University of Southern Queensland
Faculty of Health, Engineering & Sciences

Investigation of Factors Influencing Maximum Biogas Production of Abattoir Wastewater

A dissertation submitted by

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in fulfilment of the requirements of

Courses ENG4111 and ENG4112 Research Project

towards the degree of

Bachelor of Engineering (Environmental Engineering) and Science (Environment and Sustainability)

Submitted: October, 2013

Abstract

Australia has ever increasing pressure to increase its renewable and alternative energy sources from the implementation of environmental protection schemes, legislations and taxes such as the carbon tax, implemented in July 2012. There is an increasing movement globally to reduce greenhouse gas emissions and reduce the reliability upon fossil fuels.

The development of biogasification plants in sectors such as agriculture, which produces the highest volumes of methane per sector, can assist businesses to reduce emissions, reduce sludge volume, lower reliability on the power grid, decrease costs of taxes and policies and increase revenue through excess supply back to the power grid.

Abattoir wastewater, in particular, has considerable volumes of methane and carbon dioxide gases being produced through the use of anaerobic treatment ponds that reduce the organic loading of wastes. These ponds have the potential to be transformed into covered anaerobic ponds or digestion reactor tanks to produce methane in a way that it can be captured. Once obtained the methane gas can be used to produce electricity or flared.

Little research is available on the feasibility of methane capturing through the anaerobic digestion of abattoir wastewater as the wastes are hard to characterise due to their varying composition between different abattoirs. Abattoir wastewater is typically hard to digest and therefore co-digestion has been investigated to evaluate the methane potential when combined with other easily biodegradable, carbon rich sources.

This dissertation reports on the findings made from two experimental processes. The first of which looked into the feasibility of co-digestion with nutrient rich vegetable wastewater and the second looked into the impacts of inoculum to substrate ratio and temperature on the biogas production of the abattoir wastewaters alone.

The results from the feasibility test showed that co-digestion was not compatible with the abattoir wastewater. It was evident that the abattoir wastewater produced higher volumes of biogas when anaerobically digested alone, than in comparison to the volume of biogas produced through co-digestion. The blood water alone produced the highest volumes of biogas with 736.9 mL/200 mg/L DTOC closely followed by the saveall wastewater with 724.9 mL/200 mg/L DTOC. The mixture of 80% vegetable waste, and 10% of each abattoir wastewater stream gave 588.6 mL/200 mg/L DTOC followed by the glucose substrate with 308.4 mL/200 mg/L DTOC. The lowest biogas production was by the vegetable wastewater with 69.1 mL/200 mg/L DTOC.

From the second experimental process it was found that low inoculum to substrate ratios and higher temperatures produced the highest volumes of biogas. The optimum was found at an ISR of 5 and a temperature of 40 °C but further research needs to be completed for a true conformation of these results.

This research will give further insight into the potential volumes of methane and biogas that can be obtained through the optimisation of a number of variables.

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Acknowledgement

I wish to thank Dr Vasantha Aravinthan for her ongoing supervision and guidance throughout this research project. The time and knowledge she has provided me has been invaluable to my research and I would like to genuinely thank her for her support and assistance.

I would like to thank Friederike Eberhard and Raed Ahmed Mahmood for their continuing guidance and support in the laboratory for operating laboratory equipment, analysing samples and setting up various experimental apparatus.

I wish to thank Shane Richardson from the Pittsworth Sewage Treatment Plant for the provision of anaerobic sludge. I extend this thanks to the Toowoomba Wetalla Wastewater Treatment Plant for the supply of waste activated sludge and also to the helpful staff of Oakey Abattoir for the assistance in waste sample collection.

Lastly, I would like to thank my family for their ongoing support and understanding through this busy year of study and research.

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Glossary of Terms

BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
CO_2	Carbon Dioxide
CH_4	Methane
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
DO	Dissolved oxygen
DIC	Dissolved inorganic carbon
DTN	Dissolved total nitrogen
DTOC	Dissolved total organic carbon
FW	Food waste
GHG	Greenhouse gas
IC	Inorganic carbon
LCFA	Long chain fatty acids
MW	Municipal waste
NaOH	Sodium Hydroxide
SS	Suspended solids
STP	Standard temperature and pressure (0 °C, 101.325 kPa)
TN	Total nitrogen
TOC	Total organic carbon
TS	Total solids
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge

1 Introduction

The Carbon Tax, Carbon Pollution Reduction Scheme (CPRS) and the Renewable Energy Target are just a few policies that Australia currently have in place in an attempt to reduce the impact to the atmosphere from the release of harmful greenhouse gases. Through these schemes businesses and industries are being encouraged to pursue renewable energy alternatives. By employing environmentally friendly energy sources the benefits are perceived as endless. This perception then extends to the reduction in the negative environmental impacts, costs involved with the Carbon Tax and a reduced liability from the CPRS. Other benefits include energy security, positive industry image, reduced reliability on the electricity grid, incentives through the possible supply of energy to the power grid and the better utilisation of waste products.

In Australia in 2011, 53% of the nations land mass was being utilised for some form of agriculture. This agricultural land and farming contributed to 2.4% of the Gross Domestic Product (GDP) (ABS 2012). Of this agricultural production, 44% were involved in beef production. The cattle herd was approximated at 26 million head in 2012, showing the enormity of the industry (Meat & Livestock Australia 2012). For this extensive agricultural production, there is a substantial volume of waste being produced in the farming and processing phases. The waste being developed is high in organic and solid materials and treatment through anaerobic digestion allows for the production and possible capturing of a greenhouse gas, methane. Although being produced along with other smaller quantities of gases, methane can be used to produce heat or electricity in a renewable manor. As Australian agriculture is already demonstrating the use of anaerobic digestion for means of breaking down wastes, it has been for a number of reasons that the transition to methane capturing has not come sooner.

Historically, the relatively affordable and readily available fossil fuels have dominated the energy market. Until recently, the push for environmentally friendly processing was not seen as a relevant concern. With society's increasing taxes and

policies on mainstream energy sources there is an increased need for businesses to have a positive public image and new technologies making renewable energy more affordable. Processes such as methane capturing are becoming more evident throughout the World and Australia.

1.1 Purpose

The purpose of this research is to investigate the feasibility of the potential methane production from meat processing wastes of Oakey Abattoir. The purpose includes determining the effect of co-digestion on the anaerobic digestion of wastewater and the resulting methane production. Other experimental considerations include important components for digestion, such as the Carbon to Nitrogen Ratio, Inoculum to Substrate Ratio (ISR) and the operating temperature.

1.2 Aims and Objectives

Research will be conducted to maximise the amount of methane that can be produced by the actions of anaerobic digestion.

The objectives include:

- Conduct extensive research on the Biological Methane Potential (BMP) of abattoir wastewater
- Collect samples of abattoir wastewater, organic rich wastes and sludge containing anaerobic bacteria
- Conduct characterisation experiments to determine the properties of the waste samples
- Conduct experiments to test the feasibility of co-digestion with vegetable wastes and comment on the impacts of the Carbon to Nitrogen Ratio

- Produce experiments that will replicate set variables (such as the Carbon to Nitrogen Ratio, Inoculum to Substrate Ratio and temperature) decided upon using the Response Surface Method in the Minitab16 mathematical software program
- Use this mathematical software modeling to find the optimum combination of the set variables to maximise the methane yield

1.3 Scope

The scope of this project is to explore the suitability and potential for increased methane production through the use of the response surface method and the varying of the components as aforementioned.

Limitations for this project were:

- Only single samples were taken from each type of waste and sludge, meaning that daily fluctuations in the flow characteristics were not taken into consideration
- The characterisation of wastes and inoculum were limited to the equipment and time frames that were available
- Grab samples of inoculum sludge will be undertaken in winter which may reduce the effectiveness of the bacteria due to cooler temperatures
- Methane production was investigated but the conversion of this methane and uses for it will not be considered
- Experimental processes will be limited to the time and resources available

1.4 Overview of Dissertation

The dissertation chapters follow the proceeding layout:

Chapter 2 Literature Review

This chapter provides an overview on the literature available on biogas production from the anaerobic digestion of wastewaters. It includes information regarding anaerobic digestion, biogas and methane measurement procedures, optimum operating conditions and touches on variables such as co-digestion, inoculum to substrate ratio (ISR) and temperature.

Chapter 3 Biogas in Australia

Touching on the biogas status in Australia, this chapter aims to introduce some of the economic, social and environmental momentum supporting the adoption of this technology. It covers the extent and size of the meat processing industry, the wastes it produces and further identifies the problems faced for Oakey Abattoir.

Chapter 4 Methodology

This chapter covers the methodology implemented for the different experimental processes undertaken for the research involved in this dissertation. It covers characterisation of the various substrate and inoculum wastewaters, gas measurement and data acquisition.

Chapter 5 Biogas and Methane Production from the Co-digestion of Abattoir Wastes

This chapter presents and analyses the results obtained through the feasibility testing of co-digestion of abattoir wastewater with nutrient rich vegetable wastes. The results have been discussed for the biogas and methane produced and also a mass carbon balance has been performed to further determine the activity of the anaerobic bacteria.

Chapter 6 Biogas Production from Varying ISR and Temperature

Chapter 6 provides the results obtained for batch experiments undertaken to test the impacts of varied ISR and temperature on biogas production. These results have been analysed and a model implemented to predict an optimum for both variables in order to maximise gas production.

Chapter 7 Conclusions and Further Research

This chapter provides the conclusions for the research undertaken and outlines research to be undertaken in the future.

2 Literature Review

This chapter provides background information regarding methane recovery from anaerobic digestion of wastewater, various parameters that impact on biogas production, operating procedures, gas measurements and analyses.

2.1 Fundamentals of Anaerobic Digestion

Anaerobic digestion is the process in which bacteria metabolise and breakdown organic rich waste or wastewaters in the absence of oxygen. This process has been utilised to reduce the volume of waste needing to be disposed of by removing organic solids. Anaerobic digestion has the potential to be used for energy production and allows for a reduction in greenhouse gas emissions if biogas capturing occurs (Agriculture and Consumer Protection 1997). Along with these benefits it reduces the Chemical Oxygen Demand (COD) and the Biological Oxygen Demand (BOD) of organic rich wastewater. To achieve this, certain bacteria need to be present.

There are four main types of anaerobic bacteria that are required to produce methane. The first stage of the process requires hydrolytic bacteria which excrete extracellular enzymes to break down the wastes into amino acids, sugars and long chain fatty acids (Li et al. 2011). The next step is performed by fermentative bacteria which break down the wastes into short chain volatile fatty acids, hydrogen gas, carbon dioxide and acetic acid (Khanal 2008). The third stage requires acetogenic bacteria to break down the fatty acids and acetic acid into acetate, hydrogen gas and carbon dioxide. Lastly, methanogenic bacteria break down the acetate and other compounds to create methane gas. Other gases are also present as aforementioned, although the methane and carbon dioxide are the main constituents. Figure 1 shows the steps required in anaerobic digestion.

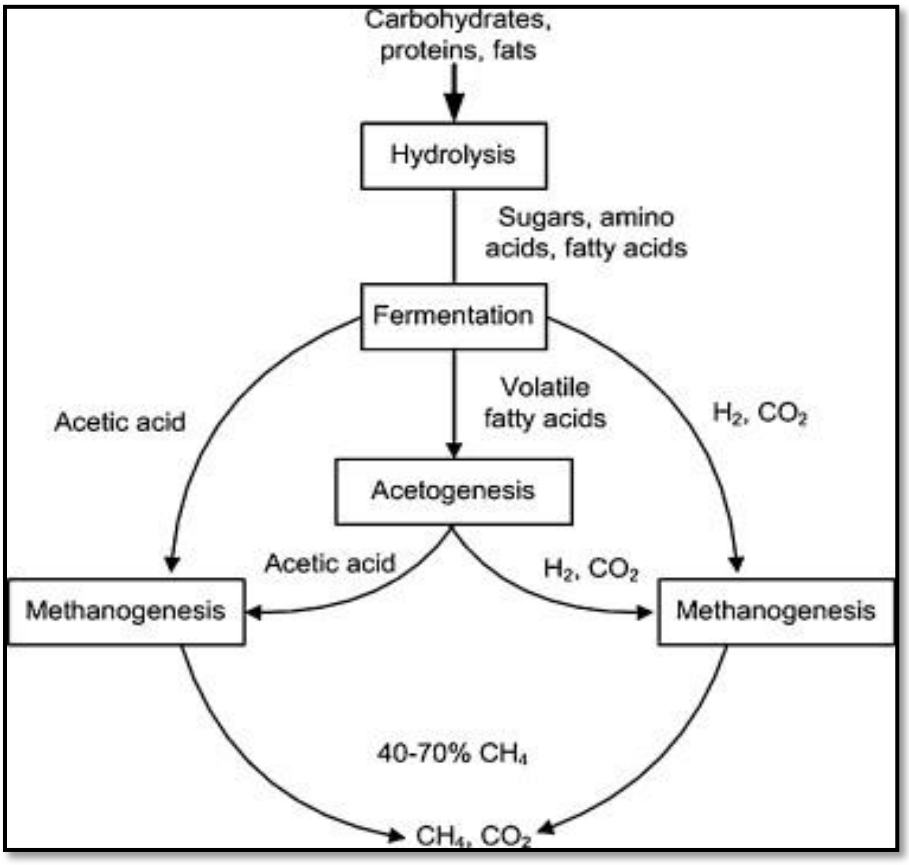

Figure 1 Flow Diagram of Anaerobic Bacteria Activity (Li et al. 2011)

An important note to make for this process is that all of these bacteria need to be working effectively, in order to create sufficient volumes of methane. Each type of bacteria is sensitive to varying inhibiting factors and the most important of which, methanogenic bacteria, is the least tolerant of the four (Chen, Cheng and Creamer 2008). Elements such as ammonia, sulphides, nitrates, nitrites, phosphates, metal ions and some organic compounds can be inhibitory to the process along with temperature, alkalinity and pH (Chen, Cheng and Creamer 2008).

2.2 Factors Influencing Anaerobic Digestion

Methane production relies on a number of environmental components but the carbon to nitrogen ratio, temperature and inoculum to substrate ratio (ISR) are essential in

creating conditions for optimum volumes of methane. The carbon to nitrogen ratio impacts on bacterial synthesis and biogas production. It is important to have both carbon and nitrogen for the synthesis and growth of new bacterial cells but excess or deficits of either can be detrimental to the bacteria and consequently the biogas and methane production. If the carbon is in excess, acidification will occur. If the carbon to nitrogen ratio is too low then excess nitrogen molecules will be present and cause an ammonia accumulation which inhibits bacterial growth (Misi and Forster, 2001).

The temperature also impacts on biogas production. There is extensive research available on the impacts that temperature can have on the gas production of anaerobic bacteria. Although there is research suggesting different temperatures may achieve optimal conditions, the gas production in a field anaerobic digester may be difficult to maintain constant and will vary with seasonal changes. The impact from these seasonal changes has not been thoroughly investigated and therefore providing a gap in the available literature.

The inoculum to substrate ratio is important when testing for methane and biogas production as it is an indication as to how much sustenance is required to maintain optimal bacterial growth and gas production without overloading the bacteria to result in inefficiencies for the entity.

Along with an optimum carbon to nitrogen ratio (C:N ratio), temperature and ISR, the type of waste will impact on the efficiency of the anaerobic bacteria. Typically bacteria need to become acclimatised to the type and diversity of waste being digested. Wastewater can vary from being high in carbon, such as waste from a fruit and vegetable processing plant, or high in nitrogen, such as blood water sourced from an abattoir. Wastes that are easily biodegradable and have higher carbon contents are easier to digest for anaerobic bacteria and those with high nitrogen levels are usually more complex and harder to digest. The right balance needs to be determined between these two elements and the impact that this has on gas production also needs to be established.

The literature available shows that although having only little development into anaerobic digestion, abattoir wastewaters are difficult to process due to their high nitrogen content. Therefore it has presented as an area requiring further investigation. One suggestion to the dilemma of carbon and nitrogen imbalances is co-digestion. Co-digestion is the process in which two or more substrates are digested together in order to provide a more varied diet and diverse nutrient supply to bacteria. Co-digestion is said to 'significantly improve the waste treatment efficiency' (Chen, Cheng and Creamer 2008) by improving the carbon to nitrogen ratio and diluting inhibitory compounds.

Along with this, it is believed that co-digestion could improve methane yields and stability through improving the C:N ratio, diluting inhibitory factors and providing easily biodegradable substrates to help with the breakdown of more difficult substances. Anaerobic digestion provides the opportunity for pollution control and energy recovery but low methane yields and instability has prevented the wider use of the technology (Chen, Cheng and Creamer 2008).

2.3 Methane Recovery from Waste

There are a number of benefits that are associated with methane recovery, the obvious being energy recovery for processing into electricity or heating. With adequate equipment, electricity can be produced and used on site or the gas can be pumped off site for further processing. The other main benefit is the reduction of greenhouse gas emissions, passing on positive impacts to the environment and reducing taxes such as the carbon tax. The biogasification process has small facility sizes, a high quality of gas produced, the ability to handle wet and dry feed stocks and the construction is relatively easy (Frank and Smith 1987). The process involves methods such as using covered anaerobic ponds or digestion tanks to trap biogas that is produced from the anaerobic bacteria. Methane has a net warming value of 35, 000 kJ/m^3 and even by burning it reduces its greenhouse gas potential by close to 98% (CSIRO Food and Nutritional Sciences 2010). This may mean that an entity

producing high volumes of methane could find it beneficial to trap and burn this biogas, to reduce the impact felt by the carbon tax or other pollution schemes. Anaerobic digesters have the potential to breakdown a number of varying wastes such as municipal, vegetal, food, meat and agricultural wastes. The literature on the current use of such technology is hard to compare due to the varying reliance on different units and classification bases. Table 1 shows the different range of gasification plants and the various classification bases of methane production.

2.4 Anaerobic Digestion of Abattoir Waste

This section will cover a number of the characteristics of abattoir wastewater and the conditions required for anaerobic digestion.

2.4.1 Abattoir Wastewater Characteristics

Abattoir waste, as previously stated, has a high nitrogen content (low C:N ratio) making it difficult to process alone. The waste passing from the slaughter floor and other areas contains high amounts of blood, fats, hair, skin cells and bone. All of these are difficult to process for anaerobic bacteria and even harder for bacteria that are not acclimatised to digesting these types of wastes. This may become a hurdle to face in the experimental processes but this will be covered in further detail later in the report.

Through the optimisation of different variables methane produced from abattoir wastewater, has the potential to be up to 70% of the total biogas produced by anaerobic digestion (CSIRO Food and Nutritional Sciences 2010). To optimise methane production of abattoir wastewater various procedures must first be undertaken such as pretreatment, analysis and bacterial development. These will be discussed in depth further in this dissertation.

Table 1 Anaerobic Reactors and Methane Production (Adapted from Bauer 2011)

Waste Type	Reactor	Feed	Methane	Methane Content	Source
Dairy manure with turkey processing water	Attached growth 15 L	Continuous	0.8 m^3 g VS^{-1}	56-70%	i
Cheese whey and dairy manure	Anaerobic reactor tank 20 L	Continuous	1.51m^3 m^3d^{-1}	60%	ii
Grass silage	Batch leach bed with USAB 1 L	Internal recirculation	0.141-0.204 m^3 kg VS^{-1}	-	iii
Apple pulp and slaughter water	101 CSTR reactor	Continuous	0.8 m^3 kg^{-1} OTS	77-80%	iv
Olive mill waste and cow manure	Anaerobic reactor tank	Continuous	0.91 L CH_4 $L^{-1}_{reactor}$ d^{-1}	-	v
IFW and WAS	Batch assays	Batch	239 mL g VS^{-1}	50-70%	vi
Cooked meat **Boiled rice** **Fresh cabbage** **Mixed food wastes**	All batch reactors	All batch	482 mL g VS^{-1} 294 mL g VS^{-1} 277 mL g VS^{-1} 472 mL g VS^{-1}	82% 72% 73% 86%	vii

i (Ogejo & Li 2010)

ii (Kavacik & Topaloglu 2010)

iii (Lehtoma ki et al. 2008)

iv (Llaneza Coalla et al. 2009)

v (Dareioti et al. 2010)

vi (Siddiqui et al. 2011)

vii (Kubaska 2010)

2.4.2 Preliminary Treatments

Preliminary treatments for abattoir waste includes the use of screens, skimmers, settlers, floatation, equalisation and catch basins to remove fats, hair, bones, manure, sand or grass that may be contaminating the wastewater. This is used to remove the solids content that is then directed to the primary and secondary treatment areas.

Preliminary treatments for the abattoir waste in the laboratory includes pH, alkalinity, Total and Total Dissolved Organic Carbon (TOC & DTOC), Dissolved Inorganic Carbon (DIC), Total and Total Dissolved Nitrogen (TN & DTN), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Solids (TS), Volatile Solids (VS), Total Suspended Solids (TSS) and Total Volatile Suspended Solids (TVSS). These treatments can then, through analysis, provide a basis for optimisation to occur.

2.4.3 Optimum Conditions for Anaerobic Digesters

In order to maximise methane production, the anaerobic digestion process should be working under optimum conditions. Anaerobic digestion requires a pH between 6 and 8 with an optimum close to neutral (CSIRO Food and Nutritional Sciences 2010). It requires an absence of oxygen with preferably bacteria that is acclimatised to the waste being digested or to a wastewater with similar chemical characteristics. There are two ranges for temperature mesophilic and thermophilic. For the experiments to be run in the laboratory, the initial chosen temperature will be mesophilic at 37±1°C as it is less sensitive to changes (Siddiqui et. al. 2011, Kubaska et. al. 2010, Labatut 2012). Further analysis will allow for temperature to be varied. There are a number of elements that can be inhibiting to the process and these will be investigated with the ion chromatography machine to discover the concentrations of ions present. The inoculum to substrate ratio (ISR) will impact on the efficiency in the laboratory experiments and the ISR has a number of literature reviews with conflicting optimums.

The carbon to nitrogen ratio is also very important and similarly to the ISR has a number of conflicting reports. The C:N ratio has been said to be within the optimum range between 15-35, ranges are shown in Table 2.

Table 2 Optimum Rates for C:N Ratio

C:N	Wastes	Reference
15:1	11% IFW[1]	Siddiqui et. al. 2011
26.31:1	Dairy, chicken manure &wheat straw	Wang et. al. 2012
15:1-19:1	Swine	Siever and Burne 1978
25:1-30:1	Unknown	Doerr and Lehmkuhl 2008
20:1-30:1	Unknown	Agstar 2012
20:1-30:1	Fruit and vegetable waste	Weiland 2006
22:1-25:1	Fruit and vegetable waste	Bouallagui et. al. 2009
20:1-35:1	Fruit and vegetable waste	Guermoud et al. 2009
20:1	Mixtures	Stephen, Szolar and Braun 1998

[1] IFW: Industrial Food Waste

If the C:N ratio is too high then acidification will occur and if the ratio is too low the accumulation of ammonia ions will inhibit the anaerobic bacteria from producing methane. Ammonia levels lower than 200 mg/L is required to prevent such accumulation and complete inhibition is caused by 8.4 g/L ammonium (Chen, Cheng and Creamer 2008, Buendia et. al 2009). The use of co-digestion can help prevent the toxicity of some accumulated ions.

2.5 Co-digestion

Co-digestion is the process in which wastes that are difficult to digest for anaerobic bacteria are digested in the presence of other easily biodegradable, nutrient rich wastes. Co-digestion helps to provide nutrients that are absent, improve the carbon to

nitrogen ratio, dilute inhibitory factors and potentially adjust or maintain a constant pH. This is summed up with Misi and Forster (2001) stating that 'The methane yields of the mixtures will always be greater than the sum of the methane yields which would be obtained if the same quantities of the individual wastes used to make up the mixture were digested separately and the yields summed'. This statement confirming that the method of co-digestion will be beneficial for experimental procedures regarding abattoir waste.

2.6 Biological Methane Potential Assays

Biological methane potential assays will be run in this experimental process as a method to determine the biodegradation of the known organic content of waste using seed sludge (inoculum). Assays will be run over a period of time until a point where a plateau effect is evident. The apparatus, procedure and gas measurements will be touched on in the following sections but will be elaborated on in section 4 Methodology. Table 3 shows the extent of different BMP assays that have been developed previously.

2.6.1 BMP Equipment and Procedures

Biochemical Methane Potential experiments can be set up as continuous digesters or batch assays. They will be run in sealed 500 mL Wheaton bottles with rubber butyl septa lids that allow for easier measurement of biogas production. The gas can be measured by volume, pressure or gas chromatography. Inoculum can be sourced from a working anaerobic digester that may or may not be acclimatised to the waste on hand. Literature available on the equipment and procedures varies considerably as the time, incubator space and the available equipment, are all limiting factors.

Table 3 BMP Assays for Various Substrates (Adapted from Bauer 2011)

Substrate	Working vol. (% of total)	Feed Type	Inoculum Source	Time (days)	Temp (°C)	Measurement Method	Mixing	Reference
Dairy manure & food waste	50%	Batch	Laboratory scale treating municipal waste	30	35 ±1	Pressure gauge	Manually for 1 minute prior to measurement	(El-Mashad & Zhang 2010)
Manure, FW, water plants	-	Batch	manure and food waste anaerobic digester from farm	30	35 ±1	Pressure transducer with data acquisition	manually every second day	(Labatut et al. 2011)
Swine & bovine slurries	49%	Batch	swine slurry anaerobic digester on laboratory scale	123-153	30	Digital manometer	continuous	(Vedrenne et al. 2008)
Manure & straw	66.7%	Continuous	biogas digester	30	35 ±1	Gas meter	-	(Demirbas 2006)
Mill textile effluent	62.5%	Batch	Acclimatised Sludge	60	35	Collected with syringe	-	(Desiana & Setiadi 2009)
Municipal waste	60%	Batch	Acclimatised Sludge	-	35 ±1	Manometer	Continuous	(Zheng et al. 2009)
Kitchen waste	-	Batch	UASB reactor treating brewery effluent	25	37	Pressure transducer	Continuous	(Neves et al. 2004)
Fruit & vegetable waste	-	Batch	CSTR	100	35 ±1	Glass syringe	-	(Gunaseelan 2004)

2.6.2 Pressure and Gas Measurement

The pressure is critical for standardising and calculating the volume of biogas being produced. In past research, gas measurements have been made by inserting a hypodermic needle into the sealed rubber butyl lid of the test vessel, extracting the biogas to store in gas bags and finally taking a sample to analyse with a gas chromatography machine. This method proves difficult as it is hard to maintain all of these elements as air tight components with little contamination to open air. As this is the case, another method to analyse the composition of methane was utilised. Firstly, the pressure is to be analysed in the test bottles and by using the Universal Gas Laws the volume of biogas can then be determined. This method involves the use of an alkaline solution, sodium hydroxide. Biogas has a composition mainly of CH_4 and CO_2. CO_2 will dissolve in the sodium hydroxide (NaOH) whereas CH_4 will not. Using this, the biogas can be passed through a solution of NaOH in order to absorb the CO_2 and allow for the volume of methane to then be recorded. A supplementary discussion is contained in the Methodology (Section 4).

2.6.3 Inoculum to Substrate Ratio

To calculate the ratio of inoculum to substrate (ISR), the strength of each substrate needs to be determined. It is hard to compare different literature for the ISR as there are many ways to characterise the strength of inoculum (total suspended solids, a ratio between total solids and volatile solids or by a volume basis). The determination of the ISR was made from the International Standards (ISO 11734: 1995 (E)). This will be covered in further detail, within the Methodology.

2.7 Summary

It is evident that the process of anaerobic digestion is a very sensitive process, from solids content, nutrient availability, pH, alkalinity, ion inhibition, temperature, oxygen contamination and bacteria efficiency. There are a multitude of factors that need to be considered for the successful production of methane gas, some have the potential to be managed and others cannot be controlled due to availability of equipment in the laboratories. The BMP assays have the potential for easy biogas assessment with the use of sodium hydroxide. The use of co-digestion with vegetable waste also seems promising for its high carbon and nutrient content. However, from the literature available, it is evident that there is information lacking on the impacts that co-digestion will have on abattoir waste specifically when using vegetable waste as the co-substrate.

3 Biogas in Australia

This chapter describes the status of biogasification and methane capturing in Australia. It also touches on the wastes produced by the meat processing industry and how this is impacted by current laws and legislations that are enforced for carbon emissions through treatment of the wastewater produced. A case study is presented for the source of the abattoir wastewater that will be used throughout this research.

3.1 Current Methane Status in Australia

Methane production through the use of anaerobic digestion is becoming more popular but is still not being widely used due to instability of processes and costs involved. As previously mentioned, the recent implementation of taxes has put more pressure on adopting biogasification plants for methane production. There are companies emerging across Australia that produce and sell biogas plants for the anaerobic digestion of farm wastes, such as BIOGAS AUSTRALIA in South Australia and Diamond Energy from north Victoria. The only biogas plant in Australia to date is the Berrybank Farm Piggery which produces 275 000 L of sewage a day and uses a two stage digester producing low voltage electricity. Of the electricity that is produced 90% is used on site and the rest sold back to the grid (Doan 2009). Australia has the technology to apply the biogas plants but has not yet begun full scale use of methane recovery.

3.2 Methane and the Meat Industry

This section details the role the Australian beef industry plays in carbon emissions and waste treatment complexities across the nation. It describes the extent of the industry, its waste production and the impact this is having on the environment.

3.2.1 Australian Beef Industry

Australians on average eat 33.7 kg of beef products a year, with the cattle herd sitting at approximately 30 million head in 2011 (Meat and Livestock Australia 2012). The nation is the 6th largest beef producer and the 2nd largest exporter of beef in the World, showing just how huge the industry is (Meat and Livestock Australia 2012). Processing livestock into portions of meat comes with a considerable amount of water use to keep the animals, yards, carcasses and infrastructure clean and hygienic. All of this wastewater that is rich in manure, stock feed, fats, blood and hair needs to be treated or reused, so it is generally sent to settling and storage ponds. These wastewaters are often treated before reuse in cattle yards as washing water or as irrigation water for feed crops. Abattoir and slaughterhouse wastes are hard to stereotype or generalise when it comes to characterising as there are a number of factors that impact on what goes into the waste products.

Some of these variables include:

- Number of stock slaughtered each day
- The breed of animals being produced
- Washing of livestock prior to entering slaughterhouse
- Reuse or recycling of cattle washing water
- The inclusion of paunch waste in wastewater
- Inclusion of slaughter floor waste in the wastewater stream or used in alternative purposes
- Filtration or treatment before the waste enters the treatment ponds

- The use of chemicals for sterilisation
- Wastes such as fat and bones being separated and reused for items such as pet food

These varying elements in each slaughterhouse make characterising abattoir waste as a whole industry nearly impossible.

3.2.2 Wastewater in the Meat Industry

Wastewater from abattoirs can be reused on site for washing down cattle before they are slaughtered, washing down yards or for use in crop irrigation. To reuse the wastewater, it is usually treated by settlers, skimmers, screens, flotation, equalisation, anaerobic digestion ponds and/or catch basins to remove fats, hair, bones, manure, sand or grass that may be contaminating the wastewater. The suspended solids in the water need to be removed and then is generally sterilised if needing to be used in the washing of cattle. Sterilisation of the wastewater is required to prevent any viral or bacterial infection to live stock. The sterilised water is generally used soon after sterilising as when it's still warm it gets mud off faster, using less water (Meat and Livestock Australia 2007). Irrigation for feed crop can occur once the suspended solids have been removed from the water to prevent blockages of irrigation pipes and pumps.

3.2.3 Greenhouse Gas Emissions in Australia

Greenhouse gas emissions in Australia for various industrial sectors can be seen in Table 4. It is evident from this table that the Agricultural sector is the largest CH_4 producer with close to double that seen by fugitive emissions from fuel. The agricultural sector represents approximately 57.5% of all methane and 77.4% of N_2O emissions across the country.

Table 4 Australia's Net Greenhouse Gas Emissions by Sector (UNFCCC 2011a)

Sector and Subsector	CO_2	CH_4	N_2O	HFCs	PFCs	SF_6	Total	Share of total
	Mt CO_2-e							%
1 All energy (combustion + fugitive)	384.0	35.4	2.6	NA	NA	NA	422.0	76.4%
Stationary energy	*290.8*	*1.4*	*1.0*	*NA*	*NA*	*NA*	*293.2*	*53.1%*
Transport	*85.6*	*0.4*	*1.6*	*NA*	*NA*	*NA*	*87.6*	*15.9%*
Fugitive emissions from fuel	*7.7*	*33.6*	*0.03*	*NA*	*NA*	*NA*	*41.3*	*7.5%*
2 Industrial Processes	22.5	0.1	2.7	7.6	0.3	0.1	33.3	6.0%
3 Solvent and other product use[a]	NA	NA	IE	NA	NA	NA	IE	IE
4 Agriculture	NA	64.8	19.4	NA	NA	NA	84.1	15.2%
6 Waste	0.03	12.4	0.4	NA	NA	NA	12.8	2.3%
Total net emissions (excluding *LULUCF*)	**406.6**	**112.6**	**25.1**	**7.6**	**0.3**	**0.1**	**552.3**	**100.0%**
5 Land use, land use change and forestry	-43.1	1.6	1.1	NA	NA	NA	-40.3	-7.3%
Total net emissions (including *LULUCF*)	**363.5**	**114.2**	**26.2**	**7.6**	**0.3**	**0.1**	**511.9**	**100.0%**

(a) confidential N_2O emissions from *solvent and other product use* are reported under *industrial processes*

To further investigate the methane production throughout Australia, Table 5 shows the various outputs, storages and capturing of methane from 1990 to 2011.

Table 5 Methane Generation and Emissions, Australia 1990:2011 (UNFCCC 2011a)

Year	Carbon additions to landfill (kt C)	Carbon loss (through emissions) (kt C)	Methane generated (Gg CH_4)[a]	Methane capture (Gg CH_4)	Net methane (Gg CH_4)
1990	2,464	1,077	714	2	712
1991	2,422	1,077	719	2	717
1992	2,398	1,082	719	11	708
1993	2,446	1,079	722	11	711
1994	2,383	1,076	721	35	686
1995	2,397	1,074	719	28	690
1996	2,319	1,075	718	91	626
1997	2,282	1,080	718	98	620
1998	2,367	1,084	721	130	591
1999	2,351	1,082	724	121	602
2000	2,444	1,082	723	129	594
2001	2,440	1,087	723	131	592
2002	2,434	1,095	726	128	598
2003	2,432	1,104	732	176	556
2004	2,474	1,105	738	197	540
2005	2,476	1,103	738	207	530
2006	2,447	1,102	737	222	515
2007	2,441	1,113	736	216	520
2008	2,465	1,126	744	205	539
2009	2,272	1,137	752	215	537
2010	2,175	1,142	759	204	555
2011	2,227	1,140	763	233	530

Source: DIICCSRTE estimates.
Note: (a) methane generated prior to oxidation.

It is seen, from the correlation of data in Table 5, that in the years 2009 to 2011 there has been a significant drop to the volume of carbon being added into landfills and an increase to the carbon lost through emissions. Although there has been a 6.9% increase in CH_4 generation over the past 21 year period there has been an increase in methane capturing of close to 120 times the capturing apparent in 1990. These patterns have lead to a 26% decrease in net methane present since 1990. This shows that some efforts are being made to reduce the impact of GHG emissions however, there is still a significant volume being released into the atmosphere which is detrimental to the health of the Earth.

Table 6 Methane Recovered as a Percentage of Industrial Wastewater Treatment 2011 (UNFCCC 2011b)

Commodity	Fraction of methane recovered/flared (%)
Dairy[b]	27%
Pulp and Paper[c]	67%
Meat and Poultry[b]	9%
Organic Chemicals[b]	6%
Sugar[c]	0%
Beer[a],[c]	23%
Wine[b]	26%
Fruit[b]	7%
Vegetables[b]	17%

Source: (a) O'Brien 2006a, (b) NGGIC 1995 (c) NGERS 2011.

Table 6 shows the factor of methane recovery and burning, for each commodity sector in Australia. The pulp and paper industry recovers the highest percentage with 67% and the sugar sector the least with no evident attempts to recover or burn off methane. The meat and poultry sector recovers only 9% methane from the industrial wastewater produced. With such a small percentage of methane being recovered or oxidised, entities will be under immense pressure from various taxes and legislations that are in place across the country.

3.2.4 Energy, Emissions and the Carbon Tax

As previously mentioned in Section 1, the carbon tax, renewable energy target and the carbon pollution reduction scheme is placing immense pressure on the industry to reduce energy consumption, increase energy renewal and reduce wastes being placed in landfill. The carbon tax is placed on the release of emissions such as carbon dioxide, methane or nitrous oxides (King 2012).

The Australian Carbon Tax was implemented on July 1 2012. It is thought that by 2013, 33 countries and 18 sub-national jurisdictions will have implemented carbon pricing. If this is the case, this would cover 850 million people, 30% of the global economy and 20% of global emissions (SBS 2013). The tax applies to companies emitting over 25 000 tonnes of carbon dioxide per year or other companies that are involved in the supply or use of natural gas. The carbon tax was implemented under a Labor Government and the current government is vowing to abolish it. Since the passing of the Carbon Tax, there has been extensive uproar over the high costs and the little need for such a tax in Australia.

The Carbon Tax is set at a slowly increasing but fixed annual price from its beginning date in July 2012 to July 2015. In its initial planning stages, figures showed an estimated cost of $29/tonne of emissions in the global market by 2015. From this and the reassurance that the cost of carbon was to be greater than $20 per tonne the initial prices in Australia were set (McGrail 2013). The initial year had a cost of $23.00; the second year (2013/2014) was to cost $24.15 per tonne and the final year a fixed price of $25.40. From July 2015 the Carbon Tax was to be then based on the global carbon pricing, with a floor rate of $15 per tonne. These costs are significantly higher than that seen in New Zealand for its initial fixed and current floating prices, the European Union's current floating prices, California's planned initial prices and the current carbon prices seen in Denmark (Politifact 2013). Although being higher than a large number of countries the prices are lower than that seen in Finland (US$30/tonne), Sweden (US $150/tonne) and British Columbia (US$30/tonne).

The expected revenue for the carbon tax implementation was estimated around $7-8 billion per year on the fixed rates (Politifact 2013) or $10.5 billion by 2015 (Benson 2013) which represents 0.005% of the total GDP.

Although creating revenue for the Australian Government, there is a significant amount of initial and ongoing costs involved in the set up and administration of the carbon tax. It is estimated that the carbon tax is costing the economy $100 million per week and putting a cost of $148 million to the Queensland Government alone in 2012 and hundreds of millions more for other government owned corporations (Hepworth 2013).

The dilemma faced with the Carbon Tax is that there has been a considerable crash in the market leaving a slump in carbon pricing. Once the Carbon Tax is set to follow global floating prices in mid 2015 it is expected that prices will be around $2 per tonne or a maximum of around $6 per tonne in comparison to the $29 per tonne expected when the tax was created. The Australian government is now expecting a price of $12.10 in the 2015/2016 year and $18.60 per tonne in the 2016/2017 year which is still 2 and 3 times greater than the expected price from the European Union of $5.57 per tonne in June 2016 (The Liberal Party of Australia 2013).

3.2.5 Environmental Impact

Methane is a naturally occurring emission through the use of anaerobic treatment ponds. When treating water, the flow entering the pond is high in fats and lipids that float on the surface off a pond. These fats and lipids form an air tight crust over time, thus creating an anaerobic pond when all of the oxygen is consumed. The bacteria that arise and thrive from this change then form methane and carbon dioxide which bubbles up and releases naturally. Considering methane has a global warming factor 21 times that of carbon dioxide, it can create a substantial account (cost wise and environmentally) when left to release into the atmosphere. Capturing methane and combusting it alone will decrease its impact on the atmosphere by up to 98% and

reduce the costs of taxes. Other than combusting, the methane can be used to create electricity and be used or pumped back into the main grid.

3.3 Case Study: Oakey Abattoir

This section will cover an introduction and case study for Oakey Abattoir, Oakey, Queensland. This abattoir will be the focus of this research dissertation.

The Oakey Abattoir was established in 1956 and is Australia's 4th largest abattoir (Nippon Meat Packers Australia). It processes on average 1150 head of grass and grain fed cattle per day (5 days a week, all year except for Christmas and Easter), with both chilled and frozen cuts of meat being produced. Of this production, approximately 80% is exported and 20% is sold locally.

Oakey Abattoir's wastewater flows firstly to an anaerobic pond, which is naturally producing methane due to the thick layer of fat floating on the top preventing air from entering the pond. This treatment removes organic compounds through anaerobic digestion. From the anaerobic pond, the water flows to a serpentine pond which uses a snake like form to remove more suspended solids from the wastewater. Finally the water is pumped under the Warrego Highway to a large evaporation pond, where some water is further pumped for the irrigation of feed crops for the stock. Samples are to be collected from the processing plant before any treatment has begun, to get an idea on the characteristics of the raw wastes that have the potential for methane production. Figure 2 shows an aerial view of the entire abattoir, three treatment ponds and the field that is irrigated with recycled abattoir wastewater. Figure 3 shows a closer view to the anaerobic digestion pond to the right and serpentine treatment pond to the left in the photo.

Figure 2 Oakey Abattoir Aerial Image (Google Maps 2013)

Figure 3 Serpentine and Anaerobic Pond of Oakey Abattoir (Google Maps 2013)

The use of sterilised slaughter floor water as washing for cattle saves the company over 0.5 ML a week (Meat and Livestock Australia 2007). The abattoir has a Dissolved Air Flotation System (DAF) that is applied to the saveall water (all wastewater excluding the slaughter floor water) which removes fats from the effluent flow to reduce the load on treatment ponds (Meat and Livestock Australia 2007). The DAF system uses skimmers to remove floating solids. The DAF system saves 3 tonnes of fat per day to be used in other products (Meat and Livestock Australia

2007). The abattoir also has an irrigation system that was implemented in 2003 to irrigate feed crops for the cattle, thus reducing pressure on the evaporation pond.

Other methods that the company has looked into for decreasing the pressure of the carbon tax and other environmental legislations are:

- Recent approvals for solar panels to be installed on the roofs of the abattoir
- Planting trees to help with soil condition, aesthetics and carbon credits
- Changing of light bulbs to energy saving

Oakey Abattoir is currently starting the process of investigating the benefits of implementing a biogasification plant to harness methane, but no substantial findings have been discovered at this point in time; however, contact has been made and any data/studies will be forwarded on.

3.4 Summary

Australia has the potential to adopt methane production and it is becoming more widespread with the introduction of the Carbon Tax and the push towards more renewable technologies. With knowledge and experience, the application of these technologies will become more popular. Oakey Abattoir alone has the potential to implement a biogasification plant with the space and waste production to make it a viable option.

4 Methodology

The method for this experimental process is quite lengthy, in depth and sensitive to a wide range of elements. From collecting the wastewater, seed sludge and vegetable waste to characterising each of these, performing preliminary experiments and finally creating the final experimental process.

4.1 Collection of Inoculum

The Inoculum has been sourced from the anaerobic digestion tank at the Pittsworth Waste Water Treatment Plant. The sewage digester operates with a trickling filter and anaerobic digester. The anaerobic digester was fed sludge from the primary clarifier approximately once a week. The sludge sample was obtained from the bottom of the digester and transported in a plastic container. Samples were collected by single grab samples and stored in sealed containers to be kept at a constant four degrees Celsius (ISO 9887:1992). Once collected, the inoculum was sieved through a 500 μm sieve to remove larger solid particles. As previously mentioned, vaccinations and safety precautions were required on handling this waste.

Figure 4 Anaerobic Digester Pittsworth

Figure 4 shows the anaerobic digestion tank at the Pittsworth Wastewater Treatment Plant.

4.2 Collection of Abattoir Waste

Permission was obtained from Oakey Abattoir to collect waste samples from the two different types of outflows- saveall wastewater and slaughter floor wastewater. Each of these samples were collected in sealed plastic containers and transported back to the University Laboratories where they were stored in the cool room at 4 °C as by Australian Standards (ISO 9887:1992). Saveall wastewater is the water that comes from all areas of the plant except the slaughter floor and contains high concentrations of fats, some blood, paunch waste, gut pit and rendering wastes. The water from the slaughter floor is called blood water due to its main constituent, blood. Figures 5 and 6, show the different areas of waste collection.

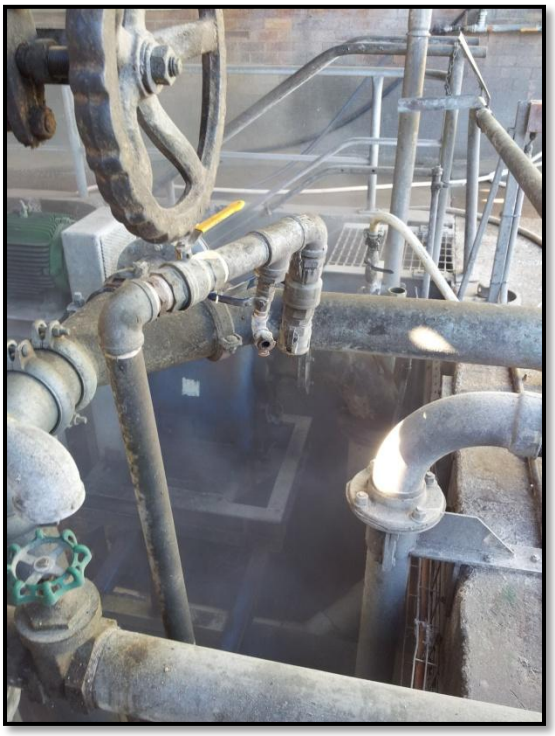

Figure 5 Outlet used to collect Saveall Water Sample Grab

Figure 6 Outlet used to collect Blood Water Sample

4.3 Collection of Vegetable Waste as Co-substrate

Vegetable waste is being investigated to develop a further understanding the impact that carbon rich vegetable waste would have on the methane production of the abattoir wastewater. The vegetable waste provides the abattoir waste with missing minerals and vitamins (El-Mashad and Zhang 2008). The vegetable waste could be sourced from a local vegetable processing site but the focus of the experiment is not to assess the methane potential of vegetable waste, only to see its impacts of co-digestion for abattoir waste. Therefore a vegetable waste was developed to maximise the carbon to nitrogen ratio to give a greater flexibility in the laboratories. The following literature is available to the nature of certain vegetables.

Table 7 C:N Ratios of Vegetables

Waste	C:N	% N weight	Reference
Carrot	27:1	1.6	(2)
Corn	37:1	1.23	(1)
Corn Leaves + Tassels	45:1	1.0	(1)
Cabbage	12:1	3.6	(2)
Potatoes	25:1	1.5	(2)
Turnips	44:1	1	(2)

(1) (Heard 2010)
(2) (Kourik 1986)

4.4 Determination of Wastewater and Inoculum Characteristics

The characteristics of the wastewater and inoculum were required to analyse and prepare mixing ratios. The sludge inoculum and wastewater were characterised on carbon, nitrogen and on the basis of total, suspended and volatile solids. Along with these necessary characteristics other parameters were determined also. These included pH, alkalinity and electrical conductivity. An ion chromatograph analysis

was also performed to determine the presence of ions in the wastewaters such as fluoride, chlorine, nitrate, nitrite, sulfates and phosphates.

4.4.1 Total Organic Carbon and Total Nitrogen

To evaluate the Total Organic Carbon and Total Nitrogen, each of the wastewater samples were filtered, transferred into the correct vials, placed in the TOC-TN machine and finally analysed. The machine being used is a TOC-VCPH/CPN analyser. Due to the limitations of this machine, the samples had to be filtered with a 45 micrometre filter before use, giving only the dissolved total organic carbon (DTOC). The results given are Soluble Total Organic Carbon, Soluble Inorganic Carbon and Soluble Total Nitrogen. These will be covered in Section 5.

4.4.2 Nitrate, Nitrite, Phosphorous and Sulphate

The levels of particular ions have been measured in order to develop further understanding to the processes that may be at work within the waste samples. The ions may be within optimum ranges or outside of natural ranges which could cause both positive and negative impacts to the anaerobic bacteria that will be consuming the waste. The levels could be useful indicating whether or not inhibition might be evident or if the ions are assisting with production. Nitrate has been suggested to ‘enhance bioconversion by altering the microflora and the physical and chemical characteristics of the process’ (Smith and Frank 1987) whereas ammonia is known to inhibit bacterial action over permitted levels. These ions were discovered through using an anion AS-18 column in an Ion Chromatograph Analyser and are presented in Chapter 5.

4.4.3 Total, Suspended and Volatile Solids

To characterise each of the substrates and in particular the seed sludge, the solids content was required. This was completed in accordance with the Standard Methods for Examination of Water and Wastewater, 21st edition (Clesceri et. al 2005).

4.4.4 Suspended Solids

The mass of suspended solids (SS) per litre indicates the portion of total solids retained on a filter paper 0.45 μm in size. The SS were calculated following standard procedures (Clesceri et. al 2005).

4.4.5 Total Solids

The total solids (TS) is the mass of solid content that remains after putting a sample in an oven at 105 °C overnight. It contains both organic and inorganic portions as well as the volatile and inorganic compounds.

4.4.6 Volatile Suspended Solids

The volatile suspended solids (VSS) are the mass of solids retained on a 45 μm filter paper after ignition at 550 °C for 20 minutes. It is important because it represents the mass of solids able to be consumed or processed by bacteria. Bacteria will not consume inorganic compounds. The suspended solids are most important when characterising the inoculum as the dissolved solids will be removed through the centrifuging and replacement with anaerobic media.

4.4.7 Total Volatile Solids

The total volatile solids (TVS) give both the suspended and dissolved organic particles.

4.5 Biochemical Methane Potential Assays- Feasibility of Co-digestion

The following method was utilised in order to assess the possibility of co-digestion of abattoir wastewater with carbon rich vegetable wastewaters.

4.5.1 Preparation of Inoculum and Anaerobic Media

The sludge requires a concentration of 1-3 g/L TS (ISO 11734:1995). The wastewater was initially filtered through a 500 μm sieve to remove larger solids such as fats, bone or hair. The inoculum was characterised by its solids content, centrifuged and the supernatant removed. The waste was re-suspended in anaerobic media and centrifuged once again (see Section 4.6.4). The supernatant is removed, anaerobic media is added and the waste is then ready to use. This is the reason that only the suspended solids within the inoculum are important because the dissolved particles will be removed with the supernatant after centrifuging.

4.5.2 Preparation of Anaerobic Media

Anaerobic media is a solution that acts to provide nutrients that may not be present in sufficient quantities in the waste substrates.

Table 8 Anaerobic Media

Chemical	Formula	Amount Required
Anhydrous potassium dihydrogenphosphate	KH_2PO_4	0.27g/L
Disodium hydrogenphosphate dodecahydrate	Na_2HPO_4-$12H_2O$	0.444g/L
Ammonium chloride	NH_4Cl	0.53g/L
Calcium chloride dihydrate	$CaCl_2$-$2H_2O$	0.075g/L
Magnesium chloride hexahydrate	$MgCl_2$-$6H_2O$	0.10g/L
Iron chloride	$FeCl_2$-$4H_2O$	0.013g/L
Sodium sulphide nonahydrate	Na_2s-$9H_2O$	0.1g/L
Resazurin		0.001g/L
Stock Solution		10 mL
Distilled Water		To 1L

800 mL of water was boiled in an Erlenmeyer flask and all chemicals are added, except for the sodium sulphide nona-hydrate. The solution is cooled, transferred to a flask and then the volume adjusted to 1 L before being transferred into the storage container. The solution is then sparged with nitrogen gas until the dissolved oxygen levels are at zero and the sodium sulphide nona-hydrate is added. The pH is measured and altered if needed and the headspace is flushed with Nitrogen gas again before the container was sealed.

4.5.3 Inoculum to Substrate Ratio

For the feasibility test of co-digestion, the ISR will be maintained at 2 g/L of volatile solids of inoculum for 200 mg/L of DTOC of substrate (ISO 1174:1995). For the experimental process varying the ISR and temperature the concentration of inoculum will be maintained at 2 g/L VS and the concentration of DTOC will be varied.

For example, if the ISR is to be for a range of 5 to 30 the following would apply.

$$ISR = \frac{Concentration\ of\ inoculum}{Concentration\ of\ DTOC}$$

$$ISR = \frac{\frac{2000mg}{L}}{400\frac{mg}{L}DTOC}$$

$$ISR = 5$$

The same calculation would apply for an ISR of 30 and then the range of DTOC concentration can be applied.

4.5.4 Preparation of Biological Methane Potential Assays

As seen in Figure 7, the BMP assays will consist of 500 mL volume Wheaton bottles sealed with a chlorobutyl lid allowing hypodermic needles to be inserted, in order to measure the gases produced.

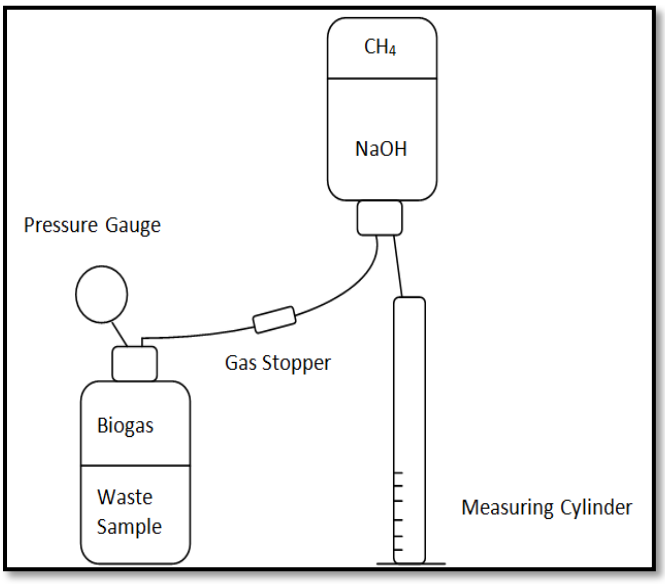

Figure 7 Biological Methane Potential Assay

The following table shows the volumes of inoculum and each substrate added to each individual test vessels. The test bottles were filled to a working volume of 420 mL with distilled water.

Table 9 Volume of Substrate and Inoculum added to each Test Bottle

No.	Type of Substrate	Glucose Volume	Vegetable Volume	Blood Volume	Saveall Volume	Inoculum Volume
1	Vegetable		115			46
2	Vegetable		115			46
3	Vegetable		115			46
4	Blood			140		46
5	Blood			140		46
6	Blood			140		46
7	Saveall				70	46
8	Saveall				70	46
9	Saveall				70	46
10	Glucose	210				46
11	Glucose	210				46
12	Glucose	210				46
13	Mix		56	14	11.5	46
14	Mix		56	14	11.5	46
15	Mix		56	14	11.5	46

The bottles will be filled with the required volume of sample, inoculum, and distilled water and flushed with Nitrogen gas in the headspace until the dissolved oxygen level reaches zero.

Figure 8 Test Bottle being Sparged while Measuring Oxygen Levels

The retention time for each of the samples will be decided during the experimental process but is assumed to be from 20-30 days, depending on the state of biodegradation of the waste and the time available. The following points show three sources with varying retention times:

- 10 days (Siddiqui 2012)
- 28 Days (Kubaska et. al. 2010)
- 30 Days (Labatut 2012)

This time will depend on the time taken for the methane production to reach a plateau.

4.5.5 Volume and Gas Measurement

Each of the bottles were prepared and kept in the incubator at 37±0.2 °C (Siddiqui et. al. 2011, Kubaska et. al. 2010, Labatut 2012) as shown in Figure 9.

Figure 9 Test Bottles being Incubated

Gas measurements were performed by having the inverted sodium hydroxide bottle on a stand (Figure 10). The bottles will be removed from the incubator one at a time to keep the temperature constant and then tested. It is important that the temperature is kept as constant as possible as it will maintain a steady pressure in the assay bottles.

Pressure measurement for the experiments was made by using a pressure gauge that is attached to a hypodermic needle with a range of 0-25 kPa and 0-150 kPa for higher ranges. The needle was inserted into each of the rubber butyl lids to measure the pressure. The volume of biogas produced each day was released daily, meaning that the pressure measurement returned to zero after each reading.

Figure 10 Biological Methane Potential Assay Apparatus

Volume measurement was undertaken using the principle of displacement. The sample bottle has another hypodermic needle inserted into its rubber butyl seal that is connected to an inverted bottle filled with 1.2 M sodium hydroxide. This bottle will have one capillary tube connected to it allowing gas to enter and another exit point where sodium hydroxide will be forced out from the increasing pressure from the gas. The gas passing through the sodium hydroxide will have a composition of methane, carbon dioxide, hydrogen, nitrous oxides and hydrogen sulphide. The volumes of hydrogen, hydrogen sulphide and nitrous oxides are all assumed negligible. Research made has shown that when this biogas passes through sodium hydroxide the methane will remain but the other gases present will dissolve. When the gas pressure displaces the sodium hydroxide into another measuring cylinder, it

is expected that the volume of displaced sodium hydroxide will be the equivalent to the volume of methane.

Mixing of the sample was required to increase the contact between inoculum and wastewater samples. This is suggested to be and was performed manually everyday (Labatut 2012). The mixing of samples also allows carbon dioxide to dissolve into the sample increasing the dissolved inorganic carbon within the sample.

The process of sampling included swirling the bottle manually for a set period of time, measuring temperature, taking a pressure reading and finally releasing the gas. The temperature was recorded by using an Infrared Thermometer. This was useful as the temperature could be recorded without needing to open the test vessels. A section of non-reflective masking tape was attached to the outside of the test jars in order to reduce errors from the thermometer.

The results for this process will be covered in Section 5.

4.6 Biogas production from Abattoir in different seasons

The main experimental process will be run to test the impact of the inoculum to substrate ratio and the temperature on the fluctuation of gas production. The process will be run in a similar manner to the experimental process in Section 4.5. However, only the pressure measurement will be taken as an indication to the volume of gas present within the headspace. The content of methane will not be taken into consideration.

4.6.1 Determining Experimental Trials using Minitab16

The program Minitab16 is a mathematical software program that allows the use of methods such as Analysis of Variance and Response Surface Method. The Response Surface Method will be used to calculate the minimum number of experimental runs that are required in order to give the most representative range of results based on the number of variables. The program applies a central composite design method to calculate the number of experimental trials. The central composite design method uses four axial and four factorial points overlaid and with a set number of central points, which are equal to the number of controls used. In the diagram below, the red point represent the axial points, blue dots are the factorial points and the yellow central dot represents the number of controls (usually 3-5).

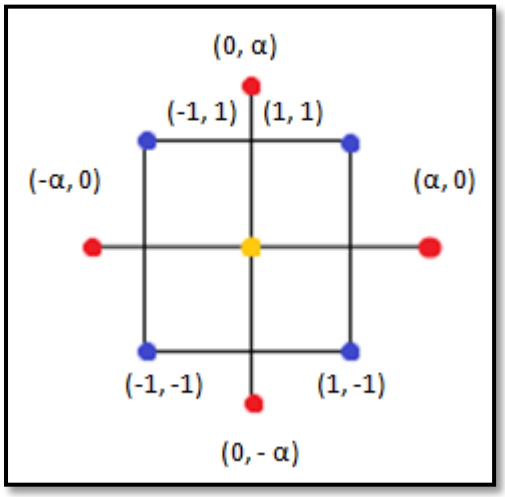

Figure 11 Central Composite Design

Each of the axial and factorial points is situated at each end of their respective domains: -1 to 1 for factorial and –α to α, for the axial points. The value of alpha is calculated from a formula based on the number of variables being applied, k.

$$\alpha = (2^k)^{0.25}$$

Table 10 Central Composite Design (Trinh & Kang 2010)

Code	Actual Value
-α	Xmin
-1	$\frac{(\alpha-1)Xmax + (\alpha+1)Xmin}{2\alpha}$
0	$\frac{Xmax + Xmin}{\alpha}$
1	$\frac{(\alpha-1)Xmin + (\alpha+1)Xmax}{2\alpha}$
α	Xmax

To calculate for the maximum amount of biogas produced there are two variables involved with this method, the inoculum to substrate ratio and the temperature. Using the formula given below, the number of experiments can be calculated:

$$Number\ of\ Trials \quad = \quad 2^k + \ 2^k + \ Number\ of\ Controls$$
$$Number\ of\ Trials \quad = \quad 13$$

(Trinh and Kang 2010)

The experiments will be run with 5 control trials and the two variables, giving 13 trials.

Once the software has been imbedded with the given number of trials and the value of each variable to include in each, the experiment can then be performed. Once the results have been recorded they can be added into the program. From the experimental results, the response surface method analysis can be run to produce an optimisation model. These results will be covered in Chapter 6.

4.6.2 Biochemical Methane Potential Assays- Varying Temperature and Inoculum to Substrate Ratio

From the calculations made using the Response Surface Method, it is then required that an experimental procedure be created like that in the preliminary experiment. The purpose of this experiment is to obtain the gas production for the varied temperature and ISR to input them into Minitab to achieve optimisation. Firstly the Minitab16 model was created. The ISR range of 5 to 30 was decided upon, along with the temperature range of 4 to 40 °C to represent the possible variations in seasons.

Figure 12 shows the variables entered into Minitab16.

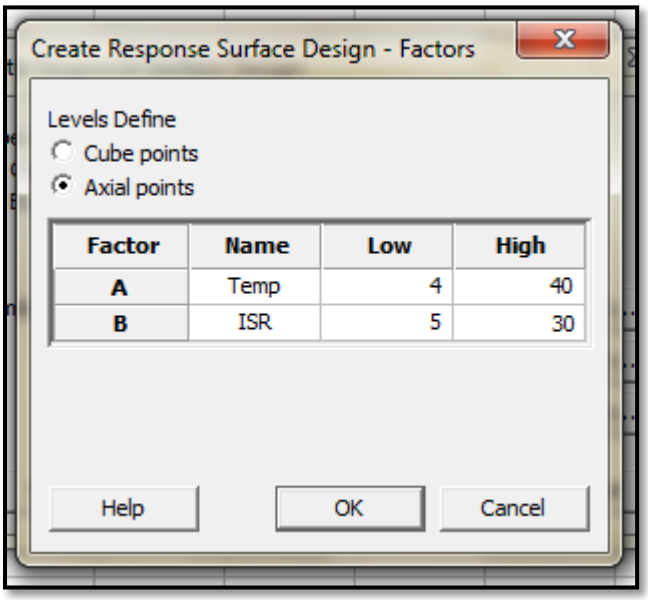

Figure 12 Defining Variables in Minitab16

Figure 13 shows the output of the experimental design. There are 13 test vessels and each will have the shown corresponding temperature (Temp) and inoculum to substrate ratio (ISR).

↓	C1	C2	C3	C4	C5	C6
	StdOrder	RunOrder	PtType	Blocks	Temp	ISR
1	11	1	0	1	22.0000	17.5000
2	13	2	0	1	22.0000	17.5000
3	12	3	0	1	22.0000	17.5000
4	2	4	1	1	34.7279	8.6612
5	6	5	-1	1	40.0000	17.5000
6	4	6	1	1	34.7279	26.3388
7	3	7	1	1	9.2721	26.3388
8	1	8	1	1	9.2721	8.6612
9	5	9	-1	1	4.0000	17.5000
10	8	10	-1	1	22.0000	30.0000
11	7	11	-1	1	22.0000	5.0000
12	9	12	0	1	22.0000	17.5000
13	10	13	0	1	22.0000	17.5000

Figure 13 Output of Experimental Design in Minitab 16

Table 13 shows the bottle number, ISR, temperature, volume of inoculum, volume of substrate, volume of anaerobic media (AM), volume of distilled water and the area each vessel will be stored in.

The inoculum was found to have 18.78 g/L VSS. This was required to be 2 g/L within the whole test vessel which had a working volume of 420 mL:

$$Volume\ of\ Inoculum \quad = \quad \frac{420\ mL}{\left(\frac{18.78 g/L}{2\ g/L}\right)}$$

$$Volume\ of\ Inoculum \quad = \quad 44.73\ \approx 45\ mL$$

The final volume of inoculum required was 45 mL.

Table 11 Volume Calculations for Main Experimental Process (mL Volume)

No.	ISR	Temp °C	Inoculum mL	Substrate mL	A.M mL	Water mL	Store Place
1	17.5	22	45	85	-	290	RT
2	17.5	22	45	85	-	290	RT
3	8.7	34	45	170	-	205	Tank
4	8.7	34	45	170	-	205	Tank
5	17.5	40	45	85	-	290	WB
6	17.5	40	45	85	-	290	WB
7	5	22	45	300	-	75	RT
8	5	22	45	300	-	75	RT
9	8.7	9	45	170	-	205	Incubator
10	8.7	9	45	170	-	205	Incubator
11	30	22	45	50	-	325	RT
12	30	22	45	50	-	325	RT
13	17.5	4	45	85	-	290	Cold Room
14	17.5	4	45	85	-	290	Cold Room
15	17.5	22	45	85	-	290	RT
16	17.5	22	45	85	-	290	RT
17	26.3	9	45	60	-	315	Inc.
18	26.3	9	45	60	-	315	Inc.
19	17.5	22	45	85	-	290	RT
20	17.5	22	45	85	-	290	RT
21	17.5	22	45	85	-	290	RT
22	17.5	22	45	85	-	290	RT
23	17.5	22	45	85	-	290	RT
24	17.5	22	45	85	-	290	RT
25	26.3	34	45	60	-	315	Tank
26	26.3	34	45	60	-	315	Tank
27	seed	22	45	0	-	375	RT
28	seed	34	45	0	-	375	Tank
29	seed	40	45	0	-	375	WB
30	blank	22	0	0	45	375	RT
31	blank	34	0	0	45	375	Tank

There will be duplicates of each of the bottles detailed in Table 11 which shows bottles 1-26. Bottles 27-29 will have only the inoculum and distilled water to measure gas output for inoculum alone as it processes any suspended organic matter in the inoculum pellet and then reaches a plateau phase or endogenous growth. The final two bottles (30 and 31) will have only distilled water and anaerobic media. The

anaerobic media was included as it is a pH buffer, so will show the effects that the media has on the substrates and inoculum. For ease, the volume of inoculum was maintained constant throughout the tests and the volume of substrate was varied in order to reach the desired ISR. The volume of distilled water is required to reach an equal volume.

Table 12 Volume Calculations for Varied ISR

ISR	Concentration of Substrate (mg/L DTOC)	Volume of Substrate (mL)	Volume of Substrate (mL) Actual added
5	400.00	298.83	300
8.7	229.89	171.74	170
17.5	11429	85.38	85
26.3	76.05	56.81	60
30	66.67	49.80	50

A sample from each test vessel was taken before sparging with Nitrogen gas and sealing. The sample was then analysed to determine the initial DTOC and DTN. Once sealed, the bottles were incubated at their respective temperatures. 24 hours was allowed for each test vessel to reach the correct temperature and the gas volume was released. From this point, the pressure and temperature will be monitored daily to calculate the daily and cumulative biogas production.

The volume of biogas production will be calculated through the use of the Universal Gas Laws, as previously seen for the first experimental process. The pressure within the test bottles will be measured and released under a fume hood daily along with manual mixing to suspend bacteria in the substrate.

4.7 Risks

There are a number of risks involved with this project as there are dealings with raw sewage wastewater and abattoir wastewater. These two types of waste can hold many harmful bacteria and viruses which can be dangerous for humans. For this reason, vaccinations were required for Hepatitis A, Tetanus and Q fever to prevent any contractions. While working with these wastes in the laboratory personal protection equipment such as gloves, laboratory coat, safety glasses, enclosed footwear and face mask were used at all times. The correct labeling of bottles and storage containers was required to ensure that the hazard was not passed on to anyone else.

Before entering the laboratory for experiments an induction was required, covering everything from safety information, introduction to all equipment, fire escape plans, emergency phone and contact numbers, chemical spill equipment and plan, body and eye wash stations, fire extinguishers and coverage of the required personal protection equipment.

When using equipment in the laboratory it was necessary to wash all glassware thoroughly and then rinse in hydrochloric acid and/or nitric acid to ensure that no cross contamination was caused. All disposable equipment was discarded into the hazardous waste bin and wastewater disposed of with care in compliance with standards and regulations.

Every chemical used required a Material Safety Data Sheet (MSDS) to be read, signed and handed into the laboratory supervisor. Along with ensuring the knowledge of the chemicals being used, a standard operating procedure was reviewed for the equipment required. These too were signed and handed into the laboratory supervisor.

Simple tasks such as cleaning surfaces with ethanol, correct labeling and correct disposal of wastes and chemicals was necessary. The storage and transport of wastewater is to be in sealed containers that are well labeled with warnings. Care

must be taken with handling anything from the autoclave, furnace or oven, making sure the correct gloves and eyewear is worn.

4.8 Summary

This chapter covers the experimental processes used to develop the biological methane potential assays. It presents the characterisation methods for each of the waste substrates and outlines the procedure undertaken for the various experiments completed. It also describes the mathematical software programming required to analyse the required results.

5 Biogas and Methane Production from the Co-digestion of Abattoir Wastes

This chapter provides the results and discussion of biogas and methane production from the co-digestion of abattoir waste with carbon rich vegetable wastewater along with the digestion of single substrates of abattoir wastewater, vegetable wastewater and an easily biodegradable carbon source, glucose. Initial experiments were performed in order to investigate the feasibility of co-digestion and to test the activity of the seed anaerobic sludge. Finally it concludes with the evaluation of the anaerobic biodegradability of all of the substrates by analysing the carbon mass balance. This mass balance allows for the determination of the percentage of biodegradation.

5.1 Raw Wastewater Characteristics and Design of Experiments

This section describes the results and discussions on the raw water characteristics and how the BMP assays for each of the substrates were designed. It discusses the design parameters and the analysis of the initial conditions found in the sample assays once the experiments had been initialised.

5.1.1 Raw Wastewater Characteristics

Preliminary characteristics were evaluated for the three types of wastewater: vegetable, saveall and blood water. These wastewaters contained both suspended as well as dissolved organic matters that can be utilised as a food source by the anaerobic microorganisms and through metabolism, are converted into biogas. A well mixed and homogenised sample was taken in set volumes to measure the total organic matters from both suspended and dissolved states. The organic constituents

present in the dissolved state were measured by filtering the samples for the analysis of various parameters further discussed in the methodology.

The results shown in Table 13 detail the average results found for a range of components along with the standard deviation where possible.

The pH and alkalinity were measured to check for extreme ranges that would inhibit bacterial growth. As aforementioned the pH range between 5.5 and 8 would be acceptable but close to neutral would be ideal. The abattoir wastewaters were both considered within acceptable ranges for both of these factors. The electrical conductivity was analysed for use in the development of calibration curves for the ion chromatography machine that analysed the levels of ions found within the samples. This will be covered further in this module.

The DTOC for vegetable, blood and saveall wastes were 1264, 607 and 730 mg/L respectively. The vegetable wastewater had the highest concentration of DTOC and also the lowest in DTN as expected due to lower nitrogen or protein content within the vegetable matter. The saveall and blood wastewaters both had very similar concentrations of carbon and nitrogen. The DTOC/DTN ratios therefore are similar between the blood and saveall wastewaters. The blood water had a slightly higher ratio than the saveall wastewater.

It is evident that the TTOC of all of the substrates have been considerably high due to higher amounts of suspended solids. Vegetable wastewater has the highest concentration of total organic carbon, although not having the highest suspended solids content. This is from the vegetable wastewater naturally having higher concentrations of carbon in relation to the abattoir wastes. Of the abattoir wastes, the saveall has the highest concentration of TTOC which correlates to higher concentrations of suspended solids. The blood water has lower concentrations of suspended solids which suggests and has confirmed that the TTOC is lower in comparison to both the vegetable and saveall wastewaters.

Table 13 Initial Wastewater Characteristics

Characteristic	Units	Vegetable Waste		Blood Wastewater		Saveall Wastewater	
		Mean	Std Dev.	Mean	Std Dev.	Mean	Std Dev.
General Chemical Attributes							
pH	-	-	-	7.71	0.10	7.08	0.06
Alkalinity	ppm	-	-	728.0	27.62	1665	161.2
Electrical Conductivity	ppm	430.4	-	322.4	-	1835	-
Soluble Characteristics							
CODs	mg/L	2750.0	4.2	1454.3	23.0	1772.8	30.0
DTOC	mg/L	1264	84.85	606.95	24.68	729.95	1.77
DIC	mg/L	0.86	0	83.91	0.07	154.2	1.13
DTN	mg/L	60.62	0.16	144.15	0.21	171.65	4.03
Scalar TC Analyser							
CODt	mg/L	4119.0	22.6	3886.0	86.0	4548.0	96.0
Total Carbon	mg/L	5500	-	4300	-	6400	-
Total Nitrogen	mg/L	200	-	200	-	400	-
TTOC	mg/L	5485.8	-	3775.2	-	5284.1	-
Solids Analysis							
TS	g/L	4.82	0.04	3.70	0.06	7.55	0.05
TSS	g/L	2.37	0.13	1.74	0.60	2.55	0.10
TVS	g/L	4.14	0.04	2.74	0.02	5.22	0.06
TVSS	g/L	2.02	0.52	1.87	1.59	2.39	1.71
Derived Parameters							
CODt/CODs	-	1.50	-	2.67		2.57	-
TOC (from CODt/CODs)	mg/L	1893.24	-	1621.78	-	1872.61	-
DTOC/DTN	-	20.85	-	4.21	-	4.25	-
TOC/TN	-	27.43	-	18.88	-	13.21	-
TVS/TS	%	85.98	-	74.05	-	69.14	-

The chemical oxygen demand refers to the amount of oxygen required under acidic conditions to oxidise organic compounds into inorganic outputs. It gives an indication to the level of contamination a water stream has. The COD can be measured for dissolved state and total state matters. The higher levels of organic content, the higher the COD will be. When looking at the total COD (CODt) the saveall waste has the highest with blood water having the lowest. The saveall water as previously discussed has a considerably high concentration of TTOC and subsequently it has the highest CODt with 4548 mg/L. The vegetable wastewater has higher concentrations of TTOC but has a slightly lower CODt than the saveall wastewater. The difference seen here is because the saveall wastewater has much higher total solids concentration, requiring more oxygen in the COD process to break down the organic fraction. The blood water has the lowest TTOC and also the lowest CODt with 3886 mg/L. The blood water has similar solids content to that of vegetable wastewater meaning it requires less to break down organic fractions into organic compounds.

The analysis of total, suspended and volatile solids was also used to understand the characteristics of suspended and dissolved solids present in the wastewaters. The saveall water had the highest levels of suspended solids. Vegetable waste had higher solids content when compared to blood water due to the plant matter contained. When looking at the percentage of volatile solids the vegetable, blood and saveall contained 86.0, 74.0 and 69.1 % respectively. The percentage of volatile suspended solids is important as it gives an indication to the particles within the wastewater that have the potential to be consumed by bacteria as it indicates the organic fraction.

The solids analysis is important for understanding the composition of each of the waste. Total solids show the total concentration in g/L of the total, suspended, dissolved, organic and inorganic fractions. The most important of these are the total, total volatile and total volatile suspended solids. The volatile solids indicate the fraction of organic material in comparison to the total concentration of solids. The organic fraction is obviously important due to bacteria only being able to metabolise and process organic materials. The saveall wastewater had the highest concentration of total solids with 7.55 g/L; of this 69% are volatile solids. The saveall is expected

to have higher concentrations of inorganic particles as it is water that has come into contact with animals that may be covered in dirt or mud. In comparison to the blood and vegetable wastewater, this lower organic fraction is seen. The vegetable waste water has the second highest concentration with 4.82 g/L with 86% organic fraction. The blood wastewater has 3.7 g/L total solids with a lower 74% organic. The blood water also has some inorganic contamination through open air contamination, dirt and soil from the slaughter floor, other particles through contamination in transportation, storage and piping.

Once all of the raw water characteristics have been investigated and analysed, the experiments have been designed so that the incubated test bottles would receive substrates having a final concentration of 200 mg/L of DTOC.

5.1.2 BMP Assay for Various Substrates

Batch assays are prepared with the addition of substrates as per calculation to have a final concentration of 200 mg/L DTOC. In developing the seed bacteria for analysis, the bacteria require washing in an anaerobic media, as explained further in the Methodology. This anaerobic media contains some ammonia which contributes to the nitrogen content within the seed bacteria, hence increasing the nitrogen found within the substrate analysis. For analysis of the substrate alone, the concentrations of carbon and nitrogen within the seed sample need to be subtracted.

Once prepared, well-mixed and sparged with Nitrogen gas to induce anoxic conditions, set samples of 20 mL volume were made in order to analyse and confirm the concentrations of various ions, total organic carbon and nitrogen.

Table 14 shows the results given for the Ion Chromatography analysis. This was performed in order to analysis the presence of extreme ranges of any particular ions that may have a considerable affect on the total biogas and methane production. Each

test run contains the given volume of substrate and seed, and the seed bacteria control is also seen in this table.

Table 14 Ion Chromatography Results for Various Substrates

	F	Cl	NO2	SO4	Br	NO3	PO4
vegetable	12.38	30.63	0.00	8.86	0.00	0.00	16.77
blood	0.00	54.81	na	4.04	0.00	0.00	17.62
saveall	0.00	52.64	na	11.72	0.00	na	21.12
glucose	0.00	22.44	na	5.14	0.00	na	14.76
mixture	5.05	33.79	na	5.86	0.00	0.00	16.50
seed	0.00	21.94	na	7.40	0.00	na	14.94
blank	0.00	0.09	na	6.72	0.00	0.00	na

There was fluoride present in the vegetable wastewater and mixture from the preparation of vegetable waste. The vegetable waste contains some portion of regular tap water which contains fluoride. Chlorides again have similar results. The seed shows that the chlorine of around 20 mg/L has been provided through the wastewater treatment plant. 10 mg/L of chlorine is provided by the tap water used in preparing the vegetable waste. This is carried through into the mixture. The blood and saveall contain higher levels of chlorine as the water used for washing in the abattoir is likely to have high concentrations for sterilisation purposes. The chlorine found in the abattoir and vegetable wastes are evident in the preparation of the mixture. Glucose has 23 mg/L of chlorine, which is sourced from the seed bacteria. There is no presence of NO_2 or bromide within any of the samples. There are some low concentrations of sulphates and phosphates present in all of the samples. The blank (deionised water only) has some contamination of sulphates but no evidence of phosphates. Overall it can be concluded that no concentration of any of the above mentioned ions will impact upon the gas production of the bacteria as they are all at low concentrations. Other major impacting elements included the total and soluble carbon and nitrogen concentrations.

Table 15 shows the results obtained from the carbon and nitrogen sample analysis.

Table 15 Calculated Initial Parameters for Co-digestion Feasibility Test

	Vegetable	Blood Water	Saveall Water	Glucose	Mixture	Seed
DTC (mg/L)	314.13	203.53	169.70	193.55	185.59	29.80
DTOC (mg/L)	301.05	161.90	129.43	180.75	163.75	16.36
DIC (mg/L)	13.08	41.63	40.28	12.80	21.84	13.44
DTN (mg/L)	40.85	68.85	64.01	23.90	40.38	25.70
DTOC/DTN	7.37	2.35	2.02	7.56	4.06	0.64
Derived TTOC from raw waste characteristics						
TTC	5500.00	4300.00	6400.00	164.35	5420.86	-
TTOC	5500.00	3420.44	4881.07	153.48	4782.96	-
TTN	200.00	200.00	400.00	0.00	228.22	-
TTOC/TTN	27.50	17.10	12.20	-	20.96	-

It was evident that the required DTOC of 200 mg/L was not exact when added to the test vessels. These discrepancies come from experimental error through measuring and the inclusion or exclusion of say large clumps of sample that would increase the carbon content. The higher vegetable concentrations and lower saveall concentrations were due to human error. This set level of carbon was required to ease the process of comparing biogas and methane production between different substrates. These values are standardised later in the analysis of gas production. The TTOC for each of the test substrates was determined by multiplying the TTC found in the raw water samples by the ratio of organic to total carbon found in the test vessels.

Although the DTOC/DTN ratios for vegetable, blood, saveall, glucose and mixtures were 7.37, 2.35, 2.02, 7.56 and 4.06 respectively, after deducting the seed samples gave the following:

- Vegetable: 18.79
- Blood water: 3.37
- Saveall water: 2.95
- Mixture: 10.04

The carbon to nitrogen ratio cannot be calculated for the glucose sample as there is no nitrogen contained within the sample. Therefore, from this analysis it is evident that the carbon to nitrogen ratio (based on DTOC/DTN) is much higher for that of vegetable in comparison to the abattoir wastewater samples. The mixture contains 80% vegetable waste (as seen in the Methodology) and it is evident the impact the high nitrogen concentrations in the abattoir wastewaters is having on the ratio within the mixture.

The final DTOC and DTN were also recorded after the digestion period had been completed. It was expected that if the bacteria were consuming the organic carbon as a food source and nitrogen to assist with cell synthesis that there will be a decrease in both concentrations for the DTOC and DTN. Although saying both concentrations will decrease, the carbon will be removed at a higher rate than the nitrogen. It is also expected that the inorganic carbon levels will increase. This is due to the bacteria respiring carbon dioxide (CO_2) and CO_2 being dissolvable in solution. Dissolved CO_2 will increase the dissolved inorganic carbon (DIC).

Table 16 Calculated Final Parameters for Co-digestion Feasibility Test

	Vegetable	Blood Water	Saveall Water	Glucose	Mixture	Seed
DTC (mg/L)	228.81	194.65	181.03	107.06	144.59	94.28
DTOC (mg/L)	147.05	42.23	44.58	30.09	31.86	23.78
DIC (mg/L)	81.8	152.4	136.45	76.97	112.7	70.50
DTN (mg/L)	73.37	121.47	101.75	56.38	82.22	60.84
DTOC/DTN	2.00	0.35	0.448	0.53	0.39	0.39
Derived Parameter						
TTC	5500.00	4300.00	6400.00	164.35	5420.86	-
TTOC	3534.75	932.81	1576.02	46.19	1194.44	-
TTN	200.00	200.00	400.00	0.00	228.22	-
TTOC/TTN	17.67	4.66	3.94	-	5.23	-

When deducting the seed from the other substrates the following DTOC/DTN ratios were obtained:

- Vegetable: 9.84
- Blood water: 0.30
- Saveall: 0.51
- Mixture: 0.38

In order to compare the initial and final carbon to nitrogen ratios Table 17 is presented.

Table 17 Initial and Final Carbon to Nitrogen Ratios for Co-digestion Analysis

	DTOC/DTN initial	DTOC/DTN final
Vegetable	18.79	9.84
Blood Water	3.37	0.30
Saveall Water	2.95	0.51
Glucose	n/a	n/a
Mixture	10.04	0.38

Theoretically, the carbon to nitrogen ratios should decrease as the bacteria consume the carbon in quantities much higher than that of nitrogen. This is evident for all of the substrates; however, some have experienced more significant changes than others. The vegetable waste water has the most obvious outlying result. While the mixture and abattoir wastewaters have had a decrease in DTOC/DTN ratio of 82-96% decrease, the vegetable waste has only decreased in DTOC/DTN ratio by 47%. This shows that there is some inhibitory element within the vegetable wastewater that is slowing or preventing the consumption of carbon.

The biogas produced from the batch assays was measured by pressure and volume. The test vessels were to hold approximately 200 mg/L DTOC and were incubated at a constant temperature of 37 °C.

5.2 Measured Biogas and Methane Yields

Using Gas Laws to standardise the volumes produced, it was then possible to compare volumes for each substrate at a constant temperature and pressure. The following results were achieved.

Volume of biogas and methane produced at standard atmospheric pressure and 37 °C (mL) over a 33 day incubation period:

5.2.1 Cumulative Biogas and Methane Production

Table 18 Cumulative Biogas and Methane Production

	Vegetable	Blood Water	Saveall Water	Glucose	Mixture
Cumulative Biogas (mL)	162.8	472.8	426.9	144.9	278.7
Cumulative Methane (mL)	99.2	337.4	312.3	86.1	185.7
% Methane	61.0	71.4	73.2	59.4	66.6

The highest volume of biogas recorded was produced by blood water, closely followed by saveall wastewater. This was opposite to the conclusion made from the literature reviews that suggested that the higher carbon to nitrogen ratios will produce the highest volume of biogas. The vegetable waste produced lower percentages of methane, whereas the blood and saveall water produced the highest percentages. It is evident that the presence of vegetable waste in the mixture is constraining the methane potential. The mixture is 80% vegetable waste, showing that the presence of abattoir wastewater is increasing the percentage of methane than of the vegetable waste alone. The glucose also produced less than expected. Being an easily biodegradable carbon substrate it was expected to also produce higher volumes of biogas and methane. Low volumes such as this may be due to human error in calculations of concentrations or the lack of acclimatisation of the bacteria to the substrate.

When looking at the percentage of methane being produced from these substrates, it is evident that some variations are present. The average methane percentage of the five substrates is 66.3% which lies between the suggested percentages of 50-70%. It is evident that the abattoir waste water actually produces higher biological methane potentials than the vegetable, glucose and mixture substrates. This shows that the bacteria were able to digest the abattoir waste with greater ease than the other substrate types. The glucose substrate had the lowest methane content of 59.4%. The biodegradation of glucose will be investigated in a later module of this dissertation but the lower methane content could be due to some difficulty for the bacteria to

consume the glucose (acclimatisation did not occur) or that the chemical structure of glucose does not provide the bacteria with the required elements to produce required methane volumes. The purpose of the glucose was to provide some indication to the activity of the bacteria if the digestion of abattoir waste was not successful. Again it is evident that the presence of a total of 20% of abattoir waste in 80% vegetable waste has increased the methane content substantially. It is still lower than the production of abattoir wastewater alone but there is an improvement evident.

Variations in these methane contents come from human error along with variations in the biodegradability and composition of substrates. Human error comes from releasing the biogas through the sodium hydroxide solution. When the biogas was released, it was not possible to regulate the flow. Increased rate of release lead to faster rate of bubbles through the solution, leaving less time for the CO_2 to be dissolved out of the biogas. The methane percentage in the biogas was also impacted by the size of the gas bubble released. Bubbles that were smaller were optimal in this situation for allowing the CO_2 to dissolve as it provides a larger surface area to volume ratio.

To show the gas production phases, cumulative biogas and methane graphs were developed. Some initial lag phases are seen while bacteria acclimatise to the substrates. This stage is then followed by a rapid increase in the production rate where the bacteria are digesting the substrates. Once the substrate is close to being consumed the production rate decreases to develop into a plateau phase. Once all of the substrate is consumed, the bacteria enter an endogenous growth phase. In the endogenous phase, bacteria begin consuming themselves so some biogas production will be seen until the remaining bacteria run out of the food source.

Figure 14 shows the cumulative volume of total biogas being produced by each substrate. This represents the biogas produced essentially by consumption of the substrate alone and does not include the biogas production of the seed alone.

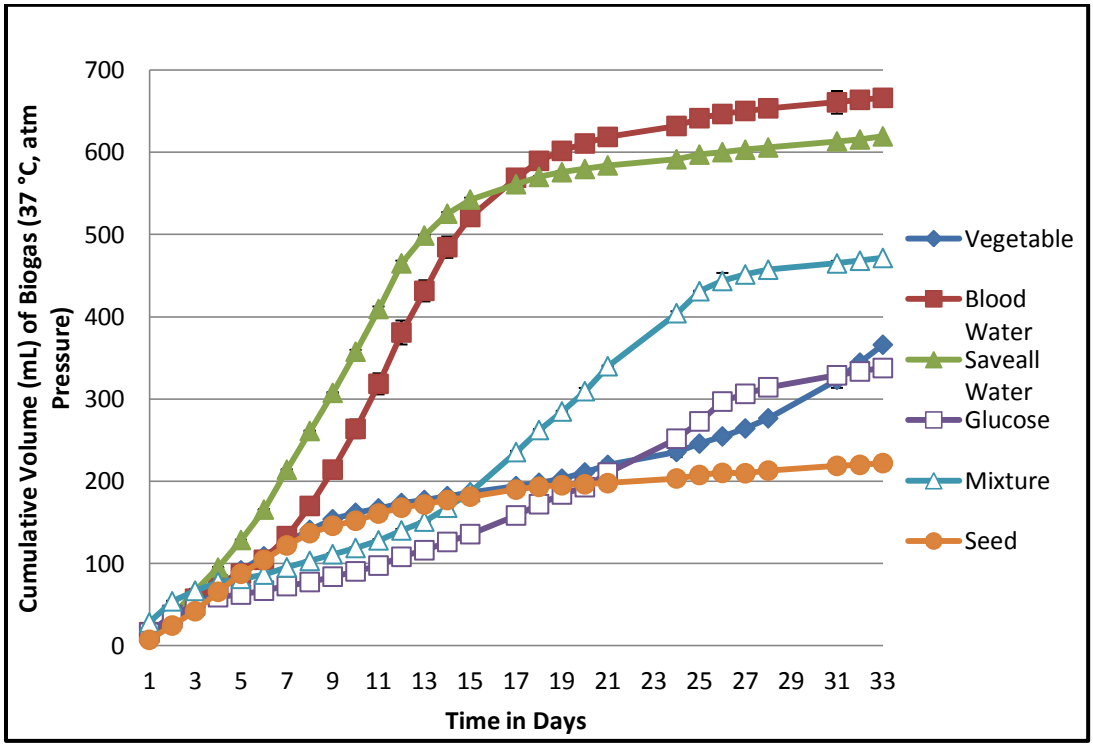

Figure 14 Volume of Cumulative Biogas Produced at 37 degrees Celsius and Std. Pressure

It is evident in Figure 14 that the blood and saveall wastewaters produced the highest volumes of biogas. They also follow relatively similar distribution with blood water having a slightly longer lag phase then the saveall wastewater and reaching a plateau phase at a later date. The mixture produced the next highest volume of biogas with approximately 472 mL of biogas being produced. The lag phase for the mixture was also longer than both of the abattoir wastewaters, showing some inhibitory or further acclimatisation taking place due to the vegetable waste present. The mixture has a very similar distribution pattern to that of the glucose. They both has a short rapid rate of production lasting 2 to 3 days followed by a lag phase of 12 to 13 days in length, a shorter rapid phase and lastly the plateau phase. The vegetable wastewater did not produce an initial lag phase and followed the distribution of the seed control test vessel until the 19th day. The vegetable waste produced slightly more biogas than the glucose and less than the mixture. It seems at day 19 a considerable increase in the gas production rate occurred.

Literature suggests that a mixture of two or more components, such as the carbon rich vegetable and nitrogen rich abattoir wastes, will produce higher volumes of

biogas when mixed together than when digested separately. Evidently this was not the case. The mixture proved more likely to produce biogas than the vegetable waste alone but less than both of the abattoir wastewaters. This suggests there were some inhibitory factors in the vegetable waste that impacted on the ability of the anaerobic bacteria to produce the biogas as desired. This could be due to lignin or other difficult to digest components of vegetable wastes. Following the cumulative volumes produced by the seed and vegetable waste suggest that the bacteria relied on the suspended solids of the inoculum added and when these were consumed then moved to consuming the vegetable wastewater. Acclimatisation to the waste might help to prevent this to some degree.

The seed alone produced approximately 222 mL of biogas, reaching a plateau phase as early as 8 to 10 days. This phase was reached much faster than that of the other substrates. The seed control vessels did not have a lag phase as there was no acclimatisation occurring.

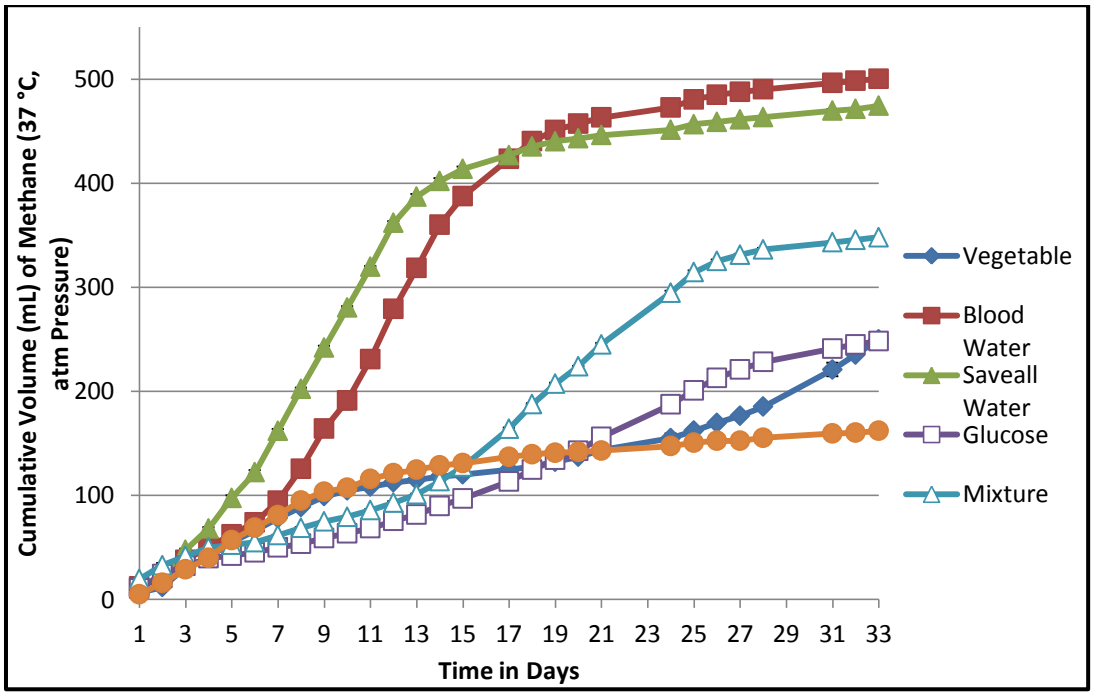

Figure 15 Volume of Cumulative Methane Produced at 37 Degrees Celsius and Std. Pressure

Figure 15 shows the distribution of methane production of each substrate. It is evident that these follow that same phases as seen in Figure 14. However, it is seen that the vegetable wastewater actually produced less methane than the seed control until around day 21. This shows that the vegetable wastewater was actually inhibitory to some extent to the production of biogas and methane.

The variations between biogas and percentage methane is shown in Figure 16. This box and whisker plot shows the range of methane percentages that were recorded for each substrate with the maximum, minimum, range and quartiles shown.

5.2.2 Methane Content in Biogas

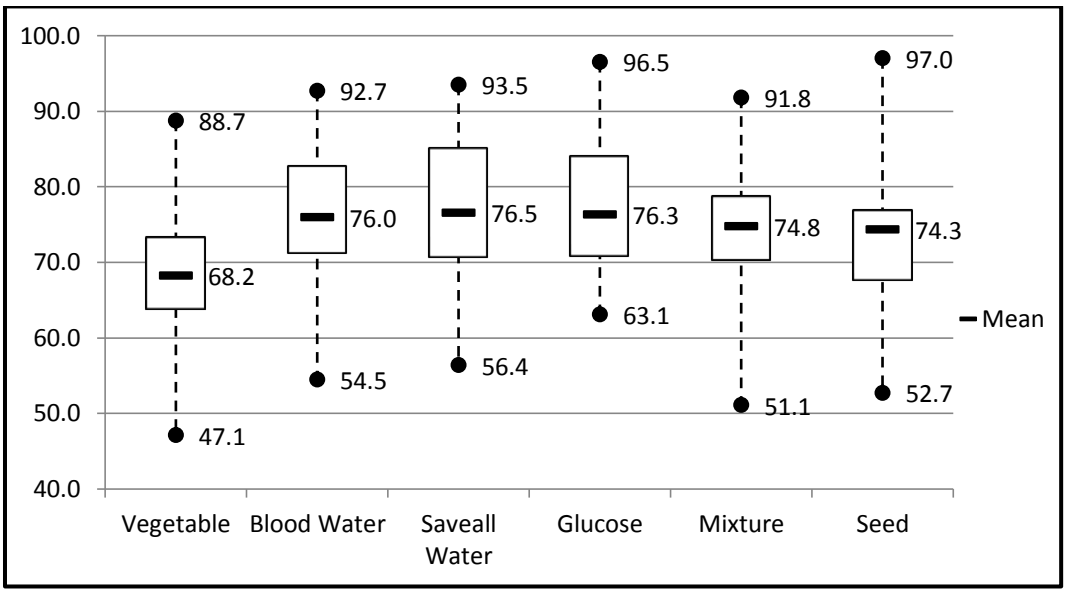

Figure 16 Percent Composition of Methane in Biogas (%)

Figure 16 shows that the biogas composition for the experiments varied but on average was found to be between 65% and 85%, thus agreeing with literature. As mentioned previously, a capillary tube was used to release the volume of biogas into the inverted bottle of alkaline solution, sodium hydroxide. All of the CO_2 will be adsorbed in the solution however, experimental errors with the capillary tube resulted in fluctuating results for methane composition.

Figure 16 clearly depicts the large variations seen in the percentage of methane recorded. Results of 47.1% to 97% methane were recorded which are highly variable, unpredictable and unreliable. This further suggests the inconsistencies with the methane recording process.

As the target organic carbon in the samples was intended to be 200 mg/L DTOC, the results were standardised per 200 mg/L of DTOC. The results are again at standard atmospheric pressure, 37 °C and for an incubation period of 33 days.

5.2.3 Standardised Results for 200 mg/L DTOC

The following Figures, 17 and 18, show the standardised biogas and methane production for each of the substrates. After standardising the results, both of the abattoir wastewaters produced very similar results with almost equal volumes of biogas being produced. The mixture, glucose and vegetable substrates all followed the same ranking when looking at the biogas production with vegetable waste being considerably lower than the other substrates.

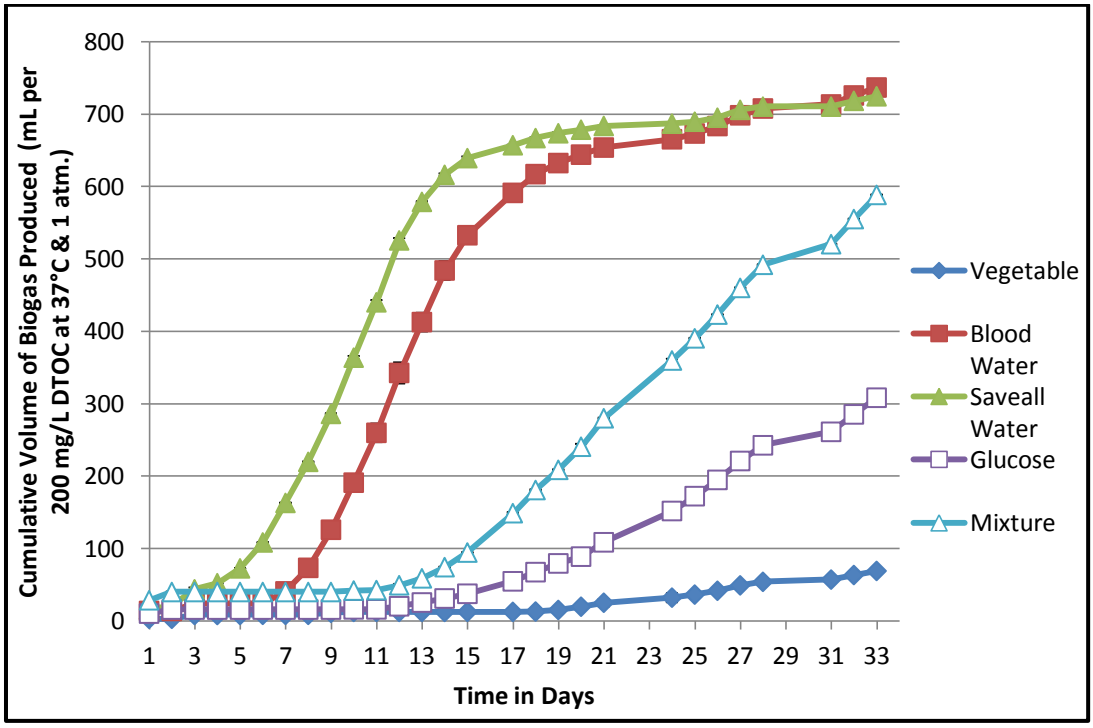

Figure 17 Cumulative Biogas Production (mL/200 mg/L DTOC at 37 Degrees Celsius and Std. Pressure)

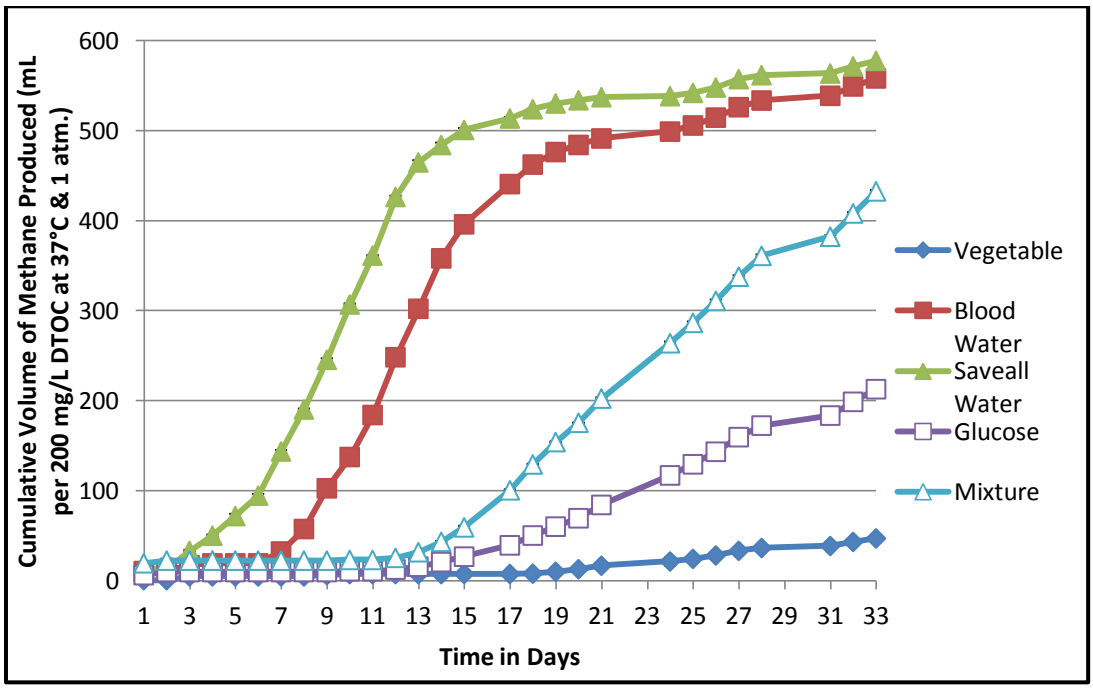

Figure 18 Cumulative Methane Production (mL/200 mg/L DTOC at 37 Degrees Celsius and Std. Pressure)

By deducting the seed and standardising to 200 mg/L DTOC there is a significant difference between each of the substrates. The blood water and saveall water produced similar volumes of biogas and methane which is explained by the fact that both water types were similar in carbon, nitrogen, cation/anion and solid particle concentrations. Although having similar volumes the saveall produced the smallest volume of biogas and the higher percentage of methane. There was no evidence of inhibition from the accumulation of volatile fatty acids or ammonia ions as suggested in literature. The mixture ranked in the same position as without standardising but the glucose was found to produce more biogas than the vegetable waste. As discussed early the vegetable obviously had some inhibiting factor or was complex in a way that nutrients and food was not available to the bacteria and therefore it is understandable that the glucose would produce more biogas as it is an easily biodegradable substrate with a simple molecular structure.

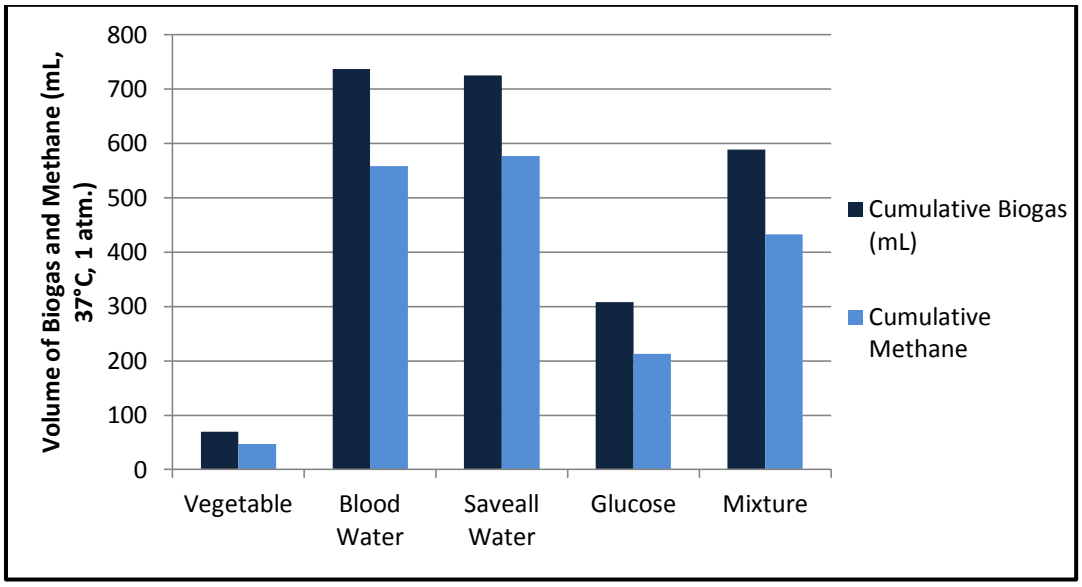

Figure 19 Cumulative Volume of Biogas and Methane (mL/200 mg/L DTOC at Std. Pressure and 37 Degrees Celsius)

Figure 19 shows the corresponding methane production with the biogas production. This image clearly depicts the lack of gas production by the vegetable wastewater.

Table 19 Cumulative Biogas and Methane Production (mL/200 mg/L DTOC at 37 Degrees Celsius and Std. Pressure)

	Vegetable	Blood Water	Saveall Water	Glucose	Mixture
Cumulative Biogas	69.1	736.9	724.8	308.4	588.6
Cumulative CH_4	47.4	557.9	577.4	213.0	432.6
% Methane	68.6	75.7	79.7	69.1	73.5

Table 19 shows the measured volumes of cumulative total biogas and cumulative methane. It also shows that methane ranges from 68.6-79.7% of the total volume of biogas. This agrees with studied literature, however, as the biogas could not be analysed using a gas chromatography analyser to provide a more accurate reading over the measured methane from the chosen method. Again, it is evident that the abattoir wastes provided the highest percentages of methane production, which is desirable. The mixture also provided relatively high percentages with close to that of blood water.

The following graphs show the behaviour of gas production over the 33 day incubation period for standardisation by volatile solids.

5.2.4 Biogas and Methane Production Standardised by Volatile Solids

The gas production per unit weight of volatile solids is useful to compare the gas production for the weight of organic fraction of wastes. Volume of biogas and methane produced by each of the substrates per gram of volatile suspended solids are shown in Figure 20.

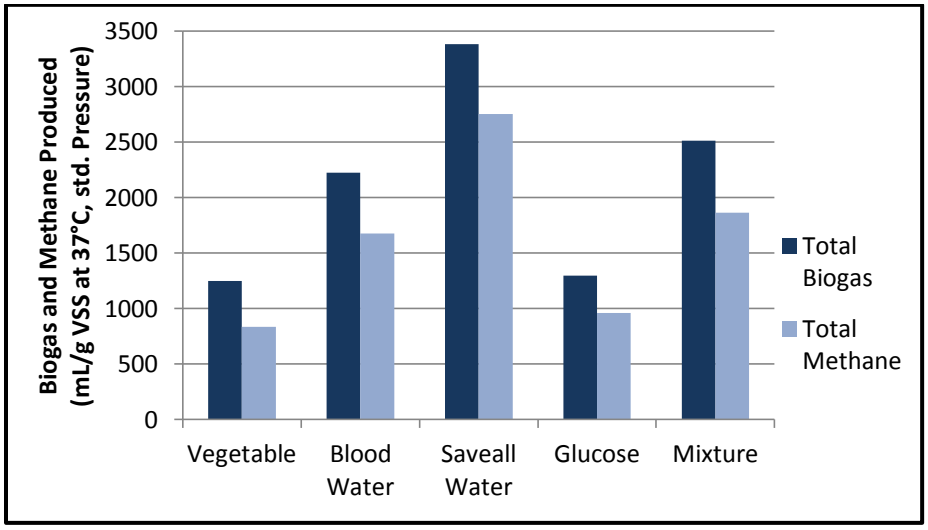

Figure 20 Comparison between Biogas and Methane Production (mL/g VSS)

The comparison of biogas production shows that the saveall wastewater provides the highest volumes of biogas and methane. This is closely followed by the mixture and then blood wastewater. The vegetable waste water again is the lowest producer.

Table 20 Cumulative Biogas and Methane Production (mL/g VSS)

Substrate	Total Biogas (mL)	Total Methane (mL)	%BM
Vegetable	1250.7	837.8	67.0
Blood Water	2223.1	1675.4	75.4
Saveall Water	3381.8	2755.7	81.5
Glucose	1299.2	962.4	74.1
Mixture	2514.8	1862.5	74.1

It is evident that the percent of methane is still within the range of 65-85%. This table shows that the saveall wastewater actually produces much higher volumes of methane per gram of total volatile solids. Thus suggesting that the saveall wastewater is preferable for digestion over the other substrates, least of all being vegetable waste.

5.3 Theoretical Biogas and Methane Yields

The relationship between biogas and methane depends on the extent of oxidation of organic matter. Biogas has the main constituents of CO_2 and methane (CH_4). Methane is the reduced state whereas the CO_2 is the most oxidised form. To estimate the theoretical biogas production from substrates such as glucose, abattoir wastewater and vegetable waste the Buswell Equation can be used.

The Buswell equation will provide ratios between methane and carbon dioxide and the Ideal Gas Law will then be used to develop this into a volume.

Buswell Equation (Sobotka 1982):

$$C_nH_aO_bN_z + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3z}{4}\right)H_2O = \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3z}{8}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3z}{4}\right)CH_4$$

The extended formula was required to include Nitrogen, due to the nitrogen present in proteins. The suggested composition of proteins, carbohydrates and lipids are as follows:

Protein:	$C_5H_7O_2N$	
Carbohydrate:	$C_6H_{10}O_5$	
Lipid:	$C_{57}H_{104}O_6$	(Raposo 2011)

The Buswell Formula provides the ratio of moles between the substrate added and the carbon dioxide and methane received. The volumes of each substrate can then be calculated from the Ideal Gas Law at standard conditions.

$$PV = nRT$$

Where P= Pressure (Pa), V= Volume (L), n= Moles, R= Molar gas constant (8314 J/mol.K), T= Temperature (Kelvin)

Table 21 Proportion of CO2 to CH4

Substrate	Formula	Moles H_2O	Moles CO_2	Moles CH_4
Glucose	$C_6H_{12}O_6$	0	3	3
Protein	$C_5H_7O_2N_1$	3	2.5	2.5
Carbohydrate	$C_6H_{10}O_5$	1	3	3
Lipid	$C_{57}H_{104}O_6$	28	17	40

This approach then allows the volume of biogas produced by 200 mg/L TOC of each substrate to be calculated for 37 °C and standard pressure. Glucose, Proteins and Carbohydrates produced equal parts of biogas and methane at 424.17 mL of total biogas and 50% methane. Lipids have a more complex structure and produced comparable amounts of biogas but, as seen in the graph below, higher percentages of methane at 70.17%.

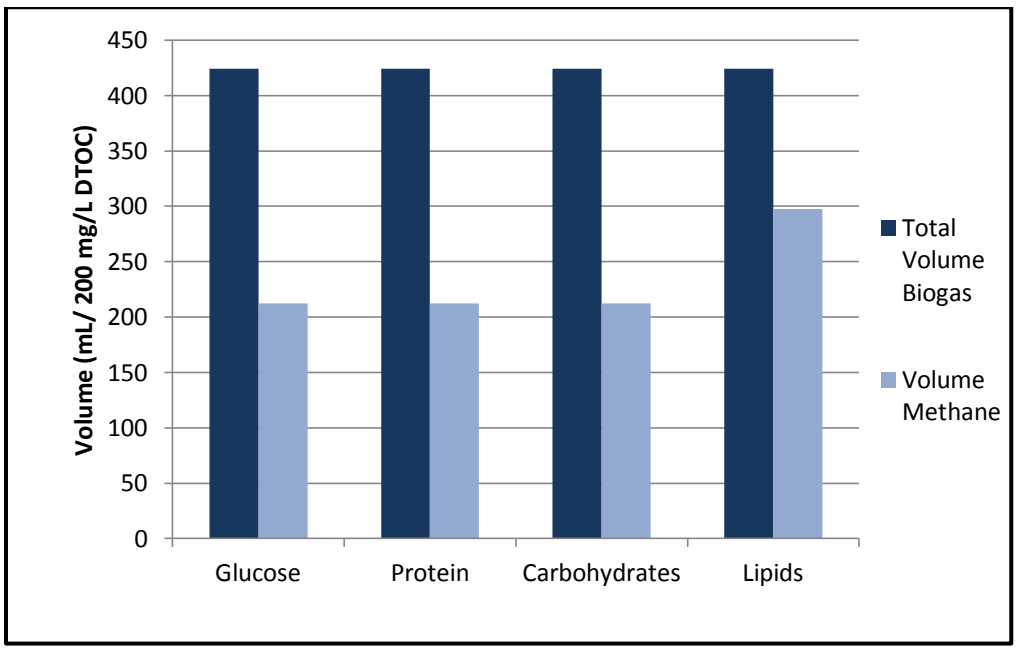

Figure 21 Theoretical Gas Production (mL/200 mg/L DTOC at 37 Degrees Celsius and Std. Pressure)

Each of these carbon chains will be found in different proportions in each of the waste substrates. It is expected that the blood water will contain some proteins whereas the saveall wastewater, from visual inspection, will contain lipids and carbohydrates due to the presence of solidified fats within the wastewater. The waste types could not be characterised to determine these properties specifically. The one substrate that is comparable here is the glucose. The glucose theoretically will produce 450 mL of biogas per 200 mg/L of DTOC of substrate. Of this, 50% will be methane. From the experimental results, 308.4 mL of biogas was produced under the same conditions. Of this 69.1% was measured methane. This shows that the bacteria are digesting waste substrates at a sufficient rate. The higher percentage of methane may be due to other nutrients and wastes that were present in the bacteria inoculum pellet that could be increasing methane content. The lower obtained volume of biogas could be caused from a lack of acclimatisation

5.4 Carbon Mass Balance and Biodegradation

Biodegradation can be calculated using standard methods and by looking at a carbon balance of what has been added to the test vessels, what is remaining within the experiments and what is being extracted. Carbon can be released as carbon dioxide and methane and it can also be dissolved within the sample, as carbon dioxide. The carbon remaining is determined by the DTOC measured at the end of analysis and finally a balance is made between the added carbon and the aforementioned releases to find the amount of unaccounted carbon. This carbon is assumed to be consumed for bacterial cell growth, adsorbed onto suspended solids or assimilated within wastes.

5.4.1 Inorganic Carbon

Within the batch assays, carbon dioxide is being produced. Not only is this CO_2 being released as biogas but it is also being dissolved within the sample, aggravation through stirring helps this CO_2 to dissolve. Hence, an increase in the CO_2 levels should be evident by the end of analysis. The increases seen in inorganic carbon was as follows:

Table 22 Variation in Inorganic Carbon Concentration (mg/L)

Substrate	Initial IC (mg/L)	Increase IC (mg/L)
Vegetable	0.0	11.62
Blood Water	28.2	53.74
Saveall Water	26.8	39.12
Glucose	0.0	7.12
Mixture	8.4	33.84

Table 22 shows that there has been a significant increase in inorganic carbon for the blood water, saveall and mixture. The mixture had the highest increase of 304%, the blood water had an increase of 91% and the saveall wastewater had an increase of 46%.

5.4.2 Organic Carbon

The total organic carbon inputs and outputs give an indication of the extent of biodegradation also. The initial and final concentrations of nitrogen were also determined but will be discussed later within the dissertation. The initial and final concentrations of DTOC were recorded and represented in Figure 22 with the extent of degradation shown also as a percentage.

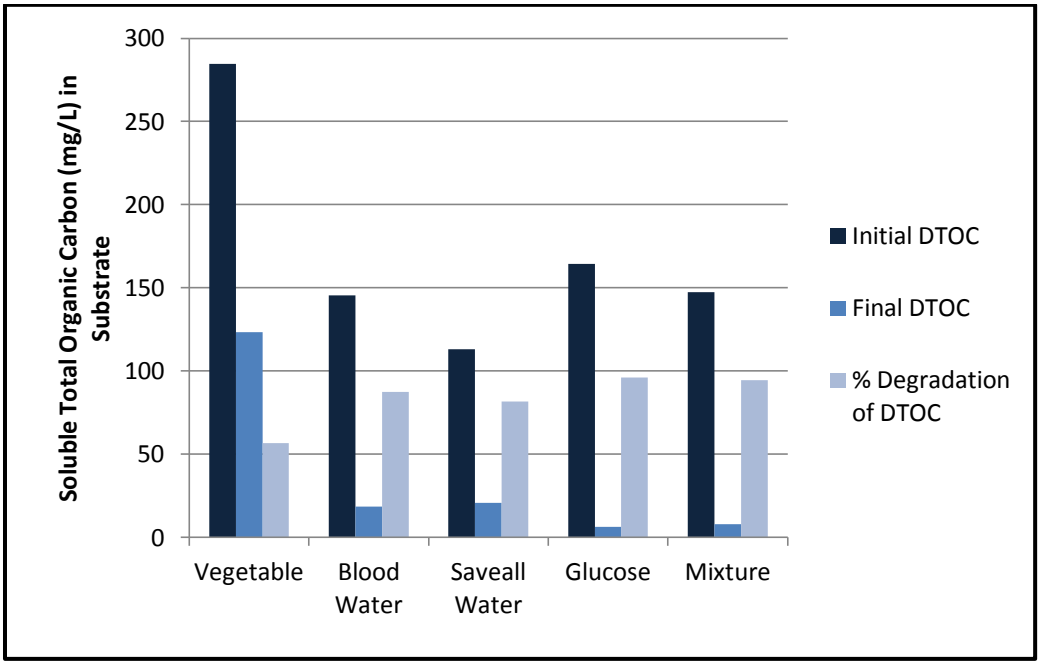

Figure 22 Comparison between Initial and Final DTOC Concentrations (mg/L)

The Figure 22 shows that the glucose shows the biggest reduction in carbon. This proves that the glucose is in fact the most easily biodegradable as previously suggested. The dissolved organic carbon in the mixture vessels had higher carbon reduction in comparison to the vegetable and abattoir wastes alone. This suggests the abattoir waste in the mixture of wastes assists in carbon consumption. Thus showing that co-digestion although did not assist in biogas and methane production, it did consequently increase the biodegradability of the carbon within the substrates.

Figure 22 shows that the carbon added to the vegetable waste test vessels was not consumed to the extent of the other substrates as it has the lowest degradation.

5.4.3 Variation of Nitrogen in Digestion

The initial, final and percentage increase in Nitrogen are seen in Figure 23 below.

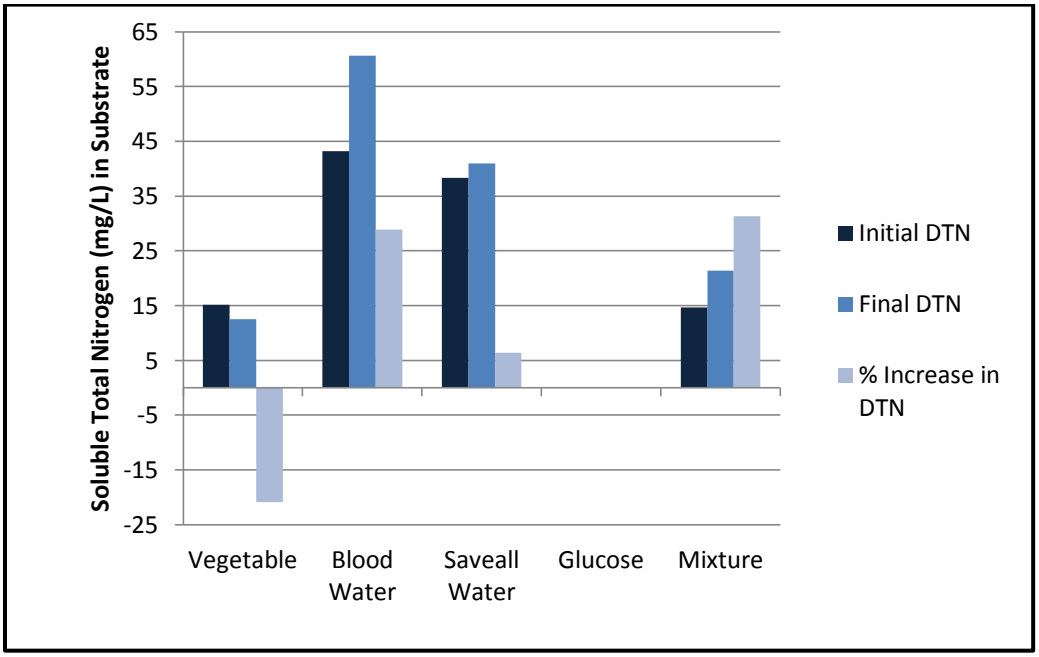

Figure 23 Comparison between Initial and Final Dissolved Nitrogen Concentrations (mg/L)

The nitrogen levels are important in determining the carbon to nitrogen ratios and assessing the impacts of nitrogen in the form of ammonia ions which can be inhibitory when accumulated. Firstly, the glucose had no nitrogen present initially or at the end of the trial. The blood water, saveall water and mixture test bottles increased in nitrogen. This is due to the breakdown of organic particles releasing nitrogen. The vegetable waste decreased in nitrogen. Microbes require nitrogen for maintained growth. It is possible that because the vegetable wastewater was initially low in nitrogen, nitrogen is not being released during digestion. Instead, the nitrogen is being consumed for cell growth.

5.4.4 Biodegradation Standard Methods

Due to equipment availability, the soluble organic carbon (DTOC) and soluble nitrogen (DTN) were found using the TOC-TN analyser. The total organic carbon (TTOC) and total nitrogen (TTN) were analysed using the scalar primacs TC/TN analyser. As the scalar primacs TC/TN machine cannot differentiate between organic and inorganic content it only provides a total carbon and nitrogen value. To calculate the biodegradation the organic fraction was required. A ratio between soluble and total carbon was useful in calculating the total organic fraction of each of the substrates.

Table 23 Ratios between TTC and DTC

Substrate	Factor of TTC/DTC
Vegetable	4.34
Blood Water	6.22
Saveall Water	7.23
Glucose	1.0
Mixture	5.07

The carbon present in the glucose mixture is completely soluble and therefore the concentrations of glucose in the filtered and unfiltered samples are equal. Glucose was assumed to have a ratio of 1 as it will only have dissolved solids and no suspended solids (in which carbon is adsorbed or assimilated). Vegetable water was also higher in dissolved particles showing that the ratio is not as large as the abattoir wastewater which has high levels of suspended particles.

The total biodegradation can be calculated by defining the mass of carbon within the liquid and headspace of the test vessels. Following the standards (ISO 1995) the procedure is as follows.

The mass of carbon in the headspace:

$$m_h = \frac{12000 * 0.1 * (P * \Delta V)}{R * T}$$

Where m_n is the mass of carbon in the headspace, P is 1013.25 millibars, ΔV is the cumulative volume of biogas produced, R is the gas constant (8314 J/mol.K) and T is the temperature at 310 K.

The mass of carbon in the liquid:

$$m_l = p_{ICnet} * V_l$$

Where m_l is the mass of carbon in the liquid, p_{ICnet} is the net change in inorganic carbon minus the blank and V_l is the volume of the liquid in the test vessel.

The total mass of gasified carbon (mg):

$$m_t = m_h + m_l$$

The carbon added to the test vessel:

$$m_v = p_{cv} * V_l$$

Where m_v is the mass of test substrate carbon, p_{cv} is the concentration of test compound carbon and V_l is the volume of the liquid.

The total biodegradation can therefore be described as:

$$D_t = \frac{m_t}{m_v} * 100$$

Table 24 Total Biodegradation of Carbon in Vessels (%)

Substrate	Dt (%)
Vegetable	15.8
Blood	64.6
Saveall	60.8
Glucose	83.3
Mixture	46.3

Evaluating the total biodegradation shows that glucose was degraded the most at 83%. This is due to glucose being an easily biodegradable substrate and this was expected. Again the two abattoir wastewater streams were degraded at similar rates. Evidence thus far supports the two streams being similar in composition although they were initially thought to respond differently to anaerobic digestion. The mixture also was degraded by close to 47% which shows that the small presence of abattoir waste is actually lifting the biodegradability of the vegetable wastewater. Vegetable wastewater was depleted the least at 16.5%, this may have been due to inhibitory factors or also the complex structure of vegetable waste in the form of lignin.

Within the sample vessel there are a number of ways carbon can be deposited and released through biogas. The initial mass of carbon added to the test vessel is required to make this analysis. Firstly, carbon is released through the biogas in two forms, methane and carbon dioxide. Secondly, CO_2 is soluble in the solution and therefore represents the inorganic fraction. The difference between initial and final inorganic carbon levels represents the mass of carbon remaining inorganically in the sample at the end of analysis. The percentage of carbon remaining in the effluent is calculated from the final concentration of the organic carbon and finally the balance remaining shows the amount of carbon unaccounted for through carbon synthesis, assimilation or the adsorption of carbon onto suspended solids. Adsorption onto suspended particles cannot be measured as the TOC/TN Analyser can only measure the carbon within solution or dissolved particles.

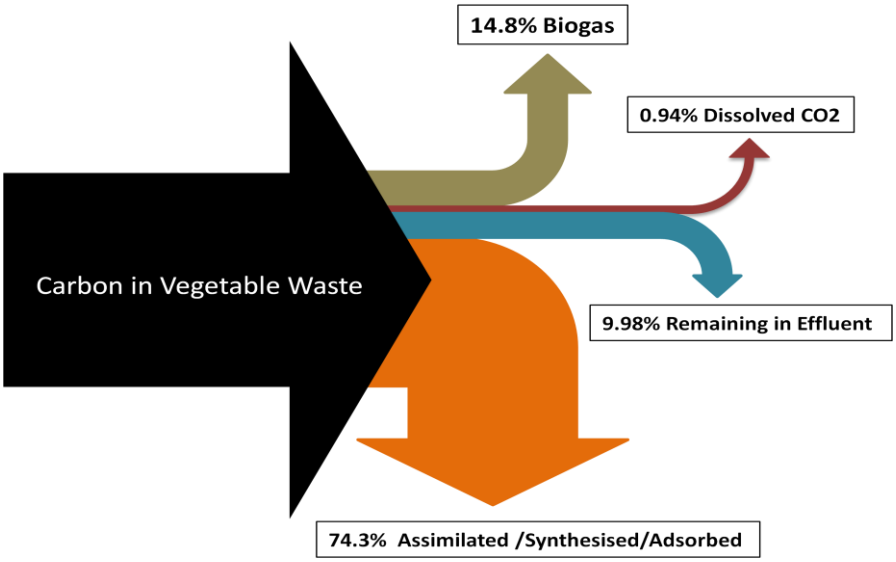

Figure 24 Carbon Balance for Vegetable Wastewater

Vegetable waste had a significant quantity of carbon that has been assimilated, synthesised or adsorbed. Only 14% was exerted through biogas and approximately 10% was remaining in the effluent. This shows that the vegetable waste was difficult to process and also that the carbon involved has mainly been utilised or adsorbed with little gas production.

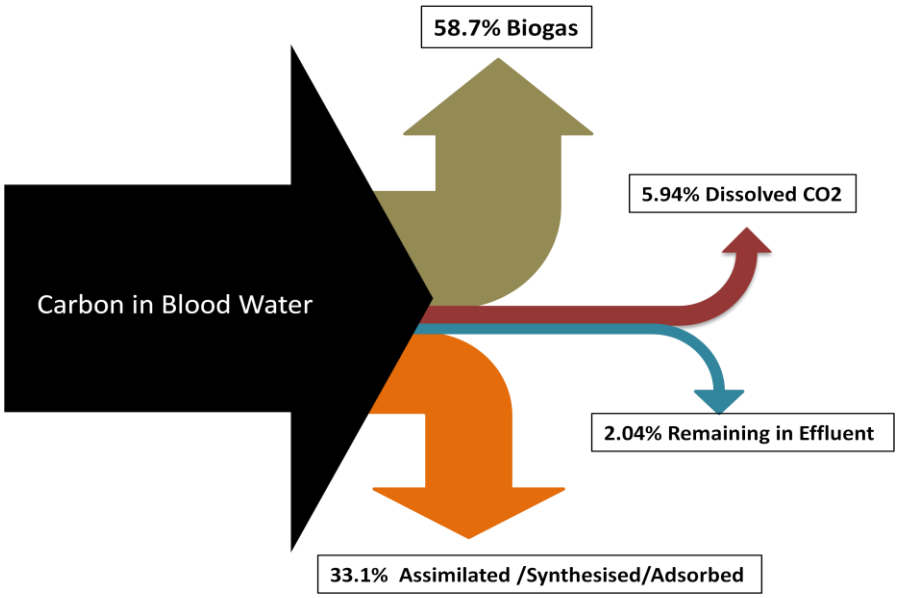

Figure 25 Carbon Balance for Blood Wastewater

Blood wastewater had a high percentage of carbon transformed into biogas and approximately a third was utilised in cell growth or adsorbed onto suspended particles. This shows that the blood water was much more productive than the vegetable wastewater. It is also evident that the majority of the wastewater was consumed as there is only around 2% of carbon remaining in the solution at the end of the preliminary trial.

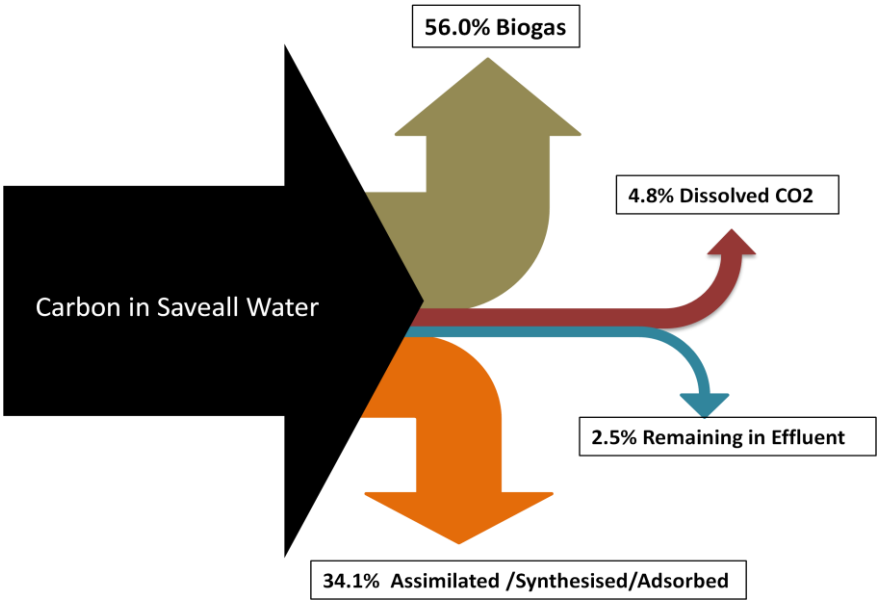

Figure 26 Carbon Balance for Saveall Wastewater

Saveall wastewater is comparable to the blood wastewater as they have very similar characteristics and consequently have resulted in very similar degradation behaviour. The results for both the blood and saveall wastewaters is desirable as a large portion is being exerted as biogas and the majority of what is remaining is then used in synthesis of new cells, assimilated or adsorbed. In practice, the carbon that is assimilated or adsorbed will be removed later in the wastewater treatment process through further settling and sedimentation processes.

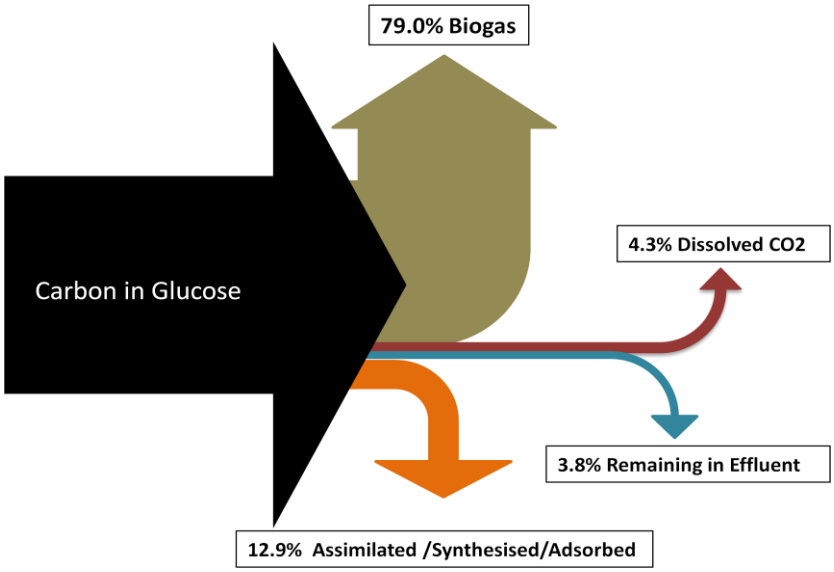

Figure 27 Carbon Balance for Glucose

It is evident that glucose is an easily biodegradable substrate as the majority of carbon has been processed and exerted as biogas. A mere 13% was assimilated, synthesised or adsorbed and only 3.8% remained in the effluent. This shows a great degree of biodegradation within the sample, which was desired.

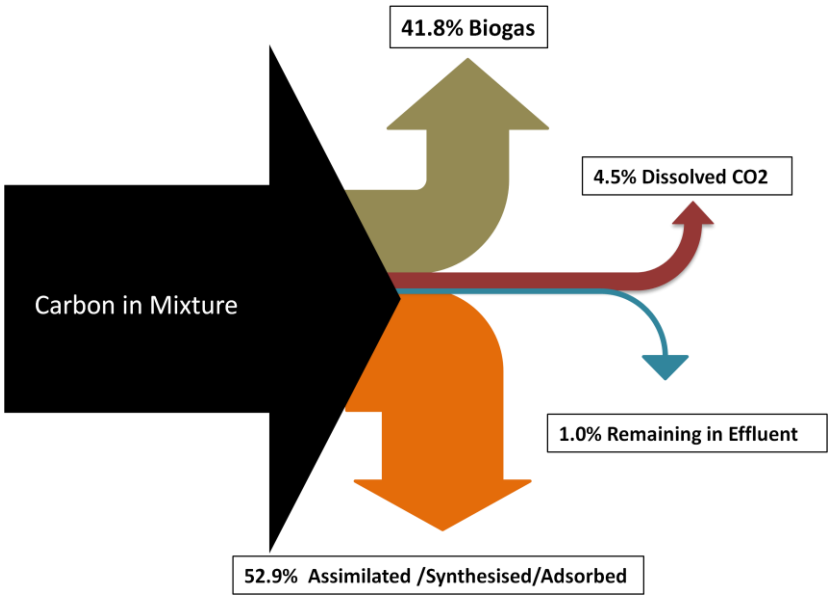

Figure 28 Carbon Balance for Mixture Wastewater

The carbon present in the mixture has also been manipulated with only 1% of carbon remaining in the effluent. The amount of dissolved carbon was similar to that achieved for the other wastewaters and the quantity released as biogas and also synthesised/adsorbed was closest to that of both of the abattoir wastewater. Although the mixture contained 80% vegetable wastewater it is evident that the presence of abattoir wastewater greatly improves the ability of the vegetable water to be digested. This would be useful in practice if the aim was to optimise the digestion of vegetable waste. It does not help, however, with the digestion of abattoir wastewater.

5.5 Summary

This chapter has reported and discussed the impacts of the Carbon to Nitrogen ratio and co-digestion on biogas and methane production of two streams of abattoir wastewater along with vegetable wastewater. This chapter has given an understanding to the volumes of biogas and methane that can be obtained from the anaerobic digestion of vegetable, blood, saveall, glucose and mixtures of abattoir and vegetable wastes. The blood water alone produced the highest volumes of biogas with 736.9 mL/200 mg/L DTOC closely followed by the saveall wastewater with 724.9 mL/200 mg/L DTOC. The mixture of 80% vegetable waste, and 10% of each abattoir wastewater stream gave 588.6 mL/200 mg/L DTOC followed by the glucose substrate with 308.4 mL/200 mg/L DTOC. The lowest biogas production was by the vegetable wastewater with 69.1 mL/200 mg/L DTOC. Thus showing that co-digestion did not improve the biogas production of abattoir wastewater by using a carbon rich substrate, such as vegetable wastewater.

6 Biogas Production from varying ISR and Temperature

As described in the methodology, the second experimental process involved varying the inoculum to substrate ratio (ISR) and temperature. This procedure involved looking at the ISR and the effects this has on biogas production along with the effect of seasonal temperature ranges. This chapter aims to present and discuss the findings of this experimental procedure.

6.1 Initial Characteristics

The DTOC is measured for further calculation of biodegradation. Table 25 shows the recorded DTOC for all of the test vessels including that of the seed controls.

Table 25 Initial Characteristics for Experimental Process Varying ISR and Temperature

ISR	Temperature	DTOC recorded
17.5	**22**	84.617
8.7	**34**	159.482
17.5	**40**	82.877
5	**22**	330.317
8.7	**9**	185.312
30	**22**	76.427
17.5	**4**	96.437
17.5	**22**	121.772
26.3	**9**	68.147
17.5	**22**	121.442
17.5	**22**	122.717
17.5	**22**	98.702
26.3	**34**	76.442
Seed	**22**	13.343
Seed	**34**	13.013
Seed	**40**	15.293

Table 26 Initial Characteristics after Deducting Controls

ISR	Temperature	DTOC of substrates	TOC required
17.5	**22**	70.7	114
8.7	**34**	145.6	230
17.5	**40**	69.0	114
5	**22**	316.4	400
8.7	**9**	171.4	230
30	**22**	62.5	67
17.5	**4**	82.6	114
17.5	**22**	107.9	114
26.3	**9**	54.3	76
17.5	**22**	107.6	114
17.5	**22**	108.8	114
17.5	**22**	84.8	114
26.3	**34**	62.6	76

Table 25 shows the measured DTOC of the substrates alone. This has the seed controls deducted.

Due to the substrate being kept in storage, there is some natural degradation of carbon as the substrate breaks down from natural processes. This is shown as the volumes of substrate gave lower concentrations of organic carbon than required. This means that the gas production will need to be standardised for the required DTOC concentration. The nitrogen content for this experiment is not important and will not impact upon the biogas production and therefore has not been taken into consideration.

6.2 Cumulative Biogas Production

Through the use of the Universal Gas Laws, the pressure recorded in the test vessels can be converted into a volume of biogas at standard pressure through the known volume of headspace and recorded temperature.

Table 27 Total Biogas Production from Varied ISR and Temperature

ISR	Temperature	Total Biogas (mL)
17.5	22	301.9
8.7	34	708.6
17.5	40	385.1
5	22	838.8
8.7	9	65.0
30	22	202.0
17.5	4	52.9
17.5	22	283.2
26.3	9	12.0
17.5	22	288.0
17.5	22	284.3
17.5	22	284.9
26.3	34	344.4
Seed	22	64.8
Seed	34	192.7
Seed	40	140.7

Table 27 shows the volumes of biogas recorded for each of the test vessels without any manipulation.

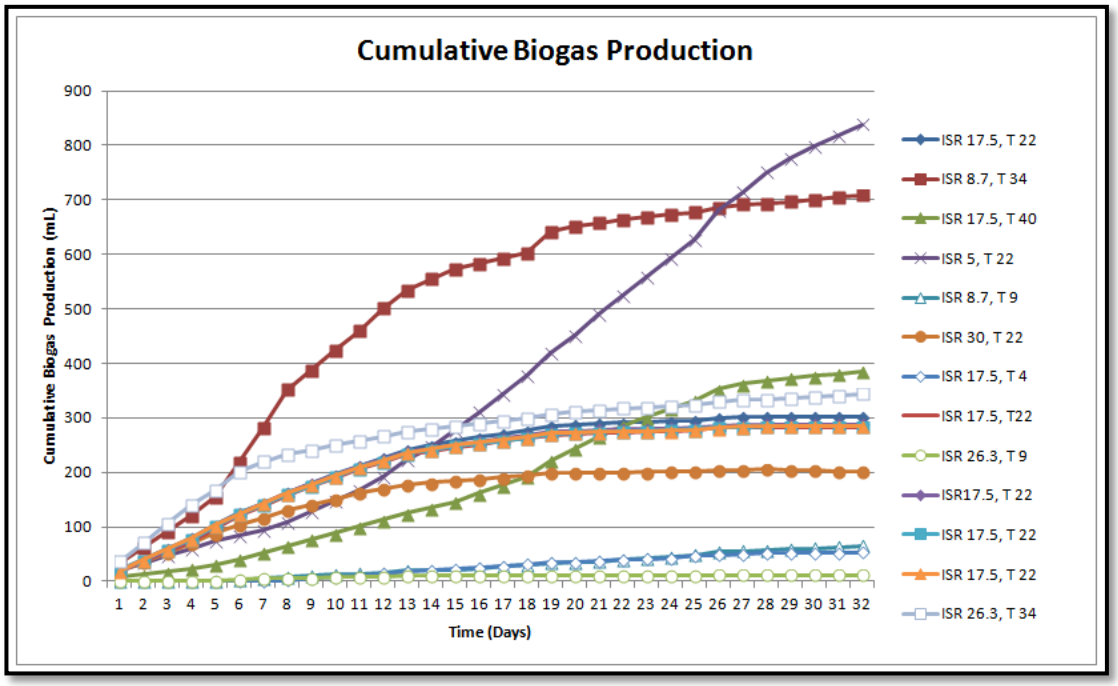

Figure 29 Cumulative Biogas Production from Varied ISR and Temperature (Raw Results)

Due to size constraints and for a clearer picture, the cumulative biogas production curve is contained in Appendix B. Appendix B shows the cumulative biogas production in milliliters over the 32 day trial period. The test vessel producing the highest volume of biogas was that at an ISR of 5 and temperature of 22 °C. The results were considerably consistent with production following all assumptions. The gas production curves are similar to that seen in the first experimental results. The three test vessels held at 4 °C and 9 °C produced the lowest amounts of biogas. The results as to which temperature and ISR will produce the higher volumes of biogas will be discussed later within this section.

When looking at the distribution of the production curves, 3 of the ISR and temperature variations gave initial lag phases. These included the following:

Table 28 Lag Phases Seen in Biogas Production

ISR	Temperature	Length of Lag Phase
17.5	40	16
5	22	6
8.7	34	5

The remaining curve plots followed the similar pattern of an initial rapid growth phase followed by a reduction in rate leading to a plateau phase.

Table 29 Time Taken to Reach Plateau Phase

ISR	Temperature	Plateau Phase Reached (Days)
17.5	**22**	14
8.7	**34**	16
17.5	**40**	25
5	**22**	32
8.7	**9**	-
30	**22**	11
17.5	**4**	-
17.5	**22**	14
26.3	**9**	-
17.5	**22**	14
17.5	**22**	14
17.5	**22**	14
26.3	**34**	8

The lag phases vary depending on the volume of substrate being provided to the inoculum. A low ISR means that the concentration of substrate is higher and a high ISR means that the concentration of substrate is lower (less food source). If the ISR is low it is expected that the plateau phase will be reached at a later stage than if the ISR is high. The plateau phase is also impacted on by the temperature. Extreme

temperature ranges are more likely to induce a faster plateau phase as the bacteria will find it more difficult to produce biogas. Very low temperatures of 4 °C and 9 °C had a gas production curve with no lag phase or initial rapid production rate; therefore no comment can be made to the plateau phase.

When producing the variable ISR and temperatures, there are 5 central points to the central composite design method. These 5 points had an ISR of 17.5 and an effective temperature of 22 °C. It is evident in this graph that these 5 points were extremely close in their biogas production, showing there was little error in the experimental process. The next step is to look at the standardised results.

These results are to be standardised for the required DTOC concentrations and specified temperature.

Table 30 Standardised Biogas Production for Varied ISR and Temperature

ISR	Temperature	Cumulative Biogas (mL)
17.5	**22**	421.8
8.7	**34**	926.6
17.5	**40**	495.6
5	**22**	995.5
8.7	**9**	70.3
30	**22**	151.6
17.5	**4**	56.1
17.5	**22**	234.5
26.3	**9**	0.0
17.5	**22**	240.5
17.5	**22**	233.0
17.5	**22**	318.1
26.3	**34**	225.7

The cumulative biogas standardised for the ISR and given temperature is shown in Table 30.

For a more accurate depiction on the results an analysis in the software modeling program, Minitab16, is required. As aforementioned in the Methodology (Chapter 4), the cumulative biogas (seen in Table 30) is inputted as the result of each variable given. This information can then be optimised through the Response Surface Method analysis model.

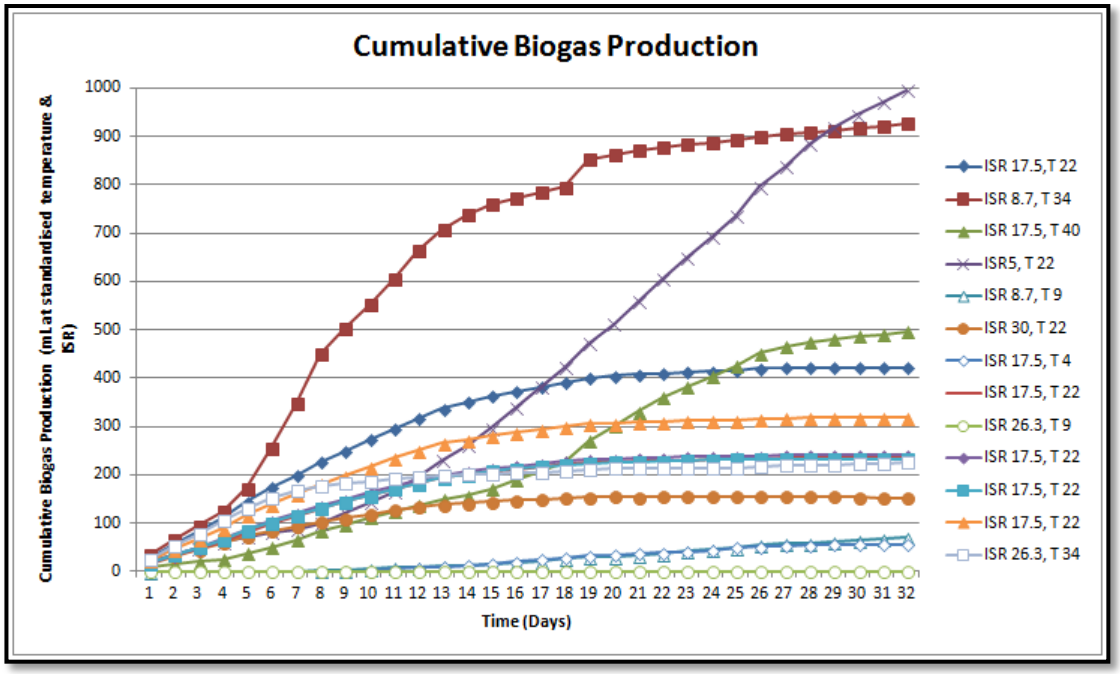

Figure 30 Cumulative Standardised Biogas Production for Varied ISR and Temperature

The above graph shows the cumulative biogas production standardised for the required ISR and temperature and at standard pressure. It is evident that the highest production came from the lowest ISR of 5 and temperature of 22 °C. The second highest biogas production was by the test vessel containing an ISR of 8.7 and temperature of 34 °C. It is apparent from this that the lower ISR's are preferable for higher gas production. It is also evident that the lower temperatures do not produce high volumes of biogas with the three lowest producing test vessels having the three

lowest temperatures. Further analysis is provided in Section 6.3 for a clearer depiction of the results.

6.3 Minitab16 Analysis

Table 31 Response Surface Method Design for Minitab16 Program

↓	C2	C3	C4	C5	C6	C7
	RunOrder	PtType	Blocks	ISR	Temp	Biogas (ml)
1	1	1	1	8.6612	34.7279	926.60
2	2	-1	1	30.0000	22.0000	151.60
3	3	-1	1	17.5000	4.0000	56.10
4	4	-1	1	17.5000	40.0000	495.60
5	5	1	1	26.3388	9.2721	0.00
6	6	0	1	17.5000	22.0000	256.53
7	7	0	1	17.5000	22.0000	234.50
8	8	-1	1	5.0000	22.0000	995.50
9	9	0	1	17.5000	22.0000	233.00
10	10	0	1	17.5000	22.0000	240.50
11	11	1	1	8.6612	9.2721	70.30
12	12	1	1	26.3388	34.7279	225.70
13	13	0	1	17.5000	22.0000	318.10

Table 31 shows the exact inputs in column C5 to C7. Column C5 and C6 were outputted as seen in Chapter 4 and C7 shows the standardised cumulative biogas production. Analysis to assess the presence of any errors was then developed.

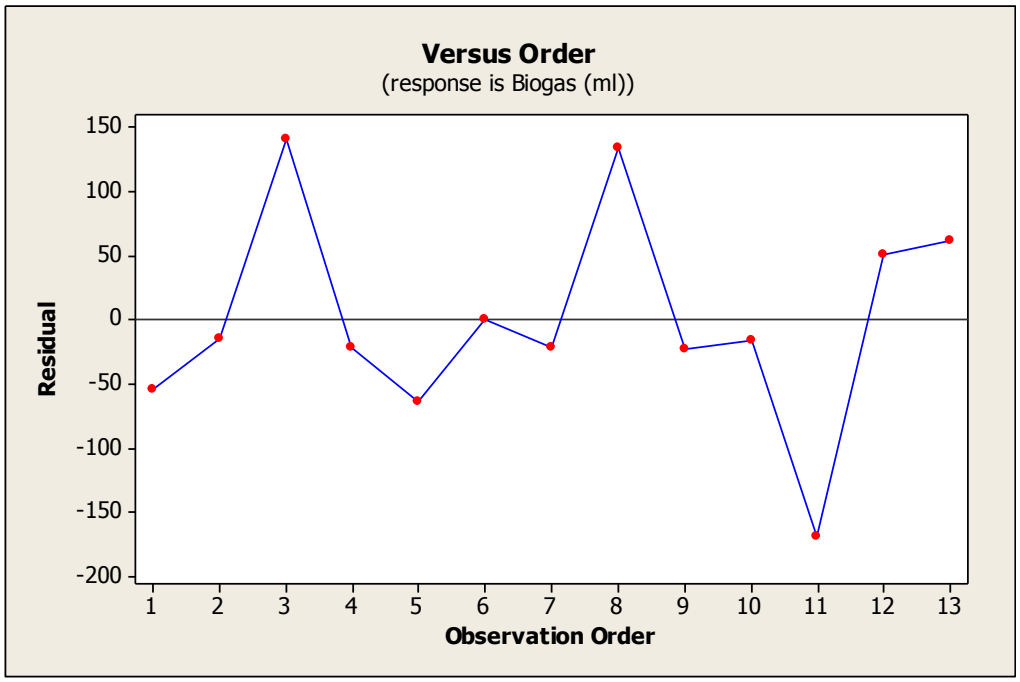

Figure 31 Observation Order vs. Residuals

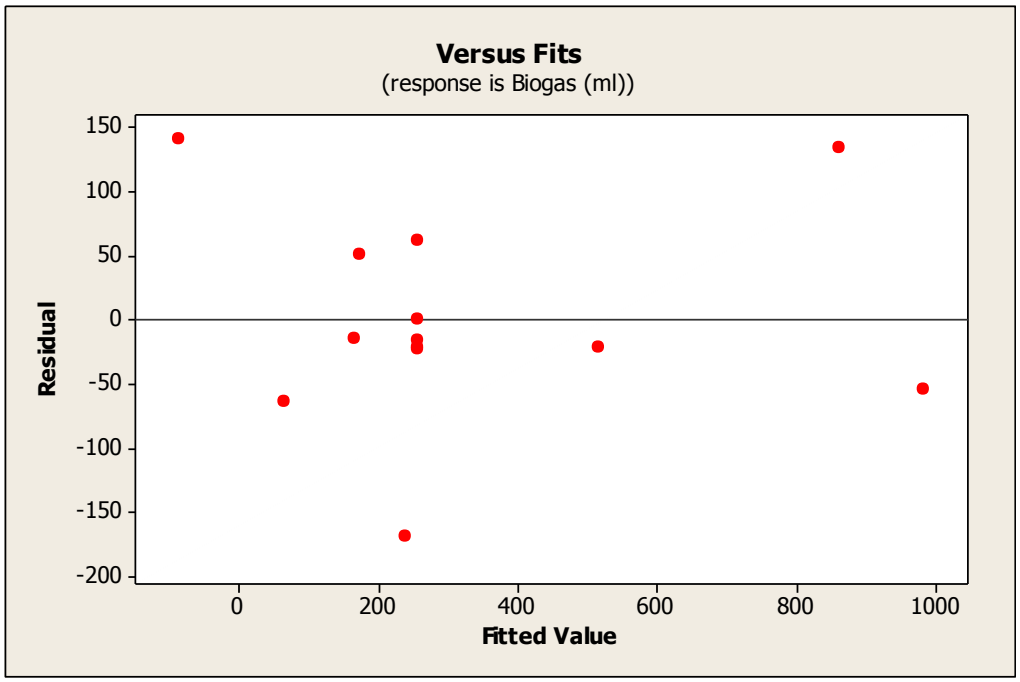

Figure 32 Fitted Values vs. Residuals

Figure 31 shows the plot of the residuals against the observation order. The residual is defined as the difference between the observed value and its fitted equivalent. If the model is appropriate for this experimental procedure the values will have no pattern or consistency indicating random distribution.

As seen in Figure 32, there is also no pattern evident between the scatter of the fitted value against the residual. This again indicates that there is no apparent error of the fitted model.

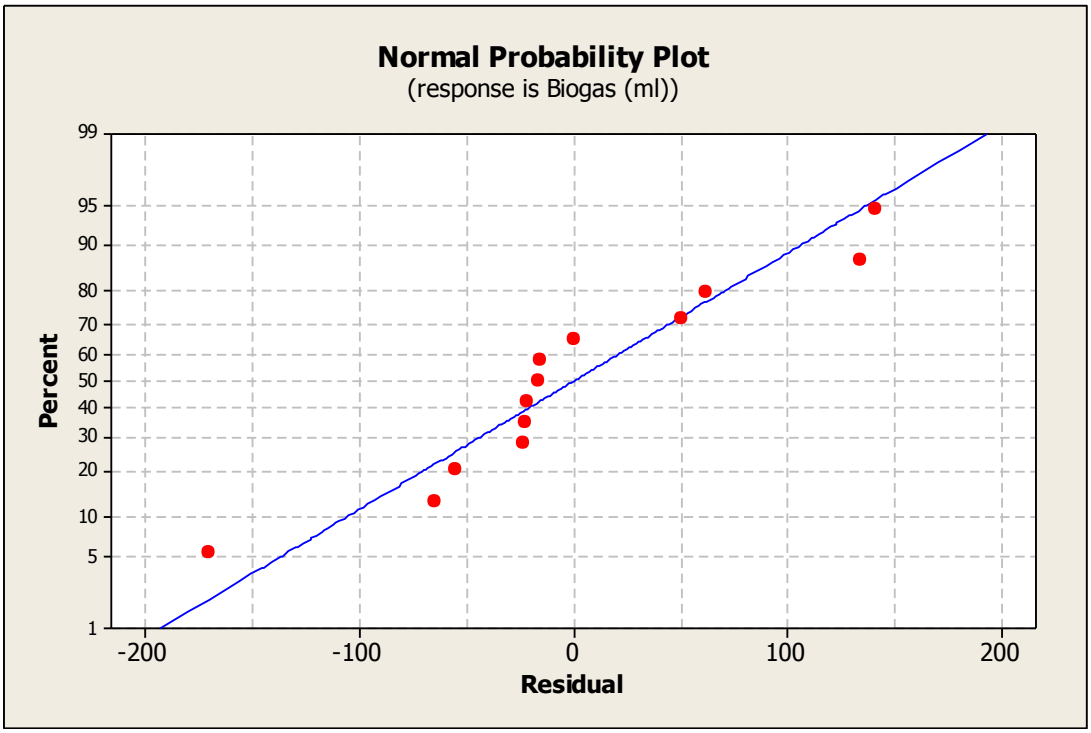

Figure 33 Residuals vs. Theoretical Normal Distribution

The experimental data and the fitted model should follow a normal distribution if developed correctly. To asses this, a theoretical normal distribution is plotted and the residuals plotted against it. If the model is suitable the values should follow the line approximately. This is evident in the graph of Figure 33.

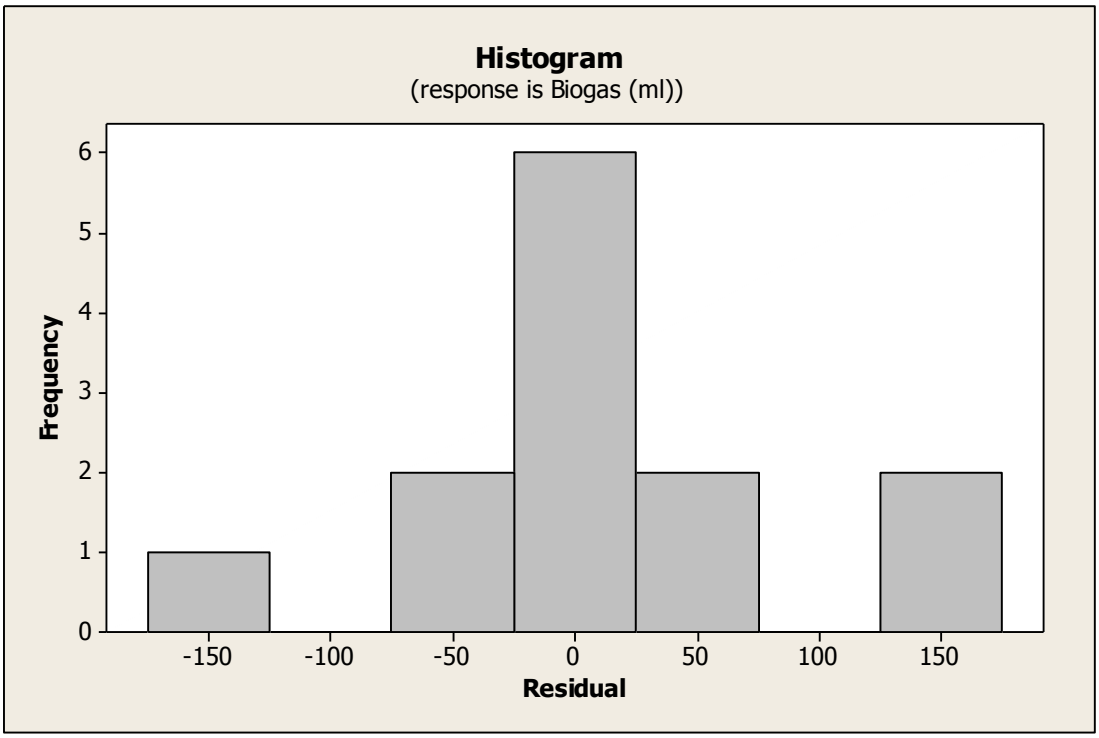

Figure 34 Residuals vs. Frequency of Residuals

The histogram shown in Figure 34 shows again an approximate normal distribution again supporting the model's fit. The above four figures show that the model used to approximate the optimum variable dosage rates will be suitable. To approximate the ISR and temperature that will produce the highest volume of biogas, a contour plot can be created.

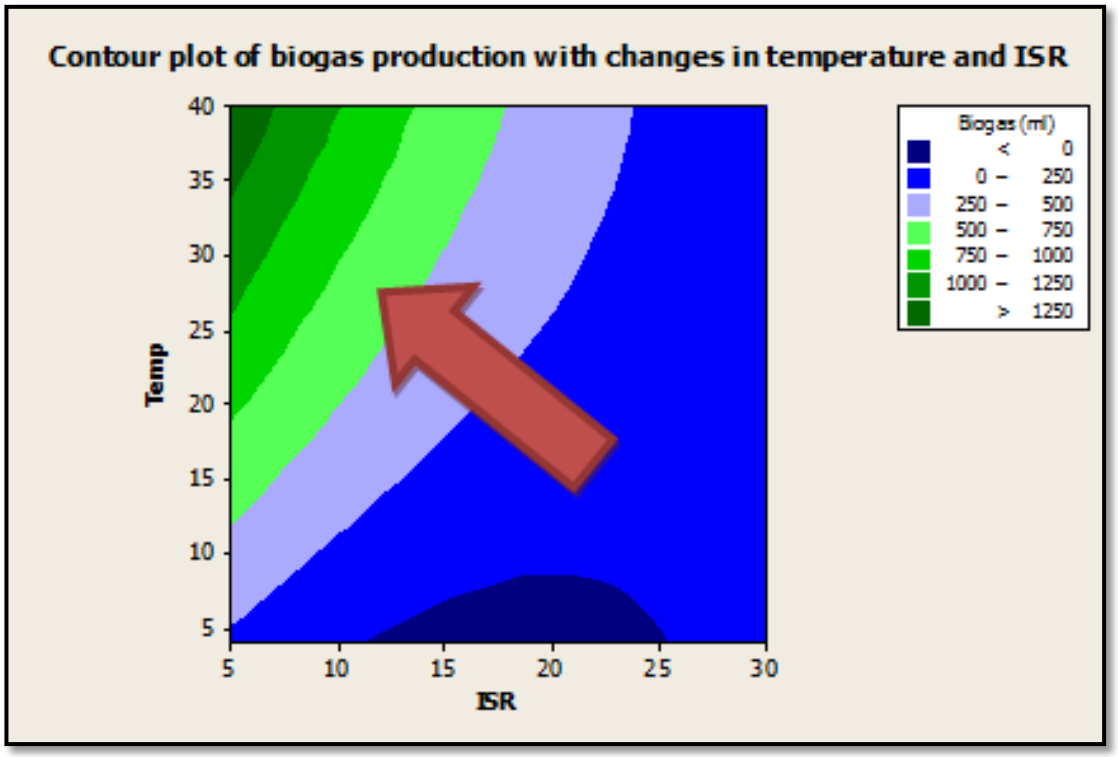

Figure 35 Contour Plot of Biogas Response

The contour above shows the pattern of growth of biogas production in the direction of the red arrow. The contour plot shows a maximum reached at the point where the ISR is approximately 5 to 7.5 and the temperature is between 34 and 40. This indicates that the bacteria prefer to be suspended in higher volumes or concentrations of substrate. It also indicates that the bacteria develop the highest volumes of biogas at higher temperatures. This outcome is desirable as higher volumes of substrate will be able to be digested with lower volumes of inoculum needing to be added and the preference for higher temperatures indicates that the bacteria will produce the higher volumes of biogas during or close to the summer months. Although this is the highest biogas production reached for the variables set in this experiment, it is evident that a full maximum contour line cannot be clearly defined. Without being able to define the total or majority of the maximum contour line it is not possible to conclude that 40 °C is actually the temperature that will return the most biogas. The graph also shows also a minimum biogas production pattern for higher ISR's and at lower temperatures. This means that the biogas produced will be less when the

bacteria have less food source and will not produce biogas during the cooler nights of the winter months.

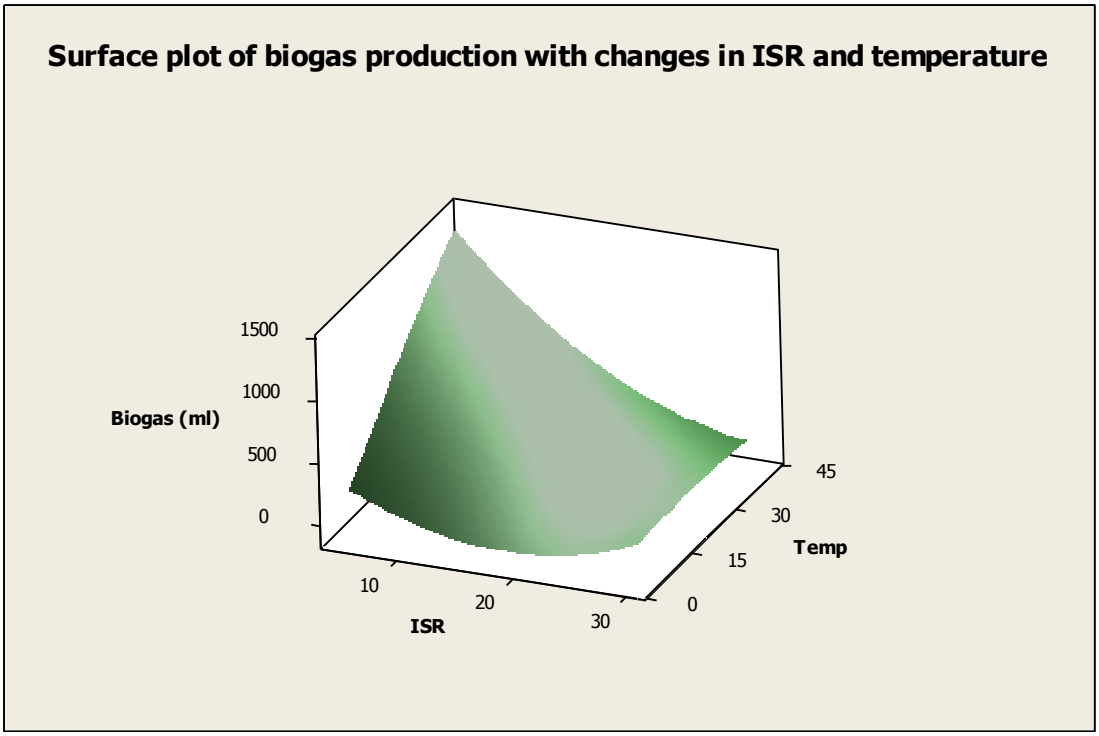

Figure 36 Surface Plot of Biogas Production with Varied ISR and Temperature

When looking at the 3D surface plot shown in Diagram 36, it is evident that the maximum biogas production has not been reached by the experimental procedure undertaken. It does not have full profile of the increasing production (should have a dome shape to see the peak of biogas production for both variables). From this analysis, it is then acceptable to conclude that further analysis needs to be completed before an optimum ISR and temperature can be concluded on.

Another method to analyse the suitability of the model is to evaluate the coefficient of determination (R^2) and the adjusted coefficient of determination (R_{adj}^2). The coefficient of determination will be above 90% to show a good model fit and the adjusted coefficient will be a value somewhat similar to this. An adjustment factor similar to the determination coefficient implies that the data has been successfully

fitted to the quadratic models. As seen in Appendix D from the Minitab16 output the following was evident:

$$R^2 = 92.86\%$$
$$R^2_{adj} = 87.76\%$$

This shows the model is suitable through a numerical method. Other variables presented through the Minitab16 analysis was through the regression and lack of fit coefficients. The regression coefficient was found to be 0.001 and the lack of fit coefficient was slightly higher at 0.007.

This indicates again that the model is sufficient for predicting the maximum biogas that can be produced by the bacteria varying the ISR and temperature.

By analysing the response surface method that was applied to this model, the central design point was an ISR of 17.5 and a temperature of 22 °C. Further analysis would require the central design point of an ISR of 5 and a temperature of 40 °C. This process would allow for the research to be continued in each direction of ISR and temperature (increase and decrease both) to see if the biogas production would continue to increase with lower ISR's and higher temperatures or if this was indeed the maximum biogas able to be obtained from the given samples and bacterial population.

6.4 Summary

This chapter reported on the results obtained through the biogas production through the variation of the ISR and temperature. It was found that although a true optimum was not determined, the model analysis method used was sufficient in determining the optimum within the ranges set. It was possible to say that from the variables and ranges set that the volume of biogas will be optimised at an ISR of approximately 5

and a temperature close to 40 °C. Further analysis would need to be undertaken to test the variables further to determine the range of variables that were not covered in testing completed.

7 Conclusions and Future Research

This chapter evaluates the final conclusions that can be made from the research completed. It will also comment on the further work that needs to be completed to further support this research.

From the first experimental process carried out it was evident that an increased carbon to nitrogen ratio did not increase the volume of methane produced as suggested by literature. It was evident that the abattoir wastewater produced higher volumes of biogas when anaerobically digested alone than in comparison to the volume of biogas produced through co-digestion.

From the second experimental process it was found that low inoculum to substrate ratios and higher temperatures produced the highest volumes of biogas. Although this was the conclusion taken from the experiment performed, an actual prediction of optimum biogas production could not be determined from the limitations to the variables in place. A model was developed to determine the optimum which through analysis proved to be a suitable and reliable model. Further research is required in order to recommend the ultimate ISR and temperature for maximum biogas production.

Further work to be completed would include:

- Extensive characterisation of vegetable wastewater to analyse the presence of inhibitory components
- Analysis of different vegetable wastewater sources to further test the feasibility of co-digestion
- The development of another experimental run, again using the central composite design method, with a central design point revolving around an ISR and temperature of 5 and 40 °C to continue the evaluation of the optimum biogas production

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Appendix A Project Specification

University of Southern Queensland
FACULTY OF ENGINEERING AND SURVEYING
ENG4111/4112 RESEARCH PROJECT
PROJECT SPECIFICATION

FOR: JAYMEE WICKS
TOPIC: Investigation of factors influencing maximum biogas production of abattoir wastewater

SUPERVISOR: Dr. Vasantha Aravinthan
ENROLMENT: ENG4111- S1, D, 2013
ENG4112- S2, D, 2013

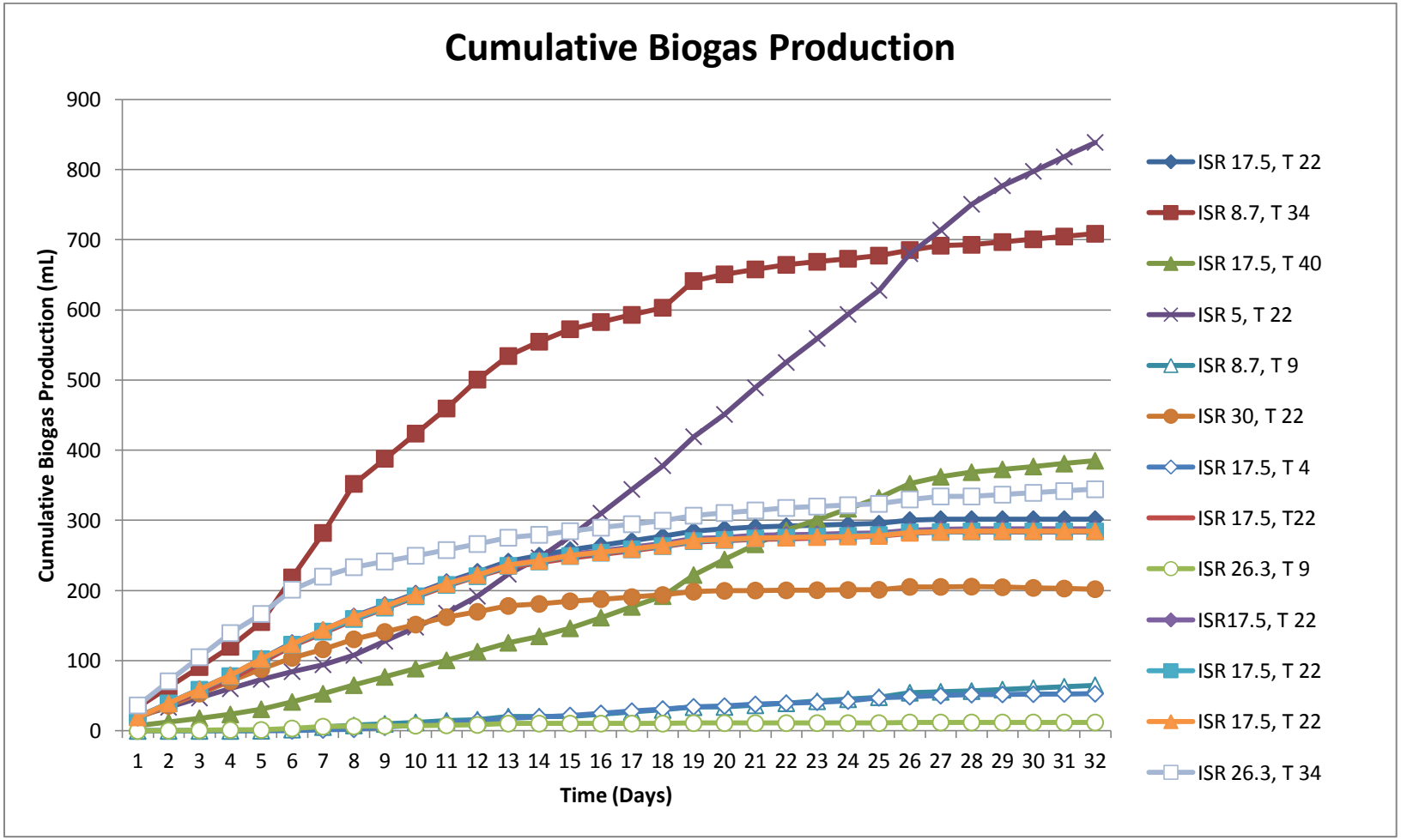
PROJECT AIM: This project seeks to analyse the impacts of co-digestion on the anaerobic digestion of complex organic wastes with carbon rich and easily biodegradable wastewater. The affects of the inoculum to substrate ratio and seasonal variations of temperature are to be analysed and optimised using mathematical software modelling.

PROGRAMME: **Issue B, 19th October 2013**

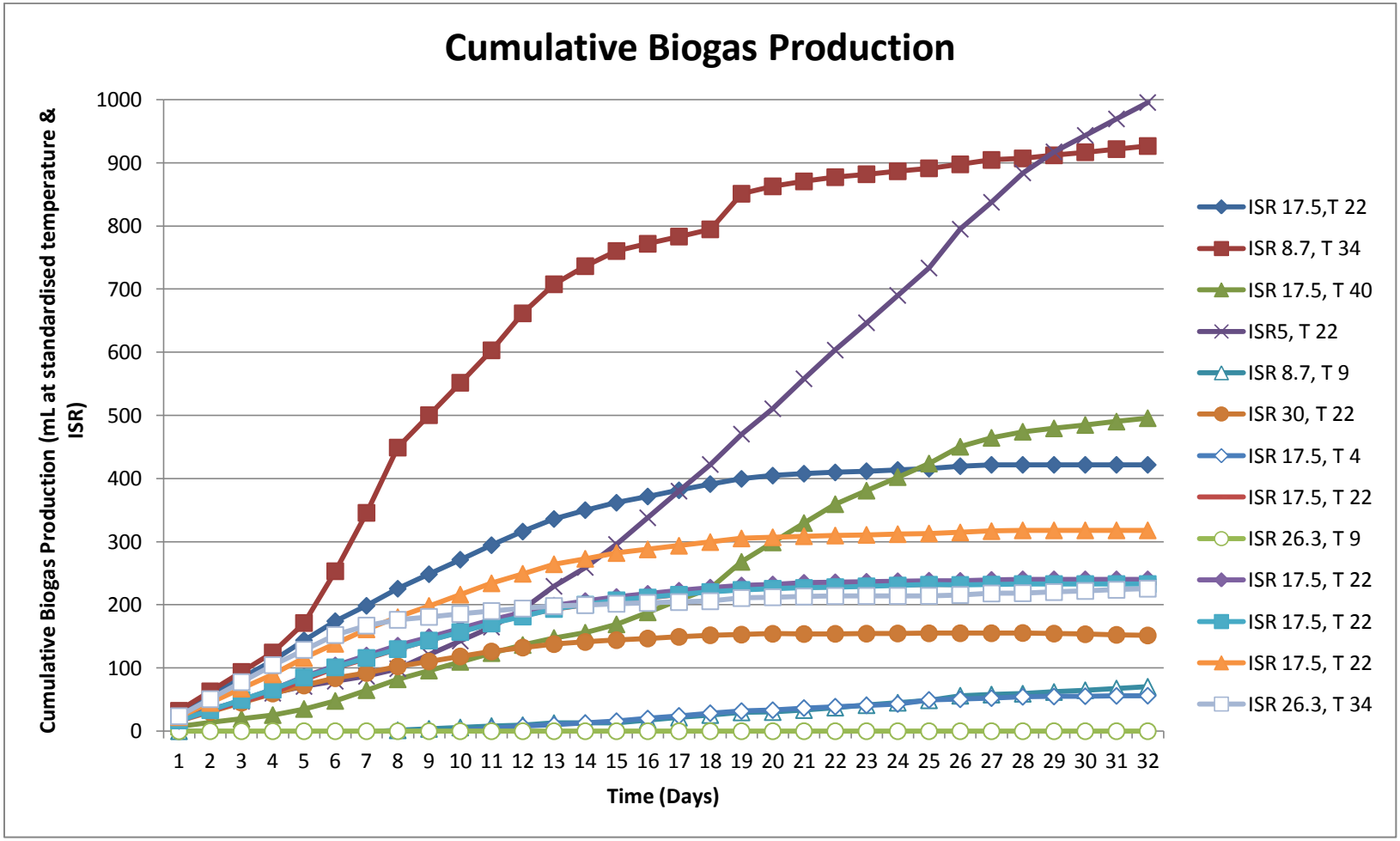
1. Conduct extensive literature review on the BMP (Biological Methane Potential) of abattoir wastewater
2. Collect samples of abattoir wastewater, other organic rich wastewater and wastewater treatment sludge containing anaerobic bacteria
3. Acquire the necessary laboratory skills and characterise the collected wastewater and inoculum on a number of parameters
4. Conduct experiments to determine the biological methane potential (BMP) from abattoir wastewater, vegetable wastewater, an easily biodegradable substrate (glucose) and a mixture of abattoir and vegetable wastewaters to analyse and evaluate the impacts of co-digestion
5. Research and decide on the range in which the ISR and temperature will be varied for maximum production of biogas and then apply the Design of Experiments (DoE) using the Response Surface Method (RSM) in Minitab16 to determine the minimum number of experimental runs
6. Conduct experiments to analyse the impacts of ISR and temperature variations on the biogas production of abattoir wastewater, measuring the biogas production daily
7. Standardise and prepare the cumulative biogas production as a response to be entered into Minitab16 in order to analyse and predict the optimum range for the two variables
8. Evaluate and comment on the findings
9. Submit an academic dissertation on the research

AGREED: JAYMEE WICKS 19/10/2013 Dr.VASANTHA ARAVINTHAN 21/10/ /2013

Appendix B Cumulative Biogas Production under the change of ISR and Temperature



Appendix C Standardised Biogas Production for Varying ISR and Temperature



Appendix D Minitab16 Output Analysis

Response Surface Regression: Biogas (ml) versus ISR, Temp

The analysis was done using uncoded units.

Estimated Regression Coefficients for Biogas (ml)

Term	Coef	SE Coef	T	P
Constant	279.816	277.185	1.009	0.346
ISR	-54.635	21.679	-2.520	0.040
Temp	46.713	14.307	3.265	0.014
ISR*ISR	1.648	0.526	3.133	0.017
Temp*Temp	-0.124	0.254	-0.489	0.640
ISR*Temp	-1.401	0.482	-2.909	0.023

S = 108.379 PRESS = 556466
R-Sq = 92.86% R-Sq(pred) = 51.69% R-Sq(adj) = 87.76%

Analysis of Variance for Biogas (ml)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	1069604	1069604	213921	18.21	0.001
Linear	2	845243	316590	158295	13.48	0.004
ISR	1	482484	74602	74602	6.35	0.040
Temp	1	362759	125227	125227	10.66	0.014
Square	2	124947	124947	62474	5.32	0.039
ISR*ISR	1	122137	115314	115314	9.82	0.017
Temp*Temp	1	2811	2811	2811	0.24	0.640
Interaction	1	99414	99414	99414	8.46	0.023
ISR*Temp	1	99414	99414	99414	8.46	0.023
Residual Error	7	82222	82222	11746		
Lack-of-Fit	3	77135	77135	25712	20.22	0.007
Pure Error	4	5087	5087	1272		
Total	12	1151826				

Obs	StdOrder	Biogas (ml)	Fit	SE Fit	Residual	St Resid	
1	3	926.600	981.350	85.681	-54.750	-0.82	
2	6	151.600	166.720	85.681	-15.120	-0.23	
3	7	56.100	-84.822	85.681	140.922	2.12	R
4	8	495.600	517.472	85.681	-21.872	-0.33	
5	2	0.000	64.300	85.681	-64.300	-0.97	
6	9	256.530	256.526	48.469	0.004	0.00	
7	12	234.500	256.526	48.469	-22.026	-0.23	
8	5	995.500	861.330	85.681	134.170	2.02	R
9	11	233.000	256.526	48.469	-23.526	-0.24	
10	10	240.500	256.526	48.469	-16.026	-0.17	
11	1	70.300	240.163	85.681	-169.863	-2.56	R
12	4	225.700	174.887	85.681	50.813	0.77	
13	13	318.100	256.526	48.469	61.574	0.64	

R denotes an observation with a large standardised residual.