primary reasons why there are seasonal fluctuations fruit waste volumes.

Because of these predicaments, models to quantify fruit waste at the farm gate and across the fruit industry have the potential to become increasingly tedious, costly and may not yield statistically representative data. Models such as the economic input-output of Gay *et al.* (1993)

are more applicable at estimating fruit waste losses along the value chain, but not the farm gate. Survey sampling methods or creation of a data logging platform on which producers can record fruit waste may yield more credible data and help inform waste management at primary production.

Khan *et al*. (2015) noted that fruit processing, which is greatly dependent on output from agricultural systems, generates large quantities of waste both in solid and liquid forms. Van Dyk (2013) estimated that between 25-35% of processed apples on a dry matter basis, 50% of citrus and 20% of grapes fruit eventually end up as waste. In addition, fruit processing generates vast volumes of wastewater that require pre-treated in order to comply and meet specific safety standards before it can be discharged into rivers or used for crop irrigation. Anaerobic digestion thus offers an excellent opportunity for the management of organic biomass waste such as fruit.

According to Burton *et al*. (2013) as cited by Khan *et al*. (2015), to extract the maximum value out of liquid waste, the addition of solid waste is essential, especially if the waste is to undergo anaerobic digestion for the generation of bio-energy. In addition, Burton *et al*. (2013) found that waste waters from fruit processing such as canning, juicing, fruit drying or vinification have great potential in wastewater treatment for the production of renewable energy. This is largely attributed to their higher concentration of soluble carbohydrates such as glucose, fructose and sucrose (Cruz-Cardenas *et al*., 2015). However, literature on the production of renewable energy from cannery waste water remains limited. Although, Sigge & Britz (2007) successfully operated a laboratory scale up-flow anaerobic sludge blanket reactor to investigate these issues, the reactor failed as a result of high saline concentrations in the wastewater and no results were reported.

Mineral salts such as Sodium (Na^+) and Nitrate compounds are commonly used in the food processing industry to either preserve, improve aesthetic value or enhance flavour of processed food. Low Sodium concentrations have been reported to be vital in the probable formation of adenosine triphosphate (ATP), oxidation of nicotinamide adenine dinucleotide (NADH) (Feijoo *et al*. 1995) and maintainace of cell osmotic pressure in AD (Rinzema *et al*., 1988). However, high concentrations between 10 000-25 000 mg l^{-1} have been documented to cause some level of inhibition in anaerobic microbial populations (Feijoo *et al*., 1995). Feijoo *et al*. (1995) further highlighted the importance of these mineral salts and how the absence of salts such as, sodium chlorine can result in a 50% reduced oxidation of volatile fatty acids. However, Sigge & Britz (2007) added that methanogens are particularity sensitive to salt accumulation, after observing that biogas quantity and quality were among the first parameters to change.

Despite, the undesirable salt concentrations in processed fruit and its waste waters, unadulterated fruit such as apples have been considered to be a good source of dietary mineral salts. Violeta *et al*. (2010) noted that apples had higher concentrations of phosphorus (P) and potassium (K). Where vast volumes of waste are produced, these minerals, if recovered have the potential to be re-utilized and provide important nutrients needed to sustain plant growth and productivity (Tiwary *et al*., 2015).

According to Khan *et al*. (2015), South Africa produces sufficient quantities of fruit waste to justify investments in bio-energy technologies. In order for fruit waste to be considered an economically feasible feedstock in the production of bio-energy, there should be sufficient quantities and that seasonal fluctuations are an important factor to consider. In addition, fruit waste should have a substantial value-addition potential to outcompete current alternative uses. Current uses of fruit waste are not well documented in literature despite the economic potential that can be derived from fruit waste. Nonetheless, composting and feeding fruit waste to livestock remain by far the largest alternative value addition practices for fruit waste at the farm level.

Before fruit waste can be used in an AD, it remains critical to determine fruit waste physical and chemical compositions. This includes measures such as carbon: nitrogen ratios, dry matter and ash analysis. These are particularly critical in calculating feedstock ratios, maintain a vigorous anaerobic microbial population and prevent process failures. Violeta *et al*. (2010) analysed the dry matter of several apple cultivars and reported an average dry matter content of 15.5% for most, however, cultivars such as ‘Prima’ and ‘Red Boskoop’ had dry matter contents 12.49% and 20.09% respectively.

According to Chagné (2014) the interaction of genotype and the environment can influence the qualities of fruit considerably. Phenotypic traits such as fruit maturation, firmness and dry matter content are important fruit quality traits. Traits such as dry matter have been documented to be significant determinants of fruit quality related to taste such as sugar and acid metabolism (Chagné, 2014). In addition Violeta *et al*. (2010) noted that soluble solids can be used as an indicator of sugar content also referred to as soluble solids in apples and that soluble solids in apple cultivars varied between 10.8% and 16.5% for cultivars such as ‘Prima’ and ‘Red Boskoop’.

While phenotypic traits such as apple colour has been found to be influenced by temperature (Lin-Wang *et al*. 2011), changes in environmental conditions have been reported to influence quality of fruit such as apples (Sugiura *et al*., 2013). This points to the need to consider the influence of environmental conditions on fruit waste that ends up as feedstock for an anaerobic reactor.

5.3. Food waste

Nahman & de Lange (2013) highlighted that the narrative of food waste may vary extensively, such that in the context of their study, the researchers defined food waste to be a loss of edible and inedible food fractions before it reaches the consumer and that which is discarded by the end consumer. According to Gustavsson *et al*. (2011), per capita food waste in sub-Saharan Africa amounts to 170 kg per person on an annual basis. Although this data can be spatially variable from one region to another, the importance of food waste is of significant interest especially in a region where most countries are net food importers with widespread levels of food insecurity and malnutrition.

According to Oelofse & Nahman (2013), per capita food waste in South Africa were 177 kg per annum in 2007, which is comparable to the 170 kg estimated by (Gustavsson *et al*. 2011) for Sub-Saharan Africa. Oelofse & Nahman (2013) however, expected a significantly higher per capita food waste figure bearing in mind that South Africa has a relatively higher gross income per capita (GDP) compared to other Sub-Sahara African countries.

At a household level, much of the food waste is mixed in with other non-organic material, which makes it difficult to manage. This predicament remains the leading reason as to why large quantities food waste are eventually discarded in landfills as the most economic waste management alternative for municipalities. Trapped in the landfill, the organic matter decays anaerobically to release harmful greenhouse gases, saline seepage, while also acting as repositories of pest and diseases. In a landfill, food waste represent a waste of scares resources such as fossil fuels, labour, fertilizer and scarce water that went into the production, processing, distribution and marketing of food. Nahman & de Lange (2013) estimated that the cost of food waste across the value chain until it reaches the landfill to be R 61.5 billion, equivalent to 2.1% of the country's GDP in 2011.

Despite several alternatives of managing food waste in a sustainable way, anaerobic digestion for the production of renewable bioenergy and nutrient rich digestate remains one of the viable alternatives. This is despite food waste bringing a whole new dimension and challenges to anaerobic digestion, because of its high variability and inconsistency. Comparable to processed fruit waste, food waste has the potential to contain high levels of soluble mineral salts and food additives that can interfere with anaerobic microbes from acclimatising well within the anaerobic environment by slowing down growth (Chen *et al*., 2008). The addition of food waste to a stable anaerobic reactor needs to be judicious and well calculated to avoid process failures that can result in a significant amount of microbial biomass loss in keystone species.

Assuming that the physical and chemical properties of food waste was consistent in South Africa, the 12.6 million tons of food waste generated per annum, as estimated by de Lange & Nahman (2015), have the potential to lock in 4.58 million tons of carbon if send to a landfill. In addition, this aggregated food waste in a landfill would trap approximately 124 000 tons of N, 57 000 tons of P and 135 000 tons of K from food production systems, which signifies a colossal economic, environmental and social cost.

Anaerobic co-digestion of dairy wastewater, fruit and food waste on semi-commercial scale, using a plug flow-reactor has not been quantified under local conditions regarding efficiency and suitability as sustainable alternative to the management of agricultural waste. Therefore, this chapter's objective is to determine the efficiency and sustainability of this anaerobic reactor to co-digest dairy manure, food and fruit waste substrates. This will be quantified addressing various variables such as temperature, EC, pH, Escherichia *coli*, water soluble minerals, phytotoxicity and microbial population dynamics.

5.4. Material and methods

5.4.1. Fruit (apple) waste moisture content

Five Golden Delicious (*Malus domestica*) apples were randomly sampled from a harvested lug. The apples were cut into four quadrants number accordingly from 1 to 4 and each sub sample weighed. The moisture content of each sub sample was then determined by drying samples in a 60 °C oven for 72 hours, after which they were weighed to determine the moisture loss. Dried samples were then grounded in a blender, after which a specified weight of approximately 2 grams (g) per sub sample was added to individual crucibles and dried in an incubator at 100 °C for 24 hours. Thereafter the crucibles were weighed again to obtain the final moisture content, before the samples were ashed in a 550 °C furnace to determine volatile solids (dry matter – ash content) of the waste according to Palmer *et al.* (2010).

5.4.2. Food waste moisture content

Food waste obtained from the University of Stellenbosch student resident catering was sampled from individual drums at Welgevallen experimental farm. After inspecting several drums, five with representative food waste mixtures were chosen compared to drums containing solely bread, pancakes or vegetable waste. Food waste in the selected drums was thoroughly mixed to obtain a more uniform composition upon, which sub samples of food waste/drum were obtained to generate one composite sample for analysis. This sample was dried at 60 °C in an oven for 48 hours, weighed and milled. After milling, 2.5 grams of food waste was weighed into crucibles before these were incubated at 100 °C for 48 hours. After the 48 hours, the crucibles were removed from the incubator, cooled in a desiccator for two hours before each crucible was individually weighed to determine the moisture content. Finally, these samples were ashed to determine the volatile solids.

5.4.3. Fruit and Food waste mineral content

Mineral and nutrient analysis of fruit and food waste were performed by a commercial laboratory (Bemlab (Pty) Ltd, Strand) at the beginning of each trial.

5.4.4. Treatments

Treatments were executed consecutively due to the AD limitations as semi-commercial plug flow-reactor that only allowed one treatment at a given time. The second treatment dairy manure wastewater and fruit waste (DF) commenced on the 11th and ended on the 30th of August 2015. To maintain a HRT of 20 days and an OLR similar to that of the preceding dairy manure wastewater of 5.078 kg/day of TSS or 3.859 kg/day VSS. Fruit waste and food waste both have a VS content of 96% compared to 76% VS of dairy manure wastewater.

To achieve a 50% co-digestion of fruit waste alongside dairy manure wastewater would require 2.43 kg/day of fruit waste and 2. 54 kg/day of food waste, with a combine OLR of 4.97 kg/day. The average VS solid content of the co-digested feedstock would thus be 86%, which is relatively higher than the 76% for dairy manure waste water only. Before feedstock was added to the reactor to be co-digested alongside dairy manure waste water, it was reduced to a smooth paste and mixed with 0.35 m^3 of dam water that had an EC content of 9.4 µS/cm. In addition, this was done to maintain an organic retention time of 0.096 (OLR = Volatile solids / Total volume of the reactor).

All variables including temperature, EC, pH, pathogenic *E.coli* enumeration, phytotoxicity, DNA extraction and PCR were analysed using similar methodologies in the previous chapter and protocols in the annexure.

5.5. Results

5.5.1. Physical and chemical analysis of fruit and food waste

Fruit waste had a mean moisture content of 83% (Table 9) and a high volatile solids (VS) concentration of 95% (Table 10). This makes fruit waste an ideal feedstock for anaerobic co-digestion with dairy manure wastewater, because of its exceptionally high carbon (51.3%) and low nitrogen (0.42%) content. Food waste had an average moisture content of 72% (Table 12), which is lower in comparison to fruit waste. In addition, food waste also had a lower C: N percentage of 36:1 than fruit waste, which had a C: N percentage of 51: 0.4. Nonetheless, food waste had a high VS content of 96% (Table 13), which is similar to that of fruit waste. Mineral nutrient concentrations of fruit and food waste are summarised in (Table 11 and 14). A comparison between the mineral concentrations of the food and fruit sources shows that food waste is notably richer in most minerals, which makes it an excellent feedstock for co-digestion.

5.5.2. Temperature and Electrical Conductivity

From the onset of the second treatment (11-30 August 2015), temperature was on an increasing trend (Figure 8), although this increase was confined within a 5 °C range (11-16 °C), but marginally higher than the 10-14 °C observed during the first treatement (Figure 1) and could have been largely due to the increase in ambient temperature with the progression of the season towards spring. Although, temperature continued to increase, EC displayed a decreasing trend (Figure 8), which is contrary to the proposed linear relationship between temperature and EC Hayashi (2004). EC in all three chambers followed a similar trend from day 1 to 9, before a noticeable decline was observed in Chamber 1 only from day 10 until day 13, where after an increase followed. The data appear to indicate some level of microbial activity in chambers 2 and 3 whereby solid molecules are digested into soluble molecules to yield high concentrations of short-chain fatty acids, which subsequently results in a higher EC (Goberna *et al*., 2009). Despite, a decreasing trend in EC, it remained relatively higher in comparison to the first treatment when only dairy manure wastewater was fed into the reactor. This high EC could also have resulted from overfeeding the reactor with fruit waste on day 1 of the experiment.

During the 3rd (Figure 9) and 4th (Figure 10) treatments, psychrophilic temperatures between 14-18 °C were observed. Although, slightly higher than temperatures observed during treatments 1 and 2, the increase in temperature did not induce an increase in EC. These findings contradict Hayashi (2004) linear relationship between temperature and EC. Interestingly, EC stabilized within the (3000-3300 µS/cm) range, which according to NRCS (1999) remained above the 2000 µS/cm threshold needed if the wastewater is to be used for irrigation, but did not exceed the toxicity threshold of 4000 µS/cm. These observations were comparable to those documented for all three feedstock when fed in a ratio of 2:1:1 dairy manure wastewater, fruit and food waste. Despite temperature-EC observations contradicting the linear relationship argued by Hayashi (2004) the findings compare with the author's counter statement that EC of natural water also has a tendency to be non-linear within a temperature range of 0-30 ºC.

5.5.3. pH

After overfeeding the reactor with fruit waste equivalent to the feeding requirements of 10 days on day 1, a sudden and drastic decline in pH was observed on day 2 (Figure 11). At pH 6.06 in chamber 1 and 5.89 in chamber 3, pH had dropped below the preferred optimal range (6.8-7.2) for optimal microbial growth. To prevent further decline in pH and avoid possible process failure, feeding of fruit waste was suspended over a time period of 10 days. This emergency strategy was adopted from recommendations by Fricke *et al*. (2007) who noted that there are various emergency alternatives to reverse a rapid decline in pH, which includes ceasing feeding the reactor or addition of acid buffers.

Kim *et al*. (2002) stated that process failures are more likely to occur if pH drops below 5.5. Despite the rapid decline, pH had not dropped below this value and it started increasing again although not into the optimal microbial growth range. The pH in chambers 2 and 3 were to a large extend influenced by advances in chamber 1, which was expected to be colonized by acid producing bacteria. Throughout this experiment, chamber 3 constantly showed the lowest pH. Assuming that chamber 3 would be colonized by methanogens, these low pH observations were not optimal for methanogens to thrive as they preferred a more neutral pH around 7.2

During the third treatment (Figure 12), pH continued to decline although, at a much slower pace. This is despite anticipating that the addition of more acidic food waste (pH = 4.1) would cause a decline in the pH. Food waste from the residences is treated with 'Bokashi which contains lactic acid forming bacteria in order to preserve and prevent pathogenic microorganism from thriving in the food waste. However, the co-digestion of food waste did not seem to influence pH due to conservatively low organic loading rate of 1.36 kg/m^3 fed into the reactor on a daily basis. In addition, the stabile pH could have resulted from an increase of buffering capacity from the digestion of nitrogen compounds to yield ammonium. Fricke *et al*. (2007) noted that ammonium concentrations at 1000 mg/l play an important role by stabilizing pH, but this was not reached in this experiment yet.

After 60 days operating at full capacity, the reactor's pH finally stabilised (Figure 13). For the very first time, pH observation in chamber 3 were higher than chamber 1 and 2 - from day 13 until the end of the experiment, on day 20. The pH in chamber 1 also stabilized within the preferred hydrolytic bacteria range of 6.2. It can thus be assumed that, during this time period, the biomass of methanogenic microbial population was adequate to sufficiently digest VFA's. The relatively higher reactor temperature could have contributed to the possible balance of microbial biomass population in the reactor. Despite, the higher reactor temperatures recorded during this experiment, it still remained within the psychrophilic range (< 20 °C) with little to no detectable biogas volumes, which made it impossible to conduct biogas quality tests.

5.5.4. Total solids reductions

The co-digestates of both fruit and food waste had mean TSS of 98% (Table 21) reduction which could indicate that the reactor was efficient enough to effectively reduce the organic solid content of feedstock. However, temperature was not ideal during these treatments such that these results could have been largely influenced by the sedimentation of solids at the bottom of the reactor.

5.5.5. *Escherichia coli* reduction in digestates

The undigested Fruit and food waste feedstock had comparable concentrations of colony forming units per millilitre (log cfu/ml) (Figure 14). This was despite anticipating a lower cfu/ml count in food waste, which was preserved with bokashi, while fruit waste was stored in emptied food waste drums that could have been colonized by pathogenic microorganisms.

Anaerobic digestion did not have a noticeable reduction of pathogenic microorganisms (Figure 15) as it remained relatively constant throughout the three co-digestion treatments. However, there was a small decline in *E.coli* colonies from log 4.82 cfu/ml to log 3.71 cfu/ml, when fruit waste was co-digested alongside dairy manure wastewater,. Nonetheless, the count remained higher than the log 3 cfu/ 100 ml legal limit for irrigation water or discharge in the environment. Côté *et al*. (2006) noted that temperature and HRT remain two of the most decisive factors that will determine the efficiency of a reactor to reduce indicator pathogenic microorganism such as *E.coli*. The relatively high counts of cfu/ml contradicts Côté *et al*. (2006) that indicated AD potential to reduce 97-100% of *E.coli* after 20 days from a batch scale anaerobic reactor. In addition, Kumar *et al*. (1999) observed that *E.coli* survived for 25 days in an anaerobic reactor at room temperatures between 18-25 °C. However, both these studies utilized sequence batch reactors and not plug-flow reactors, which could have contributed to the positive results.

5.5.6. Water soluble minerals

Co-digestion of dairy manure wastewater and of fruit waste did have a noticeable influence on the concentration of water soluble minerals present in the digestate (Tables 15 and 16). This is evident in the higher concentrations of minerals in both dairy manure wastewater- fruit waste digestates and dairy manure wastewater, fruit and food waste. Although food waste contained higher concentrations of P, K, Ca, Mg, Na, Mn, Fe, Cu and Zn than fruit waste. The digestates of dairy manure wastewater and food waste contained lower concentrations of water soluble minerals compared to dairy manure wastewater and fruit.

These results were not anticipated, especially when EC of the two effluents followed a similar trend. However, this could have been influenced by the high fat content observed in the food waste. Long chain fatty acids have been found to be inhibitory towards microorganisms at low concentration by attaching to the cell walls/membrane of gram-positive bacteria (Kabara *et al*., 1977; Rinzema *et al*., 1994). This possibly interfered with the microbes' ability to break down and digest solid organic molecules in order to release water soluble nutrients and minerals.

Despite the stark decline in water soluble mineral concentrations due to the addition of food waste in both treatment 3 and 4, calcium (Ca) and potassium (K) concentrations persisted to be high in these digestates (Tables 15 and 16). This is despite the relatively low Ca concentrations in both fruit (0.04%) and food waste (0.19%) feedstocks. These high Ca concentrations could only originated from the dairy manure wastewater (Figure 5) as Ca remains an essential postpartum nutrient in dairy cows' diets and is thus added to feed during formulation (Horst, 1986). Calcium concentrations in the digestate were below the 8000 mg/l reported by Parkin & Owen (1986) that can cause anaerobic microbial inhibition.

5.5.7. Phytotoxicity

After the seed germination data from a CRD, 5 treatments and 3 replications were subjected to SAS 9.3, the co-digestate of dairy manure wastewater and fruit waste (p value = 0.1318) does not seem to have any phytotoxicity effects as on the tomato seed germination (Table 17). The digestate can thus be used as an alternative source of liquid fertilizer. However, under soilless growing conditions such as hydroponics the NH_{4+} will have to be converted to nitrate, because tomatoes can be highly sensitive to NH_{4+} concentrations (Liedl *et al*., 2006).

The co-digestion of the three feedstock (p value = 0.0096) seemingly had a phytotoxic effect on the germination of tomato seeds. Subsequent pairwise comparison test revealed that the 100% concentration seem to have had a greater effect on seed germination than the other concentrations.

After treatment 3, there was not sufficient statistical evidence (p value = 0.08) to show that the dairy manure wastewater and food waste digestates had a significant effect on tomato seed germination. Although two of the digestates at 100% concentration were not found to exhibit any phytotoxicity towards the germination of tomato seeds, supplementary research would be crucial to validate this and ensure that the digestate is safe to use on actively growing plants.

5.5.8. Anaerobic microbial population dynamics

Treatment 2: Microbial community diversity in dairy manure wastewater and fruit waste effluent

After overfeeding the reactor with apple fruit waste on the 1st day, there was an increase in microbial diversity from day 1 to day 7 (Table 18 and Figure 16). However, after ten days, a decline in population diversity followed, which can be observed on day 13 after which the population remained stable. These observations are comparable with those presented in treatment 1. It is possible that microbial species, especially hydrolytic bacteria, responded positively to feeding as reflected by the increase in microbial diversity in chamber 1 (18 to 33 species), 7 days before the other species acclimatised to the new feedstock. It was also during days 1 - 7 where there was a noticeable decline in pH, with the reactor only recovering from day 9 to 15.

Treatment 3: Microbial community diversity in dairy manure wastewater, fruit and food waste effluent

At the commencement of the third treatment, the reactor had been in operation for more than 40 days. A relative decline in the microbial species diversity can be observed from 29 species that were observed in the last day of the second treatment to 20 species on day 1 of the third treatment, only two days after the second treatment ceased (Table 19 and Figure 17).Nonetheless, during the following days, the microbial community structure increased to stabilised between day 13 and 19. This may have been due largely to the stable pH in the acidic range 5.8- 6.6 (Figure 12) during this period. Taking cognisance of the fact that feedstock fed into the reactor on a daily basis is derived from an aerobic environment, the potential to introduce new aerobic and anaerobic microbial species into the reactor remains high. However, the stable microbial communities during this period (days 13 to 19) could have been largely composed of keystone species that were competitive enough to ensure that other species do not get an opportunity to establish themselves.

Treatment 4: Microbial community diversity in dairy manure wastewater and food waste digestate

The microbial community diversity of the dairy manure wastewater and food waste treatment (Table 20 and Figure 18) had a similar trend in comparison to the preceding treatment to that of the co-digestion of dairy manure wastewater, fruit and food waste. During the remaining period, the community structure remained fairly stable especially from day 7 to day 19 with a diversity between 32 and 33 species. Consequently, the reactors temperature was higher than in the preceding treatments and continued to rise, whereas pH in chamber 1, which is presumably colonized by hydrolytic bacteria, decline to 6.2. Consequently, the pH in chamber 3 increased consistently above that of chamber 1 and 2, which indicates that the microbial community structure in chamber 3 could be methanogens as they prefer a higher pH than hydrolytic bacteria.

5.6. Conclusion

As concluded in chapter 4, variables such as temperature, pH and organic loading rates were confirmed to be important parameters for optimal reactor performance. Temperature is particularly important for the reduction and elimination of pathogenic microorganisms such as *E.coli*, which continued to persist at unacceptably high concentrations in digestates from all treatments. This implies that the temperature inside the reactor needs to increase to eradicate *E.coli*. This is particularly important considering that continuous feeding of the reactor introduce new load of pathogenic microorganisms on a daily basis.

Unlike in the first experiment with only dairy manure wastewater as feedstock, pH remained acidic (below 6.8) with the co-digestion treatments. This could have partly been the result from overfeeding the reactor with fruit waste at the start of experiment two, which could have resulted in fast growing hydrolytic and acidogenic bacteria, colonizing the reactor. Hydrolytic and acidogenic bacteria species break down solids in feedstock to yield VFA's responsible for lowering pH.

Under psychrophilic temperature, the OLR seem to have a noticeable influence on the anaerobic microbial community dynamics, such that when the reactor was overfed with fruit waste, a decrease in the microbial community species from 27 species (Figure. 7) at the end of treatment 1 to 23 species (Figure 16) at the commencement of treatment 2. During the same period, pH declined to levels where process failure could occur. However, after feeding was ceased, the possible number of keystone microbial species increased as the reactor stabilised.

The co-digestion of dairy manure wastewater and fruit waste influenced the concentration of water soluble mineral nutrients positively by increasing the concentration of these elements in the digestate above the concentrations derived from using only dairy manure wastewater. Findings from the study suggest that, even under sub-optimal experimental conditions, fruit waste has the potential to be an excellent feedstock option for co-digestion alongside dairy manure wastewater.

In addition, the digestate from the co-digestion of dairy manure wastewater, even at 100% concentration did not show any phytotoxicity effects on the germination of tomato seeds, in contrast with phytotoxicity observed with germination using dairy manure digestate only in chapter 4. However, the digestate from co-digesting dairy manure wastewater, fruit and food waste exhibited some degree of phytotoxicity at 100 % concentration. This digestate therefore needs to be partially diluted before it can be used as an alternative source of liquid fertilizer. Although the AD system used in this study remained under suboptimal, the findings from this study seem to suggest that AD has the potential to become a sustainable alternative to organic waste management under optimal conditions.

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Table 9: Proximate analysis of fruit waste at 60˚C for 48 hours performed on 4 quadrants of 5 fruit.

Quarter	Sample weight (g)	Dry Matter weight (g)	Moisture (g)	Moisture (%)
1^{st}	205.21	33.80	171.41	84
2^{nd}	231.68	38.81	192.79	83
3^{rd}	206.28	35.55	170.73	83
4^{th}	212.96	36.09	176.87	83
Mean				83

Table 10: Dry matter, moisture content of fruit waste at 100 ˚C, including ash and volatile solids content.

Quarter	Sample weight (g)	Dry Matter (g)	Moisture %	Ash (g)	Volatiles (g)	Volatiles
1^{st}	2.00	1.81	9%	0.08	1.73	96%
2^{nd}	2.00	1.82	9%	0.06	1.75	96%
3^{rd}	2.00	1.82	9%	0.10	1.72	95%
4^{th}	2.00	1.82	9%	0.09	1.74	95%

Table 11: Nutrient and mineral analysis of fruit waste.

Element	**C**	**N**	**P**	**K**	**Ca**	**Mg**
Unit:%	51.3	0.42	0.05	0.72	0.04	0.04
Element	**Na**	**Mn**	**Cu**	**Fe**	**Zn**	**B**
Unit: mg/kg	169	21	3	3	13	49

Table 12: Dry matter and moisture content of food waste moisture at 60 ˚C after 48 hours.

Sample	Sample weight (g)	Dry Matter (g)	Moisture (g)	Moisture (%)
1	509.57	136.70	372.87	73
2	425.50	121.95	303.54	71
Mean				**72**

Table 13: Dry matter, moisture content of food waste at 100 ˚C, including ash and volatile solids content

Sample number	**Sample weight**	**Dry Matter (g)**	**Moisture (%)**	**Ash (g)**	**Volatile solids (g)**	**Volatile solids (%)**
1	2.50	2.34	7%	0.10	2.24	96%
2	2.50	2.34	6%	0.10	2.24	96%
3	2.50	2.33	7%	0.10	2.24	96%
4	2.50	2.34	7%	0.10	2.24	96%
5	2.50	2.34	7%	0.10	2.24	96%

Table 14: Nutrient and mineral analysis of food waste.

Element	**C**	**N**	**P**	**K**	**Ca**	**Mg**
Unit: %	36.36	0.98	0.45	1.07	0.19	0.14
Element	**Na**	**Mn**	**Cu**	**Fe**	**Zn**	**B**
Unit: mg/kg	8629	32	4	156	28	5

Table 15: Concentration (mg/l) of macro-minerals and nitrogen compounds of the three co-digestates.

Digestate	**P**	**K**	**Ca**	**Mg**	**NO_2-N**	**NO_3-N**	**NH_4-N**
DF	53.03	211.24	490.62	70.51	1.25	1.76	182.44
DFF	45.61	276.45	199.19	63.01	0.97	0.36	89.92
DFd	34.09	88.86	51.97	14.81	0.82	0.32	84.44

Table 16: Concentration (mg/l) of micro minerals in the three co-digestates.

Digestate	**Na**	**Mn**	**Cu**	**Fe**	**Zn**	**B**
DF	53	4	0	17	4	3
DFF	62	1	1	11	2	2
DFd	14	0	0	2	0	1

Table 17: Phytotoxicity effects of digestates from four different feedstocks on the germination percentage of tomato seeds after 72 hours.

Treatment	DM	DFd	DF	DFF
	% germination			
Control (water)	97 a	87 ns	93 ns	93 a
25% digestate	93 a	90	93	97 a
50% digestate	90 a	97	80	97 a
75% digestate	93 a	87	93	90 a
100% digestate	77 b	83	93	73 b
P >0.05	0.0361	0.0800	0.1318	0.0096

Table 18: Anaerobic microbial population diversity in dairy manure wastewater and fruit waste digestate.

Day	**Chamber**	**Species richness**
1	1	18
1	2	26
1	3	26
7	1	33
7	2	36
7	3	47
13	1	27
13	2	28
13	3	30
19	1	27
19	2	34
19	3	26

Table 19: Anaerobic microbial population diversity in dairy manure wastewater, fruit and food waste effluent.

Day	Chamber	Species richness
1	1	18
1	2	24
1	3	18
7	1	27
7	2	22
7	3	24
15	1	27
15	2	21
15	3	20
18	1	17
18	2	23
18	3	25

Table 20: Anaerobic microbial population diversity in dairy manure wastewater and food waste effluent.

Day	Chamber	Species richness
1	1	26
1	2	26
1	3	30
7	1	31
7	2	32
7	3	33
13	1	30
13	2	34
19	1	35
19	3	30

Table 21: Total solids concentration of the three co-digestates

Digestate	TSS (g/l)	VSS (g/l)	TSS Reduction
DF	0.61	0.59	97%
DFF	0.55	0.54	98%
DFd	0.54	0.52	97%

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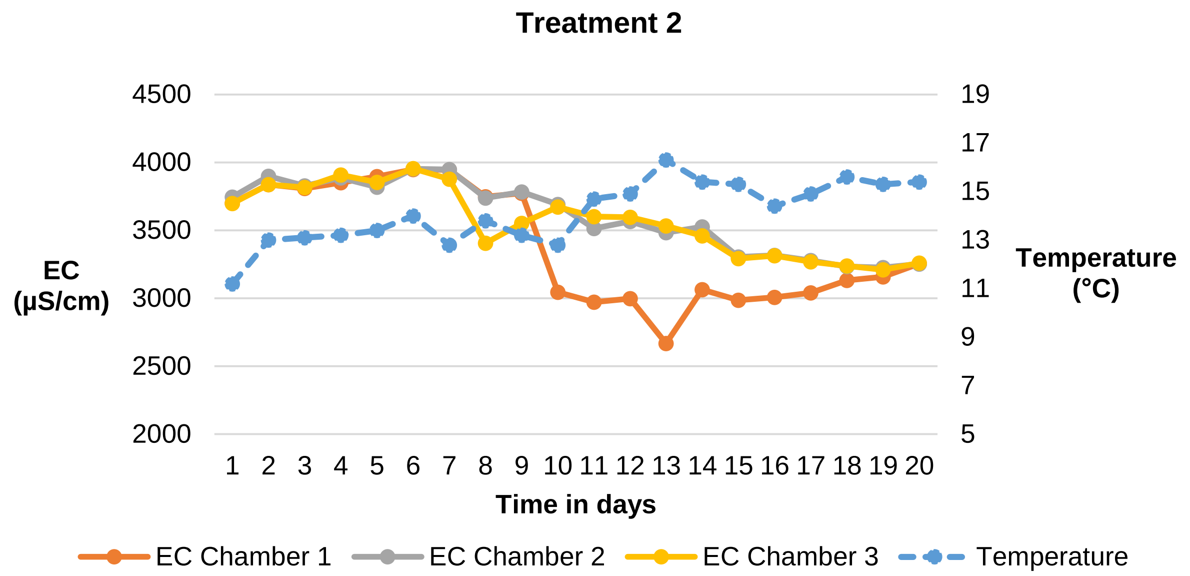

Figure 8: Temperature and EC of reactor co-digesting dairy manure waste water and fruit waste (11-30 August 2015).

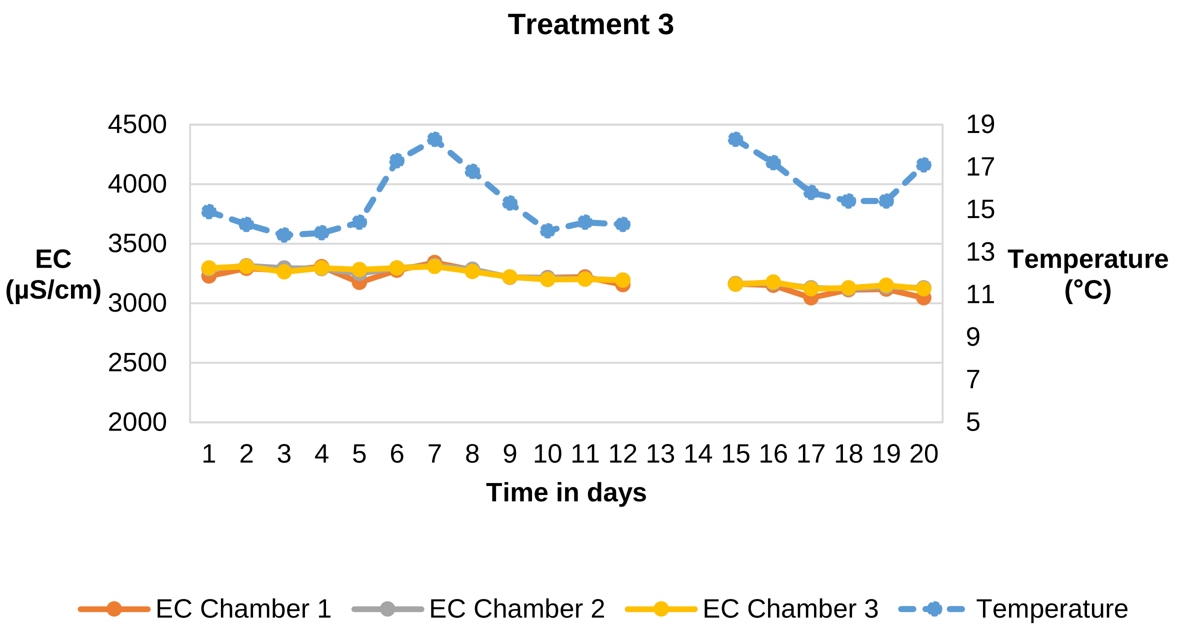

Figure 9: Temperature and EC of reactor co-digesting dairy manure wastewater, fruit and food waste (31 August - 19 September 2015).

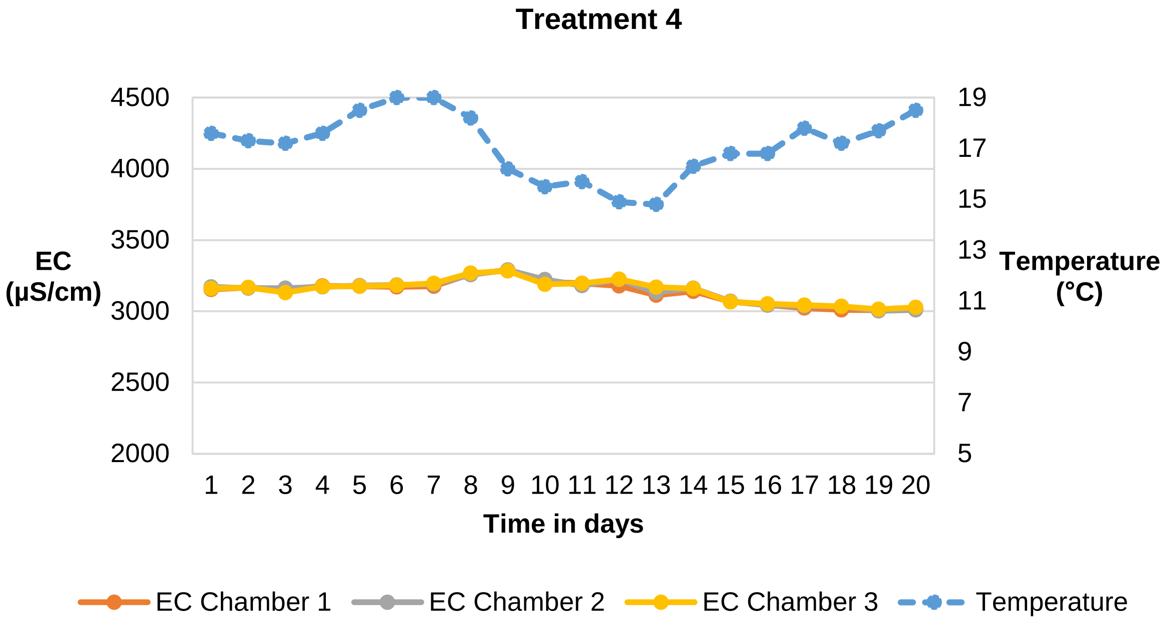

Figure 10: Temperature and EC of reactor co-digesting dairy manure wastewater and food waste (20 September - 09 October 2015).

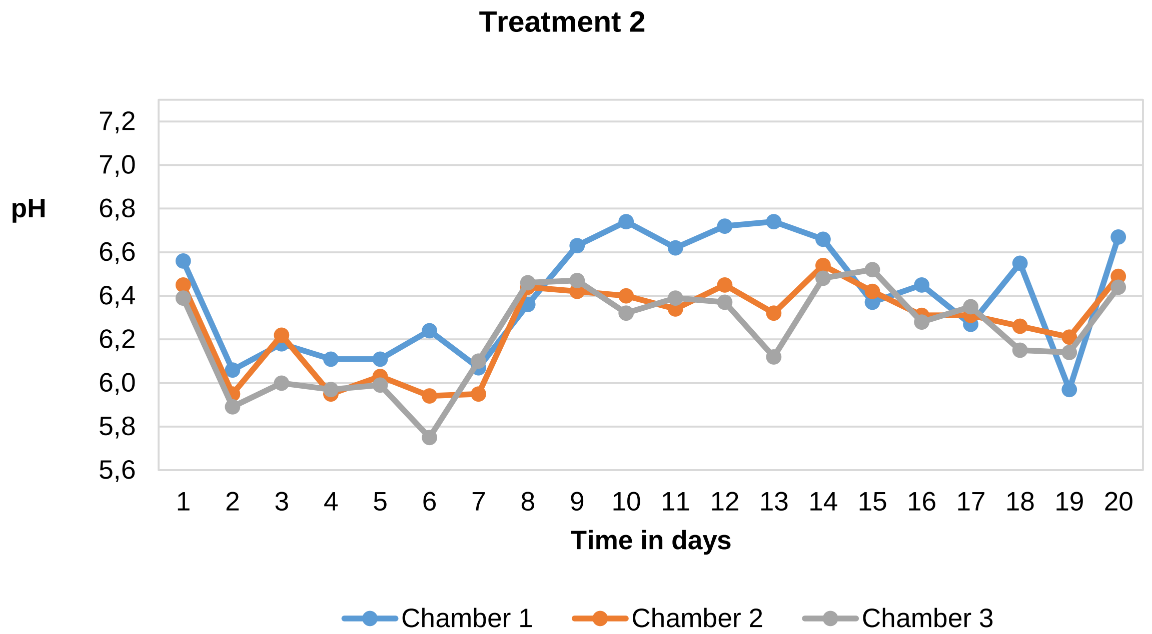

Figure 11: pH of reactor co-digesting dairy manure wastewater and fruit waste (11-30 August 2015)

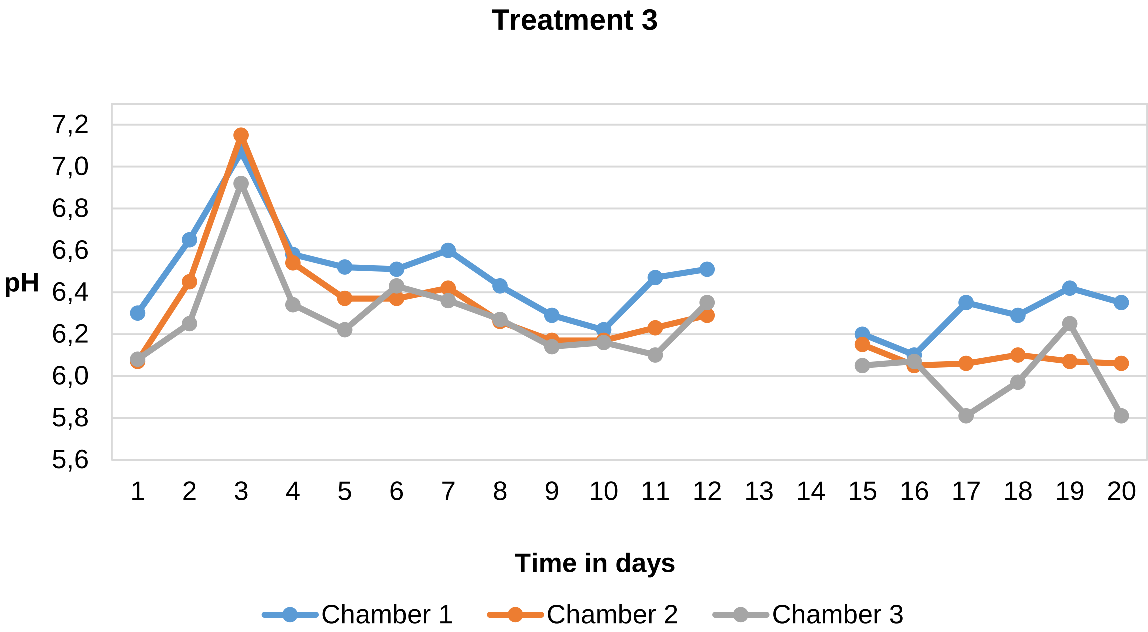

Figure 12: pH of reactor co-digesting dairy manure wastewater, fruit and food waste (31 August – 19 September 2015).

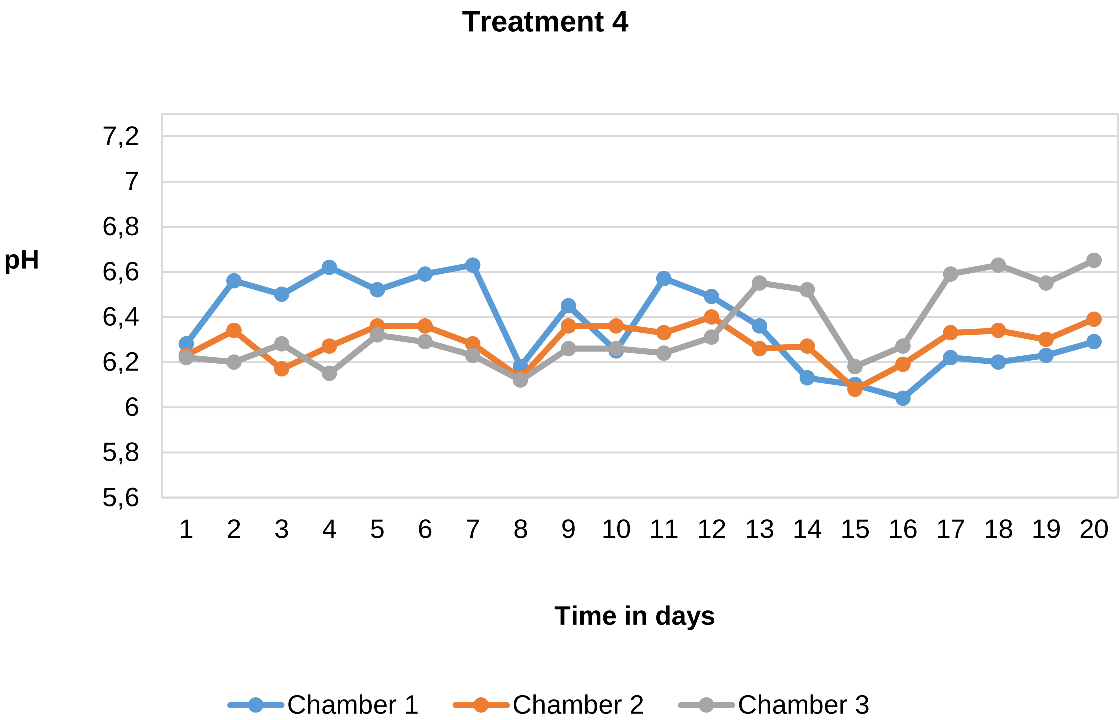

Figure 13: pH of reactor co-digesting dairy manure wastewater and food waste (20 September - 09 October 2015).

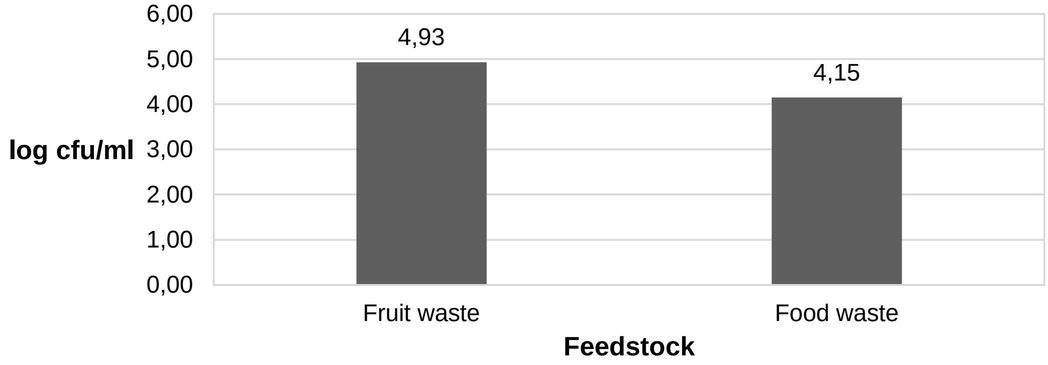

Figure 14: *E.coli* concentrations in fruit and food waste feedstocks.

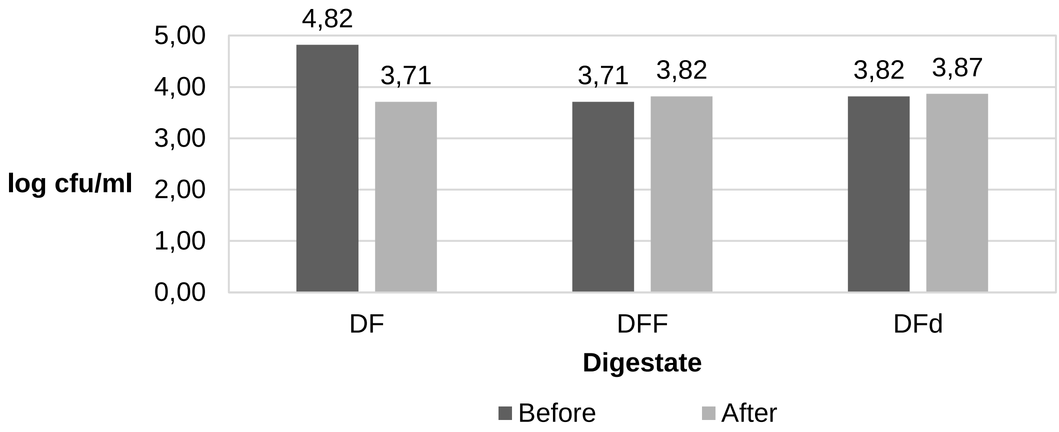

Figure 15: *E. coli* concentrations in the digestate of Dairy manure and fruit waste (DF) adapted from paper 1, Dairy manure, fruit and food waste (DFF) and Dairy manure and food waste (DFd).

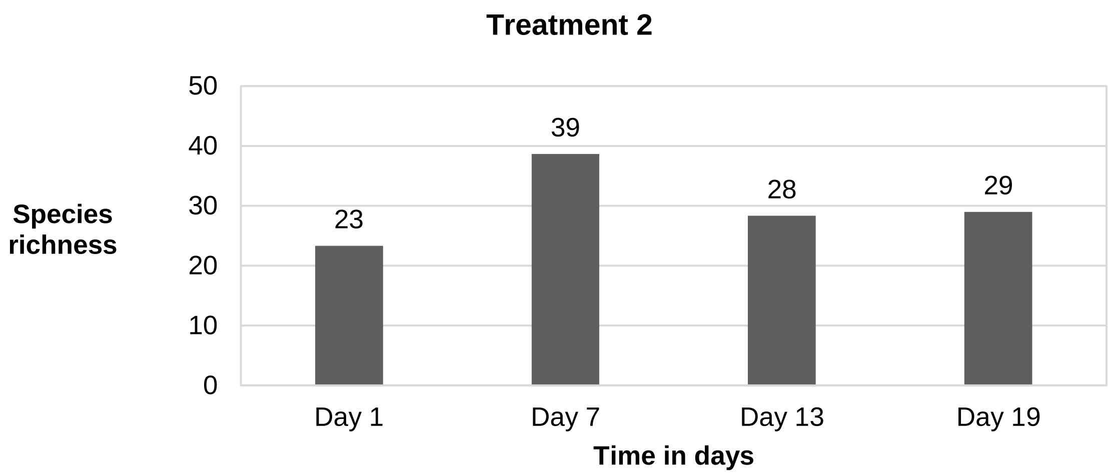

Figure 16: Means of microbial community diversity in dairy manure and fruit waste effluent.

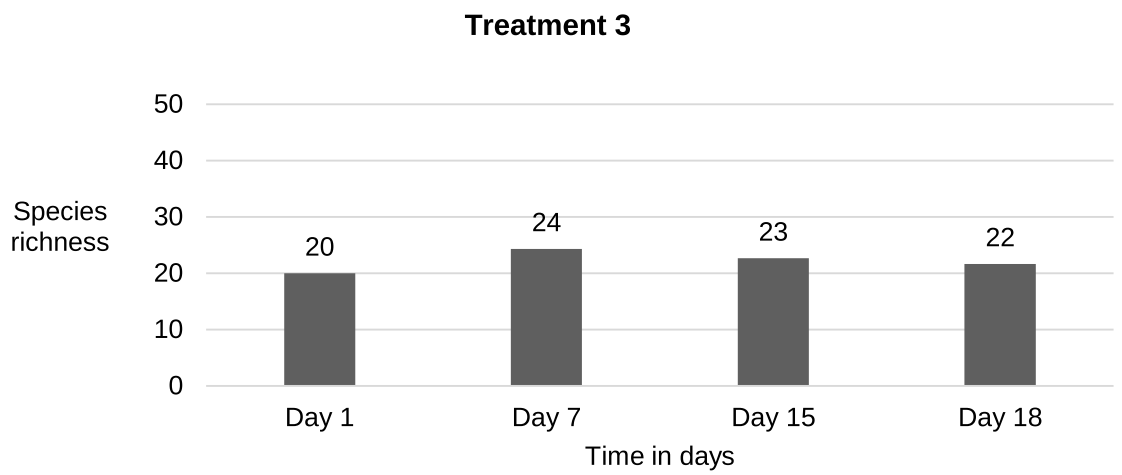

Figure 17: Means of microbial community diversity in dairy manure wastewater, fruit and food waste effluent.

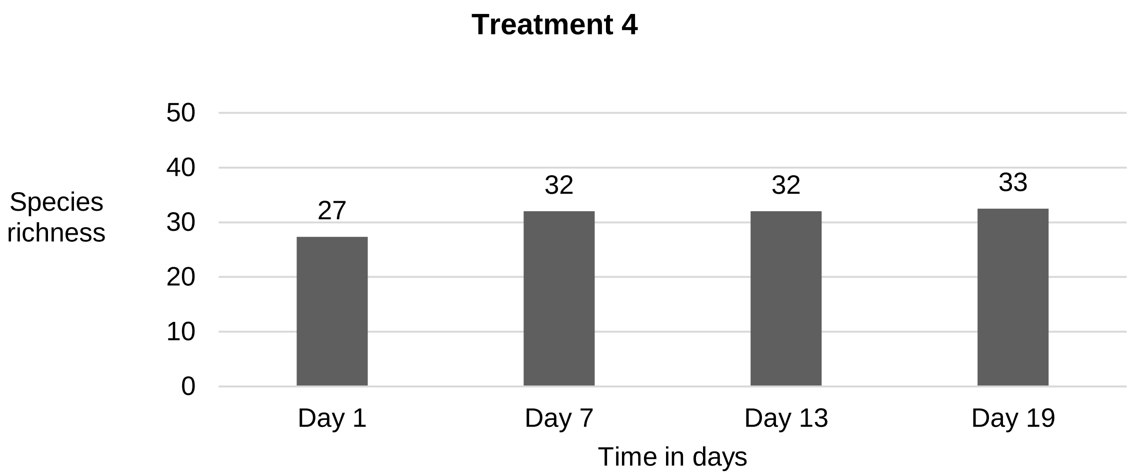

Figure 18: Means of microbial community diversity in dairy manure wastewater and food waste effluent.

Chapter 6

Economic evaluation of an anaerobic reactor to process organic waste streams at Welgevallen experimental farm, a partial budget

6.1. Background

Economic resources such as land, labour and capital are often limited with competing uses. This is particular true in agriculture when faced by non-economic factors, such as climate change, that make investment analysis much more complex, especially when compounded with aspects of sustainability (Yiridoe *et al*., 2009). However, with increasing consumer demands from an ever increasing human population, the need to remain competitive in an ever evolving market forces and the intensification of production system such as dairy, remain inevitable. However, sustainable intensification increases the requirement and considerations for waste management, which will necessitate the substitution of capital for labour.

Before any investment is made, it remains imperative to conduct an investment analysis in order to evaluate the net change in revenue before an investment decision is made. This is particularly important in agriculture where producers remain price takers on both sides of inputs and output. This is complicated further by the specialization of inputs such as machinery or infrastructure in the case of commercial anaerobic digesters. AD cannot easily be converted from one economic use to another - increasing risks associated with entry and exit barriers of any technology.

In spite of this, producers can use several financial tools to evaluate the economic cost and benefits of an investment such as the construction, operation and maintenance of an anaerobic reactor do exist (Yiridoe *et al*. 2009). Until recently, most of the semi-solid manure obtained after the water used to flush it out of the feedlots at Welgevallen experimental farm drained into the wetland or were composted. With the construction of a semi-commercial anaerobic reactor with a total capacity of 40.128 m^3in 2015, some of the dairy manure wastewater is now pumped to the reactor. Assuming that investing in an anaerobic reactor to sustainably manage all dairy manure solid and liquid waste is in direct competition for feedstock with composting, one may rightfully ask whether AD is an economically sustainable alternative to composting.

6.2. Introduction

Agriculture remains one of the largest emitters of greenhouse gases, such as methane, from livestock production systems like dairy farms (Olesen *et al*., 2006). However, gases such as methane have a high calorific value and can be exploited as a renewable source of energy for both on farm consumption (Yiridoe *et al*. 2009) and sale (Tafdrup, 1995). Unlike photovoltaic and wind energy, bio-energy in the form of methane from AD reactors can be stored and transported to suit the energy needs of consumers at a time period when they require it most. However, just like other sources of energy generation, the exploitation of AD for the generation of methane as an energy fuel requires monetary inputs for both construction and plant operation (Murphy & McKeogh, 2004).

Investment decisions on farm can either have an immediate impact or may only manifest in the long term. Nonetheless, these decision are equally important in the economic sustainability of a farm, which requires farm management to analyse investment opportunities in a way that ensures that the cost of not investing in the next best alternatives is well comprehended.

A commonly used and relatively flexible method of analysing trade-offs is the partial budget. According to Roth & Hyde (2002) to partial budgeting is a planning and decision making tool that is utilized to compare costs and benefits of competing alternatives faced a farming business. It questions what would happen on opting for the next best alternative and provides answers to such questions. Roth & Hyde (2002) further stated that partial budgeting allows farmers to better understand how a decision can affect the profitability of a farm or farming enterprise, and that partial budgeting is highly dependent on the quality of quantitative and qualitative data used in the analysis.

The economic feasibility of anaerobic reactors was quantified by several authors who used investment tools such as the net present value (NPV), internal rate of return and payback period (Laramee & Davis, 2013; Avaci *et al*., 2013) to account for economic cost and benefits derived from the technology. However, most of these studies referred to desktop studies Smith *et al*. (2014) or studies conducted in controlled environments such as laboratories (Cooney *et al*., 2007; Lin *et al*., 2011; Callaghan *et al*., 2002; El-Mashad & Zhang 2010). Data was then extrapolated to determine the economic feasibility of commercial anaerobic reactors.

Different models to achieve this exist. One such model is to utilize a break-even time budget as this evaluates the physical quantities, such as streams of costs and benefits (Dillon, 1993). However, unlike composting, the AD does not yet command clear streams of revenue or cost at this point and that assumption will be made depending on the physical and chemical properties of biogas and effluent digestates, which are the end-products of AD. However, this method of analysis regularly assumes that whatever is produced is sold and that prices remain constant, which is not reflective of volatile market were demand. In addition, this method too makes assumptions about cost and revenue in the future and thus has a tendency to extrapolate data. An alternative option is to compile a long-term capital budget which is used to determine long-term investment opportunities that can be derived over a longer time period of space. This financial tool considers variables such as risks involved, operating and maintenance costs, discount rates, depreciation and replacement of capital, exchange rates on imported equipment and material. Long-term capital budgets are however dependent on assumptions about the uncertain future, which will not be covered in this study.

However, under our conditions, the only plausible option would be to develop a partial-budget as this allows us to utilize information and data that is readily available and more reliable. The partial budget also looks at information that is most relevant and that is of interest. Nonetheless, it too can be limited by unavailable data and does not reflect the effects it will have on the overall financial structure of a farm. However, it remains a useful tool evaluating the best waste management alternative between two competing alternatives.

Therefore, the objective of this paper was to determine the economic costs and benefits derived from an anaerobic reactor compared to composting for the treatment of three different organic waste streams at Welgevallen experimental farm with the partial budget model. This is an explorative exercise on the financial evaluation comparing the current waste management practices, composting and AD, on the farm. This exercise will employ assumptions, largely as a result of limited data that needs to be gathered over short time period. Financial evaluation and not analysis will be used primarily, because the study uses primary data that is readily available and not data that has been statistically validated.

6.3. Material and methods

Important Assumptions

Unlike, vineyards and orchards where per unit area of land commands a high economic value, the average commercial dairy farms in the Western Cape are usually large enough that the opportunity cost of availing marginal land for the establishment of composting site or construction of an AD reactor can reduce the return of investment to such an extent that in undertaking such a project, the cost of land is not given much emphasis during budgeting. Nonetheless, these marginal tracks of land would still command an economic value if it was to be sold to a neighbouring farmer or developer who intends to build residential units. Compared to composting, the construction of an anaerobic reactor requires a moderately smaller portion of land unlike composting operations that would require larger and relatively flat pieces of land.

The second assumption relates to cost of labour to render time and energy at working at either a reactor plant or a composting site, based on the minimum wage for South African farm workers for 2015. This method of assessment was proposed by (Austin & Blignaut, 2008) and is practical for budgeting in an industry were there can be large variation between farm employee wages. In the context of Welgevallen experimental farm, it is assumed that the AD only requires 2.5 hours a day for labour, while composting equal the time required for hiring a tractor on a yearly basis. Labourers on Welgevallen would not only spend time at the AD or composting sites, but that they will continue to be involved in operational activities on the farm, which will contribute towards their actual wages.

The third assumption is the need to standardize the unit of measurement in terms of volume output to match the AD's daily volumetric loading rate of 2.1m^3 per day with the amount of biomass that composting processes, noting that composting is not yet commercialised to point where resources are employed on a daily basis. A comparison also needs to be drawn to indicate the time required for compost to mature during the warmer summer months when maturation occurs within 90 days whereas this process will be 180 days during the colder winter months.

The AD produces two products that have the potential to generate revenue - provided that essential infrastructure is in place. These outputs are biogas and digestates rich in several macro and micro-nutrients. The theoretical biogas yield will be estimated utilizing the simple chemical oxygen demand (COD) formula based on results obtained during the study. Dairy manure wastewater had a low COD content of 3385 mg/l, while the digestate had a COD content 2845 mg/l. In addition, it is assumed that volumetric loading rate of 2.1m^3 would yield 766.5 m^3 of liquid digestate over 365 days/ year with a COD content of 2845 mg/l.

$CH_4 = 0.35 \times COD_{destroyed.}$

$COD_{destroyed} = COD_{in} – COD_{out.}$

COD remove would thus be 540 mg/l or 0.54 kg/m^3.

0.35 × (0.54 kg/m^3) × (766.5 m^3 dairy manure wastewater) = 145 kg of Biogas per annum (Angelidaki & Ellegaard, 2003).

This would imply that, during the anaerobic digestion of dairy manure wastewater, the reactor's efficiency for COD removal was a mere 16% with a potential to yield 145 kg of biogas, which is well below the typical low 10 to 20 m^3 CH_4/t of treated manure (Angelidaki & Ellegaard, 2003). Assuming that methane sells for half the current market price of liquid petroleum gas (LPG) with a market price of R 18.98/l on the 25th of November 2015 ("News-North Coast Gas

Installations", 2015), 145 Kg of biogas with a methane content of 60%, would yield R 1 566.00 in benefits.

With a volumetric loading rate of 2.1 m^3, the reactor has the potential to produce 766.5 m^3 of liquid digestate per annum, while this would be 378 m^3 over 180 days in winter**.** However, these quantities and time periods will have to be adapted to align with the working hours of labour on the farm. Additionally, it will require to calculate and quantify the amount of compost produced, including the retail value R450.00 for compost, noting that only 25% of the initial feedstock end up as retailable compost.

In order to attach a viable economic value to the digestate, it will have to meet specific nutritional requirements such as those of a commercial organic liquid fertilizer (Nitrosol) or the hydroponic nutrient solution with current market prices at R 1.00/l for Nitrosol and R 0.20/l for the hydroponic nutrient solution (Table 22 and 23). Two of the digestates (DF: Dairy manure wastewater and fruit digestate; DFd: Dairy manure wastewater and food digestate) did not exhibit any phytotoxicity effects on tomato seed germination and were therefore selected for this analysis. However, both these solutions contain sodium (Na) concentrations that may negatively affect plant health in high concentrations and repeated application (Qados, 2011). These concentrations were 53 mg/l (DF) and 14 mg/l (DFd) which both exceeded the limit for allowable Na in a commercial liquid fertiliser like Nitrosol. The arbitrary market price for the digestates was set at R 0.20/l, provided that is chlorinated to reduce colony forming units, put through a *Nitrosomonas* bacteria bio-filter to convert ammonium to nitrite and then, a *Nitrobacter* bacteria bio-filter to yield more nitrate.

6.4. Results and Discussion

Under the current circumstances of sub-optimal functioning, AD is not economically feasible compared to windrow composting. If the composting was to cease in favour for AD, the farm would relinquish R 118 848 in revenue (Table 24). However, before any conclusion is drawn, it is imperative to take cognisance of the fact that both aerobic composting and AD are yet not fully fledged commercial activities at Welgevallen experimental farm. The composting operation still largely remains research oriented, while the anaerobic reactor during the current study period remain largely a pilot trial.

Due to technical challenges and unconducive temperatures, compounded by the low OLR during the experiment, AD still remains an attractive waste management option, especially for high moisture feedstocks such as dairy manure wastewaters. In addition, to increase the economic viability, enriching the digestate with additional nitrogen to make it a viable fertilizer for use in plant nutrition and consequently increase its economic value. In addition, financial incentives such as carbon credits for carbon neutral fuels can improve the economic viability of investing in AD technology.

6.5. Conclusion

The partial budget revealed that AD as an alternative waste management alternative to composting at Welgevallen experimental farm is not a viable alternative. This was compounded by the sub-optimal operating conditions during the experimental time, despite that AD can yield environment and social benefits that are difficult to attached an economic value. Optimally performing AD reactors can yield benefits such as odour reduction, greenhouse gas reductions without carbon trading, pathogen free wastewater and reduction of weeds in livestock manures (Minde *et al*., 2013). Despite, carbon credits having the potential to serve as an important stream of revenue, these markets are policy driven and thus remain volatile to price fluctuations. In addition, to potential economic benefits of the anaerobic digester, other benefits would include that communities utilizing the river's water to irrigate their cash crops, will be able to do so without the fear of contamination from dairy manure wastewater.

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List of tables

Table 22: Concentration (mg/l) of macro-nutrients in digestates compared to two commercial fertilizers

Digestate	P	K	Ca	Mg	NO_2-N	NO_3-N	NH_4-N
DM	36.18	328	157	74.13	0.85	3.44	123.9
DF	53.03	211	491	70.51	1.25	1.76	182.4
DFF	45.61	276	199	63.01	0.97	0.36	89.9
DFd	34.09	89	52	14.81	0.82	0.32	84.4
Nitrosol	95.99	230	22	2.40	0	52.8	72.2
Hydroponic solution	46.50	273	216	50.82	0	182	14.0

Table 23: Concentration (mg/l) of micro-nutrients in digestates compared to two commercial fertilizers.

Digestate	Na	Mn	Cu	Fe	Zn	B
DM	74	1.9	0.2	9	1.9	3.7
DF	53	4.0	0.0	17	4.0	3.0
DFF	62	1.0	1.0	11	2.0	2.0
DFd	14	0.0	0.0	2	0.0	1.0
Nitrosol	14	0.7	0.6	0.9	0.5	1.1
Hydroponic solution	0	0.6	0.1	0.9	0.3	0.4

Table 24: Partial budget comparing AD and composting at Welgevallen.

Additional Revenue	Unit	Quantity	Price	Revenue
Biogas	Litres	145	R 9.49	R 1 376
Liquid fertilizer	Litres	766500	R 0.20	R 153 300
Total Added Revenue				**R 154 676**

Additional Cost	Unit	Quantity	Price	Cost
Added depreciation (Investment)	Life span of 25 years	1	R 27 500	R 27 500
Electricity	0-350kWh/per month: Cape Town rates	4200	R 0.96	R 4 037
2 Labour hours	2.5 hours/day	912.5	R 15.00	R 13 687
Total added Costs				**R 45 224**
Profit			**Reactor**	**R 109 728**

Reduce Cost	Unit	Quantity	Price	Cost
2 Labourers	Hours	3840	R 15	R 57 600
Tractor driver	Hours	384	R 290	R 111 360
Chipper rent	Hours	32	R 720	R 23 040
Water	m³	1	R 5 000	R 5 000
Pump and piping	Lifetime of 12 years	1	R 120 000	R 10 000
Irrigation system		1	R 5 000	R 5 000
Total				**R 212 000**

Reduce Revenue	Unit	Quantity	Price	Revenue
Sale of compost	Tons	1258	R 350	R 440 300
Total				**R 440 300**
Profit compost				**R 228 300**
Changes in Profitability				**R - 118 848.00**

CHAPTER 7

General conclusion

The demand for plant and livestock products or services from an ever increasing global population will continue to influence agricultural production systems. Inevitable waste streams such as dairy manure wastewaters, fruit and ultimately food waste will continue to increase as they are directly interlinked with food production systems. Organic waste streams not only have environmental ramifications, but is intertwined with social and economic concerns. To effectively manage agricultural waste, it remains paramount to quantify available and accessible waste streams. However, a lack of well-articulated models and methodologies of estimating organic waste volumes at the farm level pose a challenge. This is particularly important especially when considering that AD technology and infrastructure can be capital intensive especially at semi-commercial and commercial scales.

In this study, temperature and pH proofed to be two important variables for optimal performance of the AD process. The ideal circumstance was to operate the reactor under mesophilic temperatures, in order to ensure the efficiency of the reactor in reducing pathogenic microorganisms to concentrations that allow the digestate to be safely used as an alternative liquid fertilizer or discharged into the environment. In addition, the digestates EC has to meet local requirements of not more than 2000µS/cm for irrigation purposes. However, the EC in all digestates was comparatively higher and would need to be diluted before application.

Although, diluted digestates proofed to have a potential to be utilized as a liquid fertilizer, they remain high in NH_4^+-N and low in NO_3 which is the primary nitrogen source for plants. The digestates will thus require to undergo denitrification to convert the NH_4^+-N to NO_3 enriched digestate and potentially make it a viable liquid fertilizer.

Due to low reactor temperature (<20 °C), the AD process did not proof efficient enough to reduce pathogenic microorganisms to concentrations below limits as stipulated by the National

Water Act 36 of 1998. This implies that the digestates from the study cannot be used for irrigating purposes especially for the production of fresh produce that are primarily consumed raw as it would pose a serious public health hazard. Future experiments would thus benefit from providing an external heating source to the reactor until such a time when the reactor has produced sufficient combustible biogas to sustain itself.

In addition, the reactor operated at a conservative OLR of 5.078 kg/day TS for dairy manure wastewater and 4.97 Kg/ day for the co-digestion treatments with fruit and food waste. This was largely attributed to technical challenges that prevented sufficient solid manure from entering the sump and eventually the reactor. However, the reactor did proof efficient enough at reducing TSS in the wastewater such that a reduction of 80% in dairy manure wastewater was observed, while the three co-digestates observed a mean TSS reduction of 97%. However, this reduction could not have resulted from digestion by AD microbes due to the low gas levels that were not sufficient enough to heat the reactor.

Anaerobic microbes play an important role in the digestion and reduction of solid organic matter into soluble organic acids that serve as precursors for the production of biogas. Although, one of the study aims was to evaluate the microbial community structure with the ultimate aim of identifying keystone microbial species within the reactor, this aim was not achieved due to time limitations. However, the study managed to quantify microbes and established that on any given day, the reactor was colonized with more than 20 species of microbes. The identification of keystone species would facilitate further research into the quantification of microbial biomass which are difficult to culture under laboratory conditions.

Despite, the sub-optimal temperatures during the entirety of the four treatments, a partial budget proofed composting to be a more profitable alternative to AD at Welgevallen experimental farm. AD under optimal conditions has the potential to be a sustainable complementary waste management to composting for high moisture organic waste.

Annexure

Annexure 1: Illustrative diagram of the three phase, plug flow anaerobic reactor at Welgevallen experimental farm.

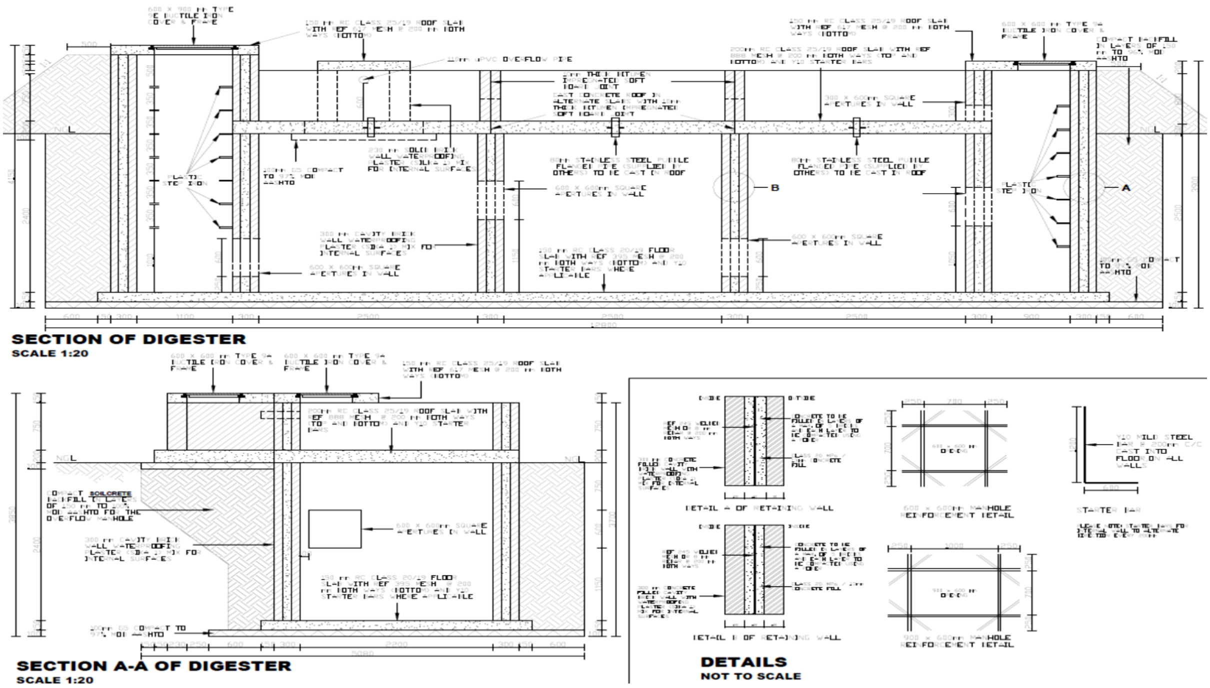

Plate 7: Three chamber, plug-flow anaerobic reactor at Welgevallen experimental farm.

Annexure 2

Preparation methodology for TSS and VSS analysis.

Step 1: Filter disc preparation: Weight A

1. Glass fibre filter discs without binder (Whatman grade 934AH), with a diameter of 70mm were inserted with the wrinkled side facing up into a filtration apparatus.
2. 60 ml of distilled water in 3 successions of 20 ml were applied to the filter disc with suction provided from a vacuum pump to remove traces of water on the filter disc.
3. The filter disk was then transferred to a porcelain dish, placed in a muffle furnace for 2 hours at 550 °C.
4. After the 2 hours and allowing the furnace to cool down for an hour, the porcelain dishes with filter discs were removed, placed in a desiccator for an hour before their weight (in grams) was recorded to 4 decimals.

Step 2: Sample transfer: Weight B

1. The filter discs from step one were inserted back into filtration apparatus and moistened with 10 ml of distilled water to set.
2. To ensure a representative sample, the dairy manure waste water in a container was well shaken, before a 20ml form the midpoint of the container was pipetted with a 3ml graduated pastette into a graduated cylinder.
3. The sample was then transferred onto the filter disc and the vacuum pumped which one, however after 10 minutes of suction and clogging of the filter disc. Another attempt of adding only 10 ml was used, but still clogged the filters, before 5 ml succeeded.
4. After applying 5ml of dairy manure wastewater, the filter discs were washed with 30 ml of distilled water in 3 successions of approximately 10 ml each, while applying vacuum suction for approximately 3 minutes before placing the individual filter discs back into the porcelain dishes.
5. The dishes were than dry in a 105 °C oven overnight, cooled in a desiccator before their weights (B) were recorded.

Step 3: Sample drying: Weight C

1. After recording weight (B) the porcelain dishes with filter discs were transferred to muffle furnace and heated to 550 °C for 3 hours.
2. Thereafter, the furnace was allowed to cool down for an hour before the porcelain dishes were removed, placed in a desiccator for 2 hours and weighted.

Annexure 3

Protocol for pour plant E.coli enumeration.

Preparation of dilution series.

1. 9% Saline solution of sodium chlorine was prepared by adding 90 grams of sodium chloride (NaCl) to 1 litre of distilled water and mixed well until all salts crystals have dissolved.
2. 90 ml of the solution was than siphoned into test tubes before they were autoclaved at °C for 2 hours.
3. After the 2 hours test tubes were allowed to cool down, before they were labelled according to 5 dilution series starting from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.
4. 1ml of dairy manure wastewater was then siphoned into dilution series 10^{-1}, from which 1 ml was obtained and siphoned into dilution series 10^{-2}.
5. The process of obtaining 1ml from each serie was repeated in a chronological fashion until dilution serie 10^{-5}.

Preparation of bacterial growth medium.

1. 40 grams of MacConkey Broth purple mixed with 12 grams of Agar Bacteriological were added to 1 litre of distilled water and the solution stirred with a magnetic strip.
2. The solution was then autoclaved for 2 hours and allowed to cool down while been stirred with a magnetic strip, before it can be poured onto petri dishes.
3. Before the bacterial growth medium was poured onto the petri dishes, they were labelled according to the dairy manure waste water dilution series.
4. 1ml was then obtained from each test tube and placed into the petri dish labelled with the identical dilution serie number.
5. This meant that 1ml from dilution serie 10^{-1} was placed into a petri dish labelled 10^{-1} and so forth.
6. The bacterial growth medium was then poured into petri dishes with the 1 ml dilution serie solution and stirred swiftly to ensure that good amalgamation of possible bacteria in the solution with the growth medium.
7. This entire process was all done in a sterile environment.
8. The growth media petri dishes were then allowed to solidify, before then were placed in an incubator at 37°C for 24 hours.

Quantification of microbial population

1. Bacterial colony plate count commence after an incubation period of 48 hours in a 37 °C incubator.
2. To achieve practical bacterial counts, only plates with bacteria colonies in a range of 30 to 300 colony forming units/ml (cfu/ml) and converted to log_{10} cfu/ml.

Annexure 4

Protocol for DNA extraction:

Zymo-Spin test kit protocol.

1. 50-100mg of solids from centrifugation was added to a ZR bashing bead tube, to which 750 µl of Lysis solution is added.
2. The tube was secured to a bead beater (Disruptor Genie) cell disruptor and processed for 5 minutes.
3. The bashing bead tubes from the Disruptor Genie were than centrifuged at 10 000 rpm for 1 minute. In some instances, the time for centrifugation had to be increased to 3 minutes to avoid colloids in the sample from clogging the Zymo-spin IV spin filters.
4. 400 µl of the supernatant was transferred to a Zymo-Spin IV spin filter (orange top), tips snapped off and held in a collection tube to collect the filtrate before this was centrifuged at 7000 rpm for 1 minute.
5. 1 200 µl of fungal/bacterial DNA binding buffer was then added to the filtrate that collected in the collection tube.
6. With 1600 µl in the collection tube, 800 µl was transferred to a Zymo-spin IIC Column in a new collection tube and centrifuged at 10 000 rpm for 1 minute.
7. The filtrated from the collection tube from step 6 was discarded before the last 800 µl was added to the Zymo-spin IIC Column and centrifuged again for 10 000 rpm for 1 minute.
8. The filtrate was discarded before 200 µl DNA Pre-wash Buffer to the Zymo-Spin IIC Column in a new collection tube. This was then centrifuged at 10 000 rpm for 1 minute and the filtrate discarded again.
9. 500 µl of Fungal/Bacterial DNA Wash Buffer was then added to the Zymo-Spin IIC Column and centrifuged at 10 000 rpm for 1 minute.
10. The Zymo-Spin IIC Column was transferred to a sterile 1.5 ml micro centrifuge tube before 100 ml DNA elution buffer to elute pure DNA was added to the column and centrifuged at 10 000 rpm for 30 seconds.

DNA detections and visualization

Reagents	Quantity
Agarose	0.3 g for small gel or 0.8 g for large gel
TAE buffer	30 ml for small gel or 80 ml for large gel
Ethidium bromide	1µl
Ladder	1µl
Loading dye	Small droplet for each DNA sample

Annexure 5

Polymerase Chain Reaction (PCR) protocol

1. During the entire preparation process, all samples were kept on ice.
2. A master mix sample was prepared by using these reagents inside an Eppendorf tube.

Component	Per sample
MilliQ	4.1µl
ReadyMix (Taq)	5.0µl
Forward primer	0.2µl
Reverse primer	0.2µl
DNA	0.5µl
Total volume	**10.0µl**

3. After these reagents were added to the Eppendorf tube, vortexed to mix thoroughly.
4. Inside a laminar flow cabinet, 9.5 µl of the master mix was than aliquoted into reaction tubes, 3 replications for each DNA sample.
5. 0.5 µl of DNA was added to the reaction tubes, 3 replications for each sample of DNA while negative control only received MilliQ.
6. The reaction tubes were than vortexed to thoroughly mix reagents before reaction tubes were placed into the PCR machine before the machine was started.
7. To ensure that the PCR was successful, PCR products were visualized through gel electrophoresis.
8. The PCR was successful only if negative control sample yielded no DNA bands after gel electrophoresis.
9. In the event of a contamination, the entire process is repeated, whereas if successful, the three replicated samples are pooled (combined into one sample) and submitted for DNA sequencing at the Stellenbosch University Central Analytical Facility (CAF) for sequencing.