University of Southern Queensland

Faculty of Engineering and Surveying

Investigation into the Biochemical Methane Potential of Abattoir Wastewater

A dissertation submitted by

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ABSTRACT

Anaerobic digestion is used as a means of treating wastewaters and producing methane for heating and energy around the world. With the cost of fossil fuels rising and the possibility of a carbon tax, Australia's agricultural and food processing industries are interested in harnessing methane from their wastewaters. There are international studies available on the volumes of methane produced from a variety of wastes and wastewaters. However, abattoir wastewaters are complex with varying compositions, therefore, individual abattoirs need to be studied separately to determine the volumes of methane that can be produced.

This dissertation presents the results from laboratory studies conducted to measure the methane yield of easily biodegradable substrates including, glucose, acetate, gelatine and powder milk and abattoir wastewaters including, yard water, blood water and saveall water. The experiments were conducted in batch mode following standard batch assay procedures and semi-continuous mode using a continuously stirred bioreactor. The batch assays had a working volume of 400mL, which included a mixture of anaerobic microorganisms, anaerobic media and the substrate being tested. The assays were incubated at 35°C and the daily gas pressure and gas volume were monitored. The bioreactor had a working volume of 4L and was fed weekly with a different wastewater. Gas chromatography was used to determine the methane content of the biogas.

Experimental results indicated that powder milk produced the most biogas, totalling 61 mL biogas/100mg/L TOC at STP, of the easily biodegradable substrates. This indicated that complex substrates were suitable for anaerobic digestion. For experiments conducted on abattoir wastewater using anaerobic media to supplement nutrients, 6.2mL CH_4/100mg/L DTOC at STP, 7.0mL CH_4/100mg/L DTOC at STP and 110.4mL CH_4/100mg/L DTOC at STP, were produced by yard water, blood water and saveall water respectively. Experiments were also conducted to analyse if the addition of anaerobic media increased or decreased methane production. The results indicated that without anaerobic media, 4.7mL CH_4/100mg/L DTOC at STP, 4.0mL CH_4/100mg/L DTOC at STP and 118mL CH_4/100mg/L DTOC at STP were produced by yard water, blood water and saveall water respectively. The results obtained using the bioreactor

indicated that a combination of the three wastewaters produced increased volumes of methane totalling, 532mL CH_4/100mg/L DTOC at STP.

This research will give insight into the volumes of methane that could be produced from abattoir wastewater and provide assistance in determining the feasibility of utilising methane as a supplement energy source.

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CERTIFICATION

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GLOSSARY

The following abbreviations are used throughout the dissertation:

BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DO	Dissolved oxygen
DIC	Dissolved inorganic carbon
DTN	Dissolved total nitrogen
DTOC	Dissolved total organic carbon
GC	Gas chromatography
GHG	Greenhouse gas
IC	Inorganic carbon
LCFA	Long chain fatty acids
SS	Suspended solids
STP	Standard temperature and pressure (0°C, 101.325kPa)
TN	Total nitrogen
TOC	Total organic carbon
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids

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CHAPTER 1 INTRODUCTION

Reduction of greenhouse gas emissions is currently an issue that requires urgent attention. With ever increasing energy costs and the introduction of the Renewable Energy Target (RET), Carbon Pollution Reduction Scheme, (CPRS) and a possible carbon tax, industries are under ever increasing pressure to reduce their green house gas emissions by utilising renewable energy alternatives. Renewable energy sources offer many clear benefits, including lowering emissions, increasing energy security, reducing liability under CPRS and, most importantly, provides a strong economic positive for industries.

Australia is a country with an agricultural based economy and about 60% of the land mass is used for some form of agricultural practice (Hogan & Morris 2010). The beef industry accounts for 44% of all agricultural activity and the Australian herd was estimated to consist of 24 million beef cattle (Sparkes et al. 2011), therefore, there are considerable volumes of waste being produced in both the farming and processing of these animals. These wastes are rich in organic matter and, when treated, through the natural process of anaerobic decomposition, produce methane which can be utilised as a renewable energy source. Methane is a dominant greenhouse gas emission from the Australian agriculture sector with substantial volumes being produced naturally. Therefore, by utilising this gas, there is an ultimate reduction in greenhouse gas emissions.

Historically, methane recovery from waste has not been widely practised in Australia, as the benefits did not match the capital and operational costs. Relatively cheap and abundant natural resources ensured that energy was easily available at an economically viable price, therefore, methane recovery was not considered as a cost effective means of producing energy. However, as the economy becomes constrained by carbon pricing, industries consuming substantial amounts of energy are compelled to investigate renewable energy options to stay economically competitive and comply with CPRS.

The meat processing industry is under ever increasing scrutiny from environmental authorities to reduce its environmental impact (Pitt & Skerman 1992). The consequences of abattoir wastewater pollution are felt by both humans and the environment with adverse affects on air quality, water quality and aquatic flora and fauna. Therefore, there are multifaceted benefits that arise from utilising methane as an energy source. These benefits include the reduction of fossil fuel consumption, the capture and subsequent utilisation of a greenhouse gas (methane), improved wastewater treatment due to increased monitoring as well as the improved public image of the industry for utilising renewable energy.

1.1 PURPOSE

Numerous studies have been published on methane production from a variety of wastes and wastewaters, however, there is a gap in research corresponding to methane production from abattoir wastewater in Australia. In addition, Abattoir wastewater characteristics are complex in nature and vary significantly between processes within the same plant and between different plants, therefore, it is difficult to compare published findings to a specific plant. Methane production is highly dependent on the organic matter content of the wastewater and the ratios of organic matter to other inhibiting substances. This dissertation reviews research relating to the anaerobic digestion of waste, specifically focusing on the anaerobic digestion of abattoir wastewater. This research gives insight into the volumes of methane produced from abattoir wastewater and could help abattoirs determine if methane utilisation is feasible.

1.2 AIMS AND OBJECTIVES

This research aimed at determining the amount of methane that could be produced from abattoir wastewater during anaerobic digestion.

The objectives of the project were to:

- Conduct an extensive literature review on methane production from abattoir wastewater and other substrates.
- Obtain and characterise wastewater samples from Kilcoy Pastoral Company.

- Obtain, characterise and prepare sludge from an operating anaerobic digester to be used as inoculum.
- Design and conduct experiments to investigate the methane potential of abattoir wastewater against other easily biodegradable substrates following standard batch assay procedures.
- Monitor the daily gas production by taking pressure and volume readings as well as analysing the gas composition with gas chromatography.
- Analyse the data and provide results showing the biodegradation curves to compare methane production potentials of different substrates.
- Design and conduct a continuous experiment in an anaerobic digester using abattoir waste based on results from batch assays.

1.3 SCOPE

The scope of this research was to analyse the suitability of using anaerobic digestion to treat the wastewater from abattoir industries while understanding the potential to generate biogas.

The limitations of the research were:

- Only single grab samples of each wastewater stream of the industry were analysed, therefore, the daily fluctuation of the strength of waste caused by changing flows and processes was not considered.
- Only one inoculum to substrate ratio was used for all trials.
- The characterisation of wastewater and gases were limited by the availability of equipment.
- Only methane production processes were investigated. Processes for utilising methane and converting it to energy were not investigated.

1.4 OVERVIEW OF DISSERTATION

The dissertation is organised as follows:

Chapter 2 Literature review

This chapter provides a review of literature relating to biogas production from wastes and wastewaters, including experimental techniques, gas measurement and available standards. An overview of the anaerobic digestion processes and optimum conditions is also covered.

Chapter 3 Biogas in Australia

This chapter gives an overview of methane utilisation in Australia. It looks at the current status of methane production at both the research and operational levels. It reviews why methane capture technology would be suitable for the meat processing industry in Australia. This chapter also provides a brief case study of Kilcoy Pastoral Company including; production capacities, water consumption, energy consumption and wastewater treatment processes.

Chapter 4 Methodologies

Chapter four provides the methodologies used for analysing wastewaters, conducting biochemical methane potential batch assays, conducting semi-continuous experiments, gas measurement and data analysis.

Chapter 5 Biogas production from easily biodegradable substrates

This chapter presents the results and discussion for the biochemical methane potential assays conducted on easily biodegradable substrates including glucose, acetate, gelatine and powder milk.

Chapter 6 Biogas and methane production from abattoir wastewater

This chapter presents the results and discussion of the biochemical methane potential assays conducted on three different waste-streams from the abattoir industry including

yard water, blood water and saveall water. A comparison is provided of trials conducted with and without anaerobic media supplying necessary additional nutrients.

Chapter 7 Biogas and methane production using a bioreactor

This chapter provides the results and discussion for batch experiments conducted in a semi-continuous anaerobic digester using different abattoir wastes.

Chapter 8 Conclusions and future work

Chapter eight provides the conclusions of this study and summarises some recommendations for future work

CHAPTER 2 LITERATURE REVIEW

This literature review covers the fundamentals of anaerobic digestion, methane production processes and reviews research conducted on the anaerobic digestion of abattoir waste including optimum operating conditions. In addition, the procedures for conducting batch and continuous anaerobic experiments are reviewed and summarised.

2.1 FUNDAMENTALS OF ANAEROBIC DIGESTION

Anaerobic digestion is a process used to decompose organic matter which utilises bacteria to metabolise organic matter in an oxygen free environment. Anaerobic technology has been used to treat a variety of wastes including agricultural wastes, food wastes and municipal wastes and has the capability to reduce chemical oxygen demand (COD) and biochemical oxygen demand (BOD) as well as produce renewable energy (Li et al. 2011). Wastewater contains complex macromolecules such as proteins, carbohydrates and lipids that are converted into methane and carbon dioxide through a number of metabolic stages and by a range of microorganisms (Khanal 2008).

Anaerobic digestion utilises four groups of microorganisms that are classified as; hydrolytic, fermentative, acetogenic and methanogenic bacteria (Veeken & Hamelers 1999). Firstly hydrolytic bacteria excrete extracellular enzymes and reduce complex organic compounds into amino acids, sugars and long chain fatty acids in a process known as hydrolysis (Li et al. 2011). Fermentative bacteria then convert these products into a mixture of short chain volatile fatty acids (VFAs), carbon dioxide (CO_2), hydrogen (H) and acetic acid (Khanal 2008). In acetogenesis the acetogenic bacteria convert the short chain fatty acids into acetate, H and CO_2 (Henze 2008). The final step is methanogenesis, where methanogenic bacteria consume the acetate, carbon dioxide and hydrogen to produce methane (Li et al. 2011). The process flow is illustrated in Figure 2.1. The characteristics and demands of the groups of bacteria are very different and it is difficult to maintain harmony and balance (Wang et al. 2009). Once the balance is broken the methanogenic process will be interrupted and this is usually attributed to the initial characteristics of the wastewater (Wang & Banks 2003).

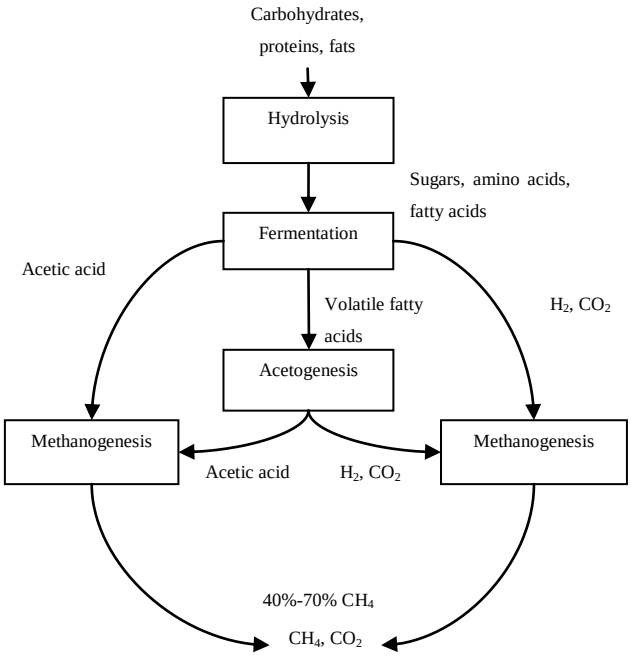

Figure 2.1: Process flow of the degradation of organic material through anaerobic digestion sourced from: (Li et al. 2011)

Table 2.1: Methane production, substrates and reactors

Substrate	**Reactor**	**Mode of feeding**	**Temp °C**	**Time, d**	**Methane production**	**Methane content**	**Reference**
Flush dairy manure with turkey processing wastewater	Attached growth 15L	Continuous	37±1	5	0.8 m^3 gVS^{-1}	56%-70%	(Ogejo & Li 2010)
Cheese whey and dairy manure	Cylindrical metallic anaerobic reactor, 20L	Continuous	34	5	1.510m^3 m^3d^{-1}	60%	(Kavacik & Topaloglu 2010)
Grass silage	Batch leach bed with second stage USAB, 1L	Internal recirculation	35±1	55	0.141-0.204m^3 kg^{-1}VS	Unknown	(Lehtomäki et al. 2008)
Apple pulp and slaughter house waste	101 CSTR reactor	Continuous	38	20	0.8m^3 kg^{-1} OTS	77%-80%	(Llaneza Coalla et al. 2009)
Olive mill wastewater and liquid cow manure	Continuous stirred tank reactor	Continuous	35	19	0.91L CH_4 $L^{-1}_{reactor}$ d^{-1}	Unknown	(Dareioti et al. 2010)
Water hyacinth (*Eichhornia crassipes)*	BPM (assays)	batch	38	Unknown	267Lbiogaskg/1 VS	50%	(O'Sullivan et al. 2010)

2.2 METHANE RECOVERY FROM WASTE

Literature shows that methane has been produced by anaerobic digestion from a large range of industries including municipal wastewater, food industry wastes, agricultural wastes and aquatic plants. Table 2.1 highlights some of substrates or organic wastes that have been tested for methane production, the reactors and experimental process used and the methane yields. Methane yields are hard to compare as the results are presented in a varying range of units.

2.3 ANAEROBIC DIGESTION OF ABATTOIR WASTEWATER

2.3.1 ABATTOIR WASTEWATER CHARACTERISTICS

Abattoir waste is difficult to characterise as different processing plants have different associated wastes due to the many procedures and facets of the meat processing industry. The contaminant loading of the wastewater discharged from an abattoir also varies seasonally, daily or even on a shift basis (Sroka et al. 2004). Typically meat processing wastewaters are classified as difficult to decompose due to their specific characteristics, irregular discharge and high content of organic, mineral, chemical and biogenic matter (Bohdziewicz & Sroka 2005). Abattoir wastewater usually has BOD, COD, suspended solids (SS), organic nitrogen, and fats at least several times higher than domestic sewage (Arvanitoyannis & Ladas 2008). It also contains high amounts of proteins that putrefy easily giving off offensive odours (Bohdziewicz & Sroka 2005).

2.3.2 PRELIMINARY TREATMENTS

Preliminary treatment is used to reduce the organic load to the primary and secondary treatment processes and mainly involves suspended solids removal. During the meat processing process, solid particles such as fat, bone, hair and meat, as well as manure grass and sand can be included in the wastewater. In the meat processing industry, preliminary treatments typically include screening, catch basins, flotation, equalisation skimmers and settlers (Mittal 2006).

2.3.3 OPTIMUM CONDITIONS FOR ANAEROBIC DIGESTERS

Methanogenic bacteria and their relative population levels depend on the wastewater characteristics and the operational and environmental conditions of the reactor. Stresses imposed on these conditions may lead to a change in bacterial species and their population levels which will be reflected in the reactor performance (Jawed & Tare 1999). A material or process can be considered inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth (Palatsi et al. 2011). Abattoir wastewater is itself an inhibiting factor when anaerobically digested. The high natural ammonia levels in manure, the high protein content of blood and the high lipid content of the fat contribute to the inhibition of anaerobic digestion processes (Chen et al. 2008). Physiochemical factors such as temperature, pH and particle size can also inhibit microbial growth.

AMMONIA

The acid forming and methane forming bacteria differ greatly in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions (Boone et al. 1993). During anaerobic digestion, the acid forming bacteria degrade proteins and lipids and this can lead to an accumulation of ammonia and long chain fatty acids (LCFA) (Chen et al. 2008).

Ammonia is essential for bacterial growth, however, in high concentrations can also inhibit growth. It is produced from the microbial degradation of nitrogen containing compounds that are mainly proteins (Resch et al. 2011). It can affect the digestion process by different levels from mild suboptimal reactor performance, where methanogens are inhibited and there is a build up of LCFAs, or sever inhibition affecting all stages of digestion (Nielsen & Angelidaki 2008). It is suggested that free ammonia (NH_3) is the active component causing ammonia inhibition as it is freely membrane-permeable causing consequent changes in the intracellular pH, potassium deficiency, an increase in the maintenance energy required and the inhibition of enzyme reactions (Siles et al. 2010). Chen et al. (2008) also reports that acclimatisation can

influence the degree of ammonia inhibition and more methane can be produced after an adaptation period.

SULPHATE

Sulphate is also a common constituent of agricultural wastewaters (Chen et al. 2008). Sulphate concentrations can also cause inhibition, as sulphate-reducing bacteria will compete with methanogens for substrates such as acetate, H and CO_2 (Hansen et al. 1999). Sulphate is reduced to sulphide which can cause inhibition as sulphide is toxic to various bacteria groups (Chen et al. 2008).

LIPIDS

Lipids are attractive for biogas production due to their high biogas yields. Oh and Martin (2010) report that substrates with a high lipid/protein ratios can produce higher methane yields than substrates with low lipid/protein ratios. However continuous anaerobic digesters fed with lipid-containing wastewaters are hindered by acute toxicity caused by LCFAs towards the bacterial consortium and by the absorption of these compounds onto the biomass, inducing sludge flotation and washout (Pereira et al. 2003). Studies have demonstrated that LCFA inhibition is reversible and that microorganisms can recover after a lag period and then efficiently methanise the LCFAs (Palatsi et al. 2010), (Cavaleiro et al. 2008), (Palatsi et al. 2009).

pH

Methanogenic and acetogenic microorganisms both have their optimal pH and failing to maintain the pH within optimal conditions could cause reactor failure. pH also affects the growth of microorganisms in wastes containing high concentrations of total ammonia nitrogen (Chen et al. 2008). Nielsen and Angelidaki (2008) found that lowering the pH helped reduce the negative effect of free ammonia via a decrease in free ammonia concentration, however, in extreme cases this further inhibited methanogenesis. Hansen et al. (1998) reported that an increase of pH from 7 to 8 will lead to an eight-fold increase in the free ammonia concentration.

TEMPERATURE

Temperature can affect both microbial growth rates and fatty acid concentrations. Chae et al. (2008) reported that a temperature increase from 25°C to 35°C produced a 17.4% increase in biogas yield for the digestion on swine manure. It has been shown that wastes with a high ammonia concentration were more easily inhibited and less stable in the thermophilic temperature range than the mesophilic temperature range (Chen et al. 2008). Hansen et al. (1998) also reported that as temperatures exceeded 37°C, there was a steady increase in free ammonia and VFAs as the temperature increased.

SOLIDS CONTENT

Izumi et al. (2010) suggested that the particle size has an effect on VFA accumulation in the anaerobic digestion of food wastes. They found that a decrease in particle size increased microbial degradation of VFAs, however, excessive reduction of the particle size of the substrate actually increased VFA accumulation. Their results suggested that there was an optimal particle size that could improve methane yields.

Inhibiting factors can be overcome by acclimatisation, monitoring and maintaining reactors at optimal conditions. Hansen et al. (1998) states that the interaction between free ammonia, VFAs and pH will lead to an inhibited steady state where the anaerobic process is still continuing however with a lower methane yield. The effects of free ammonia, temperature and pH can be controlled with the addition of water, substrate or reactor effluent to dilute the concentrations of inhibitory substances. This could increase the recovery speed back returning the reactor to optimal conditions (Nielsen & Angelidaki 2008). The effect of LCFAs can be severely inhibiting however methanogenic activity does recover after a lag period (Palatsi et al. 2011).

2.4 BIOCHEMICAL METHANE POTENTIAL ASSAYS

Biochemical methane potential (BMP) assays are a means of evaluating the biodegradability of complex organic compounds. The basic principal uses a seed sludge (inoculum) containing anaerobic bacteria to degrade a known concentration of organic

compound in a controlled environment for a period of time long enough for substantial biodegradation to occur.

2.4.1 STANDARDS

No Australian standard exists for the analysis of the biochemical methane potential of organic compounds. International Standard 11734 (ISO 1995) provides a procedure for evaluating the biodegradability of organic compounds by measuring the biogas production. This standard outlines the procedure, equipment and chemicals required for BMP assays. This standard also provides the calculation procedure to show the extent of biodegradation. Although a standard exists, different laboratories use different experimental conditions, equipment, inoculums, hydraulic retention times, inoculum to substrate ratios, and units of expressing results. This has made it difficult to compare results and (Angelidaki et al. 2009) suggested that there should be stricter protocols to unify standard procedures.

2.4.2 BMP EQUIPMENT AND PROCEDURES

Typically the test is conducted in batches in closed bottles ranging in size from 100mL-2000mL with a rubber septa that is used to remove a sample of gas. Inoculums have been obtained from full-scale operational anaerobic digesters to laboratory anaerobic digesters. Incubation temperatures range from mesophillic to thermophillic conditions. The amount of gas produced is measured by pressure, volume or gas chromatography (GC) and the results can be used to quantify the amount of biodegradation that has occurred. Table 2.2 to shows the variation in equipment and procedures that have been used.

Table 2.2: Biochemical methane potential assay experiment conditions

Test compound	Total volume (MLA)	Working volume (MLA)	Batch or continuous	Inoculum origin	Retention time (days)	Temperature (ºC)	Gas measurement	Stirring	Sparging gas	Addition of nutrients	Reference
Dairy manure and food waste	1000	500	Batch	Laboratory scale treating municipal solid waste	30	35±1	Pressure gauge	Manually shaken for 1 minute prior to gas measurement	helium	unknown	(El-Mashad & Zhang 2010)
Manure, food waste, aquatic weeds	250	unknown	Batch	Farm based anaerobic digester (manure and food wastes)	30	35±1	Pressure transducer with data acquisition	Manually every 2 days	N2	no	(Labatut et al. 2011)
Swine and bovine slurries	330 or 1170	Slurry to Headspace =0.49	Batch	Laboratory anaerobic digester treating swine slurry	123-153	30	Digital manometer	continuous	N2	Tap water with known mineral concentration	(Vedrenne et al. 2008)
Manure and straw	1800	1200	continuous	Biogas digester	30	308±1K	Gas meter	unknown	unknown	unknown	(Demirbas 2006)
Textile mill effluent	160	100	batch	Acclimatized sludge	60	35	Collected with syringe	Unknown	N2	Media solution	(Desiana & Setiadi 2009)
Municipal waste	1000	600	batch	Acclimatized sludge	unknown	35±1	U tube water displacement manometer	Continuous 50rpm	N2	unknown	(Zheng et al. 2009)
Kitchen waste	160	unknown	batch	UASB reactor treating brewery effluent	600hrs	37	Pressure transducer	Continuous 15.7 rad/s	N2	Media solution	(Neves et al. 2004)
Fruit and vegetable waste	135	unknown	Batch	CSTR	100	35±1	Glass syringe	unknown	N2 CO2	Media solution	(Gunaseelan 2004)

2.4.3 PRESSURE AND GAS MEASUREMENT

Biogas production can be quantified using a range of methods to measure the pressure or volume of gas in the headspace. Desiana and Setiadi (2009) collect gases with a syringe by allowing the pressure to push the piston of the syringe up and recording the volume. El-Mashad and Zhang (2010) use a Wal-BMP-Test system pressure gauge to measure the pressure daily. Labatut et al. (2011) and Neves et al (2004) use pressure transducers and data acquisition software. U tube displacement was used by Zheng et al. (2009) and Vedrenne et al (2008) used a digital manometer. Another common method is displacement of an alkaline solution which absorbs the CO_2 and the volume of methane is then recorded (Rico et al. 2007). An international inter-laboratory study conducted by Raposo et al. (2011) found that the most common methods for quantifying biogas production were manometrically, volumetrically and by GC and were used by 63%, 26.3% and 10.5% respectively, of the people surveyed .

The theoretical methane yield can be calculated if the composition is known using the Buswell equation to provide a comparison between theoretical and experimental methane production (Angelidaki & Sanders 2004).

2.5 SUMMARY

This chapter reviewed the literature relevant to the anaerobic digestion of abattoir wastewater. The fundamental processes of anaerobic digestion were covered as well as the optimum operating conditions. Procedures for conducting biochemical methane potential assays were reviewed and these procedures were adapted and used, as outlined in Chapter 4.

CHAPTER 3 BIOGAS IN AUSTRALIA

Methane is a natural by-product of anaerobic digestion and is utilised to provide heating and energy around the globe. The technology is very mature and turns a pollution problem into an energy resource (Rao et al. 2010). It is widely considered as a simple, adaptable and locally acceptable technology (Amigun & von Blottnitz 2010). Globally, methane production from anaerobic digestion is utilised by a range of industries from food processing, sewage treatment plants, livestock feedlots, agricultural wastes and meat processing wastes. The methane is used for electricity generation, heating, vehicle fuel or supplied to a gas grid.

Australia is typically behind the rest of the world when it comes to biogas production. Australia's abundant natural resources ensured that in the past energy was cheap and economical. However, the reliance on these natural resources has contributed to Australia having the highest green house gas emissions (GHG) in the western world (Yusaf et al. 2011). Now as Australia shifts to a carbon based economy, energy costs are increasing and therefore the cost of methane pollution is set to rise. Due to this, a recent shift has taken place and anaerobic digestion is playing a small but ever increasing role in the renewable energy mix. It has become recognised as a suitable alternative energy option for industries that produce substantial volumes of waste.

3.1 CURRENT METHANE STATUS IN AUSTRALIA

Methane is one of the most significant GHG's emitted by the rural sector. Carbon dioxide is the most important of the greenhouse gasses in Australia's inventory with a share on 73.4% (400.3Mt) of total CO_2-e emissions. Methane is the second most important contributing 20.6% (or 112.7Mt CO_2-e) of total CO_2-e emissions (UNFCCC 2011a). Total methane emissions have shown a steady increase over the past 20 years as indicated in Table 3.1. Methane capture has also increased slightly over the past 20 years but only small percentages are recovered in the dairy and meat and poultry industries as indicated in Table 3.2. Methane mitigation presents a unique opportunity as the technologies not only reduce emissions but recovered methane provides a renewable source of relatively clean energy.

Table 3.1: Methane generation and emissions, Australia: 1990-2009

Modified from UNFCCC (2011b)

Year	Carbon additions to landfill (kt C)	Carbon loss (through emissions) (kt C)	Methane generated (Gg CH_4)	Methane capture (Gg CH_4)	Net methane (Gg CH_4)
1990	2360	1132	754	2	677
1991	2317	1127	751	2	674
1992	2297	1127	751	11	666
1993	2340	1124	749	11	665
1994	2266	1119	746	35	640
1995	2277	1116	744	28	644
1996	2199	1117	745	91	588
1997	2194	1121	747	98	584
1998	2271	1126	751	130	558
1999	2248	1132	755	121	570
2000	2334	1136	757	129	565
2001	2330	1144	763	131	569
2002	2326	1152	768	128	576
2003	2329	1160	883	176	537
2004	2375	1160	773	197	518
2005	2369	1156	770	207	507
2006	2333	1156	771	222	494
2007	2322	1171	781	216	509
2008	2351	1184	789	205	526
2009	2121	1197	798	215	525

Table 3.2: Methane recovered as a percentage of industrial wastewater treatment 2009

Modified from UNFCCC (2011b)

Commodity	Fraction of methane recovered/flared
Dairy	6%
Pulp and paper	64%
Meat and poultry	6%
Organic chemicals	6%
Sugar	0%
Beer	57%
Wine	0%
Fruit	100%
Vegetables	100%

3.2 METHANE UTILISATION IN AUSTRALIA

In Australia, methane is utilised from sewage treatment plants, livestock feedlots and agricultural wastes (ABARES 2010). Methane capture from meat-processing wastes is growing in popularity as economic incentives and project timelines are addressed. There are a number of fully operational biogas plants including landfill sites, sewage treatment plants, and livestock feedlots. Figure 3.1 shows the distribution of these facilities in Australia however these are predominantly landfill and sewage biogas facilities.

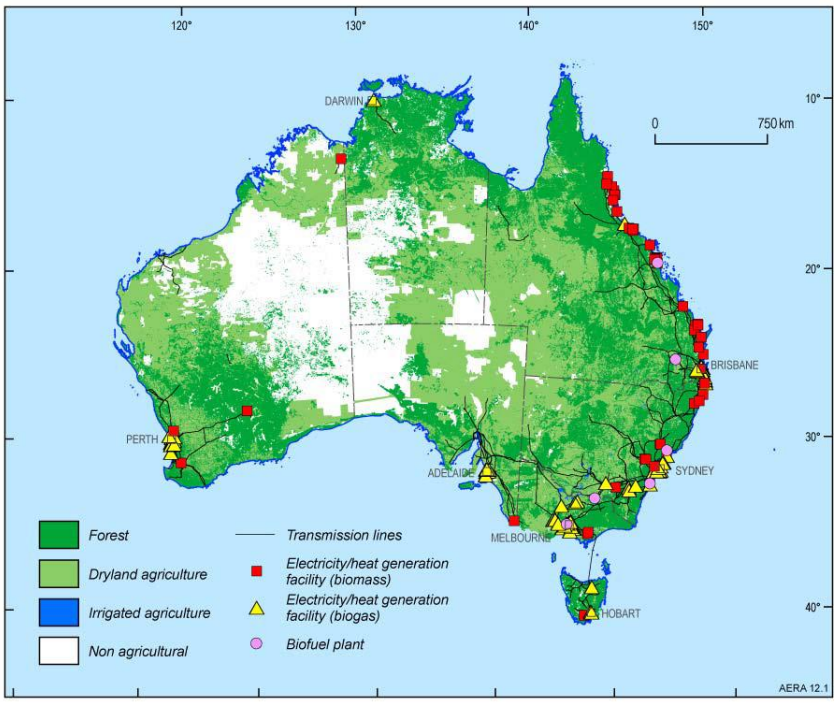

Figure 3.1: Bio energy facilities in Australia

Source: ABARES (2010)

Finding statistics on the actual number of biogas plants operating at feedlots and food processing facilities was a difficult task. In 2009 there was only one operating facility at Berrybank Farm Piggery in Ballarat (DAFF et al. 2008) and Wilkinson (2011) reported that this was the only commercial on-farm anaerobic digester in Australia. Churchill abattoir has recently begun utilising methane produced from their anaerobic ponds as shown in Figure 3.2.

Figure 3.2: Covered anaerobic pond at Churchill Abattoir

Source: AMPC (2011)

Many research projects are underway investigating anaerobic digestion performance and biogas production utilisation at a range of feedlots including Bears Lagoon piggery (Birchall 2010).

3.2.1 FACTORS INFLUENCING THE ADOPTION OF BIOGAS

A study done by Wilkinson (2011) highlighted the main factors influencing the adoption of on-farm anaerobic digestion in Australia as shown in Table 3.3. Although these factors relate specifically to on-farm application they apply to other industries as well, including food processing.

Table 3.3: Summary of factors influencing the adoption of anaerobic digestion in Australia. Modified from Wilkinson (2011)

Context	Factors
Environmental policy	• 6.5% of electrical energy from renewable. Target 20% by 2020 • 5-15% reduction in emissions below 2000 levels by 2020 depending on the nations actions • Possible carbon tax
Energy security	• Net exporter of energy • Brown coal reserves in SE Australia >500 years; Black coal second biggest export earner. • Net importer of crude oil • Natural gas reserves >60 years • No nuclear energy
Farming context	• Approx 5500 dairy farms in SE Australia. With average heard size ~ 200 head, 8% have >500 head • Rural consolidation has occurred in Australia but rural subsidies second lowest in OECD countries. • Highly competitive farming sector • Weak enforcement of animal effluent regulations
Economic incentives	• Mandatory Renewable Energy Target (MRET) expanding • Carbon Pollution Reduction Scheme (CPRS) • Possible carbon tax • $AUD500m Renewable Energy Fund for demonstration and deployment of technologies. • Australian Methane to markets in Agriculture Program (AMTMA)

3.3 METHANE AND THE MEAT INDUSTRY

3.3.1 AUSTRALIAN MEAT INDUSTRY

Beef is the most popular fresh meat at retail in Australia and Australian's eat approximately 35.7kg of beef each year (MLA 2011). Australia is a proficient producer of beef and Hogan and Morris (2010) identified that in 2008-2009 Australia was the second largest exporter of beef and veal in the world. Hogan and Morris (2010) also stated that in 2009-2010 beef and veal was Australia's largest farm export commodity.

In the 2011 march quarter 1,827,000 cattle were slaughtered producing 522,559 tonnes of meat (ABS 2011). Accompanying these high volumes of meat production are high volumes of water that end up as wastewater.

3.3.2 WASTEWATER IN THE MEAT INDUSTRY

Substantial volumes of wastewater with a high organic matter content are produced in the meat processing industry. Water usage in abattoirs has increased due to the automation in carcass dressing together with the incorporation of washing at every stage including; scalding, bleeding, evisceration and tripe treatment (Palatsi et al. 2011). It was reported in the National Inventory Report (UNFCCC 2011b) that for meat and poultry processing 13.7m^3 of wastewater is generated for every tonne of commodity product. An Industry Environmental Sustainability Review (GHD 2010), showed that in 2008-2009 the average wastewater generation for beef processing was 7.9kL per tonne of hot standard carcass weight. As methane is generated by the decomposition of organic matter, the principal factor which determines the methane generation potential of wastewater is the amount of organic matter in the wastewater stream.

3.3.3 ENERGY USE

Meat processing is an energy intensive industry consuming energy in livestock holding, slaughtering and processing, monitoring and testing, cleaning, packing and refrigeration. Refrigeration is typically the most energy intensive activity. The Industry Environmental Sustainability Review 2010 (GHD 2010) found that energy usage per tonne of meat produced has increased by 18% since 2003. In addition, of the 15 sites surveyed none reported generating their own electricity. The majority of energy is derived from electricity, natural gas and coal as shown in Figure 3.3.

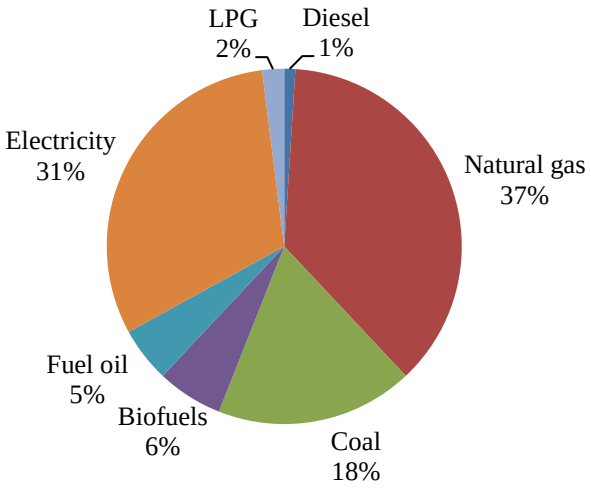

Figure 3.3: Energy use in the meat processing industry

Modified from: GHD (2010)

3.3.4 ENVIRONMENTAL IMPACT

Meat processing is an energy intensive industry, therefore, a plant has a substantial ecological footprint with regard to the amount of energy they consume and the emissions they generate. There are carbon dioxide emissions relating to energy production as well as waste emissions that are predominately methane generated from the anaerobic decomposition of organic matter. Methane has a 21 times greater global warming potential than carbon dioxide, therefore, reducing its release into the atmosphere is beneficial to reducing global warming (El-Fadel & Massoud 2001). In the meat processing industry on average 35% of greenhouse gas emissions were contributed to anaerobic wastewater treatment and 67% were energy related emissions (GHD 2010).

3.4 CASE STUDY: KILCOY PASTORAL COMPANY

This section provides a brief introduction to Kilcoy Pastoral Company to help identify where the wastewaters used in this research came from. Information outlined here came from personal communications with Les Moorhead (2011), Kilcoy Pastoral Company's By-Products/Environmental Manager.

- Kilcoy Pastoral Company is located in the Sunshine Coast hinterland at Kilcoy
- They currently kill about 750 head per day all being 100-day grain fed cattle.
- It is the largest single site beef abattoir in Australia.
- The meat is predominantly exported to Asia (60%), US (2%) Other (Middle east, Pacific, Europe)(18%) and domestic (20%).
- Estimated amount of potable water used in the processes amount to 1.25ML, with the addition of 0.25ML recycled water per day.
- Estimated amount of electricity used is about 15,000,000 kWh each year and they currently do not generate any electricity on site.
- The total water effluent per day is approximately 1.5ML. Wastewater is currently treated in anaerobic, anoxic and aerobic ponds as shown in Figure 3.4.

Figure 3.4: Treatment ponds at Kilcoy Pastoral Company

Pond 1

Is a mixture of;

- Yard water: stockyards waste containing manure and urine and wash down from the stock yards.

- Blood water: wastewater from the slaughter floor which is very lightly contaminated with blood, and water used to transport paunch contents to a 0.5mm wedge wire screen used for screening the paunch solids from the water.

Ponds 3 & 5: (Ex Saveall water)

- Contains a mixture waste water from the rendering department, which is predominantly stick water, (~4% solids) the waste form the tallow-water separation, and fat from the offal bin draining. Floor washing and wastewater from the slaughter floor, which is predominantly wastewater from the processing of intestinal material and water used to carry condemned material through pipe work. This water has a fair amount of blood in it.
- There is also wastewater from the stock yards nearest the slaughter floor.
- Flows to ponds 3 and 5 are treated in a saveall where floating fat is removed.

3.5 CHAPTER SUMMARY

Meat processors can reduce their CO_2 footprint by reducing their energy consumption or obtaining some of their energy from renewable resources. The costs of fossil fuels have risen substantially and these costs will only increase further with the implementation of the Carbon Pollution Reduction Scheme (CPRS) and Renewable Energy Targets, as well as a proposed carbon tax. Benefits to the meat processing industry for utilising renewable energy would include lowering emissions, increasing energy security, reducing liabilities under the CPRS as well as improving public image (Franklin et al. 2010).

CHAPTER 4 METHODOLOGY

This chapter outlines the procedures used to test the biochemical methane potential of easily and slowly biodegradable substrates in both batch and semi-continuous experiments. The following steps are covered in this chapter:

1. Collection of inoculum
2. Collection of abattoir wastewater
3. Determination of wastewater and inoculum characteristics
4. Gas phase characteristics
5. Biochemical methane potential assays
6. Semi-continuous experiment using A bioreactor
7. Risks

4.1 COLLECTION OF INOCULUM

The anaerobic bacteria were collected from the Pittsworth Sewage Treatment Plant operating with a trickling filter and anaerobic digester. The anaerobic digester (Figure 4.1) was fed with approximately 1000L per day of sludge from the primary clarifier. The digester had a hydraulic retention time of one month where 50% was removed. Several samples were collected over a period of two months. The digester was open to the atmosphere and methane was observed bubbling up through the scum layer as shown in Figure 4.2. The sludge was obtained from the bottom outlet of the digester. The sludge was transported in a plastic container and stored at 4°C.

Figure 4.1: Anaerobic digester at Pittsworth Sewage Treatment Plant

Figure 4.2: Scum layer with methane bubbles in anaerobic digester

4.2 COLLECTION OF ABATTOIR WASTEWATER

Wastewater was collected from Kilcoy Pastoral Company on the 15th July 2011. A sample was taken from each of the three wastewater streams that were outlined in section 3.4.

Figure 4.3 and Figure 4.4 show the collection pits of the three wastewaters.

Figure 4.3: Blood water from slaughter floor

Figure 4.4: Yard water and saveall water flows

4.3 DETERMINATION OF WASTEWATER AND INOCULUM CHARACTERISTICS

The characteristics of the inoculum and wastewaters were analysed to determine the mixing ratios. The concentration of the inoculum was determined on a total solids (TS) basis. The strength of the wastewaters was determined by the total organic carbon (TOC) and total nitrogen (TN). Wastewater and inoculum were initially filtered through 500μm sieve to remove large particles of hair, meat, fat, grass and grain.

4.3.1 TOTAL ORGANIC CARBON AND TOTAL NITROGEN

The TOC and TN was analysed using a Total Organic Carbon/ Total Nitrogen Analyser (TOC-VCPH/CPN). Samples were initially filtered through 0.45μm filter paper to ensure the analyser could function properly.

4.3.2 SUSPENDED SOLIDS

Total suspended solids (SS) is the portion of solids retained on a 0.45μm nominal pore size filter membrane. Using standard procedure (APHA 1995) the filter paper was initially weighed then the sample volume was filtered leaving a residue on the filter

paper. The filter paper and residue were dried at 100°C for two hours then placed in a desiccator to cool. The following formula was used.

$$SS = \frac{(\,filter\ paper\ and\ dry\ residue - filter\ paper\ ) \times 1000}{Sample\ volume\ (ml)}$$

Equation 4.1

4.3.3 VOLATILE SUSPENDED SOLIDS

Volatile suspended solids (VSS) is the weight loss of the solids retained on the filter paper after ignition at 500°C. Volatile suspended solids were determined following standard methods (APHA 1995). The dry residue and filter paper were heated in a furnace for 20 minutes at 500°C then cooled in a desiccator. The volatile suspended solids were calculated as follows.

$$VSS = \frac{(weight\ dry\ residue\ and\ filter - weight\ volatile\ residue\ and\ filter\ ) \times 1000}{Sample\ volume\ (ml)}$$

Equation 4.2

4.3.4 TOTAL SOLIDS

Total solids were determined following standard methods (APHA 1995). A clean evaporating dish was heated at 105°C for one hour then cooled in a desiccator. A sample was measured and dried in the dish overnight then cooled in a desiccator. The total solids were calculated as follows

$$TS = \frac{(weight\ of\ dry\ residue\ plus\ dish - weight\ dish) \times 1000}{Sample\ volume\ (ml)}$$

Equation 4.3

4.3.5 VOLATILE SOLIDS

Volatile solids (VS) were determined following standard methods (APHA 1995). The dry residue and dish were heated in a furnace for 20 minutes at 500°C then cooled in a desiccator. The volatile solids were calculated as follows.

$$VS = \frac{(weight\ of\ dry\ residue\ plus\ dish - weight\ of\ volatile\ residue\ plus\ dish\ ) \times 1000}{Sample\ volume\ (ml)}$$

Equation 4.4

4.3.6 NITRATE, NITRITE, PHOSPHOROUS AND SULPHATE

Nitrate nitrogen, nitrite nitrogen, phosphorous and sulphate were measured using Ion Chromatography system (IC, Dionex ICS 2000) using an anion (AS-18) column.

4.4 GAS PHASE CHARACTERISTICS

4.4.1 GAS CHROMATOGRAPHY (GC)

A Shimadzu gas chromatograph model GC 2014, with a thermal conductivity detector was used to analysis the composition of the biogas and provide the methane content as a percentage value. Carbon dioxide could not be analysed as the molecular sieve column could not detect this gas. The operating conditions are outlined below.

Carrier gas: Helium

Carrier gas flow: 25mL/min

Column: Molecular sieve (Grace Davison Discovery Sciences)

Column Temperature: 70ºC

Injection temperature: 120ºC

Current: 170mA

A standard gas mix of 40% CH_4 and 60% N_2 (British Oxygen Company) was used to get a known standard curve. Only one standard was used, therefore, a standard curve could not be created and the percent methane had to be derived. Each sample was then compared to this standard to get the methane content.

4.4.2 PRESSURE AND VOLUME MEASUREMENT

Pressure and volume measurement is outlined in section 4.5.7.

4.5 BIOCHEMICAL METHANE POTENTIAL ASSAYS

4.5.1 PRINCIPLE

Biochemical methane potential assays were used to determine the biodegradability of organic substrates including D-glucose, sodium acetate, powder milk, gelatine and three different abattoir wastewaters, using standard methods (ISO 1995). The substrates were mixed with an anaerobic inoculum obtained from an operating anaerobic digester. The solution was incubated in gas tight bottles with rubber septa and the daily gas production was monitored. The gas produced was quantified using pressure and volume measurement and gas composition was determined using gas chromatography. Figure 4.5 shows a schematic of the biochemical methane potential assay.

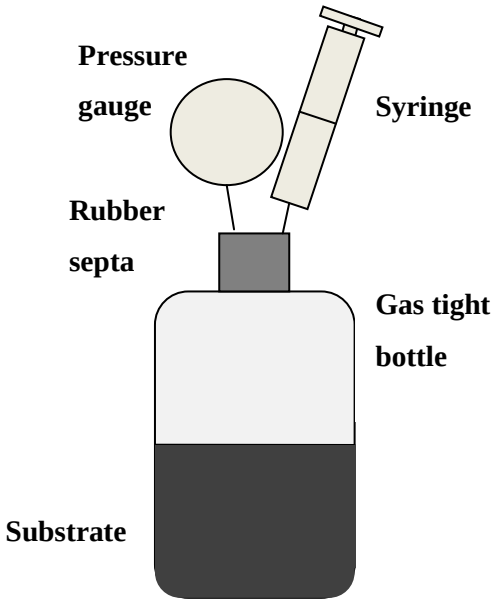

Figure 4.5: Schematic of biochemical methane potential assay

4.5.2 PREPARATION OF INOCULUM

A sludge concentration of between 1g/L and 3g/L solids was required. The sludge was initially filtered through 500μm sieve to remove large particles and foreign materials

including sand and gravel, hair, wood and large organisms. The suspended solids were determined using standard methods as describe in section 4.3.2.

EXAMPLE CALCULATION

The sludge concentration was 27.8 g/L SS, and required dilution. The dilution was calculated to be 10.5 times which gave a SS concentration of 2.6 g/L which was within the required range. The required volume of sludge was then calculated as follows

$$\frac{420ml\ working\ volume}{10.5} = 40ml\ sludge$$

The required volume of sludge was then centrifuged at 1431g for 10 minutes at 20°C. The supernatant was removed and the sludge was resuspended in anaerobic media solution and centrifuged a second time. The supernatant was again removed leaving the pellet for use as seen in Figure 4.6.

Figure 4.6: Anaerobic sludge after centrifuging showing pellet of inoculum

4.5.3 PREPARATION OF ANAEROBIC MEDIA

The anaerobic media solution provides the required minerals to maintain optimum growth conditions these include N, P, Ca, Mg, Fe, K. in addition the stock solution provides further minerals that are only required in small amounts including Mn, Zn, Cu, Co, Mo, Ni. The composition of the anaerobic media is shown in Table 4.1.

Table 4.1: Anaerobic media composition used for batch assays

Chemical	**Formula**	**Amount**
Anhydrous potassium dihydrogenphosphate	KH_2PO_4	0.27g
Disodium hydrogenphosphate	Na_2HPO_4	0.444g
Ammonium chloride	NH_4Cl	0.53g
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0.075g
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	0.10g
Iron chloride	FeCl3	0.013g
Sodium sulfide nonahydrate	$Na_2S \cdot 4H_2O$	0.1g
Stock solution		10ml
Distilled, de-oxygenated water		to 1L

A stock solution of trace elements was included in the media solution to improve anaerobic degradation processes. The constituents used in the stock solution are shown in Table 4.2.

Table 4.2: Stock solution of trace elements used in batch assays

Chemical	**Formula**	**Amount**
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0.05g
Zinc chloride	$ZnCl_2$	0.005g
Copper chloride	$CuCl_2$	0.005g
Disodium molybdite dihydrate	$Na_2MoO_4 \cdot 2H_2O$	0.001g
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	0.1g
Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	0.01g
Distilled water		To 1L

The medium was prepared by boiling 800mL of water in an Erlenmeyer flask, and adding all the media chemicals except Sodium sulfide nonahydrate. The solution was allowed to cool and transferred into a flask and the volume adjusted to 1L then transferred to the storage container. The solution in the storage container was sparged with helium for 20 minutes or until the dissolved oxygen (DO) was zero (see Figure 4.7). The sodium sulfide nonahydrate was then added to the solution to remove any residual oxygen that may be present in the solution. The pH was measured and considered suitable at 7 ± 2, therefore, did not require adjusting. Finally, the headspace of the bottle was flushed with helium for 5 minutes and the bottle was sealed.

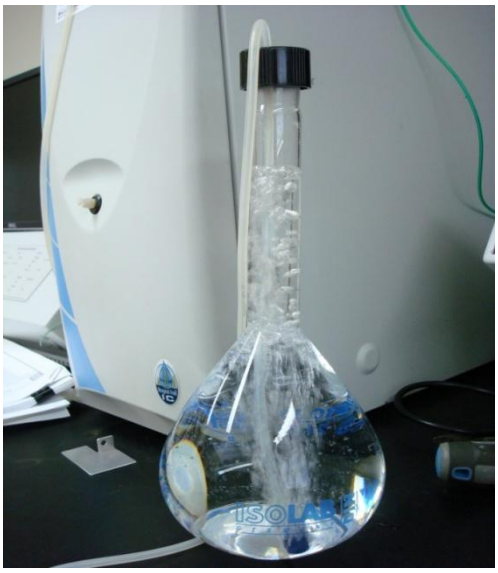

Figure 4.7: Helium sparging to remove dissolved oxygen

4.5.4 TEST SUBSTRATE PREPARATION

The test substrates were added as a solution to the bottles to give a final concentration of 100mg/L as total organic carbon (TOC). A working volume of 420mL containing 210mL substrate and 210mL media and inoculum. The required TOC in each bottle was calculated as follows,

$$\frac{100mg\ TOC}{L} \times 0.42\ L = 42mg\ TOC$$

$$\frac{42mg\ TOC}{210ml} \times \frac{1000ml}{L} = \frac{200mg\ TOC}{L}$$

PREPARATION OF D-GLUCOSE

$C_6H_{12}O_6$ = 180.16g

Carbon atomic weight

C = 12.011

$$Carbon\ content\ of\ glucose = 12.011\ \times 6 = 72.066g$$

The amount of glucose required for 1L with a final concentration of 100mg/L carbon in 420 mL volume is

$$\frac{200mg\ TOC}{L} \times \frac{180g\ glucose}{72g\ TOC}\ \times \frac{g}{1000mg} = \frac{0.5g\ glucose}{L}$$

PREPARATION OF SODIUM ACETATE

CH_3COONa = 82.03g

Carbon atomic weight

C = 12.011

$$Carbon\ content\ of\ sodium\ acetate = 12.011\ \times 2 = 24g$$

The amount of sodium acetate required for 1L with a final concentration of 100mg/L carbon in 420 ml volume is

$$\frac{200mg\ TOC}{L} \times \frac{82.03g\ acetate}{24g\ TOC}\ \times \frac{g}{1000mg} = \frac{0.684g\ acetate}{L}$$

PREPARATION OF POWDER MILK

The TOC of milk powder at a concentration of 1g/L was 376 mg/L.

The amount of powder milk required for 1L with 100mg/L carbon is

$$\frac{200mg\ TOC}{L} \times \frac{1g\ powder\ milk}{0.376g\ TOC} \times \frac{g}{1000mg} = \frac{0.53g\ powder\ milk}{L}$$

PREPARATION OF GELATINE

The TOC of gelatine at a concentration of 1g/L was 370 mg/L.

The amount of gelatine required for 1L with 100mg/L carbon is

$$\frac{200mg\ TOC}{L} \times \frac{1g\ gelatine}{0.37g\ TOC} \times \frac{g}{1000mg} = \frac{0.54g\ gelatine}{L}$$

PREPARATION OF ABATTOIR WASTEWATER

The wastewaters were filtered with 500 µm sieve to remove large particles such as hair, grain and meat. Samples were taken for dissolved total organic carbon (DTOC) analysis and filtered again with 0.45 µm filter paper. The wastewaters were then sparged with helium to remove any DO and then diluted to achieve approximately 100mg/L DTOC per assay.

4.5.5 PREPARATION OF BMP ASSAYS WITH MEDIA

Tests were prepared in duplicate in 500mL Wheaton media bottles with open top screw cap with gray chlorobutyl/50 septa and flange (Edwards Instrument Co.). A working volume of 420mL was used which allowed 20mL of solution to be removed at the beginning of the trial for TOC and IC tests. A control bottle that contained only distilled water and inoculum was prepared in duplicate to monitor residual methane production of the inoculum. In addition, a bottle with just tap water was prepared to monitor

changes in atmospheric pressure in the bottle. Two runs were undertaken with anaerobic media as outlined in Table 4.3 and Table 4.4.

Table 4.3: Substrates used in trial 1

Sample number	Substrate
1	D-glucose
2	Sodium acetate
3	Gelatine
4	Powder milk
5	Blank
6	Tap water only

Table 4.4: Substrates used in trial 2

Sample number	Substrate
1	D-glucose
2	Yard water
3	Blood water
4	Saveall water
5	Blank
6	Tap water only

The filling procedure of the bottles involved careful handling to ensure minimal contact between the inoculum and oxygen. The pellet of inoculum was resuspended in 210mL of anaerobic medium and placed into the bottle. Then 210mL of test compound was added to the bottle. A 20mL sample of the solution was removed for TOC and IC tests. The solution was then sparged with helium for 15 minutes to remove the DO and the headspace was flushed for a further 5 minutes. The bottles were then closed tightly and placed in an incubator.

4.5.6 PREPARATION OF BMP ASSAYS WITHOUT MEDIA

Trials was conducted to test the biogas production when no anaerobic media was added. The substrates that were tested are shown in Table 4.5. Glucose with media was used as the control. The assays were prepared using the same procedure as trials conducted with

anaerobic media except the inoculum was resuspended in distilled water and distilled water was added to make the working volume up to 420mL.

Table 4.5: Substrates used in trial 3

Sample number	Substrate
1	D-glucose with media
2	D-glucose no media
3	Yard water
4	Blood water
5	Saveall water
6	Blank
7	Tap water only

4.5.7 INCUBATION AND GAS MEASUREMENT

The prepared vessels were incubated at 35°C ±2°C in an incubator as shown in Figure 4.8.

Figure 4.8: Incubating bottles

The pressure was obtained using Wika capsule pressure gauges, model 611.10, standard series with a scale range of 0 - 4 Kpa and 0 - 25 Kpa (Wika Australia Pty Ltd). The gauges were connected to a brass adapter that was welded to a 26-gauge stainless steel hypodermic needle. Determining a suitable method of pressure measurement was a vital

component of the BMP tests therefore pictures of the gauges and needle adapter are shown in see Figure 4.9 and Figure 4.10

Figure 4.9: Pressure gauges fitted with hypodermic needles

Figure 4.10: Close up of hypodermic needle connection

The volume of gas was measured using a Nipro 26-gauge hypodermic needle and Terumo syringe. The needle and syringe were sealed with Stag Jointing Paste to prevent gas leaks. Both pressure and volume measurements were taken every day to ensure a reading could be obtained as the needles were subject to blockages.

Surface temperature was measured using a Fluke infrared thermometer before and after the pressure and volume measurements. An infrared thermometer had to be used as the

bottles had to stay sealed. The infrared laser was pointed at white masking tape stuck to the bottle to reduce reflection and ensure an accurate reading (see Figure 4.11). Measuring the temperature before and after pressure measurement ensured that gas changes due to temperature drops could be taken into account.

Figure 4.11: Infrared thermometer

The measurement procedure involved the following:

1. Mix the solution by shaking the bottle for approximately one minute
2. Read the temperature
3. Take a pressure reading
4. Take sample for gas chromatography (GC)
5. Remove the volume of gas until the gauge reads zero
6. Read the temperature

Initially the vessels were incubated for one hour to allow equalization and then the excess gasses were released to atmosphere until the pressure gauge read zero and the temperature was recorded. The temperature, pressure and volume were then recorded daily. In addition, a 2mL sample was taken for gas analysis using gas chromatography as outlined in section 4.4.1. Figure 4.12 shows taking a sample in the gas syringe.

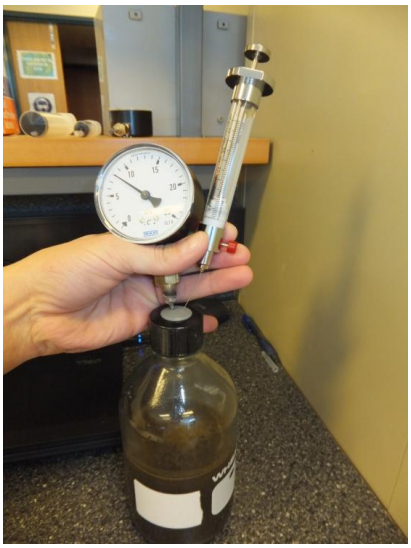

Figure 4.12: Biochemical methane potential assay: measuring gas pressure and GC sampling

4.6 SEMI-CONTINUOUS EXPERIMENT USING A BIOREACTOR

Semi continuous experiments were conducted in a Stratorius APlus bioreactor. This allowed volumes to be up scaled and continuous monitoring of pH, temperature and stirrer speed.

4.6.1 REACTOR DESIGN AND OPERATION

The reactor design consisted of the following;

A glass vessel to contain the test mixture sealed with a lid containing sample ports and fittings for the temperature probe, pH probe, DO probe and stirrer. The temperature was kept constant with a heating blanket. Gas was collected in a gas collection bag. Figure 4.13 shows a schematic of the reactor design and Figure 4.14 shows the actual set up.

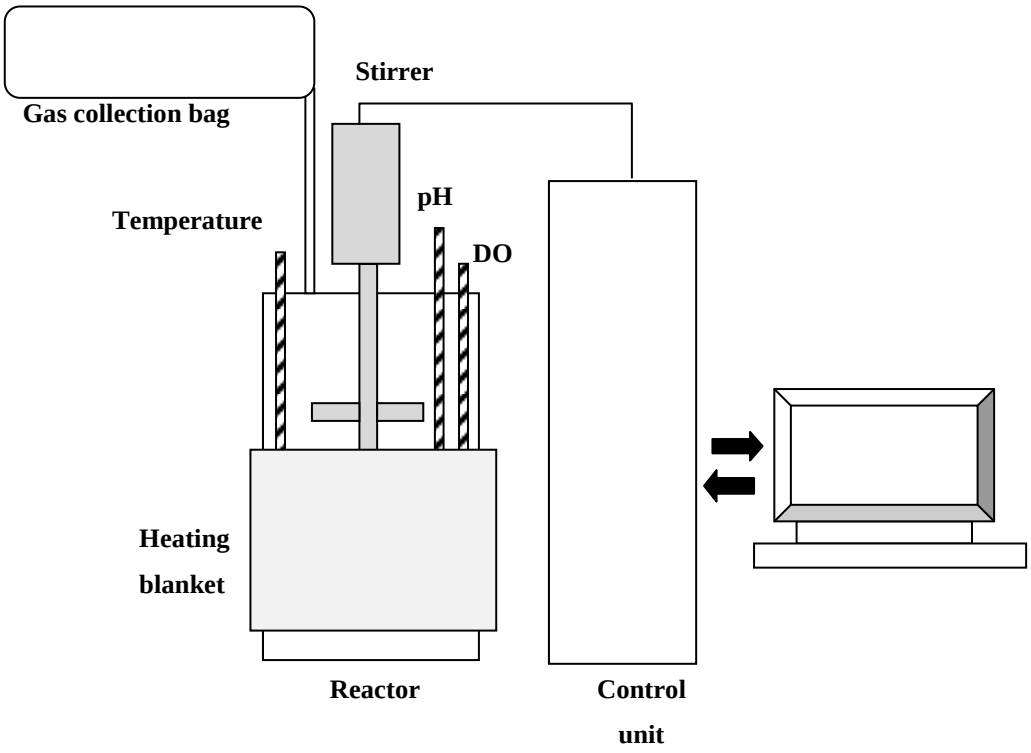

Figure 4.13: Schematic diagram of Startorius Biostat APlus bioreactor

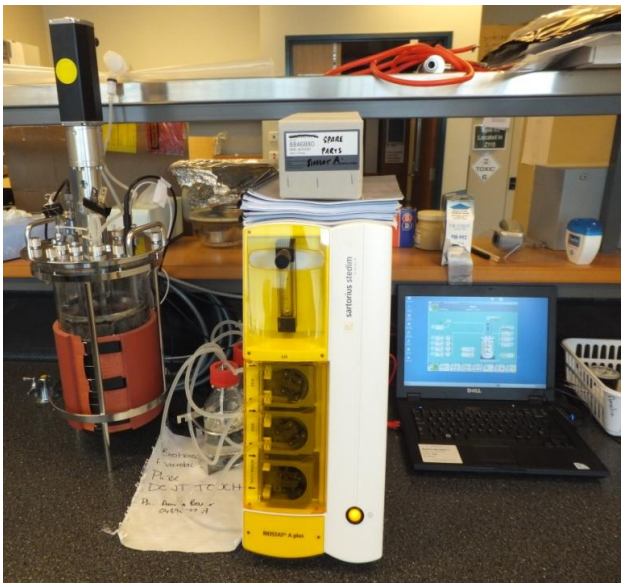

Figure 4.14: Startorius Biostat APlus bioreactor

The reactor could be easily controlled using the control panel shown in Figure 4.15. Only the temperature and stirrer highlighted in green were adjusted.

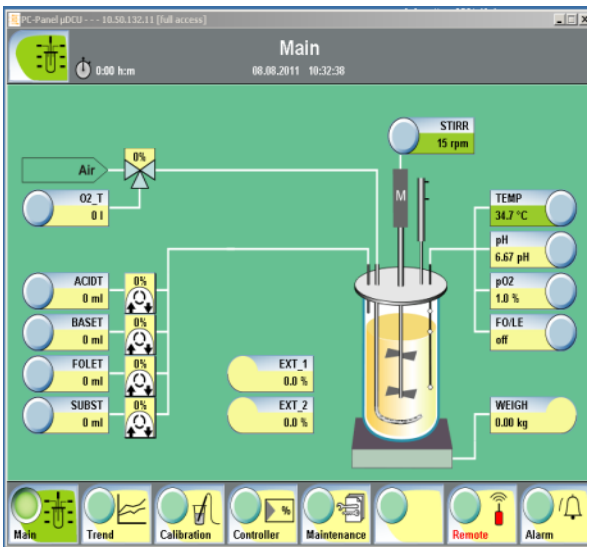

Figure 4.15: Stratorius Biostat APlus bioreactor control panel

Real time monitoring of the temperature, pH, and DO could also be displayed as shown in Figure 4.16

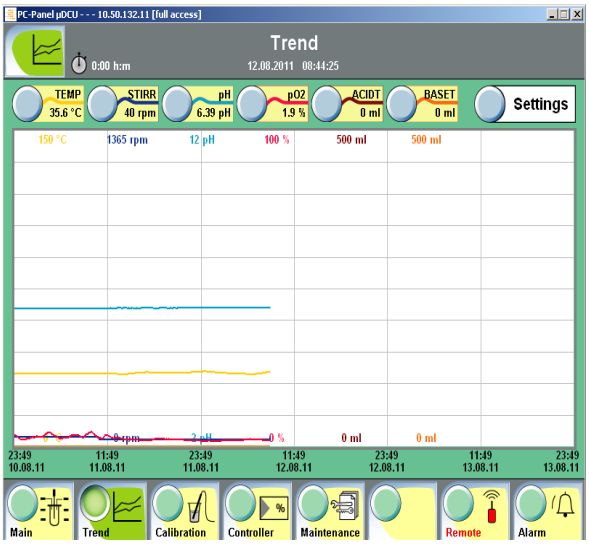

Figure 4.16: Real time monitoring

4.6.2 MEDIA PREPARATION

Anaerobic media was prepared with the constituents shown in Table 4.6 and Table 4.7 as used by Thompson (2008). This ensured an initial carbon:nitrogen:phosphorus

(C:N:P) ratio of approximately 100:8:1, which is in the range stated by Ronquest and Britz (1999) as being the ideal ratio for anaerobic bacteria.

Table 4.6: Anaerobic media used in bioreactor

Chemical	**Formula**	**Amount**
Dipotassium hydrogen phosphate	$K_2H\ PO_4$	0.296g
Ammonium chloride	NH_4Cl	1.496g
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0.264g
magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.377g
Ferric chloride hexahydrate	$FeCl_3 \cdot 6H_2O$	0.172g
Sodium sulfide nonahydrate	$Na_2S \cdot 4H_2O$	0.1g
Stock solution		10mL
Distilled, de-oxygenated water		to 1L

Table 4.7: Stock solution of trace elements

Chemical	**Formula**	**Amount**
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0.05g
Zinc chloride	$ZnCl_2$	0.005g
Copper chloride	$CuCl_2$	0.005g
Disodium molybdite dihydrate	$Na_2MoO_4 \cdot 2H_2O$	0.001g
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	0.1g
Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	0.01g
Distilled water		To 1L

4.6.3 FEED PROCEDURE

Week 1: Anaerobic sludge at a concentration of 5g/L TS, anaerobic media and distilled water were added to a working volume of 4L

Week 2: The solution in the reactor was allowed to settle for 1hour then the supernatant was removed Powder milk was added at a concentration of 16g/L.

Week 3: The solution in the reactor was allowed to settle for 1hour then the supernatant was removed leaving 2L of sludge remaining in the reactor.

2L of prepared blood water was added.

Week 4: The solution in the reactor was allowed to settle for 1hour then the supernatant was removed leaving 2L of sludge remaining in the reactor.

2L mixture of equal parts, yard water, blood water and saveall water was added.

4.6.4 OPERATING CONDITIONS

The reactor was kept at 35°C ± 2°C using the heating blanket. The reactor was continuously stirred using a paddle stirrer at 40RPM. The speed was increased to 100RPM for 5 minutes prior to sampling.

4.6.5 ANALYSIS

Gas was collected in a Tedlar bag (shown in Figure 4.17) then the daily volume of gas was removed with a syringe by opening the valve and sucking the gas out. A 2 mL sample was collected for gas chromatography using a gas syringe from the septum port in the bioreactor. A sample of 20mL, of the reactor contents, was removed daily for the analysis of DTOC.

Figure 4.17: Tedlar bag used for gas collection

4.7 RISKS

4.7.1 DISEASE PREVENTION

During the handling of municipal wastewater there is a risk of contracting Hepatitis A and B and tetanus. When entering the abattoir site and handling wastewater there is a risk of contracting Q fever. Vaccinations were required several weeks prior to handling these wastewaters. In addition, gloves, safety glasses and a lab coat were required. Hand washing and the use of disinfecting alcohol gel were also required after the handling of samples.

4.7.2 BACTERIAL MANAGEMENT

Anaerobic sludge was obtained from a digester treating the sludge from municipal wastewater and has a high possibility of containing pathogens, therefore, there was a risk of bacterial contamination. To minimise the spread of bacteria 70% ethanol was used to cleanse all surfaces after use. Appropriate warning labels were placed on all substances, bottles, equipment and experiments containing bacteria.

4.8 CHAPTER SUMMARY

This chapter discussed the procedures used to conduct the biochemical methane potential assays and semi-continuous anaerobic digester experiments. The procedures used to characterise and prepare the substrates and inoculum were outlined. Methods of collecting and analysing the gas characteristics were also outlined.

CHAPTER 5 BIOGAS PRODUCTION FROM EASILY BIODEGRADABLE SUBSTRATES

This chapter provides the results and discussion on the theoretical biogas yield, measured biogas yield and the percentage of biodegradation, of easily biodegradable substrates including, glucose, acetate, gelatine and powder milk. This initial trial was conducted to test the activity of the inoculum and provide a standard to compare other substrates too. Glucose and acetate were chosen as they are easily biodegradable. Gelatine was chosen as it is high in protein similar to abattoir wastewater. Powder milk was chosen as it is more complex than glucose, acetate and gelatine and contains carbohydrates, proteins and fats. All volumes are reported at STP unless otherwise stated.

5.1 THEORETICAL BIOGAS AND METHANE PRODUCTION

When organic matter is degraded anaerobically, the end result is carbon in its most oxidized form, CO_2, and its most reduced form, methane (CH_4). The ratio of CH_4 and CO_2 depends on the oxidation state of the carbon present in the organic material. Therefore, the more reduced the organic carbon content is, the more CH_4 will be produced. The theoretical CH_4 yields were calculated using the Buswell equation which balances the total conversion of organic material to CH_4 and CO_2 (Sobotka et al. 1982). Gas composition was not measured for this run, therefore, the total theoretical biogas yield was calculated (CH_4+CO_2) in addition to the theoretical CH_4 yield, which was then converted to volumes by the Ideal Gas Law. The following provides the formulation of the theoretical gas yield calculations.

Buswell formula

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

Equation 5.1

O'Rourke and Boyle (Sobotka et al. 1982) recommended an extension of the original Buswell formula to include N as follows;

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

Equation 5.2

The O'Rourke and Boyle's extended formula was required as the estimated formula for protein contained N. Raposo et al. (2011) provided the average formulae representing protein, carbohydrates and lipids (fats) as shown below.

Protein: $C_5H_7O_2N$

Carbohydrate: $C_6H_{10}O_5$

Lipids: $C_{57}H_{104}O_6$

Theoretical volumes were the obtained using the Ideal Gas Law. It can be shown that 1 Mole of any gas at STP occupies 22.4L. This can be confirmed by applying the ideal gas law

$$PV = nRT$$ Equation 5.3

Where

P = pressure in Pa

V= volume (L)

n = number of moles

T = temperature in K

R = the molar gas constant (8314 J/mol·K)

$$PV = nRT$$

$$V = \frac{nRT}{P}$$

$$V = \frac{1 \times 8314 \times 273.15}{101325}$$

$$V = 22.4\ L$$

Powder milk

Using the nutrition information shown below, indicating the quantity of protein, fat and carbohydrate contained in the powder milk and the empirical formulas for proteins, fats and carbohydrates outlined previously, the theoretical gas production could be calculated for each component respectively and combined to give the total gas produced for powder milk.

Nestle Sunshine instant full cream powder milk

Nutrition information:

Protein: 3.2g/13.3g powder milk

Fat: 3.7g/13.3g powder milk

Carbohydrate: 4.9g/13.3g powder milk

Gelatine

Similarly, using the nutrition information shown for gelatine indicating the quantity of protein, fat and carbohydrate it contained and the empirical formulas for protein, fat and carbohydrates the O'Rourke and Boyle equation was applied to give the theoretical gas production.

Davis Gelatine

Nutrition information:

Protein: 88g/100g gelatine

Fat: Nil

Carbohydrate: Nil

The theoretical results shown in Figure 5.1 indicate that Powder milk should produce the most biogas of 252mL/100mg/L TOC, followed by glucose producing

187mL/100mg/L TOC, acetate producing 187mL/100mg/L TOC and gelatine producing 99mL/100mg/L TOC.

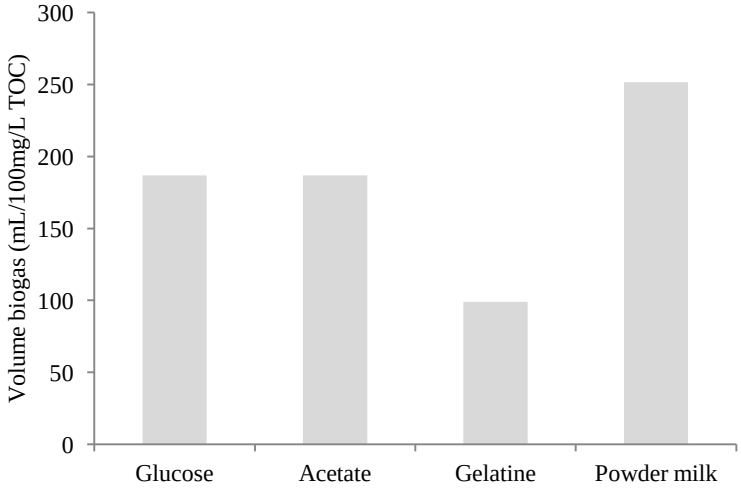

Figure 5.1: Theoretical biogas yield at STP

The theoretical biogas and theoretical CH_4 produced is shown in Figure 5.2. This indicates that the biogas produced by glucose should contain about 50% CH_4. Biogas produced by acetate should contain about 44% CH_4. The biogas produced by gelatine should contain 56% CH_4 and the biogas produced by powder milk should contain 60% CH_4.

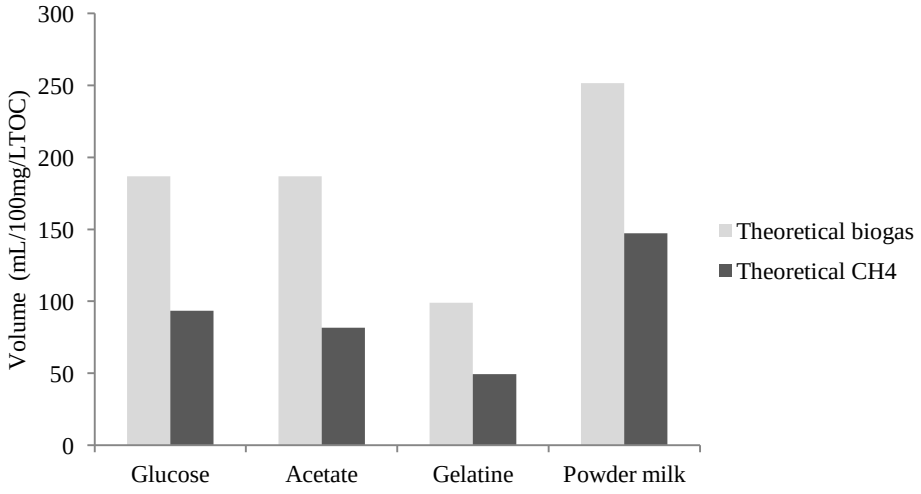

Figure 5.2: Theoretical biogas and theoretical CH_4 yield at STP

5.2 MEASURED BIOGAS PRODUCTION

The measured biogas volumes required adjusting to STP before a comparison of the volumes produced could be made. Gas laws state that

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$ Equation 5.4

Where

P_1 is the measured pressure in the bottle

V_1 is the volume of the headspace

T_1 is the measured temperature

P_2 is standard pressure (101325 pa)

T_2 is standard temperature (273.15 K)

V_2 is calculated volume

This means that the measured pressure, volume of the headspace of the bottle and measured temperature in the bottle are converted to standard pressure, standard temperature and volume of gas at these conditions. An example of the calculation procedure is shown in Appendix D, with raw data shown in Appendix E.

Figure 5.3 shows the cumulated biogas produced at STP by glucose, acetate, gelatine, powder milk and blank over the incubation period of ten days for 100mg/L TOC added. These results show cumulated biogas produced after the changes in atmospheric pressure were deducted. This shows that the blank produced some gas, therefore, the volume of gas produced by the blank had to be deducted from volumes of gas produced by glucose, acetate, gelatine and powder milk.

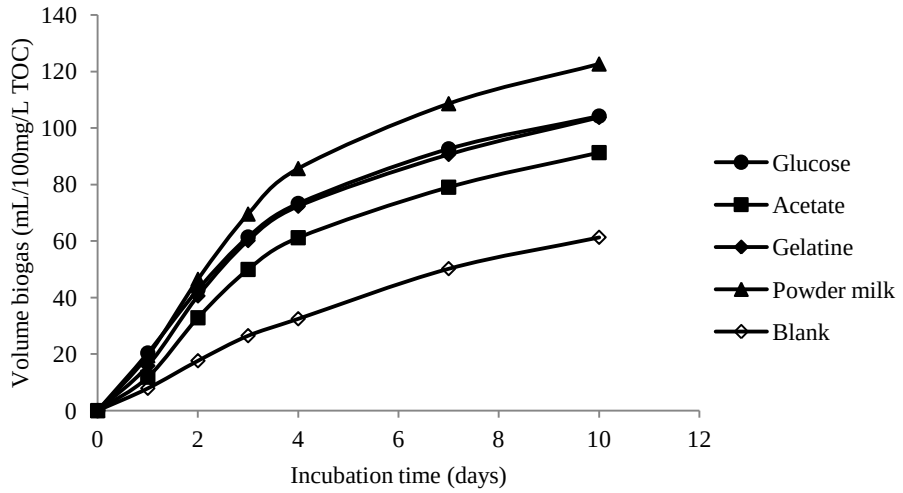

Figure 5.3: Cumulated volume of biogas produced at STP

Figure 5.4 shows the cumulated volume of biogas produced at STP by glucose, acetate, gelatine and powder milk over an incubation period of ten days. These results show total biogas produced for 100mg/L TOC added, after the background volume of biogas produced by the blanks and the changes in atmospheric pressure were deducted. All substrates produced biogas at an exponential rate until day four when they began to plateau. Acetate showed a small lag phase of one day. Powdered milk produced the most biogas totalling 61mL/100mg/L TOC at STP. Gas produced from powder milk continued to slowly increase from 53.1mL to 61mL during the plateau phase indicating that full biodegradation had not yet occurred. Glucose produced 43mL/100mg/L TOC at STP. Acetate produced 30mL/100mg/L TOC at STP. Gelatine produced 42mL/100mg/L TOC at STP.

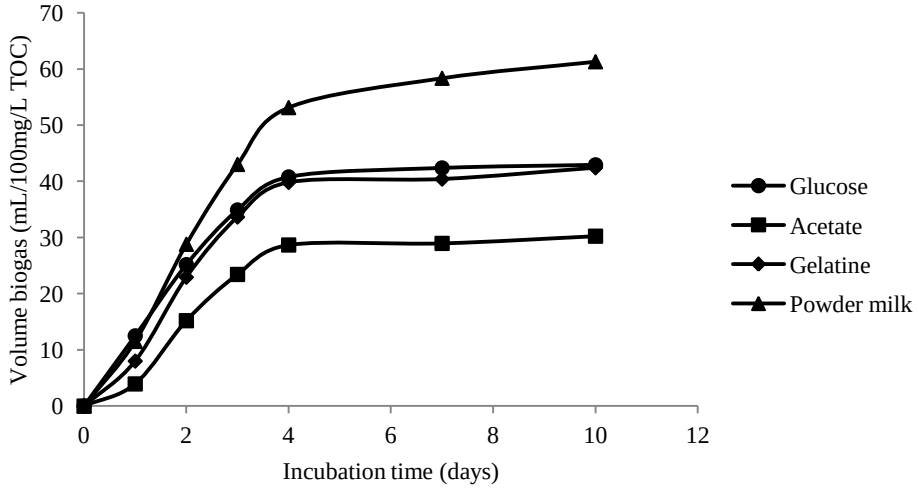

Figure 5.4: Cumulated volume of biogas produced once blanks were deducted, at STP

5.3 COMPARISON OF MEASURED AND THEORETICAL BIOGAS PRODUCTION

The measured results can be compared to the theoretical results as shown in Figure 5.5, which provides further confirmation that powder milk produces the most biogas. This can be attributed to the fact the powder milk is more complex than the other substrates and contains fats, minerals and trace elements.

The lower biodegradability of gelatine can be explained, considering the degradation of protein should be inhibited due to the accumulation of intermediates such as VFA's and free ammonia. However, gelatine preformed only slightly lower than glucose and did not display any distinct inhibition as Hansen et al (2004) observed in the anaerobic digestion of gelatine.

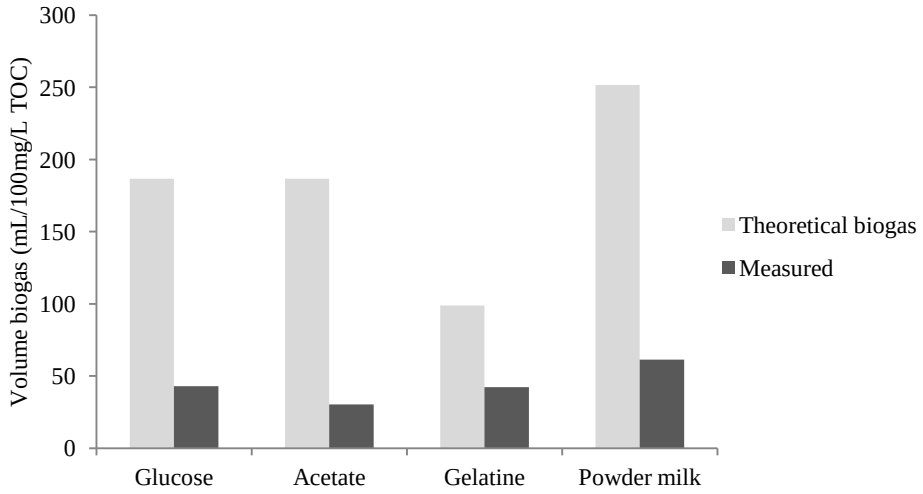

Figure 5.5: Comparison of theoretical and measured biogas yield at STP

Angelidaki and Sanders (2004) give several reasons why measured biogas is less than the theoretical biogas potential. These include;

- Some of the substrate is utilised to synthesize bacterial mass, typically 5-10% of the organic material degraded.
- At a finite retention time a fraction of the organic material will be lost in the effluent, typically 10%.
- Lignin does not degrade anaerobically.

- Part of the organic matter is inaccessible due to binding in particles or structural organic matter.
- Limitation of other nutrient factors.

Another factor that may have contributed to the lower measured biogas yields is that the inoculum may have had a poor microbial population and diversity of anaerobic bacteria. Although the inoculum came from an active anaerobic digester treating municipal sludge waste there may have been other bacteria present in the sludge, which would have reduced the concentration of the anaerobic bacteria population. Forster-Carneiro et al (2007) found that an anaerobic reactor treating municipal waste was the best reactor to source inoculum from as compared with reactors treating swine and cattle manure, kitchen waste and corn silages.

5.4 CUMULATED PRESSURE INCREASE

The course of biodegradation can also be shown by plotting the cumulated pressure increase against time. The cumulated pressure shown in mbar at 35ºC is required by standard (ISO 1995). Figure 5.6 shows the cumulated pressure for the test substrates indicating the maximum cumulated pressure for glucose was 161mbar at 35°C. Cumulated pressure for acetate was 116mbar at 35°C. Cumulated pressure for gelatine was 160mbar at 35ºC. Finally, cumulated pressure for powder milk was 226mbar at 35ºC.

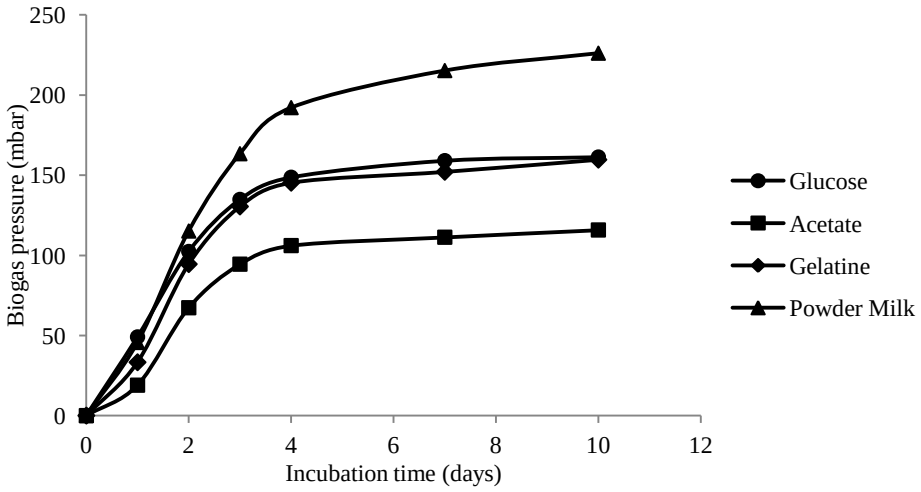

Figure 5.6: Cumulated pressure at 35ºC

5.5 BMP RATE: FIRST-ORDER KINETIC MODEL

Kinetic studies provide a mechanism of understanding anaerobic biodegradation, including lag phases and inhibition of the process. Typically, the rate of anaerobic digestion processes can be evaluated using the biogas values obtained from BMP experiments. However, the *k* value provides a good comparison of the rate of biodegradation. The degradation of each sample was assumed to follow the first-order kinetic model (Gunaseelan 2004).

$$B = B_o(1 - e^{-kt}) \qquad \text{Equation 5.5}$$

B is the cumulative biogas yield at time *t*. B_o is the maximum volume of biogas at infinite time. B_o was assumed to be maximum after 10 days of incubation. *k* was estimated by curve fitting with the measured results using Matlab's curve fitting tool box. Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10 show the measured data with the curve fit for glucose, acetate, gelatine and powder milk respectively.

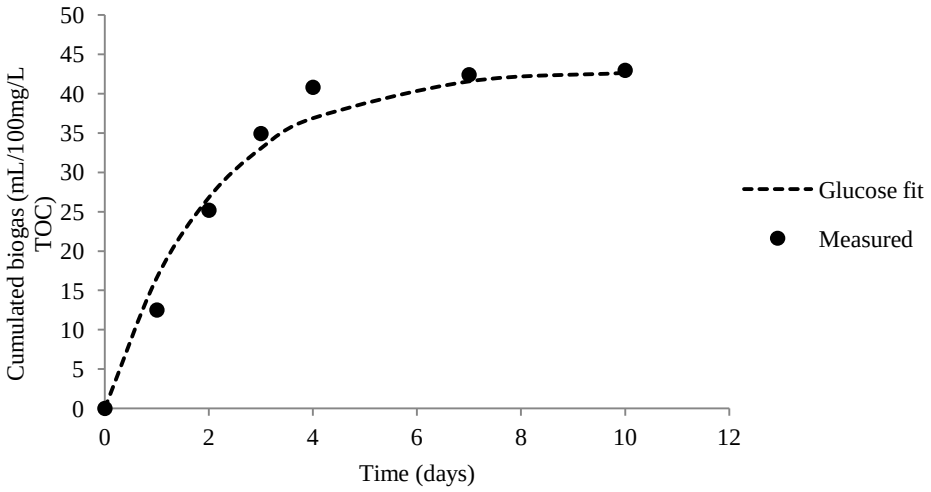

Figure 5.7: Comparison of measured and fitted data for glucose at STP

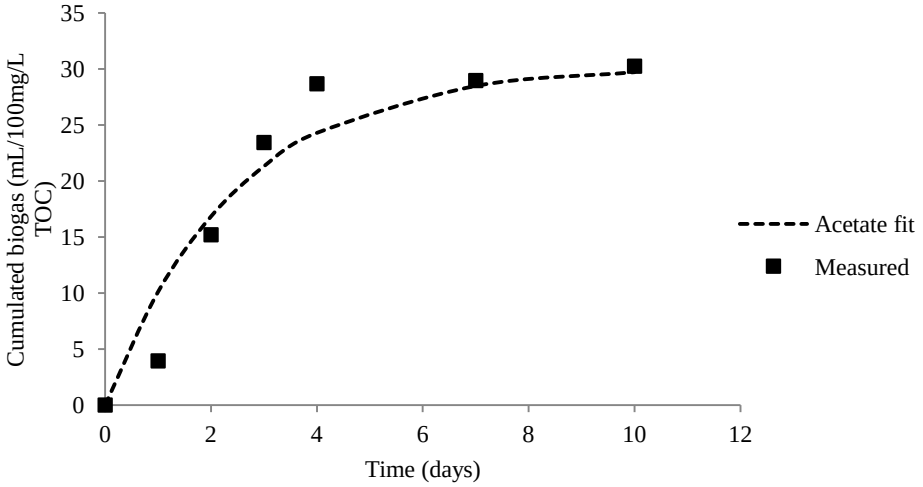

Figure 5.8: Comparison of measured and fitted data for acetate at STP

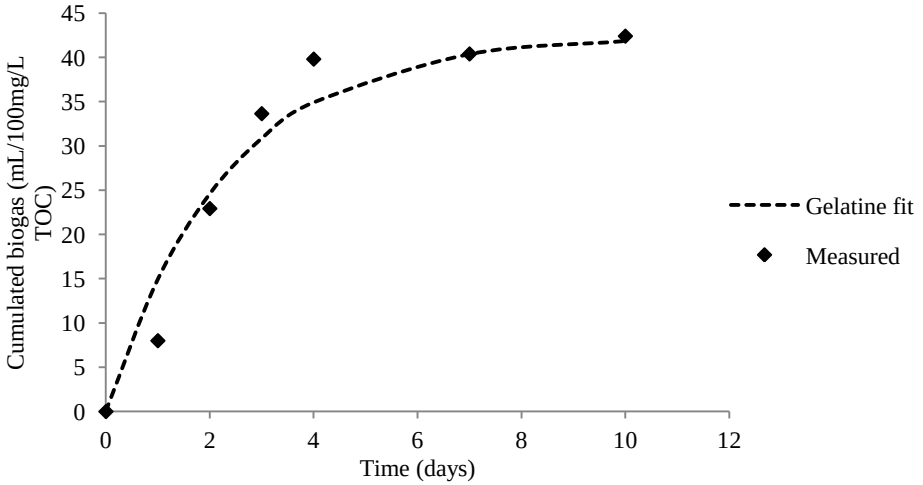

Figure 5.9: Comparison of measured and fitted data for gelatine at STP

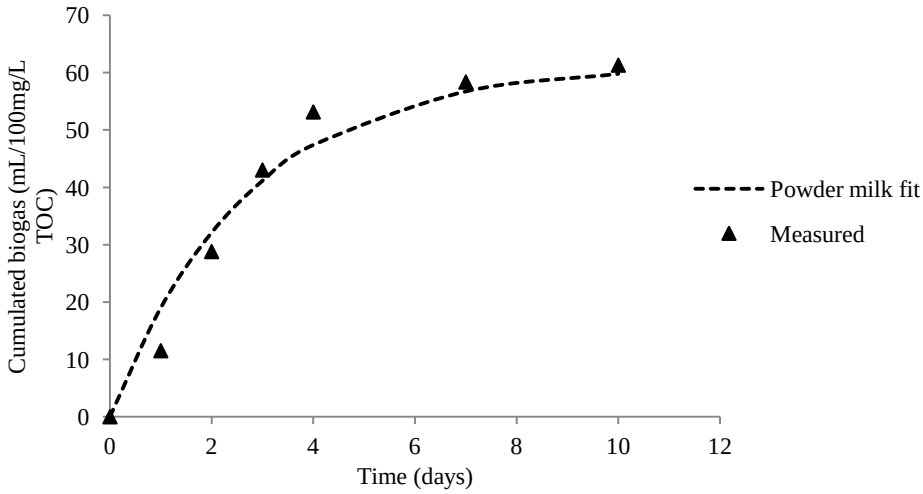

Figure 5.10: Comparison of measured and fitted data for powder milk at STP

k can be determined as the slope of the linear part of the curve and this value is characteristic of a given substrate and gives information about the time required to generate a given ratio of the ultimate methane potential (Angelidaki et al. 2009). A high *k* value produces a fast rate of degradation or steep slope in the initial days and then plateaus as the food is exhausted and maximum biodegradation has occurred. A low *k* value produces a slower steadier rate of degradation and takes substantially longer to reach maximum biodegradation.

The *k* values for glucose, acetate, gelatine and powder milk are provided in Table 5.1 below. This shows that glucose, acetate and gelatine biodegraded fairly quickly and powder milk was slower to reach maximum biogas yield, however, powder milk had a higher maximum yield.

Table 5.1: Maximum ultimate biogas yield, first order rate constant (*k*) and R^2

	B_o (mL biogas)	*k*	R^2
Glucose	42.9	0.49	0.98
Acetate	30.2	0.41	0.93
Gelatine	42.4	0.43	0.95
Powder milk	61.3	0.37	0.97

5.6 BIODEGRADATION

5.6.1 BIODEGRADATION USING STANDARD METHODS

The final content of inorganic carbon (IC) gives an indication of the biodegradation that has taken place. The CH_4 that is produced is mainly released to the gas phase, however, CO_2 is partly dissolved in the liquid phase or is converted to bicarbonate, therefore, there should be an increase in IC at the end of the trial.

The biodegradation was found using standard methods outlined in ISO 11734 (ISO 1995). The following equations were used to obtain the percentage of biodegradation.

The mass of carbon produced as gas in the headspace

$$m_n = 0.468(P \times V_h)$$ Equation 5.6

Where

m_n is the mass of carbon produced as gas

P is the cumulated pressure minus the blanks

V_h is the volume of the headspace

The mass of carbon in the liquid of the was calculated by

$$m_l = p_{ICnet} \times V_l$$ Equation 5.7

Where

m_l is the mass of carbon in the liquid

p_{ICnet} is the concentration of inorganic carbon in the liquid minus that in the blanks

V_l is the volume of liquid

The total mass of gasified carbon in each bottle was calculated from

$$m_t = m_n + m_l$$ Equation 5.8

Where m_t is the total mass of gasified carbon (mg)

The carbon in the test vessel

$$m_v = p_{cv} \times V_l$$ Equation 5.9

Where

m_v is the mass of test compound carbon

p_{cv} is the concentration of test compound carbon

V_l is the volume of the liquid

The total biodegradation was calculated as follows,

$$D_t = \frac{m_t \times 100}{m_v}$$ Equation 5.10

Figure 5.11 shows the percentage of biodegradation for glucose, acetate, gelatine and powder milk. Glucose reached 60% biodegradation, acetate 38% biodegradation, gelatine 77% biodegradation and powder milk 87% biodegradation.

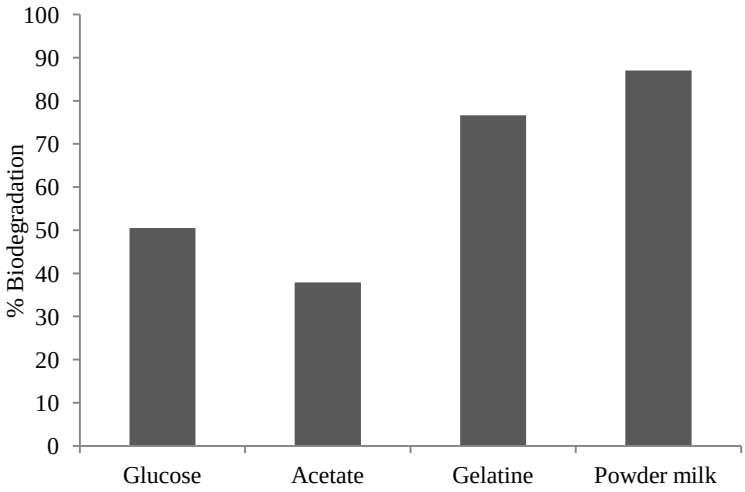

Figure 5.11: Percentage of biodegradation using standard methods

5.6.2 TOC REDUCTION

TOC reduction also provided an indication of the biodegradation that had occurred. Figure 5.12 shows the initial and final TOC concentrations. Glucose had a reduction of 51mg/L TOC, acetate had a reduction of 43mg/L TOC, gelatine had a reduction of 36mg/L TOC and powder milk had a reduction of 53mg/L TOC. The blank had an increase in TOC of 8mg/L, the possible causes of this are discussed in section 6.2.4.

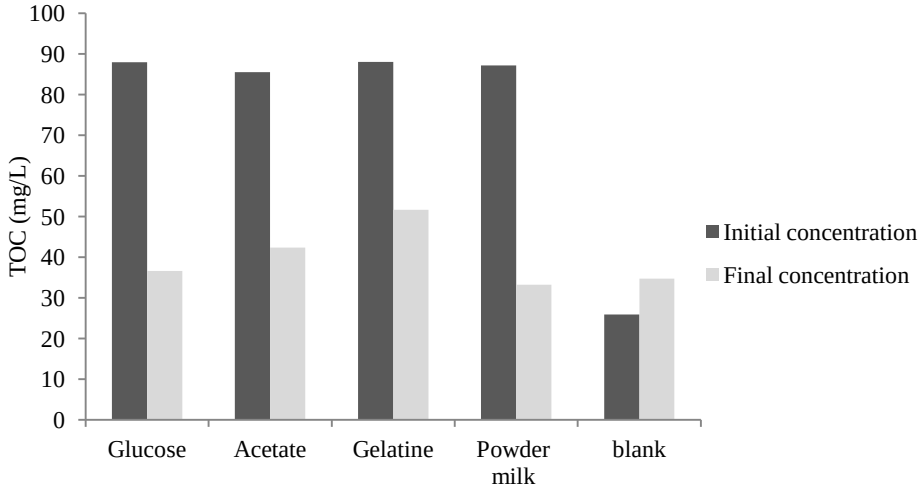

Figure 5.12: Comparison of initial and final TOC concentrations

5.6.3 COMPARISON OF BIOGAS YIELD, TOC REDUCTION AND PERCENTAGE OF BIODEGRADATION

The total biogas production, TOC reduction and percentage of biodegradation can now be compared as shown in Figure 5.13. This shows that for glucose, 42mL/100mg/L TOC of biogas was produced from 51mg/L of TOC and that approximately 51% of the total added TOC was used. For acetate, 30mL/100mg/L TOC of biogas was produced from 43mg/L TOC and 43% of the total added TOC was used. For gelatine, 42mL/100mg/L TOC biogas was produced from 35mg/L TOC and 65% of the total added TOC was used. Powder milk produced 61mL/100mg/L TOC of biogas from 53mg/L TOC and 91% of the total TOC was used. Glucose and acetate showed an expected ratio of biogas produced to TOC reduction to percent biodegradation however gelatine and powder milk showed a different trend. Both gelatine and powder milk have a lower TOC reduction and a very high percentage of biodegradation. This indicates that gas may have been produced from TOC that was not measured.

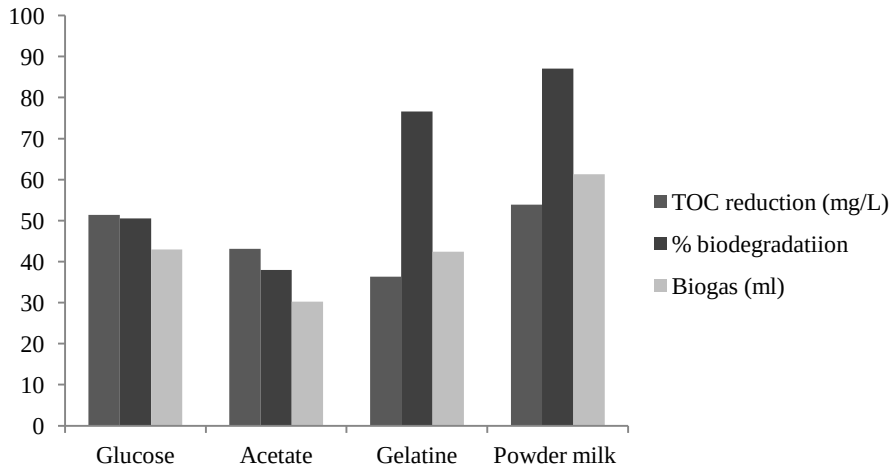

Figure 5.13: Comparison of biogas production, TOC reduction and percentage of biodegradation

5.7 SUMMARY

This Chapter summarises the biogas volume obtained from four different easily biodegradable substrates including; glucose, acetate, gelatine and powder milk. The results indicated that glucose produced 43mL biogas/100mg/L TOC at STP, acetate produced 30mL biogas /100mg/L TOC at STP. The results showed that powder milk

produced the most biogas of 61mL/100mg/L TOC at STP, indicating that good volumes can be produced from the more complex substrates. Gelatine produced 42mL/100mg/L TOC at STP, indicating that biogas can be produced from substrates high in protein. This research has also given an indication of the activity of the anaerobic inoculum showing that it is suitable to use for easily biodegradable substrates.

CHAPTER 6 BIOGAS AND METHANE PRODUCTION FROM ABATTOIR WASTEWATER

This chapter provides the results and discussion on the total biogas yield, CH_4 yield, and biodegradation of abattoir wastewater including, yard water, blood water, and saveall water. Two separate trials were conducted; the first run monitored the biogas and methane production when the substrates containing abattoir waste were mixed with anaerobic media providing necessary nutrients and minerals required for microbial growth. The second run monitored the biogas and CH_4 yield of the substrates when no such anaerobic media was added. This provided a comparison to determine if the addition minerals and nutrients improved the biodegradation and gas production of the abattoir wastewater. All volumes are given at STP unless otherwise stated.

6.1 WASTEWATER CHARACTERISTICS

The single grab samples of the three abattoir wastewaters were characterised as shown in Table 6.1. Yard water and blood water samples had low DTOC content as compared with samples taken on an earlier date. For example, yard water had a DTOC of 225mg/L and on an earlier occasion, this water had a DTOC of 1353mg/L. These earlier results are not included here however this comparison highlights how much the strength and composition of the wastewater can change on different days and times. This reinforces the fact that, where practicable, a composite sample collection would be more appropriate.

Table 6.1 shows the general composition of the three abattoir wastewaters. SS for yard, blood and saveall waters were 1.47, 0.39 and 4.81g/L respectively and this was used to give an indication of how much unmeasured TOC went into the bottles, as TOC was measured using dissolved samples.

Table 6.1: Wastewater characteristics

	Unit	Yard water	Blood water	Saveall water
Description		Manure and urine	Slaughter, paunch	Slaughter, gut pit, paunch liquid, rendering
DTC	mg/L	556.9	315.9	808.5
DIC	mg/L	331.3	23.32	56.875
DTOC	mg/L	225	292	752.5
DTN	mg/L	352	85	4.76
Nitrite	mg/L	0	N/A	0
Nitrate	mg/L	0.14	N/A	0.40
Chloride	mg/L	455	N/A	305
Phosphate	mg/L	161	N/A	233
Sulphate	mg/L	22	N/A	45
TS	g/L	2.57	0.86	7.62
VS	g/L	1.20	0.60	6.54
SS	g/L	1.47	0.39	4.81
VSS	g/L	0.86	0.14	4.80

Yard water contains manure and urine and is initially sieved on site through a 5mm screen. Rico et al (2007) found that when slurry pit water from a cowhouse was screened through 1.5mm sieve cellulose and hemicelluloses were removed however lignin was only minimally removed. Therefore, it was assumed that the SS of yard water mainly contain lignin, cellulose and hemicelluloses. Cellulose and hemicelluloses are biodegradable, however, lignin is difficult to biodegrade under anaerobic conditions. Blood water would be expected to have some protein present as indicated by the dissolved total nitrogen (DTN) content (Palatsi et al. 2011). Saveall water had the highest SS of 4.8g/L and on visual inspection it appeared as a large portion of this was solidified fat. This is consistent with the description of wastewater sources highlighted in section 3.4.

6.2 BIOGAS AND METHANE YIELD OF ABATTOIR WASTEWATER WITH ANAEROBIC MEDIA

This run involved conducting batch assays on yard water, blood water and saveall water. Glucose was used as the control. All wastes were filtered through a 500µm sieve and added to the sample bottles, so that final concentrations in the bottles remained at approximately 100mg/L DTOC. However, the total TOC in the wastewaters could not

be measured as the TOC instrument required samples to be filtered through 0.45 µm paper, therefore, unmeasured TOC went into the assays in the form of SS. The results and implications of unmeasured TOC are discussed further in this section.

6.2.1 MEASURED BIOGAS YIELD

The control assay of glucose was compared to the control assay of glucose used in the previous tests on easily biodegradable substrates (section 5.2). This ensured that similar biodegradation was taking place between all experiments. Figure 6.1 shows that the glucose control assay in this run had a higher maximum biogas yield than the glucose control assay used in tests on easily biodegradable substrates. This, however, was considered acceptable as they followed a similar rate of increase in the linear stage. This difference may be due to storage of the anaerobic sludge.

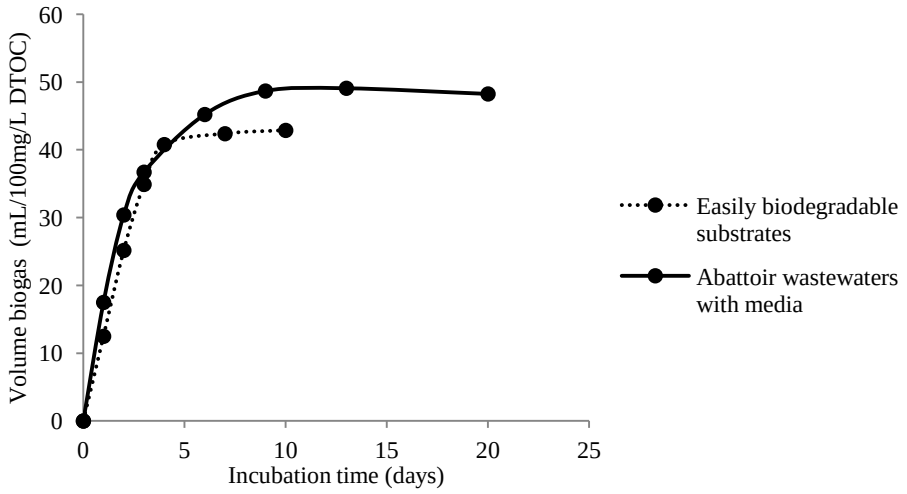

Figure 6.1: Comparison of glucose controls for trials conducted on easily biodegradable substrates and abattoir wastewater with media, at STP

Figure 6.2 shows the cumulated biogas produced at STP by glucose, yard water, blood water and saveall water over an incubation period of 20 days. These results show total biogas produced from approximately 100mg/L DTOC added, after necessary adjustments were made to include the changes in atmospheric pressure. These results show that the blank was producing substantial volumes of biogas therefore the volumes

produced in the blank had to be subtracted from the volumes produced by the other substrates.

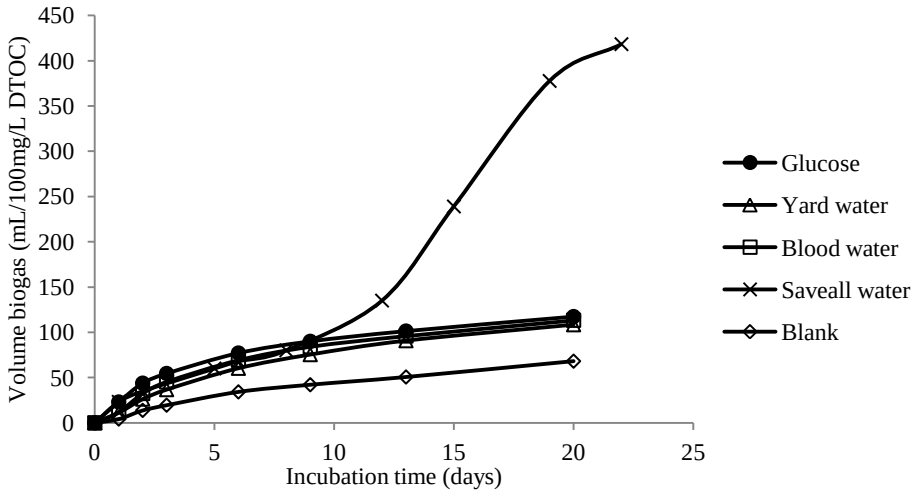

Figure 6.2: Cumulated volume of biogas produced at STP, by glucose, yard water, blood water, saveall water and blank

Figure 6.3 shows the cumulated biogas produced by glucose, yard water, blood water and saveall water once the blank had been deducted. Yard water and saveall water experienced an initial lag phase of two days. After the initial lag period, all waters produced comparable volumes of biogas until day 10, after which the saveall water started producing biogas at a higher rate as shown in Figure 6.3 finally producing 314mL biogas/100mg/ L DTOC. Blood water produced 44mL biogas/100mg/ L DTOC and yard water produced 39mL biogas/100mg / L DTOC.

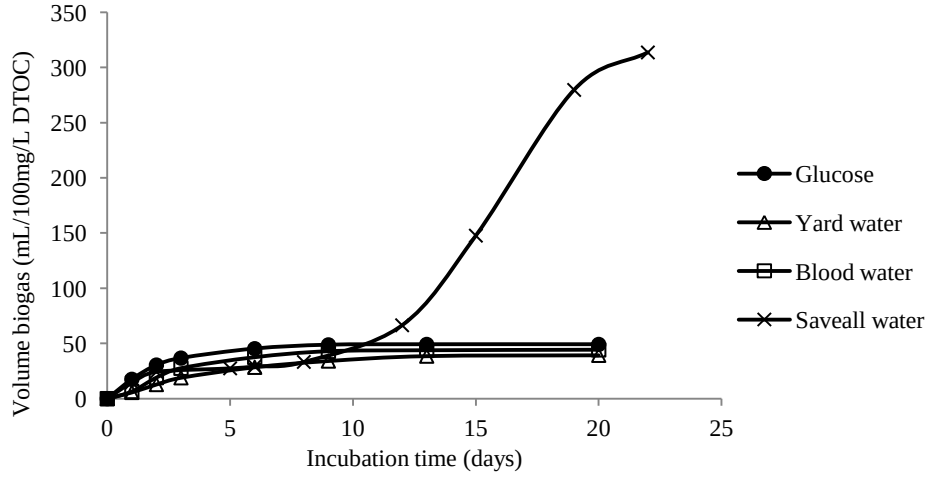

Figure 6.3: Cumulated volume of biogas produced at STP, once biogas produced by the blanks was deducted.

The saveall water exhibited different biodegradation characteristics as compared with the other wastewaters. In the first 10 days, the saveall water showed similar gas production as the yard water and blood water. However, on day 10, biogas production rapidly increased indicating that another food source was present. The first 10 days is indicative of how much biogas was produced from the hydrolysed material. Whilst, after day 10, the fats added as extra solids, that were not measured in the DTOC analysis began to biodegrade. This can be confirmed by examining the bottles at the beginning and end of the trial. Figure 6.4 shows the sample at the beginning of the trial. It can be clearly seen that fats were floating on the surface. No other substrates exhibited these characteristics providing confirmation that there were substantial amounts of fat in the saveall wastewater. Figure 6.5 shows the same bottle at the end of the trial indicating that the fats had been biodegraded.

Figure 6.4: Saveall water: First 10 days showing floating layer on top

Figure 6.5: Saveall water: Final 10 days showing floating layer has been digested

To gain a better comparison of the biogas produced between substrates, the biogas volumes were calculated in terms of volatile solids (VS) added. As mentioned previously in section 6.1, yard water had a VS concentration of 1.2g/L, blood water had a VS concentration of 0.6g/L and saveall water had a VS concentration of 6.54g/L. The total calculated biogas produced for 1g of VS added is shown in Table 6.2. This gives a better representation of the biogas produced as all the solids now are included showing that yard water produced the least biogas per gram of VS of 325mL/gVS at STP. Blood water produced 733mL/gVS at STP and saveall water produced 952mL/gVS at STP.

Table 6.2: Total biogas expressed in terms of g/L VS added at STP

Substrate	VS of substrate (g/L)	Volume substrate added (L)	Total VS added (g)	Total biogas produced (mL/gVS)
Yard water	1.2	0.1	0.12	325
Blood water	0.6	0.1	0.06	733
Saveall water	6.54	0.05	0.33	952

6.2.2 BMP RATE: FIRST-ORDER KINETIC MODEL

The first-order kinetic model was applied to the results to give an indication of the rate of biogas production. B_o was assumed to be the maximum gas yield after 20 days of incubation. Following the same procedure applied in section 5.5, k was estimated by curve fitting with the experimental results using Matlab's curve fitting toolbox. Figure 6.6, Figure 6.7, Figure 6.8 and Figure 6.9 show the measured data with the curve fit for glucose, yard water, blood water and saveall water respectively.

Glucose has a much better fit as compared with glucose tested with easily biodegradable substrates (section 5.5) indicating that this run was more stable. Yard and blood water also have smooth fits, however, the saveall water had to be fitted twice to obtain a fit for the first 10 days and a fit for the following 10 days as shown in Figure 6.9.

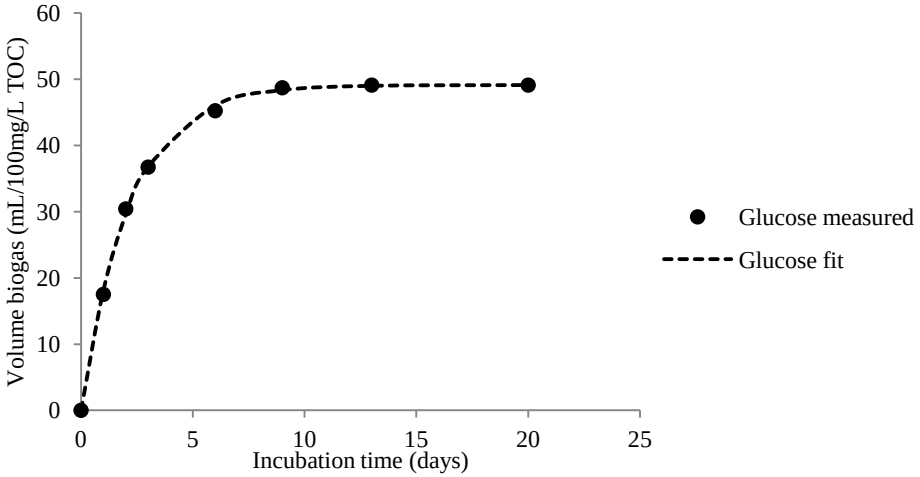

Figure 6.6: Comparison of measured and fitted data for glucose at STP

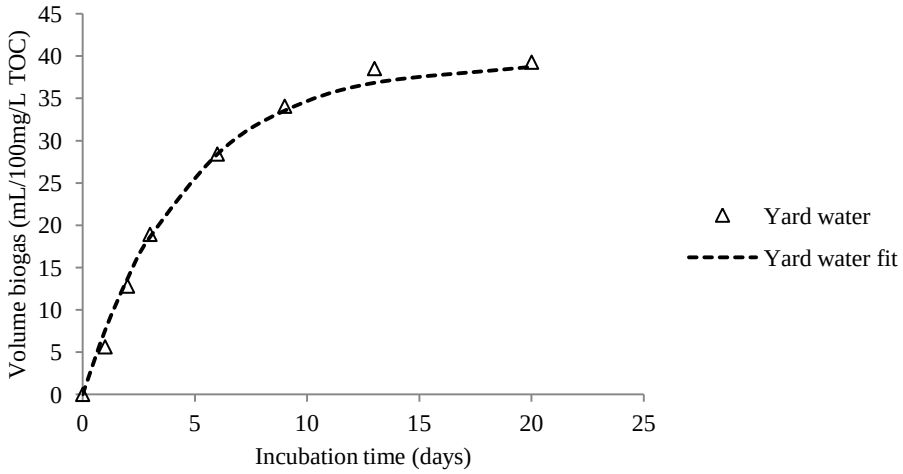

Figure 6.7: Comparison of measured and fitted data for yard water at STP

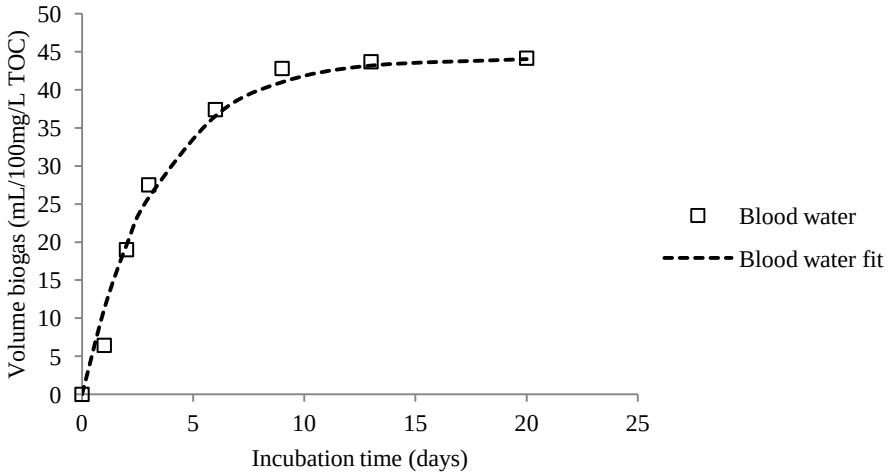

Figure 6.8: Comparison of measured and fitted data for blood water at STP

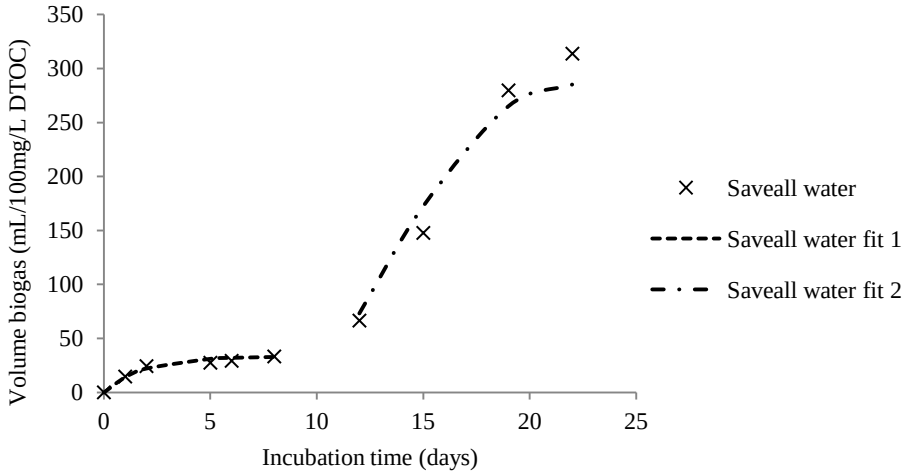

Figure 6.9: Comparison of measured and fitted data for saveall water at STP

The *k* values for glucose, yard water, blood water and saveall water are provided in Table 6.3 below. This shows that glucose biodegraded fairly quickly as did the initial 10 days of the saveall water. Yard water, blood water and the final 10 days of the saveall water biodegraded slower with *k* values between 0.2 and 0.3.

Table 6.3: Maximum ultimate biogas yield, first order rate constant (*k*) and R^2

	B_o (mL biogas)	*k*	R^2
Glucose	49.1	0.46	0.99
Yard water	39.2	0.21	0.99
Blood water	44.1	0.29	0.98
Saveall water fit 1	33.2	0.54	0.96
Saveall water fit 2	313.7	0.27	0.96

6.2.3 MEASURED METHANE CONTENT AND YIELD

The gas composition was determined by gas chromatography by comparing gas samples from the batch assays to a known standard gas. Only CH_4, N_2 and O_2 were detected due to the gas chromatographs column capability and only CH_4 results are discussed here. A sample chromatograph can be found in Appendix B.

Figure 6.10 shows the percentage of CH_4 obtained for each substrate over an incubation period of 20 days. Glucose showed a drop in CH_4 content on day 9 however increased steadily again after that. Glucose had an average CH_4 content of 17% and the biogas had the highest CH_4 content of 22% on day 13. Yard water experienced a drop in CH_4 content similar to glucose on day 9 and had an average methane content of 16% and a maximum of 22% on day 13. Blood water did not experience a drop in CH_4 content and had an average of 17% CH_4 and maximum CH_4 content of of 21% on day 9. The saveall water experienced a drop in CH_4 content on day 6, however, the CH_4 content of the biogas continued to increase steadily until day 19. Saveall water had an average CH_4 content of 24.5% and a maximum CH_4 content of 53% on day 19.

These results were typically lower that that reported in literature. Vedrenne et al. (2008) achieved between 66% and 75% methane in his research on livestock wastes. Alvarez and Liden (2008) reported between 51% to 57% methane in the co-digestion of solid slaughterhouse waste with manure and vegetable waste. Other authors reported similar ranges of between 40 % and 60% methane (Ashekuzzaman & Poulsen 2011), (Chae et al. 2008). The lower methane yields obtained from this research could be caused by many factors, however, without fully analysing the bacterial populations present this is difficult to determine. A possible explanation is the anaerobic bacteria were not fully acclimatised to the substrates. Alternatively, there may have been a low population of methanogenic bacteria in the anaerobic sludge, therefore, less methane would have been produced.

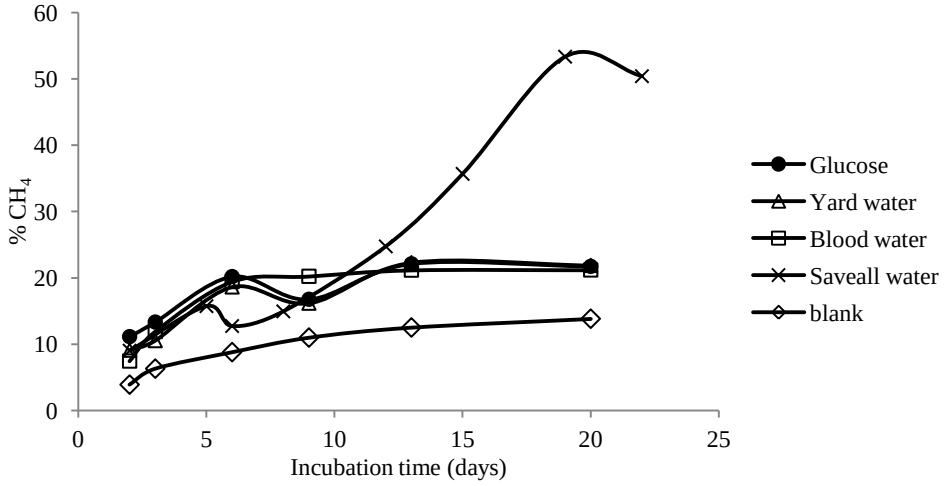

Figure 6.10: Gas composition - percent CH_4 in biogas

Figure 6.10 shows clear drops in CH_4 content on day 6 for saveall water and day 9 for glucose and yard water. This indicates there was some sort of inhibition to the methanogenic bacteria hence the volumes of methane being produced dropped.

Methanogenic bacteria are the most sensitive of the anaerobic bacteria to changes in environmental conditions (Chen et al. 2008). The presence of ammonia, sulphate and pH changes can inhibit the methanogenic bacteria and therefore a lag in methane production is observed until the bacteria acclimatise. Typically, inhibition is caused by the build up of volatile fatty acids (VFA's) produced by the acid forming bacteria however, without measuring the VFA's it is unknown what caused the decline in methane production.

The gas composition was then used to obtain CH_4 volumes as shown in Figure 6.11 below. Glucose, yard water and blood water produced similar total volumes of 7.2mL/100mg/L DTOC at STP, 6.2mL/100mg/L DTOC at STP and 7mL/100mg/L DTOC at STP respectively. Saveall water had the highest CH_4 yield of 110mL/100mg/L DTOC at STP. The higher methane yield of saveall water is mainly due to the contribution of organics from suspended solids present in the added wastewater as discussed in section 6.2.1.

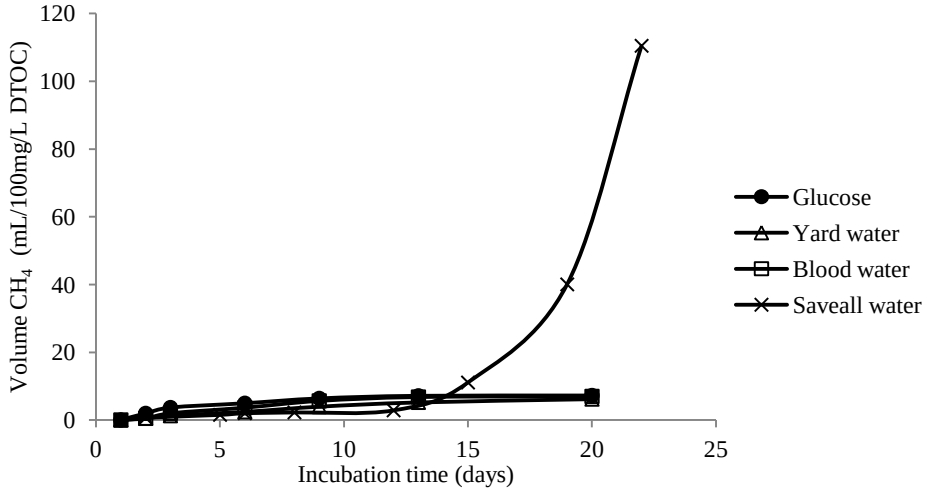

Figure 6.11: Volume of CH_4 produced at STP

To make a better comparison between substrates, the volumes of methane were calculated and expressed in terms of VS as shown in Table 6.4. Yard water produced 52mL CH_4/gVS at STP. Blood water produced 118mL CH_4/gVS at STP and saveall water produced 333mL CH_4/gVS at STP. These results are somewhat comparable to Buendia et al (2009).

Table 6.4: Volumes of CH_4 expressed in terms of VS added, at STP

Substrate	VS of substrate (g/L)	Volume substrate added (L)	Total VS added (g)	Total CH_4 produced (mL/gVS)
Yard water	1.2	0.1	0.12	52
Blood water	0.6	0.1	0.06	117
Saveall water	6.54	0.05	0.33	333

Figure 6.12, Figure 6.13 and Figure 6.14 show the comparison of total biogas and CH_4 volume for yard water, blood water and saveall water respectively. This shows that there is a substantial volume of other gases that could not be measured. This is confirmed by examining the sample gas chromatograph in Appendix B. The gas chromatograph detected high amounts of N_2 and trace amounts of O_2 in all samples. Other gasses that may have been present include CO_2, hydrogen (H) and hydrogen sulphide (H_2S), however, this could not be confirmed due to the columns capabilities.

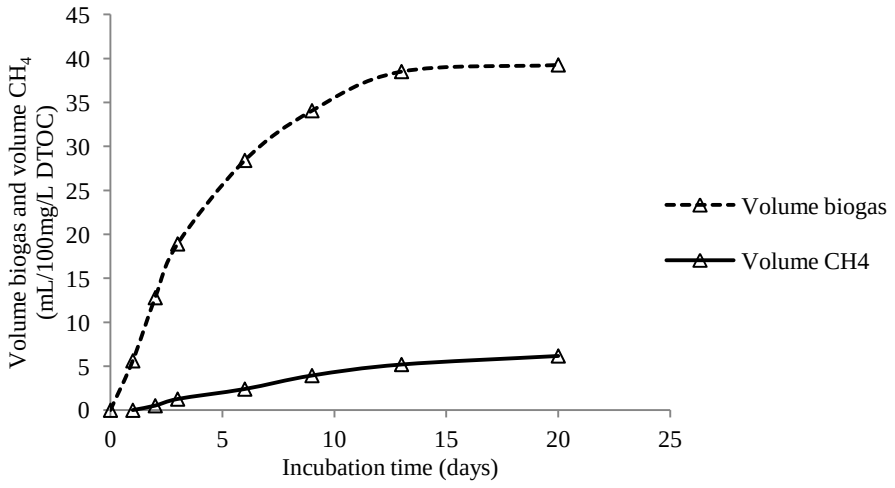

Figure 6.12: Comparison of volume of biogas and volume of methane for yard water at STP

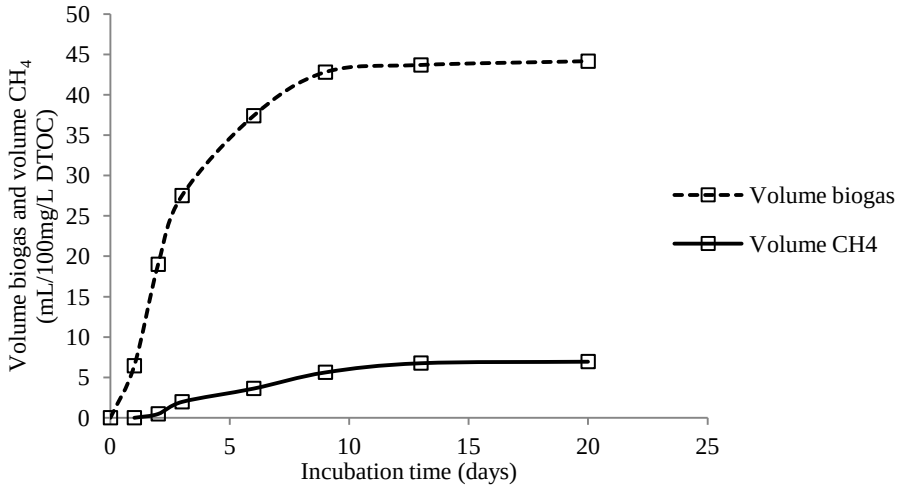

Figure 6.13: Comparison of volume of biogas and volume of CH_4 for blood water at STP

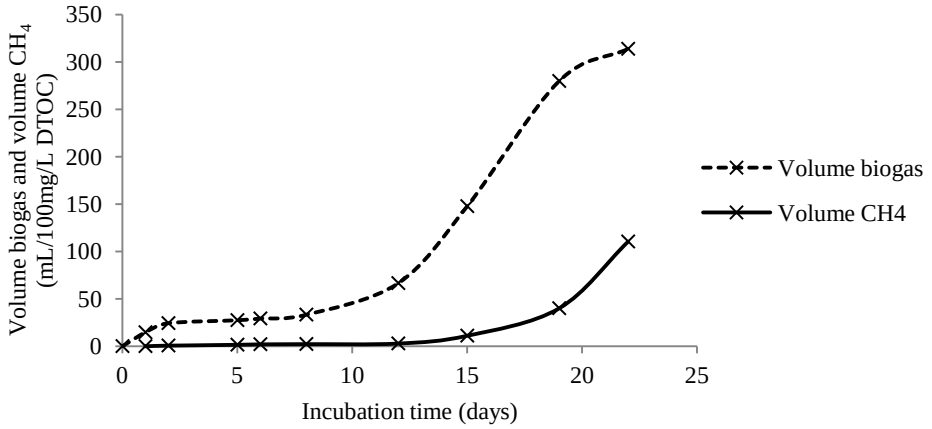

Figure 6.14: Comparison of volume of biogas and volume of methane for saveall water at STP

6.2.4 BIODEGRADATION

The biodegradation was found using standard methods outlined in section 5.6.1, to obtain the percentage biodegradation. Figure 6.15 shows the percent biodegradation for glucose, yard water, blood water and saveall water. Glucose had 64% biodegradation, however, yard water, blood water and saveall water all had over 100% biodegradation. The calculations indicated that yard water had 114%, blood water 129% and saveall water 500%. These calculations are based on the DTOC and DIC measurements, which only measures dissolved organic strength of the waste and therefore a similar problem exists as mentioned in section 6.2 with the addition of samples containing organic suspended solids. These results therefore are not representative of the biodegradation that has taken place.

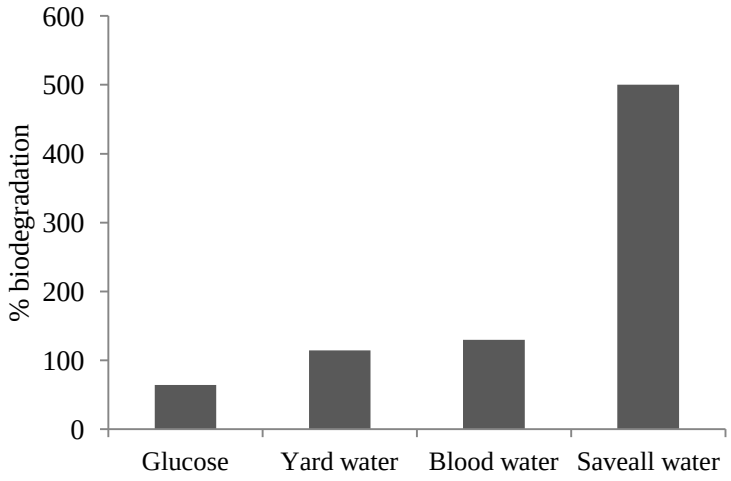

Figure 6.15: Percentage of biodegradation using standard methods

The total organic carbon (TOC) was measured at the beginning and end of the run. This gives another indication of the biodegradation that has taken place. Figure 6.16 shows the initial and final DTOC concentrations of glucose, yard water, blood water, saveall water and blank. Glucose exhibited the expected trend of a substantial reduction in DTOC of around 60%. Blood water also exhibited a slight reduction of 31%. However yard water, saveall water and the blank all exhibited an increase in DTOC. Yard water had an increase of 36% saveall water had an increase of 4% and the blank had an increase of 32%. For the yard and saveall water this can also be attributed to the addition of the extra unmeasured solids.

Yard water contained solids that consisted of difficult and un-biodegradable material such as lignin as outlined in section 6.1. These solids would have been hydrolysed as the bacteria broke down and utilised them for food. Some of this hydrolysed material would have been left, therefore, showing an increase in DTOC at the end of the test. Similar biodegradation would have taken place in the saveall water, however, the solids were predominantly fats that are easier to biodegrade and therefore, saveall water did not show as great an increase in DTOC. The blank showed a similar increase in DTOC, however, as no food was added the increase in TOC was attributed to the death and breakdown of the bacterial cells.

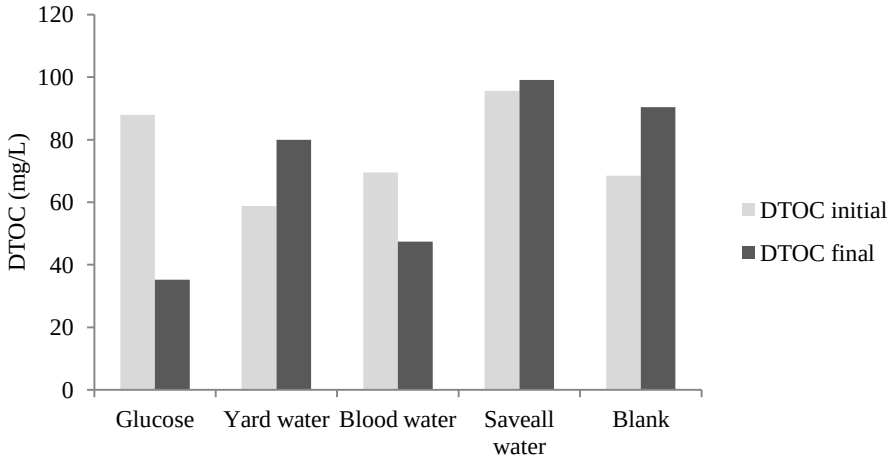

Figure 6.16: Initial and final DTOC content

The total biogas production, DTOC reduction and percentage of biodegradation can now be compared as shown in Figure 6.17. Glucose exhibited a similar pattern as in other trials (section 5.6.3) with 7.2mLCH_4/100mg/L DTOC being produced, with a reduction of 52mg/L DTOC and 64% biodegradation occurred. Yard water, blood water and saveall water all exhibited strange behaviour due to the addition of the extra organic carbon in the form of SS. As mentioned previously the percentage of biodegradation was affected by the additional gas produced by the unmeasured carbon. Similarly, the DTOC observed an increase as these SS were hydrolysed. Therefore, these results are inconclusive indicating that blood water produced 6.9mLCH_4 /100mg DTOC, with a reduction of 22.1mg/L DTOC and 130% biodegradation took place. Yard water produced 6.2mLCH_4/100mg/L DTOC with an increase in DTOC concentration of 21.2mg/L and 114% biodegradation took place. Saveall water produced 110 mL CH_4/100mg/L DTOC with an increase in DTOC concentration of 3.5mg/L and 500% biodegradation took place.

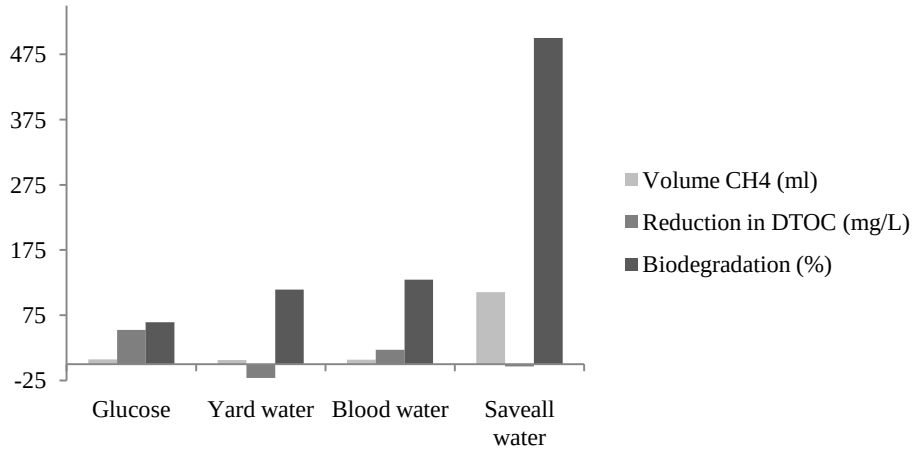

Figure 6.17: Comparison of volume of CH_4 produced, DTOC reduction and percentage of biodegradation, at STP

6.3 BIOGAS AND METHANE YIELD OF ABATTOIR WASTEWATER WITHOUT ANAEROBIC MEDIA

This run was conducted on glucose, yard water, blood water and saveall water without using anaerobic media. This trial was conducted to see if the addition of minerals and trace elements had any effect on the CH_4 production and biodegradation. This was done after it was realised that the C:N:P ratio in the standard (ISO 1995) appeared misleading. Ronquest and Britz (1999) suggested that the approximate optimum ratio of C:N:P is 100:8:1. After analysing the amount of N and P in the anaerobic media used in the standard it was found that the ratio was 0.6:0.9:1. This was a concern, as carbon and nitrogen were considerably lower than the phosphorus concentration and therefore, not providing the nutrients to the inoculum at the required ratio.

6.3.1 MEASURED BIOGAS YIELD

The control assays of glucose were compared to ensure that similar biodegradation was taking place as compared with the glucose control assay of the run conducted on easily biodegradable substrates (section 5.2) and the glucose control for the run conducted on abattoir wastewaters with anaerobic media (section 6.2.1). Figure 6.18 shows that the glucose control, used in the run conducted on abattoir wastewater with the addition of anaerobic media, had a higher maximum biogas yield and smoother biodegradation

curve than all other runs. A different sample of sludge was used in this trial, therefore, this could explain the more erratic biogas production and could be attributed to a different consortium of bacteria being present in the inoculum.

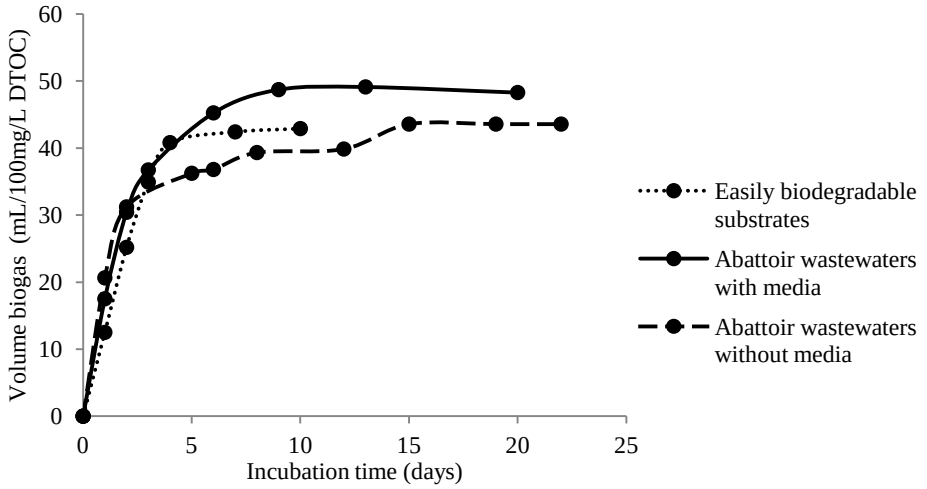

Figure 6.18: Comparison of biogas produced by the glucose control for trials conducted on easily biodegradable substrates, abattoir wastewater with media and abattoir wastewater without media

Figure 6.19 shows the cumulated biogas produced by glucose, yard water, blood water, saveall water and blank over an incubation period of 22 days at STP. These results show total biogas yield for approximately 100mg/L DTOC added, after the changes in atmospheric pressure were deducted. However, to give a better indication of the gas produced by the substrates, the gas produced by the blanks was deducted from the volumes produced by the substrates. Figure 6.20 shows the cumulated biogas produced by the substrates once the blanks have been deducted. The produced gases were 48.6 mL/100mg/L DTOC at STP, 32mL/100mg/L DTOC at STP, 34mL/100mg/L DTOC at STP and 319mL/100mg/L DTOC at STP for glucose, yard water, blood water and saveall water respectively.

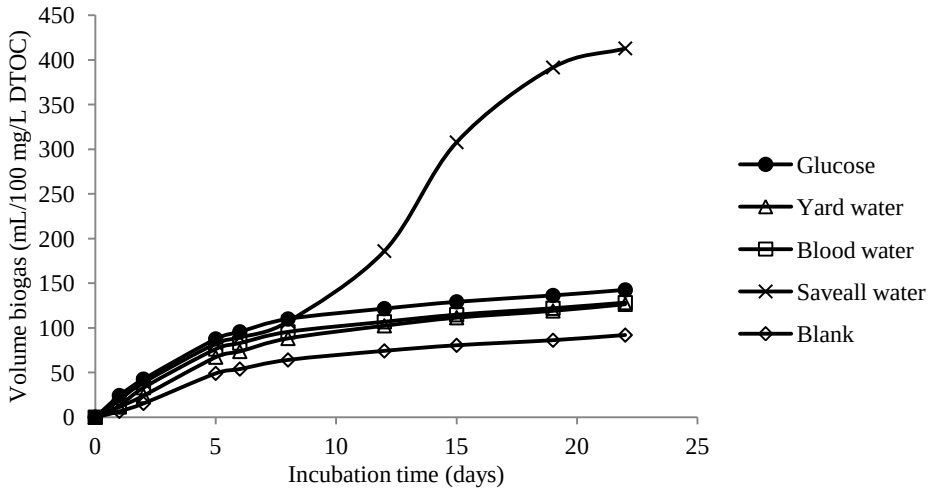

Figure 6.19: Cumulated biogas produced by glucose, yard water, blood water, saveall water and blank with no anaerobic media at STP

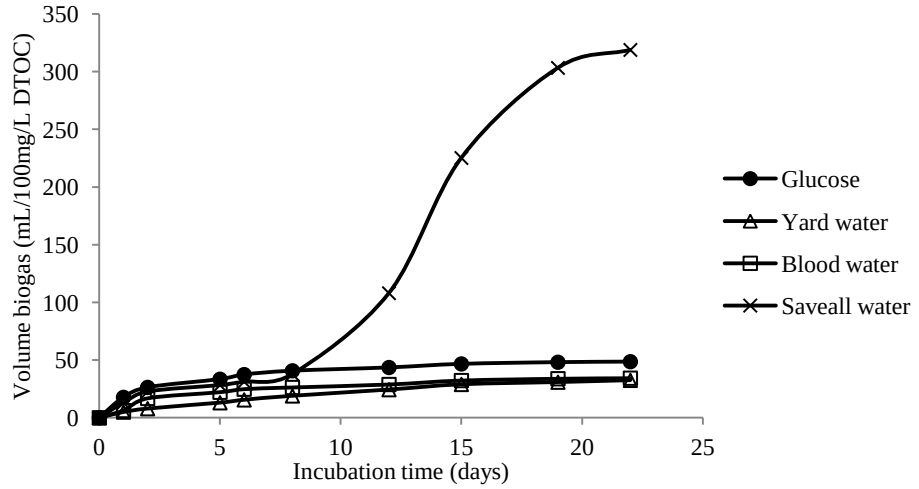

Figure 6.20: Biogas produced once blanks have been deducted

As mentioned previously in section 6.1, yard water had a VS concentration of 1.2g/L, blood water had a VS concentration of 0.6g/L and saveall water had a VS concentration of 6.54g/L. The total calculated biogas produced for 1g of VS added is shown in Table 6.5. This gives a better representation of the biogas produced as all the solids now are included showing that yard water produced the least biogas per gram of VS of 267mL/gVS at STP. Blood water produced 567mL/gVS at STP and saveall water produced 967mL/gVS at STP.

Table 6.5: Volume biogas expressed in terms of VS added, at STP

Substrate	VS of substrate (g/L)	Volume substrate added (L)	Total VS added (g)	Total biogas produced (mL/gVS)
Yard water	1.2	0.1	0.12	267
Blood water	0.6	0.1	0.06	567
Saveall water	6.54	0.05	0.33	967

6.3.2 BMP RATE: FIRST-ORDER KINETIC MODEL

The first-order kinetic model was applied to the results obtained in this trial to give an indication of the rate of biogas production. B_o was assumed to be at maximum after 22 days of incubation. Following the same procedure applied in section 5.5, k was estimated by curve fitting with the measured results using Matlab's curve fitting toolbox. Figure 6.21, Figure 6.22, Figure 6.23 and Figure 6.24 show the measured data with the curve fit for glucose, yard water, blood water and saveall water respectively.

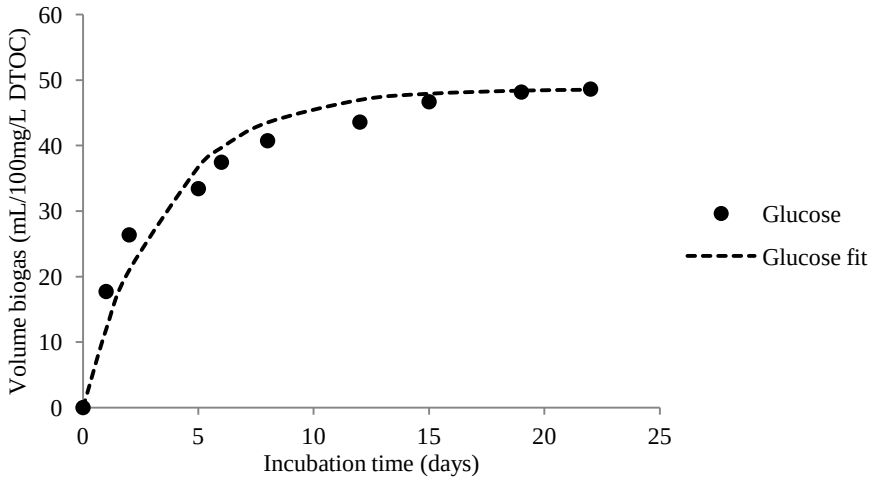

Figure 6.21: Comparison of measured and fitted data for glucose at STP

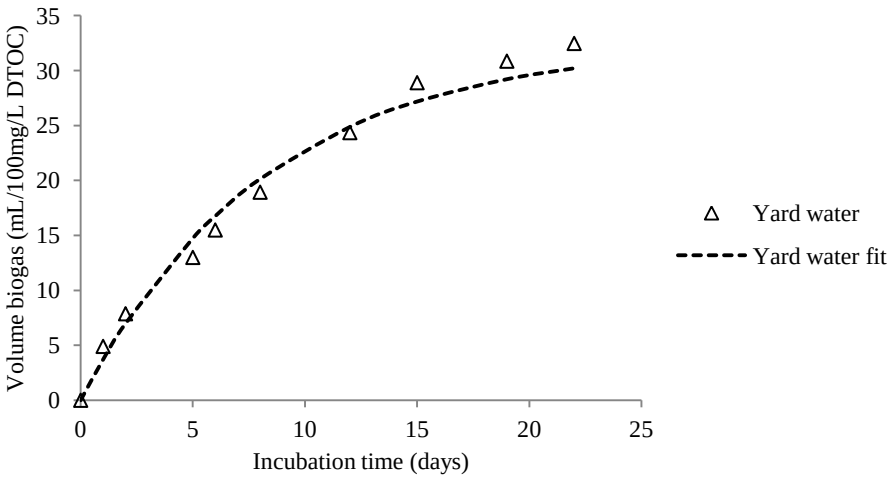

Figure 6.22: Comparison of measured and fitted data for yard water at STP

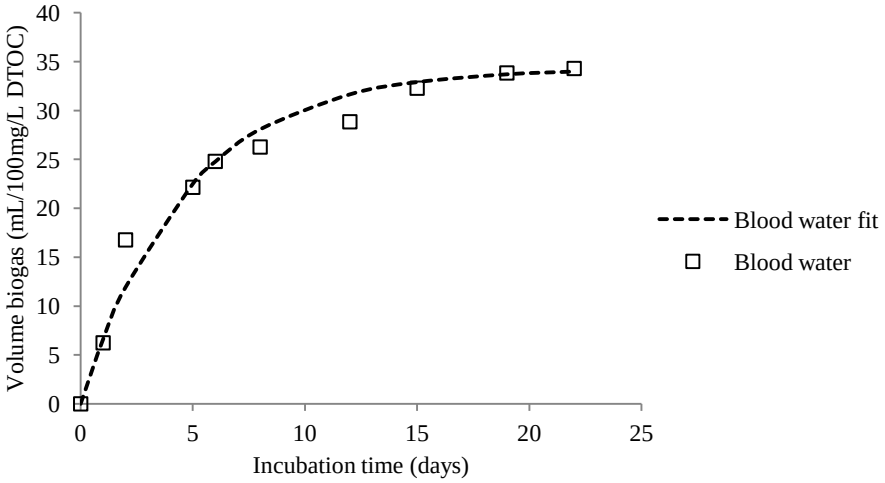

Figure 6.23: Comparison of measured and fitted data for blood water at STP

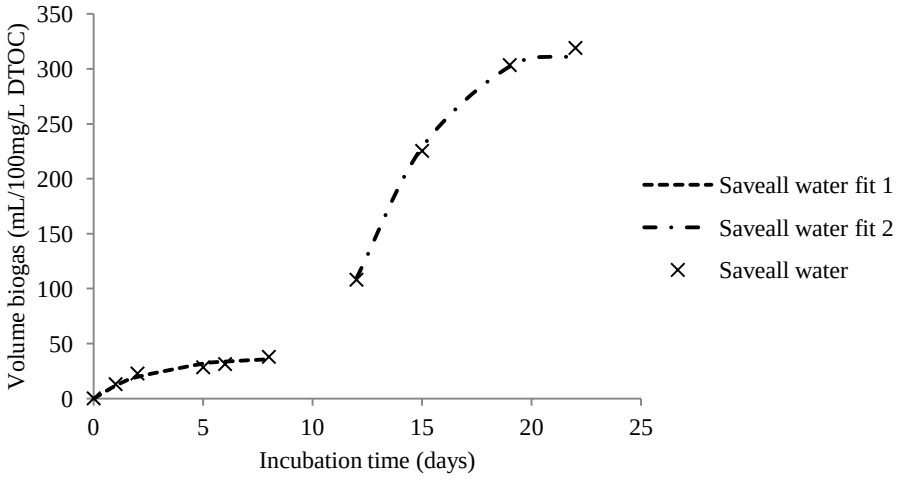

Figure 6.24: Comparison of measured and fitted data for saveall water at STP

The *k* values for glucose, yard water, blood water and saveall water are provided in Table 6.6. This shows that yard water biodegraded the slowest followed by blood water, glucose then the initial 9 days of saveall water followed by the final 14 days of saveall water.

Table 6.6: Maximum ultimate biogas yield, first order rate constant (*k*) and R^2

	B_o (mL biogas)	*k*	R^2
Glucose	48.6	0.28	0.95
Yard water	32.4	0.12	0.98
Blood water	34.3	0.21	0.97
Saveall water fit a	37.8	0.37	0.96
Saveall water fit b	318.9	0.42	0.99

6.3.3 MEASURED METHANE CONTENT AND YIELD

The CH_4 content of the biogas produced by glucose, yard water, blood water and saveall water is shown Figure 6.25. The CH_4 content of all substrates showed distinct inhibition on day 5 with an average drop of about 4%. The CH_4 content began to improve again on day 6 with the CH_4 content of the saveall water continually increasing to 56% on day 19, with a total average CH_4 content of 25%. Glucose had an average CH_4 content of 14% and a maximum of 21% on day 19. Yard water had an average CH_4 content of 14% and a maximum of 20% on day 19. Similarly, blood water had an average CH_4 content of 14% and a maximum of 21% on day 19. Glucose and blood water showed another slight inhibition on day 15.

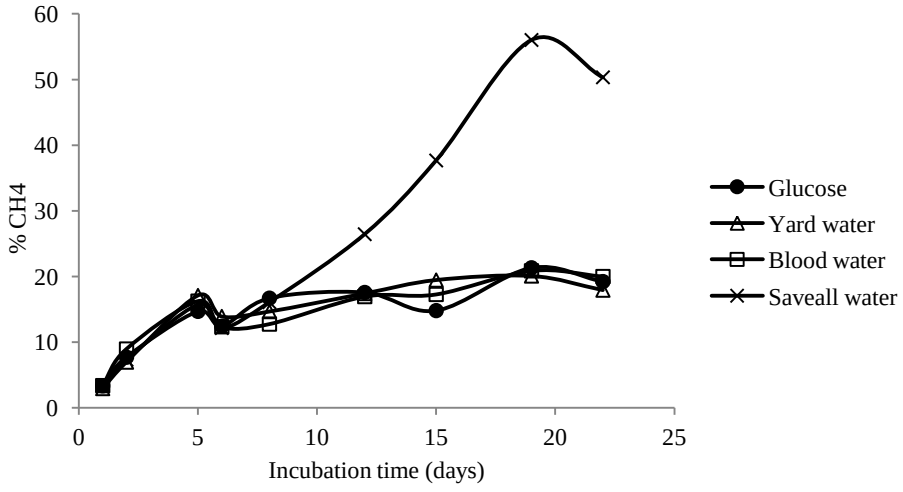

Figure 6.25: Gas composition – percent CH_4 in biogas

The gas composition was then converted to CH_4 volumes as shown in Figure 6.26. Glucose, yard water and blood water produced similar total volumes of 4.7mL/100mg/L DTOC at STP, 4.6mL/100mg/L DTOC at STP and 4mL/100mg/L DTOC at STP respectively. Saveall water had the highest CH_4 yield of 118mL/100mg/L DTOC at STP.

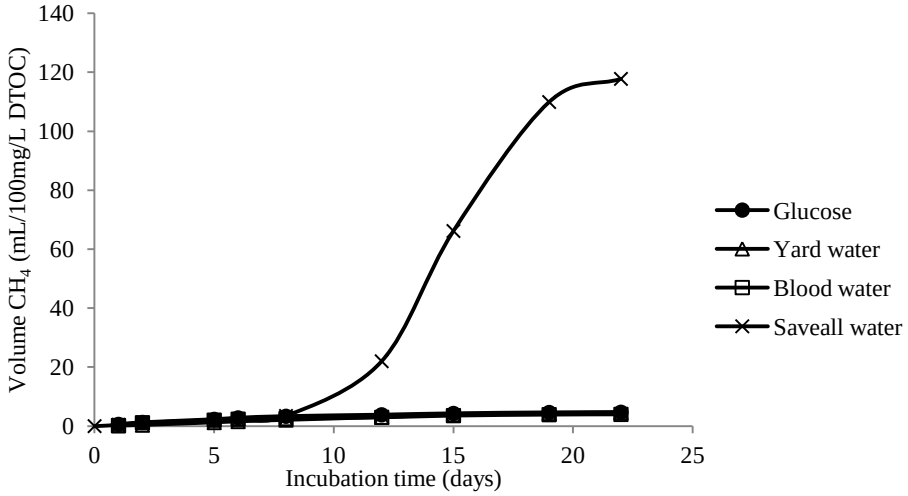

Figure 6.26: Volume of CH_4 produced with no anaerobic media at STP

Expressing in terms of VS as shown in Table 6.7 indicates that yard water produced the least CH4 followed by blood water and saveall water. Yard water produced 38mL CH_4/gVS at STP. Blood water produced 67mL CH_4/gVS at STP and saveall water produced 358mL CH_4/gVS at STP.

Table 6.7: Volume CH_4 expressed in terms of VS added, at STP

Substrate	VS of substrate (g/L)	Volume substrate added (L)	Total VS added (g)	TotalCH_4 produced (mL/gVS)
Yard water	1.2	0.1	0.12	38
Blood water	0.6	0.1	0.06	67
Saveall water	6.54	0.05	0.33	358

6.3.4 BIODEGRADATION

The biodegradation was found using standard methods outlined in section 5.6.1, to obtain the percentage of biodegradation. Figure 6.27 shows the percentage of

biodegradation for glucose, yard water, blood water and saveall water. Glucose achieved 68% biodegradation and yard water achieved 71% however, blood water and saveall water had over 100% biodegradation with the calculations indicating that, blood water had 129% biodegradation and saveall water had 453% biodegradation. These calculations are based on the DTOC and DIC measurements therefore a similar problem exists as previously mentioned, with the inclusion of unmeasured solids. Therefore, these results are not representative of the biodegradation that has taken place.

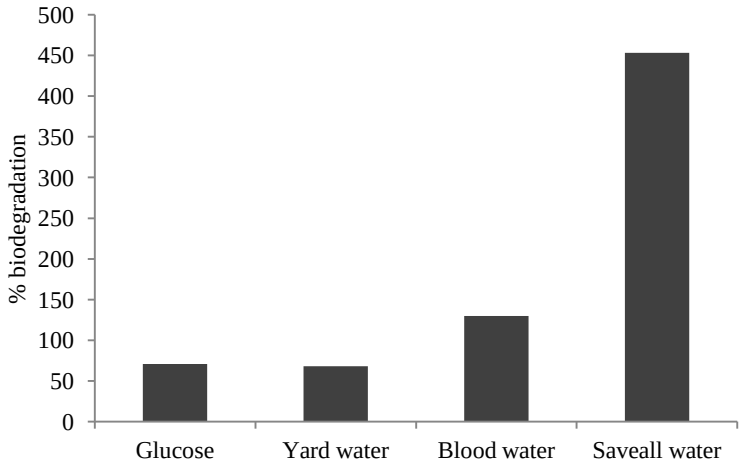

Figure 6.27: Percentage of biodegradation using standard methods

Figure 6.28 shows the initial and final DTOC concentrations of glucose, yard water, blood water, saveall water and blank with no anaerobic media. Glucose exhibited the expected trend with some reduction in DTOC of around 35%. Yard water also exhibited a slight reduction of 2%. Blood water, saveall water and the blank all exhibited an increase in DTOC. For the blood and saveall water this can also be attributed to the addition of the extra unmeasured solids. The blank showed a similar increase in DTOC, however, as no food was added this was attributed to the death and breakdown of the bacterial cells.

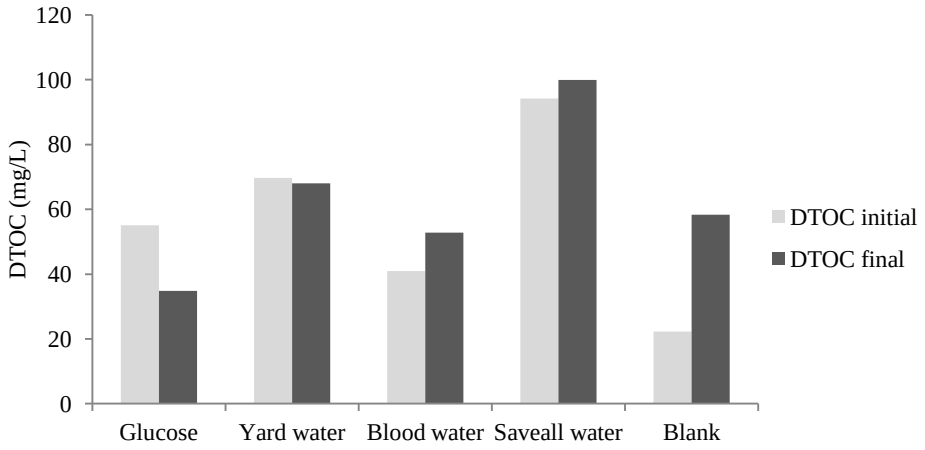

Figure 6.28: Comparison of initial and final DTOC concentrations

The total biogas production, DTOC reduction and percentage of biodegradation can now be compared as shown in Figure 6.29. Glucose exhibited a similar pattern as in other trials (section 5.6.3), with 4.7mL CH_4/100mg/L DTOC being produced with a reduction in TOC of 20.6mg/L and 70% biodegradation occurred. Yard water, blood water and saveall water all exhibited strange behaviour due to the addition of the extra carbon in the form of SS. As mentioned previously, the percentage of biodegradation was affected by the additional gas produced by the unmeasured carbon. Similarly, the DTOC observed an increase as these SS were hydrolysed. Therefore, these results are inconclusive indicating that blood water produced 4mLCH_4/100mg/L DTOC with an increase in DTOC of 11.8mg/L and 130% biodegradation took place. Yard water produced 4.6mLCH_4/100mg/L DTOC with a decrease in DTOC of 1.7mg/L and 68% biodegradation took place. Saveall water produced 118mLCH_4/100mg/L DTOC with an increase in DTOC of 5.8mg/L and 453% biodegradation too place.

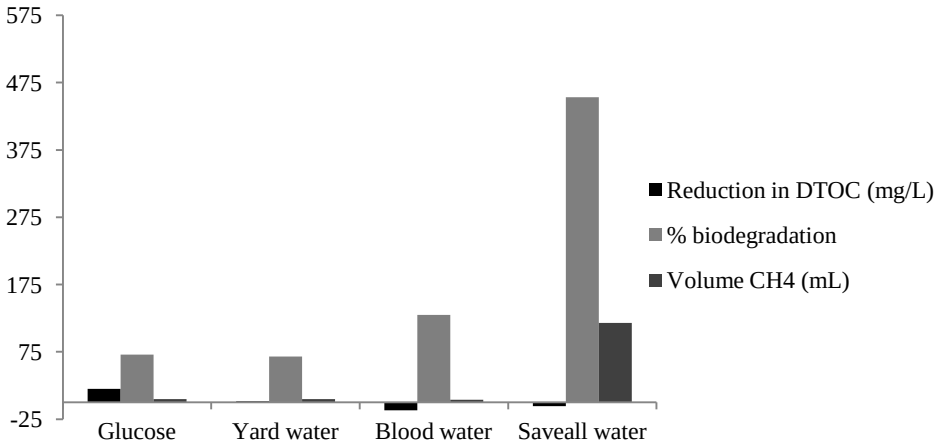

Figure 6.29: Comparison of the volume of CH_4 produced, DTOC reduction and percentage of biodegradation

6.4 EFFECTS OF ANAEROBIC MEDIA ADDITION

6.4.1 RATIO OF CARBON:NITROGEN

Comparing the DTOC and DTN at the start of the trials for abattoir wastewaters with and without anaerobic media, as shown in Figure 6.30 and Figure 6.31, indicates that there was a poor ratio of C:N. Table 6.8 shows the ratios of C:N for each of the wastewaters. When anaerobic media was added, yard water had a very high concentration of N as compared to C with a ratio of 10:24. This was concerning as high concentrations of N can cause inhibition to the methanogenic bacteria. The ratio of C:N for yard water was improved when no anaerobic media was added however was still considered outside the optimal range. All other substrates showed improved ratios of C:N when no anaerobic media was added.

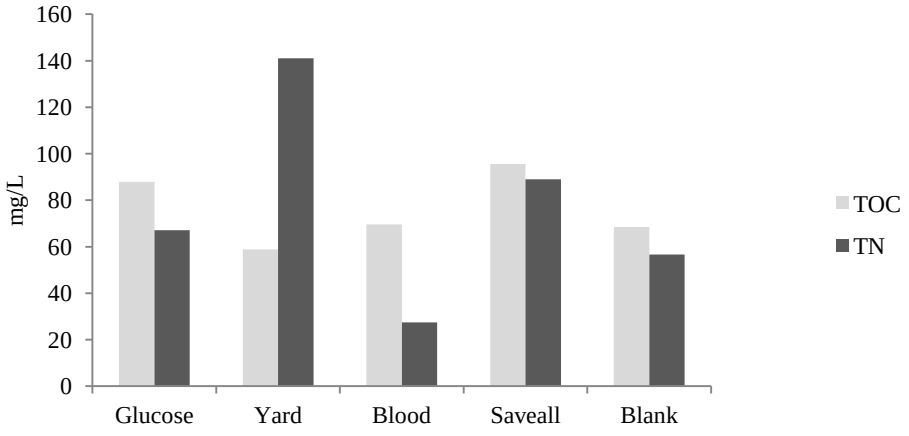

Figure 6.30: Comparison of DTOC and DTN with anaerobic media

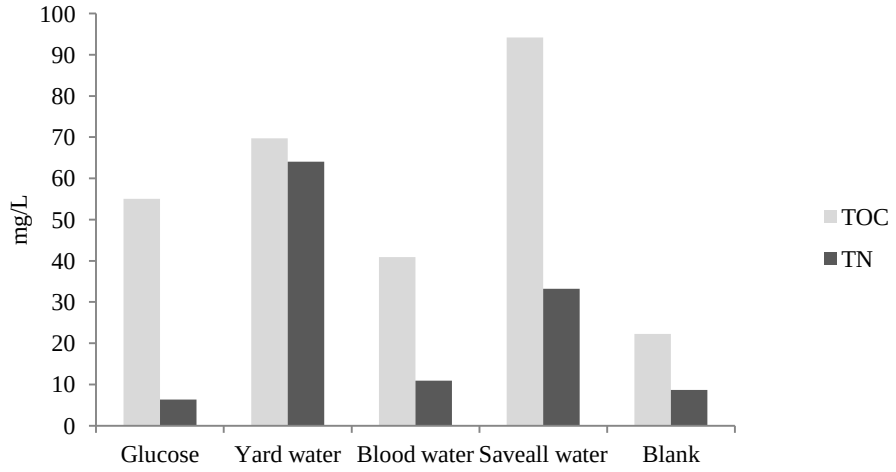

Figure 6.31: Comparison of DTOC and DTN with no anaerobic media

Table 6.8: C:N ratio of assays with media and without media

	C: N with media	**C:N with no media**
Glucose	10:8	10:1
Yard water	10:24	10:9
Blood water	10:4	10:3
Saveall water	10:9	10:4
Blank	10:8	10:4

6.4.2 COMPARISON OF BIOGAS PRODUCTION AND *k*

Results from trials with anaerobic media and trials without anaerobic media, can be compared to assess if the addition of anaerobic media was beneficial to the rate of biogas production and the ultimate biogas production. Table 6.9 shows the volumes of biogas produced for each substrate with and without media. As seen in the table glucose produced the same volume of 49mL biogas/100mg/L DTOC both with the inclusion of media and without media. Yard water produced slightly more biogas with media of 39mL/100mg/L DTOC and 32mL/100mg/L DTOC without media. Blood water produced more biogas with media of 44mL/100mg/L DTOC and 32mL/100mg/L DTOC without media. Saveall water was different and produced more biogas without media totalling 319mL/100mg/L DTOC and produced slightly less with media totalling 313mL/100mg/L DTOC

Table 6.9: Comparison of biogas production for substrates with media and without media at STP

	Biogas – with media (mL/100mg/L DTOC)	***Biogas – without media (mL/100mg/L DTOC)***
Glucose	49	49
Yard water	39	32
Blood water	44	34
Saveall water	313	319

Comparing *k* values gives an indication of the rate of biogas production. Table 6.10 shows that glucose had a higher *k* value of 0.46 with media as compared with 0.28 without media. This indicates that the rate of biogas production was faster with the addition of media even though the same volume was produced. Similarly, yard water had a higher *k* value of 0.21 with media and 0.12 without media. Blood water did not have as great a difference with a *k* value of 0.29 with media and 0.21 without media. As discussed in section 6.2.2, saveall water had to be split into and initial fit and final fit to compensate for the gas that was produced in the first 10 days and the final 10 days. In the first 10 days saveall water with media produced gas faster, with a *k* value of 0.54 as compared with no media that had a *k* value of 0.37. In the second period of gas production, the saveall water with no media produced gas faster with a k value of 0.42 as compared to saveall water with media that had a *k* value of 0.27. This indicates that acclimatisation of bacteria could produce a larger volume of biogas at a faster rate.

Table 6.10: Comparison of first order rate constant (*k*) for substrates with media and without media

	k – tests with media	***k – tests without media***
Glucose	0.46	0.28
Yard water	0.21	0.12
Blood water	0.29	0.21
Saveall water fit a	0.54	0.37
Saveall water fit b	0.27	0.42

6.4.3 COMPARISON OF CUMULATIVE METHANE PRODUCTION

Figure 6.32, Figure 6.33, Figure 6.34 and Figure 6.35 show the comparison of each substrate for assays with media and without media. Glucose, yard water and blood water all produced slightly more CH_4 with the addition of anaerobic media and saveall water produced slightly more CH_4 with no anaerobic media added. These results indicate that the addition of anaerobic media has only minimal effects on the CH_4 production from these substrates. The utilisation of media containing minerals and vitamins becomes essential to investigate the biodegradation of substrates containing a sole carbon source such as glucose, however, the waste from abattoirs might already have the necessary trace minerals and vitamins needed for microbial growth thus eliminating the need for the artificial addition of media.

It was expected that glucose would produce more gas with the addition of media as glucose is only a simple food with no nutrients or trace elements. The addition of the extra N did not seem to inhibit the CH_4 produced by the yard water. Instead, the media improved gas production indicating that yard water and blood water may be lacking in some essential nutrients that the methanogenic bacteria require.

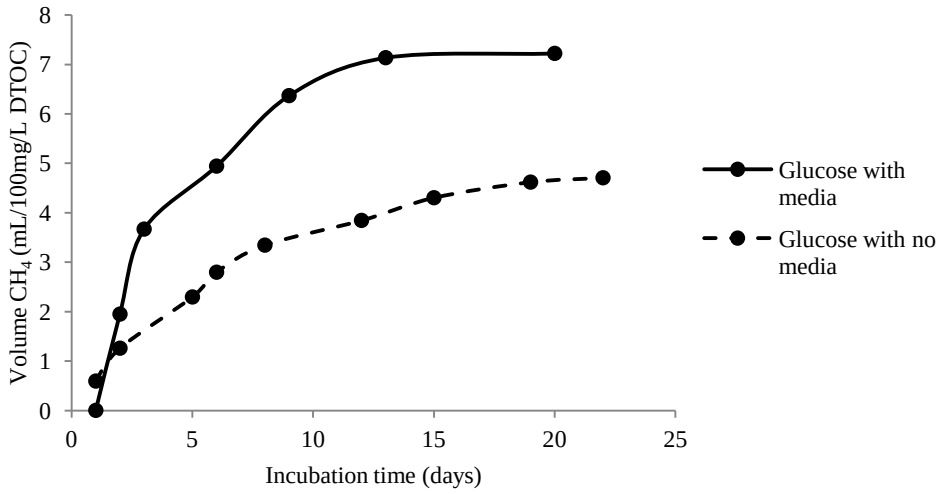

Figure 6.32: Comparison of volume CH_4 for glucose, with and without media at STP

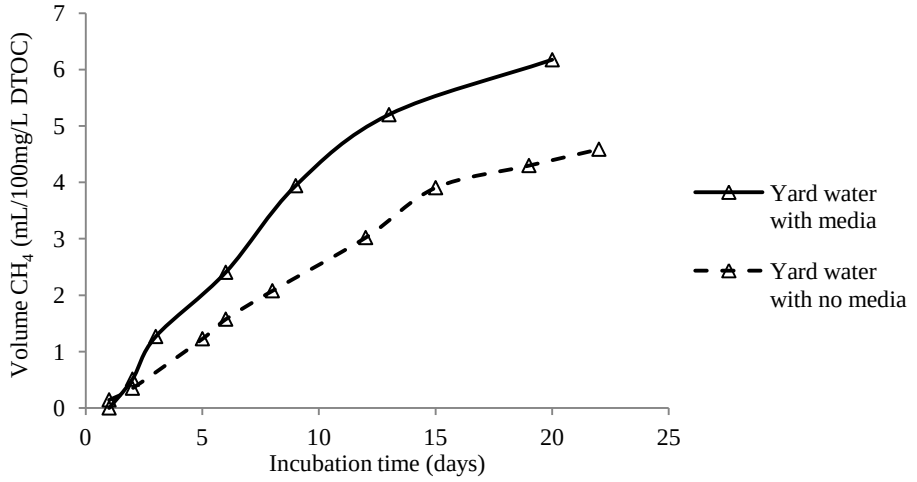

Figure 6.33: Comparison of volume CH_4 for yard water, with and without media at STP

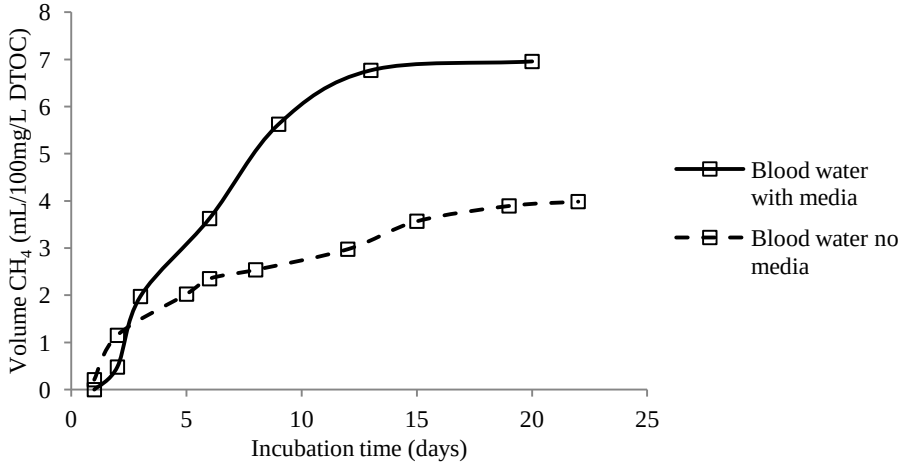

Figure 6.34: Comparison of volume CH_4 for blood water, with and without media at STP

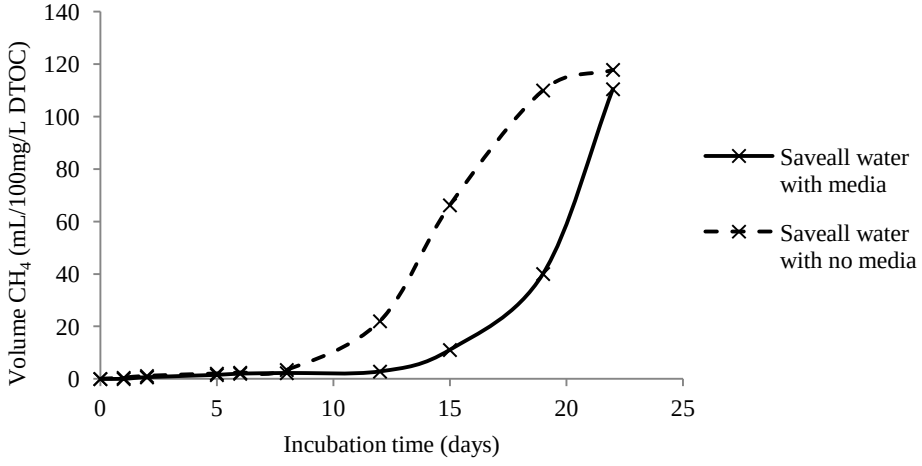

Figure 6.35: Comparison of volume CH_4 for saveall water, with and without media at STP

6.5 SUMMARY

This Chapter reported and discussed volumes of biogas and CH_4 obtained from three different streams of abattoir wastewater. This research has given insight into the volumes of CH_4 produced during the anaerobic digestion of yard water, blood water and saveall water using standard batch assay procedures. Yard water produced the least CH_4 with a total of 39.2mL/100mg/L DTOC added. Blood water produced 44.1mLCH_4/100mg/L DTOC and saveall water produced 313.7mL CH_4/100mg/L DTOC. Slightly better volumes were achieved using anaerobic media indicating that anaerobic media has the potential to improve CH_4 yields if the wastewaters are low in minerals and trace elements.

CHAPTER 7 BIOGAS AND METHANE PRODUCTION USING A BIOREACTOR

This chapter provides the results and discussion on the biogas yield and CH_4 yield for powder milk, blood water and mixed abattoir wastewater under semi-continuously fed conditions in an anaerobic bioreactor. The daily variation of DTOC was monitored in addition to the pH, that was continuously monitored using the real-time analysis software provided with the reactor. SS and VSS were also recorded before and after the addition of a new substrate.

The digester was fed weekly, in the first week it was run with only distilled water and anaerobic media to monitor the endogenous gas production of the anaerobic bacteria. The digester was then fed again following the procedures outlined in Table 7.1. The gas production and change in DTOC and DIC were monitored daily and the change in pH was continuously monitored.

Table 7.1: Bioreactor weekly feeding procedure

Week	Procedure
1	Distilled water and anaerobic media were added to the anaerobic bacteria to a working volume of 4L
2	The solution was allowed to settle and 2L of the supernatant was and replaced with 2L of powder milk solution at a concentration of 500mg/L DTOC
3	On week 3 the digester was again allowed to settle and 2L of the supernatant was removed. The digester was fed with 2L of blood water with a concentration 232 mg/L DTOC
4	On week 4 the same process was followed removing the supernatant and replacing with 2L of mixed abattoir wastewater with a DTOC of 340 mg/L

7.1 SUBSTRATE CHARACTERISTICS

The required concentration of powder milk was determined using the methods described in section 4.5.4. The combined abattoir wastewater was prepared as an equal parts mix of the yard water, blood water and saveall water. The characteristics of the blood water and mixed water are provided in Table 7.2. The substrates were added on a volume basis (2L), therefore, the wastes were diluted when they were added to the remaining 2L of sludge in the digester.

Table 7.2: Wastewater characteristics

	Blood water	**Combined abattoir wastewaters**
DTOC	232mg/L	340mg/L
IC	22.7mg/L	115mg/L
TN	63mg/L	196mg/L
TS	0.86g/L	4.21g/L
VS	0.60g/L	2.81g/L
SS	0.39g/L	2.13g/L
VSS	0.14g/L	1.17g/L
Phosphate	N/A	88.7mg/L
Sulphate	N/A	21.2mg/L
Chloride	N/A	162.0mg/L

7.2 MEASURED BIOGAS PRODUCTION

Biogas was collected in a Tedlar bag where it was removed daily by a syringe. Biogas began to be produced rapidly after the addition of each substrate then began to plateau after about 6 days. Figure 7.1 shows the cumulated biogas produced over the incubation period of 32 days. The graph has a step like shape indicating the production and plateau phase of each feed. The initial small step is the background gas produced by the sludge. The second step beginning at day 7 is powder milk, the third step beginning at day 14 is the blood water and the final step is the mixed water.

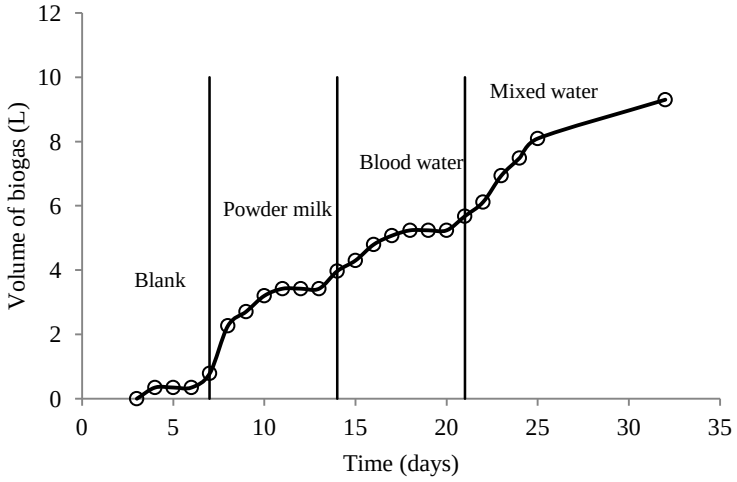

Figure 7.1: Cumulated volume of biogas at STP

The volumes of gas obtained for each substrate could not be easily compared as they were added on a volume basis and had differing DTOC contents. Therefore, they were converted to give a volume for 100mg/L DTOC added and g VS added as shown in Table 7.3. This shows that blood water produced about 0.37 L biogas/100mg/L DTOC. This is substantially more than the gas produced in earlier batch tests (section 6.2.1) where only 0.044L/100mg/L DTOC biogas was produced.

Table 7.3: Adjusted volumes of biogas at STP

Substrate	Total volume biogas (L)	Volume biogas (L/100mg/L DTOC)	Volume biogas (L/gVS)
Powder milk	3.18	N/A	N/A
Blood water	1.71	0.37	1.43
Mixed abattoir wastewater	3.63	0.53	0.64

7.3 MEASURED METHANE PRODUCTION

The CH_4 content of the biogas was typically higher than that achieved in the batch assay experiments (section 6.2.3and 6.3.3). The CH_4 content of the biogas produced by powder milk ranged between 17% and 39%. The CH_4 content of the biogas produced by blood water ranged between 9% and 33% and the CH_4 content of the mixed water

ranged between 27% and 47%. These percentages are lower than those achieved by Hejnfelt and Angelidaki (2009). Figure 7.2 shows the cumulated CH_4 production over the 32 day period. It exhibits a similar step pattern to Figure 7.1.

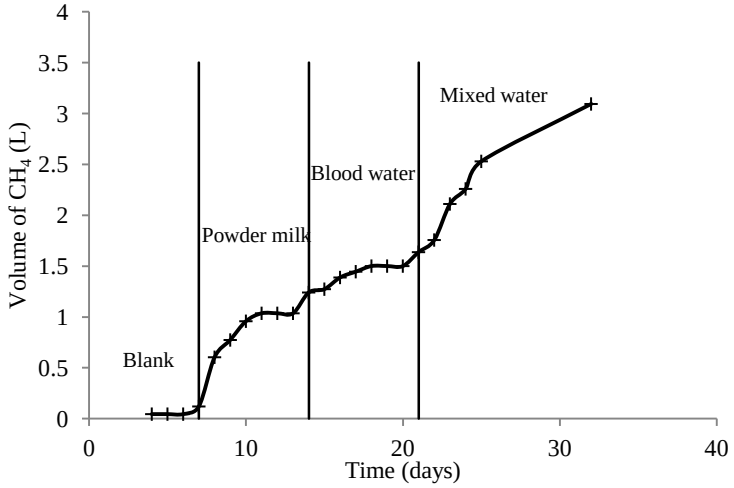

Figure 7.2: Cumulated volume of CH_4 at STP

To make a reasonable comparison between the substrates, the volumes of CH_4 had to converted to a standard measure. Table 7.4 shows the adjusted volumes.

Table 7.4: Adjusted volumes of CH_4 at STP

Substrate	**Total volume CH_4 (L)**	**Volume CH_4 (L/100mg/L DTOC)**	**Volume CH_4 (L/gVS)**
Powder milk	1.12	N/A	N/A
Blood water	0.4	0.09	0.33
Mixed abattoir wastewater	2.21	0.33	0.39

Blood water produced 0.33L CH_4/g VS added and the mixed waste produced 0.39L CH_4/g VS added as indicated in Table 7.4. These volumes are in the range achieved by Buendia et al (2009) who reported between 0.1 to 0.5L CH_4/g VS using cow manure, paunch waste and pig and cow waste slurries. Alvarez and Liden (2008) reported similar values ranging between 0.14 to 0.34L CH_4/g VS added for the co-digestion of slaughterhouse waste and manure with fruit and vegetable waste. However, Edström et

al. (2003) reported volumes ranging between 0.7 to 0.86L CH_4/g VS added for slaughterhouse wastes. This indicates that greater volumes of gas could be achievable.

7.4 pH

Monitoring the pH gives an indication of inhibition that may occur due to extremes in pH change. Table 7.3 shows the change in pH over 26 days. On day 7 after the addition of the powder milk there was a sharp drop in pH indicating that bacterial reactions were taking place. The pH increased and stabilised until the addition of the blood water on day 14. There was also a small drop in pH following the addition of the blood water, however, there was an initial lag phase of one day before the pH began to drop. The pH increased again with the addition of the mixed water on day 21 followed by another drop.

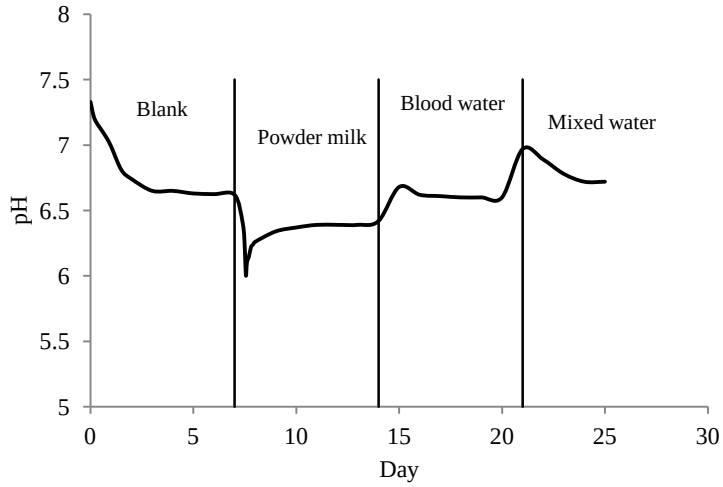

Figure 7.3: Change in pH

7.5 CHANGE IN DISSOLVED TOTAL ORGANIC CARBON

The dissolved total organic carbon (DTOC) was monitored daily to give an indication of the biodegradation that was occurring. Figure 7.4 shows the daily DTOC over the test period. The increase in DTOC on day 7 was due to the first feed of powder milk. The second feed occurred on day 14, however, no increase in DTOC was observed due to the low DTOC concentration of the blood water. The third feed occurred on day 21 and another sharp increase in DTOC can be seen on this day.

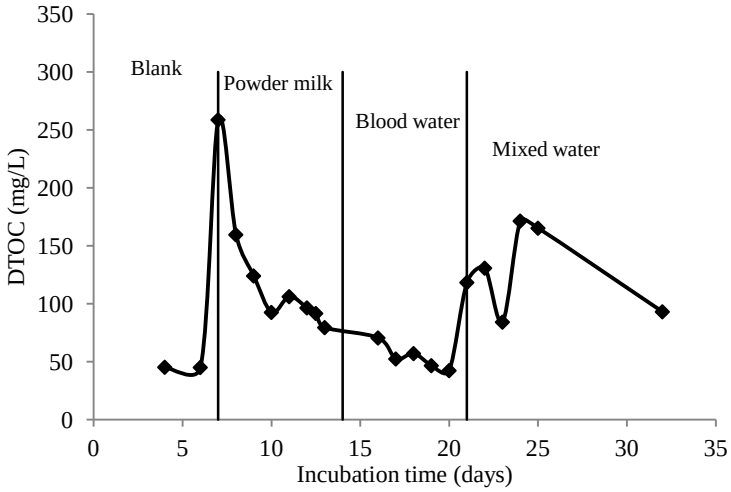

Figure 7.4: Daily DTOC for the incubation period of 32 days

Figure 7.5, Figure 7.6 and Figure 7.7 show the change in DTOC for each substrate over the one week period before the next feed. All substrates exhibited and initial decline in DTOC. All substrates also exhibited an increase in DTOC after a few days. For powder milk this occurred after 4 days. For blood water this occurred after 6 days and for the mixed water after 3 days. The mixed water had the highest increase in DTOC and this is an indication that the solids and fats were beginning to be broken down and hydrolysed by the bacteria, therefore, were able to be measured.

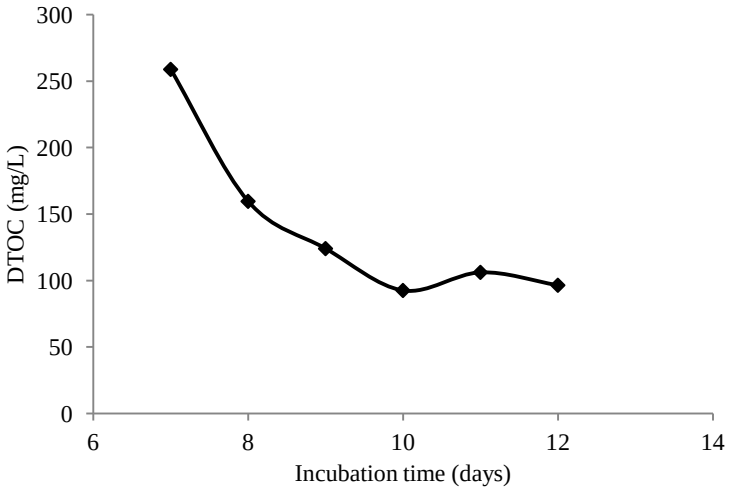

Figure 7.5: Change in DTOC for feed one - powder milk

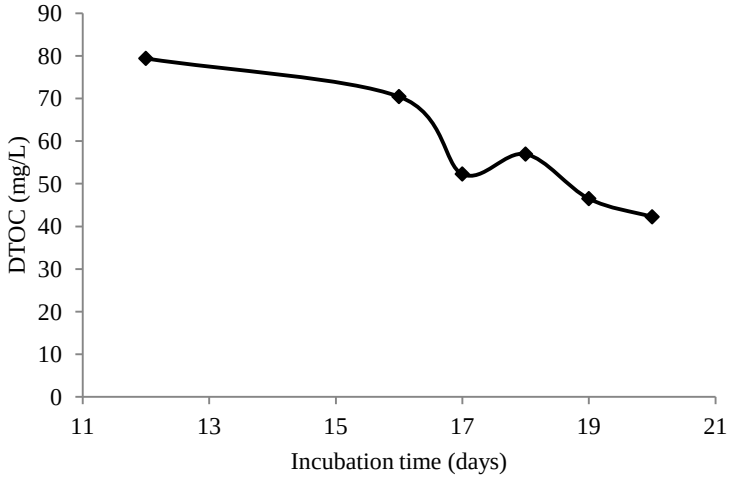

Figure 7.6: Change in DTOC for feed two – blood water

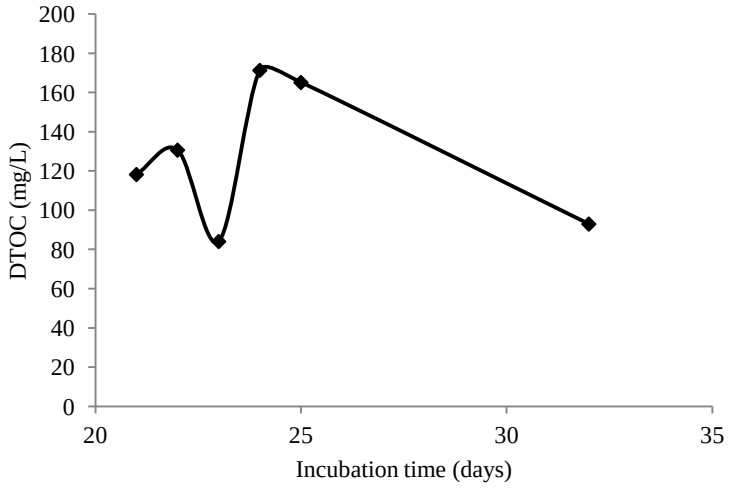

Figure 7.7: Change in DTOC for feed three – mixed water

7.6 SUSPENDED SOLIDS AND VOLATILE SUSPENDED SOLIDS

There was on overall decrease in SS over the 32 day period as indicated in Table 7.5. The SS measured here mainly indicate the bacterial mass. Typically, this needs to increase by 100mg/L, but in all these cases, it seems the biomass concentration is decreasing as shown in Figure 7.8 and Figure 7.9. This may be due to endogenous growth and decay of bacterial cells or perhaps due to experimental errors involved in these measurements.

Table 7.5: Characteristics of digester solution

	Blank	**Powder milk**		**Blood water**		**Combined abattoir wastewaters**	
		Initial	Final	Initial	Final	Initial	Final
SS (g/L),	5.07	5.17	4.99	5.5	4.34	5.81	4.65
VSS (g/L)	3.93	4.13	3.81	4.38	3.23	4.41	3.21

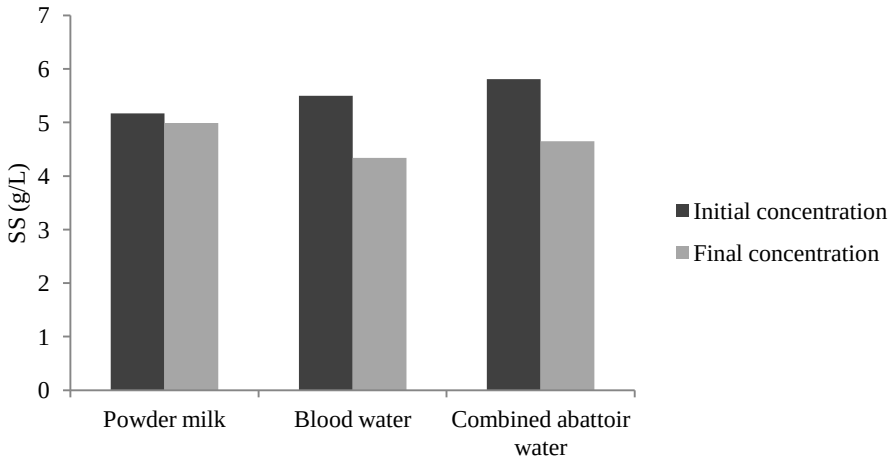

Figure 7.8: Initial and final concentrations of SS in the digester, for each substrate, for a one week period.

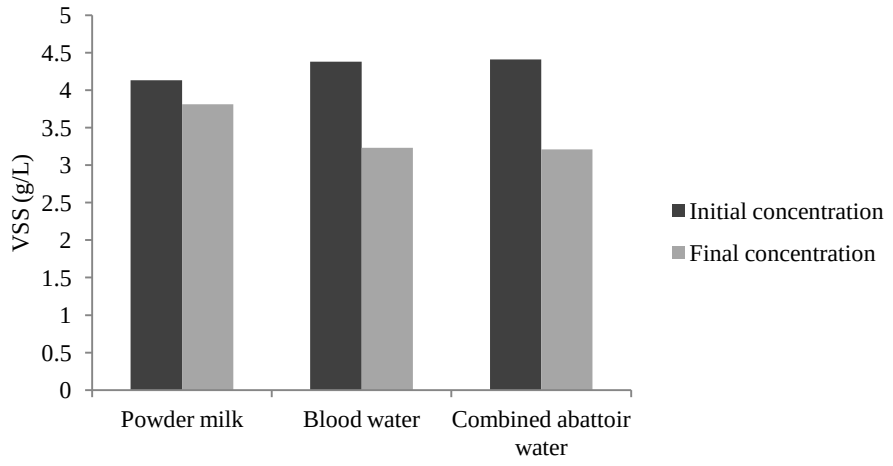

Figure 7.9: Initial and final concentrations of VSS in the digester, for each substrate, for a one week period

7.7 SUMMARY

This chapter discussed the results obtained using a continuously stirred bioreactor. It was found that increased volumes were achieved in the bioreactor as compared with batch experiments. The biogas of the mixed abattoir wastewater had the highest CH_4 content ranging between 27% and 47% with a total yield of 532mL CH_4/100mg/L DTOC at STP.

CHAPTER 8 CONCLUSIONS AND FUTURE WORK

8.1 CONCLUSIONS

In this research, anaerobic digestion was used to produce methane from abattoir wastewaters. Methane was identified as a renewable energy source suitable for use in industries that produce large volumes of wastewaters with a high organic carbon content. Methane is currently used around the world by a range of agricultural and food processing industries as a sole or supplementary energy source. Utilising methane as a renewable energy source has many benefits, including, reducing the reliance on fossil fuels, reducing greenhouse gas emissions from decomposing wastewaters and assists in the nutrient depletion of the wastewater before discharging into natural water bodies.

To evaluate the suitability of treating abattoir wastewaters by anaerobic digestion in the effective production of methane, firstly, easily biodegradable substrates including glucose, acetate, gelatine and powder milk were tested using standard batch assay procedures, with inoculums obtained from an operational anaerobic reactor. Three flows of abattoir wastewater including, yard water, blood water and saveall water, were then tested in batch assays and the volume of biogas produced and volume of methane produced were assessed. In addition, a small-scale bioreactor was used to test the volumes of methane produced when operated under continuously stirred conditions.

This research has shown that powder milk produced the most biogas totalling 61 mL biogas/100mg/L TOC at STP of the easily biodegradable substrates. Gelatine also yielded good volumes of biogas totalling 42mL biogas/100mg/L TOC at STP. These results indicated that complex substrates such as powder milk and substrates high in protein such as gelatine were suitable for anaerobic digestion. This provided a good initial indication that abattoir wastewaters could be used to produce biogas by anaerobic digestion. Comparable volumes of biogas were obtained from the abattoir wastewaters totalling 314mL biogas/100mg/ L DTOC, 44mL biogas/100mg/ L DTOC, 39mL biogas/100mg / L DTOC for saveall water, yard water and blood water respectively.

The study also showed that abattoir wastewater could successfully produce methane through anaerobic digestion. The biogas composition was analysed using gas chromatography to show the actual content of methane in the biogas. Saveall water was the most successful, producing a total of 110.4mL CH_4/100mg/L DTOC at STP, however, this was partly due to the addition of unmeasured organic carbon in the form of SS. Yard water and blood water produced smaller volumes totalling 6.2mL CH_4/100mg/L DTOC at STP, 7.0mL CH_4/100mg/L DTOC at STP respectively. This indicated that wastewaters containing fats could produce higher methane volumes as compared to yard water that possibly had a higher content of difficult to biodegrade material such as lignin.

The addition of anaerobic media had a minimal affect on the gas produced by the three abattoir wastewaters. The results indicated that increased volumes of methane could be obtained when anaerobic media was added to yard and blood water with an increase of approximately 1 to 3mL of methane. Alternatively, saveall water produced higher volumes of methane without the addition anaerobic media, with an increased methane yield of approximately 8mL. In all experiments, the methane content of the biogas was typically lower than that reported in literature. The methane content achieved in this study ranged between 3% and 53% as opposed to methane contents of 50% to 70% that have been achieved by other researches. (Ashekuzzaman & Poulsen 2011).

The results obtained using the continuously stirred bioreactor indicated that a combination of the three wastewaters produced increased volumes of methane totalling, 532mL CH_4/100mg/L DTOC at STP. The methane content of the biogas also showed improvement in the initial days with a range between 27% and 47% methane.

Overall the research has been successful, indicating that methane can be produced from individual or combined flows of abattoir wastewater. Although the methane content of the biogas and total volumes were lower than those achieved by other researches this study has given insight into the possibilities of producing methane from abattoir wastewater.

8.2 RECOMMENDATIONS FOR FUTURE WORK

8.2.1 IMPROVED WASTEWATER CHARACTERISATION

In this research, the total organic carbon was analysed using a Total Organic Carbon/ Total Nitrogen Analyser (TOC-VCPH/CPN). This provided a simple and fast means of obtaining the carbon content of the samples. However, the TOC analyser required the samples to be filtered through 0.45μm filter paper, therefore, only the dissolved organic carbon was measured. As indicated in the results, the addition of unmeasured organic carbon in the form of suspended solids obscured the produced methane volumes. It is recommended that the wastewaters be characterised by other methods such as COD or BOD so that all the organic carbon in the samples can be included in calculations and analysis of results. Alternatively reducing the size of the particles by ultrasonication would make it possible for the TOC analyser to be used.

8.2.2 ANAEROBIC BACTERIA ACCLIMATISATION

Anaerobic inoculum was either used directly after collection or after storage at 4°C, therefore, bacterial populations may have been reduced due to the shock of changing foods and temperatures. The bacteria were used to digesting sewage sludge and may not have been acclimatised properly with abattoir wastewater, therefore causing decreased methane volumes. It is recommended that experiments be conducted to determine if proper acclimatisation or inoculums obtained from successful plants treating similar waste using anaerobic digestion increase methane volumes. The results would then be more indicative of real life conditions where anaerobic reactors are fed continuously with one type of food.

8.2.3 GAS COMPOSITION

Gas analysis using gas chromatography provided an easy and fast means of obtaining the methane content of the biogas. However, only one standard gas of 40%CH_4 60% N was used and it was recommended to use three different standard gas concentrations to obtain a standard gas curve. Attempts at diluting the standard gas proved unsuccessful with high oxygen contamination. It is recommended to either purchase standard gasses

of varying concentrations or investigate more sophisticated methods of diluting the standard gas to obtain at least three different known concentrations.

The gas chromatograph was restricted to only measuring methane, nitrogen, oxygen and hydrogen due to the capability of the column. Gasses such as carbon dioxide and hydrogen sulphide could not be measured. Monitoring carbon dioxide is important when considering the efficiency at which methane is produced. Hydrogen sulphide causes serious problems in the utilisation of biogas as it is highly corrosive, therefore, it would be beneficial to monitor the volumes being produced.

8.2.4 VOLATILE FATTY ACIDS

A build up of volatile fatty acids (VFA) often leads to the inhibition of methanogenic processes, therefore, reducing the volumes of methane that are produced. Monitoring the daily VFA content could provide an indication of the methanogenic inhibition that was occurring, hence, providing an indicator of when dilution of feeding should occur.

8.3 SUMMARY

This chapter concluded by discussing the results of the dissertation and suggested some improvements to experimental processes and future research topics. It was found that abattoir wastewater produces methane through anaerobic digestion, however, improvements to experimental processes and analysis could help improve methane volumes.

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APPENDICES

APPENDIX A: PROJECT SPECIFICATION

University of Southern Queensland

FACULTY OF ENGINEERING AND SURVEYING

ENG 4111/ENG 4112 Research Project

PROJECT SPECIFICATION

FOR:	**AMELIA BAUER**
TOPIC:	INVESTIGATION INTO THE BIOCHEMICAL METHANE POTENTIAL OF ABATTOIR WASTEWATER
SUPERVISORS:	Dr. Vasantha Aravinthan
	Les Moorhead, Kilcoy Pastoral Company Limited
ENROLMENT:	ENG 4111, S1, D, 2011
	ENG 4112, S2, D, 2011
PROJECT AIM:	To investigate the methane production potential of abattoir wastewater using laboratorial procedures in both batch and semi-continuous modes.

PROGRAMME: (Issue A, 14th March 2011)

1. Conduct an extensive literature review on methane production from abattoir wastewater and other substrates.
2. Design and conduct experiments to investigate the methane potential in abattoir wastewater against other biodegradable substrates following standard batch assay procedures.
3. Statistically analyse the data and provide results showing the biodegradation curves to compare methane production potentials of the different substrates
4. Optimize the operating conditions such as pH, temperature and ideal seed to substrate ratio that give maximum methane production.
5. Design and conduct a continuous experiment on daily methane potential for an appropriate period of time in an anaerobic digester using the abattoir waste based on the experimental results obtained from batch assays.
6. Submit an academic dissertation on the research

As time permits:

1. Test different wastewater streams in the anaerobic reactor for methane production potential.

AGREED:

_____ (Student) _____ (Supervisor)

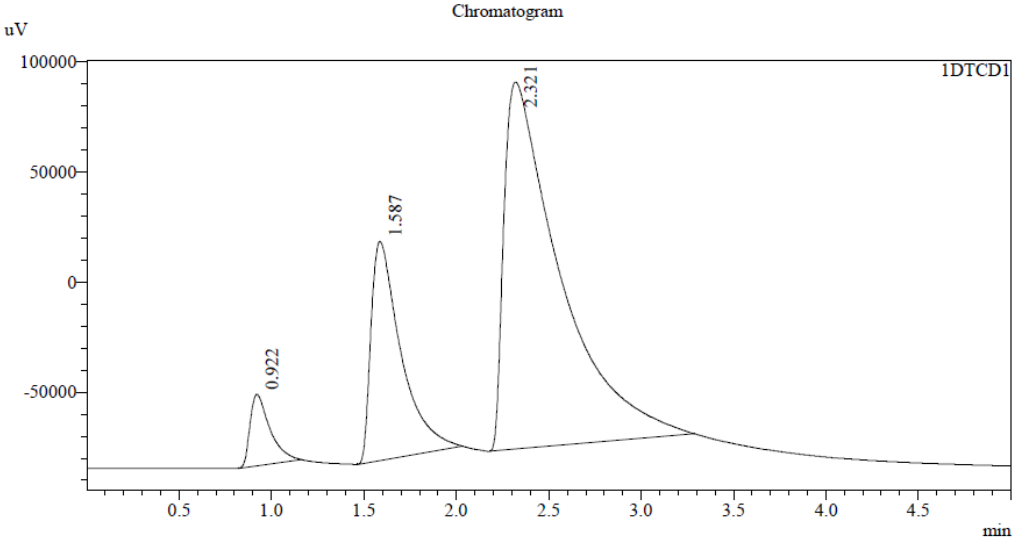
APPENDIX B: CHROMATOGRAPH

Sample chromatograph for saveall water no media

Sample Information

Sample Name	:
Sample ID	:
Vial#	: 1
Injection Volume	: 1
Data File	: R3_D19_B33.gcd
Method File	: method2_21july2011.gcm
Date Acquired	: 22/08/2011 1:17:46 PM
Date Processed	: 22/08/2011 1:22:49 PM

Chromatogram



QuantitativeResult

DTCD1

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	CH4	2.321	3514855	166504	0.000	%
Total			3514855	166504		

APPENDIX C: CURVE FITTING USING MATLAB

RUN 1

```
% mfile to load cumulated biogas data and open curve fitting tool
% data is then processed in the curve fitting tool box
% using custom equation B = Bo(1-exp^(-kt))
% k values are then saved

clc
clear
Run1 = xlsread ('run1.xls');
time=Run1(:,1);
glucose=Run1(:,2);
acetate=Run1(:,3);
gelatine=Run1(:,4);
powmilk=Run1(:,5);
cftool
%EOF
```

Glucose

General model:
val = 42.9518*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.4888 (0.3803, 0.5973)

Goodness of fit:
SSE: 39.19
R-square: 0.9766
Adjusted R-square: 0.9766
RMSE: 2.556

acetate

General model:
val = 30.2477*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.407 (0.2487, 0.5653)

Goodness of fit:
SSE: 64.8
R-square: 0.9311
Adjusted R-square: 0.9311
RMSE: 3.286

gelatine

General model:
val= 42.4199*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.433 (0.2957, 0.5704)

Goodness of fit:
SSE: 82.69
R-square: 0.9523
Adjusted R-square: 0.9523
RMSE: 3.71

powder milk

General model:
val = 61.3036*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.3707 (0.2803, 0.4611)

Goodness of fit:
SSE: 108.2
R-square: 0.9684
Adjusted R-square: 0.9684
RMSE: 4.247

Run 2

```
% mfile to load cumulated biogas data and open curve fitting tool
% data is then processed in the curve fitting tool box
% using custom equation B = Bo(1-exp^(-kt))
% k values are then saved

clc
clear
Run2 = xlsread ('run2.xls');
Run2a = xlsread ('PWR2.xls');
time=Run2(:,1);
time2=Run2a(:,2);
g=Run2(:,2);
yw=Run2(:,3);
bw=Run2(:,4);
pw=Run2a(:,1);
cftool
%EOF
```

Glucose

General model:
val = 49.1028*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.4607 (0.442, 0.4795)

Goodness of fit:
SSE: 1.867
R-square: 0.9992
Adjusted R-square: 0.9992
RMSE: 0.5165

Blood water

General model:
val= 44.1509*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.2934 (0.2431, 0.3438)

Goodness of fit:
SSE: 30.3
R-square: 0.9859
Adjusted R-square: 0.9859
RMSE: 2.08

Yard water

General model:
va = 39.2687*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.214 (0.1936, 0.2343)

Goodness of fit:
SSE: 8.011
R-square: 0.995
Adjusted R-square: 0.995
RMSE: 1.07

Saveall water

First phase
General model:
f(time) = 33.24*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.5488 (0.3653, 0.7322)

Goodness of fit:
SSE: 26.5
R-square: 0.9646
Adjusted R-square: 0.9646
RMSE: 2.302

Second phase
General model:
f(time2) = 313.7*(1-exp(-k*time2))
Coefficients (with 95% confidence bounds):
k = 0.2666 (0.1495, 0.3837)

Goodness of fit:
SSE: 1698
R-square: 0.9574
Adjusted R-square: 0.9574
RMSE: 23.79

Run 3

```
% mfile to load cumulated biogas data and open curve fitting tool
% data is then processed in the curve fitting tool box
% using custom equation B = Bo(1-exp^(-kt))
% k values are then saved

clc
clear
Run3 = xlsread ('run3.xls');
time=Run3(:,1);
g=Run3(:,2);
yw=Run3(:,3);
bw=Run3(:,4);
pw=Run3(:,5);
cftool
%EOF
```

Glucose

General model:
val = 48.6213*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.282 (0.2161, 0.3479)

Goodness of fit:
SSE: 99.38
R-square: 0.9549
Adjusted R-square: 0.9549
RMSE: 3.323

Blood water

General model:
val = 34.2959*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.2134 (0.1749, 0.2518)

Goodness of fit:
SSE: 35.43
R-square: 0.9711
Adjusted R-square: 0.9711
RMSE: 1.984

Yard water

General model:
val = 32.4651*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.1209 (0.1062, 0.1356)

Goodness of fit:
SSE: 19.37
R-square: 0.9833
Adjusted R-square: 0.9833
RMSE: 1.467

Saveall water

First phase
General model:
f(time) = 37.78*(1-exp(-k*time))
Coefficients (with 95% confidence bounds):
k = 0.3704 (0.2624, 0.4785)

Goodness of fit:
SSE: 32.68
R-square: 0.9652
Adjusted R-square: 0.9652
RMSE: 2.557

Second phase
General model:

f(time2) = 318.91*(1-exp(-k*time2))
Coefficients (with 95% confidence bounds):
k = 0.4162 (0.3765, 0.4559)

Goodness of fit:
SSE: 64.83
R-square: 0.9977
Adjusted R-square: 0.9977
RMSE: 4.649

APPENDIX D: SAMPLE VOLUME CALCULATION

Volume headspace	325	mL
Standard temp	273.15	K
Standard pressure	101.325	Kpa

Measured data					Calculated from volume measurements												From pressure measurement
	Volume	Temp Start	Temp End	Gauge Pressure	Volume of 325ml at STP	Volume of gases developed at STP	Total gas produced	Cumulated gas	Adjusted volume (gas minus blank and water)	Adjusted cumulated gas	Pressure of gases+air at 35 deg C	Pressure of headspace air at 35 dec C	pressure of gases alone at 35 deg C	Gauge pressure (measured)	Adjusted pressure (pressure minus blanks and water)	Cumulated pressure	Volume of gas produced at STP
Day	mL	deg C	deg C	Kpa	mL	mL	mL	mL	mL	mL	Kpa	Kpa	Kpa	Kpa	mbar	mbar	mL
0	0	33.4	33.4	0	289.59		0.00	0.00		0.00					0.00	0.00	
1	22	34.5	34.8	7	288.27	307.94	18.35	18.35	9.02	9.02	108.31	101.85	6.45	6.86	36.83	36.83	18.75
2	31	37.2	36.8	9.4	286.41	313.53	25.26	43.60	13.02	22.04	110.27	101.39	8.88	9.66	54.49	91.32	24.51
3	21.5	37.4	37.2	6.5	286.04	304.87	18.46	62.06	10.10	32.14	107.23	100.74	6.49	6.70	33.73	125.05	17.88
4	11	37	37	3.5	286.23	295.92	9.87	71.93	5.84	37.97	104.08	100.61	3.47	3.43	13.67	138.72	10.07
7	22.5	37	36.6	6.9	286.60	306.24	20.01	91.95	0.93	38.90	107.71	100.67	7.04	7.01	8.01	146.73	19.69
10	12	36.6	35.5	4.5	287.62	297.71	11.11	103.06	0.00	38.90	104.71	100.80	3.91	3.74	0.05	146.78	13.26

APPENDIX E: MEASURED DATA

Trial 1: Easily biodegradable substrates

Bottle 1- Glucose

	Volume	Temp Start	Temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	33.4	33.4	0
1	22	34.5	34.8	7
2	31	37.2	36.8	9.4
3	21.5	37.4	37.2	6.5
4	11	37	37	3.5
7	22.5	37	36.6	6.9
10	12	36.6	35.5	4.5

Bottle 2 - Glucose

	Volume	Temp Start	Temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	33	33	0
1	30.5	34.9	34.8	9
2	30	37	36.6	9
3	21	37.4	37.4	6.3
4	11	37.2	37	3.6
7	23.8	36.8	36.4	7.2
10	14	36.6	36.2	4.2

Bottle 3- Acetate

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	33.2	33.2	0
1	13	34.3	34.8	4
2	25.2	37.2	36.6	7.5
3	17.5	37.6	37.4	5.3
4	10.2	37.2	37.2	3.1
7	22	37	36.8	6.5
10	15	36.6	35.8	4.5

Bottle 4- Acetate

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	32.3	32.3	0
1	21	34.8	34.8	6.2
2	32	36.4	36.4	9.5
3	22	37.4	37	6.5
4	11	37.4	37.2	3.2
7	21	37.2	36.6	6.2
10	12	36.4	36	3.5

Bottle 5 - Powder milk

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	30.9	30.9	0
1	28.5	34.9	34.5	8.5
2	35.5	36.6	36.6	10.5
3	27	37.4	37.2	8
4	16.5	37.6	37.2	5
7	27	36.8	36.6	8.1
10	15	36.4	36.2	4.8

Bottle 6 - Powder milk

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	30.3	30.3	0
1	27.2	34.9	34.4	8
2	36	36.8	36.4	10.5
3	26	37.4	37.2	7.6
4	16	37.4	37.2	4.8
7	27.1	36.8	36.6	8.1
10	16.5	36.4	36.2	5

Bottle 7- Gelatine

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	29.3	29.3	0
1	24	34.6	34	7
2	33	36.4	36.2	9.7
3	22	37.2	36.8	6.5
4	11.8	37.2	36.8	3.5
7	22	36.8	36.6	6.5
10	14	36.4	36	4

Bottle 8 - Gelatine

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	30.1	30.1	0
1	25	34.5	34.3	7
2	33	36.4	36	9.7
3	23	37	36.6	6.7
4	12.1	37.4	36.8	3.5
7	21.3	35.8	35.6	6.5
10	16	36.2	35.8	4.8

Bottle 9 - Blank

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	29	29	0
1	15	34.6	34.3	4.5
2	13.5	36.2	35.8	4
3	11	36.8	36.6	3.5
4	7	37	36.6	2.05
7	19.5	36.4	36.2	5.8
10	12	36	35.8	3.9

Bottle 10 - Blank

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	27.3	27.3	0
1	16	34.2	34.1	4.2
2	12.3	36.2	35.6	3.9
3	11	36.6	36.4	3.4
4	7	36.6	36.6	2
7	19.5	36	35.6	5.8
10	13	35.8	35.8	3.9

Water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0	0	30.9	30.9	0
1	5	34.1	34	1.2
2	0	36	35.8	1.5
3		36.6	36.4	0.05
4	-2	36.6	36.6	
7		36.2	35.8	0.28
10		35.8	35.8	0.05

Trial 2: Abattoir wastewater with media

Bottle 1: Glucose

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0	0	33.8	33.6	0
1	31	36.6	36.2	9.1
2	20	34.6	34.4	6.5
3	11.5	37.8	37.6	4.5
6	21.5	36.8	36.4	6.8
9	12.2	36.8	36.8	4
13	15	37.2	37	4.3
20	20	36.8	36.2	6

Bottle 2: Glucose

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		33.6	33.6	
1	31	36.6	36.2	9.1
2	27	36.8	35.2	8.5
3	15	37.4	37	4.5
6	24	36.8	36.4	7.2
9	12	37.4	36.8	3.9
13	14	37	36.8	4.5
20	20	36.4	36.4	6

Bottle 3: Yard water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		31.4	31.4	
1	20	36.4	36.2	5.9
2	17.5	36.4	36.2	5.8
3	12	37.4	37	3.6
6	24	36.4	36.2	7.2
9	14	37.2	37.2	4.2
13	19	37	36.6	5.6
20	22	36.4	36	6.5

Bottle 4: Yard water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		30.2	30.2	
1	21	36.6	36.2	6.1
2	19	36.6	36.2	5.9
3	10	36.8	36.6	3.2
6	24	36.4	36	7.5
9	16	37	36.8	4.8
13	18	36.6	36.4	5.5
20	22	36.4	36.2	6.6

Bottle 5: Blood water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		28.4	28.4	
1	21.5	36.6	36.4	8
2	24	36.6	36.2	7.1
3	13	37	36.6	3.9
6	24.2	36.2	36.2	7.5
9	14	37.2	36.8	4.1
13	14.5	36.8	36.4	4.6
20	21	36.2	35.8	6.1

Bottle 6: Blood water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		27.8	27.8	
1	27.5	36.6	36.2	8.1
2	24.5	36.4	36.2	7.2
3	14	36.8	36.6	4.5
6	25.1	36.4	36.2	7.9
9	15	37	36.8	4.5
13	14.5	36.4	36.4	4.5
20	22	36	36	6.5

Bottle 7: Saveall water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		32.4	32.4	
1	32	35.2	35.6	9.2
2	28	36.2	36.2	8.2
5	42	36.2	36.4	12
6	10	35.8	35.6	2.8
8	16	36.2	36	4.5
12	50.5	36.2	35.8	15
15	100	35.6	35.8	
19	158	36.2	35.2	
22	43	36	35.6	12

Bottle 8: Saveall water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		32.6	32.6	
1	29	35.8	35.8	8
2	29	36.4	36.2	8
5	42	36.2	36.2	11.9
6	10	36.2	36	2.9
8	15.5	36.4	36.2	4.3
12	47.5	36.2	36	13.5
15	97	36.2	35.8	
19	156	36.4	36	
22	50	36.4	36.2	14

Bottle 9 : Blank

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		31.6	31.6	
1	11	36.4	36.2	3.2
2	12	36.2	35.8	4.5
3	5	35.6	35.6	1.8
6	16	35.6	35.6	5
9	12.2	36.4	36.2	3.6
13	12.2	36.2	35.8	3.6
20	19	35.8	35.8	5.8

Bottle 10: Blank

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		30.8	30.8	
1	12	36.2	35.8	3.5
2	6	36.4	35.8	2.9
3	4.5	35.8	35.4	2.1
6	16	35.8	35.4	4.9
9	11	36.4	36.4	3.5
13	11.5	35.6	35.6	4.6
20	20	36.6	36.2	6.5

Water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		35.2	35.2	
1	2.5	35.8	35.6	1
2	1	35.6	35.6	0.2
3	-1.6	35.8	35.6	
6	-2	35.8	35.6	
9	-2.4	36.4	36.2	
13	1.3	36.2	36	
20	2	35.8	35.6	

Trial 3: Abattoir wastewater without media

Bottle1: Glucose with media

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		35.2	35.2	
1	35	36.8	36.6	10.2
2	27	35.6	35.6	8
5	45	36.2	35.6	12.9
6	10	36.6	36.3	3
8	14	36.6	36.2	4
12	12.5	36.2	36	4.5
15	10	35.6	35.6	3
19	7	35.8	35.8	2
22	7	36.4	36.2	2

Bottle 2: Glucose with media

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		35.2	35.2	
1	36	36.6	36.2	10.2
2	30	36.4	36.2	8.8
5	43.5	36.4	36.2	12.6
6	9	36.4	36	2.5
8	14	36.8	36.4	4
12	11	36.4	36.2	3.2
15	11.2	36	36	3.4
19	7	36.2	36	2
22	7	36.6	36.4	2

Bottle 3: Glucose no media

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		35.4	35.4	
1	29	36.8	36.4	8.5
2	24	37	36.6	7.2
5	43.5	36.6	36.2	12.8
6	11	36.6	36.4	3.2
8	14	36.8	36.8	4.2
12	13	36.6	36.4	4.1
15	9.5	36.2	35.8	2.8
19	9	36.4	36.2	2.8
22	8	36.6	36.6	2.3

Bottle 4: Glucose no media

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		35	35	
1	31	36.6	36.6	9
2	26	36.8	36.6	7.7
5	46	36.4	36.2	13.1
6	12	36.8	36.6	3.2
8	14	36.8	36.6	4
12	12	36.6	36.4	3.7
15	11	36.4	36	4
19	8	36.4	36.2	2.3
22	7	36.8	36.4	2.2

Bottle 5: Yard water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		33.6	33.6	
1	18	36.6	36.4	5.5
2	17	36.2	35.4	5.1
5	46	36.2	35.8	13.9
6	10.5	36.2	36	3.2
8	15	36.2	36.2	4.5
12	17	36.2	35.8	5
15	12.5	36.2	35.6	3.8
19	9	35.4	35.8	3.9
22	9.5	36.2	36.2	3

Bottle 6: Yard water

Day	Volume ml	Temp Start deg C	temp End deg C	Gauge Pressure kpa
0		33.6	33.6	
1	16	36.4	36.4	4.5
2	19	36.6	36.4	5.6
5	40	36.4	36	11.6
6	9	36.6	36.4	2.8
8	13	36.6	36.2	3.9
12	14	36.2	36.2	4.5
15	12	36.2	36.2	3.5
19	8	36	36	2.2
22	8	36.4	36.2	2.5

Bottle 7: Blood water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		33.2	33.2	
1	19	36.6	36.4	5.5
2	26	36.4	36.4	7.8
5	43	36.2	36.4	12.5
6	9	36.4	36.2	2.6
8	12.5	36.2	36.4	3.6
12	12	36.4	36	3.5
15	10	36.2	36.2	3
19	8	35.8	35.8	2.2
22	8	36.6	36.2	2

Bottle 8: Blood water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		33	33	
1	19	36.4	36.2	5.5
2	28	36.6	36.4	8
5	43	36.4	36.2	12.5
6	10	36.4	36.2	2.6
8	10.5	35.8	35.8	3.2
12	13	36.2	36.2	3.8
15	12	36	36.2	3.2
19	8	36.4	36.2	2
22	7	36.2	36.6	2.2

Bottle 9: Saveall water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		32.2	32.2	
1	28	35.8	35.6	8
2	26	35.6	35.2	7.8
5	43.5	35.8	35.6	12.5
6	11	36.2	35.8	3
8	18	36	35.8	5
12	93	35.6	35.4	28
15	148	35.4	35.2	
19	88	35.6	35.4	
22	25	35.8	35.6	7

Bottle 10: saveall water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		32	32	
1	26	36.2	35.6	7.5
2	26	36.2	36	7.8
5	44	36.4	36.2	12.8
6	9	36.2	36	2.9
8	16.5	35.8	35.4	4.3
12	85	36.2	35.8	27
15	131	36	35.6	
19	102	36	35.8	
22	24	36.2	36	7

Bottle 11: Blank no media

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		34.8	34.8	
1	10	36.2	36.4	3
2	14	36.6	36.2	4.2
5	32.5	36.6	36	9.9
6	8	36.6	36	2.1
8	9.5	36.4	36.4	2.8
12	11	36.4	36.2	3.2
15	8.5	36.2	36.2	2.5
19	7	36.4	36.2	2
22	7	36.4	36.4	1.9

Bottle 12: Blank no media

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		34.2	34.2	
1	10	35.6	35.6	2.9
2	14	35.8	35.8	4.2
5	32	36	36	9.5
6	7	35.8	35.4	2
8	9.5	36	36	2.8
12	11	36.2	35.8	3.1
15	9.5	35.8	35.6	2.5
19	6	36.2	35.2	1.9
22	7	35.8	35.8	1.9

Bottle 13: blank with media

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		32.4	32.4	
1	13	35.6	35.6	4.7
2	16.5	36	35.6	4.5
3	33.5	36	36	9.8
6	10	36.4	36.2	2.8
8	10	36.6	36	2.8
12	12	36.4	36.2	3.5
15	9	35.8	35.8	2.5
19	7	36.4	36.2	2.2
22	8	36.4	35.8	2

Bottle 14: Blank with media

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		30.8	30.8	
1	16.5	35.2	35.2	4.6
2	16	35.6	35.4	4.5
5	34	35.6	35.6	10
6	10	36.6	36	2.9
8	10	36.4	36.4	2.8
12	14	36.2	36	3.9
15	8	36.2	35.8	2
19	9	36.4	36.4	2.6
22	7	36.4	36.2	2

Water

	Volume	Temp Start	temp End	Gauge Pressure
Day	ml	deg C	deg C	kpa
0		34.6	34.6	
1	1	35.6	35.6	0.5
2	1	35.6	35.6	
3	5	35.8	35.6	1
6	0.2	36.8	36.8	
8	0.5	36.4	36.2	
12	-2	35.8	35.8	
15	-2	35.8	35.8	
19	0	35.8	35.8	
22	0.5	36.2	36.4	