University of Southern Queensland
Faculty of Engineering & Surveying

CO-DIGESTION OF ABATTOIR PAUNCH AND STICK WATER UNDER MESOPHILLIC AND AMBIENT TEMPERATURES: A CASE STUDY

A dissertation submitted by

Jamie Edward Hunt

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ABSTRACT

Anaerobic digestion (AD) has gained favour in the red meat processing industry for its ability to treat wastewater while generating biogas, a fuel alternative to natural gas (World Biogas Association 2021). Paunch, the stomach content of cattle, is an organic-rich waste material. Digestion of paunch is not widely adopted and is traditionally landfilled despite biogas production being considered best practice (ARENA, 2017). Rising utility costs has pushed abattoirs to revisit paunch AD as an alternative energy source and to reduce disposal fees (Ramirez et al. 2021). Effective paunch AD has limitations as it contains recalcitrant lignocellulosic material not available for degradation. Abattoir treatment processes are often not heated and increased temperature enhances methanogenic bacterial growth and increases biogas production rates.

The project aims to enhance the anaerobic degradation of paunch through two objectives: 1) increasing temperature from on-site ambient to 37°C, and 2) optimisation of carbon-to-nitrogen (C:N) ratio through co-digestion with stickwater. Biochemical methane potential testing was conducted to test both co-digestion and temperature impacts. Temperate was varied from the case study ambient of 28°C to the optimum of 37°C. Feedstocks were co-digested in ratios representing production availability, and C:N ratios of 15, 20, 25, 30, & 35. Optimising the temperature enhanced methane yeild by 13.6%, the degradable fraction by 15.9%, and time of digestion by 39.9%. Biogas yield was highest at a C:N of 15, while the ideal ratios in the literature are between 20–30. Additionally, the degradable fraction was greatest in production ratio mixes, performing better than other mixes for both temparture ranges with degradable fraction of 67% at 28°C and 75% for 37°C.

Although no effect of C:N ratio was observed, significant benefit was obtained from optimising digestion temperature. The data supports the heating of digestion systems to manage paunch waste for energy production and the diversion of wastes to landfill.

Keywords: *Anaerobic Digestion, Biogas, Bioenergy, Slaughterhouse, Energy Recovery, Circular Economy*

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Jamie Hunt

[redacted]

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Abbreviations

ABR	Anaerobic baffled reactor	HRT	Hydraulic retention time
AD	Anaerobic digestion	HSCW	Hot standard carcass weight
AF	Anaerobic filter	ISR	Inoculum-to-sludge ratio
AFBBR	Anaerobic fluidised bed biofilm reactor	LCFA	Long chain fatty acids
AMPTS II	Automatic methane potential test system, II	MCFA	Medium chain fatty acids
AnMBR	anaerobic membrane reactor	O^2	Oxygen
AnSBR	Anaerobic sequential batch reactor	OLR	Organic loading rate
BMP	Biochemical methane potential	RMP	Red meat processor
C:N	Carbon-to-nitrogen ratio	SCFA	Short chain fatty acid
CAL	Covered anaerobic lagoon	SMP	Specific methane potential
CH_4	Methane	SRT	solids retention time
CN30	Carbon neutral: 2030	TA	Total alkalinity
CO_2	Carbon dioxide	TN	Total nitrogen
COD	Chemical oxygen demand	TOC	Total organic carbon
CSTR	Continuously stirred tank reactor	TS	total solids
DAF	Dissolved air floatation	UASB	Upflow anaerobic sludge blanket
FOG	Fats, oils, and grease	VFA	Volatile fatty acids
H_2	Hydrogen	VS	Volatile solids

Chapter 1 Introduction

1.1 Background

Australian red meat processing (RMP) contributes 19.2 billion AUD to the Australian economy and is an energy and resource intensive industry. In 2022, 6.1 million cattle were processed, resulting in approximately 2 million tons of hot standard carcass weight (tHSCW) for local and export meat market (Australian Beef Sustainability Framework 2023). This production accounted for the consumption of 6.84 petajoules of energy, the generation of 16.6 gigalitres of wastewater, and 25.4 kilotonnes of solid waste (Brad Ridoutt 2022; MLA 2022). Although the industry has been improving efficiency for many years now, the cost of business is still increasing.

Being a resource intensive industry, red meat processors are feeling the pinch of rising utility and waste management costs. Australian energy utility prices have recently seen record wholesale prices and are projected to continue increasing up to 30% (Australian Energy Regulator 2022). Meanwhile, the introduction of state based landfill levies, the cost for waste disposal to landfill has also increased, estimated to be approximately 12% of an abattoirs utility costs and 0.7% of total operational cost (Ramirez et al. 2021). Finally, as Australia legislated an emissions reduction target of 43% of 2005 levels by 2030 and a move towards a net zero target by 2050 (Department of Climate Change, Energy, the Environment and Water), the Australian red meat industry has responded by setting the industry's target to become carbon neutral by 2030 (CN30) (MLA). Alternative sources of energy will be required to address both the costs of energy and waste management, while also addressing the industry impact on climate change.

Anaerobic digestion (AD) has gained favour in the RMP industry for its ability to treat wastewater while generating biogas, consisting primarily of methane (~60 - 70%) and carbon dioxide (~30 - 40%). The methane has a lower heating (LHV) of 36 $MJ \cdot m^{-3}$, making the energy available in biogas between 21.6 – 25.2 $MJ \cdot m^3$ (Meghvansi & Goel 2022) and portrays biogas as a low-emissions fuel alternative to natural gas (World Biogas Association 2021). The AD technology favoured by the RMP industry has been anaerobic lagoons – large ponds absent of active heating and mixing which degrade organics slowly but accommodate for a large hydraulic load (Harris & McCabe 2020). However, while anaerobic lagoons are relatively cheap to install and operate in comparison with their engineered counterparts, there are several RMP wastes which pose problems to this technology.

The stomach contents of slaughtered cattle (i.e. paunch), is an organic rich waste material and has been a particular problem for processors to manage. In anaerobic lagoons, paunch floats to the surface of

the lagoon alongside fats and other materials to form a fatty crust across the surface of the lagoon (McCabe 2017). The crust layer restricts the degradation of material, reduces the functional volume of a reactor, promotes short circuiting which lowers the potential to treat wastewater, and in extreme cases can prevent biogas from exiting the wastewater (Harris & McCabe 2015). To overcome obstacles of paunch AD, a range of treatment processes are available for consideration ranging from physical, chemical, and thermal pre-treatment technologies. In addition to pre-treatment options, increasing biogas yield can also be enhanced by mixing feedstock materials to increase degradation via co-digestion of available waste streams (Harris & McCabe 2015). These treatment considerations are pertinent to paunch digestion as the material contains a recalcitrant portion, high in lignocellulose, denying complete degradation (Dowd et al. 2022). The recalcitrant lignocellulosic material degrades slowly and yields lower methane in single substrate digestion than other RMP wastes (Astals et al. 2014). Although biogas production is considered best practice (ARENA, 2017), due to the aforementioned problems, the AD of paunch has not been widely adopted and traditionally disposal of paunch in landfill is standard practice.

Improving industry uptake of paunch digestion will contribute to the movement towards a circular economy. Biogas generated from wastewater treatment can be utilized to generate a combination of heat and power, where both energies can be utilised within the production facility to offset electricity and natural gas consumption (ARENA 2019). Integration of AD for paunch material can increase the economic and environmental sustainability of a facility and unlock operational savings in the realms of carbon emission reporting, offsetting buy-in energy costs, and decreasing waste disposal costs.

1.2 Aims and Objectives

This project was aimed at exploring potential enhancement of biogas yield from the anaerobic digestion of paunch material at the laboratory scale to inform practices at an Australian abattoir case study. The aim can be summarised as:

1) Exploring the effect of co-digestion of paunch with other RMP residues and,

2) Investigating the effect of temperature optimisation on the digestion of paunch, bloodmeal stickwater and co-digestion mixes.

To realise the project aim, the specific objective for this research includes an extensive literature review of AD requirements specific to RMP and the feedstock material in focus, their methane potential, and relevant anaerobic digestion treatment parameters and processes. In conjunction with the literature review, experimentation on the biochemical methane potential of paunch and stickwater is completed for differing temperatures and mixes with appropriate results and discussion.

Chapter 2 Literature Review

2.1 Overview of Anaerobic Digestion Process

Anaerobic digestion is a wastewater treatment process where a complex consortium of microorganisms metabolise organic feedstock in an oxygen free environment to produce biogas. Saha et al. (2020) describes AD as a symbiosis between hydrolytic and fermentative bacteria, syntrophic bacteria and methanogenic archaea that together convert organic matter into biogas. This symbiotic digestion process occurs in four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1) (Angelidaki 2011). These steps are the pathway for degradation of organic matter into biogas, and each biological process has different requirements for optimum conditions. Hydrolysis involves the degradation of complex macronutrients (i.e., carbohydrates, proteins, and fats) converted into their soluble components (i.e., sugars, amino acids and fatty acids). The rate of hydrolysis is imperative to the overall AD process and is commonly the rate-limiting step. While carbohydrates are relatively readily hydrolysed, proteins and lipids require longer for hydrolysis to complete (Airton Kunz 2022). Recalcitrant nutrients can also be present in materials which is not hydrolysed by biological processes. Lignin present in plant matter is recalcitrant and lowers a feedstocks ability to produce methane closer to theoretical values (Taherzadeh 2008). Acidogenesis involves the conversion of products from hydrolysis into alcohols and volatile fatty acids (VFA). Hydrogen, carbon dioxide and ammonia are also created in this stage (Saha et al. 2020). Acetogenesis involves conversion of short-, medium- and long-chain acid compounds (SCFA, MCFA, LCFA) into acetate – a viable food source for methanogens for methane production (Saha et al. 2020). Methanogenesis involves the formation of methane. Two classes of archaea, acetoclastic methanogens and hydrogenotrophic methanogens, convert metabolites from the previous steps into methane (Duncan Mara 2003). While acetoclastic methanogens consume acetate to produce CH_4 and CO_2, hydrogenotrophic methanogens consume CO_2 and hydrogen (H_2) to produce CH_4.

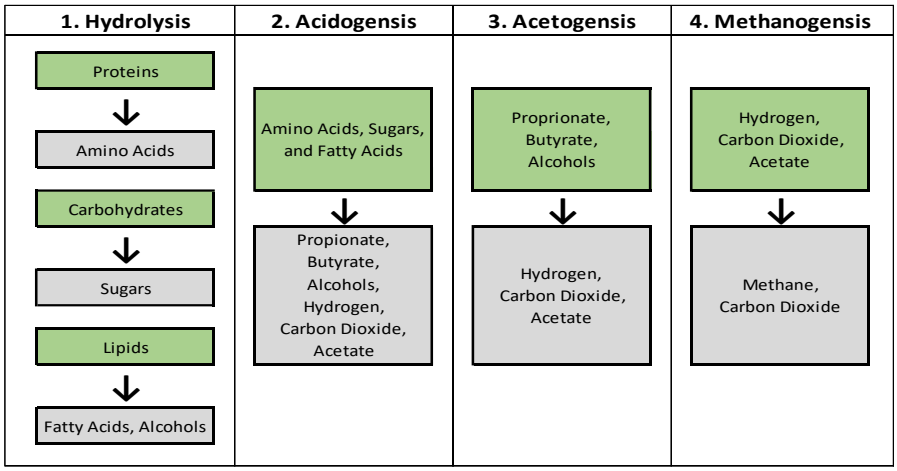


Figure 1: Stages in anaerobic digestion process, adapted from Ngan et al. (2020).

2.2 Anaerobic Digestion in Red Meat Processing Plants

The wastewater encountered at RMP plants is considered to have a high strength, containing solid organic waste material from the washing down, sterilising, and processing of meat and by-products (Schmidt et al. 2018; Shende & Pophali 2021). Anaerobic digestion has become a standard treatment process incorporated into RMP wastewater treatment systems, favoured by the industry for its ability to effectively process high-strength wastewaters with greater energy efficiency than aerobic processes, and being a net energy producing process (Metcalf & Eddy et al. 2013; Shende & Pophali 2021). The AD technology most commonly utilised by RMP is anaerobic lagoons – large ponds capable of effectively treating the full hydraulic load of the plant (Schmidt 2019). When designed for biogas capture, the treatment lagoons are covered with a HDPE liner and are termed a covered anaerobic lagoon (CAL), however they are devoid of any heating or mixing apparel. Without the ability to control the process via homogenous mixing or temperature control, the efficiency CAL's cannot be maintained (Harris & McCabe 2020). Anaerobic lagoons are typically designed with large hydraulic retention times for the purpose of ensuring effective treatment is administered to wastewater with a process that requires minimal attention for operation, often resulting in CAL's which are not optimized (Jensen et al. 2014).

2.3 Feedstock Material

Feedstock is the organic material which is to be biologically degraded within the AD process. It can be characterised by its physical and chemical properties which can impact digester performance. Different feedstocks exhibit different characteristics such as organic fractions, bioavailability, and solid/liquid or slurry like consistencies, and they can be partly characterised by these features to describe suitability for digestion and likely degradability. The VDI 4630 (2016) manual describes the predominant features of a substrate for characterisation as being: total solids (TS), organic content i.e., volatile solids (VS) and chemical oxygen demand (COD); macronutrient makeup; and chemical makeup. The VS and COD describes organic content of a substrate and is often used to describe a substrate's BMP in terms of grams CH_4 per gram of VS or COD. Assuming only organic compounds are oxidized, 1g of COD can be converted into 350 mL of CH_4 (VDI 4630 2016) although COD contains fractions which are not degradable, being either inorganic or insoluble. Similarly, VS may contain components which are recalcitrant and which AD processes lack the enzymes to degrade.

2.3.1 Abattoir Feedstock Material

Abattoirs possess numerous waste streams, each with unique characteristics. The variability in the physiochemical characterisation each waste stream can be seen in Table 1. Note that the four highest

strength streams are currently diverted away from wastewater treatment: Float material from the dissolved air floatation (DAF) is recovered from the wastewater and processed into low grade tallow; blood is processed into bloodmeal in an attempt to recover as much protein as possible from the waste stream; gut material is processed in the rendering plant to produce meat meal; and paunch is recovered for landfill disposal (AMIC 2007).

Table 1: Abattoir feedstock characteristics.

		Paunch Solids [a, c, g]	Paunch Liquid [a, c]	Blood Raw [a, e, i]	Blood Stickwater [i]	Red Stream [c]	Green Stream [c]	DAF Float [g, h]	Boning Room [c]	Cattlewash / Yards [a, c]	Guts [a]	Saveall overflow [a, d]	Combined [a-f]
TS	%	29.6 - 33.92	0.6 - 0.72	11 - 23.24	1.2	0.3	0.48	36	0.1	0.2 - 0.45	38.08	0.38	0.17 - 0.84
VS	%TS	95.89	69.8 - 70.49	95.95	n/a	21	35	98.32	45.2	48.6 - 67.4	96.5	85.83	70.24
pH		n/a	n/a	n/a	n/a	7.1	6.9	4.4	7.2	8.7		n/a	6.5 - 7.2
COD	mg L^{-1}	28,700 - 433,100	9,700 - 10,160	291,000 - 375,000	11,342	5713	11,922	469,000 - 1,053,000	542	2,530 - 5,200	534,000	7,100	5,031 - 12,893
FOG	mg L^{-1}	n/a	1,013	n/a	n/a	470	1060	10,500 - 265,000	96	129	n/a	<1,000	100 - 3,350
TN	mg L^{-1}	160	28	430* - 20,600	1,203	150	235	1,200 *	0	300	n/a	300	114 - 450

a - Barnes and Forde (2020), b - Jensen et al. (2014), c - Schmidt (2018), d - UNSW (1998), e - Johns (1995), f - Schmidt, McCabe and Harris (2018), g - Jensen (2013), h - Harris et al. (2018), i - (Jensen 2012). * Total Kjeldahl Nitrogen (TKN)

Green Stream

Wastewater streams within an abattoir are classified as either red or green streams. The green stream consists of the stomach contents of processed animals (i.e., paunch) – grass, dry feed, grain etc. and the water which transports it. Other contributions to the green stream are made from tripe processing. Solid paunch waste removed from the green stream is collected onsite and periodically transported to landfill incurring handling and disposal costs. Whether the paunch is from grass or gain fed cattle, the material exhibits >92.9 volatile solids as a percentage of total solids (Dowd, McDonnell & Tuohy 2022) and up to 95.89% VS/TS as reported in Table 1, and as such retains a high potential for decomposition. The green stream contains higher concentrations of total nitrogen (TN) and fats, oils, and grease (FOG) than the red stream, however the separation of the paunch solids from the stream reduces the nitrogen concentration. The green stream contributes approx. 67 – 92% of the total COD as a percentage of the combined streams (Table 1). This wide range for COD contribution can be attributed to data sourced from different plants with varying operations and treatment processes.

Red Stream

The red stream is a combination of flows from various abattoir processes. It contains flows from the slaughter floor, boning room, and other plant processes with bloodmeal stickwater being a major contributor to the stream. Stickwater is the liquid portion of coagulated blood which has been mechanically

separated into solid and liquid phases. The solid phase is processed into bloodmeal – a protein rich saleable by-product, however the liquid phase is diverted through to the onsite wastewater treatment systems. Stickwater characteristics can vary with respect to the time of day and the process stages– whether at startup or shutdown of either the cooking process or production floor operations, and the age of blood i.e., aged blood can exhibit better coagulation and protein recover during separation, resulting in a 'cleaner' stickwater with fewer totals solids. The quality of stickwater can be affected by process parameters such as steam coagulation pressure and temperature setpoint, temperature of initial blood for coagulation, cleanliness, and setup of decanting centrifuge, and the quality of blood collection and mixing.

2.3.2 Feedstock Characteristics

Total and Volatile Solids

Total solids (TS) is the sum of the total suspended solids and the total dissolved solids, and is the measure of the solid percentage of either a liquid or solid material with all moisture removed, sometimes referred to as the dry matter (Metcalf & Eddy, Burton & Stensel 2013). Wastewater streams in liquid viscosities will have a TS of between 1 – 2%, slurry like material such as sludge will exhibit TS in the range of 3 – 4%, and solids will have a TS greater than 7% (Angelidaki 2011). Different wastewater streams within an abattoir will have majorly varying TS values ranging from boning room wastewater consisting primarily of knife sterilisation water at 0.1%, to raw blood at 11%. Being a solid material, paunch has a TS up to 33.9% (Table 1). The total solids is an important parameter used to calculate the solids loading rates of treatment processes and to inform the process operation.

Volatile solids (VS) is presented as the percentage of TS and is volatised and burned off when ignited at 550°C. The mass which is volatised is considered to be the measurement of organic matter in the sample, however some organics are not burned, and some inorganic material are volatised at high temperatures (Metcalf & Eddy, Burton & Stensel 2013). The VS portion TS in RMP waste streams can range from between 21% measured in the red stream prior to stickwater addition, to 95.95% for the liquid portion from paunch screening (Table 1). Determining the VS of a waste stream and waste material is important for describing the requirements for effective treatment and reduction of the volatile material, i.e., reduction of organic material required prior to discharging wastewater to receiving bodies. It is also a useful parameter to both determine the organic loading rate of a treatment process in terms of kg VS m^{-3} d^{-1} and to standardise the reporting of methane produced from feedstock material, units being L_N $CH_4 \cdot$ kg VS^{-1}.

Recalcitrance

The presence of recalcitrant material is a major source of resistance for the RMP industry uptake of paunch digestion which can cause a build-up of fibrous material in AD processes (Dowd, McDonnell & Tuohy 2022). Despite having a high organic content and COD, paunch waste from the rumen of cattle is difficult to anaerobically digest in comparison to other feedstocks. Paunch contains lignocellulosic material which is naturally evolved to protect the plant cell structures to protect against cell degradation. Lignocellulosic fibres provide strength to a plant and protection to the inside of the plant cells, protecting the cellulose with a matrix of heteropolymers (namely hemicellulose, pectin and lignin) (Taherzadeh 2008). Although not inhibitive to the AD process, this gives the paunch a resistance to decomposition through microbial and enzymatic breakdown, which is known as biomass recalcitrance (Mudhoo 2012), leaving unrecoverable energy trapped within the material (Dowd, McDonnell & Tuohy 2022). This and the associated low digestibility rate are the foremost hurdles for paunch digestion in terms of biogas yield.

There are some pre-treatment technologies that focus on increasing the bioavailability of paunch contents, thus reducing recalcitrance. Nkemka et al. (2015) was able to increase methane production from paunch by 32% by pre-treating the material in a sodium hydroxide (NaOH) solution for 3days and then increasing the temperature to 100°C for 24 hours. Despite the benefits, the author noted that pre-treatment process to increase the hydrolysis of paunch material is not economically viable. Dowd, McDonnell and Tuohy (2022) also came to the same conclusion in their research regarding pre-treatment.

Chemical Oxygen Demand

The strength of a wastewater stream is generally depicted as the concentration of chemical oxygen demand (COD). It is a measurement of the amount of oxygen to chemically oxidise organic carbon, used to estimate an equivalence of the amount of organic matter present in a waste stream (Metcalf & Eddy, Burton & Stensel 2013). The organic matter present is indicative of the degradation the treatment process must achieve for effective treatment. Abattoir liquid waste streams can have COD concentrations up to 8,500 $mg \cdot L^{-1}$, paunch material up to 433,000 $mg \cdot L^{-1}$, gut material can be towards 534,000 $mg \cdot L^{-1}$, and DAF sludge float in the range of 1,000,000 $mg \cdot L^{-1}$ (Table 1). In comparison to municipal sewage containing typical concentrations of around ~400 – 600 $mg \cdot L^{-1}$, abattoir wastewater is considered extremely high in terms of strength (Johns 1995; Pons et al. 2004). As noted in section 2.3.1 – abattoir feedstock material, these extremely high strength wastes are diverted away from wastewater for processing or disposal, reducing the overall waste stream COD concentration to 5-12,000 $mg \cdot L^{-1}$. Like VS, COD is also a useful parameter to report on the solids loading of a digester (as kg COD $m^{-3} \cdot d^{-1}$) and to describe the methane production in terms of $L_N\ CH_4 \cdot kg\ COD^{-1}$.

Macronutrients

Research notes that the carbohydrate, protein, and lipid fractions make up the majority of abattoir feedstock materials and affects the methane percentage in the biogas produced (Astals et al. 2014; Nwokolo et al. 2020). Paunch is high in carbohydrates and low in protein, whereas stickwater has opposing features (Astals et al. 2014). These qualities correlate to the feedstock analysis of total carbon and nitrogen contents, carbohydrates being measured in total organic carbon, and protein content represented as total nitrogen. The theoretical methane yield from each macronutrients found in abattoir streams is 370 mL_N $CH4 \cdot g\ VS^{-1}$ for carbohydrates, 740 mL_N $CH4 \cdot g\ VS^{-1}$ for proteins, and 1,014 mL_N $CH4 \cdot g\ VS^{-1}$ for lipids (Buswell 1930; Wan et al. 2011). However, be reminded these values reported are theoretical and achieving the full AD degradation into methane can be difficult due to specific digestibility of feedstocks, the rate of degradation in relation to process designs, and the possible presence of inhibitory environments. Different abattoir waste streams exhibit different macronutrient characteristics, for example paunch being high in carbohydrates correlated with high carbon content, blood being high in protein correlated with high nitrogen content, and DAF sludge being high in lipids comprised of FOG (Jensen 2013). These macronutrient characteristics and their ratios in feedstock material play a vital role in the methane generation process and degradation of waste materials. The varying macronutrient makeup of each waste stream gives characteristics that have dictated the treatment required to process each respective stream.

Carbon-to-Nitrogen Ratio

Both carbon and nitrogen are vital to microbial cell growth and cell functions. The ratio of carbon to nitrogen (C:N) in AD systems is an important parameter to monitor and control, contributing to the process health and overall performance. Ammonia is produced within the AD process due to the degradation of nitrogenous matter and is utilised in the synthesis of amino acids, proteins, and nucleic acids (Khanal et al. 2019). Carbon on the other hand, is necessary as a structural unit for cell growth and is an energy source for microbes (Khanal, Tirta Nindhia & Nitayavardhana 2019). Too much carbon resulting in a high C:N ratio indicates there is not sufficient nitrogen to support the function of cells, slowing microbial growth and reduces biogas production (Tg et al. 2022).

Monnet (2003) reports the optimum C:N ratio is between 20 – 30, however Airton Kunz (2022) indicates this is the required range for methanogenesis, and a range of 10 – 45 is ideal for hydrolysis and acidogenesis. To cater for the whole AD process, Hoover (2016) recommends maintaining a C:N ratio between 16 – 25 for optimum efficiency. Galván et al. (2021) found that a C:N of 15 increased biogas by 41% and methane by 25% compared to C:N ratio of 20.

Feedstocks with a low C:N ratio can cause increased ammonia production and can inhibit methane production (Hoover 2016). Abattoir waste streams are generally high in nitrogen, drastically lowering the C:N ratio well below optimum levels (Harris & McCabe 2020). The presence of ammonia in high concentrations is an implication of abattoir wastewaters, as methanogenic activity may reduce up to 56.5% at concentrations of 4,051-5,734 mg L^{-1}, and growth cycles may be halted at higher concentrations (Chen et al. 2008). Paunch waste however, has a low nitrogen content resulting in a high C:N ratio (Jensen 2013). The co-digestion of the materials of high and low C:N ratios is used to balance the C:N ratio for optimum degradation of feedstocks and biogas creation (Aworanti et al. 2023; Hoover 2016; Monnet 2003).

Trace Elements

In addition to macronutrients that biodegrade into biogas, micronutrients, referred to as trace elements, are essential for healthy biological life in the digestion process. The biological organisms in the AD process utilise trace elements for cell growth, with iron copper, zinc, magnesium, molybdenum, and vanadium being essential (Airton Kunz 2022). Bayr et al. (2012) recorded the effects of trace element additives to AD experiments and reported that process stability was improved, and the organic loading rate (OLR) was able to be increased without compromising biogas yield whilst biogas production in the control reactor began to decrease after 123 days. Similar results are reported by Demirel (2011), Ek (2011), and Aworanti et al. (2023) where biogas yield is increased as an effect of maintaining optimum trace elements to support process health and stability.

Schmidt (2018) analysed an abattoir wastewater stream with regards to trace elements and found that only two elements were well within the ideal ranges set out in Table 2. Iron and Zinc in abattoir wastewater were characterised as being within the ideal range at 1458 and 159 mg·kg^{-1} TS. Nickel, cobalt, and molybdenum were measured at the low end of the ideal range at 2.4, 0.61, and 1.49 mg·kg^{-1} TS, and manganese, tungsten and selenium were all below the ideal minimum concentration. Results from their research, confirmed by Takashima (1990), shows that the addition of trace elements can help guard an AD reactor against VFA accumulation. Supplementation of trace elements can also regulate AD reactors operating at low temperatures and reactors subject to high fat, oil, and grease (FOG) loading.

Table 2: Ideal ranges for elements attributing to a healthy AD process, adapted from (Lemmer 2008).

Element			***Ideal range***	***Abattoir concentration***
Iron	**Fe**	**mg/kg TS**	750 - 5,000	1458
Nickel	**Ni**	**mg/kg TS**	4 - 30	2.48
Cobalt	**Co**	**mg/kg TS**	0.4 - 10	0.61
Molybdenum	**Mo**	**mg/kg TS**	0.05 - 16	1.49
Tungsten	**W**	**mg/kg TS**	0.1 - 30	< 0.001
Manganese	**Mn**	**mg/kg TS**	100 - 1,500	0.61
Selenium	**Se**	**mg/kg TS**	0.05 - 4	<0.01
Zinc	**Zn**	**mg/kg TS**	30 - 400	159

Inhibition

Wastewater generated in abattoirs is generally difficult to treat not only due to the high strengths, but also for the constituents of the wastewater. The presence of fats, oils, and grease (FOG) typically found in abattoir wastewater can cause physical issues within the process, such as the clogging of pipes and the formation of crusts in lagoons. The adhesion of FOG to sludge inhibits the mass-transfer of nutrients and can float sludge attached to it, resulting in the washing out of sludge (Batstone et al. 2000; Long et al. 2012). As discussed regarding the implications of maintaining a healthy C:N ratio, the inherent activity of a balanced AD process produces ammonia. However, if the feedstock and process become unbalanced and ammonia increases, the methanogenic activity can slow and even halt (Chen, Cheng & Creamer 2008). Also pertaining to a balanced system, if organic loading of an AD reactor is not well managed, there becomes a risk of VFA accumulating, resulting in a lowering of pH below ideal ranges for microbial growth, and can in extreme cases can cause death of the AD microorganisms (Ngan et al. 2020; Vega De Lille 2015).

Methanogens are also subject to inhibition and toxicity from inorganic compounds. Toxicity in AD processes does not fully incapacitate an AD process, rather the presence of the compounds can affect the reaction rates within the process. Some of the chemicals possibly present in abattoir waste streams and their inhibiting concentrations are: sodium >3,500 $mg \cdot L^{-1}$, potassium >2,500 $mg \cdot L^{-1}$, calcium >2,500 $mg \cdot L^{-1}$, magnesium > 1,000 $mg \cdot L^{-1}$, ammonia-nitrogen >1,650 $mg \cdot L^{-1}$, and sulphide >200 $mg \cdot L^{-1}$. Other inhibitory and toxic compounds to the AD process not specific to abattoirs are copper, chromium (Cr(VI and Cr(III)), nickel, and zinc. There are a number of organic compounds that are also harmful to AD (Metcalf & Eddy, Burton & Stensel 2013).

2.4 Anaerobic Digestion Process control in Red Meat Processing Plants

The efficacy of the AD process can be supported through the optimisation of process designs and parameters to enhance process stability and increase biogas production. Parameters that are particularly influential to anaerobic digestion include process temperature and pH, and feedstock composition including carbon to nitrogen (C:N) ratio and trace element concentrations (Abbasi et al. 2012; Airton Kunz 2022). Each parameter has an optimum, healthy, and extreme range. Outside of extreme ranges, AD systems may face irreversible microbial death and require re-inoculation. By optimising the process for desirable conditions, the health of the microbes can be maintained, and the longevity and productivity of an AD reactor can be enhanced.

Hydraulic & Solids Retention Time

Effective anaerobic degradation relies heavily on the correct sizing of the treatment system to provide sufficient residence time for the solid and liquid fractions of wastewater (Metcalf & Eddy, Burton & Stensel 2013). The average time liquid is held in the system is the hydraulic retention time (HRT), measured in days (Equation.1). Correct design for HRT enables slow growing methanogens to multiply in the digester while an increase in HRT leads to higher degradation of organic matter (Preethi et al. 2022; Romero Garcia et al. 2021). Minimum HRT times are required to achieve sufficient treatment of the wastewater and can affect the volatile solids destruction %. Ideal ranges for HRT depend on the treatment technology employed and the operating temperature. For example, the required HRT for a completely mixed digester at 35℃ (considered "high-rate") is between 10-15 days, whereas "low-rate" digesters require a much larger HRT of 40 – 50 days (Abbasi et al. 2012). These low-rate digesters are poorly mixed and operate at lower temperature which effects treatment efficiency. Some unmixed and unheated anaerobic lagoons require HRT up to 120 days (Pal 2017). Research by Harris and McCabe (2020) proposed that some low technology AD solutions such as anaerobic lagoons commonly utilised in RMP plants are prone to reducing their HRT. Over time, they can develop dead space in the form of a floating crust and settled sludge. The crust layer developed in abattoirs consists of uncaptured FOG and if added to the digester, paunch material. This, as well as sludge accumulations, reduce the effective volume of the reactor, thus reducing the HRT and treatment capacity (Figure 2).

Equation 1: Calculating the hydraulic retention time.

$$HRT\ (days) = \frac{Volume\ of\ reactor\ (m^3)}{Flowrate\ (m^3 \cdot d^{-1})}$$

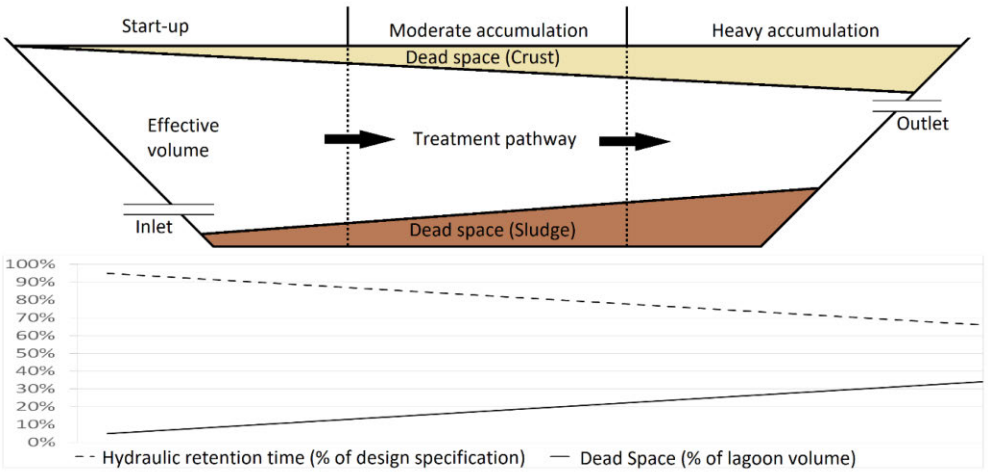


Figure 2: The accumulation of crust and sludge layers reduce the volume of an anaerobic lagoon, reducing the HRT (Harris & McCabe 2020).

The average time the solids are held in the digestion process is the solids retention time (SRT), also measured in days. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages are directly linked to SRT and each require a minimum SRT. An increase in SRT increases the extent of each of these stages and vice versa. If SRT drops below the minimum requirements for each stage, the AD process will eventually fail (Metcalf & Eddy, Burton & Stensel 2013). Biogas production can be hindered with a SRT of less than 10 days as the microbiome is less stable. Sustaining a higher SRT will maintain the biodiversity of AD microorganisms, increasing process resilience and stability (Zhang et al. 2022). To calculate SRT for soluble substrates, the mass of the digester can be divided by the mass of solids removed daily. In conventional low-rate digesters and continuously stirred reactors, solids are not retained in the system, and as such, the SRT = HRT. High-rate systems are able to achieve SRT up to three times HRT by retaining solids and micro-organisms with attached or suspended growth treatment processes (Abbasi et al. 2012).

Organic Loading Rate

The organic loading rate (OLR) is the mass rate at which substrate is added into a treatment reactor per unit of volume (Metcalf & Eddy, Burton & Stensel 2013). The measurement of OLR is kilograms of either volatile solids or chemical oxygen demand added per cubic meter of reactor volume per day, as highlighted in Equation 2 (kg $VS \cdot m^{-3} \cdot d^{-1}$ or kg $COD \cdot m^{-3} \cdot d^{-1}$) (Airton Kunz 2022). Organic loading rates for different processes vary and are specific to each to the technology employed , the temperature, and feedstock characteristics (Schmidt 2019). Reactors with a high OLR generally will have a lower feedstock degradation efficiency (Harris & McCabe 2020; Schmidt 2019; Taherzadeh 2008). Typical VS feed rates in anaerobic lagoons for cool, warm, and hot climates as reported by Australian Pork Limited (2015) is 0.45, 0.6, and 0.75 kg $VS \cdot m^{-3} \cdot d^{-1}$, and COD loading is less than 2 kg $COD \cdot m^{-3} \cdot d^{-1}$ (Metcalf & Eddy, Burton & Stensel 2013). Organic loading rates for mixed mesophilic digesters is typically 1 - 2 kg $VS \cdot m^{-3} \cdot d^{-}$ (Batstone & Jensen 2011) and less than 4 kg $COD \cdot m^{-3} \cdot d^{-1}$ (Metcalf & Eddy, Burton & Stensel 2013), whereas high-rate thermophilic processes are able to achieve an organic loading between 5 – 9.7 kg $VS \cdot m^{-3} \cdot d^{-1}$ (Vandevivere et al. 2002). Organic loading of abattoir wastewater can be 2-4 times greater than reported literature, indicating an imbalance of reporting and correct representation of the various processes in the industry (Jensen et al. 2014). The variance in abattoir OLR is highlighted by Pittaway (2011) who reports research on eight abattoir AD processes witch revealed OLR ranging from 0.0125 to 3.4 kg $VS \cdot m^{-3} \cdot d^{-1}$. Theoretical calculations on waste activated sludge completed by Hoover and Porges (1952) provides a COD/VS relationship ratio of 1.42 for estimation of one parameter from the other. (Ahnert et al. 2021) proposes the theoretical COD/VS ratios for protein, carbohydrate, and lipid materials to be 1.4-1.5, 1.07-1.18, and >2 – typically 2.9. A relationship between the two values can be established using site-specific laboratory analysis.

Schmidt (2019) investigated the effects of season temperature fluctuations on abattoir wastewater biogas production and showed that a temporary increase in OLR increased biogas yield. However the increase in OLR decreased HRT which saw an accumulation of VFA, leading to the failure of the anaerobic process. Taherzadeh (2008) also reported seeing a temporary increases in biogas production at higher OLR before AD failure. The maximum OLR causing failure in AD systems is unique to each process, temperature, and feedstock composition, with failure at maximum OLR presenting as either ammonia overload, excessive VFA/low pH, or the accumulation of inhibitory substances (Vandevivere, Baere & Verstraete 2002).

Equation 2: Calculating the organic loading rate in a reactor.

$$OLR\ (kg_{VS} \cdot m^{-3} \cdot d^{-1}) = \frac{Flow\ rate\ (m^3 \cdot d^{-1}) \times VS\ concentration\ (kg \cdot m^{-3})}{Reactor\ volume\ (m^3)}$$

$$OLR\ (kg_{COD} \cdot m^{-3} \cdot d^{-1}) = \frac{Flow\ rate\ (m^3 \cdot d^{-1}) \times COD\ concentration\ (kg \cdot m^{-3})}{Reactor\ volume\ (m^3)}$$

Temperature

Optimisation of temperature enhances biogas production, decreases the required HRT and increases the OLR potential (Abbasi et al. 2012; Airton Kunz 2022). Anaerobic digestion is commonly conducted at ambient temperature or optimised for mesophilic or thermophilic microorganisms with optima at 37°C and 55°C respectively (Figure 2). Temperature affects microbial growth rates (van Lier 1997) and is directly linked with microbial metabolism and, consequently, optimising microbial metabolism is achieved by optimisation of process temperature (Teferra & Wubu 2018). Below optimum temperatures, microbial metabolism slows and results in reduced capacity for microorganisms to consume organics, and the process may destabilise (Schmidt 2019). Psychrophilic temperatures at 15°C experience low microbial growth rates, and can upset the symbiotic balance (Airton Kunz 2022). Too high above optimum temperature will result in microbial death and process failure.

Abattoir wastewater typically contains residual heat from hot water washdown, knife sterilization, steam processes and other plant operations. Although the inclusion of hot water contributes to increasing the digestion temperature, the ambient temperature of the reactors generally operates below the mesophilic optimum. This operation range increases the required HRT for sufficient waste treatment and can lead to the formation of a crust layer which floats on the surface of the lagoon. Crusts in an RMP AD lagoon made up FOG from beef grease and tallow are not readily digested and can reduce the usable volume in the reactor

(Harris & McCabe 2015). Beef grease and tallow has a melting point between 36 – 42 °C, meaning the operation of a CAL can be better managed and the longevity of the treatment pond is ensured under optimum mesophilic conditions (Harris & McCabe 2020). As most abattoir AD processes are anaerobic lagoons without a heating source, designation as being a mesophilic process is generally accepted in Australian conditions, but seasonal temperature changes can cause the treatments to operate well below optimum mesophilic conditions.

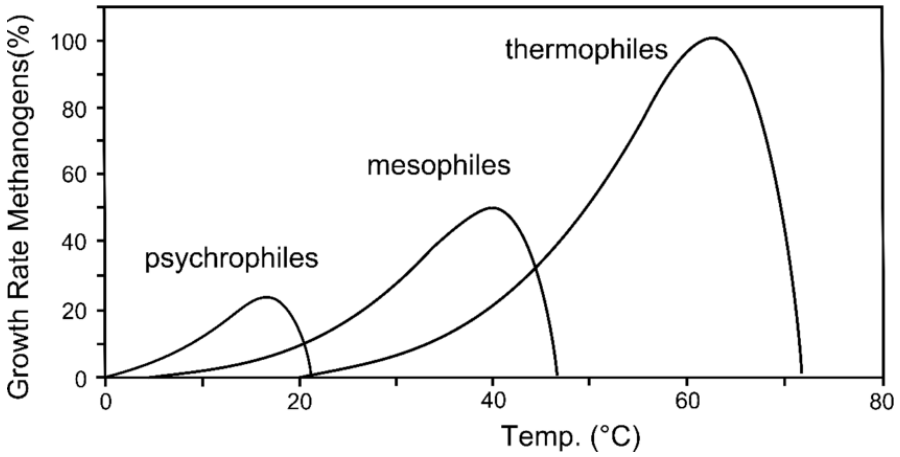


Figure 2: Growth rate of methanogens at different temperature profiles (van Lier 1997).

Some AD treatment plants are designed and operated with a heat source to maintain an operational temperature. Such plants are optimised for temperature and as a result, biogas yield is increased, degradation of feedstock can be increased, organic loading rates can be increased, and overall time for digestion can be decreased (Abbasi et al. 2012; Airton Kunz 2022). Other plants do not have temperature control and rely on any residual heat in the waste stream as well as ambient climatic temperatures dictated by the plant's geographical location. Ambient climatic temperature incorporates seasonal changes which can have dramatic effects on the performance of AD (Schmidt 2019). Many low-rate systems such as anaerobic lagoons are typically operated under ambient conditions without a source of active heating, relying on passive heating through solar radiation and heat from incoming waste streams to warm the digester. These systems are consequently incapable of maintaining optimal conditions resulting in decreased biogas yield (Harris & McCabe 2020). Some plants may not require temperature control as year-round climate averages can be commensurate with ideal treatment processes (i.e., mesophilic ranges), whereas plants in locations where temperatures can drop below freezing during wintertime would witness decreased treatment efficiency. It is noteworthy that temperatures do not change the biochemical methane potential of a substrate, rather the rate of degradation and methane generation (Holliger et al. 2016). Schmidt (2019) investigated the impact of temperature on an anaerobic lagoon operating at an RMP facility operating in Queensland, Australia. By comparing a difference in operating temperature of 25 to 38°C (Figure 3), the

authors noted the higher temperature improved process stability, enabling an increase in organic loading rate of up to 23% volatile solids (VS) and 36% chemical oxygen demand (COD), and increasing biogas yield from 40% up to 80%. Similar results have been reported by Deago et al. (2023) and Babaei A (2019). Consequently, recycling of waste heat on-site at an abattoir may yield significant benefits to lagoon systems.

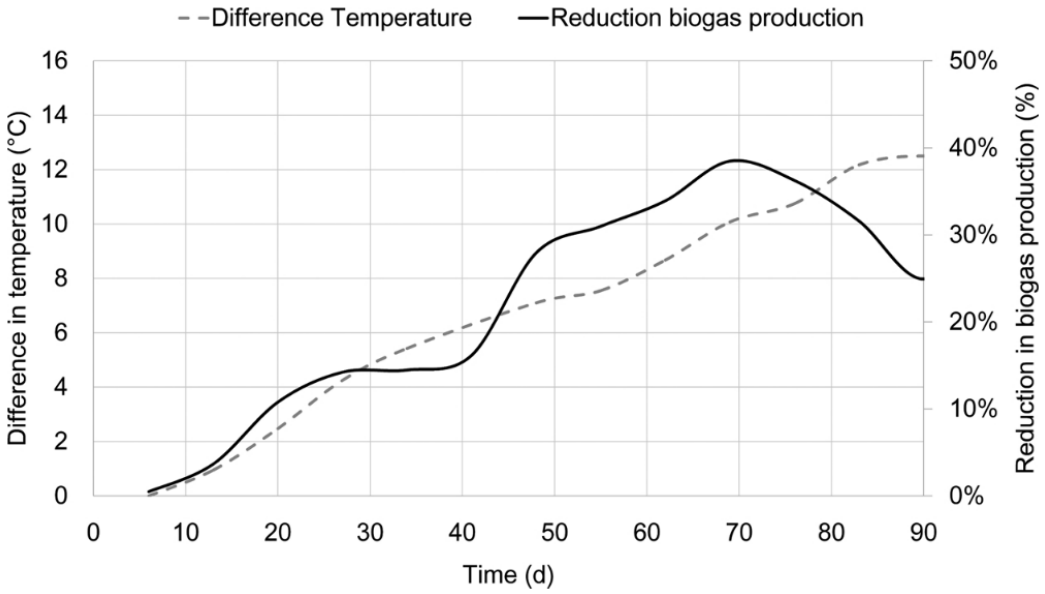


Figure 3: Effect of process temperature drop on biogas production (Schmidt 2019).

pH & Alkalinity

Control over process pH is critical to prevent digester failure. Anaerobic digestion can be broadly categorised into two stages, fermentation and methanogenesis, which differ greatly by optimum pH. The fermentation stage incorporates the hydrolysis, acidogenesis and acetogenesis steps (Figure 1), with acids and acetic acid being the major products in this stage. Consequently, while fermentative micro-organisms are capable of surviving at a neutral pH, they are adapted to an optimum pH of 5.7 – 6.5 (Yu 2002). By comparison, the methanogenesis stage occurs at an optimum pH of 7 with an extreme pH range of 6.6 – 8 (Monnet 2003; Ngan et al. 2020), outside which the methanogenic consortium will die. Consequently, an AD process in a single tank cannot be optimised for both processes and is typically operated at a pH of 7.

Correct management and control of the pH within an AD process is also linked to increased biogas yield at optimum pH concentrations (Vega De Lille 2015). Experimenting with varying substrate pH by Gopal (2021) revealed a higher methane production at a pH 7.2 than 6.8 and 7.4 concentrations. The effect of pH on methane production by Liu (2008) confirms the increased yield experienced when the digester is operated in optimum conditions (Figure 4).

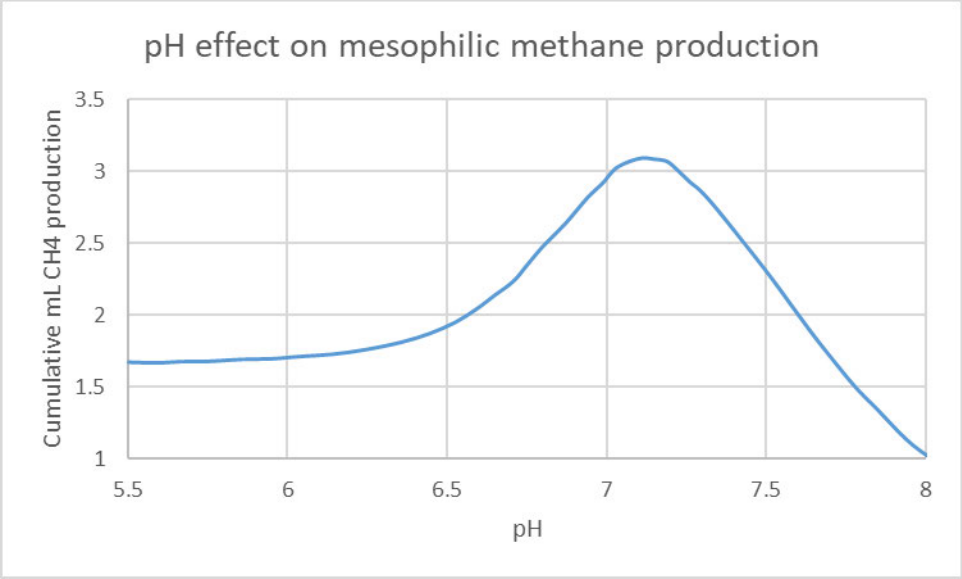


Figure 4: Effect of pH on methane yield, adapted from Liu (2008).

Alkalinity represents the digester's capacity to buffer acids (i.e., VFA), consequently resisting changes in pH and promoting process stability. Buffering capacity is necessary in the process considering acidogenic bacteria grow relatively faster than methanogens, creating VFA imbalance tending to lower pH (Anderson & Yang 1992). Total alkalinity (TA) is a measure of concentration of mainly carbonates, bicarbonate, and hydroxide compounds expressed as $mg \cdot L^{-1}$ of calcium carbonate ($CaCO_3$). The bicarbonate ion (HCO_3^-) is the primary source of buffering to maintain a pH of ~7 (Labatut & Pronto 2018). As pH changes and concentrations of hydrogen (H) and hydroxide (OH) ions fluctuate, the conjugate acid/base conversions of buffering agents occur (Moosbrugger 1993). The concentration of TA acts towards providing a buffer against changes in pH by neutralising acids, with higher concentrations of 1,500 – 5,000 mg L^{-1} $CaCO_3$ giving greater resistance against pH change and contributing to high process stability (Schnaars 2012). For example, carbonic acid dissociates into bicarbonate i.e., $H + HCO_3^- \leftrightarrow H_2CO_3$ and mitigates pH change (John Moore). Consequently, sufficient buffering capacity gives an AD operator a degree of control over the process and enables much higher loading of organics to increase biogas yield.

The ratio of VFA to TA (VFA:TA) is a critical tool in measuring process stability. In a well buffered system, the accumulation of VFA will occur with minimal change in pH. Consequently, utilising pH as a monitoring tool can be insufficient. Routine measurement of VFA:TA allows an operators to identify increasing acids relative to buffering capacity and react before a substantial change in pH occurs. The optimum VFA:TA ratio is between 0.3 and 0.4. Values above this range indicate the system is overloaded, and below indicates an underloaded system (Airton Kunz 2022). As the acidogenic bacteria's growth rate is higher than that of methanogens, if an AD process was to become overloaded, VFA will accumulate and

lower the pH. This will inhibit methanogens and VFA's will quickly rise, lowering pH further (Ngan et al. 2020; Vega De Lille 2015).

2.5 Anaerobic Digestion Technologies

Different AD treatment processes are available for consideration when exploring feedstock digestion for biogas production. The theory behind all process technologies is the same (Shende & Pophali 2021), however they are designed with varying degrees of engineering to exploit one or more AD process parameters in order to manipulate biodegradation favourably (Monnet 2003). The wide array of technologies caters for different feedstocks and are designed to treat organic waste material in either 'wet' or 'dry' AD process conditions. Anaerobic digesters are characterised by their feeding regime – batch or continuous, form of feeding – upward or laminar, total solids concentration of feedstock – wet digestion <10%, semi-solid 10-15% and solid (dry) digestion >20%, optimum temperature – psychrophilic (15°C), mesophilic (37°C), or thermophilic (60°C), and lastly the mixing intensity – complete, partial or no mixing (Airton Kunz 2022). Consideration for a suitable AD process for abattoir wastewater treatment is designed for the characteristics of feedstock material present.

The design of an effective solution is based on parameters such as temperature, OLR, HRT, and SRT to provide ideal organic reduction and methane production rates (Saha et al. 2020). There exists a wide variance in OLR, HRT, and SRT between technologies which correlate to the ability of mixing, attached biofilm growth capability, feeding style, and the capacity to retain biomass. Besides the popular anaerobic lagoon, digester technologies typically utilised in abattoirs are completely stirred tank reactor (CSTR) (Nkemka, Marchbank & Hao 2015), up-flow anaerobic sludge blanket (UASB), anaerobic baffled reactor (ABR), anaerobic filter (AF), anaerobic hybrid reactor (AHR), anaerobic fluidised bed biofilm reactor (AFBBR), anaerobic sequential batch reactor (AnSBR), and anaerobic membrane bioreactor (AnMBR) (Shende & Pophali 2021) (Table 3). Note that OLR in Table 3 are presented in terms of COD, comparison of OLR's in terms of VS can be calculated by establishing a relationship between COD/VS similar to the theoretical proposition by Hoover and Porges (1952).

Different treatments also will have vastly different footprints which will affect suitability, anaerobic lagoons require large areas to achieve the high retention times and may not be practical for some abattoirs, compared to compact high-rate treatment options. Operability and maintenance aspects also increase with the increasing technologies. Anaerobic lagoons require little maintenance, whereas high-rate reactors will require greater attention to maintain both the equipment and the process parameters to ensure a stable process (Airton Kunz 2022). For example, UASB reactors can experience sludge blanket washout if the

flowrate is increased, lowering the HRT (Romero Garcia et al. 2021), whereas processes with attached biofilms are more resistant to shock loading due to the increased density of biomass and resistance to washout (Pittaway 2011). Treatment technologies incorporating mixing in their design increase their rate of degradation and increase biogas production. This is achieved by keeping the solids in suspension as a homogeneous mixture with the anaerobic biota, having a positive effect on methane production rate by facilitating and enhancing the interaction between the feedstock and anaerobic microorganisms (Lindmark et al. 2014).

Table 3: Different AD reactors suited to treating abattoir waste streams. Adapted from Metcalf & Eddy, Burton and Stensel (2013), Nasir et al. (2012), Shende and Pophali (2021), Technologien and Wirtschaftsberatung (2001), and Wang et al. (2016).

Digester Type	Operation	OLR (kg COD·m-3·d-1)	HRT (days)	SRT (days)	Description
Aanerobic lagoons, covered (CAL)	Batch, continuous, semi-continuous	2	20-50	50-100	Unmixed and unheated with suspended/ floculating biomas. Capable of handling a wide variety of feedstodks.
Completely stirred tank reactor (CSTR)	Batch, continuous, semi-continuous	4	15 - 30	= HRT	Completely mixed system to treat wastes ustilising suspended anerobicbiomass. As the system is fully mixed, the sludge does not accumulate and is washed out in the effluent.
Up-flow anaerobic sludge blanket (UASB)	Continuous	5 - 20	4 - 20 hours	20-30	Influent is fed from the bottom and flows upward through the sludge blanket made up of suspended solids and bacterial growth.
Anaerobic baffled reactor (ABR)	Semi continuous, continuous	0.62 - 10	2.5 hrs - 3.8 days	>30	Contains a series of chambers with vertical baffles to ensure sequiential flow through the reactor, icreasing feedstock contact time with microorganisms.
Anaerobic filter (AF)	Continuous	0.9 - 20	1 - 5	1 - 10	Unmixed system. Influent feedstock flows through submerged fixed media with attached biofilm. Media accounts for 50-70% reactor volume.
Anaerobic hybrid reactor (AHR)		*Varying, dependant on design*			A combination of anaerobic technologies employing a combination of UASB and anaerobic filter to achieve a high biomass concentration and high organic removal rates.
Anaerobic fluidised bed biofilm reactor (AFBBR),	Continuous	20 - 54	2 - 4		Biomass growth is attached to small inert particles to form a dense sludge mass which is suspended by high upward velocities. Suitable for easy to degraded small particulates.
Anaerobic sequential batch reactor (AnSBR)	Batch	0.9 - 2.4	6 - 24 hours	50 - 200	A mixed suspended growth reactor with solids/liquid separation in the same vessel. The process sequences between feeding, reacting, settling, and decanting effluent.
Anaerobic membrane bioreactor (AnMBR)	Semi continuous, continuous	5 - 15	2 - 4	high	A mixed reactor with suspended biomass and a synthetic membrane for liquids/solids separation of effluent, including sludge recycle.

Some dry digestion technologies are suitable for feedstock with varying characteristics and can handle higher organic loading rates such as solid wastes (i.e. paunch), however they exhibit lower

degradation rates and typically produce lower biogas yield compared to wet digestion technologies processing the same feedstock material (Rocamora et al. 2020). In leachate bed reactors, an inoculum of anaerobic bacteria (percolate) is sprayed over the material to initiate decomposition (Fu et al. 2018). The container/vessel remains a closed system until the digestion process has completed. Other dry digester designs may include a plug flow operational design, where intermittent feeding of new feedstock material caused the outfeed of the same amount of digested material (Bristola 2023).

2.6 Co-digestion of Abattoir Feedstocks

An opportunity exists to enhance methane production of a mixture, surpassing that of individual feedstocks by establishing a synergistic relationship (Astals et al. 2014). The synergistic relationship created by mixing two or more feedstocks for simultaneous anaerobic digestion is called co-digestion. Ideally, the feedstocks considered for co-digestion will have complementary characteristics as their properties combine to create a mixture favourable for AD in terms of: C:N ratio, nutrient concentration, dilution of inhibitive qualities, and optimising the moisture content for improved digestibility and biogas yield (Jensen 2013). Depending on the individual feedstock characteristics, the interactions of their compositions, and the ratio of mixing, biogas production can be enhanced anywhere from 25 – 400% (Shah et al. 2015). This broad claim covers co-digestion in all its facets, including procuring and supplementing with organic feedstocks from outside an abattoir's waste stream, the co-digestion of paunch within the existing process, isolated co-digestion mixtures, and feedstock mixing for pretreatment.

Abattoirs have numerous opportunities to combine waste streams for co-digestion; Harris, Schmidt and McCabe (2018) were able to demonstrate a 7.08% increase in methane yield by mixing bovine bile with DAF sludge; waste activated sludge (WAS) can be co-digested with FOG material to potentially increase biogas yield of the WAS AD by 350% (Li et al. 2011); mixing blood and cow manure with paunch waste, Thomas et al. (2022) achieved a 37% increase in methane production from paunch material. A co-digestion study of paunch and DAF float by Jensen (2013) revealed that all co-digestion mixtures of the feedstock materials resulted in increased methane production. Specific mixing of feedstocks to create ratios of 50% carbohydrates to 50% lipids, and also 17% carbohydrates, 17% lipids, and 66% protein produced methane 15% higher than prediction models. The presence of paunch in abattoir AD has a general inclination to increase biogas production due to the introduction of carbohydrates, balancing the C:N ratio for more idealistic ranges (Astals et al. 2014).

Safeguarding of the AD process against ammonia and LCFA inhibition is an additional beneficial result from effective feedstock mixing of feedstocks (Astals et al. 2014). Jensen (2013) observed a synergistic effect from the mixing of highly degradable abattoir waste material with slowly degradable

paunch material. The author reported an improvement in the process kinetics as a result of the feedstock mixture mitigating LCFA inhibition and also improved methane yield by the mixing of paunch with sludge from dissolved air floatation (DAF) treatment process.

2.7 Specific Methane Potential

Understanding a waste stream's methane production potential is instrumental for the industry in the move towards carbon neutral 30 – CN30. As different feedstocks degrade at different rates and yield varying degrees of methane, a test procedure is required to determine and differentiate methane production. The biochemical methane potential (BMP) test is a laboratory scale analysis used to determine the specific methane potential (SMP) of a feedstock material under anaerobic conditions (Bioprocess Control 2014). The use of BMP testing is appropriate for determining the SMP for singular or mixed feedstocks and is standardised to allow comparison of the SMP results of other experiments. The specific methane potential (SMP) of a substrate is a critical parameter for determining economic viability. This parameter refers to the amount of methane that can be produced per unit of organic material, reported in mL of methane under normal conditions (i.e., 20°C and 101.325kPa) per gram of volatile solids. For example, the SMP of cattle manure can be between 84 – 100, dairy manure – 240, food scraps – 290, and food grease – 810 $mL_N\ CH_4\ g^{-1}$ VS (B. Moody et al. 2011). This variance is likely due to the distribution of macronutrients within the feedstock. Each macronutrient has a theoretical methane potential, being 370 $mL_N\ CH_4\ g^{-1}$ VS for carbohydrates, 740 $mL_N\ CH_4\ g^{-1}$ VS for proteins, and 1,014 $mL_N\ CH_4\ g^{-1}$ VS for lipids (Buswell 1930; Wan et al. 2011). The SMP for the individual waste streams of an abattoir can vary widely from 50 up to 650 $mL_N\ CH_4\ g^{-1}$ VS as reported by (Ware & Power 2016) and the mixed waste streams can have methane yields ranging from 200 to over 1,000 $mL_N\ CH_4 \cdot g\ VS^{-1}$ dependant on the individual plant's waste stream makeup (Jensen 2013). Applying the COD/VS ratio of 1.42, this corresponds to approximately 284 – 1,420 $L_N\ CH_4 \cdot kg\ COD^{-1}$.

Chapter 3 Methodology

3.1 Case Study Data Collection

This case study is based on the data and waste streams generated form and Australian beef abattoir slaughtering 800 – 850 head per day. Accuracy of the data collection is pertinent to the quality of the BMP results, and in turn feeds the ability of the data to reliably inform decisions regarding real world applications. To design the BMP experiments and accurately calculate the biogas capabilities of stickwater and paunch material, careful collection of site-specific data was obtained. Site-specific data is essential for scaling the results obtained from the BMP experimentation to actual waste production rates and enables any findings to be applied to real work applications.

To ensure that the feedstock data collected is relative, care must be taken to ensure that the units are standardised to abattoir operations. Reporting in units typical of the industry enables results to be applicable and relatable. Site data from the case study site is collected daily and reported weekly, with units for solid material reported in kilograms or metric tonnes, and liquids being in cubic meters (NB. m^3 = kL). To be useful to industry, the data must be comparable with a parameter which corresponds to the production rate of the facility. Data obtained from the case study is commonly collected on a per head slaughtered basis, but as the weight of each beast is variable with respect to seasons, breed, climatic conditions, and origin, results from this research will be presented as per (unit) of hot standard carcass weight (HSCW).

3.1.1 Paunch & Stick Water Volumes

Paunch

Due to a change in process during the data collection phase of the research, paunch was removed daily from site rather than bulk removal at irregular intervals. This increased the reliability of determining average daily paunch availability. Data was collected over a three-week period which includes a Saturday half-production shift making up for a public holiday and four days where maintenance downtime prevented full production. Despite these variances in production variables, collected paunch per head is still indicative of the paunch capture rate per head.

Stickwater

In the absence of flowmeters, the stickwater flow was measured with a 20L bucket and the time recorded to fill the bucket. Flow data was recorded at regular intervals throughout regular production to gain data representative of normal operation. Liaison with the plant operator was undertaken to gain insight

into the plants operation to increase confidence that the data recorded was representative of normal plant operations and that of a typical day's production.

3.1.2 Historic Data

Historic data for CAL temperature and paunch volumes was used to inform the design of the BMP experiment and to verify the results are relevant. To determine the ambient temperature conditions for the BMP experiment design, the average temperatures of the case study's CAL were used. Temperature of the effluent leaving the CAL was recorded daily by the wastewater treatment operator. The yearly average was used to determine the ambient temperature for the BMP testing.

Historic records of paunch data from the case study site consisted of the periodic transportation of stockpiled paunch to landfill. This method required the tonnage removed to be averaged out per head or tHSCW over the collection period. Also necessitating the need for historic cross-reference of data is the poor operation of the paunch press for the majority of 2022. For this reason, recent daily paunch collection per head is verified against 2020 – 2021 data for annual paunch removed and head slaughtered.

3.2 Sample Collection & Storage

The German standard VDI 4630 (2016) – Characterisation of the substrate, sampling, collection of material data, fermentation tests, provides a comprehensive methodology for the proper sampling, handling, preparation and assessment of samples. For a sample representative of the process and available feedstocks, adherence to VDI 4630 (2016) by taking several time-proportional grab samples and combined to produce a composite sample. For this case study, 12 paunch samples and 22 stickwater grab samples were collected over two days to represent the feedstock. The two days were determined to represent normal operation with respect to blood plant operation and the grass/grain fed cattle received during the period being consistent with historic data. Samples were refrigerated initially to lower the temperature to an ideal 4°C to preserve their integrity. The samples' integrity was also ensured by undertaking sampling no more than 5 days in advanced of setting up BMP tests (VDI 4630 2016). Samples were transported in eskies with ice bricks to maintain 4°C during transfer to the bioscience laboratory sample storage fridge. These sampling limitations decrease the effects of time related chemical changes within the samples.

3.3 Feedstock Characterisation

Feedstock characterisation is used to describe chemical and physical properties of a substrate, identify the presence/concentration of potentially inhibiting substances, and to allow the correct process

design, operation, and optimisation. As such, feedstock characterisation is an important factor in assessing a feedstock's ability for methane generation. Physical and chemical analysis are useful to help with the determination of degradable fractions of a feedstock material and to describe how much potential the feedstock has for biogas production (Sören Weinrich 2018). The measurement of these parameters allows the design of AMPTS II experiment and for the correlation of data after BMP has been completed. This will then allow the data to be a more useful guide for future estimations of similar feedstocks.

Characterisation can be used to flag a substrates' likelihood to either be inhibitive to the AD process or whether it will possess ideal parameters for optimum digestion. High level characterisation can be performed by identifying the carbohydrate, protein, and lipid makeup of a feedstock, however this is rarely done and appropriate characterisation for designing BMP experiments is completed by reporting on the TS, VS, COD, VFA, and C:N ratio as per VDI 4630 (2016). Trace elements can also be analysed to ensure assays are within ideal ranges to support AD health and will not cause any inhibition to methane generation.

3.3.1 External Laboratory Analysis

An external laboratory was contracted for the analysis of the feedstock materials. The assays requested were chosen for their ability to characterise feedstock and to give insight into the micronutrient concentration of the materials. The environmental laboratory engaged were not experienced in performing COD analysis on solid samples, so a modified COD method was specified to perform COD analysis on paunch material outside of normally offered testing procedures, aligning with the methods presented in VDI 4630 standard (2016). The modified method for COD involved drying the sample whilst recording the fresh mass and moisture content of the sample, grinding the dry material to a powder, and reintroducing a known weighed sample back into a water solution for COD analysis.

The results obtained from external laboratory analysis were not standardised and consequently were converted to a standard unit ($mg \cdot kg^{-1}$). Laboratory results were reported 'as received' with no correction for moisture content, and units for stickwater from the laboratory were reported in $\mu g \cdot L^{-1}$. Conversion of the units was necessary for the data to be accurately comparable with literature. To carry out the conversion of units, the result needs to be divided by the TS to reveal the $mg \cdot kg^{-1}$ concentration, however the laboratory failed to report on the TS characteristic of the materials presented for analysis. The TS and VS ratio is highly conserved, meaning there is little variation between samples. To exploit this characteristic and to calculate the TS of the sample analysed in the laboratory, in-house TS and COD analysis of the stickwater was performed and a ratio was applied to estimate the laboratory TS, as depicted in equation 3. The result is used to correct the laboratory data into units compatible for comparison.

Equation 3:Determination of lab TS using COD ratios.

$$TS_{lab} = \frac{COD_{lab}}{COD_{inhouse}} \times TS_{inhouse}$$

3.3.2 In-house Analysis

Total and Volatile Solids

Analysis of TS follows standard method 2540 G standard methods for the examination of water and wastewater (Baird et al. 2017). Briefly, crucibles are heated at 550°C for 1 hour in a muffle furnace (Nabertherm P330) to ensure no organics remain and are cooled in a desiccator to ensure no water is present. Crucibles are pre-weighed and subsequently fresh matter is weighed into the crucibles (Figure 5). Samples are dried at 105°C in a laboratory oven (Labec ODWF18) for 24 hours to remove water, resulting in the total solids content (Eq 4). Dry content is combusted at 550°C in a muffle furnace for 2 hours to produce the ash content. The difference between the total solids and the ash content is the volatile solids content (Eq 5). After each instance of heating, samples are cooled to room temperature in a desiccator to prevent hygroscopic action accumulating water weight from atmospheric humidity. The solids lost during ignition of the sample are mostly attributed to the decomposition of organic materials (Baird, Eaton & Rice 2017). It is the inverse of fixed solids and does not differentiate between organic and inorganic matter as some losses in weight can be attributed to the volatilization of mineral salts. For this reason, total organic carbon (TOC) and COD testing is also performed to increase the accuracy of feedstock characterisation.

Equation 4: Determination of solids content.

$$total\ solids, \% = \frac{Mass\ of\ dried\ sample}{Mass\ of\ wet\ sample}$$

Equation 5:Determination of volatile solids content.

$$volatile\ solids, \% = \frac{Mass\ of\ dried\ sample - Mass\ remaning\ after\ ignition}{Mass\ of\ wet\ sample}$$

Figure 5: Paunch, stickwater, cellulose, and inoculum samples prepared for TS/VS analysis.

Chemical Oxygen Demand (COD)

Determination of COD is primarily used for liquid samples, however modifications to the sample preparation method were made for the COD analysis of paunch material. A sample of paunch material was manually chopped with scissors for size reduction, and ground as much as practicably possible with a mortar and pestle. After manual size reduction, a sample of paunch was diluted at 1 in 100 w/w with deionized water. The dilution mixture was then subjected to further size reduction with a handheld stick blender. A stick water sample was diluted with a 1 in 10 w/w mixture for analysis. Both samples were analysed using a Merck Spectroquant Pharo 100 spectrophotometer, and a Merck Spectroquanta 500 – 10,000 COD cell test kit (item code 1.14555).

The COD cell test kits are pre-filled with potassium dichromate ($K_2Cr_2O_7$) to oxidise all oxidizable material. One mL of prepared diluted sample was added via pipette to the test vial, mixed vigorously, and is placed in a thermo-reactor for 120 minutes at 148°C. Samples were then cooled for 10 mins, swirled, and cooled for a further 20 minutes. After cooling, the concentration of Cr^{3+} is measured photometrically to determine the equivalence of COD, with the relationship being 1 mole of $K_2Cr_2O_7$ = 1.5 mole of O_2 and expressed as COD mg/L O_2. To relate the meter reading back to original sample, multiply the result by the inverse of the dilution factor to obtain the corrected value as expressed in equation 6.

Equation 6:COD dilution correction factor.

$$sample\ actual\ COD\ concentration = spectrophotometer\ result \times \frac{sample\ weight}{dilution\ weight}$$

Volatile Fatty Acids

Volatile fatty acids are a component of volatile solids but are commonly lost during the drying phase of TS & VS determination via standard method 2540G. Consequently, VFA must be determined separately and added onto the VS concentration determined by standard method 2540G. The parameters were tested for and determined using a TitrtaLab AT1000 series automatic titrator by HACH. A sample of stickwater was centrifuged and the supernatant was taken for the input into the TitraLab AT1000, which was then automatically titrated with a 0.1N sulphuric acid solution. The equivalence point was not found for stickwater TA analysis as the maximum ordinate point for the sample was reached. For VFA analysis, the results are presented as $mg \cdot L^{-1}$ equivalence of acetic acid ($mg \cdot L^{-1}$ CH_3COOH).

3.4 Biochemical Methane Potential

Biochemical methane potential testing was conducted in-house using an automatic methane potential test system II (AMPTS II) (Bioprocess Controls, Sweden) (Figure 4). The AMPTS largely automates the determination of methane production from an inoculated organic substrate. Digestion temperature was maintained at 37±1°C in a thermostatic water bath housing 15x 500mL reactors with an electric agitator (Figure 6, left). Biogas produced by the system passes through CO_2 scrubbers containing 3M sodium hydroxide (NaOH) solution which removes CO_2 from the gas mixture released from the samples (Figure 6, middle). Methane exiting the system is recorded by tipping counters (Figure 6, right). The system automatically logs data for the volumetric accumulation of methane in normalised mL (mL_N) i.e., 273.15 K (0°C), 1 atmosphere (101.325 kPa) and 0% humidity. To monitor for failure due to acidification, a pH measurement of each reactor was made at the start and ending of the experiment.

Figure 6: AMPTS II system for BMP testing - 3 piece testing equipment (Bioprocess Control 2014).

The method for BMP analysis is congruent with the method description in the AMPTS II user manual, Appendix A. The method is as follows:

1. Determine feedstock TS & VS.
2. Determine inoculum to substrate ratio (ISR).
3. Calculate mass of inoculum and substrate to add.
4. Connect the AMPTS unit to the monitoring computer via ethernet and enter experiment data into the software.
5. Prepare 3M solution of NaOH for CO_2 fixation, with 0.4%Thymolphthalein pH indicator.
6. Fill gas tipper-measuring device with water to indicator line.
7. Start the experiment AMPTS monitoring software.
8. Set up the experiment and enter the substrate parameters.
9. Flip each gas tipper to empty each flow cell and ensure signal is being received by software.
10. Mark up reactors with identification and fill with pre-determined substrate and inoculum.
11. Fasten the stirring stick and mixing motor to each reactor.
12. Install in series, tubing from the reactors to the CO2 fixation bottles to the gas tipping-measuring device. Keeping note to maintain correct connections, i.e., reactor #1, to bottle #1, to tipper #1
13. Ensure that tubes to atmosphere are sealed with correctly installed shut off valves.
14. Set the thermostatic water baths temperatures and allow to come up to temperature.
15. Start the data logging program.
16. Connect power supply to mixing motors and turn on.

3.4.1 Inoculum Sourcing, Preparation, & Inoculum to Substrate Ratio

The inoculum is the anaerobic material used to start the process which contains the necessary cultures of organisms to begin digesting the feedstock. To enable a BMP result representative of large-scale digestion of the feedstock being assessed, an inoculum should be ideally sourced from an existing AD plant processing similar feedstock material (Bioprocess Control 2014). If the feedstock is not currently processed in AD plants or not readily available, digestate from municipal wastewater treatment plants is recommended due to its diverse microbial community (Sören Weinrich 2018).

The inoculum sourced should be sampled as fresh as practically possible, then transported and stored correctly to maintain anaerobic conditions. A fresh sample of inoculum was sourced from a nearby wastewater treatment plant (WWTP). Considering the recommendations to utilise an inoculum adapted to the substrate, the WWTP inoculum was mixed 50% w/w with inoculum originating from a wastewater treatment plant which has been fed on abattoir substrates. This ensures a good quality sludge adapted to abattoir waste and will be suitable for both temperature conditions. Preparation of the inoculum included

straining to remove large inorganic and organic particles and characterising as per the feedstock material by testing and recording the TS & VS using equations 4 & 5.

In preparation for the addition of inoculum to an experiment, the inoculum to substrate ratio (ISR) must first be established. The mixture of ISR is based on the VS of each material, and typical ranges are between 3:2 – 2:1 (Bioprocess Control 2014). An ISR of 1:1 tends to have lower methane yields and 2:1 tends to produce more methane, whereas an ISR of 3:1 has a regulated methane production (Lekgoba & Muzenda 2020). If the feedstock is known to have inhibitive qualities, a higher ISR should be adopted. (Bioprocess Control 2014). For this research, an ISR of 3:1 was accepted to be adequate for compatibility with the feedstock.

3.4.2 Experiment Validation

Biochemical methane potential testing was conducted with both negative and positive controls. To ensure the inoculum exhibits adequate biological activity, a positive control is included in the testing regime to validate the test (VDI 4630 2016). Microcrystalline cellulose was used as a standard test substrate as it has a known specific methane potential (SMP) of 375 mL_N $CH_4 \cdot g$ VS^{-1} and is used as a positive control indicator. For the experiment to be validated, ≥ 80% of the theoretical SMP must be attained.

To isolate the methane produced from the feedstock material, an inoculum blank is included in the AMPTS II experiment as a negative control by taking the difference of methane produced by the inoculum and from the feedstock production per unit of inoculum added (Equation 7).

Equation 7: Calculation to adjust SMP.

$$Feedstock\ mL_N CH_4 g^{-1} VS = \frac{total\ mL_N CH_4 \times (mL_N CH_4\ g^{-1}{}_{inoculum\ added} \times g_{incolulum\ added})}{g\ VS_{added\ feedstock}}$$

3.4.3 Determination of Endpoint

Biochemical methane potential tests can generally run for between 30 to 60 days, depending on feedstock material, inoculum quality, ISR utilised, and the methane producing capabilities of each (Holliger et al. 2016). The test run time is also a function of the experiment's temperature range, considering the heightened activity at optimum temperatures.

The AMPTS user manual determines the experiments endpoint to have been reached when methane production is less than 5 mL per day for three consecutive days. The VDI 4630 (2016) standard stipulates a minimum test time of 25 days and suggests an endpoint indicator for terminating the BMP is when daily

biogas production rate is less than 0.5% of the accumulated volume of methane produced of three consecutive days. Holliger et al (2016) recommends to not predetermine an end point for the BMP test, rather terminating the test when daily methane production is less than 1% of the accumulated volume of methane produced ($BMP_{1\%}$ d^{-1}). Being an experiment designed to inform industry and for practical applications, the endpoint will be when CH_4 production is <1% of the accumulated total.

3.4.4 Experiment Design

The setup of BMP experiments was designed to expose: 1. The amount of methane each mixture can produce, and at what rate, 2. The optimal mixture of substrate materials to exploit the benefits of co-digestion, and 3. The effects of temperature on each mixture, tested under both ambient and mesophilic conditions. To satisfy the optimum temperature objectives of this research, two concurrent experiments were conducted. To achieve this goal, four AMPTS II systems were utilised for experimentation. Two set up at the case-study ambient temperature (28°C) and two set up identically for mesophilic conditions (37°C) (Table 4). The five spaces in the first AMPTS host the inoculum and positive control and the experiment setup allocates an analysis to 100% paunch, 100% stick water, and 100% of each feedstock as available in production ratios. Full utilisation of the second AMPTS hosts the full range of ideal C:N ratios (20 – 30), including a mixture above and below the range at 15 and 35 CN ratio mixes.

Table 4: BMP plan – Dual experiment design using four AMPTS II's.

	28 Degrees		***37 Degrees***	
cells	***AMPTS 1***	***AMPTS 2***	***AMPTS 3***	***AMPTS 4***
1, 2, 3	Cellulose	C:N 15	Cellulose	C:N 15
4, 5, 6	Inoculum	C:N 20	Inoculum	C:N 20
7, 8, 9	100 % paunch	C:N 25	100 % paunch	C:N 25
10, 11, 12	100 % stickwater	C:N 30	100 % stickwater	C:N 30
13, 14, 15	100 % production ratios	C:N 35	100 % production ratios	C:N 35

3.4.5 Experiment Setup

The instructions in the AMPTS II user manual suggest that experiments be set up with 400 grams of sample, portioned to include both the substrate and the inoculum. The inoculum to substrate ratio is the

deciding factor in the volume of material to add into test reactors and is determined by the VS of the substrate and inoculum to achieve an ISR of 3:1. The first step was to calculate the amount of substrate required for each mixture.

Determination of the VS% for each desired C:N ratios of 15, 20, 25, 30, 35 was completed by setting the paunch C:N ratio per unit of weight as static and using the Microsoft Excel solver function to scale the mass of the stickwater required to determine the VS% of each C:N ratio. The VS was then used in Equation 8 to calculate the amount of inoculum required to establish a test with an ISR of 3:1. After determining the required inoculum, the total amount of substrate mixture required is 400g minus the result gained from Equation 8. The percentages of paunch and stickwater weights required to add to the test reactor is scaled down and calculated from the ratios of the VS% and applied to the total substrate required (Equation 9).

Equation 8: Calculation for required inoculum volume.

$$Required\ inoculum, g = \frac{\left(\frac{substrate\ mixture\ VS\%}{inoculum\ VS\%} \times ISR\right)}{\left(\frac{substrate\ mixture\ VS\%}{inoculum\ VS\%} \times ISR\right) + 1} \times 400mL$$

Equation 9: determining VS% pertaining to each substrate ratio.

$$VS\%_{Total} = \%\ required_{paunch} \times VS\%_{paunch} + \%\ required_{stickwater} \times VS\%_{stickwater}$$

3.4.6 AMPTS II BMP results

The software incorporated with the AMPTS II monitoring and analysis software generates raw data with the necessary information to analyse and graph the accumulated methane generated from all samples, including inoculum used to correct the data. The data accumulated from the system was downloaded in csv format and collated using Microsoft Excel. The raw data was used to calculate the daily mL_N accumulation of methane produced from each sample, corrected for the methane produced by the inoculum blank. The result is then divided by the VS value derived for that sample to give the $mL_N\ CH_4 \cdot g\ VS^{-1}$. The completion percentage is also calculated to determine the day that practical completion is reached, being the daily CH_4 produced is less than 1% of the accumulated total.

3.5 Statistics & Data Analysis

Statistical analysis of the results is useful in discerning if there is a probability of results happening by chance or whether there is a real relationship between two results separated by a single variable (Tenny 2022). To establish whether there is statistical significance in the relationship between temperature and BMP, and also C:N and BMP, the null and alternative hypotheses for both relationships analysed are presented as:

$H_{0,\,T}$ = There is no difference in biogas production as a result of temperature.

$H_{A,\,T}$ = Temperature has a positive effect on the biogas production.

And;

$H_{0,\,CN}$ = There is no difference in biogas production as a result of C:N ratio.

$H_{A,\,CN}$ = CN ratio has a positive effect on the biogas production.

In order to reject the null hypothesis and accept the alternative, the alpha value for the p-value developed as a result of statistical analysis must be $\alpha<0.05$ tolerance level to be considered as a statistically significant result. All statistic tests were performed in Microsoft Excel using the Real Statistics ad-in for statistical analysis.

ANOVA test (Analysis of Variance) and Kruskal-Wallis

An analysis of variance (ANOVA) test was used to determine if there was any variance between the 28°C and 37°C date sets with a single variable between them, i.e., temperature, C:N, and time for completion. The one-way ANOVA test is a quite robust and requires a larger sample with some assumptions made about the data set, being that it is evenly distributed, and all populations have a common variance (Zaiontz). The alpha value adopted as the threshold for statistical significance was $\alpha=0.05$. If the assumptions for the ANOVA test are satisfied, then the results of that test should be accepted, however as the experiment samples were run in triplicates, a follow up test is completed using Kruskal-Wallis H non-parametric test which is then also followed with a pairwise t-tests to pinpoint where the differences between samples are.

Pairwise T-Test and Mann-Whitney test

The results from a pairwise t-test was used to identify the variance within a single sample group, i.e., the experiment results which have a relationship, and it flags the relationship for further statistical

analysis using t-testing. A t-test using the two independent samples was then conducted and the non-parametric equivalent Mann-Whitney test. The null hypothesis was able to be rejected and the alternative accepted if the Kruskal-Wallis test and the pairwise t-test returns a statistical significance result i.e., $\alpha<0.05$, and if subsequent t-test with Mann-Whitney results are all $\alpha<0.05$.

3.6 Gas Composition

Gas composition was determined at the end of the experiment by recirculating the headspace of the reactor using a Biogas 5000 gas analyser (Geotech, UK). The composition of the headspace gas is used to determine the fraction of CO_2, CH_4, and H_2S in the gas produced in each reactor. The measurement was recorded when values were stable for 30 seconds. Measurements included an oxygen percentage which was present from connecting the gas analyser. As true anaerobic process does not contain oxygen gas except for in trace concentrations, Equations 10 and 11 is used to correct the gas composition to represent concentrations for CO_2 and CH_4 as percentages.

Equations 10 & 11: Gas composition correction for oxygen.

$$CH_4\% = \frac{CH_4}{CH_4 + CO_2 + H_2S}$$

$$CO_2\% = \frac{CO_2}{CH_4 + CO_2 + H_2S}$$

3.7 Modelling and Process Kinetics

Modelling the AD of a substrate enables the fitting of a curve to describe biogas production yield by using time and B_0 (SMP) to determine the production rate and the lag phase duration. The resulting curve can be utilised for process design and optimisation (Ben Khedher et al. 2022). The Gompertz model is a sigmoidal (s-shaped) function and is one of the most frequently used models to describe growth, commonly used in biology to describe the growth characteristics of a wide range of systems from bacteriological to plant growth (Tjørve & Tjørve 2017). As an extension of the Gompertz equation, the model was modified by Zwietering et al. (1990) to describe the biogas production as a function of methanogenic organisms growth rate (Equation 12). It is considered a simple yet accurate model in describing the SMP, producing a good fitting curve with low fitting error and being comparable to more complex models such as first order kinetics, logistic model, Richards model, and more (Ben Khedher et al. 2022; Yono et al. 2014). It has been

widely adopted for its tendency to fit experimental data closely, and for its ease of application in comparison to other models.

Equation 12: Modified Gompertz (Zwietering et al. 1990).

$$B(t) = B_0 \exp^{-\exp^{[\frac{\mu m}{B_0}(\lambda - t)+1]}}$$

Where:

B = cumulative biogas output, mL g^{-1} VS (SMP)
B_0 = biogas production potential at finish, mg g^{-1} VS
μ_m = maximum biogas production rate, mL g^{-1} d^{-1}
λ = lag phase period, days
t = time, days

3.7.1 Curve Fitting

The modified Gompertz equation was set up in Excel adjacent to the measured daily data to model the methane generation using B_o, μ_m, t, and λ as the inputs. The difference between the measured biogas production potential and the output of the modified Gompertz equation was determined and squared. The sum of the squares was calculated as Σr^2. The Excel solver tool was used to determine values for the B_o, μ_m, and λ as the variable inputs to equation 12 with the goal to minimise the Σr^2. For the solver tool to obtain an accurate result, initial values must be close to the expected values.

Chapter 4 Results & Discussion

4.1 Case Study

The case study site has an opportunity to increase its biogas production, reduce disposal costs, and decrease utility usage. The opportunity can be realised by diverting paunch waste away from landfill and understanding the potential for valorising the energy within paunch waste, with the recovered energy playing an important role in the industry's move with towards carbon neutral 2030 – CN30. The anaerobic treatment utilised at the site is an unmixed and unheated 20ML covered anaerobic lagoon (CAL) (Figure 7) to reduce organic loading of the wastewater before an aerobic biological nutrient removal (BNR) process. The site captures biogas produced from the CAL for use as supplementary fuel source for onsite steam generation. The influent volume averages 2.7 ML per production day producing a HRT of 7.4 days with a maximum organic loading measured as COD as 11,080 $mg \cdot L^{-1}$ and average of 5,285 $mg \cdot L^{-1}$. The average OLR is 0.7, peaking at 1.5 kg $COD \cdot m^{-3} \cdot d^{-1}$, however data is not available to produce an OLR in terms of VS. Comparing with literature, the HRT is below reported ideal ranges suggesting a possibility of incomplete digestion, although the site achieves up to 97% COD reduction efficiencies with stable effluent results. The OLR is less than reported figures, indicating a slight underloading and the potential to cater for increased organic loading. However, there is excessive crusting within the CAL and there are suspicions of significant sludge and grit buildup which decreases the functional volume of the reactor, further reducing HRT and increasing OLR. The site is also heavily active with a utilities reduction program (URP), meaning that when water consumption is reduced through usage efficiency initiatives, COD concentrations are increased, resulting in HRT and OLR being increased. The increased HRT sees a potential to utilise the CAL to the best of its design capabilities.

The minimum process temperature in the winter is 20.8℃ and biogas is produced at approximately 3,299 $m3 \cdot d^{-1}$ correlated with production values to be represented as 84.85 $m^3 \cdot tHSCW^{-1}$. In contrast with the summer months, the process reaches a maximum of 36.2℃ and produces an average 4,950 $m3 \cdot d^{-1}$, 117.17 $m^3 \cdot tHSCW^{-1}$. Biogas composition is consistent throughout the year averaging 69.3±2.5% CH_4. The average effluent temperature recorded from the sites' CAL discharge between January 2022 – January 2023 was 28.8±3.5°C. The temperature adopted for use as ambient for the BMP experimentation was 28°C. The effect that seasonally fluctuating ambient process temperature has on biogas sees a reduced production volume of 82,331 m^3 Autumn-Spring, in comparison to Summer biogas production (Table 5).

Table 5: Reduced biogas production as a result of seasonal ambient temperature variations.

	biogas, m^3	difference
Average Summer	146,856	
Average Autumn	129,364	-17,492
average Winter	101,154	-45,702
Average Spring	127,720	-19,137
	total	***82,331***

Figure 7: Case study plant's CAL

4.2 Feedstock Availability and Characterisation

Paunch Production

Annual paunch production was estimated by combining available waste disposal data with production data. The paunch disposal to landfill was determined to be 10.63 kg collected per head, with a standard deviation of ±3.34 (Appendix 6). Combining this paunch value with the 2021 – 2022 production data of 209,490 head slaughtered averaged out for 52 weeks production, 5 days per week equalling 806 head·day^{-1}, the average paunch retrieved is 8,567.8 kg·day^{-1}. This yields an average 2.2 kilotons of paunch collection per year. This is verified against the number of head slaughtered for fiscal years 2020 through to 2023 (Table 6). Cross referencing with tHSCW production data shows an average of 41 ±1.2kg·tHSCW^{-1}. This data was used to inform the experiment designed to replicate feedstock co-digestion at 100% production ratio availability.

Table 6: Paunch data per fiscal year.

fiscal year	head slaughtered	tHSCW	kiloton of paunch #	kg paunch / tHSCW
20-21*	207,816	52,228.05	2.21	42.3
21-22	209,490	55,767.68	2.23	40.0
22-23	204,138	52,624.53	2.17	41.2
Average	207,148	53,540	2.20	41
Std. dev.	2737.8	1939.3	0.031	1.2

** Data for 20-21 extrapolated from 22 weeks of data*

 # Based off 10.63kg/head

Stickwater Production

Determination of the stickwater available in production flow volumes is integral to the experiment design. Results from the measurement of stickwater flow rate is shown in Table 7, revealing a mean flowrate of 1.02 $L \cdot s^{-1}$ = 61.2 $L \cdot min^{-1}$. As the bloodmeal production process does not operate continually throughout the shift due to raw blood availability, the 'on' time was determined to calculate the daily stickwater flow rate. The operational records over 18 shifts (Appendix 7) showed the average 'on' time was 312 mins per shift (Table 8), resulting in an average flow volume for the recording period of 19.09 kL stickwater volume per day. To reveal the average stickwater flowrate per head, the average flow measurement was combined with the average of 812 head slaughtered for the period and produced a production rate of 23.5 $L \cdot head^{-1}$ (Appendix 7). During the flowrate testing period, the average HSCW was 0.258 $t \cdot head^{-1}$. Combining the measured stickwater flow rate with this value yields 91.09 $L \cdot tHSCW^{-1}$.

Table 7: Stickwater flow rate measurement.

test no.	1	2	3	4	5	6	7	8	9	10
t, sec	19.5	19.0	21.5	22.0	15.5	15.0	16.0	24.5	22.5	28.0
Q, L · s^{-1}	1.03	1.05	0.93	0.91	1.29	1.33	1.25	0.82	0.89	0.71

Average	Std. Dev. ±
1.02 L · s^{-1}	0.20 L · s^{-1}

Table 8: Blood valve on times correlating to daily stickwater volume.

	Max.	Min.	Average	Std. Dev
Blood valve 'on' time, mins	367	238	312	36.9
Head	839	777	812	16.6
Estimated stickwater L · day^{-1}	22,460	14,566	19,091	2259.0
Estimated stickwater L · head^{-1}	26.8	18.7	23.5	2.9

Feedstock Characterisation

Results of in-house analysis characterises the fresh feedstock samples, inoculum, and cellulose required to inform the design the BMP tests (Table 9). The inhouse COD and TS testing data is used to correct the laboratory analysis to inform design of BMP experiment by allowing comparison of paunch and stickwater, with COD being the tying relationship between samples.

Table 9: Characterisation of fresh feedstock samples, inoculum, and cellulose.

Average	Moisture %	TS %	VS %	COD, mg/L
Paunch	72.73	27.27	24.65	22,906
Stickwater	97.94	2.06	1.38	294,823
Cellulose	98.24	1.76	94.57	
Inoculum	95.81	4.19	2.60	

4.3 Biochemical Methane Potential Test

4.3.1 AMPTS II Experiment Setup

Correct setup of the experiment is pivotal in obtaining reliable data translatable from laboratory to real world application. Table 10 outlines the calculated portions of feedstock and inoculum masses to achieve the designated C:N mixes whilst maintaining an ISR of 3:1. The actual mass added to each reactor is recorded in Appendix 8 for both temperature ranges, which includes calculation of the actual ISR and also the pH of each reactor before and after the experiment. Daily paunch production of 8,567.8 $kg \cdot d^{-1}$ and stickwater production of 19.09 $kL \cdot d^{-1}$ were used to determine the ratio of feedstock weight added to represent production ratios, which created a mixture with a C:N ratio of 32.85.

Table 10: Feedstock mix ratios, mixed feedstock VS%, and the inoculum/substrate required for 3:1 ISR.

C:N	*P %*	*SW %*	*VS %*	*P, grams*	*SW, grams*	*Total substrate*	*Inoculum*
Cellulose	--	--	95.39	--	--	3.17 g	396.83 g
Paunch	100%	--	24.66	11.99	--	11.99 g	388.01 g
Stickwater	--	100%	1.38	--	142.54	142.54 g	257.46 g
15	8.62%	91.38%	3.38	6.34	67.21	73.54 g	326.46 g
20	13.97%	86.03%	4.63	7.9	48.64	56.53 g	343.47 g
25	20.00%	80.00%	6.03	8.97	35.89	44.86 g	355.14 g
30	26.83%	73.17%	7.62	9.75	26.59	36.34 g	363.66 g
32.85*	31.16%	68.84%	8.63	10.11	22.34	32.45 g	367.55 g
35	34.65%	65.35%	9.44	10.35	19.52	29.86 g	370.14 g

**** Production ratio mix***

4.3.2 Failed Replicates, Data Rejection, and Validation

During the experiment, some of the valves designed to isolate the reactor from the atmosphere were not closed properly, hence methane did not exit the experiment through the tipping measuring devices. Paunch and stickwater experiments at 37℃ and C:N 25 ratio at 28℃ had one replicate failing to produce data and the paunch experiment at 28℃ had two failed replicates, leaving one sample to represent the SMP of paunch. Also, the first inoculum replicate at 37°C was not loaded with a reactor as there was a known problem with the flow recording cell.

Analysis of the data revealed an anomaly with the inoculum replicates in the 28°C sample. Replicates two and three recorded an unrealistic production of methane of 642.3 and 618.5 mL_N whereas the first replicate was 63.1, which was validated against the two inoculum results from the 37°C experiment, being 73.5 and 56.3 mL_N. The source of the anomaly is unknown, and skewed the SMP calculations so severely that the two abnormal results were omitted form the analysis. The experiments were deemed validated and the integrity of the was inoculum verified as the cellulose positive control samples from each experiment exceeded 80% degradation of the theoretical SMP.

4.3.3 Specific Methane Potential and Kinetic Modelling

The specific methane production results were obtained from the experiment and reported in table 11. From initial inspection, note is taken that all average SMP values for 37℃ exceed the 28℃ results. Stickwater mono-digestion experiments all produced the highest methane yield.

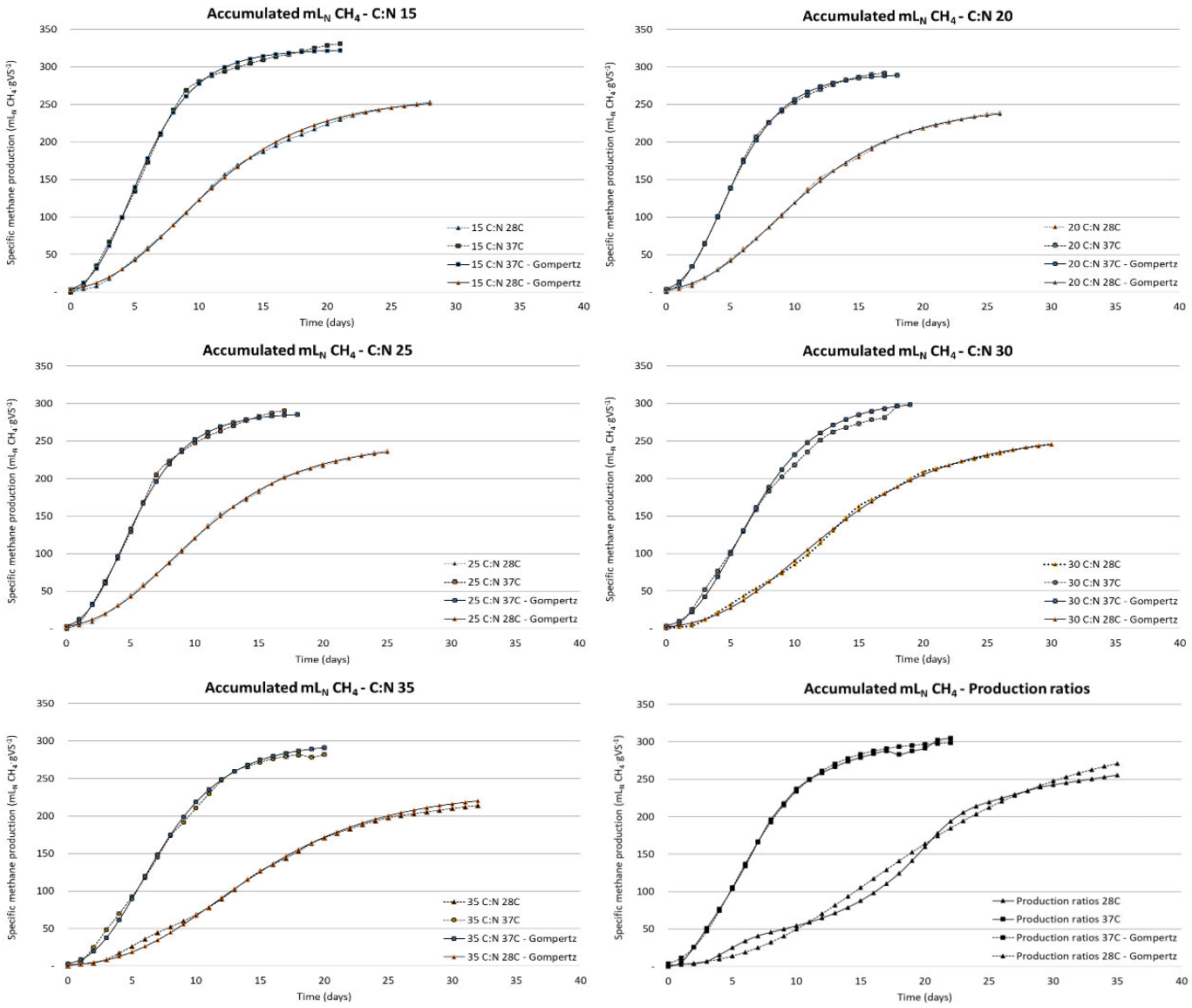
There is a wide range of standard deviations for the suite of experiments which is attributed to the relatively small sample size and sample heterogeneity. Precision of the balance for sample measurement and the AMPTS II also contributed errors which effected the accuracy of the result. The large standard deviation for production ratio (PR) test at 28℃ indicates re-testing would be required to produce reliable results. There is no way to verify the accuracy of the single paunch result at 28℃ without a comparison, however the results follow the trend of the 37℃ experiments, being paunch having the lowest methane yield.

Table 11: Specific methane production, $mL_N\ CH_4 \cdot g\ VS^{-1}$.

	28℃								37℃							
Specific Methane Production mLN CH4 / g VS	**Paunch**	**Stick water**	**PR**	**C:N 15**	**C:N 20**	**C:N 25**	**C:N 30**	**C:N 35**	**Paunch**	**Stick water**	**PR**	**C:N 15**	**C:N 20**	**C:N 25**	**C:N 30**	**C:N 35**
replicate 1	220	276	357	275	232	234	234	230	--	--	302	323	297	282	302	294
replicate 2	--	272	225	275	244	242	252	220	236	442	294	318	297	297	294	302
replicate 3	--	286	211	244	242	--	252	219	291	388	305	342	290	294	256	275
Average	220	278	264	265	239	238	246	223	263	415	300	328	295	291	284	290
SD	0	6	66	15	5	4	8	5	28	27	5	10	3	6	20	11

For the curve fitting of the modified Gompertz kinetic model, variables for each replicate were determined to fit the kinetic model to the measured data by reducing the sum of the residual squares (Appendix 9). The average values for the sum of squares were 896±872, with the maximum result being 3,651. This wide array of values is due to some experiments experiencing abnormal methane accumulation in the final days of the experiment, namely C:N ratios 15 and 35. The modified Gompertz input variables were averaged and applied to Equation 12 to plot the curve to the measured data (Figure 8, a - f).

Figure 8, a - f: BMP results plotted with kinetic model.



4.3.4 Theoretical Methane Yield

The theoretical methane production for a feedstock is calculated under the assumption that all COD is organic and is converted into CH_4 and CO_2. As indicated in section 2.3, one gram of COD is converted into 350 mL_N of CH_4 and calculated providing measured COD in mg/L is equal to mg/kg. Considering the fact there is a of 271,917 $mg \cdot L^{-1}$ COD difference between feedstocks, there is only a difference between the maximum and minimum theoretical methane potentials of 94.5 mL CH_4 (Table 12). This is due to the design of the experiment, but special note is taken to consider this when analysing the results for the fraction degradable.

Table 12: Theoretical methane production as a function of COD.

Feedstock	P (g)	P %	SW (g)	SW %	COD mg/g	g COD	Theoretical mL CH4
Paunch	11.99	100%	0	0%	294,823	3.53	1,237
Stickwater	0	0%	142.54	100%	22,906	3.27	1,143
15 CN	6.34	9%	67.21	91%	46,345	3.41	1,193
20 CN	7.9	14%	48.64	86%	60,899	3.44	1,205
25 CN	8.97	20%	35.89	80%	77,277	3.47	1,213
30 CN	9.75	27%	26.59	73%	95,861	3.48	1,219
35 CN	10.35	35%	19.52	65%	117,126	3.50	1,224
Production ratio	10.11	31%	22.34	69%	107,623	3.49	1,222
					Average	3.45	1207.2
					Std dev.	0.08	27.32

4.4 Effects of Temperature

4.4.1 Specific Methane Potential results.

Increasing temperature from ambient to optimum mesophilic had a positive effect on the AD of experimental feedstocks. Due to the increased biological growth and activity at elevated temperatures, all experiments produced a higher methane yield at 37°C than their 28°C counterparts, reduced the time for completion, and increased the fraction degraded for each feedstock. There was statistical significance ($\alpha < 0.05$) observed in all C:N mixes besides C:N 25, allowing the rejection of the null hypothesis and acceptance of the alternative hypothesis that temperature did have an effect on biogas production. Figure 9 visualises the increase in SMP across all C:N ratio experiments because of an increased digestion temperature. Considering the difficulties of paunch AD, mono- digestion of paunch yielded 18% increased methane at 37°C and reduced the days for completion by 51.9% (Table 13). Stickwater appears to be readily degraded at both temperature ranges seeing that raising the temperature reduced the completion time by only 5%. Although despite this, the methane production of the stickwater was enhanced by 40% from 278 to 415 $mL_N CH_4 \cdot g\ VS^{-1}$, the highest recording from this experiment corresponding with an increase in the fraction

degradable of 39.6%. The same effect due to temperature was seen when comparing the methane production of C:N ratio mixtures 35, 15, 20, 25, 30 which increased by 26, 21, 21, 20, and 14% respectively. The production ratio mixture's methane yield was increased by 13% as an effect of increasing the temperature. Overall increases of SMP increase seen in co-digestion mixtures was between 35.9 – 67.3 $CH_4 \cdot g\ VS^{-1} \approx$ 50.98 – 95.57 $CH_4 \cdot g\ COD^{-1}$

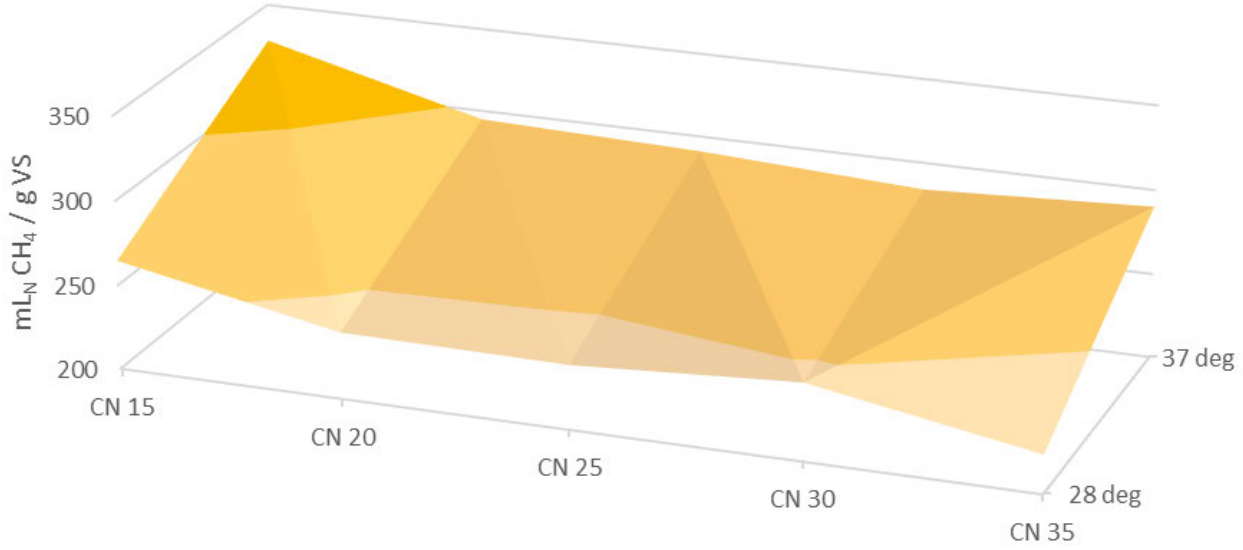


Figure 9: Effect of temperature on SMP at different C:N ratios.

Table 13: Improved SMP, fraction degraded, and digestion completion time due to temperature.

	SMP, $mL_N \cdot g^{-1}$ VS			Degradability, %			Completion Time, days		
	28℃	37℃	% increase	28℃	37℃	% increase	average 28℃	average 37℃	% decrease
Paunch	220.3	263.4	**17.8%**	62.8%	52.5%	17.8%	34.0	20.0	-51.9%
Stick water	278.0	415.1	**39.6%**	71.3%	47.7%	39.6%	25.3	24.0	-5.4%
Production ratios	264.3	300.2	**12.7%**	70.7%	62.1%	12.9%	31.0	20.3	-41.6%
15 C:N	264.6	328.0	**21.4%**	67.7%	54.8%	21.1%	27.3	19.3	-34.3%
20 C:N	239.3	294.8	**20.8%**	63.7%	51.6%	20.8%	26.0	17.3	-40.0%
25 C:N	238.0	291.1	**20.1%**	64.6%	52.8%	20.1%	25.5	17.3	-38.1%
30 C:N	245.8	284.0	**14.4%**	58.0%	55.6%	4.3%	30.0	18.0	-50.0%
35 C:N	222.8	290.1	**26.2%**	67.0%	51.4%	26.3%	32.0	20.7	-43.0%

4.4.2 Fraction Degraded

A welcomed benefit of increasing the temperature into the optimum mesophilic range included the improved degradability of feedstocks. Due to the increased microbial growth rates and heightened activity, an overall average increase of 20.35% fraction degraded was seen across all the experiments. The average degradability of all samples is higher in comparison to co-digestion mixtures (17.6%) due to the large

increase in the fraction degraded of 39.6% seen in the stick water sample. The percentage of paunch mono-digestion samples as reported in Table 13 also increased by 17.8%. The increase seen in the degraded fraction of feedstock material is the source of increased methane production.

4.4.3 Completion Time

Not only was the maximum CH_4 yield by an increased temperature, but also the rate of production was substantially enhanced, resulting in a reduced time for completion. The average completion time for the 37℃ experiment was 19.6 days over all assays, compared to 28.9 days for the 28℃ experiments – a reduction of 38%. The earliest completion was the C:N 20 and 25 experiments at 37℃, producing less than 1% of accumulated methane after only 17.3 days. Shown in Table 13 that in all cases, the completion time was reduced for co-digestion mixes compared to paunch and stickwater mono digestion. Completion time for paunch at the higher temperature decreased completion time by 52%, whereas stickwater only saw a reduction of 5%. Comparing both temperature experiment's CN ratios 15, 25, 20, 35, 30 reductions of 34, 38, 40, 43, 50% were observed respectively, and production ratios (C:N 32.85) was reduced by 42%. As with the increase of degradability, the increase is due to the enhanced microbial growth and biological activity at mesophilic optimum temperature.

4.4.4 Effect of Temperature on Gas Composition

Unlike other results in this study, the methane content appeared to be undesirably affected by temperature. Gas composition analysis of the experiment reactor headspaces revealed that all samples produced methane in the range of 68.78 – 84.95% (Figure 10). Mesophilic experiments produced an average of 71% ±1.79% whereas the ambient temperature experiments exhibited an averaged 38% higher methane fraction at 78.7% ±2.18% (Appendix 10). The CH_4 fraction of the 37℃ experiments are reasonable and conform to literature, however considering the 28℃ experiments, the higher CH_4 fractions are not regular and are not easily explained, especially the high 84% result which does not conform with literature. Although outside of the scope of this research, speculation can be made towards whether the effect was seen as a result of small-scale continuously stirred reactors, or that the lower temperature effected the solubility or CO2 in the substrate/inoculum mixture.

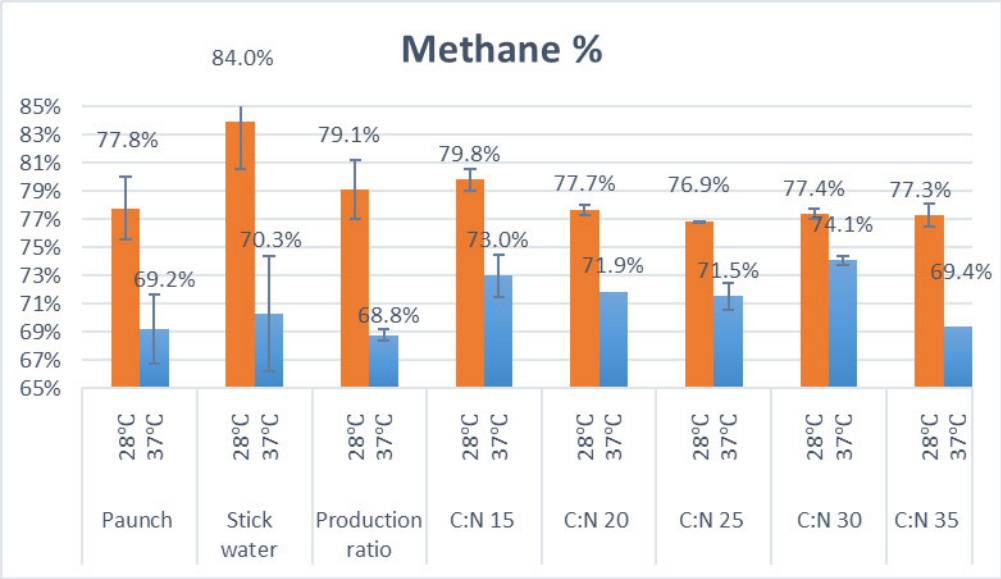


Figure 10: Methane percentage for each test assay

4.5 Effects of Co-Digestion

Contrary to literature, definite effects of co-digestion were not seen in the results of the experiment. Both experiments exhibited a decline in SMP rate as the C:N ratio increased as depicted in Figure 11. However, despite this apparent relationship, $\alpha < 0.05$ was not able to be produced through statistical analysis and as such there was no evidence found to reinforce C:N effects on methane production with statistical significance. Therefore, the null hypothesis is accepted that there was no effect seen because of paunch and stickwater co-digestion. In both experiments, paunch material produced the least methane whereas stickwater alone produced the highest methane fraction in all experiments. The highest methane producing mixture in both experiments was C:N 15 at 328 $mL_N\ CH_4 \cdot g\ VS^{-1}$ at 37°C and 265 $mL_N\ CH_4 \cdot g\ VS^{-1}$ at 28°C, which lies outside the common literature recommendations of a ratio range of 20-30 C:N, but aligns with the findings of Malik et al. (1987). Explanation for this observation is outside the scope of this research, however the most likely reason for this would be the nature of the experiment being a short-term batch experiment. Positive effects due to co-digestion are more likely to be evident in a continuous digestion experiment. This eliminates any residual nutrients introduced from the inoculum and microorganisms will acclimate to the feedstock to develop the synergistic relationships required to reap the benefits of co-digestion.

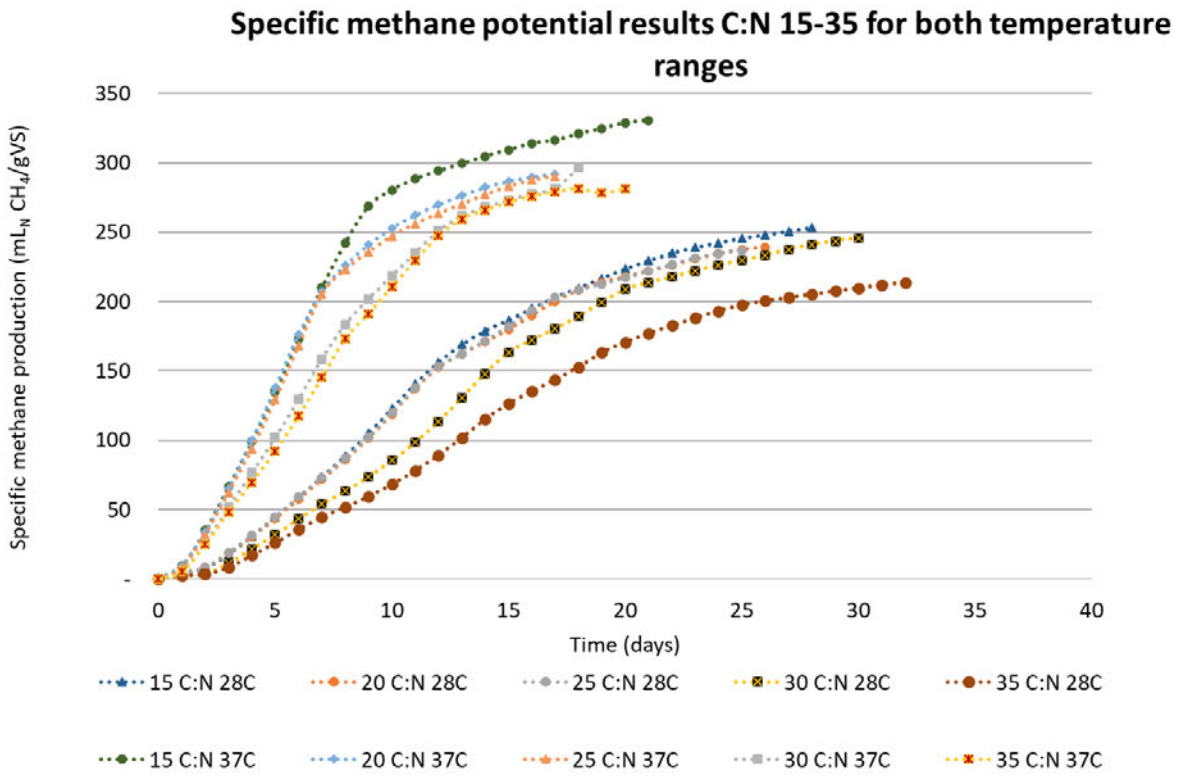


Figure 11: All SMP results at both temperatures from BMP testing.

4.6 Implications on Future AD Infrastructure

Diverting paunch from landfill and towards onsite AD inherently increases the organic loading of a waste stream and requires assurance that facilities are sufficiently sized to process the waste material. To ensure infrastructure is not overloaded, consideration is taken around the capacity and likely chance of overloading, which leads to an inefficient process prone to inhibition and failure. The addition of paunch to the case study waste stream increases COD loading by 2,526 kg COD·d^{-1}. This increases the OLR from 0.7 to 0.84 kg COD·m^{-3}·d^{-1}. Depending on the density of paunch material added to the treatment, the 8,567.8 kg material generated daily will increase the 2.7 ML·d^{-1} inflow, decreasing the already low HRT of 7.4 days. Future infrastructure will need to be designed to cater for these parameters.

To achieve optimum mesophilic conditions, process heating is required. In Summer, the minimum difference in temperature to raise the process to optimum mesophilic is 0.8°C, whereas the maximum temperature difference in Winter is 16.2°C. Conjointly, a heat source is required additional to heat exchanging infrastructure to introduce heat to the process. Waste heat from biogas conditioning

refrigeration units is a potential source of heat, as are combined heat and power (CHP) engines which convert biogas into electricity whilst capturing the waste heat for utilisation.

Additionally, the co-digestion of paunch generating more biogas will require infrastructure to condition, monitor, and use the increased biogas availability. For example, co-digestion at available production ratios can potentially generate 1.06 $m^3 \cdot tHSCW^{-1}$ at 28°C and 1.21 $m^3 \cdot tHSCW^{-1}$ at 37°C. Combining with the case study yearly average of 53,540 tHSCW, this computes as 56,752 – 64,783 $m^3 \cdot CH_4$ $year^{-1}$ of methane requiring utilisation (Table 14). Noting that this figure is CH_4 production and depending on CH_4:CO_2 ratios, biogas volumes will be greater. At 36 $MJ \cdot m^{-3}$ this is converted to represent an additional energy availability of 2,043 $GJ \cdot year^{-1}$ at 28°C and 2,332 $GJ \cdot year^{-1}$ at 37°C. Paunch mono-digestion indicates a production of 2.23 m^3 CH_4 $tHSCW^{-1}$ due to the higher VS in the feedstock (24.65%) compared to stickwater (1.38%).

Table 14: Potential CH4 production, $m3 \cdot tHSCW^{-1}$.

$tHSCW^{-1}$	28°C		37°C	
	m^3 CH_4	MJ	m^3 CH_4	MJ
Paunch	2.23	80.17	2.66	95.84
Stick water	0.35	12.58	0.52	18.79
Production ratios	1.06	38.19	1.21	43.38
15 C:N	0.53	19.24	0.66	23.85
20 C:N	0.60	21.48	0.74	26.46
25 C:N	0.72	25.93	0.88	31.71
30 C:N	0.89	32.13	1.03	37.13
35 C:N	0.96	34.67	1.25	45.15

4.6.1 Suitable AD treatment Technologies

Bearing in mind the nature of paunch material, suitable AD technologies for consideration need to be capable of treating solid organic matter. High-rate treatment processes designed for soluble matter, processes with SRT lower than paunch degradation, unmixed, and decanting processes are not suitable for efficient paunch digestion. A treatment process with mixing capabilities ensures paunch material does not float and enables continuous contact of the organic matter with the biomass. This maximises the opportunity for increased hydrolysis of the degradable organic fraction by maintaining a homogenous mix and allows the feedstock to remain in constant contact with anaerobic microorganisms. Heating capabilities are also required to ensure treatment is carried out efficiently without the requirement for large reactors and long SRT/HRT requirements. Suitable AD reactor technologies for paunch digestion are: CSTR, UASB, AnMBR,

and AnSBR (Figure 12, a-d). An anaerobic hybrid reactor (AHR) is also an apt contender for consideration with its treatment capability drawn from the combination of other technologies. These reactor types are capable of ensuring high feedstock degradation is achieved by having sufficient OLR capacity and an increased SRT which prolongs each of the AD process stages, especially hydrolysis of feedstocks.

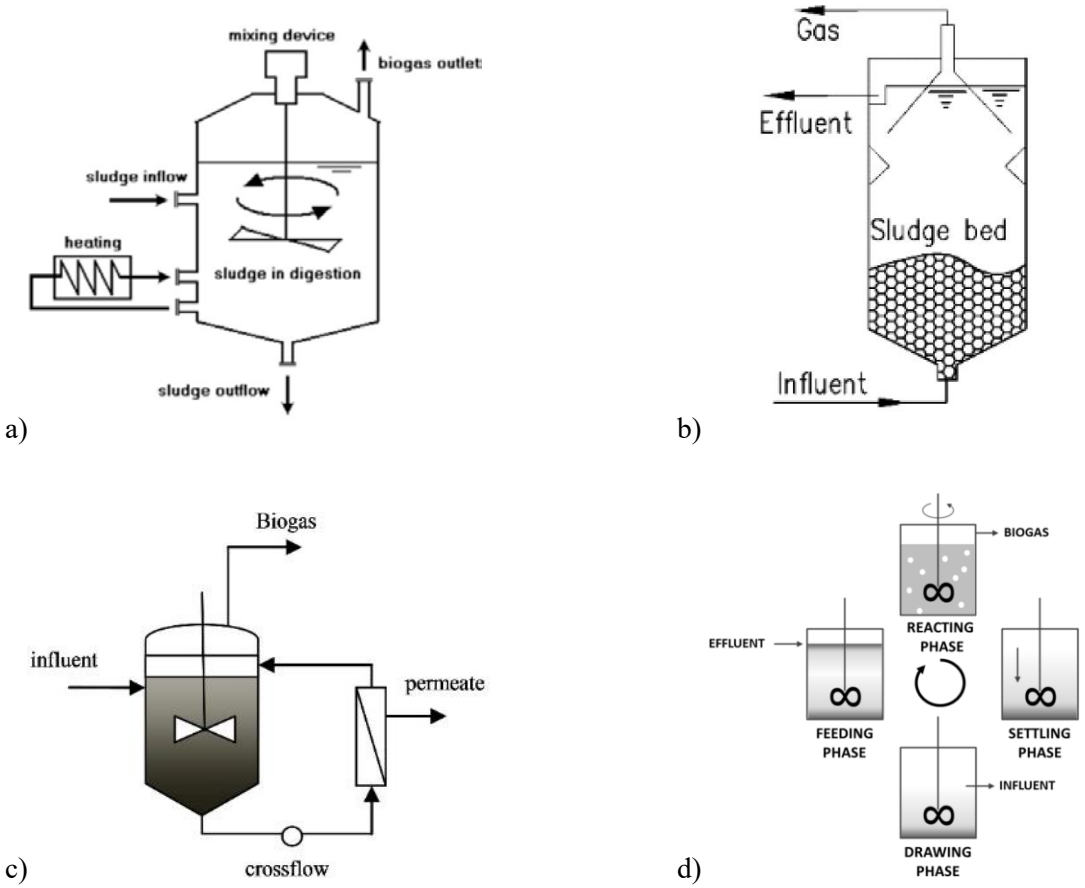


Figure 12: Suitable AD reactors: a- CSTR with sludge heating, b- UASB, c- AnMBR, d- AnSBR. (Chang 2014; de Lemos Chernicharo 2007; Metcalf & Eddy, Burton & Stensel 2013; Ripoll et al. 2023)

4.6.2 Implications to Current Plant Design

Anaerobic digestion of paunch in the current CAL may be possible, but not without barriers. Firstly, the addition of paunch increasing the OLR to 0.84 kg COD·m^{-3}·d^{-1} raises no concern as the value is below literature recommendations of 2 kg COD·m^{-3}·d^{-1}. However, this is assuming availability of the full reactor volume, and the tendency of paunch to float in unmixed processes will quicky build upon the already heavy crust layer. The excessive crust layer will then drastically reduce the functional volume, increasing OLR and further reducing an already low HRT. Being an unheated process, the increased OLR and lowered HRT will not be sufficient to degrade the paunch material to a satisfactory standard. This has a carry-on effect of

loading the subsequent aerobic BNR process, increasing energy consumption for aeration to remove organic material not fully degraded in the CAL.

An opportunity exists for the enhancement of the CAL through modifications to optimise the process for optimum mesophilic conditions. Due to seasonal changes in ambient temperature, there is an opportunity to produce 82,331 m^3 of additional biogas per year by maintaining the process temperature to at least the summer average (Table 15). Additional increase in temperature to optimum mesophilic will further degrade feedstocks, and also begin to liquify the crust build-up made of FOG material. Waste heat generated by the biogas conditioning refrigeration units to condense entrained water can be utilised as a heat source to enhance the AD process. Also, utilisation of peripheral sludge withdrawal pipes installed on the floor of the CAL and inspection ports can allow the conversion of the CAL into an AHR (Figure 13). This can provide an opportunity for mixing of the lagoon, an ideal point for heat exchanger installation, and allow the influent to be introduced to the reactor from the bottom as in UASB reactors. The outlet pit from the CAL has a penstock valve which can be upgraded with an automated actuator to convert the process into an AnSBR. The flow characteristics of an abattoir make this feasible, with most of the daily flow happening during day production hours. These theoretical modifications could possibly enhance the digester process to a degree where mixing and temperature control enable crust management, the possibility of maintaining a high SRT, and the ability to process a higher OLR (including solids feedstock material) with a lower HRT requirement.

Table 15: Reduction in biogas production due to seasonal changes in the process ambient temperature.

	biogas, m^3	***difference***
Average Summer	146,856	
Average Autumn	129,364	-17,492
average Winter	101,154	-45,702
Average Spring	127,720	-19,137
	total	***82,331***

Figure 13: Sludge draw off and inspection port.

4.7 Limitations & Recommendations

4.7.1 Further Investigations and Recommendations

Abattoir wastewater streams vary vastly in composition, volumes, and concentrations. As this case study was isolated for paunch and stickwater BMP analysis, it is recommended to characterise all waste streams both individually and in composite to determine the BMP interaction of paunch co-digestion with the entirety of an abattoir's wastewater. Recommendations are to complete analysis to characterise each waste stream to identify C:N content, pH, organic loading, and also volumetric flow analysis. Determination of trace element concentration in the composite waste stream is useful for determining a healthy process and should be completed to assess any major shortfalls. This enables a more thorough understanding on the digestion rates of paunch when interacting with waste streams and develops a clear understanding of methane generation capabilities. To gain a clear characterisation of the feedstock materials, it is necessary to continue collecting organic loading data of the waste streams in terms of currently monitored COD data and to also begin developing a history of VS data.

Acknowledgement is made that the design of the BMP test is not entirely representative of an unmixed anaerobic lagoon. Being a completely mixed process, the BMP test results are for understanding the maximum methane a feedstock can be expected to make, however the rate of methane production and time to reach <1% of accumulated production in a CAL will vary from experimental results. Investigation is required to establish a relationship between measured methane in BMP testing and actual methane produced in the CAL. Future methane production studies should be carried out to find a correlation with reported literature around C:N ratio. To reveal the true C:N interactions of paunch co-digestion, a continuous experiment should be developed to minimise the effect of inoculum in a batch test carrying its own C:N which may distort feedstock C:N ratios, in addition to not being well adjusted to the feedstock. This will enable a clearer understanding around the co-digestion of available feedstocks and a longer test duration will better inform the decision-making process regarding either design of new infrastructure or current process modifications.

Lastly, reliable knowledge of the current process capabilities is crucial in ascertaining suitable process limits. Further investigation is recommended to reveal the extent of effective volume reduction in the CAL due to severe crusting and the presence of sludge/grit. After establishing the effective volume, subsequent determination of the actual HRT and OLR of the process can be calculated. This will increase the accuracy of feasibility analysis on the co-digestion of paunch with current waste streams.

4.7.2 Early Economic Assessment

Capital expenditure is required whether new digestion reactors are constructed, or if existing infrastructure will receive upgrading modifications. Included in the capital cost is the gas handling and utilisation equipment. A cost-benefit analysis is necessary to determine the economic viability of capital expenditure. Analysis relies on determining the capital cost of infrastructure which for this case study is unknown. Cost-benefit analysis tools such as net present value (NPV), internal rate of return (IRR), and payback period are ways to calculate the return on capital investment. The monetary benefits of paunch digestion are mostly realised by the cost avoidance experienced: elimination of disposal costs for 2.2 kilotons paunch to landfill annually, and utility reduction by utilisation of up to 2,332 GJ from CH_4 annually. Dependant on the price per GJ of electricity or natural gas, site specific comprehensive analysis is required to ascertain the most beneficial utilisation of methane energy.

Chapter 5 Conclusions

5.1 Biogas Production from Paunch

Red meat processing plants rely on large quantities of energy for successful operation and production and under normal operating conditions RMP plants produce wastes in the form of wastewater and solid material, namely paunch. These waste materials vary in strength and volumes, but all contain fractions of organic material available for conversion into a renewable energy source in the form of methane. Some energy is currently recovered from the wastewater streams using anaerobic digestion, however conversion of paunch organic matter into methane is not presently realised in many abattoirs. This dissertation investigated the co-digestion of a case study abattoir's paunch and bloodmeal stickwater waste stream and reports on the SMP effected by mixing feedstocks at differing C:N ratios and increasing digestion temperature to optimum mesophilic.

Temperature was found to have a significant impact on SMP. Anaerobic co-digestion at the optimum mesophilic temperature of 37°C showed an average increase in methane yield by 21.6±8%, an increase in the fraction degraded by 20.4±10%, and a decrease in experiment completion time of 38±13%. These results emphasise the important role of maintaining consistent temperatures within an anaerobic treatment process, and more so, the results are transferrable from the laboratory to real-world abattoir processes for the valorisation of renewable energy from paunch waste material.

Despite literature evidence regarding the clear importance of C:N ratios on the AD process, this research did not find statistical evidence that varying C:N ratios affected methane production. It is possible that the batch nature of the BMP testing may have contributed to the lack of significance where residual nutrients present in the inoculum, and an inoculum not well adjusted to the feedstock influenced the results. The discrepancy highlights the need for future research to determine the interactions between paunch and abattoir wastewater. It is recommended to experiment with a continuous digestion system over an extended experimentation period.

In summary, RMP abattoirs are in a unique position with access to a renewable energy source. The valorisation of paunch via co-digestion enables abattoirs to gain economic benefit by reducing their waste to landfill, reducing grid energy consumption, and mitigating their environmental footprint. These benefits can be realised by the case study in the realms of 2.2 kilotons of paunch waste diverted from landfill towards methane production, equating to 2,332 GJ of energy annually.

In conclusion, this dissertation has provided some valuable insights into the potential of methane production from paunch waste and provides the groundwork for future study to build on and improve on the findings. The potential for methane production from paunch material is not only possible but is a promising waste-to-energy strategy for increasing the industry's sustainability and move towards a carbon neutrality.

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APPENDICES

APPENDIX 1: PROJECT SPECIFICATION

ENG4111/4112 Research Project

Project Specification

For: BENH – Bachelor of Engineering (Honors)

Title: *Co- Digestion of Abattoir Paunch & Stick Water under Mesophilic and Ambient Temperatures: A Case Study*

Major: Environmental

Supervisors: Peter Harris

Enrolment: ENG4111 – EXT S1, 2023
ENG4112 – EXT S2, 2023

Project Aim: A case study to investigate the potential for enhancing biogas production of paunch by anaerobic co-digestion with blood meal stick water at various temperatures.

Programme: Version 2, 15th March 2023

1. Collect data on the chemical make-up and composition analysis of both paunch and bloodmeal stick water feedstocks through both published research, and site-specific analysis.
   Assays would include:
   a. Moisture content %, total solids, volatile solids
   b. Total chemical oxygen demand of each sample
   c. pH & nutrient analysis
2. Determine the maximum volumes of each feedstock material likely available in the processing plant. Perform a mass balance to depict the nutrient and organic contents of each feedstock and determine their theoretical biogas potential.
3. Collect samples of feedstock material and schedule 3 site visits to UniSQ to set up and finalise BMP tests & assess the results, with samples to represent BMP of:
   a. Each feedstock separately in optimum mesophilic (37°C) & also current onsite average temperature conditions
   b. Various practicable ratio mixes of feedstocks in above conditions
4. Use data to describe the digestibility kinetics of the feedstocks and the effect of mixing ratios.
5. Evaluate experimental data and compare both it and the theoretical calculated BMP max. against COD data to determine the biodegradability of each.
6. Relate the data derived to determine to opportunity for integration in current treatment processes, benefits of process modifications to enable mesophilic conditions, and compare to a standalone design for a separate digester.

If time and resource permit:

7. Analyse the wastewater stream from the night cleaning shift and cross reference with cleaning chemicals used. Use data to propose a pre-treatment process by utilising chemicals present in waste stream.
8. Analyse the feedstock collection processes and determine the temperatures and heat contact times of each stream. See if there is any practicable use of waste heat within each process to extend the heat intensity and contact time in the effort to break down lignocellulosic fibres.
9. Sample aged/stockpiled paunch material and perform BMP test to compare with fresh sample.

APPENDIX 2: PROJECT RESOURCES

ENG4111/4112 Research Project

Project Resources

For: BENH – Bachelor of Engineering (Honors)

Title: *Co- Digestion of Abattoir Paunch & Stick Water under Mesophilic and Ambient Temperatures: A Case Study*

Major: Environmental

Supervisors: Peter Harris

Proposed resources required for the completion of this dissertation include:

Laboratory access for the purpose of:

- COD analysis
- Biochemical methane potential
- Volatile Solids analysis
- Total solids analysis

Dates and durations:

- 5th – 7th July – for feedstock characterisation and AMPTS setup
- 5th – 7th August (possible – to take down AMPTS tests)

Required access to laboratory equipment:

- Automated Methane Potential Testing System (AMPTS)
- Oven
- Furnace
- Spectrophotometer
- Blender

APPENDIX 3: RISK ASSESSMENT

NUMBER	RISK DESCRIPTION	TREND	CUR-RENT	RESID-UAL
2476	ENG4111/4112 Research - BMP Co-Digestion Test-ing		Medium	Medium

DOCUMENTS REFERENCED
Biosafety Procedure USQ Laboratory& Workshop Safety Manual VDI 4630 - Fermentation of Organic Materials (2016) AMPTS II User Manual Sodium Hydroxide 3M SDS

RISK OWNER	RISK IDENTIFIED ON	LAST REVIEWED ON	NEXT SCHEDULED RE-
Jamie Hunt	22/05/2023	23/05/2023	23/11/2023

RISK FACTOR(S)	EXISTING CONTROL(S)	PROPOSED CONTROL(S)	OWNER	DUE DAT
Sample collection - Paunch	**Control:** Sample jars used and contact with material avoided. Gloves to be worn to limit skin exposure. **Control:** Sample Jars washed after collection.	**No Control:**		
Sample collection - Paunch	**Control:** Use ladder to take paunch sample from trailer - do not attempt to over ex-tend and capture directly from press discharge on top of platform	**No Control:**		
Sample collection - Stickwater. Stick water is discharged from decanting centrifuge at temper-atures up to 90 Degrees.	**Control:** Use Sample Jars to collect samples	**No Control:**		

	Control: Don elbow length gauntlet gloves. Wear apron, spats, and protective eyewear.	
Transport - Taking samples to Laboratory. Driving to Toowoomba to set up AMPTS II	**Control:** Follow road regulations: Licensed driver only, Registered Vehicle Stop for 15 minute rest breaks every 2 hrs of driving **Control:** Wear seat belt **Control:** Ensure car is in good working condition and all safety features are working.	**No Control:**
Complete in house Laboratory work - TS & VS.	**Control:** Samples contained in furnace / ovens. Hot crucibles are handled with crucible tongs and stored in a desiccator until cool (~15 minutes for 105C and 30 minutes for 550C) **Control:** Training as per USQ laboratory and workshop manual **Control:** Gloves and eye wear to be worn. Closed in shoes	**No Control:**
Complete in house Laboratory work - BMP, TS & VS. Biohazardous materials include sample material and inoculum.	**Control:** Training as per USQ laboratory and workshop manual	**No Control:**

	Control: Appropriate lab safety equipment to be worn, including gloves, protective eyewear, closed in shoes **Control:** Fume cupboard to contain aerosols **Control:** Hands are washed and sanitised upon completion of work. Ensure vaccination against key wastewater pathogens including Q Fever, Hepatitis A and Hepatitis B.	
Use of chemicals in preparations of BMP samples	**Control:** SDS's available for all used substances and read by users. **Control:** All appropriate PPE to protect against dangers outlined by SDS to be worn.	**No Control:**
Cuts and lacerations	**Control:** If broken, glassware, crucibles and desiccators pose a high risk for cuts and lacerations. Wear gloves when using glassware. **Control:** Two hands used to transport glassware, and tongs used to remove crucibles from oven. **Control:** Set up work area as to minimise or fully avoid	**No Control:**

	the need to move samples far distances.	
Slips, Trips & Falls	**Control:** And spills or dropped equipment should be cleaned up immediately. **Control:** If spillage occurs - Wet floor sign to be erected as soon as practicable to identify slip hazard. **Control:** Work area and access to be kept clean and tidy at all times.	**No Control:**
Analysing results and writing dissertation	**Control:** Correct posture and computer setup. Take regular breaks. Complete regular stretching exercises. **Control:** Ergonomic chair and computer peripherals	**No Control:**

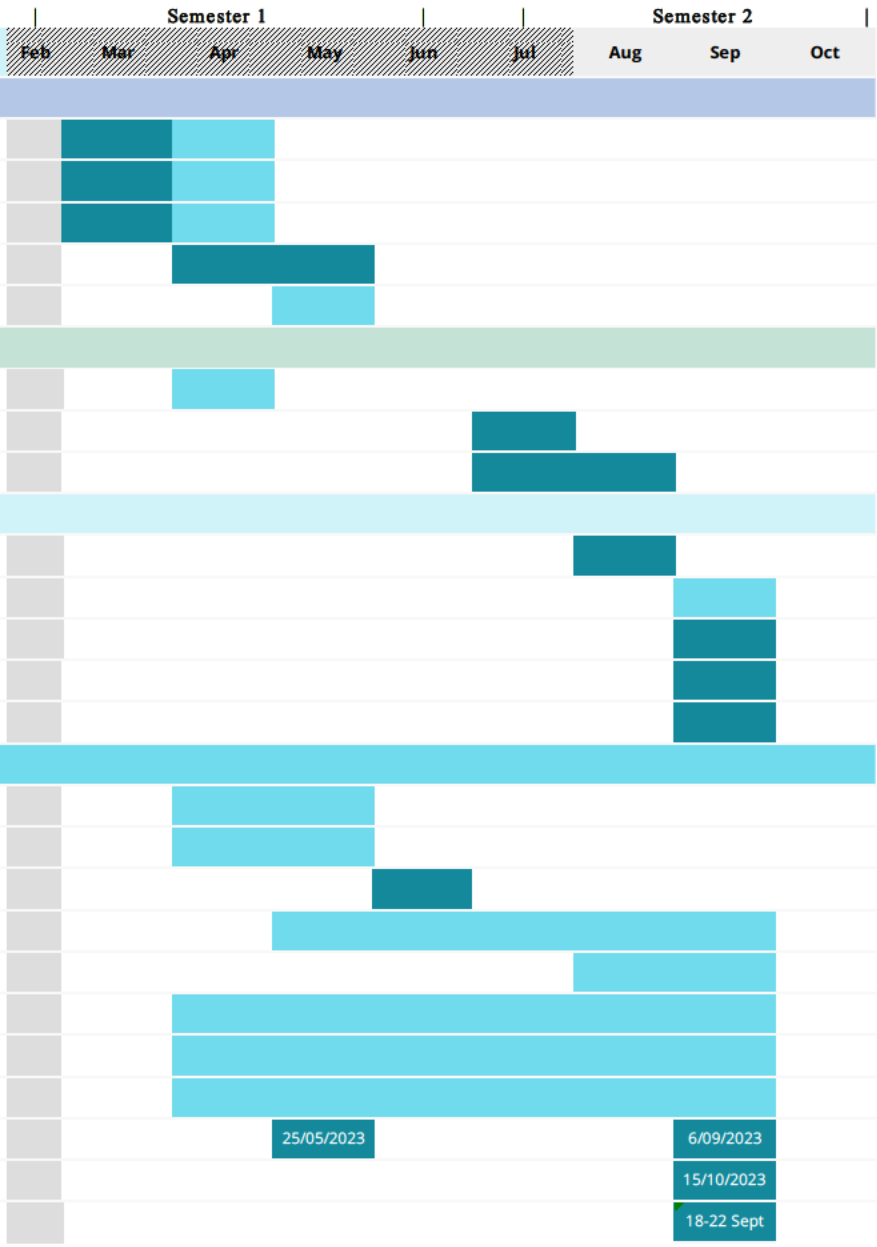
APPENDIX 4: PROJECT TIMELINE

Project Timeline - Jamie Hunt Capstone 2023

Jamie Hunt [redacted]
BENH - ENV

Days until submission - **0**

Task	Start	End	Days until due	Progress
Data Collection				100%
Feedstock published research	16-Mar-23	01-Apr-23	-197	100%
Feedstock volumes data	16-Mar-23	01-Apr-23	-197	100%
CAL operating Temps.	16-Mar-23	01-Apr-23	-197	100%
Site specific data	15-Apr-23	30-Apr-23	-168	100%
Calculate theoretical BMP	30-Apr-23	30-May-23	-138	100%
Feedstock testing				100%
Chemical Analysis	01-Apr-23	15-Apr-23	-183	100%
BMP test setup	04-Jul-23	07-Jul-23	-100	100%
BMP test results and analysis	07-Aug-23	23-Aug-23	-53	100%
Data Analysis				100%
Test result compilation	23-Aug-23	27-Aug-23	-49	100%
Graph results	28-Aug-23	03-Sep-23	-42	100%
Kinetic assessment of digestion	04-Sep-23	10-Sep-23	-35	100%
Compare to COD	04-Sep-23	10-Sep-23	-35	100%
Determine biodegradability	04-Sep-23	10-Sep-23	-35	100%
Dissertation Paper				100%
Project Aims	20-Mar-23	10-May-23	-158	100%
Litrature review	20-Mar-23	10-May-23	-158	100%
Methadology	27//03/23	30-Jun-23	-107	100%
Results and discussion	24-Apr-23	06-Sep-23	-39	100%
Conclusions	23-Jul-23	30-Sep-23	-15	100%
Table of Contents, Figures, Tables	15-Mar-23	30-Sep-23	-15	100%
Appendixes	15-Mar-23	30-Sep-23	-15	100%
References	15-Mar-23	30-Sep-23	-15	100%
Draft Dissertation Submission			-39	Deadline
Final Dissertation submission			0	Deadline
ENG4903 Practice school				



APPENDIX 5: Paunch and stickwater chemical makeup laboratory analysis with internal conversions and corrections.

	Laboratory as reported		*Laboratory converted*		*In-house corrected*	
Assay	***Units***	***Stick water***	***Units***	***Stick water***	***Paunch***	***Stick water***
TS		N/A	%	3.69	27.27	2.06
pH		8.4		8.40	6.10	8.40
COD	*mg O2/L*	41,000	*mg O2/L*	1,111,917	293,362	621,146
TOC	*mg/L*	22,000	*mg/kg d.b.*	596,639	600,000	333,298
TOC			*mg/kg W.b.*	22,000	163,621	6,865
TN	*mg/L*	22,000	*mg/kg d.b.*	59,664	10,000	33,330
TKN	*mg/L*	22,000	*mg/kg d.b.*	59,664	10,000	33,330
C:N		10		10	60	10
S	*mg/L*	130	*mg/kg d.b.*	3526	1,200	1,969
P	*mg/L*	120	*mg/kg d.b.*	3254	1,800	1,818
K	*mg/L*	380	*mg/kg d.b.*	10306	720	5,757
Fe	*µg/L*	23000	*mg/kg d.b.*	624	700	348
Ni	*µg/L*	<2	*mg/kg d.b.*	0.05	6.00	0.03
Co	*µg/L*	<2	*mg/kg d.b.*	0.05	1.00	0.03
Mo	*µg/L*	210	*mg/kg d.b.*	5.70	3.00	3.18
W	*µg/L*	22	*mg/kg d.b.*	0.60	1.00	0.33
Mn	*µg/L*	25	*mg/kg d.b.*	0.68	110.00	0.38
Cu	*µg/L*	180	*mg/kg d.b.*	4.88	7.00	2.73
Se	*µg/L*	35	*mg/kg d.b.*	0.95	2.00	0.53
Zn	*µg/L*	540	*mg/kg d.b.*	14.64	110.00	8.18

APPENDIX 6: Daily paunch collection data vs head slaughtered.

Date	Paunch t/day	Head killed/day	paunch kg/hd	paunch kg/t HSCW
31/05/2023	9.46	851	11.12	39.410
1/06/2023	10.4	809	12.86	45.513
2/06/2023	7.24	841	8.61	30.321
5/06/2023	9.84	836	11.77	41.405
6/06/2023	5.86	854	6.86	24.354
8/06/2023	7.08	846	8.37	29.547
9/06/2023	5.22	818	6.38	22.479
13/06/2023	8.42	645	13.05	44.380
14/06/2023	8.94	873	10.24	36.430
15/06/2023	7.2	850	8.47	29.654
16/06/2023	7.52	820	9.17	32.203
17/06/2023	6.32	411	15.38	53.657
21/06/2023	3.38	439	7.70	25.533
22/06/2023	8.34	547	15.25	52.882
23/06/2023	10.72	620	17.29	62.492
23/06/2023	6.5	859	7.57	26.908
average	7.653	744.938	10.63	37.323
std. dev ±	1.98	158.39	3.34	11.86

APPENDIX 7: Blood plant operational data

stickwater flowrate estimate	61.2	*Pump set at 48Hz*

Date:	2/05/2023	TUESDAY
Blood valve on	Blood valve off	Time (hours)
7:02:00	9:21:00	2:19:00
10:36:00	12:28:00	1:52:00
12:57:00	13:32:00	0:35:00
14:03:00	15:09:00	1:06:00
Total time		5:52:00
Total time, mins		352
Bloodmeal, kg		2823
Head		823
Stickwater estimate, L		21542.4
Stickwater estimate, L/hd		26.17545565

Date:	3/05/2023	WEDNESDAY
Blood valve on	Blood valve off	Time (hours)
6:50:00	8:43:00	1:53:00
10:25:00	12:32:00	2:07:00
13:30:00	14:51:00	1:21:00
		0:00:00
Total time		5:21:00
Total time, mins		321
Bloodmeal, kg		2830
Head		836
Stickwater estimate, L		19645.2
Stickwater estimate, L/hd		23.49904306

Date:	4/05/2023	THURSDAY
Blood valve on	Blood valve off	Time (hours)
7:05:00	9:28:00	2:23:00
10:51:00	12:00:00	1:09:00
12:44:00	13:10:00	0:26:00
		0:00:00
Total time		3:58:00
Total time, mins		238
Bloodmeal, kg		2396
Head		824
Stickwater estimate, L		14565.6
Stickwater estimate, L/hd		17.67669903

Date:	5/05/2023	FRIDAY
Blood valve on	Blood valve off	Time (hours)
7:20:00	9:07:00	1:47:00
10:25:00	12:24:00	1:59:00
13:05:00	13:35:00	0:30:00
14:20:00	14:47:00	0:27:00
Total time		4:43:00
Total time, mins		283
Bloodmeal, kg		2750
Head		806
Stickwater estimate, L		17319.6
Stickwater estimate, L/hd		21.48833747

Date:	6/05/2023	SATURDAY KILL
Blood valve on	Blood valve off	Time (hours)
7:10:00	9:14:00	2:04:00
10:38:00	12:23:00	1:45:00
13:23:00	13:50:00	0:27:00
14:15:00	15:44:00	1:29:00
Total time		5:45:00
Total time, mins		345
Bloodmeal, kg		
Head		791
Stickwater estimate, L		21114
Stickwater estimate, L/hd		26.69279393

Date:	8/05/2023	MONDAY
Blood valve on	Blood valve off	Time (hours)
8:27:00	10:08:00	1:41:00
10:38:00	11:10:00	0:32:00
11:40:00	12:45:00	1:05:00
13:30:00	14:45:00	1:15:00
Total time		4:33:00
Total time, mins		273
Bloodmeal, kg		1974
Head		812
Stickwater estimate, L		16707.6
Stickwater estimate, L/hd		20.57586207

Date:	12/05/2023	FRIDAY
Blood valve on	Blood valve off	Time (hours)
6:20:00	9:40:00	3:20:00
11:40:00	13:09:00	1:29:00
13:32:00	14:00:00	0:28:00
14:40:00	15:30:00	0:50:00
	Due to downtime two days prior - more blood	
Total time		6:07:00
Total time, mins		367
Bloodmeal, kg		3460
Head		784
Stickwater estimate, L		22460.4
Stickwater estimate, L/hd		28.64846939

Date:	15/05/2023	MONDAY
Blood valve on	Blood valve off	Time (hours)
8:25:00	10:35:00	2:10:00
11:00:00	11:50:00	0:50:00
12:30:00	13:20:00	0:50:00
13:37:00	14:00:00	0:23:00
14:20:00	14:45:00	0:25:00
Total time		4:38:00
Total time, mins		278
Bloodmeal, kg		2080
Head		813
Stickwater estimate, L		17013.6
Stickwater estimate, L/hd		20.92693727

Date:	17/05/2023	WEDNESDAY
Blood valve on	Blood valve off	Time (hours)
7:25:00	9:10:00	1:45:00
10:15:00	12:10:00	1:55:00
12:47:00	13:17:00	0:30:00
13:38:00	14:00:00	0:22:00
14:20:00	14:50:00	0:30:00
Total time		5:02:00
Total time, mins		302
Bloodmeal, kg		2691
Head		813
Stickwater estimate, L		18482.4
Stickwater estimate, L/hd		22.73357934

Date:	18/05/2023	THURSDAY
Blood valve on	Blood valve off	Time (hours)
7:30:00	9:15:00	1:45:00
10:15:00	12:10:00	1:55:00
13:00:00	14:42:00	1:42:00
0:00:00	0:00:00	0:00:00
Total time		5:22:00
Total time, mins		322
Bloodmeal, kg		2617
Head		800
Stickwater estimate, L		19706.4
Stickwater estimate, L/hd		24.633

Date:	19/05/2023	FRIDAY
Blood valve on	Blood valve off	Time (hours)
7:10:00	9:13:00	2:03:00
10:35:00	12:10:00	1:35:00
12:40:00	13:41:00	1:01:00
14:22:00	15:23:00	1:01:00
Total time		5:40:00
Total time, mins		340
Bloodmeal, kg		3171
Head		777
Stickwater estimate, L		20808
Stickwater estimate, L/hd		26.77992278

Date:	22/05/2023	MONDAY
Blood valve on	Blood valve off	Time (hours)
8:35:00	10:10:00	1:35:00
10:50:00	12:00:00	1:10:00
12:35:00	13:20:00	0:45:00
13:55:00	14:45:00	0:50:00
Total time		4:20:00
Total time, mins		260
Bloodmeal, kg		2053
Head		807
Stickwater estimate, L		15912
Stickwater estimate, L/hd		19.71747212

Date:	23/05/2023	TUESDAY
Blood valve on	Blood valve off	Time (hours)
7:00:00	9:15:00	2:15:00
10:20:00	11:53:00	1:33:00
13:00:00	14:45:00	1:45:00
0:00:00	0:00:00	0:00:00
Total time		5:33:00
Total time, mins		333
Bloodmeal, kg		2862
Head		813
Stickwater estimate, L		20379.6
Stickwater estimate, L/hd		25.06715867

Date:	24/05/2023	WEDNESDAY
Blood valve on	Blood valve off	Time (hours)
7:20:00	10:20:00	3:00:00
11:20:00	13:15:00	1:55:00
13:50:00	14:55:00	1:05:00
0:00:00	0:00:00	0:00:00
Total time		6:00:00
Total time, mins		360
Bloodmeal, kg		2835
Head		829
Stickwater estimate, L		22032
Stickwater estimate, L/hd		26.57659831

Date:	25/05/2023	THURSDAY
Blood valve on	Blood valve off	Time (hours)
7:10:00	9:27:00	2:17:00
10:36:00	12:10:00	1:34:00
13:00:00	13:50:00	0:50:00
14:15:00	14:45:00	0:30:00
Total time		5:11:00
Total time, mins		311
Bloodmeal, kg		2727
Head		798
Stickwater estimate, L		19033.2
Stickwater estimate, L/hd		23.85112782

Date:	26/05/2023	FRIDAY
Blood valve on	Blood valve off	Time (hours)
7:07:00	9:10:00	2:03:00
10:20:00	12:00:00	1:40:00
12:43:00	13:24:00	0:41:00
14:00:00	15:27:00	1:27:00
Total time		5:51:00
Total time, mins		351
Bloodmeal, kg		3084
Head		824
Stickwater estimate, L		21481.2
Stickwater estimate, L/hd		26.06941748

Date:	29/05/2023	MONDAY
Blood valve on	Blood valve off	Time (hours)
8:00:00	10:05:00	2:05:00
10:40:00	11:36:00	0:56:00
12:10:00	13:10:00	1:00:00
13:47:00	14:46:00	0:59:00
Total time		5:00:00
Total time, mins		300
Bloodmeal, kg		2240
Head		839
Stickwater estimate, L		18360
Stickwater estimate, L/hd		21.88319428

	Average	*Max.*	*Min.*	*Std. Dev ±*
Blood valve 'on' time, mins	312	367	238	36.9
Bloodmeal, kg	2,662	3,460	1,974	406.6
Head	812	839	777	16.6
Estimated stickwater L/day	19,091	22,460	14,566	2259.0
Estimated stickwater L/head	23.5	26.8	18.7	2.9

APPENDIX 8: AMPTS II Setup

28 °C		Substrate						Inoculum		pH		Actual
Sample	Replicate	Target	Paunch target	Paunch actual	SW target	SW actual	Actual total	Target	Actual	Pre-dig	Post-dig	ISR
Inoculum	1	0					0	400	400	7.725	7.724	-
Inoculum	2	0					0	400	400	7.766	7.729	-
Inoculum	3	0					0	400	399.99	7.741	7.777	-
Celluose	1	3.17					3.1679	396.83	396.84	7.769	7.628	3.00
Celluose	2	3.17					3.1722	396.83	396.86	7.775	7.693	2.93
Celluose	3	3.17					3.1736	396.83	396.9	7.763	7.688	3.00
Paunch	1	11.99	11.99	11.98			11.98	388.01	388.07	7.744	7.579	3.01
Paunch	2	11.99	11.99	11.97			11.97	388.01	388.06	7.757	7.712	3.01
Paunch	3	11.99	11.99	11.95			11.95	388.01	390.14	7.761	7.651	3.01
Stickwater	1	142.54			142.54	142.6	142.6	257.46	257.47	7.835	7.686	3.01
Stickwater	2	142.54			142.54	142.59	142.59	257.46	257.51	7.852	7.740	3.00
Stickwater	3	142.54			142.54	142.52	142.52	257.46	257.48	7.848	7.715	3.01
Production ratios	1	32.45	10.11	10.15	22.34	22.31	32.46	367.55	367.56	7.765	7.548	3.00
Production ratios	2	32.45	10.11	10.12	22.34	22.41	32.53	367.55	367.54	7.749	7.574	3.00
Production ratios	3	32.45	10.11	10.17	22.34	22.47	32.64	367.55	367.54	7.762	7.612	3.00
C:N 15	1	73.54	6.34	6.31	67.21	67.26	73.57	326.46	326.45	7.823	7.634	3.12
C:N 15	2	73.54	6.34	6.31	67.21	67.19	73.5	326.46	326.47	7.836	7.615	3.01
C:N 15	3	73.54	6.34	6.38	67.21	67.25	73.63	326.46	326.48	7.805	7.621	2.97
C:N 20	1	56.53	7.9	7.93	48.64	48.64	56.57	343.47	343.53	7.831	7.570	3.01
C:N 20	2	56.53	7.9	7.94	48.64	48.66	56.6	343.47	343.53	7.827	7.575	3.01
C:N 20	3	56.53	7.9	7.92	48.64	48.66	56.58	343.47	343.53	7.805	7.569	3.00
C:N 25	1	44.86	8.97	8.95	35.89	35.97	44.92	355.14	355.18	7.811	7.571	3.01
C:N 25	2	44.86	8.97	8.95	35.89	35.85	44.8	355.14	356.5	7.803	7.551	3.01
C:N 25	3	44.86	8.97	8.95	35.89	35.9	44.85	355.14	355.11	7.811	7.656	3.00
C:N 30	1	36.34	9.75	9.76	26.59	16.61	26.37	363.66	363.66	7.815	7.564	3.01
C:N 30	2	36.34	9.75	9.75	26.59	26.6	36.35	363.66	363.67	7.81	7.559	3.01
C:N 30	3	36.34	9.75	9.73	26.59	26.63	36.36	363.66	363.7	7.833	7.555	3.01
C:N 35	1	29.86	10.35	10.39	19.52	19.53	29.92	370.14	370.16	7.839	7.549	3.00
C:N 35	2	29.86	10.35	10.35	19.52	19.55	29.9	370.14	371.97	7.821	7.560	3.00
C:N 35	3	29.86	10.35	10.36	19.52	19.55	29.91	370.14	370.16	7.844	7.565	3.01

37°C		Substrate						Inoculum		pH		Actual
Sample	Replicate	target	Paunch target	Paunch actual	SW target	SW actual	Actual total	Target	Actual	Pre-dig	Post-dig	ISR
Inoculum	1	0					0	400	400.01	7.741	0.000	-
Inoculum	2	0					0	400	400.01	7.741	7.734	-
Inoculum	3	0					0	400	400.01	7.74	7.732	-
Celluose	1	3.17					3.1762	396.83	396.86	7.786	7.629	3.01
Celluose	2	3.17					3.173	396.83	386.91	7.801	7.570	3.00
Celluose	3	3.17					3.1717	396.83	396.86	7.772	7.490	3.00
Paunch	1	11.99	11.99	11.96			11.96	388.01	388.07	7.763		3.01
Paunch	2	11.99	11.99	11.98			11.98	388.01	387.98	7.752	7.496	3.01
Paunch	3	11.99	11.99	11.98			11.98	388.01	388.1	7.743	7.448	3.03
Stickwater	1	142.54			142.54	142.52	142.52	257.46	257.47	7.82		3.00
Stickwater	2	142.54			142.54	142.59	142.59	257.46	257.48	7.835	7.418	3.01
Stickwater	3	142.54			142.54	142.57	142.57	257.46	257.51	7.842	7.446	3.01
Production ratios	1	32.45	10.11	10.1	22.34	22.38	32.48	367.55	367.55	7.766	7.418	3.00
Production ratios	2	32.45	10.11	10.19	22.34	22.33	32.52	367.55	367.57	7.772	7.513	3.00
Production ratios	3	32.45	10.11	10.18	22.34	22.35	32.53	367.55	367.56	7.806	7.432	2.99
C:N 15	1	73.54	6.34	3.65	67.21	67.24	70.89	326.46	326.46	7.82	7.408	3.01
C:N 15	2	73.54	6.34	6.33	67.21	67.22	73.55	326.46	326.49	7.799	7.617	3.01
C:N 15	3	73.54	6.34	6.38	67.21	67.98	74.36	326.46	326.45	7.803	7.531	3.00
C:N 20	1	56.53	7.9	7.87	48.64	48.65	56.52	343.47	343.5	7.799	7.486	3.00
C:N 20	2	56.53	7.9	7.89	48.64	48.61	56.5	343.47	343.47	7.808	7.501	3.00
C:N 20	3	56.53	7.9	7.896	48.64	48.7	56.596	343.47	343.48	7.801	7.562	3.00
C:N 25	1	44.86	8.97	8.93	35.89	35.88	44.81	355.14	355.32	7.814	7.422	3.00
C:N 25	2	44.86	8.97	8.97	35.89	35.86	44.83	355.14	355.25	7.809	7.515	3.02
C:N 25	3	44.86	8.97	8.97	35.89	35.96	44.93	355.14	355.13	7.808	7.492	3.01
C:N 30	1	36.34	9.75	9.74	26.59	26.57	36.31	363.66	363.68	7.818	7.488	4.14
C:N 30	2	36.34	9.75	9.76	26.59	26.57	36.33	363.66	363.69	7.812	7.465	3.01
C:N 30	3	36.34	9.75	9.76	26.59	26.6	36.36	363.66	363.7	7.839	7.511	3.01
C:N 35	1	29.86	10.35	10.36	19.52	19.57	29.93	370.14	370.16	7.823	7.431	3.00
C:N 35	2	29.86	10.35	10.39	19.52	19.52	29.91	370.14	370.17	7.813	7.408	3.02
C:N 35	3	29.86	10.35	10.33	19.52	19.51	29.84	370.14	370.15	7.835	7.421	3.00

APPENDIX 9: BMP and modified Gompertz modelling results.

37 °C	paunch	paunch	paunch	paunch	stickwater	stickwater	stickwater	stickwater	roduction rati	roduction rati	roduction rati	roduction rati	C:N15	C:N15	C:N15	C:N15
Day	Average Gompertz	Paunch 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	SW 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	PR 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN15 37 SMP	Std. Dev.	Avg Completion
0	2.2212	0.0000	0.0000	#DIV/0!	6.2879	0.0000	0.0000	#DIV/0!	3.6333	0.0000	0.0000	#DIV/0!	3.0252	0.0000	0.0000	#DIV/0!
1	9.2596	5.9765	2.6369	1.0000	14.9395	6.3957	0.0274	1.0000	10.9933	5.3762	0.5238	1.0000	11.7858	9.3294	1.2908	1.0000
2	25.1446	27.2508	5.8589	0.7919	29.6950	28.3527	1.6130	0.7737	25.1945	25.5596	1.9757	0.7898	30.8987	34.8566	3.7014	0.7332
3	50.5864	54.4898	10.7729	0.5017	51.2295	59.1985	2.4362	0.5214	46.8927	50.9839	3.1145	0.4991	61.1790	66.7058	6.3234	0.4780
4	82.5034	84.1417	18.3999	0.3493	78.9820	87.7220	3.5903	0.3252	74.6799	76.5482	5.4140	0.3335	99.2788	99.4696	10.7588	0.3284
5	116.1785	116.8462	27.6691	0.2766	111.3716	116.3678	6.2227	0.2457	105.8254	104.0472	8.9550	0.2633	139.9170	134.5521	17.0385	0.2591
6	147.6205	145.0730	29.2439	0.2005	146.3023	146.3543	10.6650	0.2038	137.4009	134.1420	13.9542	0.2228	178.4357	172.8793	23.5175	0.2208
7	174.5563	165.5817	20.9058	0.1323	181.6777	177.8382	16.6368	0.1754	167.0837	166.3248	18.9097	0.1925	211.9981	209.9390	22.1040	0.1790
8	196.2772	188.1525	13.0910	0.1234	215.7555	210.8353	22.7875	0.1552	193.4477	195.9616	19.7267	0.1523	239.5415	242.0851	11.4074	0.1350
9	213.0645	206.0251	1.9057	0.0873	247.3030	243.2566	25.4972	0.1336	215.8871	217.6436	14.5956	0.1016	261.2030	268.7668	2.4676	0.0995
10	225.6582	215.0400	4.1692	0.0414	275.5991	267.5826	15.8689	0.0934	234.3850	236.8269	10.5499	0.0816	277.7312	280.3678	1.2287	0.0414
11	234.9109	221.7796	9.4287	0.0294	300.3490	293.2808	3.3354	0.0881	249.2724	249.8173	10.6675	0.0521	290.0747	288.3668	1.2864	0.0277
12	241.6101	227.9394	13.4163	0.0261	321.5693	319.5195	10.7272	0.0807	261.0403	258.8169	12.2515	0.0346	299.1538	294.1022	1.8373	0.0195
13	246.4112	233.9225	16.1667	0.0249	339.4770	343.3210	23.5160	0.0671	270.2178	266.9262	13.5525	0.0302	305.7600	299.4477	2.5919	0.0178
14	249.8274	240.6818	17.6634	0.0278	354.4000	357.0028	27.0554	0.0378	277.3032	273.8662	13.7827	0.0254	310.5302	304.6237	3.6759	0.0170
15	252.2461	246.2716	19.0573	0.0224	366.7121	366.0306	25.6513	0.0251	282.7322	279.3308	13.4733	0.0197	313.9559	309.3003	4.3115	0.0151
16	253.9524	250.9597	19.6029	0.0186	376.7896	373.6876	24.8339	0.0207	286.8683	284.0818	13.0657	0.0168	316.4068	313.8832	3.4394	0.0146
17	255.1533	254.9690	19.8599	0.0157	384.9863	379.8317	24.2716	0.0163	290.0062	287.8287	12.2908	0.0132	318.1553	316.4880	3.0055	0.0148
18	255.9970	256.9282	21.2001	0.0072	391.6198	385.1945	24.2703	0.0140	292.3792	283.3396	1.6240	0.0145	319.4004	320.8352	3.8680	0.0135
19	256.5890	281.8855	0.0000	0.0133	396.9670	391.1344	24.8295	0.0152	294.1694	287.8385	0.7374	0.0156	320.2858	324.8372	4.4492	0.0123
20	257.0041	285.3712	0.0000	0.0122	401.2637	397.5586	25.1083	0.0162	295.5176	291.6426	0.4027	0.0130	320.9148	328.8792	5.4324	0.0122
21	257.2950	289.0762	0.0000	0.0128	404.7076	401.7891	25.7444	0.0105	296.5316	294.8551	1.0302	0.0109	321.3613	339.2253	0.0000	0.0145
22	257.4987	291.1124	0.0000	0.0070	407.4625	406.8354	26.6098	0.0123	297.2933	298.8839	0.0000	0.0100	321.6782	342.3997	0.0000	0.0093
23					409.6628	411.3975	27.0929	0.0111	297.8653	301.8111	0.0000	0.0097				
24					411.4180	415.1442	27.0665	0.0091								

37 °C	C:N20	C:N20	C:N20	C:N20	C:N25	C:N25	C:N25	C:N25	C:N30	C:N30	C:N30	C:N30	C:N35	C:N35	C:N35	C:N35
Day	Average Gompertz	CN20 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN25 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN30 37 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN35 37 SMP	Std. Dev.	Avg Completion
0	3.6767	1.0697	1.5127	#DIV/0!	3.3211	0.0000	0.0000	#DIV/0!	3.1241	0.0000	0.0000	#DIV/0!	2.6190	0.0000	0.0000	#DIV/0!
1	13.5682	9.9136	2.1609	1.0000	12.4195	8.1618	0.8577	1.0000	9.6090	5.4992	1.0723	1.0000	8.1581	5.3627	0.7287	1.0000
2	33.9041	33.8780	0.7572	0.7536	31.4485	32.4192	1.0880	0.7486	22.4355	25.6655	5.4441	0.7845	19.3346	24.9051	3.0821	0.7850
3	64.4484	65.1270	1.0560	0.4802	60.5093	62.6163	1.7019	0.4823	42.5461	51.8723	10.5818	0.5059	37.2334	48.2974	7.0377	0.4825
4	101.1275	100.0187	3.1874	0.3357	95.9463	94.0507	3.1750	0.3341	68.9618	76.4061	17.6205	0.3164	61.2447	69.8046	12.4281	0.3034
5	138.7074	137.9343	4.3106	0.2745	132.7556	129.2482	4.8638	0.2722	99.2902	101.8822	26.7875	0.2422	89.3740	92.1977	19.9555	0.2350
6	173.1212	176.2451	2.5560	0.2286	166.8746	168.2290	6.3739	0.2317	130.7295	129.4464	37.9949	0.2037	119.0880	117.4481	29.6203	0.2057
7	202.2360	206.9751	2.2763	0.1569	196.0484	205.2564	6.4018	0.1806	160.8924	158.7075	48.8209	0.1787	148.0941	145.2503	40.3374	0.1835
8	225.5317	226.1129	5.6288	0.0726	219.6100	223.2223	4.9058	0.0807	188.1832	183.3933	52.3363	0.1388	174.7567	173.2777	49.0346	0.1600
9	243.4542	240.9975	7.1214	0.0547	237.8873	235.7111	5.2722	0.0530	211.8042	202.0871	49.3192	0.1030	198.1687	191.4345	48.0317	0.1044
10	256.8679	253.0532	7.6973	0.0454	251.6675	247.0450	5.5537	0.0459	231.5748	218.3445	42.0382	0.0854	218.0225	210.4971	42.1038	0.1019
11	266.7147	262.3489	7.6819	0.0347	261.8508	256.0498	5.9776	0.0351	247.7085	235.3188	31.4399	0.0803	234.4190	229.5637	31.5011	0.0920
12	273.8453	269.8377	7.0431	0.0286	269.2702	263.4114	6.3037	0.0279	260.6247	250.9567	20.2084	0.0665	247.6901	247.2367	19.4293	0.0760
13	278.9601	276.4600	5.6819	0.0263	274.6223	270.2100	6.5124	0.0252	270.8167	261.8474	14.5956	0.0429	258.2684	259.6335	13.8191	0.0491
14	282.6044	282.3366	4.3087	0.0251	278.4561	276.9966	6.9893	0.0245	278.7717	267.9793	15.2296	0.0228	266.6029	266.0606	14.8872	0.0240
15	285.1888	286.6360	4.6247	0.0191	281.1886	282.8170	6.6028	0.0206	284.9296	273.0758	16.5463	0.0184	273.1117	271.6095	16.2224	0.0202
16	287.0156	289.7891	4.3869	0.0132	283.1295	287.5609	6.0025	0.0165	289.6669	278.0341	17.6904	0.0176	278.1607	276.2489	16.8565	0.0167
17	288.3038	291.8351	4.1486	0.0077	284.5047	290.3132	6.1579	0.0095	293.2943	281.3359	18.2757	0.0116	282.0574	279.0747	17.1233	0.0101
18									296.0619	296.5604	5.1114	0.0093	285.0532	281.4473	17.2064	0.0084
19													287.3495	278.2435	17.8666	0.0104
20													289.1058	281.6127	17.5831	0.0121
21													290.4468	284.9426	17.0340	0.0118
22													291.4694	272.0169	0.0000	0.0151
23													292.2483	274.7086	0.0000	0.0098

28°C	paunch	paunch	paunch	paunch	stickwater	stickwater	stickwater	stickwater	roduction rati	roduction rati	roduction rati	roduction rati	C:N15	C:N15	C:N15	C:N15
Day	Average Gompertz	Paunch 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	SW 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	PR 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN15 28 SMP	Std. Dev.	Avg Completion
0	2.7265	0.0000	0.0000	#DIV/0!	1.0158	0.0000	0.0000	#DIV/0!	2.4114	0.0000	0.0000	#DIV/0!	3.2095	0.0000	0.0000	#DIV/0!
1	4.4562	2.7238	0.0000	1.0000	3.2055	1.6610	0.0478	1.0000	3.7662	2.9545	0.0276	1.0000	6.5439	3.7797	1.0366	1.0000
2	6.9029	3.3104	0.0000	0.1772	7.9832	5.2917	0.4956	0.6843	5.6517	3.6012	0.0584	0.1795	11.8855	7.7194	2.7136	0.4897
3	10.1943	5.8308	0.0000	0.4323	16.4750	15.7711	0.6254	0.6651	8.1778	6.8405	0.7536	0.4680	19.5946	17.8801	4.4292	0.5809
4	14.4279	15.1658	0.0000	0.6155	29.2860	29.7324	0.8984	0.4697	11.4473	15.4950	1.1292	0.5596	29.7864	30.8251	5.3045	0.4279
5	19.6601	24.6036	0.0000	0.3836	46.2414	46.7872	1.0408	0.3646	15.5476	25.0913	0.6371	0.3831	42.3040	44.4077	6.7112	0.3082
6	25.9001	34.1760	0.0000	0.2801	66.4567	65.7100	1.1758	0.2880	20.5445	34.0550	0.8103	0.2631	56.7568	58.9071	8.3185	0.2472
7	33.1094	42.4593	0.0000	0.1951	88.6343	86.5062	2.1941	0.2403	26.4771	40.9160	1.4709	0.1673	72.6006	73.7173	10.1670	0.2013
8	41.2062	48.8164	0.0000	0.1302	111.4045	107.5532	2.8729	0.1957	33.3551	45.6114	2.0142	0.1026	89.2297	88.6189	12.2537	0.1681
9	50.0731	54.9661	0.0000	0.1119	133.5822	130.5666	3.1977	0.1763	41.1582	49.9149	2.7006	0.0857	106.0577	104.8963	14.4161	0.1553
10	59.5674	61.0448	0.0000	0.0996	154.2954	155.5305	3.4878	0.1605	49.8380	54.3165	3.4068	0.0805	122.5739	122.8850	16.1317	0.1471
11	69.5317	67.7957	0.0000	0.0996	173.0061	179.7228	3.8444	0.1346	59.3211	59.0809	4.3386	0.0799	138.3734	140.7264	15.2518	0.1289
12	79.8043	74.9672	0.0000	0.0957	189.4656	198.4546	3.7452	0.0944	69.5139	64.7378	5.3660	0.0867	153.1658	156.3737	10.6823	0.1024
13	90.2273	82.9084	0.0000	0.0958	203.6428	215.1358	4.4099	0.0775	80.3076	71.3043	6.5148	0.0914	166.7679	169.0907	4.9948	0.0763
14	100.6542	91.6903	0.0000	0.0958	215.6514	219.6431	4.4401	0.0205	91.5836	78.9436	7.7369	0.0962	179.0879	178.9880	2.8606	0.0553
15	110.9537	101.2439	0.0000	0.0944	225.6887	223.0536	4.6169	0.0153	103.2191	87.7479	8.8944	0.1000	190.1063	186.6852	4.1728	0.0411
16	121.0137	112.2086	0.0000	0.0977	233.9900	227.0605	4.7940	0.0176	115.0913	98.4870	9.8224	0.1092	199.8565	195.0928	6.0658	0.0428
17	130.7415	123.2411	0.0000	0.0895	240.7983	230.4387	5.0495	0.0146	127.0818	110.4500	10.1849	0.1089	208.4083	203.3051	7.6795	0.0402
18	140.0643	135.0874	0.0000	0.0877	246.3450	234.4286	5.4528	0.0170	139.0795	124.3857	9.8181	0.1129	215.8532	209.8291	6.0556	0.0313
19	148.9282	148.0527	0.0000	0.0876	250.8400	239.4720	5.5815	0.0211	150.9830	141.1972	8.2634	0.1201	222.2942	216.8851	3.4553	0.0327
20	157.2960	161.4588	0.0000	0.0830	254.4673	245.4158	5.9992	0.0242	162.7021	159.7445	5.4468	0.1166	227.8375	223.6138	0.8773	0.0301
21	165.1458	174.2176	0.0000	0.0732	257.3848	252.1717	6.4268	0.0268	174.1591	178.0828	6.7158	0.1021	232.5875	229.4509	3.0606	0.0253
22	172.4682	183.7859	0.0000	0.0521	259.7251	258.9750	6.9919	0.0262	185.2882	193.7998	14.6032	0.0785	236.6427	234.9357	6.6950	0.0229
23	179.2645	191.3526	0.0000	0.0395	261.5985	266.7797	7.2375	0.0293	196.0362	205.5574	21.7522	0.0543	240.0941	239.0099	7.3772	0.0170
24	185.5443	194.5451	0.0000	0.0164	263.0956	274.1429	7.1667	0.0269	206.3610	214.0688	24.7394	0.0387	243.0242	242.4095	7.7296	0.0140
25	191.3238	197.2629	0.0000	0.0138	264.2904	277.6835	6.1478	0.0128	216.2317	219.7516	26.6198	0.0252	245.5062	245.6108	8.4110	0.0130
26	196.6240	199.5747	0.0000	0.0116	265.2430	275.5095	0.0000	0.0032	225.6268	224.9621	28.8132	0.0223	247.6049	249.7396	10.5163	0.0123
27	201.4692	201.9873	0.0000	0.0119					234.5336	229.9092	31.9273	0.0202	249.3768	252.9519	11.6452	0.0125
28	205.8862	204.6034	0.0000	0.0128					242.9466	234.6192	35.7655	0.0182	250.8709	269.3002	0.0000	0.0175
29	209.9025	207.4907	0.0000	0.0139					250.8671	252.9041	42.3703	0.0179	252.1294	272.8367	0.0000	0.0130
30	213.5465	210.7181	0.0000	0.0153					258.3013	257.1652	43.9417	0.0127	253.1885	275.1740	0.0000	0.0085
31	216.8460	213.4358	0.0000	0.0127					265.2599	260.7279	44.9957	0.0110				
32	219.8284	215.9501	0.0000	0.0116					271.7572	263.8448	45.8890	0.0098				
33	222.5197	218.3288	0.0000	0.0109					277.8098	266.9794	46.6218	0.0098				
34	224.9451	220.3355	0.0000	0.0091					283.4366	270.3488	47.6579	0.0102				
35										273.1868	48.5688	0.0088				
36											0.0000	0.0310				
37											0.0000	0.0084				
39											0.0000	0.0090				

28°C	C:N20	C:N20	C:N20	C:N20	C:N25	C:N25	C:N25	C:N25	C:N30	C:N30	C:N30	C:N30	C:N35	C:N35	C:N35	C:N35
Day	Average Gompertz	CN20 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN25 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN30 28 SMP	Std. Dev.	Avg Completion	Average Gompertz	CN35 28 SMP	Std. Dev.	Avg Completion
0	3.2711	0.0000	0.0000	#DIV/0!	3.3741	0.0000	0.0000	#DIV/0!	2.1886	0.0000	0.0000	#DIV/0!	1.5407	0.0000	0.0000	#DIV/0!
1	6.6168	4.5419	0.3671	1.0000	6.8155	4.6521	0.2025	1.0000	4.2810	2.3477	0.0013	1.0000	2.9589	2.6506	0.8118	1.0000
2	11.9346	8.4416	0.8155	0.4610	12.2703	8.5786	0.0943	0.4574	7.6199	3.8132	0.0664	0.3841	5.2209	3.7511	1.4891	0.2622
3	19.5557	18.4846	1.6565	0.5436	20.0634	19.0582	0.1624	0.5498	12.5064	11.4823	0.4547	0.6676	8.5575	8.5604	4.2443	0.4615
4	29.5689	30.5849	2.4292	0.3961	30.2685	31.5318	0.3816	0.3956	19.1449	21.6284	0.7566	0.4692	13.1546	17.1127	6.1833	0.5386
5	41.7996	43.6204	3.2390	0.2991	42.6912	44.8606	0.6007	0.2971	27.6036	32.0788	1.0415	0.3258	19.1234	26.0927	8.3551	0.3566
6	55.8523	57.8092	4.0836	0.2456	56.9161	59.2027	0.8010	0.2423	37.8032	43.2843	1.3259	0.2589	26.4821	35.8378	10.5478	0.2796
7	71.1909	72.0107	5.0246	0.1973	72.3908	73.2743	1.0015	0.1920	49.5319	53.8397	1.5085	0.1961	35.1546	44.5004	12.9558	0.1956
8	87.2282	86.0845	6.1578	0.1634	88.5178	87.1044	1.3502	0.1588	62.4793	63.4184	1.9810	0.1510	44.9817	51.8746	15.6835	0.1380
9	103.4020	101.5629	7.3664	0.1523	104.7316	102.3424	1.8651	0.1489	76.2787	73.6462	2.4362	0.1388	55.7428	59.5923	18.6681	0.1252
10	119.2279	119.0116	8.6185	0.1466	120.5495	119.8251	2.4904	0.1459	90.5485	85.3571	2.9248	0.1372	67.1810	68.2937	22.0749	0.1234
11	134.3263	137.3441	9.0581	0.1338	135.5974	138.0230	2.7826	0.1319	104.9263	98.4209	3.3106	0.1328	79.0277	78.0655	25.8511	0.1217
12	148.4281	152.4721	6.8660	0.1000	149.6145	153.1598	2.0579	0.0989	119.0930	113.5742	3.9168	0.1334	91.0235	89.3311	30.1269	0.1234
13	161.3671	162.3131	6.2903	0.0607	162.4432	162.3720	2.1114	0.0567	132.7860	130.4524	4.4883	0.1294	102.9338	101.8574	34.7872	0.1210
14	173.0638	170.7939	6.9521	0.0496	174.0122	171.7585	2.7755	0.0546	145.8045	148.1385	4.9572	0.1194	114.5588	115.2042	39.1332	0.1166
15	183.5064	179.9194	7.7328	0.0506	184.3174	182.0555	3.3468	0.0565	158.0072	163.1861	4.6268	0.0923	125.7375	126.4892	41.3615	0.0944
16	192.7324	190.2227	8.5967	0.0541	193.4025	193.5198	3.6958	0.0592	169.3064	172.3397	4.2991	0.0532	136.3486	135.2982	40.7398	0.0754
17	200.8127	200.1052	8.7957	0.0494	201.3431	202.9310	2.3617	0.0465	179.6599	180.4817	4.5338	0.0451	146.3078	143.7416	39.6914	0.0676
18	207.8379	208.2801	6.2170	0.0396	208.2335	208.1238	2.0645	0.0250	189.0617	189.3101	4.5639	0.0467	155.5640	152.8579	38.8742	0.0665
19	213.9083	213.9848	5.6463	0.0267	214.1764	212.7800	2.3040	0.0219	197.5333	199.4507	4.8829	0.0508	164.0934	163.0253	38.2598	0.0679
20	219.1270	217.8442	5.8269	0.0177	219.2764	217.1587	2.4141	0.0202	205.1161	208.7481	6.0368	0.0444	171.8950	170.6789	35.9558	0.0508
21	223.5940	221.9931	6.1649	0.0187	223.6345	221.9875	2.5796	0.0217	211.8646	213.7498	6.6647	0.0233	178.9847	176.9084	32.6008	0.0407
22	227.4040	226.0623	6.1821	0.0180	227.3456	226.6188	3.0781	0.0204	217.8411	217.5739	6.9526	0.0176	185.3914	182.6923	29.8227	0.0355
23	230.6438	230.6479	6.4898	0.0199	230.4963	231.4126	3.0770	0.0207	223.1115	222.1412	7.3592	0.0205	191.1525	188.1421	27.5452	0.0317
24	233.3918	234.4440	5.9064	0.0163	233.1647	234.7079	2.7248	0.0141	227.7425	226.0825	7.5278	0.0174	196.3109	193.2013	25.9214	0.0280
25	235.7177	237.4754	5.7159	0.0128	235.4199	237.0596	2.9463	0.0099	231.7988	229.7948	8.0200	0.0161	200.9127	197.4455	24.2401	0.0230
26	237.6829	236.0732	4.3100	0.0087	237.3226				235.3424	233.3180	8.2739	0.0151	205.0047	200.3555	24.0831	0.0149
27	239.3408								238.4308	237.5843	8.5445	0.0179	208.6331	202.8256	24.1664	0.0123
28									241.1172	241.0562	8.3045	0.0145	211.8425	205.1068	24.1227	0.0113
29									243.4500	243.6491	8.3374	0.0106	214.6754	207.4827	23.9555	0.0117
30									245.4727	245.7757	8.4726	0.0086	217.1712	209.7786	23.7198	0.0112
31													219.3666	199.9819	19.7814	0.0114
32													221.2949	182.8762	0.0000	0.0146
33													222.9866	185.5875	0.0000	0.0146
34													224.4692	188.2659	0.0000	0.0142
35													225.7673	191.1085	0.0000	0.0149
36													226.9030	216.7989	0.0000	0.1185
37													227.8958	218.8224	0.0000	0.0092

		37℃							28℃						
		λ	*B0*	*μm*	*sum of r2*	*actual NmL CH4*	*mL CH4/g VS*	*Average days for completion*	*λ*	*B0*	*μm*	*sum of r2*	*actual NmL CH4*	*mL CH4/g VS*	*Average days for completion*
Paunch	***average***	1.57	257.97	33.88	380.84	776.78	263.42	20	4.36	245.76	10.45	1301.29	649.79	220.34	34
	± std. dev	0.41	44.45	6.98	140.96	114.58	39.16	2.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Stickwater	***average***	1.88	418.25	35.52	1986.84	814.42	415.14	24	3.12	268.95	22.82	1283.72	545.34	277.97	25.3
	± std. dev	0.50	54.26	0.31	1231.44	75.37	38.28	0	0.08	7.19	0.71	184.63	14.46	7.37	0.6
Production ratios	***average***	1.68	299.58	31.84	432.72	843.14	300.21	20.3	6.42	347.39	12.01	3650.98	741.33	264.28	32.7
	± std. dev	0.12	9.14	3.43	260.79	17.23	5.75	3.1	1.85	90.64	2.23	3244.93	225.94	80.78	6.8
C:N 15	***average***	1.57	322.45	40.84	818.81	815.55	327.98	19.3	2.71	258.73	16.86	364.22	660.08	264.63	27.3
	± std. dev	0.22	8.24	3.88	521.06	32.45	12.78	3.1	0.60	21.69	0.87	185.84	41.56	18.27	2.5
C:N 20	***average***	1.25	296.39	36.72	374.20	772.34	294.81	17.3	2.62	248.05	16.21	134.76	626.53	239.34	26
	± std. dev	0.17	11.50	1.40	101.14	10.42	3.97	0.6	0.18	6.26	0.93	10.06	17.57	6.68	1
C:N 25	***average***	1.41	287.81	37.10	354.83	787.27	291.07	17.3	2.56	247.28	16.26	103.29	643.17	237.98	25.5
	± std. dev	0.03	7.73	1.24	89.70	20.37	7.90	0.6	0.00	3.81	0.39	1.13	14.98	5.47	0.7
C:N 30	***average***	1.85	304.74	31.55	928.20	710.36	284.04	18	3.71	258.20	14.40	293.56	680.47	245.78	30
	± std. dev	0.42	16.35	8.76	891.90	104.16	24.24	1	0.04	11.73	0.42	38.68	29.10	10.38	0
C:N 35	***average***	2.01	294.72	29.83	1237.72	819.05	290.08	20.7	4.42	234.66	12.01	688.77	628.66	222.77	32
	± std. dev	0.59	6.23	5.84	1721.69	39.34	13.96	2.5	1.61	8.82	2.89	730.79	17.80	6.05	4.6

APPENDIX 10: Gas compositions

37°C		CH_4			CO_2		
	n	*Average*	*±*	*Std. Dev.*	*Average*	*±*	*Std. Dev.*
Blank	***2***	68.84%	±	0.20%	31.16%	±	0.20%
Cellulose	***3***	74.54%	±	0.47%	25.46%	±	0.47%
Paunch 37C	***2***	69.22%	±	1.74%	30.78%	±	1.74%
Stick water 37C	***2***	70.28%	±	1.55%	29.72%	±	1.55%
Production ratios 37C	***3***	68.78%	±	3.32%	31.21%	±	3.31%
15 C:N 37C	***3***	72.99%	±	2.74%	27.01%	±	2.74%
20 C:N 37C	***3***	71.87%	±	0.32%	28.13%	±	0.32%
25 C:N 37C	***3***	71.53%	±	1.72%	28.47%	±	1.72%
30 C:N 37C	***3***	74.07%	±	1.25%	27.96%	±	1.25%
35 C:N 37C	***3***	69.38%	±	0.64%	30.62%	±	0.64%

28°C		CH_4			CO_2		
Sample	*n*	*Average*	*±*	*Std. Dev.*	*Average*	*±*	*Std. Dev.*
Blank	***3***	81.57%	±	4.71%	18.43%	±	4.71%
Cellulose	***3***	83.13%	±	0.63%	16.87%	±	0.63%
Paunch 28C	***1***	77.80%	±	0.00%	22.20%	±	0.00%
Stick water 28C	***3***	83.95%	±	0.29%	16.05%	±	0.29%
Production ratios 28C	***3***	79.09%	±	0.78%	20.91%	±	0.78%
15 C:N 28C	***3***	79.82%	±	0.01%	20.18%	±	0.01%
20 C:N 28C	***3***	77.70%	±	0.26%	22.30%	±	0.26%
25 C:N 28C	***3***	51.63%	±	35.67%	48.37%	±	35.67%
30 C:N 28C	***3***	77.39%	±	0.13%	22.83%	±	0.13%
35 C:N 28C	***3***	77.26%	±	0.67%	22.74%	±	0.67%

Methane %	*Paunch*	*Stick water*	*PR*	*C:N 15*	*C:N 20*	*C:N 25*	*C:N 30*	*C:N 35*	*Paunch*	*Stick water*	*PR*	*C:N 15*	*C:N 20*	*C:N 25*	*C:N 30*	*C:N 35*
triplicate 1	--	--	64.43%	69.13%	71.69%	69.18%	75.14%	69.06%	77.80%	83.54%	78.13%	79.82%	77.47%	77.11%	77.20%	76.54%
triplicate 2	70.96%	71.83%	72.48%	75.15%	72.33%	73.25%	72.32%	68.82%	--	84.20%	79.10%	79.80%	78.07%	76.59%	77.44%	77.09%
triplicate 3	67.48%	68.73%	69.44%	74.70%	71.60%	72.15%	74.74%	70.27%	--	84.11%	80.03%	79.83%	77.57%	--	77.52%	78.16%
Average	69.22%	70.28%	68.78%	72.99%	71.87%	71.53%	74.07%	69.38%	77.80%	83.95%	79.09%	79.82%	77.70%	76.85%	77.39%	77.26%
SD	1.74%	1.55%	3.32%	2.74%	0.32%	1.72%	1.25%	0.64%	0.00%	0.29%	0.78%	0.01%	0.26%	0.26%	0.13%	0.67%

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