ANAEROBIC DIGESTION OF PRE-TREATED SLAUGHTERHOUSE WASTE

A Thesis submitted by

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Abstract

Low-rate covered anaerobic lagoons (CALs) offer the Australian red meat processing (RMP) industry an attractive wastewater treatment option with the added benefit of capturing methane-rich biogas that can be combusted to offset onsite fossil fuel consumption. Whilst high-strength, high-fat wastewater generated by the RMP industry provides excellent potential for biogas production, it also presents operational problems and can reduce the performance of anaerobic digestion (AD) systems. Fats, oils and greases, and other solids present in the wastewater are responsible for pipe blockages, degradation of lagoon covers, inhibition of mass transfer of nutrients, and sludge flotation and washout.

This thesis presents an investigation of pre-treatment on AD of high-fat waste cattle slaughterhouse using dissolved air flotation (DAF) sludge as a standard substrate. The first phase of work evaluated four pre-treatment options using biomethane potential (BMP) tests. The pre-treatment methods assessed were thermobaric, chemical, thermochemical and bovine bile as a novel bio-surfactant. Phase 2 examined thermobaric pre-treatment in continuous digestion.

Under batch digestion, thermobaric pre-treatment demonstrated the greatest improvement in the digestion process. Thermobaric pre-treatment was also the most practical for implementation at slaughterhouses, with potential for heat-exchange to reduce pre-treatment cost. Soluble chemical oxygen demand was enhanced from 16.3% in the control to 20.84% (thermobaric), 40.82% (chemical), and 50.7% (thermochemical). Pre-treatment altered volatile fatty acid concentration by -64% (thermobaric), 127% (chemical) and 228% (thermochemical). Lag phase was reduced by 20% in the thermochemical group, and 100% in the thermobaric group. Specific methane production (SMP) was enhanced by 3.28% (chemical), 8.32% (thermobaric), and 8.49% (thermochemical) as a result of pre-treatment.

Bovine bile was dosed at arbitrary concentrations from 0.2-6 g/L. At 0.6 g bile/L, methane yield increased by 7.08%. Doses above 2 g bile/L produced negative impacts on SMP, kinetics and digestion profile. At 6 g/L bile produced a 6% decrease in specific methane production and up to 79% additional inhibitory duration, delayed time of peak methane production 74%, and slowed total digestion time 65%. Reaction kinetics declined linearly with respect to bile addition, reaching half the control value at 6 g/L bile concentration. Subsequent anaerobic toxicity assays using bile in the range of 1-6 g/L revealed the inhibitory nature of bile at higher doses. Economic feasibility assessment showed that, when compared to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile to 220% of its current sale value.

Based on the batch BMP results, thermobaric-treated substrate was used for continuous digestion experiments. Thermobaric-treated DAF sludge combined with abattoir wastewater was fed to lab-scale continuous stirred tank reactors (CSTR) for 49 days. While pre-treatment under batch digestion improved methane yield and inhibition, methane yield was decreased by 12.1%, pH was consistently lower, and H_2S concentration was 56% higher on average in continuous digestion mode. Under the conditions of this investigation, the benefits measured under batch digestion were not reproduced under continuous digestion. This highlights the value of continuous digestion experiments in evaluating substrates for industrial application.

Certification of Thesis

This Thesis is the work of Peter Harris except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Bernadette McCabe

Associate Supervisor: Thomas Schmidt

Associate Supervisor: Mark Lynch

Student and supervisors signatures of endorsement are held at the University.

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List of Abbreviations

AD	Anaerobic digestion
AMPTS II	Automated methane potential test system II
AnMBR	Anaerobic membrane reactor
AUD	Australian dollars
B	Cumulative SMP at time (t)
B_0	Cumulative SMP at end of digestion
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
BR	Bioreactor
BRS	Bioreactor simulator
$Ca(OH)_2$	Calcium hydroxide
CaO	Calcium oxide
CH_4	Methane
CHP	Combined heat and power
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CoHRAL	Covered high-rate anaerobic lagoon
CSTR	Continuous stirred-tank reactor
DAF	Dissolved air flotation
DAI	Data acquisition instrument
DB	Database
DMDO	Dimethyldioxirane
DS	Dry solids
EGSB	Expanded granular sludge bed reactor
$FeCl_2$	Iron chloride
FOG	Fat, oil and grease
FS	File storage
GC-FID	Gas chromatography with a flame ionisation detector
GHG	Greenhouse gas
H_2O_2	Hydrogen peroxide
$H_2SO_4^-$	Sulphuric acid
H_3PO_4	Phosphoric acid

HCl	Hydrochloric acid
HNO_3	Nitric acid
HPH	High-pressure homogenisation
HRT	Hydraulic retention time
IBC	Intermediate bulk container
ISR	Inoculum to substrate ratio; I:S
k	Rate constant of logistic equation
KOH	Potassium hydroxide
L	Litre, 1 cubic decimetre; 1 dm^3
LCFA	Long-chain fatty acids
M	Molar; moles/litre
mEq	Milli equivalents
$Mg(OH)_2$	Magnesium hydroxide
M_I	Mass of inoculum
min	Minutes
mL_N	Normal Millilitres; 1 atm. 0°C, corrected for water vapour
MJ	Megajoules
mM	Millmolar
M_R	Final mass of reactor liquid components
M_S	Mass of substrate
Mt	Megatonnes
MWh	Megawatt hours
N	Normality
NaOH	Sodium Hydroxide
NH_4-N	Ammonium-nitrogen
NOx	Nitrogen oxides
O_3	Ozone
OLR	Organic loading rate; g (VS or COD)/L/day
P	Phosphorus
PL-250	Pancreatic lipase 250
POMS	Peroxymonosulphate
psi	Pounds per square inch
RMP	Red meat processing

sCOD	Soluble chemical oxygen demand
tCOD	Total chemical oxygen demand
SMP	Specific methane production
t	Time in days
T_{EQ}	Time of equivalent methane yield in days
T_{FIN}	Time in days of digestion finish in days
tHSCW	Tonnes of hot standard carcass weight
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
U	Rate constant of Gompertz equation
U/mg	Units of activity per mg of enzyme
UASB	Upflow anaerobic sludge blanket
USA	United States of America
v/v	% volume per unit of volume
VFA	Volatile fatty acids
V_R	Reactor volume
VS	Volatile solids
VS_I	VS of inoculum
VSS	Volatile suspended solids
VS_S	VS of substrate
w/v	% weight per units of volume
WAS	Waste activated sludge
λ	Lag phase in Gompertz equation

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Statement of Authorship

This thesis is based on the following papers.

I. Harris, P & McCabe, B 2015, 'Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater', *Applied Energy*, vol. 155, pp. 560-75.

II. Harris, PW, Schmidt, T & McCabe, BK 2017, 'Evaluation of chemical, thermobaric and thermochemical pre-treatment on anaerobic digestion of high-fat cattle slaughterhouse waste', *Bioresource Technology*, vol. 244, pp. 605-10.

III. Harris, PW, Schmidt, T & McCabe, BK 2018, ‘Bovine bile as a bio-surfactant pre-treatment option for anaerobic digestion of high-fat cattle slaughterhouse waste’, *Journal of Environmental Chemical Engineering*, vol. 6, pp 444-50.

IV. Harris, PW, Schmidt, T & McCabe, BK 2018, ‘Impact of thermobaric pre-treatment on the continuous anaerobic digestion of high-fat cattle slaughterhouse waste’, *Biochemical Engineering Journal*, vol 134, pp. 108-13.

The thesis is concerned with evaluating pre-treatment of high-fat slaughtrehouse waste with the aim of improving anaerobic digestion. The experimental work focused on biochemical methane potential and continuous digestion in lab-scale continuous stirred-tank reactors.

Paper I is a review of the literature which critically examines various pre-treatment options to improve anaerobic digestion across a broad range of substrates. This review identified two main knowledge gaps in the literature. Firstly, a lack of standardisation across investigations into anaerobic digestion makes drawing meaningful comparison difficult. This is also a hinderance in generating cost/benefit analyses to better inform industry on the how to optimise digestion of their substrates. Secondly, literature regarding the anaerobic digestion of fat-rich substrate and abattoir waste and wastewater in general, and the pre-treatment of such substrates are largely absent from the literature.

Paper II and III report on the effect of pre-treatments on the anaerobic digestion, as determined by biochemical methane potential (BMP) testing, of dissolved-air flotation (DAF) sludge, a substrate used in this study to reflect the high fat content of red meat processing wastewater. **Paper II** reports on the application of thermobaric, chemical and thermochemical pre-treatment methods and the subsequent effect on the anaerobic digestion of this material. Thermobaric pre-treatment was the most effective pre-treatment in terms of improving methane yield and eliminating lag-phase inhibition. **Paper III** reports on the effect of a novel bio-surfactant pre-treatment in the form of bovine bile to DAF sludge. **Papers II and III** briefly explore cost/benefit analyses of pre-treatment application. **Paper IV** reports on the application of thermobaric pre-treatment to lab-scale continuous stirre- tank reactors to monitor and assess the suitability of pre-treatment during long-term continuous digestion.

Contributions to papers

Paper I: I wrote the paper. Edits were made by Bernadette McCabe

Author contributions:	Peter Harris	80%
	Bernadette McCabe	20%

Paper II: I performed all experimental work, I wrote the paper. Edits were made by Bernadette McCabe and Thomas Schmidt.

Author contributions:	Peter Harris	75%
	Bernadette McCabe	20%
	Thomas Schmidt	5%

Paper III: I performed all experimental work, I wrote the paper. Edits were made by Bernadette McCabe and Thomas Schmidt.

Author contribtuions:	Peter Harris	75%
	Bernadette McCabe	20%
	Thomas Schmidt	5%

Paper IV: I performed the majority of experimental work, with contibutions by Seonmi Lee, Thomas Schmidt and Bernadette McCabe on various occasions. I performed the majority of analyses, with contributions from Thomas Schmidt and

Seonmi Lee. I wrote the paper, edits were made by Bernadette McCabe and Thomas Schmidt.

Author contributions:	Peter Harris	75%
	Bernadette McCabe	20%
	Thomas Schmidt	5%

Structure of the Thesis

This thesis is structured as per the guidelines set forth by the University of Southern Queensland with respect to Thesis by Publication. The thesis is organised into four chapters that derive from **Papers I-IV** listed above, and 4 appendices which contain the published **Papers I-IV** in full journal format. Chapter one provides an introduction, review of the literature and outlines the research aims and objectives. Chapter two includes a brief overview of the methodology, for which the full methodology can be found in **Papers II-IV**. Chapter three provides an in-depth discussion of **Papers II-IV**, and chapter four provides conclusion drawn from **Papers II-IV**.

I

Introduction

Global processing of cattle has intensified consistently over the past 50 years, increasing by 36.29 Mt from 27.69 Mt in 1961 to 63.98 Mt in 2013 (FAOSTAT 2015) (Figure 1). While production has more than doubled, waste mitigation techniques have lagged behind the ever increasing accumulation of waste.

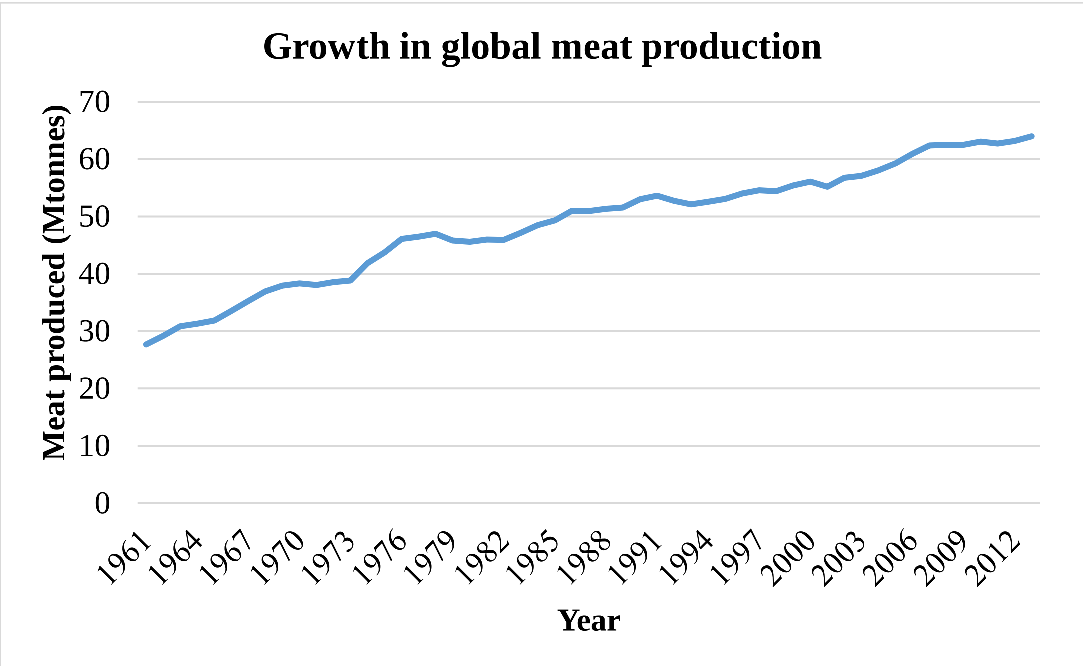

Figure 1: Growth in global meat production from 1961-2013 (FAOSTAT 2015)

Processing livestock is an energy and cost intensive process. An environmental sustainability review of the Australian red meat processing (RMP) industry conducted by AMPC and MLA (2010) revealed that 9.8 kL of water was used to generate a single tonne of hot standard carcass weight (tHSCW) during 2008-2009 and generated 8.7

kL of wastewater. Per tHSCW, this consumed 4108 MJ of energy from various sources, and committed 11.3kg of solid waste to landfill, while greenhouse gas (GHG) emissions averaged 554kg $CO_{2\text{-eq}}$/tHSCW. Of total energy emissions, 67% were related to electricity use, and 35% of emissions contributed by anaerobic wastewater treatment (AMPC & MLA 2010). For the year of 2014-15, with 8.76 million cattle harvested resulting in the production of 2.42 million tHSCW, the industry generated approximately 20.8 gigalitres of wastewater, consumed 9.94 petajoules of energy, committed 27.35 Mt of solid waste to landfill, and emitted 1.34 Mt of CO2-eq of GHG emissions (AMPC 2015; Australian Bureau of Statistics 2016). The terms 'wastewater' and 'waste' will be used interchangeably in this thesis. Any differentiation between solid and liquid waste will be clearly stated.

The Australian RMP industry is currently working on a range of measures in an effort to reduce carbon pollution and improve energy efficiency through actively seeking renewable sources of energy and water recovery. This has been largely in response to a variety of factors including prolonged drought, tightened water restrictions, increasing costs of water, fuel and energy, improved community focus and environmental awareness, and rising GHG emissions (AMPC & MLA 2010). Several knowledge gaps have been identified in which research is needed to reduce the industry's emissions and energy costs (AMPC & AMIC 2012). One of the technologies identified as a potential solution reducing emission and energy costs is anaerobic digestion (AD). It has been demonstrated that AD technology can play a major role in waste management and the production of biogas in the abattoirs (Ortner *et al.* 2014). The methane (CH_4) produced can be combusted to generate heat and electricity (CHP), or can be refined into renewable natural gas and transport fuels (Stucley *et al.* 2012). In addition, AD can be used to manage waste and reduce GHG emissions, and the digestate may be used or sold as a valuable organic fertilizer substitute or soil amendment (Appels *et al.* 2011).

Red meat processors have embraced the uptake of AD systems to treat high-strength wastewater and thereby reduce emissions. In Australia, AD systems typically take the form of low-rate anaerobic lagoons, which are well suited to the vacant land space available, with a move to covered anaerobic lagoons to capture methane and reduce GHG emissions (CSIRO, 2010). While it has been noted that anaerobic lagoons are not optimised treatment strategies, they are low-capital investments which can affect a large degree of organic degradation and methane generation (Jensen *et al.* 2014).

The high-strength wastewaters produced in Australian abattoirs tend to contain high levels of fat, oil and grease (FOG) with values ranging between 5 and 4570 mg/L in grab samples (McCabe *et al.* 2012). While AD is effective for the degradation of many substrates, FOG present several challenges. Before waste reaches the digester, FOG can adhere to pipe walls and begin accumulating to form blockages. In the case of covered anaerobic lagoons, FOG typically has two fates; accumulation as fatty crust, or hydrolysis and digestion to form methane. In the first instance, accumulation of FOG, hair and cellulosic material from paunch float to the lagoon surface and coalesce into increasingly thicker masses to form the crust. (UNSW 1998; Mayo 2011; McCabe *et al*. 2013; White, Johns & Butler 2013). In the second instance, fat particles that are hydrolysed to long-chain fatty acids (LCFA) may subsequently adhere to the surface of the sludge microbes. These LCFA form a layer over the microbial surface, producing reversible inhibition of mass-transfer between the microbes and the medium (Long *et al.* 2012).

Australian abattoirs stand to benefit substantially if an appropriate pre-treatment method can be developed to improve the bioavailability and subsequent conversion of FOG to methane. McCabe *et al.* (2014) has shown that biogas production can potentially vary tenfold depending on factors such as lagoon efficiency and operational practices. With exception to anaerobic membrane reactor technology (Dasa *et al.* 2016) and Lipothan reactor technology (ACS-Umwelttechnik 2017) which are yet to be rigorously tested, no other AD system currently deals with FOG effectively, typically the more sophisticated the anaerobic digestion technology, the less capable they are of handling FOG loads (Appels *et al.* 2008; Jensen *et al.* 2014).

1.1 Brief overview of anaerobic digestion

Anaerobic digestion is a natural process by which a consortium of micro-organisms operates synergistically to break down organics to produce biogas in the absence of oxygen (Gerardi 2003). The four steps of anaerobic digestion include hydrolytic, acidogenic, acetogenic and methanogenic activity (Figure 2; Appels *et al.* 2008). Biogas produced from this process consists primarily of methane (60-80%) and carbon dioxide (20-40%) (Di Bella 2010).

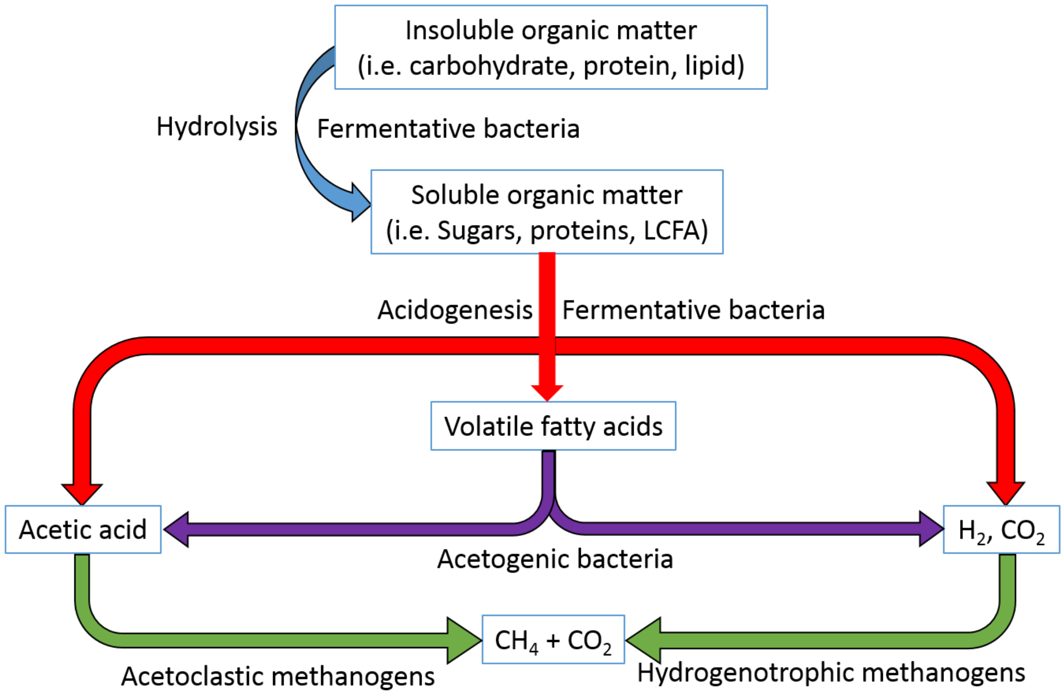

Figure 2: Stages of anaerobic digestion, modified from Appels et al. (2008).

For complex substrates, hydrolysis is the rate limiting step in the AD process (Appels *et al.* 2008). The role of hydrolyic enzymes is to degrade large insoluble carbohydrates, proteins and lipids to their soluble metabolites. Carbohydrates are degraded from polysaccharides to di- or mono-saccharides, proteins break down to amino acids, and lipids break down to form LCFA. The next stage of digestion, acidogenesis, further degrades the products of hydrolysis to form volatile fatty acids (VFA), hydrogen and carbon dioxide, and some other by-products. Acetogenesis involves the degradation of VFA and alcohols to produce acetic acid, hydrogen and carbon dioxide. These products are consumed by two groups of methanogenic archae to produce methane. While acetoclastic methanogens consume acetic acid and produce methane and carbon doixide, hydrogenotrophic archae utilise hydrogen and carbon dioxide and produce methane (Appels *et al.* 2008), and some archae utilise both pathways.

1.2 Characteristics of abattoir wastewater

The main types of wastes from abattoirs include organic solid wastes generated during meat processing and wastewaters from washing at various stages of the process. Australian RMP wastewater is generated at high volumes and characterised as having high organic, fat and nutrient loading. Volumes are typically around 850kL/day with organic content of 5700kg chemical oxygen demand (COD) per day (MLA 2002). In Australia, a typical abattoir is defined as processing 150 tHSCW per day, equivalent to 625 head of cattle (MLA 2002). Production is assumed to take place 5 days a week, 250 days per year, including boning and rendering (MLA 2002). While Johns (1993) determined typical values for abattoir wastewater, case studies have reported pollutant concentrations far greater than the typical (McCabe et al. 2013; UNSW 1998; Table 1). Abattoir wastewater becomes high-strength due to the accumulation of constituents including blood, fat, paunch, protein and excrement in the water. The composition of Australian RMP wastewaters may vary significantly from abattoir wastewaters in other countries due to the fully integrated facilities in Australia which include slaughter, boning and rendering processes at the same plant (Johns 1995). In contrast, German abattoirs, for example, are required by law to perform rendering in an off-site facility (UNEP & DEPA 2000). Furthermore, the high-strength wastewaters produced in Australian abattoirs tend to contain high levels of FOG compared with their non-integrated equivalents. For this reason, care must be taken when comparing reports from various abattoirs around the world. While large integrated beef slaughterhouses in the USA show excellent similarities with data from Australian abattoirs, Australian abattoirs tend to generate higher volumes of higher-strength wastewaters than their European counterparts (Johns 1995; MLA 2002). Although high-strength wastewaters typically contribute well to biogas production, the FOG component tends to be problematic (Wan *et al.* 2011).

Table 1: Concentrations of parameters of high-strength wastewater produced by abattoirs.

Parameter (mg/L)	Typical abattoir raw wastewater (all meats) [b]	King Island (beef) [c]	Southern Meats wastewater ex DAF (sheep) [d]	Churchill Abattoir (Beef) [e]
BOD	1600-3000	3000	~1/2 COD	163-7020
COD	4200-8500	7250	3100-11500	1040-12100
FOG	100-200	120	290-2670	5-2110
TSS	1300-3400	2000	1150-5700	457-6870
VSS	n/a	n/a	1040-5300	n/a
TN	114-148	450	180-440	296-785
NOx	n/a		0.01 – 0.12	n/a
NH_4-N	65-87	250	18-135	23.8-349[f]
Total P	20-30	45	26.4-60	n/a
VFA	175-400	n/a	61-600	1020-1980
Alkalinity	350-800	n/a	340-700	70-906

[a] Benefield (2001); [b] Johns (Johns 1993); [c] White; Johns and Butler (2013); [d] UNSW (1998); [e] McCabe *et al.* (2013); [f] Value is for NH_3-N; n/a indicates not available
BOD – biochemical oxygen demand; TSS – total suspended solids; VSS – volatile suspended solids; TN – total nitrogen; NOx – nitrogen oxides; NH_4-N – ammonium as nitrogen; P - phosphorus

1.3 Wastewater parameters associated with biogas production

The wastewater parameters which are of particular interest to this work are those which could be logically associated with increased biogas production, including COD, soluble COD (sCOD), volatile solids (VS), FOG, fat particle size, and VFA (Appels *et al.* 2008; Nakhla *et al.* 2003; Pilli *et al.* 2011). Pre-treatments are often assessed with respect to sCOD release and degradation (Amani, Nosrati & Sreekrishnan 2010). As treatments rupture cells, the intracellular contents are released into the extracellular medium, contributing to the soluble fraction of COD (Gronroos *et al.* 2005). As a measure of pre-treatment impact on substrate degradation, sCOD appears to be useful (Kim et al. 2003; Rincón et al. 2013). However, while sCOD may increase in response to a pre-treatment, the relationship between sCOD and biogas production is complex, and as such, does not necessarily indicate an increase in biogas production (Carrere *et al.* 2010). Therefore, if biogas production is to be reported with respect to sCOD degradation, further information must be collected to support findings.

Although less commonly investigated as a measure of pre-treatment impact, specific methane production is regularly reported with respect to VS added (Luste & Luostarinen 2010). Also known as organic solids, VS is made up of carbohydrates, proteins and fats, typically derived from organisms, but may also include artificial organic compounds. Consequently, there is a strong correlation between VS degradation and biogas production (Appels *et al.* 2008). Given this strong correlation, measuring VS as an indicator of pre-treatment impact may be more valuable than measuring sCOD. However, while drying a sample for VS determination, there may be an initial loss of volatiles such as alcohols and VFA. Due to the lack of standardization in the reporting of pre-treatment impact on AD performance, this chapter will cover the majority of common measurements.

This chapter is particularly focused on the degradation of FOG, either during the pre-treatment process, or during the AD process as a result of pre-treatment. In batch digestions, measurement of FOG content can be done before and after pre-treatment, and post-digestion. Fat particle size reduction is another favourable outcome of pre-treatment. A reduction in particle size increases the surface area to volume ratio of the fat content, increasing the area susceptible to chemical and enzymatic interaction (Mshandete *et al.* 2006). Logically, this should increase the rate of methane production, but may result in temporary inhibition due to increased LCFA concentration. Further degradation of LCFA will produce VFA, which are also of interest as these are an end products of the acidogenic and acetogenic pathways of anaerobic digestion, and a feedstock for methanogenic archaea. While VFA at concentrations of 6.7-9 mM are toxic to methanogens, if a pre-treatment were capable of degrading triglycerides and LCFA to VFA, the process could significantly enhance reaction kinetics (Batstone *et al.* 2000).

1.4 Impact of fat, oil and grease in anaerobic digestion

The FOG component of high-strength wastes, such as those created in abattoirs, can induce several problems including clogging of pipes, adhesion to sludge causing both inhibition of mass-transfer of nutrients and sludge flotation with subsequent washout (Girault *et al.* 2012; Long *et al.* 2012). Anaerobic lagoons can receive large volumes of FOG and continue to function for long periods of time before the lagoon fails. This is likely due to the lack of mixing in lagoons, allowing FOG to float to the lagoon surface along with lignocellulosic material to form a fatty crust. While this accumulation is far from ideal, a managed crust does offer some benefit in odour reduction, pond insulation, and FOG locked up in crust is relatively unavailable to cause process inhibition (AMPC 2012; Golder Associates Pty Ltd 2009).

In continuously fed anaerobic lagoons this process can be unsustainable, where accumulation of FOG as crust outweighs FOG consumption. If FOG accumulation is not monitored and dealt with accordingly, crust can accumulate to several meters thick with surprising density as shown in Figure 3 (McCabe *et al.* 2013). Not only does this make crust removal from large lagoons difficult and expensive, the issue of how to deal with waste FOG after removal has not been addressed (Mayoh 2011).

Figure 3: Section of crust removed from an anaerobic lagoon by an excavator after desludging indicating crust thickness (McCabe et al. 2013).

In time, accumulation of crust on the lagoon surface heavily restricts the functional volume of the lagoon through the generation of dead space, resulting in short circuiting (Shilton & Harrison 2003). Figure 4 depicts a schematic diagram of the impact of crust accumulation on the functional volume of an anaerobic lagoon. Furthermore, the organic material itself is largely unavailable for degradation by the anaerobic consortium, as very little surface area with respect to crust volume is accessible by hydrolytic enzymes.

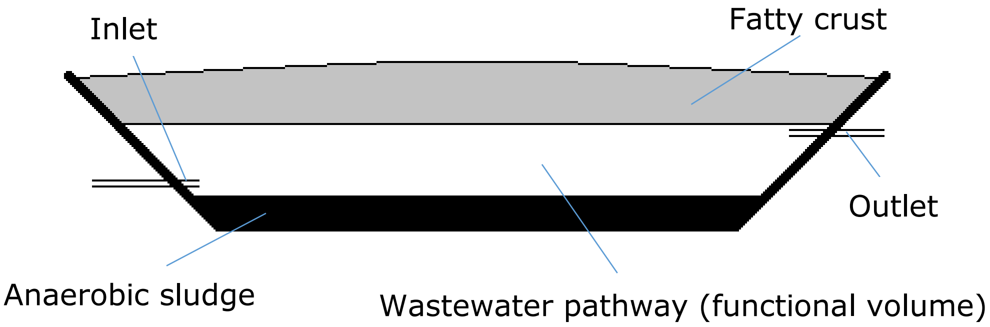

Figure 4: Illustration of dead space contributed by crust and sludge volume resulting in a large reduction in functional pond volume.

In addition to affecting the functional volume of a digester, covered anaerobic lagoons suffer further complications due to FOG. Thick crust material can significantly inhibit gas permeation and subsequently reduce gas capture by the cover (McCabe et al. 2013). Cover materials that come into contact with FOG are subject to chemical attack which can compromise the material integrity and result in ruptures, or gas leakage (Golder Associates Pty Ltd 2009). As crust accumulates and thickens, such as in Figure 4, floating raft-style covers can be flexed and bent out of shape, compromising the ability of the cover to capture gas.

Alternatively, high rate systems with active heating and mixing bring microbes into greater contact with FOG and LCFA. Subsequently, high rate AD systems that utilise granular sludge are more sensitive to FOG loadings and are at a greater risk of resulting failure than anaerobic lagoons (Jensen et al. 2015; Dereli *et al.* 2012). While microbes can be acclimated to FOG loadings this is a typically slow process with the time required to acclimate increasing with FOG loading (Fernandez, Sanchez & Font 2005). A move toward covered high rate anaerobic lagoon (CoHRAL) technology to treat abattoir wastewater which incorporates novel waste water distribution and settling systems is underway with the recent commissioning of the first CoHRAL system in the Australian RMP industry (Condon, 2014). The monitoring of this type of system will be particularly useful in assessing the overall impact of FOG loading and AD performance.

While anaerobic lagoons are currently considered the most suitable digester type for handling wastes with high FOG content, new research into anaerobic membrane reactor (AnMBR) technology has shown great promise in wastewater treatment, especially in wastes with high FOG loads. Christian *et al.* (2011) reported on the first two years of treating high-strength industrial wastewater at Ken's Foods in Massachusetts, USA. This AnMBR, the largest in the world in 2011, had a design of 475 m^3/d with COD, BOD and TSS loadings of 39000 mg/L, 18000 mg/L and 12000 mg/L respectively. The AnMBR produced consistently high-quality effluent with non-detectable TSS, and average COD and BOD concentrations of 210 and 20 mg/L, indicating removal efficiency of 99.4% and 99.9% respectively. Furthermore, AnMBR reactors have been loaded with COD in the order of 5-30 kg COD/m^3/d, and FOG loading of up to 4-6 kg/m^3 with removal rates of 97% and 100% removal efficiency respectively (Dereli *et al.* 2012; Diez, Ramos & Cabezas 2012). However, few investigations have involved large FOG loadings being treated using AnMBR technology. Given that high-rate AD systems are typically sensitive to FOG loadings, more research should be conducted to investigate the feasibility of FOG digestion using AnMBR technology (Long *et al.* 2012).

1.4.1 Enhancing biogas yield through co-digestion

While FOG have typically been viewed as a problematic substrate they have much to offer AD operations. Addition of FOG to an AD system has the potential to significantly increase biogas production (Zhu, Hsueh & He 2011). When the theoretical methane potential with respect to the stoichiometry of the macromolecules is compared, lipids are capable of yielding more methane at 1014 L/kg VS than both proteins at 480 L/kg VS and carbohydrates at 370 L/kg VS (Buswell & Neave 1930; Wan *et al.* 2011). These theoretical values were supported by Labatut (2012), with observed specific bio-methane yields ranging from 903.9-1101.2 L/kg VS for lipids, 302.5-407.3 L/kg VS for proteins and 191.8-359.3 L/kg VS for carbohydrates digested under mesophilic conditions. Indeed, co-digestion of substrates with FOG has produced significant increases in biogas production. Li, Champagne, and Anderson (2011) compared the biogas produced from digestion of waste activated sludge (WAS) co-digested with FOG using BMP tests. While the WAS control produced 117 ± 2.02 mL/g total volatile solids (TVS), the reactor co-digesting WAS with 0.35 g FOG at an S:I ratio of 0.46 produced 418 ± 13.7 mL/g TVS. This represents more than 350% increase in biogas production attributed to the addition of FOG. Similarly, Silvestre *et al.* (2011) co-digested sewage sludge with trapped grease waste. Not only did this study result in increased biogas production by 138%, but found that acetic and β-oxidation syntrophic acetogenic activities were 2.5 and 3.75 times higher than the initial inoculum respectively. This suggested that sludge could become acclimatised to greater FOG loads over time, and that this could be an effective strategy for improving fat degradation and reducing the inhibitory effects of LCFA. Table 2 lists several investigations which support the conclusion that co-digestion with FOG can significantly improve methane yields by considerable volumes.

Table 2: Effect of co-digesting substrates with FOG-rich co-substrates on methane yield.

Main substrate	Co-substrate	CH_4 volume	CH_4 %	Reference
Sewage sludge (100% VS)	Grease trap sludge (0% VS)	278 m^3/t VS added	63	Luostarinen, Luste and Sillanpaa (2009)
Sewage sludge (54% VS)	Grease trap sludge (46% VS)	463 m^3/t VS added (+66% CH_4 yield)	62	Luostarinen, Luste and Sillanpaa (2009)
Sewage sludge (100% VS)	Grease trap sludge (0% VS)	271 m^3/t VS added	65	Davidsson *et al.* (2008)
Sewage sludge (70% VS)	Grease trap sludge (30% VS)	344 m^3/t VS added (+27% CH_4 yield)	69	Davidsson *et al.* (2008)
Pig slurry (100% v/v)	Waste sardine oil (0% VS)	0.43 m^3 CH_4/m^3 digester/d	72	Ferreira, Duarte and Figueiredo (2012)
Pig slurry (95% v/v)	Waste sardine oil (5% VS)	1.61 m^3 CH_4/m^3/digester/d (+274% CH_4 yield)	70	Ferreira, Duarte and Figueiredo (2012)
Poultry manure (100% v/v)	Olive oil mill wastewater (0% v/v)	0.43 L/(V_R/d)	74.1	Gelegenis *et al.* (2007)
Poultry manure (75% v/v)	Olive oil mill wastewater (25% v/v)	0.52 L/(V_R/d) CH_4 yield ↑ 21%	71.8	Gelegenis *et al.* (2007)
Sewage sludge (77% VS)	Grease trap waste (23 % VS)	CH_4 yield ↑ 138%		Silvestre *et al.* (2011)
Municipal primary sludge (21% VS)	Thickened WAS (31% VS) and FOG (48% VS)	CH_4 yield ↑ 195%		Kabouris et al. (2009)

V_R – Reactor volume; ↑ - original value has increased, beyond 100%, by the given percentage.

However, co-digestion is dependent on access to available waste streams. Investigation of co-digestion using Australian abattoir wastewater is only in its infancy and is noted to be a multifaceted issue which goes beyond simply sourcing feedstocks for AD. The Australian RMP industry consists of medium to large enterprises which are often not located within close proximity to other agro-industrial waste streams. Subsequently, co-digestion is currently not an economically viable option for Australian abattoirs. Thus, Australian RMP industries which employ biogas facilities use abattoir wastewater as a monosubstrate. Ortner *et al.* (2015) exemplifies the situation of developing a reliable monodigestion process using slaughterhouse waste as the sole substrate. Beyond co-digestion, pre-treatment of FOG offers the next step to enhancing the AD process.

1.5 Pre-treatment of substrates for anaerobic digestion

In the context of this work, pre-treatment refers to the treatment of the waste or wastewater to enhance the availability of the substrate components to microbial enzymes, and thereby improve the removal of organics, increase reaction kinetics, and or total biogas production (Figure 5). Substrate availability may be enhanced through several mechanisms, resulting in liberation of sequestered organics, enhance surface area to volume ratio, or hydrolysis of macromolecules. The two reactions of primary interest are hydrolysis and β-oxidation. As hydrolysis is the first reaction involved in the degradation of complex substrates, this is general considered to be the rate limiting step (Luo, Yang & Li 2012). However, for the degradation of substrates high in FOG, LCFA degradation through β-oxidation is the slowest reaction, and controls the overall degradation kinetics (Ma et al. 2015). There are several different pre-treatment methods available to enhance digestion, including biological, mechanical, thermal, chemical, enzymatic, and biochemical approaches (Appels *et al.* 2008; Nakhla *et al.* 2003). While this chapter contains collated literature data on various pre-treatment methods, due to non-standardised reporting and great variability between research projects, direct comparison is difficult. Although projects that report on methane and biogas production are preferred, projects which report on other variables such as VS and sCOD have been included as they are valuable to inform further research.

Figure 5 illustrates the effect of pre-treatments on rate of anaerobic digestion (i.e. reaction kinetics; pre-treatment b) and increase the methane yield (pre-treatment c). Both effects will improve the operation of a biogas plant. However, depending on when a BMP test is ended, different interpretations are possible: t1: pre-treatment b - double the methane yield; t2: none of the pre-treatment methods increase methane yield; t3: pre-treatment c - increased the methane yield by 25% (Montgomery & Bochmann 2014).

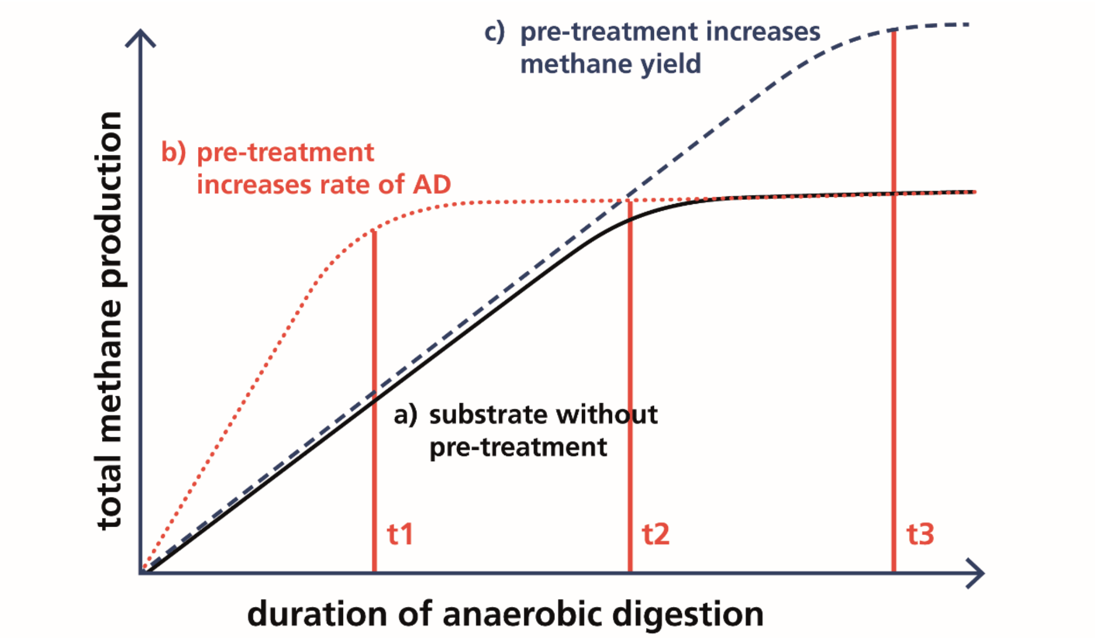

Figure 5: Effect of pre-treatments on reaction rate and methane yield from anaerobic digestion (Montgomery & Bochmann 2014).

Biogas production kinetics are used to describe and evaluate the anaerobic digestion of batch digestions by fitting the biogas production data to various kinetic equations (Ghatak & Mahanta 2014). Ghatak and Mahanta (2014) compiled a list of kinetic equations developed by various researchers, and described the evolution of kinetic equations from a simple linear equation, through logarithmic growth curves, Gaussian equations, through to logistic growth equations and finally the modified Gompertz equation. While these equations relay varying degrees of information to the researcher, the modified Gompertz equation is quite comprehensive for batch digestions. By curve fitting this equation to collected data, a researcher can reliably measure the rate constant and lag phase of a digestion which, like most complex substrates, produce a sigmoid curve of cumulative biogas production (Ghatak & Mahanta 2014).

This information is particularly useful for the investigation of co-digestion and pre-treatment in which reaction rates can be improved through various mechanisms. It is within the interests of an AD plant to enhance these reaction rates to produce as much biogas in as short a time as possible. A decrease in lag phase is indicative of a substrate which requires a lesser degree of hydrolysis from the AD consortium. This reduction in las phase typically results in an overall reduction in time required to complete digestion. This may allow an operator to decrease the hydraulic retention time (HRT) of a reactor, and or increase the organic loading rate (OLR). An increase in rate constant indicates that the substrate is more readily degradable due to pre-treatment or co-digestion, and the rate of biogas production is increased, typically resulting in shorter digestion times, and potentially, increased biogas yield.

Carlsson, Lagerkvist & Morgan-Sagastume (2012) reviewed pre-treatments in literature applied to different substrate categories in lab-, pilot- and full-scale studies as well as discussed in reviews (112 papers from 1978-2011). The pie-chart (Figure 6) illustrates the number of times each substrate-type occurs in combination with a pre-treatment; the total number of occurrences is larger than the number of articles since several articles discuss more than one pre-treatment type. The bar-charts illustrate the distribution among the different pre-treatments for each substrate-type. The literature was selected so as to cover as many different types of substrates, pre-treated with as many processes and/ or technologies as possible.

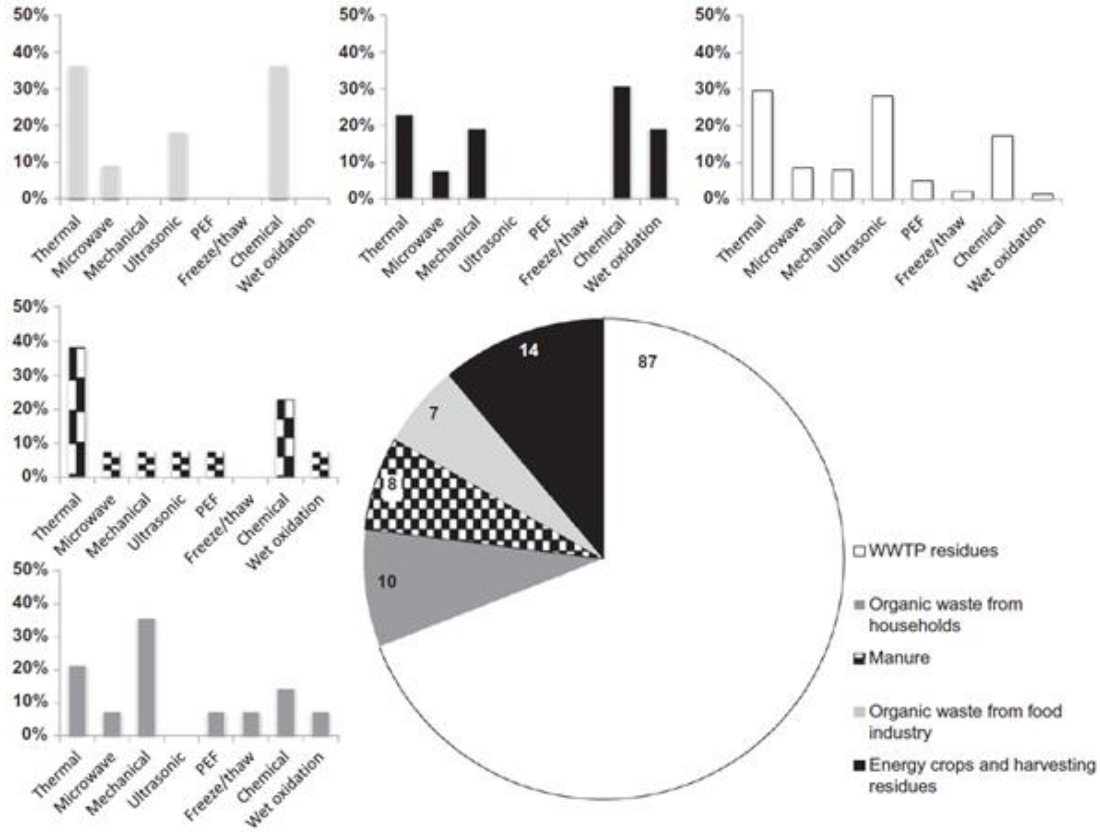

Figure 6: Pre-treatments and substrates in the reviewed literature. Substrate pre-treatments applied to different substrate categories in lab-, pilot- and full-scale studies as well as discussed in reviews (112 papers from 1978-2011). The pie-chart illustrates the number of times each substrate-type occurs in combination with a pre-treatment; the total number of occurrences is larger than the number of articles since several articles discuss more than one pre-treatment type. The bar-charts illustrate the distribution among the different pre-treatments for each substrate-type. The literature was selected so as to cover as many different types of substrates, pre-treated with as many processes and/or technologies as possible.) (Carlsson, Lagerkvist & Morgan-Sagastume 2012).

1.5.1 Mechanical degradation of feedstocks

Mechanical pre-treatments are commonly used to enhance digestion of cellular wastes such as sludges (e.g. WAS), cellulosics (e.g. crop waste), and other similar wastes. The aim of these pre-treatments is to rupture the cell walls of the cellular organisms in these feedstocks, a process which can be reduced from days to minutes through mechanical pre-treatment (Kopp *et al.* 1997). High-pressure homogenisation (HPH) and ultrasonication are two mechanical methods of potential benefit to FOG digestion. More in-depth review of mechanical pre-treatments can be found in **Paper I**.

High-pressure homogenisation works by compressing and projecting waste at high speed against an impact ring (Figure 7). The turbulence, cavitation and shear stresses applied to the waste disintegrate the cells, releasing cellular contents into the medium (Appels *et al.* 2008). While this technology has been successfully applied to disintegration of algal biomass and heavily utilised in the field of sludge disintegration, there is little available literature which considers HPH for pre-treatment of lignocellulosic biomass or fatty substrates. While some investigations have assessed the effect of HPH on substrates that are suitable for AD, they have focussed on the impact to the substrate, and not on the AD process. Subsequently, it is unknown how the changes in these substrates would impact a BMP test.

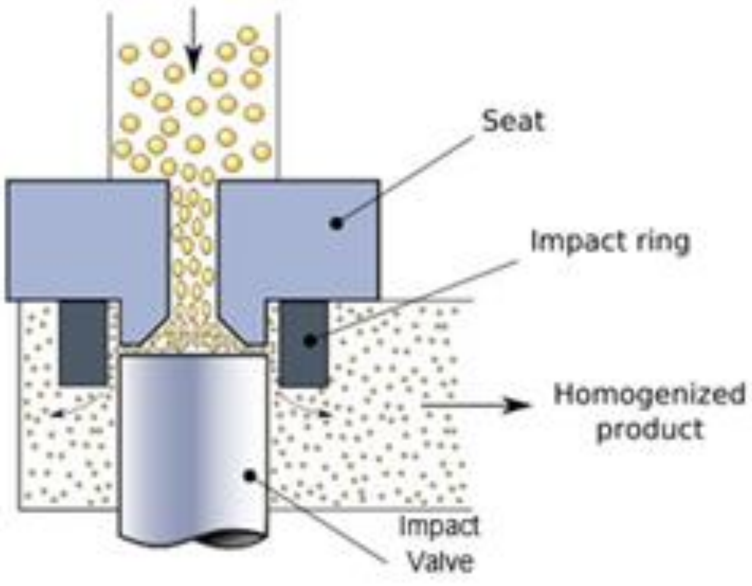

Figure 7: Diagrammatic disintegration of waste activated sludge by high-pressure homogenisation (Genizer 2009)

Ultrasonication has also been applied sparingly to FOG-rich substrates. The mode of action of ultrasonication is more sophisticated than HPH. As ultrasound waves propagate through the medium they create regions of compression and rarefaction. Microbubbles formed in this process grow in successive cycles and reach an unstable diameter at which they violently collapse in a process known as cavitation. Cavitational collapse produces intense local heating and high pressure (around 5000°C and over 500 atmospheres with a lifetime of a few microseconds) on a liquid-gas interface, and, turbulence and high shearing phenomena in the liquid phase (Erden, Buyukkamaci & Filibeli 2010; Pilli *et al.* 2011). Furthermore, cavitation produces highly reactive $H^{\bullet}$ and $OH^{\bullet}$ radicals which facilitate chemical reactions for destroying organic materials. These chemical reactions are further favoured by the high temperature and pressure generated at the site of cavitation (Dewil 2006). Table 3 lists several mechanical pre-treatment methods and summarises the conditions and results of numerous investigations.

Table 3: Mechanical pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Substrate	Results	Reference
Ball mill			
	WAS	• sCOD content ↑ 42% • Gas yield ↑ 20-50%	Baier and Schmidheiny (1997)
High-pressure homogenisation			
30 – 50 bar	WAS	• sCOD content ↑ 551% • Soluble protein ↑ 86% • VS removal ↑ 11-15%	Choi, Hwang and Shin (1997)
150 – 600 bar	WAS	• Biogas yield ↑ 30%	Onyeche (2007)
600 bar	WAS	• Biogas yield ↑ 28-54%	Engelhart *et al.* (2000)
Mechanical jet			
	WAS, (30 bar)	• sCOD content ↑ 500%	Nah *et al.* (2000)
Sonication			
	WAS	• No improvement in VS removal	Sandino *et al.* (2005)
	WAS	• sCOD content ↑ 11-39%	Khanal *et al.* (2006)
6000 kJ/kg TS	WAS	• Hydrolysis constant (*k*) ↑ 30-80%	Braguglia, Tomei and Mininni (2006)
120 MJ/kg TS	Meat processing effluent	• Oil removal ↑ 55.9% • COD removal ↑ 14.73%	Erden, Buyukkamaci and Filibeli (2010)
750 MJ/kg TS	Meat processing effluent	• COD removal ↑ 76.74%	Erden, Buyukkamaci and Filibeli (2010)
0.5 W/mL , 5 min	WAS	• Particle size ↓ 92%	Biggs and Lant (1998)
0.1-0.4 W/mL, 30-60 min	Municipal solid waste	• Biogas yield ↑ 24% • sCOD content ↑ 71.8%	Cesaro *et al.* (2012)

↑ - original value has increased, beyond 100%, by the given percentage.

Table 3 continued.

Pre-treatment	Substrate	Results	Reference
Sonication			
2000 kJ/kg TS	Waste vegetable oil,	Organic content ↑ 41932% (emulsification)	Moisan (2012)
Microwave			
0.3-300 GHz, 15 min	WAS	• sCOD content ↑ 22% CH_4 yield ↑ 79%	Park *et al.* (2004)
Electrical Field			
8000 kJ/kg DS	WAS	Sludge digestion ↑ 9%	Kopplow, Barjenbruch and Heinz (2004)

DS – Dry Solids; ↑ - original value has increased, beyond 100%, by the given percentage.

1.5.2 Thermal hydrolysis

The concept behind thermal pre-treatment is to expose substrates to elevated temperatures for long enough to promote chemical reactions and solubilisation of larger biomolecules. While temperatures typically range between 150-220°C under pressures of 600-2500 kPa, lower temperature pre-treatments have also been investigated (Appels *et al.* 2008; Gavala *et al.* 2003). However, many European researchers are required to adhere to the EC 1069/2009 regulation for the treatment of animal by-products not intended for human consumption.

Thermal pre-treatment of WAS has been heavily investigated, while other applications such as manure, abattoir waste, lignocellulosics and even algal biomass have received little attention (Appels *et al.* 2008; Carlsson, Lagerkvist & Morgan-Sagastume 2012; Cuetos *et al.* 2010; Mladenovska *et al.* 2006; Sims 2013). Furthermore, there have been few investigations into thermal pre-treatment of FOG-rich wastes. Fortunately, these investigations have yielded some encouraging results. Hiraoka *et al.* (1985), pre-treated substrates high in triglyceride content, and measured the decomposition of glyceride fatty acids to produce significant increases in acetic, propionic, butyric and valeric acid following thermal pre-treatment. Subsequent digestion displayed an increase in biogas production of 30%. Similar results were measured by Wilson, Novak and Murthy (2009), with pre-treatment at 170°C vastly enhancing acetic acid content of feed sludge. Equivalent increases in biogas production have also been supported in research by Li and Jin (2015). Table 4 lists the conditions and results of numerous investigations into thermal pre-treatment.

Table 4: Thermobaric pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Substrate	Results	Reference
Thermobaric			
70°C, 1-7 days	WAS	• CH_4 yield ↑ 19.8-85.9%	Gavala *et al.* (2003)
121°C, 30 minutes	WAS	• Biogas yield ↑ 32%	Kim, Ahn and Speece (2002)
121°C, 60 minutes	WAS	• Biogas yield ↑ 20%	Barjenbruch and Kopplow (2003)
170°C, 60 minutes	WAS	• CH_4 yield ↑ 45%	Valo, Carrere and Delgenes (2004)
170°C, 60 seconds	WAS	• Biogas yield ↑ 49%	Dohányos *et al.* (2004)
175°C, 40 minutes	WAS	• TSS removal ↑ 65%	Graja *et al.* (2005)
130°C, 30 minutes	WAS	• VSS/TSS ratio ↓ 70-80%	Bougrier, Delgenes and Carrere (2006)
170°C, 30 minutes	WAS	• CH_4 yield ↑ 51%	Bougrier, Delgenes and Carrere (2007)
110°C, 30 minutes	WAS	• VVS/TSS ratio ↑ 464%	Bougrier, Delgenes and Carrere (2008)
135°C, 35 minutes	WAS	• sCOD content ↑ 34%	Bougrier, Delgenes and Carrere (2008)
190°C, 50 minutes	WAS	• sCOD content ↑ 46%	Bougrier, Delgenes and Carrere (2008)
116°C, 38-73 minutes	WAS	• VSS/TVS ratio ↑ 383-429%	Bougrier, Delgenes and Carrere (2008)
122°C, 20-90 minutes	WAS	• VSS/TVS ratio ↑ 306-1410%	Bougrier, Delgenes and Carrere (2008)
128°C, 38-73 minutes	WAS	• VSS/TVS ratio ↑ 814-1441%	Bougrier, Delgenes and Carrere (2008)
134°C, 55 minutes	WAS	• VSS/TVS ratio ↑ 1104%	Bougrier, Delgenes and Carrere (2008)
165°C, 30 minutes	WAS	• Biodegradability ↑ 47-61%	Mottet *et al.* (2009)
170°C, 30 minutes	WAS	• sCOD content ↑ 765%	Wang *et al.* (2009)
100°C, 1 hour	Pig manure	• Biogas yield ↑ 31%	Rafique *et al.* (2010)

↑ - original value has increased, beyond 100%, by the given percentage.

Table 4 continued.

Pre-treatment	Substrate	Results	Reference
Thermobaric			
133°C, 20 min, >3 bar	Slaughterhouse waste	• Formation of refractory compounds. Unsuccessful in enhancing biodegradability of lipids and nitrogen-rich waste	Cuetos *et al.* (2010)
60, 80, 100°C	WAS	• Biogas yield ↑ 30%	Ho (2010)
90-120°C, 50-70 minutes	Kitchen waste	• Retention time required for acidification ↓ 5 days • Propionic acid was the dominant VFA produced • Biogas yield ↑ 31.7%	Li and Jin (2015)
80°C, 1.5 hours	Food waste	• Methane yield ↑ 52% • Extra yield can supply energy required for pre-treatment	Ariunbaatar et al. (2014)
Steam explosion			
170 – 230°C, 5 – 15 minutes	Salix	• CH_4 yield ↑ 50%	Estevez, Linjordet and Morken (2012)
134°C	Gravity thickened WAS	• sCOD content ↑ 4829-7987% • Total soluble nitrogen ↑ 2190% • Soluble NH_4^+-N content ↑ 1371%	Gianico *et al.* (2013)
	Dynamic thickened WAS	• sCOD content ↑ 2317-3289% • Total soluble nitrogen ↑ 3862% • Soluble NH_4^+-N content ↑ 771%	Gianico *et al.* (2013)
220°C, 30 seconds	WWTP sludge	• Biogas yield ↑ 80% • TS solubilised ↑ 55%	Zheng *et al.* (1998)
Hydrothermal			
170-220°C, 1.7-2.0 MPa, 30 minutes	Poultry slaughterhouse waste	• TS loss of 73.1-77.2% • TCOD loss of 57.8-68.3% • COD solubility increased from 2.2% to 98.2% • NH_4^+-N content ↑ 104.8% • VFA content ↑ 405.7-482.9%	Park et al. (2017)

↑ - original value has increased, beyond 100%, by the given percentage.

1.5.3 Acid and alkali and oxidative pre-treatments

Addition of acids and bases to AD feedstocks have been heavily investigated across a range of substrates including sludges, wastewater treatment plant (WWTP) residues, organic waste, plant residues and manures (Appels *et al.* 2008; Carlsson, Lagerkvist & Morgan-Sagastume 2012). Acidic pre-treatment has been performed using acids such as HCl, H_2SO_4, H_3PO_4 and HNO_3, and is indicated to be more effective in treating lignocellulosic biomass (Zhen et al. 2017). The main mechanism in this application is the acid hydrolysis of hemicellulose to release monomeric sugars and soluble oligomers from the cell wall into the digestate, and thereby improving the bioavailability of the substrate to exoenzymes and microorganisms (Zhen et al. 2017). Conversely, alkali addition is generally more efficient at enhancing the AD process (Jan *et al.* 2008). Beyond substrate degradation, alkali addition carries the added benefits of improving the system buffering capacity, specific methanogenic activity, and process stability (Zhen et al. 2017). Of the alkaline pre-treatments which have been investigated, sodium hydroxide (NaOH) is the most effective for enhancing organics hydrolysis and the AD process (Kim *et al.* 2003). NaOH aids in the degradation of substrates through solvation and saponification, inducing depolymerisation and cleavage of complex structure and subsequent solubilisation of smaller molecular weight compounds (Zhen et al. 2017).

Sodium hydroxide pre-treatment has been optimised for the enhancement of WAS digestion. Kim *et al.* (2003) determined that optimal dosing with NaOH was 7 g/L, bringing the solution to pH 12. The duration at which the substrate was held at pH 12 was not mentioned. This pre-treatment increased sCOD content by approximately 478% from 2250 mg/L to around 13000 mg/L. Digestion resulted in greater sCOD removal from 1136 mg/L in the control to 4941 mg/L after treatment, an increase of 335%. Degradation of VS was also improved from 20.5% up to 29.8% in the chemically treated sample. Both biogas production and methane content increased in response to the treatment, with increases of 13.4% and 12.8% respectively.

Alkali pre-treatment of pork fat has also been investigated. Massé, Kennedy and Chou (2001) studied the effect of NaOH pre-treatment on the solubilisation and size reduction of pork fat particles in abattoir waste. While sCOD was not impacted by addition of 50-400 mEq NaOH/L, the authors measured a 73 ± 7% reduction in particle size at concentrations ranging from 150-300 mEq/L. Although the fat particles were then smaller, they were still hydrophobic and would float on the surface of a digester, unavailable for immediate consumption. However, this reduction in particle size and subsequently increased surface area should increase the rate of degradation due to exoenzymes produced by the sludge, or could be utilised to improve the efficiency of subsequent pre-treatment methods, such as enzymatic pre-treatment.

This impact on degradation rate was noted by Battimelli, Carrere and Delgenese (2009). These researchers investigated the effect of NaOH pre-treatment on biogas production from fatty abattoir waste. While this pre-treatment affected little change in the total biogas produced, it did slightly enhance the initial reaction kinetics. These findings support the previous assertion that reduction of particle size due to alkaline hydrolysis could be exploited for additional benefit through further pre-treatment.

The third type of chemical pre-treatment is oxidative pre-treatments. These methods involve the use of oxygen at temperatures of ~260°C and pressures of 10 MPa (Amani, Nosrati & Sreekrishnan 2010). However, odour, corrosion and high energy consumption restrict practical application of this process (Appels *et al.* 2008). Alternatively, powerful oxidants including ozone (Ariunbaatar et al. 2014; Bougrier et al. 2006), and peroxides peroxymonosulphate (POMS) and dimethyldioxirane (DMDO) (Dewil et al. 2007), have also been investigated, with the latter being the most promising options. Table 5 lists the pre-treatment conditions and results of various chemical methods investigated in the literature.

Table 5: Literature results for the effects of chemical pre-treatment on various substrates

Pre-treatment	Substrate	Results	Reference
Alkali			
NaOH (1%), 7 d	Cattle dung	• Digestibility ↑ 31-42% • Biogas yield ↑ 100%	Dar and Tandon (1987)
NaOH, 130°C	WAS	• Biogas yield ↑ 20%	Tanaka *et al.* (1997)
NaOH, 0.01 N, 4 d	WAS	• Improved sludge thickening	Saiki *et al.* (1999)
NaOH, 20-80 mEq/L, 25°C, 10 h	WAS	• sCOD content ↑ 31%	Chang, Ma and Lo (2002)
NaOH, 45 mEq/L, 25-55°C, 4 h	WAS	• sCOD content ↑ 28-38%	Heo *et al.* (2003)
NaOH 20 mEq/L, 24 h	WAS	• Biogas yield ↑ 83%	Ray, Lin and Rajan (1990)
NaOH 7 g/L (175 mEq/L)	WAS	• sCOD content ↑ 31.7%	Kim *et al.* (2003)
KOH	WAS	• sCOD content ↑ 28.5%	Kim *et al.* (2003)
$Mg(OH)_2$	WAS	• sCOD content ↑ 2.7%	Kim *et al.* (2003)
$Ca(OH)_2$	WAS	• sCOD content ↑ 7.2%	Kim *et al.* (2003)
CaO	WAS	• No observed improvement	Carballa, Omil and Lema (2004)
Oxidation			
0.2 g O_3/g COD	Primary-secondary sludge	• CH_4 yield ↑ 112%	Weemaes *et al.* (2000)
0.16 g O_3/g SS	WAS	• SS removed ↑ 22%	Battimelli *et al.* (2003)
0.015-0.05 g O_3/g TS	WAS	• TS removed ↑ 28%	Goel, Tokutomi and Yasui (2003)
0.06 kg O_3/kg TSS	WAS	• sCOD content ↑ 16%	Sievers, Ried and Koll (2004)
0.1 g O_3/g TS	WAS	• No improvement in TS removal	Bernal-Martinez *et al.* (2007)
0.068 g O_3/g TS	Food waste	• Methane yield ↑ 8.7%	Ariunbaatar et al. (2014)
0.07 g Fe^{2+}/g H_2O_2, 50g H_2O_2, 1 h	WAS	• COD content ↑ 494%	Dewil *et al.* (2007)
60 g POMS/kg DS, 1 h	WAS	• COD content ↑ 406%	Dewil *et al.* (2007)
660 mL DMDO/kg DS, 1 h	WAS	• COD content ↑ 589%	Dewil *et al.* (2007)

N – Normality; SS – Suspended Solids; ↑ - original value has increased, beyond 100%, by the given percentage.

1.5.4 Thermochemical pre-treatment

Several researchers have combined thermal and chemical pre-treatments to produce more favourable results than either individual pre-treatment (Table 6). Again, WAS is a prime candidate for thermochemical pre-treatment. Kim *et al.* (2003) demonstrated the effects of thermochemical pre-treatment with 7g NaOH/L. This pre-treatment enhanced COD solubilisation by 85.4% over the control, over 40% greater than chemical pre-treatment alone, and increased VS reduction by 30% (Figure 8). Furthermore, when Tanaka *et al.* (1997) treated WAS with 0.3 g NaOH/L at 130°C in an autoclave for 5-200 minutes, they recorded an increase in VSS solubilisation of 40-50% and an increase in methane production by greater than 200% over the control. Valo *et al.* (2004) treated WAS at 170°C for 15 minutes in an autoclave and recorded an increase in TS reduction of 59%, with 92% higher gas production. While pre-treatment of WAS has been heavily investigated, there is little literature regarding FOG pre-treatment. One exception to this is an investigation conducted by Li, Champagne and Anderson (2013) in which co-digested FOG and kitchen waste were pre-treated thermochemically. Pre-treatment enhanced biogas production by 9.9 ± 1.5% over the control.

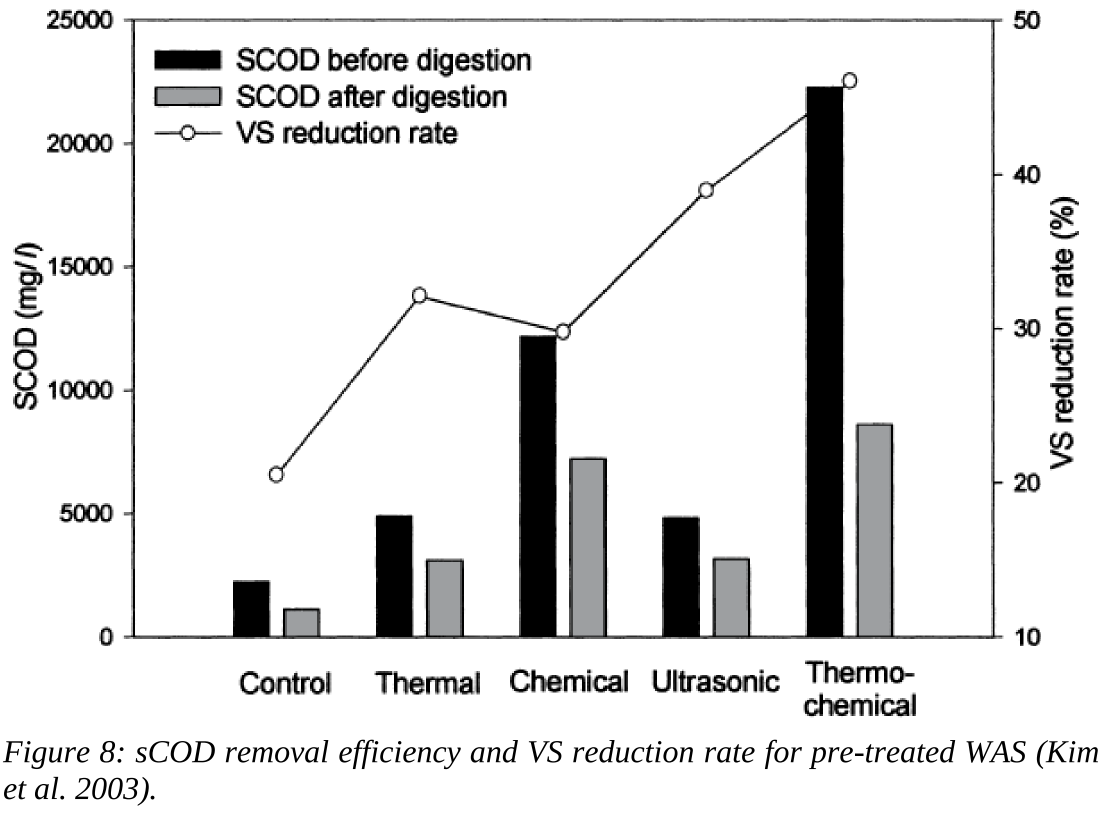

Figure 8: sCOD removal efficiency and VS reduction rate for pre-treated WAS (Kim et al. 2003).

Table 6: Combined pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Substrate	Results	Reference
Thermo-chemical			
50-90°C, Lime	WAS	• VSS content ↑ 46% • CH_4 yield ↑ 30%	Vlyssides and Karlis (2004)
$Ca(OH)_2$ for 1 h, 70°C, 1 h, HCl for 2 h	Pig manure	• Biogas yield ↑ 86%	Rafique *et al.* (2010)
60°C, 0.6 mg H_2O_2+1.5 mg $FeCl_2$/mg S^{2-}, 30 min	WAS	• sCOD content ↑ 157% • Soluble protein content ↑ 167% • Soluble carbohydrate content ↑ 250% • total VFA content ↑ 20% • CH_4 yield ↑ 20% • COD removal ↑ 10% • sCOD removal ↑ 20%	Dhar *et al.* (2011)
NaOH 7 g, 121°C, 30 min	WAS	• sCOD content ↑ 77.3% • VS removal ↑ 25.6% • CH_4 yield↑ 34%	Kim *et al.* (2003)
KOH 65 mEq/dm^3, 170°C, 15 min	WAS	• Biogas yield ↑ 54% • sCOD ↑ 80% • COD removal ↑ 71%	Valo, Carrere and Delgenes (2004)
0.156 g NaOH/g VS, 3 hours 60,120,150°C		• Biodegradation improvement • Bioavailability increase	Battimelli et al. (2010)
0.04 mol NaOH/g COD, 70°C, 1 hour		• Lipid hydrolysis efficiency ↑89% • Increased bioavailability of solid fatty waste	Affes et al. (2013)
pH 10, 55°C	Waste kitchen oil and kitchen waste	• Biogas yield ↑ 9.9 ± 1.5%	Li, C., Champagne, P. and Anderson, B. C. (2013)
Chemical-mechanical			
Lime, vacuum (0.02 bar), 30 min	WAS	• sCOD content ↑ 33%	Abbassi (2003)
Thermo-Enzymatic			
120°C, 5 minutes, Alkaline endopeptidase, 2-10g/L	Feathers	• Methane yield ↑ 37-51%	Salminen and Rintala (2002)

KW – kitchen waste; ↑ - original value has increased, beyond 100%, by the given percentage.

1.5.5 Biological pre-treatment of AD feedstocks

Biological pre-treatment includes methods that utilise pre-digestion, enzymes and bio-surfactants to enhance digestion (Table 7). Pre-digestion, involves two-stage digestion - a digestion stage prior to the main digestion process. By subjecting the waste to different digestion parameters prior to the main AD process, researchers aim to improve the digestibility of the waste. Peng *et al.* (2014) investigated the use of an oil-degrading *Bacillus* species. Prior to AD, oily wastewater was subject to a 24 hour digestion with *Bacillus*. During this time, exoenzymes were released by the bacteria to cleave triglycerides, diglycerides and LCFA, and increase the concentration of VFA present. This results in greater contact between microbes and the VFA substrates, significantly enhancing mass transfer of soluble nutrients into the sludge. This pre-digestion process resulted in an increase in methane yield by 16%, and an increase in the methane content of the biogas produced by 8% from 52-60%. However, unlike the other forms of pre-treatment, pre-digestion is rarely reported in the literature.

As the focus of this work is on abiotic pre-treatments which alter the substrate, as opposed to a series of digestions in which the inoculum is changed, this review will not go into depth with respect to biological pre-treatments.

Table 7: Biological pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Substrate	Results	Reference
Pre-digestion			
30-35°C, 1-2 d	Cattle slurry	• Biogas yield ↑ 17-19% • CH_4 content ↑ 7-11%	Singh, Jain and Tauro (1983)
Pre-hydrolysis			
70°C	Primary sludge	• SS removal ↑ 12%	Lu *et al.* (2008)
Aerobic digestion			
bacterium type SPT2-1	WAS	• Biogas yield ↑ 50%	Hasegawa *et al.* (2000)
Geobacillus sp. strain AT1	WAS	• Biogas yield ↑ 210%	Miah, Tada and Yang (2005)

↑ - original value has increased, beyond 100%, by the given percentage.

Table 7 continued.

Pre-treatment	Substrate	Results	Reference
Enzymatic			
42°C, 2 d	WAS	Biogas yield ↑ 10%	Mayhew *et al.* (2003)
HRT 2 d	WAS	CH_4 yield ↑ 60%	Davidsson *et al.* (2007)
Porcine pancreas lipase 0.5% w/v and 10mM Ca^{2+}, pH 8 (1M NaOH), 37°C, 4, 8, 12, 24 h	Lipid-rich dairy wastewater	• Free fatty acid content ↑ 1240% • Lipids hydrolysed ↑ 39.5 ± 6.8% • Glycerol content ↑ 65% • Proteins hydrolysed ↑ 32.7% • Biogas yield ↑ 162-292% COD removed ↑ 30-40.9%	Mendes, Pereira and de Castro (2006)
Pancreatic lipase 250 (PL-250), 25°C, 5.5 h	Slaughterhouse waste	• 35% of fat hydrolysed during pre-treatment • Digestion time ↓ 5% More effective on beef fat	Massé, Kennedy and Chou (2001); Masse and Massé (2003)
Lipase-producing *Staphylococcus xylosus*, 6 days	Poultry slaughterhouse waste	• Lipid degradation correlated well with sCOD increase. Increased biogas yield	Affes et al. (2017)
Bio-surfactant			
BOD-Balance™, 100, 250 and 500 mg/L	Raw and high FOG rendering wastewater	**Raw:** • pCOD removal ↑ 59-96% • sCOD removal ↑ 74-100% **High FOG:** • COD removal rate coefficient ↑ 164-238% +164-247% pCOD removal rate coefficient	Nakhla *et al.* (2003)

pCOD – Particulate COD; ↑ - original value has increased, beyond 100%, by the given percentage.

1.5.5.1 Enzymatic pre-treatment of AD feedstocks

Use of enzymes to enhance hydrolysis of macromolecules, and thereby enhance the AD process, has been under investigation for many years. Through this method, enzymes specific to the type of substrate being degraded are used to cleave macromolecules such as polysaccharides (e.g. with amylase enzyme), proteins (e.g. with pepsin enzyme) and fats (e.g. with lipase enzyme) into their lower molecular weight products, ideally to monomers. While enzymatic pre-treatment of FOG has received the greater deal of research into FOG pre-treatment, the majority of enzymatic pre-treatment research has been focused on cellular feedstocks (Higgins & Swartzbaugh 1986; Nagle *et al.* 1992; Romano *et al.* 2009; Sonakya, Raizada & Kalia 2001). Cammarota and Freire (2006) have performed a review of hydrolytic enzymes in the treatment of wastewater with high oil and grease content and conclude that further investigation is needed to determine the efficacy of these pre-treatments to improve degradation of the relatively recalcitrant and problematic FOG component of dairy and slaughterhouse wastewater.

Hydrolysis of pork and beef fat through enzymatic pre-treatment has been demonstrated by Masse, Massé and Kennedy (2003). This investigation involved the pre-treatment of abattoir waste with pancreatic lipase 250 (PL-250) at 25°C for 5.5 hours. Pre-treatment alone resulted in the hydrolysis of 35% of fat, while subsequent digestion achieved 80% reduction in neutral fat and LCFA concentration 5% faster than the controls. Methane content of biogas was unaffected by PL-250 pre-treatment. Furthermore, Massé, Kennedy and Chou (2001) have stated that PL-250 is more effective in the treatment of beef fat particles than treating pork fat particles.

Mobarak-Qamsari *et al.* (2012) investigated the effect of enzyme extract preparation from *Pseudomonas aeruginosa* on synthetic dairy wastewater with 1000 mg/L total fat content. A treatment of 10% v/v with a lipase activity of 0.3 U/mL was effective in enhancing removal efficiency of COD by 24%, and biogas production after 13 days of digestion by 102%. The researchers noted that these results indicate potential to accelerate the digestion of FOG in the AD process. Mendes, Pereira and de Castro (2006) also investigated enzymatic pre-treatment of lipid-rich dairy wastewater. The lipase used was a crude preparation of porcine pancreas lipase with activity of 1770 U/mg solid. Treatment with enzyme at 0.5% w/v affected increases in lipid hydrolysis, free fatty acid content, glycerol content, protein hydrolysis, COD removal and biogas production by 39% ± 6.8%, 1240%, 65%, 35.45% ± 5.45%, and 227% ± 65% respectively.

1.5.5.2 Biochemical emulsification of AD feedstocks

Bio-surfactants are typically used to pre-treat wastes high in FOG. These substances contain both hydrophilic and hydrophobic structural components which facilitate interactions between polar and non-polar compounds (Liu et al. 2015), in this instance, fatty residues and the aqueous digestate.

A study by Nakhla *et al.* (2003) evaluated a cactus-derived bio-surfactant, 'BOD-balance', in the treatment of FOG-rich rendering wastewater prior to AD. With a dose of 500 mg/L, BOD-balance affected reductions in tCOD and sCOD of 63.42% and 73.21% respectively, an improvement of 29.71% and 36.07% respectively over the controls. When trialled at full-scale, the addition of BOD-balance at 130-200 mg/L affected a dramatic increase in biogas production and a drop in pH (amended with sodium bicarbonate). The concentration of FOG and COD decreased by 84.6 and 40.9% respectively, and COD removal efficiency was noted to have increased from 20% to 64%. Furthermore, the authors of Nakhla *et al.* (2003) note that the concentrations of bio-surfactant used in this study are very high due to very high FOG content, as well as past accumulation of FOG in the digester. Accordingly, long-term dosage may be lower than employed in this study. While biogas production was not reported, methane content was measured to be 73%.

1.6 Relative performance of pre-treatment options

A number of factors need to be considered when selecting a pre-treatment technology, including the relative performance, advantages / disadvantages of each technology, and associated costs. Although pre-treatment has the potential to improve anaerobic digester performance in the Australian RMP industry, there is significant variation in biogas production reported in the literature for each technology (Poschl, Ward & Owende 2010). Major sources of variation can be categorised as reporting, digester, pre-treatment, and feedstock variations.

A general assessment of the advantages and disadvantages of different pre-treatment methods with respect to a specific substrate are presented in Table 8. It is important to note that the advantages and disadvantages listed in Table 8 are relative to the substrate being treated. Without standardised reporting, the current state of the literature does not allow for any reasonable degree of comparison of pre-treatment methods across substrates.

Table 8: Advantages and disadvantages of pre-treating WAS with different technologies (originally adapted from Taherzadeh et al. (2008); Hendriks and Zeeman (2009); further modified from Montgomery and Bochmann (2014)).

Process	Advantages	Disadvantages
Mechanical		
Milling	• Increases surface area • Makes substrate easier to handle • Often improves fluidity in digester	• Increased energy demand • High maintenance costs / sensitive to stones etc.
High-pressure homogenisation	• Increases surface area • Organic solvent free method • Well established technology on large scale	• High heat and energy demand • Complex equipment required
Ultrasonication[(a)]	• Increases surface area • Increased methane production • No chemical addition • Low maintenance cost	• Increased energy demand • Probes require replacement every 1.5-2 years
Thermal		
Hot water	• Increases the enzyme accessibility	• High heat demand • Only effective up to certain temperature
Steam explosion	• Breaks down lignin and solubilises hemicellulose	• High heat and electricity demand • Only effective up to certain temperature
Extrusion	• Increases surface area	• Increased energy demand • High maintenance cost / sensitive to stones etc.
Chemical		
Acid	• Enhances organics hydrolysis	• High cost of acid • Corrosion problems • Formation of inhibitors, particularly with heat
Alkali	• Enhances organics hydrolysis • Reduces fat particles	• High alkali concentration in digester • High cost of chemical
Ozonation	• Destruction of pathogens • Flexible operation	
Biological		
Microbial	• Low energy consumption	• Slow • No lignin breakdown
Enzymatic	• Low energy consumption	• Continuous addition required • High cost of enzyme
Bio-surfactant	• Dissolution of lipids • Less toxic than anionic surfactants	• High cost of bio-surfactants • Low commercial production

[(a)] Appels *et al.* (2008); [(b)] Focus is on lipids; Saharan, Sahu and Sharma (2011)

Assuming standardised reporting of methane production, it remains difficult to produce a blanket energy assessment for pre-treatments. Every industry brings with it a unique and challenging feedstock – Some of these include plant residues including but not limited to lignocellulosics and pulps, WAS, municipal WWTP, manures from livestock and poultry, FOG from kitchen waste, grease trap waste and oily products, meat processing effluent, vegetable waste, slurries, offal, biosolids, cheese whey and algal wastes (Dereli *et al.* 2012; Dhorgham, Sakthipriya & balasubramanian 2012; Graja *et al.* 2005; Heo *et al.* 2003; Kopplow, Barjenbruch & Heinz 2004; Li & Jin 2015; Martinez-Soza *et al.* 2009; Massé, Kennedy & Chou 2001; Methanogen Ltd. 2010; Mladenovska *et al.* 2006; Taherzadeh & Karimi 2008; Zhu, Hsueh & He 2011). Each of these substrates varies in composition (Labatut, Angenent & Scott 2011). Within each industry, wastes are still subject to significant variation between individual processors (UNSW 1998). In the RMP industry, variation will include the degree of primary treatment, including the number, size and efficiency of screens, DAF, contra sheers, screw presses, sterilisation and rendering (AMPC 2012). Other factors that will impact waste include the degree of product recovery; size of a slaughterhouse; water: waste ratio (i.e. dilution - not to be confused with moisture content); species processed; and operating climate, and differences down to the week, day and shift (Bauer 2011). Each waste source presents a novel characteristic profile – carbohydrate: protein: lipid ratios, VS, TS, alkalinity and VFA content to name a few (Alkaya & Demirer 2011). The impact of individual pre-treatment methods across a range of feedstocks will vary due to the nature of the feedstock (Kim *et al.* 2003). Unless the goal is to compare the effect of a static pre-treatment method across feedstocks, it is unsuitable to compare the impact of multiple pre-treatment methods unless the substrate is controlled. Furthermore, pre-treatment methods between researchers can vary significantly. Consequently, this becomes a determination of what parameters are most effective within a pre-treatment type on a specific feedstock.

Prior to digestion, pre-treatment may be applied at the discretion of the operator. Pre-treatments, as discussed, include thermal, chemical, thermochemical, mechanical and biological methods which are more or less suitable given the application (Figure 6). Not only may a pre-treatment be unnecessary, one risk of pre-treatment is that by increasing the amount of available compounds, a digester may experience inhibition (Poschl, Ward & Owende 2010). This is a real potential, for example, in high-protein wastes with ammonia formation, and FOG-rich wastes which break down to potentially inhibitory concentrations of LCFA and VFA (Batstone *et al.* 2000; Chen, Cheng & Creamer 2008). Furthermore, the degree of impact of a pre-treatment depends on the waste that the pre-treatment method is applied to (Engelhart *et al.* 2000). As a result of pre-treatments being targeted to a specific waste source, it is difficult in the case of a review to draw appropriate material together for a reasonable comparison.

Following pre-treatment, digestion methods also vary significantly. Digesters are divided into either low-rate or high-rate systems. Low-rate anaerobic systems include batch digestions, plug-flow reactors and lagoons and typically require a high hydraulic (5-120 days) and solids retention time. Alternatively, high-rate anaerobic systems include up-flow anaerobic sludge blanket (UASB), continuous stirred tank reactors (CSTR), expanded granular sludge bed (EGSB) and AnMBR systems among others (Appels *et al.* 2008; Dereli *et al.* 2012; van Lier 2008). These systems are heated to either the mesophilic or thermophilic optimum temperatures of ~37°C and ~55°C respectively, and receive active stirring or mixing. These high-rate systems typically involve a de-coupling of the solids and hydraulic retention time and as such, can treat equivalent volumes of wastewater with a HRT ranging from hours to days (Dereli *et al.* 2012). Several things need to be taken into consideration when comparing energy yield here. An important factor to consider is that some pre-treatments actively improve reaction kinetics without impacting total biogas production (Labatut, Angenent & Scott 2011). Energy production must then be compared as a function of time, not simply total methane produced.

1.7 Merit of pre-treatment methods in abattoir waste in Australia

Australian abattoirs stand to benefit substantially if an appropriate pre-treatment method can be developed to improve the bioavailability and subsequent conversion of FOG to methane. While no anaerobic digestion system currently deals with FOG effectively, typically the more sophisticated the anaerobic digestion technology, the less capable they are of handling FOG loads.

With the increasing popularity of overseas technologies being introduced to Australian RMP plants it is important to note that the quality and biodegradability of the effluent is key to maximise performance of these AD technologies. This is particularly important in light of the high strength nature of the waste water and volumes produced in this industry. This is quite significant when the scale of capital investment is considered which can be regarded as one of the largest inhibitors of uptake of foreign AD technologies. The use of cost-effective pre-treatments to improve the biodegradability of the wastewater will enable additional energy recovery with a concomitant reduction in GHG emissions. The actual energy balance and costs is dependent on a number of factors highlighted in the previous section. Further research is needed to fully understand the economics of AD systems to meat processors. The value of biogas, recovered non-renewables, treated water, and GHG mitigation to a meat processor must be understood in order to put forward a strong financial case for an AD system. Only once this is known, can an AD system and subsequent pre-treatment of wastes for AD be valued.

Researched and speculated actions of the pre-treatment of effluents rich in fats and oils from several origins presented in this chapter show new and promising applications for the enhancement of the AD process. Of all the pre-treatments discussed, ultrasonic, thermochemical and biochemical have shown greatest potential in the degradation of high fat waste water in addition to some studies describing the degradation of fats and oils by alkaline/acid/enzymatic hydrolysis. The greatest increase in biogas production covered in this chapter was 227% ± 65% using enzymatic pre-treatment of lipid-rich dairy waste; however, it should be noted that several articles investigating pre-treatment methods which do not concern themselves with AD and biogas production have been reviewed. Regardless, there is evidence from these investigations that these pre-treatment methods affect considerable substrate degradation, and are subsequently worth investigation as pre-treatment methods for FOG-rich AD substrates. Although carbohydrates and protein are relatively easily digested, the challenge is to develop a pre-treatment method which greatly improves FOG digestion to produce methane, and developing a digestion protocol to optimally include FOG to improve biogas production while limiting the inhibitory impacts associated with FOG-rich substrates.

Treatment efficiency and nutrient recovery of waste streams can also be optimised through treatment of separate fractions of the waste stream (Deng *et al.* 2014). Aptly, Jensen *et al.* (2014) suggest that this concept be investigated in cattle abattoirs, with treatment of individual waste streams. While this may indeed result in a greater degree of organic removal and nutrient recovery, this could be a relatively expensive operation compared with digestion of a combined waste. However, this could also provide excellent conditions by which FOG could be separated from the primary waste streams, perhaps by dissolved air floatation, pre-treated and suitably introduced to an AD system.

1.8 Summary of the literature

The Australian RMP industry is under pressure to reduce GHG emissions and optimise energy consumption. Wastewater produced from fully integrated abattoirs in Australia is high-strength and FOG-laden and contributes significantly to abattoir GHG emissions. Although pre-treatment of wastes such as lignocellulosics and WAS are commonplace, investigation of pre-treatment of FOG for AD is relatively rare. Given the significantly higher theoretical methane content of FOG over carbohydrates and proteins, it is surprising that FOG are only now being considered for pre-treatment.

Despite the fact that FOG has the potential to significantly enhance biogas yield from AD systems, FOG can also produce several problems. Pre-treatment may be critical in reducing problems caused by FOG, including pipeline blockages, adhesion to sludge, and inhibition of mass transfer of nutrients, problems which ultimately lead to anaerobic lagoon failure. However, there is potential that pre-treatment may worsen problems, in particular inhibition of mass-transfer due to LCFA adhesion to sludge. This may be overcome by diluting pre-treated fatty substrates with co-substrates. While it remains to be seen whether pre-treatment of FOG is economically viable, investigation must first be conducted to identify suitable pre-treatment methods for an optimised process. Once a process is optimised, FOG digestion will help to ease the impact of rising electricity and water prices in industry, as well as reduce GHG emissions.

This chapter highlights several knowledge gaps in the literature. There is a distinct lack of standardisation when reporting on AD investigations. This makes meaningful comparison across the literature a difficult task. Also prominent is the lack of investigations that focus on FOG-rich wastes, regardless of the potentially enormous benefit from enhanced methane production. Once standardised reporting has been established across the literature, it will be possible to produce a reliable cost/benefit analysis to better advise industry on the best course of action to provide optimal digestion of their waste, and subsequently, optimal methane production. While there are some investigations into pre-treatment of FOG-rich wastes, further research is needed to understand the mechanisms by which pre-treatments impact the FOG component of wastes – investigations which would benefit greatly from standardised reporting. There is little-to-no literature which advises industry on how to handle crust material once it has accumulated. While AnMBR reactors represent a possible solution to digest FOG-rich wastes and avoid the complications associated with crust formation, more research is needed to understand the fate of FOG in these reactors. These knowledge gaps need to be addressed in order to improve performance and further the development of AD technology through industrial uptake.

1.9 Objectives of the study

The comparative review of various pre-treatments revealed that there is merit in applying these methods to high-fat slaughterhouse waste in an effort to increase AD performance and overcome associated operational issues. Hence the research described in this thesis was concerned with evaluating pre-treatments to improve the performance of high-fat abattoir wastewater in an anaerobic digestion system.

The scope of this investigation encompassed two main objectives:

- To compare the biochemical methane potential of high-fat slaughterhouse waste when subjected to four different pre-treatment methods, namely chemical, thermobaric, thermochemical and bovine bile (as a novel bio-surfactant);
- To apply the best pre-treatment as deemed from the results of BMP tests and assess continuous anaerobic digestion performance of high-fat slaughterhouse waste in a lab scale study.

II

Methodology

To address the objectives, the experimental design followed a 2-phase, 5-stage approach (Figure 9). For full methodology refer to **Papers II, III and IV** in Appendices B-D.

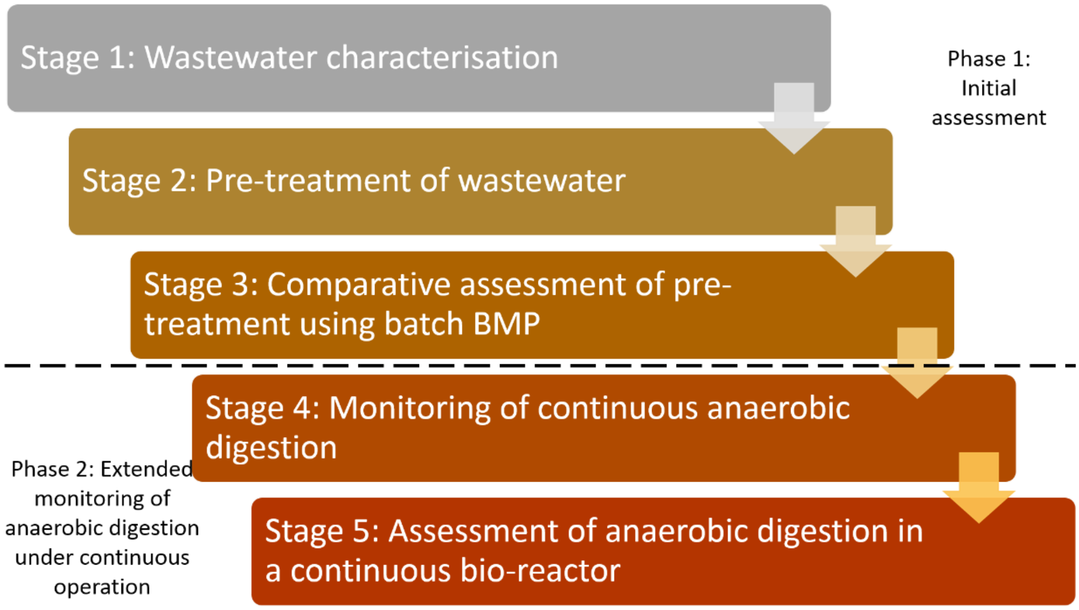

Figure 9: General outline of project experimental design.

The first phase (stages 1-3) represented the initial assessment of pre-treatment effect on the substrate using BMP testing, while phase 2 (stages 4-5) was concerned with assessing the performance of the substrate using a single pre-treatment under continuous digestion. In stage 1, waste materials were characterised to provide a baseline to measure the effect of pre-treatment on the substrate. Stage 2 involved application of pre-treatment to the substrate, and subsequent analysis of substrate characteristics to measure change due to pre-treatment. Under stage 3, substrate was subject to BMP testing and results were analysed for the effect of pre-treatment on specific methane production and digestion profile (i.e. change in reaction kinetics, inhibition finish time). To conclude phase 1, the most promising pre-treatment was selected for use in continuous digestion experiments.

Phase 2 involved continuous digestion experiments, the next progression after BMP analysis in investigating a substrate for suitability in anaerobic digestion. This progression allowed for regular feeding intervals, with control over hydraulic retention time, organic loading rate, and the ability to investigate the health of the digestion system with respect to pH, VFA concentration, and buffering capacity. Stage 4 involved monitoring of continuous digestion of pre-treated high-fat abattoir waste in a BioReactor Simulator (BRS; Figure 10; BioProcess Control, Sweden). During this stage, anaerobic reactors were operated for 70 days. Monitoring included daily substrate addition, digestate collection, and regular analysis. Biogas flow rate and volume was measured in real time. pH was measured daily, while biogas composition, VFA, total alkalinity, total and volatile solids, ammonium content, and fat, oil and grease content were measured twice weekly. Stage 5 was conducted in parallel to stage 4, and involved the critical analysis of the data collected in stage 4.

2.1 Methodology overview

This overview of methodology contained in this section is to supplement the detailed information provided in **Papers II-IV**.

2.1.1 Inoculum, substrate and bile collection

For **Papers II and IV**, inoculum was collected from the recirculation pump servicing a covered anaerobic lagoon as a nearby cattle slaughterhouse. For **Paper III**, inoculum was sourced initially from the same site as for **Paper II**, but due to on-site complications, inoculum quality was compromised, and was no longer capable of achieving the benchmark of 80% in the microcrystalline cellulose control as specified by Verein Deutscher Ingenieure (2006). An alternate inoculum was sourced from a wastewater treatment plant prior to sludge thickening. Once collected, inoculum was transferred back to the laboratory and incubated at 37°C until use, typically 4-10 days later.

The substrate used in **Papers II-IV** was DAF sludge sourced from a nearby cattle slaughterhouse. DAF sludge was collected from the weir of a DAF unit treating green stream waste - a collection of paunch wash, tripe wash, boning, stick water, bone chip and render waste. In **Paper IV** DAF sludge was combined with green stream waste. Substrate was transferred back to the laboratory and stored at 4±1°C until use.

Bile was collected from below the kill floor of the red meat processing plant. During the slaughter process, the animal is eviscerated, and the gall bladder is removed from the liver. The gall bladder is slashed and bile is drained into a collection drain which exits above a 1 m^3 intermediate bulk container (IBC). Bile for these experiments was collected from this drain, above the IBC. Bile was transferred back to the lab on ice and stored at 4±1°C until use. While bile was dosed per unit of reactor volume in **Paper III**, supplementary table 2 at the end of Appendix C shows these dosage calculated as bile addition per unit of FOG.

2.1.2 Biochemical methane potential

Batch BMP tests were conducted using the Automated Methane Potential Test System II (AMPTS II) in accordance with the guidelines set forth in Verein Deutscher Ingenieure (2006). No trace elements, vitamins or nutrients were added to digesters in addition to what is contained in the substrate. While BMPs are conventionally performed at an inoculum to substrate ratio (ISR) of 2:1 on the basis of VS, an ISR of 3:1 was used in this work. This ratio gave good results in preliminary experiments, reducing inhibition and foaming, and providing a margin by which to avoid overloading with fatty or inhibitory substrates. Gas produced by the reactors is passed through scrubbers of 3M sodium hydroxide, designed to remove carbon dioxide from the gas. Scrubbed gas passes to flow cells in a data acquisition instrument (DAI) which measures the amount of volumetric methane and produces an output corrected to standard temperature and pressure (0°C, 1 atmosphere) and is corrected for moisture content. Results are captured as normal millilitres (mL_N CH_4), corrected for VS load, and reported as SMP (mL_N CH_4/g VS).

As digesters are loaded on basis of VS, the masses of substrate and inoculum loaded into digesters has not been reported. These values were considered inconsequential, as inoculum and substrate VS content is can be dynamic across a broad range, and reporting masses would make reproduction of the work difficult. However, given the reported inoculum and substrate VS, as well as the ISR and final mass of the reactor liquid, the following equations (I) and (II) can be used to calculate the masses added.

$$M_I=M_R/(((VS_I/ISR)/VS_S)+1) \quad (1)$$

$$M_S=M_R-M_I \quad (2)$$

Where M_I is the inoculum mass, M_S is the substrate mass, M_R is the reactor liquid mass, VS_S is the % VS of fresh matter of the substrate, VS_I is the % VS of the inoculum fresh matter, and ISR is the inoculum to substrate ratio on basis of VS.

As the VS content of a substrate can be altered as a result of pre-treatment, it was important that reactors be loaded based on the VS content of the untreated substrate. This allowed for any change in BMP resulting from pre-treatment to be accounted for. For **Paper III**, supplementary table 1 lists the TS and VS content of the inocula and substrates as a percentage of fresh matter.

2.1.3 Curve fitting and reaction kinetics

Results from BMP tests were assessed for reaction kinetics using two equations; a growth curve logistic equation (Equation 3), and a modified Gompertz Equation (Equation 4; Ghatak & Mahanta 2014). Curves were fitted to the data to acquire rate constants and lag periods using SciPy optimisation curve-fit routine.

$$B = \frac{B_0}{1+e^{-k(t-t_0)}} \quad (3)$$

From equation 3, B is the cumulative specific methane potential (SMP; mL_N CH_4/g VS) at time t (days); B_0 is the maximum SMP achieved by end of digestion; *k* is the rate constant; T_0 is the time at which maximum production rate occurs. The function is weighted using standard deviation to achieve a better fit.

$$B = B_0 e^{-e(\frac{Ue}{B0}(\lambda-t)+1)} \quad (4)$$

From equation 4, B is the cumulative SMP at time t; B0 is the maximum SMP achieved by end of digestion; U is the kinetic constant of methane production rate; λ is the duration of lag phase in days, used here to represent inhibition. Equation is unweighted.

2.1.4 Continuous digestion

While batch digestions are effective at determining the specific methane potential of a substrate, they do little to elucidate the long-term sustainability of an anaerobic digester treating the substrate in question. Continuous digestion experiments are the next progression after batch BMP experiments. These systems allow researchers to investigate the large-scale application and potential of a substrate. Substrates for continuous digestion should be chemically analysed for macromolecule content, total organic carbon, total nitrogen, as well as a suite of elements including phosphorus, sulphur, iron, nickel, cobalt, molybdenum, tungsten, manganese, coper, selenium and zinc (Schmidt et al. 2014).

Continuous digesters are controlled for temperature, stirring/agitation, and experiments are designed with an OLR and HRT in mind to simulate industrial performance. Under these conditions, reactors can be acclimatised to new substrates, and their OLR and HRT can be modified over time to optimise biogas yield while maintaining a high degree of substrate degradation. Reactors can be fed continuously, or at regular intervals, and digestate is collected from the reactors as a result.

Regularly collected digestate allows for process monitoring, in which pH, VFA, alkalinity, ammonium, and various other parameters can be measured to assess digester performance.

Continuous digestion experiments were conducted using the BRS system (Bioprocess Control, Sweden; Figure 10). This system consists of 6x2 L bioreactors (BR), temperature controlled by a thermostatic water bath, and stirred by an agitation system attached to the reactor. Gas produced by the system is measured automatically by the DAI flow cells in an accompanying water bath. Each flow cell sends data to the database (DB), which is then accessed by the user through the website. Data is stored remotely on file storage for later access.

By operating continuous digesters in lab-scale, researchers can simulate the operation of large-scale industrial reactors. These digesters are typically temperature controlled, stirred systems in which the OLR, HRT and solids retention time (SRT) can be controlled more strictly than in an industrial application. For the continuous digestion work outlined in this thesis, a HRT of 8 days was used to emphasise the effect of the pre-treatment by rapidly turning over the digestate with fresh substrate. Furthermore, SRT was decoupled from HRT by allowed sludge to settle prior to digestate collection. This allowed for a retention of active biomass within the digester and consequently promoting degradation. Continuous systems also allow for regular measurement of key parameters to observe for changes in digester performance. These parameters include pH, VFA, alkalinity, ammonium, VS and TS, COD, FOG, and any other parameters a researcher may be interested in.

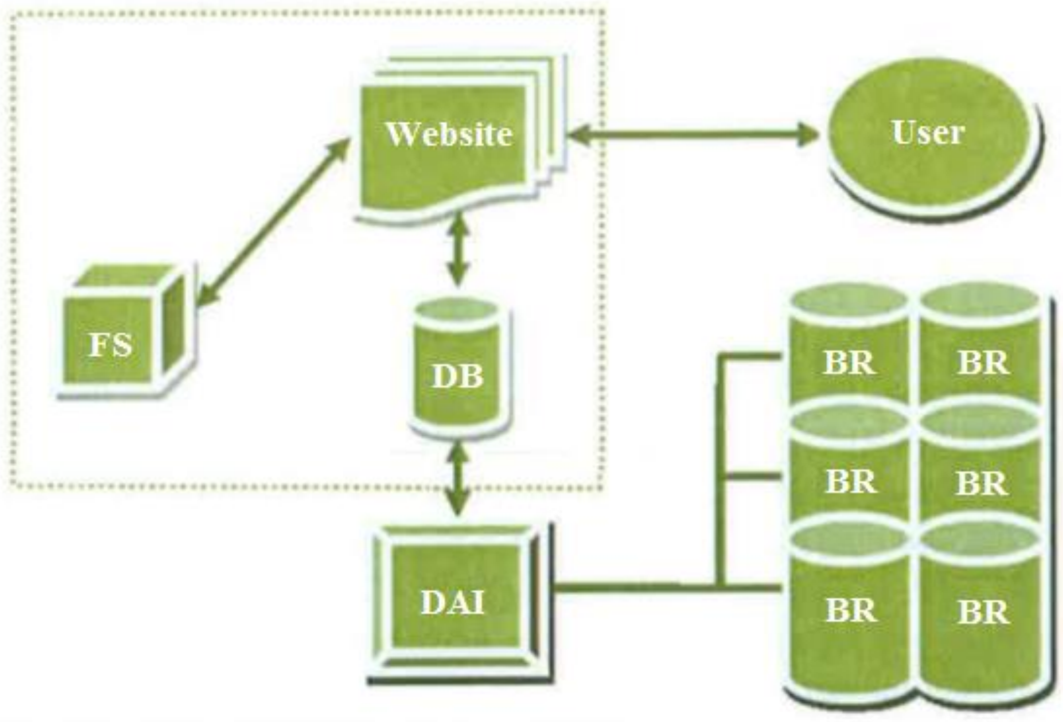

Figure 10: Visual representation of the BioReactor Simulator (Strömberg et al. 2012). BR – Bioreactor; DAI – Data Acquisition Instrument; DB – Database; FS – File storage.

III

Results & Discussion

3.1 Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater

The literature review presented as **Paper I** was required to identify technologies and methods which showed particular promise in the treatment of substrates which contain high concentrations of FOG. **Paper I** identified that, while fatty material has a large potential to generate methane, the problems associated with utilising such feedstocks in anaerobic digestion tend to be more of a hindrance than a benefit. As a consequence, very little research has been conducted on the pre-treatment of fatty substrates, and instead research has tended to lean toward co-digestion (Li, Champagne, & Anderson 2013). The exploration of pre-treatment methods and technology in this chapter enabled the research to focus on technologies which were considered more likely to be viable candidates in which pre-treatment would generate a favourable outcome. Accordingly, thermobaric, chemical, thermochemical, ultrasound, enzymatic and bio-surfactant methods identified as potentially beneficial pre-treatment methods. Due to expense of enzymatic pre-treatment was excluded from further investigation, and while ultrasonic pre-treatment was investigated, complications with the equipment prevented publication of the results. Consequently, thermobaric, chemical, thermochemical and bio-surfactant pre-treatments were utilised in work moving forward.

3.2 Evaluation of chemical, thermobaric and thermochemical pre-treatment on anaerobic digestion of high-fat cattle slaughterhouse waste

Stoichiometry and co-digestion experiments in the literature both indicated that addition of fat in anaerobic digestion systems can increase methane yields. However, fat is a generally problematic material. Pre-treatment has potential to not only reduce the problematic aspects of fat addition, but also further increase methane yields. While a large body of work exists concerning the pre-treatment of a wide range of substrates, there has been little work regarding the pre-treatment of high-fat waste, with particular rarity in the context of RMP waste. **Paper I** identified 6 pre-treatment categories that have potential to enhance biogas yield. From these, thermobaric, chemical, thermochemical, and bio-surfactant pre-treatment methods were used to pre-treat high-fat abattoir waste prior to anaerobic digestion.

It was hypothesised that application of these pre-treatments to a FOG-rich substrate would aid in the anaerobic degradation of the substrate. **Paper II** documents the investigation of thermobaric, chemical and thermochemical pre-treatment of DAF sludge, and subsequent batch BMP testing. DAF sludge is a high-fat cattle slaughterhouse waste stream that is generally sent to a rendering plant for conversion into tallow, and is of identical chemical composition to the fat which remains uncaptured by fat-removal technology. Results from this investigation were therefore considered translatable to pre-treated fat on-site. The effect of pre-treatment on the substrate was assessed by measuring COD solubilisation and VFA formation. BMP testing was conducted to assess methane production and the effect of pre-treatment on the digestion profile with respect to inhibition, rate kinetics, equivalent digestion time, and total digestion time.

3.2.1 Thermobaric pre-treatment

The results reported in **Paper II** were encouraging. The effect of thermobaric pre-treatment was in accordance with the alternative hypothesis that thermobaric treatment aided in the anaerobic digestion of high-fat abattoir waste. sCOD, reported as a percentage of total COD, increased from 16.3% to 20.84%, an indication that larger, insoluble macromolecules have been hydrolysed to lower molecular weight, and soluble products. As hydrolysis is the rate limiting step for complex macromolecules in anaerobic digestion (Appels *et al.* 2008), this should reduce lag phase inhibition. Indeed, BMP testing produced a digestion profile completely free of lag-phase inhibition. Although the rate constant was reduced in the thermobaric treatment, linear digestion began 5 days earlier than the control, resulting in much greater methane yield at all times during digestion. Total digestion time was increased from 12 days to 14 days in the thermobaric treatment, allowing for an increase in methane yield by 8.32%. However, the thermobaric treatment achieved equivalent methane yield to the controls by roughly day 9, around 25% earlier than the controls. When considered for continuous digestion, this presents the operator with 2 options. In an industrial context, if the primary interest is to reduce organic content, an operator could allow this digestion to continue to completion at 14 days and achieve the 8.32% increase in methane yield. However, the thermobaric trials required only 9 days to break even with the final yield achieved by the controls at day 12. Therefore, the thermobaric trials required 5 days to achieve only 8.32% extra methane yield. If the primary interest is to produce energy, a reduction in HRT would take advantage of much greater reaction kinetics in the thermobaric treatment, utilising the 5 day period to gain much more methane (i.e. 440 mL) than completing digestion (77 mL) (Table 9). Alternatively, given that the system is capable of degrading the same amount of organics in a reduced time-frame, increasing the OLR would allow for much greater methane yield to be achieved within the original 12 day completion time of the control. This, in effect, is similar to decreasing the HRT. Similar results were reported by Li and Jin (2015) in which thermal pre-treatment reduced the retention time necessary for acidification by 5 days.

In industrial application, it is common for OLR to be dictated by volumetric throughput, not by adjusting the organic content in the waste stream. Consequently, OLR and HRT tend to be linked, and an increase in OLR coincides with a decrease in HRT. An economic analysis of thermobaric pre-treatment indicated that active heating of the substrate would not be economically viable. However, heat exchange would significantly reduce the cost of active heating, and would improve the economic viability of such a pre-treatment.

Table 9: Effect of pre-treatments on substrate and AD parameters.

Treatment	Thermal	Chemical	Thermochemical	Bile 0.6 g/L
SMP	+8.32%	+3.28%	+8.49%	+7.08%
Lag phase	-100%	0%	-20%	0%
Rate constant	↓	-	↓	-5.66%
sCOD	+4.50%	+31.9%	+34.40%	0%
VFA	-64%	+27%	+128%	0%
T_{EQ}	-16.67%	-16.67%	-8.33%	-12.12%
T_{FIN}	+8.33%	0%	+8.33%	+3.03%

T_{EQ} – Time required to achieve a methane yield equivalent to the control at T_{FIN}

While methane yield and reaction kinetics were influenced positively by pre-treatment in this investigation, economic assessment produced a less favourable outcome. Pre-treatment with sodium hydroxide would result in a net loss of 51% of operating cost. With respect to thermobaric pre-treatment, economic assessment indicated that, depending on water content, losses ranged from 97% to 61% of operating cost. However, this assessment was based entirely on active heating, and ignored potential for heat-exchange, or for the value of minimising problematic interactions with fatty material. Consequently, there may be value in thermobaric pre-treatment, and these outcomes could be supported by further investigation.

There remains some concern about the reaction vessel used in these experiments remaining sealed during the thermobaric pre-treatment. Schott bottles are designed so that under sufficient pressure, the lid will become loose to release the pressure to prevent the glass bottle from exploding. Under the conditions of thermobaric pre-treatment (121°C, 15 psi, 20 min.), if the seal of the reaction vessel became compromised, loss of VFA would be inevitable, and result in a loss of biogas potential. Similar losses in organic content were measured by Park et al. (2017), in which following pre-treatment at temperatures ranging from 170-220°C under 1.7-2.0 MPa respectively, TS was reduced from 20.4% w/w in the untreated substrate to 6.1-7.2% w/w, and TCOD was reduced from 26.8 g/L to 8.5-11.3 g/L following hydrothermal pre-treatment. In this instance, the researchers note that following pre-treatment, the residual steam was discharged from the reactor, and reaction products were removed. This could be solved by acquiring a pressure vessel and performing pre-treatment such as in Wilson and Novak (2009)

It would also have been valuable to assess the effect of pre-treatment on the species of LCFA and VFA produced as a result of pre-treatment. Wilson and Novak (2009) demonstrated that LCFA respond better to thermobaric pre-treatment with increasing degree of unsaturation. Furthermore, the authors also demonstrated that fatty acid with a higher degree of unsaturation degrade to form more acetic and propionic acids, while saturated fatty acids tend to produce more valeric, caproic and heptanoic acid (Wilson & Novak 2009). As approximately half of the LCFA found in beef tallow is unsaturated, and the majority of this is only mono-unsaturated, it is likely that a large degree of valeric, caproic and heptanoic acid may have been produced by the pre-treatment process. While the results of research into the inhibitory effect of various species of VFA vary, it is clear that the health of a reactor cannot be defined by a generic VFA concentration (Franke-Whittle *et al.* 2014).

3.2.2 Chemical pre-treatment

The performance of the chemical pre-treatment was consistent with the alternative hypothesis, that pre-treatment would enhance anaerobic digestion of the high-fat substrate. Soluble COD was increased from 16.3% to 48.2% (Table 9). This increase in soluble organics is likely due to the saponification of fatty material to form sodium salts of LCFA, although Kim *et al.* (2003) demonstrated a significant capacity for chemical pre-treatment with sodium hydroxide to solubilise protein. Treatment had also degraded organics to yield VFA, indicated by an increase in VFA by 27%. Pre-treatment reduced inhibition by approximately 1 day, and similar to the thermobaric pre-treatment, achieved an equivalent yield to the control 2 days faster. Total digestion time was prolonged by 1 day, for a methane yield increase by 3.28%. This improvement lends the same benefits as discussed with respect to thermobaric pre-treatment, regarding OLR and HRT.

3.2.3 Thermochemical pre-treatment

The effect of thermochemical pre-treatment was consistent with the alternate hypothesis that pre-treatment would improve the anaerobic digestion of high-fat abattoir waste. Like the chemical treatment, SCOD was increased from 16.3% to 50.7% (Table 9). The combination of chemical and thermal aspects greatly improve VFA content by 128%. These results indicate that saponification of the fats, and solubilisation of protein has occurred, as in the chemical trial, with the enhanced hydrolysis seen in the thermobaric trial. Lag-phase inhibition was reduced by 20%, with a distinct increase in the rate of gas production. However, it appears that the chemical component of the thermochemical treatment limits the ability of the thermal component to reduce inhibition. Total digestion time was extended by 2 days for an increased methane yield by 8.49%. Thermochemical treatment achieved the final yield of the control around 1.5 days in advance, opening up the opportunities with respect to adjusting HRT and OLR to increase yields under continuous digestion.

3.2.4 Economic assessment of chemical, thermobaric and thermochemical pre-treatments

A simple economic assessment was conducted for chemical, thermobaric and thermochemical pre-treatments in **Paper II**. A number of assumptions were made for the simple economic assessment. First, the assessment considers ongoing costs, but not the capital required for infrastructure. Secondly, the flow-on effects that pre-treatment may have on digester operation, such as greater treatment efficiency, impacts on crust accumulation and sensor fouling, etc., are not considered here. Thirdly, the value of extra heat generated from CHP, is not considered here.

The economic assessment for the chemical pre-treatment based on the application of 7 g NaOH/L as used in this study. Sodium hydroxide pellets could be purchased for $467 Australian dollars (AUD) per 1000 kg, enough to treat 143 m^3 of FOG-rich waste. With an improvement in biogas yield of 3.28%, this would be worth AUD $185 as electricity, or AUD $229.60 to offset natural gas. This is insufficient to cover the cost of sodium hydroxide, and is likely not an economically viable pre-treatment option.

Experimentally, thermobaric pre-treatment yielded an extra 8.32% methane yield. Treating 143 m^3 of FOG-rich waste, the same volume of waste as determined in the chemical pre-treatment, this would yield an extra 28172 MJ. Converting to electricity with 40% efficiency provides 3130 kWh. The value of this as electricity is AUD $470, and used to offset natural gas would be worth AUD $230. However, the cost of performing this pre-treatment is heavily dependent on the water content of the waste. With a specific heat capacity of 4.18 J/g/°C, water is energetically expensive to heat, and the economics of the pre-treatment could be improved through dewatering. For instance, with a moisture content of 85.44%, 117.2 MWh of electricity would be required to heat 143 m^3 of material from 40 to 100°C. At an estimated cost of AUD $0.15/kWh, this would cost AUD $17580. In contrast, if 90% of the moisture content were removed, the cost to heat would be around AUD $3045. These calculations highlight that active heating of the material is not a viable option to take advantage of the effects of pre-treatment in this situation. However, utilisation of waste heat from CHP or from other plant processes could significantly reduce the need for active heating, and improve viability of thermobaric pre-treatment in an industrial setting.

Like the thermobaric and chemical pre-treatments, the economic viability of thermochemical pre-treatment is subject to the cost of heating and the cost of sodium hydroxide. Given that these separate treatment methods are not viable, thermochemical treatment will also require either cheaper cost of treatment, or better return on investment to become economically viable.

3.2.5 Limitations and future work

- Batch digestion

The work presented in this section represents a small fraction of the potential work in this field, and there are many other pre-treatment options that may produce benefits under BMP testing. From the literature review, ultrasound, enzymatic, microwave and advanced oxidative techniques pre-treatments were also identified as having potential to enhance the anaerobic digestion of high-fat substrates. Furthermore, there have been a host of microbial bio-surfactants identified, which may be valuable pre-treatment options for high-fat substrates. Each of these experiments should investigate a range of pre-treatment conditions, i.e. a range of temperatures, doses/concentrations, energy inputs, exposure time, etc. to find the optimal conditions for treatment.

With respect to the thermobaric, chemical and thermochemical pre-treatment methods which were the focal point of **Paper II,** these methods should be further investigated to identify the optimal conditions for these pre-treatments to be conducted under. In particular to chemical pre-treatment, although sodium hydroxide has been identified as the most effective alkali for the degradation of waste activated sludge, other alkalis could be tested to determine the best chemical for the degradation of lipid.

- Quantitation of free fatty acid liberation from bound fatty acids

Quantitation of the degradation of bound fatty acids (e.g. triglycerides, diglycerides) to free fatty acids (FFA) is useful to understanding the effect of a pre-treatment. This is difficult to achieve, as methods typically cleave LCFA from glycerol prior to derivatisation to fatty acid methyl esters, and are thereby inappropriate for extracting FFA. Likely due to the specific nature of the experiment, these tests are not performed commercially in most instances.

Attempts were made in this study to extract free fatty acids from a mixture of free and bound fatty acids. Known quantities of water and lard were mixed to simulate an environmental sample. Aqueous samples were acidified to below pH 2 with acid. Attempts were made with both hydrochloric and sulphuric acids. Following acidification, the lipid soluble fraction was extracted in hexane. Extraction of free fatty acids from the hexane was attempted with base. Attempts were made with both 0.1 M sodium hydroxide, and 0.1 M potassium hydroxide. The aqueous layer was collected and acidified to below pH 2, and lipids were extracted into the hexane. Solvent was evaporated under a compressed air stream, and vacuum dried in a desiccator with sodium hydroxide pellets. Dried sample was trans-esterified with 14% boron tri-fluoride in methanol for 24 hours at 50-55°C. Samples were analysed using a Shimadzu GC-2010 gas chromatograph with mass spectrometer GCMS-QP2010 plus gas chromatograph mass spectrometer, with an RTX-5 capillary column (30 m x 0.25 mm x 0.25 µm, serial number 801339)

Recovery of the free fatty acids proved difficult. Attempts to analyse FFA extracted from lard, as a more controlled material, and later DAF sludge, produced insufficient signal-to-noise ratios for the peaks to be detected, indicating a failure in the extraction process. Repeated failures to achieve FFA extraction from a mixed sample led to seeking to outsource the method to a commercial lab. While lipid profiling is common in commercial laboratories, the separation and quantitation of FFA and bound fatty acids is not routinely performed. The concept of separating FFA from bound fatty acids was consequently abandoned in favour of producing lipid profiles as a far simpler, yet much less informative alternative.

Although lipid profiles were produced, quantitation of the fatty acids of interest was difficult. Commercial analysis of the LCFA standard was performed using a 100m column. By comparison, a 30m column was used for these analyses. While separation of peaks for the most part was good, separation of C18:1, C18:2 and C18:3 was not possible. Attempts to achieve peak separation included decreasing the temperature ramp rate surrounding the elution time of these fatty acids, and introducing isothermal periods at the expected elution times. Despite several attempts to achieve peak separation, all efforts were ineffective.

An effort should be made to separate bound fatty acids (i.e. tri-, di-, mono-glycerides) from free fatty acids in environmental samples. This would help to determine how effective a pre-treatment is at degrading fatty substrates, and learn how the fats are degraded with respect to pre-treatment. Furthermore, this would help to discern how pre-treatments aid or detract from the AD process.

- Particle size analysis

Determination of particle size, particularly micellar diameter is of particular interest with respect to FOG pre-treatment. One aspect of FOG which makes digestion difficult is the property of hydrophobicity and the tendency for lipids to group together, as either clumps or micelles. This grouping reduces the surface area to volume ratio of the mass of fat, and consequently reduces the area available for enzymatic cleavage to occur. Particle size analysis aids in the understanding of the mode of action of the pre-treatment, or whether a method has been effective at improving the degradability of the substrate. Particle size analysers were considered for this study but were not available.

3.3 Bovine bile as a bio-surfactant pre-treatment option for anaerobic digestion of high-fat cattle slaughterhouse waste

In addition to the thermobaric, chemical and thermochemical pre-treatment methods investigated in **Paper II**, **Paper I** also identified bio-surfactant addition as a potentially viable pre-treatment method. Bile is a novel bio-surfactant which *in vivo* acts to improve the surface area-to-volume ratio of lipids for the purpose of improving the rate of enzymatic degradation of these lipids to long-chain fatty acids, and subsequently, volatile fatty acids. It was this action for which bile was considered for pre-treatment for the anaerobic digestion of high-fat abattoir waste.

Paper III investigates the use of bovine bile as a novel bio-surfactant to aid in the anaerobic digestion of DAF sludge. As the pre-treatment is novel, a suite of doses were determined arbitrarily, however, inspiration was drawn from Nakhla *et al.* (2003) with their use of 'BOD-balance', a bio-surfactant extracted from cacti that yielded favourable outcomes. It was hypothesised that the addition of bile to high-fat abattoir waste would benefit the anaerobic digestion process. This would be realised in an improvement to the digestion profile of the high-fat waste, measured by either a decrease in inhibition and digestion time, an increase in reaction kinetics, or an increase in methane yield. Three individual digestions were performed to collect the data for this investigation, and highlighted that the effect of pre-treatment on the anaerobic digestion process depends significantly on the composition of the substrate and quality of the inoculum.

The effect of bile dosed at 0.2-1 g/L was consistent with the alternate hypothesis that bile addition would enhance the anaerobic digestion of high-fat abattoir waste. While there was no improvement in the digestion profile, an increase in methane yield of 7.08% was measured with a bile dose of 0.6 g/L. Addition of bile showed no improvement in solubilising COD, nor did it increase VFA content (Table 9). The mode of action was likely emulsification of fatty material.

Bile dosed at 0.2-1 g/L with sludge acquired from a WWTP, treating substrate with a DAF sludge with very high fat content produced a significant increase of up to 7.08%. Conversely, bile dosed at 1-6 g/L with sludge acquired from a red meat processing facility treating a DAF sludge with relatively low fat content produced negligible influence with 1-2 g bile/L. At concentrations of 3-6 g/L, bile produced inhibition that increased exponentially with increasing dose. Reaction kinetics declined linearly with increasing dose, declining to half the control value with a dose of 6 g bile/L. Lag-phase inhibitory duration increased by up to 79%, time required to achieve peak methane production was delayed by up to 74%, and total digestion time was slowed by up to 65%. At a dose of 6 g bile/L, methane yield was reduced by 6%.

An anaerobic toxicity assay was also performed to assess the effect of bile dosed at 1-6 g/L to reactors digesting cellulose as a standard substrate. Although WWTP sludge was used for the toxicity assay, the results of the high-dose BMP were replicated, albeit to a lesser extent.

3.3.1 Economic assessment of bile pre-treatment

The economic viability of using bile as a bio-surfactant was briefly assessed. In comparison to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile, through biogas production, to 220% of its current sale value. In contrast with the pre-treatment options trialled in **Paper II**, bile was the only option which produced a positive economic outcome under the conditions outlined in **Paper II.**

3.4 Impact of thermobaric pre-treatment on the continuous anaerobic digestion of high-fat cattle slaughterhouse waste

The results of **Papers II and III** formed the basis for the next stage of work, **Paper IV**, in which bile, chemical, and thermochemical pre-treatments were eliminated as viable options for pre-treatment of DAF sludge. Low-dose bile produced up to 7.08% increased methane yield, while high-dose bile pre-treatment resulted in decreased methane yields, reduced reaction kinetics, and increased inhibitory effect. While chemical treatment enhanced methane yields, the increase was minor in comparison to that obtained by thermobaric and thermochemical pre-treatments options. Although the thermochemical pre-treatment produced marginally more methane than the thermobaric pre-treatment, the addition of sodium hydroxide appeared to be a largely ineffective component of the pre-treatment process. Subsequently, thermobaric pre-treatment, with an increase in methane yield by 8.32%, and 100% reduction of inhibition, was selected to progress to continuous digestion experimentation (**Paper IV**).

While the simple economic assessment was not favourable for thermobaric pre-treatment, the reduction in treatment time and increased rate of methane production may allow for more consistent use of gas-fired boilers, and offset consumption of coal, or other fossil fuels to yield a positive economic outcome.

It was hypothesised that thermobaric-treated DAF sludge would improve substrate utilisation under continuous digestion conditions, resulting in either increased methane yield and/or increasing substrate degradability (**Paper II**). While an increase in methane potential would be a good outcome, an increase in substrate degradability appeared to be the most beneficial aspect of the thermobaric treatment.

This would allow for more regular feeding intervals (i.e. a reduced HRT), or conversely, an increase in OLR, and subsequently a higher daily rate of gas production.

Thermobaric pre-treatment of DAF sludge and subsequent digestion in CSTR reactors was not beneficial to the digestion process. Treatment resulted in reduced biogas and methane yields by approximately 12%, which may be a result of VFA loss during pre-treatment. Such losses were also exhibited by Park et al. (2017). Reactors digesting thermobaric-treated DAF sludge experienced greater instability in pH, VFA and VFA:TA ratio, greater accumulation of FOG, and a higher production of hydrogen sulphide. VFA content was higher in the reactors receiving thermobaric-treated substrate over the first 30 days, which may be a result of a more readily degradable substrate, and contributed to a consistently lower digester pH over the first 44 days.

H_2S concentrations were 56% greater on average, indicating a greater degradation of protein in the thermobaric-treated substrate. The increased FOG and decreased OLR produced with the fresh batch of substrate from day 34 onward caused the digesters to fail by day 43. Addition of $Mg(OH)_2$ rapidly recovered digester pH, biogas production and significantly reduced H_2S concentrations. Extraction of fully-mixed effluent samples from day 48 onward induced a critical loss of active biomass, ultimately causing digester failure. It is possible that the addition of trace elements to the reactors could have both improved reactor stability and prolonged digestion under the conditions of this experiment (Schmidt et al. 2014). It was speculated that the large variations seen in substrate characteristics between all stages of investigation played a large role in influencing the effect of pre-treatment.

3.5 Limitations and future work

- Continuous investigations

In comparison to the batch digestion, while work performed in **Paper II** yielded encouraging results, application of thermobaric-treated DAF sludge to continuous digestion was not beneficial. Such conflicting results have been reported previously. For example, Schwede *et al.* (2013) thermally treated microalgae and produced a 185% increase in methane yield under batch conditions. However, under continuous digestion, an increase of only 108% was recorded. Similarly, Zhang, Su and Tan (2013) measured on average 29% less methane produced from substrate digested in continuous systems when compared with batch systems.

Although simple BMP tests give a good indication of the amount of biogas and methane that can be ultimately produced from a substrate, these tests do not accurately reproduce the conditions of a large-scale AD system under continuous or continuous operation (Carrere *et al.* 2016). Given that laboratory investigation to understand a substrate's biogas potential is critical in making business and design decisions regarding the implementation of AD technology, it is important to consider the limitations of BMP tests, and the advantages and shortcomings of batch and continuous digestion investigations.

As shown in **Paper III,** bile under BMP testing has potential to increase methane yield up to 7.08% at a dose of 0.6 g/L. At the more conservative dosage of 0.2 g bile/L, which is also the more viable dosage for industry, the measured increase was reduced to 5.71%. At this more modest increase, through the generation of methane, the value of bile is 220% greater than current use as a sale product. Investigation of bile addition under continuous digestion conditions should be conducted to assess the viability of bile addition in a full-scale industrial system. Promising candidates from BMP investigation of other pre-treatment options should also be subjected to continuous digestion experimentation. If steady state digestion is achieved, researchers should look to vary the OLR and HRT to achieve optimal digestion conditions. Digester effluent should be regularly analysed for the accumulation of VFA species and other inhibitors.

- Quantitation of VFA produced from hydrolysis of lipid

Quantitation of VFA species using GC-FID was conducted early in the project as a way of measuring VFA as acetic acid equivalence. At the time, there was no interest in measuring the quantities of individual VFA, but more interest in generating VFA as an indicator of the pre-treatment enhancing hydrolysis. It is now understood that VFA play a role in digester inhibition, and can be used as an indicator for digester failure, but the inhibitory concentration of these VFAs is a subject of ongoing research. For future research, it would be preferable to quantify the degradation of individual macromolecules to VFA such as was performed by (Wilson & Novak 2009).

- Control over substrate characteristics

Research into, and operation of AD systems, is heavily influenced by the variation and inconsistency in substrates and inocula (Schmidt, McCabe & Harris

2018). The nature of uncontrolled industrial samples influenced by on-site activities and fluctuations undermines the quality of research outcomes. Waste characteristics vary considerably, as demonstrated in **Paper 1**, and are subject to variation with respect to species slaughtered, seasonal change, weekly, daily, and even between shifts (Bauer, 2011). Due to these sources of variation, substrate and inoculum characteristics can vary significantly at any given time.

Control over industrial substrate characteristics is a difficult problem to overcome. Some approaches to substrate control include: Composite sampling, collecting large grab samples, and using a synthetic substrate. Composite sampling aims to limit variation between grab samples by collecting material at intervals, or with respect to flow volume, throughout the day. While this produces a more consistent substrate, the variation is not eliminated, but may allow for more consistent experimentation throughout a long-term investigation, where multiple batches of substrate are needed. In contrast, for short-term experimentation, depending on the research question, it may be suitable to collect a large grab sample. While this ensures that sub-sampling from this well-mixed grab sample will yield reproducible results, eventually, the batch will be either depleted, or become overgrown with contaminating organisms, and subsequent grab samples will likely vary greatly from the previous. Finally, these issues can be solved through production of a synthetic substrate. However, producing a synthetic substrate is more difficult than the previous options. Importantly, the synthetic substrate should be as identical to the real substrate as possible, so that results are relatable to industry. Therefore, production of a synthetic substrate should begin with characterisation of the substrate which is to be mimicked. Carbohydrate, protein and lipid content should be matched, and effort should be made to provide identical macromolecular constituents, as for instance, different lipids are more degradable, while others are more inhibitors. Beyond this, the synthetic substrate must contain micro-nutrients/trace elements for continued support of the microbial community. For a complex waste stream such as an abattoir wastewater, this may be achieved simply by adding bovine blood in a controlled manner. The result is a substrate which can be reproduced with minimal variation over numerous batches, and, once the recipe is created, should be simple to create in a timely and cost-effective manner. It would have been greatly beneficial to analyse substrate characteristics for total carbon, total nitrogen, phosphorus and sulphur, as well as trace elements including iron, zinc, nickel, cobalt, copper, selenium, tungsten, molybdenum and manganese.

With a desire for reproducibility in mind, creation of a synthetic substrate was considered to overcome this problem. However, AD systems were considered too complex to consider all of the biological necessities to create a sufficiently suitable synthetic substrate. Instead, Baxter beef flavoured dog food was trialled in this project as a synthetic substrate for a continuous digestion experiment, with the aim of increasing fat content by adding lard to determine the critical point before digester failure due to FOG loading. At this point lard was to be reduced to a sustainable loading and the substrate was to be pre-treated to commence the second stage of the experiment. Unfortunately, following lard addition, digesters immediately began to fail, and despite considerable effort, the digesters were unrecoverable. While the goal was to determine the impact of pre-treatment on the digestibility of the lipid fraction, this substrate was considered too far removed from slaughterhouse waste, and the change to DAF sludge was made for experiments detailed in papers II, III and submitted manuscript IV.

With respect to inoculum consistency, weather events, shock loadings, feedstocks and operational inconsistencies significantly impact anaerobic sludge quality. Consequently, a number of inoculum sources were utilised throughout this project, and made comparison of results difficult.

In order to limit this variation, and consequently improve future data quality and confidence in the results, effort should be made to produce both a controlled inoculum and substrate. Consistency in inoculum quality could be controlled by producing sludge in-house with controlled substrate addition, temperature control, stirring and monitoring.

IV

Conclusions

This investigation demonstrated that anaerobic digestion of high-fat abattoir waste can be enhanced through pre-treatment under batch conditions. Batch digestion of DAF sludge pre-treated with 0.2-1 g/L bile, chemical, thermochemical, and thermobaric pre-treatment each produced beneficial outcomes in the AD of high-fat abattoir waste. The most significant improvements were achieved through thermobaric pre-treatment, with an 8.32% increase in methane yield, a complete elimination of lag-phase inhibition, and equivalent yield to the control achieved 3 days earlier. The results using thermobaric pre-treated DAF sludge under continuous digestion were contrary to those achieved under batch digestion. Unlike earlier work, continuous digestion did not show increases in specific methane production but revealed important information related to the negative impacts that a heterogeneous, high-fat slaughterhouse waste has on anaerobic digestion performance. Under continuous digestion, thermobaric pre-treatment resulted in reduced methane yield by 12.1%, a consistently lower pH, and 56% increased hydrogen sulphide content. This reduction in methane yield is speculated to be due to loss of volatile organics during the pre-treatment process given the lack of a pressure vessel. The study was carried out using varying levels of fats, oils and greases at different organic loading rates and highlighted the importance of close process control and monitoring, particularly when the substrate is used in mono-digestion rather than co-digestion. It has been concluded that while pre-treatment can have significant benefits to the digestion process, consistency and quality of sludge and inoculum are essential elements in deriving benefit from pre-treatment. Consequently, industries which experience great variation in substrate characteristics should take great care in sampling and subsequent analysis of substrates for the planning of AD installations.

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Appendix A

Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater

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Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater

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HIGHLIGHTS

- We review pre-treatment options applicable to wastewater high in fats and oils.
- The unique characteristics of abattoir wastewater are summarised.
- Pre-treatments are evaluated for their potential to improve anaerobic digestion.
- Appropriate pre-treatment technologies are considered on the basis of performance.
- Limitations and future research opportunities in this area are presented.

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ABSTRACT

This paper explores pre-treatment options for the anaerobic digestion (AD) of high-fat cattle slaughterhouse wastewater by assessing and attempting to compare pre-treatment methods used to treat various waste streams. The central focus on cattle slaughterhouse wastewater stems from the problematic nature of high fat, oil and grease (FOG) present in Australian red meat processing (RMP) waste water.

Fully integrated abattoirs such as those operating in Australia typically produce wastewaters that carry high FOG loads of 100–4000+ mg/L. While excessive levels of fat can be inhibitory to the AD process, these fats contain a very high theoretical methane potential of 1014 L CH_4/kg VS when compared with carbohydrates at 370 L CH_4/kg VS and proteins at 740 L CH_4/kg VS. However, due to the hydrophobic and inhibitory nature of fat, oil and grease, accessing this methane potential is difficult. This article serves as a review of the literature in the field of pre-treatment of wastewaters and subsequent anaerobic digestion with the goal of increasing biogas yield, with an emphasis on digestion of wastes high in fat, oil and grease. This review covers mechanical pre-treatments including high-pressure homogenisation, ultrasonication and electrokinetic disintegration, and other forms of pre-treatment including thermal, chemical, thermochemical, and enzymatic hydrolysis, and biochemical emulsification. Biological pre-treatments, also known as pre-hydrolysis and two stage digestion are briefly reviewed. The most significant considerations for selecting a pre-treatment technology are the energy balance and costs. Therefore, this review will also provide a commentary on the advantages and disadvantages of the pre-treatment methods reviewed and conclude by evaluating their relative worth in pre-treating FOG.

Abbreviations: BMP, biomethane potential; COD, chemical oxygen demand; DMDO, dimethyldioxirane; DS, dissolved solids; FOG, fat, oil and grease; GHG, greenhouse gasses; GHz, gigahertz, billion hertz; HPH, high-pressure homogenisation; HRT, hydraulic retention time; KW, kitchen waste; LCFA, long-chain fatty acids; MHz, megahertz, million hertz; MJ, megajoules, million joules; MPa, megapascals; NH_4–N, ammonium as nitrogen; NOx, oxides of nitrogen; pCOD, particulate chemical oxygen demand; PL-250, pancreatic lipase 250; POME, palm oil mill effluent; POMS, peroxymonosulphate; RMP, red meat processing; SBM, stirred ball mill; sCOD, soluble chemical oxygen demand; SS, suspended solids; tCOD, total chemical oxygen demand; tHSCW, tonnes of hot standard carcass weight; TOC, total organic carbon; WWTP, wastewater treatment plant.

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Contents

1. Introduction

Global processing of cattle has intensified consistently over the past 50 years, increasing by 36.29 Mt from 27.69 Mt in 1961 to 63.98 Mt in 2013 [1] (Fig. 1). While production has more than doubled, waste mitigation techniques have lagged behind the ever increasing accumulation of waste.

Processing livestock is an energy and cost intensive process. An environmental sustainability review of the Australian red meat processing (RMP) industry conducted in 2010 revealed that 9.8 kL of water was used to generate a single tonne of hot standard carcass weight (tHSCW) during 2008–2009 and generated 8.7 kL of wastewater. This consumed 4108 MJ of energy from various sources, and committed 11.3 kg of solid waste to landfill, while greenhouse gas (GHG) emissions averaged 554 kg CO_2/tHSCW. Of total energy emissions, 67% were related to electricity use, and 35% of emissions contributed by anaerobic wastewater treatment [2].

The Australian RMP industry is currently working on a range of measures in an effort to reduce carbon pollution and improve energy efficiency through actively seeking renewable sources of energy and water recovery. This has been largely in response to a variety of factors including prolonged drought, water restrictions and rising water costs, rising fuel and energy costs, increased community focus, and GHG emissions. Several knowledge gaps have been identified in which research is needed to reduce the industry's emissions and energy costs [3]. One of the technologies identified as a potential solution reducing emission and energy costs is anaerobic digestion (AD). It has been demonstrated that AD technology can play a major role in waste management and the production of biogas in the abattoirs [4]. The methane produced can be combusted to generate heat and electricity (CHP), or can be refined into renewable natural gas and transport fuels [5]. In addition, AD can be used to manage waste and reduce greenhouse gas emissions, and the digestate may be used or sold as a valuable organic fertilizer substitute or soil amendment [6].

Fig. 1. Growth in global meat production from 1961–2013 [1].

Red meat processors have embraced the uptake of AD systems to treat high-strength wastewater and thereby reduce emissions. In Australia, AD systems typically take the form of low-rate anaerobic lagoons, which are well suited to the vacant land space available with a move to covered anaerobic lagoons to capture methane and reduce GHG emissions [7]. While it has been noted that anaerobic lagoons are not optimised treatment strategies, they are low-capital investments which can affect a large degree of organic degradation and methane generation [8].

The high-strength wastewaters produced in Australian abattoirs tend to contain high levels of fat, oil and grease (FOG) with values ranging between 5 and 4570 mg/L in grab samples [9]. While AD is effective for the degradation of many substrates, FOG present several challenges. Before waste reaches the digester, FOG can adhere to pipe walls and begin the accumulating to form blockages. In the case of covered anaerobic lagoons FOG typically has two fates; accumulation in fatty crust or hydrolysis and digestion to form methane. It has been observed that a large portion of the fatty material floats to the liquid surface along with cellulosics from paunch material to form a fatty crust [10–13]. Fat particles that are hydrolysed to long-chain fatty acids (LCFA) may subsequently adhere to the surface of the sludge microbes. These LCFA form a layer over the microbial surface, producing reversible inhibition of mass-transfer between the microbes and the medium [14].

Australian abattoirs stand to benefit substantially if an appropriate pre-treatment method can be developed to improve the bioavailability and subsequent conversion of FOG to methane. McCabe et al. [15] has shown that biogas production can potentially vary tenfold depending on factors such as lagoon efficiency and operational practices. While no current AD system currently

Table 1
Concentrations of parameters of high-strength wastewater produced by abattoirs.

Parameter (mg/L)	Residential-strength[a]	Typical abattoir raw wastewater (all meats)[b]	King Island (beef)[c]	Southern Meats wastewater ex DAF (sheep)[d]	Churchill abattoir (beef)[e]
BOD	100–400	1600–3000	3000	~1/2 COD	163–7020
COD		4200–8500	7250	3100–11,500	1040–12,100
FOG	50–150	100–200	120	290–2670	5–2110
TSS	100–400	1300–3400	2000	1150–5700	457–6870
VSS		n/a	n/a	1040–5300	n/a
TN		114–148	450	180–440	296–785
NOx		n/a		0.01–0.12	n/a
NH_4–N		65–87	250	18–135	23.8–349[f]
Total *P*		20–30	45	26.4–60	n/a
VFA		175–400	n/a	61–600	1020–1980
Alkalinity		350–800	n/a	340–700	70–906

n/a indicates not available.
[a] Benefield [19].
[b] Johns [18].
[c] White et al. [13].
[d] UNSW [10].
[e] McCabe et al. [12].
[f] Value is for NH_3–N.

deals with FOG effectively, typically the more sophisticated the anaerobic digestion technology, the less capable they are of handling FOG loads [16,8].

In the context of this review, pre-treatment does not refer to the action of screens, dissolved air flotation systems, contra shears and screw presses which act to capture and separate solids from the waste streams. Here, pre-treatment refers to the treatment of the wastewater to enhance the availability of substrates to microbes and thereby improve the removal of organics and increase reaction kinetics and or total biogas production. The aim of this review is to identify available methods that have commonly been used to treat various substrates that may translate well to FOG pre-treatment, including but not limited to, waste activated sludge (WAS), kitchen waste and grease trap sludge. Biological pre-treatments, also known as pre-hydrolysis and two-stage digestion, are briefly reviewed, as this process is concerned with changing the inoculum as opposed to changing the waste feedstock to enhance digestion. Pre-treatment of FOG is rarely investigated, typically in the form of enzymatic pre-treatments, and little comparison between the effects of various pre-treatment types has been reported. This review investigates several forms of pre-treatment in their ability to enhance methane production. Where possible, investigations into pre-treatment of FOG or high-fat substrates are cited in preference to investigations which treat substrates more removed from abattoir wastewater. The most significant considerations for selecting a pre-treatment technology are the energy balance and costs. Therefore, this review will also provide some preliminary commentary on the advantages and disadvantages of the pre-treatment methods reviewed and conclude by evaluating their relative worth in pre-treating FOG.

2. Characteristics of abattoir wastewater

The main types of wastes from abattoirs include organic solid wastes generated during meat processing and wastewaters from washing at various stages of the process. Australian RMP wastewater is generated at high volumes and characterised as having high organic, fat and nutrient loading. Volumes are typically around 850 kL/day with organic content of 5700 kg COD/day. In Australia, a typical abattoir is defined as processing 150 tHSCW per day, equivalent to 625 head of cattle. Production is assumed to take place 5 days a week, 250 days per year, including boning and rendering [17]. While Johns [18] has determined typical values for abattoir wastewater, case studies have reported pollutant concentrations far greater than the typical [12,10]; Table 1). Abattoir wastewater becomes high-strength due to the accumulation of constituents including blood, fat, paunch, protein and excrement in the water. The composition of Australian RMP wastewaters may vary significantly from abattoir wastewaters in other countries. This is due to the fully integrated facilities in Australia which include slaughter, boning and rendering processes at the same plant [20]. In contrast, German abattoirs, for example, are required by law to perform rendering in an off-site facility [21]. Furthermore, the high-strength wastewaters produced in Australian abattoirs tend to contain high levels of FOG compared with their non-integrated equivalents. For this reason, care must be taken when comparing reports from various abattoirs around the world. While large integrated beef slaughterhouses in the USA show excellent similarities with data from Australian abattoirs, Australian abattoirs tend to generate higher volumes of higher-strength wastewaters than their European counterparts [20,17]. While high-strength wastewaters typically contribute well to biogas production, the FOG component tends to be problematic [22].

3. Wastewater parameters associated with biogas production

The wastewater parameters which are of particular interest to this review are those which could be logically associated with increased biogas production, including soluble COD (sCOD), volatile solids (VS), FOG, fat particle size, and volatile fatty acids (VFA) [16,23,24]. Pre-treatments are often assessed with respect to sCOD release and degradation [25]. As treatments rupture cells, the intracellular contents are released into the extracellular medium, contributing to the soluble fraction of COD [26]. As a measure of pre-treatment impact on substrate degradation, sCOD appears to be useful. However, while sCOD may increase in response to a pre-treatment, the relationship between sCOD and biogas production is complex, and as such, does not necessarily indicate an increase in biogas production [27]. Therefore, if biogas production is to be reported with respect to sCOD degradation, further information must be collected to support findings.

Although less commonly investigated as a measure of pre-treatment impact, specific methane production is regularly reported with respect to degradation of VS [28]. Also known as organic solids, VS is made up of carbohydrates, proteins and fats, typically derived from organisms, but may also include artificial organic compounds. Consequently, there is a strong correlation between VS degradation and biogas production [16]. Given this strong correlation, measuring VS as an indicator of pre-treatment impact may be more valuable than measuring sCOD. Due to the lack of standardization in the reporting of pre-treatment impact on AD performance, this review will cover the majority common measurements.

This review is particularly focused on the degradation of FOG, either during the pre-treatment process, or during the AD process as a result of pre-treatment. Measurement of FOG content can be done before and after pre-treatment, and post-digestion. Fat particle size reduction is another favourable outcome of pre-treatment. A reduction in particle size increases the surface area to volume ratio of the fat content, increasing the area susceptible to chemical and enzymatic interaction [29]. Logically, this should increase total methane production, but may result in temporary inhibition due to increased LCFA content. Further degradation of LCFA will produce VFA, which are also of interest as these are an end product of the acidogenic and acetogenic pathways of anaerobic digestion, and a feedstock for methanogenic bacteria. While VFA at concentrations of 6.7–9 mM are toxic to methanogens, if a pre-treatment were capable of degrading triglycerides and LCFA to VFA, the process could significantly enhance reaction kinetics [30].

4. Impact of fat, oil and grease in anaerobic digestion

The fat, oil and grease component of high-strength wastes, such as those created in abattoirs, can induce several problems including clogging of pipes, adhesion to sludge causing both inhibition of mass-transfer of nutrients and sludge flotation with subsequent washout [31,14]. Covered anaerobic lagoons suffer further complications, with FOG reacting with cover materials to compromise the material integrity, inhibition of gas permeation and therefore capture by the cover. This crust material can significantly reduce the effective volume of the digester and is largely unavailable to the anaerobic consortium as very little is accessible by hydrolytic enzymes.

Anaerobic lagoons can receive large volumes of FOG and continue to function for long periods of time before the lagoon fails. This is likely due to the lack of mixing in a lagoon, allowing FOG to float to the lagoon surface along with lignocellulosic material to form a fatty crust. The accumulation of crust on the lagoon surface heavily restricts the functional volume of the lagoon in time, through the generation of dead space, resulting in short circuiting [32]. Fig. 2 depicts a schematic diagram of the impact of crust accumulation on the functional volume of an anaerobic lagoon. In a continuously fed anaerobic lagoon this process is unsustainable, and accumulation of FOG as crust has typically outweighed FOG consumption. If FOG accumulation is not monitored and dealt with accordingly, crust can accumulate to several meters thick with surprising density as shown in Fig. 3 [12]. Not only does this make cleaning from large lagoons difficult and expensive, the issue of how to deal with waste FOG after removal has not been addressed

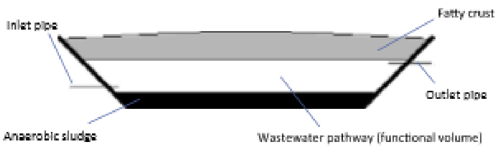

Fig. 2. Illustration of dead space contributed by crust and sludge volume resulting in a large reduction in functional pond volume.

Fig. 3. Section of crust removed from an anaerobic lagoon by an excavator after desludging indicating crust thickness [12].

[11]. While this accumulation is far from ideal, a managed crust does offer some benefit in odour reduction, pond insulation, and FOG locked up in crust is relatively unavailable to cause process inhibition [33,34].

Alternatively, high rate systems with active heating and mixing bring microbes into greater contact with FOG and LCFA. Subsequently, high rate AD systems are more sensitive to FOG loadings and are at a greater risk of resulting failure than anaerobic lagoons [35]. While microbes can be acclimated to FOG loadings this is a typically slow process with time required to acclimate increasing with FOG loading [36]. A move towards covered high rate anaerobic lagoon (COHRAL) technology to treat abattoir waste water which incorporates novel waste water distribution and settling systems is underway with the recent commissioning of the first lagoon in Australia [37]. The monitoring of this type of system will be particularly useful in assessing the overall impact of FOG loading and AD performance.

While anaerobic lagoons are currently considered the most suitable digester type for handling wastes with high FOG content, new research into anaerobic membrane reactor (AnMBR) technology has shown great promise in wastewater treatment, especially in wastes with high FOG loads. Christian et al. [38] reported on the first two years of treating high-strength industrial wastewater at Ken's Foods in Massachusetts, USA. This AnMBR, the largest in the world in 2011, has a design 475 m3/d with COD, BOD and TSS loadings of 39,000 mg/L, 18,000 mg/L and 12,000 mg/L respectively. The AnMBR produces consistently high-quality effluent with non-detectable TSS, and average COD and BOD concentrations of 210 and 20 mg/L, giving removals of 99.4% and 99.9% respectively. Furthermore, AnMBR reactors have been loaded with COD in the order of 5–30 kg COD/m^3/d, and FOG loading of up to 4–6 kg/m^3 with removal rates of 97% and 100% removal efficiency respectively [35,39]. However, few investigations have involved large FOG loadings being treated using AnMBR technology. Given that high-rate AD systems are typically sensitive to FOG loadings, more research should be conducted to investigate the feasibility of FOG digestion using AnMBR technology [14].

4.1. Enhancing biogas yield through co-digestion

While FOG have typically been viewed as a negative influence they have much to offer AD operations. Addition of FOG to an AD system has the potential to significantly increase biogas production [40]. When the theoretical biochemical methane potential (BMP) of macromolecules is compared, lipids are capable of

Table 2
Effect of co-digesting substrates with FOG-rich co-substrates on methane yield.

Co-substrates	Feed ratio	CH_4 volume	CH_4 %	Reference
Sewage sludge and grease trap sludge	100:0 54:46	278 m^3/t VS added 463 m^3/t VS added (+66% CH_4 yield)	63 62	Luostarinen et al. [45]
Sewage sludge and grease trap sludge	100:0 70:30	271 m^3/t VS added 344 m^3/t VS added (+27% CH_4 yield)	65 69	Davidsson et al. [46]
Pig slurry and waste sardine oil	100:0 95:5	0.43 m^3 CH_4/m^3 digester/d 1.61 m^3 CH_4/m^3/digester/d (+274% CH_4 yield)	72 70	Ferreira et al. [47]
Poultry manure and olive-oil mill wastewater	100:0% v/v 75:25% v/v	0.43 L/(L_R/d) 0.52 L/(L_R/d) (+21% CH_4 yield)	74.1 71.8	Gelegenis et al. [48]
Sewage sludge and grease trap waste	77:23 VS	+138% CH_4 yield		Silvestre et al. [44]

yielding more methane at 1014 L/kg VS than both proteins at 740 L/kg VS and carbohydrates at 370 L/kg VS [41,22]. These theoretical values were supported by Labatut [42], with observed specific biomethane yields ranging from 903.9 to 1101.2 L/kg VS for lipids, 302.5–407.3 L/kg VS for proteins and 191.8–359.3 L/kg VS for carbohydrates digested under mesophilic conditions. Indeed, co-digestion of substrates with FOG has produced significant increases in biogas production. Li et al. [43] compared the biogas produced from digestion of WAS with WAS co-digested with FOG using BMP tests. While the WAS control produced 117 ± 2.02 mL/g TVS (total volatile solids), the reactor co-digesting WAS with FOG produced 418 ± 13.7 mL/g TVS. This represents more than 350% increase in biogas production attributed to the addition of FOG. Similarly, Silvestre et al. [44] co-digested sewage sludge with trapped grease waste. Not only did this study result in increased biogas production by 138%, but found that acetic and β-oxidation syntrophic acetogenic activities were 2.5 and 3.75 times higher than the initial inoculum respectively. This suggested that sludge could become acclimatised to greater FOG loads over time, and that this could be an effective strategy for improving fat degradation and reducing the inhibitory effects of LCFA. Table 2 lists several investigations which support the conclusion that co-digestion with FOG can significantly improve methane yields by considerable volumes.

However, co-digestion is dependent on access to available waste streams. Investigation of co-digestion using Australian abattoir wastewater is only in its infancy and is noted to be a multi-faceted issue which goes beyond simply sourcing feedstocks for AD. The Australian RMP industry consists of medium to large enterprises which are often not located within close proximity to other agro-industrial waste streams. Subsequently, co-digestion is currently not an economically viable option for Australian abattoirs. Thus, Australian RMP industries which employ biogas facilities use abattoir wastewater as a monosubstrate. Ortner et al. [49] exemplifies the situation of developing a reliable monodigestion process using slaughterhouse waste as the sole substrate. Beyond co-digestion, pre-treatment of FOG offers the next step to enhancing the AD process.

5. Pre-treatment of substrates for anaerobic digestion

In the context of this review, pre-treatment refers to the treatment of the wastewater to enhance the availability of substrates to microbes and thereby improve the removal of organics and increase reaction kinetics and or total biogas production. Substrate availability may be enhanced through several mechanisms, resulting in liberation of sequestered organics, enhance surface area to volume ratio of, or hydrolysis of macromolecules. To achieve these goals, pre-treatments typically aim to enhance hydrolysis, the rate-limiting step of the AD process [50]. There are several different methods available to achieve this, including biological, mechanical, thermal, chemical, enzymatic, and biochemical approaches [16,23]. While this review contains collated literature data on various pre-treatment methods, due to non-standardised reporting and the great variability within between research projects, direct comparison is difficult. Although projects that report on methane and biogas production are preferred, projects which report on other variables such as VS and sCOD are valuable to inform further research.

Fig. 4 illustrates the effect of pre-treatments on rate of anaerobic digestion (i.e. reaction kinetics; pre-treatment b) and increase the methane yield (pre-treatment c). Both effects will improve the operation of a biogas plant. However, depending on when a BMP test is ended, different interpretations are possible: t1: pre-treatment b double the methane yield; t2: none of the pre-treatment methods increase methane yield; t3: pre-treatment c increases the methane yield by 25%, but pre-treatment b has no effect [51].

Carlsson et al. [52] reviewed pre-treatments in literature applied to different substrate categories in lab-, pilot- and full-scale studies as well as discussed in reviews (112 papers from 1978 to 2011). The pie-chart (Fig. 5) illustrates the number of times each substrate-type occurs in combination with a pre-treatment; the total number of occurrences is larger than the number of articles since several articles discuss more than one pre-treatment type. The bar-charts illustrate the distribution among the different pre-treatments for each substrate-type. The literature was selected so as to cover as many different types of substrates, pre-treated with as many processes and/or technologies as possible.

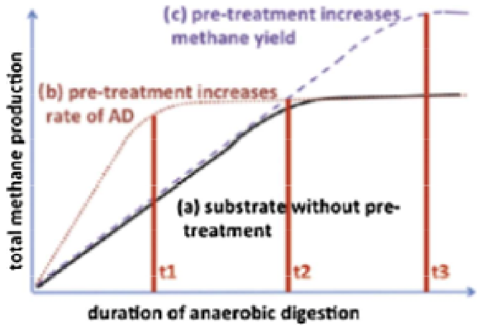

Fig. 4. Effect of pre-treatments on rate of anaerobic digestion and increase the methane yield [51].

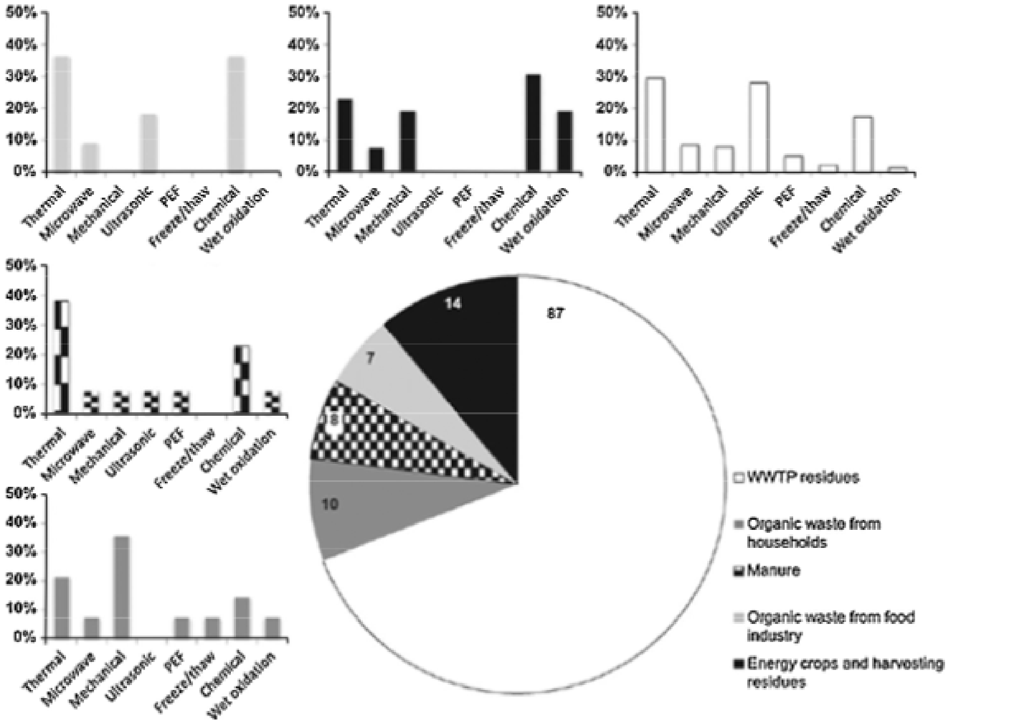

Fig. 5. Reviewed pre-treatments in literature applied to different substrate categories in lab-, pilot- and full-scale studies as well as discussed in reviews (112 papers from 1978–2011) [52].

Table 3
The effect of various pre-treatment methods on substrates.

Pre-treatment	Substrate	Treatment		Effect	Source
		Conditions	Time		
Ultrasonic	Waste vegetable oil	2000 kJ/kg TS	N/A	+41,932% organic content (emulsification)	Moisan [53]
Thermal	WAS	60, 80 and 100 °C	Not disclosed	+30% biogas produced	Ho [54]
Chemical	WAS	7 g NaOH/L	Not disclosed	+31.7% sCOD content	Kim et al. [50]
Thermo-chemical	Waste kitchen oil + KW	pH 10, 55 °C Stirred	90 min	+9.9 ± 1.5% biogas produced	Li et al. [55]
Biochemical	FOG	500 mg/L	N/A	+87 ± 13% sCOD removal	Nakhla et al. [23]

KW – kitchen waste; sCOD – soluble chemical oxygen demand; TS – total solids.

Table 3 gives an indication of some of these pre-treatment methods applied to substrates, with an emphasis on FOG-rich wastes. It is important to note that the effects of various pre-treatments have not been reported in a standard format. As such, while investigations have been conducted using various pre-treatment methods, it is difficult to compare their impact in terms of altering biogas yield.

5.1. Mechanical degradation of feedstocks

Mechanical pre-treatments are commonly used to enhance digestion of cellular wastes such as sludges (e.g. WAS), cellulosics (e.g. crop waste), and other similar wastes. The aim of these pre-treatments are to rupture the cell walls of the cells in these feedstocks, a process which can be reduced from days to minutes through mechanical pre-treatment [56]. This branch of pre-treatments requires the action of units such as, but not limited to, stirred-ball mills (SBM), high-pressure homogenisers (HPH), ultrasonicators, and less commonly, units which emit microwaves and electrical fields. While SBM is not expected to be effective in pre-treating FOG, HPH has been applied to dairy milk with some encouraging results, and therefore is the mechanical pre-treatment which will be covered in this review. Table 4 at the end of this section lists several mechanical pre-treatment methods and summarises the conditions and results of numerous investigations.

5.1.1. High-pressure homogenisation

Popular in large-scale operations, HPH works by compressing waste and projecting at high speed against an impact ring. The turbulence, cavitation and shear stresses applied to the waste disintegrate cells, releasing cellular contents into the medium (Fig. 6;

Table 4
Mechanical pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Pre-treatment conditions	Results	Reference
Ball mill	WAS	+42% sCOD content	Baier and Schmidheiny [57]
		+20–50% gas produced	
HPH	30–50 bar	+551% sCOD content	Choi et al. [58]
		+86% soluble protein	
		+11–15 VS removal	
	150–600 bar	+30% biogas produced	Onyeche [59]
	600 bar	+28–54% biogas produced	Engelhart et al. [60]
Mechanical jet	WAS, (30 bar)	+500% sCOD content	Nah et al. [61]
Sonication	WAS	No improvement in VS removal	Sandino et al. [62]
	WAS	+11–39% sCOD content	Khanal et al. [63]
	WAS, 6000 kJ/kg TS	+30–80% hydrolysis constant value	Braguglia et al. [64]
	Meat processing effluent, 120 MJ/kg TS	+55.9% oil removed	Erden et al. [65]
		+14.73% COD removed	
	Meat processing effluent, 750 MJ/kg TS	+76.74% COD removed	
	WAS, 0.5 W/mL, 5 min	−92% particle size	Biggs and Lant [66]
	Municipal solid waste, 0.1–0.4 W/mL, 30–60 min	+24% biogas produced	Cesaro et al. [67]
		+71.8% sCOD content	
Microwave	WAS, 0.3–300 GHz, 15 min	+22% sCOD content	Park et al. [68]
		+79% CH_4 produced	
Electrical field	WAS, 8000 kJ/kg DS	+9% sludge digestion	Kopplow et al. [69]

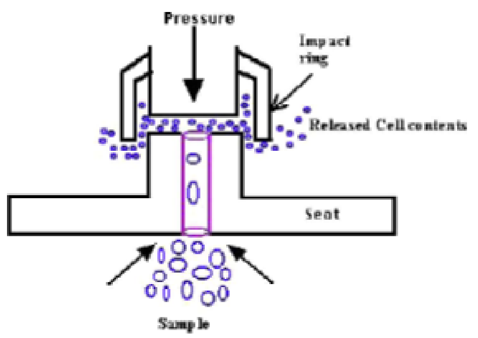

Fig. 6. Diagrammatic disintegration of waste activated sludge by high-pressure homogenisation [70].

[70,16]. While this technology has been successfully applied to disintegration of algal biomass and heavily utilised in the field of sludge disintegration, there is little available literature which considers HPH for pre-treatment of lignocellulosic biomass or fatty substrates. Choi et al. [58] investigated the ability of HPH to disrupt bacterial cells in sludge, assessed on the basis that the majority of the bacterial cytoplasm consists of protein, and subsequent release of protein was caused by cellular disruption. Pressures of 0.5, 1, 2, 3 and 5 MPa were investigated for their ability to increase protein concentration. After a single treatment, protein concentration increased significantly. Each sample was treated a further four times at the nominated pressure, and assessed for protein concentration following each treatment. Protein concentration continued to increase for each subsequent treatment. After the full five treatments, protein concentration increased from 14% untreated, to 35%, 55%, 75%, 80% and 86% in the 0.5 MPa, 1 MPa, 2 MPa, 3 MPa and 5 MPa treatments respectively. Choi et al. [58] also report on the effect of a single treatment event on the concentration of sCOD, total organic carbon (TOC) and alkalinity as mg of calcium carbonate ($mgCaCO_3$) as a representation of system buffering capacity. After a single treatment at 5 MPa, the average concentrations increased from: sCOD – 152 mg/L to 1250 mg/L; TOC – 90 mg/L to 1010 mg/L; and alkalinity – 229 to 280 $mgCaCO_3$/L.

High-pressure homogenisation operations have also been conducted at much greater pressures. Engelhart et al. [60] investigated the effect of HPH under pressures including 5 MPa, 30 MPa and 60 MPa on the disintegration of excess sludge. Disintegration was quantified by measuring COD release and oxygen consumption, while other notable measurements include total solids (TS), VS and ammonium–nitrate (NH_4–N). As seen with Choi et al. [58], Engelhart et al. [60] also measured an increase in several treatment parameters with increasing pressure. Release of COD increased from 4.1 ± 0.9% in the 5 MPa treatment to 21.7 ± 4.8%, and 43.4 ± 11.2% in the 30 MPa and 60 MPa treatments respectively. Oxygen consumption also increased with pressure from 6.6 ± 1.4% in the 5 MPa treatment, to 41.0 ± 15.4% and 79.2 ± 4.6% in the 30 MPa and 60 MPa treatments respectively. Ammonium–nitrate measurements indicating the digestion of protein indicated that protein liberation increased significantly with greater pressures. The 5 MPa treatment resulted in ammonium–nitrogen production of 68.7 ± 4.9 mg/L, while the 30 MPa and 60 MPa treatments produced 199.2 ± 129.0 mg/L and 290.3 ± 117.7 mg/L respectively. Initially, total and volatile solids were not significantly impacted by these treatments. However, once subject to digestion, VS degradation was enhanced up to 25%, resulting in a higher specific biogas production by 12% with respect to VS eliminated. Interestingly, while there is no literature that investigates the use of HPH as a pre-treatment for FOG-rich wastes, HPH is widely used for treatment and emulsification of dairy products. Hayes and Kelly [71] investigated the effect of HPH on raw whole bovine milk on globule size among other properties. Fat globules in milks treated with HPH (50–200 MPa) were significantly smaller than those in conventionally-homogenised samples. However, subsequent investigation suggested that achieving low fat globule size was more dependent on having the fat in a liquid state when exiting the primary homogenisation valve than the actual homogenisation pressure. However, Thiebaud et al. [72] investigated impacts of HPH on dairy milk, and determined that HPH reduces the particle size of fat globules in dairy milk. Particle size reductions of 79%, 83% and 90% were measured at 4 °C, 14 °C and 24 °C respectively under a treatment pressure of 200 MPa (Fig. 7).

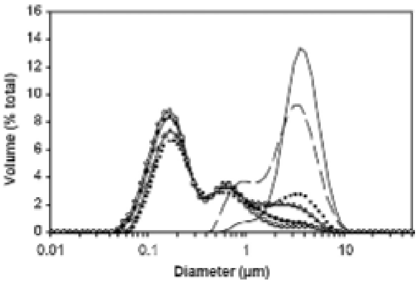

Fig. 7. Effect of homogenisation pressure on the size distribution of fat globules in milk, at an inlet temperature of 4 °C: 0.1 MPa (Control) (-), 100 MPa (--), 150 MPa (...), 200 MPa (Δ), 250 MPa (•), and 300 MPa (O). Clustering was not observed at any homogenisation pressure used [72].

While some investigations have assessed the effect of HPH on substrates that are suitable for AD, they have been focussed on the impact to the substrate, and not on AD. Subsequently, it is unknown how the changes in these substrates would impact the BMP achievable through digestion.

5.1.2. Ultrasonic pre-treatment

Like HPH, ultrasonication has also been applied sparingly to FOG-rich substrates. The mode of action of ultrasonication appears to be more sophisticated than SBM and HPH. As ultrasound waves propagate through the medium they create regions of compression and rarefaction. Microbubbles formed in this process grow in successive cycles and reach an unstable diameter at which they violently collapse in a process known as cavitation. Cavitational collapse produces intense local heating and high pressure (around 5000 °C and over 500 atmospheres with a lifetime of a few microseconds) on a liquid–gas interface, and, turbulence and high shearing phenomena in the liquid phase [65,24]. Furthermore, cavitation produces highly reactive H· and OH· radicals which facilitate chemical reactions for destroying organic materials. These chemical reactions are further favoured by the high temperature and pressure generated at the site of cavitation [73].

While sonication is effective in the degradation of many cellular wastes, including for example crop silage, wheat, hay and sludge [53,24,74], there are few investigations which report on the effect of sonication on FOG digestion [55]. Moisan [53] investigated the effects of ultrasonication on paper sludge, switch grass, hay, wheat straw and FOG as spent vegetable oil. For each instance, sonicated substrates released more organic carbon into solution than unsonicated substrates. This effect was exaggerated in the case of oil treatment. While the treatment of paper sludge, switch grass, hay, and wheat straw result in the degradation of cells and the release of organic contents into the solution, the effect of treatment on oil was to create an emulsion. Unsonicated, the oil–water solution presented an organic carbon content of 4.832 ± 0.79 mg/L. Following sonication, the solution was creamy in texture and presented an organic carbon content of 2031 ± 151 mg/L. The author noted that this effect is due to the dissolution of long-chain fatty acids in the water, and that this may have influenced the AD results obtained. Biogas production for the sonicated and unsonicated samples were measured, the sonicated sample produced a much greater rate of biogas production over the first 250 h of digestion. Beyond 250 h, the production of biogas from the sonicated FOG mirrored that of the unsonicated oil. By 825 h, unsonicated oil produced biogas equivalent to 1.56 m^3/kg VS, and sonicated FOG produced 1.66 m^3/kg VS at an assumed CH_4 content of 60%. Similarly, Li et al. [55] reported that not only was ultrasonic pre-treatment ineffective in enhancing biogas production, but such pre-treatment introduced inhibitory effects on FOG co-digestion. However, the results by Moisan [53] suggest a possible procedural exploit which may be taken advantage of to degrade FOG more quickly in low-retention digesters. The extreme temperatures and pressures generated during cavitational collapse, aided by the formation of H· and OH· radicals create an environment which would favour degradation of FOG to LCFA and VFA. This would explain the early spike in biogas production, which tapered off to ultimately produce similar biogas yields as seen by Moisan [53].

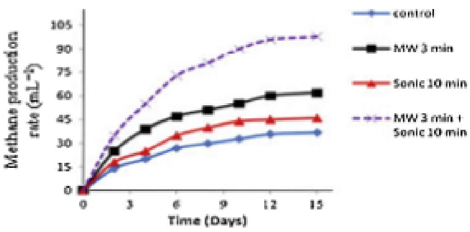

Fig. 8. Rate of methane production after microwave and ultrasonic pre-treatment [75].

Saifuddin and Fazlili [75] investigated ultrasonic pre-treatment of 2% palm oil mill effluent (POME) in distilled water. Samples of 200 mL were sonicated at 20 kHz and 100 W for 10 and 30 min. Sonication for 30 min at 0.2 W/mL affected an 18% increase in SCOD from 11% to 29%, and increased methane production by 19% from 37 mL to 44 mL. These treatments were later combined with microwave treatment for more encouraging results (Fig. 8).

5.1.3. Pre-treatment of feedstocks using microwave and electrokinetic disintegration

Microwave pre-treatment operates by irradiating a sample with an oscillation frequency 0.3–300 GHz. The alternating electric field by microwave irradiation causes rapid alignment and realignment of dipoles in a polar solvent, with continuous repetitions generating friction, and thereby local heat generation [75]. Microwaves are reported as effective for solubilising sludge, household organic waste, energy crops/plant residues, food industry waste and manure, and is generally considered effective in enhancing biogas production from AD [52,76]. Although it is possible that the heat generated from microwave pre-treatment may result in thermal hydrolysis of FOG, microwave pre-treatment of FOG-rich waste is rarely investigated.

Saifuddin and Fazlili [75] investigated the effects of microwave pre-treatment on palm oil mill effluent. While POME contained a high FOG content of 4000 mg/L, POME was diluted to 2% TVS in distilled water. Samples were irradiated using a 2450 MHz, 700 W microwave oven for 3 and 7 min. The 3 min treatment consumed 252 kJ/L of sludge, and produced increases in temperature from 10 °C to 59.4 °C, in sCOD content from 11% to 21% and in BOD5/sCOD ratio from 0.44 to 0.56, and an increase in methane production by 57% from 37 mL to 58 mL. Irradiation for 7 min consumed 588 kJ/L and increased temperature from 10 to 91.8 °C, sCOD from 11% to 45%, and BOD_5/sCOD ratio from 0.44 to 0.93. Both the 3 min microwave, and the 10 min ultrasonic pre-treatment mentioned previously were combined for treatment of a single sample. The total input energy for this treatment was 372 kJ/L of sludge. By combining pre-treatment methods, methane production was increased by 164% from 37 mL to 98 mL (Fig. 8).

The effect of combined microwave-ultrasonic pre-treatment on the anaerobic biodegradability of primary sludge and excess activated sludge was investigated by Yeneneh et al. [77]. Microwave treatment was conducted at 2450 MHz, 800 W for 3 min, followed by ultrasonication with a density of 0.4 W/mL, 90% amplitude, 150 W intensity, pulse of 55/5 for 6 min. Pre-treatment produced an increase in methane yield in both primary sludge and excess activated sludge trials, with increases from 7.9 to 11.9 mL/g tCOD (+50.6%) and 20.7 to 66.5 mL/g/tCOD (+221%) respectively. While this microwave-ultrasonic pre-treatment method has been effective in microbial cell-based substrates, it is difficult to extrapolate these results to other substrates.

Electrokinetic disintegration is mainly used for the treatment of sewage sludge. The process aids AD by breaking apart flocs and aggregates which are formed by negatively charged molecules on microbial extracellular polymeric substances that result in the formation of ionic bonds with cations. These ionic bonds are disrupted through the application of an electrical field, breaking apart the flocs. Furthermore, it is also likely that electrical fields disrupt cells by changing the charge of the cell membranes. Electrical fields have been applied in pre-treatment to several substrates. However, positive outcomes appear to be restricted to sludge and manure wastes [52]. As is a recurring theme among the mechanical pre-treatment methods, the effects of electrokinetic pre-treatments on FOG have not been investigated.

5.2. Thermal hydrolysis

The concept behind thermal pre-treatment is to expose substrates to elevated temperatures for long enough to promote chemical reactions and solubilise larger biomolecules. While temperatures typically range between 150 and 220 °C under pressures of 600–2500 kPa, lower temperature pre-treatments have been investigated [16,78]. Many European researchers are required to adhere to the EC 1069/2009 regulation for the treatment of animal by-products not intended for human consumption. Thermal pre-treatment of WAS has been heavily investigated, while other applications such as manure, abattoir waste, lignocellulosics and even algal biomass have received little attention [16,52,79–81]. There have been few investigations into thermal pre-treatment of FOG-rich wastes. Fortunately, these investigations have yielded some encouraging results. Hiraoka et al. [82], pre-treated substrates high in triglyceride content and measured the decomposition of glyceride fatty acids to produce significant increases in acetic, propionic, butyric and valeric acid following thermal pre-treatment. Subsequent digestion displayed an increase in biogas production of 30%. Similar results were measured by Wilson et al. [83], with pre-treatment at 170 °C vastly enhancing acetic acid content of feed sludge. Equivalent increases in biogas production have also been supported in research by Li and Jin [84]. Table 5 lists the conditions and results of numerous investigations into thermal pre-treatment.

5.3. Acid and alkali and oxidative pre-treatments

Addition of acids and bases to AD feedstocks have been heavily investigated across a range of substrates including sludges, wastewater treatment plant residues, organic waste, plant residues and manures [16,52]. While acid hydrolysis has been investigated using sulphuric and hydrochloric acids, the addition of alkalis are more efficient at enhancing the AD process [99]. Of the alkaline pre-treatments which have been investigated, sodium hydroxide (NaOH) is the most effective for enhancing organics hydrolysis and the AD process [50].

Kim et al. [50] determined that optimal dosing of WAS with sodium hydroxide was 7 g/L NaOH, bringing the solution to pH 12. The duration at which the substrate was held at pH 12 was not mentioned. This pre-treatment increased sCOD content by approximately 478% from 2250 mg/L to around 13,000 mg/L. Digestion resulted in greater sCOD removal from 1136 mg/L in the control to 4941 mg/L, an increase of 335%. Degradation of VS was also improved from 20.5% up to 29.8% in the chemically treated sample. Both biogas production and methane content

Table 5
Thermal pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Pre-treatment conditions	Results	Reference
Thermal	WAS, 70 °C, 1–7d	+19.8–85.9% CH_4 produced	Gavala et al. [78]
	WAS, 121 °C, 30 min	+32% biogas produced	Kim et al. [85]
	WAS, 121 °C, 60 min	+20% biogas produced	Barjenbruch and Kopplow [86]
	WAS, 170 °C, 60 min	+45% CH_4 produced	Valo et al. [87]
	WAS, 170 °C, 60 s	+49% biogas produced	Dohányos et al. [88]
	WAS, 175 °C, 40 min	+65% TSS removal	Graja et al. [89]
	WAS, 130 °C, 30 min	−70–80% VSS/TSS ratio	Bougrier et al. [90]
	WAS, 170 °C, 30 min	+51% CH_4 produced	Bougrier et al. [91]
	WAS, 110 °C, 30 min	+464% FVS/TSS ratio	Bougrier et al. [92]
	WAS, 135 °C, 35 min	+34% sCOD content	
	WAS, 190 °C, 50 min	+46% sCOD content	
	WAS, 116 °C, 38–73 min	+383–429% FVS/TVS ratio	
	WAS, 122 °C, 20–90 min	+306–1410% FVS/TVS ratio	
	WAS, 128 °C, 38–73 min	+814–1441% FVS/TVS ratio	
	WAS, 134 °C, 55 min	+1104% FVS/TVS ratio	
	WAS, 165 °C, 30 min	+47–61% biodegradability	Mottet et al. [93]
	WAS, 170 °C, 30 min	+765% sCOD content	Wang et al. [94]
	Pig manure, 100 °C, 1 h	+31% biogas produced	Rafique et al. [95]
	Slaughterhouse waste, 133 °C, 20 min, >3 bar	Formation of refractory compounds. Unsuccessful in enhancing biodegradability of lipids and nitrogen-rich waste	Cuetos et al. [79]
Steam explosion	Salix, 170–230 °C, 5–15 min	+50% methane produced	Estevez et al. [96]
	Gravity thickened WAS, 134 °C	+4829–7987% sCOD content +2190% total soluble nitrogen +1371% soluble NH_4^+–N content	Gianico et al. [97]
	Dynamic thickened WAS	+2317–3289% sCOD content +3862% total soluble nitrogen +771% soluble NH_4^+–N content	
	220 °C, 30 s	+80% biogas produced +55% TS solubilised	Zheng et al. [98]

increased in response to the treatment, with increases of 13.4% and 12.8% respectively.

Massé et al. [100] investigated the effect of alkaline pre-treatment on the solubilisation and size reduction of pork fat particles in abattoir waste. While sCOD was not impacted by addition of 50–400 meq NaOH/L, the authors measured a 73 ± 7% reduction in particle size at concentrations ranging from 150 to 300 mEq/L. Although the fat particles were then smaller, they were still hydrophobic and would float on the surface of a digester, unavailable for immediate consumption. However, this reduction in particle size and subsequently increased surface area should increase the rate of degradation due to exoenzymes produced by the sludge, or could be utilised to improve the efficiency of subsequent pre-treatment methods, such as enzymatic pre-treatment.

This impact on degradation rate was noted by Battimelli et al. [101]. These researchers investigated the effect of NaOH pre-treatment on biogas production from fatty abattoir waste. While this pre-treatment affected little change in the total biogas produced, it also enhanced slightly the initial reaction kinetics. These findings support the previous assertion that reduction of particle size due to alkaline hydrolysis could be exploited for additional benefit through further pre-treatment.

Oxidative pre-treatments can involve the use of oxygen at temperatures of ~260 °C and pressures of 10 MPa. However, odour, corrosion and high energy consumption restrict practical application of this process [16]. Alternatively, powerful oxidants including ozone, and peroxides peroxymonosulphate (POMS) and dimethyldioxirane (DMDO), with the latter being the most promising options. Table 6 lists the pre-treatment conditions and results of various chemical methods investigated in the literature.

5.4. Thermochemical pre-treatment

Several researchers have combined thermal and chemical pre-treatments to produce more favourable results than either individual pre-treatment (Table 7). Again, WAS is a prime candidate for thermochemical pre-treatment. Kim et al. [50] demonstrated the effects of thermochemical pre-treatment with 7 g NaOH/L. This pre-treatment enhanced COD solubilisation by 85.4% over the control, and over 40% greater than simple chemical pre-treatment and increased VS reduction by 30% (Fig. 9). Furthermore, when Tanaka et al. [103] treated WAS with 0.3 gNaOH/L at 130 °C in an autoclave for 5–200 min, they recorded an increase in VSS solubilisation of 40–50% and an increase in methane production by greater than 200% over the control. Valo et al. [87] treated WAS at 170 °C for 15 min in an autoclave and recorded an increase in TS reduction of 59%, with 92% higher gas production. While pre-treatment of WAS has been heavily investigated, there is little literature regarding FOG pre-treatment. One exception to this is an investigation conducted by Li et al. [55] in which co-digested FOG and kitchen waste were pre-treated thermochemically. Pre-treatment enhanced biogas production by 9.9 ± 1.5% over the control.

5.5. Biological pre-treatment of AD feedstocks

Biological pre-treatment includes methods that utilise pre-digestion, enzymes and bio-surfactants to enhance digestion (Table 8). Pre-digestion, involves two-stage digestion – a digestion stage prior to the main digestion process. By subjecting the waste to different digestion parameters prior to the main AD process, researchers aim to improve the digestibility of the waste. Peng et al. [126] investigated the use of an oil-degrading *Bacillus* species. Prior to AD, oily wastewater was subject to a 24 h digestion with *Bacillus*. During this time, exoenzymes were released by the bacteria to cleave triglycerides, diglycerides and LCFA, and increase the concentration of VFA present. This results in greater contact between microbes and the VFA substrates, significantly enhancing mass transfer of soluble nutrients into the sludge. This pre-digestion process resulted in an increase in methane yield by 16%, and an increase in the methane content of the biogas produced by 8% from 52 to 60%. However, unlike the other forms of pre-treatment, pre-digestion is rarely reported in the literature.

5.5.1. Enzymatic pre-treatment of AD feedstocks

Using enzymes to enhance hydrolysis of macromolecules, and thereby enhance the AD process has been under investigation for many years. While enzymatic pre-treatment of FOG has received the greater deal of research into FOG pre-treatment, the majority of enzymatic pre-treatment research has been focused on cellular feedstocks [127–130]. Cammarota and Freire [131] have performed a review of hydrolytic enzymes in the treatment of wastewater with high oil and grease content and conclude that further investigation is needed to determine the efficacy of these pre-treatments to improve degradation of the relatively

Table 6
Chemical pre-treatments, wastes used, conditions, and results from the literature.

Pre-treatment	Pre-treatment conditions	Results	Reference
Alkali	Cattle dung, NaOH (1%), 7 d	+31–42% digestibility +100% biogas produced	Dar et al. [102]
	WAS, NaOH, 130 °C	+20% biogas produced	Tanaka et al. [103]
	WAS, NaOH, 0.01 N, 4 d	Improved sludge thickening	Saiki et al. [104]
	WAS, NaOH, 20–80 mEq/L, 25 °C, 10 h	+31% sCOD content	Chang et al. [105]
	WAS, NaOH, 45 mEq/L, 25–55 °C, 4 h	+28–38% sCOD content	Heo et al. [106]
	WAS, NaOH 20 mEq/L, 24 h	+83% biogas production	Ray et al. [107]
	WAS, NaOH 7 g/L (175 mEq/L)	+31.7% sCOD content	Kim et al. [50]
	WAS, KOH	+28.5% sCOD content	
	WAS, $Mg(OH)_2$	+2.7% sCOD content	
	WAS, $Ca(OH)_2$	+7.2% sCOD content	
	WAS, CaO	No observed improvement	Carballa et al. [108]
Oxidation, ozone	Primary-secondary sludge, 0.2 g/g COD	+112% CH_4 produced	Weemaes et al. [109]
	WAS, 0.16 g/g SS	+22% SS removed	Battimelli et al. [110]
	WAS, 0.015–0.05 g/g TS	+28% TS removed	Goel et al. [111]
	WAS, 0.06 kg/kg TSS	+16% sCOD content	Pilli et al. [112]
	WAS, 0.1 g/g TS	No improvement in TS removal	Bernal-Martinez et al. [113]
	WAS, 0.07 g Fe^{2+}/g H_2O_2, 50 g H_2O_2, 1 h	+494% COD content	Dewil et al. [114]
	WAS, 60 g POMS/kg DS, 1 h	+406% COD content	
	WAS, 660 mL DMDO/kg DS, 1 h	+589% COD content	

Table 7
Combined pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Pre-treatment conditions	Results	Reference
Thermo-chemical	WAS, 50–90 °C, Lime	+46% VSS content +30% CH_4 produced	Vlyssides andKarlis [115]
	Pig manure, $Ca(OH)_2$ for 1 h, 70 °C, 1 h, HCl for 2 h	+86% biogas production	Rafique et al. [95]
	WAS, 60 °C, 0.6 mg H_2O_2 + 1.5 mg $FeCl_3$/mg S^{2-}, 30 min	+157% sCOD content +167% soluble protein content +250% soluble carbohydrate content +20% total VFA content +20% methane produced +10% COD removal +20% sCOD removal	Dhar et al. [116]
	WAS, NaOH 7 g, 121 °C, 30 min	+77.3% sCOD content +25.6% VS removal +34% CH_4 produced	Kim et al. [50]
	WAS, KOH 65 mEq/dm³, 170 °C, 15 min	+54% biogas produced +80% sCOD +71% COD removed	Valo et al. [87]
Chemical–mechanical	WAS, lime, vacuum (0.02 bar), 30 min	+33% sCOD content	Abbassi [117]

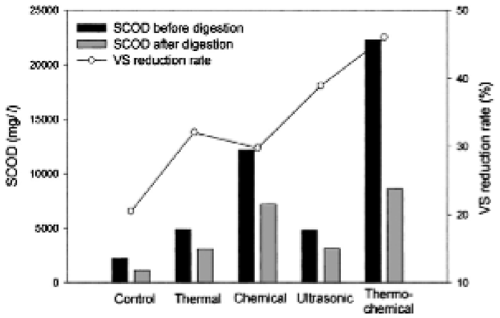

Fig. 9. SCOD removal efficiency and VS reduction rate for pre-treated WAS [50].

recalcitrant and problematic FOG component of dairy and slaughterhouse wastewater.

Hydrolysis of pork and beef fat through enzymatic pre-treatment has been demonstrated by Masse et al. [125]. This investigation involved the pre-treatment of abattoir waste with pancreatic lipase 250 (PL-250) at 25 °C for 5.5 h. Pre-treatment alone resulted in the hydrolysis of 35% of fat, while subsequent digestion achieved 80% reduction in neutral fat and LCFA concentration 5% faster than the controls. Methane content of biogas was unaffected by PL-250 pre-treatment. Furthermore, Massé et al. [100] have stated that PL-250 is more effective in the treatment of beef fat particles than treating pork fat particles.

Mobarak-Qamsari et al. [132] investigated the effect of enzyme extract preparation from *Pseudomonas aeruginosa* on synthetic dairy wastewater with 1000 mg/L total fat content. A treatment of 10% v/v with a lipase activity of 0.3 U/mL was effective in enhancing removal efficiency of COD by 24%, and biogas production after 13 days of digestion by 102%. The researchers noted that these results indicate potential to accelerate the digestion of FOG in the AD process. Mendes et al. [124] also investigated enzymatic pre-treatment of lipid-rich dairy wastewater. The lipase used was a crude preparation of porcine pancreas lipase with activity of 1770 U/mg solid. Treatment with enzyme at 0.5% w/v affected increases in lipid hydrolysis, free fatty acid content, glycerol content, protein hydrolysis, COD removal and biogas production by 39 ± 6.8%, 1240%, 65%, 35.45 ± 5.45%, and 227 ± 65% respectively.

5.5.2. Biochemical emulsification of AD feedstocks

Bio-surfactants are typically used to pre-treat wastes high in FOG. A study by Nakhla et al. [23] evaluated BOD-balance in the treatment of FOG-rich rendering wastewater prior to AD. With a dose of 500 mg/L, BOD-balance affected reductions in tCOD and sCOD of 63.42% and 73.21% respectively, an improvement of 29.71% and 36.07% respectively over the controls. When trialled at full-scale, the addition of BOD-balance at 130–200 mg/L affected a dramatic increase in biogas production and a drop in pH (amended with sodium bicarbonate). The concentration of FOG and COD decreased by 84.6% and 40.9% respectively, and COD removal efficiency was noted to have increased from 20% to 64%. Furthermore, the authors also note that the concentrations of bio-surfactant used in this study are very high due to very high FOG content, as well as past accumulation of FOG in the digester. Accordingly, long-term dosage may be lower than employed in this study. While biogas production was not reported, methane content was measured to be 73%.

6. Relative performance of pre-treatment options

A number of factors need to be considered when selecting a pre-treatment technology including the relative performance, advantages/disadvantages of each technology and the costs. Although pre-treatment has the potential to improve anaerobic digester performance in the Australian red meat processing industry, there is significant variation in biogas production reported in the literature for each technology [133]. Major sources of variation can be categorised as reporting, digester, pre-treatment, and feedstock variations.

A general assessment of the advantages and disadvantages of different pre-treatment methods with respect to a specific substrate are presented in Table 9. It is important to note that the advantages and disadvantages listed in Table 9 are relative to the substrate being treated. Without standardised reporting, the current state of the literature does not allow for any reasonable degree of comparison of pre-treatment methods across substrates.

Assuming standardised reporting of methane production, it remains difficult to produce a blanket energy assessment for pre-treatments. Every industry brings with it a unique and challenging feedstock – Some of these include plant residues including but not limited to lignocellulosics and pulps, WAS, municipal WWTP, manures from livestock and poultry, FOG from kitchen waste, grease trap waste and oily products, meat processing effluent, vegetable waste, slurries, offal, biosolids, cheese whey and

Table 8
Biological pre-treatments, wastes treated, conditions, and results from the literature.

Pre-treatment	Pre-treatment conditions	Results	Reference
Pre-digestion	Cattle slurry, 30–35 °C, 1–2 d	+17–19% biogas produced +7–11% CH_4 content	Singh et al. [118]
Pre-hydrolysis	Primary sludge, 70 °C	+12% SS removal	Lu et al. [119]
Aerobic digestion	WAS, bacterium type SPT2-1 WAS, *Geobacillus sp.* strain AT1	+50% biogas produced +210% biogas produced	Hasegawa et al. [120] Miah et al. [121]
Enzymatic	WAS, 42 °C, 2 d WAS, HRT 2 d Lipid-rich dairy wastewater, porcine pancreas lipase 0.5% w/v and 10 mM Ca^{2+}, pH 8 (1 M NaOH), 37 °C, 4, 8, 12, 24 h	+10% biogas produced +60% CH_4 produced +1240% free fatty acid content +39.5 ± 6.8% lipids hydrolysed +65% glycerol content +32.7% proteins hydrolysed +162–292% biogas produced +30–40.9% COD removed	Mayhew et al. [122] Davidsson et al. [123] Mendes et al. [124]
	Slaughterhouse waste, pancreatic lipase 250 (PL-250), 25 °C, 5.5 h	35% of fat hydrolysed during pre-treatment −5% digestion time More effective on beef fat	Masse et al. [125]
Bio-surfactant	Raw and high FOG rendering wastewater, BOD-Balance™, 100, 250 and 500 mg/L	Raw: +59–96% pCOD removal +74–100% sCOD removal High FOG: +164–238% COD removal rate coefficient +164–247% pCOD removal rate coefficient	Nakhla et al. [23]

Table 9
Advantages and disadvantages of pre-treating WAS with different technologies (originally adapted from Taherzadeh and Karimi [134]; Hendriks and Zeeman [135]; further modified from Montgomery and Bochmann [51]).

Process	Advantages	Disadvantages
Mechanical		
Milling	• Increases surface area • Makes substrate easier to handle • Often improves fluidity in digester	• Increased energy demand • High maintenance costs/sensitive to stones etc.
High-pressure homogenisation	• Increases surface area • Organic solvent free method • Well established technology on large scale	• High heat and energy demand • Complex equipment required
Ultrasonication[a]	• Increases surface area • Increased methane production • No chemical addition • Low maintenance cost	• Increased energy demand • Probes require replacement every 1.5–2 years
Thermal		
Hot water (TDH)	• Increases the enzyme accessibility	• High heat demand • Only effective up to certain temperature
Steam explosion	• Breaks down lignin and solubilises hemicellulose	• High heat and electricity demand • Only effective up to certain temperature
Extrusion	• Increases surface area	• Increased energy demand • High maintenance cost/sensitive to stones etc.
Chemical		
Acid	• Enhances organics hydrolysis	• High cost of acid • Corrosion problems • Formation of inhibitors, particularly with heat
Alkali	• Enhances organics hydrolysis • Reduces fat particles	• High alkali concentration in digester • High cost of chemical
Ozonation	• Destruction of pathogens • Flexible operation	
Biological		
Microbial	• Low energy consumption	• Slow • No lignin breakdown
Enzymatic	• Low energy consumption	• Continuous addition required • High cost of enzyme
Bio-surfactant[b]	• Dissolution of lipids • Less toxic than anionic surfactants	• High cost of bio-surfactants • Low commercial production

[a] Appels et al. [16].
[b] Focus is on lipids; Saharan et al. [136].

algal wastes [35,137,89,106,69,84,138,100,139,80,134,40]. Each of these substrates varies in composition [140]. Within each industry, wastes are still subject to significant variation between individual processors [10]. In the RMP industry, variation will include the degree of primary treatment, including the number, size and efficiency of screens, DAFs contra sheers, screw presses, sterilisation and rendering [33]. Other factors that will impact waste include the degree of product recovery; size of a slaughterhouse; water: waste ratio (i.e. dilution – not to be confused with moisture content); species processed; and operating climate. Each waste source presents a novel characteristic profile – carbohydrate: protein: lipid ratios, VS, TS, alkalinity and VFA content to name a few [141]. The impact of individual pre-treatment methods across a range of feedstocks will vary due to the nature of the feedstock [50]. Unless the goal is to compare the effect of a static pre-treatment method across feedstocks, it is unsuitable to compare the impact of multiple pre-treatment methods unless the substrate is controlled. Furthermore, pre-treatment methods between researchers can vary significantly. Consequently, this becomes a determination of what parameters are most effective within a pre-treatment type on a specific feedstock.

Prior to digestion, pre-treatment may be applied at the discretion of the operator. Pre-treatments, as discussed, include thermal, chemical, thermochemical, mechanical and biological methods which are more or less suitable given the application (Fig. 5). Not only may a pre-treatment be unnecessary, one risk of pre-treatment is that by increasing the amount of available compounds, a digester may experience inhibition [133]. This is a real potential, for example, in high-protein wastes with ammonia formation, and FOG-rich wastes which break down to potentially inhibitory concentrations of LCFA and VFA [30,142]. Furthermore, the degree of impact of a pre-treatment depends on the waste that the pre-treatment method is applied to [60]. As a result of pre-treatments being targeted to a specific waste source, it is difficult in the case of a review to draw appropriate material together for a reasonable comparison.

Following pre-treatment, digestion methods also vary significantly. Digesters are divided into either low-rate or high-rate systems. Low-rate anaerobic systems include batch digestions, plug-flow reactors and lagoons and typically require a high hydraulic (5–120 days) and solids retention time. Alternatively, high-rate anaerobic systems include up-flow anaerobic sludge bed (UASB), continuous stirred tank reactors (CSTR), expanded granular sludge bed (EGSB) and AnMBR systems among others [16,35,143]. These systems are heated to either the mesophilic or thermophilic optimum temperatures of ~37 °C and ~55 °C respectively, and receive active stirring or mixing. These high-rate systems typically involve a de-coupling of the solids and hydraulic retention time and as such, can treat equivalent volumes of wastewater with a hydraulic retention time (HRT) ranging from hours to days [35]. Several things need to be taken into consideration when comparing energy yield here. An important factor to consider is that some pre-treatments actively improve reaction kinetics without impacting total biogas production [140]. Energy production must then be compared as a function of time, not simply total methane produced.

7. Merit of pre-treatment methods in abattoir wastewater in Australia

Australian abattoirs stand to benefit substantially if an appropriate pre-treatment method can be developed to improve the bioavailability and subsequent conversion of FOG to methane. While no anaerobic digestion system currently deals with FOG effectively, typically the more sophisticated the anaerobic digestion technology, the less capable they are of handling FOG loads.

With the increasing popularity of overseas technologies being introduced to Australian RMP plants (e.g. [37]) it is important to note that the quality and biodegradability of the effluent is key to maximise performance of these AD technologies. This is particularly important in light of the high strength nature of the waste water and volumes produced in this industry. This is quite significant when the scale of capital investment is considered which can be regarded as one of the largest inhibitors of uptake of foreign AD technologies. The use of cost-effective pre-treatments to improve the biodegradability of the waste water will enable additional energy recovery with a concomitant reduction in GHG emissions. The actual energy balance and costs is dependent on a number of factors highlighted in the previous section. Further research is needed to fully understand the economics of AD systems to meat processors. The value of biogas, recovered non-renewables, treated water, and GHG mitigation to a meat processor must be understood in order to put forward a strong financial case for an AD system. Only once this is known, can an AD system and subsequent pre-treatment of wastes for AD be valued.

Researched and speculated actions of the pre-treatment of effluents rich fats and oils from several origins presented in this review show new and promising applications for the enhancement of the AD process. Of all the pre-treatments discussed, ultrasonic, thermochemical and biochemical has shown greatest potential in the degradation of high fat waste water in addition to a few studies describing the degradation of fats and oils by alkaline/acid/enzymatic hydrolysis. The greatest increase in biogas production covered in this review was 227 ± 65% using enzymatic pre-treatment of lipid-rich dairy waste; however, it should be noted that several articles investigating pre-treatment methods which do not concern themselves with AD and biogas production have been reviewed. Regardless, there is evidence from these investigations that these pre-treatment methods affect considerable substrate degradation, and are subsequently worth investigation as pre-treatment methods for AD substrates. Although carbohydrates and protein are relatively easily digested, the challenge is to develop a pre-treatment method which greatly improved FOG digestion to produce methane, and developing a digestion protocol to optimally include FOG to improve biogas production while limiting the inhibitory impacts associated with FOG-rich substrates.

Treatment efficiency and nutrient recovery of waste streams can also be optimised through treatment of separate fractions of the waste stream [144]. Aptly, Jensen et al. [8] suggest that this concept be investigated in cattle abattoirs, with treatment of individual waste streams. While this may indeed result in a greater degree of organic removal and nutrient recovery, this could be a relatively expensive operation compared with digestion of a combined waste. However, this could also provide excellent conditions by which FOG could be separated from the primary waste streams, perhaps by dissolved air floatation, pre-treated and suitably introduced to an AD system.

8. Conclusions

The Australian RMP industry is under pressure to reduce GHG emissions and optimise energy consumption. Wastewater produced from fully integrated abattoirs in Australia is high-strength and FOG-laden and contributes significantly to abattoir GHG emissions. Although pre-treatment of wastes such as lignocellulosics and WAS are commonplace, investigation of pre-treatment of FOG for AD is relatively rare. Given the significantly higher theoretical methane content of FOG over carbohydrates and proteins, it is surprising that FOG are only now being considered for pre-treatment.

Despite the fact that FOG has the potential to significantly enhance biogas yield from AD systems, FOG can also produce several problems. Pre-treatment may be critical in reducing problems caused by FOG, including pipeline blockages, adhesion to sludge, and inhibition of mass transfer of nutrients, problems which ultimately lead to anaerobic lagoon failure. However, there is potential that pre-treatment may worsen problems, in particular inhibition of mass-transfer due to LCFA adhesion to sludge. This may be overcome by diluting pre-treated fatty substrates with co-substrates. While it remains to be seen whether pre-treatment of FOG is economically viable, investigation must first be conducted to identify suitable pre-treatment methods for an optimised process. Once a process is optimised, FOG digestion will help to ease the impact of rising electricity and water prices in industry, as well as reduce GHG emissions.

This review highlights several knowledge gaps in the literature. There is a distinct lack of standardisation when reporting on AD investigations. This makes meaningful comparison across the literature a difficult task. Also prominent is the lack of investigations that focus on FOG-rich wastes, regardless of the potentially enormous benefit from enhanced methane production. Once standardised reporting has been established across the literature, it will be possible to produce a reliable cost/benefit analysis to better advise industry on the best course of action to provide optimal digestion of their waste, and subsequently, optimal methane production. While there are some investigations into pre-treatment of FOG-rich wastes, further research is needed to understand the mechanisms by which pre-treatments impact the FOG component of wastes – investigations which would benefit greatly from standardised reporting. There is little-to-no literature which advises industry on how to handle crust material once it has accumulated. While AnMBR reactors represent a possible solution to digest FOG-rich wastes and avoid the complications associated with crust formation, more research is needed to understand the fate of FOG in these reactors. These knowledge gaps need to be addressed in order to improve performance and further the development of AD technology through industrial uptake.

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Appendix B

Evaluation of chemical, thermobaric and thermochemical pre-treatment on anaerobic digestion of high-fat cattle slaughterhouse waste

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Evaluation of chemical, thermobaric and thermochemical pre-treatment on anaerobic digestion of high-fat cattle slaughterhouse waste

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ABSTRACT

This work aimed to enhance the anaerobic digestion of fat-rich dissolved air flotation (DAF) sludge through chemical, thermobaric, and thermochemical pre-treatment methods. Soluble chemical oxygen demand was enhanced from 16.3% in the control to 20.84% (thermobaric), 40.82% (chemical), and 50.7% (thermochemical). Pre-treatment altered volatile fatty acid concentration by −64% (thermobaric), 127% (chemical) and 228% (thermochemical). Early inhibition was reduced by 20% in the thermochemical group, and 100% in the thermobaric group. Specific methane production was enhanced by 3.28% (chemical), 8.32% (thermobaric), and 8.49% (thermochemical) as a result of pre-treatment. Under batch digestion, thermobaric pre-treatment demonstrated the greatest improvement in methane yield with respect to degree of pre-treatment applied. Thermobaric pre-treatment was also the most viable for implementation at slaughterhouses, with potential for heat-exchange to reduce pre-treatment cost. Further investigation into long-term impact of pre-treatments in semi-continuous digestion experiments will provide additional evaluation of appropriate pre-treatment options for high-fat slaughterhouse wastewater.

1. Introduction

The Australian red meat processing industry consist of more than 150 slaughterhouses which for the financial year of 2013–4 produced 20.8 gigalitres of untreated wastewater (AMPC, 2015; Australian Bureau of Statistics, 2016). This wastewater contained high concentrations of pollutants, with average concentrations of 2657 and 1780 mg L^{-1} for biochemical oxygen demand (BOD) and fat, oil and grease (FOG) respectively (AMPC, 2015). As a result, 37 kilotonnes of FOG entered waste streams. Subsequently, this waste requires several treatment interventions prior to discharge to sewage (Bustillo-Lecompte & Mehrvar, 2015; McCabe et al., 2013).

A comprehensive list of primary, secondary and tertiary treatment technologies used by Australian red meat processors is published by Meat and Livestock Australia (MLA, 2002). Primary treatment options listed include static and rotary screens, screw presses, dissolved air flotation (DAF), and collection pits. These options can result in significant reductions in wastewater pollutant concentrations.

Secondary treatment involves biological treatment as either aerobic or anaerobic digestion, or a combination of both. Anaerobic digestion (AD) is a four-stage process which involves the action of microbes to digest organic waste to produce biogas – a combination of typically 20–50% carbon dioxide, and 50–80% methane gas. The capture of methane from Australian slaughterhouse wastewater via anaerobic digestion has gained momentum over the past two decades (IEA, 2015).

In order for AD systems to perform optimally, it is essential to focus on process stability. Control over influent stream is necessary to reduce the frequency and magnitude of shock loadings, and regulate FOG loading. In Australian slaughterhouses, recovery of FOG for sale as tallow is key for value adding. Following recovery of FOG as tallow, the remaining fat often collects in the anaerobic digesters. There are a number of potential drawbacks to FOG addition to AD feedstocks, including digester foaming, pipe blockages, clogging of gas collection and handling systems, crust formation, sludge flotation and washout, and digester inhibition (Long et al., 2012).

While these drawbacks have been acknowledged, FOG remains a potentially desirable substrate due to a relatively high theoretical methane potential of 1014 L per kg of volatile solids (VS) when compared with protein and carbohydrate at 480 L kg VS^{-1} and 370 L kg VS^{-1} respectively (Buswell & Neave, 1930; Verein Deutscher Ingenieure, 2006; Wan et al., 2011). Consequently, research has been focused on mechanisms to enhance FOG bioavailability, while avoiding digester inhibition (Chen et al., 2008). Many research projects have utilised co-digestion to varying degrees of success, producing encouraging results. For example, Gelegenis et al. (2007) combined poultry manure with olive-oil mill wastewater at 3:1 v/v and yielded 21% more methane

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than the poultry manure control, while Davidsson et al. (2008) observed a 9–27% increase in methane yield when adding 10–30% grease trap sludge on a VS basis respectively. While co-digestion in Australia has received little investigation, the country is actively pursuing research in this area (Astals et al., 2014).

Alternatively, various pre-treatment methods have been identified and used to good effect in the degradation of waste activated sludge (WAS) prior to anaerobic digestion (Appels et al., 2008). Pre-treatments are aimed at augmenting hydrolysis, the rate limiting step, by improving the surface area to volume ratio of the substrates (Carrere et al., 2016). Methods such as thermobaric, chemical and bio-surfactant all achieve this increase in surface area to volume ratio of organics to varying degrees (Kim et al., 2003; Li & Noike, 1992; Mouneimne et al., 2003). Hydrolysis could also be enhanced by improving conditions for chemical reactions to occur, or introducing reagents that allow hydrolysis to occur more rapidly. For instance, thermobaric treatment will increase the rate at which hydrolysis occurs, while sodium hydroxide produces a saponification reaction to cleave long-chain fatty acids (LCFA) from glycerol (Mouneimne et al., 2003).

While much of the research on pre-treatments of high FOG substrates has centred on WAS, no studies have been performed on the effect they have on slaughterhouse wastewater to date (Harris & McCabe, 2015). This paper presents the results of three pre-treatment methods, namely chemical, thermobaric, and thermochemical, conducted on slaughterhouse DAF sludge as a first step in evaluating its effectiveness in enhancing anaerobic digestion.

2. Materials and methods

2.1. Inoculum and substrate

The inoculum was anaerobic sludge sourced from a covered anaerobic lagoon at a local slaughterhouse. Sludge was stored in an incubator at 37 ± 1 °C for 5 days prior to use. The DAF sludge used as substrate is the concentrated FOG residues collected by the dissolved air filtration unit. This material was used for its high FOG content and is representative of the fatty material entering the anaerobic digestion system of red meat processors. Substrate was collected as a 2 L grab sample from the DAF of a local red meat processing plant. Samples were immediately returned to the laboratory and stored at 4 °C. DAF sludge was stirred to achieve homogeneity before portioning waste into bottles for pre-treatment, or into reactors for digestion. The following DAF sludge characteristics were measured: pH, total solids (TS), VS, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), FOG, volatile fatty acids (VFA), measured as acetic acid equivalence per litre (HAcEq L^{-1}) (Table 1).

2.2. Pre-treatments for DAF sludge

Pre-treatment options were selected based on reported research which use WAS as a substrate (Li et al., 2015). For this current study, methods were selected that were expected to produce a positive impact on the degradation of FOG component of the wastewater (Harris & McCabe, 2015).

Chemical pre-treatment using 7 g NaOH/L was adapted from Kim et al. (2003) and allowed to react with the substrate for 24 h prior to digestion. Thermobaric pre-treatment was conducted using an autoclave at 121 °C for 30 min, and allowed to cool for 24 h before use (Kim et al., 2003). Thermochemical treatment was a combination of chemical and thermochemical treatment, with NaOH addition prior to autoclaving.

2.3. Biochemical methane potential testing

Tests were conducted using the Automated Methane Potential Test System II (AMPTS II; Bioprocess Control, Lund, Sweden). Inoculum and substrate were added at a ratio of 3:1 respectively on the basis of VS to avoid overloading the inoculum. Substrate was portioned based on weight, and rinsed into reactors with distilled water. Final reactor volume was approximately 400 mL of liquid with the remaining volume as head space in a 500 mL Schott bottle. Reactors were maintained at a constant temperature of 37 ± 1.5 °C in a water bath. Seven sets of triplicate were tested, including a sludge blank, cellulose control, raw wastewater control and five treatment groups. Carbon dioxide was removed from the biogas using 3 M sodium hydroxide scrubbers, and resulting methane was measured by the AMPTS II gas measurement unit and corrected for standard temperature and pressure (0 °C and 1 atm.). Digestions were considered finished on the day that daily biogas production was less than 1% of the total yield (Verein Deutscher Ingenieure, 2006).

2.4. Cost and energy calculations

The results of the lab-scale investigations detailed in this paper were used in a preliminary evaluation of the treatment options for suitability in a red meat processing context. For the assessment of cost, this section will not consider initial capital investment required to facilitate on-going pre-treatment. Alternatively, this section will focus on the approximate on-going cost of pre-treatment and the anticipated benefits, as well as assess the appropriateness of the pre-treatment operation on-site at a red meat processing facility. For the calculation of financial values, the waste parameters measured in this investigation will be utilised, a combined heat and power (CHP) unit with electrical conversion efficiency of 40% will be assumed, and an electricity cost of $0.15 AUD kWh^{-1} and a natural gas cost of $8.15 AUD GJ^{-1} will be used (AEMO, 2017). This does not take into account the use of heat from the CHP unit. Furthermore, this work does not consider the flow-on benefits to the AD system that may be established as a result of pre-treatment, as further work using semi-continuous digesters is needed to identify such benefits.

2.5. Analytical methods

Various parameters were investigated on anaerobic sludge and DAF sludge prior to digestion. VS and TS were analysed using a modification to standard method 2540G with a 20 h residence time at 105 °C (Standard methods for the examination of water & wastewater, 2005). Volatile fatty acids were analysed using photometric measurement of Merck volatile organic acid test kits (Cat. No. 101809) and FOG content was measured using a Wilks Infracal 2 analyser. Total COD was measured using Merck test kits following a dilution series. Soluble chemical oxygen demand was determined using centrifugation at 13,000*g* for 10 min and subsequent photometric measurement of the supernatant with Merck test kits both before and after biochemical methane potential (BMP) investigation.

Table 1
Characteristics of inoculum and untreated DAF sludge used in this workND – not determined.

Sample	pH	TS (%)	VS (%)	VS/TS (%)	TCOD (g L^{-1})	SCOD (g L^{-1})	FOG (mg L^{-1})	VFA (mg L^{-1})
Inoculum	6.82	2.6 ± 0.00	1.99 ± 0.00	76.41 ± 0.02	ND	ND	ND	ND
Substrate	4.16	14.56 ± 0.61	14.21 ± 0.59	97.57 ± 0.01	205	33.5	90000	6400

2.6. Statistical analyses

One factor analysis of variance (ANOVA) was used to detect a difference between trials. Due to small sample size, the non-parametric equivalent, the Kruskal-Wallis test was employed in an attempt to improve the resolution of the statistical investigation. In the event that both the ANOVA and Kruskal-Wallis tests were significant with $P < 0.05$, T-tests were used to further investigate between groups with the non-parametric Mann-Whitney test used to help account for low sample sizes. The T-test outcome has been reported where statistical significant was identified. Standard deviations are provided for values with n greater than 1.

3. Results and discussion

3.1. Qualitative effects of pre-treatment on DAF sludge

Pre-treatment produced varying effects on substrate consistency, from creating a more gelatinous product in the thermochemical and chemical treatments, to a more liquid and particulate substrate in the thermobaric treatment. In particular, this had implications for the uniform portioning of thermobaric substrate into digesters.

3.2. Effect of pre-treatment on COD solubilisation

Thermobaric treatment slightly enhanced COD solubility from 16.3% to 20.84%. Kim et al. (2003) reported similar results with SCOD increased from 8.1% to 17.6% following thermobaric treatment. Thermochemical pre-treatment produced the greatest change, increasing the soluble fraction of COD from 16.3% to 50.7% (Table 2). This was indicative of the hydrolysis and subsequent solubilisation of organic residues in the DAF sludge.

Solubilisation of COD was also enhanced following chemical pre-treatment with 7 $g\,L^{-1}$ NaOH. Chemical pre-treatment increased SCOD content from 16.3% to 48.2%. Similar results were reported by Kim et al. (2003) in which WAS treated with sodium hydroxide exhibited an increase in SCOD from 8.1% to 39.8%. Karlsson (1990) further supports this outcome with an increase in% SCOD from approximately 13% to 38% when treated with NaOH at pH 11 at 90 °C. In contrast, when similar treatment was attempted by Massé et al. (2001) using sodium hydroxide at concentrations of 2–16 $g\,L^{-1}$ on pork slaughterhouse waste, the authors reported no increase in SCOD after a reaction time of four hours. However, average size of fat particles was reduced to 73 ± 7% of the initial average. It is likely that, as the reactions were performed at room temperature, the surface area for reaction was poor. A modest increase in reaction temperature to melt apart the fat globules may have yielded a greater impact on SCOD. Massé et al. (2001) also discuss the results of Karlsson (1990), in which it was reported that NaOH was far more effective at hydrolysing proteins than lipids, and cite this as the mechanism by which SCOD is increased in similar pre-treatment investigations. Results by Kim et al. (2003) support the findings that NaOH is effective at solubilising protein, but do not elaborate on its effect specifically on lipids.

Table 2
Comparative effects of pre-treatments on SCOD, VFA and specific methane potential.

Treatment	SCOD ($mg.L^{-1}$)	[VFA] ($mg.L^{-1}$)	SMP(L_N CH_4.kg VS $^{-1}$)
Control	35,000	6400	759 ± 4
Thermobaric	35,000	2300	823 ± 97
Chemical	147,500	14510	783 ± 6
Thermochemical	156,000	20976	822 ± 11

3.3. Volatile fatty acids production from pre-treatment

Thermochemical treatment increased VFA content by +228%, chemical treatment increased VFA content by +127%, while a loss of VFA content by -64% was measured in the thermobaric treatment (Table 2).

In contrast to Wilson et al. (2009), a large decrease in VFA content was measured after thermobaric treatment of the substrate. This can possibly be explained by loss of volatiles during the autoclaving process, as the vessel seal may have become compromised under the intensity of the autoclaving process. However, under the conditions of this experiment, similar losses should have been observed in the thermochemical treatment.

The increase in VFA content produced by the chemical and thermochemical groups was attributed to the addition of sodium hydroxide. Similar results were obtained by Mouneimne et al. (2003) in which solid fatty residues from a wastewater treatment plant were degraded using sodium hydroxide and potassium hydroxide to yield VFA. This result demonstrated that treating with sodium hydroxide effectively enhanced hydrolysis of macromolecules to form organic acids. VFA liberation was greater in the thermochemical treatment possibly due to elevated temperature increasing the rate of both saponification and steam hydrolysis. This suggests that the majority of VFA production from sodium hydroxide addition occurred post-autoclaving.

3.4. Biochemical methane potential of treated DAF sludge

Anaerobic sludge was assessed for activity using a cellulose control, which achieved 80% of its theoretical specific methane production (SMP) by day 6. Thermobaric-treated DAF sludge produced an 8.49 ± 12.75% greater than the control ($P = 0.0821$); the chemical treatment group enhanced the SMP of DAF sludge by 3.28 ± 0.81% ($P < 0.05$); and the thermochemical treatment group 8.32 ± 1.40% ($P < 0.05$; Table 2).

The increase in SCOD and VFA concentration observed in the chemical (+31.9% SCOD, +127% VFA, +3.28% SMP) and thermochemical (+34.4% SCOD, +228% VFA, +8.32% SMP) treatments provided the basis that improvement in digestion parameters such as methane production or reaction kinetics should occur (Kim et al. 2003). Results obtained from the thermobaric treatment (+4.54% SCOD, -64% VFA, +8.49% SMP) appear to contradict this concept. Subsequently, positive relationships between SCOD or initial VFA concentration, and methane production or reaction kinetics were not demonstrated under the conditions of this experiment.

3.4.1. Thermobaric pre-treatment of DAF sludge

Although displaying the greatest variability, thermobaric treatment performed best in BMP testing (Fig. 1). While it is possible that thermobaric treatment of lipid-rich wastes to form inhibitory concentrations of LCFA could occur, such inhibition was not observed in this experiment. The lag period of 5 days in the control group was not observed, indicating that the thermobaric treatment produced effective hydrolysis that did not result in inhibition (Fig. 1). These results are supported by Wilson et al. (2009) who reported similar results in which thermobaric treatment of lipids did not produce LCFA at previously reported inhibitory levels.

Time required to degrade the pre-treated substrate was similar to the control. However, the thermobaric treatment had produced equivalent gas volume as the controls by approximately day 9, effectively improving digestion time by 3 days (Fig. 1). Furthermore, Gianico et al. (2013) suggested that the increased organic solubilisation resulting from thermobaric pre-treatment had likely converted a fraction of recalcitrant material to a more degradable form. This explanation supports the greater production of methane in the thermobaric and thermochemical groups.

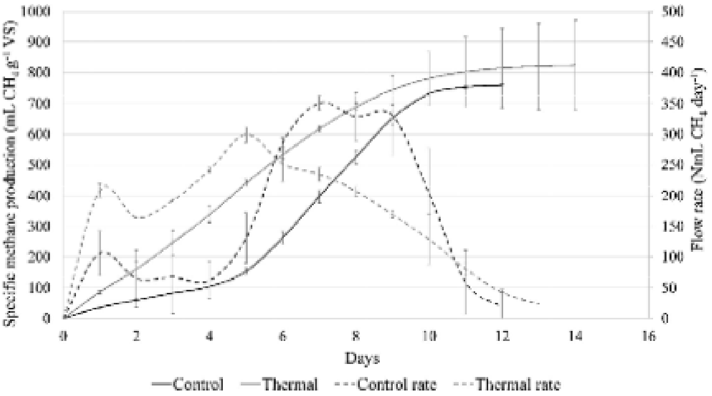

Fig. 1. Specific methane production and flow rates for controls and thermobaric treated DAF sludge measured during biochemical methane potential test. (Avg ± SD; Control $n = 3$, thermobaric $n = 6$).

3.4.2. *Chemical pre-treatment of DAF sludge*

Chemical treatment was expected to saponify the lipid component of the DAF sludge, and subsequently induce LCFA inhibition (Wilson et al., 2009). However, treatment did not extend the lag period exhibited in the control group, and even appeared to reduce any inhibitory impact (Fig. 2). While treated reactors completed digestion after 12.67 ± 0.58 days, methane production equivalent to the control was achieved by day 10, effectively reducing digestion time by 2 days.

3.4.3. *Thermochemical pre-treatment of DAF sludge*

Thermochemical-treated DAF sludge performed similar to thermobaric and chemical treatments. While the SMP was comparable to thermobaric-treated DAF sludge, the digesters still experienced a 4 day lag period (Fig. 3). The profile of rate of gas production retains the inhibitory phase in the first 4 days of digestion, after which the profile appears to follow more comparable to the thermobaric treatment (Fig. 3). Methane production in the treatment group was equivalent to the control group end point by day 11, producing an effective improvement in digestion time by 1 day.

3.5. *Implications for use of pre-treatments in slaughterhouse industrial applications*

3.5.1. *Chemical pre-treatment*

While pre-treatment with 7 g NaOH L^{-1} demonstrated a high degree of COD solubilisation, the economic outcome of increasing methane production was minimal at 3.28%. Sodium hydroxide pellets could be purchased for approximately $467 AUD per 1000 kg. Assuming infrastructure were in place to remove residual FOG from waste streams to be made available for pre-treatment, this would allow for the treatment of 143 m^3 of FOG-rich waste. With an improvement of 3.28%, this would be worth $185 AUD as electricity, or could offset natural gas worth $229.60 AUD. This is insufficient to cover the cost of sodium hydroxide pre-treatment and is likely not a viable option. Furthermore, following pre-treatment, this material would likely require neutralisation with acid prior to dosing to the anaerobic digester. However, this does not take into account the flow-on effects of greater treatment efficiency, and the effects on anaerobic digester operation.

3.5.2. *Thermobaric pre-treatment*

Given the 8.32% increase observed from thermochemical pre-treatment, and a load of 143 m^3 of FOG-rich waste would generate an extra 28172 MJ. Converting to electricity with a 40% conversion efficiency provides 3130 kWh. The value of this as electricity is $470 AUD, and used to offset natural gas would be worth $230 AUD.

The cost of performing this treatment is heavily dependent on the water content. With a specific heat capacity of 4.18 J g^{-1} °C^{-1}, water is energetically expensive to heat, and the economics of the treatment could be improved through dewatering (Table 3). With a TS content of 14.56, and a water content of 85.44%, 117.2 MWh of electricity would be required to heat 143 m^3 of material from 40 to 100 °C. At an estimated cost of $0.15 AUD kWh^{-1}, this would cost $17580. In contrast, if

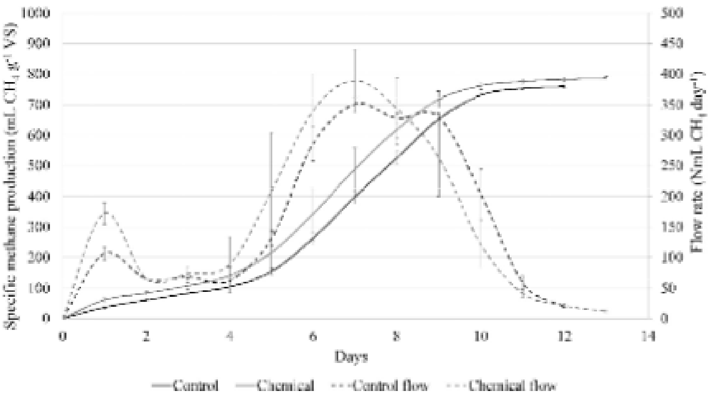

Fig. 2. Specific methane production and flow rates for controls and chemical-treated DAF sludge measured during biochemical methane potential test. (Avg ± SD; $n = 3$).

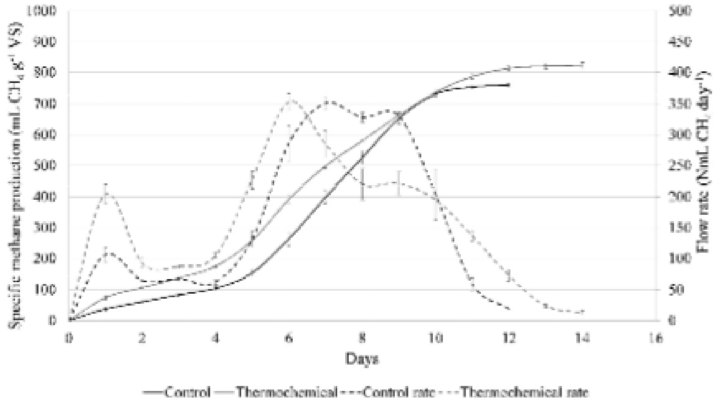

Fig. 3. Specific methane production and flow rates for controls and thermochemical DAF sludge measured during biochemical methane potential test. (Avg ± SD; $n = 3$).

Table 3
Scenarios identifying estimates on the energy required to heat fatty waste from 40 to 100 °C based on different levels of de-watering.

	Water content					
	85.44%	70%	50%	30%	10%	0%
Volume treated (m3)	143	120.42	91.82	63.22	34.62	20.32
Volume of water (m3)	122.18	100.10	71.50	42.90	14.30	0
Volume of fat (m3)	20.32	20.32	20.32	20.32	20.32	20.32
VS (%w/w)	14.21	16.87	22.13	32.14	58.69	100.00
Mass treated (t)	140	118	90	61	32	18
Mass of fat treated (t)	18	18	18	18	18	18
Specific heat capacity (kJ/kg)[a]	3.87	3.81	3.70	3.48	2.90	2.00
Required heating (MJ)[b]	32557	27018	19861	12718	5625	2163
MWh needed	117.2	97.3	71.5	45.8	20.3	7.8

[a] Specific heat capacity of tallow obtained from Cameo Chemical (1999).
[b] Energy required to heat substrate from 40 to 100 degrees Celsius.

90% or 100% of water were removed, the cost to heat would be around $3045 AUD and $1170 AUD respectively.

These calculations highlight that active heating of the material is not a viable option to take advantage of the effects of pre-treatment in this situation. Utilisation of heat-exchange from CHP, or from other plant processes, such as re-using waste heat from the stacks could significantly reduce the need for active heating, and improve viability of thermobaric pre-treatment in an industrial setting.

3.5.3. *Implication summary*

Thermobaric pre-treatment is the most viable option for the pre-treatment of DAF sludge under the conditions of this investigation. Pre-treatment efficacy can be greatly enhanced through reasonable dewatering to limit the amount of heat wasted heating water, and utilisation of heat exchange to reduce active heating costs. Utilisation of CHP technology will further improve the economics of thermobaric pre-treatment.

4. Conclusions

This work identifies that methane yields can be enhanced by 3.28%, and 8.49% by chemical and thermochemical treatments respectively. SCOD and VFA concentrations can also be greatly increased. Early inhibition was reduced by thermochemical (-20%) and thermobaric (-100%) pre-treatments. Preliminary assessment of economic viability identified thermobaric as the most viable pre-treatment technology for industrial application under the conditions of this investigation. Thermobaric pre-treatment efficacy can be greatly enhanced through utilisation of heat exchange, and substrate dewatering. CHP technology could further improve the economics of thermobaric pre-treatment. Semi-continuous investigations are necessary to assess on-going benefits of thermobaric pre-treated DAF sludge.

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Appendix C

Bovine bile as a bio-surfactant pre-treatment option for anaerobic digestion of high-fat cattle slaughterhouse waste

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Bovine bile as a bio-surfactant pre-treatment option for anaerobic digestion of high-fat cattle slaughterhouse waste

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ABSTRACT

Bovine bile was assessed as a novel bio-surfactant pre-treatment to enhance anaerobic digestion of lipid-rich dissolved air flotation (DAF) sludge using biochemical methane potential (BMP) tests. Bile was dosed at arbitrary concentrations from 0.2–6 g/L. At 0.6 g bile/L, methane yield increased by 7.08%. Doses above 2 g bile/L produced negative impacts on SMP, kinetics and digestion profile. At 6 g/L bile produced a 6% decrease in specific methane production and up to 79% additional inhibitory duration, delayed time of peak methane production by up to 74%, and slowed total digestion time by up to 65%. Reaction kinetics declined linearly with respect to bile addition, reaching half the control value at 6 g/L bile concentration. Subsequent anaerobic toxicity assays between 1 and 6 g bile/L revealed that bile has an inhibitory effect under BMP testing at these higher doses. The economic viability of using bile as a bio-surfactant was assessed. In comparison to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile to 220% of its current sale value. The promising results of bile dosed at 0.6 g/L under BMP testing warrant further investigation into long-term impact of bile pre-treatments of high-fat slaughterhouse wastewater in semi-continuous digestion experiments.

1. Background & introduction

The high concentrations of fat, oil and grease (FOG) in red meat processing (RMP) water can be problematic in anaerobic digestion (AD) systems. Lipids affect digesters in many ways, including pipe blockages, crust formation and short-circuiting, sludge flotation and washout, and reversible inhibition of mass-transfer of nutrients induced by long-chain fatty acids (LCFA) [1]. This is particularly relevant when sludge is less active; situations where slaughterhouse waste is used in monodigestion or the AD technology does not incorporate temperature control and stirring. While FOG may be difficult to utilise as a substrate, altering the material with pre-treatment prior to entering an AD system may improve its bio-availability, and reduce either the frequency and or severity of complications [2].

Pre-treatment of a substrate involves the application of a treatment to the substrate prior to digestion in an attempt to improve substrate degradability [3]. The desired effect of this is to improve biogas yields, while improving or maintaining stable digester operation. While there have been many investigations into the pre-treatment of waste activated sludge, lipid pre-treatment has been a largely undeveloped field [4,5]. Pre-treatment options of particular interest include thermobaric, chemical, thermochemical, ultrasound, and biochemical methods. Of these, biochemical methods have been investigated the least, and literature regarding bio-surfactant pre-treatment methods is scarce [2].

Bio-surfactants are naturally-derived, typically non-toxic, and biodegradable surface active agents which improve the solubility of lipids into an aqueous solution, thereby increasing the interaction between microbial enzymes and lipids, and consequently enhancing hydrolysis, the rate-limiting step of anaerobic digestion [6–8]. However, this also increases the risk of foaming [9,10]. Saharan et al. [8] identified a number of potential bio-surfactants derived from microbiological and plant sources, although few have been investigated for application in anaerobic digestion. Some successful applications of bio-surfactants include use of 'BOD-balance' by Nakhla et al. [11], which is a combination of bio-surfactant and enzyme used by Damasceno et al. [12].

Investigation of BOD-balance by Nakhla et al. [11] as a pre-treatment to aid in the digestion of wastewater high in FOG yielded promising results. With a dose of 500 mg BOD-balance/L, the researchers measured no change in chemical oxygen demand (COD) solubilisation following pre-treatment, but did record a significant improvement in particulate COD (PCOD) soluble COD (SCOD) degradation. Bio-surfactant addition increased PCOD removal by 96%, and SCOD by 100%, while also increasing COD biodegradation rate coefficient of 164–238%. The authors note that the increase in PCOD removal is due

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to a reduction in surface tension induced by the bio-surfactant, which helps solubilize hydrophobic organics, including FOG and colloidals [11]. Unfortunately, there was little focus on methane production during the investigation by Nakhla et al. [11]. However, it was noted that bio-surfactant addition appeared to reduce methane yield.

Bile is a natural product which is formed in the liver and stored in the gall bladder. It is a by-product of meat processing, and while there are pre-existing markets in cosmetics, pharmaceuticals and biological media [13], bile may be of value in enhancing the anaerobic digestion of high-fat wastes and aid in the operation of on-site AD systems in red meat processing plants. *In vivo*, bile acts as a surfactant to reduce large fat globules into smaller globules and thereby increase the surface area to volume ratio, consequently increasing the surface area available for enzymatic degradation.

This article presents novel work conducted using bovine bile as a bio-surfactant pre-treatment of high-fat cattle slaughterhouse. The aim of the work was to assess the effectiveness of bile, a readily available by product of meat processing, in enhancing anaerobic digestion of abattoir wastewater using batch biochemical methane potential (BMP) tests.

2. Materials and methods

2.1. Inoculum and substrate

Three batches of inocula were used in this experiment. The inoculum for the first BMP test using bile at 1–6 g/L was collected from the sludge recirculation pump servicing an anaerobic digester at a red meat processor. Due to unforeseen operational issues at the initial site of inoculum collection, the quality of the inoculum decreased markedly, and in subsequent testing was no longer able to produce > 80% of theoretical methane potential within 10 days when digesting cellulose. Consequently, subsequent batches of inoculum were collected from a new source at a wastewater treatment plant, prior to sludge thickening. Two separate samples were collected to conduct the second BMP testing bile at 0.2–1 g/L, and an anaerobic toxicity assay (ATA). Sludge was immediately transported back to the lab and stored in an incubator at 37 °C.

The substrate was dissolved air flotation (DAF) sludge, a concentrated source of FOG residues produced by the DAF process that is representative of the fatty material entering the anaerobic digestion system of red meat facilities [14]. Substrate was collected from the outlet of a DAF unit, and refrigerated at 4 °C until use. Avicel microcrystalline cellulose powder was used as a control substrate to measure sludge activity.

Bile was collected fresh from the abattoir and refrigerated at 4 °C until use. The characteristics of the inocula, substrates and bile used in this investigation are presented in Table 1.

Table 1
Characteristics of inocula, DAF sludge, cellulose and bile used in digestions.

	pH	VS (% of TS)	COD (mg/L)	FOG (mg/L)
Low-dose BMP: 0.2–1.0 g bile/L				
Inoculum	7.48	63.01	ND	ND
DAF sludge	4.40	98.32	469,000	85,000
High-dose BMP: 1–6 g bile/L				
Inoculum	6.86	76.86	ND	ND
DAF sludge	4.28	95.82	469,800	10,900
Anaerobic Toxicity Assay				
Inoculum	7.48	76.41	ND	ND
Cellulose	ND	95.38	ND	ND
Bio-surfactant				
Bile	6.74	81.7	ND	ND

ND = not determined; BMP = Biochemical methane potential; ATA = Anaerobic toxicity assay.

2.2. Pre-treatment of DAF sludge

Bile was dosed to reactors immediately prior to beginning the BMP digestion. Concentrations for bile addition were determined arbitrarily due to the novelty of the pre-treatment. Consequently, bile was dosed 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5 and 6 g/L of final liquid volume prior to commencing the BMP test. Bile characteristics are presented in Table 1.

2.3. Biochemical methane potential and anaerobic toxicity assay

BMP tests were conducted using the Automated Methane Potential Test System II (AMPTS II; Bioprocess Control, Lund Sweden). Final reactor volume was 400 mL, with an inoculum to substrate ratio of 3:1 based on volatile solids (VS) to avoid overloading. Reactor temperature was maintained at 37 ± 1.0 °C in a water bath. Biogas was scrubbed of carbon dioxide using 3 M sodium hydroxide, and resulting methane was measured by the AMPTS II gas measurement unit. Cellulose controls were used to confirm sludge activity of > 80% of its theoretical maximum [15], and a bile control was used to account for methane yield from bile VS. Digestions were considered complete on the day that daily methane production dropped below 1% of the total methane production (T_{FIN}) [15]. Results are reported as normal millilitres (mL_N), normalised to 0 °C and 1 atm and corrected for water vapour.

An anaerobic toxicity assay was performed to elucidate the non-specific, overall inhibitory effect of high-dose bile addition. The ATA was performed using the AMPTS II as above, with cellulose as a standard substrate and bile was dosed at 1, 2, 3, 4, 5 and 6 g/L of final reactor volume. Inoculum to substrate ratio was 3:1 to be consistent with the BMPs. Kinetic analysis was used to quantify the effect of bile addition on methane formation rate kinetics, and inhibition with respect to lag phase, delay in reaching peak methane production, and time required to complete digestion (Table 3).

2.4. Analytical methods

Parameters included: pH, VS and total solids (TS) using standard method 2540G [16]. COD was measured using Merck colorimetric test kits type 5000–90000 mg COD/L with a Spectroquant Pharo 100 spectrophotometer. FOG content was measured using a Wilks Infracal II, with sample workup similar to the user manual. Briefly, sample material was acidified using HCl to pH < 2, shaken, mixed 10:1 with hexane, and shaken again for 1 min. Emulsified hydrophobic component was extracted and centrifuged at 18000*g* for 5 min to break the emulsion. Hexane component was measured on the Wilks Infracal II O&G unit. Samples requiring dilution for COD (1 in 10, V/V) and FOG (1 in 100–1000, V/V) analysis were diluted with distilled water prior to application to the analytical method.

2.5. Kinetic analysis

Kinetic analysis was applied to the collected data to determine the rate constant (k, U) of linear gas production and better estimate the lag period (λ) for each treatment to assess the degree of inhibition due to bile pre-treatment. Two equations were fitted to the data to acquire values for rate constants and lag periods. Eq. (1) was a standard growth curve logistic function, while equation 2 was a modified Gompertz equation [17]. Equations were fitted using SciPy optimization curve-fit routine [18]. In order for the equations to be applied, data must fit a sigmoid shape. With exception to the cellulose and controls, sigmoid-shaped graphs were achieved by excluding data obtained from days 0–3, with day 4 considered to be day 0 for subsequent curve fitting. This offset was then added back to the equation outputs to obtain the true value for variables such as inhibitory period and time of maximum production.

Eq. (1): Growth curve logistic equation

$$B = \frac{B_0}{1 + e^{-k(t-t_0)}} \tag{1}$$

From Eq. (1): B is the cumulative specific methane potential (SMP; mL CH_4/g VS) at time t (days); B_0 is the maximum SMP achieved by end of digestion; k is the rate constant; t_0 is the time at which maximum production rate occurs. The function is weighted using standard deviation to achieve a better fit.

Eq. (2): Modified Gompertz equation [17].

$$B = B_0 e^{-e^{\left(\frac{Ue}{B_0}(\lambda - t)+1\right)}} \tag{2}$$

From equation (2): B is the cumulative SMP at time t; B_0 is the maximum SMP achieved by end of digestion; U is the kinetic constant of methane production rate; λ is the duration of lag phase in days, used here to represent inhibition. Equation is unweighted.

2.6. Statistical analyses

One factor analysis of variance (ANOVA) was used to detect a difference between groups in BMP tests. Due to small sample sizes of $n = 3$, the non-parametric equivalent, the Kruskal-Wallis test was employed in an attempt to improve the resolution of the statistical investigation. In the event that both the ANOVA and Kruskal-Wallis tests were significant with $P < .05$. T-tests were used to further investigate between groups, with the non-parametric Mann-Whitney test used to help account for low sample sizes. Where P values are given, all four of these tests have returned a significant result, and the T-test result has been reported.

3. Results and discussion

3.1. Biochemical methane potential of bile-treated DAF sludge

3.1.1. BMP of DAF sludge treated with bile at 0.2–1 g/L dosage

Addition of bile at 0.2–1 g/L improved biogas production from the outset of digestion (Fig. 1). With respect to the final methane yield attained by the control, bile treatments achieved equivalent methane yield 4 ± 0.71 days earlier and yields corresponded to the theoretical maximum yield from fat of 1014 mL/g VS. Impact to rate kinetics were negligible (Table 2).

Addition of bile at dosage of 0.2–1 g/L produced a significant increase in SMP in the range of 5.71%–7.08% with $P < .05$ in all cases. Although bile addition increased SMP at these doses, a dose-response relationship was not demonstrated. The increase in biogas production from the bile control was negligible, and coupled with the lack of dose-response relationship, this indicated the increase in SMP achieved by the digesters was not related to the additional VS or COD in the form of bile. It may be possible that 0.2 g bile/L was sufficient to saturate the fatty material, aid solubility of fats, and subsequently improve digestion such that further increases in bile dose would produce minimal improvement.

3.1.2. BMP of DAF sludge treated with bile at 1–6 g/L dosage

Bile addition at dosage of 1–6 g/L had negligible influence on biogas production. However, the impact on lag phase and T_{FIN} was significant and was prolonged with increasing doses of bile (Fig. 2; Table 3). An inhibitory duration of 7.1 ± 0.2 days was determined for the controls using the Gompertz equation. Addition of bile further increased the inhibitory duration by 10% ± 3% (3 g bile/L), 14% ± 3% (4 g bile/L), 37% ± 4% (5 g bile/L), and 79% ± 6% (6 g bile/L) (Fig. 2; Table 3). Similar outcomes were recorded by Feitkenhauer and Meyer [19] in which alcohol sulfate, an anionic surfactant was added to batch digestions. Although the concentration of surfactant used by Feitkenhauer and Meyer [19] was much lower at 50–500 mg/L, the researchers observed significantly prolonged inhibition. Lag phase was doubled in the lowest dose, and time required to finish digestion was also extended. The curves displayed in Fig. 2 are the average of 3 replicates, with error bars removed to improve clarity. Standard deviations ranged from 3 to 11 mL_N CH_4/g VS with an average of 8 mL_N CH_4/g VS.

Peak methane production rate was achieved by 10.1 ± 0.1 days in the controls, with similar results in the 1 and 2 g bile/L groups. Increased dosage produced statistically significant delays at 13 ± 2% (3 g/L), 19 ± 2% (4 g/L), 39 ± 2% (5 g/L) and 74 ± 3% (6 g/L) respectively (Table 3). Completion of digestion followed a similar trend. At doses of 0 and 1 g bile/L trials were complete after 17 ± 0 days, with a negligible increase at 2 g/L to 17.7 ± 0.6 days. At doses of 3–6 g bile/L, T_{FIN} was significantly delayed by 16 ± 7% (3 g/L), 22 ± 7% (4 g/L), 37 ± 7% (5 g/L), and 65 ± 6% (6 g/L) (Table 3). Reaction kinetics declined linearly with respect to bile addition ($R^2 = 0.9634$). From the logistic equation, a rate constant of $k = 0.73 \pm 0.01$ was determined for the controls. Addition of bile impacted rate constants by −3 ± 3% (1 g bile/L), −7 ± 1% (2 g bile/L), −22 ± 3% (3 g bile/

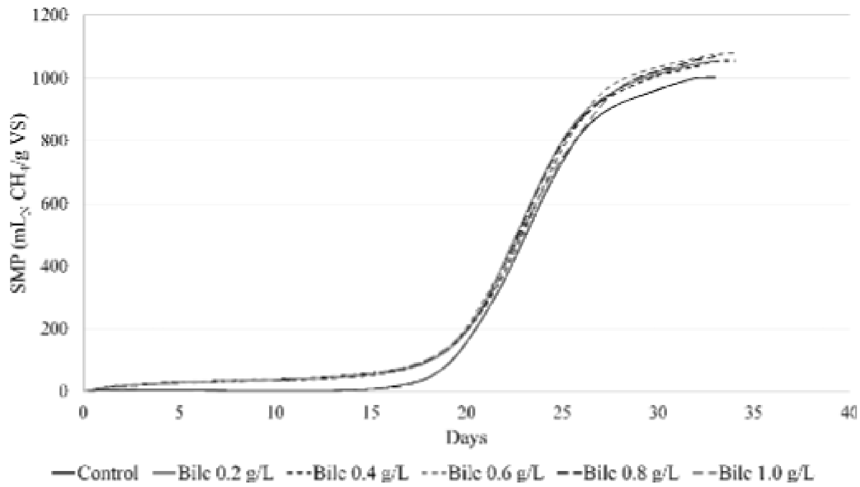

Fig. 1. Effect of low-dose bile on SMP and anaerobic digestion profile of DAF sludge.

Table 2
Kinetics modelling of SMP curves from low-dose bile BMP using a standard growth curve logistic equation and a modified Gompertz equation.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	B_0 NmL CH_4/g VS	k	U NmL CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose[a]	3.2 ± 0.1	5.8 ± 0.1	14 ± 1.2	348 ± 2	0.81 ± 0.04	71 ± 2	0.997	0.998
Control	19.2 ± 0.1	23.2 ± 0.0	33 ± 0.6	999 ± 7	0.53 ± 0.01	136 ± 2	1.000	0.999
Bile 0.2 g/L	19.1 ± 0.1	23.1 ± 0.1	33 ± 0.6	1056 ± 22	0.51 ± 0.01	139 ± 3	0.999	0.998
Bile 0.4 g/L	19.2 ± 0.1	23.4 ± 0.1	34 ± 0.0	1056 ± 3	0.48 ± 0.01	135 ± 9	0.999	0.997
Bile 0.6 g/L	19.1 ± 0.1	23.3 ± 0.2	33 ± 0.6	1080 ± 12	0.50 ± 0.01	135 ± 4	1.000	0.997
Bile 0.8 g/L	19.1 ± 0.1	23.3 ± 0.1	33 ± 0.6	1068 ± 4	0.50 ± 0.01	136 ± 4	0.999	0.998
Bile 0.1 g/L	19.0 ± 0.1	23.2 ± 0.3	33 ± 1.7	1056 ± 12	0.50 ± 0.03	129 ± 4	1.000	0.997

[a] Cellulose is provided as a reference for comparison, logistic equation was unweighted to achieve curve fit.

L), −27 ± 4% (4 g bile/L), −40 ± 2% (5 g bile/L), and −52 ± 5% (6 g bile/L) (Table 3).

Bile dosed at 1 and 2 g/L produced negligible impact on inhibitory duration. Given the minimum inhibitory concentrations of oleic acid (C18:1) from the literature of 0.443 mM [20], to 2.4 mM [21], and the composition of beef tallow of 37–47% C18:1 [22,23], the FOG load of 1245 mg/L equates to 1.73–2.15 mM C18:1. This is well within the reported range of inhibitory concentrations, and given sufficient solubilisation, LCFA inhibition should result.

While doses of 1 and 2 g bile/L appeared to have negligible impact on inhibitory duration, doses between 3 and 6 g bile/L induced significant inhibition. At doses of 3, 4, 5 and 6 g/L, inhibition increased by 9.8%, 14.1%, 36.6% and 78.87% respectively. The inhibition observed was consistent with descriptions of inhibition by LCFA adsorption, as the inhibition was reversible and overcome to produce roughly equivalent methane yields in all doses with exception to 6 g/L [24]. At 6 g bile/L, methane yield was reduced by an average of 6% ($P < .05$, $n = 3$).

The methane yields from the BMP trialling doses of 0.2–1 g bile/L were much greater than those obtained from the BMP trialling doses of 1–6 g bile/L. Yields in the low-dose BMP were very close to the theoretical maximum yield from fat of 1014 mL/g VS, and was likely due to the much greater FOG content of the DAF sludge used. Conversely, the yields from the high-dose BMP were much lower, which was consistent with a much lower FOG content.

While it is standard for BMP experiments to be conducted at an I:S ratio of 2:1, Li et al. [25] identified that the optimum I:S ratio for FOG digestion lies between 4:1 and 1.33:1. For this work, an I:S ratio of 3:1 was used with the intent to limit overloading and subsequent inhibition typically associated with FOG digestion. As 4:1 was the highest ratio investigated by Li et al. [31], I:S ratios greater than 4:1 may further optimise FOG digestion, increasing SMP and/or reducing inhibition.

3.2. Evaluation of bile inhibition using anaerobic toxicity assay

The ATA produced small changes in digestion profile (Fig. 3). As bile dose increased from 0 to 6 g/L, inhibition increased by up to 16.67% with a good linear correlation to dose ($R^2 = 0.94$). The time required to reach T_0 was delayed by up to 10%, and also correlated well with bile dosage ($R^2 = 0.88$). Bile dosage showed no effect on time required to complete the digestion. Similarly, the rate constant, k, showed little change between the control and 4000 mg/L dose, but began to reduce with doses of 5000 and 6000 mg/L by 3% and 5% respectively (Table 4). The curves displayed in Fig. 3 are the average of 3 replicates, with error bars removed to improve clarity. Standard deviations ranged from 1 to 17 mL_N CH_4/g VS with an average of 5 mL_N CH_4/g VS.

In comparison to the results produced by the ATA, where sludge response at doses of 0–6 g bile/L was linear, the response observed in the BMP is much more typical of a logarithmic growth curve, in which 6 g/L is beginning to severely delay the process (Fig. 4). This variation could be a result of the much greater sludge quality in the ATA.

The anaerobic toxicity tests demonstrate that bile had an inhibitory effect during BMP testing at doses of 3–6 g bile/L. As evidenced by Girault et al. [26] and Martin-Gonzalez et al. [27], once an anaerobic consortium had overcome initial LCFA inhibition, the rate of biogas production increases to a similar rate as the controls. While the ATA was digesting cellulose, the recovery of the rate kinetics reproduced in the ATA indicated that bile produced reversible inhibition, while a dose of 6 g bile/L induced the first signs of decline in reaction kinetics. However, in the BMP, the rate kinetic began to decline significantly from addition of 3 g/L. This inhibition could be caused by susceptibility to free fatty acids [28], or bile [29], but possibly due to the compounding effect of both. Bile is known to be toxic to various bacteria, in particular, gram-positive bacteria [29]. While population composition varies, gram-positive can account for a considerable fraction of active anaerobic biomass [30]. It is therefore possible that bile toxicity could have played a major role in reducing the rate of biogas production.

3.3. Comparison of bile pre-treatment at low (0.2–1 g/L) and high (1–6 g/L) doses

The high-dose trial utilised a FOG-acclimatised inoculum, with a substrate relatively low in FOG content. The resulting impact of bile addition was found to be largely negligible or negative depending on the dose. In comparison, the low-dose trial used an inoculum that was unaccustomed to high-fat substrates, and was combined with a substrate much higher in FOG content, yet produced an increase in SMP.

Table 3
Kinetics modelling of SMP curves from high-dose bile BMP using a standard growth curve logistic equation and a modified Gompertz equation.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	B_0 NmL CH_4/g VS	k	U NmL CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose[a]	1.3 ± 0.1	2.9 ± 0.1	10.0 ± 0	312 ± 6	1.30 ± 0.13	102 ± 4	0.991	0.998
Control	7.1 ± 0.2	10.1 ± 0.1	17.0 ± 0	765 ± 11	0.73 ± 0.01	121 ± 6	0.999	0.999
Bile 1 g/L	7.3 ± 0.1	10.3 ± 0.1	17.0 ± 1	764 ± 12	0.71 ± 0.02	121 ± 6	0.999	0.995
Bile 2 g/L	7.0 ± 0.2	10.1 ± 0.1	17.7 ± 0.6	761 ± 3	0.68 ± 0.01	109 ± 5	0.993	0.994
Bile 3 g/L	7.8 ± 0.2	11.4 ± 0.2*	19.7 ± 1.2*	756 ± 7	0.57 ± 0.02*	96 ± 4*	0.997	0.994
Bile 4 g/L	8.1 ± 0.2	12.0 ± 0.2*	20.7 ± 1.2*	755 ± 8	0.53 ± 0.02*	88 ± 4*	0.994	0.989
Bile 5 g/L	9.7 ± 0.3*	14.0 ± 0.2*	23.3 ± 1.2*	741 ± 3	0.44 ± 0.01*	75 ± 4*	0.989	0.987
Bile 6 g/L	12.7 ± 0.4*	17.6 ± 0.3*	28.0 ± 1*	720 ± 11*	0.35 ± 0.02*	65 ± 3*	0.986	0.986

* Statistically significant at $P < .05$, $n = 3$.
[a] Cellulose is provided as a reference for comparison, logistic equation was unweighted to achieve curve fit.

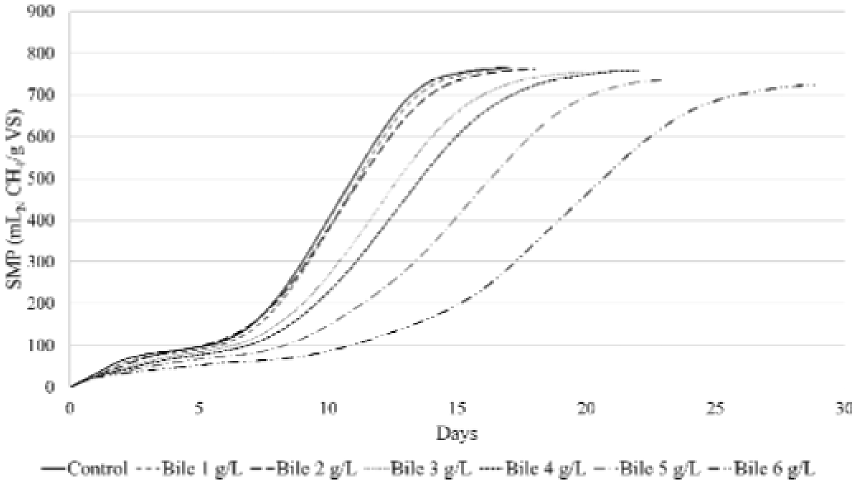

Fig. 2. Effect of high-dose bile on SMP and anaerobic digestion profile of DAF sludge.

The data indicate that there is potential for beneficial outcomes from low-dose bile addition. However, the data also support that the influence of bile on the digestion process at these lower doses is also reliant on other factors, indicated by the varying result of the 1 g/L trial, which overlaps both BMP investigations. It is likely that variation in inoculum or substrate is responsible for this inconsistency. Subsequently, the cellulose controls for each BMP and the ATA were compared (Fig. 5). The sludge used for the low-dose investigation, while slower to complete digestion, produced 9% and 12% more biogas than the inoculum used for the ATA and high-dose BMP respectively. It is likely that the superior sludge quality used in the low-dose BMP was responsible for the positive response to bile addition observed in the low-dose BMP.

Bile is a complex mixture of components, with a range of critical micellar concentrations (CMC). Surfactant compounds within bile, sodium deoxycholate, sodium chenodeoxycholate and sodium cholate, have CMCs of 5.3, 7.0 and 18.4 mM respectively [31]. Of the bile doses trialled, 5 g/L and 6 g/L have potential bile salt concentrations above 5.3 mM, with 5.6 and 6.7 mM respectively. Measured differences in inhibition, SMP, finish time and reaction kinetics, as shown in Figs. 2 and 4, and Tables 3 and 4 indicate that degradative effects are appearing as early as 3 g/L, with a potential bile salt concentration of 3.6 mM. While these effects don't appear to correlate with CMC, these degradative effects do appear to become more severe at concentrations above the CMC of sodium deoxycholate, and micelle formation may induce more significant inhibitory effects.

3.4. *Economic considerations of bile pre-treatment of wastewater at meat processing facilities*

Bile is a by-product of red meat processing and was used in this study to assess the relative merit as onsite treatment of processing wastewater to improve anaerobic digestion. The current use of bile at

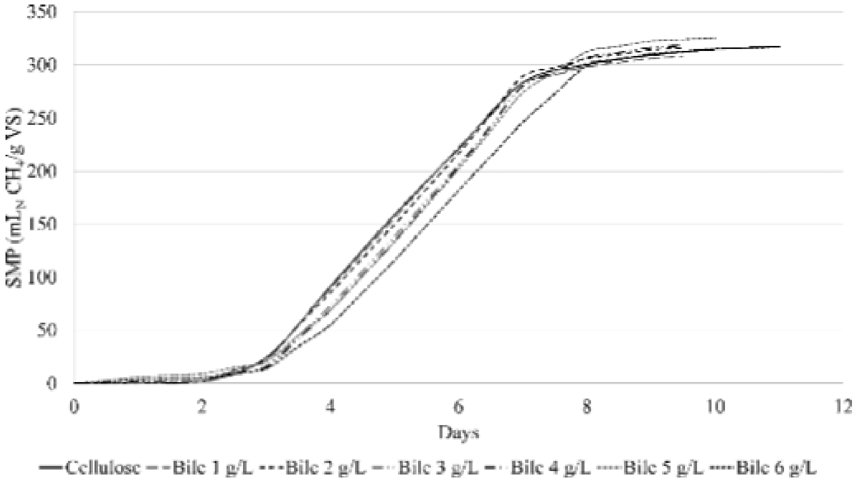

Fig. 3. Anaerobic toxicity assay with cellulose as a standard substrate, and bile as the substance in question.

Table 4
Anaerobic toxicity assay with cellulose and bile.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	SMP mL_N CH_4/g VS/day	k	U mL_N CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose	3.0 ± 0.1	5.1 ± 0.1	10 ± 0	318 ± 2	1.03 ± 0.05	83 ± 3	0.998	0.998
Bile 1 g/L	3.0 ± 0.1	5.0 ± 0.0	10 ± 0	309 ± 2	1.07 ± 0.03	84 ± 3	0.998	0.997
Bile 2 gL	3.1 ± 0.1	5.1 ± 0.0	10 ± 0	318 ± 9	1.05 ± 0.03	84 ± 3	0.998	0.995
Bile 3 g/L	3.3 ± 0.1	5.3 ± 0.0	10 ± 0	313 ± 2	1.07 ± 0.03	85 ± 3	0.998	0.995
Bile 4 g/L	3.4 ± 0.1	5.4 ± 0.0	10 ± 0	322 ± 1	1.06 ± 0.03	86 ± 3	0.998	0.995
Bile 5 g/L	3.5 ± 0.1	5.5 ± 0.0	10 ± 0	318 ± 1	1.04 ± 0.03	83 ± 3	0.998	0.995
Bile 6 g/L	3.5 ± 0.1	5.7 ± 0.0	10 ± 0	318 ± 1	1.00 ± 0.03	80 ± 3	0.998	0.993

Australian RMP facilities is as a sale product to pharmaceutical industries. The effort of collecting and preparing bile for this purpose is considerable. Although collection by specialised equipment can yield approximately 96% of bile, simple slashing of the gall bladder and draining bile by workers can result in loss of up to 50% is common [32]. Collected bile must then be heated to concentrate the bile to 75% solids. This carries the benefits of preserving the product and avoiding the cost of preservatives, while eliminating large quantities of water to reduce shipping costs [32]. At a throughput of 500 head of cattle per day for a medium sized Australian RMP facility, with a bile volume of 0.4 L and a solids content of 10%, this equates to 20 kg of solids available per day [32]. At a value of 25 AUD/kg (Dennis King, pers.comm. 1/11/2017) [37], bile is worth 500 AUD/day or 125,000 AUD/year (250 days) to the processor. Assuming 50% loss of bile, this value is reduced to 250 AUD/day or 62500 AUD/year. This return is so low that many processors consider the return on invested effort and energy does not warrant collection (Dennis King, pers.comm. 1/11/2017).

By comparison, dosage to the waste stream does not require concentration. At a throughput of 500 cattle, 200 L of bile is recoverable. While the maximum methane increase measured in this investigation was 7.08%, 0.6 g bile/L, the corresponding quantity would require 600 L of bile to treat 1 ML of wastewater. Alternatively, a dose of 0.2 g bile/L would require 200 L of bile, and is possible for an increase in methane yield of 5.71%, assuming 100% bile recovery.

In comparison with the financial implications considered in Harris *et al.* [14], an SMP of 759 m^3 CH_4/kg VS was measured for a DAF sludge containing 14.21% VS, presumed to be primarily FOG. For a 143 m^3 load of waste, the equivalent FOG load was calculated to be around 20.3 m^3, or, with a density of approximately 0.7861 g/mL, a rough mass of 15.96 tons of FOG solids. The treatment of this volume of wastewater with bile would require 28.6 L of bile. This mass, with an SMP of 759 m^3/kg VS would produce 12105119 m^3 CH_4. An additional 5.71% equates to 691202 m^3 CH_4. With an energy content of 35.75 MJ/m^3, this volume contains 19334 MJ. At 3.6 kWh/MJ, and an electrical conversion efficiency of 40% for a combined heat and power plant, this would result in approximately 2148 kWh of usable electricity. At a rate of 0.15 AUD/kWh, this would be worth 322 AUD. If used to offset natural gas, at a rate of 8.15 AUD/GJ, this would be worth 158 AUD. With respect to the volume of bile generated per day, around 200 L, the treatment of 1 ML of such waste would bring the value of bile up to around 1102 AUD/day based on these values. In comparison with the collection and preparation of bile for sale to the pharmaceutical industry, bile for wastewater treatment is dosed directly to the waste stream with no other treatment, reducing the effort and energy investment. Conclusion

This work identified that bile dosed at 0.6 g/L produced a 7.08% increase in methane yield. Higher doses of bile ranging between 3 and 6 g/L resulted in reduced methane yield, increased inhibition by up to 79%, and reduced reaction kinetics by 52%. The economic viability of using bile as a bio-surfactant was assessed. In comparison to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile, through biogas production, to 220% of its current sale value. The quality of inoculum and substrates were important factors when assessing the effect of bile as a pre-treatment option.

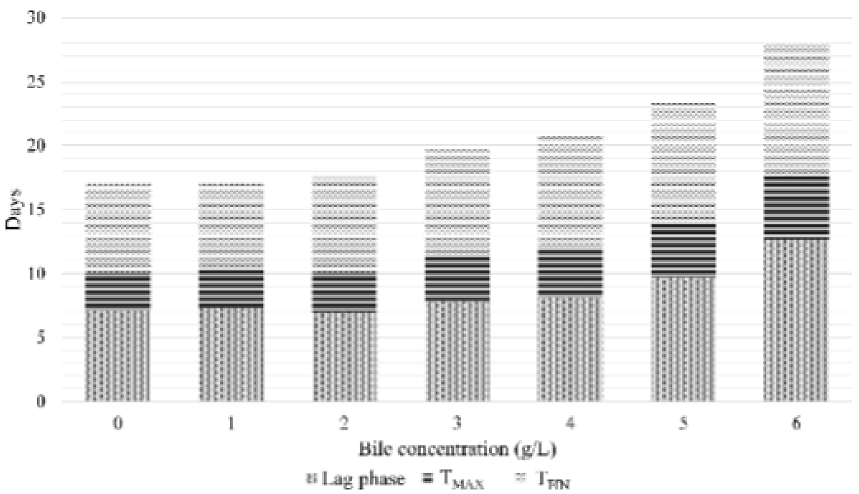

Fig. 4. Inhibition period with respect to bile addition in BMP testing. T_{MAX} represents the time between recovering from lag phase inhibition and achieving maximum methane production rate. T_{FIN} represents the time between achieving T_{MAX} and completing digestion with methane production < 1% of total yield.

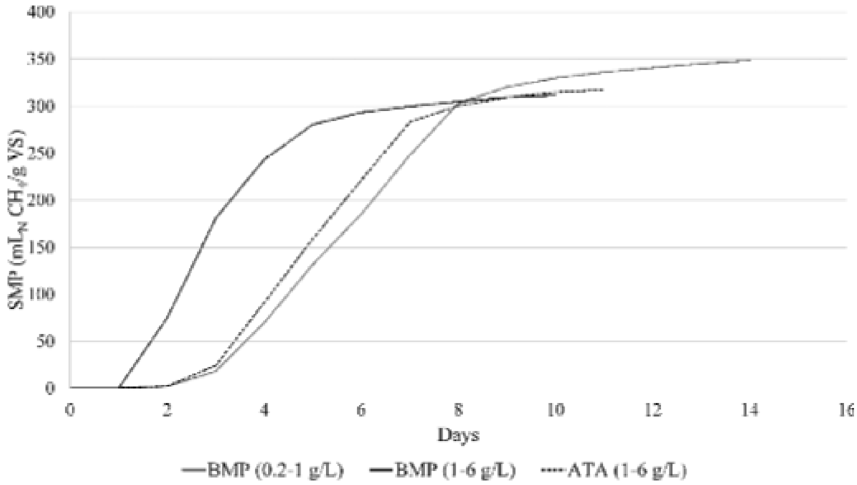

Fig. 5. Comparison of cellulose controls from BMP and ATA investigations.

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Supplementary Tables

Supplementary Table 1: Characteristics of inocula, DAF sludge, cellulose and bile used in digestions.

	pH	TS (% FM)	VS (% FM)	VS (% of TS)	COD (mg/L)	FOG (mg/L)
Low-dose BMP: 0.2-1.0 g bile/L						
Inoculum	7.48	5.08	3.20	63.01	ND	ND
DAF sludge	4.40	28.67	28.19	98.32	469,000	85,000
High-dose BMP: 1-6 g bile/L						
Inoculum	6.86	2.44	1.88	76.86	ND	ND
DAF sludge	4.28	9.33	8.94	95.82	469,800	10,500
Anaerobic Toxicity Assay						
Inoculum	7.48	2.60	1.99	76.41	ND	ND
Cellulose	ND	100	95.38	95.38	ND	ND
Bio-surfactant						
Bile	6.74	9.63	7.87	81.7	ND	ND

ND = not determined; BMP = Biochemical methane potential; ATA = Anaerobic toxicity assay; FM = Fresh matter

Supplementary Table 2: Conversion of volumetric dosing of bile to dosage per unit of FOG.

	Low-dose bile BMP					High-dose bile BMP					
Dose (g bile/L)	**0.2**	**0.4**	**0.6**	**0.8**	**1**	**1**	**2**	**3**	**4**	**5**	**6**
Inoculum mass (g)	385.4	385.4	385.4	385.4	385.4	373.8	373.8	373.8	373.8	373.8	373.8
Substrate mass (g)	14.6	14.6	14.6	14.6	14.6	26.2	26.2	26.2	26.2	26.2	26.2
Bile added (mg)	80	160	240	320	400	400	800	1200	1600	2000	2400
FOG (mg)	1239.6	1239.6	1239.6	1239.6	1239.6	275.1	275.1	275.1	275.1	275.1	275.1
Bile dose (mg/mg FOG)	0.065	0.129	0.194	0.258	0.323	1.454	2.908	4.362	5.816	7.270	8.723

Appendix D

Impact of thermobaric pre-treatment on the continuous anaerobic digestion of high-fat cattle slaughterhouse waste

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Regular article

Impact of thermobaric pre-treatment on the continuous anaerobic digestion of high-fat cattle slaughterhouse waste

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ABSTRACT

Thermobaric pre-treatment of a combination of dissolved air flotation (DAF) sludge and slaughterhouse wastewater was evaluated for performance over 50 days of continuous anaerobic digestion. Continuous digestion was conducted over three phases represented by varying fat, oils and grease (FOG) concentrations and organic loading. In comparison with earlier biochemical methane potential (BMP) investigations using thermobaric treated substrate by Harris, Schmidt and McCabe (Harris et al., 2017) which yielded an 8.32% increase in specific methane production, pre-treated DAF sludge produced negative impacts on digestion under continuous conditions. Average pH was consistently lower by 0.04, and loss of volatile organics during pre-treatment reduced methane yield by 12.1%. H_2S concentration was 56% higher on average with 795 ppm compared with 510 ppm in the controls owing to enhanced protein degradation. Alkalinity was low due to insufficient replacement from the substrate. Fresh substrate containing double the fat content (236 mg/L) and reduced organic loading rate (OLR) caused both control and treatment reactors to fail, highlighting the need for consistent substrate characteristics. Magnesium hydroxide addition effectively recovered both pH and biogas production within digesters rapidly, addressing the underlying complication of insufficient alkalinity contribution by the substrate.

1. Introduction

Low-rate covered anaerobic lagoons (CALs) offer the Australian red meat processing (RMP) industry an attractive wastewater treatment option with the added benefit of capturing methane-rich biogas that can be combusted to offset onsite fossil fuel consumption [2]. The RMP industry produces high-strength, high fat wastewater which has excellent potential for biogas production. In comparison with carbohydrates and proteins which have theoretical methane potentials of 370 and 480 m^3/kg VS, fat has a potential of 1014 m^3/kg VS [3,4], and thereby has the potential to significantly enhance methane yields from AD systems. However, fats also present operational problems, and can reduce the performance of anaerobic digestion (AD) systems [5]. Fat has potential to cause problems such as blockages, foaming, cover damage, reversible inhibition, sludge flotation, and sludge washout [6,7]. Co-digestion and pre-treatment are two avenues of research which work to overcome these problems and increase methane yields [8]. While co-digestion in RMP facilities in Australia has received little investigation, the country is actively pursuing research in this area [9].

Pre-treatment options for FOG-rich substrates typically fall into the categories of thermal, chemical, thermochemical, mechanical, enzymatic and surfactant methods [10]. Harris, Schmidt & McCabe [1] investigated the effects of thermobaric, chemical and thermochemical pre-treatment on dissolved air flotation (DAF) sludge. Under batch digestion, thermobaric pre-treatment demonstrated improvement in methane yield, increasing specific methane production (SMP; mL_N CH_4/g VS) of DAF sludge by 8.32%, achieving equivalent methane yield 4 days earlier than the controls, and completely eliminated lag phase inhibition.

While BMP tests provide good information regarding the amount of methane that can ultimately be produced from a feedstock, these experiments are not entirely representative of industrial scale AD systems [11]. Lab-scale continuous digestion experiments are the next progression from BMP tests which can elucidate further information regarding the digestion of a substrate, and it's suitability for large-scale AD. Lab-scale continuous digestion experiments allow the operator to optimise organic loading rate (OLR) and hydraulic retention time (HRT), while monitoring for the accumulation of potential inhibitory compounds, or the gradual loss of necessary components (i.e. trace elements, alkalinity, etc.) [3]. One of the assumptions that is made with a BMP test, and challenged with continuous digestion experiments, is the

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Table 1
Initial parameters of sludge, and substrate batches.

	pH	TCOD (mg/L)	VS (%)	FOG (mg/L)	TN (mg/L)	VFA (mg/L)
Inoculum	7.12	ND	2.00 ± 0.01	ND	ND	ND
	n = 1		n = 2			
Phase I: Days 0–33						
Control	7.32	7450 ± 134	1.01 ± 0.04	917	86	151 ± 0
Thermal	7.40	7875 ± 502	1.01 ± 0.07	ND	ND	178 ± 0
	n = 1	n = 2	n = 3	n = 1	n = 1	n = 2
Phase II & III: Days 34–49						
Control	7.42 ± 0.25	7034 ± 2.7	0.85 ± 0.03	1886	102	150 ± 1.15
Thermal	7.50 ± 0.26	6989 ± 2.7	0.86 ± 0.05	ND	ND	180 ± 4.04
	n = 7	n = 9	n = 17	n = 1	n = 1	n = 2

Table 2
Substrate mixtures.

Phase	DI water (ml)	Green stream (ml)	DAF sludge (ml)	Total (ml)
I	459	230	11.34	700
II, III	520	173	6.16	700

supply of a substrate of consistent composition, and the impact that this variation has on the digestion process. The chemical and physical composition of wastewater in the RMP industry varies significantly owing to the degree of primary treatment, including the number, size and efficiency of screens, DAF units, contra sheers, screw presses, sterilisation and rendering [12], as well as species slaughtered, seasonal changes, and variation down to the week, day and even between shifts [13].

Although batch investigations provide valuable information, there is often disparity between results obtained from batch and continuous digestion investigations. Schwede, Rehman, Gerber, Theiss and Span [14] thermally treated microalgae and produced a 185% increase in methane yield under batch conditions. However, under continuous digestion, an increase of only 108% was recorded. Similarly, Zhang, Su and Tan [15] measured on average 29% less methane produced from substrate digested in continuous systems when compared with batch systems.

This current study is a progression of work performed by Harris, Schmidt and McCabe [1], and aimed to investigate the impact of thermobaric-pre-treatment on the anaerobic digestion of a combination of DAF sludge and slaughterhouse wastewater during continuous digestion, with respect to methane yield and process stability.

2. Materials and methods

2.1. Inoculum and substrate

The inoculum was anaerobic sludge sourced from a CAL at a local slaughterhouse (Table 1). Sludge was immediately portioned into 6 × 2L continuous stirred-tank reactors (CSTRs), incubated at 37 ± 1 °C for 4 days to allow residual organics to digest.

The substrate was a semi-synthetic combination of distilled water, wastewater collected from the slaughterhouse green stream, and DAF sludge (Table 1), with the intent to limit substrate variability and contribute to a greater C:N ratio. DAF sludge is a concentrated source of FOG residues collected by the DAF process. This material was used for its high FOG content and has a fatty acid composition representative of the fatty material entering the AD system of the red meat processor [1]. Green stream waste is a combination of tripe wash, render, stick water, paunch wash, cattle wash and green wash waste. Green stream waste was diluted to allow for the majority of COD to be contributed by DAF sludge, while attempting to retain some degree of nitrogen to contribute to alkalinity. Both wastewater and DAF sludge were collected as grab samples and immediately returned to the laboratory for storage at 4 °C, and subsequent pre-treatment prior to use. DAF sludge was blended using a 600 W stick blender to improve uniformity prior to portioning into storage containers. Waste components were combined to produce a substrate of 8 g COD/L to allow for an OLR of 1 g COD/L/day while retaining a HRT of 8 days (Table 2).

2.2. Pre-treatment and continuous digestion set-up

Thermobaric pre-treatment of DAF sludge was performed as per Harris, Schmidt & McCabe [1]. Anaerobic reactors were BioProcess Control bioreactor simulator (BRS) reactors (BioProcess Control, Sweden). As described by Strömberg et al. [16], the BRS consists of 5 main parts. Gas from the bioreactors (BR) is measured by a data acquisition instrument (DAI). Information from the DAI is transmitted to the database (DB), and through the website to file storage (FS). The user then accesses this data through the website.

Reactors were operated at a working volume of 1.8 L with a HRT of 8 days. Reactors were fed daily with 225 mL of semi-synthetic substrate, and 225 mL of effluent digestate was recovered for subsequent analysis. Reactors were maintained at 37 ± 1 °C with a thermostatic water bath. Table 3 details the feeding regimes for the reactors. Phase I spanned days 0–32, in which reactors were fed with the first batch of substrate. Phase II spanned days 33–43, in which reactors were fed with a second batch of substrate. Phase III spanned days 44–49, in which reactors were fed with the sec-

Table 3
Operational details of CSTR continued.

Phase	Days	Stirring (hours on/day)[a]	OLR (g COD/L/day)	FOG load (mg/L/day)	$Mg(OH)_2$ (g/ml/day)
I	0–32	20.5	1	115	0
II	33–43	20.5	0.85	236	0.005
III	44–49	23.83	0.85	236	0.005

[a] Stirring interval is 1:5 min on/off.

ond batch of substrate, but dosed with magnesium hydroxide ($Mg(OH)_2$).

Stirring was paused for 2.5 h prior to feeding in phases I and II allowed for biomass settling. In response to gradually increasing sludge flotation observed visually over the course of phases I and II, stirring protocol was modified in phase III. Stirrers were stopped 10 min prior to feeding to allow for sludge settling while limiting sludge flotation. Reactors are stirred intermittently at 80% of motor capacity, with 1 min on and 5 min off. Volumetric gas measurement was normalised to 0 °C, 1 atmosphere and corrected for water vapour internally by the DAI, and is reported as normal millilitres (mL_N). Adjustment of pH was performed using magnesium hydroxide at a dose of 0.005 g $Mg(OH)_2/mL_{SUBSTRATE}$/day on days 43–49.

2.3. Analytical methods

Effluent digestate samples were collected in parallel to daily substrate addition. pH was measured daily. Total solids (TS) and VS were measured twice a week in accordance with standard method 2540G [17]. Merck test kits were used to measure ammonium, total nitrogen (TN), COD at 25–1500 mg/L, 500–10000 mg/L, and 5000–90000 mg/L and were subsequently analysed on a Spectroquant Pharo 100 spectrophotometer. Volatile fatty acids (VFA) and TA were measured by FOS/TAC titration [18], samples were centrifuged at 2345g for 30 min and filtered through a Whatman grade 4 (20–25 μm) filter paper to remove particulate lipid prior to titration.

Biogas volume was measured continuously by the BRS, and gas composition was analysed for methane (CH_4), carbon dioxide (CO_2), oxygen, and hydrogen sulphide (H_2S) twice a week by recirculating the digester headspace through a Biogas 5000 gas meter (Geotech, United Kingdom). FOG was measured using a Wilks Infracal II analyser, using the extraction method outlined in the user manual, with modifications outlined in [1].

2.4. Statistical analyses

Single factors analysis of variance (ANOVA) was used to detect statistical significance between samples.

3. Results and discussion

3.1. Biogas production and composition

The onset of daily biogas production generally occurred sooner in the controls, with production increasing within 15 min of substrate addition. By comparison, production began within 1 h of substrate addition in the treatment group. This indicated that the control substrate was either more readily accessible by the microbes than the thermobaric substrate, or contained substances which were more rapidly degradable, i.e. VFA potentially lost during pre-treatment as seen in Harris, Schmidt and McCabe [1].

Daily biogas production typically remained greater in the control group throughout the 24 h period (Fig. 1). The decrease in biogas production at 21–22 h was due to the cessation of stirring prior to feeding. This was contrary to the results obtained by BMP analysis of substrate collected from the same location and subject to the same treatment in Harris, Schmidt and McCabe [1], in which treated DAF sludge produced greater methane yield and reduced lag phase inhibition. This was expected to translate to a reduced delay between daily substrate addition and subsequent gas production under continuous digestion.

Specific biogas production (SBP; mL_N/g VS/day) in the control averaged 930 ± 142 mL_N/g VS, while the treatment reactors averaged 814 ± 128 mL_N/g VS. This was significantly lower than the theoretical value for tallow at 2009 mL_N/g VS [4,19], indicating that the substrate was not fully degraded within the 24 h period.

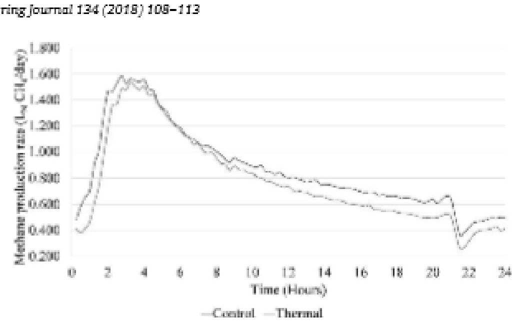

Fig. 1. Average daily biogas production from control and thermal groups ($P<0.05$, $n=49$).

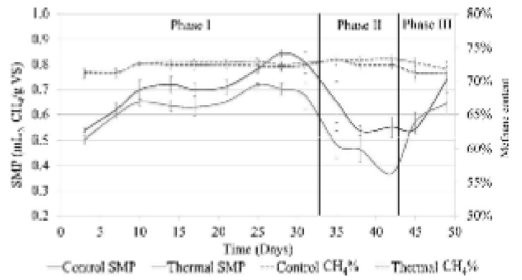

Fig. 2. Gas flow rates and methane content. Values are the average of 3, with error bars representing one standard deviation.

SBP was consistently higher in the control group by an average of 12.8% ($P<0.05$), as was specific methane production (SMP; mL_N CH_4/g VS/day) by 12.1% ($P<0.05$) (Fig. 2). It was expected that in the absence of beneficial effects of pre-treatment, that the biogas yield from the treatment group should mirror the controls. This was not the case. As pre-treatment was conducted in a Schott bottle, it is suspected that the vessel seal was broken, and some of the reduction in gas yield resulted from the loss of organic content, specifically as VFA, from DAF sludge during the pre-treatment process as reported in Harris, Schmidt and McCabe [1]. This problem could be overcome with the use of a pressure vessel as was conducted in Wilson & Novak [20]. The reduction in yield may also be in part due to reduced substrate degradability, or increased inhibition perhaps induced by increased LCFA content [20]. This reduced yield is consistent with the results of Schwede, Rehman, Gerber, Theiss and Span [14] and Zhang, Su and Tan [15] in that pre-treated substrate produced a greater result under batch conditions than under continuous digestion conditions. However, the pre-treatments investigated by Schwede, Rehman, Gerber, Theiss and Span [14] and Zhang, Su and Tan [15] still produced an increase in methane yield in comparison to the controls, and in this respect, the results of this investigation are inconsistent with the literature.

Methane and carbon dioxide content remained largely consistent throughout the experiment. Controls averaged $72.1 \pm 0.6\%$ CH_4 and $27.6 \pm 1.15\%$ CO_2, while the thermobaric treatment averaged $72.5 \pm 0.6\%$ CH4 and $27.5 \pm 1.15\%$ CO_2 (Table 4). Conversely, H_2S content varied greatly (Table 4; Fig. 3). Thermobaric-treated substrate produced 56% more H_2S until day 43, indicative of increased protein degradation. Magnesium hydroxide ($Mg(OH)_2$) addition on day 43 significantly reduced H_2S concentrations in both the control and treatment groups. The mechanism for this reduction in

Table 4
Biogas production and composition.

Reactor	SBP mL_N/g VS	SMP mL_N CH_4/g VS	CH_4 (%)	CO_2 (%)	H_2S (%)
Control 1	932 ± 141	665 ± 103	71.3 ± 1.12	27.6 ± 1.15	413 ± 210
Control 2	945 ± 161	687 ± 118	72.4 ± 1.16	27.5 ± 1.15	576 ± 304
Control 3	909 ± 126	656 ± 93	72.0 ± 0.94	27.8 ± 0.92	541 ± 270
Thermal 1	852 ± 111	616 ± 80	72.0 ± 1.18	27.8 ± 1.14	830 ± 303
Thermal 2	834 ± 127	604 ± 92	72.1 ± 1.11	27.8 ± 1.11	746 ± 305
Thermal 3	759 ± 197	550 ± 148	72.4 ± 1.70	27.4 ± 1.70	796 ± 321

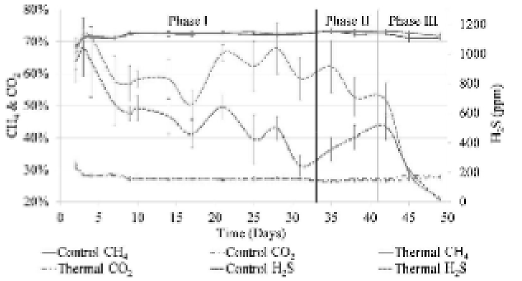

Fig. 3. Average biogas composition ± standard deviation with respect to CH_4, CO_2 and H_2S content.

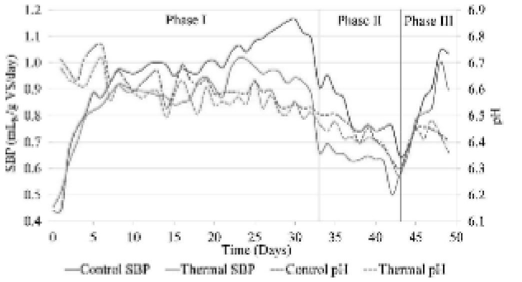

Fig. 4. Digester response to reduced pH, and recover with $Mg(OH)_2$. Phase I involved the digestion of the first batch of substrate. Phase II indicates the change to a new batch of substrate on day 33. Phase III indicates the addition of $Mg(OH)_2$ on day 43.

H_2S content is two-fold. Firstly, $Mg(OH)_2$ reacts with H_2S in solution to produce hydrosulphide (HS^-), a non-volatile product [21]. Secondly, raising the pH from 6.3 to a more alkaline environment increased the capacity of the water to solubilise H_2S, resulting in reduced gas release [22].

3.2. *Process stability – VFA, TA and NH_4-N*

3.2.1. *Effect of pH on biogas production*

Reactor pH steadily decreased from 6.71 ± 0.05 to 6.51 ± 0.02 in the control group during phase I (Table 3; Fig. 4). A change in substrate to a fresh batch with around double the FOG content saw a rapid pH decrease to 6.30 ± 0.03 by the end of phase II. A similar trend was exhibited by the treatment group, decreasing from 6.68 ± 0.04 to 6.47 ± 0.03 during phase I, followed by a rapid decrease to 6.27 ± 0.02 by the end of phase II. Continued biogas production at such a low pH was unexpected, but not unprecedented [23]. This rapid decrease in pH was accompanied by an extreme decrease in biogas production in all reactors. At this point, $Mg(OH)_2$ was added to the substrate to recover the digesters and indicated the beginning of phase III. Although pH recovered slowly, biogas production recovered rapidly. Overall, the treatment group maintained a lower pH at almost all times in comparison to the control group. Indeed, signs of decline had begun to show by day 23 in the treatment group, while the control group was still increasing in biogas production.

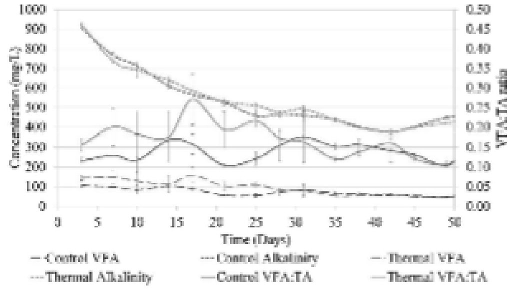

Fig. 5. Comparison of volatile fatty acids, total alkalinity, and VFA:TA ratio. Values are given as average ± one standard deviation. $Mg(OH)_2$ was dosed to digester from day 43 onward.

3.2.2. *Effect of VFA and alkalinity content on pH*

While VFA content was 64% lower in the substrate following thermobaric pre-treatment in the BMP investigation [1], this was inconsistent with the literature, and reinforces the possible loss of organics from the substrate through pre-treatment. A similar investigation by Wilson, Novak and Murthy [24] demonstrated that thermobaric treatment at 170 °C increased VFA yield by 89% over the yield achieved at 150 °C. Wilson and Novak [20] further elucidated the effects of temperature on LCFA degradation. Regarding saturation, lipids with greater degrees of saturation responded at lower temperatures, and VFA yield was far greater at each temperature trialled than their more saturated counterparts. While the results of Wilson and Novak [20] are encouraging, it should be noted that C18:1 accounts for almost half of the fatty acid profile of beef tallow, which only began to show improvements at 190 °C. The VFA profile produced by thermal-treated LCFA in the Wilson and Novak study was also dependant on LCFA saturation. Thermal treatment of C18:0 effected a small increase in acetic, propionic and butyric acids, and a relatively large generation of valeric, caproic and heptanoic acids. Conversely, thermal treatment of C18:3 generated a profile almost opposite to C18:0, with acetic acid and propionic representing roughly 85% of all VFA

Under continuous digestion, VFA concentrations were on average 54% higher in the thermobaric-treated digester effluent for the first 25 days (3 HRT) of digester operation. Beyond day 25, VFA concentrations reduced to levels equivalent to the controls, at approximately 60 ± 18 mg/L in both groups (Fig. 5). Higher VFA content within the thermobaric reactors indicated that organics were more easily degraded to form VFA following pre-treatment. This explains the reduced pH of the treatment group with respect to the control group over the first 28 days of digestion. Given that accumulation of VFA was not detected throughout the experiment, the decrease in pH was found to be a result of reduced buffering capacity due to washout and insufficient replacement by the substrate.

A total alkalinity of 903 mg $CaCO_3$/L and 923 mg $CaCO_3$/L was present in the control and treatment reactors respectively at the beginning of digestion, contributed by the inoculum. Alkalinity decreased almost linearly during phases I and II to 375 ± 7 mg $CaCO_3$/L in the control group, and 374 ± 8 mg $CaCO_3$ in the thermal group. This decrease was due to a low TN concentration in the undiluted green stream waste of 198 mg/L, and loss through washout. Green stream waste was diluted 1:2 in phase I, and 1:3 in phase II and III to achieve a high proportion of pre-treated DAF sludge as COD in the substrate. While the DAF sludge contained a high TN of 3300 mg/L, at such a small loading, the TN in the prepared substrate was only 86 mg/L in phase I, and 102 mg/L in the phase II and III. Consequently, with a HRT of 8 days, ammonium, which contributes significantly to alkalinity [25], was quickly diluted and discharged from the system, with insufficient replacement.

Addition of magnesium hydroxide in phase III steadily increased alkalinity until the final measurement on day 49. Control reactors increased from a minimum of 375 ± 7 mg $CaCO_3$/L on day 42 up to 446 ± 6 mg $CaCO_3$/L by day 49. This resulted in a corresponding increase in digester pH from 6.30 ± 0.03 to $6.41 \pm 6.41 \pm 0.03$. Pre-treated reactors also increased from a minimum of 374 ± 8 mg $CaCO_3$/L on day 42 up to 421 ± 5 mg $CaCO_3$/L by day 49, resulting in a pH increase from 6.27 ± 0.02 up to 6.36 ± 0.04 (Fig. 4).

VFA:TA ratio remained below the optimum range of 0.3–0.4:1[26], with averages of 0.14 ± 0.02:1 in the control group, and 0.17 ± 0.04:1 in the treatment group. It is important to note that VFA:TA ratio is dynamic, with acid concentrations increasing shortly after substrate addition as hydrolysis and acidogensis take place, and decreasing as digestion proceeds to completion.

Ultimately, pH was heavily reliant on the buffering capacity. Although increased protein degradation was indicated by the increase in H_2S production, there appeared to be little impact on ammonium content. Total nitrogen in the substrate was too low at 50–66 mg/L to replace the ammonium washed out during substrate addition. While VFA concentration exhibited little fluctuation with 73 ± 20 mg/L in the control group and 95 ± 40 in the treatment group, alkalinity continually decreased as ammonium was lost from the system ($R^2 = 0.977$). Although pH decreased gradually with respect to alkalinity, the VFA:TA ratio remained low, indicating that VFA accumulation did not cause digesters to fail.

These data indicated that digestion was not completed within the 24 h timeframe. OLR could have been increased, either by decreasing the dilution of the green stream component of the substrate and thereby increasing the nitrogen contribution to the digester, or by increasing the DAF sludge component of the wastewater. Following addition of magnesium hydroxide, pH and gas production increased markedly.

3.3. Operational aspects

3.3.1. Inhibition/overloading

Both control and pre-treated groups experienced a significant reduction in biogas production during phase II, corresponding with the addition of a fresh batch of substrate (Fig. 6). During phase II, SBP decreased by 45% from a high of 2096–1.172 mL_N/g VS/L/day in the control group, and by 47% from 1700 to 909 mL_N/g VS/L/day in the treatment group. With respect to the change of substrate characteristics in phase II, HRT remained static at 8 days, while OLR was reduced to 0.85 ± 0.03 g/L/day, and the FOG load to the reactors doubled from 115 to 236 mg/L/day. Although TN was greater in the second batch of substrate, the pH of the reactors further declined, and intervention with magnesium hydroxide was necessary to prevent digester failure. As pH recovered, SBP recovered until the collection of completely mixed digestate was implemented on day 48.

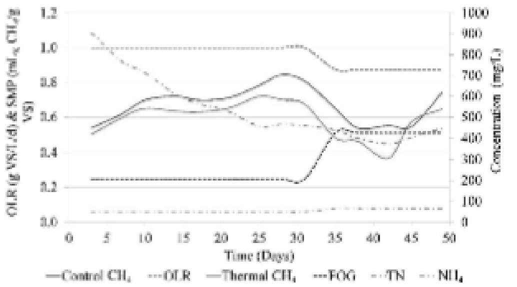

Fig. 6. Effect of substrate changeover on SMP.

As reported by Harris and McCabe [10], wastewater composition in RMP plants can vary significantly. The effect of substrate variation and the minor degree of shock loading demonstrated in Fig. 6 is indicative of the effect of substrate variation on the performance of industry-operated anaerobic digesters. This highlights the need for consistent feedstock for optimum anaerobic digestion. While some full scale AD facilities make use of an equalizing tank to reduce the severity of the peaks and troughs in wastewater characteristics over time, these fail-safes are not perfect, and there may be potential for pre-treatment to aid in the formation of more consistent feedstocks.

In order to reduce substrate variability, the substrate used in this study was semi-synthetic. Although elements of industrial waste streams were utilised, these components were diluted and added in proportions that achieved a desirable OLR. Despite this, the degree of control over feedstock variability was limited, and shock loading of FOG in the second batch was still enough to significantly affect AD performance. Hanaki, Matsuo and Nagase [27] reported similar inhibitory actions from shock loadings of LCFA in a system treating synthetic waste, where the addition of FOG to the reactors induced a lag phase in the degradation of acetate, LCFA and n-butyrate, and reduced the rate of methane production from hydrogen [27].

3.4. Comparison of batch vs continuous digestion

While simple BMP tests give a good indication of the amount of biogas and methane that can be ultimately produced from a substrate, these tests do not accurately reproduce the conditions of large-scale AD systems under continuous or semi-continuous operation [11]. Given that laboratory investigation to understand the biogas potential of a substrate is critical in making business and design decisions regarding the implementation of AD technology, it is important to consider the limitations of BMP tests, and the advantages and shortcomings of batch and continuous digestion investigations. Continuous digesters are more sophisticated systems that accommodate for the monitoring of operational parameters such as OLR, HRT, solids retention time, and critical parameters which reflect process stability, such as pH, organic acid load and system buffering capacity, and the effect of accumulation of various compounds. Consequently, many of the observations in this experiment would not have been possible under batch digestion.

Protein degradation appeared to be enhanced by pre-treatment, as indicated by the H_2S content in the biogas. However, pH in the thermobaric reactors was consistently lower than the controls, VFA:TA ratio was more variable, and response to change in substrate characteristics was more severe. Measurement of VFA:TA and ammonium concentrations indicate that digesters were subject to continual washout of buffer from the system, without a reasonable source of replacement such as proteinaceous substrate.

The effect of substrate variation also had distinct impact on AD performance in this experiment. Shock FOG load induced immediate digester failure, although digesters were recovered with $Mg(OH)_2$. This exemplifies the necessity for the Australian RMP industry, which is subject to extraordinary variation in substrate characteristics [10], to better regulate digester substrate characteristics in order to guarantee reliable performance in both methane yield and substrate degradation [28]. This can be achieved through use of an equalizing tank with a short HRT, allowing for the reduction of peaks and troughs in substrate characteristics [29].

While mono-digestion of porcine slaughterhouse waste has been demonstrated by Ortner et al. [30], BMP testing of individual and combined waste streams demonstrated that although the majority of methane was produced within 15–20 days, reactors required 48–81 days to complete digestion. Subsequent continuous digestion performed well under mesophilic conditions, with an OLR of typically 1.5–2.5 kg VS/m^3/d, and a HRT within a reasonable range of 20–40 days for the majority of the experiment [30]. However, under psychrophilic conditions of 25 °C, a more representative temperature for unheated and low-rate anaerobic digesters, digestion of the substrate seemed difficult. OLR was reduced to around 0.35, and HRT was increased up to 165 days [30]. Such operation is too slow and ineffective for systems such as covered high-rate anaerobic lagoons to be worthwhile as an energy source. Accordingly, slaughterhouse waste may lend itself better as an optimised substrate as in the case of co-digestion, and further potential for pre-treatment to significantly enhance degradation rates for such slowly degrading substrates.

4. Conclusion

Previous BMP investigation of thermobaric-treated DAF sludge yielded an 8.32% increase in SMP and eliminated lag-phase inhibition [1]. Subsequent investigation of thermobaric-treated substrate under continuous digestion resulted in reduced methane yield by 12.1% due to lost volatile organics, consistently lower digester pH, and enhanced protein degradation produced 56% higher H_2S concentrations. Alkalinity was consistently washed out of reactors, with insufficient replacement from the substrate, leading to steady pH decline during phase I. Although phase II decreased OLR, a concurrent increase in FOG loading caused digesters to fail. Addition of $Mg(OH)_2$ rapidly recovered digester pH, biogas production and significantly reduced H_2S concentrations.

Acknowledgement

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