INTEGRATING BIO-BASED RESOURCE RECOVERY AND TREATMENT INTO MUNICIPAL SOLID WASTE MANAGEMENT IN DEVELOPING COUNTRIES: A FOCUS ON ANAEROBIC DIGESTION

Thesis presented for the degree of Master of Science in
Engineering in the Department of Chemical Engineering

University of Cape Town

September 2012

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DECLARATION

I know the meaning of plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own.

Signed by candidate

Gracia Munganga

September 2012

ACKNOWLEDGMENTS

Foremost, I would like to praise God for His wonderful blessings and for making this dream, which was once out of reach a few years back, possible. I cannot express my gratitude enough for Your grace; I thank You for the strength, courage, comfort, support and wisdom without which I could have never gotten to this point in my life.

I would also like to express my deepest gratitude to my supervisor, Prof Harro von Blottnitz who constantly provided the support and structure needed as a masters student. Many thanks for your sound advice, enthusiasm for this field, patience, willingness to help and great efforts to guide me through my work, and explain things clearly and simply. I thank you again your warmth, your humility, and making your research group, a home away from home for so many of us. I have certainly learned a lot both academically and as a person, and believe this would not have been possible had our paths not crossed in my 3rd year (2007). Many thanks again for sticking with me through the lengthy process of my write-up.

I am also deeply grateful to the Department of Chemical Engineering for funding my postgraduate studies.A big thank you to my colleagues and friends from our research group i.e. the Environmental and Process Systems Engineering (E&PSE), in the persons of Mymoena, Carol and others. Special thanks to Ms Thabi Melamu from E&PSE, for your constant support, encouragement and guidance throughout my degree. Your constant warmth, efforts and willingness to help,and friendship have certainly made this journey easier, less lonely and more pleasant.

I would like to acknowledge the support and contribution of Solid Waste Department of the City of Cape Town, in the person of Mr. Melumzi Nontangana and Ms. Sumaya, throughout the duration of the sampling period. Many thanks as well to Mr. Saliem Haider, Noel Johannesen and their team for organising and supporting through the characterisation study at both Athlone Transfer Station (ARTS) and Bellville Compost (BC).

I am indebted to the Centre for Bioprocess Engineering Research (CeBER) from Chemical Engineering and, the Water Research Laboratories from Civil Engineering at UCT, in particular to Mrs Fran Pocock, Emmanuel Ngoma, Hector Mafungwa and Taliep Lakay, for hosting the laboratory work and for providing invaluable advice on laboratory technique and practice.

My most profound gratitude to my loving parents, Robert and Justine Munganga, and my wonderful brothers, Ravic, Gregory and Herve, who have been with me every step of the way through this challenging journey. Thank you, papa and maman, for all the sacrifices, for your kindness, your unconditional love, genuine support, prayers, and encouragements despite being far. I feel very blessed to have you in my life, and feel privileged to be part of this wonderful family. I dedicate this work to my family, particularly my late twin sister, Muriel. I wish you lived to see this day but believe you are in a better place.

I would also like to thank all my wonderful friends, particularly Linus Naik and Seun Oyekola for your support during my write-up, and the time invested despite your busy schedule.

SYNOPSIS

Management of municipal solid waste (MSW) in developing economies is often poor and unsustainable.The MSW in developing countries is characteristically dense, has a high moisture content, higher organic fraction and lower calorific value compared to that in developed countries. The most common method of waste disposal in developing countries is disposal in dumpsites or landfillsdue to the operative ease, the low financial costs, or the absence of planning and investment into infrastructure. Landfills, especially open and uncontrolled dumps, have a heavy environmental footprint, including the release of landfill gas rich in methane (CH_4) with high global warming potential, odours from volatile organic compounds (VOCs), smoke from uncontrolled burning, and contaminated or toxic leachate. Although disposal practices in developed countries have evolved from simple open dumps to engineered, sanitary landfills, limited available land and its irreversible loss after use makes landfilling unsustainable in the long run.

Many authors, along with the United Nations Environment Programme (UNEP), argue that biological treatments such as anaerobic digestion (AD) and composting would be suitable in developing cities due the highly organic nature of their waste. AD entails the degradation of organic substances in the absence of oxygen, and its main products are CH_4-rich biogas and the remaining sludge. AD occurs in four main phases (hydrolysis, acidogenesis, acetogenesis and methanogenesis) and is a complex process, where microbiological, biochemical and physico-chemical phenomena are closely linked. Although composting the organic fraction of MSW (OFMSW) has been the preferred treatment method due to the low economic costs and operative ease, the production of biogas - a renewable energy source - has now positioned AD as the more energy efficient process as opposed to composting which requires energy inputs.

A clear identification and understanding of the different organic waste streams available, their respective biogas potential, the engineering considerations and process limitations are needed to ensure good performance of AD plants. Location-specific data on the behaviour of different organic wastes in AD are, however, scarce, particularly in Africa. This dissertation is an attempt to generate Africa-relevant engineering knowledge through laboratory scale experiments of AD of organic waste streams typically disposed off in landfills. The main objectives of this dissertation are to (i) investigate the suitability for treatment by AD of typical organic waste streams in MSW, and (ii) consider the interplay between the nature of the available wastes and the temporal evolution of various process parameters. Finally, this dissertation aims tomake sound recommendations from the data generated, building a preliminary source of knowledge for waste management officials and independent entrepreneurs.

The laboratory work was organised in two phases. The first phase was based on a contracted study with the City of Cape Town (CoCT), investigating the biomethane potential (BMP) of 17 different organic waste streams, as already reported on to the City of Cape Town (Munganga *et al.*, 2010). Experiments were carried out at 100 mL scale and under mesophilic conditions. The outcome of BMP tests has been presented in four main groups, viz. garden wastes, fines and paper sludge, kitchen wastes and, abattoir and fisheries wastes.

An analysis of variance (ANOVA) generally indicated good replicability for the triplicates within each waste types, and significantly different biogas yields between them. Fine waste from mixed sources and garden waste (with two exceptions) showed the best performance; whilst among food wastes and high-nitrogen wastes only one substratein each case produced significant methanogenic activity. The highest CH_4 yields in the four categories were: fruits and vegetables biomass from Cape Town's Market with 119 mL CH_4/gVS, garden waste from the Bellville Compost plant with 223 mL CH_4/g VS, fines from the same plant with 194 mL CH_4/g VS and fisheries wastes with 104 mL CH_4/g VS. The biomethane potential (BMP) evaluations for the food waste experiments, whilst generally with statistically acceptable variability between triplicates, were not successful.The CH_4 yields achieved were very low and acidic conditions prevailed. The food: inoculum ratio in these batch tests were relatively high at around 4-5, which is considerably higher compared to values reported in the literature as suitable (i.e. 0.5-1).

The second phase of experiments was carried out under fed-batch conditions in 2 L reactors for garden, kitchen and blood wastes since it was not possible to monitor regularly experimental parameters such volatile fatty acids (VFAs), NH_3 and pH, at 100 mL scale due to the small reactor size. The main goals of the second phase of experiments were firstly, to build on BMP results, secondly to monitor the temporal evolution of experimental parameters, and thirdly to investigate the performance of AD under different and real life conditions (semi-continuous, limited agitation).The effect of the feeding regime was also investigated with kitchen waste, where a flat and a progressive feeding regime were compared. Experiments at 2 L scale were operated at ambient temperature (~17°C) initially and it appears that psychrophilic conditions were not suitable for biodegradation at start-up, from the negligible biogas production for most bioreactors during the first two-week period.

The experiments were then continued at 35°C. The highest cumulative biogas yield was from the kitchen waste on a flat feeding regime with 23 L (107.5 mL/g VS), and the lowest was from blood waste with only 3L (14 mL/g VS). The average cumulative biogas, and yields for kitchen-progressive and garden waste were respectively 12.4 L (56 mL/g VS) and 13.2 L (59 mL/g VS). The progressive feed performed poorly compared to the flat feed once an excessively high organic loading rate (OLR) was imposed.

This emphasises the crucial role a sustainable OLR plays in the success of AD systems. Generally, AD of cellulosic materials exhibited a more stable behaviour relative to easily biodegradable feeds such as kitchen waste. This can be attributed to the slower hydrolysis rates, which reduce the accumulation of intermediate products and allow a balance between the activities of different microbial groups. Although BMP results showed that higher CH_4 percentages were generally observed for pH values between 6.5 and 7.5, this was not always applicable as the variability of CH_4 composition was significant between sampling days. Following from the above, little can be inferred about the CH_4 content of the biogas in the fed-batch reactors which could unfortunately not be measured during that experiment. Consequently, the performance of the digesters was evaluated based on biogas, pH, VFAs and NH_3 where applicable. Gas volumes presented are corrected by deducting the maximum volumes of CO_2that could have been produced from the speciation of bicarbonate ions (HCO_3^-) into CO_2 from the buffer used.

The suitability of co-digestion of protein-rich wastes (viz. abattoir and fisheries wastes), with carbon-rich wastes (e.g. garden wastes, paper sludge) was investigated to adjust the composition (C:N ratio) of the substrate and overcome the lower biogas and CH_4 yields commonly reported with such wastes. The laboratory work was carried out both at 100 mL and under fed-batch conditions at 2 L scale. Results of the co-digestion experiments showed higher biogas yields, lower VFAs and NH_3 concentrations compared to those of abattoir and/or blood waste.

Expected values of biogas yields for co-digestion were determined as the weighted average of yields of each substrate; the experimental results were generally considerably lower than the expected values. This category of experiments thusexhibited limited microbial activity. The possibility of ammonia inhibition was investigated but inhibitory levels seem not to have been reached. There are a number of other factors, not investigated in this study, such as the acclimatisation of the microbial consortia to the substrate, and other pollutants (e.g. LCFAs, H_2S) from the degradation of slaughterhouse wastes that could have affected these AD systems.

Monitoring the levels of the different VFAs allowed for advanced insights into the dynamics of the AD process. There was no apparent link between accumulation of propionic acid and inhibition to methanogens in this study, contrary to the literature where propionic acid is reported as the main inhibitor (Wang *et al.*, 1999). Butyric and iso-butyric acid were the main forms of VFAs observed across the fed-batch experiments, and not acetic acid by contrast to many studies. There was a high level of isomerisation observed for VFAs with a carbon chain higher than four (C_4), and high concentrations of butyric acid appeared to correlate to higher biogas yields for kitchen waste and co-digestion experiments.

The main variables confirmed as good process indicators, to monitor and optimise the performance of AD systems, were: the CH_4 composition combined with biogas yield, pH, the feed regime (F:I ratio) and OLR, the concentration of individual VFAs combined, and for slaughterhouse wastes the concentration of inhibitory substances.

The main recommendations for further research are to gain a better understanding of the kinetics of the AD of garden refuse, building on its better performance compared to other substrates. And, to optimise the performance of co-digestion systems by looking individually at the different operating parameters such as the mixing ratio, the adequate feeding regime, suitability of the sludge used, and concentration of pollutants (e.g. NH_3, H_2S).

Recommendations to waste management officials are to pursue the value of integrating AD technology into MSW management, taking note ofthe important factors confirmed in this dissertation to consider when planning an AD plant.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations

MSW: municipal solid waste

OFMSW: organic fraction of municipal solid waste

BMP: biomethane potential

VFA: volatile fatty acid

FSA: free and saline ammonia

TKN: total kjedhal nitrogen

BC: Bellville Compost

ARTS: Athlone Refuse Transfer Station

UNEP: United Nations Environmental Programme

ASTM: American Society for Testing and Materials

ISWMP: integrated solid waste management plan

IWMP: integrated waste management plan

VOC: volatile organic compound

GHG: greenhouse gas emissions

US EPA: United Stated Environmental Protection Agency

AD: Anaerobic digestion

GDP: gross domestic product

WWTW: waste water treatment works

1 INTRODUCTION

1.1 Background

Management of municipal solid waste (MSW) in developing countries, especially in Africa, is becoming a major cause of environmental and social concern for communities, as well as local and national governments. This is a consequence of increasing waste generated per capita as cities become more developed and industrialised. There is also a general population migration from rural to urban settings. This, coupled with rapid population growth, overpowers the capacity of many cities to provide even the most basic of municipal solid waste management (MSWM) services (Couth and Trois, 2010). Although globally, urban solid waste management has evolved from simple collection for disposal into landfills to an integrated approach, the main challenges experienced in developing cities are often directly linked to a lack of financial and human resources and consequently, a lack of various support and technological services (Barton *et al.,* 2008).

An integrated approach to waste management puts a strong focus on strategies such as waste reduction at source, treatment of waste, resource recovery or recycling and finally disposal in landfills; together these make up an integrated solid waste management plan (ISWMP). An ISWMP uses complementary strategies (technologies or practices) to effectively handle MSW. The focus of any ISWMP lies on the reduction of waste generation at source, re-use and recycling of valuable material to be recovered (Palczynski, 2002; US EPA, 2002).

Disposal of MSW in dumpsites or landfills is the most common method of waste disposal in developing countries, because of the operative ease and the low financial costs attached to landfill disposal (Domingo and Nadal, 2008; UNEP, 1996).However, the environmental risks attached to landfills are significant, including: the release into the environment of the landfill gas, odors from volatile organic compounds (VOCs), and contaminated or toxic leachate. Landfill gas is rich in methane (up to ~50%), which bears risks of explosion and is a significant contributor (up to 4%) of the national greenhouse gas (GHG) emissions (Cointreau-Levine, 1996; Domingo and Nadal, 2008; Pognani *et al.,* 2009). Limited available land in the vicinity of cities, and its irreversible loss after use as a landfill, makes landfilling unsustainable in the long run, despite the fact that landfill designs have evolved from simple open dumps to engineered sanitary landfills which facilitate the capture of landfill gas and treatment of the collected leachate.

The composition of MSW is influenced by different factors such as: income levels, type of domestic fuel used, climate, and seasonal variations in food supplies (Wells, 1994; Wang and Nie, 2001; Metin *et al.,* 2003; World Bank, 2003; Buenrostro and Bocco, 2003 cited in Troschinetz and Milheclic, 2009).

The organic fraction in MSW (OFMSW) in developing countries is usually high and varies between 22-61% according to a study by Diaz *et al.* (2007).Results of a study carried out by Troschinetz and Milheclic (2009) on the composition of MSW for 19 different developing economies, showed that the organic fraction varied between 17% and 80% of the total MSW, with an average of 55%. This agrees with research carried by Collivignarelili *et al.* (2007), which states that the organic content in the solid waste decreases with increasing gross domestic product (GDP) generally. For example, research from US EPA (2003) and Eurostat (2003) showed that the average composition of the organic fraction in MSW is approximately 30% and 25% respectively for the USA and the European Union, compared to 55% for the 19 developing countries examined (Troschinetz and Milheclic, 2009). Treating and recovering useful resources such as energy and nutrients from the OFMSW could therefore help divert ~55% on average of the MSW generated, where applicable, from landfill disposal in developing countries. Biological treatments, including composting and anaerobic digestion (AD) of various organic waste streams are already popular in developed countries, particularly Europe. AD entails the degradation of organic substances in the absence of oxygen; producing biogas (rich in methane), and anutrient-rich sludge.

The United Nations Environmental Programme (UNEP, 2002)speculates that biological treatments would be suitable in developing cities due to the highly organic nature of their waste. While composting has been the method of choice in the past due to the low operating costs; AD has the additional advantage of energy-recovery. Furthermore, new regulations to encourage production of renewable energy often include biogas from waste, making AD a more attractive strategy (Abdullahi *et al.,* 2008). The use of AD to treat and stabilise organic wastes is therefore increasingly being considered for developing countries as solid waste management solution for both rural and urban activities.

1.2 Problem Statement

There exists significant knowledge on the occurrence of MSW in developing economies. However, the characteristics of the biodegradable fraction of MSW and how these would relate to energy recovery in the form of biogas, are less well studied, particularly in Africa. The bulk of the literature consists of studies carried out in other regions such as China (Jiang *et al.,* 2007; Li *et al.,* 2011 and Lianhua *et al.,* 2010) and India (Rao *et al.*, 2010; Chanakya *et al.,* 2009;Gunaseelan, 2003 and Viswanath *et al.,* 2008).

The author of this dissertation was the lead contributor of a team that completed a study of the biomethane potential (BMP) of different organic waste streams for the solid waste department of the City of Cape Town (Munganga *et al.,* 2010).

The study had two main components: firstly, an attempt was made to characterise variability of a residual (post resource recovery) stream at the Athlone Refuse Transfer Station (ARTS) and of the received MSW at the Bellville Compost (BC) plant, especially of the organic fraction. Secondly, laboratory work was done to determine the BMP of samples from these two mixed OFMSW sources, as well as from a range of source-specific organic wastes generally disposed off in landfills.

Whilst this study reported BMP values for 17 different types of waste, it did not extend beyond empirical observations, largely ignoring limitations experienced. It also did not investigate how other characteristics of the studied wastes would affect engineering considerations for the planning and design of anaerobic digesters, as part of an integrated waste management plan for the City.

Furthermore, the waste management official of the City of Cape Town had indicated a particular concern with landfill disposal of slaughterhouse wastes. Many studies report the challenges experienced with AD of slaughterhouse wastes (e.g. Chen *et al.*, 2007; Cuetos *et al.*, 2007; Salminen and Rintala, 2002). The possibility of co-digesting this waste with other organic materials, to overcome lower biogas yields, was introduced but not elaborated in the 2010 report to the City of Cape Town.

1.3 Dissertation Objectives and Scope

This dissertation extends from the previous study for the City of Cape Town, into a critical evaluation of the experimental variables, their interrelationships, the challenges encountered with AD of the organic wastes investigated, and the strategies to overcome them, especially. the strategy of co-digestion for recalcitrant slaughterhouse wastes.

The objectives of this dissertation are therefore to:

1) Investigate the suitability for treatment by AD of typical organic waste streams in MSW, which are often disposed of in landfills, by critically re-analysing the prior bio-methane potential study.
2) Consider the temporal evolution of various process parameters that play a key role in anaerobic digestion of such organic wastes, so as to develop knowledge needed for the design and operation of AD plants, going beyond the bio-methane potential.

3) Make sound recommendations from the data generated, and build a preliminary source of knowledge for waste management officials and independent entrepreneurs, of important factors to consider when planning for an urban waste-based biogas plant.

This dissertation revolves around the experimental investigation of the AD of different typically urban, organic waste fractions. The waste materials investigated varied, from pure industrial and commercial waste streams, to mixed organic wastes found after the processing at the material recovery facility (MRF) at refuse transfer stations. The focus was on determining the BMP of the different waste streams and identifying the respective challenges experienced during the digestion of such wastes based on experimental variables, as well as ways to overcome them. The dissertation also addresses the shortcomings identified relative to the data generated and how those should be dealt with for future undertaking of such research.

The technological applications, social aspects and economic feasibility of the AD of the different wastes are beyond the scope of this dissertation. The laboratory work was organised into two phases. Firstly, AD of16 different waste streams sampled was carried out in batch experiments at 100 mL. The second phase investigated AD of three different waste streams, under fed-batch conditions at 2 L. The co-digestion of high protein wastes, with carbon-rich substrates was investigated under both conditions.

1.4 Dissertation Outline

The remainder of the dissertation is structured as follows: **Chapter 2** presents the literature review, which is organised into three main sections. In the first section, background information is given on the concepts of MSW, and an overview of the milestones achieved with its management. The challenges faced by developing economies in terms of solid waste management are also highlighted, with a focus on the regulatory framework for waste management in South Africa. In the second part, a review of the literature is presented on the microbiology and fundamentals of AD; the third section discusses the main parameters identified as inhibitory, the reported thresholds in the literature, and strategies to overcome the inhibition where applicable.

Chapter 3 presents the research questions which formed the basis of the laboratory investigation to meet the dissertation's objectives. A detailed description of the laboratory set-up and methods used for AD of the various organic wastes, under batch and fed-batch conditions, is presented. The 'Activated Biomass Concept', used to design the feeding regime for the AD experiments under fed-batch conditions at 2 L, is also discussed.

Chapter 4: Results of the batch experiments on the BMP of the 16 organic waste samples are presented. The BMP potential is presented per waste category i.e. (i) garden wastes, (ii) fines and paper sludge, (iii) food wastes, (iv)and abattoir wastes and fisheries wastes.The evolution of experimental variables parameters investigated is also presented. The performance of the different wastes is based on the methane (CH_4) yield, and an analysis of variance was performed to analyse the variability across the different categories. The chapter ends with a discussion on the data generated, and organic load destruction achieved.

Chapter 5: results of AD under fed-batch conditions of kitchen, garden and blood wastes are presented. Results extend from comparison with the batch results of the three waste streams investigated, to the evaluation of parameters investigated throughout the digestion period viz. effect of the feeding regime (g VS/day), biogas productivity (mL/day), pH, concentration of volatile fatty acids (VFAs), and the concentration of free and saline ammonia (FSA).

A general discussion of the key parameters that affected the performance of AD is presented in the last section, with reference with the relevant literature reported. A simulation of the food-to-inoculum ratio, as well as validation with experimental data, of the ABC model used is presented to evaluate the performance of the fed-batch reactors.

Chapter 6 presents results of the co-digestion experiments under batch at 100 mL and fed-batch conditions at 2 L. The VFAs and free and saline profiles (FSA) of the bioreactors at 2 L are also discussed relative to other process variables measured, and the relevant literature reviewed.

Chapter 7 presents general conclusions from the work completed, and recommendations.

2 LITERATURE REVIEW

This chapter is organised into three main sections. The first section presents a general overview of MSW, starting with the milestones achieved in waste management in industrialised nations. The review then proceeds to the state of waste management and the challenges faced in developing countries, from which a strong argument is developed in favour of the key role that biological treatment options can play as part of an effective integrated solid waste management plan (ISWMP). The first section concludes that AD followed by composting is a more efficient option than composting alone. The second section then focuses on presenting AD in more details. A review of the literature is presented on the parameters that influence AD, microbial groups involved, and parameters that influence the process. The third section discusses inhibitory substances and mechanisms identified in literature for AD. The section focuses on inhibitory substances identified in literature e.g. volatile fatty acids (VFAs), ammonia (NH_3), etc.

2.1 Municipal Solid Waste Management Practices: An Overview

2.1.1 What is municipal solid waste?

MSW consists of everyday items that are used and thrown away in a solid or semi-solid form, such as kitchen waste, packaging products, containers, newspapers, grass clippings and miscalleneous inorganic wastes (clothing, furniture, appliances, batteries, tires, etc.). The main sources of MSW are residential homes, commercial businesses, hospitals and other commercial sources. Its collection is normally organised by the local municipality. Generally, MSW does not include industrial and agricultural wastes or sewage sludge. A waste management system often includes the following main categories: collection of the waste, sorting and treating at designated refuse transfer stations, and final disposal of the the waste into municipal landfill sites (US EPA, 2011).

Management of MSW can be challenging due to the vast volumes of waste and its diverse nature; seasonal variations also affect the composition and quantities of waste. The different types of waste can be identified as follows:

- Biodegradable waste: food and kitchen waste, green waste, paper
- Recyclable materials: paper, glass, cans, metals, certain plastics, etc.
- Inert wastes: construction and demolition rubble, dirt, bricks, debris, etc.
- Composite wastes: clothing, tetra packs, certain plastics, etc
- Household hazardous waste and toxic wastes: e-waste, paints, chemicals, batteries, pesticide, etc. (Braber, 1995).

2.1.2 General trends and the waste management hierarchy and MSW

The ‘Waste Management Hierarchy’ originated when environmentalists questioned the common practices of disposal-based waste management in the 1970s. They argued that MSW is not homogenous and should be handled as an ensemble of different materials and treated separately. The concept evolved later in the 1990s, within the framework of industrial management systems and cleaner production, when ‘end-of-pipe’ treatments were found to be inadequate, because of their long term impacts. The waste hierarchy suggests a fundamental change in the nature of environmental interventions. Preference should be given to avoidance, elimination, prevention and considerable reduction in the causes of environmental problems, instead of managing the waste emissions and impacts from the life cycle of a service/product (Gertsakis and Lewis, 2003).

The ‘Waste Management Hierarchy’ has since evolved as a philosophy and core strategy of MSW management for both industrialised and developing economies. Its main principles are prevention and waste minimisation via re-use, recycling, energy recovery and only then final disposal of the residual waste (Figure 1). In general, the extent to which a country/city focuses on different aspects of the ‘waste hierarchy’ depends on factors such as socio-economic circumstances, environmental laws, density of the population and infrastructure, etc (Sakai *et al.,* 1996, Palczynski, 2002).

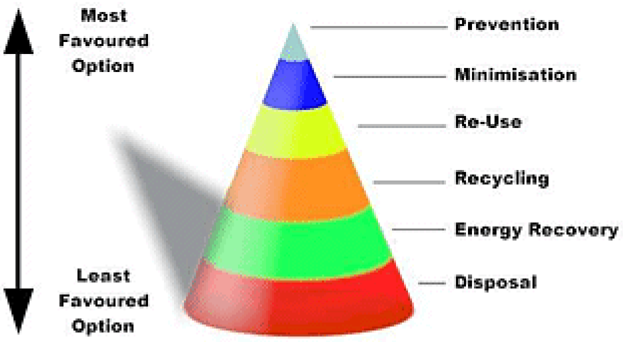


Figure 1: Hierarchy of integrated solid waste management (greenstar, 2009)

Waste minimisation’ applies heavily to the manufacturing sector, where waste avoidance and higher efficiencies in the production process are encouraged by authorities. The objectives of ‘waste minimisation’ are therefore to optimise the use of raw materials, reduction of packaging requirements, and avoidance of costs from handling and managing the waste generated. The consumption aspect of ‘waste minimisation’ is to alter people’s attitude towards consumerism by encouraging re-use and recycling with emphasis on values such as ‘environmental-friendliness’, product quality and durability, etc.

Material recovery is achieved through re-use and re-cycling, which are two different concepts, even though they are often used interchangeably. Re-use of materials leads toreduction in waste generation, even though the materials still need to be handled, processed/managed and transported. Recycling, on the other hand, is the use of wastes as raw materials to produce an identical or new product. Recycling for most materials serves to preserve materials and prevent waste for treatment or disposal. It is considered beneficial, as the environmental impacts, caused by extraction of raw materials and waste management, are reduced (Sakai *et al.,* 1996).

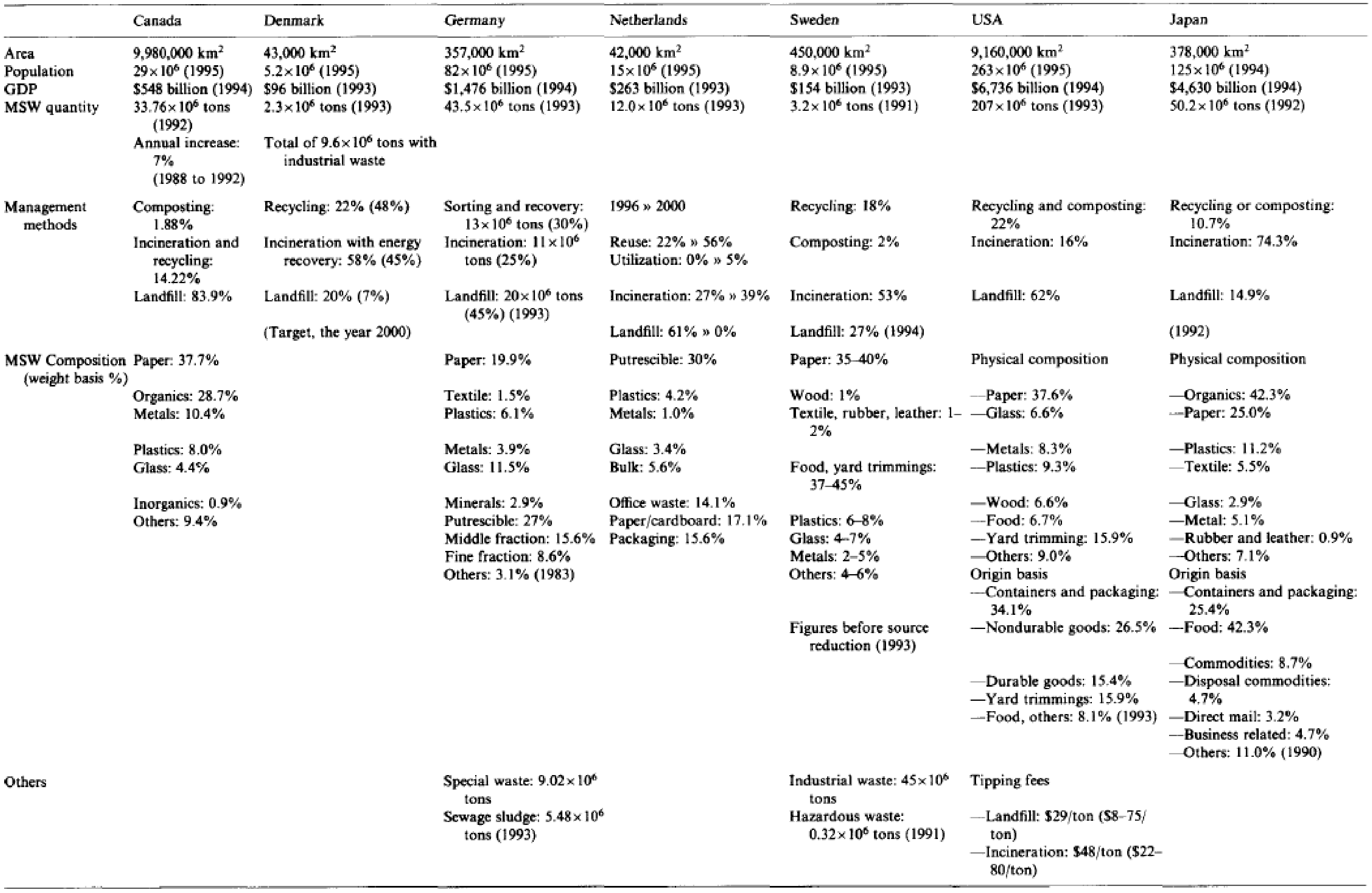
A clear understanding of the characteristics of the waste (composition and volumes) from each country is essential prior to planning policies for an effective waste management strategy. This is particularly relevant when focusing on initiatives, such as recycling of specific waste materials (e.g. paper, plastics, bottles, etc.).

2.1.3 General trends in MSW management in industrialised countries

The comparison of waste data from different countries can be problematic due to the ambiguity associated with defining the term MSW within each administrative entity. The ways in which data are collected and compositional classification vary considerably (Sakai *et al.,* 1996). Sakai *et al.* (1996) compiled waste data recognising these differences, from 7 developed countries, Canada, Denmark, Germany, Japan, the Netherlands, Sweden and the United States over a decade ago (Table 1). The waste characteristics, quantities and common treatment options in each country are outlined when waste minimisation initiatives, based on the waste hierarchy, were initiated about 14 years before the study was conducted. Landfilling has been the main method for handling MSW for most countries, except when the bulk of the waste is incinerated as is the case for Japan for example (Table 1).Landfill designs have evolved from open dumps to engineered sanitary landfills; where landfill gas is captured, and the leachate generated is treated to mitigate greenhouse gas (GHG) emissions and minimise the environmental effects. The gas generated at landfills is from the biodegradation of the putrescibles and is rich in methane (CH_4), a GHG and contributor to global warming (Domingo and Nadal, 2008). However, limited land areas make the option of landfilling unsustainable in the long run for any country despite the level of environmental control achieved with sanitary landfills.

The waste in high-income countries tends to have a high calorific value and low moisture content, which makes options such as incineration a viable option for waste treatment and energy recovery due to lower energy demand (US EPA, 2002; Thomas, 2006; Mohee, 2002).Incineration has been the treatment of choice for the waste, rather than other initiatives such as composting or recycling for developed countries. For example, the amount of waste incinerated varies from at least 14% in Canada to 74% in Japan. The main reason for using incineration is the high percentage of volume reduction (up to 90%) and sterilisation that can be achieved with the waste through incineration (Table 1).

Table 1: Overview of the MSW and main treatment options in 7 developped countries (Sakai *et al.,* 1996)



	Canada	Denmark	Germany	Netherlands	Sweden	USA	Japan
Area	9,980,000 km^2	43,000 km^2	357,000 km^2	42,000 km^2	450,000 km^2	9,160,000 km^2	378,000 km^2
Population	29×10^6 (1995)	5.2×10^6 (1995)	82×10^6 (1995)	15×10^6 (1995)	8.9×10^6 (1995)	263×10^6 (1995)	125×10^6 (1994)
GDP	\$548 billion (1994)	\$96 billion (1993)	\$1,476 billion (1994)	\$263 billion (1993)	\$154 billion (1993)	\$6,736 billion (1994)	\$4,630 billion (1994)
MSW quantity	33.76×10^6 tons (1992)	2.3×10^6 tons (1993)	43.5×10^6 tons (1993)	12.0×10^6 tons (1993)	3.2×10^6 tons (1991)	207×10^6 tons (1993)	50.2×10^6 tons (1992)
	Annual increase: 7% (1988 to 1992)	Total of 9.6×10^6 tons with industrial waste					
Management methods	Composting: 1.88%	Recycling: 22% (48%)	Sorting and recovery: 13×10^6 tons (30%)	1996 » 2000	Recycling: 18%	Recycling and composting: 22%	Recycling or composting: 10.7%
	Incineration and recycling: 14.22%	Incineration with energy recovery: 58% (45%)	Incineration: 11×10^6 tons (25%)	Reuse: 22% » 56% Utilization: 0% » 5%	Composting: 2%	Incineration: 16%	Incineration: 74.3%
	Landfill: 83.9%	Landfill: 20% (7%)	Landfill: 20×10^6 tons (45%) (1993)	Incineration: 27% » 39%	Incineration: 53%	Landfill: 62%	Landfill: 14.9%
		(Target, the year 2000)		Landfill: 61% » 0%	Landfill: 27% (1994)		(1992)
MSW Composition (weight basis %)	Paper: 37.7%		Paper: 19.9%	Putrescible: 30%	Paper: 35–40%	Physical composition	Physical composition
	Organics: 28.7% Metals: 10.4%		Textile: 1.5% Plastics: 6.1%	Plastics: 4.2% Metals: 1.0%	Wood: 1% Textile, rubber, leather: 1–2%	—Paper: 37.6% —Glass: 6.6%	—Organics: 42.3% —Paper: 25.0%
	Plastics: 8.0% Glass: 4.4%		Metals: 3.9% Glass: 11.5%	Glass: 3.4% Bulk: 5.6%	Food, yard trimmings: 37–45%	—Metals: 8.3% —Plastics: 9.3%	—Plastics: 11.2% —Textile: 5.5%
	Inorganics: 0.9% Others: 9.4%		Minerals: 2.9% Putrescible: 27% Middle fraction: 15.6% Fine fraction: 8.6% Others: 3.1% (1983)	Office waste: 14.1% Paper/cardboard: 17.1% Packaging: 15.6%	Plastics: 6–8% Glass: 4–7% Metals: 2–5% Others: 4–6%	—Wood: 6.6% —Food: 6.7% —Yard trimming: 15.9% —Others: 9.0% Origin basis —Containers and packaging: 34.1%	—Glass: 2.9% —Metal: 5.1% —Rubber and leather: 0.9% —Others: 7.1% Origin basis —Containers and packaging: 25.4%
					Figures before source reduction (1993)	—Nondurable goods: 26.5%	—Food: 42.3%
						—Durable goods: 15.4% —Yard trimmings: 15.9% —Food, others: 8.1% (1993)	—Commodities: 8.7% —Disposal commodities: 4.7% —Direct mail: 3.2% —Business related: 4.7% —Others: 11.0% (1990)
Others			Special waste: 9.02×10^6 tons Sewage sludge: 5.48×10^6 tons (1993)		Industrial waste: 45×10^6 tons Hazardous waste: 0.32×10^6 tons (1991)	Tipping fees —Landfill: \$29/ton (\$8–75/ton) —Incineration: \$48/ton (\$22–80/ton)	

Modern incineration facilities, particularly in Europe, are designed with energy recovery either in the form of process steam for heating, or electricity generation. However, the capital costs of incineration facilities have escalated due to the requirements for further treatment of the flue gas and ash generated as a consequence of ever-increasing rigid environmental regulations (Sakai *et al,*. 1996).

The efforts and initiatives made to shift to more sustainable practices in waste management, based on the waste hierarchy, are evident in waste data, particularly from the European Union (EU) countries. It can be observed in Figure 2 that the amount of waste going to landfills has been significantly reduced compared to Sakai *et al.,* 1996 data (Table 1). For example, the amount of waste to landfills was reduced from 45, 53 and 61% to less than 10% in the Netherlands, Germany and Sweden respectively (Eurostat, 2009).

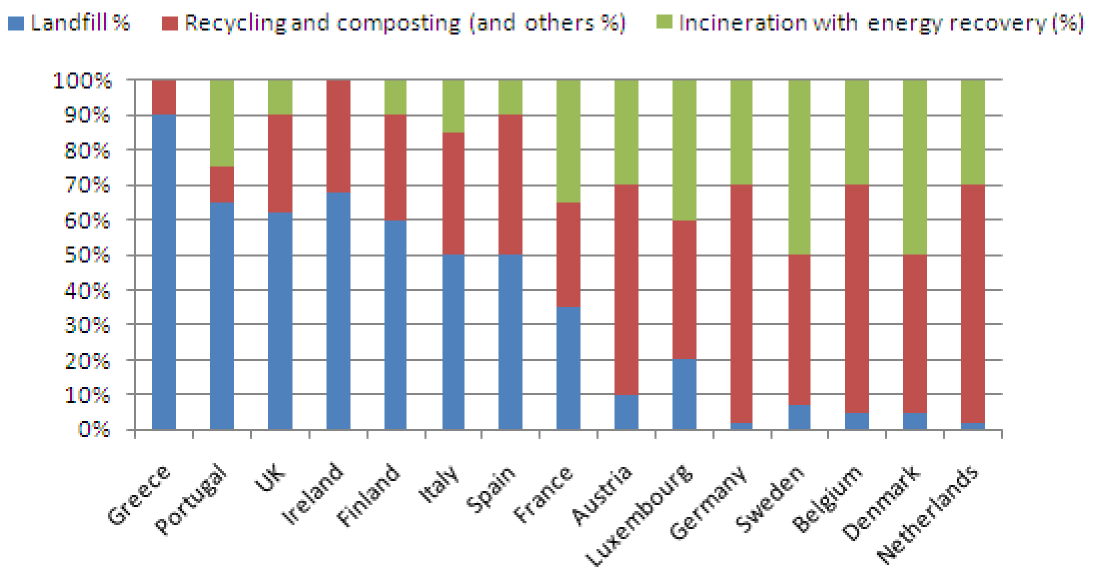


Figure 2: MSW management in the European Union in 2006 (Adapted from Eurostat, 2009)

Waste minimisation initiatives can only target a fraction of the waste and further management options are required for the residual waste. The study by Sakai *et al.,*(1996) points out the organic and paper wastes still constitute the major fraction of waste streams for most countries despite, the respective variability of their wastes (Table 1). Biological treatments, such as composting and AD, are increasingly considered as viable options for treating the biodegradable fraction of the waste and recovery useful products. Many European countries are implementing changes in their regulation to encourage high-end quality products (Sakai *et al.,* 1996). For example, the drive to achieve high quality compost products, has led to the implementation of separation of the waste at source on a ‘dry-wet basis’, to reduce contamination in the final compost product (Sakai *et al.,* 1996). Source separation of the bio-waste from other recyclables has been encouraged in a number of other countries since.

Experience in Switzerland, Germany, the Netherlands and Denmark has shown that separation at source has been successful. For example, 50-70% of the OFMSW is collected and recovered with minimal contamination, enabling the production of good quality compost (Braber, 1995). The alternative to ‘separation at source’ is mechanical separation of the waste. The organic fraction of OFMSW collected is separated from other contaminants before treatment using rotary drums, sieves, etc. However, compost produced from a mixed waste stream will often not meet the required standards for use as soil conditioner (Braber, 1995).

Composting has been the method of choice in the past due to the low operating costs. However, composting is reported as a net energy consuming process. The energy consumed per ton of waste is 30-35 kWh on average for composting, while AD is a net energy producer (100-150 kWh per ton of waste is produced on average). Furthermore, high energy prices and increasing levies on landfill site disposal are positioning AD as the more viable and interesting alternative (Sakai *et al.,* 1996; WASTE, 2009; Braber *et al.,* 1995). This is corroborated by recent work which argues that biological treatment of MSW would be optimised by first producing biogas through AD, and then subjecting the residual sludge to composting(UNEP, 2002).

In the past, the nature of the organic fraction determined the suitable treatment method i.e. aerobic composting was preferred for yard trimmings and garden wastes since these are rich in ligno-cellulosic material hard to biodegrade. AD on the other hand, was found more suitable for wet organic waste without structure such as kitchen waste. AD of paper has also been controversial despite a few trial experiments that indicate its viability both as the sole substrate or when co-digested (Braber, 1995).

2.1.4 Overview of solid waste management in developing countries

Many cities in developing countries have experienced rapid urbanisation in the last 50 years or so, and the African continent is no exception e.g. countries like Nigeria, Kenya, Mauritius and South Africa have experienced continuous growth of their cities. The tremendous urbanisation has resulted in rapid population growth coupled with increased purchasing power per capita, and waste generation rates overall. This altogether often overpowers the capacity of most municipalities to provide even the most basic of solid waste management services.

The common characteristics of solid waste management in developing countries are inadequate service levels, poor waste and environmental awareness of the communities, inefficient practices of waste collection, illegal and uncontrolled dumping, scavenging and littering due to limited resources and lack of financial planning.

Disposal of MSW in landfills is the most common method of waste management in developing countries, including Africa. The operative easiness and the low financial costs attached to landfill disposal make this an attractive option (Domingo and Nadal, 2008). The majority of landfills are simple open dumps, where the landfill gas and leachate generated are released to the surrounding environment, as opposed to sanitary landfills in with industrialised nations. Open burning of waste material is often practised, releasing toxic fumes. Landfills in developing cities, are therefore, a major cause of environmental and public health concern for surrounding communities due to the exposure to pathogens, toxic substances and release of various gases to the surrounding environment (Al-Khatib *et al.,* 2007; Barton *et al.,* 2008).

Resource recovery for recycling is often described as an informal activity, accounting for limited amounts of waste recovered in most urban areas in developing countries (Agarwal *et al.,* 2004). Such descriptions must be critiqued as somewhat naive, as the practices of informally operating "scavengers" invariably forms part of a supply chain involving formal enterprise in collecting the waste, and industry in processing. The urban waste management challenge is to meet high standards in service delivery with limited resources and lack of adequate data.Most integrated solid waste management plans (ISWMP) being developed have adopted the 'waste hierarchy' (as explained in details in Section 2.1.2), from which strategic initiatives are derived.

It is important to note that the current technology does not allow the exclusion of landfilling from any waste management system as the residual waste would still need to be disposed off. The main goal of integrating the waste management hierarchy into any solid waste management system is therefore to shift the waste management pyramid as illustrated in Figure 3. This will not only reduce the amount of waste to landfills, but also limit landfilling only to the residual waste fraction that cannot be recovered further after recycling, treatment and energy recovery (Mohee, 2002; Thomas, 2006).

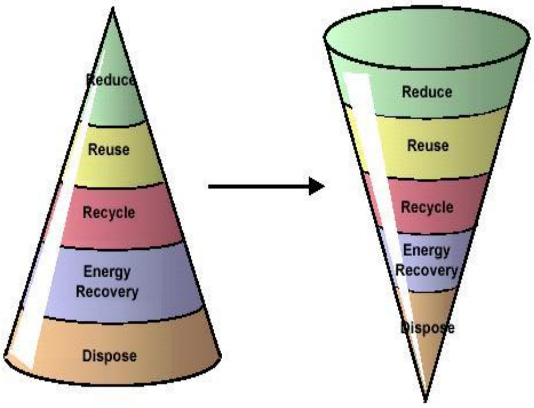


Figure 3: Desired shift in waste management (Fermanagh District Council, 2010)

The focal point of any ISWMP to be instituted in developing economies should therefore revolve around the reduction of waste generation, re-use and recycling of material that can be recovered, and the integration of AD and/or composting of the OFMSW; and only resort to landfills for disposal of the residual fraction of the MSW (Palczynski, 2002).The amount and nature of the waste (general vs. hazardous), its potential to generate significant leachate volumes in relation to the local water balance usually determine the design and operation of the waste disposal facilities. MSW in developing countries is different from that of developed countries e.g. it is dense, richer in organics (kitchen, vegetables, etc.), has a higher moisture content, and lower calorific value compared to that in developed countries. The calorific value (CV) of MSW is a function of the moisture content and hydrogen content of the waste. Hence, the CV of materials such as waste tyres and plastics would be higher than that of soft organics e.g. kitchen scraps, garden wastes (UNEP, 2009).

Supporting this, a net increase in paper, glass, metals consumption and a decrease in kitchen waste is observed when comparing MSW data in developed versus industrialised countries as illustrated in Figure 4 and Table 2 (Asomani-Boateng, 1999; Troschinetz and Mihelcic, 2009).

MSW generation rates increase with gross national product (GNP) in general; the waste per capita in developing countries is therefore considerably lower than in industrialised nations as presented in Table 2. For example, the average waste per capita for developing countries is 0.5 kg/day compared to 1.25 for developed countries. The high organic fraction of MSW in developing countries can be explained by the fact that other materials (glass, plastics, metals, etc.) are often retrieved and reused from the waste streams mostly by the informal sector (Thomas, 2006).

Table 2: Characteristics of MSW by level of industrialisation (Adapted from Thomas, 2006)

	Developing countries	**Middle-income countries**	**Industrialized countries**
Waste generation	0.4-0.6	0.5-0.9	0.7-1.8
MSW wet density (kg/m3)	250-500	170-330	100-170
H_2O %	40-80	40-60	20-30
Wt % composition			
Paper	1.0-10	15-40	15-40
Glass, Ceramics	1.0-10	1.0-10	4.0-10
Metals	1.0-5	1.0-5	3.0-13
Plastics	1.0-5	2.0-6	2.0-10
Leather, Rubber	1.0-5	–	–
Wood, Bones, Straw	1.0-5	–	–
Textiles	1.0-5	2.0-10	2.0-10
Putrescibles	40-85	20-65	20-50
Inerts (Miscallenous)	1.0-30	1.0-30	1.0-20

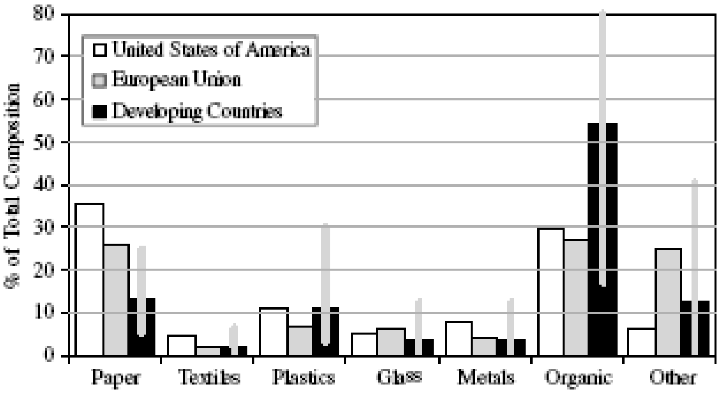


Figure 4: Comparison of MSW composition between developed and developing countries (Troschinetz *et al.*, 1999)

Waste treatment options such as incineration of MSW are generally considered inappropriate technology for developing economies, despite the fact a volume reduction between 80 and 95% can be achieved (Rand *et al.,* 2000). The main reasons provided in literature are linked to the high initial financial capital and operational costs required, lack of technical expertise, spare parts and repair technology.

These factors altogether act as barriers for successful implementation of the technology. The bulk of the costs is associated with mitigation of the environmental hazards. For example, scrubbers for post-treatment of the flue gas, and adequate disposal of the ash generated have become mandatory for incineration facilities due to stringent environmental regulations (UNEP, 1996 cited in Zerbock and Candidate, 2003).

Focusing on the OFMSW in developing countries could help to divert a sizeable portion of the MSW generated from being disposed off in landfills. It is argued that biological treatments, particularly AD could be suitable in developing cities due the highly organic nature of the waste, the added benefit or production of renewable energy, the simplicity and flexibility of the technologies, as they can be adapted to smaller and decentralised applications (UNEP, 2002; Ponsa *et al.,* 2008).

2.1.4.1 Solid waste management and South Africa

The Department of Environmental Affairs and Tourism reports that the average middle class South African generates 0.7 kg per day on average (DEAT, 2006 cited in Pegels,2010). This translates to about 2.7 million tonnes, generated per year by the increasing middle class. The organic fraction constitutes about 40% generally, and MSW is disposed off in landfills without any prior treatments. The waste system and legislation focused on the “concentrate and contain” approach in the past. However, the Polokwane Declaration signed during the first South African Waste Summit in 2001 has set new standards towards “reduction of waste generation and disposal by 50% and 25 % respectively by 2012 and the development of a ‘zero waste’ plan by 2022”.

Solid waste disposal in South Africa is regulated by the minimum requirements for disposal of waste by landfill, published by the department of water affairs and forestry (DWAF, 1998).Research by Matete and Trois (2007) shows that the waste hierarchy in South Africa is now centered on the 3R principle (i.e. Reduce-Reuse-Recover- Dispose) which was introduced by some local governments in the form of “by laws” in 2000 to reach sustainability goals. It is within the context of the waste hierarchy, that ‘waste minimisation’ and ‘zero waste’ emerged as tangible tools. Strategies for waste minimisation include reducing the amount of waste generated, reuse and recovery; while the concept of zero waste goes beyond as it ensures that new products are reused, repaired or recycled back into nature or on the market.

Matete and Trois (2007) developed a model based on different strategies to attain ‘waste minimisation/ zero waste’ for post consumer wastes in local urban communities, using the city of Durban as a case study. Results show that two main steps can be adopted to achieve ‘zero waste’: the first step should encourage strong efforts towards waste minimisation at point of purchase and reuse at a household level for products.

The second step should be to pioneer the implementation of a ‘wet-dry’ separation system to enable efficient recovery of the remainder waste.

In light of this for example, the city of Cape Town (CoCT) initiated in 2007 the ‘Think Twice’ campaign as a pilot project area to divert recyclables from landfills in specific. Wet (kitchen, and garden wastes) and dry waste (recyclables mainly) are collected separately and the dry waste sent to material recovery facilities for reprocessing and re-using (City of Cape Town, 2011).Further work by Trois *et al. (*2007) pursues this second step, arguing that the important criteria for selecting viable technologies and waste treatment options in South Africa should be low-cost infrastructure, labour-intensive for job creation, low input of energy, and applicability in daily landfill operations. Trois *et al.* (2007) suggested that composting of the organic waste in windrows using passive aeration, and post-composting mechanical treatment to separate the OFMSW would be the ideal scenarios for South Africa due to the low capital costs, energy inputs and the benefits of job creation since the process would be labour intensive. Dome aerated technology (DAT) was selected as the technology of choice for the first pilot project in Durban. However, the success of the windrow composting was limited due to the lack of suitable substrates, structural material for composting, and a poor market for the finished product (Trois *et al.,* 2007; Griffith, 2005).

2.1.4.2 Potential value of a waste-to-energy (WtE) sector in South Africa

Encouraging renewable energy (RE) technologies, waste-to-energy included,could provide additional supply to the grid and help reduce the carbon intensive economy in South Africa.

The energy sector in South Africa faces the following challenges:

- power shortages, as a result of the limited capacity of electricity supply and the narrow reserve margin
- immense pressure to increase the electricity supply as studies project a doubled demand within the next 15 years
- limited resources available for extension programmes of the grid to meet the demand within the next decade.Eskom, the sole power supplier in South Africa is underfunded but has predicted that it would require an estimated ZAR 300 billions over the next decade for the required infrastructure upgrade to increase electricity supply to the projected demand
- the need to develop a low carbon economy to meet sustainability goals and tackle climate change. Coal still constitutes 86% of the electricity mix and South Africa contributes 40% of GHGs emission in Sub-Sarahan Africa (Pegels, 2010).

The high costs of RE technologies relative to the low electricity costs in SA, have been the main barrier for the setbacks and reluctance experienced in the deployment of RE in the past. For example, despite the increase in electricity prices since the first power outages experienced in 2007/2008, the equivalent price of electricity in South Africa is EUR 0.03/kWh compared to a minimum cost of EUR 0.05/kWh from wind power (Pegels, 2010).

However, efforts from the government such as the “White Paper on Renewable Energy” published by the South African Department of Mineral and Energy (DME) are providing a framework to overcome the barriers and help establish RE projects (Pegels, 2010). The ‘White Paper on Renewable Energy’, represent the vision of the South Africa’s government on the principles, objectives and strategic goals on the introduction and endorsement of RE (DME 2003, cited in Pegels, 2010). The document stated South Africa’s target of installing 10 GW of renewable energy (RE) by 2013. Following from this, the introduction of the Renewable Energy Bidding Tariffs (REBID in August 2011, by the Department of Energy will support independent power producers for the first roll-out of large scale grid-connected RE). This coupled with the recent increase in the electricity price are also motivations that will drive further the deployment of RE technologies and implement energy efficiency (EE) projects.

Landfill gas, biomass and biogas are also included in the procurement process and would be generated at a cheaper price compared to other technologies considered viz. Mainly wind and solar photovoltaics (for example, R0.6/kWh for biogas versus R1.15/kWh for wind). This altogether has repositioned anaerobic digestion as a viable technology for production of power from biomass. Biogas also has the potential to increase the energy access in remote areas since the technology is suitable for small-scale applications (Pledges, 2010; Amigun and von Blottnitz, 2010).

2.1.4.3 Anaerobic digestion in South Africa

AD is an established technology in South Africa i.e. it is commonly being used in wastewater treatment works/facilities (WWTWs) to reduce the amount of sludges. Unfortunately, most of the digesters are either not operated properly, or the biogas is simply flared and not recovered (Snyman *et al.,* 2006; Ross *et al.,* 1992).Anaerobic digestion of organic waste, could therefore be viable and be implemented at reduced costs using the digesters already installed at WWTWs as has been the case for Russia (Greben *et al.,* 2008).

Recently, Greben *et al.* (2008) investigated at a laboratory scale the co-digestion of primary sludge generated at the WWTWs with kitchen waste at different ratios. This was to demonstrate that revalorising the digesters in place, co-digesting the organic wastes with the sludge to produce biogas can be feasible. Results showed that the highest biogas yield was generated when a mixture 30% of kitchen waste, with 70% primary sludge was used.

2.2 Biological Treatment of Municipal Solid Waste: A Focus on AD

Anaerobic digestion is not a new technology, the first record of industrialised operations dating back to the 1895 in Exeter (England) at a sewage treatment facility (McCabe *et al.*, 1957). It occurs naturally in the rumen of cows, and the process itself has been exploited for many uses; particularly to stabilise the sludge generated at wastewater treatment plants (Janssen, 2010). The designs of the first pilot plants to anaerobically digest the organic fraction of municipal solid wastes (OFMSW) emerged in the early 1990s (Karagiannidis and Perkoulidis, 2009; Braber, 1995).

Biogas from organic wastes (kitchen, crop waste, manures, etc.) has also gained popularity in rural areas of China and India for cooking and lighting (Feng *et al.,* 2009; Bhatia, 1990). Experience in China and India, demonstrate the value in extending the use of AD to the treatment of different organic wastes in other developing economies, particularly on the African continent. As discussed in Section 2.1, MSW is largely landifilled without prior treatment in Africa (Domingo and Nadal, 2008); whilst at the same time energy provision is often problematic or reliant on traditional means. Yielding biogas from OFMSW could possibly be an ideal technology for addressing both energy provision and waste management (Greben *et al.,* 2008).

2.2.1 Process overview

AD constitutes the biodegradation of organic materials (biomass, sewage sludge, etc.) under the action of fermentative micro-organisms, in the absence of oxygen. The outputs are a methane rich (50 – 70 %) gas stream which is the renewable energy source, and an effluent sludge, which has a high nutrient value and can be used as soil conditioner if not contaminated. The general composition of biogas is presented later in Table 3 (Section 2.2.5).

Equation 1(Chynoweth *et al.*, 1987) illustrates the degradation process; the biogas produced consists mainly of carbon dioxide (CO_2) and methane (CH_4), literature reports that the ratio of CO_2:CH_4 can vary between 1:1 to 1:3, depending on the feedstock used and the process variables. Water (H_2O), ammonia (NH_3), and hydrogen sulphide (H_2S) are also produced as by-products in trace amounts. The average heating value of the biogas is reported to be 23MJ/Nm^3 depending on the amount of methane. Biogas can be further treated to remove contaminants such as H_2S; however it is suitable for use without much pre-treatment (Appels *et al.*, 2008).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad \textbf{Equation 1}$$

Where: *n, a, b* =stoichiometric coefficients

The process itself is complex as it involves microbial and physico-chemical mechanisms and reactions. All these mechanisms are closely linked and need to be optimised in order to ensure stability of the AD system (Greben *et al.*, 2008).

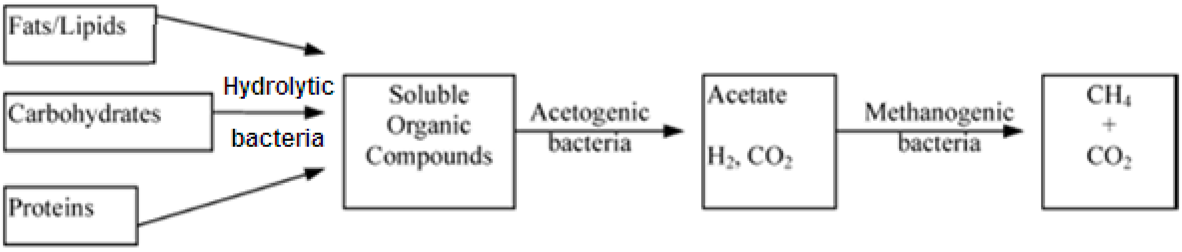
2.2.2 Microbial processes in anaerobic digestion

There are four main groups of microbes identified which operate in a synergetic way in AD. The different groups: hydrolytic, acidogenic and acetogenic bacteria which constitute the first three groups, and the last group is the methanogenic archaea responsible for CH_4 generation. Hydrolysis is the first step in AD, where insoluble complex organic compounds are broken down into smaller soluble monomers. Organic acids are then produced using the monomers from hydrolys in acidogenesis. In acetogenesis, the acids produced are used to produce mainly acetate, hydrogen (H_2) and CO_2. Finally CH_4 and CO_2 are produced in methanogenesis using acetate as the main substrates as illustrated in Figure 5.

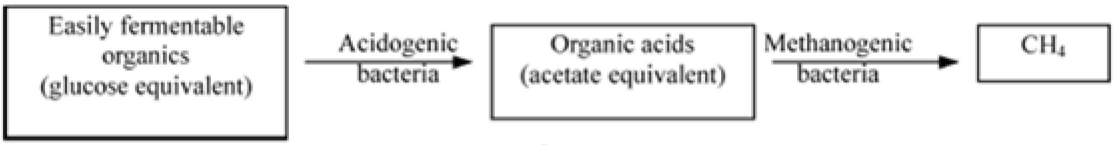
2.2.2.1 Hydrolytic bacteria

Hydrolytic bacteria break down complex organics such as fats, proteins and polysaccharides (e.g. starch, cellulose) into soluble monomers such glucose, fatty and amino acids (Figure 5). Hydrolysis is generally considered the rate-limiting step in AD for complex organic molecules as it provides substrate materials for the subsequent phases of AD (Chynoweth *et al*., 1987). The hydrolysis rate depends mainly on the physical structure and chemical composition of substrates to be digested. For example, for cellulosic materials (e.g. garden wastes), the action of hydrolytic enzymes is hindered by the presence of complex organic molecules such as cellulose, hemicelluloses and lignin which do not biodegrade easily. While hydrolysis occurs faster with easily fermentable substrates such as sugars, smaller molecules found in kitchen scraps, etc (Chynoweth *et al.* 1987; Ward *et al.*, 2008).

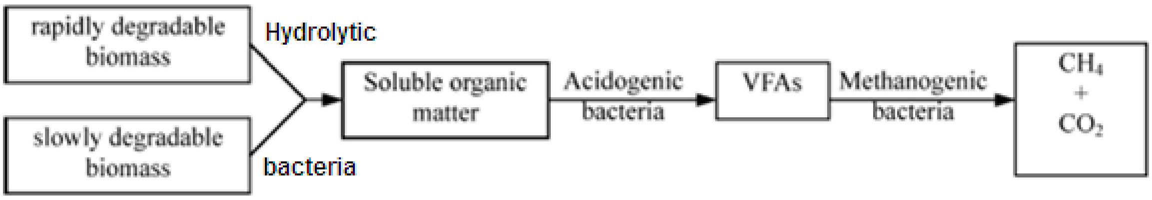
A phase may be slower than other when a complex process such as AD is composed of sequences of biological reactions. The slower phases in AD of organic wastes are generally considered to be either methanogenesis (Section 2.2.2.3), or alternatively hydrolysis of solids or complex organic molecules (Vavilin *et al.*, 1996).



Schematic biochemical process stages of anaerobic digestion (Kleinstreuer and Powegha, 1982)



Flow chart of Moletta et al. (1986) model



Flow chart of Smith et al. (1988) model

Figure 5: Different models of the schematic biochemical process stages of AD. Adapted from (Kleinstreuer and Powegha, 1982; Moletta *et al.,* 1986; Smith *et al.,* 1988 cited in Lyberatos and Skiadas, 1999)

2.2.2.2 Acidogenic and acetogenic bacteria

Acidogenic bacteria convert the organic monomers from hydrolysis (glucose, amino and fatty acids) into organic acids (Equations 2 and 3, adapted from Chynoweth *et al.*, 1987). In general, the main organic acids generated vary between C2-C6 chain lengths; these are acetic, propionic, butyric, valeric and caproate acids (Wang *et al.*,1999). The effect of the different organic acids is discussed later in detail in Section 2.3.1.During acetogenesis organic acids, synthesised previously, are converted to acetate, CO_2 and hydrogen (H_2) as illustrated in Figure 5, model developed by Kleinstreuer and Powegha (1982).

$$Sugars \rightarrow CH_3CH_2COOH + 2H_2O$$ **Equation 2**

$$Amino.Acids \rightarrow Alcohols/L.Acid/F.Acids$$ **Equation 3**

The extensive published literature on AD of easily degradable organic materials agree that the first three stages of AD tend to occur relatively faster compared to methanogenesis due to the slower growth and regeneration rate of the methanogenic archaea.

For example, Deublein and Steinhauser (2008) report that the regeneration rate of methanogens, mainly responsible for production of CH_4 gas, is 5-16 days compared to 24 hours, and 80-90 hours for the bacteria involved acidogenesis and acetogenesis respectively. This is particularly the case for easily biodegradable organic molecules where hydrolysis is not rate limiting.

Based on the previous argument, the biogas peak observed in the early days of AD in the published literature are generally attributed to the effect of acidogenesis and acetogenesis where Hydrogen gas (H_2) and CO_2 are released from the reduction of the hydrolysed monomers and organic acids.

2.2.2.3 Methanogenic archaea

The methanogenic archaea are often found in deep sediments or in the rumen of herbivores, they are responsible for the production of CH_4 and CO_2. Methanogens are classified into 2 main groups: the methylotropic methanogens which digest acetate, methanol, methylated amines. The non-methylotropic methanogens belong to the second group. These use H_2, CO_2 and formate to produce CH_4(Equations 4 and 5), Chynoweh *et al.*, (1985). Research carried out on this group of bacteria has identified up to 33 different methanogens depending on the the specific substrate they transform (Chynoweth *et al.*, 1987).

In general, 70-73% of the CH_4 produced is from acetate. The remaining fraction of CH_4 is mostly from the reaction of H_2 and CO_2 from the other substrates such as formate, methanol and mono-, di-and trimethylamines (Chynoweth *et al.*, 1987; Chen *et al.*, 2008). The growth of methanogens is stimulated by trace elements such as vitamins,minerals (e.g Coa and Ni) and nutrients (e.g fatty acids).The action of methanogens is generally hindered in acidic conditions that can result from hydrolysis and acidogenesis and, therefore, reduce the methane yield (Chynoweth *et al.*, 1987).

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \textbf{Equation 4}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \textbf{Equation 5}$$

2.2.3 Important parameters in anaerobic digestion

The success of an AD system lies heavily on the effective growth of microbes and the respective biochemical reactions in a symbiotic manner. It is therefore essential to monitor process parameters to optimise the biogas, CH_4 yield and biodegradation. This section discusses some of the common control parameters reported in literature.

2.2.3.1 Waste composition (carbon-to-nitrogen ratio)

The composition of the organic wastes influences the performance of AD systems. The carbon-to-nitrogen (C:N) ratio is a useful indicator of the waste composition and an important parameter in AD. The amount of carbon (C) constitutes the source of energy for the microorganisms while nitrogen (N) is essential for microbial growth for nucleic and amino acids (Salminen and Rintala, 2002).An insufficient amount of N will result in a smaller bacterial population and longer periods for the digestion process.

Generally, C is consumed 30-35 times faster than N. For this reason, Monnet (2003) proposes that the ideal range of the C:N ratio for substrates should be between 20 and 30. Similarly, Resh *et al.* (2011) argues that a C:N between 16 and 25 would result in a satisfactory performance. C: N ratios above the recommended range (i.e. an excess of carbon) slow down the degradation process since there is excess of degradable substrate for the microorganisms. While, C:N ratios below the recommended range (i.e. an excess of N beyond the microbial requirements) result in conversion of the extra N into ammonia (NH_3) under anaerobic conditions. High concentrations of NH_3can lead to inhibition in the AD process (Bernal *et al.*, 2008). This will be further elaborated in Section 2.3.5.The C:N ratio can be expressed differently. For example, Resch *et al.* (2011) used the Chemical Oxygen Demand-to-Total Khedjal Nitrogen (COD:TKN) as an indication of the C:N ratio while investigating AD of slaughterhouse wastes.

Co-digestion of different but complementary waste streams has generally been used to adjust the C:N ratio and provide suitable substrates. There have been extensive studies on the co-digestion of sewage sludge with kitchen waste and OFMSW. Nitrogen-rich materials such as urea, clean sewage sludge and manure can be used as sources of additional N (Sosnowski *et al.*, 2003; Gomez *et al.*, 2006; Cuetos *et al.*, 2006; Luste *et al.*, 2010).

2.2.3.2 Temperature

AD most commonly occurs under mesophilic conditions (i.e. 20–45°C), or under thermophilic conditions (i.e. 50 –65°C); the temperature is an important factor since AD microbes are temperature dependent. The optimal temperatures reported for AD are between 30 and 38°C for mesophilic conditions, and between 44 and 57°C for thermophilic conditions. Biodegradation and biogas production rates generally increase with temperature (Igoni *et al.*, 2008).

Thermophilic conditions lead to higher performance such as higher biogas yield, solids and chemical oxygen demand (COD) reduction (Coelho, 2011; Gannoun, 2009). However, the process generally becomes unstable under thermophilic temperatures because thermophilic bacteria are very sensitive to temperature changes.

The overall effect often requires higher energy inputs to maintain the digester constantly at higher temperatures, which can ultimately reduce the digester's efficiency (Dugba and Zhan, 1998). Tchobanoglous *et al.* (2003) also report that temperatures between 25-35°C are optimal for microbial growth, biological reaction rates and maintaining a stable process. Running AD digesters under mesophilic temperatures, is still the preferred choice for plant operators, since decent biogas, CH_4 yield and process stability can be maintained. Typical AD systems in European countries (about 62% of plants) operate at mesophilic conditions (Igoni *et al.*, 2008; Karagiannidis and Perkoulidis, 2009).

It is common practice that small-scale AD, especially in rural areas, is carried out at ambient temperatures, which may fall into the psychrophilic conditions i.e. between 15 and 20°C (Nohra *et al.*, 2003). There are conflicting views about psychrophilic AD. For example, the work by Tchobanoglous *et al.* (2003) on AD of MSW claims that lower temperatures do not allow for the degradation of long chain fatty acids, leading to foaming and process inhibition. However, work by Connaughton *et al.* (2006) on the AD of waste stream from breweries observed similar yields between psychrophilic conditions and mesophilic conditions; and allowed a higher organic loading rate for AD of brewery waste.

2.2.3.3 pH

The concentration of protons (H^+) in the digester can be inhibitory to the growth, especially of the methanogenic archaea, when levels of acidity are high (Viessman and Hammer, 1993; Eckenfelder, 2000). The products of intermediate phases influence the pH; particularly the production of volatile fatty acids (VFAs) by acidogens. Accumulation of VFAs generally lowers the pH in the reactor media and can lead to prevalence of acidic conditions.

The pH should be self-stabilising in a perfectly balanced system i.e. there will naturally be an increase in the overall pH of the system when the VFAs generated in hydrolysis are consumed by methanogens. Moreover, the pH-dependent conversion of soluble CO_2 into hydrogen carbonate ions (HCO_3-) in acidogenesis contributes to the buffering effect and tends to restore the pH, thus contributing to self-stabilising effect.

An imbalance between the rate of production of VFAs and their consumption, generally leads to accumulation of VFAs, which results in a drop in pH, inhibit the methanogens and lowers the quality of biogas (Igoni *et al.,* 2008).Achieving a stable pH state can be very challenging with easily biodegradable materials such as kitchen waste, fruits and vegetable biomass, etc. This is because hydrolysis occurs more quickly compared to other phases of AD; which generally leads to VFAs accumulation and inhibition of methanogens (Igoni *et al.*, 2008).

Ward *et al.* (2008) showed that the CH_4 yield is optimised when the pH is between 6.5 and 7.5; and demonstrated that a 35% increase in the cumulative methane yield can be achieved within the optimal pH range as illustrated in Figure 6.

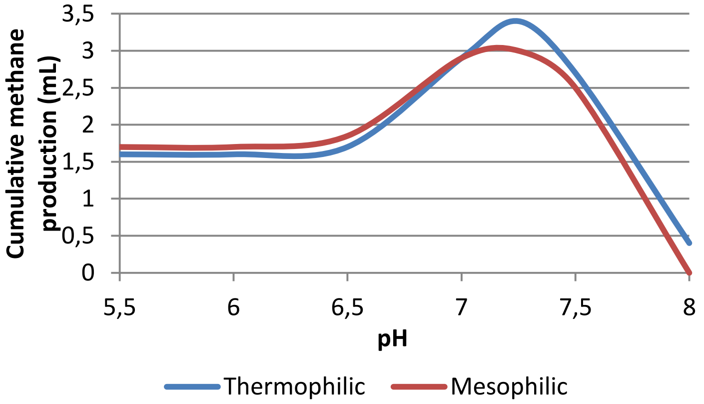


Figure 6: Optimal range of pH for anaerobic digestion of organic waste (Adapted from Ward *et al.*, 2008)

Research on the suitability of different alkaline buffers showed that sodium bicarbonate ($NaHCO_3$) is a better buffer compared to other alkaline substances at a ratio of 0.06 solids to buffer ratio under dry conditions, compared to $Ca(OH)_2$, Na(OH), $CaCO_3$(Brummeler and Koster, 1989). The chemistry of the buffering system with bicarbonate (HCO_3^-) ions is presented in Equations 6 and 7. The HCO_3^- ions react with a proton (H^+) to produce carbonic acid (H_2CO_3). The hydrating constant of dissociation of carbonic acid is very low ($[H_2CO_3]/[CO_2]= 1.7*10^{-3}$), which means that CO_2 form will prevail under normal conditions (American Chemical Society, 1979).

$HCO_3- + H+ \leftrightarrow H_2CO_3$ Equation 6

$H_2CO_3 \leftrightarrow CO_2 + H_2O$ Equation 7

Figure 7 illustrates the relation between the speciation of CO_2 into carbonate (CO_3^{2}-) and HCO_3^- depending on pH (The Pool Water Treatment Advisory Group-PWTAG, 1999).The speciation of CO_2 increases with pH i.e. it is observed that at pH~7, there will be an equal split between CO_2 and HCO_3-, at 7.5 ~10% will be CO_2 and 90% HCO_3^-.

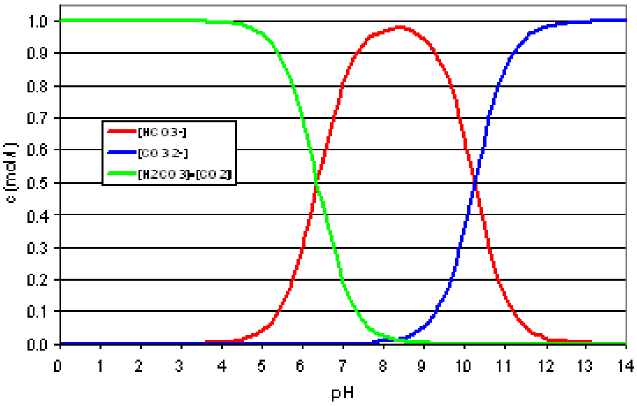


Figure 7: pH/ CO_2 equilibrium in aqueous solutions (PWTAD, 1999)

2.2.3.4 Waste particle size

Particle size affects the rate of biological transformation of waste, and other logistical factors such as the size of the equipment to be used. Reducing the size of the waste particles increases the surface area available for contact between microbes and the substrate, and ultimately both the rate and extent of biodegradation (Izumi *et al.*, 2010;Mshandeteet *al.*, 2005). Shredding and grinding are generally used to reduce the waste particle size to improve the contact area for microbes and process efficiency (Igoni *et al.*, 2008; Agunwamba, 2001).

2.2.3.5 Organic loading rate and hydraulic retention time

The organic loading rate (OLR) is a measure of the biological conversion capacity of the AD system. It is a particularly relevant control parameter in continuous systems, as it affects the performance of a digester (Rincon *et al.*, 2007). It is defined as follows:

Equation 8

The maximum OLR is a measure of the biological conversion capacity of an AD system. It is often expressed in g VS/cm^3/day or kg VS/m^3/day (Equation 6; Chaudhary, 2008), where volatile solids (VS) represents the fraction of organic material that is biodegradable. Feeding a system above its sustainable OLR generally results in low biogas yields due to accumulation of inhibiting substances in the digester media such as VFAs. The feeding rate to the system must be reduced in such a case. It is important to ensure the amount of substrate and AD bacteria are in balance in the digester to optimise performance of AD systems. The food-to-inoculum (F:I) ratio is also an indication of that balance, and is affected by the OLR (Igoni *et al.*, 2008).

The hydraulic retention (HRT) time is an indication of the time that the liquid spends in the reactor. It is an important parameter as HRT indicates the time available for conversion of the substrate to biogas and the bacterial growth achieved. HRT can be calculated according to Equation 9 (Chaudhary, 2008; Dennis and Burke, 2001).

The required retention time for completion of the AD reactions varies with differing technologies, process temperature, and waste composition. The retention time for wastes treated in mesophilic digester ranges from 10 to 40 days (Chaudhary, 2008).

– **Equation 9**

Where HRT: Hydraulic retention time (days), V: volume of digester (m^3), Q: Volumetric flow rate (m^3/day)

2.2.3.6 Mixing

Mixing inside a biogas digester is beneficial because it enables the maintenance uniform conditions everywhere in digester (temperature, concentration of substrate, pH, etc.). Moreover, mixing is particularly important for batch systems to release the biogas produced as it is generally trapped in gas pockets (Karim *et al.*, 2005).

It is common practice in larger industrial digesters to use mechanical stirrers, gas or liquid re-circulation for mixing in AD systems. Stirrers are more suited for high solids systems and gas re-circulation for wet ADsystems (Stafford, 2003; Igoni *et al.*, 2008). However, millions of small biogas systems operate without mixing, mostly of the fixed dome type.

2.2.4 Types of anaerobic digestion systems

The choice of digester type depends on a variety of factors such as the nature of the waste, its solid content, etc. Digesters can be operated as dry or wet systems; batch, semi-batch or continuous systems depending on the feeding regimes and solids content of the reactor. In batch systems, the substrate is fed and the reactor is sealed until completion of the degradation process, without further addition of fresh substrates. On completion of a run, the digester is emptied and refilled with fresh substrate (Chaudhary, 2008). While in continuous processes, the substrate is fed and removed from the bioreactor on a continuous basis (Buekens, 2005; Verma, 2002).It is common practice that 10-15% of digestate material be left as a seed culture for the next batch on completion of one AD cycle in continuous systems (Igoni *et al.*, 2008).

The digestion period can therefore vary considerably depending on the AD system; the average retention time however, in industrial applications, is about 30 days. Generally, a waste reduction of 56% on a mass basis and biogas yield of 572 L biogas per dry kg can be achieved with OFMSW (Ponsa *et al.,* 2008).

Wet AD systems have 10-15% total solid (TS) or dry matter (DM), while dry systems generally contain 25-40% TS. In wet digestion systems, water is used to adjust the TS of the waste to 10-15% TS. Commonly, sewage sludge from wastewater treatment plants is mixed with OFMSW for co-digestion (Chaudhary, 2008; Karagiannidis and Perkoulidis, 2009). The disadvantage of wet systems is the extra costs linked to the bigger reactor size required due to the large amount of water used.Dry anaerobic systems use feedstocks with 25-40% TS, and are becoming increasingly popular to treat municipal waste. Their main advantage is the lower water requirements, reduced leachate generation, digester size and energy efficiency, since the amount of energy required in a digester is directly related to the moisture content of the feedstock (Forster-Carneiro and Perez, 2009).

In dry systems, the waste is fed into the reactor with leftover digested material from another reactor as seed culture. Leachate is generally collected from the bottom and re-circulated to distribute AD micro-organisms and nutrients, as pre-inoculation and to maintain moisture levels (Luning *et al.*, 2006).

AD can be operated in single or multi-stage digesters, with various feedstocks. In one-phase systems, the biodegradation reactions take place in a single reactor. The advantages of a single stage reactor are: simpler reactor design, smaller capital investment and less frequent technical failures (Forster-Carneiro and Perez, 2009; Luning *et al.,* 2006).

Multi-stage AD systems often involve the use of two or more tanks for the AD process. The first tank generally features hydrolysis, acidogenesis and acetogenesis, while the second optimises methane forming conditions from VFAs (Karagiannidis and Perkoulidis, 2009; Chynoweth *et al.,*1987). The motivation for multi-stage systems is the optimisation of methane yield and the avoidance of inhibition to methanogens experienced from accumulation of intermediate products (such as VFAs, NH_3, etc). Despite the higher methane yields achieved with multi-stage systems, single stage systems are still preferred in industry due to lower capital costs, simpler plant designs and less maintenance is required compared to multi stages systems (Appels, 2008; Igoni *et al.,* 2008).

Common technologies have been developed for treatment of organic wastes, mainly from Europe. The most patented European digesters are Wassa, Valorga, Dranco, Kompogas and BTA (Karagiannidis and Perkoulidis, 2009). Anaerobic digesters can be either vertical or horizontal, with regards to their geometry.

2.2.5 Uses of biogas

Biogas is rich in CH_4, and can generally be used in all the applications developed for natural gas. It has the advantage of being produced from a renewable source and can be stored relatively easily at the place of generation, often in the digester itself, thereby ensuring an adequate supply pressure. Biogas is flammable and poses explosive risks if not managed adequately (Igoni *et al.*, 2008).

Many studies advocate the potential benefit of the reduction of greenhouse gases (GHG) emissions that can be achieved by integrating AD technology in waste management (Zglobisz *et al.*, 2010). This mitigation achieved is attributable to avoided emissions from landfills, fossil fuel replacement when the biogas is used, and replaced fertilizer when the sludge is used as a soil additive (Kaparaju and Rintala, 2011). For example, biogas generation in the US contributes 1% to the electric grid and reduces the power generation industry's emissions by 10% (Igoni *et al.*, 2008).Table 3 presents the general composition of biogas.

Table 3: General composition of biogas (adapted from Igoni *et al.*, 2008)

Constituent	Composition
Methane (CH_4)	**55-75%**
Carbon dioxide (CO_2)	**30-45%**
Hydrogen Sulphide (H_2S)	**1-2%**
Nitrogen (N_2)	**0-1%**
Hydrogen (H_2)	**0-1%**
Carbon monoxide (CO)	**Traces**
Oxygen (O_2)	**Traces**

The main uses of biogas reported in the literature are cooking, heating, vehicle fuel, and generation/co-generation of electricity. A successful example of application of AD technology in South Africa is the electricity generation project undertaken by the Ethekwini municipality in Durban. The landfill gas is harvested to fuel microturbines and generates electricity that is added to the electrical grid. The project has earned carbon credits since a renewable energy source is produced and GHGs emissions from the landfill sites are mitigated (Strachan *et al.*, 2007 cited in Greben *et al.,* 2008).

Figure 8 illustrates the different pathways of biogas use. Biogas can only be used without any treatments at the point of generation or if diluted into natural gas pipelines; further treatment would be required to transport biogas in large pressure tanks over longer distances.

The treatment required would generally include compression and removal of CO_2 and other contaminants H_2O, hydrogen sulphide (H_2S), etc. to increase its energy value (Greben *et al.*, 2008).

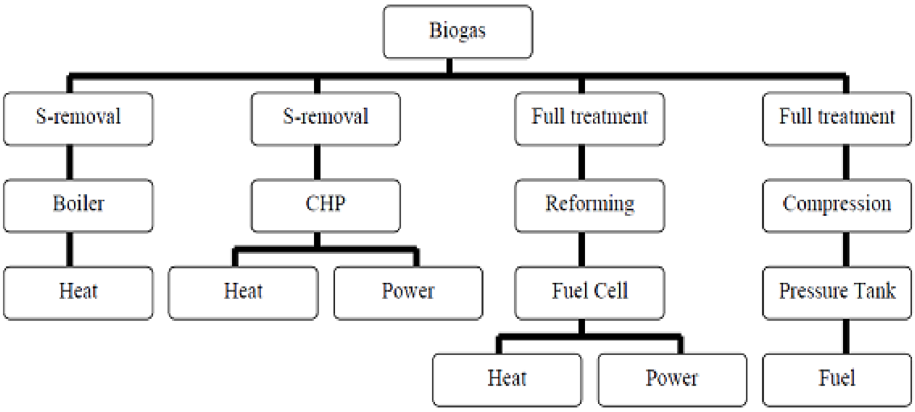


Figure 8: Utilisation pathways of biogas (Appels *et al.*, 2008)

2.3 Inhibitory Substances and AD Process Indicators

AD is a complex process, which some claim cannot be optimised without adequate monitoring and control (though millions of small-scale installations appear to operate satisfactorily). Optimisation of AD refers to attaining a stable process with high CH_4 yields and biodegradation rates. The challenge is the selection and measurement of adequate process indicators, without which control is ineffective. A suitable indicator should be directly measured and be a reflection of the status in the biodigesters (Boe *et al.*, 2010).

The biogas yield is the most common indicator used, since it indicates the overall process performance and is relatively easy to measure. However, biogas yield alone is not enough as it does not indicate rapidly a process imbalance, and is only reduced when the process is already inhibited (Moletta *et al.,* 1994, Boe *et al.*, 2010). The pH can also be used as an indicator; however, the pH response is less sensitive to the VFAs concentration in well buffered systems.

Boe *et al.* (2010) assessed the suitability of different indicators, reported in literature, such as the individual VFA concentration, biogas production, dissolved H_2, pH, alkalinity, CH_4 composition and gaseous H_2, etc. Results showed that the indicators with the fastest response were respectively the amount of dissolved H_2, pH, the concentration of acetic and then butyric acid (for digestion of glucose), the amount of propionic acid (to detect overload in the system) and finally biogas.

Boe *et al.* (2010) therefore advise that biogas production together with the concentration of individual VFAs such as acetic and propionic acid, be combined as process-indicators for an in-depth understanding of the process status and performance.

The inhibition experienced in AD is a result of the imbalance between the different microbial groups. For example, AD microbes responsible for hydrolysis and acidogenesis are rapidly growing and relatively insentitive to pH, while acetogens and methanogens grow slowly and are very sensitive to pH (Wang *et al.,* 1999; Deublein and Steinhauser, 2008). A substance becomes inhibitory when it leads to an unfavourable shift in microbial activity or growth. Inhibition can be detected by a decrease in biogas production rate from the 'assumed steady state' value, accumulation of organic acids manifested by a drop in pH, or when no further intermediate products and biogas are generated (Kroeker *et al.*, 1979).

The literature on the inhibitory substances tends to vary considerably, due to their different focus and experimental set-ups i.e. substance and substrates investigated, inocula used, methods, waste composition, etc. Chen *et al.* (2008) provides a detailed description of inhibitory substances for AD systems, the mechanisms responsible and a review of the milestones achieved in this field.

The common inhibitors in AD identified by Chen *et al.* (2008) were: NH_3, light and heavy metals, organics (organic acids, long chain fatty acids i.e. LCFAs, lignin, etc), and sulphides.The next two sections will focus on the inhibition experienced from NH_3 and VFAs which are more commonly encountered. Emphasis is given to understanding the inhibition mechanism and the concentrations reported as inhibitory in the literature. The last section presents an overview of the characteristics, and related challenges experienced with typical OFMSW waste streams investigated in this dissertation.

2.3.1 Volatile fatty acids (VFAs)

2.3.1.1 General introduction

Many authors agree that the concentration of VFAs is a crucial indicator, since VFAs are the main intermediate products, and their accumulation indicates a process imbalance (Jacobi *et al.*, 2009; Molina *et al.*, 2009, Ahring *et al.*, 1995, Hill *et al.,* 1987). Babel *et al.* (2004) argue that the inhibition caused by VFAs is directly linked to the concentration of undissociated acids (UAs) since pH is directly affected by the concentration of UAs. Accumulation of VFAs, coupled with a drop in pH is generally considered the main cause leading to inhibition, and eventually process failures.

The inhibition experienced usually increases with acidic conditions i.e. the concentration of UAs increases with decreasing pH. For example, Babel *et al.* (2004) observed that the inhibitory levels of UAs were: 2300, 650 and 120 mg/L at pH of 5, 6 and 7 respectively for the same concentration of total VFAs. The concentration of UAs can be determined fromEquation 10 (Babel *et al*., 2004) below:

———**Equation 10**

Where [HA] is the concentration of UAs (mole/L), Ct is the total acid concentration (mole/L), [H+] is the hydrogen ion concentration (mole/L); Ka is the ionisation constant of each acid.

The occurrence of individual VFAs generally decreases as the chain length increases. Formic acid is usually limited because of its rapid conversion to other substances, such as CH_4 and CO_2. For this reason, research carried out often focuses on the effect and behaviour of the VFAs with a carbon chain length between C_2 and C_6 as illustrated in Table 4, where the chemical formulas of the different acids are presented. VFAs with carbon chains longer than four tend to isomerise. The isomerisation occurs through migration of the carboxyl group ($-CO_2H$) to the next carbon atom, and in the presence of co-enzymes B12. Butyric acid is generally degraded into iso-butyric and acetic acid, while iso-butyric acid degrades directly into acetic acid during AD (Wang *et al.,* 1999).

Wang *et al.* (1999) observed that the degradation rate of butyric acid was very rapid, and as a result could not be detected. Results also showed that the decomposition of both butyric and iso-butyric acids were greater than the degradation of valeric and caproate acid (Wang *et al.,* 1999).

Table 4: Chemical formula of individual VFAs between C_2-C_6 (Wang *et al.,* 1999)

	Name of Compound		Chemical Formula
1	Acetic Acid	C_2	CH_3COOH
2	Propionic Acid	C_3	$(CH_3)_2CHCOOH$
3	Buturic Acid	C_4	$CH_3(CH_2)_2\text{-}COOH$
4	Iso-butyric acid	C_4	$(CH_3)_2CHCOOH$
5	Valeric Acid	C_5	$CH_3(CH_2)_3COOH$
6	Iso-valeric Acid	C_5	$(CH_3)_2CH\text{-}CH_2COOH$
7	Caproate Acid	C_6	$CH_3(CH_2)_4COOH$
8	Iso-Caproate Acid	C_6	$(CH_3)_2CH(CH_2)_2COOH$

Acetic acid is the primary product from which CH_4 is generated. The other VFAs (i.e. propionic, burtyric, iso-butyric, valeric, iso-valeric and caproate and iso-caproate acids) are first converted to acetic acid before being converted to biogas.The conversion rate of other VFAs to acetic acids depends on the methanogenic population, the degradation rate of acetic acid and the methane yield. Wang *et al.* (1999) observed that the degradation rates of the different acids were respectively as as follows: butyrate > acetate / caroate / valerate / iso-butyrate > propionate / iso-valerate > iso-caproate (Figure 9).

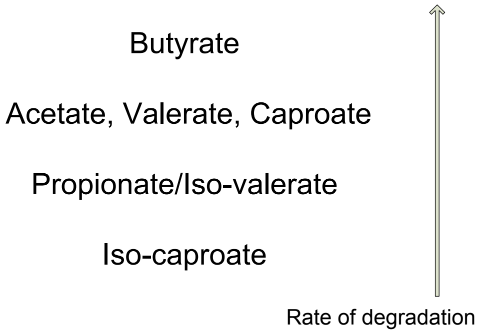


Figure 9: Order of degradation of the different VFAs observed by Wang *et al.* (1999)

The biodegradation of all individual VFAs generally follows first order kinetics. Table 5 presents the degradation reactions of individual VFAs, as well as their standard Gibbs free energy at 1 atm and 25°C and pH ~7. Reactions (ii) and (iii) represent the biodegradation of propionic and butyric acids into acetic acid and propionic acid respectively. These reactions are not thermodynamically feasible, since they have positive Gibbs free energy values. Many authors agree that VFAs predominantly inhibit methanogens (Azan et al., 2005, Babel et al., 2004; Wang *et al.*, 1999). However, there are few studies that report inhibition of hydrolysis (Anderson *et al.,* 1982, Mawson *et al.,* 1991; Kroeker *et al.,* 1979). For example, Babel *et al.* (2004) demonstrated using a two-phase system that acid-making bacteria are also inhibited. Similar studies observed inhibition of hydrolytic bacteria.

Table 5: Standard free energy and equation of biodegradation of different VFAs (adapted from Wang *et al.*, 1999)

	Reaction	ΔG (kJ) at 25°C
(i)	$CH_3COO^-+H_2O\rightarrow HCO_3^-+CH_4$	-31
(ii)	$CH_3CH_2\ COO^-+3H_2O\rightarrow CH_3COO+ HCO_3^-+H^++3H_2$	+76.1
(iii)	$CH_3CH_2CH_2\ COO^-+H_2O\rightarrow 2CH_3COO+ H^++2H_2$	+48.1
(iv)	$CH_3CH_2CH_2CH_2COO^-+2H_2O\rightarrow CH_3COO+ H^++2H_2$	+25.1
(M)	$4H2+ HCO_3^-+H\rightarrow CH4+3H2O$	-135.6
(ii+M)	$4CH_3CH_2\ COO^-+3H_2O\rightarrow 4CH_3COO+ HCO_3^-+ H^++3CH_4$	-102.4
(iii+M)	$2CH_3CH_2CH_2\ COO^-+ HCO_3^-+H_2O\rightarrow 4CH_3COO+ H^++CH_4$	-39.4

It is often difficult to determine a threshold value beyond which inhibition occurs, since the values in literature vary considerably due to the different systems used. For example, experiments carried out on methanogens *Clostridium thermoaceticum* showed that the bacteria remained viable at VFAs concentration of 15 and 20 g/L at pH between 6 and 7 (Anderson *et al.*, 1982; Mawson *et al.*, 1991). These results are similar to those obtained by Babel *et al.* (2004), where a maximum VFA concentration of ~13-14 g/L could be achieved with a pH value of 6, and 20 g/L at pH of 7. On the other hand, Azan *et al.* (2005) report that VFAs concentrations between 4 and 6 g/L do not affect the performance of the reactor, and recommend a ratio of propionic acid to acetic acid (P/A) of~1.4 for good performance.

2.3.1.2 Effect of propionic acid

Many researchers agree that the accumulation of propionic acid inhibits the activity of methanogens and leads to failure of the fermentation process. Propionic acid is produced through β-oxidation of odd-numbers carbon atoms and has a slow degradation rate. Lactic acid is also degraded to propionic acid under anaerobic conditions. It is therefore not desirable as a fermentation product as it would increase the propionic acid concentration (Wang *et al.*, 2009).Ren *et al.* (1994) observed that an increasing concentration of propionic acid was linked with restrained growth of acid-forming bacteria and lower bio-degradation of VFAs, and the build up effect altogether reduced the activity of methanogens. Wang *et al.* (2009) also showed that the amount of propionic acid plays a pivotal role in the inhibition experienced by methanogens. The microbes' concentration decreased from $6x10^7$ to 0.6-$1x10^7$/mL when the concentration of propionic acid increased above 900 mg/L. Similarly, Barredo and Evison (1991) demonstrated that the bacteria population decreased as the concentration of propionic acid increased. Yeole *et al.* (1996) also showed a 22-38%

decrease in methane yield when the concentration of propionic acid concentration was 5000 mg/L at a pH of 7.

VFA inhibition is experienced differently by the various groups of methanogens. Angelidaki *et al. (*1999) observed that aceticlastic methanogens have a higher growth rate than propionate degraders (i.e. 0.6 versus 0.49 per day). For this reason, many authors argue that the concentration of propionic acid is a better indicator of process stress (Boe *et al.*, 2010; Nielsen *et al.*, 2007).

The inhibition process occurs in the following sequence (Wang *et al.*, 2009):

- Accumulation of propionic acid and othet VFAs
- Decrease in pH
- Repression of acid forming bacteria, and inhibition to methanogens
- Low methane yield
- Further accumulation of VFAs, and complete cessation of the methanogenic activity

The concentration of propionic acid increases with decreasing pH and the inhibition experienced is often irreversible since the activity of the bacteria will not reconvert as reported by Wang *et al.* (2009).

The inhibition threshold of propionic acid can vary considerably depending on the system investigated. For example, Wang *et al.* (2009) observed methanogens were inhibited at 900 mg/L; while Pratap *et al.* (2001) report a concentration of up to 2750 mg/L. Demirel *et al.* (2002) also observed that a concentration of propionic acid above 951 mg/L inhibit the growth of methanogens. The growth rates of the different AD bacteria were not affected with PA concentrations below 300 mg/L (Wang *et al.,* 2009).

On the other hand, Demirel *et al.* (2002) observed that adding butyric acid improved the inhibition to some extent. Wang *et al.* (2009) also observed high concentrations of butyric acid enhanced the methane yield; while the concentration of acetic acid did not affect the system. Boe *et al.* (2010) observed that the amount of propionic acid increased with OLRs in a continuous system. Initially, acetic and butyric acids were the predominant forms and later propionic acid became the dominant form from day 20 onwards as inhibition built up, which led to a decrease in biogas yield.

2.3.2 Inhibition from light metal ions

Light metals ions (i.e. calcium, potassium, sodium and magnesium) are present in the influent of anaerobic digesters; they can be released through the biodegradation of organic matter or through added chemicals for pH control. They are required in moderate amount to stimulate bacterial growth, however high concentration can have the opposite effect and even cause severe inhibition because high salt levels cause the dehydration of cells due to osmotic pressure (Chen *et al.,* 2008).

2.3.2.1 Sodium (Na^+) inhibition

Soto *et al.* (1991/1992) showed that mesophilic microbes performed better and can adapt at high salinity concentrationscompared to thermophilic ones. Sodium (Na^+) toxicity generally decreases in the following order: lignocellulose-degrading>acetate-utilising>propionate-ultilising> H_2/CO_2-utilising bacteria (Chen *et al.,* 2008). Soto *et al.* (1991/1992) also showed that propionic acid-utilising are most sensitive to high Na + toxicity compared to other VFAs-utilising bacteria microbes.

The optimal concentration of Na^+ for mesophilic microbes, reported in literature differ e.g. 230 mg Na^+/L (Chin, 1971), 350 mg Na^+/L (Patel and Roth, 1977). Most authors agree however that concentrations ranging 3500-5500mg Na+/L are moderately inhibitory, and values above 8000 mg Na^+/L are strongly inhibitory (Chen *et al.,* 2008; Cheng, 2010).

The antagonistic effects of Na^+relative to other elements, results show that the inhibition of Na^+ was directly related to the amount of magnesium (Mg^{2+}). For example, 0.35 mg Na^+/L were found completely inhibitory at Mg^{2+} concentration of 0.05 mM, while more Na^+ was required at higher Mg^{2+} concentration (Chen *et al.,* 2008). The inhibitory effects of Na+ would ultimately depend on adaptation period, antagonistic/synergy effects, substrate and reactor configuration (Chen *et al.,* 2008).

2.3.3 Inhibition from organics

There is a wide range of organic compounds that can inhibit AD. Organic compounds with a low solubility for aqueous solutions, or that are adsorbed on the sludge's surface can easily accumulate to high concentration levels. The most common ones reported as toxic are:some long chain fatty acids (LCFAs), phenols, alkanes, halogenated benzenes, ethers, carboxylic acids, etc (Chen *et al.,* 2008).

Thermophiles have been reported to be more sensitive to LCFAs than mesophiles due to their different composition of cell membranes, and some studies have reported that LCFAs inhibition can be reduced through the formation of insoluble salts by addition of calcium (Ca^+), (Chen *et al.,* 2008).

The following parameters affect the toxicity of organic compounds: concentration of biomass, adaptation, loading rate patterns, cell age, temperature and exposure time to toxic substance (Yang and Speece, 1986).

2.3.3.1 Inhibition from long chain fatty acids (LCFAs)

The treatment of fatty substrates is generally hindered by LCFAs inhibition. The LCFAs inhibition mechanism occurs via adsorption onto the cell wall/membrane and interference with the transport of protective function (Chen *et al.,* 2008). The more common LCFAs in literature are: oleic acid, cypric acid, lauric acid, capric acid and mystiric acid.

LCFAs inhibition at low concentrations has been reported for gram-positive microbes but not for gram-negative ones. Methanogens are inhibited by LCFAs as their cell walls resemble gram-positive microbes (Chen *et al.,* 2008).

2.3.4 Sulfate reducing bacteria (SRB) and sulphide inhibition

The sulphate reducing bacteria (SRB) are a group of micro-organisms, associated with acidogenesis, responsible for the reduction of sulphate into sulphide using hydrogen and other organic molecules as electron donors during the fermentation of amino acids (Salminen and Rintala, 2002). The SRB competes directly with the methanogenic archaea as these microbes use the same substrates as methanogens and acetogens such as organic acids (e.g. acetate, propionic acid, lactic acid, butyric acid, etc) and hydrogen (H_2). SRB have a thermodynamic and kinetic advantage over the corresponding acetogens and methanogens in AD. The affinity of SRB for substrates goes in this order generally: H_2> propionate> and other organic electron donors (Chen *et al.,* 2010).

Fermentative bacteria are less affected by sulphide produced than SRB and the methanogenic archaea since the sulphide ions are toxic to the later groups of microbes. The combination of these two effects combined, ultimately has an inhibitory effect on the methanogenic archaea and lead to reduced production of CH_4 (Wang *et al.,* 2007).

H_2S can penetrate the microbial cells' membrane, and as a result interfere with the ability of microbes to assimilate sulphur, denature native proteins through the formation of sulphide and (Equation 11) disulphide, and interfere with various co-enzymes. Scientists agree that H_2S is the toxic form of for the reasons cited above (Cheng, 2010). Research by Hansen *et al.* (1999) report that the concentration of sulphide (~23 mg S_2^-/L) may increase NH_3 inhibition in AD systems, while activate carbon (2.5 %w/w) could decrease sulphide inhibition by adsorbing the suphide onto its surface (Salminen and Rintala, 2002).

H_2S (aq)↔H^+ + HS^-, pKa~7.04 (18°C) **Equation 11**

The ratio of undissociated H_2S to sulphide ion depends on pH in AD systems. The range reported in literature as inhibitory for sulphide ion is 100-800 mg/L, and 50-400 mg/L for undissociated H_2S (Cheng, 2010). The speciation of H_2S according to pH in aqueous sollutions, is presented in Figure 10.

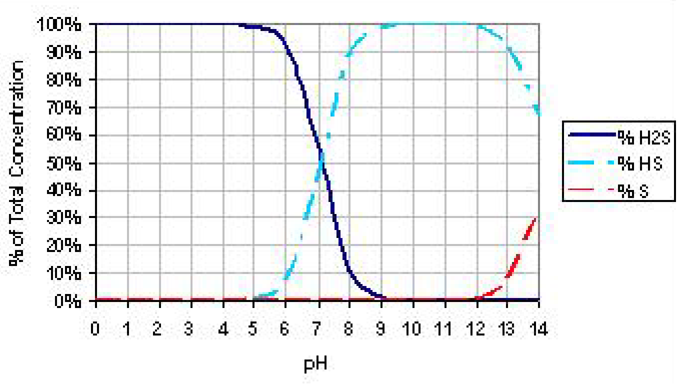


Figure 10: Speciation of H_2S depending on the pH (Adapted from Chastain, 2008)

Although the literature published reports a wide range of pH for the activity of SRB (i.e. pH~5.0-9.0), research by Postgate (1979) and Visser *et al.* (1996) showed that the activity of SRB was optimised at a pH range of 7-8 (Gutierrez *et al.,* 2009). Similarly Koster *et al.*(1986) shows that sulphide inhibition increases with pH (Chen *et al.,* 2008).

2.3.5 Ammonia inhibition

2.3.5.1 General introduction

NH_3 is produced from the degradation of nitrogen compounds (e.g. urea, proteins, etc.) under anaerobic conditions. Equation 12(Chen *et al.*, 2008)presents its stoichiometric equation in AD systems (Kayhanian, 1999; Tchobanoglous *et al.,* 1993 cited in Chen *et al.*, 2008).

Equation 12

Where a, b, c and d: stoichiometric coefficients

Ammonium ion (NH_4^+) and free ammonia (FA) are the main forms of inorganic nitrogen in digesters; however FA has been recognised as the main cause of inhibition since it easily penetrates membranes (Kroeker *et al.*, 1979; de Baere *et al.*, 1984). Inhibition is due to the proton imbalance and/or potassium deficiency caused when FA diffuses into the cell in the first case (Sprott and Patel, 1986; Gallert *et al.,* 1998). Two main mechanisms have been identified as inhibition pathways: direct inhibition of FA to activities of methanogens, or the conversion of NH_3 to NH_4^+ through cell diffusion. In the second case, inhibition occurs through an intracellular change in pH, which inhibits a specific enzyme reaction (Hansen *et al.,* 1998).

Methanogens are the most sensitive to NH_3 compared to the other groups of AD bacteria as their growth and activity is hindered at high NH_3 concentrations (Kayhanian, 1994). These results were confirmed by a study by Koster and Lettinga (1988) which showed that acidogenic population were not affected, while the activity of methanogenicbacteria wasreduced by 56% when the NH_3 concentration was increased from 4051 to 5734 mg NH_3-N/L.

It is not clear from literature whether it is the hydrogen-consuming methanogens, or the acetate-consuming methanogens that are the most affected by high NH_3 concentrations. Zeeman *et al.*(1985) report that aceticlastic bacteria were the most affected, based on methane yield and bacterial growth rate, while other authors argue otherwise (Zhang *et al.,* 2011;Calli *et al.* 2005). Similarly Angelidaki and Ahring (1993) also reported a higher sensitivity of the acetotrophic methanogens. Further research published showed that the specific growth rate was halved for the acetotrophic compared to the hydrogenotrophic (3.5 versus 7 g/L) when the NH_3 concentration was increased (Koster and Lettinga, 1984). The former observation is supported by Angenent *et al.* (2002) who demonstrated that at high NH_3 concentration, the methane produced is mainly from hydrogen. This confirms the fact that the inhibition experienced is mostly to acetate-consuming methanogens.

Consequently, NH_3 inhibition impacts considerably on the biogas and methane yield since 73% of the carbon flow in an AD system is converted through the acetotrophic pathway (Garcia *et al.*, 2000). Jarrell *et al.* (1987) identified methanogenic bacteria *Methanospirillum hungatei* as the most sentitive to NH_3 amongst other methanogens. The others identified were: *Methanosarcina barkeri, Methanobacterium thermoautotrophicum, Methanospirillum hungatei, and Methanobacterium formicicum* to name a few.The concentration of FA depends on many factors such as pH, temperature, concentration of total ammonia-nitrogen (TAN), etc.

The main parameters that factors identified to play a role in NH_3 inhibition are discussed in more details below:

Concentration

It is difficult to identify a threshold value beyond which inhibition occurs because results from literature vary from different experimental conditions such as the types of inocula, organic wastes, temperatures, pH and adaptation periods (Chen *et al.*, 2008). A wide range of values have been reported as inhibitory; research by Kroeker *et al.* (1979) and Chen *et al.* (2007) report any values between 1.7 and 14 g/L of TAN depending on the system examined. While, the work done by Farina *et al.* (1998) also observed that AD of swine and poultry manure was inhibited at 1.1-4 g-N/L. Many authors agree that non-adapted cultures are inhibiting within the 1.5-2.5 of NH_4+-N/L range (Koster and Lettinga, 1984; Hashimoto, 1986; Van Velsen, 1979 cited in Luste, 2011).

For example, Buendia *et al.* (2009) reported a concentration of 1.13 g NH_4+-N/L which caused a 50% reduction in the CH_4 produced. The inhibition threshold can increase significantly by adaptation if the system is exposed to increased NH_3 concentration by gradually increasing the OLR (Luste, 2011).

Adaptation

The level of the NH_3 inhibition is influenced by the adaptation period of the fermentation microbes. Melbinger and Donnellon (1971) were amongst the first to investigate the effect of adaptation on methanogens by exposing the culture to increasing concentrations of NH_3.

The methanogens can remain viable and active at NH_3 concentrations far exceeding the initial inhibition treshhold value once adapted (Kroeker *et al.* 1979; Bhattacharya and Parkin, 1989; Angelidaki and Ahring, 1993).For example, Koster and Lettinga (1988) showed that adapted methanogens were still active at 11 g-N/L while the inititial inhibition occurred at 1.9-2 g-N/L. Hashimoto (1986) also observed a decrease in sensitivity to higher NH_3 concentration of acclimated thermophilic as opposed to non-acclimated methanogens. Parkin and Miller (1983) demonstrated as well that methane yield was not affected for acclimated cultures with TAN concentrations as high as 8-9 g/L.

The positive outcome of allowing for adaptation can be attributed to internal changes such as a shift in the methanogens population or in the predominant methanogen species. The biogas/methane yield of AD systems without NH_3 accumulation is generally higher than that from digestion of high protein wastes; however, it is possible to achieve a stable performance through adaptation of the AD culture (Koster and Lettinga, 1988; Borja *et al.,* 1996 a).

pH

The pH in AD digesters affects both the growth of microbes and the concentration of FA in systems treating N-rich substrates. FA, a fraction of TAN, has been identified as the toxic substance. Generally, higher pH favour the speciation of NH_4+ into free FA, while lower pH lead to higher NH_4+ as illustrated by Equation 13 (Clegg and Whitefield,1995)

$NH_4^+ \leftrightarrow NH_3\ (aq) + H^+$ **Equation 13**

The equilibrium will be displaced to the left at lower pH. NH_3 is a weak base, and usually contributes to the buffer system in digesters. As the biodegradation occurs, more NH_3 is synthetised and the pH increases by maintaining high level of bicarbonate in the digester initially (Salminen and Rintala, 2002; Speece, 1996). High NH_3 concentrations inhibit methanogens, and subsequently lead to accumulation of VFAs as the intermediate products are no longer consumed by methanogens (Section 2.3.5). This effect acts against the alkaline effect of NH_3 and lowers the pH temporarily, particularly when the buffer capacity of the system is insufficient.

Reducing the pH in high protein systems can reduce the NH_3 inhibition experienced as it reduces the amount of FA, and can improve process efficiency. Chen *et al*. (2008/2011) observed that the temporary pH drop because of VFAs accumulation, favours lower FA concentrations in AD of N-rich substrates. This theory was validated by a research with AD of crab water, where lower pH conditions improved digester performance i.e. higher methane yield and COD destruction were achieved (Chen *et al.*, 2011). Similarly, Boardman and McVeigh (1997) also showed that the biogas yield increased four times after decreasing the pH from 7.5 to 7.0 with AD of cow manure.

AD systems treating N-rich substrates often experience ‘inhibited steady state’ as a result of the interaction between pH, VFAs and NH_3. In this state, digesters are stable but run sub-optimally (Angelidaki and Ahring, 1993; Angelidaki *et al.,* 1993). Generally, the concentration of VFAs is a good indicator of inhibition since they correspond to transitional digestion products in the AD process. Accumulation of VFAs is always a sign of inhibition from different compounds, or an imbalance in the microbial interactions. A high concentration of VFAs in the end product is an indication of a loss of biodegradable material not converted to biogas (Resch *et al.*, 2011).

Temperature

Temperature affects microbial growth and the concentration of FA in digesters. An increase in temperature generally leads to a higher metabolism, and higher concentrations of FA. Several studies have demonstrated that AD of N-rich substrates is more inhibited under thermophilic conditions compared to mesophilic conditions (Braun *et al.*, 1981; Parkin and Miller, 1983).

2.3.5.2 Techniques to overcome ammonia inhibition

There is limited information about the challenges experienced with AD of high protein (N-rich) substrates besides batch experiments and pilot scale applications of AD such as utilisation limits, process options, etc. (Resch *et al.*, 2006/2011). Two main approaches to overcome NH_3 inhibition have been identified from the literature: the first approach consists of physically removing the NH_3 directly from the digester to reduce the quantity below inhibitory values. In the second approach, the C:N ratio in the feed substrate is adjusted to reduce the amount of excess N available to be converted to NH_3 by microbes (Chen *et al.*, 2007). The main techniques used to physically remove the NH_3 in solution are air stripping and chemical precipitation of the NH_3. Both methods have proven to be efficient and feasible at high NH_3 concentration (Chen *et al.*, 2008).

The addition of a carbon-rich substrate to adjust the C:N ratio has also been tested (Kayhanian, 1999). Co-digestion of different waste streams is generally used to to generate a suitable substrate and adjust/increase the C:N ratio to the desirable range between 20 and 30 (Igoni *et al.*, 2008 , Rosenwinkel and Meyer,1999; Salminen *et al.*, 2003).

For example, a study by Schanmugam and Horan (2009) demonstrated that increasing the C:N ratio from 5 to 15 through mixing municipal organic wastes and wastewater from the leather industry increased the biodegradation from 24.36% to 44.31%. Furthermore, many studies report the improved performance and higher biogas yield from laboratory scale experiments of the co-digestion of high protein wastes (such as slaughterhouse wastes) with sewage sludge (Luste and Luostarinen. 2010; Luostarinen *et al.* 2009), OFMSW (Cuetos *et al.*, 2007), and agricultural wastes (Murto *et al.*, 2004). The positive effect of co-digestion can be attributed to the fact that inhibitive substances are diluted, and the nutrient content in the substrates is increased.

The overall result is a synergetic effect between the different substrates, which leads to better overall process performance (Mata-Alvarez *et al.*, 2000). However, there are mixed opinions about the benefits of co-digestion in the literature. For example, Nielsen and Angelidaki (2008) reported that reducing the concentration of NH_3 already present in the digesters using techniques such as steam stripping is more efficient. Similarly, Resch *et al.* (2011) showed that direct air stripping of the NH_3 from the digestion gave better results than co-digestion with glycerine, a pure carbon source. Resch *et al.* (2011) argues co-digestion may not necessarily solve the problem, as the extra source of carbon might be digested but if the NH_3 concentration is high and the conversion rate of VFAs is slower, inhibition will still prevail.

Separation of the AD phases is also a technique used to improve performance sinceinhibition is mainly experienced by methanogens (Wang and Banks, 2003; Cuetos *etal.*, 2007). Wang and Banks combined a hydraulic flush and anaerobic filter as reactors.Results showed improved performance compared to a single stage reactor, the two phasesystem remains active at higher loading rates and a 66% compared to 41% solids reductionwas achieved with the two-phase system.

2.4 Recalcitrant Substances

This section presents general characteristics of the common organic wastes streams found in MSW and investigated in this dissertation, these are: high protein wastes, garden waste, paper sludge and food wastes. An overview of the characteristics of each type of substrates, as well as the general challenges experienced with their AD is presented.

2.4.1.1 Food wastes

Kitchen wastes and wastes from the food process industry are rich in organic materials making them ideal for AD. Food wastes are easiliy biodegradable, and tend to be relatively high in protein and lipids. The fast hydrolysis step may lead to accumulation of VFAs and to inhibition of the methanogens from acidic conditions.

There are however few reports of AD plants running solely on source separated kitchen waste, because AD of pure food waste still suffers many setbacks due to the many operational problems reported (Archer *et al.*, 2005). Some food wastes are rich in protein, and have a low C:N ratio which can also result in NH_3 inhibition from the degradation of extra organic nitrogen, as discussed in Section 2.3.5.The level of inhibition can vary tremendously depending on the nature of the AD bacteria as well as the period of adaptation (Fricke *et al.*, 2007). The average biogas yield for food remains reported in the literature is 500-600 mL/g VS, with a CH_4 composition between 70 and 80%, equivalent to ~400 mL CH_4/g VS (Steffen *et al.*, 1998; Banks *et al.*, 2011).

2.4.1.2 Garden wastes

A variety of crops is reported as suitable for AD (Kalra and Panwar, 1986). However, there are many inhibiting factors identified for AD of garden wastes. Speece (1987) argues that although microorganisms might adapt to the inhibitors, kinetics of the process might still be affected. The challenges identified with AD of garden waste can be associated with the fact that such wastes

- are rich in cellulosic material and lignin content, making them difficult to biodegrade
- have a high C:N
- can experience potential inhibition from herbicides or pesticides that could influence the kinetics of the digestion process (Khalil *et al.*, 1991; Chakraborty *et al.*, 2002);
- can release inhibitory substances such as formic, levulinic acids, and hydromethyl fufural (Speece, 1987)
- depending on the plant, secrete resin that protects them from biodegradation (Speece, 1987)

These factors altogether can result in lower reaction rates and biogas yields with garden waste. Pre-treatments such as acid and base hydrolysis are generally used to increase the biogas yield of garden wastes (Speece, 1987).

2.4.1.3 High protein substrates

The main types of waste that belong to this category are: animal wastes i.e. poultry and livestock, wastewater, juices from silage; slaughterhouse wastes, etc. These types of wastes can be substantial contributors to pollution as they have high levels of pathogens, can contaminate drinking water and affect wetlands if not managed adequately (Chen et al., 2007). The amount of total ammonia in animal wastes is usually very high due to the presence of proteins and urea that release NH_3 upon anaerobic treatment (Zeeman *et al.*, 1985; Krylova *et al.*, 1997; Hansen *et al.,* 1998). The main challenge experienced with anaerobic digestion of such wastes is the instability caused by NH_3 inhibition (Chen *et al.*, 2008), as explained in details in Section 2.3.2.

The literature values reported as highest for the OLR of co-digestion of meat processing wastes is 1-2.9 kg VS/m^3/day (Alvarez and Liden, 2008; Rosenwinkel and Meyer, 1999), and 3.9-4.2 kgVS/m^3/d for the mechanically pre-treated materials (Murto et al., 2004). Higher OLR generally lead to lower biogas yields (Luste, 2011). The characteristics of meat industry by products, cattle manure and sewage sludge-usually referred to as protein rich substrates for AD are presented in Table 6 .

Table 6: Characteristics (%) of meat processing wastes, cattle manure and sewage sludge (Luste, 2011)

Content	Meat-processing wastes	Cattle manure	Sewage sludge
VS	92	72	59-75
Lipids	55	3.5	4.5-12
Cellulose	−	17	7
Hemicellulose	−	19	−
Lignin	−	6.8	−
Protein	29	19	32-41
Ash	8	28	25-41

The level of NH_3 inhibition depends varies significantly in the literature reviewed according to different systems. For example, Hansen *et al.* (1998) investigated the inhibition experienced under different NH_3 concentrations in a 118 mL batch reactor loaded with 6 mL of manure (~3 g VS/L). Results showed that the system was inhibited at a free ammonia concentration of 1.1 g-N/L at pH 8. The concentration of NH_3 in the reactors was increased by adding ammonium chloride (NH_4Cl) solution.

2.4.1.4 Paper sludge

Waste streams from the paper industry are a cause of concern due to their high organic strength. For example, the waste pulp generated by the paper industry exhibits high COD concentrations, that correspond to ~40-45% of the original wood weight; and generally contain higher a volatile fraction (Ali and Sreekrishnan, 2001; Lin *et al.,* 2009). Generally, the composition of pulp and paper sludge (PPS) is as follows: lignin (20-58%), carbohydrate (0-23%), protein (22-52%), cellulose (2-8%) and lipid (2-10%); (Lin *et al.,* 2009).

The temperature of the waste generated is usually warm (around 35°C) which makes anaerobic digestion a viable treatment method for paper pulp (Ali and Sreekrishnan, 2001). Satisfactory pilot projects and lab scale anaerobic digestion of wastes from the paper industry has been reported (Rintala *et al.*, 1991). A number of studies report low methane yields generally experienced with paper sludge due to the longer residence times required resulting from its slow biodegradable organics, e.g. lignin (Lin *et al.,* 2009).

The common inhibitors reported for AD of effluent wastes from the paper industry are: tannins, resin acids, sulphide, halogens, LCFAs (Ali and Sreekrishnan, 2001).Tannins contribute up to 50% of the COD in the debarking process water, and are known to be toxic to methanogens depending on the extent of polymerisation (Field *et al.*, 1988). LCFASs and other acids originate from the pulping process and are particularly inhibitors to the acetic consuming methanogens (Hanaki *et al.*, 1981; Koster and Cramer, 1987). In addition, halogens have been known to originate from the bleaching process and their toxicity in AD systems has been well documented.

Most of the organic inhibitors biodegrade to a certain extent, however there is limited knowledge on the origins and types of all the contaminants as well as their degree of toxicity (Ali and Sreekrishnan, 2001).

2.5 Conclusions from the Literature Review

The following important observations can be retained from the literature review:

2.5.1 Trends in municipal solid waste (MSW) in developing economies

Inadequate service levels, poor waste and environmental awareness of communities, inefficient practices of waste collection, illegal and uncontrolled dumping, scavenging and littering due to limited resources and lack of financial planning characterise solid waste management in developing countries (Onu, 2000). Landfill disposal remains the most common method of waste disposal and is unsustainable in the long term due to the limited amount of available land, even in the case of engineered sanitary landfills (Domingo and Nadal, 2008; Thomas, 2006).

Municipal solid waste (MSW) in developing countries is highly organic (~55%), very dense with a lower calorific value and high moisture (40-80%) content compared to that in developed (Troschinetz *et al.*, 1999; Thomas, 2006). The suitability of advanced physico-chemical waste treatment options such as incineration for developing economies is questionable despite the high volume reduction that the technology can achieve. This is mainly due to high capital and operational costs to ensure that such installations are indeed a cleaner option, lack of spare parts and technical expertise available locally (UNEP, 2002; Asomani-Boateng, 1999).

Biological treatments of the organic fraction of MSW (OFMSW) such as composting and AD are increasingly popular in industrialised nations. They currently offer the only way to recycle the biodegradable fraction of the waste and recover valuable end products such as biogas and soil conditioner. The United Nations Environmental Programme (UNEP, 2002) recommends that biological treatments are more suitable in developing cities due the highly organic nature of the waste, and operational ease of the technology.

2.5.2 Biological waste treatments for MSW

Composting the organic fraction of municipal solid waste (OFMSW) has been the preferred treatment method for developing cities in the past compared to AD due to the low economic costs and operative easiness. However, the production of biogas has positioned AD as the more energy efficient process as opposed to composting where energy inputs are required. In AD, 75-150 kWh is recovered per ton of MSW treated, compared to composting where 50-75 kWh is required per ton of MSW treated (Braber, 1995).

AD occurs in four main phases (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis) and is a complex system, where microbiological, biochemical and physico-chemical phenomena are closely linked. In hydrolysis, organic molecules are biodegraded into smaller monomers. It is often considered the rate-limiting step. The major intermediate products, volatile fatty acids (VFAs), are produced in acidogenesis, and further transformed into acetate and hydrogen in acetogenesis. Biogas is synthesised from acetate in the final stage, methanogenesis.

2.5.3 Important parameters of AD and challenges experienced

The symbiotic growth of various groups of microbes not only influences the stability of the process, but is also influenced by a number of parameters (Greben *et al.*, 2008). Importantly, while the hydrolytic and acidogenic bacteria remain relatively pH-insensitive; the performance of the methanogenic archaea is only favoured in the neutral range i.e. between 6.5 and 7.5 (Ward *et al.*, 2008; Chynoweth *et al*., 1987).

The important parameters identified in the literature for AD are: the pH, the chemical composition (C:N ratio), temperature, the organic loading rate (OLR) and hydraulic retention time (HRT), (Chynoweth *et al.*, 1987).Mesophilic conditions (i.e. 30-38°C) are preferred over higher temperatures for anaerobic digestion due to the decent biogas yield and degradation achieved under stable conditions (Tchobanoglous *et al.*, 2003; Igoni *et al.*, 2008; Karagiannidis *et al.*, 2009).

The pH should be self-stabilising in systems where the activity of the different microbial groups is balanced. Under neutral conditions, it is expected that 50-80% of the CO_2 produced during acidogenesis will be speciated into HCO_3^- ions, and will raise the pH by reacting with a proton (H^+). Moreover, additional $HCO_3^-$are produced by methanogens when VFAs are consumed to generate methane as illustrated in Table 5.A pH imbalance occurs when the rate of production of VFAs surpasses the action of acetogens and methanogens. This is generally observed with substrates such as kitchen wastes, fruits and vegetable biomass, etc., which are easily fermentable. The fast hydrolysis step leads to an excess of intermediate products such as VFAs from the action of acidogens and acetogens.

This would lead to a pH drop and acidic conditions would limit the action of the methanogenic archaea, which ultimately reduces the productivity, and CH_4 content of the biogas (Igoni *et al.,* 2008).

The organic loading rate (OLR) is the measure of the biological conversion capacity of the AD system, and an important control parameter particularly for continuous systems. Feeding a system above its sustainable OLR often results in low biogas yield due to accumulation of inhibiting substances in the digester media such as VFAs (Rincon *et al.*, 2007).

The OLR also affects the Food-to-inoculum ratio (F:I), which can be used as as an additional control parameter to ensure the amount of substrate and AD bacteria are balanced (Igoni *et al.*, 2008).The inhibition experienced in AD systems can be attributed to a wide range of variables. The main challenges reported in with AD are accumulation of VFAs, which result in acidic conditions and inhibition to methanogens; inhibition from pollutants such as $NH_3$and H_2S experienced with the degradation of proteins, and LCFAs from lipids depending on the OLR and pH of the AD systems.

The chemical composition and nature of the waste affect its biodegradability, and can influence the extent of VFAs accumulation in AD systems e.g. easily fermentable substrates versus ligno-cellulosic materials would lead to increased activity of acidogens and consequently accumulation of intermediate products (VFAs). Similarly, overfeeding the system above its sustainable OLR, and interaction with other inhibitors of the methanogenic archaea such as NH_3 would lead to an accumulation of VFAs.

2.5.4 Benefits of co-digestion

The C:N ratio is reported to be a useful indicator of the characteristics of the substrate and an important parameter in AD. Carbon is consumed 30-35 times faster than nitrogen, the ideal range of the C:N ratio for substrates is reportedly between 20 and 30 to promote microbial growth and for suitable performance. Higher C:N ratios slow down the degradation process since there is excess of degradable substrate for the microorganisms; while an excess of N beyond the microbial requirements results in conversion of the extra N into gaseous Ammonia (NH_3). The literature reviewed reports NH_3 inhibition as particularly challenging, and common with AD of high protein wastes such as urines-manures mixtures, slaughterhouse wastes, etc (Bernal *et al.*, 2008).

Co-digestion of different waste streams has been used for many years to provide a suitable substrate for AD. Using co-digestion to overcome lower biogas yields experienced with substrates such as sewage sludge and slaughterhouse wastescould be effective as it would add a carbon source to the digester mixture and result in higher C:N ratios (Kayhanian, 1999; Igoni *et al.*, 2008 , Rosenwinkel and Meyer,1999; Salminen *et al.*, 2003).

The positive effect of co-digestion can be attributed to the fact that inhibitive substances are diluted, the nutrient content in the substrates is increased and there is a synergetic effect between the different substrates that often results in better overall process performance (Mata-Alvarez *et al.*, 2000).

3 RESEARCH QUESTIONS AND METHODOLOGY

The literature review has elaborated the argument already presented in chapter 1 for the potential benefits of integrating AD technology into MSW management in developing economies. Chapter 2 has also reviewed the various challenges generally experienced with AD technology.

The success of AD technology cannot be achieved without a clear identification and understanding of the different organic wastes streams available, their respective biogas potential and the engineering considerations of the process limitations. This chapter develops four specific research questions to address this problem based on the literature review and in support of the dissertation objectives (Section 1.3). The remainder of the chapter presents the methodology adopted to answer these questions.

3.1 Research Questions

In order to address the set objectives, the following key questions were raised, informed by the challenges identified in the literature related to the AD of different organic wastes materials:

1. Several studies have pointed to AD as one of the biological options suitable for treating solid waste in developing cities, due its highly organic composition (Mohee, 2002; Thomas, 2006; Pegels, 2010; Amigun and von Blottnitz, 2010). It has also been noted that the performance of AD is directly linked to the amount of biodegradable materials available versus the level of contamination in wastes fractions, as is the case with composting (Thomas, 2006; Mensah, 2003).
   Consequently, **the first research questio**n is: What is the biomethane potential of different pure, and mixed, organic wastes streams typically found in an urban setting in developing economies? Furthermore, to what extent does the nature of the substrate influence the digestion process and the biogas yield?

2. Aerobic composting has been the preferred choice for yard trimmings and garden wastes in the past. Such wastes are rich in ligno-cellulosic compounds, which are recalcitrant to biodegradation. AD, on the other hand was found more suitable for wet organic wastes, without structure, such as kitchen waste (Braber, 1995). However, lab-scale studies on AD of different types of garden wastes show satisfactory performance despite their ligno-cellulosic structure (Kalra and Panwar, 1986).

   The production of biogas, a renewable energy source from AD, the global efforts to reach sustainability goals and reduce GHG emissions have also positioned AD as the more energy efficient process compared to composting where energy is consumed (Braber, 1995). It is expected that the AD of garden waste will require longer digestion periods to yield comparative biogas yields due to its cellulosic nature.

Accordingly, **the second research question** is: How does AD of garden waste compare to that of non-cellulosic wastes, in terms of key parameters of AD, viz. biogas and CH_4 yields per g VS, and stability during digestion?

3. Some studies report lower biogas yields from the AD of slaughterhouse wastes, due to their chemical composition, especially their low C:N ratio compared to suitable values for microbial growth, and potential inhibitors generated during the biodegradation of proteins and fats,esp. NH_3, H_2S and LCFAs (Saliminen and Rintala, 2002; Chen *et al,* 2008). Many studies advocate improved performance and higher biogas yield when protein-rich wastes such as slaughterhouse wastes are co-digested with sewage sludge (Luste and Luostarinen, 2010; Luostarinen *et al.,* 2009), OFMSW (Cuetos *et al.,* 2007), or agricultural wastes (Murto *et al.,* 2004), etc.
The third research question is therefore: To what extent can co-digestion with a rich carbon source improve the performace of digesters treating slaughterhouse wastes and overcome the lower biogas yields generally experienced?

4. AD technology still suffers many setbacks despite the abundance of wastes available, due to challenges experienced with the existing plants viz. unstable conditions, suboptimal yields and low efficiencies, which may eventually lead to process failures (Chen *et al.*, 2008). AD is a complex system i.e. microbiological, bio-chemical and physico-chemical systems are closely linked, and a symbiotic balance is required to ensure bacterial growth and a stable process (Greben *et al.,* 2008).
Process control would be ineffective without suitable indicators (Moletta *et al.*,1994, Boe *et al.*, 2010).Previous work suggests a number ofsuitable process indicators, which should be measured directly to reflect status in the bioreactors. One of the most common indicators used as a proxy for overall process performance, which is relatively easy to measure is the biogas yields. Somewhat more complicated is the regular determination of methane biogas composition to determine methane yield. Consequently, **the last research question** is, besides the biogas yield and methane yields, does the measurement and monitoring of other parameters indeed allow for more advanced insights and effective control of the AD process, as advocated in literature?

3.2 Approach Taken to Answer the Questions, and Chapter Overview

Laboratory experiments were setup to investigate the AD of different waste streams typically found in OFMSW disposed off in landfills. The different waste samples were sourced from Cape Town. The organic waste fractions sampled varied from pure, homogeneous waste streams to mixed waste from refuse transfer stations. The experimental investigation was carried out in two phases. The first phase is a direct response to questions one and two, where 16 different types of waste streams were sampled and their BMP evaluated in 100 mL batch reactors.

In the second phase of experiments, AD was investigated under fed-batch conditions in 2 L reactors for garden, kitchen and blood waste. The aims of the second phase of the laboratory work were to: (i) validate results from the batch experiments, (ii) monitor the temporal evolution of measured parameters and, (iii) investigate the performance of AD systems under real life conditions (fed-batch, ambient temperature, etc). This second phase allowed a better understanding of the AD process to answer question four.The answer to question three was derived from experiments of the co-digestion of nitrogen-rich and carbon-rich substrates, which was investigated under both batch and fed-batchconditions.

The rest of this chapter is organised in six sections. In the first section, the sampling technique used to collect the waste is discussed, and the second section presents the physical and chemical characteristics of the different substrates used. The experimental set-ups of the BMP and fed-batch tests respectively are discussed in the next two sections. Co-digestion experiments are discussed in the next section; and finally analytical methods used to collect data are presented in the last section.

3.3 Sampling of the Different Organic Waste Fractions for Batch Experiments

The batch experiments came as a result of the contracted study for the Solid Waste Department of the City of Cape Town (CoCT) to carry out a preliminary waste characterisation and evaluate the BMP of different mixed and source-separated organic waste streams in the city (Munganga *et al.*, 2010). The waste characterisation was carried out at two of the city’s refuse transfer stations viz at Athlone refuse transfer station (ARTS) and at Bellville Compost (BC).The waste characterisation at ARTS was carried out over 5 days, to ensure the data collected was representative, since both residential and commercial, waste are collected on different days for the areas serviced by the municipality. The BC plant has a smaller capacity compared to ARTS, and was undergoing maintenance shutdown during the sampling period. For this reason, the waste characterisation study was only carried out over 3 days. Samples from the organic fraction at ARTS and BC were retained for BMP analysis.

3.3.1 Equipment for sampling

The sampling was carried out using the following equipment:

- A shovel, to collect in depth composite samples wherever required from a waste pile. This was particularly relevant for sampling at the refuse transfer stations for waste piles accumulated post a conveyor belt.
- A scale, to record the weight of the samples collected. The samples collected were then preserved in plastic containers at 4°C in a cooling box to prevent further biodegradation during the sampling trip.

- 240 L wheelie bins for collection of the waste at the refuse transfer stations post the conveyor belts at ARTS and BC.
- A perforated table in triplex sheets with 4 cm holes punched. The table was used to allow smaller waste particles to filter through for waste characterisation at ARTS and BC. These waste particles, with a diameter of <4 cm, are referred to as 'fines' in this dissertation.
- Sampling sheets to record waste data at collection, sample waste sheets are presented in Appendix (Section 9.3.1).

3.3.2 Sampling of pure industrial organic waste streams

The method used for sampling was a stratified random approach, developed according to the *General Planning for Waste Sampling from the American Society of Standards (ASTM) book, method D4687-95*. A random approach ensures that every element is represented in the waste sample extracted, and that results are an unbiased estimate of the average waste concentration. Random sampling is also generally used for highly heterogeneous wastes as was the case in this study. The number of samples collected was influenced by the variability of the waste pile except, when dealing with homogeneous wastes such restaurant, fisheries and abattoir wastes.

Sample collection was achieved by both discrete (at the surface) and vertical composite grid sampling. Discrete grab sampling consists of collecting composite samples on the surface from pre-destined locations within the grid (called compass points), Figure 11. While, vertical composite sampling consists of collecting the sample to a specified depth (as far as the hole can be advanced into the pile with the shovel).

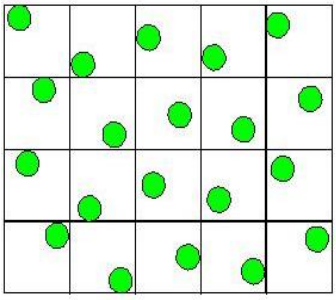


Figure 11: illustrating grid sampling on a waste pile (ASTM, method D4687-95)

The number of grid points on the waste pile adequate for stratified random sampling, is usually at least ten times the number of grid points as the number of samples required (ASTM, method D4687-95).

In this study, a minimum number of four samples per site were taken to achieve 80% confidence level in the representativity of the waste samples, *Solid Waste Guidelines for Data Collection and Analysis*, (UNEP, 2009) for both commercial and residential wastes as illustrated in Table 7 below.

Table 7: Number of samples for waste composition. Adapted from UNEP (2009)

Materials	C.L 95%		C.L 90%		C.L 80%		C.L 70%	
	Residential	Commercial	Residential	Commercial	Residential	Commercial	Residential	Commercial
Newsprint	224-2397	695-3563	55-600	170-991	16-150	45-223	9-58	21-101
Cardboard	899-1955	55-997	225-499	134-250	58-123	35-64	27-66	17-50
Aluminium	275-1437	754-4399	70-350	191-1100	19-92	60-275	10-42	23-125
Ferrous	194-554	552-3411	50-139	138-953	14-37	36-214	8-18	17-97
Glass	145-619	596-2002	39-155	149-501	19-61	39-126	6-19	19-58
Plastic	261-1100	422-783	67-275	107-195	18-70	28-61	10-32	14-24
Organic	12-47	26-92	5-14	8-25	3-5	4-8	3-4	3-5

Where CL: confidence Level

The different waste fractions, the number of samples collected and their sources are presented in Table 8. The most homogeneous wastes collected were from the Vineyard Hotel, Roelcor abattoirs, Premier Foods and Nampak. On the other hand, the most heterogeneous waste samples were from both transfer stations (ARTS and BC) as was expected since the waste stream is highly mixed. The sampling at Wynberg drop off occurred at the end of the conveyor belt after branches and leaves were chipped mechanically, before collection for compost manufacture (Figure 12). The samples were later frozen at -27°C in the laboratories until used for analysis and BMP tests.

Figure 12: Sampling at Wynberg Drop-Off for garden refuse

Table 8: The various organic waste fractions collected, their sources and the No. of samples

	Substrate	Source	No. Sample(s) for analysis (s)
1	Market Waste	Cape Town Market	1
2	Restaurant waste	Cavendish Square	1
3		Vineyard Hotel	1
4	Garden waste	Wynberg Drop off	1
5	Water hyacinth	Cape Flats Sewage waste	1
6	Blood waste	Stickland abattoir	1
7	Abattoir waste	Stickland abattoir	1
8	Fisheries waste	Houtbay fisheries	1
9	Cereal waste	Premier Foods	1
10	Waste paper pulp	Nampak	1
11	Garden waste	ARTS	5X1
12		BC	3X1
13	Fines	ARTS	5X1
14		BC	3X1
15	Food Waste	ARTS	5X1
16		BC	3X1
17	Partially compost product	BC	1

3.3.3 Waste characterisation at the refuse transfer stations

3.3.3.1 Athlone refuse transfer station (ARTS)

The ARTS receives on average 900 tons of waste per day, and sometimes more during peak periods. The facility is equipped with a Material Recovery Facility (MRF) operated by a private company. This facility helps divert about a third of the waste received by manually recycling paper products, glass and other recyclables. The rest of the waste is then accumulated on the apron floor until collection for disposal in the municipal landfill. It was the post-separation waste that was sampled for characterisation, from which the organic fraction (garden, fines and kitchen wastes) was retained for BMP analysis. The samples taken on any particular day would represent the incoming waste of the previous day since the waste received only gets processed after 24 hours.

Two 240 L wheelie bins were used to collect waste post, the MRF off the conveyor belt, and the weight of the waste collected in each bin was recorded. Each run consisted of sampling the waste off the conveyor belt in duplicate (two bins), sorting the waste and data collection.

The waste was evenly spread over the perforated sampling table, and the rest of the contents were separated into 7 categories i.e. paper products, soft organics, green waste, mixed recyclables, sanitary and medical waste, and others as illustrated in Figures 13 and 14 below.

(13) **(14)**

Figure 13: Sampling at the end of the conveyor belt at ARTS, Figure 14: Waste characterisation at ARTS with perforated table

3.3.3.2 *Bellville Compost Plant*

The Bellville Compost (BC) facility is currently the only facility available for composting of MSW, which is operated and owned by the City of Cape Town. Its average incoming waste stream varies throughout the year, but averages at ~800 tons per month. The composting process occurs in three main phases: in the first phase, big non-degradable materials are removed from the raw waste, the waste is then trommelled with a 2mm sieve in the second phase, and finally re-arranged into aerobic windrow that are processed into finished compost product over 6 weeks.

The sampling at BC was scheduled over the month of December, during which the waste received is significantly less than all the other months. It is also the time when plant closes down for maintenance, and concentrates on sales of the finished compost product. The waste characterisation, done over three days, was done using stockpiles of waste that were received on the preceding days, from which grab samples were taken for BMP analysis. Samples were collected using a shovel, from static stockpiles that arrived on the preceding days prior to treatment for compost production. The two 240 L wheelie bins were filled with waste and emptied onto the perforated table for sorting as was done at ARTS. A sample from the partially composted waste or intermediate compost was also retained for BMP analysis.

Results of the waste characterisation at ARTS and BC are not reproduced in this dissertation, since the focus of the dissertation is on the AD process of the waste samples. Complete results of the study carried out are available in the report submitted to the solid waste department in the city of Cape Town (Munganga *et al.*, 2010). AD experiments were set up with a number of repeats due to the variability of these mixed waste streams. The impact of the variability is analysed further in the BMP result chapter, and an analysis of variance is performed based on the biogas productivity (mL/g VS) for the different categories of wastes, with reference to stream variability reported to the City of Cape Town.

3.4 Organic Waste Streams Used as Substrates

3.4.1 Biomethane potential (BMP) tests under batch conditions

The 16 waste fractions collected can be separated into pure industrial waste streams (or source separated) such as restaurant waste from the Vineyard Hotel; and mixed organic wastes from the refuse transfer stations (i.e ARTS and BC). Garden waste, food waste and fines from ARTS and BC were retained for BMP analysis. The inoculum used, was from the Cape Flats Sewage plant. Table 9 presents more details about the different organic waste fractions sampled for BMP analysis, their source and description.

The physical and chemical characteristics of each substrate are presented in Table 10. Note that only four of the five samples collected at ARTS were digested (1-4) for each type of waste, while only one representative sample from the BC waste was digested for each category. Garden waste and paper sludge are generally rich in carbon i.e. had a higher carbon-to-nitrogen (C:N) ratio, while abattoir and fisheries wastes are richer in nitrogen (i.e. had the lowest C:N ratio).

Table 9: Description of the 16 organic waste samples collected

	Waste(s)	**Source**	**Description/ Appearance**
1	Market waste	Cape Town Market	Fresh and semi- rotten fruits and vegetables
2	Restaurant waste	Vineyard Hotel	Homogeneous paste of cooked food waste
3		Cavendish Square	More heterogeneous food waste e.g. dough, fries, bread, etc.
4	Garden waste	Wynberg Drop-Off	Chipped leaves and branches
5	Water Hyacinth	Cape Flats Sewage Plant	Water hyacinth leaves growing in water ponds onsite
6	Blood waste	Stikland abattoir	Blood waste collected after cow slaughtering
7	Abattoir waste	Stikland abattoir	Meat, fats, intestines, skin,etc.
8	Fish waste	Houtbay fisheries	Crude flesh, bones, scales, intestines,etc.
9	Cereal waste	Premier Foods	Homogenous mixture of all the cereal types produced
10	Paper pulp	NAMPAK	Homogeneous grey paste rich in $CaCO3$
11	Garden waste	ARTS	Mixed garden waste entrained with sand and other contaminants (food waste traces, recyclable debris, etc.)
12		BC	Same as above
13	Fines	ARTS	Any particle with a diameter less than 4 cm a mixture of organic, recyclables and non-recyclables materials.
14		BC	Same as above
15	Food waste	ARTS	Mixed cooked food, vegetables, etc. entrained with sand and recyclables, etc
16		BC	Same as above

Table 10: Physical and chemical characteristics of each substrate and inoculum for batch tests

	Substrate		% TS	%MC	%VS	pH	C:N
1	Fruit & vegetables wastes waste	E	11.4	88.6	89.6	4.7	22.1
2	Vineyard Hotel food waste	VH	25.7	74.3	96.9	4.6	22.9
3	Cavendish Square food waste	CAV	23.4	76.6	74.5		30.2
4	Wynberg Drop-Off garden waste	WDO	31.8	68.2	92.6	NA	NA
5	Water Hyacinth	WHW	6.6	73.4	76.7	6.3	10.7
6	Abattoir waste	AW	29.4	70.6	96.9	6.2	11.1
7	Blood waste	BW	13.9	86.1	93.5	7.6	3.6
8	Fisheries waste	FW	35.8	64.2	95.4	7.5	4.7
9	Waste paper pulp	PW	32	68	98.5	6.7	21:0
10	Cereal waste	CW	91.0	9	97.7	NA	61
11	Garden waste	AGW1	45.1	54.9	77.3	NA	51.3
		AGW2	53.8	46.2	79.7	NA	32.4
		AGW3	41.5	58.5	74.3	NA	80.6
		AGW4	43.6	56.4	68.5	NA	102.3
12		BC GW	47.6	52.4	61.7	NA	36.6
13	Fines	AFi1	56.8	43.2	54.9	NA	86.2
		AFi2	55.4	44.6	45.6	NA	11.7:0
		AFi3	51.3	48.7	88.2	NA	9.9:0
		AFi4	51	49	68.2	NA	59.1
14		BC Fi	50.8	49.2	43	NA	6.9:0
15	Food waste	AFW1	17.4	82.6	82.3	5.6	20
		AFW2	15.9	84.1	89.8	5.6	21.5
		AFW3	17	83	88.2	5.4	27.9
		AFW4	21.7	78.3	68.2	5	17.5
16		BC FW	39.6	59.4	81	4.4	39.9
17	Inoculum	I	3.5	96.5	82.7	7.2	NA

Where NA: Not Analysed

3.4.2 Fed-batch anaerobic digestion tests

The aims of this second phase of laboratory work are presented in detail in Section 3.2. The substrates selected were therefore similar to that used in the BMP experiments i.e. restaurant waste from the Vineyard Hotel, garden waste from the Wynberg Drop-Off and blood waste from the Stickland abattoirs.Batch results of general abattoir waste were nearly identical to that of blood waste (as presented later, Section 4.1.4).For this reason, pure blood was used in the second experimental phase instead of general abattoir waste since it is easier to handle due to its homogeneous nature.

The inoculum used was from a mixture of waste activated sludge (WAS) from the Athlone Sewage treatment plant and, inoculum from a domestic biodigester at the Goedgadacht Estate in Riebeek Valley (~70:30% mixture respectively). The biodigester at the Goedgadacht operated on a mixture of kitchen waste and toilet waters, under ambient temperatures. The description of the waste sampled for anaerobic digestion tests is presented in Table 11 below.

Table 11: Description of the three organic waste samples for fed-batch tests

	Appearance/Description
Kitchen Waste	Moist restaurant waste i.e. gravy, egg shells, cooked meat and vegetables, etc.
Garden Waste	The garden waste collected was a mixture of fresh leaves and partially composted.
Blood waste	Pure / animal blood

The physical characteristics of the substrates and inoculum used are presented in Table 12. Generally, the C:N ratio of the substrates used are similar to those of the substrates used for the BMP tests (Table 10, Section 3.4.1).

Table 12: Physical and chemical characteristics of various substrates and Inoculum under fed-batch conditions

	TS	MC	VS	C:N
Kitchen waste	0.25	0.75	0.94	26.8
Garden waste	0.42	0.58	0.67	40.5
Blood waste	0.20	0.80	0.96	3.0
Garden+Blood	NA	NA	NA	12.6
Inoculum	0.01	0.99	0.79	N/A

Where NA: Not Analysed

3.4.3 Pre-treatment of the various organic waste samples

Pre-treatment of waste samples was essential before the inoculation to provide a suitable and refined substrate for AD. The pre-treatment entailed removing non-degradable materials (glass, plastics, metals and large unbreakable wood chips for Wynberd Drop-Off waste) from the waste samples, particularly for the highly heterogeneous waste from ARTS and BC as adapted from the research by Forster-Carneiro *et al*. (2008) and Fernandez *et al.* (2008).

The food wastes, with the exception of homogeneous substrates such as cereal waste, were diluted with distilled water and mashed to a homogeneous paste using a kitchen blender. While, fines and garden refuse were dried to a higher total solids (TS) content over 24 hours, then crushed and sieved to a particle size of <2800 µm. No pre-treatment was required with waste paper pulp since the waste collected was already a homogeneous paste.

3.5 Batch tests for the Biomethane Potential

Pyrex serum vials with a working volume of 100 mL were used as bioreactors, in a controlled temperature chamber under mesophilic conditions (37°C) for 60 days (Figure 15); the tests were carried out in triplicate. The experimental set-up, presented in Tables 76-77(Appendix, Section 9.3.2.1), was scaled down from conditions used by Forster-Carneiro (2008) i.e. dry conditions (20% TS and 30% inoculum) to 5% TS and 30% inoculum on a volume basis. Each vial used was sparged with nitrogen gas (N_2) for 2-3 minutes to create anaerobic conditions. The reactors were then sealed with rubber stoppers and aluminium crimps.

Trace element solutions were added to the bioreactors to provide nutrients for microbial growth as recommended by the ASTM book of Standards. Table 13 presents the concentration and volumes of the solutions of trace elements solution added. Sodium hydrogen carbonate ($NaHCO_3$), was used as a buffer, at a buffer-to-substrate ratio of 0.06 on a wet mass basis in the BMP experiments to achieve a neutral pH (~7) before inoculation. This is supported by research conducted by Brummeler and Koster (1988) which showed that $NaHCO_3$ was the most effective buffer for AD compared to other alkali chemicals at the cited ratio. A control experiment with distilled water and only the inoculum was set up additionally, to determine the amount of biogas generated by the inoculum solution.

Table 13: Concentration of trace elements solution added to each bioreactor

Trace metals	concentration (g/L)	mL in 100 mL volume
KH_2PO_4	27	1
$NaHPO_4$-$12H_2O$	112	1
NH_4Cl	53	1
$CaCl_2$-$2H_2O$	7.5	1
$MgCl_2$-$6H_2O$	10	1
$FeCl_2$-$4H_2O$	2	1
Resazurin (O_2 indicator)	0.1	1
Na_2S-$9H_2O$	5	2

The biogas volumes were measured through negative displacement of water, according to Angelidaki *et al.* (2006) as illustrated in Figure 15 below. Red food colourant was added for visibility, and the water was acidified to a pH ~4 to conserve biogas volumes and minimise dissolution of carbon dioxide gas (CO_2). The difference in water level was recorded as the volume of the biogas produced. The biogas produced was collected on a daily basis for the first 3-4 weeks and, less frequently thereafter since minimal amount of biogas were produced.

Figure 15: Illustration of experimental set-up of batch anaerobic digesters

3.6 Progressive Feeding Model

The feeding regime used for the second phase of experiments was based on the research carried out by Angelidaki *et al.* (2006) with AD of the OFMSW under thermophilic conditions (~55°C) to ensure an effective start-up. Results from this study showed that a gradual progressive feed based on the 'activated biomass concept' is a preferred start-up strategy compared to feeding daily a constant amount as the second option leads to higher biogas yields and more stable process conditions.

These findings are supported by research conducted by Lepitso and Rintala (1995) on the investigation of ways to reduce inhibition experienced by the build-up of intermediate products such as VFAs, which generally lead to reactor failure at high concentrations.

Following Angelidaki's work, limited amount of inoculum (i.e. ~15% of final volume) were used at the start-up phase. Experiments were carried out under fed-batch conditions for 60 days, except for the co-digestion experiments which lasted 50 days. The progressive feed was used with all the three substrates (i.e. kitchen, garden and blood waste), adapted from Angelidaki *et al.* (2006), based on the 'activated biomass concept' (discussed in detail later in the next session, Section 3.6.1). The organic loading rate values variedbetween 1.7 and 15 g VS/day for the progressive feed rate and, were 5.7 g VS/day for the flat feeding rate with kitchen waste in Angelidaki's research.

Moreover, the effect of the feeding regime on the performance of AD systems was investigated using kitchen waste, where both feeding regimes were used (i.e. progressive and flat feeding regime) .A total of 10 reactors were therefore set-up and, all experiments were run in duplicate. An additional reactor was setup, as a control, with the inoculum and only distilled water. This reactor was dismantled when no biogas was produced after 2 weeks at ambient temperature.

3.6.1 Activated biomass concept

The initial amount of inoculum at start-up is considered as 'active biomass' and it is assumed that the amount of substrate alimented daily to the reactor is gradually converted to 'active biomass' over a period of seven days. The conversion factors of the fresh biomass achieved over the seven days are presented in Table 14 where 25% of the fresh feed is converted to activated biomass during the first day, then 20%, then 15% and so on. The amount of inoculum ('active biomass') as a function of time could be determined algebraically using Equation 14

$$\mathrm{AB}_n = \mathrm{AB}_{n-1} + \sum_{i=1}^{7} \mathrm{F}_{n-i} a_i,$$

Angelidaki *et al.* (2006) **Equation 14**

Where AB= activated biomass; F= daily feed; n= day number; a_i*=conversion percentage, i=1-7.*

The 'activated biomass concept' recommends an exponential organic loading rate to follow the gradual growth of AD microbes, which should prevent overloading the bioreactors.

Angelidaki's results showed that the gradual progressive feeding regime (i.e. with initial low organic loading rates and minimum inoculum) was a more effective start-up strategy to avoid acid build-up and allow the progressive adaptation of the AD microbes (Angelidaki *et al.,* 2006).

Table 14: Percentage conversion of fresh feed to "active biomass" based on the feed activation concept for loading at a progressive increasing rate, adapted from Angelidaki *et al.*(2006)

Feed activation concept		
Days	ai	Conversion (%)
1	a1	25
2	a2	20
3	a3	20
4	a4	15
5	a5	10
6	a6	5
7	a7	5
Total		100

ai: conversion factor during the first 7 days after feeding

3.7 Investigation of AD of Organic Wastes under Fed-Batch Conditions

Experiments were carried out in 2L bioreactors for 60 days under fed-batch conditions. Experiments were to be carried out at ambient temperature originally (i.e. 17°C) and with no agitation. However, the temperature was later increased to 35°C from week~2due to the poor performance observed at ambient temperature.The experimental set-up of the bioreactors at start-up is presented in Table 15.Distilled water was added at start-up to the total working volume. The water was then gradually replaced by the daily amount of substrate solution alimented to the reactors.

Table 15: Reactor set-up at the start of experiments

Experimental Set-Up	
Progressive Feeding Rate, gVS/day	0.97-8.6
Flat Feeding Rate, g VS/day	3.26
Inoculum Start-up, mL	(300 mL)~15% total volume
Water, mL	~1700
Buffer start-up ($NaHCO_3$),g	~100-150
Total working volume, mL	2000

3.7.1 Reactor design

Erlenmeyer flasks were used as bioreactors; the headspace had an equivalent volume of ~500mL; this space was used to enable the accumulation of the biogas produced. The flasks were sealed with rubber stoppers and lined with silicon to ensure the system was gas tight and minimise leaks. The rubber stoppers were designed with three outlets i.e. the main feeding port, the gas outlet and the sampling port. Bioreactors were also equipped with an overflow outlet at the 2L level (i.e. once the flask was 2L full), to ensure the total working volume remained constant. Hoffman clamps were used to close the bioreactors' outlets. A long feeding pipe was used to reach the bottom of the flask, to minimise loss of material through the overflow. The sampling port ran halfway down the flask to ensure the samples taken were a representative mixture (Figure 16).

Negative liquid displacement was also used to measure the amount of biogas volumes (Figure 16). The water was saline (~10 g/L of NaCl was added) and acidified to minimise loss of biogas and the dissolution of CO_2 in water as reported by literature (Angelidaki *et al.*, 2006; Walker *et al.*, 2009). Food colorant was also used to increase its visibility. All reactors were buffered at start-up with $NaHCO_{3;}$ and additional buffer was used later to control the pH for food waste and garden waste (Brummeler and Koster, 1988).

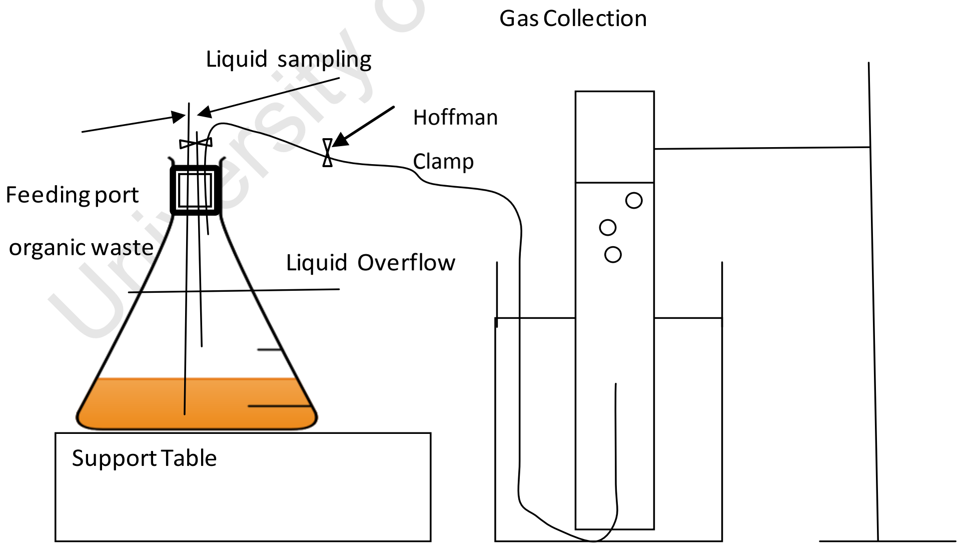


Figure 16: Reactor design of fed batch anaerobic digestion tests

A water bath with a heating resistance was used to raise the temperature of the experiments from ambient temperature to mesophilic conditions at ~35°C from the second week. The heating resistance was equipped with a pump to circulate the water and ensure the temperature was uniform. Polystyrene pellets were also added to the water bath as a top layer to minimise vaporisation of the water (Figure 16).

Figure 17: Experimental set-up of the fed batch anaerobic digestion tests

3.7.2 *Important design parameters*

The following main parameters were used as design parameters during experiments: organic loading rate (OLR), the total solids percentage in the feed (%TS), the hydraulic retention time (HRT) and solid retention time (SRT). The feed was diluted to a 5% TS on a mass basis (w/w), and the SRT was infinite. The system was based on these general assumptions: the amount of solids lost in the overflow was minimal, and as a result, the risks of bacteria wash out in the overflow were minimal, since the microbial population entrained in the sludge generated, settled at the bottom of the reactor.

The Organic loading rate (OLR) of the bioreactors varied from 0.49 kg VS/m^3/day to 4.3 kgVS/m^3/day for the progressive feeding rate, and was 1.63 kg VS/m^3/day for the flat feeding rate assuming the working volume remained constant at 2L (Equation 9, Section 2.2.3.5). The OLR was reduced to the constant flat feeding rate value of 1.63 g VS/m^3/day for all reactors from day ~45 since it was suspected that the reactors were overfed from the lower biogas volumes, and unstable conditions observed. The HRT in these experiments was calculated from Equation 9 (Section 2.2.3.5) from 25 days (OLR=3.25 kg VS/m^3/day) to 60 days (OLR=0.49 g VS/m^3/day) for the progressive feed rate; and was ~40 days for the flat feeding rate (OLR=1.63 kg VS/m^3/day). Sample calculations are presented in the Appendix (Section 3.5.1).

3.8 Co-digestion experiments

Anaerobic co-digestion means the digestion of two or more raw materials together in one process. In this study, co-digestion experiments were conducted both under batch and fed-batch conditions to investigate ways of overcoming the lower biogas and CH_4 yields observed with abattoir and blood wastes compared to others.

The rationale behind the experiments was to generate a substrate with a C:N ratio close to the ideal range for AD to reduce the inhibition experienced and improve process performance (CH_4 and biogas yield, concentration of volatile fatty acids, and NH_3 generated from the degradation of protein).

Many studies agree on the positive effect of co-digestion e.g. a 80-400% increase in CH_4 production is achievable when co-digesting manure and/or sewage sludge with other organic wastes streams (Braun *et al.*, 2003 cited in Luste *et al.*, 2002).

NH_3 inhibition generally experienced with AD of nitrogen-rich substrates, by co-digestion with a rich carbon source. These experiments were set up because of the low biogas yields obtained from abattoir and fisheries wastes relative to others with the BMP tests. Codigestion is also reported to improve various parameters which affect AD, such as nutrient balance, improved TS content, C:N -ratio and dilution of inhibiting substances,etc (Mata-Alvarez et al., 2000; Mata-Alvarez, 2003).

The optimum range for the C:N ratio is between 25 and 35, since 30 is the required C:N ratio for optimum microbial growth in anaerobic digestion. Lower C:N ratios imply an excess of N per degradable C, which leads to excess inorganic N as NH_3 whose accumulation can lead to inhibition of the methanogenic archaea (Bernal *et al.*, 2008), as discussed earlier in Section 2.3.5. Higher C:N ratios slow down the degradation process since there is an excess of degradable substrate for the microorganisms.

The garden wastes and fines were mixed at a ratio of 3:1 to blood and abattoir waste while paper/ blood or abattoir waste, and the paper sludge was mixed at ratio of 1:5 on a wet mass basis. The mixing ratio used, was estimated sufficient to provide a substrate with an adequate C:N ratio based on similar C:N ratio values from literature (Washington State University, 2010), as presented in Table 16.

Table 16: Literature values of the C:N ratios used to determine mixing ratio (Adapted from Washington State University, 2010)

Waste types	C:N ratio
Newspaper, shredded	175:1
Manures	15:1
Garden waste	30:1

3.9 Data Acquisition

The following experimental variables were monitored throughout the duration of the experiments: biogas volumes, pH, TS, VS, chemical oxygen demand (COD), methane (CH_4) composition, VFAs, free and saline ammonia (FSA) and the C:N ratio. The biogas volumes were generally measured after swirling/shaking the reactors to release the biogas trapped.

3.9.1 Batch BMP tests

The biogas was measured on a daily basis; while the pH, COD, CH_4 composition, TS and VS were measured on a weekly basis. The biogas volumes were measured less frequently after week 4 since the biogas production reduced significantly thereafter. Samples were extracted using a syringe, for the remaining analyses.

3.9.2 Fed-batch anaerobic digestion tests

The main parameters investigated in the second phase of experiments were: the pH, biogas volumes, VFAs and FSA concentration, TS and VS. The CH_4 was not measured due to technical problems with the Gas Chromotograph (GC) during the time experiments were carried out. Biogas volumes and pH were measured on a daily basis, while the concentration of VFAs, TS and VS were measured three times a week.

The FSA concentration was measured on a weekly basis for the bioreactors digesting pure blood and the mixture of garden waste with blood. The amount of biogas produced at ambient temperature during the first two weeks was minimal; hence biogas readings were only recorded from the 2nd phase of experiments onwards. The gas outlet tubing was left closed for the biogas to accumulate in the headspace and only opened to measure the biogas produced daily. The pH was measured from a sample collected from the overflow using a standard calibrated pH probe.

3.9.3 Analytical methods

3.9.3.1 Volatile fatty acids (VFAs)

VFA analyses were performed using a High Pressure Liquid Chromatography (HPLC). The machine was used with the Breeze 2 software system, equipped with a BIO-RAD organic acids analysis column (dimensions: 300mm×7.8mm) and a UV detector (210 nm wavelength). The system's pressure was around ~1500 psi on average and sulphuric acid (0.01M H_2SO_4) at a flow rate of 0.6 mL/min, was used as a mobile phase.

The following acids were analysed: acetic, lactic, propionic, butyric and valeric acids, and the iso-forms of the later two. Standard solutions of each acid were made at different concentrations of 0, 0.25, 0.5, 0.75 and 1 g/L.

Calibration curves between the acid concentration and the peak area were used to identify and quantify the various acids in the bioreactors. The resulting standard curves are presented in the Appendix,Section 9.1.1.The samples extracted were poisoned with mercury to prevent further biodegradation and stored at 4°C until analysed. The samples were filtered to a particle size <0.45 µm prior to analysis and only 100 µL were injected into the column.

3.9.3.2 Chemical oxygen demand (COD)

The COD reading analyses were performed using the high range (500-10000 mg/L) MERCK COD solutions A and B, where solution B is the potassium dichromate solution in sulphuric acid and Solution A is mercury (II) sulphate in sulphuric acid. The samples were diluted with de-ionised, distilled water, with a diluting factor between 20 and 25 where necessary before being oxidised.

One mL of substrate solution was added to 2.2 mL and 1.8 mL of solution A and B respectively in glass vials. The mixture was digested in a heating block (HANNA C9800) for two hours at 150◦C. The digested mixture was transferred to cuvettes and inserted into a Helios α UV-Vis Spectrophotometer, where the COD was then measured at a wavelength of λ 610 nm. Alternatively, the absorbance of the samples was measured and the COD was determined using a standard curve of the absorbance versus COD concentration of potassium hydrogen phthalate (KHP). Standard KHP solutions of 0; 0.25; 0.5; 0.75 and 1 g/L were prepared to derive the standard curve (Appendix, Section 9.1.2).

The biggest challenge experienced with COD measurements was with the precision of the readings due to the highly heterogeneous nature of the waste examined such as with garden waste and fines.

3.9.3.3 *Total solids (TS), volatile solids (VS)*

The TS and VS were measured according to the *Standards Methods for theExamination of Water and Waste Water (1995).*

3.9.3.4 *Free and saline ammonia (FSA)*

Total nitrogen, referred to as Total Kjedahl Nitrogen (TKN) is the combination of ammonia and organically bound nitrogen in waste waters. TKN represents the bulk of the nitrogen in bioreactors; it is defined as the sum of organic nitrogen compounds that are converted to ammonium sulfates [$(NH_4)_2SO_4$] and, free ammonia (FA), Benton Franklin Health District (2002). It is the free and saline ammonia (FSA) that was measured in these experiments i.e. ammonium (NH_4^+) ions and free ammonia (NH_3). The literature suggests that the NH_3 inhibition experienced with AD of high proteins waste is mostly caused by FA (Kroeker *et al.*, 1979; de Baere *et al.*, 1984).

FSA analyses were carried out by titration and colorimetry, according to standard procedures from the Laboratory Procedures-Waste Water Treatment Laboratory (Lakay *et al.*, 2000) and *Standards Methods for the Examination of water and wastewater* (1995). Analyses were carried as follows: 5 mL of the bioreactor sample was mixed with 10 mL of pre-made solution of sulphuric acid (H_2SO_4), mercury sulphate ($HgSO_4$) and potassium sulphate (K_2SO_4).

The mixture was then distilled using a steam distillation unit where ~7 mL of Sodium hydroxide (NaOH) was added to the mixed sample solution, evaporated and condensed into an Erlenmeyer flask with 25 mL of Boric acid (H_3BO_3). The sample was then allowed to distil until the volume of the Boric acid solution in the flask increased to ~40-50 mL. This solution was then titrated using standardised 0.001N H_2SO_4 solution until a colour change to deep purple. The amount of acid used was recorded, and Equation 15 (Lakay *et al.*, 2000) was used to determine the FSA amount from experimental details.

$$ - \quad \frac{}{} \qquad \textbf{Equation 15} $$

Where,

a: mL 0.001 N H_2SO_4 for sample , N = actual normality of 0.001 N H_2SO_4 (i.e. =1), A= sample size (mL)

3.9.3.5 *Methane analysis using gas chromatography*

A Perkin Elmer gas chromatograph with a flame ionisation detector (FID) was used to analyse samples of the biogas produced on a weekly basis for batch tests.

The GC used is equipped with a capillary polar column (polarity 5Z, 30 M X 0.32 MM ID-SolGel-WAX 0.25 UM). The column material was SOLGEL-WAX with fused silica tubing coating.

GC syringes of 100 µl and 50 µl were used to collect the gas sample directly from the bioreactors and then inserted into the machine for analysis, the operational conditions used are presented in Table 17 . Nitrogen gas (N_2) was used as carrier gas. Standard methane (Air Liquide) gas with 52.5 % CH_4, was used as calibration for the system. Sample chromatographs, and calibration calculation are presented in the Appendix, Section 9.1.3.

Table 17: Gas chromatography conditions

Oven Temperature	40°C
FID Detector temperature	280°C
Injector Temperature	240°C
FID Pressure	60 Kpa
Carrier Flowrate	1.5 mL/min
Hydrogen Flowrate	~45 mL/min
Air Flowrate	~450 mL/min

3.9.3.6 *Elemental analysis (C: N ratio)*

The elemental analysis was outsourced and carried out by the Microanalysis laboratory in Chemistry Department, using an elemental CHNS-O Themo (carbon, hydrogen, nitrogen and sulfur) machine to determine the elemental composition of the samples. The C:N ratio was obtained by dividing the amount of carbon by the amount of nitrogen, where the relationship between C and N to mass was assumed to be linear . Sample calculations used to determined the resulting C:N ratio is presented in the Appendix, Section 9.1.5.

4 BATCH TESTS TO EVALUATE THE BIOMETHANE POTENTIAL OF DIFFERENT ORGANIC WASTES

Results of the biomethane potential (BMP) tests carried out under batch and mesophilic conditions at 100 mL scale, for 16 different substrates, are presented in this chapter. The following parameters were monitored to evaluate the performance of the different substrates: biogas productivity (mL/day) and yield (mL/g VS), CH_4 composition, pH, COD and VS destruction over the 60 days digestion period. The control BMP tests with only water and the inoculum produced negligible biogas (~3 mL/g VS cumulative).This was expected since the inoculum was de-gassed prior to inoculation.

This chapter is organised into three main parts, first presenting the BMP results, followed by a comparative discussion, andpresenting an analysis of the efficiency of the batch test, based on COD and VS profiles, in the last part. It should be noted that the repeat samples from the Athlone Refuse Transfer Station (ARTS) correspond to samples collected over four days of sampling (i.e. AFW1-AFW4 represent the food waste fraction at ARTS on the four successive days).

4.1 BMP of the different organic waste fractions investigated

Results of the analyses conducted for the 16 different waste samples are presented in four categories: (i) garden waste, (ii) fines, paper sludge and partially composted waste; (iii) food waste; and (iv) abattoir and fisheries waste.Each section begins with results of the biodegradation and relevant profiles (graphs), proceeds to present analyses of variance (ANOVA) based on biogas yields, then analyses gas quality and a possible effect of the additional bicarbonate buffering before finally estimating the bio-methane potentials as mL methane per g VS, the commonly used metric in the literature. A brief discussion derived from integrating various trends observed per waste category is also included.

4.1.1 Results of garden wastes

The following samples are included in this category: garden refuse from Bellville Compost (BCGW), Water hyacinth leaves (WHW), garden waste from Wynberg Drop-off (WDO), and repeat samples of garden refuse from ARTS viz. AGW1, AGW2, AGW3 and AGW4.

Anaerobic digestion of garden wastes

The daily biogas volumes and biogas yields for the different garden wastes are presented in Figures18 and 19 respectively. The highest productivity (distinct peak in mL/day) is observed within the first 10 days for most samples with 123, 213, 157, 168 and 127 mL/day for BC FW, WHW, WDO, AGW 1 and AGW 3 respectively.

The gas productivity decreases after day 19 for most waste samples. As a result, the biogas volumes were only measured every 3-5 days thereafter, except for WHW and BC GW. The cumulative biogas yields are presented in Figure 19 as a ratio of the cumulative daily volumes (mL) divided by the amount of loaded VS viz. mL/gVS (sample calculation are presented in the Appendix, Section 9.1.8).The highest biogas yield in this category was from garden refuse from BC with 546.1 mL biogas/g VS, and the lowest biogas yield was from water hyacinth (WHW) with only 265.2 mL biogas/ g VS.The biogas yield of WDO was 368.8 mL biogas/g VS and the average biogas yield for the repeat samples from ARTS (AGW 1-4) was 384±25 mL/g VS.

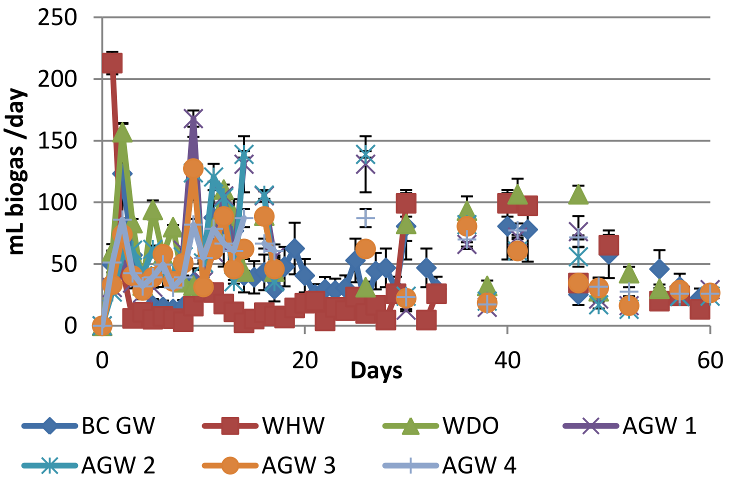


Figure 18: Daily biogas volumes of AD of garden wastes

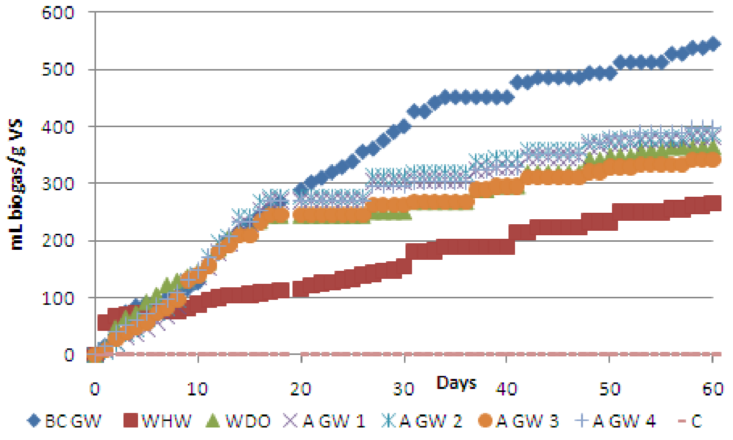


Figure 19: Cumulative daily biogas production from different garden wastes

Analysis of variance

An analysis of variance was performed for the cumulative biogas yields (cumulative biogas in mL/g VS) to evaluate the level of variability across these waste samples statistically. The analysis was done using the two-way ANOVA without replication in *Microsoft Office Excel 2007*(method outlined in details inAppendix, Section 9.1.11).

The analysis was performed using the biogas yields for the different samples in this category, and also for the repeat samples from ARTS in particular. Tables 18 and 20 present the individual yields per triplicate for repeat samples from ARTS, and the data for all waste samples in this category. The average of ARTS data (e.g. AGW1-4) was used for to analyse the variability in the second analysis, Table 21. This was to examine the 'actual' variability per source rather than per sample since ARTS results had already been examined in detail separately.

Table 18: Biogas yields for repeat samples of garden waste from ARTS (mL / g VS fed)

	AGWI	AGW2	AGW3	AGW3
I	356.08	363.99	277.76	388.98
II	348.07	363.74	344.78	346.64
III	412.46	405.91	380.30	439.78

Table 19 below presents results of the ANOVA for ARTS samples. Rows represent the triplicates (viz. I,II, and III), and the columns represent samples collected over four days. Results show that the P-value for variation between rows, is smaller than 0.5 and F is greater than F-crit, which means that the difference between the triplicates for each day is statistically significant. On the other hand, the difference between the means from sample from each of the 4 days, is not statistically significant (i.e. P-value> 0.05 and F<F-crit). This meansresults of samples collected over four days were relatively similar on average (i.e. 384.1±25 mL/g VS) despitethe fact the variability between samples collected per day was significant.

Table 19: ANOVA results for repeat garden waste samples from ARTS

Source of Variation	*F*	*P-value*	*F crit*
Rows	20.4	2.E-02	10.1
Columns	0.1	0.5	9.3
Error			
Total			

Results of the ANOVA performed for all garden waste samples are presented in Table 21 Rows represent the triplicates and the columns the different waste samples.

The difference across the waste samples is statistically significant (i.e. P-value< 0.05 and F>F-crit), while the difference between the triplicates, within each waste type is not statistically significant (i.e. P-value> 0.05 and F< F-F-crit). This shows good reproducibility of results, as the experimental set-up of the triplicates was identical.

Table 20: Biogas yields of garden wastes in triplicates

	WHW	BC GW	WDO	AGW
I	299.8	572.2	339.9	346.7
II	312.6	524.3	371	350.8
III	229.8	587.8	353.9	409.6

Table 21: ANOVA results for different garden wastes samples

Source of Variation	*F*	*P-value*	*F crit*
Rows	0.03	0.97	5.1
Columns	28.5	6E-2	4.73
Error			
Total			

Productivity and yield of methane

The CH_4 compositions for garden waste are presented in Figure 20. The highest CH_4composition was from BC GW with up to ~90% CH_4; and the lowest CH_4 composition was from WDO (i.e. it remained below 10%, except for week 5 where it increased to 71.5 % and dropped again).

There are two high regions for the CH_4 composition i.e. around week 2 with BC GW and AGW3 with 90% and 80% respectively, and later in week 6 with WHW and AGW3 viz. 88% and 85%. These values are higher than those generally reported in literature based on stoichiometry of AD i.e. 50-75% $CH_4$for biogas. This phenomenon is not uncommon and can occur when CO_2 is retained in the system from the speciation of CO_2 into bicarbonate (HCO_3^-) favoured at high pH and pressure, as discussed in the literature review (Section 2.2.3.3). Methane is hardly soluble in aqueous solutions, and as a result the ratio of CH_4: CO_2 increases. The CH_4 composition of WHW remained below 20% and increased drastically in week 5 to 85 and 80% in week 6 to decrease thereafter (Figure 20). This period also corresponds to an increase in the biogas productivity from ~25 mL/day to ~100 mL/day between day 35 and 42 and to a decrease in pH from from 9 (week 2) to 8 (week 4 onwards), as discussed later in this section.

The variability of the CH_4 content was at times high among triplicates, as witnessed by the large error bars. By contrast, the variability in the data collected for ARTS was relatively low compared to other samples as their CH_4 profiles are similar (Figure 20). The very low CH_4 composition on day 35 (week 5) for most waste corresponds to periods with little biogas produced (less 50 mL/day).

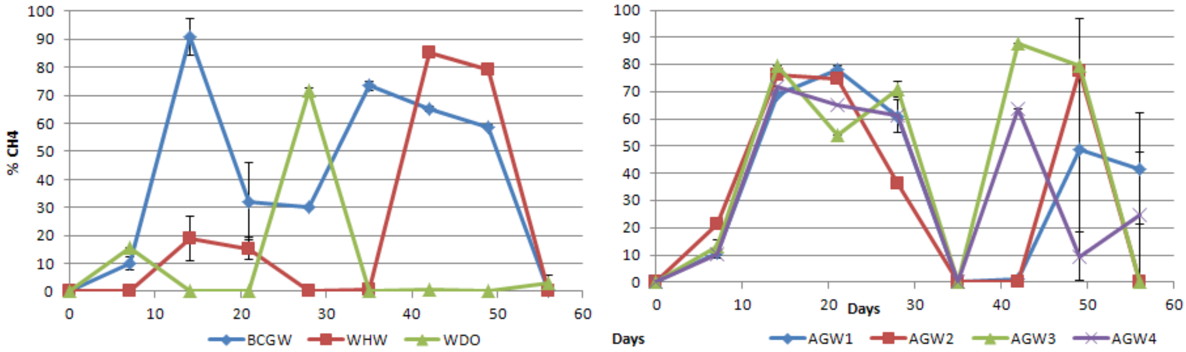


Figure 20: CH_4 content in biogas for garden waste samples

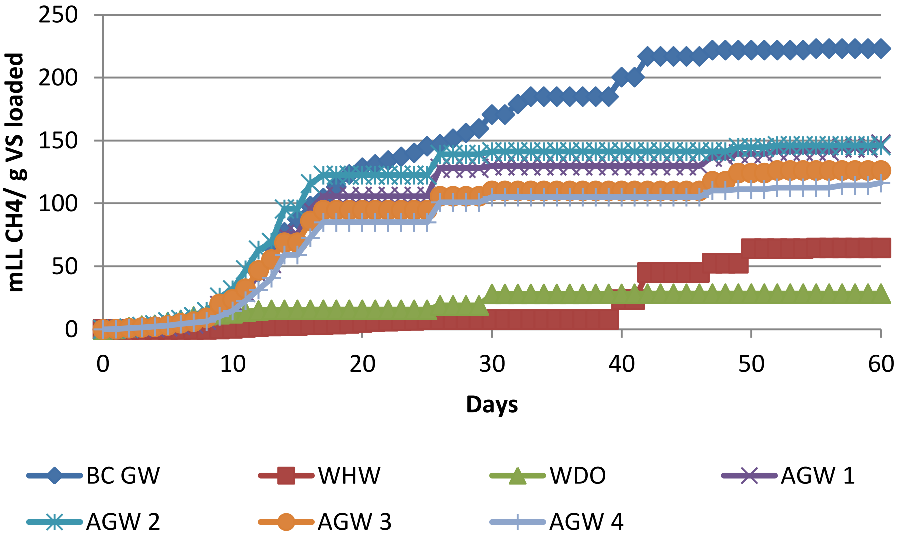


Figure 21: Estimated CH_4 yield of garden waste samples

Profiles of the estimated CH_4 yields for each waste samples, are presented in Figure 21 above. The CH_4 volumes were calculated from the product of biogas volume and estimated CH_4 composition, using linear interpolation for the CH_4 composition data between weeks. The highest CH_4 yield was from BC GW with 224 mL CH_4/g VS and the lowest was from WDO with 28.4 mL CH_4/g VS.

The average CH_4 yield for repeat samples from ARTS was 134±15.2 mL CH_4/g VS. The CH_4 yield curves exhibit an' S' shaped curve viz. with an initial lag phase, followed by a period of fast increase and finally levelling off towards the end, though with lag phases of differing length for different substrates – and no real methanogenic activity for WDO.

pH control

The pH was relatively stable for the different garden waste digestions, except for a pH drop observed in the first week. The pH recovered and remained in the neutral range thereafter, with the exception of WHW which was alkaline throughout the digestion period. The pH dropped to ~6 and ~5 for BC GW and repeat samples from ARTS, but the system was buffered with additional $NaHCO_3$ which helped with pH control (Figure 22).

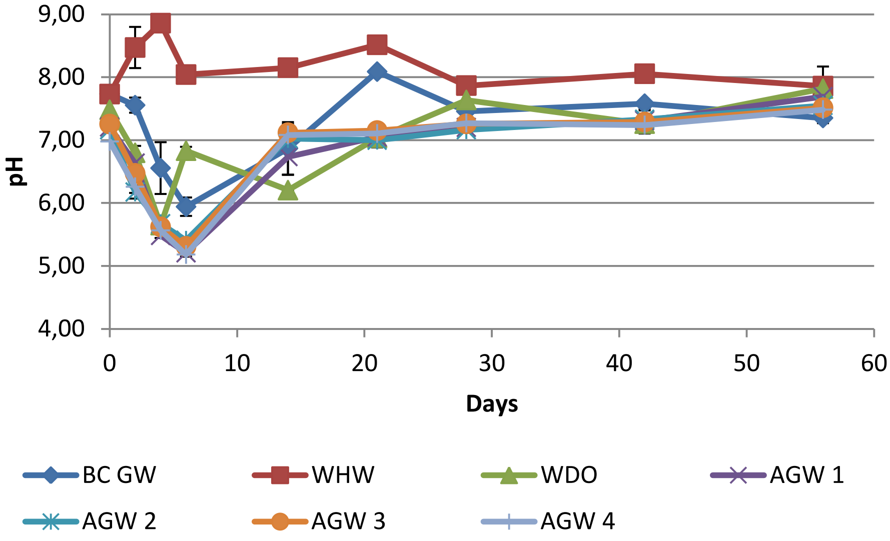


Figure 22: Temporal evolution of pH for garden wastes

Table 22 presents an estimation of the maximum CO_2 that could have been released from the buffering system if all the $NaHCO_3$ added at start-up and later on, had reacted at 35°C and atmospheric pressure as illustrated in Equations 16 and 17. Solution thermodyamics for CO_2 in aqueous solution predict that the volume of $CO_2(g)$ released from the buffer is a function of the pH and pressure, with CO_2 release more pronounced at lower pH,as presented in Figure 7 in the literature review (American Chemical Society, 1979; Section 2.2.3.3).

$$HCO_3^- + H+ \leftrightarrow H_2CO_3 \qquad \textbf{Equation 16}$$

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad \textbf{Equation 17}$$

The buffer was added at a buffer to substrate ratio of 0.06 per gram of VS adapted from the published research by Brummeler and Koster (1988), as discussed earlier in Section 3.5.2. Hence, the amount added per waste sample depended on the characteristics of each waste type i.e. TS and VS (Table 76-77, Appendix- Section 9.3.2.1). The fraction of maximum chemical CO_2 release compared to the total biogas volumes for BC GW and WDO were 37.8% and 16.8% respectively. This inferred fraction was generally lower (i.e. 6.8% ±0.8) for repeat samples from ARTS since less buffer was added.

The fraction of CO_2 produced biologically over the total CO_2 is also presented in Table 22; this was calculated as the ratio of the likely minimum CO_2 from biodegradation over the total CO_2 volumes estimated using the CH_4 yield values (sample calculation are presented in Appendix, Section 9.1.9.). For BC GW it is estimated that at least 36% of the total CO_2 released was from biological degradation. The range for the other samples (except WHW) was between 82-91%. BC GW had the highest estimated CH_4 yield in this category (224 mL CH_4/g VS). The amount of CO_2 possibly chemically released was relatively significant compared the 'biogas' volumes measured, aligning well with the big difference between measured biogas yields and estimated methane yields.

Methane production forWHW was estimated at 54.8 mL CH_4/ g VS, despite the theoretical possibility that the maximum of CO_2 released chemically could have exceeded the gas volumes recorded (i.e. 134%), Table 22. This is an indication that the actual CO_2 released chemically was less than the calculated value (as it should have at these high pH values); there isthus evidence that the methanogenic archaea were active. It is also likely that the alkaline conditions in WHW limited the activity of the methanogenic archaea which resulted in low CH_4 content and yield. Results of Ward *et al.* (2008) support the above statement, as the author report a 65% decrease in CH_4 yield was observed when the pH increased from 7.5 to 8 in the AD.

Table 22: Estimation of the maximum CO_2 produced from the buffer

	g buffer	No. moles	Vol chemical CO_2, mL	Total cum biogas, mL	% X	% Y
BC GW	2.1	2.5E-02	637.9	1685	37.8	36
WHW	**4.6**	**5.4E-02**	**1369.0**	**1017**	**134.6**	
WDO	0.9	1.1E-02	282.8	1681	16.8	82
A GW 1	0.3	3.8E-03	96.3	1507	6.4	89.8
A GW 2	0.3	3.7E-03	93.3	1564	6.0	91
A GW 3	0.3	3.7E-03	93.3	1295	7.2	88.9
A GW 4	0.4	4.3E-03	108.3	1387	7.8	89.6

*Where **X:** maximum fraction of chemical CO_2 over total cumulative biogas for each sample, **Y:** minimum fraction of 'biological' CO_2 over total CO_2*

It is noteworthy that the amount of buffer added to WHW was in excess relative to other substrates (~4 g in 100 mL, Table 22), thereforethe alkaline pH of WHW values observed results from the excess amount of buffer added in the digester.However, the buffer added in the other digesters was relatively lower (~2 g in 100 mL). The lower values from Table 22 estimating the fraction of ‘chemical CO_2’ relative to gas volumes collected, give confidence that most of the gas volumes measured were actual biogas, particularly for repeat samples from ARTS and WDO.

Figure 23 presents a scatter plot of all pH and CH_4 composition values for all waste samples in this category. Data from week 1 (i.e. %CH_4=0) was omitted, as well as values that corresponded to biogas volumes smaller than 20 mL per day. It is observed that CH_4 contents higher than 50% are never observed at pH values below 6.8. A pH within the neutral range thus seems to be a necessary condition for good biogas quality, as emphasised in the published literature. However, it is not a sufficient condition, as there are some data points showing low CH_4 composition values when solution pH was 7 or above, e.g. for WDO. At least some of these may include start-up conditions under buffering, where some chemical and/or respiratory CO_2 is released whilst methanogens are not yet active.

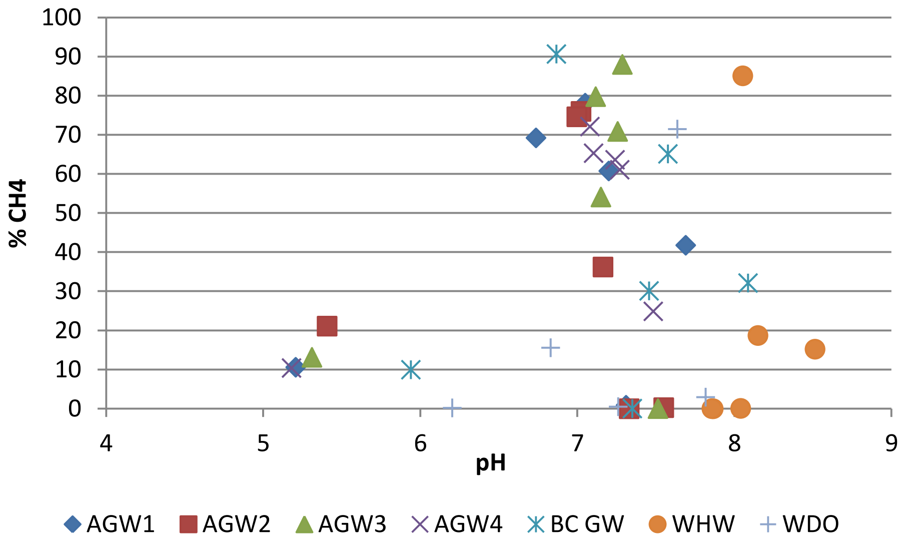


Figure 23: Scatter plot of CH_4 composition and pH

In concluding this presentation and interpretation of results for garden wastes, it is noted that reporting the measured “biogas” volumes is an overestimation. The actual biogas produced was less, due in part to the possibility that some CO_2 was released chemically from the buffering system. That notwithstanding, with a neutral pH regime generally maintained and reasonable CH_4 yields achieved, there is evidence that the methanogenic archaea became reasonably active for all samples except WDO.

Of the estimated bio-methane potentials reported in Figure 21, the value for water hyacinth should be regarded with caution due to the inhibitory pH regime in that experiment. These BMP results demonstrate that garden refuse could be suitable substrate for AD and not just composting as reported by previous research (Papadimitriou, 2010; Braber, 1995), and discussed earlier in Sections 2.1.3 and 2.3.1.

4.1.2 Fines and paper sludge

The following samples are included in this category: waste paper pulp from Nampak (PW), fines from Bellville Compost (BC Fi) andrepeat samples of fines from ARTS viz. AFi1, AFi2, AFi3 and AFi4.

Anaerobic digestion of fines and paper sludge

The daily biogas productivity (mL/day) and biogas yields (mL/g VS) for this category are presented in Figure 24 and 25 respectively. The highest productivity is observed between day 5 and 15 for most samples, with the exception of BC Fi where the highest production was achieved on day 3 with 73 mL/day. BC Fi had the highest biogas yield with 493 mL biogas/g VS, and PW had the lowest biogas yield with 140.9 mL biogas/ g VScompared to others. The biogas productivity generally decreases after the second week for most samples. BC Fi exhibits two high productivity periods i.e. between day 5 and day 15, and later between day~30 and 42.

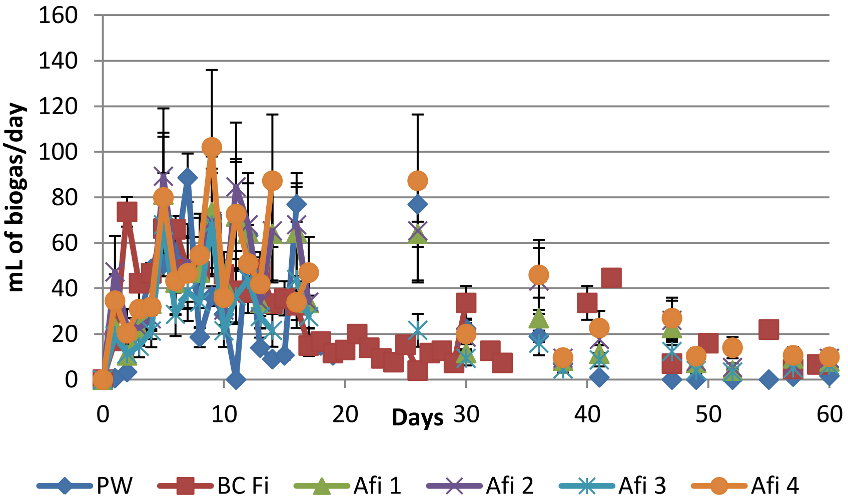


Figure 24: Daily biogas volumes from AD of fines and paper sludge

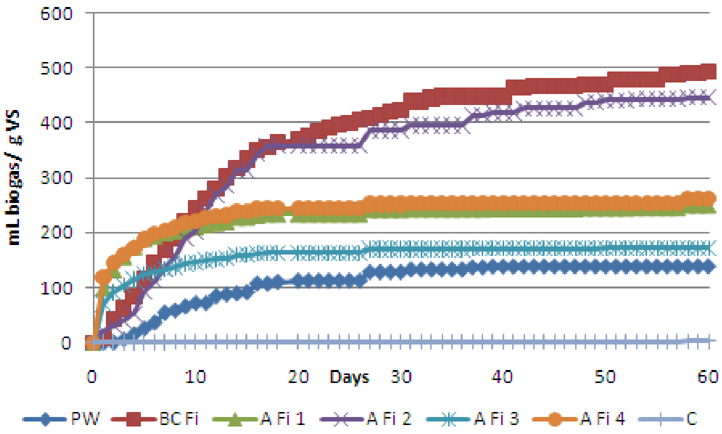


Figure 25: Cumulative biogas yield of fines and paper sludge

Analysis of variance (ANOVA)

Results of the analysis of variance performed are presented here. The analysis was carried out similarly to that of garden waste i.e. separately for repeat samples from ARTS, and across different waste samples in this category. Table 23 represents the average of results for AFi 1-4. Rows represent the triplicates for each sampling day, while columns represent samples collected from day 1-4.

The ANOVA results show that the variability within the triplicates from ARTS is not statistically significant (i.e. P-value> 0.05 and F<F-crit), while the variability across samples from different days is statistically significant. The high variability experienced with fines can be associated to the nature of the waste i.e. fines were highly heterogeneous as they constitute of any waste particle with a diameter smaller than 4 cm (food, plastic, paper, garden waste, etc). For example, the average biogas yield for the repeat samples (i.e. AFi1-AFi4) was 285.7 ±117 mL/g VS (Figure 25). It is therefore expected that some samples would be richer in biodegradable organics compared to others which explains the discrepancy observed in the results.

Table 23: Biogas yields for repeat samples from ARTS

	1	2	3	4
AFi	284.3	484.2	199.5	412.8
	350.6	433.7	230.4	428.7
	338.6	388.5	276.0	391.1

Table 24: ANOVA results for repeat samples from ARTS

Source of Variation	*F*	*P-value*	*F crit*
Rows	0.2	0.9	5.1
Columns	14.6	3.70E-03	4.8
Error			
Total			

Table 25 presents the biogas yields of the different waste samples in this category, and results of the ANOVA analysis performed are presented in Table 26 below. Rows represent the triplicates, while columns represent the different waste sample. The variability across samples is statistically significant (i.e. P-value> 0.05 and F> F-crit) while the variability within the triplicates for each waste sample is not statistically significant (i.e. P-value<0.05 and F<F-crit) as expected.

Table 25: Biogas yields for fines and paper sludge

	PW	BC Fi	Afi
I	124.4	478.3	345.2
II	155.5	384.2	360.9
III	135.5	688.6	348.5

Table 26: ANOVA results for fines and paper sludge

Source of Variation	*F*	*P-value*	*F crit*
Columns	12.3	1.90E-02	6.9
Rows	0.8	0.51	6.9
Error			
Total			

Productivity and yield of CH_4

Figure 26 presents the CH_4 composition of biogas for this category; it is observed that the overall CH_4 composition across waste samples followed a similar trend, despite the significant variation observed within the triplicates as illustrated by the large error bars. The highest CH_4 composition achieved was from AFi3 with up to 95% on week 7. Results remained generally high over the digestion period.

The period with low CH_4 compositions (i.e. lower than 25%) observed between days 21 and 35, corresponds generally to periods of low biogas productivity (i.e. lower than 20 mL/day) for most samples. Similarly, the high gas productivity periods observed with BC FW correspond to period with the higher CH_4 composition (60-75% between day 5 and 15, and 48-55% between day 35 and 49).

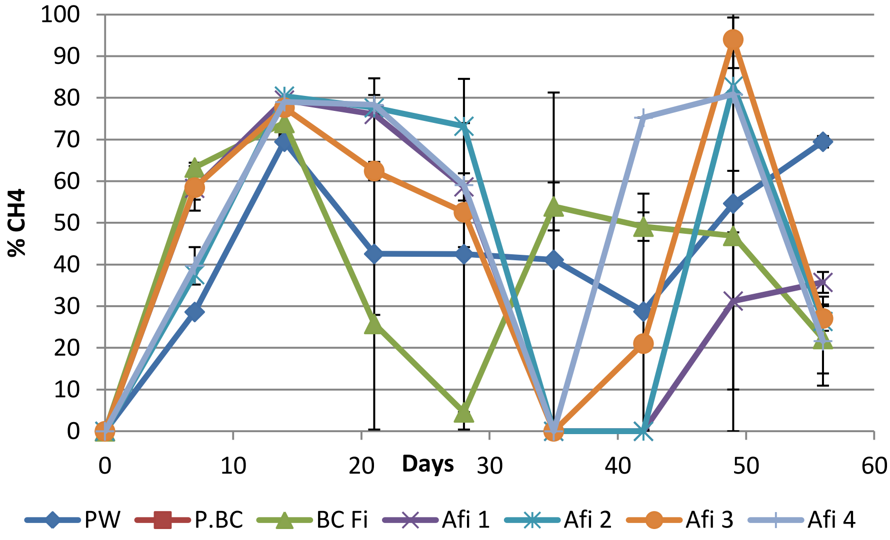


Figure 26: CH_4 content in biogas produced from fines and paper sludge

Figure 27 presents the estimated CH_4 yield of samples in this category. The high variability of the CH_4 composition also emphasises the need for regular sampling as more data points could provide better understanding /insight of the dynamics of the CH_4 as AD proceeds.

The profile of CH_4 yield follow from the CH_4 composition profiles, where the highest CH_4 yield is from BC Fi with 240.6 mL /g VS, and the lowest is from PW with 54.6 mL/g VS (Figure 3). The average CH_4 yield for the repeat samples from ARTS was 190 ±36.7 mL CH_4/g VS. The CH_4 profiles also exhibit an ‘s’ shaped form, the lag phase observed for methanogens isshorter (~8 days), this period is then followed by a linear increase until the activity is reduced further with the CH_4 productivity, as discussed earlier with garden wastes.

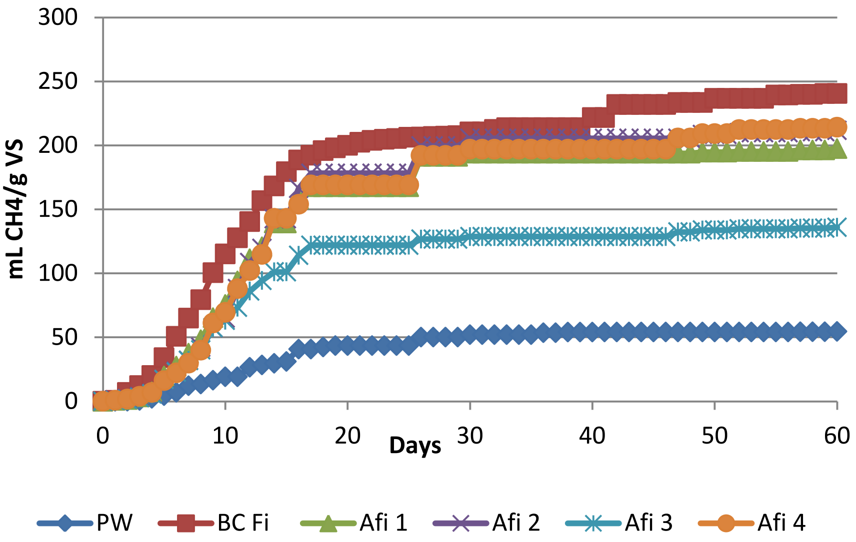


Figure 27: Estimated CH_4 yields for fines and paper sludge

Figure 28 presents a scatter plot of all pH and CH_4 composition for the different wastes in this category. Data points corresponding to low biogas productivity are not included on the graph. A pH higher than 6.5 again seems to be a necessary but insufficient condition for high CH_4 content in the biogas. The high CH_4 compositions (> 75%) occurred in a pH range of 7-~7.7) - conditions at which, the speciation of CO_2 into HCO_3^- was favoured.

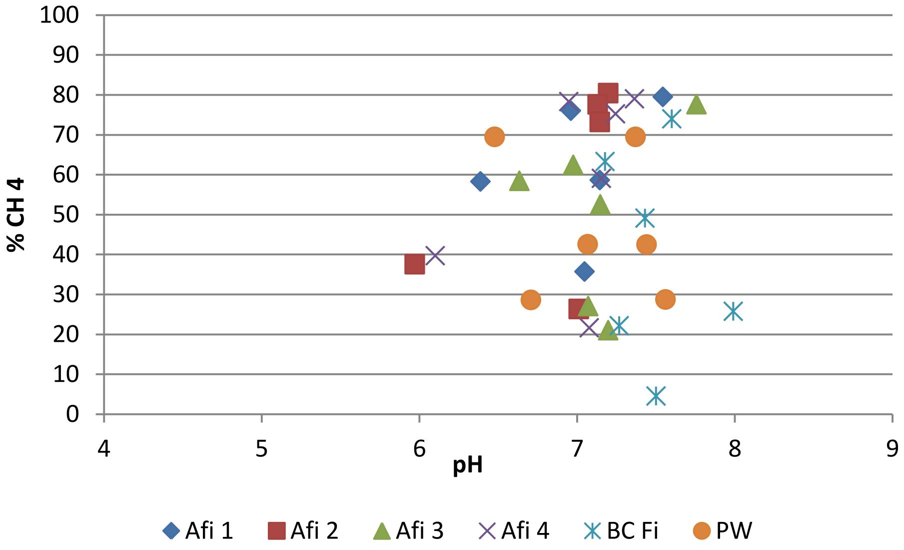


Figure 28: Scatter plot of CH_4 composition versus pH for fines and paper sludge

pH control

The pH in this category was fairly stable for all substrates i.e.the pH dropped during the first week and then increased above 6.5 above for the duration of experiments (Figure 29).

Table 27 presents the maximum amount of CO_2 that could have been released from the buffer ($NaHCO_3$). The maximum amount of chemical CO_2 released for PW constitutes ~41.5% of the 'biogas' measured. This fraction is 16.8% for BC Fi, and the average fraction for repeat samples from ARTS is 13.1 ±3.1 %. It is noteworthy that PW with the highest CO_2 released chemically, also had lower CH_4 composition and the lowest CH_4 yield.

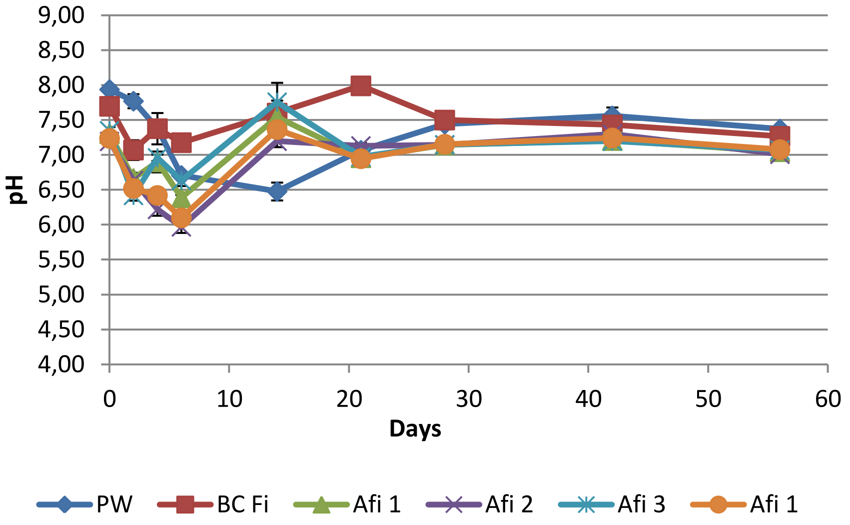


Figure 29: Temporal evolution of pH for fines and paper sludge

Table 27: Estimation of the maximum CO_2 produced from the buffer

	g buffer	No. moles	Vol chemical CO_2, mL	Total cum biogas, mL	%X	% Y
PW	0.94	1.1E-02	282.8	681.7	41.5	31.5
BC Fi	0.59	7E-03	177.5	1059.2	16.8	65.7
A Fi 1	0.3	3.6E-03	90.3	928.8	9.7	79.8
A Fi 2	0.47	4.4E-03	111.3	1025.3	10.9	83.8
A Fi 3	0.32	3.8E-03	96.3	618.5	15.6	65.
A Fi 4	0.33	3.9E-03	99.3	604.3	16.4	

Where ***X:*** maximum fraction of chemical CO_2 over total cumulative biogas for each sample, ***Y:*** minimum fraction of 'biological'CO_2 over total CO_2

It is noted that the measured “biogas” volumes are an overestimation, and the actual biogas produced were lower resulting from the chemical release of CO_2 from the buffering system. Results however generally showed stable conditions with pH that remained mainly within the neutral range for the digestion period, with the highest CH_4 content and yields for BC Fi. The good performance of waste samples in this category can possibly be associated to their mixed composition, as essential nutrients which may be limiting in one substrate might be supplied by another, improving hydrolysis rates and overall performance(Liu *et al.*, 2007).

It is noteworthy that PW results were lower than values reported in literature for paper sludge i.e. ~50 mL CH_4/g VS compared to ~150 mL CH_4/g VS (Lin *et al.*, 2009). Many authors agree that lower performance of digesters treating paper sludge can be associated with the composition of the waste, as it is rich in non-biodegradable organics such as lignin, and potential chemical inhibitors present in the waste e.g tannins, resin acids, sulphide, halogens, LCFAs (Ali and Sreekrishnan, 2001; Lin *et al.,* 2009), as explained earlier in Section 2.4.1.4.

4.1.3 Food waste samples

This category includes the following samples: the soft organic fraction (or food waste) from Bellville Compost (BC FW), repeats of samples of the soft organic fractions from the residual waste at ARTS i.e. mostly food waste (AFW1, AFW2, AFW3 and AFW4), fruits and vegetables biomass from the Cape Town market (EW), restaurant waste from Vineyard hotel (VHW), and restaurant waste from Cavendish Square (CAV) and cereal waste from Premier Foods plant (CW).

Biological degradation of food wastes

The daily biogas volumes measured are presented in Figure 30, and the biogas yields (mL/ g VS loaded) are presented in Figure 31. The ‘biogas’ productivity was at its highest during the first week and sharply decreased thereafter to remain low (i.e. below 50 mL/day) for the remainder of the digestion period, except for EW where the average productivity is ~93 mL/day on day 31 and 41.

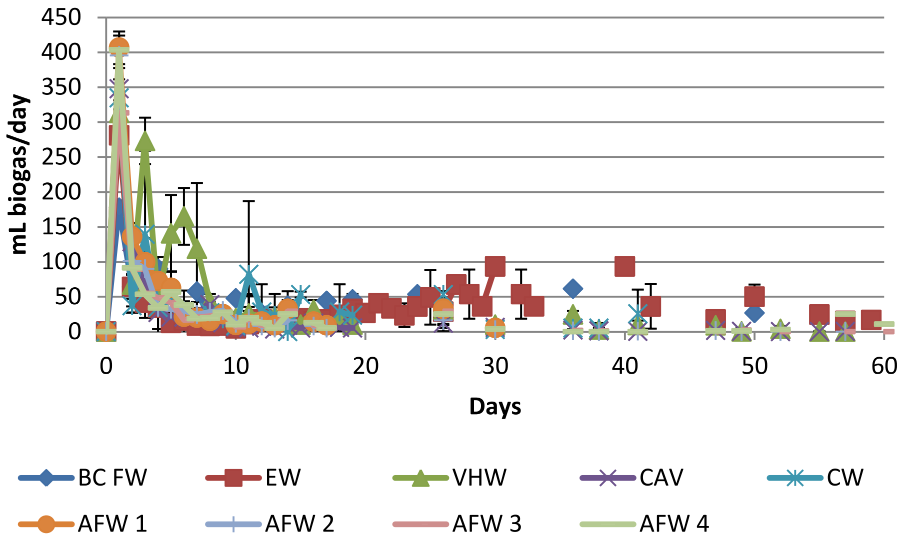


Figure 30: Daily biogas volumes of different food waste samples

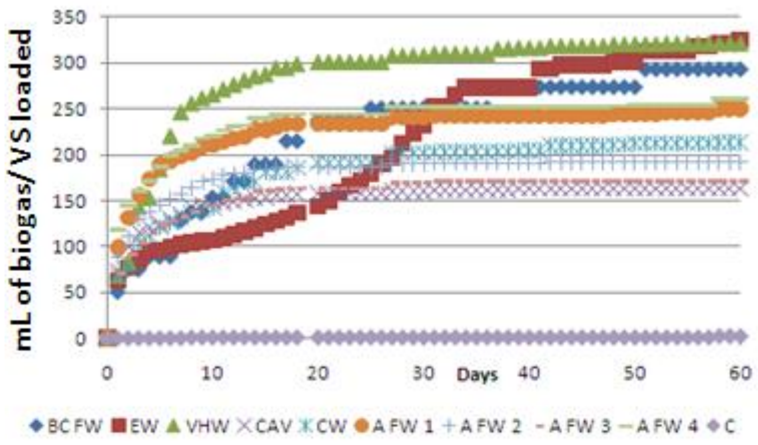


Figure 31: Cumulative biogas production of the different food wastes

The highest gas yield was from the Cape Town Market's biomass (EW) with 325.2 mL biogas/g VS, followed by restaurant waste from the Vineyard Hotel (321.9 mL biogas/g VS) and food waste from BC (BC FW, 292.9 mL biogas/g VS), Figure 31. The lowest gas yield was from food waste from CAV (163.1 mL biogas/g VS). The biogas yield of restaurant waste from CAV was almost half (166.5 mL/ g VS) of the waste from the VHW, despite the fact that they are expected to have a similar nature and would both yield similar results.

The trend exhibited by the biogas production curve from the Cape Town market is different from the others, viz. an 's' shaped curve is observed compared to others, which exhibit a linear increase for the first 10 days and a levelised curve thereafter.

Analysis of variance

An analysis of variance was performed to evaluate the variability between different food waste samples versus the variability within the triplicates based on biogas yields. Results of the analysis performed for repeat samples from ARTS and across all waste samples in this category are presented in Tables29 and 31 respectively.

Table 28: Cumulative biogas yields for repeat samples from ARTS

	1	2	3	4
AFW	244.4	182.1	190.2	245.8
	217.7	182.3	175.2	263.5
	294.2	200.9	142.9	258.5

Table 29 present ANOVA results for repeat samples from ARTS, where columns represent the samples collected over the four day sampling period and the rows represent the triplicate for each day. The variability between both the samples collected during days 1-4, and within the triplicates is not statistically significant (i.e. F< F-crit and P-value> 0.05) for rows and columns. This means that results across the 4 sampling days and between the triplicates were similar contrasting results of fines and garden waste from ARTS.

Table 29: ANOVA results for food waste from ARTS (i.e. AFW1-AFW4)

Source of Variation	*F*	*P-value*	*F crit*
Rows	0.29	0.76	5.14
Columns	3.58	0.09	4.76
Error			
Total			

Table 31 presents the results of the ANOVA across the different waste samples in this category. The columns represent the waste samples while the rows represent the triplicates for each waste sample. The variability across the different waste types is statistically significant since F> F-crit and P-value< 0.05, while the difference is not statistically significant within the triplicates i.e. F< F-crit and P> 0.05. ANOVA results show that triplicatesyielded reproducible results.

Table 30: Total cumulative biogas in triplicate per food waste samples

	EW	VH	CAV	CW	BC FW 5	AFW
I	1558	1323	723.5	507	686	875.1
II	1566.5	1465	778	658.5	788	846.4
III	1396.0	1604	834.5	1505	793	906.3

Table 31: ANOVA results for food waste analysis

Source of Variation	*F*	*P-value*	*F crit*
Columns	7.31	3E-2	3.36
Rows	1.66	0.24	4.10
Error			
Total			

where F: statistical test, P-value=probability, F-crit= value is the number that the test statistic must exceed to reject the test, alpha (α=0.05)=confidence level i.e. generally set up at 0.05 by convention

Productivity and yield of CH_4

The average CH_4 composition is generally low for this category compared to garden waste and fines (Figure 32). The CH_4 starts low, increases to the highest value between week 2 and week 4, and decreases thereafter for most samples. However, the highest CH_4 composition for CAV, AFW 1 and CW are respectively observed week 8 (50%) for both CAV and AFW1, and in week 7(40%) for CW.

The CH_4 composition of EW is distinctively different from the other waste samples viz. it increases to a maximum of 95% during week 5 (day 35) and remains above 75% thereafter (Figure 32). The CH_4 composition of EW is the highest in this category and is also higher than conventional values reported in literature derived from stoichiometry. The high CH_4 compositions observed might be explained by the speciation of CO_2 into HCO_3^- at higher pH as discussed earlier.

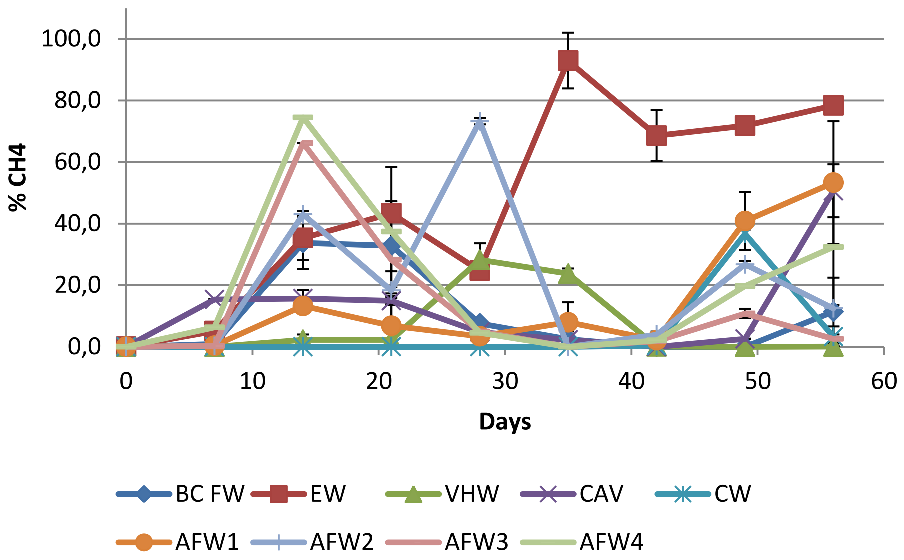


Figure 32: CH$_4$ composition of different food waste samples

The CH$_4$ yield for this category was generally very low for most samples compared to garden and fines results (i.e. below 15 mL CH$_4$/gVS), with the exception of EW which achieved ~119 mL CH$_4$/g VS as illustrated in Figure 33 below.Hence, Figure 3 only presents data of EW.Sample calculations are included in Appendix 9.1.8.

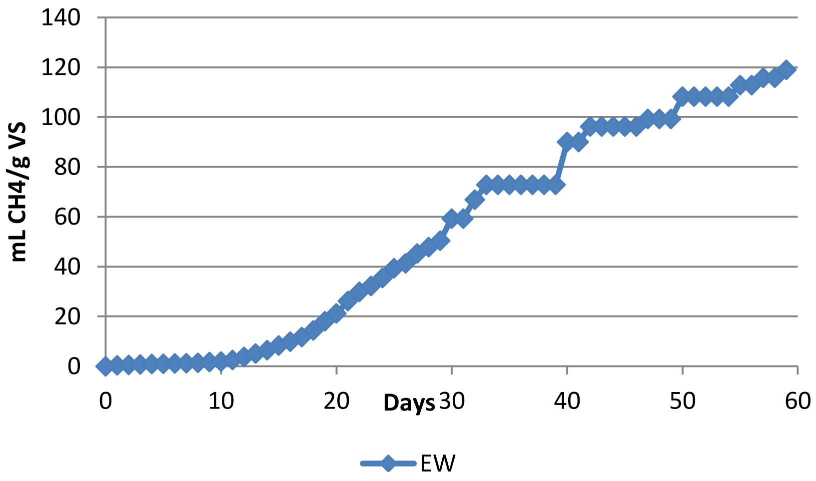


Figure 33: Estimated CH$_4$ yield of EW

pH control

Figure 34 presents the pH profiles of the different food waste samples. Generally, the pH dropped within the first week and settled around 6.5 for most waste samples with the exception of EW which recovered to above 7 after week 2 and BC FW which remained above 7 and then decreased with time.

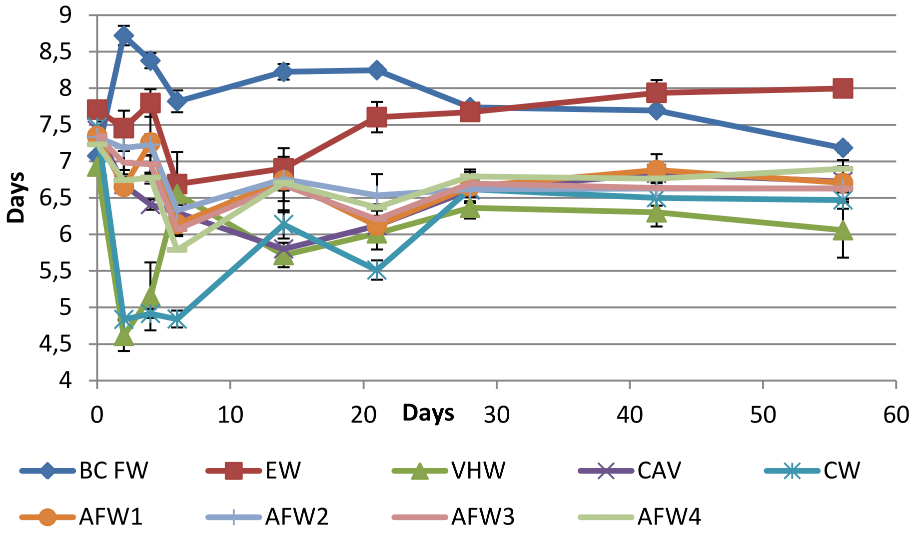


Figure 34: Temporal evolution of pH

Additional chemical buffer ($NaHCO_3$) was used to increase the pH in the first week since the ideal pH range for the methanogenesis archaea is between 6.5 and 7.5 (Ward *et al.*, 2008). The buffering system was unsuccessful since the average pH beyond week 1 was ~6.5 and below for most samples despite the added $NaHCO_3$, except for BC FW and EW which remained neutral.Table 32 presents the maximum of CO_2 that could have been released from the buffer for this category at 100% conversion rate.

Table 32: Estimation of the maximum CO_2 produced from the buffer

	g buffer	No.moles	Vol chemical CO_2, mL	Total volgas measured, mL	%X	%Y
BC FW	0.8	9E-3	228.7	755.7	30	68
EW	2.6	3.1E-2	788.3	1457.3	54	15
VHW	1.2	1.4E-2	352	1464	24	75
CAV	1.3	1.6E-2	394.1	781.7	50	48
CW	0.3	4E-3	99.3	1041.8	10	90
AFW 1	1.8	2.1E2	529.5	1056.5	50	49
AFW 2	1.9	2.3E-2	568.7	860.5	66	33
AFW 3	1.8	2.1E-2	532.6	756	70	26
AFW 4	1.4	1.6E-2	415.2	901.8	46	51

*Where **X:** maximum fraction of chemical CO_2 over total cumulative biogas for each sample, **Y:** minimum fraction of 'biological'CO_2 over total CO_2*

Results show that a significant fraction of the biogas produced could be chemical CO_2 from the buffer i.e. from 30-70% with the exception of CW (10%). The first week is characterised by the highest gas yields (Figures 30), very low CH_4 composition (15% and 20% for CAV and BC FW respectively, and below 5% for the remaining waste samples, Figure 32) and acidic conditions particularly for VHW (pH~4.6) and CW (pH~4.8), Figure 34. Also note that the 'biogas' peak observed during the first week constitute ~74% of the maximum CO_2 released chemically on average. These results are consistent with the low CH_4 composition and the pH profile (Figure 32) where acidic condition prevailed for the first week, as low pH (particularly for VH and CW i.e. pH~4.6) would favour the release of CO_2 (g) from the buffering system, as discussed earlier in Section 2.2.3.3.

It is noteworthy that of the CO_2 volumes estimated, about ~51±23 % is expected to have been from biodegradation (%Y, Table 32). This fraction is considerably lower for EW and AFW3 with 20% and 26% respectively where most of the CO_2 was released from the buffer; compared toCW and VHW with higher percentages (viz. 90% and 75% respectively).

Figure 35 below present the relationship between pH and CH_4. Generally, CH_4 composition higher than 50% were achieved at pH> 6.5, but this is not observed for all food waste samples. There isn't strong evidence for a relationship between the pH and CH_4 composition for food waste samples.

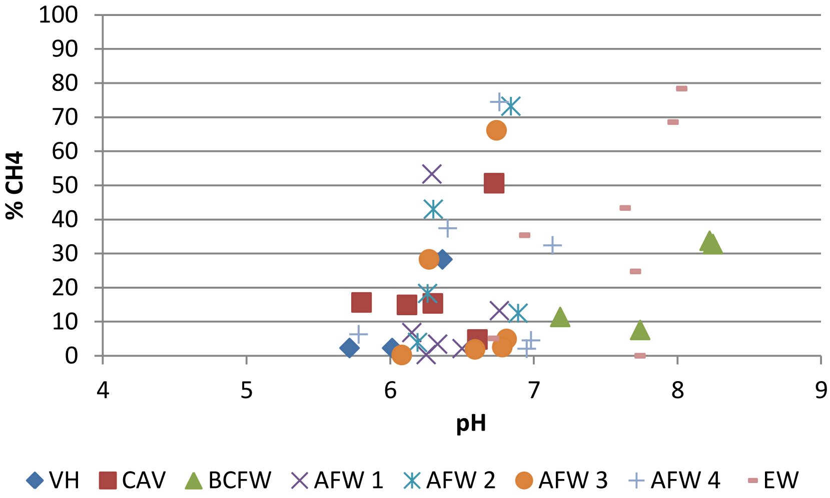


Figure 35: Scatter plot of CH_4 composition versus pH values

It is worth noting that despite the low fraction of chemical CO_2 compared to the biogas produced for CW (i.e. 10%), the CH_4 composition remained among the lowest (i.e. the CH_4 composition was almost null except for day 49, with ~40% methane), Figure 35.

It is more likely that the low CH_4 composition for CW resulted from the acidic conditions that prevailed in the bioreactor and as a result hindered the action of methanogens i.e. the pH of CW remained below 6 for the first 25 days and settled at 6.5 until the end of experiments (day 60). The pH drop wasprobable since cereals are made of easily degradable sugars (e.g. glucose) which would be hydrolysed rapidly and lead to the accumulation of volatile fatty acids (VFAs), during thelag phase experienced before methanogens convert the intermediate products into biogas,and consequently inhibit the activity of methanogens.

On the other hand, the pH profiles of EW show an increase above 7 after week 2, which correspond to higher biogas productivity rates and CH_4 composition. This trend is consistent with the literature on the optimal pH conditions for methanogenic activity (Igoni *et al.,* 2008; Viessman and Hammer, 1993; Eckenfelder, 2000).The pH stabilises when there is a balance between the activity of the various microbial groups (after the lag phase for the growth and adaptation of methanogens, an increase in the activity of methanogens would lower concentrations of available organic acids viz. LCFAs and VFAs, released in acidogenesis (Igoni *et al.*, 2008).The link between pH and CH_4 however it is not evident for BC FW, since it appears the activity of methanogens remained limited from the low CH_4 composition profile throughout the duration of experiments (below 40%) despite the neutral pH maintained for the digestion period.

The only waste samples which showed credible results for interpretation based on the biogas productivity and yield, CH_4 composition and pH profiles are EW for good performance compared to others.And, CW with limited amount of buffer added where the poor CH_4 yield achieved can be linked to the inhibition of methanogens from the acidic conditions that prevailed in the digester. It is hard to draw any conclusions from the data of the remaining samples in this category due to poor performance, which probably resulted as a combination of factors e.g. limited microbial activity beyond day 10, unsuccessful buffering and lower pHs which led to the release chemically of significant volumes CO_2 from the buffering system. .

4.1.4 Abattoirs and fisheries wastes

The following waste samples are included in this category: blood (BW) and abattoir wastes (AW) and fisheries waste (FW). Abattoir and fisheries waste are rich in protein i.e. their C:N is significantly lower (3.6 -11.1) compared to the other categories examined(Table 10, Section 3.4.1).

Anaerobic digestion of fines

The daily biogas volumes are presented in Figure 36, the productivity of AW and BW were the lowest compared to other categories i.e. the highest (~70 mL/day) was observed in the first week and the productivity decreased thereafter (below ~10 mL/day) for both waste types. The productivity of FW however was comparable to that of garden and fines i.e the highest observed was ~85 mL/day versus for example ~100 mL/day for fines.

The biogas yields, presented in Figure 37, of this category of wastes were the lowest compared to the others analysed i.e. the biogas yield of abattoir, blood and fisheries waste was respectively 77.7, 79.0 and 129.8 mL biogas/g VS. The biogas yield for fisheries waste was nearly twice as that of abattoir and blood waste despite their similar characteristics (Table 10, Section 3.4.1), and that the biogas profiles of blood and abattoir waste were nearly identical as expected.

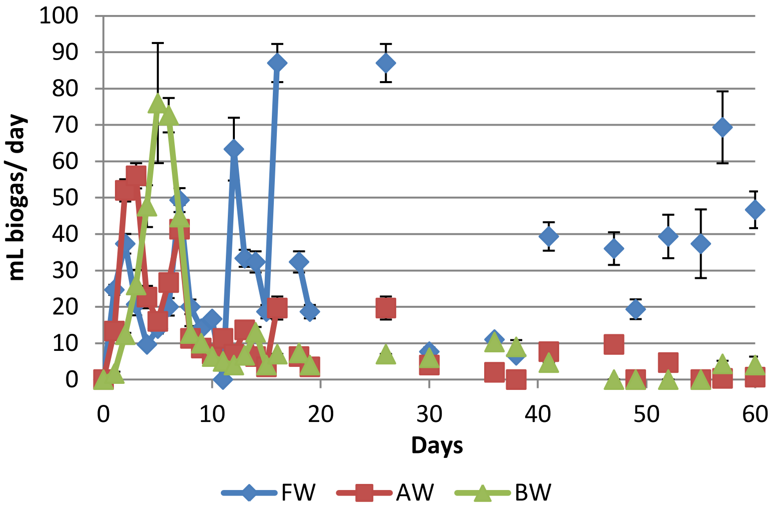


Figure 36: Biogas productivity for abattoir and fisheries wastes

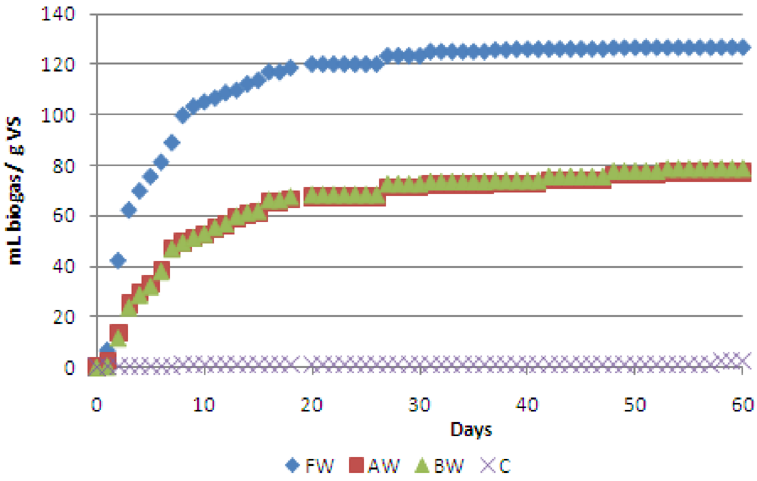


Figure 37: Biogas yield for abattoir and fisheries wastes

Analysis of Variance (ANOVA)

Results of the ANOVA analysis are presented in this section. The biogas yields in mL/g VS are presented in Tables33 and 35 of repeat samples from ARTS and for all waste samples in this category. Columns represent the different waste samples, while the rows represent the triplicate for each waste type. The variability across the difference waste samples is statistically significant (i.e. p-value <0.05 and F> F-crit) while the difference between triplicates for each sample is not statistically significant (i.e. P-value >0.05 and F< F-crit)., Table 34 The difference across samples indicates that the higher yields achieved with FW are statistically significant, since biogas yields from AW and BW were nearly identical.

Table 33: Biogas yields for repeat samples from ARTS

	AW	BW	FW
I	75	89.8	228.2
II	77	98.3	287.8
III	80.3	75.3	203.5

Table 34: ANOVA results for abattoir and fisheries wastes

Source of Variation	*F*	*P-value*	*F crit*
Columns	44.3	1.90E-03	6.9
Rows	1.6	3.10E-01	6.9
Error			
Total			

Productivity and yield of methane

The profiles of the CH_4 composition and yields are presented in Figures 38 and 39 respectively. The CH_4 composition of AW and BW was the lowest;AW and BW produced very little CH_4 throughout the duration of experiments i.e. the CH_4 remained insignificant and then increased to 60% for BW on day 42 (week 6), and to 30% in the last week for AW. The CH_4 profile of FW was significantly higher than of AW and BW i.e. the CH_4 increased to ~70% on week 3, and decreased to zero on week 7 to then sharply increased to ~82% in last week. The higher CH_4 composition for FW corresponds to the higher biogas productivity (i.e. 88 mL/day with 70% CH_4 and 70 mL/day with 82% CH_4). The estimated CH_4 yield of FW is 88 mL CH_4/g VS, while that of of AW and BW was almost insignificant i.e. less than 4.5 mL CH_4/ g VS (Figure 39).

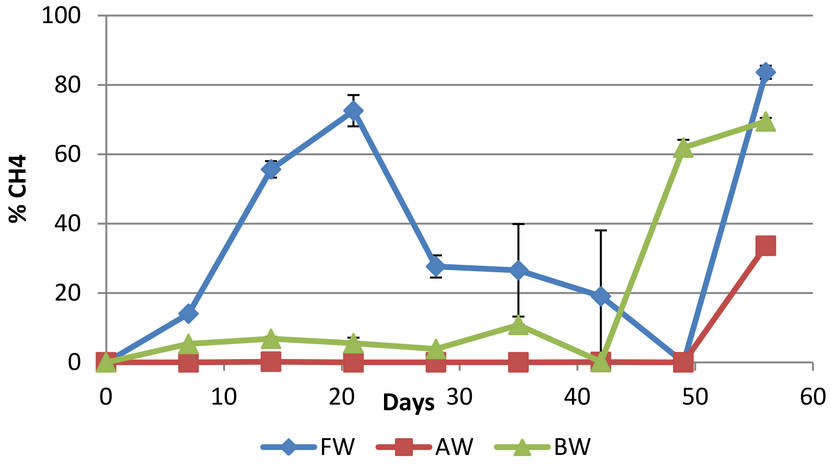


Figure 38: CH_4 composition of abattoir and fisheries wastes

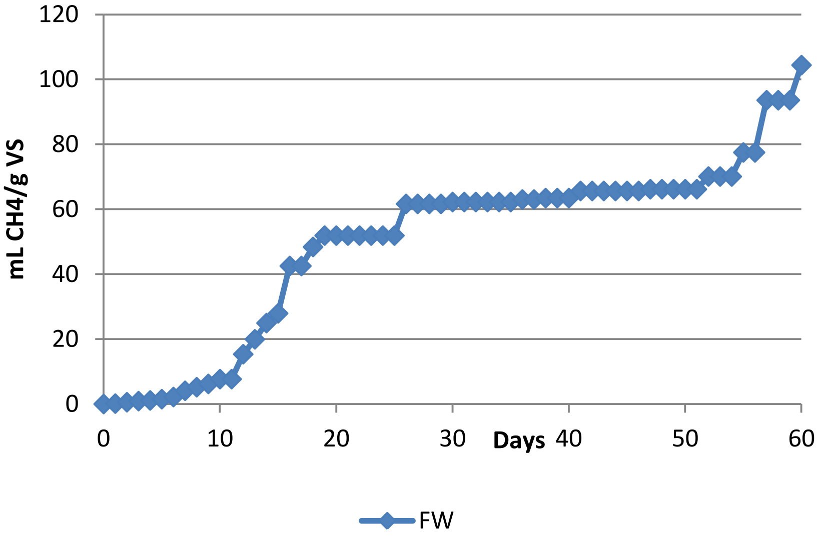


Figure 39: CH_4 yield of FW

pH control

The pH of N-rich substrates generally remained stable around 7 in general i.e. the lowest value the pH dropped to was 6.86 with abattoir waste (AW), Figure 40; the alkaline conditions in the reactor media can be associated to the basic effect of N-compounds such as $NH_3$and H_2S, formed from the degradation of protein in such wastes as illustrated Equation 18 (Deublein and Steinhauser, 2008).Moreover, alkaline conditions can also be associated to the amount of buffer added, particularly for AW and BW which showed limited microbial activity and therefore limited products would have been generated from degradation.

Proteins dedradation: $C_{13}H_{25}O_7N_3S+6H_2O\rightarrow 6.5\ CO_2+6.5\ CH_4+3NH_3+H_2S$ Equation18

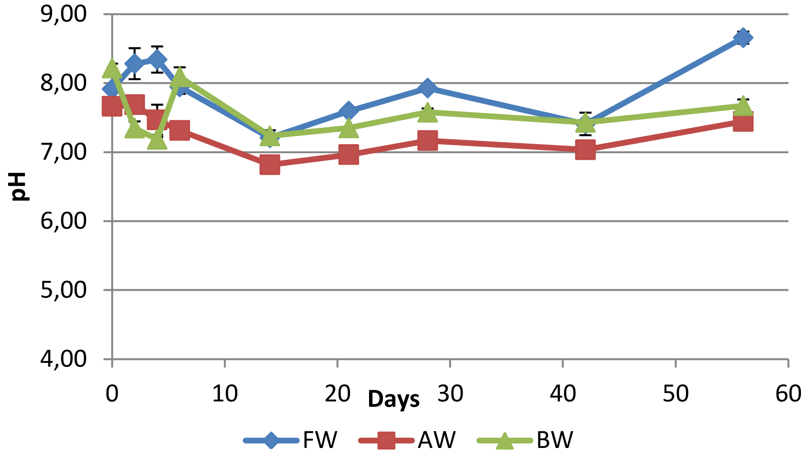


Figure 40: pH profiles of abattoir and fisheries wastes

Table 35 present the maximum CO_2 released from the buffer. The fraction of CO_2 released chemically surpasses the total gas measured for FW and BW. Note however that FW produced significant volumes of CH_4 despite release of chemical CO_2. This is an indication that the methanogenic archaea remained active and that the actual chemical CO_2 released was lower than the calculated value.

Table 35: Estimation of the maximum CO_2 released chemically from the buffer

	g buffer	No. moles	Vol chemical CO_2, mL	Total cum biogas, mL	% X	%Y
FW	1.8	2.1E-2	538.6	458.7	117.4	
AW	1	1.2E-2	306.9	375	81.8	22
BW	2.1	2.5E-2	637.9	363.3	175.6	

Where ***X:*** *maximum fraction of chemical CO_2 over total cumulative biogas for each sample,* ***Y:*** *minimum fraction of 'biological'CO_2 over total CO_2*

The performance of these waste samples was the lowest compared to other categories on average. It appears that the activity of the methanogenic archaea was severely limited for BW and AW. Further research is needed to investigate different factors that would have impacted on the reaction dynamics of biological degradation of these protein-rich substrates. The only sample that showed fair methanogenic activity was FW.

4.1.5 General discussion on BMP results

This section aims to consolidate, compare and discuss trends or observations in the results presented earlier for the different wastes. Fines and garden waste (with the exception of WHV and WDO) showed best performance, whilst among food wastes and high-N wastes only one experiment (EW and FW, respectively) produced reasonable methanogenic activity.

The variability between waste samples is generally not statistically significant between triplicates, while that between waste samples is. This was expected, as the experimental set-up of triplicates was identical. Table 36 below presents the average CH_4 yield for each category. Only waste sample who showed fair methanogenic activity are considered.

Table 36: Average CH_4 yield for the different waste categories

	Samples considered	Avg CH_4 yield, mL/g VS	Comments
Garden wastes	BC GW, AFW1-AFW4	145±46.8	WDO~28.4 and WHW~63 mL CH_4/g VS
Fines and paper sludge	BC Fi, AFi1-AFi4	200±39	PW~54 mL CH_4/g VS
Food Wastes	EW	119	
Abattoir+Fisheries wastes	FW	104.4	

The CH_4 yield curves exhibit an 'S' shape for most waste samples which showed good performance. An initial lag phase of 6-10 days is observed where the CH_4 production is very low, this period is then followed by a sharp linear increase between days 10-20 on average, and the CH_4 production then levels off for the remainder of the experiments. This trend was not observed with WHW, where the microbial activity was minimal until day~40, and increased thereafter. The published literature shows that the regeneration rate of methanogens is between 10 and 14 days (Deublein and Steinhauser, 2008), as discussed earlier in Section 2.2.2.2.

The CH_4 yield curves are aligned with the above statement, as they exhibit a lag phase of ~10 day characterised with little CH_4 before the methanoogenic activity increases. The CH_4 is expected to level off when the amount of organic substrate left for degradation becomes the limiting factor in batch systems.

It is noteworthy that the gradient of the CH_4 curves of EW, BC Fi and FW however, was still increased towards the end of experiments compared to others. This shows that values for the BMP reported could be higher. The literature reviewed also report inhibition of the methanogenic activity due to high concentrations of cations such as solium (Na^+). For example, the literature published report that concentrations ranging 3500-5500mg Na+/L are moderately inhibitory, while values above 8000 mg Na+/L are strongly inhibitory (Chen *et al.* 2008, Cheng, 2010). The average concentrations of Na^+ in this studied varied between 1 and 5 g-Na^+ /L, which is below the threshold identified as inhibitory with the exception of WHW (~12 g-Na^+ /L) and EW (~7g-Na^+ /L). It is difficult to deduce any robust conclusions about the Na^+toxicity in the systems studied since most of the buffering was done at start-up.

It appears however from integrating the low biogas and CH_4 yield of WHW, with the pH profiles that the activity of methanogens for this waste sample was limited at under alkaline conditions as discussed earlier. There is some consistency in WHW data set as a pH drop to 8, led to more favourable conditions for the methanogenic archae and a higher CH_4 composition (from zero to ~80%). It is encouraging however that despite the heavy buffering, the biogas and CH_4 yields achieved for WHW (265 biogas mL/g VS and 54 mL CH_4/g VS) are similar to those reported in literature at 35°C i.e. 267 mL biogas/g VS and 52 mL CH_4/g VS (O'Sullivan *et al.* 2010).The estimated CH_4 yield achieved for mixed fines was higher compared to others i.e. 200 mL CH_4/g VS versus 145, 104 and 119 mL CH_4/g VS for garden, fisheries and biomass from Cape Town's market as presented in Table 36 above.

The average F:I ratio for food wastes, presented in experimental set-up (Tables 76-77,Appendix 9.3.2.1), was ~5.5 (g VS/g VS) which is higher than suitable values reported in literature. For example, Liu *et al.* (2009) showed that the biogas yields were halved when the F:I increased to ~5 under both mesophilic and thermophilic conditions. Angelidaki and Sanders (2004) suggest that depending on the organic loading, that high inoculum concentration viz. 60-90% (w/w) be used, if the sole purpose is to determine the maximum biogas yield achievable to avoid any accumulation of intermediate products such as organic acids, ensure there is a balance between the amount of substrate to be degraded and microbial consortia, and ultimately avoid any inhibition.

The low pH and CH_4 profiles of food waste are consistent with these recommendations, i.e. it is very likely that easily fermentable organics were hydrolysed; acidogens and acetogens produced excess intermediate products, mainly organic acids, which then led to an accumulation of VFAs and lowered the pH, limiting the activity of methanogens.

The activity of the microbial consortia in AW, BW and food waste, with the exception of EW, CW and BC FW appeared limited. For abattoir and fisheries wastes, there is no apparent relationship between biogas productivity, pH profiles and the significant increase in CH_4 composition for AW and BW which questions the credibility of these last values since the productivity was very small (i.e. less than 5 mL/ day).

4.2 Removal efficiency of volatile solids and chemical oxygen demand

The efficiency of the biodegradation process is presented here based on the magnitude of volatile solids (VS) and chemical oxygen demand (COD) destruction for the digestion period. The COD percentage reduction achieved is plotted against the VS reduction in Figure 41. The highly heterogeneous nature of the waste posed a challenge to obtaining representative samples from the bioreactors particularly for fines and garden waste. Inconsistent results of COD and VS analyses of the following samples were discarded i.e. BC FW, VH, BC GW, P.BC for VS and BC FW, VHW, CAV, AFW1 and P.BC for COD. The error in analysis of the data can be further linked to the small reactor size and the syringe employed in sampling as it was not always possible to extract solid particles in suspension from the reactors. The average percentage reduction in VS observed with food wastes was ~20% with the exception of EW and CW, where a 57% and 52 % reduction was achieved respectively.

The VS and COD reduction achieved with EW are consistent with the biogas and CH_4 profiles, since EW achieved the highest biogas and CH_4 yield in its class. Moreover, the highest CH_4 yield among food wastes (an estimated 94 mL CH_4/g VS) was linked to the highest COD removal (70%). Similarly, the destruction achieved with VS for CW is also consistent with the data presented earlier i.e. biogas and CH_4 profiles showed that acidic conditions limited the activity of methanogens, which resulted in a CO_2 rich biogas. Moreover, Table 32 shows that 90% of the biogas volumes estimated where from the AD of cereals, which confirm the VS and COD destruction achieved.

The average VS reduction for WDO, WHWand PW was ~10%; while the average reduction for repeat samples of fines and garden waste from ARTS was 20% and 35% respectively (Figure 41). FW had 27.4% VS reduction. The lowest VS reduction achieved was from BW with 15%; this was expected as its biogas yields was also the lowest compared to others.

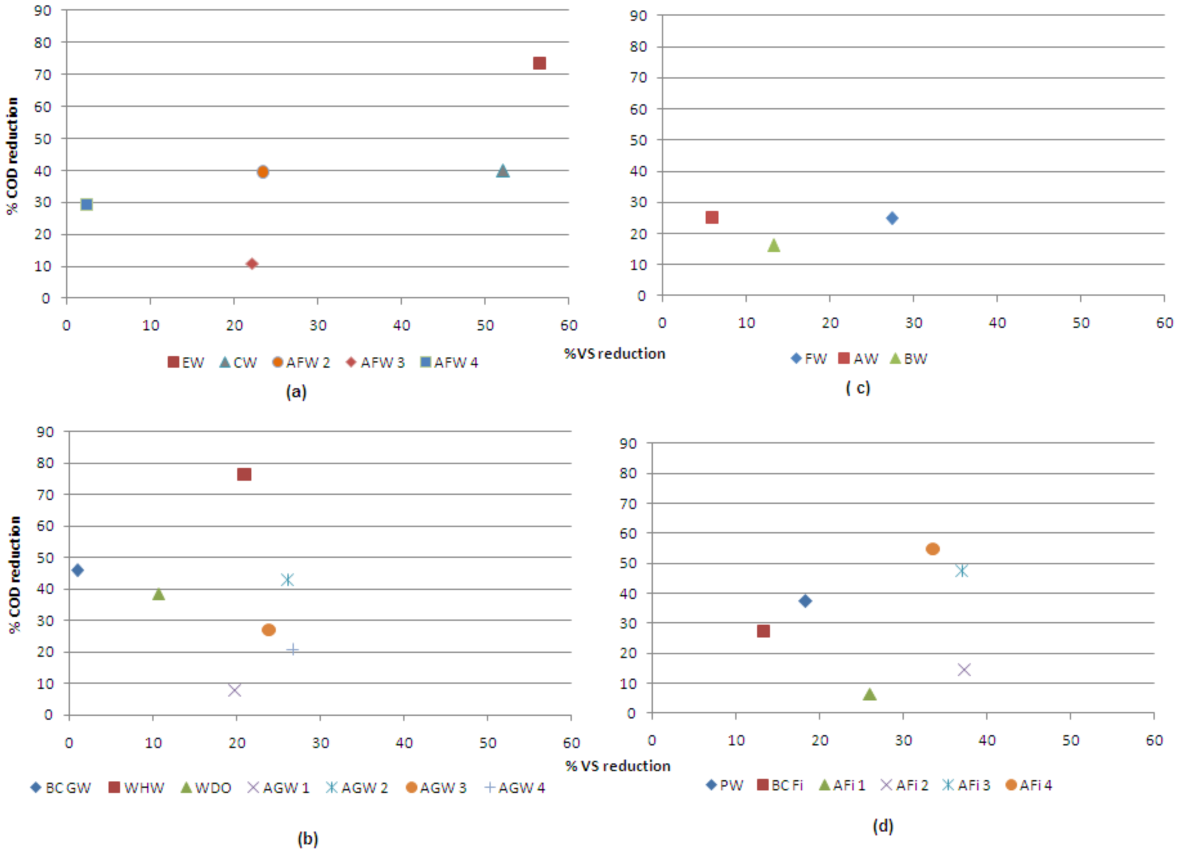


Figure 41: % COD reduction versus %VS reduction in AD of (a) food wastes, (b) garden wastes, (c) abattoir and fisheries wastes and, (d) fines and paper sludge

There was no apparent link between the CH_4 yield versus VS and COD reduction for fines, garden waste, and abattoir waste in particular where the highest biogas and CH_4yields of BC GW and BC Fi did not correlate to the highest COD reduction. The sampling method indicates that soluble COD and not total COD was measured. This could have led to an overestimation of COD reduction, and as a result account for the inconsistencies observed. The above can be justified by the challenges experienced with both extracting representative samples of these heterogeneous wastes, measuring accurate COD of solid particles and the complex nature of the substrates.

The average COD reduction with fines, garden and abattoir waste was 24%, 40% and 20% respectively; and the waste sample with the highest removal in the latter two categories were water hyacinth (WHW) in garden wastes with 75.3 % and FW with 25 % (Figure 41). Results of FW are consistent with the biogas and CH_4 profiles as FW with the highest biogas yield in the category corresponds to the highest COD reduction.

Figure 42 presents the comparison between ‘calculated CH_4’ volumes and experimental values for food waste based on the extent of COD destruction. These values were calculated using the specific CH_4 yield reported in literature based on stoichiometry per gram of COD destroyed i.e. 0.35 L CH_4/kg COD destroyed at standard temperature and pressure (Barthakur *et al.,* 1991).

This literature value can vary depending on the nature and composition of substrates (i.e. fraction of carbohydrates, fats or proteins in the subtrate). It is worth noting that calculated values would carry the level of error from the COD measurements. For example, data for AGW1, AFi1 and AFi 2 are inconsistent since the experimental values are greater than the calculated values. Only FW and EW and CW data are presented from their respective categories as they were the only ones showing incidences of fair to good methanogenic activity. For CW, the significant measured COD destruction should have been accompanied by measurable methane production; however, due to the low pH throughout the experiment, the methanogens were inhibited, and CO_2 was produced instead, as discussed earlier.

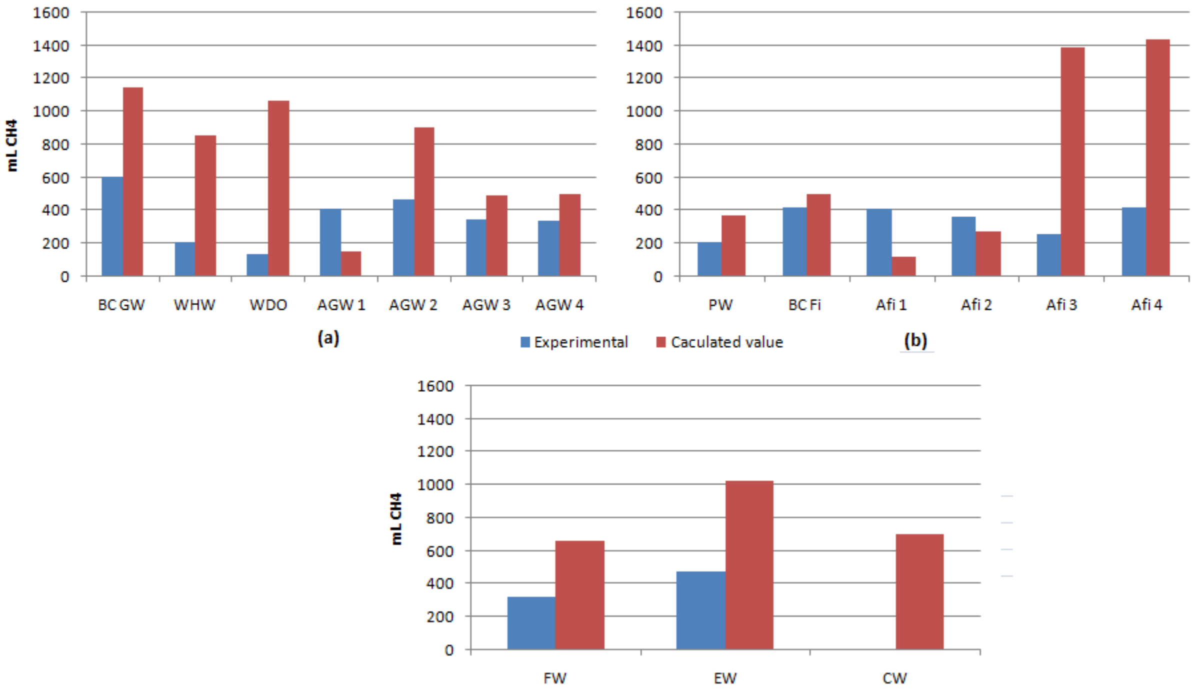


Figure 42: Comparison of theoretical CH_4 and experimental values for (a) garden wastes, (b) for fines and paper sludge and (c) for biomass from Cape Town’s market and fisheries wastes

This calculation provided a benchmark to evaluate the experimental values obtained for CH_4 yield. For example, CW shows that very little CH_4 was produced compared to the calculated value from the COD reduction achieved (Figure 42).

These results are consistent with biogas, CH_4 composition and pH profiles presented earlier for CW. It is likely that the acidic conditions that prevailed in the reactor from accumulation of organic acids limited the activity of methanogens; and the VS and COD reduction achieved are probably a result of the action of acidogens and acetogens which produce mostly CO_2 and H_2 (Chynoweth *et al.*, 1987; Deublein and Steinhauser, 2008). Data of AGW 1 and AFi are inconsistent and can be linked to analytical error.

4.3 Conclusions from the BMP experiments

The BMP of 16 different waste streams that would normally be disposed off in landfills was evaluated. Results showed that generally, mixed organic waste streams achieved higher biogas and CH_4yields compared to pure commercial waste streams.

Slaughterhouse wastes (viz blood and abattoir wastes) performed poorly compared to other wastes samples. The wastes samples which showed credible results EW, FW and mixed waste samples from the garden waste and fines category. Generally, fines and garden waste showed better performance compared to others. With the exception of the bmp evaluation for EW wastes, the food waste experiments, whilst generally with statistically acceptable variability between triplicates, were not successful at evaluating the potential of energy from food wastes, since the CH_4 yields achieved were very low from theoretical considerations.

It was not possible to monitor daily experimental variables such as VFAs, NH_3 and pH to better understand and control the process due to the small reactor size employed in this study. Investigating the influence of these factors in larger systems to validate the results obtained at 100 mL should be valuable in optimising the performance of anaerobic digestion (biogas yield, stability, etc.). This was the rationale for devising AD testwork for some of the substrates at a larger scale, discussed in the following chapter.

Although the CH_4 composition profiles varied significantly between weeks, results showed that higher CH_4 composition generally corresponded to pH values between 6.5 and 7.5. However, whilst a pH in this range appeared to be a necessary condition for good quality biogas, it was not a sufficient condition. As a closing note, it would be valuable in the future to address the shortcomings experienced in this study by repeating the food waste experiments, except for EW, with better buffering and with lower VS loading as the F:I ratio was suboptimal in this study. Due to the high variability in the data collected for CH_4profiles, measurements of pH, CH_4 composition and biogas volumes should be performed daily.

There was evidence of limited microbial activity beyond the first week for most food waste samples and AW, BW; consequently, performing tests to ensure each microbial group is represented in the inoculum, and evaluating the effect of different microbial consortia as well as their adaptation capacity on the performance of the digestion of the different substrates would be valuable, and could be the subject for further research.

5 ANAEROBIC DIGESTION EXPERIMENTS UNDER FED-BATCH CONDITIONS

This section presents results from the second phase of the laboratory investigation of AD of organic wastes under fed-batch conditions. Duplicate experiments were carried out in 2L bioreactors over 60 days for the chosen substrates. The duplicate bioreactors used for blood and garden waste broke on day ~ 36. These runs were re-started and only run for ~25 days until all other experiments were ceased, results of these re-started experiments are omitted in this dissertation since they were not operated sufficiently long. The main objectives of this second phase were to validate the BMP tests, investigate AD under real life conditions and gain a better understanding of the influence of experimental variables on the performance of AD systems, as stated in Section 3.2.The samples included in these experiments were kitchen waste on a flat (K. Flat) and progressive (K. Prog) feed regime, garden refuse (G), abattoir blood and its co-digestion with garden waste (G+B) which is discussed in Chapter 6. The characteristics of the different waste fractions used are discussed in Section 3.4.2, and the experimental set-up for these experiments is presented earlier in Section 3.7.1. The progressive feed was based on the ‘activated biomass concept’, explained in Section 3.6.1, and the different analytical methods used to collect data are presented in Section 3.9.

5.1 Overview of results

The values of the cumulative daily biogas (mL) from the duplicate reactors (a and b) are presented in Figure 43 (a-b) for the different substrates. Results of kitchen waste on the flat (K. Flat) and progressive (K. Prog) feeding regime are presented in Figure 43 a; and results of the other substrates are presented in Figure 43 b.

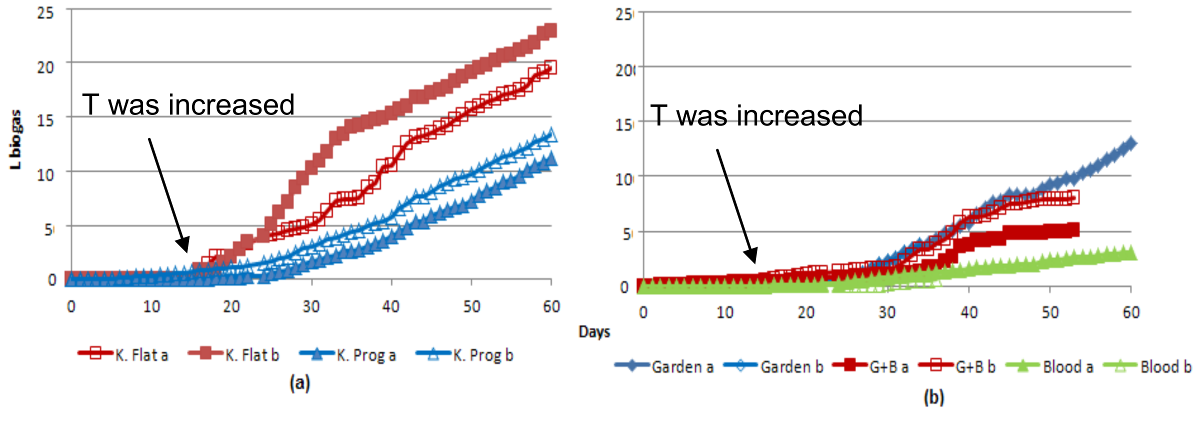


Figure 43: Cumulative biogas of (a) kitchen waste, and (b) other substrates for the fed-batch AD experiments

The biogas production was negligible within the first two weeks; this period corresponds to the time where bioreactors were run at ambient temperature, under psychrophilic conditions (~17°C) before being heated up to 35°C (Figure 43). The highest biogas yield was from the kitchen waste on a flat feeding (K. Flat b) regime with up to 23 L, and the lowest was from blood waste with only 3L. The average biogas results for kitchen-progressive (K-Prog), and garden waste were respectively 12.4 and 13.2 L.

Table 37 presents the comparison of the specific biogas yields in mL/g VS for these runs in duplicate, with the BMP batch tests. The BMP results refer to the kitchen waste from the Vineyard Hotel (VHW), garden waste from Wynberg Drop-off (WDO) and blood waste from the Stickland abattoirs. The variability between the duplicate reactors fluctuates between 14-17% for kitchen waste, and is considerably higher for the G+B digesters (~39%), where the cumulative biogas for G+B a was 5 L (~22 mL/g VS), versus 8 (~36 mL/g VS) for G+B a. Results of the kitchen waste show a decent level of reproducibility, compared to the more heterogeneous substrates such as G+B.

Table 37: Comparison of the biogas yield achieved from the BMPs at 100 mL and under fed batch conditions

		Specific measured gas yield (mL/g VS fed)	Specific gas and CH_4 yield
K. Flat	A	99	321.9 mL/g VS (~6.3 mL CH_4/g VS)
	B	115.9	
K. Prog	A	51	321.9 mL/g VS (~6.3 mL CH_4/ g VS)
	B	61	
Blood	A	14.13	79.3 mL/g VS (~4.5 mL CH_4/g VS)
	B	NA	
Garden	A	59.5	362.9 mL/g VS (~23.7 mL CH_4/g VS)
	B	NA	

The gas yields of the fed-batch experiments are significantly lower than those of the BMP tests at 100 mL scale, with yields between 15 and 30% of those expected, (Table 37). It is worth noting however, that the CH_4 yields achieved for this waste samples in the batch tests, were minimal and show poor performance.

5.2 Kitchen Waste

The experiments considered in this section are kitchen waste under flat (K. Flat) and progressive (K. Prog) feeding regimes. A net amount of 3.27 g VS/ day was fed for K. Flat, while the organic loading rate was increased from 0.97 to 6.25 g VS/day until day 46 for K. Prog; and then reduced to 3.27 g VS since it was suspected, from their poor performance, that reactors on the progressive feed were overfed. The VFAs examined in these experiments were lactic, acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acid. Note that the y-axes of Figures 47 and 52 are on a logarithmic scale.

5.2.1 Kitchen waste on a flat feeding rate (K. Flat)

Biogas productivity and pH profiles

The biogas productivity (mL biogas/day) of the duplicate reactors is presented in Figure 44 below.There is a high productivity period between days~ 30 and 45, and days ~19 and 35 for K. Flat a, and b respectively; associated with the highest daily gas volumes. The highest peak observed were 1420 mL/day and 1080 mL/day for K. Flat a, and K. Flat b respectively. Thereafter, the gas productivity generally decreased to below 400 mL/day (Figure 44).K. Flat b achieved a higher biogas yield compared to K. Flat b despite similar reactor set-up.

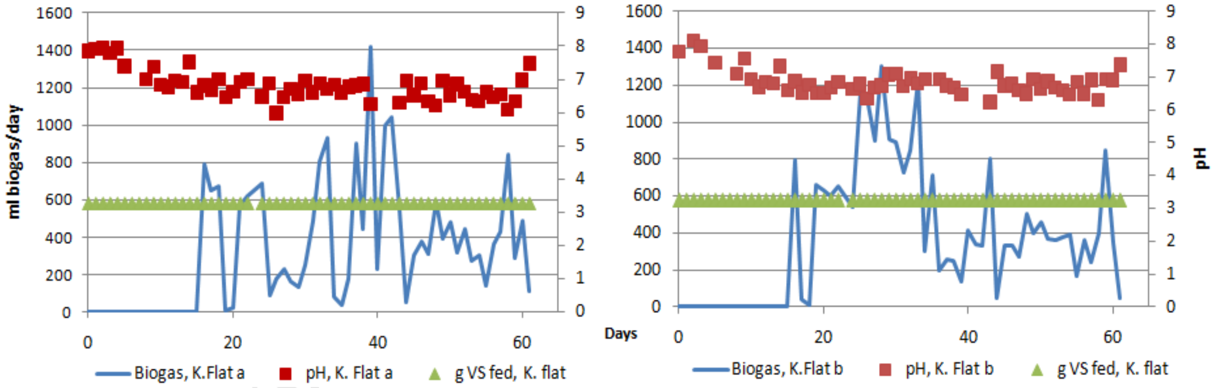


Figure 44: Biogas yield, pH and daily feed for AD of kitchen waste on a flat feeding regime

The pH profiles of both K. Flat bioreactors are presented in Figure 45 . It is worth noting that the start-up conditions were intentionally made slightly alkaline (pH~8) based on the experience with BMP tests, as acidic conditions prevailed particularly in batch reactors with kitchen wastes. The pH profiles of kitchen waste show that the pH would not have remained neutral without additional buffering to ensure optimal conditions for the methanogenic archaea i.e. pH~6.5-7.5(Ward *et al.,* 2008). Additional buffering using $NaHCO_3$was required; the arrows on the graph indicate the days on which the bioreactors were buffered. A gradual pH drop was observed during the first 20 day from ~8 to ~6.5, and later between days 37 and 41 from ~6.8 to ~6.3. $NaHCO_3$ was therefore added on days 21, 28, 42 to maintain the reactor media under neutral conditions.

The total amount of buffer added in the bioreactors, and consequently the estimated amount of the maximum CO_2 released chemically from the speciation of CO_2, is presented in Table 38. The volume of CO_2 that could have been chemically released constitutes ~20% on average of the total cumulative biogas volumes measured.

Table 38: Estimation of CO_2 released chemically from $NaHCO_3$ for K. Flat reactors

	Total g, buffer added	moles	v (T= 35°C)	Total biogas produced, L	%
K. Flat a	14.3	0.2	4.3	19.7	21.8
K. Flat b	14.6	0.2	4.4	23	19.0

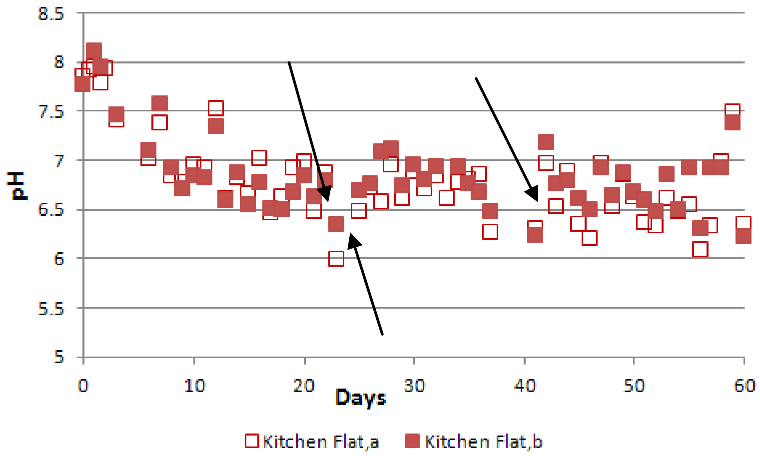


Figure 45: Temporal evolution of pH of AD of duplicate reactors of K. Flat reactors

There is a correlation between the high biogas peak and the days on which $NaHCO_3$ was added in bioreactors (Figures 44 and 45). For example, ~2.46 g of buffer were added on day 28, and which corresponds to an increase in biogas productivity from 160 to 250-930 mL/day for days 30-33, and the productivity dropped again to 80 mL/day thereafter. This trend is consistent throughout for 'buffering' periods with the other bioreactors. This shows evidence that the 'biogas' peak observed were, at least partially, combined with release of CO_2 chemically. The pH profiles for day 21 and 42, are in line with the above argument since the highest biogas peak correspond to period where the pH was between 6 and 6.5, which would have favoured the speciation of HCO_3^- into CO_2 gas.

Accumulation of substrate in digesters

Figure 46 presents a comparison between the amount of VS fed, and the g VS measured in the reactor media; the values presented are the average for the duplicate reactors. The values in the reactor were calculated by scaling up amount of g VS from the sample size to 2L (Appendix, Section 9.1.12). Reactors were operated as a fed-batch, and the material loaded would generally settle at the bottom. Hence, reactors were shaken before sampling, to attempt collecting a representative sample of the reactor media. The cumulative amount of biomass loaded is presented (i.e. ~198 g VS were loaded over the 60 days period). It is observed that the amount of VS in the reactors increased steadily, and was considerably higher than that fed on a daily basis, which reflect a build-up of biomass in the system (e.g. ~15 g VS versus ~3 g VS for days 24-30).

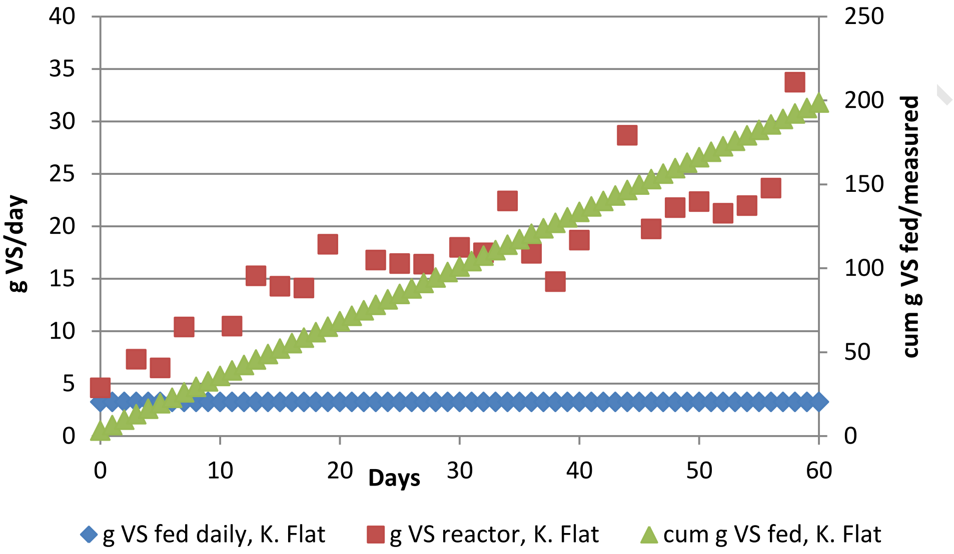


Figure 46: comparison between g VS fed, and g VS in the reactor

Temporal evolution of volatile fatty acids (VFAs) profiles

The concentration profile of the different VFAs measured is presented in Figure 47. The occurrence order of the different acids was for the K. Flat reactors were as follows: iso-butyric>acetic/propionic/butyric>Iso-valeric>valeric> and lactic acid; minimal amounts of lactic acid were detected (less than 0.6 g/L on average).The VFAs concentration generally increases sharply in the first ~14 days and stabilises for days 14-55, when the biogas production commences. It is noteworthy that it is unusual that the biogas productivity exhibit high variability between days 14 and 45, while the VFAs profiles were relatively stable.

The sharp decrease in concentration of VFAs observed between days ~33 and 47 for

K. Flat b, also coincides with one of the highest biogas productivity periods, despite the fact that some of the gas volumes measured would be chemically released CO_2 from the buffer. A significant increase in the concentration of propionic acid is then observed towards the end of the digestion period (day 55). The high concentrations of VFAs are in line with the VS profiles in the reactor (Figure 46 above), where a net accumulation of biomass in the system is observed.

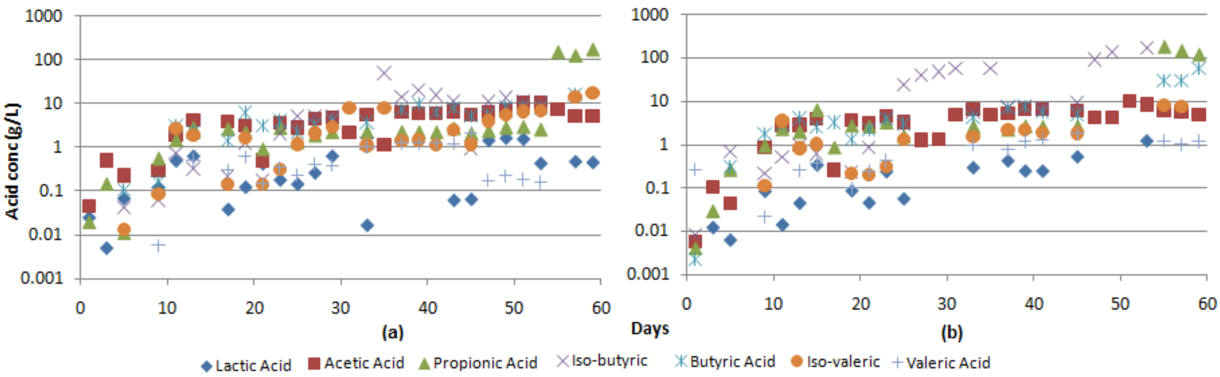


Figure 47: (a) Temporal evolution of the different VFAs for K. Flat, and (b) for K. Flat b

Many authors agree that propionic acid generally tends to accumulate because its degradation is thermodynamically unfavourable since the Gibbs free energy of the reaction is positive (Wang *et al.*, 1999/2009). Experimental results are aligned with the above statement i.e. the concentrations of propionic acid in both K. Flat bioreactors increased considerably from day ~55. The concentration of propionic acid increased from ~3 g/L on day 49 to ~153 on day 55 for K. Flat a, and from ~2 g/L on day 45 to 179.9 on day 55 for K. Flat b.

Acetic acid was not the predominant VFAs detected for K. Flat reactors. Butyric was the predominant form until day ~20, iso-butyric then became the most predominant until day~55 for both K. Flat reactors. The concentration of propionic acid increased to become prevalent after day~55.These results are similar those of Boe *et al.* (2010) where butyric acid was predominant initially when the organic load was increase with glucose, and proprionic acid became predominant towards after an overload towards the end of the digestion process.

Figure 48 highlights the relationship between the total VFAs (TVFAs), pH and feeding rate for the K. Flat bioreactors. Generally, the highest contribution to TVFAs was from iso-butyric, and propionic acid became the predominant form in the end for both reactors (Figure 48). The TVFAs remained relatively low until day~55 for K. Flat a and ~45 for K. Flat b. The concentration of propionic acid increased considerably (~above ~150 g/L) thereafter for K. Flat a, and both iso-butyric and propionic acid increased for K. Flat b.

It is worth noting that pH was not affected despite the fact that the TVFAs concentration almost doubled above day 55. This either suggests that the buffering capacity of the system was high from the bicarbonate added, or that these values could be analytical error resulting from entrained amount pollutant in the column.

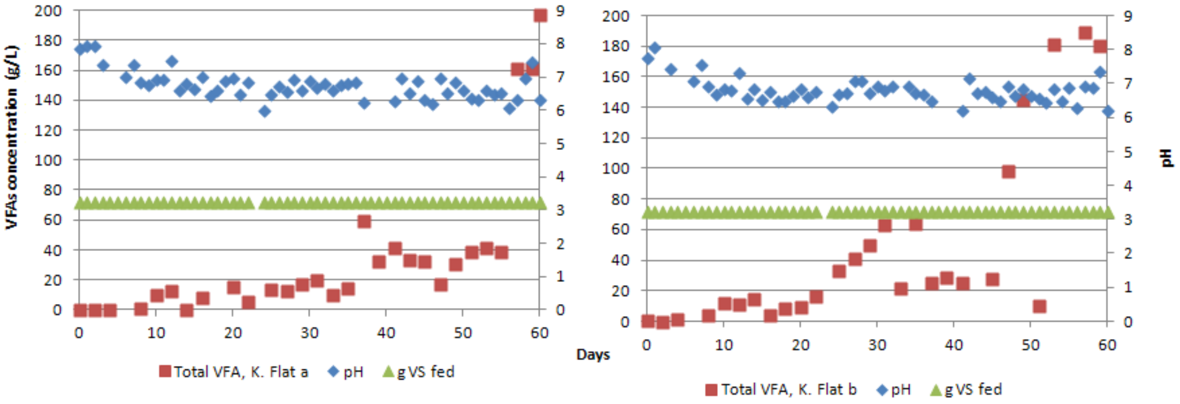


Figure 48: Relationship between total volatile fatty acids (TVFAs), pH and the feeding rate (g VS/day) for K. Flat

Many studies agree on the inhibitory effect of propionic acid to methanogens in AD systems, even though reported toxic values differ considerably (Boe *et al.*, 2010; Nielsen *et al.*, 2007; Wang *et al.*, 2009), as discussed earlier in Section 2.3.1.2. By contrast, experimental values did not show any correlation between the high concentrations of propionic acid in both reactor as the gas productivity decreased earlier on day ~40. The maximum values for total VFA concentration in the reviewed published literature (Section 2.3.1.2) are considerably lower than values achieved in these experiments (>100 g/L) which is cause of concern. Anderson *et al.* (1982) and Mawson *et al.* (1991) report 15-20 g/L for a pH between 6 and 7, while Babel *et al.* (2004) report values of 13-14 g/L achieved for a pH of 6, and 20 g/L for a pH of 7 before inhibition of the system.

Wang *et al.* (2009) showed that while propionic acid led to inhibition, butyric acid improved degradation and acetic acid had no effects (Section 2.3.1). Experimental results are aligned with the above statement i.e. higher concentration of iso-butyric acid observed in K. Flat b (i.e. 171 g/L versus 50.6 g/L), also correspond to higher biogas yield (mL/g VS fed) achieved compared to K. Flat b (i.e. 107 versus 56 mL/ g VS).

Many researchers agree that about 70% of the biogas generated originates from acetate (i.e. from acetic acid) by acetoclastic methanogens; the remaining VFAs are firstly converted to acetic acid before being used by acetogens and methanogens (Wang et al., 1999), as discussed earlier in in Section 2.3.1.

It could be argued, among other factors, that the lower biogas yields in this study compared to BMP values could be linked to the lower concentration of acetic acid. Organic materials were converted to other VFAs that were not necessarily converted into acetic acid, and later not converted into biogas (CH_4 and CO_2).

The increasing VFAs profiles are an indication that hydrolytic and acidogenic bacteria remained active, producing organic acids which accumulated in the system. Little can be inferred about methanogens since the CH_4 content of the gas was not measured, the activity of methanogens must have been limited which then slowed down the conversion rate of intermediate products into biogas, compared to the previous AD phases for the first 20 days period. The relative stability of the VFAs profiles thereafter are an indication that either a steady state was reached between the early phases of AD, and methanogenesis; or alternatively that the rate of hydrolysis-acetogenesis decreases beyond day~20.

5.2.2 Kitchen waste on a progressive feeding rate (K. Prog)

Biogas productivity and pH profiles

Figure 49 presents the biogas productivity (mL/day) for the K. Prog reactors. By visual inspection, the variability in the daily biogas volumes appeared lowered in K. Prog reactor than the K. Flat reactors.

The biogas productivity for K. Prog reactors was generally lower than that achieved in K. Flat reactors. The lower biogas volumes achieved in K. Prog, compared to the K. Flat reactors, despite the higher OLRs, was cause of concern, and led the believe that the system was overloaded and running sub-optimally. For this reason, the OLR was reduced from 6.25 g VS/day to the flat feeding rate 3.27 g VS/day on day 46, which led to an increase in the specific biogas yields in the K. Prog reactors. The average daily biogas production increased from 89 mL/g VS to ~100 mL/g VS fed, with 144 mL/g VS as the highest daily biogas productivity.

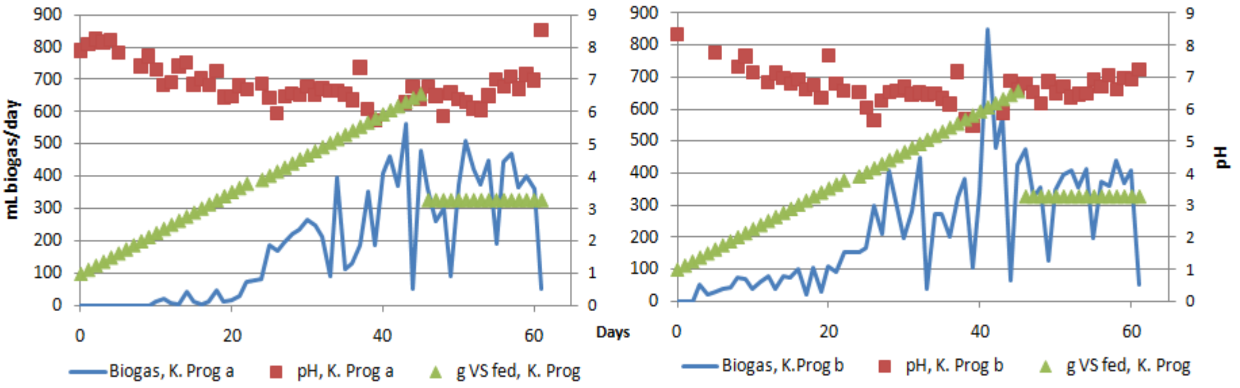


Figure 49: Biogas yield, digester pH and daily feed of AD of kitchen waste on a progressive feeding regime

The pH profiles of K. Prog are presented in Figure 50; it is observed that the pH of K. Prog in those reactors did not stabilise throughout the duration of the experiments, and required additional chemical buffering. The arrows indicate the days on which the biodigesters were buffered.

It is worth noting that the pH drop observed with K. Prog bioreactors was the highest compared to K. Flat i.e. the pH dropped to ~5.5 on days 23 and 41, Figure 50. $NaHCO_3$ was added on days 21, 28, and 42 to increase the pH when it dropped below 6.5, as illustrated by the arrows (Figure 50).

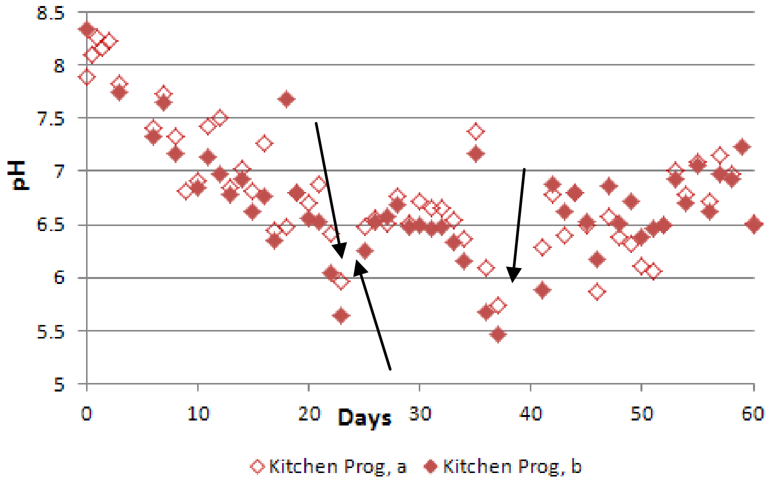


Figure 50: Temporal evolution of pH of AD of duplicate reactors of K. Prog reactors

Table 39 present the estimation of the maximum CO_2 released chemically from the buffer at 35°C and atmospheric pressure, which constitute ~40-48% of the biogas volumes measured. The corresponding pH around days 22-23 and 37, was ¬5.5-6 which would have favoured the speciation of HCO_3^-into CO_2 .The biogas peaks observed, particularly on day 41-43 for K. Prog b,are in agreement with the above statement as they correlate to period close when the reactors were buffered.

Table 39: Estimation of CO_2 released chemically from $NaHCO_3$ for K. Prog reactors

	Total g, buffer added	moles	v (@ 35°C)	Total biogas produced, L	%
K. Prog a	18	0.2	5.4	11.3	47.9
K. Prog b	18	0.2	5.3	13.5	39.6

Accumulation of substrate in digesters

Figure 51 presents the comparison between g VS fed against the amount of VS accumulated in the reactors, the cumulative amount of substrate fed over the 60 days period is also presented (~225 g VS). The amount of biomass (g VS) in the bioreactors is similar to the amount fed, and little accumulation was observed in the reactors for the first 20 days. The g VS in reactor surpassed the amount fed significantly (e.g. 25 g VS versus 3.3 g VS on day 50) thereafter, and a net accumulation of biomass was observed.

The first 20 days correspond to the period when reactors were running under psychrophilic conditions with minimal biogas was produced. No accumulation of g VS in reactor implies that the feeding rate of fresh substrate appears to be similar to its removal rate from the system due to microbial degradation. Experimental results do show some biogas volumes produced for K. Prog b (~60 mL/day average). However, it is hard to draw any solid conclusion as some of the gas volumes recorded could be chemically released CO_2 from the buffer. CH_4 and CO_2 measurement of the biogas volumes could have given more confidence in confirming that the some of the gas volumes measured resulted indeed from biological degradation.

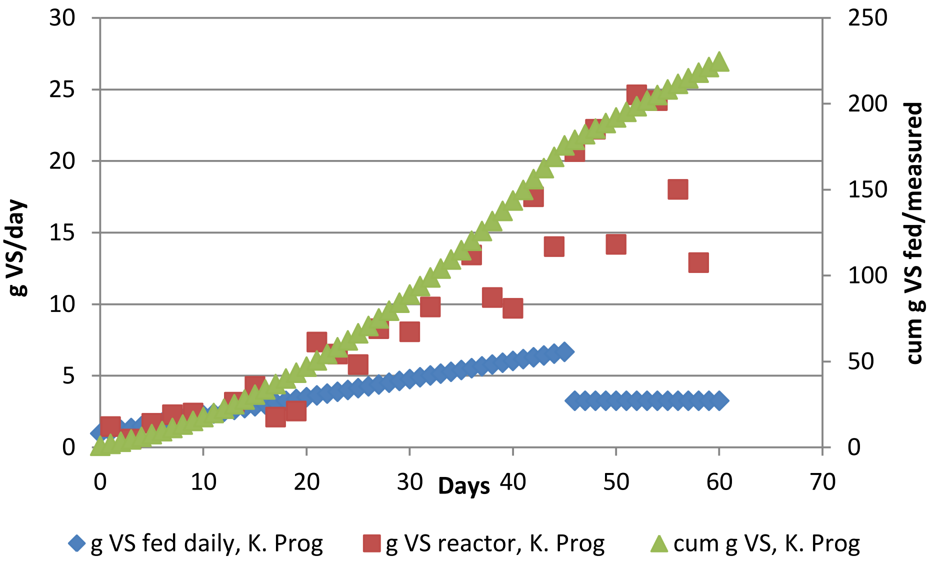


Figure 51: Comparison between g VS fed and g VS in reactor

The periods with higher biogas productivity correspond to the time when biomass started accumulating in the system. It is also observed from day 46 onwards that reducing the OLR, may have increased the biogas yields, but was not sufficient to reverse the accumulation of biomass. The accumulation of biomass observed in K. Prog varied significantly towards the end (i.e. between day 45 and 60). It is worth noting that the the accumulation of biomass commenced when beyond day 18, when the OLR of K. Prog reactors became similar to that of K. Flat and increased further.

The VS variations observed beyond day 40 are unlikely since their magnitude is greater than the amount of substrate fed to the reactor. This can be attributed to non-homogeneous systems and poorly mixed sample collected for analysis.

Temporal evolution of volatile fatty acids (VFAs) profiles

Figure 52 presents the VFAs profiles of both K. Prog reactors. The order of occurrence for the progressive feeding rate is iso-butyric > butyric ≥ acetic/propionic/iso-valeric ≥ valeric acid> and, lactic acid. Butyric and iso-butyric acid were the most predominant forms as observed in K. Flat. The isoforms of butyric and valeric acids were generally more predominant than their normal forms with K. Prog reactors, as was the case with the K. Flat reactors.The VFAs profiles of K. Prog were different from K. Flat, and exhibited two periods of sharp increase in VFAs concentration, noticeable by double-hump curves (Figure 52).

Each period is respectively followed by a decrease in VFAs concentration (i.e. between days ~20 and 35, and later between days ~42 and days 55). The drop in VFAs is an indication that the organic acids were consumed in the system, the acetogenic and methanogenic archaea are responsible for consumption of organic acids to produce biogas (mostly CO_2 and CH_4).

Results are aligned with the above statement since the first period with lower VFAs correspond to the increase in the biogas productivity beyond day ~20. The second period corresponding to lower VFAs concentration is characterised by relatively steady daily biogas volumes (~400 mL/g VS for days 42-55). It is acknowledged that some of the volumes measured would be chemically CO_2 volumes released from the speciation of HCO_3^-into CO_2 gas, particularly since the pH (~6) would have favoured it. However, the reduced VFAs concentration observed give confidence that the combined effect of the increased biogas volumes and VFAs reduction was the result of the action of acetogenic bacteria and the methanogenic archaea.

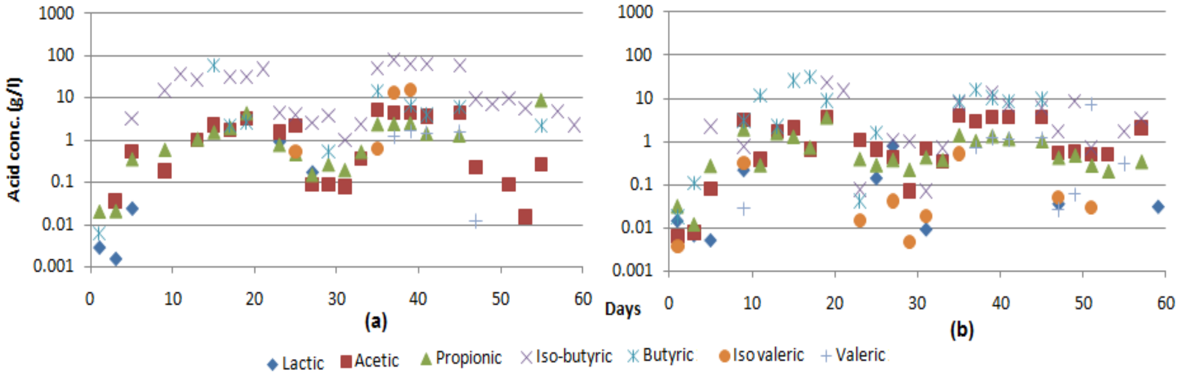


Figure 52: (a) Temporal evolution of the different VFAs for K. Prog a, and (b) for K. Prog b

The relationship between pH, the TVFAs, and the daily amount of substrate fed is presented in Figure 53. The TVFAs levels of K. Prog are lower than K. Flat reactor, and varied between duplicates. The highest concentration of TVFAs was ~100 and 40 g/L for K. Prog a and b respectively, but there are periods between days 20-35 and after day 47 with low to optimal TVFA values.

The profiles of biogas productivity and VFAs for K. Prog do not seem to correlate. The significant drop in TVFAs observed between days 20 and 45 (i.e. from 50 g/L to less than 10 g/L) does correspond distinctively to a significant increase in biogas yield. The highest peak is observed later on day 45. This is cause of concern even though generally, a gradual increase in biogas productivity is observed between day 20 and 45. For example, the average daily biogas yield between day 20 and 35, was ~200 mL/day (~36 mL/g VS fed), compared to only ~50 mL/day (~60 mL/g VS fed) that was produced initially for the first 20 days, for reactor b.

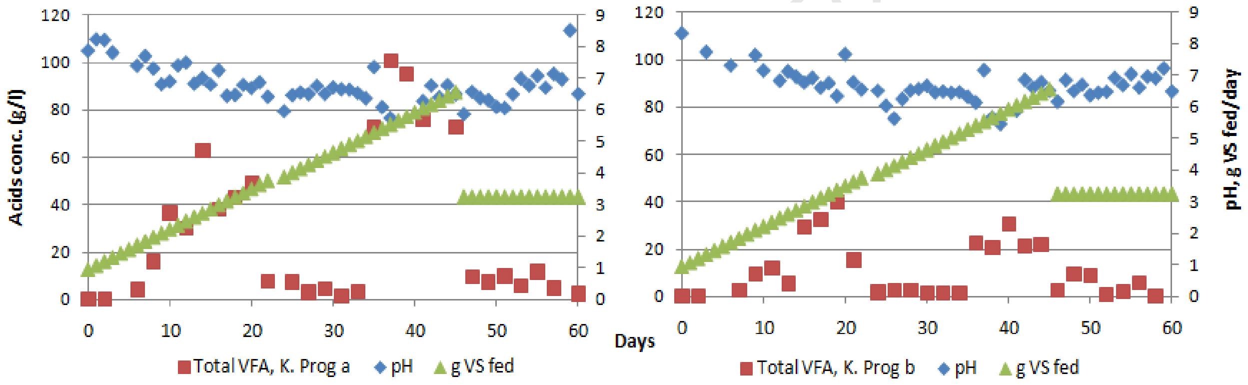


Figure 53: Relationship between TVFAs, pH and the feeding rate (g VS/day)

Figure 54 below presents the comparison between the TVFAs of K. Prog and K. Flat. It is observed that the VFAs concentration of K. Prog remains below 50 g/L, while that of K. Flat steadily increased from ~25 g/L to ~200 g/L beyond day 42.

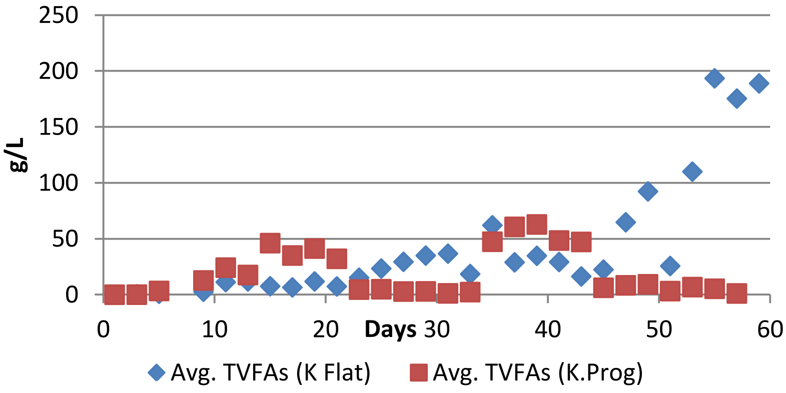


Figure 54: average values of the TVFAs of duplicate reactors of K. Flat and K. Prog

There is a correlation between the biogas and VFAs profiles of K. Flat reactors.The daily biogas productivity of K. Flat appear to have dropped (e.g. from ~1000 mL/day to ~400 mL/day) after the concentration of TVFAs increased significantly (from day 42 onwards), and became similar to those of K. Prog reactors. The drop in biogas probably could result from accumulation of VFAs in the system, generated from degradation of easily fermentable substrates present in kitchen waste, which led to a limited inhibition activity of the methanogenic archaea. The above statement is hard to confirm as the pH recovered after buffering on day 42, and it appears the buffering capacity of the system prevented a further drop until the end of experiments. This suggests that factors other than inhibition from VFAs accumulation were at play.

5.2.3 Concluding remarks on kitchen wastes

The daily biogas volumes for K. Prog were generally lower than that achieved by K. Flat except between day 45 and 60, where similar values were observed (~400 mL/day). It is not evident from the volumes calculated for the chemically released CO_2, that the order of the actual biogas productivity and yields would be different. The fact that the biogas yields of K. Prog remained lower than K. Flat,is also cause of concern.

It is worth noting that the pH drop observed with K. Prog bioreactors was higher compared to K. Flat. In general, there was no clear correlation between pH and VFAs. This can be attributed to the buffering effect of the system via $NaHCO_3$ addition. However, there are clear inverse proportionalities between pH and VFAs up to the first injection of buffer for experiments (around day ~23) for both K. Flat and K. Prog, where a general increase of the concentration of VFAs corresponded to a drop in pH, with the exception of the high concentration observed towards the end of experiments since it did not affect the pH.

Although the biogas yield increased when the feeding regime was reduced from 6.25 to 3.27 g VS/ day, completely stopping the digester feed could have proved more successful at reversing the effects of overfeeding and biomass accumulation in the K. Prog reactors.

There was a high level of isomerisation observed with kitchen waste; the reason can be that the normal forms of VFAs were consumed too fast. This would is similar to research carried by Wang *et al.* (1999) where the rate of degradation of butyric acid was so fast, it could not be detected. In general, the amount of propionic acid remained lower than that of butyric and iso-butyric acid, and the quantity of lactic acid was insignificant (less than 0.1 g/L).

The values of the total VFA data generated, particularly towards the end of experiments were considerably higher than values reported in literature, which is cause of concern and an indication that VFA accumulation resulted from the overloaded feeding regime (as explained later in Section 5.4.3).

5.3 Garden Waste

5.3.1 Biogas and pH profiles

Figure 55 present the biogas productivity of the reactors with garden waste. The highest daily biogas productivityachieved with garden waste was ~800 mL/day from reactor a, similar to values achieved with K. Prog. The reactors were operated under a progressive feeding regime until day 46; the feed rate was then reduced to a constant 3.25 g VS/day thereafter. Data recorded for reactor b is up to day~36 when the reactor broke. Garden waste produced a very similar biogas yield to kitchen waste on the same progressive feed regime (K. Prog~56 mL/g VS versus 59. 5 m/g VS fed for garden waste).

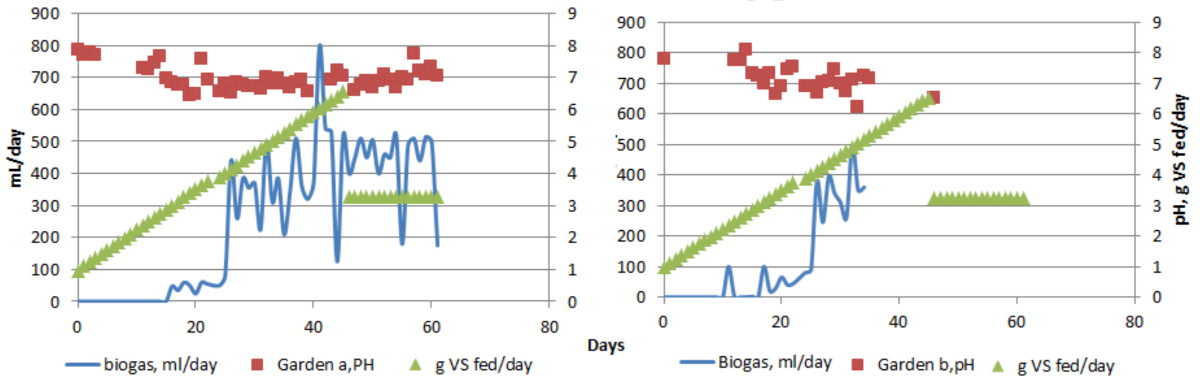


Figure 55: Biogas yield, digester pH and daily feed of AD of garden waste

Figure 56 presents the pH profiles of the bioreactors with garden wastes. Addition of chemical buffer ($NaHCO_3$) was stillrequired to maintain the pH under neutral conditions and was added on days 16, 23 and 42 respectively as illustrated by the arrows in Figure 56.

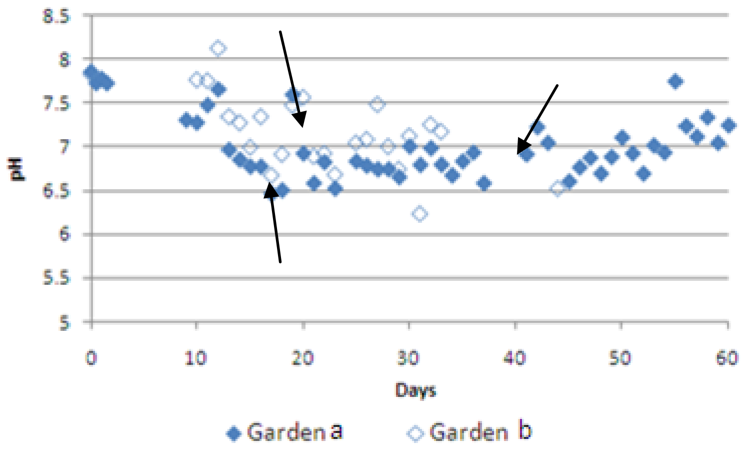


Figure 56: Temporal Evolution of pH for AD of garden waste assays

Table 40 presents the estimation of the maximum amount of CO_2 that could be generated from the buffer. The CO_2 chemically released could constitute up ~35.7% of the gas volumes measured for reactor a.

Table 40: Estimation of CO_2 chemically released from $NaHCO_3$ for garden wastes

	Total g, buffer added	No. moles	V, L (T= 35°C)	Total biogas produced, L	%
Garden	15.6	0.2	4.7	13.2	35.7
Garden	15.6	0.2	4.7	3.7	

Accumulation of substrate in digesters

The comparison between g VS fed, and the g VS in the bioreactor is presented in Figure 57The cumulative feed is also presented (~224 g VS) over the 60 days period. It is observed that the amount of VS in the reactor were generally lower than the amount fed until ~day 46. This shows that the rate of removal of substrate by the micro-organisms was higher than the feeding rate. A net accumulation of biomass is observed thereafter. The challenge experienced with garden waste was collection of a homogeneous and representative sample from the reactor media. It is also worth noting that the amount of VS available is considerably less than that of K. Flat and K. Prog (i.e. 8 g VS versus 35 and 25 g VS respectively).

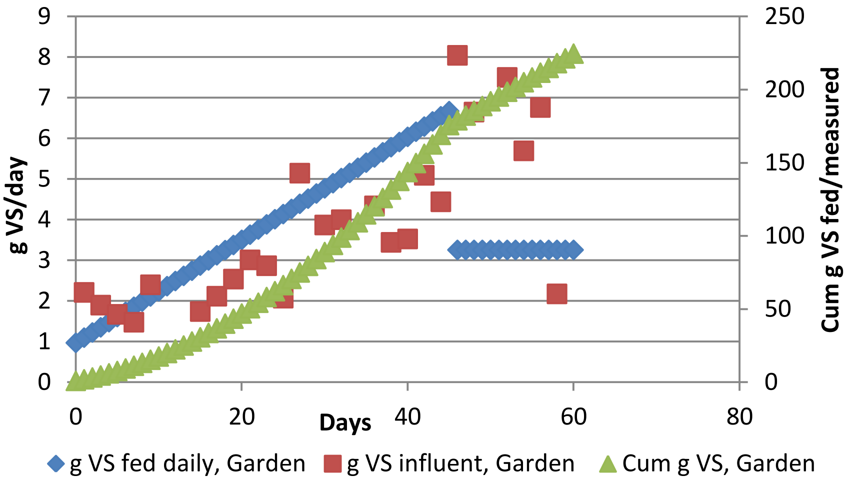


Figure 57: Comparison between g VS fed and g VS in reactor for garden waste

Temporal evolution of individual volatile fatty acids (VFAs)

The profiles of the individual VFAs examined are presented in Figure 58 for the duplicate reactors (a and b); the y-axis is on a logarithmic scale. The predominant VFAs present in reactor a until day 31 were acetic, butyric, iso-butyric and iso-valeric acid. Iso-butyric then became predominant until day~53, and the concentration of lactic acid increased to become the highest VFA towards the end of experiments.The concentration of lactic acid was the lowest as was the case with K. Flat and K. Prog.

The order of occurrence in reactor b was as follows: butyric/iso-butyric>acetic/iso-valeric/valeric> propionic > and lactic acid until day~36 (Figure 40). The concentration of propionic acid remained relatively low (i.e. below 4 g/L) for both reactors, and it did not become the predominant form at any stage when compared to both experiments with kitchen waste (K. Flat and K. Prog).

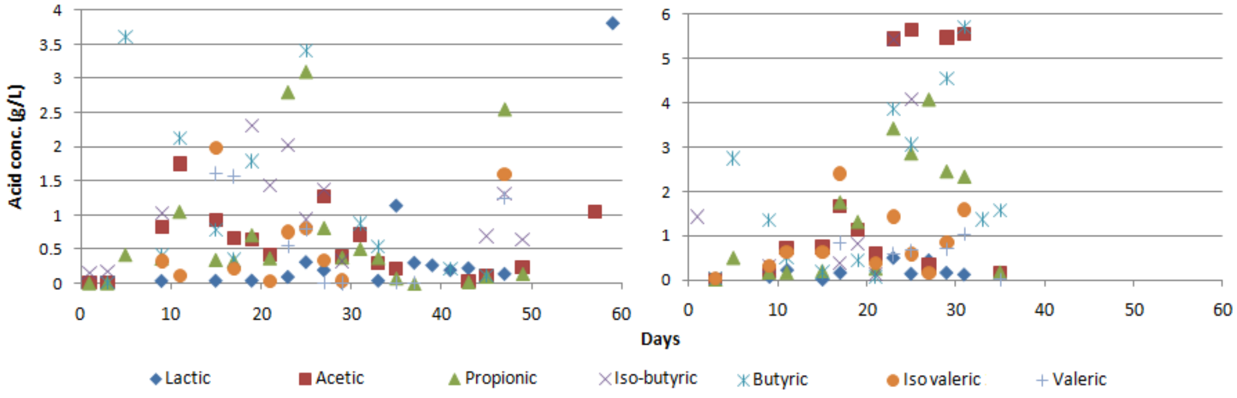


Figure 58: Temporal Evolution of the individual VFAs for Garden refuse digesters

The relationship between pH, total VFAs (TVFAs) and the amount of food waste fed is presented in Figure 59. Generally, the concentration of TVFAs increased with the loading rate,suggesting the easily biodegradable would be hydrolysed first, and converted to organic acids by acidogenic bacteria. As a result, the VFAs concentration would increase as more VFAs are produced by acidogens from the hydrolysed monomers.

The concentration of TVFAs remained relatively low compared to kitchen waste (both K. Flat and K. Prog), and similar to values reported in literature as suitable for AD digesters for a pH of 6 and 7 i.e. 13-14 g/L (or 15 g/L) and 20 g/L respectively (Babel *et al.*, 2004; Anderson *et al.*, 1982; Mawson *et al.*, 1991), as outlined in Section 2.3.1.For example, the maximum TVFAs was ~25 g/L compared to ~200 g/L and ~60 g/L for K. Flat and K. Prog respectively.

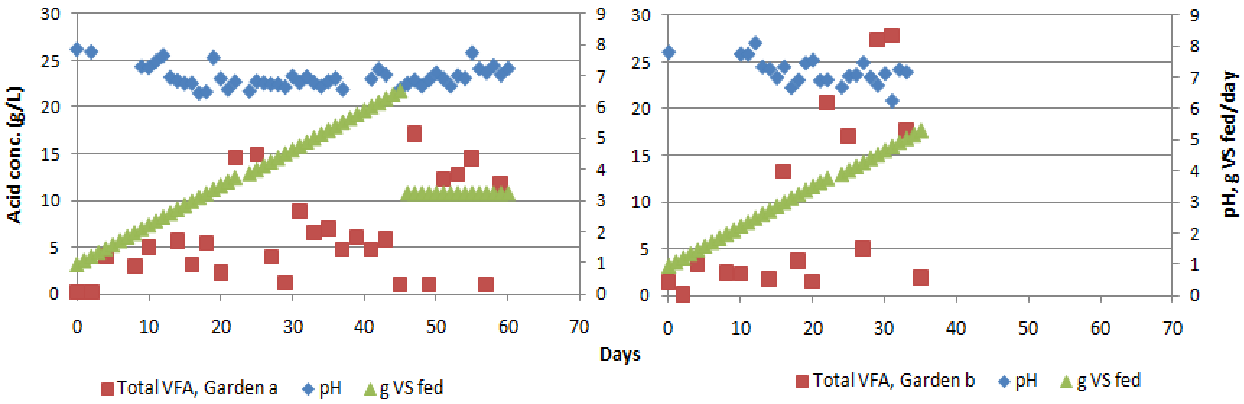


Figure 59: Relationship between the TVFAs, pH and amount of food fed for garden waste

The lower TVFAs profiles experienced with garden waste (i.e. 15 g/L for reactor a, versus 100 g/L for K. Prog b) suggest that hydrolysis was the limiting step for these experiments. The literature reviewed report garden wastes are rich in ligno-cellulosic materials which is recalcitrant to the action of micro-organisms and slows down the biodegradation process (Speece, 1987; Kalra and Panwar, 1986). Inhibition of hydrolysis is not uncommon either and has been reported in literature, where acidic conditions, amino-acids and some sugars can act as inhibitors (Sanders, 2001; Kadam *et al.*, 2004). Garden waste can also contain substances identified as inhibitory such as resins or herbicides, which can impact the AD process negatively (Khalil *et al.*, 1991; Chakraborty *et al.*, 2002), as discussed earlier in Section 2.4.1.2.

A slower hydrolysis step would therefore result in lower accumulation of intermediate products such as VFAs, relatively stable conditions in the reactor media, longer adaptation period,and a balance between the different groups of bacteria. Supporting this, the biogas and pH profiles of garden waste appeared more stable than those from kitchen wastes. For example, the biogas of Garden a, was relatively stable (i.e. between 400 and 500 mL /day) throughout experiments, while the variability in the reactors with kitchen waste appeared significant. Similarly, the pH drop experienced with garden waste was subtle compared to that of K. Prog despite similar biogas yields i.e. the pH dropped to 6.5/6.23 compared to 5.5 for kitchen waste (K. Prog b).

It is also interesting to note that garden waste with lower TVFAs compared to K. Prog (i.e. 100 and 40 g/L versus 15 and 25 g/L) achieved a higher biogas volumes and yields than K. Prog. This suggests that acetogens and methanogens remained active, and the biodegradation process in garden waste was more efficient.

5.4 Experimental variables identified as key for the monitoring of the AD process

The second objective of this dissertation (Section 1.3) was to indentify/confirm among the experimental parameters, the key ones that influence the performance of AD systems. This section presents a discussion on the parameters identified as most influential during the course of the digestion process for BMP and fed-batch experiments.

5.4.1 Biogas yield and pH

Results of the batch tests showed that the buffering was not successful to maintain the pH in the desired range for methanogens, this was particularly evident for kitchen wastes. Batch tests provided an opportunity to learn, and the pH monitoring in the fed-batch experiments was more successful, with the pH drop experienced being lower than those observed with batch tests. Sufficient additional $NaHCO_3$ was added to generally maintain the pH between 6.5-7.5.Still, due to the overfeeding, the pH did at times drop below 6.5, requiring additional buffer addition, which was then followed by significant additional CO_2 releases, skewing the measured gas yields.

The biogas yield (mL/ g VS) was used as the benchmark to compare the performance of different substrates. However, little can be deduced from the pH profiles about the quality of the biogas produced since the CH_4 composition in batch test exhibit a high variability between sampling days even for substrates which showed better performance such as garden waste and fines.The challenge experienced with the gas volumes measured is also linked to the fact that some of the ‘biogas’ were CO_2 released chemically from the buffer during the speciation of HCO_3^- into CO_2 gas. Hence, data about CH_4 composition would have been more appropriate to compare performance of the different digesters.

It is is not evident however that the order of magnitude of results would change for the actual biogas volumes produced as illustrated in Table 41, where an estimation of the actual biogas volumes is presented. The actual biogas volumes were calculated as the difference between the gas measured and the CO_2 volumes estimated.

Table 41: Comparison between gas volumes measure, CO_2 volumes released chemically and actual biogas volumes

	Gas volumes measured , L	Maximum CO_2 from buffer, L	Actual biogas volumes
K. Flat a	19.7	4.3	15.4
K. Flat b	23	4.4	18.6
K. Prog a	11.3	5.4	5.9
K. Prog b	13.5	5.3	8.2
Garden a	13.2	4.7	8.5
Garden b			

The literature reviewed reports that high salt concentrations can cause severe inhibition because since it leads to the dehydration of cells due to osmotic pressure (Chen *et al.* 2008).For example, Soto et al. (1991/1992) showed that propionic acid-utilising are most sensitive to high Na + toxicity compared to other VFAs-utilising bacteria microbes. Most authors agree however that concentrations ranging 3500-5500mg Na^+/L are moderately inhibitory, and values above 8000 mg Na+/L are strongly inhibitory (Chen *et al.,* 2008, Cheng, 2010). The concentration used in this study is in the range reported as inhibitory for microbes (i.e. 1.45 g Na^+/L), which is well below values reported as inhibitory.Hence, the low biogas yields experienced with the fed-batch reactors cannot be associated with Na+ toxicity.

5.4.2 *VFA concentration profiles*

Process variables (i.e. indicators) that can measure intermediate products are more effective for control. Consequently, Boe *et al.* (2010) argue that the biogas yield alone is not enough despite the fact that it is the most common indicator reported in literature. This is because it does not indicate well a process imbalance and is only reduced when the process is already inhibited (Moletta *et al.*, 1994). Many authors therefore proffer that the concentration of VFAs is a crucial process indicator since VFAs are the main intermediate products and their accumulation generally indicate a process imbalance (Jacobi *et al.*, 2009; Molina *et al.*, 2009, Ahring *et al.*, 1995; Hill *et al.*, 1987), as discussed earlier in Section 2.3.

The VFAs profile provided an in-depth understanding of the digestion process. Acetic acid is the main substrate for methane generation; other acids get converted to acetic acid prior to being converted to biogas. Butyric acid degrades into iso-butyric and acetic acid, while iso-butyric acid degrades directly into acetic acid (Wang *et al.*, 1999). However, it is butyric and iso-butyric acids rather than acetic than acetic acid that were the most predominant acids in these experiments for all substrates. A high level of isomerisation was also observed, since the isomer forms of butyric and valeric were predominant. This is not uncommon as VFAs with a carbon chain higher than four (i.e. C_4) tend to isomerise by migration of the carboxyl group to the next carbon (Wang *et al.*, 1999).

The presence of butyric acid in the experiments appeared to have improve biodegradation in research by (Wang *et al*, 1999; Boe *et al.*,2006). This was evident with the K. Flat experiments where the highest biogas yield was from reactor b, with the highest concentration of Iso-butyric acid in spite of the fact that both duplicate reactors had similar concentration of propionic acid (~180 versus 175 g/L). This is in agreement with a study by Wang *et al.* (1999), where results showed that biodegradation was improved with increasing concentrations of butyric acid.

Although, propionic acid is reported as the main inhibitor in literature, there was no apparent inhibition threshold in the K. Flat reactors observed in this study, when the concentration of propionic acid increased drastically from ~2 g/L to ~200 g/L from day 55. The significant increase in propionic acid seems unlikely biologically as microbial degradation occurs gradually, moreover the fact that the pH and biogas profiles were not affected by the increase, lead to believe that these values could be linked more toanalytical error. Figure 60 below presents a comparison of the HPLC chromatograph for days 53 (a) with each peak related to the retention time of a particular VFAs (Section 3.2.1), and 55 (b) for K. Flat a, with a single elevated peak corresponding the amount of propionic acid. The magnitude of the area under the curve for day 55 is 5 times that of day 53. The error could be associated with pollutant from previous runs, as the machine was commonly used by many students. It is also feasible that the main peak observed in Figure 60 (b) masks other peaks referring to differents VFAs in Figure 60 (a) due to a too concentrated sample. In this case, further dilution would have been required to check the validity of this data for these experiments.

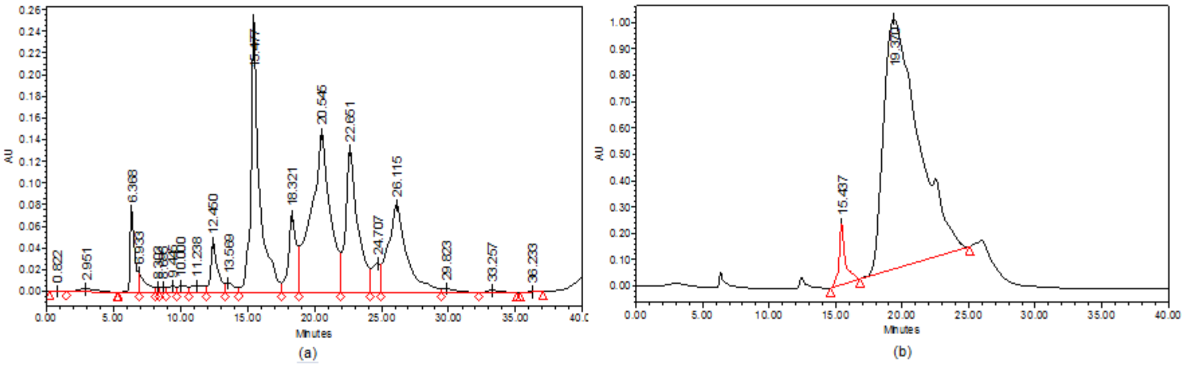


Figure 60: presenting the comparison between day 53 (a) and day 55 (b) data

Generally, the inhibition experienced from VFAs accumulation can be reduced if the pH is maintained in the neutral range as it reduces the concentration of dissociated acids, responsible for the inhibition experienced, increases with lower pH (Ahring *et al.*, 1993, Hill *et al.*, 1987). This was observed with the K. Flat which had more stable pH conditions and achieved a higher biogas yield (K. Flat b~23 L) compared to K. Prog reactors (K. Prog~12.4 L).

5.4.3 Food-to-inoculum ratio in fed-batch experiments

The literature published on the optimal F:I ratio for good performance of AD systems differs considerably, since different systems were investigated under varying operating conditions. For example research by Chynoweth *et al.* (1993) on AD of herbaceous feedstock, showed that the optimal F:I ratio was between 0.5-1. Similarly, Raposo *et al.*(2006) also reported that ratio between 0.5-1 on g VS basis is recommended for AD systems.

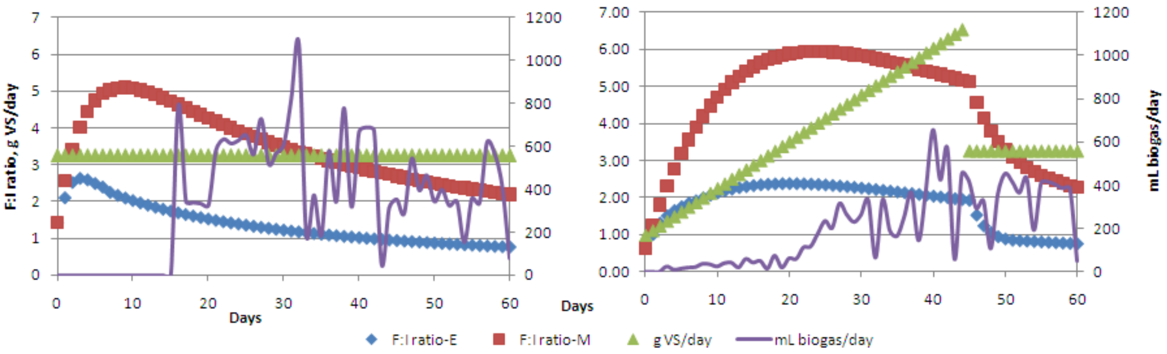
Despite the different numbers in general, most authors agree that high ratios lead to inhibition e.g. Liu *et al.* (2009) showed that the biogas yields were halved from the original value when the F:I increased to ~5 under both mesophilic and thermophilic conditions.

Computer models were developed using Excel and Matlab to investigate the temporal evolution of the Food-to-Inoculum (F:I) ratio in the fed-batch experiments, as it could not be directly measured. Modelling results are presented in details in Appendix (Section 9.2).

The Excel model was developed using the 'Activated Biomass Concept' (ABC, Section 3.4), which assumes a 100% conversion rate of the substrate into inoculums within 7 days. The ABC concept alone could not give accurate representation of the digestion process because it does not account for the conversion of organic materials into biogas as well as other process limitations. Hence, a model that combined AD kinetics and the 'Activated Biomass Concept' was developed. The kinetic model was developed using MATLAB software, assuming first order kinetics and using kinetic constants reported by Vavilin *et al.* (2003) in Table 69 (Appendix, Section 9.2).The second model developed will be referred to as the

MATLAB model in this dissertation. In the MATLAB model, the cumulative amount of inoculum in the reactors was determined using the ABC, while the cumulative substrate available for digestion was determined using kinetics, which also account for the conversion of biomass into biogas. More details about each model, calculations and assumptions are presented in the Appendix (Setion 9.2).

Figure 61 below illustrates results from both models developed for kitchen waste on a flat and progressive feeding rate. It is observed that the F:I ratio using the ABC model (F:I ratio-E) is considerably lower than that from the MATLAB model (F:I ratio-M), confirming that the ABC alone would not be accurate. The F:I ratio-M increases rapidly to≥~5 within the first 10 days regardless of the feeding regimes; values higher than what is reported in the literature as ideal (Liu *et al.* 2009).



(a) Flat Feeding rate

(b) Progressive Feeding rate

Figure 61: Illustrating the temporal evolution of the F:I ratio for reactors with kitchen waste fed on a flat (left) and progressive (right) feeding regime

Where: F:I ratio-E, Model developed using the ABC in Excel ; F:I ratio-M, Model which combines ABC and kinetics and developed in MATLAB

Results of the simulation show that (i) higher F:I ratios achieved with the MATLAB model which indicate that the ABC model is indeed over idealistic about the conversion rate of fresh feed; and (ii) F:M ratio-M increases within the first 10 days above 5, reported as detrimental to good performance for AD systems (Liu *et al.*, 2009; Chynoweth *et al.*, 1993). The F:I ratio in the K. Flat however, appears to drop quicker than in K. Prog reactors, indicating that the balance between micro-organisms and the biomass was improved.

It is then evident from the high F:I ratios that the reactors, particularly under the progressive feeding regime were overfed. Since all the other reactors with the remaining substrates investigated were on a progressive feeding regime, it could be assumed that this was among the causes for the low biogas yields that were achieved.

Experimental data agree best with the MATLAB model presented,for kitchen waste (Appendix, Section 9.1.2).

5.5 Conclusions from the fed-batch experiments

The following conclusions can be deduced from the laboratory investigation of AD of kitchen and garden waste under fed-batch conditions:

- Psychrophilic start-up conditions appeared not suitable for biodegradation, since the biogas production was undetectable when the bioreactors were operated at ~17°C for most substrates.

- The estimated F:I ratio were~5, and indicate that reactors under both feeding regime were overloaded with organic materials. However, the F:I ratio in the K. Flat reactors decreased more quickly to values reported in literature as suitable, compared to the K. Prog reactors.

- The biogas yields reported may well be an overestimation since a fraction of the gas volumes measured would be CO_2 chemically released from the speciation of HCO_3^- added with the buffer. Hence, data about the CH_4 composition would have been more suitable to evaluate system's performance, and methanogenic activity.

- The progressive feed performed poorly compared to a daily flat feed once an excessively high loading rate was imposed. This emphasises the crucial role that adaptation and a sustainable OLR plays in the success of AD systems.

- AD of cellulosic materials exhibited a more stable behaviour relative to the easily biodegradable kitchen waste. This could be attributed to the slower hydrolysis rates, which limited the amount of intermediate products (e.g. VFAs) available for the methanogenic archaea, allowing a balance between the activities of the different microbial groups to avoid VFAs accumulation and potential inhibition.

- The VFA values measured particularly with kitchen waste were higher than those reported in the reviewed literature, which possibly puts in doubt the experimental design and accuracy of the analyses carried out. However, the above was not observed for VFAs values of garden waste.

- Butyric and iso-butyric acids were the predominant forms of VFAs, and correlated to higher biogas yields. It appears that the concentration of iso-butyric enhanced biodegradation, this was particularly evident with the K. Flat reactors where reactor b achieved a higher biogas yield compare to reactor a, despite identical reactor set-up. There was no apparent link between accumulation of propionic acid, pH or reduced biogas volumes contrary to the literature published which identifies propionic acid as a cause of inhibition.

- Finally, the biogas yields for the fed-batch experiments were considerably lower than those from the BMP tests for all substrates. However, the CH_4 yield of these BMP samples is considerably low, showing poor performance for the BMPs (Table 37). It is difficult to draw any robust conclusions based on the biogas yield as these values are overestimates. It is noteworthy that comparing batch and fed-batch experiments based on mL CH_4/g VS (/g COD) destroyed would have allowed a more accurate comparison of the systems' efficiency. This exercise should be carried out in future research.

- There are a number of factors that could have influenced results such as the psychrophilic conditions for the first two weeks and, the F:I ratio or the different microbial population used. It would be interesting to repeat these experiments at 35°C from start-up, with particular attention to buffer addition, measuring the CH_4 composition, digesting BMP and fed-batch test with the inoculums from the same source and investigating the suitability of different inoculums.

6 CO-DIGESTION

The co-digestion experiments of nitrogen-rich with carbon-rich substrates are presented in this chapter. The materials used for the BMP tests were: blood and abattoir wastes from the Stikland abattoirs, garden waste from the Bellville Compost plant (BC Garden), fines from BC (BC Fines) and paper sludge. The substrates used for the fed-batch experiments were: blood from the Stikland abattoirs, and garden waste from Wynberg Drop-off (G). Details about the source and characteristics of substrates were presented earlier in Sections 3.4.1 and 3.4.2.

6.1 Batch Test Results

6.1.1 Observed gas yields

Figure 62 presents the gas productivity (mL/day) of the co-digestion of abattoir and blood waste with garden waste, paper sludge and garden waste respectively. The maximum productivity is observed during the first 10 days for blood waste, and 20 days with abattoir wastes. The activity of the microbial consortia almost ceased beyond the first 10-20 days as limited biogas volumes were produced. The maximum biogas productivity (mL/g VS) varies between 60 and 80 mL/day for blood and abattoir waste.

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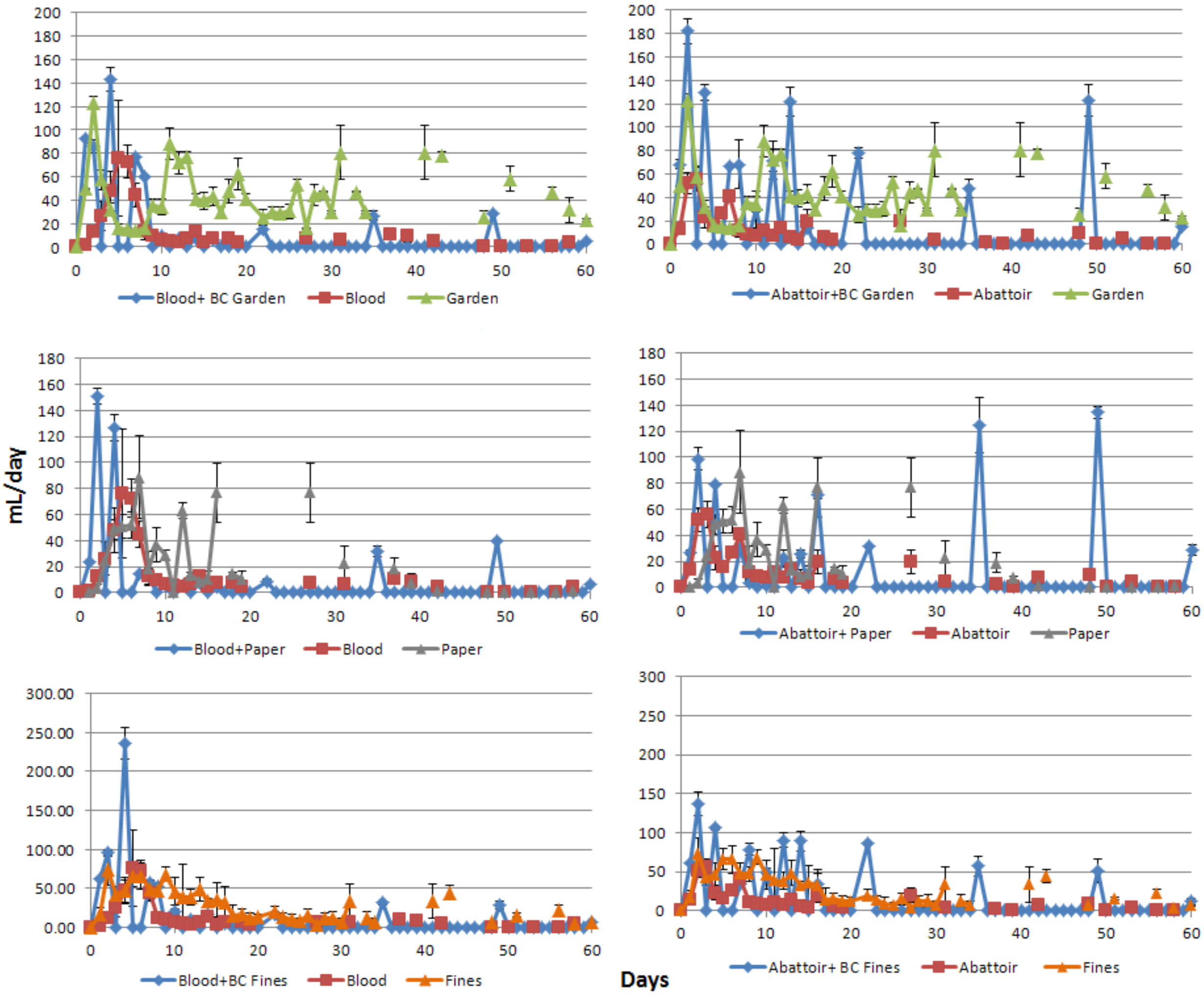


Figure 62: Biogas productivity (mL/day) of the pure waste streams and co-digested substrates in 100 mL batch tests

The biogas yields of the different substrates are presented in Figure 63. The biogas yields of the pure waste streams are: 77, 79, 546,140.9 and 493 mL/ g VS for abattoir, blood waste, garden refuse (BC GW), paper sludge and fines (BC Fi) respectively as discussed earlier (Chapter 4).

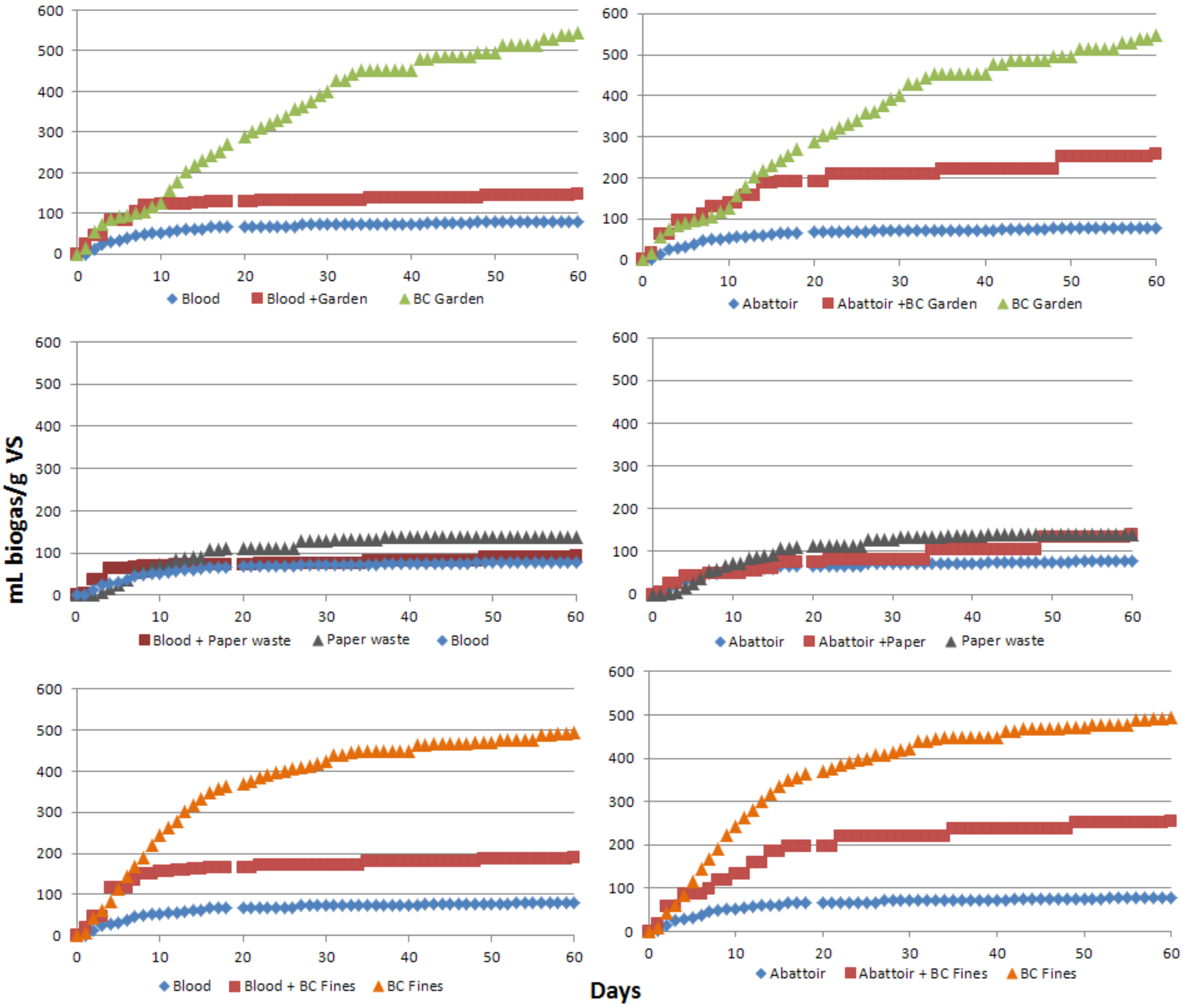


Figure 63: Cumulative yields of garden, paper sludge, abattoir, blood and their co-digestion for BMP test

The gas yield of the co-digestion of abattoir wastes with garden waste (Abattoir+BC Garden), paper sludge (Paper sludge+ Abattoir) and fines (Abattoir+ BC Fines) were respectively 256, 139 and 255 mL/g VS (Figure 63).while, the gas yields of the co-digestion with blood were respectively 147, 190 and 92 mL/ g VS for garden waste (Blood+ BC Garden), paper sludge (Blood+ Paper sludge) and fines (Blood+ BC Fines).

6.1.2 Analysis of variance (ANOVA)

Results of the analysis of variance (ANOVA) performed on the gas yields (mL/g VS) are presented in this section, this analysis was carried out separately for co-digestion with abattoir and blood waste substrates. Table 42 presents the biogas yields of the co-digestion with blood waste, and results of the ANOVA are presented in Table 43.

The columns represent the different digesters, and the rows represent each triplicate sample. Results show that the variability of the triplicates within each substrates type is not statistically significant (i.e. P-value> 0.05, and F>F-crit). By contrast, the variability between the co-digestion with the different wastes (garden waste, paper sludge and fines) is statistically significant. This indicates that the difference in magnitude between the mean values of the co-digestion with paper sludge (~102 mL/g VS) versus the others (~155 mL/gVS and ~200 mL/g VS for garden waste and fines respectively) is significant.

Table 42: Cumulative biogas yields of different substrates co-digested with blood waste

	BW+ GW	BW+PW	BW+Fi
I	164.75	98.32	191.69
II	144.13	101.47	202.08
III	158.49	106.53	206.53

Table 43: ANOVA results for the co-digestion of different substrates with blood waste

Source of Variation	*F*	*P-value*	*F crit*
Rows	0.73	0.54	6.94
Columns	105.56	3.46E-4	6.94
Error			
Total			

Table 44 presents the specific biogas yields of the co-digestion of different substrates with abattoir wastes, and the ANOVA results are presented in Table 45. The columns represent different substrates, while the rows represent each triplicate within the group. Results show that the variability between triplicates was not very large that it is not possible to conclude formally that the different co-digestion formulations yielded different gas volumes (i.e. P-value>0.05 and F<F-crit).

Table 44: Cumulative biogas yields of co-digestion of different substrates with abattoir waste

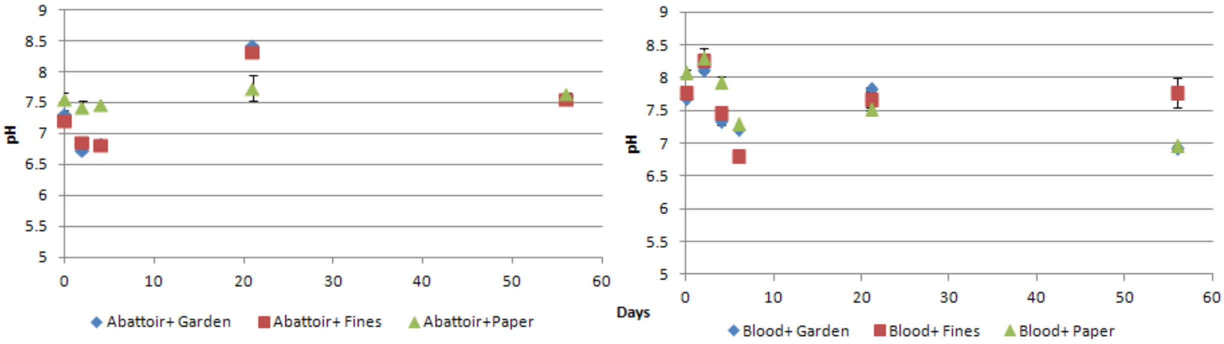
	AW+ GW	AW+PW	AW+Fi
I	324.49	168.03	247.85
II	342.93	192.21	243.27
III	207.58	157.38	328.37

Table 45: ANOVA results for the co-digestion of abattoir wastes with garden waste, paper sludge and fines

Source of Variation	*F*	*P-value*	*F crit*
Rows	0.16	0.85	6.94
Columns	3.33	0.14	6.94
Error			
Total			

6.1.3 pH control

Figure 64 presents the pH profiles of co-digestion experiments of garden waste, paper sludge and fines, with abattoir (left), and with blood waste (right). The pH of these waste streams remained relatively stable throughout the duration of the experiments. There is a slight decrease in the pH observed within the first week (from ~7.5 to ~6.8), but the pH increases above 7.0 for most experimentsthereafter(Figure 64). The pH conditions reported with high protein wastes are usually associated with the buffering effect of N-compounds from the degradation protein wastes in the literature (Salminen and Rintala, 2002; Speece, 1996).



(a) co-digestion with abattoir wastes

(b) co-digestion with blood waste

Figure 64: Temporal evolution of pH of the different co-digestion substrates

Table 46 presents the estimation of the maximum CO_2 released chemically from the original amount of buffer added. The lowest amount of buffer added was in the bioreactors with paper sludge where the maximum CO_2 released chemically constitute between ~25.8% and 38.6% of the gas volumes measured.

The digesters with the highest potential of CO_2 released were those co-digesting blood and fines, and abattoir and fines (the CO_2 volumes released could constitute ~70% and ~115% respectively, of the gas volumes measured).

Table 46: Estimation of the maximum CO_2 produced from the buffer

	g buffer	No. moles	Vol chemical CO_2, L	Total cum biogas produced, mL	% X	%Y
Blood+ Garden	1.32	0.02	0.40	563.33	70.50	20.46
Blood+ Fines	1.27	0.02	0.38	639.00	59.80	NA
Abattoir+Garden	0.87	0.01	0.26	890.33	29.40	62.07
Abattoir+ Fines	1.68	0.02	0.51	438.33	115.32	NA
Blood+ Paper	0.87	0.01	0.26	678.67	38.57	55.58
Abattoir+ Paper	0.87	0.01	0.26	1016.33	25.76	73.18

Where ***X:***maximum fraction of chemical CO_2 over total cumulative biogas for each sample,

Y:minimum fraction of 'biological'CO_2 over chemical CO_2 ,NA: non-applicable

It is expected however that only ~20% of the maximum amount would have been released as CO_2 since alkaline conditions (pH>7.5) favour the speciation of CO_2 molecules into its bicarbonate/carbonate form as discussed earlier in Section in 2.2.3.3 (Figure 7; American Chemical Society, 1979).

The 'Y' ratio in Table 46 is an expression of the fraction of the 'actual' CO_2 from biodegradation over the estimated CO_2 volumes. For example, for the co-digestion of abattoir and paper sludge, at least ~73% of the estimated CO_2volumes produced resulted from biodegradation.

6.1.4 Methane composition and productivity

The CH_4 composition of the co-digested substrates is presented in Figure 65 below. The readings measured were null beyond the third week. This is consistent with the biogas productivity profiles which limited biogas volumes produced beyond day~10.

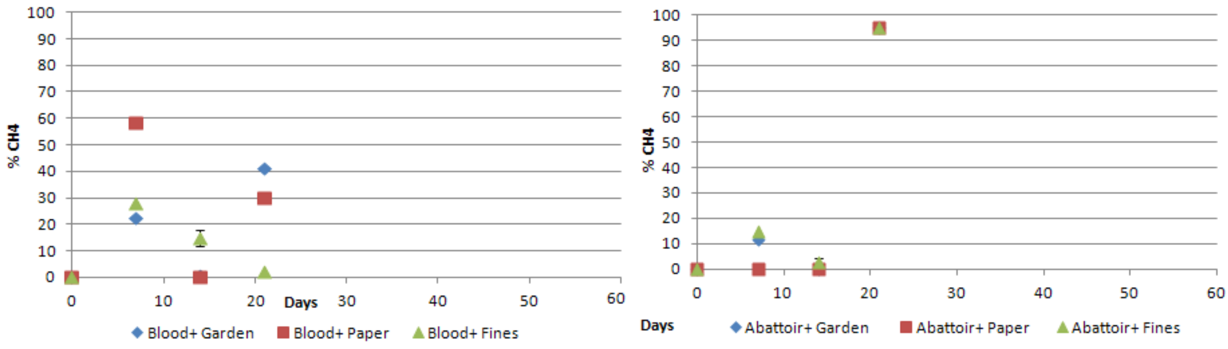


Figure 65: CH_4 composition of co-digested substrates

The CH_4 content for abattoir wastes is generally lower than the blood ones, with the exception of day~21. The highest CH_4 composition achieved was ~58% for the co-digestion with blood and paper sludge in the second week. While the highest observed was ~95% on week 3 for all the co-digested substrates with abattoir wastes.

Although the signficant increase (to 95%) in CH_4 composition seems unlikely, it is consistent with the biogas productivity profiles which exhibit a distinct peak on day~21 for co-digestion with abattoir wastes (Figure 62).The higher CH_4 can be linked to the retention of CO_2 in the system favoured under alkaline conditions from the speciation of CO_2 (g) into HCO_3^-.The pH profile is aligned with the above statement as the pH was ~8 on day 21.

Figure 66 presents the estimated cumulative CH_4 yields (mL/g VS) of the different co-digested substrates. The highest CH_4 yield is from the co-digestion of abattoir with garden with ~50 mL CH_4/g VS. This is consistent with the biogas yields profiles i.e. the co-digestion of abattoir waste with garden waste had the highest biogas yield (~256.4 mL/g VS). The CH_4 yield achieved in digesters with blood are considerably lower than those with abattoir wastes i.e. the average CH_4 yield for the co-digestion with blood is lower than ~30 mL/g VS for all the substrates compared to co-digestion with abattoir wastes. The production of CH_4 is an indication that the methanogenic archaea were active during the first three weeks. Their activity does however appear to have been limited/restricted.

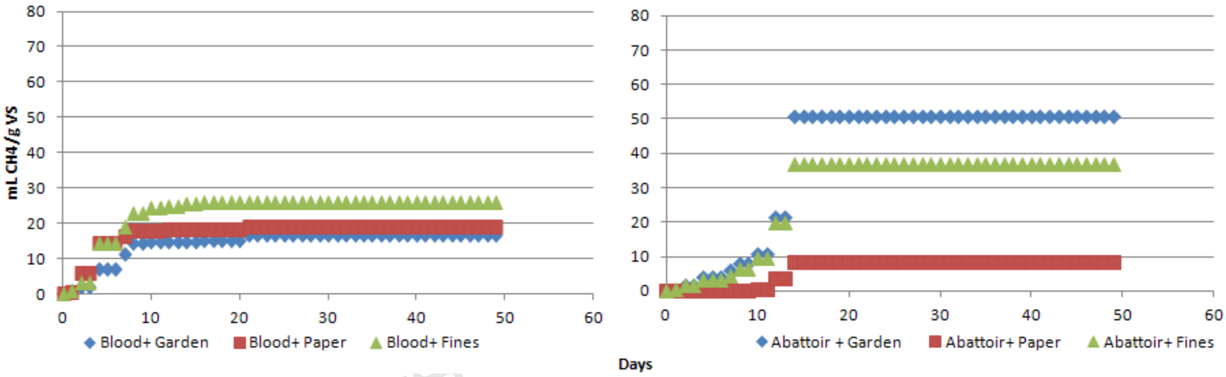


Figure 66: Cumulative CH_4 yield for the different co-digestion substrates

The values expected for CH_4 yields from the co-digestion experiments were determined as the weighted average of the individual CH_4 yields achieved (i.e. nitrogen and carbon-rich substrates), sample calculation are presented in the Appendix, Section 9.1.7. The mixing ratio of blood and abattoir waste to garden waste and fines was 1:3 on a mass basis, and 5:1 for paper sludge (Section 3.8). The comparison of the expected versus experimental results is presented in Figure 67.Determining the expected CH_4 yield provided a baseline from which the benefits/effects of co-digestion could be evaluated i.e. the extent to which co-digestion improved the overall CH_4 yield.

Generally, experimental results are significantly lower than the expected results, except for co-digestion with paper sludge where the biogas yields of the co-digestion with both pure blood and abattoir wastes were as poor as for the mono-digestions.

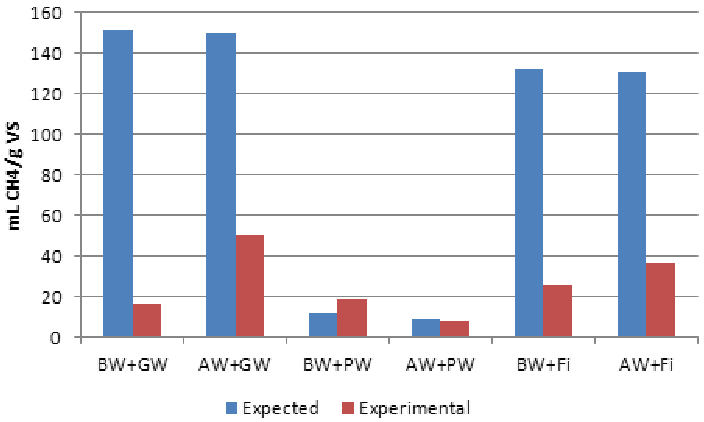


Figure 67: Expected versus experimental results of the co-digestion of high protein waste with a rich carbon source

Where B+PW=blood and paper sludge, A+PW=abattoir and paper sludge, B+GW=blood and garden waste, A+GW=abattoir and garden waste, B+Fi=blood and fines, A+Fi=abattoir and fines.

6.1.5 Discussion

The biogas and CH_4 were used as the basis of comparison of the performance of the co-digestion experiments, however the activity of microbes appeared to be inhibited after day 10 and day 20 for co-digestion with blood, and abattoir wastes respectively. The VS and COD destruction of this batch of experiments was not quantified, it would be valuable for further work to repeat these tests and evaluate the performance of these co-digestion system based on organic content destruction, as well as a mass balance based on theoretical values after a detailed characterisation of the substrates (proteins, carbohydrates and fats content) based on the stoichiometry of AD from the literature (Equations 19-21, Deublein and Steinhauser, 2008).

Table 47: C:N ratio of the various substrates

	C:N ratio
Abattoir waste	11.1
Blood waste	3.2
Bellville Compost (BC) garden waste	36.6
Bellville Compost (BC) fines	6.9:0
Paper Sludge	21:0 (*)
EW	22.1

(*), the ratio is equal to infinity i.e. no parts of nitrogen was detected or the amount of nitrogen relative to carbon was insignificant

Carbohydrates: $C_6H_{12}O_6 \rightarrow 3CO_2+3CH_4$ **Equation 19**

Fats: $C_{12}H_{24}O_6+ 3H_2O \rightarrow 4.5CO_2+7.5CH_4$ **Equation 20**

Proteins: $C_{13}H_{25}O_7N_3S+6H_2O \rightarrow 6.5CO_2+6.5CH_4+3NH_3+H_2S$ **Equation 21**

Many authors agree that the lower yields generally experienced with the AD of protein-rich substrates such as slaughterhouse wastes, is linked to the potential accumulation of high concentration of NH_3 which is inhibitory to the methanogenic archaea. The range of values reported in literature as inhibition treshold varies signficantly depending on the system used, adaptation period of the microbial sludge, the OLR and retention periods (Chen *et al.*, 2007; Farina *et al.,*1998; Kroeker *et al.,*1979). An increase in pH with high protein systems is undesirable as the concentration of free ammonia (FA), identified main inhibitor, increases with pH (Borja *et al.*, 1996; Chen *et al.*, 2008/2011; Boardman and McVeigh, 1997).

Some authors have also linked lower biogas and methane yields to the action of sulphate reducing bacteria (SBR), which compete for intermediate products used by the methanogenic archaea and generate sulphide (H_2S) during biodegradation as illustrated by Equation 3 above. Methanogens are more sensitive to sulphide inhibition than fermentative bacteria (Wang *et al.* 2007; Deublein and Steinhauser, 2008), as discussed earlier in the literature review (Section 3.1). The range reported in literature as inhibitory for sulphide ion is100-800 mg/L, and 50-400 mg/L for undissociated H_2S (Cheng, 2010). The undissociated form of sulphide (HS-) is reported as the main inhibition form, and its concentration increases with pH (i.e. 50% of the H_2S will exist as HS- at pH~7, and that ratio increase to 90% at pH~8; Chastain, 2008).

Following from the above arguments, the estimated concentrations of NH_3 and H_2S generated from the digestion of AW and BW, are presented in Table 48 (sample calculation are presented in Appendix, Section 9.1.10). These values were determined using the C:N ratio measured, the general formula of the stoichiometric degradation of a protein (Equation 21) and the VS destruction rate achieved, and represent overestimated values for and NH_3 and H_2S, assuming there were a population of SRB in the microbial consortia.

Table 48: Estimation of the NH_3 and H_2S generated from biodegradation of protein for AW, BW and EW

	C:N	g VS loaded	VS destruction ,%	NH_3 (100 mL), mg	NH_3, g/L	H_2S (100 mL), mg	H_2S, g/L
AW	11.1	4.84	5	24	0.24	16.2	0.16
BW	3.2	4.58	13	82.7	0.83	55.16	0.55
EW	22.1	4.48	56	131	1.32	87.9	0.88

Results show that based on the VS destruction, the concentration of NH_3 in the batch tests were ~240 mg/L for AW, and 830 g/L for BW. The NH_3 concentrations for AW and BW are well below concentrations reported in the literature reviewed i.e. 1.7-14 g/L of NH_3 (Wang *et al.*, 2009). Hansen *et al.* (1998) however report NH_3 concentration between 100 and 150 mg-N/L for non adapted cultures, which could be applicable in this case. Nonetheless, the case for NH_3 accumulation and inhibition from biodegradation is not evident based on the above argument. This is particularly valid for AW and EW as the NH_3 concentrations are an overestimation (Section 9.1.10) and would be expected to be considerably lower.

The calculated concentration of H_2S generated would be 0.16 and 0.55 g/L for AW and BW respectively. It is expected that 60-80% of the H_2S calculated would be undissociated (HS-), from the alkaline pH conditions (pH~7.5-8). The H_2S concentration for AW and BW are in the rage of values reported in literature as inhibitory (i.e. 100-800 mg/L for H_2S). It is hard to draw any robust findings however, because the concentration was not monitored relative to other parameters such as the biogas and CH_4 productivity during the digestion period.

It is expected that the H_2S and NH_3 concentration would be lower in the co-digestion reactors as the protein-rich substrate is diluted. The biogas and CH_4 yields of the co-digested substrates remained lower than those of the carbon source (i.e. paper sludge, garden waste and fines). The poor performance is cause of concern, and might best be explained by a mismatch of inocula and substrates, as it appears NH_3 inhibition is only probable for BW does not seem to have occurred for others based on estimated degration extent of the substrate. And H_2S inhibition could occur based on the calculated values, however the data generated is not sufficient to substantiate the above argument. Moreover, food waste samples such as EW had similar H_2S concentration, under similar pH values but yet achieved significantly higher biogas and CH_4 yields; suggesting at the very least that other factors were at play for the lower microbial activity and lower performance.

6.2 Co-digestion experiments under fed-batch conditions

The following samples are included in this category: blood waste from the Stickland abattoirs, garden waste from the Wynberg Drop-off, and the co-digestion of blood and garden waste.

6.2.1 Biogas and pH profiles

Figure 68 presents the relationship between pH, daily biogas yield and the amount of substrate fed daily for the duplicate reactors with blood wastes. Results of its co-digestion with garden waste is presented in Figure 69. The biogas productivity (mL/day) was also negligible under psychrophilic conditions before the digesters were heated to ~35°C, similarly to other substrates (kitchen and garden waste). The biogas productivity for blood waste then increased to ~150 mL/day between days ~25 and 35 and dropped to ~100 mL/day thereafter. The highest biogas peak is 195 mL/day, observed on day 49.

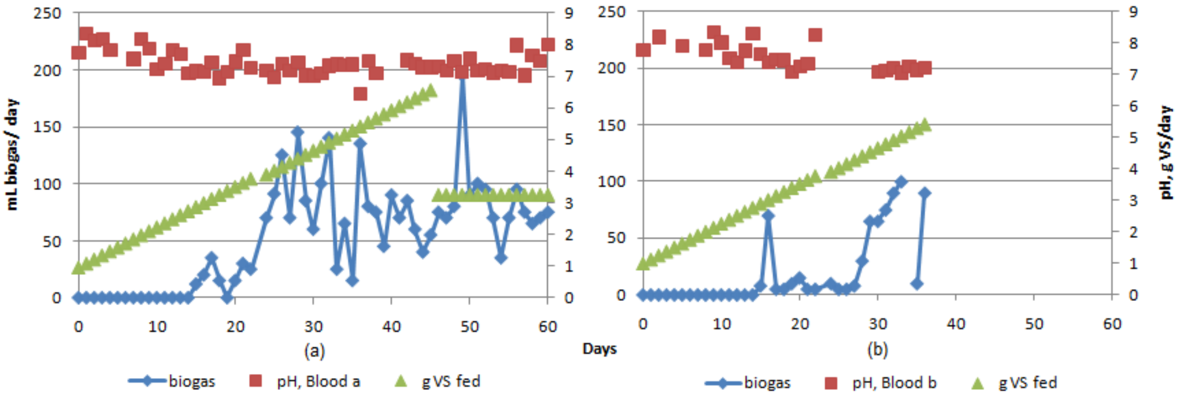


Figure 68: Biogas yield, pH and amount of g VS fed daily with AD of blood

The biogas productivity (mL/day) for the co-digestion of blood and garden waste, is generally higher compared that of pure blood. For example, the highest daily biogas volume measured for co-digestion is ~1000 mL/g VS compared to 195 mL/g VS for blood waste. The biogas productivity remained very low for the first 30-36 days except for the peaks observed between days 1 and5, then doubled on average (from below 200 mL/day to ~400 mL/day) until day~45, and decreased thereafter.The early peak observed could be associated with the degradation of any easily fermentable substrate available from the action of acidogens and acetogens.

The highest productivity period also corresponds to the time after the feeding regime was changed from progressive (6.25 g VS/day) to a flat amount of 3.257 g VS/day (i.e. on day~37), which is an indication that reducing the OLR improved the performance of the bioreactors.

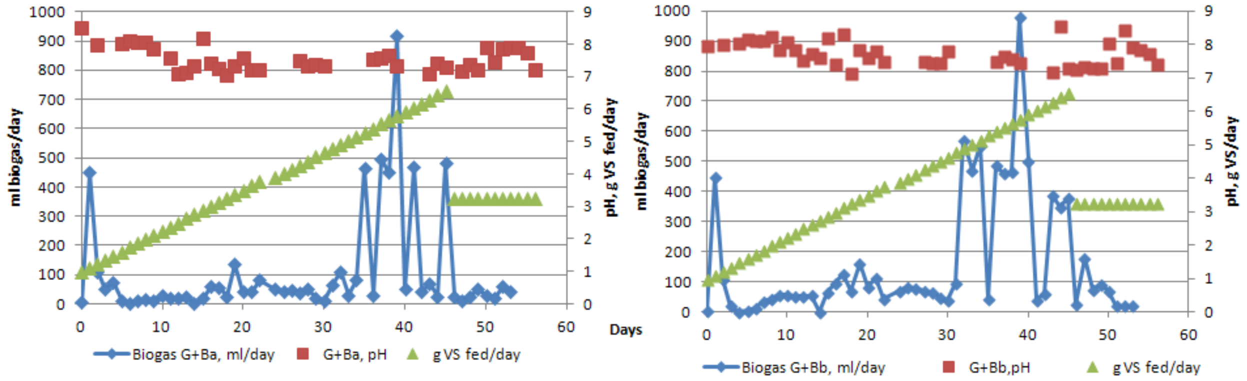
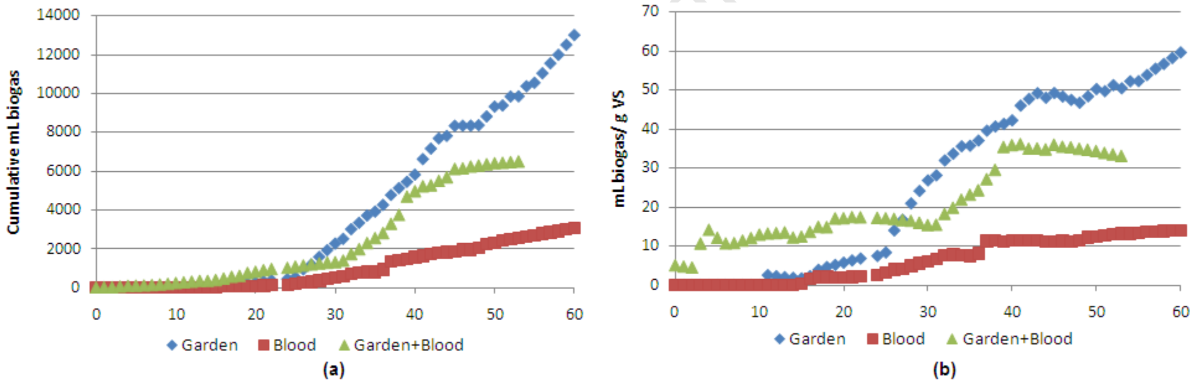


Figure 69: Biogas yield, pH and amount of g VS fed daily in the co-digestion assays

Figure 70 presents the average cumulative biogas yield in mL and mL/g VS for the different substrates. The cumulative biogas yields (cumulative mL of biogas/cumulative g VS loaded) of garden, blood and the co-digestion of garden with blood were respectively: 13,174 mL (60 mL/g VS); 3,128 mL (14 mL/g VS) and 6,499 mL (33 mL/g VS). The biogas yield of blood was the lowest compared to others (garden waste and its co-digestion with blood) similarly to the BMP results.



(a) (b)

Figure 70: Biogas yield of blood, garden and co-digestion of blood with garden waste

The pH profiles are presented in Figure 71. The pH remained in the neutral-alkaline range (i.e. between ~7 and 8.5) for both blood waste and its co-digestion. Table 49 presents the maximum amount of CO_2 chemically released from the initial amount of buffer added ($NaHCO_3$). No additional chemical buffer was required to maintain the pH neutral for optimal methanogenic activity for the remainder of the digestion period.

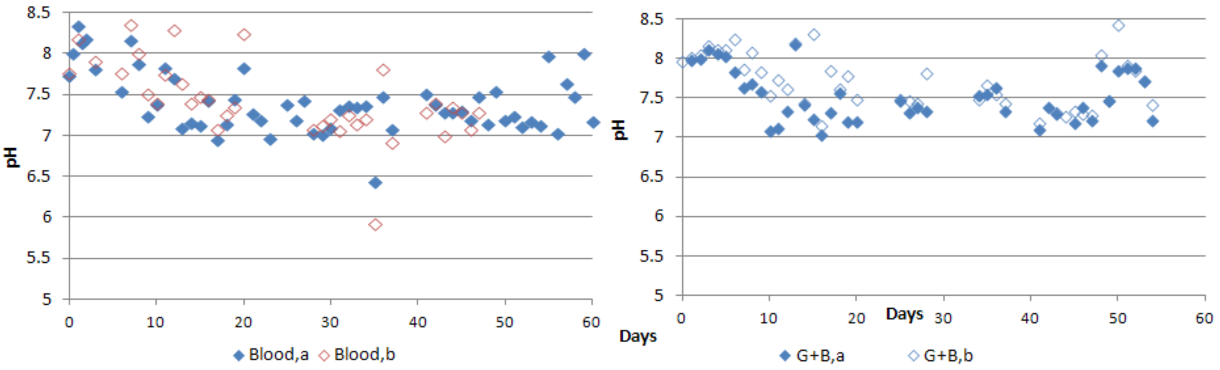


Figure 71: Temporal evolution of pH for blood waste, and its co-digestion with garden wastes

Results show that the maximum CO_2 that could be released chemically would constitute ~40-63% of the gas volumes measured for the co-digestion digesters, and more than~100% of the gas volumes measured for blood waste could be from the buffer. It is expected however that only 20-50% of the maximum CO_2 volumes would have actually been released as CO_2 (g) when considering the fact that pH varied between ~ 7-8.5, which would have favoured the speciation of CO_2 into HCO_3^- as discussed earlier (Figure 7; American Chemical Society, 1979).

Table 49: Estimation of CO_2 released chemically from $NaHCO_3$ from the blood and its co-digestion with garden waste

	g buffer	No. moles		Vol CO_2, from $NaHCO_3$ at 35°C	Total cum biogas vol, mL	%
Blood a	10.57	2.9	3.18	3.13	101.7	101.7
Blood b	–	–	–	–	–	–
G+Ba	10.57	0.1	2.99	3.18	5.01	63.4
G+B b	10.57	0.1	2.99	3.18	7.98	39.9

It is noteworthy that the period with the highest biogas yields also corresponded the one with a lower pH (~7.3); as was observed with the BMP results of the co-digestion experiments. This is similar with research by Boardman and McVeigh ,1997 and Chen *et al.* (2008/2011)on the positive effect of lower pH of AD systems with high protein wastes due to pH effects which limit the speciation of pollutants such as H_2S and NH_3 from biodegradation, into their inhibitory forms. The pH drop probably resulted from the production of VFAs from the action of acidogens.

6.2.2 Accumulation of biomass in the digesters

Figure 72 presents the comparison between the amount of VS loaded and the experimental values measured in the digesters, the method to generate the data is identical to that discussed earlier in chapter 5. The total cumulative amount of biomass fed (g VS) is also presented, a total of ~224 g VS was fed based on the progressive feed..

Results of the blood waste (left) show a clear accumulation of biomass in the system very early on compared to the co-digestion data (right), where the accumulation is only observed beyond day ~50. The magnitude of the accumulation in co-digestion digesters is also smaller compared to the blood one (i.e. the highest value is ~11 g VS for blood versus ~7 g VS for the co-digestion digesters).

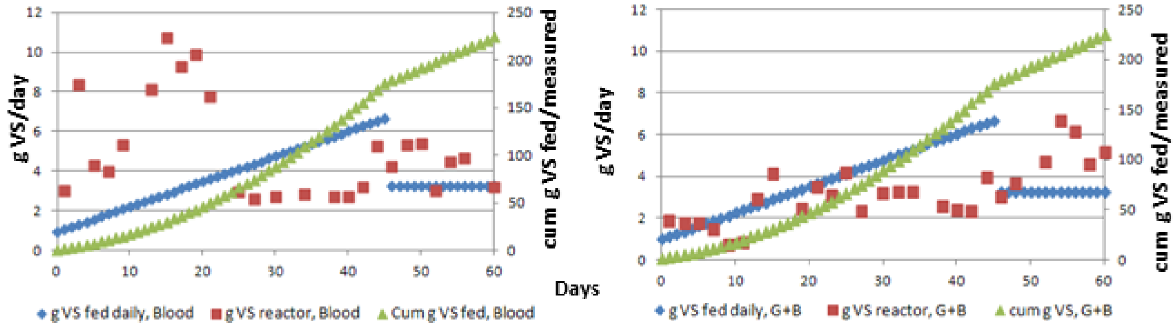


Figure 72: comparison between g VS fed, and g VS in the reactor

These results are aligned with the profiles of the simulated the food-to-inoculum (F:I) ratio for the progressive feeding regime (Figure 61, Section 3.2) which showed that fed-batch were overloaded. For example, the F:I ratio under the progressive feeding regime remained between 5 and 6 from days 20-50, despite the reduction in organic load from 6.25 g VS/day to the flat rate of 3.27 g VS/day. This value (i.e. F:I~5-6) is well above the recommended range (F:I~0.5-1) reported in the reviewed literature for optimal digester’s performance (Chynoweth *et al.*,1993; Raposo *et al.,* 2006).

6.2.3 Temporal evolution of individual volatile fatty acids (VFAs)and NH_3 profiles

The profiles of individual VFAs are presented in Figures 73 and 75for the AD of blood waste and its co-digestion with garden waste respectively; the y-axes are on a logarithmic scale. The general order of occurence of the different VFAs in the blood reactors was as follows: iso-butyric acid/butyric acid>acetic acid>iso-valeric acid>propionic acid>valeric acid>lactic acid until day ~47 (Figure73). Propionic acid became prevalent thereafter with a concentration of up to 159 g/L.

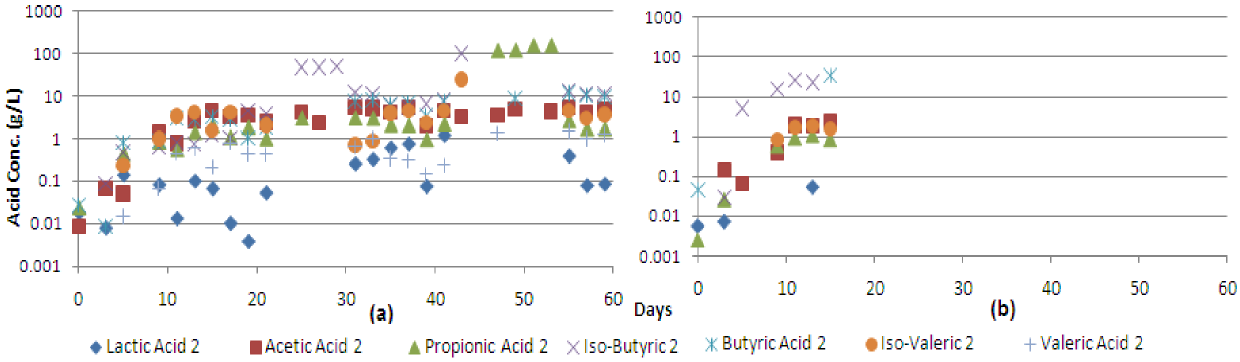


Figure 73: Temporal evolution of individual VFAs for AD of Blood waste

The concentration of propionic acid increased significantly from 2.3 g/L to ~120-158 g/L between days 47 and 53, and dropped again to 2.7 g/L thereafter. The increase in propionic acid corresponds with a pH drop from ~7.5 to 7 between days 41 and day 57 for reactor a; and a drop in biogas productivity from ~150 mL/day to ~100 mL/day (Figure 73). However, while the decrease of the concentration of propionic acid beyond day 57 relates to an increase in pH; no trend is observed in the biogas profiles.

The concentration of NH_3 in the fed-batch experiments was monitored on a weekly basis.Figure 74presents the temporal evolution of the average free and saline ammonia (FSA) concentration in the bioreactors of blood (Figure 74a) and its co-digestion with garden waste (Figure 74 b). The main forms of ammonia measured would be ammonium ions (NH_4^+) and free ammonia (FA i.e. NH_3). The average concentration of both FSA and FA are presented for the respective digesters. The speciation of NH_4^+ into NH_3 is dependent on pH. Sample calculation of FA concentration are presented in Section 9.1.4 (Appendix).The FSA and FA concentration gradually increased during experiments, and the highest concentration of FSA was ~2.9 g/L (FA~35 mg/L) for AD of blood and ~450 mg (FA~11 mg/L) for its co-digestion with garden waste.

The experimental values for blood wastes beyond the second week, are similar to values reported in the literature as inhibitory by Farina *et al.* (1998) i.e. 1.1-4 g/L of total ammonia nitrogen (TAN) for the AD of swine and poultry manure. The FSA concentration increased significantly from ~1g/L to ~2.9 g/L beyond day ~41 towards the end of experiments for blood wastes. This period also corresponds to the time when the concentration of propionic acid increased significantly. However, the sudden drop in propionic acid beyond day 57 is irregular, which suggest this sudden change can be associated with analytical error as was the case with the K. Flat reactors.

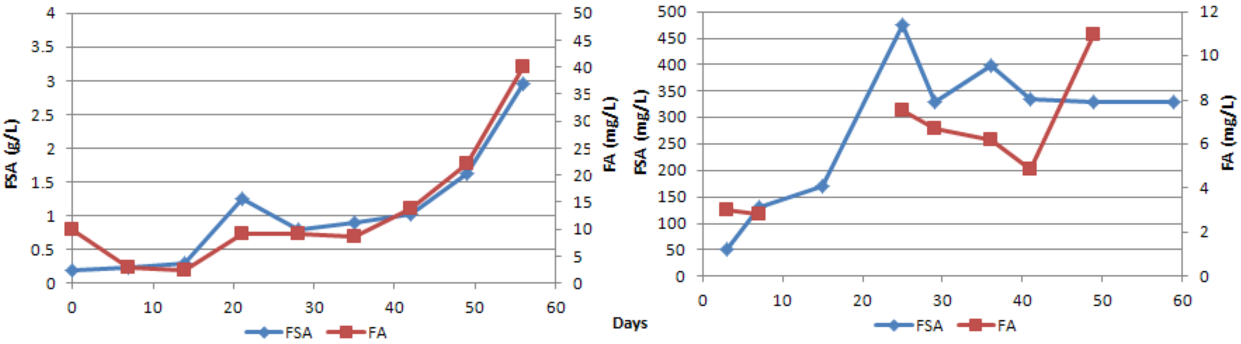


Figure 74: Temporal evolution of the average FSA and FA (NH_3) concentration in AD assays of (a) pure blood, (b) co-digestion of blood with garden waste

Figure 75 presents the individual VFAs profiles for the co-digestion of blood with garden waste. The order of occurence of the different acid was as follows for both duplicates: iso-butyric/butyric>acetic acid>iso-valeric>propionic acid> and lactic acid.

Iso-butyric was initially predominant in reactor a until day 25, and butyric acid then became the predominant form. The interchange between the two acids (butyric and iso-butyric) can be linked to the high level of isomerisation observed generally in the fed-batch experiments. It is noteworthy that reactor b with a higher biogas yields, also had higher concentration of iso-butyric acid (Figure 75), as was observed with the K. Flat reactors.

The VFAs profiles exhibit a period with a general decrease in the concentration of VFAs for both reactors i.e. between days 21 and 35 for reactor a, and between days 22 and 35 for reactor b, indicating the consumption of VFAs in the system.

However,this trend is not aligned with the biogas productivity where the higher biogas productivity periods are observed later between days day 35 and 45 for reactor a, and between day 30 and 45 for reactor b.

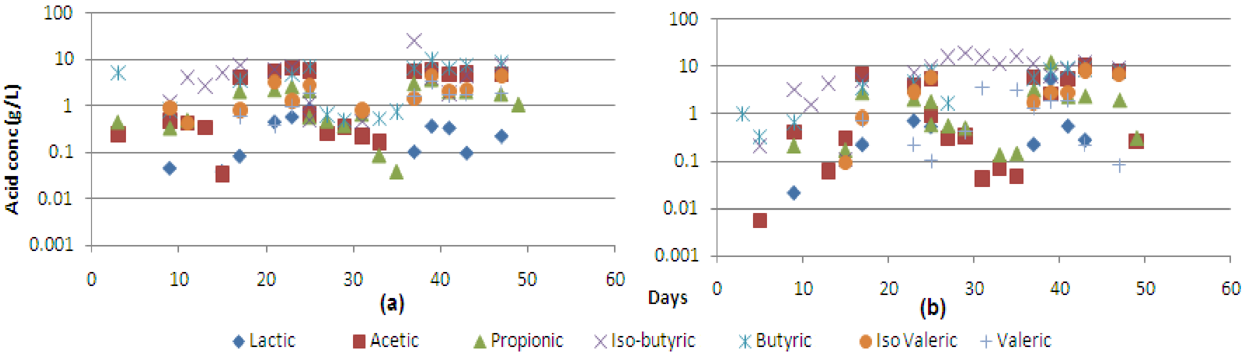


Figure 75: Temporal evolution of individual VFAs for AD of co-digestion of blood with garden waste for reactors a, and b

The accumulation of VFAs was considerably higher in the blood experiments compared to the co-digestion data. For example, the accumulation of propionic acid -whose accumulation is reported as sign of inhibition in the literature (Wang *et al.*, 2009)-in the co-digestion experiments, was minimal compared to the AD of blood waste. Propionic acid did not become predominant throughout the digestion period with the co-digestion assays and its concentration remained low in the co-digestion digesters (i.e. ~2 g/L compared to 100 g/L on average for the blood reactors).

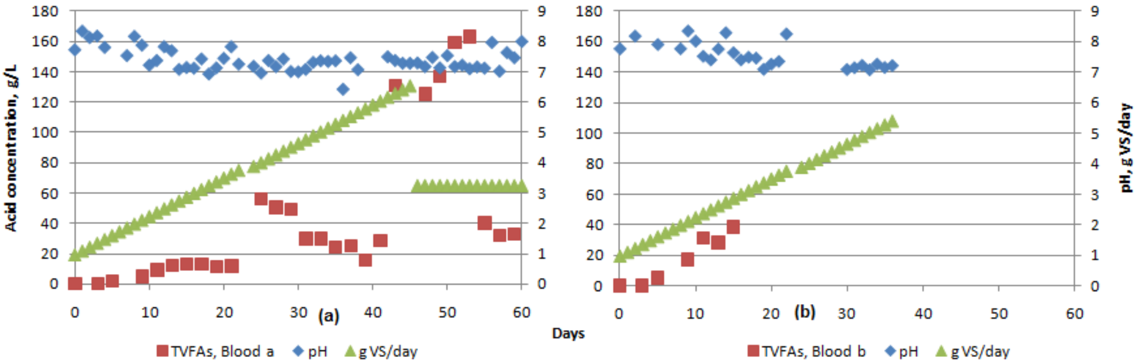
The lower concentration of NH_3 and VFAs, coupled with higher biogas yields achieved with the co-digestion experiments indicate improved performance from mixing complementary waste streams.This was confirmed by the elemental analysis carried out on the co-digested substrates which show that mixing these substrates, diluted the feed and increased the C:N to 12.6 (Table 50).

Table 50: presents the C:N ratio of the various substrates investigated

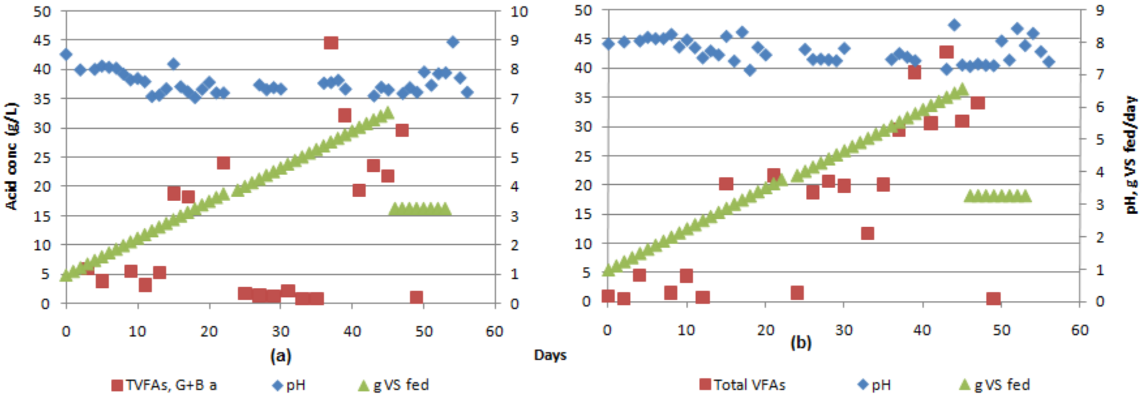
	C: N ratio
Food waste	26.8
Blood waste	3.0
Garden wastes	40.5
Garden+blood	12.6

6.2.4 Relationship between pH,TVFAs, daily feed

Figure 76 illustrates the relationship between the pH, the TVFAs and g VS of substrate fed for AD of blood waste and its co-digestion with garden waste for the duplicate reactors a and b. There is a significant difference in magnitude for the accumulation of VFAs between AD of blood waste and its co-digestion. The highest concentration of TVFAs recorded in blood AD was160 g/L, while 45 g/L was the maximum concentration in the co-digestion experiments.



Blood



Co-digestion

Figure 76: Relationship between total VFAs, pH and amount fed on a daily basis for blood (top) and its co-digestion with garden waste (bottom).

Figure 77 correlates the TVFAs profiles, with biogas productivity for blood waste, and its co-digestion with garden waste. The lag phase observed with the co-digestion was longer compared to that of blood waste (~20 versus 10 days), which suggest that either AD microbes required a longer adaptation period, and/or that the activity of microbes was limited from the start. The limited activity could result from a combination of factors e.g. the fact that reactors were overloaded, as the biogas productivity increased when the feeding rate was reduced from 6.27 to 3.25 g/day, the use of a sub-optimal microbial consortia for the substrate.

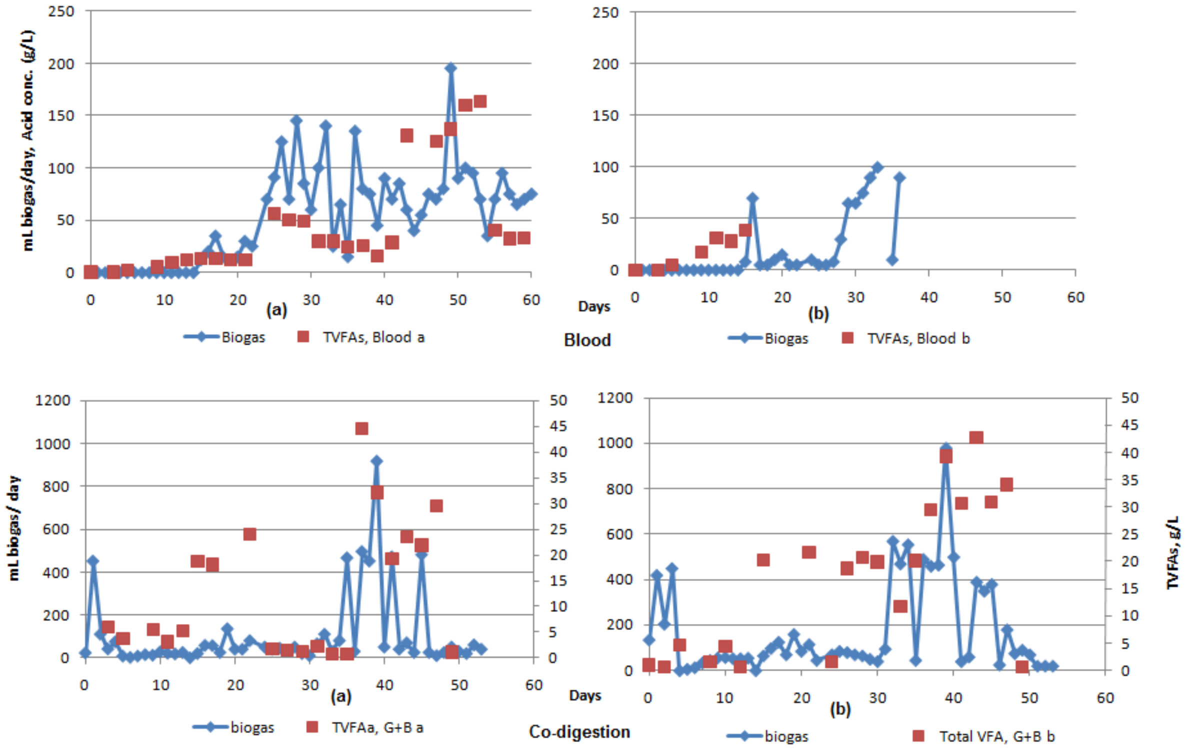


Figure 77: Relationship between biogas productivity, and total VFAs for blood (top) and its co-digestion with garden waste (bottom).

The main VFA accumulated in the blood was propionic acid, whose accumulation is reported in the literature reviewed as an indication of inhibitory mechanisms taking place in AD systems (Cuetos *et al.*, 2008). This trend was not evident in the blood waste reactors i.e. a drop in the daily biogas volumes from ~150 mL/day to ~100 mL/day is observed. However, the highest biogas peak is observed (~200 mL/day) on day ~51 corresponding to ~150 g/L of propionic acid. It can be argued that the biogas peak observed resulted from the chemical release of CO_2 from the buffer, since the pH drop observed during that period (from 7.5 to 7) would favour the speciation of HCO_3^- into CO_2 (g). The CH_4 composition profiles would have provided a robust mean of evaluating methanogenic activity; it is hard to draw any robust conclusions about the effect of propionic acid in the blood digestersfollowing from the above argument.

Co-digestion enhanced the biogas productivity, but had no effect on the lag phase of the digestion period. These results are contradictory to research carried by Buendia *et al.* (2009) on the co-digestion of meat industry wastes with waste sludge at 35°C, where the duration of lag phase generally decreased with co-digestion (i.e. a 1- 90% reduction of the lag phase was observed depending on the mixing ratio, and type of meat wastes used).

The low biogas volumes observed for blood throughout the digestion period, are cause of concern even if many authors report that most systems operate under suboptimal conditions as a result of the interaction between pH, VFAs and NH_3 (Angelidaki and Ahring, 1993; Angelidaki *et al.*, 1993).

6.2.5 Effect of co-digestion and performance of batch digesters relative to BMP tests

Figure 78 below presents the cumulative biogas volumes (mL) of garden waste (left) and its co-digestion with blood waste (right) versus the cumulative feed (g VS). The slopes of the curves represent the different specific biogas yields (mL/g VS) achieved throughout the duration of the digestion period. This exercise provides a benchmarck for comparison of the performance of AD of garden waste and its co-digestion with garden waste for the digestion period. The data from blood was omitted since blood assays had the poorest performance.

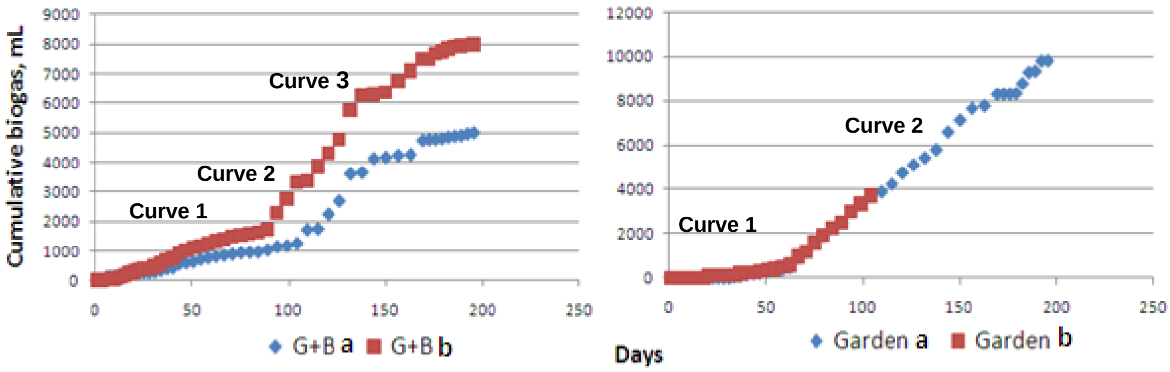


Figure 78: Cumulative biogas (mL) vs. cumulative amount fed (g VS) for co-digested mixture (left) and garden waste (right)

The values of the slopes of the different curves are presented in Table 51. The specific biogas yield increases, and the biogas productivity remains relatively constant for garden waste (~66.2 mL/g VS) throughout the duration of the digestion period.Except when the digesters were operated at room temperature, while there appears three distincts biogas yields observed for the co-digestion assays for the digestion period. The specific biogas under psychrophilic conditions (~17°C) was considerably lower with ~5.5 and ~15 mL/gVS on average for both garden waste and its co-digestion with blood respectively.

Table 51: Slopes of the various biogas curves of the cumulative biogas yield versus cumulative feed

	G+B 1	G+B 2	Garden 1/2
	mL biogas/ g VS		
Curve 1	12.2	19.4	5.5
Curve 2	63.8	93.2	66.2
Curve 3	18.4	31.5	

The gas yield of the co-digestion experiments increased to 63.8 and 93.2 mL/g VS for reactor a and b respectively, when the temperature was raised to 35°C, with a corresponding cumulative feeding was ~100-150 g VS. The productivity declined thereafter to 18.4 and 31.5 mL/ g VS for reactors a and b respectively, when the the cumulative feed was greater than ~150 g VS/day (day ~40). This period also correspond to an increase in FSA concentration from ~150 to ~350 mg/L between days~20 and 40 (Figure 50, Section 6.2.2).One could argue among other factors that, organic overload as well as an increase in FSA concentration at pH~7-7.5, contributed to the drop in biogas productivity.Supporting this research by Hansen *et al.* (1998) report values between 100 and 150 mg-N/L as NH_3 inhibition threshold for non-adapted cultures.

It is noteworthy that during the period where the cumulative feed was between ~100 and 150, the biogas productivity of the co-digestion experiments(~78 mL/g VS) was higher on average than that of garden waste (~66 mL/g VS). This shows that the biogas yield of the co-digested mixture was higher than that of the carbon source at some point during the digestion period, suggesting the performance of co-digestion systems can be improved by looking at optimising different experimental variables (e.g. the mixing ratio).

Table 52 presents the comparison between the expected (theoretical value and experimental results). Experimental results show a better correlation to the expected values compared to values from the BMP tests of co-digestion of blood with garden waste (i.e. ~70% versus 40% of the expected value was achieved).

Table 52: Comparison between expected and experimental results of the co-digestion of blood with garden waste under fed-batch conditions

	Mixing ratio	Expected results, mL/g VS	Experimental results, mL/g VS
Blood+Garden	1:3	49	33

Table 53 below presents the comparison between the specific biogas yields (mL/g VS) of BMP and fed-batch results.

Table 53: Comparison between results of BMP tests and fed-batch experiments for the co-digestion of blood with garden waste

Substrate	BMP batch tests		Fed Batch reactors, mL/g VS
	Biogas, mL/g VS	mL CH_4/g VS	
Garden waste	363	23.7	60
Blood waste	79	4.5	14
Co-digestion	–	–	33

It is noteworthy that data for the co-digestion are not presented as garden waste from Bellville Compost, and not Wynberg Drop-off was used in the BMP tests. The biogas yields from the fed batch reactors were significantly lower than results from the batch systems (Table 53), similarly to what was also observed with the other substrates (garden and kitchen). However, the performance of the BMP tests remained poor based on the CH_4 yield. The data generated showed that a number of factors were at play in the fed batch experiments. For example, the NH_3 profiles of blood waste provide mild evidence that methanogenic activity could have been limited by the high concentrations of NH_3, however this is not the case for its co-digestion with garden waste.

Results of the simulation of the F:I ratio in the digesters (Sections5.4.3 and 9.2) showed that the digesters, particularly under the progressive feed, were overloaded. Moreover, while the pH control was better compared to BMP tests, the concentration of Na^+ ion was high and close to values reported in literature between moderately-strongly inhibitory. The estimation of H_2S in the BMP tests showed that the amount generated was close to values reported as inhibitory in the literature, however H_2S concentration were not measured in the fed-batch experiments.

The starting temperature for the first two weeks proved inadequate for microbial activity for all the substrates examined. Although the speciation of HCO_3- into CO_2 gas would be limited in these tests as the pH remained alkaline (~7-8) for the digestion period, the biogas yields-on which the comparison of performance of substrates is based-as presented in this study would be an overestimation.

Moreover, research by *Calli et al.* (2005) showed that sludge from swine manure was a better inoculum than conventional AD sludge as it acclimatised to higher NH_3 concentration (~2.4 g-N/L) and remained active without any signs of inhibition compared to normal AD (~1 g-N/L) often used in the research published on AD of slaughterhouse wastes.

The lower biogas yield of nitrogen-rich substrates could also have resulted from unsuitable sludge since the same inoculum was used for all substrates. Hence, it expected that the combination of the above factors in the fed-batch experiments probably led to the poor biogas yields observed compared to the BMP tests.

6.3 Findings and future work

The effect of co-digestion of high protein wastes with substrates rich in carbon was investigated, based on the analysed C:N ratio. To a limited extent, the co-digestion experiments showed improved performance in both the BMP tests and the fed-batch experiments i.e. higher biogas yields, lower VFAs and NH_3 concentrations relative to that of the nitrogen-rich substrate used (viz. abattoir and blood waste). Comparison of the experimental and expected results proved to be a simple and effective way to evaluate the impact of co-digestion. Generally, the experimental results were considerably lower than the expected values, with the exception of co-digestion with paper sludge where all experiments failed.

It would be useful to repeat these experiments while addressing the shortcoming identified from the data generated. For example, it would be valuable to repeat the fed-batch experiments under mesophilic conditions from start-up, and maintain the progressive feed within values reported as suitable in literature for meat-industry by-products i.e. 1-2.9 g VS/L/day (Luste, 2011). Little can be inferred about the CH_4 content of these tests as data generated for BMPs showed that CH_4 composition was highly variable between weeks, hence the CH_4 composition should be measured daily if this work is repeated. A detailed characterisation of each substrates (protein, fats, etc.), and regular sampling (every second day) of the reactor media and effluent with relevant test (TS, VS and COD, NH_3, H_2S) would help quantify the efficiency of the system based of organic content destruction, and by a material balance around each digester.

7 CONCLUSION AND RECOMMENDATION(S)

7.1 Achievement of objectives

This dissertation was developed with three main objectives, stated in the introduction (Section 1.3). The laboratory work conducted was developed from four formulated key questions (Section 3.1) which formed the basis of the approach taken to meet the cited objectives. This section discusses results obtained to meet these objectives.

The **first objective**, to study the suitability of AD for treating a range of typical municipal organic wastes, was investigated through sampling of different pure commercial organic, and mixed waste streams. Sixteen different waste samples were retained for BMP analysis in 100 mL bioreactors in the first phase of experiments, and fed-batch experiments were carried out in 2L reactors for the second phase of the laboratory work. The substrates used in the second phase, were similar to those used for the BMP analyses; these were kitchen waste, garden waste and blood waste. Results of the first and second phase are presented in Chapters 4 and 5 respectively. The effect of co-digestion of slaughterhouse wastes with carbon-rich substrates (e.g. garden waste, paper sludge and fines), as a means to adjust the C:N ratio, and overcome the lower biogas yields generally experienced with such wastes, was also investigated both for BMP tests, and fed-batch experiments; results are presented in Chapter 6.

The **second objective**, to observe and analyse the temporal evolution of key process parameters, was achieved through analysing and monitoring different experimental variables throughout the digestion period at both 100 mL and at 2 L scale. Moreover, two different feeding regimes were used with kitchen waste to investigate the effect of the feeding regime on the performance of AD systems, informed by research conducted by Angelidaki *et al.* (2006).Results of the different analyses carried out, are spread over chapters 4, 5 and 6. The second phase of experiments allowed more analyses to be carried out, as samples could be extracted from the bioreactors for analysis, since experiments were conducted at a larger scale and under fed-batch conditions. The data generated enabled identification of key process parameters, as well as gaining a better understanding through careful analysis, with reference to the relevant literature of their interrelation and impacts. A discussion of the relevant parameters identified, is presented at the end of Chapters 4-6 (Sections 4.15, 5.4 and 6.3), and concluding remarks on the key parameters identified are presented later in Section 7.2.4

The **third objective**, to draw sound conclusions and build a knowledge base for practitioners, has been met through the first and second objective, and careful consideration of the gaps and shortcomings identified from the trends observed in the data generated, and the laboratory work conducted. The recommendations that emanated, for both further research and to waste management officials, are presented later in Section 7.4.

7.2 Answers to research questions

Four key questions were formulated in Section 3.2 to meet the objectives of this dissertation, after presenting a review of relevant literature. Based on the results obtained and the interpretive discussions, these questions can now be answered.

7.2.1 Key question 1

What is the bio-methane potential of different organic wastes streams typically found in an urban setting in developing economies?

Results are presented from Sections 4.1.1-4.1.4. The BMP data is organised in four main categories i.e. (i) garden waste, (ii) fines and paper sludge, (iii) kitchen wastes and (iv) abattoir and fisheries wastes. High protein wastes (abattoir and fisheries wastes) had the lowest biogas and CH_4yields, and garden refuse had the highest ones compared to other categories. An analysis of variance (ANOVA) was performed to investigate the statistical significance of the variability for the different waste samples, within each category.

The highest biogas and CH_4yields, for each category were as follows from the: Cape Town Market's biomass (EW) with 325 mL/g VS (119 mL CH_4/g VS), garden refuse from Bellville Compost (BC GW) with 546 mL/g VS (223 mL CH_4/g VS), fines from BC (BC Fi) with 493 mL/g VS (240 mL CH_4/g VS) and fisheries waste (FW) with 130 mL/g VS (104 mL CH_4/g VS).

The biogas yields reported are an overestimation since CO_2 was released chemically from the speciation of bicarbonate ions (HCO_3^-) occurredfrom the buffer. The amount of CO_2generated, would depend on the amount of buffer added (g), and the pH of the system. Hence, the CH_4 composition proved to be an efficient indicator to evaluate the methanogenic acitivity. Acidic conditions prevailed with the BMPs of kitchen waste, and the amount of CO_2 released chemically varied between 30-70%. The above arguments, combined with very low CH_4profiles for kitchen waste indicate that most experiments in that category had to be regarded as failed, with the exception of EW and CW which exhibit conventional AD behaviour based on experimental variables.

Additional experiments were carried out with kitchen, garden and blood wastes at 2L and under fed-batch conditions building from BMP results. These results are presented in Chapter 5. The biogas yields achieved were significantly lower than those of the BMP tests (only ~15-30% of the BMP values on average).

The poor performance of the fed-batch experiments compared to the batch tests is the result of a combination of factors that impacted the AD process (some of which were intentionally designed into the experiments to mimic real rather than ideal conditions), such as overloading of reactors (from the high F:I ratios estimated), limited agitation, psychrophilic conditions at start-up, and limited microbial activity observed particularly with blood and co-digestion experiments.

However the performance of these BMP tests was relatively poor based on their CH_4 yield indicating that biogas volumes per g VS loaded was not necessarily the best parameter to compare both systems. Quantification of the organic load destruction in the fed-batch systems, would have allowed more accurate comparison of the system's performance and efficiency.

Furthermore, to what extent does the nature of the substrate influence the digestion process and biogas yield?

The nature of a substrate plays a pivotal role in the performance of AD systems. This has been particularly evident with the effect of pH in AD of kitchen waste. This was due to the accumulation of VFAs resulting from these wastes' composition of easily fermentable materials, and faster early phases of AD relative to methanogenesis thereof It was found that pH control using a chemical was necessary to maintain stable and neutral pH, with kitchen waste under batch for the fed-batch reactors.

In general, organic wastes from mixed samples produced better results than those taken directly from industrial or commercial waste generation points. For example, the highest biogas and CH_4 yield achieved for BMP test were garden waste and fines from the Bellville composting plant with 546 mL/g VS (223 mL CH_4/g VS) and, 493 mL/g VS (240 mL CH_4/g VS) respectively. AD of cellulosic materials also exhibited a more stable behaviour relative to the easily biodegradable such as kitchen waste. This could be attributed to the slower hydrolysis rates, which limited the amount of intermediate products (e.g. VFAs) available for the methanogenic archaea, allowing a balance between the activities of the different microbial groups to avoid VFAs accumulation and potential inhibition.

The chemical composition, i.e. the C:N ratio, also plays an important role in anaerobic digestion. This is particularly applicable to nitrogen-rich (i.e. abattoir and blood wastes) rather than carbon-rich substrates where lower biogas and CH_4 yields were observed despite similar potential with kitchen waste for example i.e. blood waste had similar starting COD values for BMP tests, and VFAs concentration for fed-batch experiments yet much lower biogas and CH_4profiles.

The literature reviewed associated the poor performance of AD of slaughterhouse wastes to the accumulation of pollutants (e.g. NH_3, H_2S, LCFAs) generated through the degradation of such wastes (Kroeker *et al.*, 1979; de Baere *et al.*, 1984;Hansen *et al.*,1998; Kayhanian,1994;Salminen and Rintala, 2002; Chen *et al.*, 2008).

7.2.2 Key question 2

How does AD of garden waste compare to that of non-cellulosic wastes based on the main experimental variables investigated (viz biogas and CH_4 yields per g VS, stability of the digestion based on pH and concentration of VFAs)?

Anaerobic digesters with garden wastes generally performed better compared to other substrates investigated in this study both for BMP tests, and under fed-batch conditions. The biogas and calculated CH_4yields of garden waste samples were the highest under batch conditions (546 mL/gVS, and ~223 mL CH_4/g VS for a garden waste sample from a mixed source, although the BMP experiments for one of the pure sources, WDO, failed). BMP results also showed that AD of garden waste was more stable based on the pH profiles, which remained within the neutral range for most of the digestion compared to kitchen waste for example.

In the fed-batch experiments, the day to day variability of biogas yield was much lower for garden waste compared to kitchen wasteand less pH control was required. The biogas yield of garden waste was similar to kitchen waste under the same feeding regime (60 mL/g VS versus 56 mL/g VS for kitchen waste) despite lower concentrations of VFAs, indicating better performance and efficiency. For example, the concentration of VFAs in fed-batch experiments was the lowest with garden waste (~25 g/L) compared to kitchen waste (~40-100 g/L) and blood waste (~160 g/L), Sections5.2.1-5.2.3.The lower VFAs accumulation can be attributed to the slower degradation experienced due to the cellulosic nature of the waste (Sanders,2001, and Kadam *et al.,* 2004) as discussed in the previous section.

7.2.3 Key question 3

To what extend can co-digestion with a rich carbon source improve the chemical composition (i.e. C:N ratio), and overcome the lower biogas yields generally experienced with slaughterhouse wastes for improved performance based on experimental variables?

Results of co-digestion experiments are presented in Sections 6.1 and 6.2. The biogas and CH_4yields of the co-digested mixture were generally higher than that of the nitrogen-rich substrate for BMP results, but lower than the carbon-rich source. Similarly, the biogas yields of co-digestion were higher for fed-batch reactors, with improved performance i.e. co-digestion experiments exhibited lower VFA accumulation and NH_3 concentration. For example, the highest concentration of TVFAs in the blood reactors was ~160 g/L compared to ~45 g/L in the co-digestion reactor.

The concentration of free and saline ammonia (FSA) was lower in the co-digestion reactors (~0.45 g/L) compared to blood waste (~2.9 g/L). Moreover propionic acid, identified in the literature as an inhibitor, did not become prevalent in the co-digestion reactors throughout the digestion period compared to the blood experiments.
The expected biogas yield from the co-digestion experiments was determined as the weighted average of the individual biogas yields of both substrates; and in most experiments, co-digestion yielded significantly lower biogas amounts than expected results (i.e. 37-67% of the expected value). The lower experimental values despite improved performance remain a cause of concern.

Many researchers have linked the lower biogas yields achieved with slaughterhouse wastes to its composition, and inferred accumulation of NH_3 which inhibits the methanogenic archaea (Kroeker *et al.*,1979; de Baere *et al.*,1984; Hansen *et al.*,1998; Kayhanian,1994;Salminen and Rintala, 2002).However, the case for NH_3 inhibition was not evident in this study based on the data generated since the NH_3 values measured were generally lower than the inhibitory thresholds identified in the literature; except for blood waste experiments. Limited microbial activity appeared to be the dominant causefor poor performance, which is an indication other factors not investigated in this study, were at play and influenced the digestion process e.g. loading rate, other pollutants (e.g. H_2S and LCFAs), suitability of the microbial consortia, etc.

7.2.4 Key question 4

Besides the biogas yield and methane yields, does the measurement and monitoring of other parameters indeed allow for more advanced insights and effective control of the AD process, as advocated in literature?

It was evident from results that the biogas yield is insufficient for process control.This is because it does not indicate an intermediate state or a process imbalance in bioreactors and only decreases once the process is already inhibited (Moletta *et al.,* 1994). Moreover, the biogas values reported in this study are generally an overestimation as some volumes of CO_2 were released from the speciation of HCO_3^- ions from the buffer used for pH control in this study. The CH_4 composition proved to be a more accurate measure of methanogenic activity in the BMP tests. The high variability observed between sampling periods, and between different substrates for CH_4 composition, suggests regular daily sampling for analyses would have been more adequate.

The influence of pH in the BMP testswas more evident with waste from the biomass from Cape Town's Market (EW), where both the biogas yields and CH_4 composition increased with pH once neutral conditions were restored. This trend agrees with research carried out by Ward *et al.* (2008) where the methane yield was increased by 35% with a pH between 6.5 and 7.5.

The pH is a valuable indicator but is not always sufficient, particularly with systems with a high buffering capacity, such as those fed with high protein wastes and kitchen waste in the fed-batch experiments. Moreover, results show that pH and biogas alone are insufficient as process indicators since lower biogas yields prevailed in fed-batch reactors with kitchen waste, despite the better pH control, compared to BMP tests.

Although propionic acid is reported as the main sign of inhibition in literature (Wang *et al.*, 2009, Ren *et al.*1994;Barredo and Evison, 1991), the inhibitory effect/threshold of propionic acid was not evident in this study. The concentration of butyric/iso-butyric acid correlated with improved bioreactor performance in the fed-batch experiments with kitchen waste on a flat feeding regime, and co-digestion of garden and blood waste.

Therefore, the other parameters considered for process control purposes were: the chemical composition (C:N ratio), the concentration of VFAs, concentration of FSA for nitrogen-rich substrates, the pH if interpreted relative to the feed regime and accumulation of other pollutants (LCFAs, H_2S), and the OLR relative to the food-to-inoculum (F:I) ratio. This latter was found to be a very important predictor of sub-optimal performance

7.3 Conclusions

The following conclusions can be drawn from this current research:

- BMP tests performed at the 100 mL scale resulted in mixed organic waste streams producing more biogas and more biomethane compared to pure commercial waste streams. The lower biogas yields for pure streams are, however, not necessarily an indication of a lower biomethane potential; rather they may well have resulted from the difficulty of achieving optimum conditions in the experimental set-up used in this study, specifically the use of a high substrate : inoculum ratio. This was exemplified by the poor performance of kitchen waste, where acidic conditions prevailed and the methanogenic activity was limited.
  The biogas volumes reported in this study are an overestimation of bio-methane potential, due to the pH-dependent speciation of HCO_3^- into CO_2 from the buffer used.Supporting this, CH_4 composition proved to be critical for evaluating methanogenic activity.

- Cellulosic materials such as garden refuse appear to besuitable substrates for AD. Higher biogas and CH_4 yields, than for fast hydrolysing wastes, were achieved at the 100 mL batch scale, and better efficiency based on VFAs profiles was observed for the fed-batch experiments. At both scales, stable values for key process parameters viz. pH, VFA concentration and accumulation of VS in digesters were lower compared to kitchen waste, and to blood waste.

- The organic loading rate (OLR) is an important design and control parameter. It dictates the food-to-inoculum (F:I) ratio and considerably influences the performance of AD systems. This was evident from the fed-batch results at 2L, using the model developed to estimate the F:I ratio under the two feeding regime investigated. While reactors under both regimes were overloaded, the model shows that the F:I ratio under the flat feeding rate decreases more rapidly from 5 to ~1.5-2, closer to the range of values reported as suitable (Liu *et al.*, 2009, Chynoweth *et al.*, 1993). The better performance of the reactors of a flat feeding regime (K. Flat) can be attributed to more suitable ratios between the biomass to be degraded, and the microbial sludge.

- Co-digestion of nitrogen-rich substrates with a carbon-rich source is frequently advocated in the literature as a viable technique to improve the performance of digesters treating high protein wastes such as slaughterhouse wastes.Results obtained showed better performance under batch and fed-batch experiments than for pure protein-rich wastes (i.e. higher biogas yield, lower FSA and VFA concentrations). Comparison of the experimental and expected biogas yields, proved to be a simple and effective method to evaluate performance of co-digestion systems.

- The experimental values were, however, significantly lower than theoretically expected values, except for the co-digestion with paper sludge, for which all the BMP experiments failed. This leaves significant room for improving performance of co-digestion systems, e.g. by reconsidering the inoculum sludge used, mixing ratios, and monitoring of additional pollutants (Na^+, H_2S, etc.).

- The pH is a valuable indicator of process performance, but is not always relevant with highly buffered systems such as digesters receiving protein wastes, or with added alkalinity from buffer for pH control as was the case with kitchen waste. For example, the pH profiles of K. Prog remained within the neutral range (~6.5-7.5), while the concentration of VFAs was relatively high. The absence of VFA data would not have allowed to detect the imbalance taking place between acidogens and the methanogenic archaea, manifested by the high VFAs concentrations. The parameters that proved to be more effective are those indicating intermediate states in digesters such as the NH_3 (FSA) concentration for high protein wastes, and more importantly the VFA concentration. It is important to note, however, that the high VFA concentrations (particularly propionic acid) reported in this research with kitchen and blood wastes are questionable.

  Consequently, it can be confirmed that the key parameters for monitoring the performance of AD systems as identified previously in the literature, and based on the laboratory work carried out, are mainly: (i) the biogas yield multiplied by the CH_4 composition, (ii) the pH, (iii) concentration of C1-C6 VFAs, (iv) the feed-to-inoculum ratio, and (v) the concentration of pollutants generated during the degradation of proteins e.g. in slaughterhouse wastes, such as NH_3 , H_2S, and LCFAs.

7.4 Recommendations

7.4.1 Recommendations for further work

The work done in this dissertation provides a preliminary overview of harnessing the biogas potential of organic wastes in developing cities. The laboratory work provided some understanding of the potential challenges that would be experienced in an AD treatment facility relative to the different waste fractions.This dissertation has presented the data generated with special mention to the shortcomings and challenges identified, and reported in the body of this dissertation.

The following recommendations can be made, based on the work completed:

- Garden waste showed better performance compared to other substrates (higher biogas yield and methane composition, stable pH and lower accumulation of VFA). It raises interesting research questions and it is recommended this topic be explored for further research tounderstand the biological and bio-chemical reasons behind its good performance in terms of reaction kinetics, types of microbes and their adaptation.

- It is noteworthy that for the fed-batch co-digestion experiments with blood-garden waste at 2L scale, between days 33 and 42, the cumulative biogas production in mL/g VS were higher than that of garden waste and of the expected value determined (~78 versus 66 mL/g VS), Section 6.2.5. This is an indication that the biogas yields of co-digested systems can be improved. Further research is recommended to optimise the performance of co-digestion systems by looking individually at the different operating parameters such as the mixing ratio, the adequate feeding regime, suitability of the sludge used, etc.

- It is evident from the data generated that (i) the organic load was inadequate the BMP experiments with most kitchen wastes, and later in the fed-batch reactors; (ii) certain experimental variables that were not analysed, could have provided better understanding of microbial activity such as daily analysis of the CH_4 composition, measuring additional pollutants (LCFAs, H_2S,etc), quantification of organic load destruction for fed-batch reactor . Finally, AD under psychrophilic conditions failed, and might have prejudiced the performance of and quality of data collected from fed-batch reactors.
  It is therefore recommended in future undertaking of this research that these tests be repeated while addressing the main shortcomings identified above. For example, it would be valuable to repeat the BMP tests with kitchen waste with a lower organic load ensuring the F:I ratio remains within the desired range (0.5-1, Chynoweth *et al.*,1993; Raposo *et al.,* 2006) to avoid VFAs accumulation of VFAs, from the fast hydrolysis generally reported with such wastes.

Similarly, Angelidaki*et al.* (2006) recommends a 70% (v/v) inoculation to determine the BMP of easily fermentable organic substrates, to ensure the amount of substrate and inoculum is balanced and limit the risks of inhibition from accumulation of intermediate VFAs.

- The AD of slaughterhouse wastes and their co-digestion showed that factors other than NH_3 inhibition were at play, contrary to what is commonly reported in the literature.

  Should this study be repeated, it is recommended that the concentration of LCFAs and H_2S be monitored regularly (every second day) in addition to the CH_4 composition. It would be valuable to investigate AD of these nitrogen-rich wastes with a different inoculum. For example, *Calli et al.*, (2005) reported that sludge from manure performed better with nitrogen-rich substrates such as slaughterhouse wastes and sewage sludge as it is already acclimatised to high NH_3 concentration.

- It is important to understand the need to develop technical solutions that optimise biogas yield for the specific mix of substrates occurring locally. Results from this dissertation can be used as a benchmark for comparison, particularly for garden wastes and mixed waste streams such as fines, for anyone with the intention of developing this research further. Values generated can then be used later to develop a techno-economical model that would link viable digester technologies and biogas yield. This would be valuable to determine the technical and economical feasibility of integrating AD technology as a waste management and energy recovery strategy for developing cities. One such model is the feasibility model in the form of a spreadsheet developed for the SA Cities Network by AGAMA Pty (Ltd), (2009).

7.4.2 Recommendations for industry practitioners and solid waste management officials

The third objective of this dissertation was to generate a set of data to inform decision making by different stakeholders, particularly by officials in waste management. Integrating bio-based technology into solid waste management would be beneficial not only to stabilise the waste, but also to generate a renewable source of energy in the form of biogas. Results indicated that a vast range of waste streams (kitchen waste, garden waste, fines from a transfer station and high protein wastes) currently being disposed off in landfills, could possibly be treated via AD.

Results also showed that the co-digestion of abattoir wastes with a rich carbon source such as garden wastes is feasible, but not simple and will require specialist expertise. AD of high protein waste either at a municipal level, or individually by farmers or abattoirs should be encouraged by officials. This could be particularly valuable since, such wastes fall under the hazardous category and their disposal is costly to both the municipality and the business owner.

It is evident from the results and shortcomings experienced in this study, that careful planning, design, and the inclusion of process control are required to ensure the AD process is successful. The factors that emerged as crucial for the planning phase were: the type of substrate and chemical composition, the temperature at which the digester is to operate (psychrophilic, mesophilic or thermophilic), and the feeding regime for continuous systems especially with a view to avoid overfeeding.
A combination of the following factors is recommended for an effective monitoring and control system: the biogas yield, CH_4 content, pH, the feeding regime, concentration of VFAs, and of other pollutants for protein-rich wastes (FSA, H_2S, LCFAs, etc.).

Overall, it is recommended that waste management officials in developing cities start paying more attention to the value contained in organic wastes, and equip themselves with knowledge required to valorise such wastes through a combination of anaerobic digestion for energy recovery and composting for nutrient recycling.

8 REFERENCES

Abdullahi, Y. A., Akunna, J. C., White, N. A., Hallett, P. D., and Wheatley, R. (2008).Investigating the effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of municipal solid waste as soil amendment.*Bioresource Technology*, 99(18), 8631-8636

AGAMA Biogas (Pty) Ltd. (2009). Sustainable Cities: Biogas. *A feassibilitystudy and guidelines for implementing/technical report*, 27-30.

Agarwal, A., Singhmar, A., Kulshrestha, M., Mittal, A.K., 2004. Municipal solid waste recycling and associated markets in Delhi, India. *Resources Conservation and Recycling* 44, 73–90.

Agunwamba, JC (2001). Waste: Engineering and Management Tools. Enugu, Nigeria: *Immaculate Publications Ltd*., p. 572.

Ahring, B.K., (2003). Biomethanation II. In: Scheper, T. (Ed.), *Advances in Biochemical Engineering/Biotechnology*, vol. 82. Springer, Berlin, p. 220.

Ahring, B.K., Sandberg, M.and Angelidaki, I., (1995).Volatile fatty acids as indicators of process imbalance in anaerobic digestors.*Applied Microbiology and Biotechnology*.43, 559-565.

Ali, M., and Sreekrishnan, T.R.,(2001). Aquatic toxicity from pulp and paper mill effluents: a review. *Advances in Environmental Research*. 5, 175–196.

Al-Khatib, I. A., Arafat, H. A., Basheer, T., Shawahneh, H., Salahat, A., and Eid, J., (2007). Trends and problems of solid waste management in developing countries: A case study in seven palestinian districts. *Waste Management*, 27(12), 1910-1919

American Chemical Society (1979).*Chemical Modeling in Aqueous Systems*. Washington D.C: American Chemical Society. 771-775.

Amigun,B. and von Blottnitz,H. (2010). Capacity-cost and location-cost analyses for biogas plants in Africa.*Resources, Conservation and Recycling*.55 , 63-73.

Anderson GK, Donnelly T, Mckeown KJ (1982).Identification and control of inhibition in the anaerobic treatment of industrial wastewater.*Process Biochemistry*.17,(4):28–32.

Andrews University. (2011). *General Statistics: Analysis of Variance - ANOVA, Comparing more than 2 population means .* Available: http://www.pindling.org/Math/Statistics/Textbook/Chapter10_ANOVA/ANOVA.htm. Last accessed 19 December 2011.

Angelidaki,I;Alves, M; Bolzonella, D.; Borzacconi,L; Campos,J.L;Guwy,A.J; Kalyuzhnyi,S.; Jenicek, P. and van Lier, J.B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology*. 59 (5), 927-938.

Angelidaki, I.; Chen, X.; Cui, J.; Kaparaju,P.; and Ellegaard, L. (2006). Thermophilic anaerobic digestion of source-sorted organic fraction of household municipal solid waste: Start-up procedure for continuously stirred tank reactor. *Water Research*. 40 (1), 2621 – 2628.

Angelidaki, I. and Sanders, W. (2004).Assessment of the anaerobic biodegradability of macropullutants.*Reviews in Environmental Science and BioTechnology*. 3, 117-129.

Angelidaki, I., Ellegaard, L. and Ahring, B.K. (1999). A comprehensive model of anaerobic bioconversion of complex substrates to biogas. *Biotechnology and Bioengineering*. 63, 363-372

Angelidaki I. and Ahring B. K. (1993). Thermophilic anaerobic digestion of livestock waste: effect of ammonia.*Applied Microbiologyand Biotechnology*. 38, 560-564.

Angelidaki, I., Ellegaard, L., Ahring, B.K., (1993). A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. *Biotechnology and Bio-engineering*. 42, 159–166.

Angenent, L. T., Sung, S., and Raskin, L. (2002). Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Water Research*, 36(18), 4648-4654.

Appels, L., Baeyens, J., Degrève, J., and Dewil, R. (2008).Principles and potential of the anaerobic digestion of waste-activated sludge.*Progress in Energy and Combustion Science*, 34(6), 755-781.

Asomani-Boateng, R., and Haight, M. (1999).Reusing Organic Solid Waste in Urban Famring in African Cities: A Challenge for Urban Planners.

Azan, T.H.; Faridah, A.H.A and Hasnain, M.A..(2005). Relationship between Volatile Fatty Acid and Alkalinity in Anaerobic Digestion of Food Waste.School of Civil Engineering.Universiti Sains Malaysia (1), 1.

Babel, S., Fukushi, K., and Sitanrassamee, B. (2004).Effect of acid speciation on solid waste liquefaction in an anaerobic acid digester.*Water Research*, 38(9), 2417-2423.

de Baere, L. (2000). Anaerobic digestion of solid waste: state-of-the-art. *Water science and technology*. 41 (3), 283-290.

de Baere, L.A., Devocht, M., van Assche, P. and Verstraete, W., (1984). Influence of high NaCl and NH_4Cl salt levels on methanogenic associations. *Water Research*. 18, 543–548.

Banks, C.J.; Chesshire,M.; Heaven, S.; Arnold, R. (2011). Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. *Bioresource Technology*. 102, 612-620.

Barton, J. R., Issaias, I. and Stentiford, E. I. (2008). Carbon – making the right choice for waste management in developing countries. *Waste Management*, 28(4), 690-698.

Barredo MS, Evison LM (1991).Effect of propionate toxicity on methanogen-enriched sludge, methanobrevibactersmithii, and methanospirillum-hungatii at different pH values.*Applied Environmental Microbioly*, 57,1764–1779.

Barthakur,A., Bora,M. and Devendra, H. Singh. (1991). Kinetic Model for Substrate Utilization and Methane Production in the Anaerobic Digestion of Organic Feed. *Biotechnology Progress*.7 , 369-37.

Benton Franklin Health District . (2002). *Several forms of nitrogen occur in wastewater.* Available: http://www.bfhd.wa.gov/info/tkn.php . Last accessed April, 2011.

Bernal, M.P., Alburquerque, J.A. and Moral, R.(2008). Composting of animal manures and chemical criteria for compost maturity assessment.A review.*Bioresource technology*.100,5444-5453.

Bhatia, R. (1990). Diffusion of renewable energy technologies in developing countries: A case study of biogas engines in india. *World Development*, 18(4), 575-590.

Bhattacharya, S.K., and Parkin, G.F., (1989). The effect of ammonia on methane fermentation process.*Water Pollution Control Federation*. 61, 55–59.

Blischke, J., (2004). Combining anaerobic digestion with enclosed tunnel composting.*BioCycle*, 45 (4), 49.

Bo, Z., Wei-min, C., and Pin-jing, H. (2007). Influence of lactic acid on the two-phase anaerobic digestion of kitchen wastes. *Journal of Environmental Sciences*, 19(2), 244-249.

Boardman, G.D., and McVeigh, P.J.(1997). Use of UASB technology to treat crab processing wastewaters.*Environmental Engineering*. 123, 776–785.

Boe,K.; Batstone,D. J.; Steyer,J.P. and Angelidaki,I. (2010). State indicators for monitoring the anaerobic digestion process.*Water Research*. 44 (1), 5973-5980.

Borja, R., Sanchez, E., Duran, M.M., (1996). Effect of the clay mineral zeolite on ammonia inhibition of anaerobic thermophilic reactors treating cattle manure.*Environmental Science and Health*, part A. 31 (2), 479–500.

Bouallagui, H., Lahdheb, H., Ben Romdan, E., Rachdi, B., & Hamdi, M. (2009).Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition.*Journal of Environmental Management*, 90(5), 1844-1849.

Bouallagui, H., Torrijos, M., Godon, J. J., Moletta, R., Ben Cheikh, R., Touhami, Y., et al. (2004).Two-phases anaerobic digestion of fruit and vegetable wastes: Bioreactors performance. *Biochemical Engineering Journal*, 21(2), 193-197.

Braber, K. (1995). Anaerobic digestion of municipal solid waste: A modern waste disposal option on the verge of breakthrough. *Biomass and Bioenergy*, 9(1-5), 365-376.

Braun, B., Huber, P., and Meyrath, J.(1981).Ammonia toxicity in liquid piggery manure digestion.*Biotechnology*.Letters. 3, 159–164.

Brummeler, E. T., and Koster, I. W. (1989). The effect of several pH control chemicals on the dry batch digestion of the organic fraction of municipal solid waste. Resources, *Conservation and Recycling*, 3(1), 19-32.

Buendía,I.M; Fernández, F.J.; Villaseñor,J. and Rodríguez,L. (2009). Feasibility of anaerobic co-digestion as a treatment option of meat industry wastes. *Bioresource Technology*. 100 (1), 1903-1909.

Buekens, A., (2005). Energy Recovery from Residual Waste by means of Anaerobic Digestion Technologies.Conference ‘‘The Future of Residual Waste Management in Europe’’, 17–18 November, Luxemburg.

Calli, B., Mertoglu, B., Inanc, B., and Yenigun, O. (2005). Effects of high free ammonia concentrations on the performances of anaerobic bioreactors.*Process Biochemistry*, 40(3-4), 1285-1292.

Chakraborty, N., Sarkar, G.M., and Lahiri, S.C., (2002).Effect of pesticide (Tara-909) on biomethanation of sewage sludge and isolated methanogens.*Biomass Bioenergy*. 23, 75–80.

Chastain, J.R. (2008). Hydrogen sulphide in water systems: what's that smell? *Consultant’s Update: Chastain-Skillman publications*. 3, 1-3.

Chaudhary, B. K (2008).Dry continuous anaerrobic digestion of municipal solid waste in thermophilic conditions .*Master of Engineering in Environmental Engineering and Management*. Asian Institute of Technology; School of Environment, Resources and Development (Thailand)

Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, 99(10), 4044-4064 .

Chynoweth D.P; Isaacson R. (1987). Ch 1-6. In: Isaacson, H. R; Chynoweth, D. P; Hungate, R.E; Erickson, P. R; McCarty, P. L; Smith, P. H; Basilico, J.V Anaerobic Digestion of Biomass. London: Elsevier Applied Science. p1-107.

City of Cape Town. (2011). *Think Twice* . Available: http://www.capetown.gov.za/en/solidwaste/Pages/ThinkTwice.aspx . Last accessed 24th June 2011.

Cobb, S.A. and Hill, D.T., (1991).Volatile fatty acid relationships in attached growth anaerobic fermenters. Transactions of the ASAE 34 (6), 2564-2572.

Coelho, N. M. G., Droste, R. L., & Kennedy, K. J. (2011). Evaluation of continuous mesophilic, thermophilic and temperature phased anaerobic digestion of microwaved activated sludge. *Water Research*, 45(9), 2822-2834.

Cointreau-Levine, S. 1996.Sanitary landfill siting and criteria. Washington DC: World

Bank Infrastructure Notes, Urban No.UE-12.

Connaughton, S., Collins, G., & O'Flaherty, V. (2006). Psychrophilic and mesophilic anaerobic digestion of brewery effluent: A comparative study. *Water Research*, 40(13), 2503-2510.

Cuetos, M.J; Gómeza, X; Oterob, M and Morána, A. (2007). Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochemical Engineering Journal*. 40 (1), 99-106.

Couth,R. and Trois,C.(2010). Carbon emissions reduction strategies in Africa from improved waste management:a review. *Waste Management*. 30 (1), 2336–2346.

DEAT(DepartmentofEnvironmentalAffairsandTourism),2005.Globalclimate changeandozonelayerprotection—vulnerabilityandadaptation:thesituation in SouthAfrica;online:/http://www.deat.gov.za/ClimateChange2005/The_Situation_in_South_Africa.htmS (accessed05November2009).

Decision Maker's Guide. Washington, DC: The International Bank for Reconstruction and Development, World Bank.

Demirel B, Yenigun O. The effects of change in volatile fatty acid (VFA) composition on methanogenic upflow filter reactor (UFAF) performance (2002). *Environmental Technology*; 23:1179–1187.

DepartmentofMineralsandEnergy (DME, 2003). White paper on the renewable energy policy of the Republic of SouthAfrica;online: /http://unfccc.int/files/ meetings/seminar/application/pdf/sem_sup1_south_africa.pdf accessed 18 May 2009.

Domingo , J.L and Nadal,M. (2008). Domestic waste composting facilities: A review of human health risks. *Environment International*. 35 (1), 382-389.

Deublein, D. and Steinhauser, A. (2008).*Biogas from Waste and Renewable Resources*. Weinheim: Wiley-VCH. 49-85.

Department of Water Affairs and Forestry (DWAF,1998). *Minimum requirements for waste disposal by landfills* . 2nd ed. Republic of South Africa: CTP Book Printers, Cape.

Dugba, P. N., and Zhang, R. (1999). Treatment of dairy wastewater with two-stage anaerobic sequencing batch reactor systems — thermophilic versus mesophilic operations.*Bioresource Technology*, 68(3), 225-233.

Eckenfelder, Jr. WW (2000). *Industrial Water-Pollution Control*. Boston Burr Ridge: McGraw-Hill Higher Education. pp. 394–411.

Fdez.-Güelfo, L.A.; Álvarez-Gallego,C.; Sales Márquez, D. and Romero García, L.I. (2011). Biological pretreatment applied to industrial organic fraction of municipal solid wastes (OFMSW): Effect on anaerobic digestion. *Chemical Engineering Journal*.172 , 321-325.

Feng, T., Cheng, S., Min, Q., & Li, W. (2009). Productive use of bioenergy for rural household in ecological fragile area, panam county, tibet in china: The case of the residential biogas model. *Renewable and Sustainable Energy Reviews*, 13(8), 2070-2078.

Fermanagh District Council. (2010). *Waste Management Plan* .Available: http://www.fermanagh.gov.uk/index.cfm?website_Key=47&Category_key=133&Page_Key=324 . Last accessed 25 2011

Fernández,J.; Pérez, M and Romero, L.I. (2008). Effect of substrate concentration on dry mesophilic anaerobic digestion of organic fraction of municipal solid waste (OFMSW) . *Bioresource Technology*. 99 (14), 6075-6080 .

Field, J.A., Leyendecker, M.J.H., Sierra-Alvarez, R., Lettinga, G., and Habets, L.H.A., (1988). The methanogenic toxicity of bark tannins and the anaerobic biodegradability of water soluble bark matter. *Water Science and Technology*. 20, 219–240.

Forster-Carneiro,T; Perez, M and Romero, L.I. (2008). Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. *Bioresource Technology.* 99 (1), 6994–7002.

Forster-Carneiro, T. and Pérez, M. (2009).Thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste*. Bioresource Technology*. 91(15), p6763-6770

Fricke, K., Santen, H., Wallmann, R., Hüttner, A., and Dichtl, N. (2007).Operating problems in anaerobic digestion plants resulting from nitrogen in MSW.*Waste Management*, 27, 30-43.

Gallert, C., Bauer, S., Winter, J., (1998). Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population.*Bioresource Technology*. 50, 495–501.

Gannoun, H., Bouallagui, H., Okbi, A., Sayadi, S., & Hamdi, M. (2009). Mesophilic and thermophilic anaerobic digestion of biologically pretreated abattoir wastewaters in an upflow anaerobic filter. *Journal of Hazardous Materials*, 170(1), 263-271.

Garcia, J.-L., Patel, B.K.C., Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea.*Anaerobe* 6, 205–226.

Gertsakis,J. and Lewis,H. (2003). Sustainability and the Waste Management Hierarchy.*EcoRecycle Victoria*, 7.

Gómez, X., Cuetos, M. J., Cara, J., Morán, A., & García, A. I. (2006). Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: Conditions for mixing and evaluation of the organic loading rate. *Renewable Energy*, 31(12), 2017-2024.

Greben, H. ; Burke, L. M. and Szewczuk,S. (2008). Biogas, as a renewable energy source, produced, produced during the anaerobic digestion of organic wastes. Council for Scientific and industrial Research, Division for Natural Resources and the Environment.

Greenstar. (2005). *3Rs.* Available: http://www.greenstar.ie/htm/education/3rs.htm. Last accessed 23rd June 2011.

Griffith, M.R., 2005. An investigation into mechanical biological pretreatment of waste and its effect on landfill emissions in South Africa.MScEng Dissertation, University of KwaZulu-Natal, Faculty of Engineering, Durban, South Africa.

Hanaki, K., Mastsuo, T., and Nagase, M., (1981).Mechanism of inhibition caused by long chain fatty acids in anaerobic digestion process.*Biotechnology and Bioengineering*. 23, 1591–1610.

Gutierrez, O; Park, D; Sharma, K. R and Yuan, Zhiguo.(2009). Effecs of long term pH elevation on the sulphate-reducing and methanogenic activities of anaerobic sewer biofilms.*Water Research*. 43, 2549-2557.

Hansen, K.H., Angelidaki, I. and Ahring, B.K. (1998). Anaerobic digestion of swine manure: inhibition by ammonia. *Water Research*. 32, 5–12.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1999. Improving thermophilic anaerobic digestion of swine manure.*Water Research*. 33, 1805–1810

Hashimoto, A.G., (1986). Ammonia inhibition of methanogenesis from cattle waste.*Agricultural Wastes*. 17, 241–261.

Hill, D. T., & Holmberg, R. D. (1987). Long chain volatile fatty acid relationships in anaerobic digestion of swine waste. *Biological Wastes*, 23(3), 195-214.

Igoni, A.H., Ayotamuno, M. J., Eze, C. L., Ogaji, S. O. T., & Probert, S. D. (2008). Designs of anaerobic digesters for producing biogas from municipal solid-waste.*Applied Energy*, 85(6), 430-438.

Ike, M., Inoue, D., Miyano, T., Liu, T. T., Sei, K., Soda, S., and Kadoshin,S.(2010). Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in kyoto eco-energy project. *Bioresource Technology*, 101(11), 3952-3957.

Inglesbly, A.E, 2011; ‘Anaerobic digestion of algal biomass for bioenergy production-a feasibility study’, Msc thesis, University of Cape Town, South Africa.

Izumi, K., Okishio, Y., Nagao, N., Niwa, C., Yamamoto, S., & Toda, T. (2010). Effects of particle size on anaerobic digestion of food waste. *International Biodeterioration and Biodegradation*, 64(7), 601-608.

Jacobi, H.F., Moschner, C.R., and Hartung, E., (2009). Use of near infrared spectroscopy in monitoring of volatile fatty acids in anaerobic digestion.*Water Science and Technology*. 60 (2), 339-346.

Janssen, P.. (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology* . 160 (1), 1-22.

Jarrell, K.F., Saulnier, M. and Ley, A., (1987).Inhibition of methanogenesis in pure cultures by ammonia, fatty acids, and heavy metals, and protection against heavy metal toxicity by sewage sludge.*Microbiology*. 33, 551–555.

Kamlet, M.J., Doherty, R.M., Veith, G.D., Taft, R.W., Abraham, M.H., 1986. Solubility properties in polymers and biological media.An analysis of toxicant properties that influence inhibition of bioluminescence in Photobacterium phosphoreum (the microtox method).*Environmental Science and Technology*. 20, 690–695.

Karim,K., Hoffmann,R., Klasson,T. and Al-Dahhan,M.H.(2005), Anaerobic digestion of animal waste: Waste strength versus impact of mixing. *Bioresource Technology*. 96, 1771–1781.

Kaparaju, P., & Rintala, J. (2011). Mitigation of greenhouse gas emissions by adopting anaerobic digestion technology on dairy, sow and pig farms in Finland. *Renewable Energy*, 36(1), 31-41.

Karagiannidis, A. and Perkoulidis, G. (2009).A multi-criteria ranking of different technologies for the anaerobic digestion for energy recovery of the organic fraction of municipal solid wastes.*Bioresource technology.*100(8):2355-2360

Kalra, M.S. and Panwar, J.S., (1986). Anaerobic digestion of rice crop residues.*Agriculatural Wastes*.17, 263–269.

Kayhanian, M., (1999). Ammonia inhibition in high-solids biogasification: an overview and practical solutions. *Environmental Technolology*.20, 355–365.

Kayhanian, M., (1994).Performance of a high-solids anaerobic digestion process under various ammonia concentrations.*Chemical Technology Biotechnology*. 59, 349–352.

Kayhanian, M., (1999). Ammonia inhibition in high-solids biogasification: an overview and practical solutions. *Environmental Technology*. 20, 355–365.

Khalil, E.F., Whitmore, T.N., Gamaleldin, H., Elbassel, A., and Lloyd, D.(1991).The effects of pesticides on anaerobic digestion processes.*Environmental Technology*. 12 (6), 471–475.

Kleinstreuer, C. and Poweigha, T. (1982).Dynamic simulator for anaerobic digestion process.*Biotechnology and Bioengineering*, 24, 1941-1951.

Koster, I.W., Lettinga, G., 1984.The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge.*Agricultural Wastes*. 9, 2.5–216.

Koster, I.W., and Cramer, A., (1987).Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids.*Applied and EnvironmentalMicrobiology*. 53 (2), 403–409.

Kroeker E.J., Schultze, D.D., Sparling, A.B.and Lapp HM (1979).Anaerobic treatment process stability.*Water Pollution Control Federation*51(4), 718–727.

Kristjansson, J.K *et al.* (1982). "Different Ks values for hydrogen of methanogenic bacteria and sulfate-reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate". *Archae Microbiology.***131**: 278–282

Krylova, N.I., Khabiboulline, R.E., Naumova, R.P., and Nagel, M.A.(1997). The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure.*Chemical Technology and Biotechnology*. 70, 99–105.

Lakay MT, Wentzel MC and Ekama GA (2000) Laboratory procedures – wastewater treatment laborat ory, Water Research Group, Department of Civil Engineering, University of Cape Town, Rondebosch 7701, South Africa.

Lin, Y., Wang, D., Wu, S., and Wang, C. (2009). Alkali pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. *Journal of Hazardous Materials*, 170, 366-373.

Luning, L., Zundert, E. H. M. van, Brinkmann, A. J. F (2006). Comparison of dry and wet digestion for solid waste.*Water Science and Technology/Abstract*.

Liu, C-F; Yuan, X-Z; Zeng, G-M, Li , W-W and Li , J. (2007). Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource Technology*. 99, 882–888.

Liu, T. and Sung, S., (2002). Ammonia inhibition on thermophilic aceticlastic methanogens.*Water Science and Technology*. 45, 113–120.

Luostarinen, S., Luste, S. and Sillanpää, M. (2009).Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant.*Bioresource Technology*, 100(1), 79-85.

Luste, S. 2011, ‘Anaerobic digestion of organic by-products from meat-processing industry: the effect of pre-treatments and co-digestion’, PhD thesis, University of Eastern Finland.

Luste, S. and Luostarinen, S. (2010).Anaerobic co-digestion of meat-processing by-products and sewage sludge – effect of hygienization and organic loading rate.*Bioresource Technology*, 101(8), 2657-2664.

Lyberatos,G. and Skiadias, I.V. (1999). Modelling of anaerobic digestion.*Global NEST*. 1 (1), 63-76.

Mata-Alvarez, J., Macé, S., and Llabrés, P. (2000). Anaerobic digestion of organic solid wastes.an overview of research achievements and perspectives.*Bioresource Technology*, 74, 3-16.

Matete, N., Trois, C., 2008. Towards Zero Waste in emerging countries – a South African Experience. *Waste Management* 28 (8), 1480–1492.

Mawson AJ, Earle RL and Larsen VF (1991). Degradation of acetic and propionic acids in the methane fermentation.*Water Research*, 25(12):1549–54.

McCabe,J; Eckenfelder,W. eds (1975). *Biological treatment of sewage and industrial wastes.*Two volumes. New York: Reinhold Publishing.

Melbinger, N.R. and Donnellon, J., (1971). Toxic effects of ammonia-nitrogen in high rate digestion.*Water PollutionControl Federation*. 43, 1658–1670.

Mensah,A; Cofie;O and Montangero,A. (2003). Lessons from a pilot co-composting plant in Kumasi, Ghana.*29th WEDC International Conference*, 1-3.

Mohee, R. (2002). Assessing the recovery potential of solid waste in Mauritius.*Resources,conservation and recycling*. 36 (1), 33-43.

Moletta, R., Escoffier, Y., Ehlinger, F., Coudert, J.P., Leyris, J.P., (1994). On-line automatic control system for monitoring an anaerobic fluidized-bed reactor: response to organic overload. *Water Science and Technology*, 30 (12), 11-20.

Moletta, R., Verrier, D., & Albagnac, G. (1986). Dynamic modelling of anaerobic digestion.*Water Research*, 20(4), 427-434.

Molina, F., Garcı´a, C., Roca, E., Lema, J.M., Molina, F., and Castellano, M., (2009). Selection of variables for on-line monitoring, diagnosis, and control of anaerobic digestion processes. *Water Science and Technology*, 60 (3), 615-622.

Monnet, F(2003). An introduction to anaerobic digestion of organic wastes. Report by Remade Scotland Pty(Ltd) , pp 7-9.

Mshandete, A., Kivaisi, A., Rubindamayugi, M., & Mattiasson, B. (2004). Anaerobic batch co-digestion of sisal pulp and fish wastes. *Bioresource Technology*, 95(1), 19-24.

Munganga, G. Melamu, R., Von Blottnitz,H. and Haider, S. (2010). An assessment of the bio-methane potential of seventeen types of organic fraction of the municipal solid waste (OFMSW) in Cape Town.

Murto, M., Björnsson, L., and Mattiasson, B. (2004).Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure.*Environmental Management*, 70(2), 101-107.

Nielsen, H.B. and Angelidaki, I., (2008). Strategies for optimizing recovery of the biogas process following ammonia inhibition. *Bioresource Technology*. 99, 7995–8001.

Nielsen, H.B., Uellendahla, H., Ahring, B.K., (2007). Regulation and optimization of the biogas process: propionate as a key parameter. *Biomass and Bioenergy*, 31 (11-12), 820-830.

Nohra, J. A., Barrington, S., Frigon, J. C., & Guiot, S. R. (2003). In storage psychrophilic anaerobic digestion of swine slurry.*Resources, Conservation and Recycling*, 38(1), 23-37.

Palczynski, Richard. (2002). Study on Solid Waste Management Options for Africa.African Development Bank, Abidjan, Côte d'Ivoire.

Papadimitriou, E.K (2010). Hydrolysis of organic matter during autoclaving of commingled household waste.*Waste Management*. 30

Pegels,A.(2010). Renewable energy in SouthAfrica: Potentials, barriers and options for support. *Energy Policy*.38, 4945–4954.

Pognani, M., Barrena, R., Font, X., Scaglia, B., Adani, F., and Sánchez, A. (2010). Monitoring the organic matter properties in a combined anaerobic/aerobic full-scale municipal source-separated waste treatment plant.*Bioresource Technology*, 101(17), 6873-6877.

Ponsá, S., Gea, T., Alerm, L. and Cerezo, J. and Sanchez, A.(2008). Comparison of aerobic and anaerobic stability indices through a MSW biological treatment process.*Waste Management.*28(12):2735-2742.

The pool water treatment advisory group-PWAD. (1999). *Carbon dioxide in water equilibrium.*Available: http://www.pwtag.org/researchdocs/Used%20Ref%20docs/52%20Carbondioxide%20in%20water%20equilibrium.pdf#search='carbon dioxide in water equilibrium'. Last accessed 13 January 2012.

Pratap, C.P.; David, P.C.; Gerasimos, L. and Spyros, A. S. (2001). Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid.*Bioresource Technology*;8:165–169

Rahn, T., and Gandolfi, P.B., (2007). Modification and Optimisation of Existing MBT Plants using BTA Technology. 2nd International Symposium MBT, Hanover, Germany, May, 177–186.

Ren, N.Q.; Liu, M.; Wang, A.J.; Ding, J. and Hong, M. (2003). Organic acids conversion in methanogenic - phase reactor of the two phase anaerobic process.*Environmental Science* 2003; 24:89–93.

Resch, C., Wörl, A., Waltenberger, R., Braun, R., & Kirchmayr, R. (2011).Enhancement options for the utilisation of nitrogen rich animal by-products in anaerobic digestion.*Bioresource Technology*, 102(3), 2503-2510.

Resch, C., Grasmug, M., Smeets, W., Braun, R., and Kirchmayr, R. (2006).Optimised anaerobic treatment of house-sorted biodegradable waste and slaughterhouse waste in a high loaded half technical scale digester.*Water Scienceand Technology*. 54, 231–236.

Rincon,B.; Borja,R.; Gonzalez,J.M. and Portillo,M.C. and Saiz-Jimenez , S. (2007). Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue. *Biochemical Engineering Journal*. 40 (1), 253–261.

Rintala, J., Martin, J.L.S., and Lettinga, G. (1991). Thermophilic anaerobic treatment of sulphate rich pulp and paper integrate process water. Water Science and Technology. 24, 149–160.

Rosenwinkel,K.-L and Meyer,H(1999). Anaerobic treatment of slaughterhouse residues in municipal digesters,Water Science and Technology. 40,101–111.

Ross, W.R., Novella, P.H., Pitt, A.J., Lund, P., Thomson, B.A., King, P.B. and Fawcett, K.S. (1992) “Anaerobic Digestion of Wastewater Sludge”. WRC Project no 390, TT 55/92, Pretoria, South Africa.

Sakai,S.;Sawell, S.E;Chandler,A.J; Eighmy,T.T, Kosson,D.S; Vehlow,J.; van der Sloot, H.A; Hartlen and Hjelmar(1996). World trends in municipal solid waste management.*Waste management.* 16 (5/6).

Salminen,E. and Einola,J. and Rintala,J (2003). The methane production of poultry slaughtering residues and effects of pretreatments on the methane production of poultry feather, *Environmental Technology*. 24, 1079–1086.

Salminen, E. A., and Rintala, J. A. (2002). Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: Effect of hydraulic retention time and loading. *Water Research*, 36(13), 3175-3182.

Salminen,E. and Rintala,J (2001). Anaerobic digestion of organic solid poultry slaughterhouse waste – a review.*Bioresource Technology*. 83, 13–26.

Schanmugam, P.and Horan, N.J. (2009).Optimising the biogas production from leather fleshing waste by co-digestion with MSW.*Bioresource Technology*. 100, 4117–4120.

Smith, P.H., Bordeaux, F.M., Goto, M., Shiralipour, A., Wilke, A., Andrews, J.F., Ide, S. and Barnett, M.W. (1988).Biological production of methane from biomass. In: Methane from biomass. A treatment approach. Smith, W.H. and Frank, J.R. (Eds.), Elsevier, London, 291-334.

Snyman, H., Van Niekerk, A.M. and Rajasakran, N. (2006). “Sustainable wastewater treatment – What has gone wrong and how do we get back on track?” Proceedings of the WISA 2006 Conference.

Sosnowski, P., Wieczorek, A., & Ledakowicz, S. (2003). Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes.*Advances in Environmental Research*, 7(3), 609-616.

Speece RE (1987) Nutrient requirements. Anaerobic Digestion of Biomass (In: Chynoweth DP and Isaacson, R. Eds) .*Elsevier Applied Science* , pp 109-129.

Speece, R.E., (1996). Anaerobic Biotechnology for Industrial Wastewater. Tennessee Archae Press, Nashville.

Soto, M., Mendez, R., Lema, J.M., (1992).Characterization and comparison of biomass from mesophilic and thermophilic fixed bed anaerobic digesters.*Water Science and Technology*. 25, 203-212.

Soto, M., Mendez, R., Lema, J.M, (1991).Biodegradability and toxicity in the anaerobic treatment of fish canning wastewaters.*Environmental Technology*. 12, 669-677

Sprott, G.D. and Patel, G.B., (1986). Ammonia toxicity in pure cultures of methanogenic bacteria.*Systematic and Applied Microbiology*. 7, 358–363.

Stafford, D.A., 1981. The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance.*Tribune de Cebedeau*. 456, 493–500

Standard Methods Standard methods for the examination of water and wastewater , 16th ed.(1995). AmericanPublic Health Assoc., 1015 15th Str. NW, Washington DC 200005, USA.

Strachan, L.J., Wright, M., Broomfield, M., Couth, B and Pass, J. (2007).Using landfill gas. Resource, 9: No 1.

Tchobanoglous G, Burton FL (1991). Waste-water Engineering: treatment disposal and reuse. 3rd edition. New York: McGraw-Hill, p.1334.

Tchobanoglous, G., Theisen, H. and Vigil, S., (1993). Integrated Waste Management: Engineering Principles and Management Issues. McGraw-Hill, New York.

Thomas,K.(2006).Integrated solid waste management : strategies for its use in Africa.Sustainable Development Research Competition.

Trois,C.; Griffith,M. Brummack,J.; Mollekopf,N.. (2007). Introducing mechanical biological waste treatment in South Africa:A comparative study. *Waste Management* . 27 (1), 1706–1714.

Troschinetz, A.M. and Mihelcic, J.R. (2009).Sustainable recycling of municipal solid waste in developing countries.*Waste Management*. 29(2):915-923

United Nations Environment Programme.(2009). Waste Characterization and Quantifi cation with Projections for Future.*Developing Integrated Solid Waste Management Plan: Training Manual*1.

United Nations Environmental Programme, UNEP. (2009). Solid Waste: Guidelines for Data Collection and Analysis (3), 8.

UNEP.(1996). International Source Book on Environmentally Sound Technologies for

Municipal Solid Waste Management. UNEP Technical Publication 6, Nov. 1996.

United Nations Environmental Programme (UNEP).(2002). International Source Book on Environmentally Sound Technologies for Municipal Solid Waste Management.UNEP Division of Technology, Industry, and Economics.

USEnvironmental Protection Agency (USEPA) Solid Waste and Emergency Response (2002). What is Integrated Solid Waste Management.United States Environmental Protection Agency, Washington, DC.

U.S EPA.(2011). *Wastes - Non-Hazardous Waste - Municipal Solid Waste.*Available: http://www.epa.gov/epawaste/nonhaz/municipal/index.htm . Last accessed 7 th March 2011.

Vavilin, V.A; Fernandez, B.; Palatsi, J. and Flotats,X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate.*Waste Management*. 28, 939-951.

Vavilin, V. A; Rytov, S. V and Lokshina, L. Ya. (1996). A Description of Hydrolysis Kinetics in Anaerobic Degradation of Particulate Oranic Matter. *Bioresource Technology*. 56, 229-237.

Verma,S.(2002). Anaerobic digestion of biodegradable organics in municipal solid waste.*Master of Science Degree in Earth Resources Engineering*. School of Engineering & Applied Science (Columbia University).

Viessman Jr W and Hammer MJ (1993).Water Supply and Pollution Control. New York: Harper Collins College Publishers. pp. 513–679.

Visser, A. Hulshoff Pol, L.W, Lettinga, G.,(1996). Competition of methanogenic and sulfidogenenic bacteria.*Water Science and Technology*.33 (3), 99-110.

Visvanathan,C;Trankler,J and Chiemchaisri,C. (2005). Mechanical-biological treatment of municipal solid waste in Asia.*International Symposium MBT*, 99-108.

Walker, M., Banks, C. J., and Heaven, S. (2009). Two-stage anaerobic digestion of biodegradable municipal solid waste using a rotating drum mesh filter bioreactor and anaerobic filter. Bioresource Technology, 100(18), 4121-4126.

Wang, Y.; Zhang, Y., Wang, J. and Meng, L..(2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria.*Biomass and Bioenergy*. 33, 848 – 853.

Wang, A; Ren, N; Wang, X and Lee, D. (2008).Enhanced sulphate reduction with acidogenic sulphate-reducing bacteria.*Journal of Hazardous Materials*. 154, 1060-1065.

Wang, Z., and Banks, C. J. (2003). Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes. *Process Biochemistry*, 38(9), 1267-1273.

Wang, Q., Kuninobu, M., Kakimoto, K., I.-Ogawa, H., & Kato, Y. (1999).Upgrading of anaerobic digestion of waste activated sludge by ultrasonic pretreatment.*Bioresource Technology*, 68(3), 309-313.

Wang, Q., Kuninobu, M., Ogawa, H. I., & Kato, Y. (1999).Degradation of volatile fatty acids in highly efficient anaerobic digestion.*Biomass and Bioenergy*, 16(6), 407-416.

Ward , A. J; Hobbs, P.J; Holliman, P.J and Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources.*Bioresource Technology*. 99 (1), 7928–7940

Wellinger, A., Wyder, K., Metzler, A.E., (1993). Kompogas - a new system for the anaerobic treatment of source separated waste. *Water Science and Technology*, 27 (2), 153–158.

Washington State University. (2011). *Compost needs (materials and methods to ensure quality compost).*Available: http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm .Last accessed 20 February 2010.

Williams, R.B., Jenkins, B.M., Nguyen, D., (2003). Solid Waste Conversion: A Review and Database of Current and Emerging Technologies. Final Report.Department of Biological and Agricultural Engineering.University of California, Davis.

Yang, J., Speece, R.E., 1986.The effects of chloroform toxicity on methane fermentation.Water Research. 20,1273-1279.

Yeole TY, Gokhale S, Hajarnis SR, Ranade DR (1996). Effect of brackish water on biogas production from cattle dung and methanogens.*Bioresoure Technology* ;58:323–5.

Zeeman G., Wiegant W. M., Koster-Treffers M. E. and Lettinga G. (1985) The influence of the total ammonia concentration on the thermophilic digestion of cow manure. *Agricultural. Wastes.*14, 19-35.

Zerbock, Olar. (2003). Urban Solid Waste Management: Waste Reduction in Devleopling Nations. School of Forest Resources and Environemenal Science, Michigan Technological

University.CE 5993 Field Engineering in the Developing World, Master’s International Program.

Zglobisz, N., Castillo-Castillo, A., Grimes, S., & Jones, P. (2010). Influence of UK energy policy on the deployment of anaerobic digestion. *Energy Policy*, 38(10), 5988-5999.

TABLE OF CONTENT FOR APPENDIX CHAPTERS

9 APPENDIX

9.1 Methodology

9.1.1 Sample standard curves for VFAs methods

The different concentration of the individual VFA were calculated using standard curves of the peak area (measured by the HPLC) versus the acid concentration as explained earlier in Section 3.9.3.1(Figures 55a-d). Retention times of the individual acid, presented in Table 54, were used to identify the VFA.The concentration of the remaining VFAs were determined using equations from previous research on the same machine (Inglesby, 2011).

Table 54: Retention time of the different VFA

	Retention time (min)
Lactic Acid	13.0
Acetic Acid	15.7
Butyric Acid	23.1
Valeric Acid	33.9

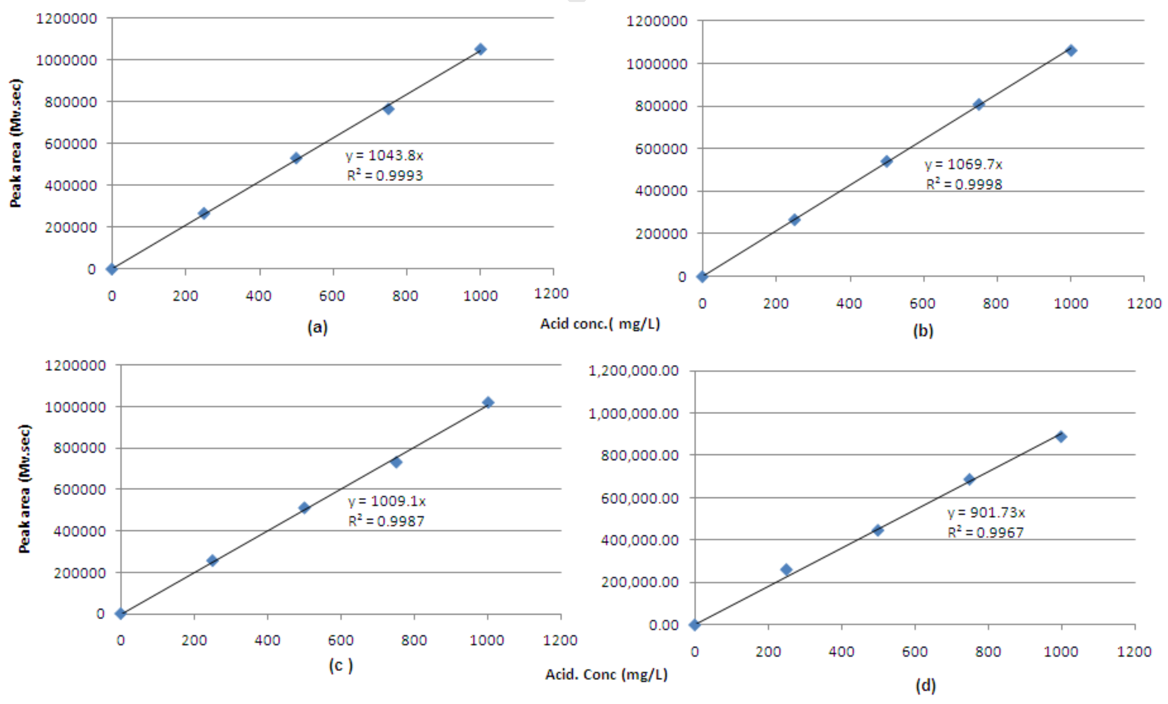


Figure 79: standard calibration curves for (a) acetic acid, (b) butyric acid, (c) lactic acid and (d) valeric acid

9.1.2 Standard curves for COD method

The equation used to calculate the COD of the fed-batch experiments is presented in Figure 56, where x is the absorbance of the different samples from bioreactors, examined.

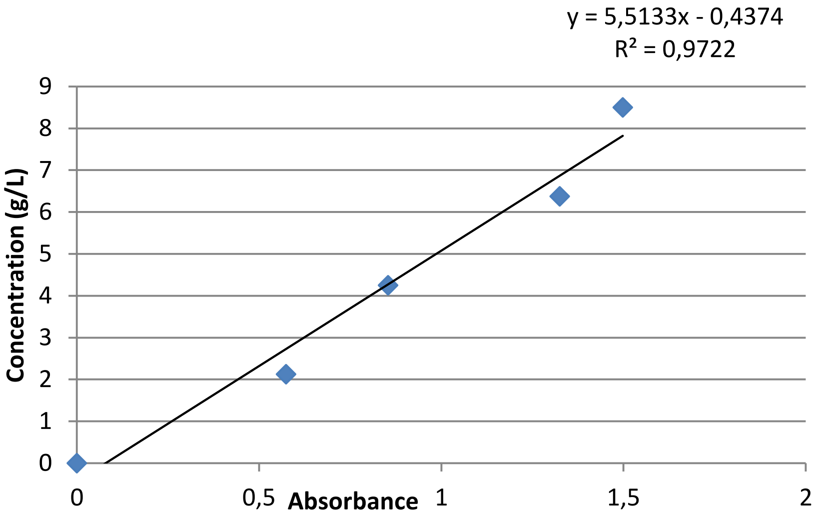


Figure 80: Calibration curve used for COD experiments

Sample values for the absorbance, derived COD of the fed-batch experiments are presented in Table 55 and Table 56.

Table 55: Absorbance of the fed-batch experiments

	49	53	55	57	59
K. Flat	0.85	0.75	0.58	1.12	1.34
	0.63	0.87	0.75	1.18	1.25
Avg	**0.74**	**0.81**	**0.67**	**1.15**	**1.29**
Stdev	**0.16**	**0.08**	**0.12**	**0.04**	**0.06**
Blood	1.15	0.75	1.08	0.61	0.16
	0.63	1.1	0.75	0.02	
Avg	**0.89**	**0.92**	**0.91**	**0.31**	**0.16**
Stdev	**0.37**	**0.25**	**0.23**	**0.42**	
K. Prog	0.85	1.11	1.03	0.31	0.21
	1.03	1.22		0.39	0.87
Avg	**0.94**	**1.17**	**1.03**	**0.35**	**0.54**
Stdev	**0.13**	**0.08**		**0.06**	**0.47**
Garden	0.17	1.12	1.1	0.38	1.05
		1.38	1.16	0.23	1.06
Avg	**0.17**	**1.25**	**1.13**	**0.31**	**1.06**
Stdev		**0.18**	**0.04**	**0.11**	**0.01**
G+B	0.06	0.3	1.08	0.35	1.31
	0.9		1.27	0.32	1.06
Stdev	**0.59**		**0.13**	**0.02**	**0.18**
Avg	**0.48**	**0.3**	**1.17**	**0.34**	**1.19**

Table 56: COD values derived from the absorbance and the equation in Figure 80, for the fed-batch experiments

	49	53	55	57	59
K. Flat	8.5	7.51	5.78	11.21	13.37
	6.3	8.71	7.53	11.8	12.49
Avg	**7.4**	**8.11**	**6.66**	**11.51**	**12.93**
Stdev	**1.56**	**0.85**	**1.24**	**0.42**	**0.62**
Blood	11.51	7.47	10.78	6.07	1.56
	6.33	10.98	7.47	0.18	
Avg	**8.92**	**9.23**	**9.13**	**3.13**	**1.56**
Stdev	**3.66**	**2.48**	**2.34**	**4.16**	
K. Prog	8.5	11.09	10.25	3.09	2.08
	10.25	12.24		3.87	8.74
Avg	**9.38**	**11.67**	**10.25**	**3.48**	**5.41**
Stdev	**1.24**	**0.81**		**0.55**	**4.71**
Garden	1.72	11.2	11.01	3.83	10.46
		13.81	11.59	2.3	10.64
Stdev		**1.85**	**0.41**	**1.08**	**0.13**
Avg	**1.72**	**12.51**	**11.3**	**3.07**	**10.55**
G+B	0.64	2.97	10.78	3.53	13.11
	8.96		12.65	3.2	10.61
Stdev	**5.88**		**1.32**	**0.23**	**1.77**
Avg	**4.8**	**2.97**	**11.72**	**3.37**	**11.86**

9.1.3 Sample calculations for CH_4 analysis

The CH_4 composition of the various BMP assays was calculated as illustrated below for biomass from Cape Town Market, using the peak areas measured by the GC (Table 57).

Table 57: Peak area for standard test (calibration) of CH_4 analysis

	Peak Area (µV. S)
1	6364585
2	6451831
3	6309631
4	6215223
Avg	6335318
Stdev	99187.4
y CH_4	0.525
% CH_4	52.5

- CH_4 Calculation for waste from the Cape Town Market (E) as illustrated inTable 58 below

Average Peak area (AE): 2586071.2 μv/s

Standard average peak area for calibration gas (i.e. yCH_4=52.5%; AC) =6335317.5 μv/s

yCH_4, E= (AE/AC)*0.525 → % CH_4,E= yCH_4,E *100

Table 58: Sample GC analysis for week # 4 of the various substrates

	1	2	3	% CH_4	Stdev (%)
EW	2586071			21.5	
VHW	4681617	2897053	2655439	28.3	9.17
CAV	616995.2	612868.3	497372.2	4.8	0.56
FW	3113175	2804223	4086214	27.6	5.54
PW	7219642	110370.7	6087487	37.1	31.65497
AW	781.9	0	0	0	
BW	532565.5	460776.6	407743.9	3.9	0.52
CW	0	486269.4	118279	1.7	2.16
WHW	182	0	0	0	
BC FW	912302.1	0	0	7.6	
A FW 1	98543	575825	560560.5	3.4	2.25
A FW 2	212013.4	739980.7	664001.3	4.5	2.37
A FW 3	757192.5	445455	580580.3	4.9	1.29
A FW 4	951759.8	334960.5	345246.6	4.5	2.92
BC Fi	548020.6			4.5	
A Fi 1	6624349	7863097	6744469	58.6	5.66
A Fi 2	6901571	8749939	10850203	73.2	16.37
A Fi 3	6343316	5961520	6720742	52.6	3.14
A Fi 4	9145184	8695721	3547478	59.1	25.77
BC GW	3630316		3095752	30.1	3.13
A GW 1	7279701	7389900		60.8	0.64
A GW 2	0	8695421	49498.1	36.2	50.66
A GW 3	8261516	9289672	8112541	70.9	5.31
A GW 4	8066460	8167777	5883270	61.1	10.69
WDO	8472488	8780102	0	71.5	1.80

9.1.4 Sample calculation for FSA analysis

This section present sample calculations used to determine the concentration of FSA in the blood and co-digestion experiments.

A: 14000, sample size= 2.5 mL, concentration of H_2SO_4=0.001

Table 59: Amount of acid required for titration, for the blood experiment

Days	0	7	14	21	28	35	42	49	56	63
	6.7	9.8	12.7	52.6	30.7	31.6	41.2	40.7	133.2	182
	6.9	6.8	8	36	26.3	32.8	32	76	78	20.1
Stdev	0.14	2.12	3.32	11.74	3.11	0.85	6.51	24.96	39.03	114.48
AVG	**6.8**	**8.3**	**10.35**	**44.3**	**28.5**	**32.2**	**36.6**	**58.35**	**105.6**	**101.05**

For example average FSA concentration for day 3, amount of acid =9.3 mL

FSA (mg N/L)=(0.001*14000*9.3)/2.5

=52.1 mg N/L

The concentration of FA was calculated using Equation 3 below (Hafner *et al.*, 2009), depending on the pH

FA= (Ka*FSA Concentration) / (Ka+10^{-pH})Equation22

Where Ka=5.6 E-10

Table 29 presents the FSA concentrations for days 7-59**.**

Table 60: Concentration of FSA, calculated from acid concentration and Equation 15 (Section 3.9.3.4) for the blood experiment

Days	0	7	14	21	28	35	42	49	56	63
	187.6	274.4	355.6	1472.8	859.6	884.8	1153.6	1139.6	3729.6	5096
	193.2	190.4	224	1008	736.4	918.4	896	2128	2184	562.8
Stdev	3.96	59.40	93.06	328.66	87.12	23.76	182.15	698.90	1092.90	3205.46
AVG	**190.4**	**232.4**	**289.8**	**1240.4**	**798**	**901.6**	**1024.8**	**1633.8**	**2956.8**	**2829.4**

Tables 61-62 present the raw data of amount of acid, and FSA concentration for the co-digestion experiments

Table 61: Amount of acid required for titration, for the co-digestion experiments

Days	0	3	7	15	25	29	36	41	49	59
		9.3	23.5	26.4	41.8	44.8	83.7	48.2	58.7	60.6
				34.1	128	72.8	75.4	55.4	57.7	61.7
Stdev				5.44	60.95	19.80	5.87	5.09	0.71	0.78
Avg		**9.3**	**23.5**	**30.25**	**84.9**	**58.8**	**79.55**	**51.8**	**58.2**	**61.15**

Table 62: Amount of acid required for titration, for the co-digestion experiments

Days	0	3	7	15	25	29	36	41	49	59
		52.1	131.6	147.8	234.1	250.9	468.7	269.9	328.7	339.4
				191	717	407.7	422.2	310.2	323.1	345.5
Stdev				30.55	341.46	110.87	32.88	28.50	3.96	4.31
Avg		**52.1**	**131.6**	**169.4**	**475.4**	**329.3**	**445.5**	**290.1**	**325.9**	**342.4**

9.1.5 Sample calculation to illustrate how the C: N, and mixing ratio was determined

Example of calculation used for abattoir waste with garden waste and paper sludge. It was assumed that the C:N ratio of manures would be similar to that of abattoir wastes.

- C:N ratio-manure=1:15; C:N ratio-garden waste=30:1; C:N ratio-newspaper=175:1
- Mixing ratio (abattoir: garden waste) =1:3, Mixing ratio (abattoir: paper sludge)= 5:1
  - Expected C:N ratio (abattoir+garden)=((15)+(30*3))/4= ~26.5
  - Expected C:N ratio (abattoir+paper sludge) = ((175)+(15*5))/6= ~ 42

9.1.6 Data points used to determine the slopes presented in Section 6.2.5 (Effect of co-digestion)

Table 63: Data points used to calculate the the slopes presented for reactor a, of co-digestion experiments

	Cumulative feed (g VS)	Cumulative biogas (mL)
	0.0	0
Data points	104.2	1267
Data points	149.9	4182
Data points	195.2	5017
Slope 1	12.2	
Slope 2	63.8	
Slope 3	18.4	

Table 64: Data points used to calculate the the slopes presented for reactor b, of co-digestion experiments

	Cumulative feed (g VS)	Cumulative biogas (mL)
	0.0	0
Data points	89.0	1726
Data points	137.7	6261
Data points	192.2	7981
Slope 1	19.39020828	
Slope 2	93.2436878	
Slope 3	31.54724567	

Table 65: Data points used to calculate the slopes presented for reactor a, of experiments with garden waste

	Cumulative feed (g VS)	Cumulative biogas (mL)
	0	0
Data points	50.6	279
Data points	195.2	9849
Slope 1	5.51	
Slope 2	66.21	

9.1.7 Calculation to determine the theoretical (expected) biogas yield for co-digestion

Biogas yield of BC GW= 546 mL/g VS, Biogas yield of abattoir waste=79 mL/g VS

Mixing ratio (abattoir: garden)= 1: 3

Expected biogas yield = (0.75*546)+(0.25*79)= 429 mL/g VS

9.1.8 Sample calculation for the biogas and CH_4 yield

Biogas yield for batch test= cumulative volume (mL)/g VS loaded

Biogas yield (fed batch) =Cumulative volume (mL)/Cumulative g VS loaded

Daily CH_4 volumes= Daily biogas volumes (mL)* % CH_4

CH_4 yield= Cumulative daily CH_4/ g VS loaded

9.1.9 Sample calculation to determine the maximum CO_2 released chemically, and the minimum CO_2 resulting from AD

Table 66: Sample estimation of the maximum CO_2 produced from the buffer

	g buffer	No. moles	Vol chemical CO_2, mL	Total cum biogas, mL	% X	% Y
BC GW	2.1	2.5E-02	637.9	1685	37.8	36

%X= (637.9/1685)*100=37.8 %

Total Cumulative CH_4, mL for BC GW =688.8

%Y = (1685-637.9-688.8)/(1685-637.9)=36 %

9.1.10 Sample calculation to estimate the amount of NH_3 and H_2S in slaughterhouse waste

Table 67: Estimated concentration of NH_3 and H_2S for abattoir wastes

	C:N	g VS loaded	VS destruction ,%	NH_3 in 100 mL, mg	NH_3, mg/L	H_2S in 100 mL, mg	H_2S, mg/L
AW	11.1	4.84	5	2.78	27.79	1.85	18.5

The values for BW were determined using the general formula of a protein ($C_{13}H_{25}O_7N_3S$) as the C:N ratio of BW is similar to that of protein (i.e. 3.2 compared to 3.7) on a mass basis. However this was not the case for AW and EW, where values calculated are overestimated due to the assumption that the bulk of the molecules was carbon and nitrogen only. It is recommended that a full analysis of chemical composition be carried out to determine the empirical formula of substrates used in further research.

Proteins: $C_{13}H_{25}O_7N_3S+6H_2O \rightarrow 6.5CO_2+6.5CH_4+3NH_3+H_2S$

g nitrogen =4.84 *[parts of nitrogen/ (parts of carbon + parts of nitrogen)]= 0.4 g

Mm, protein=367 g/mol; Mm, N_3=42 g/ mol; Mm, NH_3=17 g/mol

X (ratio of g N/g protein)=42/367=0.114

Mass protein degraded= (g nitrogen/x)* VS destruction =0.17

No. moles Proteins degraded=Mass protein/Mm, protein=4.8E-04

From stoichiometry, and integrating VS destruction = No moles ammonia

=3XNo. Moles protein =1.4E-03

Mass ammonia =No moles, NH_3 X Mm, NH_3

=2.4E-02 g in 100 mL

Concentration NH_3 (mg/L) =~245 mg/L

9.1.11 Analysis of variance (ANOVA) of BMP results

A ‘TWO-way ANOVA’ without replication was used in Excel 2007, to compare biogas volumes and composition. This analysis was carried out to evaluate the variation achieved across different types of substrates and within the triplicate for each samples. The test was done per category of substrate i.e. food waste, garden waste, fines and paper sludge and abattoir wastes separately. The test was also carried out to analyse all waste samples from the Athlone Refuse Transfer Station (ARTS) using a TWO-way ANOVA with replication since each category had four samples, representing the different sampling days (Andrews University, 2011).

Anova uses an F test to compare means of group, which indicates the relationship between two variables. Where F represent the quotient of the variance of the group means over the variance within the group as presented in Equation 23 (Andrews University, 2011).

F-test value=variance of the group means / mean of the within group variances Equation 23

The software does not indicate however the nature of the relationship(i.e. ANOVA only evaluate whether the means of groups are differentfrom a group, but cannot indicate which ones are). The main output values are the **F-test value, P Value, F critical and alpha**.

The test is based on the null hypothesis, which occurs when F=0. When F=0, there is no relationship between the different means as they are all equal. When F≠0, the null hypothesis fails indicating that there is a relationship between the means and they are different, the output table indicates which one is higher/lower (Andrews University,2011).

alpha=threshold value/confidence level i.e. generally set up at 0.05 by convention, or 95% confidence level interval of the mean

p-value: it is a probability, usually ranging from 0 to 1, expressing the likelihood of obtaining a result at least as significantly different/extreme as the one observed if the null hypothesis holds (i.e. means of the groups are equal).

When P-value>alpha, or F < F-critical: the difference between the means is not statically significant. If P-value<alpha, the difference between the means is statically significant. Where samples represent categories (e.g. EW, CAV, VH, etc) and column represent results of the triplicate (i.e. I, II and III).

9.1.12 Sample calculation of amount of VS in fed-batch reactors

Total working volume = 2000mL

Let X=measured TS fraction, Y= measured VS fraction, z= wt mass of reactor media sample (g), T= volume of reactor media sample collected for analysis (mL)

Mass of VS in sample collected=M=X*Y*z

Mass of VS in reactor = M*(T/2000)

9.2 Simulation of the F:I ratio in fed-batch reactors

9.2.1 Algorithm used to model/simulate the F:I ratio under the flat and progressive feeding regime for the fed-batch reactor

Excel model

The feeding regime was adapted from Angelidaki *et al.* (2006) as presented in Table 68.The estimated daily amount of inoculum was calculated using the conversion coefficient from the 'activated biomass concept' (ABC), using Equation 14 (Chapter 3, Section 3.6.1).

A= daily g VS inoculum based on the ABC (using measured TS and VS values)

B= daily g VS feed from scaled from the literature in Table 68

Table 68: illustrates literature data versus experimental data to investigate the influence of feeding regime on performance of AD systems.

	Literature Values (Angelidaki et al. 2006)	Experimental Data
Reactor size (working volume)	3.5 L	2 L
Progressive feeding rate	1.7-15	0.97-8.5 g VS/day
Flat feeding rate	5.7 g VS/day	3.26 g VS/day
Incremental increase (∆)	0.45 g VS/day	0.13 g VS/day
Digestion period	30 days	60 days
Temperature	55°C	35°C
Initial inoculums volume	350 mL	300 mL

Volume %	10%	15%

The daily F:I ratio based on the ABC was therefore a ratio of the two variables defined above, as illustrated in Equation 24.

F:I ratio (Excel) = B/A   Equation 24

Matlab model

The amount of inoculum was identical to that used in the Excel model (=A, i.e. based on the ABC concept). The amount of substrate available for biodegradation was determined using first order kinetic model in MATLAB ®, the hydrolytic kinetic constants used are presented in Table 68.

Table 69: Kinetic coefficients of the first-order rate of hydrolysis (Vavilin *et al.*, 2007)

Substrate	Hydrolytic/kinetic constants (k, day -1)	T (°C)
Kitchen waste	0.34	35
Slaughterhouse wastes	0.35	35
Crops and crop residue	0.009-0.094	35

Figure 81 presents a standard curve, and biodegradation equation generated for a starting concentration of 15 g VS/day for kitchen wastes. The standard curves generated for the biodegradation of food waste, garden waste and abattoir wastes are presented in Table 70 below. The coefficient to the exponential curve varied with the initial organic load, and did not impact on the overall equation.

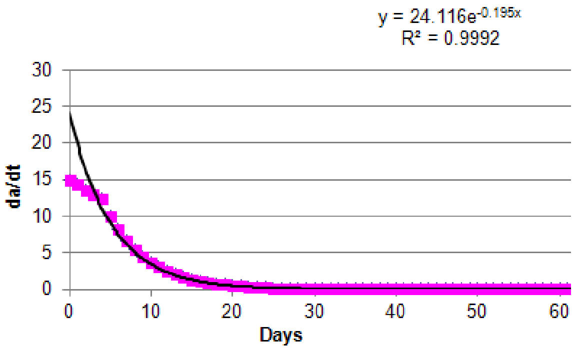


Figure 81: Biodegradation of food waste using first order kinetics

Table 70: Equation of biodegradation extracted from MATLAB for food waste, garden wastes and abattoir waste

	Biodegradation based on kinetics
Food wastes	Y=m*exp(-0.141*x)
Garden wastes	Y=m* exp(-0.197*x)
Abattoir wastes	Y=m*exp(-0.195*x)

Where m= loaded biomass in reactor (g VS), y= g VS/day, and x= day No.

Note m=amount of mass loaded based on the literature (Table 68). Hence, the daily F:I ratio based on first order kinetic was calculated using Equation 25 below.

F:I ratio (Matlab)= Y/A **Equation 25**

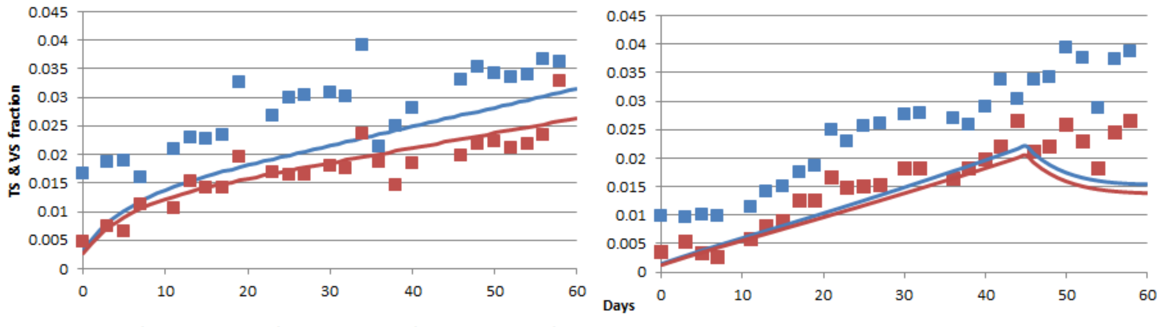
Sample of modelling code used for abattoir wastes

```
function da = abattoirwaste (t,a)
da = zeros(1,1);
m= 0.35; % m=0.35 for slaughterhouse waste @ 35 deg. C; Valvilin et
al.2004
da(1) = -m*a(1);
%starting concentration S=15 g VS/day
%options= odeset('RelTol',1e-4, 'AbsTol',[1e-4 1e-4 1e-5]);
```

9.2.2 Validation of the model

The MATLAB model developed was used to simulate the evolution of total and volatile (TS and VS) for the different substrates. These were then compared to experimental data to evaluate its validity.

Figure 82 presents experimental and theoretical values for kitchen wastes. There is generally a good correlation between experimental and theoretical results for kitchen waste for both K. Flat and K. Prog reactors. Lower theoretical TS and VS values in the digester imply higher conversion rates of the fresh feed into biogas. Following this, experimental values were then expected to be higher than the theoretical values as was the case for kitchen waste (Figure 82).



(a) Kitchen waste, Flat

(b) Kitchen waste, Progressive

Figure 82: Experimental and model TS and VS profiles for kitchen waste on (a) flat and (b) progressive feeding regime

The TS and VS profiles of garden waste are presented in Figure 4. The TS & VS profiles of the progressive feeding regime exhibit different shape of the curve i.e. the TS & VS profiles under the flat feeding regime increase gradually while that of the progressive feed increases, reaches a peak and then decreases.The solids content in the bioreactors increases sharply until day ~36 for the co-digestion of blood and garden, and day~46 for others.

The sudden drop in the solids content can be associated with the change in feeding regime from progressive from ~6.5 g/day to a flat rate of 3.26 g VS/day. From Figure 83 below, it is observed that whereas kitchen waste showed a good correlation between experimental and theoretical values; the experimental results and model values diverged quite considerably for the other substrates. A clear example of this is the lack of similar trends observed, and the lower values achieved with experimental results compared to the model values.

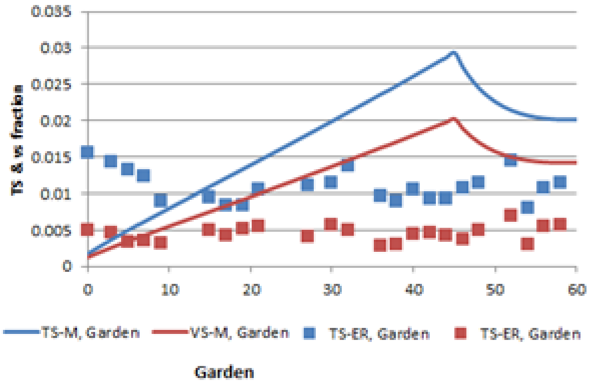


Figure 83: Experimental and model TS & VS profiles for garden waste

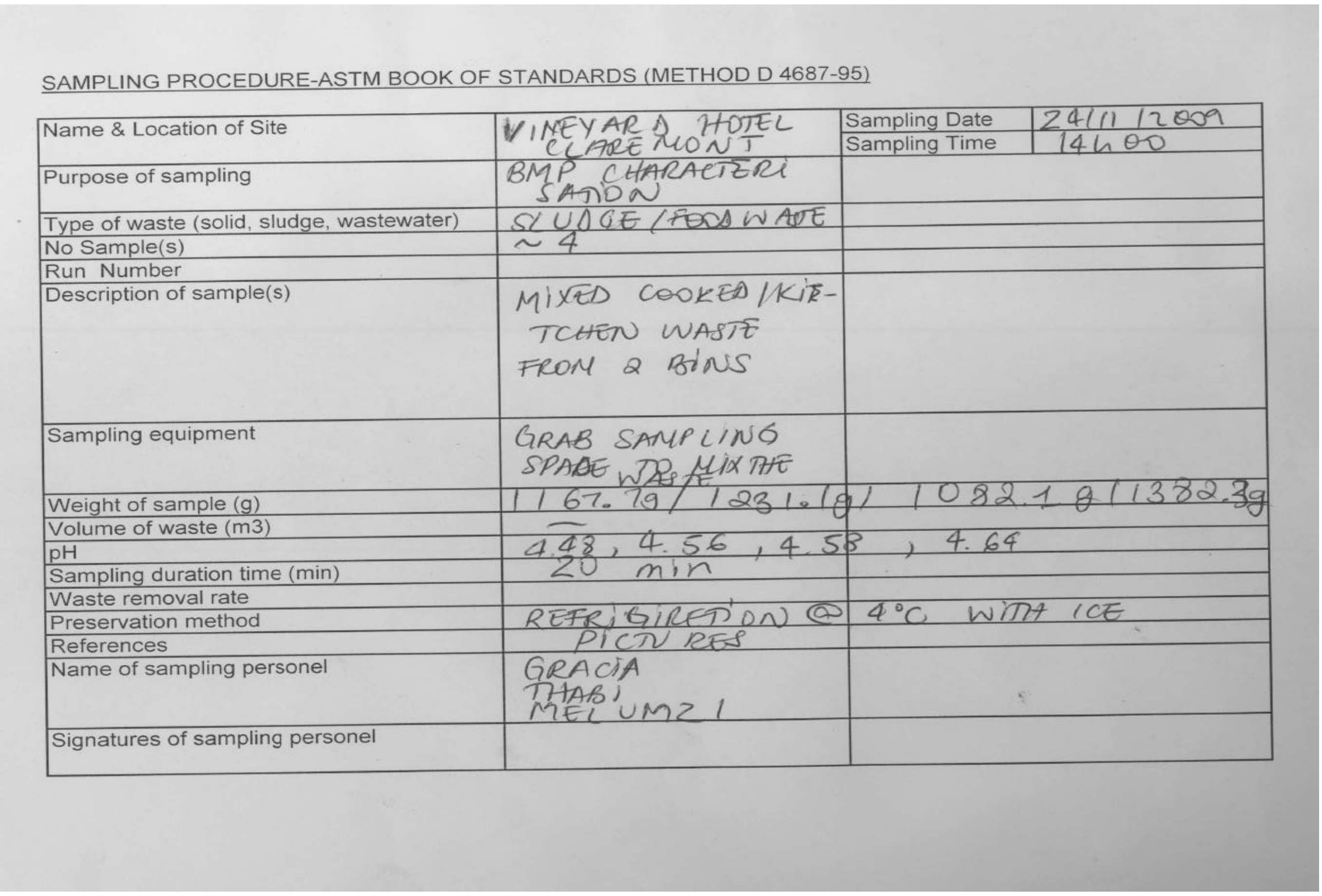
Lower experimental values are unlikely as they imply a better performance than theoretical values; these values can be linked with flaws/errors in the sampling techniques. The discrepancy can either be associated with the kinetic constants used (Table 69) or to the fact that the sample extracted were very dilute and misrepresented the reactor mix.

The second reason is most probable, particularly for garden waste because of the nature of the waste i.e. these substrates were highly heterogeneous and solid particles settled often at the bottom. It was therefore hard to ensure a representative sample was extracted from the reactors when sampling.

9.3 Raw Data

9.3.1 Sample sheets of data collection during waste sampling

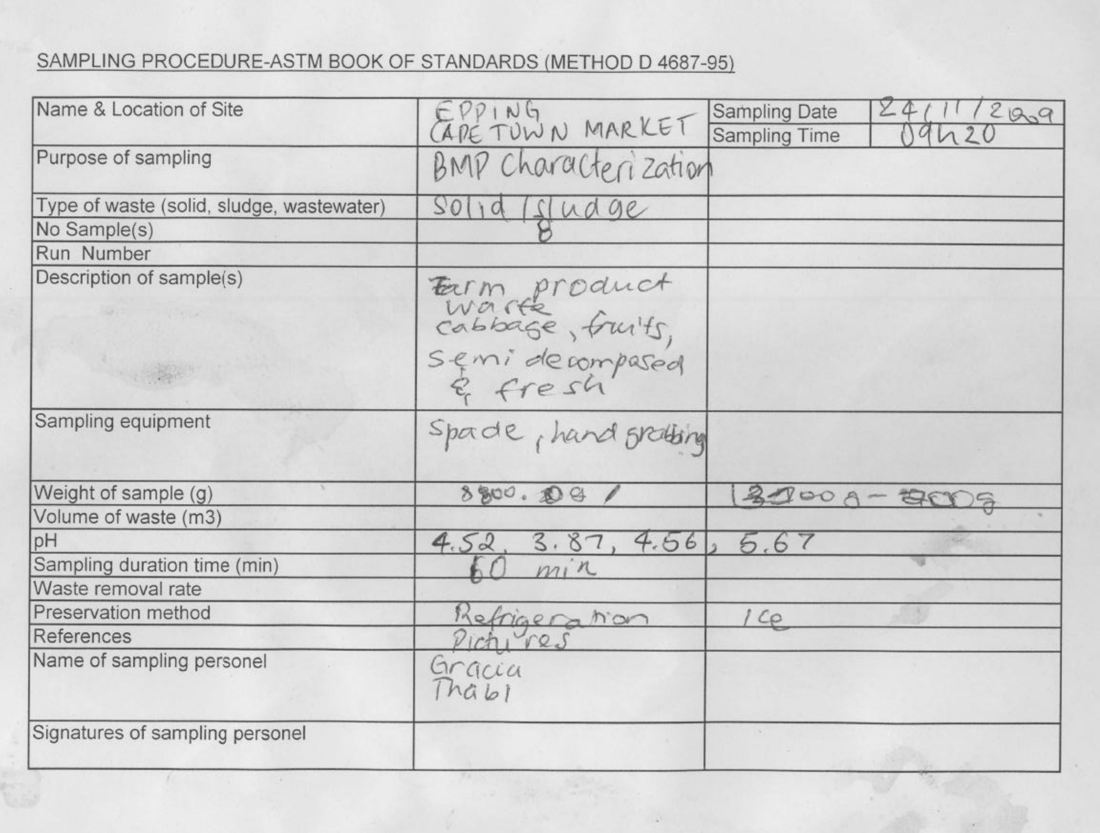
Table 71: Sampling sheet for the Vineyard Hotel



SAMPLING PROCEDURE-ASTM BOOK OF STANDARDS (METHOD D 4687-95)

Name & Location of Site	VINEYARD HOTEL CLAREMONT	Sampling Date	24/11/2009
		Sampling Time	14h00
Purpose of sampling	BMP CHARACTERISATION		
Type of waste (solid, sludge, wastewater)	SLUDGE/FOOD WASTE		
No Sample(s)	~ 4		
Run Number			
Description of sample(s)	MIXED COOKED/KITCHEN WASTE FROM 2 BINS		
Sampling equipment	GRAB SAMPLING SPADE TO MIX THE WASTE		
Weight of sample (g)	1167.7g/1231.1(g)/	1082.1 g/1382.3g	
Volume of waste (m3)	—		
pH	4.48, 4.56, 4.58	, 4.64	
Sampling duration time (min)	20 min		
Waste removal rate			
Preservation method	REFRIGIRETION @	4°C WITH ICE	
References	PICTURES		
Name of sampling personel	GRACIA THABI MELUMZI		
Signatures of sampling personel			

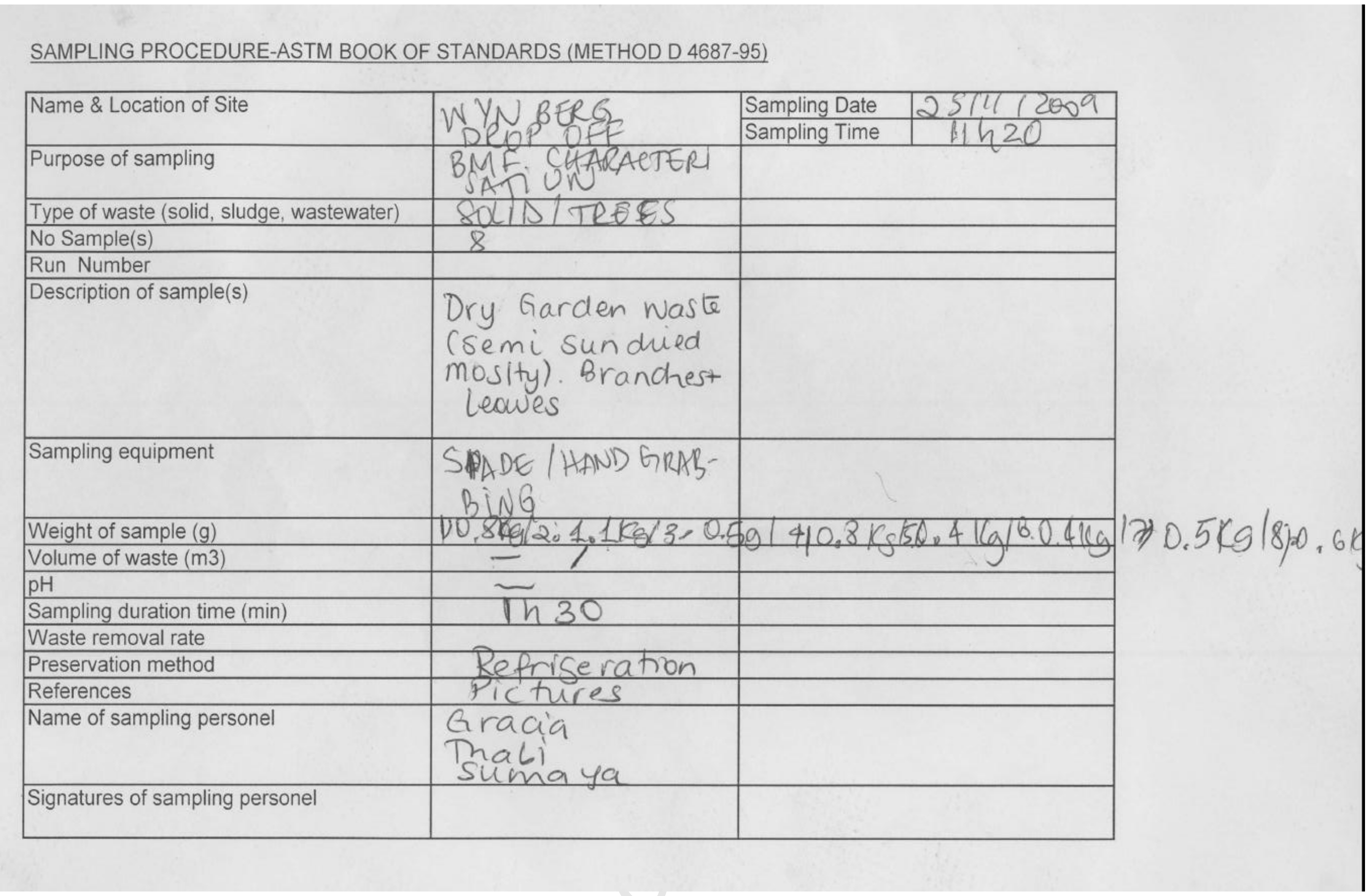
Table 72: Sampling sheet for the fruits and vegetables biomass from the Cape Town Market



SAMPLING PROCEDURE-ASTM BOOK OF STANDARDS (METHOD D 4687-95)

Name & Location of Site	EPPING CAPE TOWN MARKET	Sampling Date	24/11/2009
		Sampling Time	09h20
Purpose of sampling	BMP Characterization		
Type of waste (solid, sludge, wastewater)	Solid/sludge		
No Sample(s)	8		
Run Number			
Description of sample(s)	Farm product waste cabbage, fruits, semi decomposed & fresh		
Sampling equipment	Spade, hand grabbing		
Weight of sample (g)	8800.0g /	13000g – 9000g	
Volume of waste (m3)			
pH	4.52, 3.87, 4.56,	5.67	
Sampling duration time (min)	60 min		
Waste removal rate			
Preservation method	Refrigeration	Ice	
References	Pictures		
Name of sampling personel	Gracia Thabi		
Signatures of sampling personel			

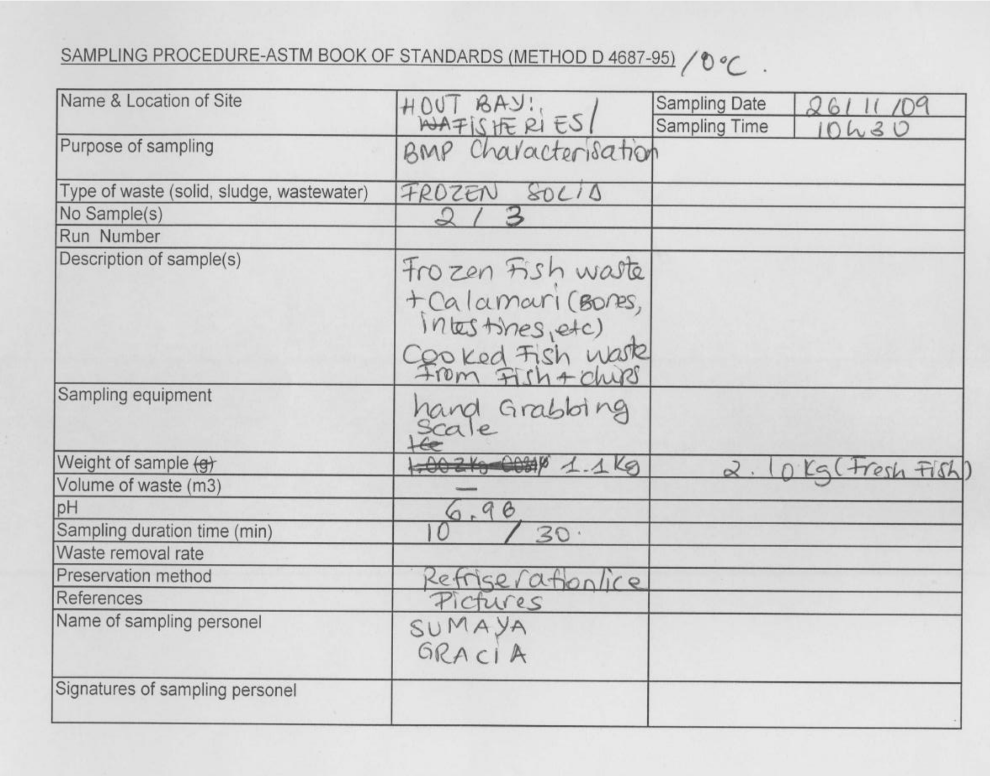
Table 73: Sampling sheet for garden waste from the Wynberg Drop-Off



SAMPLING PROCEDURE-ASTM BOOK OF STANDARDS (METHOD D 4687-95)

Name & Location of Site	WYNBERG DROP OFF	Sampling Date Sampling Time	25/11/2009 11h20
Purpose of sampling	BMF CHARACTERISATION		
Type of waste (solid, sludge, wastewater)	SOLID/TREES		
No Sample(s)	8		
Run Number			
Description of sample(s)	Dry Garden waste (semi sundried mosity). Branches+ Leaves		
Sampling equipment	SPADE/HAND GRAB-BING		
Weight of sample (g)	1) 0.8kg/2: 1.1kg/3- 0.5g/ 4) 0.3 kg/5) 0.4 kg/6. 0.4kg/7) 0.5kg/8) 0.6k		
Volume of waste (m3)	— /		
pH	—		
Sampling duration time (min)	1h 30		
Waste removal rate			
Preservation method	Refriseration		
References	Pictures		
Name of sampling personel	Gracia Thali Sumaya		
Signatures of sampling personel			

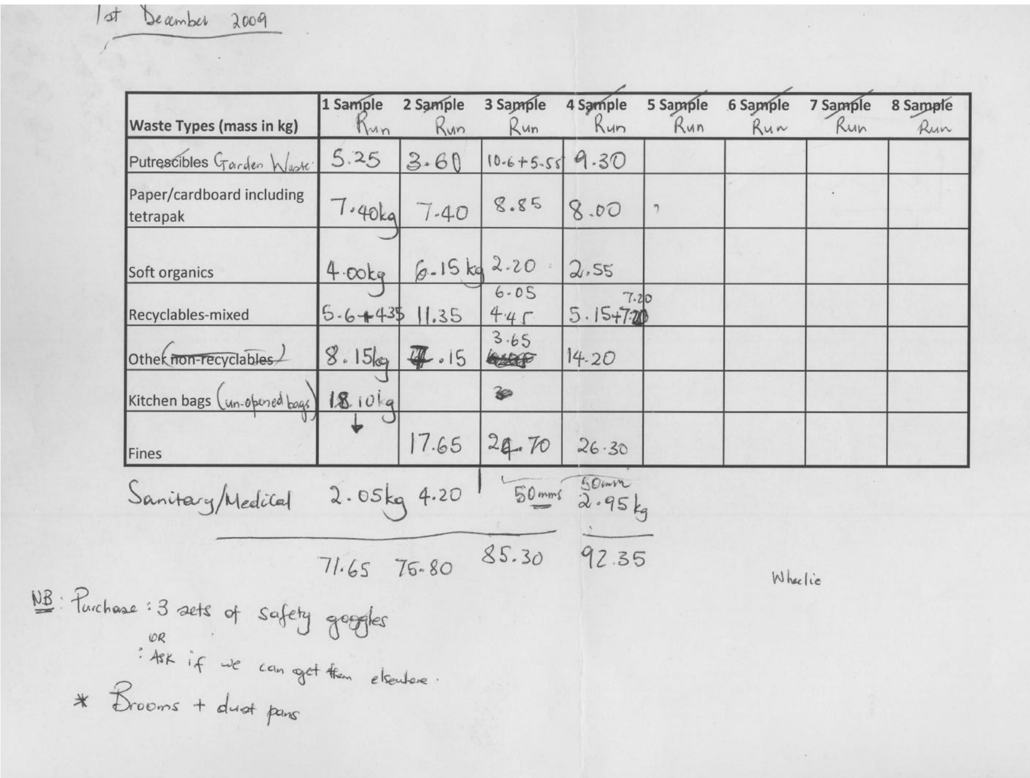
Table 74: Sampling sheet for the waste from the Hout Bay fisheries



SAMPLING PROCEDURE-ASTM BOOK OF STANDARDS (METHOD D 4687-95) /0°C .

Name & Location of Site	HOUT BAY: ~~WA~~FISHERIES/	Sampling Date	26/11/09
		Sampling Time	10h30
Purpose of sampling	BMP Characterisation		
Type of waste (solid, sludge, wastewater)	FROZEN SOLID		
No Sample(s)	2 / 3		
Run Number			
Description of sample(s)	Frozen Fish waste + Calamari (Bones, intestines, etc) Cooked Fish waste from Fish + chips		
Sampling equipment	hand Grabbing Scale ~~Ice~~		
Weight of sample ~~(g)~~	~~1.002Kg 0031~~ 1.1Kg	2.10Kg (Fresh Fish)	
Volume of waste (m3)	—		
pH	6.98		
Sampling duration time (min)	10 / 30.		
Waste removal rate			
Preservation method	Refrigeration/ice		
References	Pictures		
Name of sampling personel	SUMAYA GRACIA		
Signatures of sampling personel			

Table 75: Sample data collection sheet



1st December 2009

Waste Types (mass in kg)	1 ~~Sample~~ Run	2 ~~Sample~~ Run	3 ~~Sample~~ Run	4 ~~Sample~~ Run	5 ~~Sample~~ Run	6 ~~Sample~~ Run	7 ~~Sample~~ Run	8 ~~Sample~~ Run
~~Putrescibles~~ Garden Waste	5.25	3.60	10.6+5.55	9.30				
Paper/cardboard including tetrapak	7.40kg	7.40	8.85	8.00				
Soft organics	4.00kg	6.15 kg	2.20	2.55				
Recyclables-mixed	5.6+4.35	11.35	6.05 4.45	7.20 5.15+7.20				
Other (~~non-recyclables~~)	8.15kg	4.15	3.65	14.20				
Kitchen bags (un-opened bags)	18.10kg ↓							
Fines		17.65	24.70	26.30				

Sanitary/Medical 2.05kg 4.20 | 50mms 50mm 2.95kg

71.65 75.80 85.30 92.35

Wheelie

NB: Purchase : 3 sets of safety goggles
OR
: Ask if we can get them elsewhere.

* Brooms + dust pans

9.3.2 Raw data for BMP tests

9.3.2.1 Experimental set-up

Table 76: Experimental Set-up of the BMP assays for each different fraction of organic waste

	EW	VHW	WDO	CAV	FW	AW	CW	Paper sludge	WHW	BC FW	BC Fi	BC GW	Blood Waste
Moisture content	0.89	0.74	0.68	0.77	0.83	0.71	0.09	0.68	0.93	0.60	0.49	0.52	0.86
Fraction of TS	0.11	0.26	0.32	0.23	0.17	0.29	0.91	0.32	0.07	0.40	0.51	0.48	0.14
Fraction of VS	0.90	0.91	0.93	0.96	0.72	0.97	0.98	0.98	0.77	0.81	0.43	0.62	0.94
Total working volume, ml	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.00
Hypothetical mass,g	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.00
OFMSW (g VS)	4.48	4.55	4.63	4.79	3.61	4.84	4.88	4.92	3.83	3.238	2.15	3.09	4.58
Wet mass of OFMSW	43.71	19.44	15.75	21.78	29.90	17.01	5.49	15.63	75.81	9.99	9.84	10.50	35.35
Mass of seed	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.42
Seed Volume	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	26.88
Buffer g per 100 mL	2.62	1.17	0.94	1.31	1.79	1.02	0.33	0.94	4.55	0.76	0.59	0.63	2.12
Trace Metals Solns, mL	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
g VS inoculum	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.79
F:I	**5.53**	**5.61**	**5.72**	**5.91**	**4.45**	**5.98**	**6.03**	**6.08**	**4.73**	**4.01**	**2.65**	**3.81**	**5.78**

Table 77: Experimental set-up of the BMP assays for food waste, garden waste and fines collected at ARTS

	AFW 1	**AFW 2**	**AFW3**	**AFW 4**	**A Fi 1**	**A Fi 2**	**A Fi 3**	**A Fi 4**	**A Gw 1**	**A Gw 2**	**A Gw 3**	**A Gw 4**
Moisture content	0.83	0.84	0.83	0.78	0.02	0.18	0.06	0.08	0.05	0.04	0.04	0.16
Fraction of VS	0.17	0.16	0.17	0.22	0.98	0.82	0.94	0.92	0.95	0.96	0.96	0.84
Fraction of TS	0.82	0.90	0.88	0.68	0.55	0.46	0.50	0.49	0.77	0.80	0.74	0.68
Total working volume, ml	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Hypothetical mass,g	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
OFMSW (g VS)	4.12	4.49	4.41	3.41	2.74	2.28	2.50	2.44	3.87	3.98	3.72	3.42
Wet mass of OFMSW	29.34	31.53	29.43	23.02	5.08	6.12	5.31	5.45	5.26	5.19	5.21	5.95
Mass of seed	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98	27.98
Seed Volume	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43	27.43
Buffer g per 100 ml	1.76	1.89	1.77	1.38	0.30	0.37	0.32	0.33	0.32	0.31	0.31	0.36
Trace Metals Solns, mL	9	9	9	9	9	9	9	9	9	9	9	9
g VS inoculum	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
F: I	**5.08**	**5.55**	**5.44**	**4.21**	**3.39**	**2.81**	**3.09**	**3.02**	**4.78**	**4.92**	**4.59**	**4.23**

9.3.2.2 pH readings

Table 78: pH of different food wastes used for BMP tests

Days	0	2	4	6	14	21	28	42	56
EW	7.71	7.45	7.80	6.69	6.90	7.60	7.68	7.94	8.00
Stdev	**0.07**	**0.41**	**0.33**	**0.22**	**0.11**	**0.36**	**0.09**	**0.30**	**0.04**
VHW	6.93	4.61	5.15	6.55	5.72	6.01	6.36	6.30	6.06
Stdev	**0.09**	**0.36**	**0.81**	**1.00**	**0.29**	**0.38**	**0.26**	**0.34**	**0.65**
CAV	7.42	6.68	6.41	6.30	5.80	6.12	6.61	6.83	6.72
Stdev	**0.21**	**0.12**	**0.12**	**0.18**	**0.01**	**0.11**	**0.15**	**0.19**	**0.26**
CW	7.47	4.84	4.92	4.84	6.14	5.51	6.62	6.50	6.47
Stdev	**0.04**	**0.01**	**0.15**	**0.28**	**0.47**	**0.32**	**0.04**	**0.49**	**0.30**
BC FW	7.16	6.32	6.41	5.93	7.76			7.57	
Stdev	**0.04**	**0.21**	**0.10**		**0.07**			**0.10**	
AFW1	7.34	6.65	7.26	6.14	6.74	6.12	6.65	6.88	6.70
Stdev	**0.02**	**0.13**	**0.98**	**0.16**	**0.76**	**0.37**	**0.35**	**0.38**	**0.39**
AFW2	7.33	7.18	7.23	6.33	6.76	6.54	6.62	6.63	6.63
Stdev	**0.02**	**0.53**	**0.25**	**0.33**	**0.52**	**0.50**	**0.33**	**0.41**	**0.32**
AFW3	7.34	6.98	6.96	6.07	6.68	6.21	6.70	6.63	6.63
Stdev	**0.04**	**0.28**	**0.20**	**0.14**	**0.14**	**0.08**	**0.21**	**0.19**	**0.25**
AFW4	7.23	6.74	6.78	5.79	6.71	6.37	6.79	6.76	6.90
Stedv	**0.01**	**0.08**	**0.07**	**0.04**	**0.06**	**0.08**	**0.16**	**0.20**	**0.21**

Table 79: pH of different garden wastes used for BMP tests

Days	0	2	4	6	14	21	28	42	56
WHW	7.73	8.47	8.86	8.04	8.15	8.51	7.87	8.05	7.86
Stdev	**0.10**	**0.57**	**0.25**	**0.05**	**0.13**	**0.15**	**0.10**	**0.11**	**0.21**
BC GW	7.73	7.55	6.55	5.94	6.87	8.09	7.46	7.58	7.35
Stedv	**0.06**	**0.21**	**0.71**	**0.25**	**0.72**	**0.06**	**0.06**	**0.05**	**0.11**
AGW1	7.21	6.63	5.48	5.21	6.74	7.05	7.20	7.31	7.69
Stdev	**0.04**	**0.19**	**0.07**	**0.02**	**0.51**	**0.29**	**0.13**	**0.18**	**0.53**
AGW2	7.16	6.17	5.67	5.41	7.02	7.00	7.16	7.33	7.55
Stdev	**0.04**	**0.18**	**0.21**	**0.06**	**0.16**	**0.08**	**0.07**	**0.28**	**0.49**
AGW3	7.25	6.47	5.62	5.31	7.12	7.15	7.26	7.29	7.51
Stdev	**0.12**	**0.05**	**0.03**	**0.07**	**0.24**	**0.10**	**0.16**	**0.32**	**0.34**
AGW4	6.98	6.26	5.56	5.18	7.08	7.10	7.27	7.24	7.48
Stedv	**0.05**	**0.17**	**0.07**	**0.06**	**0.13**	**0.13**	**0.08**	**0.17**	**0.03**
WDO	7.48	6.80	5.65	6.83	6.20	7.04	7.64	7.26	7.82
Stdev	**0.25**	**0.19**	**0.05**	**0.11**	**0.02**	**0.09**	**0.17**	**0.10**	**0.61**

Table 80: pH of different fines and other wastes, used for BMP tests

Days	0	2	4	6	14	21	28	42	56
PW	7.94	7.77	7.38	6.71	6.48	7.07	7.44	7.56	7.37
Stdev	**0.11**	**0.17**	**0.17**	**0.03**	**0.22**	**0.09**	**0.13**	**0.21**	**0.06**
AFi1	7.31	6.66	6.90	6.39	7.54	6.96	7.14	7.21	7.05
Stdev	**0.04**	**0.06**	**0.26**	**0.07**	**0.31**	**0.11**	**0.08**	**0.06**	**0.05**
Afi2	7.19	6.63	6.22	5.97	7.20	7.13	7.14	7.30	7.01
Stdev	**0.02**	**0.19**	**0.16**	**0.15**	**0.15**	**0.07**	**0.06**	**0.10**	**0.03**
AFi3	7.35	6.42	6.96	6.63	7.76	6.98	7.15	7.20	7.07
Stdev	**0.09**	**0.13**	**0.08**	**0.14**	**0.48**	**0.05**	**0.08**	**0.17**	**0.01**
AFi4	7.24	6.52	6.42	6.10	7.36	6.95	7.15	7.24	7.08
Stdev	**0.05**	**0.17**	**0.03**	**0.18**	**0.02**	**0.09**	**0.03**	**0.11**	**0.05**
BC Fi	7.70	7.07	7.38	7.18	7.60	7.99	7.50	7.43	7.27
Stdev	**0.04**	**0.25**	**0.39**	**0.08**	**0.31**	**0.07**	**0.11**	**0.09**	**0.05**

Table 81: pH of high protein wastes, and their co-digestion with garde waste, paper sludge and fines

	0	2	4	6	14	21	28	42	56
FW	7.91	8.28	8.34	7.94	7.21	7.59	7.93	7.41	8.66
Stdev	**0.06**	**0.39**	**0.33**	**0.18**	**0.09**	**0.06**	**0.05**	**0.28**	**0.15**
AW	7.66	7.69	7.47	7.31	6.82	6.96	7.17	7.03	7.44
Stdev	**0.11**	**0.17**	**0.06**	**0.24**	**0.14**	**0.02**	**0.09**	**0.05**	**0.15**
BW	8.22	7.35	7.18	8.09	7.23	7.35	7.58	7.43	7.67
Stdev	**0.10**	**0.09**	**0.38**	**0.03**	**0.06**	**0.06**	**0.05**	**0.20**	**0.08**
BW+PW	8.07	8.30	7.94	7.30	7.53	0.00	0.00	6.97	
Stdev	**0.18**	**0.42**	**0.25**		**0.05**			**0.01**	
BW+GW	7.68	8.12	7.33	7.22	7.82	0.00	0.00	6.93	
Stdev	**0.05**	**0.14**	**0.17**		**0.11**			**0.01**	
BW+Fi	7.77	8.26	7.46	6.80	7.66	0.00	0.00	7.77	
Stdev	**0.10**	**0.10**	**0.25**		**0.18**			**0.65**	
AW+GW	7.32	6.74	6.84	0.00	6.89	7.63	0.00	7.60	
Stdev	**0.17**	**0.13**						**0.06**	
AW+Fi	7.22	6.87	6.82	0.00	8.33	7.73	0.00	7.56	
Stdev	**0.20**	**0.07**			**0.03**			**0.07**	
AW+PW	7.57	7.44	7.48	0.00	7.73	7.85	0.00	7.65	
Stdev	**0.28**	**0.27**			**0.62**			**0.02**	

9.3.2.3 VS readings

Table 82: Average VS measurements at start and end of BMP experiments in triplicate (n=3)

	VS Start		VS End		
	%	Stdev,%	%	Stdev,%	% Reduction
	FOOD WASTE				
BC FW	71.1	3.92	50.3	1.42	29.2
AFW 1	68.8	3.44	55.6	4.32	19.2
AFW 2	75.7	4.22	57.9	2.42	23.5
AFW 3	75.1	1.66	58.5	0.65	22.1
AFW 4	64.8	0.90	63.3	22.43	2.3
EW	84.2	1.29	36.6	1.45	56.5
VH	57.3	6.26	64.4	0.55	NA
CAV	71.1	2.94	58.3	1.53	17.9
CW	84.2	1.82	40.3	3.56	52.1
	GARDEN WASTE				
BC GW	43.3	5.50	53.8	5.50	NA
AGW 1	73.9	4.93	59.3	4.93	19.7
AGW 2	73.8	1.87	54.6	1.87	26.1
AGW 3	68.2	0.92	52.0	0.92	23.8
AGW 4	82.6	9.18	60.5	9.18	26.7
WDO	71.6	3.99	64.0	3.99	10.7
WH	45.7	2.26	36.2		20.9
	FINES AND WASTE PULP				
BC Fi	47.5	1.34	41.2	1.34	13.3
AFi 1	72.0	1.67	53.3	1.67	26.0
AFi 2	68.7	0.89	43.1	0.89	37.3
AFi 3	71.6	5.61	45.1	5.61	37.0
AFi 4	69.9	1.50	46.5	1.50	33.5
PW	31.5	0.19	25.7	0.19	
ABATTOIR AND FISHERIES WASTES					
AW	85.2	1.82	80.2	1.82	5.9
BW	74.3	0.76	64.5	0.76	13.3
FW	61.3	6.02	44.5	6.02	27.4

9.3.2.4 COD readings

Table 83: COD readings of the various BMP assays at the start and at the end, dilution factor(df)=20-25

	Start		End	
	Avg	Stdev	Avg	Stdev
	g COD/l	g COD/l	g COD/l	g COD/l
AFW 1	4.81	0.72	5.5	0.99
AFW 2	7.4	1.65	4.47	0.22
AFW 3	6.63	1.49	5.92	1.26
AFW 4	5.19	1.97	3.67	0.72
BC FW	7.55	0.48	5.54	0.60
CAV	4.3	1.17	4.57	0.87
VH	4.45	0.87	5.52	1.31
EW	4.65	1.38	1.23	0.25
CW	9.4	0.88	5.65	0.71
ABATTOIR AND FISHERIES WASTE				
FW	7.59	1.79	5.7	1.23
AW	5.28	0.43	3.95	0.65
BW	5.15	0.25	4.31	0.24
GARDEN WASTE				
BC GW	7.1	3.02	3.83	1.19
AGW 1	5.59	1.40	5.15	1.62
AGW 2	5.97	2.13	3.4	0.60
AGW 3	5.22	0.69	3.81	0.31
AGW 4	6.83	3.86	5.41	0.99
WHW	3.21	0.37	0.76	0.01
WDO	7.85	2.06	4.82	0.51
PAPER PULP AND FINES				
PW	2.86	0.35	1.79	0.77
AFi 1	5.28	2.89	4.94	0.52
AFi 2	5.36	2.73	4.58	0.23
AFi 3	8.31	1.74	4.35	1.98
AFi 4	7.47	3.62	3.37	1.41
BC Fi	5.27	0.66	3.83	1.19

9.3.2.5 Biogas readings

Table 84: (a) Daily biogas readings of BMP tests for different food wastes

Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30
EW	0	281	64	41.3	34.3	12.3	15.7	8.7	8	8.7	4.7	10.7	17.3	16.3	14.3	19	15	17.3	24	32.5	26.7	40.7	33.3	23.3	36	49	28.5	67.3	53.7	36.2
Stdev	**0**	**30.05**	**28.00**	**3.06**	**30.92**	**10.97**	**5.77**	**5.77**	**2.00**	**3.06**	**1.53**	**5.69**	**10.21**	**8.31**	**4.73**	**4.58**	**5.00**	**5.77**	**10.39**	**3.04**	**6.11**	**12.06**	**6.11**	**17.01**	**9.00**	**39.00**	**6.36**	**10.50**	**35.16**	**8.81**
VHW	0	313	67.3	273	45.3	140.7	165	118.7	42.7	26	19.3	26	22	21.7	19.8	10	31.3	0	19.8	10	0	0	0	0	0	0	31.3	0	0	0
Stdev	**0**	**18.08**	**2.31**	**33.18**	**61.33**	**54.93**	**40.63**	**94.16**	**11.02**	**7.21**	**8.62**	**3.61**	**4.00**	**12.90**	**1.26**	**0.00**	**13.50**	**0.00**	**1.26**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**13.50**	**0.00**	**0.00**	**0.00**
CAV	0	347.7	128.7	72	27.3	20.7	20.7	28	38.7	12.7	6.7	5.3	7.7	3.7	9	5.2	12	0	6	5.2	0	0	0	0	0	0	12	0	0	0
Stdev	**0**	**29.67**	**16.65**	**21.07**	**5.03**	**4.16**	**1.15**	**6.00**	**4.16**	**4.16**	**1.15**	**1.44**	**1.15**	**6.35**	**1.41**	**1.44**	**3.00**	**0.00**	**5.29**	**1.44**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**3.00**	**0.00**	**0.00**	**0.00**
CW	0	334.7	37	139.3	34.7	44	28.7	22.7	27	18	8.5	82	28	23.5	0	53	0	0	28	23.5	0	0	0	0	0	0	53	0	0	0
Stdev	**0**	**62.3**	**9.9**	**119.6**	**43.9**	**42.1**	**30.1**	**20.0**	**1.4**	**0.0**	**0.0**	**104.7**	**39.6**	**30.4**	**0.0**	**4.2**	**0.0**	**0.0**	**39.6**	**30.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**4.2**	**0.0**	**0.0**	**0.0**

(b) Daily biogas readings of BMP tests for different food wastes (ctd.)

Days	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
EW	11.37	0.00	35.16	8.81							11.37		31.75					5.77			17.32					12.10		5.51		3.79	
Stdev	**11.37**		**35.16**	**8.81**							**11.37**		**31.75**					**5.77**			**17.32**					**12.10**		**5.51**		**3.79**	
VHW	10						24.7		3			9.3						9		0.3			4								0.7
Stdev	**3.46**						**5.03**		**2.00**			**6.43**						**3.46**		**0.58**			**2.00**								**1.15**
CAV	6						2.3		1			0.3						1.7		0.3								0.3			0.7
Stdev	**0.00**						**0.58**		**1.73**			**0.29**						**1.53**		**0.58**								**0.58**			**1.15**
CW	3						5		5			25.5						6.5		4			3.8					2.5			1
Stdev	**4.24**						**7.07**		**7.07**			**34.65**						**7.78**		**5.66**			**4.60**					**0.71**			

Table 85: (a) Daily biogas readings of BMP tests for different from ARTS and BC

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30
BC FW	0.00	667.30	154.70	118.70	82.70	48.70	30.70	25.70	19.70	27.30	28.70	32.70	30.00	25.00	8.80	3.20	2.00	2.00	3.30	2.00	16.30	10.70	0.00	1.70	0.00	2.00	0.70	3.30	3.00	0.20
Stdev	0.00	54.12	54.93	43.00	42.44	5.13	11.68	0.58	3.79	22.33	32.72	21.55	12.49	8.66	3.88	0.29	1.73	2.00	1.15	1.73	4.04	3.06	0.00	2.08	0.00	1.00	0.58	3.21	2.00	0.29
A FW 1	0.00	406.70	136.00	99.30	72.30	62.70	21.30	20.00	15.30	25.00	9.50	11.30	14.30	9.00	32.70		14.30	9.00									32.70			
Stdev	0.00	27.30	74.32	19.01	36.12	17.01	4.16	5.29	6.11	21.93	8.49	11.37	8.14	7.55	16.17		8.14	7.55									16.17			
A FW 2	0.00	359.00	108.00	108.00	34.00	38.00	30.00	30.00	36.00	29.00	9.00	0.00	1.00	3.00	11.00		1.00	3.00									11.00			
Stdev	0.00	34.24	18.90	43.86	9.02	7.21	1.00	4.62	6.93	1.41	4.24	32.19	8.19	2.00	5.00		8.19	2.00									5.00			
A FW 3	0.00	337.50	92.00	52.00	42.00	30.00	24.00	22.00	23.00			38.00	16.00	9.00	62.00		16.00	9.00									62.00			
Stdev	0.00	28.37	8.33	16.17	15.14	13.32	12.70	2.31	0.58	6.36	6.08	16.77	6.03	3.51	28.62		6.03	3.51									28.62			
A FW 4	0.00	380.00	26.00	30.00	30.00	50.00	32.00	22.00	28.00	0.00	0.00	59.00	26.00	13.00	48.00		26.00	13.00									48.00			
Stdev	0.00	20.43	64.04	20.82	6.93	5.77	5.29	4.16	7.57	3.54	0.00	33.77	11.59	7.00	19.92		11.59	7.00									19.92			

(b) Daily biogas readings of BMP tests for different from ARTS and BC

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
BC FW	8.30	0.00	3.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	8.30	0.00	51.30	0.00	0.00	0.00	0.00	13.30	0.00	0.00	32.00	0.00	0.00	0.00	0.00	36.00		46.30		28.00	
Stdev	2.08		2.00	0.29							2.08		24.66					11.34			7.21					15.00		32.08		18.36	
A FW 1	5.30						0.70		0.70			0.70						6.70		0.70			3.30					20.00			27.00
Stdev	1.53						1.15		0.76			1.15						4.62		1.15			5.77					25.46			29.70
A FW 2	2.70						1.00					0.70						1.30													
Stdev	1.53						1.00					1.15						2.31													
A FW 3	3.70						1.70		0.70											2.00											
Stdev	0.58						1.53		1.15											3.46											
A FW 4	3.70																	1.00		1.00			3.00					25.00			10.70
Stdev	1.15																	1.73		1.73			5.20								11.55

Table 86: (a) Daily biogas readings of BMP tests for different garden wastes

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30	31
BC GW	0.00	49.70	123.30	57.30	32.30	16.30	15.30	13.70	15.70	35.00	34.00	87.70	72.00	77.00	40.70	39.30	43.30	29.70	48.00	62.70	40.70	25.30	29.70	28.70	30.70	53.00	16.00	44.30	47.00	29.80	80.70
Stdev	0.00	1.53	16.04	26.35	14.84	8.33	2.52	4.51	4.73	17.58	20.66	40.72	29.60	13.08	15.31	21.73	24.44	12.66	30.20	40.45	13.32	19.43	15.01	17.79	18.58	14.18	1.41	26.58	2.65	3.82	69.29
A GW 1	0.00	31.00	52.30	34.70	26.70	31.30	49.30	40.30	61.30	168.00	36.00	59.30	105.30	55.30	131.00		105.30	55.30									131.00				12.70
Stdev	0.00	3.61	6.66	4.16	3.06	1.15	8.33	2.08	3.06	19.80	1.41	3.06	14.05	5.03	68.20		14.05	5.03									68.20				2.52
A GW 2	0.00	27.30	56.70	61.30	40.70	62.00	42.70	41.30	56.00	124.00	51.00	120.70	104.00	35.70	139.00		106.00	35.70									139.00				24.00
Stdev	0.00	3.06	5.03	8.08	8.33	9.17	33.55	6.43	8.72	8.49	1.41	32.02	9.17	4.51	7.94		9.17	4.51									7.94				7.94
A GW 3	0.00	33.30	74.00	40.70	28.70	39.30	58.00	36.00	50.70	127.50	31.50	61.70	88.70	46.00	62.30		88.70	46.00									62.30				22.30
Stdev	0.00	4.16	6.93	7.02	1.15	1.15	6.00	5.29	4.16	77.07	6.36	8.74	16.17	7.21	5.86		16.17	7.21									5.86				4.93
A GW 4	0.00	51.30	86.00	42.70	32.00	39.30	49.70	31.00	39.30	82.00	55.00	78.70	66.70	60.70	87.30		66.70	60.70									87.30				23.30
Stdev	0.00	24.11	3.46	36.90	2.00	7.57	31.37	3.00	4.16	14.14	5.66	39.31	12.06	15.95	22.03		12.06	15.95									22.03				1.53
WDO	0.00	60.30	157.00	83.30	32.70	94.00	57.30	80.00	35.30	32.70	37.30	79.70	110.50	89.30	44.00		89.30	44.00									31.30				82.70
Stdev	0.00	2.08	20.22	9.87	12.06	23.07	9.45	5.29	3.06	3.06	6.11	17.62	12.02	8.33	1.73		8.33	1.73									13.50				41.49
WHW	0.00	213.00	46.00	6.00	11.00	5.30	7.70	5.80	3.30	16.00	27.30	27.00	17.70	11.30	2.40	5.50	10.70	8.00	6.30	14.50	18.30	20.00	4.00	15.00	12.30	23.30	10.00	16.70	4.70	26.00	99.30
Stdev	0.00	27.34	6.93	1.00	4.58	3.21	2.08	4.65	1.04	9.54	11.72	16.70	11.02	5.77	1.85	1.32	2.89	3.46	1.76	3.04	3.51	5.29	6.93	4.36	2.52	7.64	1.41	8.50	8.08	14.18	32.52

(b) Daily biogas readings of BMP tests for different garden wastes

	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
BC GW	47	29.8							80.7		78					25.3			58					46		31.7		22.7	
Stdev	2.65	3.82							69.29		10.58					16.20			31.75					15.87		31.50		4.16	
A GW 1					66.00		15.00	0.00		72.00						76.30		21.70			16.30					24.30			29.30
Stdev					5.29		3.00			5.29						37.82		6.03			2.31					2.31			4.16
A GW 2					66.00		15.00			72.00						76.30		21.70			16.30					24.30			29.30
Stdev					3.46		3.00			15.14						24.25		8.89			3.21					2.08			3.46
A GW 3					80.70		18.70			60.70						34.70		30.70			16.30					29.00			26.70
Stdev					3.06		4.04			3.06						18.45		25.48			2.08					5.57			2.31
A GW 4					70.00		17.30			77.30						71.70		31.70			27.70					26.00			26.00
Stdev					23.07		4.51			17.01						4.93		20.60			10.97					14.42			4.00
WDO					93.30		32.30			106.70						106.70		28.00			42.70			30.00		0.00			0.00
Stdev					34.95		13.05			37.81						20.82		6.56			15.53			10.44					
WHW	4.70	26.00							99.30		97.30					34.70			65.30					20.00		24.30		13.30	
Stdev	8.08	14.18							32.52		21.94					5.03			25.17					18.52		17.93		6.43	

Table 87: (a) Daily biogas readings of BMP tests for fines and paper sludge

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30
BC Fi	0.00	17.00	73.70	42.30	46.70	66.30	66.00	48.30	47.70	68.30	46.30	39.00	38.30	48.50	33.30	35.70	32.70	15.00	16.70	11.70	13.00	20.00	14.00	9.30	7.70	15.30	4.00	11.70	12.70	7.30
Stdev	0.00	8.54	19.40	10.41	14.01	13.80	17.35	12.29	9.29	9.07	18.18	41.58	10.41	15.55	4.16	22.48	19.43	13.86	6.43	6.81	4.36	7.21	5.29	7.57	3.79	7.57	0.71	7.37	8.33	4.54
A Fi 1	0.00	22.70	10.70	22.30	33.30	81.30	42.70	47.30	47.00	73.50	42.00	71.70	64.70	33.70	64.00		64.70	33.70									64.00			
Stdev	0.00	3.06	9.02	0.58	2.31	18.15	4.16	9.45	13.00	24.75	18.38	22.01	15.14	3.21	25.51		15.14	3.21									25.51			
A Fi 2	0.00	47.30	23.30	22.70	26.70	89.30	47.70	51.30	53.30	69.50	30.50	84.70	68.00	34.00	65.30		68.00	34.00									65.30			
Stdev	0.00	1.15	8.33	6.11	4.16	17.01	2.52	8.08	6.11	2.12	2.12	38.59	7.21	0.00	20.03		7.21	0.00									20.03			
A Fi 3	0.00	19.30	11.30	14.70	21.30	68.00	28.70	38.70	34.00	68.00	21.50	36.70	44.00	27.70	21.70		44.00	27.70									21.70			
Stdev	0.00	4.04	2.31	3.06	3.06	2.00	5.03	1.15	4.00	8.49	3.54	14.98	5.29	1.53	18.50		5.29	1.53									18.50			
A Fi 4	0.00	34.70	20.30	31.00	32.00	80.00	43.30	46.70	54.70	102.00	36.00	72.70	51.00	42.00	87.30		34.00	47.00									87.30			
Stdev	0.00	4.62	4.51	9.64	4.00	7.21	3.06	6.11	7.02	18.38	12.73	35.23	1.41	14.00	26.84		29.46	14.00									26.84			
PW	0.00	0.70	3.30	24.30	48.70	50.70	52.30	88.70	18.70	36.70	28.70	0.00	63.30	14.00	9.00	10.70	77.00		15.00	10.70							77.00			
Stdev	0.00	0.76	2.89	4.51	7.57	9.24	10.02	31.90	13.32	12.86	4.16	0.00	5.86	1.73	4.58	5.69	22.91		1.00	5.69							22.91			

(b) Daily biogas readings of BMP tests for fines and paper sludge

	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
BC Fi	33.70		12.70	7.30							33.70		44.70					7.00			16.00	0.00	0.00			22.00		4.70	
Stdev	22.12		8.33	4.54							22.12		9.02					1.73			2.00	#DIV/0!	#DIV/0!			6.08		0.58	
A Fi 1	11.70						27.00		8.30			11.30						22.70		7.30			4.00					9.30	
Stdev	2.08						7.94		5.77			4.16						7.37		2.52			1.00					0.58	
A Fi 2	18.70						43.30		9.30			17.70						26.00		8.00			5.30					6.70	
Stdev	12.58						12.22		1.15			1.53						1.00		1.73			0.58					1.53	
A Fi 3	9.30						16.00		4.70			8.70						12.30		4.00			3.30					5.00	
Stdev	7.65						2.00		1.15			0.58						2.08		2.65			0.58					0.00	
A Fi 4	20.00						46.00		9.70			22.70						27.00		10.30			14.00					10.70	
Stdev	6.00						2.00		1.53			1.15						2.65		1.53			2.65					2.08	
PW	22.30						19.00		7.00			1.00																1.30	
Stdev	13.32						7.55		2.00			1.00																2.31	

Table 88: (a) Daily biogas readings of BMP tests for abattoir and fisheries wastes

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30
AW	0.0	13.3	52.0	56.0	22.7	16.0	26.7	41.3	11.3	8.7	7.0	11.3	7.0	13.7	6.3	3.5	19.7		6.3	3.5	0.0	0.0	0.0	0.0	0.0	0.0	19.7	0.0	0.0	0.0
Stdev	0.0	1.5	9.2	10.4	9.2	2.0	1.2	4.2	5.1	1.2	1.0	1.2	1.0	4.0	1.5	1.3	9.5		1.5	1.3							9.5			
BW	0.0	1.7	12.3	26.0	47.7	76.0	72.7	44.7	12.7	10.0	6.3	5.0	4.0	6.7	13.0	4.0	7.0		7.3	4.0							7.0			
Stdev	0.0	1.5	1.5	12.5	17.2	49.5	14.2	10.1	6.1	2.0	2.1	2.6	4.1	1.2	4.4	3.0	0.0		3.1	3.0							0.0			
FW	0.0	24.7	37.3	20.7	9.7	14.0	20.0	49.3	20.0	14.0	16.7	0.0	63.3	33.3	32.3	18.7	87.0		32.3	18.7							87.0			
Stdev	0.0	4.2	8.1	9.0	1.5	4.0	7.2	9.9	6.0	2.0	3.1	#DIV/0!	25.9	7.0	8.7	5.5	15.7		8.7	5.5							15.7			

(b) Daily biogas readings of BMP tests for abattoir and fisheries wastes

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
AW	4.00						2.00					7.70						9.70		0.00								0.30			0.70
Stdev	1.00						1.73					1.53						1.15		0.00								0.58			0.58
BW	6.00						10.30		9.00			4.70								0.00								4.30			4.00
Stdev	0.00	1.53	1.53	12.49	17.24	49.52	14.19	10.07	6.11	2.00	2.08	2.65	4.09	1.15	4.36	3.00	0.00		3.06	3.00											
FW	7.70						11.00		6.70			39.30	0.00	0.00	0.00	0.00	0.00	36.00		19.30			39.30			37.30		69.30			46.70
Stdev	0.00	4.16	8.08	9.02	1.53	4.00	7.21	9.87	6.00	2.00	3.06	#DIV/0!	25.89	7.02	8.74	5.51	15.72		8.74	5.51							15.72				

Table 89: (a) Daily biogas readings of BMP tests for the co-digestion of blood and abattoir wastes, with garden waste, fines and paper sludge

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30
BW+ PW	0.00	24.00	150.70		126.70			14.70	12.70		5.00		5.30		7.30		4.70		720.00	0.00	0.00	8.70								
	0.00	2.00	17.93		29.48			1.15	2.89		1.00		3.06		3.06		1.15					4.62								
BW+ Fi	0.00	62.00	95.30		236.30			57.70	52.00		20.30		9.70		17.30		4.70					17.00								
	0.00	4.00	13.50		59.16			8.50	1.00		10.12		1.15		7.02		0.58					7.94								
BW+ GW	0.00	92.70	86.00		143.30			77.00	60.00		9.30		7.00		6.70		6.30					15.00								
	0.00	35.85	17.78		20.03			8.54	7.21		10.69		1.73		4.16		3.21					5.57								
AW + Fi	0.00	61.30	136.70		106.00			34.70	79.00		50.30		90.70		89.30		34.70					87.30								
	0.00	7.02	44.60		6.93			4.51	24.27		13.05		29.01		39.31		37.86					4.62								
AW + PW	0.00	26.70	98.70		79.30			31.00	3.70		1.70		22.30		25.30		70.70					31.30								
	0.00	5.03	25.79		1.15			3.61	4.04		2.89		19.40		9.02		8.08					2.31								
AW+ GW	0.00	68.00	182.00		130.00			66.70	68.30		30.00		71.70		121.70		14.00					78.00								
	0.00	12.17	32.19		20.00			2.31	61.71		46.86		50.08		36.12		2.65					14.42								

(b) Daily biogas readings of BMP tests for the co-digestion of blood and abattoir wastes, with garden waste, fines and paper sludge

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
BW+ PW						32.00																								
Stdev						12.49																								
BW+ Fi						31.30																								
Stdev						9.45																								
BW+ GW						26.70																								
Stdev						6.11																								
AW + Fi						57.30																								
Stdev						37.65																								
AW + PW						124.70																								
Stdev						63.13																								
AW+GW						47.3																								
Stdev						23.86																								

9.3.3 Data collected for the fed-batch experiments

9.3.3.1 *pH readings*

Table 90: pH readings of continuous experiments at 2 L scale.

Days	0	0.5	1	1.5	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Kitchen Flat,a	7.85	7.91	7.95	7.78	7.93	7.4			7.01	7.37	6.84	6.77	6.95	6.92	7.52	6.61	6.82	6.66	7.01	6.46	6.63	6.91	6.99
Kitchen Flat,b	7.77		8.1	7.94		7.45			7.09	7.57	6.92	6.7	6.84	6.82	7.34	6.59	6.87	6.54	6.77	6.51	6.5	6.67	6.84
Blood, a	7.73	8	8.34	8.13	8.18	7.81			7.54	8.17	7.88	7.23	7.38	7.83	7.7	7.09	7.15	7.13	7.43	6.94	7.14	7.45	7.83
Blood, b	7.76		8.18			7.9			7.76	8.35	8.01	7.51	7.39	7.75	8.29	7.63	7.39	7.48	7.45	7.08	7.25	7.34	8.25
Kitchen Prog, a	7.89	8.09	8.25	8.16	8.23	7.83			7.41	7.72	7.32	6.82	6.91	7.42	7.51	6.84	7.02	6.81	7.26	6.45	6.48	6.8	6.7
Kitchen Prog, b	8.34					7.75			7.32	7.65	7.16		6.84	7.13	6.97	6.78	6.93	6.62	6.76	6.35	7.68	6.79	6.56
Garden, a	7.85	7.72	7.77	7.72								7.3	7.27	7.47	7.65	6.96	6.85	6.77	6.77	6.46	6.5	7.59	6.92
Garden, b	7.82												7.76	7.75	8.12	7.34	7.27	6.99	7.34	6.67	6.91	7.47	7.56
G+B, a	8.51		7.98		8	8.11	8.07	8.04	7.84	7.64	7.69	7.58	7.08	7.12	7.34	8.18	7.41	7.24	7.04	7.32	7.56	7.2	7.2
G+B, b	7.96		8.02		8.05	8.16	8.12	8.12	8.25	7.86	8.08	7.84	7.53	7.74	7.62	8.19	7.43	8.32	7.15	7.85	7.62	7.79	7.48

Table 91: pH readings of experiments at 2 L scale (ctd.)

Days	21	22	23	25	26	27	28	29	30	31	32	33	34	35	36	37	41	42	43	44
Kitchen Flat,a	6.48	6.87	5.99	6.47	6.72	6.57	6.95	6.6	6.89	6.71	6.83	6.6	6.77	6.81	6.86	6.26	6.3	6.96	6.53	6.89
Kitchen Flat,b	6.63	6.79	6.35	6.69	6.75	7.08	7.11	6.73	6.95	6.8	6.93	0	6.94	6.75	6.68	6.48	6.24	7.17	6.75	6.79
Blood, a	7.26	7.19	6.97	7.38	7.18	7.43	7.02	7.01	7.09	7.32	7.37	7.35	7.37	6.44	7.47	7.08	7.5	7.38	7.29	7.29
Blood, b							7.07	7.13	7.21	7.06	7.25	7.14	7.2	5.93	7.81	6.91	7.28	7.4	7	7.34
Kitchen Prog, a	6.88	6.42	5.96	6.47	6.56	6.51	6.77	6.51	6.72	6.66	6.65	6.54	6.37	7.37	6.09	5.74	6.28	6.78	6.4	6.79
Kitchen Prog, b	6.52	6.05	5.64	6.26	6.53	6.58	6.68	6.47	6.5	6.46	6.48	6.33	6.15	7.17	5.68	5.47	5.89	6.87	6.62	6.79
Garden, a	6.58	6.82	6.52	6.83	6.78	6.74	6.74	6.65	7	6.79	6.98	6.792	6.67	6.83	6.93	6.58	6.91	7.22	7.04	
Garden, b	6.89	6.92	6.68	7.04	7.08	7.48	7	6.74	7.12	6.23	7.25	7.17								6.52
G+B, a				7.47	7.31	7.38	7.33						7.53	7.55	7.63	7.33	7.1	7.39	7.3	
G+B, b				7.49	7.47	7.44	7.82						7.48	7.66	7.55	7.44	7.18	8.54	7.31	7.265

Table 92: pH readings of experiments at 2 L scale (ctd.)

Days	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Kitchen Flat,a	6.34	6.2	6.97	6.52	6.86	6.63	6.37	6.33	6.61	6.47	6.55	6.08	6.33	6.98	7.48	6.34
Kitchen Flat,b	6.61	6.49	6.92	6.64	6.87	6.67	6.59	6.47	6.85	6.49	6.91	6.29	6.92	6.91	7.37	6.21
Blood, a	7.3	7.18	7.48	7.14	7.54	7.18	7.23	7.11	7.17	7.13	7.97	7.03	7.64	7.47	8	7.17
Blood, b	7.28	7.08	7.29													
Kitchen Prog, a	6.5	5.87	6.58	6.38	6.31	6.1	6.06	6.5	7	6.78	7.09	6.71	7.15	6.98	8.54	6.51
Kitchen Prog, b	6.52	6.18	6.86	6.51	6.71	6.38	6.46	6.49	6.93	6.7	7.05	6.62	6.97	6.92	7.23	6.5
Garden, a	6.6	6.76	6.87	6.69	6.88	7.1	6.92	6.69	7.01	6.93	7.74	7.23	7.11	7.33	7.04	7.24
Garden, b																
G+B, a	7.18	7.38	7.22	7.91	7.46	7.85	7.88	7.89	7.71	7.22						
G+B, b	7.34	7.3	7.29	8.05	7.46	8.43	7.91	7.85	7.72	7.41						

9.3.3.2 *Biogas and feeding regime of the different substrates*

Table 93: (a) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor a, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas K. Flat a, mL/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	790.00	650.00	670.00
g VS fed/day	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	790.00	1440.00	2110.00
Cumulative VS (g VS)	3.26	6.51	9.77	13.03	16.29	19.54	22.80	26.06	29.31	32.57	35.83	39.09	42.34	45.60	48.86	52.11	55.37	58.63	61.89
Biogas, mL/g VS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.27	24.56	34.10

(b) Biogas yield and amount fed for kitchen waste on a flat feeding rate for reactor a, days 19-37

	19	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas K. Flat a, mL/day	10	570	620	690	90	175	230	160	130	250	490	805	930	80	40	180	900
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	2120	2715	3335	4025	4115	4290	4520	4680	4810	5060	5550	6355	7285	7365	7405	7585	8485
Cumulative VS (g VS)	65.14286	71.65714	74.91429	78.17143	81.42857	84.68571	87.94286	91.2	94.45714	97.71429	100.9714	104.2286	107.4857	110.7429	114	117.2571	120.5143
Biogas, mL/g VS	32.54386	37.88876	44.51754	51.4894	50.53509	50.65789	51.39701	51.31579	50.92257	51.78363	54.96604	60.97177	67.77645	66.50542	64.95614	64.68689	70.40659

(c) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor a, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Biogas K. Flat a, mL/day	440	1420	230	1000	1045	550	55	300	380	310	590	395	480	320	440	270	300	140
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	8925	10345	10575	11575	12620	13170	13225	13525	13905	14215	14805	15200	15680	16000	16440	16710	17010	17150
Cumulative VS (g VS)	123.7714	127.0286	130.2857	133.5429	136.8	140.0571	143.3143	146.5714	149.8286	153.0857	156.3429	159.6	162.8571	166.1143	169.3714	172.6286	175.8857	179.1429
Biogas, mL/g VS	72.10873	81.43837	81.16776	86.67629	92.25146	94.03305	92.2797	92.27583	92.80606	92.85648	94.69572	95.2381	96.2807	96.31923	97.06478	96.79742	96.71053	95.73365

(d) Biogas yield and amount fed for kitchen on a flat feeding regime for reactor a, days 56-60

	56	57	58	59	60
Biogas K. Flat a, mL/day	360	430	840	290	490
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	17510	17940	18780	19070	19560
Cumulative VS (g VS)	182.4	185.6571	188.9143	192.1714	195.4286
Biogas, mL/g VS	95.99781	96.62973	99.41016	99.23431	100.0877

Table 94: (a) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor b, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas K. Flat b, ml/day	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	795	41	10
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	795	836	846
Cumulative VS (g VS)	3.257143	6.514286	9.771429	13.02857	16.28571	19.54286	22.8	26.05714	29.31429	32.57143	35.82857	39.08571	42.34286	45.6	48.85714	52.11429	55.37143	58.62857	61.88571
Biogas, mL/g VS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.35759	14.25926	13.67036

(b) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor b, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas K. Flat b, ml/day	660	630	600	650	540	1170	1132	900	1300	910	890	730	845	1230	300	710	195	260
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	1506	2136	2736	3386	3926	5096	6228	7128	8428	9338	10228	10958	11803	13033	13333	14043	14238	14498
Cumulative VS (g VS)	65.14286	68.4	71.65714	74.91429	78.17143	81.42857	84.68571	87.94286	91.2	94.45714	97.71429	100.9714	104.2286	107.4857	110.7429	114	117.2571	120.5143
Biogas, mL/g VS	23.11842	31.22807	38.18182	45.19832	50.22295	62.58246	73.54251	81.05263	92.41228	98.85965	104.6725	108.5257	113.2415	121.2533	120.396	123.1842	121.4254	120.3011

(c) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor b, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Biogas K. Flat b, ml/day	250	140	410	340	335	800	50	330	330	270	500	400	455	370	360	380	390	170
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	14748	14888	15298	15638	15973	16773	16823	17153	17483	17753	18253	18653	19108	19478	19838	20218	20608	20778
Cumulative VS (g VS)	123.7714	127.0286	130.2857	133.5429	136.8	140.0571	143.3143	146.5714	149.8286	153.0857	156.3429	159.6	162.8571	166.1143	169.3714	172.6286	175.8857	179.1429
Biogas, mL/g VS	119.1551	117.202	117.4189	117.101	116.7617	119.7583	117.3854	117.0283	116.6867	115.9677	116.7498	116.8734	117.3298	117.2566	117.1272	117.1185	117.167	115.9856

(d) Biogas yield and amount fed for kitchen waste on a flat feeding regime for reactor b, days 55-61

	56	57	58	59	60	61
Biogas K. Flat b, ml/day	360	245	400	850	350	50
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	21138	21383	21783	22633	22983	23033
Cumulative VS (g VS)	182.4	185.6571	188.9143	192.1714	195.4286	198.6857
Biogas, mL/g VS	115.8882	115.1747	115.3063	117.7751	117.6031	115.9268

Table 95: (a) Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor a, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas K. Prog a, ml/day	0	0	0	0	0	0	0	0	0	0	10	20	5	2	40	10	2	10	45
g VS fed/day	0.971429	1.098095	1.224762	1.351429	1.478095	1.604762	1.731429	1.858095	1.984762	2.111429	2.238095	2.364762	2.491429	2.618095	2.744762	2.871429	2.998095	3.124762	3.251429
Cumulative biogas, mL	0	0	0	0	0	0	0	0	0	0	10	30	35	37	77	87	89	99	144
Cumulative VS (g VS)	0.971429	2.069524	3.294286	4.645714	6.12381	7.728571	9.46	11.3181	13.30286	15.41429	17.65238	20.01714	22.50857	25.12667	27.87143	30.74286	33.74095	36.86571	40.11714
Biogas, mL/g VS	0	0	0	0	0	0	0	0	0	0	0.566496	1.498715	1.554963	1.472539	2.762686	2.829926	2.637744	2.685422	3.589488

(b) Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor a, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas K. Prog a, ml/day	10	15	30	70	80	188	170	195	220	235	265	245	210	90	395	110	130	185
g VS fed/day	3.378095	3.504762	3.631429	3.758095	3.884762	4.011429	4.138095	4.264762	4.391429	4.518095	4.644762	4.771429	4.898095	5.024762	5.151429	5.278095	5.404762	5.531429
Cumulative biogas, mL	154	169	199	269	349	537	707	902	1122	1357	1622	1867	2077	2167	2562	2672	2802	2987
Cumulative VS (g VS)	43.49524	47	50.63143	54.38952	58.27429	62.28571	66.42381	70.68857	75.08	79.5981	84.24286	89.01429	93.91238	98.93714	104.0886	109.3667	114.7714	120.3029
Biogas, mL/g VS	3.540617	3.595745	3.930365	4.945805	5.988919	8.62156	10.64377	12.7602	14.94406	17.04815	19.25386	20.97416	22.11636	21.9028	24.61365	24.43158	24.41374	24.829

(c)Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor a, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Biogas K. Prog a, ml/day	350	185	410	460	370	560	50	480	360	260	300	90	375	510	420	375	450	190
g VS fed/day	5.658095	5.784762	5.911429	6.038095	6.164762	6.291429	6.418095	6.544762	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	3337	3522	3932	4392	4762	5322	5372	5852	6212	6472	6772	6862	7237	7747	8167	8542	8992	9182
Cumulative VS (g VS)	125.961	131.7457	137.6571	143.6952	149.86	156.1514	162.5695	169.1143	172.3714	175.6286	178.8857	182.1429	185.4	188.6571	191.9143	195.1714	198.4286	201.6857
Biogas, mL/g VS	26.49234	26.73332	28.56372	30.56469	31.77632	34.0823	33.04432	34.60382	36.03846	36.8505	37.85657	37.67373	39.03452	41.06391	42.55546	43.76665	45.31605	45.52628

(d) Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor a, days 55-61

	56	57	58	59	60	61
Biogas K. Prog a, ml/day	445	470	365	400	360	50
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	9627	10097	10462	10862	11222	11272
Cumulative VS (g VS)	204.9429	208.2	211.4571	214.7143	217.9714	221.2286
Biogas, mL/g VS	46.97407	48.49664	49.47575	50.58816	51.48381	50.95183

Table 96: (a) Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor b, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Biogas K. Prog b, ml/day	0	0	0	50	20	30	40	45	75	70	40	60	80	40	80	75	100	20
g VS fed/day	0.971429	1.098095	1.224762	1.351429	1.478095	1.604762	1.731429	1.858095	1.984762	2.111429	2.238095	2.364762	2.491429	2.618095	2.744762	2.871429	2.998095	3.124762
Cumulative biogas, mL	0	0	0	50	70	100	140	185	260	330	370	430	510	550	630	705	805	825
Cumulative VS (g VS)	0.971429	2.069524	3.294286	4.645714	6.12381	7.728571	9.46	11.3181	13.30286	15.41429	17.65238	20.01714	22.50857	25.12667	27.87143	30.74286	33.74095	36.86571
Biogas, mL/g VS	0	0	0	10.76261	11.43079	12.939	14.79915	16.34551	19.54467	21.40871	20.96035	21.48159	22.65804	21.891	22.60379	22.93216	23.85825	22.37852

(b) Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor b, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas K. Prog b, ml/day	30	110	90	155	155	165	300	210	410	295	195	280	450	40	270	270	200	325
g VS fed/day	3.378095	3.504762	3.631429	3.758095	3.884762	4.011429	4.138095	4.264762	4.391429	4.518095	4.644762	4.771429	4.898095	5.024762	5.151429	5.278095	5.404762	5.531429
Cumulative biogas, mL	960	1070	1160	1315	1470	1635	1935	2145	2555	2850	3045	3325	3775	3815	4085	4355	4555	4880
Cumulative VS (g VS)	43.49524	47	50.63143	54.38952	58.27429	62.28571	66.42381	70.68857	75.08	79.5981	84.24286	89.01429	93.91238	98.93714	104.0886	109.3667	114.7714	120.3029
Biogas, mL/g VS	22.07138	22.76596	22.91067	24.17745	25.22553	26.25	29.13112	30.34437	34.03037	35.80488	36.1455	37.35355	40.19704	38.55984	39.24542	39.82018	39.68758	40.56429

(c)Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor b, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Biogas K. Prog b, ml/day	380	105	340	850	480	575	65	425	475	325	355	125	345	395	410	355	415	195
g VS fed/day	5.658095	5.784762	5.911429	6.038095	6.164762	6.291429	6.418095	6.544762	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	5260	5365	5705	6555	7035	7610	7675	8100	8575	8900	9255	9380	9725	10120	10530	10885	11300	11495
Cumulative VS (g VS)	125.961	131.7457	137.6571	143.6952	149.86	156.1514	162.5695	169.1143	172.3714	175.6286	178.8857	182.1429	185.4	188.6571	191.9143	195.1714	198.4286	201.6857
Biogas, mL/g VS	41.75897	40.72239	41.44355	45.61738	46.94381	48.73474	47.21057	47.8966	49.74722	50.67513	51.73694	51.49804	52.45415	53.64228	54.86824	55.77148	56.94744	56.99462

(d)Biogas yield and amount fed for kitchen waste on a progressive feeding regime for reactor b, days 56-61

	56	57	58	59	60	61
Biogas K. Prog b, mL/day	375	360	440	370	410	50
g VS fed/day	3.257143	3.257143	3.257143	3.257143	3.257143	3.257143
Cumulative biogas, mL	11870	12230	12670	13040	13450	13500
Cumulative VS (g VS)	204.9429	208.2	211.4571	214.7143	217.9714	221.2286
Biogas, mL/g VS	57.91858	58.74159	59.91758	60.73187	61.70533	61.02286

Table 97:Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor a, days 0-19

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
biogas Garden a, ml/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	48.00	35.00	60.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25
Cumulative biogas, mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	49.00	84.00	144.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12
Biogas, mL/ g VS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	1.45	2.28	3.59

(b) Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor a, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
biogas Garden a, ml/day	50.00	25.00	60.00	55.00	50.00	80.00	440.00	260.00	385.00	355.00	370.00	225.00	500.00	310.00	385.00	210.00	345.00	510.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28	5.40	5.53
Cumulative biogas, mL	194.00	219.00	279.00	334.00	384.00	464.00	904.00	1164.00	1549.00	1904.00	2274.00	2499.00	2999.00	3309.00	3694.00	3904.00	4249.00	4759.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37	114.77	120.30
Biogas, mL/ g VS	4.46	4.66	5.51	6.14	6.59	7.45	13.61	16.47	20.63	23.92	26.99	28.07	31.93	33.45	35.49	35.70	37.02	39.56

(c) Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor a, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
biogas Garden a, ml/day	360.00	320.00	370.00	800.00	540.00	530.00	125.00	520.00	400.00	450.00	510.00	450.00	505.00	400.00	460.00	450.00	520.00	180.00
g VS fed/day	5.66	5.78	5.91	6.04	6.16	6.29	6.42	6.54	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	0.00	0.00
Cumulative biogas, mL	5119.00	5439.00	5809.00	6609.00	7149.00	7679.00	7804.00	8324.00	8724.00	9174.00	9684.00	10134.00	10639.00	11039.00	11499.00	11949.00	12469.00	12649.00
Cumulative VS (g VS)	125.96	131.75	137.66	143.70	149.86	156.15	162.57	169.11	172.37	175.63	178.89	182.14	185.40	188.66	191.91	195.17	195.17	195.17
Biogas, mL/ g VS	40.64	41.28	42.20	45.99	47.70	49.18	48.00	49.22	50.61	52.24	54.14	55.64	57.38	58.51	59.92	61.22	63.89	64.81

(c) Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor a, days 56-61

	56	57	58	59	60	61
biogas Garden a, ml/day	490.00	510.00	440.00	515.00	500.00	175.00
g VS fed/day	0.00	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	13139.00	13649.00	14089.00	14604.00	15104.00	15279.00
Cumulative VS (g VS)	195.17	198.43	201.69	204.94	208.20	211.46
Biogas, mL/ g VS	67.32	68.79	69.86	71.26	72.55	72.26

Table 98: (a) Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor b, days 0 -18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
10 Biogas Garden b, ml/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	2.00	0.00	100.00	20.00	30.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25	3.38
Cumulative biogas, mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	102.00	102.00	202.00	222.00	252.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12	43.50
Biogas, mL/ g VS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	4.44	3.98	3.59	3.32	3.02	5.48	5.53	5.79

(b) Biogas yield and amount fed for garden waste on a progressive feeding regime for reactor b, days 19-35

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35
11 Biogas Garden b, ml/day	30.00	65.00	40.00	45.00	80.00	90.00	380.00	245.00	395.00	340.00	310.00	260.00	495.00	350.00	360.00	0.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28
Cumulative biogas, mL	252.00	317.00	357.00	402.00	482.00	572.00	952.00	1197.00	1592.00	1932.00	2242.00	2502.00	2997.00	3347.00	3707.00	3707.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37
Biogas, mL/ g VS	5.79	6.74	7.05	7.39	8.27	9.18	14.33	16.93	21.20	24.27	26.61	28.11	31.91	33.83	35.61	33.90

Table 99: (a) Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor a, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas blood a,ml/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	20.00	35.00	15.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25
Cumulative biogas, mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	32.00	67.00	82.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12
Biogas, mL/ g VS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.95	1.82	2.04

	0	0	0	0	0	0	0

(b)Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor a, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas blood a,ml/day	0.00	15.00	30.00	25.00	70.00	91.00	125.00	70.00	145.00	85.00	60.00	100.00	140.00	25.00	65.00	15.00	135.00	80.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28	5.40	5.53
Cumulative biogas, mL	82.00	97.00	127.00	152.00	222.00	313.00	438.00	508.00	653.00	738.00	798.00	898.00	1038.00	1063.00	1128.00	1143.00	1278.00	1358.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37	114.77	120.30
Biogas, mL/ g VS	1.89	2.06	2.51	2.79	3.81	5.03	6.59	7.19	8.70	9.27	9.47	10.09	11.05	10.74	10.84	10.45	11.14	11.29

(c) Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor a, days 38-55

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Biogas blood a,ml/day	75.00	45.00	90.00	70.00	85.00	60.00	40.00	55.00	75.00	0.00	80.00	195.00	90.00	100.00	95.00	70.00	35.00	70.00
g VS fed/day	5.66	5.78	5.91	6.04	6.16	6.29	6.42	6.54	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	1433.00	1478.00	1568.00	1638.00	1723.00	1783.00	1823.00	1878.00	1953.00	1953.00	2033.00	2228.00	2318.00	2418.00	2513.00	2583.00	2618.00	2688.00
Cumulative VS (g VS)	125.96	131.75	137.66	143.70	149.86	156.15	162.57	169.11	172.37	175.63	178.89	182.14	185.40	188.66	191.91	195.17	198.43	201.69
Biogas, mL/ g VS	11.38	11.22	11.39	11.40	11.50	11.42	11.21	11.10	11.33	11.12	11.36	12.23	12.50	12.82	13.09	13.23	13.19	13.33

(d)Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor a, days 56-61

	56	57	58	59	60	61
Biogas blood a,ml/day	95.00	75.00	65.00	70.00	75.00	60.00
g VS fed/day	3.26	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	2783.00	2858.00	2923.00	2993.00	3068.00	3128.00
Cumulative VS (g VS)	204.94	208.20	211.46	214.71	217.97	221.23
Biogas, mL/ g VS	13.58	13.73	13.82	13.94	14.08	14.14

Table 100: (a) Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor b, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas blood b, ml/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.00	70.00	5.00	0.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25
Cumulative biogas, mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.00	78.00	83.00	83.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12
Biogas, mL/ g VS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	2.31	2.25	2.07

(b) Biogas yield and amount fed for blood waste on a progressive feeding regime for reactor b, days 19-36

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36
Biogas blood b, ml/day	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	65.00	65.00	75.00	90.00	100.00	0.00	10.00	90.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28	5.40
Cumulative biogas, mL	83.00	83.00	83.00	83.00	83.00	83.00	83.00	83.00	83.00	148.00	213.00	288.00	378.00	478.00	478.00	488.00	578.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37	114.77
Biogas, mL/ g VS	1.91	1.77	1.64	1.53	1.42	1.33	1.25	1.17	1.11	1.86	2.53	3.24	4.03	4.83	4.59	4.46	5.04

Table 101: (a) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor a, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas G+B a, ml/day	5.00	5.00	5.00	50.00	75.00	8.00	2.00	8.00	15.00	12.00	28.00	21.00	18.00	25.00	0.00	20.00	58.00	57.00	25.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25
Cumulative biogas, mL	5.00	10.00	15.00	65.00	140.00	148.00	150.00	158.00	173.00	185.00	213.00	234.00	252.00	277.00	277.00	297.00	355.00	412.00	437.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12
Biogas, mL/g VS	5.15	4.83	4.55	13.99	22.86	19.15	15.86	13.96	13.00	12.00	12.07	11.69	11.20	11.02	9.94	9.66	10.52	11.18	10.89

(b) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor a, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas G+B a, ml/day	135.00	40.00	40.00	80.00	50.00	40.00	45.00	35.00	50.00	20.00	10.00	65.00	110.00	30.00	80.00	465.00	30.00	495.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28	5.40	5.53
Cumulative biogas, mL	572.00	612.00	652.00	732.00	782.00	822.00	867.00	902.00	952.00	972.00	982.00	1047.00	1157.00	1187.00	1267.00	1732.00	1762.00	2257.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37	114.77	120.30
Biogas, mL/g VS	13.15	13.02	12.88	13.46	13.42	13.20	13.05	12.76	12.68	12.21	11.66	11.76	12.32	12.00	12.17	15.84	15.35	18.76

(c) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor a, days 38-53

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Biogas G+B a, ml/day	450.00	915.00	50.00	470.00	40.00	70.00	25.00	480.00	25.00	10.00	25.00	50.00	30.00	20.00	60.00	40.00
g VS fed/day	5.66	5.78	5.91	6.04	6.16	6.29	6.42	6.54	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	2707.00	3622.00	3672.00	4142.00	4182.00	4252.00	4277.00	4757.00	4782.00	4792.00	4817.00	4867.00	4897.00	4917.00	4977.00	5017.00
Cumulative VS (g VS)	125.96	131.75	137.66	143.70	149.86	156.15	162.57	169.11	172.37	175.63	178.89	182.14	185.40	188.66	191.91	195.17
Biogas, mL/g VS	21.49	27.49	26.67	28.82	27.91	27.23	26.31	28.13	27.74	27.28	26.93	26.72	26.41	26.06	25.93	25.71

Table 102: (a) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor b, days 0-18

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biogas G+B b, ml/day	5.00	5.00	5.00	20.00	0.00	6.00	13.00	34.00	45.00	56.00	58.00	53.00	53.00	55.00	0.00	65.00	98.00	125.00	70.00
g VS fed/day	0.97	1.10	1.22	1.35	1.48	1.60	1.73	1.86	1.98	2.11	2.24	2.36	2.49	2.62	2.74	2.87	3.00	3.12	3.25
Cumulative biogas, mL	5.00	10.00	15.00	35.00	35.00	41.00	54.00	88.00	133.00	189.00	247.00	300.00	353.00	408.00	408.00	473.00	571.00	696.00	766.00
Cumulative VS (g VS)	0.97	2.07	3.29	4.65	6.12	7.73	9.46	11.32	13.30	15.41	17.65	20.02	22.51	25.13	27.87	30.74	33.74	36.87	40.12
Biogas, mL/ g VS	5.15	4.83	4.55	7.53	5.72	5.30	5.71	7.78	10.00	12.26	13.99	14.99	15.68	16.24	14.64	15.39	16.92	18.88	19.09

(b) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor b, days 19-37

	19	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Biogas G+B b, ml/day	160.00	85.00	115.00	45.00	70.00	85.00	80.00	70.00	65.00	50.00	40.00	95.00	570.00	470.00	555.00	45.00	490.00	460.00
g VS fed/day	3.38	3.50	3.63	3.76	3.88	4.01	4.14	4.26	4.39	4.52	4.64	4.77	4.90	5.02	5.15	5.28	5.40	5.53
Cumulative biogas, mL	926.00	1011.00	1126.00	1171.00	1241.00	1326.00	1406.00	1476.00	1541.00	1591.00	1631.00	1726.00	2296.00	2766.00	3321.00	3366.00	3856.00	4316.00
Cumulative VS (g VS)	43.50	47.00	50.63	54.39	58.27	62.29	66.42	70.69	75.08	79.60	84.24	89.01	93.91	98.94	104.09	109.37	114.77	120.30
Biogas, mL/ g VS	21.29	21.51	22.24	21.53	21.30	21.29	21.17	20.88	20.52	19.99	19.36	19.39	24.45	27.96	31.91	30.78	33.60	35.88

(c) Biogas yield and amount fed for the co-digestion of blood with garden waste on a progressive feeding regime for reactor b, days 38-53

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Biogas G+B b, ml/day	465.00	980.00	500.00	40.00	60.00	390.00	350.00	380.00	25.00	180.00	75.00	90.00	70.00	20.00	20.00	20.00
g VS fed/day	5.66	5.78	5.91	6.04	6.16	6.29	6.42	6.54	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26
Cumulative biogas, mL	4781.00	5761.00	6261.00	6301.00	6361.00	6751.00	7101.00	7481.00	7506.00	7686.00	7761.00	7851.00	7921.00	7941.00	7961.00	7981.00
Cumulative VS (g VS)	125.96	131.75	137.66	143.70	149.86	156.15	162.57	169.11	172.37	175.63	178.89	182.14	185.40	188.66	191.91	195.17
Biogas, mL/ g VS	37.96	43.73	45.48	43.85	42.45	43.23	43.68	44.24	43.55	43.76	43.39	43.10	42.72	42.09	41.48	40.89

11.1.1.1 Raw data of VFAs analyses of fed-batch experiments

I. Kitchen waste on a flat feeding regime

Table 103: (a) Concentrations of the different VFA analysed for K. Flat a from day 1-29

	1	3	5	9	11	13	15	17	19	21	23	25	27	29
Lactic acid a	0.02	0.00	0.07	0.12	0.48	0.61		0.04	0.12	0.41	0.18	0.14	0.26	0.62
Acetic acid a	0.05	0.51	0.23	0.30	1.94	4.16		3.89	3.15	0.50	3.70	2.82	4.53	4.86
Propionic acid a	0.02	0.15	0.01	0.57	1.51	2.85		2.70	2.27	0.93	2.82	1.33	1.92	2.26
Iso-butyric acid a		0.00	0.04	0.06	0.73	0.33		0.22	1.28	0.18	2.14	5.18	5.19	5.28
Butyric acid a		0.00	0.11	0.17	3.06	2.28		1.45	6.19	3.19	4.01	2.26	3.36	4.10
Iso-butyric acid a			0.01	0.09	2.78	1.86		0.14	1.67	0.14	0.32	1.19	2.11	2.91
Valeric acid a				0.01		0.50		0.30	0.60	0.14	0.35	0.23	0.41	0.38

(b) Concentrations of the different VFA analysed for K. Flat a from day 31-59

	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59
Lactic acid a	0.00	0.02					0.06	0.06	1.40	1.58	1.51	0.42	0.00	0.46	0.44
Acetic acid a	2.19	5.49	1.14	6.41	5.91	5.96	6.65	5.61	6.44	6.85	10.60	10.68	7.40	5.29	5.15
Propionic acid a		2.30		2.29	2.28	2.26	2.76	2.12	2.41	2.90	2.97	2.60	153.85	125.53	174.14
Iso-butyric acid a	0.00	1.24	50.56	14.04	20.50	15.97	11.45	0.95	10.93	13.81	11.05	10.41			
Butyric acid a		3.75		7.30	9.81	6.53	8.14	5.15	5.54	8.33	8.24	7.82		16.03	
Iso-butyric acid a	8.00	1.10	8.00	1.44	1.49	1.15	2.53	1.20	4.07	5.51	6.75	6.83		14.31	17.27
Valeric acid a		1.04		1.25	1.25	1.27	1.22	2.19	0.18	0.22	0.18	0.16			

Table 104: (a) Concentrations of the different VFA analysed for K. Flat b from day 1-29

	1	3	5	9	11	13	15	17	19	21	23	25	27	29
Lactic acid b	0.00	0.01	0.01	0.09	0.01	0.05	0.35		0.09	0.05	0.24	0.06		
Acetic acid b	0.01	0.11	0.05	0.85	2.84	2.90	4.05	0.26	3.80	3.13	4.65	3.35	1.29	1.38
Propionic acid b	0.00	0.03	0.26	0.93	2.27	1.99	6.23	0.85	2.72	2.61	3.20	1.55		
Iso-butyric acid b	0.01	0.00	0.71	0.22	0.52	1.02	0.62		0.23	0.87	4.05	24.34	39.86	48.31
Butyric acid b	0.00		0.31	1.84	2.92	4.15	2.56	3.32	1.34	2.28	4.02	3.11		
Iso-butyric acid b	0.00			0.11	3.55	0.84	1.04		0.22	0.20	0.31	1.33		
Valeric acid b	0.27			0.02		0.26	0.37		0.10	0.25	0.43			

(b), Concentrations of the different VFA analysed for K. Flat b from day 31-59

	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59
Lactic acid b		0.30		0.43	0.25	0.25		0.54				1.26			
Acetic acid b	5.07	6.61	4.85	5.28	6.79	6.61		6.07	4.27	4.38	10.11	8.46	6.15	5.76	4.89
Propionic acid b		2.61		2.20	2.65	2.56		2.67					179.93	144.90	117.80
Iso-butyric acid b	58.29	5.76	59.62	7.39	7.78	6.92		9.51	94.37	141.25		171.52			
Butyric acid b		4.30		6.98	7.52	5.80		5.19					30.05	29.92	57.07
Iso-butyric acid b		1.53		2.25	2.26	1.95		1.87					8.42	7.63	
Valeric acid b		0.99		0.77	1.21	1.35		1.82					1.23	1.02	1.20

Table 105: TS and VS fraction in bioreactors

		0	3	5	7	11	13	15	17	19	23	25	27	32	34	36	38	40	44	46	48	50	52	54	56	58
TS	1	0.018	0.021	0.021	0.015	0.023	0.022	0.023	0.023	0.037	0.026	0.031	0.030	0.030	0.049	0.025	0.024	0.030	0.003	0.033	0.033	0.034	0.032	0.034	0.038	0.038
	2	0.015	0.017	0.017	0.016	0.019	0.024	0.023	0.024	0.029	0.027	0.029	0.030	0.030	0.030	0.018	0.025	0.026	0.028	0.033	0.038	0.034	0.035	0.034	0.035	0.034
VS	1	0.248	0.424	0.388	0.570	0.490	0.589	0.629	0.630	0.647	0.627	0.583	0.568	0.602	0.613	0.589	0.598	0.690		0.633	0.638	0.674	0.643	0.621	0.654	0.654
	2	0.307	0.361	0.304	0.439	0.512	0.747	0.623	0.573	0.551	0.633	0.519	0.520	0.562	0.591	1.162	0.585	0.624		0.570	0.599	0.634	0.626	0.666	0.628	1.164

II. *Kitchen waste on a progressive feed*

Table 106: (a) Concentrations of the different VFA analysed for K. Proga from day 1-29

	1	3	5	9	11	13	15	17	19	21	23	25	27	29
Lactic acid a	0.00	0.00	0.02								0.91		0.18	
Acetic acid a		0.04	0.54	0.19		1.04	2.32	1.76	3.34		1.58	2.22	0.09	0.09
Propionic acid a	0.02	0.02	0.36	0.60		1.08	1.59	1.99	4.51		0.79	0.48	0.15	0.27
Iso-butyric a		0.00	3.27	15.16	36.41	27.68		31.98	32.29	48.87	4.37	4.01	2.61	3.78
Butyric acid a	0.01						59.11	2.22	2.69					0.55
Iso-valeric acid a												0.54		
Valeric a														

(b), Concentrations of the different VFA analysed for K. Prog a from day 31-59

	31	33	35	37	39	41	45	47	49	51	53	55	57	59
Lactic acid a														
Acetic acid a	0.08	0.37	5.15	4.47	4.46	3.65	4.49	0.23		0.09	0.02	0.27		
Propionic acid a	0.20	0.54	2.46	2.50	2.61	1.47	1.32					9.25		
Iso-butyric a	1.00	2.42	49.65	79.19	64.33	65.08	59.33	9.43	7.24	9.76	5.80		4.81	2.31
Butyric acid a			14.49		6.45	4.02	5.96					2.18		
Iso-valeric acid a			0.65	13.32	15.55									
Valeric a				1.19	1.64	1.48	1.54	0.01						

Table 107: (a) Concentrations of the different VFA analysed for K. Prog b from day 1-29

	1	3	5	9	11	13	15	17	19	21	23	25	27	29
Lactic acid b	0.01	0.01	0.01	0.22								0.14	0.80	
Acetic acid b	0.01	0.01	0.08	3.25	0.39	1.70	2.12	0.67	3.66		1.11	0.66	0.45	0.07
Propionic acid b	0.03	0.01	0.28	1.95	0.29	1.58	1.31	0.74	3.64		0.40	0.29	0.38	0.23
Iso-butyric acid b		0.00	2.29	0.78					23.58	15.46	0.08		1.09	1.01
Butyric acid b	0.02	0.11		3.08	11.42	2.27	25.88	30.88	8.94		0.04	1.59		
Iso-valeric acid b	0.00			0.32							0.01		0.04	0.00
Valeric acid b				0.03										

(b), Concentrations of the different VFA analysed for K. Prog b from day 31-59

	31	33	35	37	39	41	45	47	49	51	53	55	57	59
Lactic acid b	0.01							0.04						0.03
Acetic acid b	0.69	0.36	3.98	3.00	3.81	3.70	3.66	0.56	0.60	0.50	0.51		2.11	
Propionic acid b	0.44	0.38	1.42	1.04	1.34	1.16	1.04	0.43	0.49	0.28	0.21		0.35	
Iso-butyric acid b	0.07	0.71	7.72		13.88	6.81	6.18	1.75	8.47	0.71	0.00	1.69	3.49	
Butyric acid b			8.79	15.99	10.45	8.48	9.75							
Iso-valeric acid b	0.02		0.53					0.05		0.03				
Valeric acid b				0.75	1.21	1.14	1.20	0.03	0.06	7.32		0.31		

III. Garden waste

Table 108: (a) Concentrations of the different VFA analysed for reactor a with garden waste from day 1-31

	1	3	5	9	11	15	17	19	21	23	25	27	29	31
Lactic a	0.01			0.03		0.03		0.03		0.09	0.30	0.19	0.01	
Acetic acid a	0.01	0.01		0.82	1.75	0.92	0.66	0.64	0.42	4.01	5.57	1.27	0.39	0.71
Propionic acid a	0.01	0.01	0.42	0.37	1.05	0.35	0.33	0.71	0.38	2.80	3.10	0.82	0.39	0.51
Iso-butyric acid a	0.15	0.17		1.02				2.31	1.43	2.03	0.94	1.38	0.32	6.73
Butyric acid a		0.01	3.61	0.41	2.13	0.79	0.35	1.79		4.38	3.40			0.87
Iso-valeric acid a				0.33	0.11	1.99	0.21		0.02	0.76	0.80	0.33	0.04	
Valeric a						1.60	1.56			0.55	0.79	0.01	0.00	

(b), Concentrations of the different VFA analysed for reactor a with garden waste from day 33-59

	33	35	37	39	41	43	45	47	49	51	53	55	57	59
Lactic a	0.03	1.13	0.29	0.26	0.19	0.21		0.13			12.83	9.14		3.80
Acetic acid a	0.30	0.22				0.03	0.11	5.63	0.24	4.09			1.05	
Propionic acid a	0.38	0.08	0.00			0.03	0.10	2.55	0.15					
Iso-butyric acid a	5.28	5.56	4.52	5.83	4.43	5.56	0.69	1.32	0.64	8.25		5.38		7.96
Butyric acid a	0.54				0.21		0.12	4.66						
Iso-valeric acid a								1.60						
Valeric a		0.01	0.00					1.25						

Table 109: Concentrations of the different VFA analysed for reactor b with garden waste from day 1-35

	1	3	5	9	11	15	17	19	21	23	25	27	29	31	33	35
Lactic acid b				0.08	0.23	0.01	0.17			0.50	0.15	0.45	0.17	0.13		
Acetic acid b		0.01		0.19	0.73	0.74	1.66	1.14	0.59	5.44	5.65	0.34	5.48	5.56		0.17
Propionic acid b		0.02	0.51	0.18	0.17	0.21	1.76	1.32	0.27	3.41	2.86	4.07	2.45	2.33		0.18
Iso-butyric acid b	1.43	0.04		0.31			0.38	0.81	0.14	5.42	4.08		13.20	11.46	16.24	
Butyric acid b		0.03	2.75	1.36	0.52	0.17	6.05	0.44	0.08	3.87	3.07		4.54	5.71	1.37	1.58
Iso-valeric acid b		0.03		0.31	0.64	0.62	2.41		0.39	1.43	0.59	0.17	0.85	1.58		
Valeric b							0.84			0.59	0.67		0.70	1.04		0.01

Table 110: TS and VS measurement for garden waste (reactor a)

	0	3	5	7	9	15	17	19	21	23	25	27	30	32	36	38	40	42	44	46	48	52	54	56	58
TS	0.016	0.014	0.013	0.012	0.009	0.010	0.008	0.008	0.010	0.011	0.011	0.011	0.011	0.014	0.010	0.009	0.011	0.009	0.009	0.011	0.011	0.014	0.008	0.011	0.011
VS	0.316	0.322	0.259	0.283	0.348	0.520	0.515	0.619	0.534			0.369	0.496	0.364	0.286	0.331	0.428	0.503	0.450	0.350	0.433	0.483	0.387	0.513	0.502

IV. *Blood waste*

Table 111: (a) Concentrations of the different VFA analysed for reactor a with blood waste from day 1-31

	0	3	5	9	11	13	15	17	19	21	25	27	29	31
Lactic acid a	0.02	0.01	0.14	0.08	0.01	0.10	0.07	0.01	0.00	0.05				0.26
Acetic acid a	0.01	0.07	0.05	1.47	0.78	2.55	4.47	3.37	3.63	2.48	4.12	2.43	0.00	5.35
Propionic acid a	0.02	0.00	0.44	0.84	0.56	1.44	2.09	1.11	1.88	1.02	3.12	0.00	0.00	3.15
Iso-butyric acid a		0.09	0.47	0.62	0.82	0.75	1.22	1.03	4.67	3.82	48.62	47.97	49.11	12.48
Butyric acid a	0.03	0.01	0.82	0.87	3.09	2.75	3.21	2.95	1.02	1.91				7.34
Iso-butyric acid a			0.24	0.99	3.46	4.11	1.60	4.15	0.00	2.08				0.72
Valeric acid a			0.01	0.07	0.45	0.59	0.21	0.81	0.43	0.42				0.65

(b), Concentrations of the different VFA analysed for reactor a with blood waste from day 33-59

	33	35	37	39	41	43	47	49	51	53	55	57	59
Lactic acid a	0.327	0.611	0.762	0.075	1.231						0.391	0.078	0.085
Acetic acid a	5.220	4.261	5.432	2.022	4.580	3.213	3.617	4.962		4.370	5.394	4.183	4.987
Propionic acid a	3.097	2.129	2.091	0.964	2.272		120.412	123.026	159.442	158.779	2.695	1.742	1.741
Iso-butyric acid a	11.376	6.444	5.463	6.635	7.885	102.533					13.761	11.432	11.425
Butyric acid a	8.010	6.247	6.682	3.456	7.590			8.801			11.888	10.738	9.913
Iso-butyric acid a	0.885	4.015	4.483	2.414	4.634	24.886					4.552	3.041	3.723
Valeric acid a	1.011	0.343	0.302	0.146	0.240		1.362				1.488	1.009	1.262

Table 112: Concentrations of the different VFA analysed for reactor b with blood waste from day 1-15

	0	3	5	9	11	13	15
Lactic acid b	0.01	0.01				0.05	
Acetic acid b		0.15	0.06	0.40	1.97	1.89	2.41
Propionic acid b	0.00	0.03		0.61	0.96	1.12	0.87
Iso-butyric acid b		0.03	5.23	15.45	26.53	23.19	
Butyric acid b	0.05						33.73
Iso-valeric b				0.87	1.73	1.93	1.52
Valeric acid b							

Table 113: TV and VS measurement for blood (reactor a)

	0	3	5	7	9	13	15	17	21	25	27	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
TS	0.009	0.013	0.011	0.011	0.011	0.015	0.017	0.014	0.015	0.014	0.016	0.011	0.012	0.012	0.018	0.010	0.011	0.012	0.013	0.018	0.010	0.011	0.019	0.012	0.011	0.011	0.013
VS	0.356	0.642	0.382	0.379	0.533	0.563	0.641	0.654	0.666	0.683	0.707	0.629	0.699	0.667	0.370	0.690	0.754	0.821	0.819	0.821	0.691	0.742	0.869	0.745	0.783	0.833	94.338

V. *Co-digestion of blood waste with garden waste*

Table 114: (a) Concentrations of the different VFA analysed for G+B a from day 3-29

	3	5	9	11	13	15	17	21	23	25	27	29
Lactic acid a			0.05			0.04	0.08	0.47	0.60			0.00
Acetic acid a	0.24		0.48	0.42	0.35	0.03	4.16	5.64	6.62	0.65	0.26	0.36
Propionic acid	0.45		0.34	0.50			2.09	2.27	2.64	0.58	0.48	0.38
Iso-butyric a			1.23	4.13	2.77	5.17	7.54	6.13	7.01	0.50		
Butyric acid a	5.21		0.81				3.49		4.84		0.66	0.49
Iso-valeric a			0.90	0.43			0.82	3.24	1.28			
Valeric acid a							0.61	0.39	0.98			

(b), Concentrations of the different VFA analysed for G+B a from day 31-49

	31	33	35	37	39	41	43	25	47	49
Lactic acid a				0.10	0.38	0.34	0.10	0.87	0.23	
Acetic acid a	0.23	0.17		5.60	6.10	4.78	5.03	5.84	4.86	
Propionic acid	0.68	0.09	0.04	3.11	3.85	2.09	2.06	2.16	1.82	1.09
Iso-butyric a	0.46			25.98	4.07	1.90	4.66	1.16	7.72	
Butyric acid a		0.56	0.77	6.63	9.84	6.39	7.62	7.15	8.72	
Iso-valeric a	0.81			1.48	4.55	2.06	2.30	2.80	4.38	
Valeric acid a				1.63	3.37	1.71	1.80	1.86	1.86	

Table 115: (a) Concentrations of the different VFA analysed for G+B b from day 3-31

	3	5	9	11	13	15	17	21	23	25	27	29	31
Lactic acid b			0.02				0.23		0.72				
Acetic acid b		0.01	0.42		0.06	0.31	6.85		3.94	0.95	0.31	0.34	0.04
Propionic acid b			0.21			0.17	2.80		2.05	0.59	0.56	0.50	
Iso-butyric b		0.22	3.26	1.53	4.37	0.11	5.08		7.27		16.16	19.39	16.20
Butyric acid b	0.98	0.32	0.66				3.68		4.54		1.69		
Iso-valeric b						0.10	0.82		2.90				
Valeric b							0.73		0.23			0.41	3.60

(b),Concentrations of the different VFA analysed for G+B b from day 33-49

	33	35	37	39	41	43	25	47	49
Lactic acid b			0.23	5.37	0.55	0.28	0.54		
Acetic acid b	0.07	0.05	5.98	2.60	5.38	10.17	5.37	8.13	0.27
Propionic acid b	0.14	0.15	3.21	12.23	2.32	2.36	1.79	1.93	0.31
Iso-butyric b	11.45	16.70	11.32	6.27	8.57	12.62	9.53	9.29	
Butyric acid b			5.69	8.18	8.99	8.84	7.68	7.89	
Iso-valeric b			1.74	2.79	2.79	8.24	5.85	6.74	
Valeric b		3.20	1.31	1.81	1.94	0.23	0.10	0.08	

Table 116: TS and VS fraction for co-digestion of blood waste with garden waste

		0	3	5	7	9	11	13	15	19	23	25	27	30	32	34	38	40	42	44	46
TS	1	0.014	0.014	0.015	0.014	0.013	0.011	0.012	0.012	0.011	0.010	0.010	0.010	0.011	0.009	0.011	0.007	0.006	0.008	0.010	0.008
	2	0.013	0.011	0.011	0.016	0.011	0.011	0.012	0.011	0.011	0.010	0.011	0.010	0.009	0.008	0.009	0.006	0.006	0.007	0.008	0.007
VS	1	0.187	0.332	0.182	0.253	0.355	0.347	0.257	0.420	0.391	0.508	0.523	0.569	0.544	0.548	0.660	0.594	0.605	0.708	0.719	0.701