Enhanced Energy Recovery in Australian Industry through Anaerobic Co-digestion

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NOMENCLATURE

AD	Anaerobic digestion
AcoD	Anaerobic co–digestion
BMP or B_0	Biochemical methane potential (L CH_4.kg^{-1} VS fed)
base substrate	The main waste being digested by a CAP or anaerobic digester
CH_4	Methane gas
CO_2	Carbon dioxide gas
CAP	Covered anaerobic pond
COD	Chemical oxygen demand, a measure of chemical energy (also tCOD for total fraction, or sCOD for soluble/filtered fraction)
co-substrate	The other waste elected to be co-digested with the base substrate
C/N	Carbon to nitrogen ratio
FOG	Fat Oil and Grease
GC	Gas chromatography
GHG	Greenhouse gas
H_2	Hydrogen gas
HA	Humic acid
HRT	Hydraulic retention time
ISR	Inoculum to substrate ratio
K_a	The acid-base equilibrium coefficient
k	First-order kinetic rate coefficient (d^{-1})
I_i	Inhibition term, describing the fraction of the maximum metabolic uptake rate that is measured under inhibited conditions, with a value between 0 for completely inhibited and 1 for no inhibition.
N_2	Nitrogen gas
Na^+	Sodium ion
NH_3	Free ammonia
OTU	Operational taxonomic unit
QLD	Queensland, A state in Australia
RMP	Red Meat Processing
SMA	Specific methanogenic activity (g COD_{CH4}.g^{-1} $VS_{inoculum}$.d^{-1})
S_i	Concentration of dissolved compound i
SRT	Solids retention time
NaCl	Sodium chloride
NH_4Cl	Ammonium chloride
t_d	Initial start-up time delay for anaerobic digestion (d)
T	Temperature (˚C)
TS	Total solids
TAN	Total ammonical-nitrogen
VFAs	Volatile fatty acids
VS	Volatile solids
X_i	Concentration of particulate compound i

1 EXECUTIVE SUMMARY

Project "Enhanced Energy Recovery in Australian Industry through Anaerobic Co-digestion" is a collaboration between the Australian Meat Processor Corporation, Queensland Urban Utilities, Pork CRC, Melbourne Water Corporation and The University of Queensland. The primary focus of the project is to improve the economics of biogas projects by maximizing renewable energy recovery (and revenue) from anaerobic digestion infrastructure. Co-digestion involves the simultaneous anaerobic digestion of two or more wastes. At a Red Meat Processor (RMP), other wastes (termed co-substrates) might be added together with combined wastewater (termed base substrate) into a covered pond or paunch solid waste (also termed based substrate) in an in-vessel digester to boost methane production. Gate fees charged for receiving/treating wastes may provide additional revenue. Of key concern is the microbial health of a digester or covered pond when co-digesting. Poor digestion performance can limit methane production, cause odour, and increase residues for post-handling and disposal. To prevent unhealthy digesters or covered ponds, safe volumetric and organic loading limits should not be exceeded. Of key concern was a current poor knowledge of the impacts of waste mixture composition and operating temperature on co-digestion loading capacity. To address this, the project was structured into two sub-projects. Sub-project 1 conducted fundamental laboratory studies via two PhD projects (1A and 1B), and Sub-project 2 validated the laboratory results by testing various industrial wastes and monitoring co-digestion trials at full-scale.

Sub-project 1A investigated the effect of waste mixture composition on co-digestion performance, and found that;

1) Carbon to nitrogen (C/N) ratio, as a lumped parameter describing waste mixtures composition, could not be reliably used to anticipate co-digestion performance. Instead, performance was influenced by the macro-composition in the wastes being digested (carbohydrate, lipid, protein).
2) Co-digestion performance would likely be reasonable if:
   a) adequate amounts of essential nutrients were available, but inhibition thresholds for ammonia were not exceeded; and
   b) loading limits were not exceeded, e.g. by adding too much carbon of a particular type.
3) Lipid/fat was a preferred co-substrate when treatment times are long enough for near-complete digestion. Carbohydrates were the next strongest co-substrate candidate. Proteins ranked last because of a high risk of ammonia inhibition.
4) When operating a mixed heated in-vessel digester at a loading of 2-3 $gCOD.L^{-1}.d^{-1}$;
   a) fat/lipid co-substrates could be added at 1 $kg.m^{-3}.d^{-1}$ (additional organic loading of 2.7-3 $gCOD.L^{-1}.d^{-1}$) with no anticipated negative impact and increasing methane production by 100-150% of the baseline amount.
   b) carbohydrate co-substrates can be added at 2.5 $kg.m^{-3}.d^{-1}$ (additional organic loading of 2.5-2.7 $gCOD.L^{-1}.d^{-1}$) with no anticipated negative impact and increasing methane production by 100-125% of the baseline amount. Higher loadings may be possible, but would likely increase undigested residue requiring post-treatment and disposal. If soluble sugars are used, these readily ferment and organic acids should be carefully monitored to prevent build-up.
   c) protein co-substrates can be added to increase organic loading, but negative impacts are likely. Methane production may improve by up to 100%, but protein co-substrates cause high ammonia concentrations which destabilises digestion performance.

Sub-project 1B investigated the effect of lower temperatures (15-25°C) on co-digestion capacity, as is relevant for ambient temperature covered ponds. This PhD project found that;

1) Microbial community structure in a digester (or covered pond) depends on the waste feed type, and that these different communities have different digestion capacities.
2) A decrease in temperature decreased the rates of biological processes, meaning that digestion will be slower at colder temperatures.

Anaerobic digestion (and co-digestion) involves a balance between upstream biological reactions that break down complex and particulate organic matter into dissolved intermediates and ferment these into organic acids, and downstream biological reactions that convert fermentation products ultimately into methane and carbon dioxide (i.e. biogas). Faster upstream and slower downstream reactions can cause a build-up of organic acids and digestion instability, ultimately leading to digestion failure.

3) A temperature decrease affected some biological reactions more than others, increasing the risk of imbalance and digestion failure at lower temperatures.
4) Protein-based wastes have a high risk of digestion failure and this risk increases at cooler temperatures.
5) Carbohydrate-based wastes also have increased process risk at cooler temperatures, particularly for soluble sugar-based wastes.
6) Lipid-based wastes were comparatively low risk, because upstream reactions remained slower than downstream reactions, even at cooler temperatures.

For practical application, maximum co-substrate loads could occur during Spring-Summer when a covered pond operates at warmer temperatures. During Spring-Summer there is more flexibility to include protein, but low protein amounts are generally recommended. Co-substrate loads should be reduced during Autumn-Winter at cooler operating temperatures. During cooler months, protein-based wastes and soluble sugar based wastes should be avoided or at least minimised. Complex cellulosic wastes may be applied, but at roughly 50% lower loading in cooler months than in warmer months. Lipids are generally slow to digest and likely require long treatment times.

<u>The applied research in Sub-project 2 tested 30 wastes as possible co-substrates and found that:</u>

1) Glycerol (GLY) and Fat Oil and Grease (FOG) ranked highest in most scenarios due to very high concentration (high space loading possible) and good biological performance. GLY and FOG addition would need to be controlled to prevent overload and failure, but these risks can be managed. The settling/floating behaviour of FOG was not assessed and may impact co-digestion in covered ponds.
2) Macerated food waste and other food industry wastes were generally strong candidates for co-digestion due to rapid digestion, low impact on residual solids and low inhibition risk. However, these wastes are relatively dilute and volumetric loading constraints may limit their use.
3) Agricultural samples had mixed rankings. Energy dense agricultural wastes such as Dissolved Air Floatation sludge or protein and lipid-rich animal screenings could be suitable and could be digested completely if retained for adequate treatment times. Lower energy agricultural samples, such as paunch, waste activated sludge and pig manure ranked poorly unless used as base substrate at the sites where they are produced. These lower ranked substrates could be co-digested with high energy co-substrates.

<u>Sub-project 2 also tracked two full-scale co-digestion trials:</u>

Trial 1 was conducted at a municipal wastewater treatment plant co-digesting pre-treated sewage sludge (base substrate) with beverage wastewater (co-substrate). During Trial 1, co-substrate dosing was intermittent and ad hoc and was a small fraction of the total organic load to the digester. Consequently, changes in methane production were limited. The results were consistent with findings from the laboratory testing in Sub-project 1. Trial 2 was conducted at a piggery co-digesting pig manure (base substrate) and dewatered cattle paunch (co-substrate) in stirred heated in-vessel digesters. Although cattle paunch was a poorly ranked co-substrate in the assessments above, Trial 2 was in a rural area where co-substrate options were limited. During Trial 2, co-substrate addition was more consistent than in Trial 1 and the co-substrate was 60-100% of the total solids loading. Improvements in methane production ranged from 80% to 100% depending on the mass of paunch added. The results were consistent with findings from the laboratory testing in Sub-project 1. These project outcomes were translated into a Microsoft Excel-based co-digestion simulation tool that estimates anticipated methane production, residual solids, digestate/sludge properties, and risk of exceeding digestion capacity.

Overall, from an economic perspective, co-digestion needs to balance improvements in biogas revenue with increased residue disposal costs and increased nutrient management costs. The loading capacity for co-substrates depend on carbon types (carbohydrates/lipids/proteins), operating temperature and digester configuration (covered pond vs. mixed heated digester).

2 INTRODUCTION

2.1 Anaerobic co-digestion purpose

Anaerobic digestion (AD) is an established technology utilizing naturally occurring microorganisms to produce methane-rich biogas from organic wastes. Anaerobic co-digestion (AcoD) involves the simultaneous AD of two or more substrates, with the objectives being to process available wastes in a more environmentally sustainable manner, to maximize biogas yields and to improve the stability of the AD process. The increase in methane production by AcoD of high-energy co-substrates with the base substrate (e.g. sewage sludge, pig manure, meat processing wastewater) can leverage existing anaerobic processes and infrastructure (Mata-Alvarez et al., 2011). For example, existing covered ponds (CAPs) or anaerobic digesters could be dosed with AcoD substrates, such as solid wastes (e.g. crop residues), liquid wastes (e.g. Fat Oil Grease (FOG)), by-products from local industry (glycerol, whey, algae), or other. In the case of glycerol, for example, organic loading rates and biogas production can increase by 20-50%, even with a moderate increase of 1-2% in the volumetric loading of the digester or CAP. Glycerol, is highly concentrated and nearly 100% convertible into methane (Mata-Alvarez et al., 2014). The biggest concern with glycerol and other similar high-strength wastes, is a lack of knowledge about potential inhibitors present in such wastes and the high risk of exceeding safe organic loading limits (Mata-Alvarez et al., 2014). For example, glycerol can be inhibitory due to the presence of methanol from biodiesel production or monovalent cations (e.g. sodium). It is also very important that co-substrates are suitable for the type of digestion system being used, e.g. high solid wastes may not be suitable for CAPs, and high solids wastes likely require dilution before addition to a mixed vessel digester.

AcoD could promote linkages and cooperation between industries, in dealing with wastes from one industry in a more environmentally sustainable manner by becoming the co-feedstock for co-digestion in another industry. Such linkages broaden the potential applications for AD by exporting wastes from

smaller facilities where standalone AD is not cost-feasible, to another larger industry where it is feasible. This contributes to sustainable management of nutrients, such as nitrogen and phosphorus. For these reasons, the quality of residue remaining after AcoD (termed digestate) is also important (Mata-Alvarez et al., 2014). Ideally digestate is beneficially reused as a nutrient fertilizer. If digestate quality is compromised, then it needs to be disposed of at additional cost. For example, when sewage sludge is co-digested with manure, it improves biogas yield and process stability (Al Seadi et al., 2013), but strict requirements can limit the application of digestate as a fertilizer, because of heavy metals, persistent organic pollutants and pathogens (Al Seadi et al., 2013). The cost feasibility of AcoD is largely determined by the costs of transport to bring wastes to the site (Mata-Alvarez et al., 2014).

Waste diversion away from landfill can save landfill space, reduce whole of life impacts of landfilling and reduces post-closure maintenance of landfills (Nghiem et al., 2017). However, in Australia, landfill levies and diversion targets are implemented on a state by state basis (Edwards et al., 2015). State-based legislation is informed by a collaborative committee consisting of members of state governments and the federal government (Edwards et al., 2015). The landfill levy is still a new approach in Australia, but is already helping diversion rates, especially in New South Wales (Edwards et al., 2015). Unfortunately, a general lack of clear, robust and nation-wide waste diversion policy is a major hurdle to widespread adoption of AcoD in Australia (Nghiem et al., 2017).

2.2 Anaerobic co-digestion microbiology

Anaerobic digestion and AcoD are reliant on a balance of biological reactions carried out by various functional microorganism groups progressively breaking down complex composite organic matter into simpler intermediate products and ultimately into methane, carbon dioxide and other trace by-products (Figure 1). Upsets and failure in the AD or AcoD process are typically caused by an imbalance between (Chen et al., 2008):

(a) upstream acid-forming microbial reactions carried out by bacteria responsible for the upstream reactions of hydrolysis, acidogenesis and acetogenesis; and
(b) downstream methane-forming reactions carried out by methanogenic archaea.

An imbalance in upstream and downstream microbial reactions typically leads to an accumulation of volatile organic acids (Ahring et al., 1995), which can depress pH and cause inhibition, ultimately resulting in AcoD failure (Chen et al., 2008; Demirel & Scherer, 2008; Sträuber et al., 2012). It can be difficult or costly to recover from such failures, because it may require alkali dosing to increase pH of the liquid content in the failed CAP or digester (Tait et al., 2009). This imbalance and system failure can result from poor selection of AcoD waste mixtures or by excessively increasing the organic loading rate of the CAP or digester beyond safe limits. Therefore it is important to understand the impact of substrate mixtures on AcoD and to understand safe organic loading capacities.

Additional risks of AcoD include substrate mixtures being incompatible, again leading to overload, instability or potentially process failure. For example, it is important that wastes selected for AcoD would digest quicker than the base substrate for which the digestion system was originally designed. Otherwise, the retention time and treatment capacity may be inadequate to accommodate the slower degrading waste (Al Seadi et al., 2013). Some wastes rich in FOG, are excellent methane boosters because of high methane potential, but require very long treatment times to be converted into methane (Al Seadi et al., 2013). This is especially true at low operating temperatures, such in CAPs during winter months, because biological reactions dramatically slow down at lower temperatures

(Batstone et al., 2002). The selection of AcoD mixtures is very important to ensure that the digestion remains stable and reasonable.

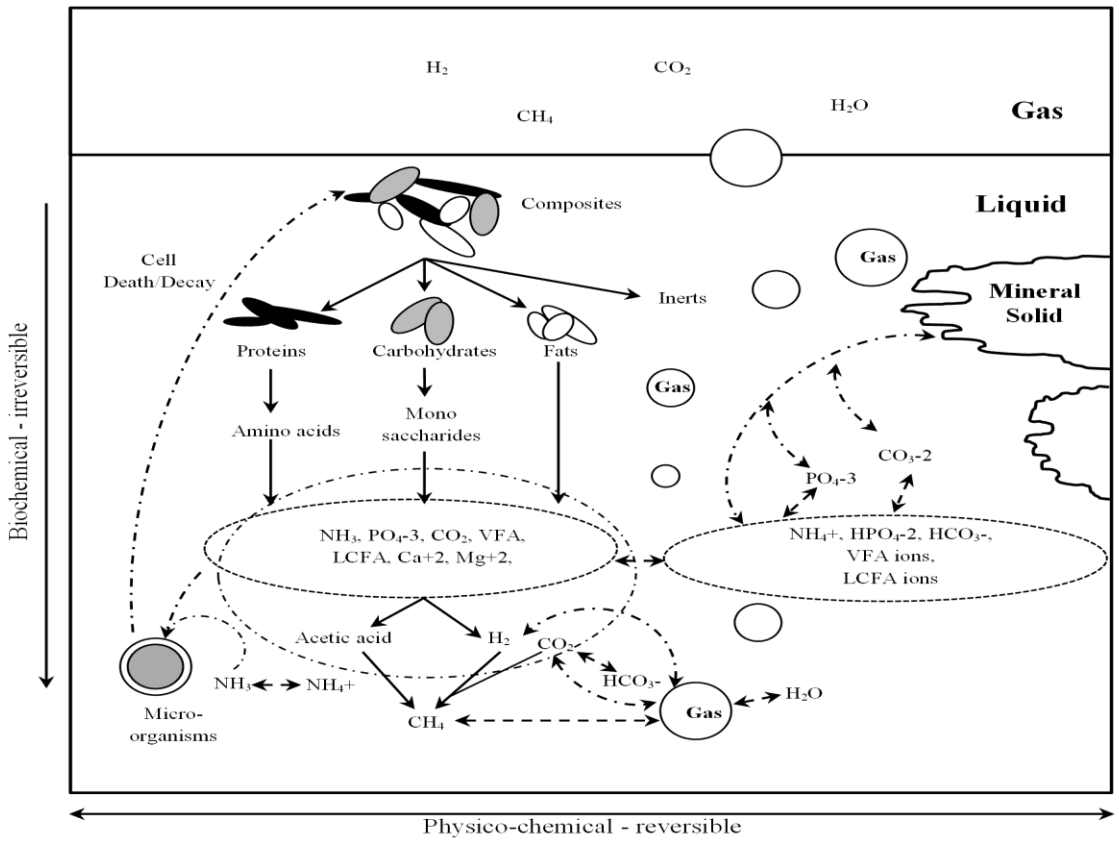

Figure 1: A schematic of key anaerobic digestion reactions, progressively breaking down composite particulate wastes into soluble intermediate products and ultimately into methane. Adapted from (Batstone, Keller et al. 2002).

2.3 Anaerobic co-digestion waste selection

Classified by sector, waste types that can be co-digested include vegetable by-products and residues, industrial organic wastes, by-products and residues from agricultural industrial origin, food wastes, fodder and brewery wastes, organically rich wastewaters from industrial sectors, and organic by-products from biofuel production and biorefineries (Al Seadi et al., 2013). Co-digestion mixtures can reduce inhibition and enhance AD. This is achieved by co-digesting a high nitrogen waste (e.g. pig manure) with preferably carbon-rich wastes, i.e. wastes having a high carbon to nitrogen ratio or C/N ratio (Astals et al., 2011). When such wastes are co-digested, the nitrogen-rich waste can increase alkalinity, stabilizing digestion pH, and providing macro or micro-nutrients, whilst the other wastes dilute strong inhibitors such as ammonia (Mata-Alvarez et al., 2014) and increase active biomass (Al Seadi et al., 2013). Overall, these effects can make AcoD more resilient to inhibitors, thereby enhancing digestion stability and performance, and increasing methane production (Al Seadi et al., 2013). In general, the use of residues is probably preferred over energy crops (as used in parts of Europe, e.g.

Germany), so that methane (energy) production would not compete with food production (Pietsch, 2014).

The waste type that can be co-digested also depends on the type of digestion system being used (Al Seadi et al., 2013; Batstone & Jensen, 2011). For example, additional wastes being added with AcoD may exacerbate sludge formation or increase digestate volumes after AcoD, increasing post-treatment and/or disposal costs. Some particulate wastes may be completely unsuitable for CAPs, because of float layers forming under the pond cover being inaccessible to microbes, or may interfere with biogas collection (Bochmann & Montgomery, 2013) or exacerbate sludge accumulation.
Co-digestion can also influence important properties of digestate or sludge, changing for example dewaterability (i.e. ability to remove moisture to produce a stackable product), but currently these impacts are poorly understood (Nghiem et al., 2017).

Australia generates over 14 million wet tonnes of organic waste per year (excluding animal and crop wastes) and approximately 50% is currently disposed to landfill. Currently, co-substrates are available from a broad range of industries that generate organic wastes. The physical, chemical and biological properties of co-substrates can be very different and are therefore suited to different AD technologies and different waste mixtures. Estimated national organic waste production for selected industries is summarised in Figure 2.

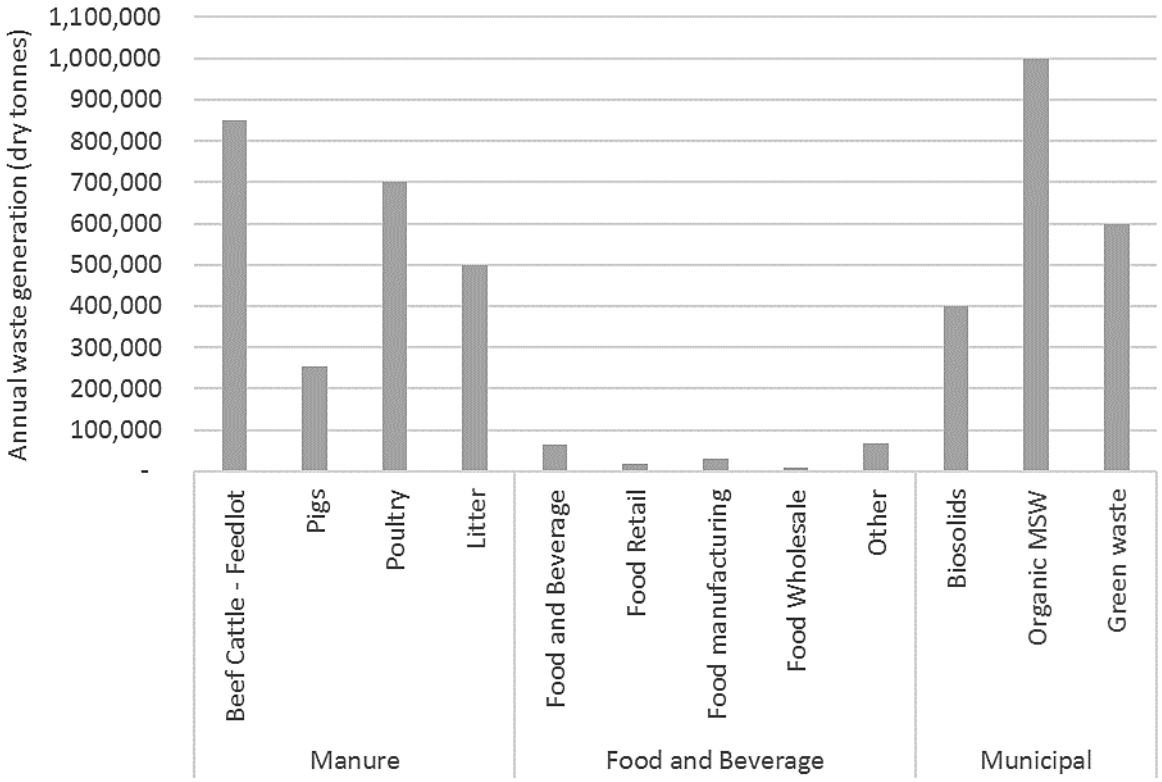

Figure 2: Estimated Australian organic waste production for selected industries. Note manure streams from animals in pasture are not collected and therefore not included in this figure (dairy cattle, sheep, beef cattle pasture).

National databases are increasingly being used to document the location and quantities of biomass suitable for anaerobic digestion. See for example, the Biomass Producer (http://biomassproducer.com.au/) and AREMI (http://nationalmap.gov.au/renewables/). Detailed

geographical waste mapping is being conducted as part of ongoing projects funded by the Clean Energy Finance Corporation, however the outcomes were not available at the time of writing this report.

The selection of co-substrates should generally focus on:

i. compatibility of the waste with the biogas technology,
ii. availability and/or variability in the waste stream,
iii. revenue (biogas, fertilizer value of residue, potential tradewaste income for taking waste),
iv. cost (residual solids disposal, residual nutrient removal, transport, receival facility capital, potential cost for purchase of waste)

The economics of co-digestion are generally highly variable (Table 1). For example, concentrated high energy wastes such as grease trap waste or glycerol can be economically attractive, however these wastes have multiple re-use options and so can often be in demand. Therefore, in some cases the biogas gas facility may actually instead be charged a fee to purchase such wastes. Whereas, lower energy wastes such as manures, crop residues or green wastes may be less attractive due to lower biogas revenue and higher residual disposal costs. However, in some cases, the biogas gas facility may receive an income stream (i.e. charge a gate fee) to accept and process such wastes.

Table 1: Economic benefit of biogas produced per tonne of co-digestion substrate

Substrate	Methane Yield (m^3 per t)	Volatile solids (VS) destruction (%)	Energy Value ($ per t)	Disposal Cost ($ per t)	Total Benefit ($ per t)	Carbon Offset (t CO_2-e per t)
FOG	1,000	80	$231	$40	**$190**	**4**
Glycerol	460	100	$106	$0	**$106**	**1.8**
Paunch	250	60	$58	$100	**$-40**	**1**
Feedlot Manure	200	40	$44	$171	**$-130**	**0.8**

Basis for calculation:
1. Energy content of methane = 34 MJ/m^3
2. Conversion to electricity = 0.35
3. Electricity value: 3.6 MJ = 1 kW.h and 1kW.h = $0.07
4. Disposal/beneficial reuse of residuals: $300 $tonne^{-1}$ (ANZBP)

Transport costs are difficult to predict, but can be excessive and very important for economic feasibility. Therefore, co-substrates should be selected from locally available material. Waste production is known to be region specific and dependent on local industries and population densities (food waste is approximately 0.8 tons $person^{-1}$ $year^{-1}$).

2.3.1 General Considerations in Anaerobic Co-digestion

Adding co-substrate to a continuous anaerobic digester or CAP needs careful consideration. When a co-substrate is added to an existing digester or CAP, the organic loading rate (OLR) of the digester or CAP generally increases. While this increased OLR can greatly enhance biogas production, it also decreases the hydraulic retention time (HRT). This can be problematic for digestion infrastructure that are operating close to design loads, because a shortened HRT reduces the time for degradation and microbial growth to occur and can consequently impact on digester performance, biogas production and digestate quality. Accordingly, wastes of high organic strength and low volume are often targeted, as a small increase in volumetric loading can considerably increase the OLR of the digester, leading to enhanced biogas production. Also, ideal co-substrates are also expected to be highly biodegradable with fast degradability kinetics. AcoD with poorly biodegradable co-substrates can adversely impact on digestate biosolids quality to the degree where the cost for handling and post-treatment can outweigh the economic benefits from enhanced biogas production.

It is worth noting that co-substrate selection can be base-substrate dependent. For example, paunch grass (a waste product from slaughterhouses) usually features low biodegradability due to the presence of lignocellulosic material and would not be an ideal co-substrate from a biodegradability perspective. However, when paunch grass is co-digested with fats, the lipid-degrading organisms in paunch can improve fat biodegradability, resulting in a synergistic effect (Astals et al., 2014). The impact of paunch grass on secondary properties, such as digestate dewaterability, has not been previously considered and is needed to evaluate overall feasibility.

The speed of co-substrate degradation is also an important factor, especially for hydrolysis controlled AD systems. For example, Wastewater Treatment Plant (WWTP) digesters are normally designed for a minimum HRT based on specific requirements for the stabilisation of sewage sludge. Therefore, it is vital to select a co-substrate which digests at the same speed or faster than sewage sludge, to ensure sufficient destruction of organic matter and acceptable biosolids quality. Otherwise, AcoD of slowly degraded co-substrate relative to the main substrate is likely to result in increased un-degraded organic matter in the digestate, which will incur extra costs to treat or dispose of. In the case of AcoD at municipal WWTPs, co-substrates of inherently low nitrogen and phosphorus contents would be preferred. With increasingly stringent regulations for nutrient discharge limits (e.g. lower effluent nitrogen and phosphorus concentrations), costs associated with power consumption for nitrification and adding external carbon sources for de-nitrification are on the rise. This means that co-digesting a waste of high nitrogen content can lead to nitrogen-rich supernatant returning to the mainstream wastewater treatment chain for biological nitrogen removal. The same applies to high-phosphorus co-substrates, which can result in increased costs for coagulants to remove phosphorus. Therefore, weighing the extra biogas benefit against the costs for high-nitrogen and/or high phosphorus supernatant treatment is necessary.

At full-scale, the ease of obtaining a co-substrate and economics of implementing AcoD with the co-substrate can be a dominating factor (Parry, 2013). This means that selection of substrate mixtures at full-scale is often ad hoc, and optimisation of dosing strategies based on specific waste characteristics is needed. Numerous lab-scale research studies have investigated the technical aspects of waste mixtures, and optimising carbon-to-nitrogen (C/N) ratio of substrate mixture has been regarded a critical factor in the literature. A detailed discussion follows.

2.3.2 C/N ratio in the selection of AcoD mixtures

Published literature often considers C/N ratio an important parameter for optimizing waste mixtures and blend ratios for enhanced biogas production. The concept is based on the carbon content of a waste representing potential for methane production (hence energy recovery); and the nitrogen content representing nutrients available for microbial growth (Smith and Holtzapple, 2011). Waste mixtures where the C/N is excessively high may cause nutrient limitations that prevent maintenance of the microbial population, while a low C/N typically represents high ammonia and increased inhibition risk. Therefore, from an energy-nutrient balancing perspective, C/N ratio has been proposed as one of the most important factors by many research studies for selection of co-substrates.

In a practical sense, utilizing C/N for design of co-digestion mixtures has generally involved mixing a nitrogen-rich substrate such as animal manure (Liu et al., 2015; Mata-Alvarez et al., 2014) with a carbon-rich substrate such as fruit waste (Fonoll et al., 2015) and glycerol (Jensen et al., 2014), so that the final mixture falls within the C/N range between 20 to 70 (Mata-Alvarez et al., 2011). This is typical of the majority of studies investigating C/N ratios which aim to establish an optimal substrate mixture C/N ratio at which biogas production or/methane production rate is maximised. If an optimal ratio exists, the C/N ratio of a co-substrate would need to complement that of the base substrate to achieve optimal C/N for the mixture. However, optimal C/N ratios reported in literature are not consistent and cover large ranges. A key challenge when assessing the impact of C/N ratio is the different measurement techniques and ratios used to represent C/N in the literature. This makes comparison of optimal C/N ratios difficult. Table 2 highlights some different measurement methods, and inconsistencies in the identified optimum C/N values (Mao et al., 2015, Puyuelo et al., 2011, Ramos-Suárez and Carreras, 2014). The AcoD review by Mata- Alvarez et al. (2011) reports a broad optimum C/N ratio spectrum (20-70).

Inconsistent C/N ratio optima reported in literature may also indicate that C/N ratio alone is not the most critical factor in balancing co-digestion mixtures and that the type of carbon present may be an important factor. Unfortunately, very few studies have considered the relative importance of waste macro-compositions (carbohydrate, protein, and lipids) and C/N ratios on digester performance. For example, adjusted to a common C/N ratio, AcoD mixtures of different carbon sources would impact the process kinetics and methane yield differently due to different AD degradation pathways and methane potential of the carbon forms, rather than due to C/N ratio. Therefore, it is not clear if C/N ratios should be a priority in designing co-substrate mixtures or if guidelines should be industry-specific or universally applied. This has highlighted the significance of investigating substrate mixture compositions and how they would impact AcoD process.

2.3.3 Substrate macro-composition in the selection of AcoD mixtures

The composition of organic matter can be categorised into 3 main groups based on the components and chemical structure, namely carbohydrates, proteins and lipids. The relative fractions of carbohydrates, protein, and lipids represent the macro-composition. As shown previously in Figure 1, carbohydrates, protein and lipids degrade through different metabolic pathways and often require different microbial consortia. However, macro-composition also impacts on methane potential (and therefore biogas revenue) and process kinetics (influencing treatment times and overload risks) (Astals et al., 2014).

Carbohydrates, protein and lipids possess different methane potential due to their chemical structure and carbon form. The theoretical COD values, and more specifically the COD to VS ratios represent the

chemical composition and give an indication of their energy density (Henze et al., 2008) and theoretical methane potential. Examples of theoretical methane potential for model substrates are shown in Table 3, with lipids generally having a very high methane potential, protein a moderate methane potential and carbohydrates a lower methane potential. In many cases, complex carbohydrates will occur as lignocellulosic materials (such as the structural material in plants). Lignin is not biologically degradable and this further reduces the practical methane potential of many lignocellulose/carbohydrate materials.

Although energy dense co-substrates (e.g. lipid-based wastes) can increase theoretical methane potential, the organic loading limits (OLL) to an AD process will actually impact on biogas production from the process. This is because if co-substrate dosage exceeds safe limits or excessively shortens HRT, then the AcoD process could be overloaded or could fail, which instead reduces or stops biogas production.

Organic acid accumulation resulting from high organic loading has been reported as a key process risk for AcoD (Wang et al., 2013). Excessive organic acids impact AD processes through multiple mechanisms. High acid concentrations reduce system pH. At lower pH organic acids become un-dissociated and can diffuse into microbial cells causing toxicity. Therefore, organic acid inhibition is linked with the pH and the alkalinity of the system (Chen et al., 2008). Inhibitory concentrations of organic acids are variable and more closely linked to balancing the kinetics of acid-producing steps (acetogenesis) with acid-consuming steps (methanogenesis). Adapted microbial consortia are able to tolerate higher organic acid levels. But in general, limited or distributed dosing for rapidly degradable co-substrates is required to avoid overloading. In general proteins and carbohydrates have faster co-digestion rate kinetics (e.g. digestion rates k=0.5 to 2.0 d^{-1}) than fats (k= 0.1 – 0.7 d^{-1}) (Siegrist et al., 2002). Therefore, loading rates would likely be dependent on macro-composition.

Ammonia nitrogen (TAN) is one of the most common inhibitory compounds managed in AD processes, and is primarily related to the protein macro-composition. Ammonia is formed during the anaerobic digestion of proteins, urea and nucleic acids which are prevalent in AD feedstocks from Agri-industries (Rajagopal et al., 2013). Free ammonia (NH_3) is generally the inhibitory form of ammonia and exists in equilibrium with ammonium ions (NH_4^+). Two key parameters which affect this equilibrium are pH and temperature (Emerson et al., 1975). AcoD can reduce ammonia inhibition through i) dilution, ii) lowering pH via rapidly biodegradable co-substrates and Volatile Fatty Acid (VFA) accumulation or iii) altering alkalinity by substrate addition (Mata-Alvarez et al., 2014).

Long chain fatty acids (LCFA) are another key compound that must be managed in AD, related to the lipid macro-composition. LCFA are produced during lipid hydrolysis and can inhibit AD microbiology by adsorbing onto the surface of microbial cell membranes, affecting membrane functionality and cellular transport processes. Due to the adsorbing nature, the toxicity of LCFAs is more correlated to substrates' physical characteristics, such as specific surface area and size distribution than to the biological characteristics (Chen et al., 2008). Mesophiles are more resistant to LCFAs compared to Thermophiles, potentially due to different cell membrane composition (Hwu and Lettinga, 1997), or reduced solubility of lipids at mesophilic temperatures limiting LCFA-membrane contact.

Table 2: Summary of C/N ratio based AcoD mixture selection for improved methane production

C and N measurement	Substrate mixture	Optimal C/N ratio(s)	references
Elemental analysis	portions of microalgae residuals with paper sludge, *Opuntia maxima*, glycerol	~7	(Ramos-Suárez & Carreras, 2014)
TC[a]:TN[b]	SS+ lime pre-treated bagasse	~30	(Rughoonundun et al., 2012)
Elemental analysis	Pig manure+ rice straw	~25	(Li et al., 2015a)
TOC[c]:TKN[d]	food waste+ rice husk	20	(Haider et al., 2015)
TC:TN (elemental analysis)	steam exploded Salix+ cattle manure	35-40	(Estevez et al., 2012)
TOC:TKN	(dairy&chicken manure)+wheat straw	~27	(Wang et al., 2012)
TOC:TAN	reed+(faeces+kitchen waste)	22-25	(Wang et al., 2015)
Organic Carbon (dry):Total nitrogen (dry)	sisal pulp+ fish waste	16	(Mshandete et al., 2004)
TC:TN	goat manure+ three crop residues	20-35	(Zhang et al., 2013)
TOC:TN	(Dairy manure& chicken manure)+ rice straw	25 at 35°C; 30 at 55°C	(Wang et al., 2014)
COD:TKN	Pig manure+ corn stalks/wheat straw/oat straw	20	(Wu et al., 2010)
TC:TKN	algal sludge+ waste paper	20-25	(Yen & Brune, 2007)
TOC:TKN	soybean protein isolate+ maize starch	30	(Liu et al., 2008)
(TOC-VFA C):TN	office paper+ wet chicken manure (extra urea addition)	37	(Smith & Holtzapple, 2011)
(TOC-lignin C):TKN	dairy manure+ glucose/cellulose	25	(Hills, 1979)
C(%TS)/N(%TS)	Industrial food waste+ sewage sludge	15	(Siddiqui et al., 2011)

[a] total carbon; [b] total nitrogen; [c] total organic carbon; [d] total kjeldahl nitrogen

Table 3: Theoretical characteristics of typical substrate components (Angelidaki & Sanders, 2004)

	Composition	COD/VS ($gCOD.gVS^{-1}$)	CH_4 yield ($L\ CH_4.gCOD^{-1}$)	CH_4 yield ($L\ CH_4.gVS^{-1}$)	Biogas composition ($\%CH_4$)
Carbohydrate	$(C_6H_{12}O_6)_n$	1.07	0.35	0.373	50
Protein	$C_5H_7O_2N$	1.42	0.35	0.496	55
Lipids	$C_{57}H_{104}O_6$	2.90	0.35	1.014	70
Ethanol	C_2H_6O	2.09	0.35	0.730	75
Acetate	$C_2H_4O_2$	1.07	0.35	0.373	50
Propionate	$C_3H_6O_2$	1.51	0.35	0.530	58

Overall, the inhibition mechanisms resulting from acid accumulation, free ammonia and LCFA are different, and inhibitory concentrations can be highly variable depending on adaptation of biomass. Importantly, each of these common inhibition scenarios are linked to the macro-composition of AD and AcoD mixtures.

2.4 Temperature impacts on the selection of AcoD mixtures and AcoD performance

Temperature is known to significantly impact on biochemical and physio-chemical processes in AD (De Vrieze et al., 2015; Vanwonterghem et al., 2015). Metabolic steps in AD (Figure 1) can be affected by temperature to different extents (Ge et al., 2011; Ho et al., 2014; Van Lier et al., 1996). This means that a change in temperature could exacerbate or resolve imbalances between upstream and downstream metabolic processes, and therefore can increase or decrease AcoD process risk.

Previous studies (Ban et al., 2013; Ge et al., 2011; Mingardon et al., 2011; Van Lier et al., 1996) have mostly focused on temperature impacts with individual process rates rather than for the complete microbial activity profile relevant to AcoD mixtures. Also, most prior studies have studied systems fed with model substrates (Ban et al., 2013; Van Lier et al., 1996) rather than complex AcoD mixtures.

Knowledge of the temperature-dependency of the complete activity profile and relative process rates allows identification of biological process bottlenecks. For example the ratio of hydrolytic activities (upstream) to downstream metabolic activities (e.g. propionate degradation) could be different at 37°C as compared to 15°C, which can increase or decrease process risk from adding complex substrate mixtures. Whilst, significant previous research has been undertaken for mesophilic (37°C) and thermophilic (55°C) AD (Li et al., 2015b; Zhao et al., 2018), studies on psychrophilic AD are few (Massé et al., 2003). This represents a significant risk to Australian industry where a number of sites are operating CAPs at ambient conditions, with seasonal fluctuations in temperature of up to 20°C (Skerman & Collman, 2012). A greater fundamental understanding of temperature impacts could allow seasonally optimised AcoD mixtures and loading with such ambient temperature CAPs.

3 PROJECT OBJECTIVES

To address the key research questions identified in the literature, the project aimed to develop co-digestion strategies (and a co-digestion manual) to improve energy recovery from anaerobic digestion while maintaining process stability. Specific objectives that address this aim include:-

(a) Investigate novel co-substrates for WWTP and agri-industry
(b) Optimize dose strategy to balance maximum biogas production while maintaining process stability, develop co-digestion manual/recommendations.
    - Determine maximum co-digestion dosage rates and compare for different base substrates
    - Predict impact of process conditions such as temperature, pH on optimal dose strategy
    - Determine changes in AD microbial communities at each dose rate
    - Determine failure conditions and recovery time to regain process stability after overdose/failure
(c) Validate co-digestion model and contribute to cost benefit analysis.
(d) Establish if co-digestion will have adverse impacts on secondary biosolids properties (pathogen levels, dewaterability and additional volatile solids destruction).

The project structure includes detailed fundamental analysis leading to development of a co-digestion manual (Sub-project 1), and validation of outcomes through full-scale co-digestion trials (Sub-project 2).

3.1 Sub-Project 1: Fundamental Knowledge

Sub-project 1 focused on characterizing the degradation properties of organic materials based on substrate and co-substrate compositions, and a consideration of substrate interactions, synergisms and antagonisms. The experimental platform initially focused on batch testing and will expand to continuous reactor operation and modelling. Proposed activities to develop fundamental knowledge in sub-project 1 included:

a) Determine degradation rates of model substrates representing carbohydrates, proteins, lipids. Develop model parameters and examine using complex substrates from partner industry;
b) Investigate impact of substrate macro-composition on degradation rate by adding model substrates (Carb, protein, FOG) to a well characterised base substrate (e.g. Waste Activated Sludge);
c) Determine the overload/inhibition threshold of model co-substrates and the inhibition mechanism.

3.2 Sub-Project 2: Applied Anaerobic Co-digestion

Sub-project 2 tested the fundamental knowledge and predictive models using complex substrates. Activities included:

(a) Investigate novel co-substrates for WWTP and agri-industry using batch tests;
(b) Establish substrate interactions, synergies and antagonisms, investigate the mechanisms;
(c) Optimize dose strategy of promising candidates to maximize biogas production while maintaining process stability;
(d) Test optimal dose strategies against different base substrates from partner industries (e.g. WAS, pig manure, slaughterhouse effluent).

4 METHODOLOGY

4.1 Analytical Methods

Table 4 provides a summary of analytical methods used in this project. For measurement of soluble COD (sCOD), TAN and PO_4-P, samples were centrifuged (5 min at 4,000 rpm) and filtered through a syringe filter (0.45 µm PES membrane) prior to analysis. For total COD (tCOD) and total nutrients and metals, samples were analysed as collected.

Table 4: Summary of general analytical methods used in the co-digestion project

Analysis	Description
Biogas composition	H_2, CH_4, CO_2 analysed using gas displacement, and gas chromatography with a Shimadzu GC-2014 equipped with a thermal conductivity detector (GC-TCD), electronic gas sampling valve (1 mL loop) and a HAYESEP Q 80/100 packed column (2.4 m length; 1/800 outside diameter, 2 mm inner diameter). The chromatograph injector, oven and detector temperatures are set at 75, 45 and 100 °C, respectively and Argon (99.99%) was the carrier gas at 28 mL min^{-1} and 135.7 kPa.
Total Solids (TS) Volatile solids (VS)	Total solids (TS) and volatile solids (VS) were measured in accordance with standard methods procedure 2540G (Franson, Eaton et al. 2005).
VFAs	Individual VFAs (acetate, propionate, butyrate, valerate, and caproate) and alcohols (methanol, ethanol, and butanol, where relevant) were analysed with Agilent 7890A gas chromatograph with Agilent DBFFAP column.
Chemical Oxygen demand (COD)	Estimates the organic content of a sample. Also an order of magnitude estimate of chemical energy present in the sample (i.e. the energy released by each gCOD converted to CO_2 and H_2O by being chemically oxidised). Chemical oxygen demand (COD) was measured using Merck Spectroquant® cell determinations and a SQ 118 Photometer (Merck, Germany).
Total Kjeldahl Nitrogen and Phosphorous (TKN and TKP) Key Soluble Nutrients (NO_3^-, NO_2^-, amoniacal nitrogen, PO_4^-)	Nutrients (solid form and soluble). Nutrient content is related to resource recovery opportunity. Nutrient content may also impact downstream processing requirements (i.e. secondary treatment after AD). Total Kjeldahl nitrogen (TKN), total phosphorus (TP), total ammoniacal nitrogen (TAN), and phosphate-phosphorus (PO_4-P) were measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee).
Metals (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg,	Trace metals in a sample impacts on both the digestate quality and reuse potential. Trace metals may also provide resource recovery opportunities. Trace

Analysis	Description
Mn, Mo, Na, Ni, P, Pb, S, Se, & Zn)	metals were measured using Inductively Coupled Plasma Optical Emission Spectrometry.
Alkalinity	Measured by titrating a volume of sample with HCl to end points of pH 5.7 and pH 4.3. Partial alkalinity was determined using the pH 5.7 endpoint and represents alkalinity contributed by hydroxides, ammonia, carbonate and bicarbonate. Intermediate alkalinity was determined as the difference between alkalinity to pH 5.7 and alkalinity to pH 4.3 and represents the contribution by organic acids. The alkalinity ratio (α) is defined as the ratio of partial alkalinity to intermediate alkalinity; with ratios <0.3 representing a healthy process (Ripley, Boyle et al. 1986).
Biochemical methane potential (BMP)	BMP measures methane potential recoverable from AD of a sample under ideal conditions with excess presence of an active and balanced microbial inoculum. BMP was assessed using methods developed in conjunction with the IWA Anaerobic biodegradability, Activity and Inhibition Task Group (Angelidaki, Alves et al. 2009).

4.2 Digestate Dewaterability

Dewaterability was assessed for specific samples using a centrifuge method developing in conjunction with Bucknell University. Initially, 100 mL of conditioned solids was free drained through belt filter fabric for 1 min to establish a filter cake. The cake was scraped into a custom designed belt filter cup (example shown in Figure 3) and centrifuged as follows:

1. 200 times gravity for 2 min.
2. 500 times gravity for 2 min.
3. 3000 times gravity for 10 min.

The cake was mixed and redistributed across the filter cloth between each centrifuge cycle. After centrifugation, the moisture/solids content of the cake as for TS (Table 4).

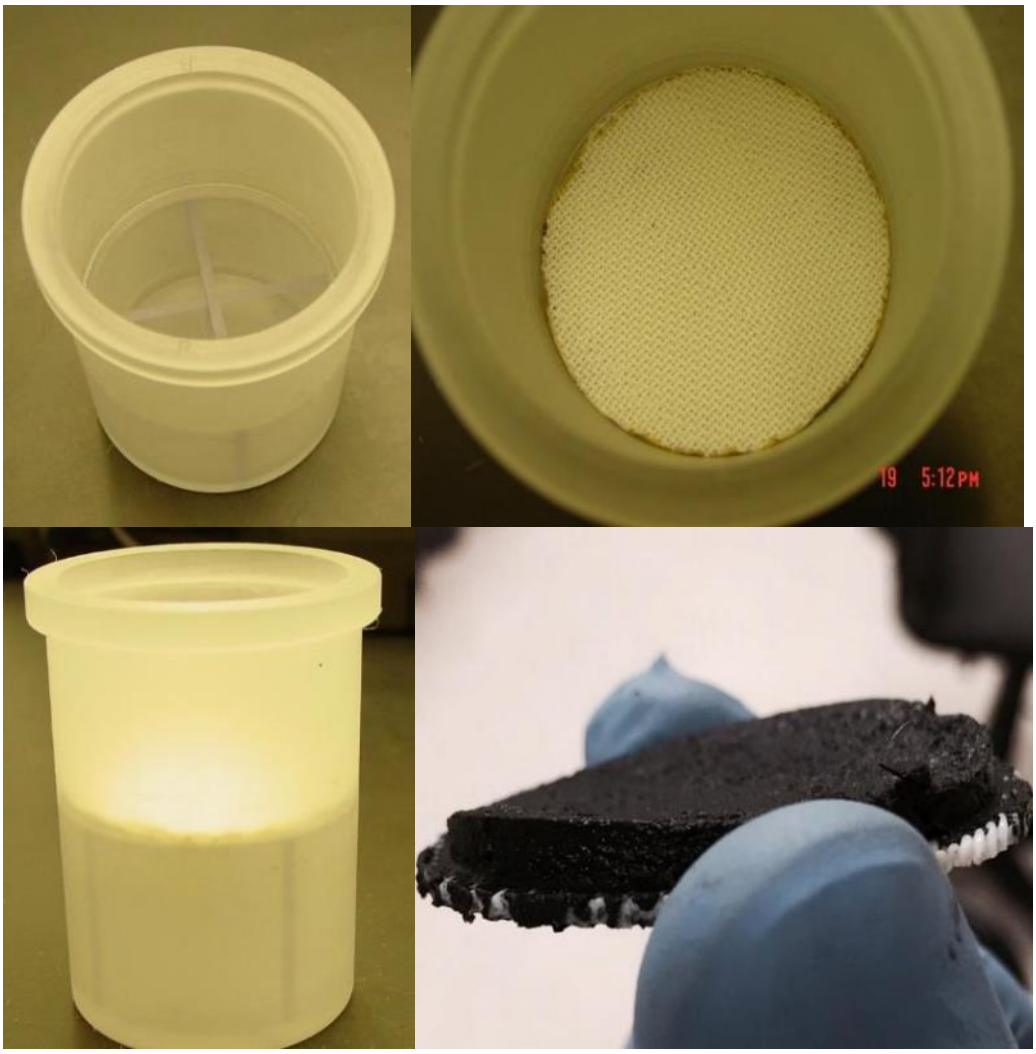

Figure 3: Custom-designed belt filter cups for dewaterability analysis

4.3 Biochemical methane potential testing

Biochemical methane potential (BMP) was assessed using methods developed in conjunction with the IWA Anaerobic biodegradability, Activity and Inhibition Task Group (Angelidaki, Alves et al. 2009). BMP tests were conducted in a minimum of 3 replicates in 160 mL serum bottles (approx. 100 mL working volume). The selection of inoculum, the inoculum to substrate ratio and the presence of nutrient medium are key design parameters in the BMP test. General test AWMC recommends:

The BMP tests assess feed degradability (Figure 1). Normally it is used to assess apparent first order hydrolysis rate (khyd), as well as ultimate degradability (fd). An example result is shown in Figure 5.

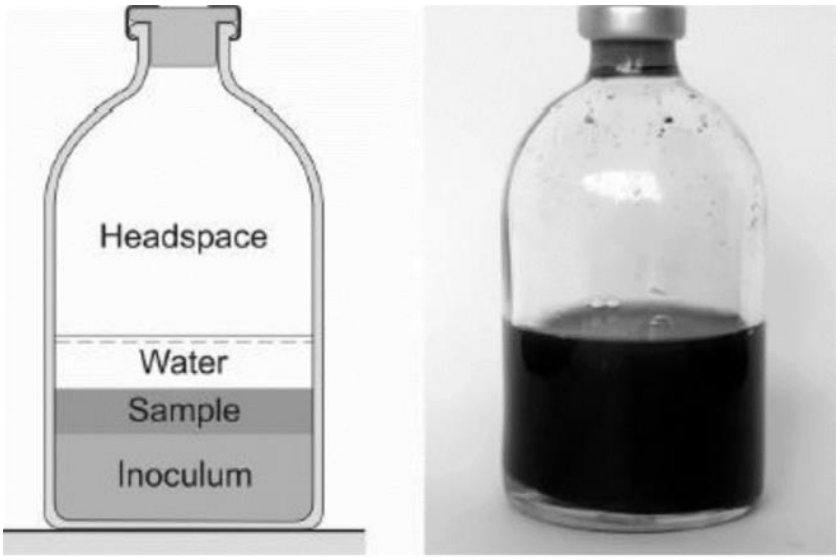

Figure 4: **Example of batch biochemical methane potential assay.**

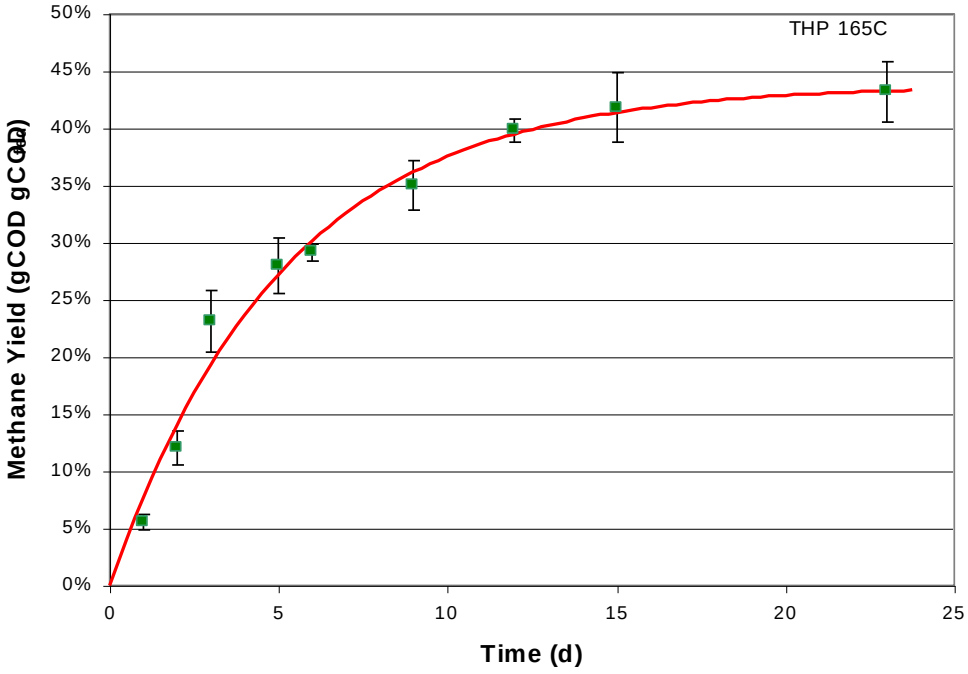

Figure 5: **Example output from biological methane potential (BMP) test. Error bars indicate 95% confidence errors from triplicate batches. The line indicates the model used to return key parameters.**

4.4 Model Based Analysis

4.4.1 Batch Model Analysis

Batch BMP tests will be used to generate independent degradability parameters fd and khyd for the inoculum and the test samples. Parameters will be determined using a simple first order kinetic model as expressed as:

$$\frac{dS_t}{dt} = -k_{hyd} \times S_t \qquad (1)$$

Where t is the incubation time, S is the degradable portion of substrate remaining at time t, and khyd is the first order hydrolysis rate constant. The parameter estimation will be done using model based software (Aquasim 2.1d). Kinetic parameters can be used to estimate the size and capital costs of process equipment (e.g. reactors).

3.2.1 Inhibition Modelling

In some tests, more complex modelling may be applied to determine specific inhibition characteristics of a substrate, such as the inhibition constant (K_I). Hydrolysis is generally the rate limiting step in AD, therefore first order degradation equations are typically applied. A first order model with inhibition function is shown as Equation 2. Where soluble compounds are the primary substrate Monod kinetics with an inhibition function (shown in Equation 3) would be an alternative approach for inhibition modelling (but unlikely to apply in this project). The inhibition function is based on non-competitive inhibition as per Equation 4:

$$\frac{dS_{CH4}}{dt} = k_{hyd} S\, I \qquad (2)$$

$$\frac{dS_{CH4}}{dt} = B_o k_{m,} \frac{1}{1+K_S/S} I \qquad (3)$$

$$I = \exp\left[-2.77\left(\frac{S - K_{I,\min}}{K_{I,\max} - K_{I,\min}}\right)^2\right] \qquad (4)$$

Where $K_{I,min}$ represents the concentration where inhibit commences and $K_{I,mas}$ represents the concentration where microbial activity is completely inhibited.

5 PROJECT OUTCOMES: FUNDAMENTAL KNOWELDGE

5.1 Impact of Substrate Carbon to Nitrogen (C/N) Ratio

5.1.1 Background

This work, conducted as part of Mike Meng's PhD project, tested the importance of C/N ratio during the selection of AcoD substrate mixtures. The specific objective was to better understand the impact of C/N ratio for different substrate macro-compositions (e.g. carbohydrates, protein, and lipids). Hydrolysis, acidogenesis, acetogenesis and methanogenesis are the 4 key metabolic steps in AcoD. These steps occur in sequence with interconnected microbial functionalities (Figure 1). However, with complex organic substrates, such as particulate AcoD substrates, hydrolysis is often most important to convert complex organic matter into accessible soluble substrates (Batstone and Jensen, 2011). For this reason, the present work focussed on C/N ratio influence specifically of hydrolytic capacity.

5.1.2 Methods

Hydrolytic activity tests were conducted using the BMP method in Section 4.1, with tests in 160 mL glass serum bottles at 37°C. In all cases, the inoculum was sludge from a healthy mesophilic digester at a South-East Queensland municipal WWTP fed with a 1:1 mix of primary and waste activated sludge (on a volume basis). This digester operated at a hydraulic retention time (HRT) of 23-24 days and a temperature of 35-37°C. The inoculum and substrate (Table 1) were added to each BMP test bottle at a VS ratio of 2 (inoculum VS: substrate VS). Once added, the initial pH of the mixture was measured, the headspace of the bottle was flushed for 1 min (4 $L \cdot min^{-1}$) with 99.99% nitrogen gas, the bottle was promptly sealed with a rubber stopper and aluminium crimp, and placed in a temperature controlled incubator. Periodically, each test bottle was removed from the incubator, mixed by gently swirling, and samples of headspace gas collected using a gas-tight syringe and a fine-gauge needle. The headspace gas pressure was measured as an overpressure on the collected gas sample volume in a bench-top liquid displacement manometer, and the composition of the gas sample was subsequently determined by GC-TCD (Section 4.1). A separate control test was run with inoculum only and no substrate, to determine background methane production from added inoculum. This background methane was later subtracted from methane produced by test bottles to give net methane produced over time from digestion of the added substrate. The net cumulative methane produced by each test bottle was normalised to the amount of substrate VS added to the test bottle.

Two sets of experiments were performed. In the first experiment, either α-cellulose ($C_6H_{10}O_5$) or oleic acid ($C_{18}H_{34}O_2$) was added as carbon substrate, and an amount of AR grade ammonium chloride was added to achieve a test C_{added}/N_{added} ratio of 1, 5, 20, 40, and 80. **Note that background nitrogen and carbon of added inoculum was NOT included in calculated C/N ratios, which instead are based on the added substrate and ammonium salt.** Due to different chemical formulas of substrates, external N added was different for the cellulose and oleic acid tests, resulting in different added total ammoniacal nitrogen (TAN) concentrations (Table 5). A second experimental set was conducted using the same substrates and a new batch of inoculum from the same source. In this second set, ammonium chloride was added to give added TAN at 0.5, 1.0, 1.5, 2.0 and 3.0 $g.L^{-1}$ for both cellulose and oleic acid as substrates (Table 5).

Table 5: Summary of conditions for batch experiments testing the effect of C/N ratio and ammonia concentration on anaerobic digestion of cellulose and oleic acid as model carbon substrates at 37°C. The initial pH data given are average values ± error estimate at the 95% confidence level. The TKN concentration of the inoculum in Experiment 1 was 2.4±0.3 g.L-1 and in Experiment 2 was 1.9±0.6 g.L^{-1} (including 1.3±0.3 g TAN.L^{-1}).

Carbon substrate	$C_{substrate}/N_{added}$ ratio	TAN added (g.L^{-1})	Initial pH
	Experiment set 1		
Cellulose	-	Cellulose control	7.5 ± 0.1
	1	4.1	7.2 ± 0.1
	5	0.9	7.5 ± 0.1
	20	0.2	7.6 ± 0.1
	40	0.1	7.6 ± 0.1
	80	0.06	7.5 ± 0.1
Oleic acid	-	Oleic acid control	7.0 ± 0.3
	1	6.3	6.6 ± 0.2
	5	1.4	6.8 ± 0.1
	20	0.4	6.8 ± 0.1
	40	0.2	6.9 ± 0.1
	80	0.1	6.9 ± 0.1
	Experiment set 2		
Cellulose	-	Cellulose control	7.6 ± 0.1
	-	0.5	7.6 ± 0.1
	-	1.0	7.6 ± 0.1
	-	1.5	7.6 ± 0.4
	-	2.0	7.5 ± 0.2
	-	3.0	7.5 ± 0.1
Oleic acid	-	Oleic acid control	7.3 ± 0.2
	-	0.5	7.3 ± 0.2
	-	1.0	7.2 ± 0.1
	-	1.5	7.2 ± 0.1
	-	2.0	7.2 ± 0.1
	-	3.0	7.2 ± 0.1

"-" means not applicable

5.1.3 Results and Discussion

Figure 6 shows methane production results from the first experiment. The results indicated that digestion is insensitive to C/N ratio over a broad range of values (5-80 for cellulose; 20-80 for oleic acid). For cellulose there was no significant difference in net methane production from the added substrate at C/N ratios of 5 to 80, with overlap of 95% confidence intervals in Figure 6. Only at very low C/N ratios did tests with cellulose show deterioration in digestion performance, likely due to ammonia inhibition because of high TAN (Table 5). Similarly, for oleic acid, there was no significant difference in net methane production from the added substrate at C/N ratios of 20 to 80. However, recoverable inhibition was observed at a C/N ratio of 5, causing a slowing in the initial rate of methane production. As expected, the methane yield from oleic acid was much greater than for cellulose, because of the higher energy content of oleic acid.

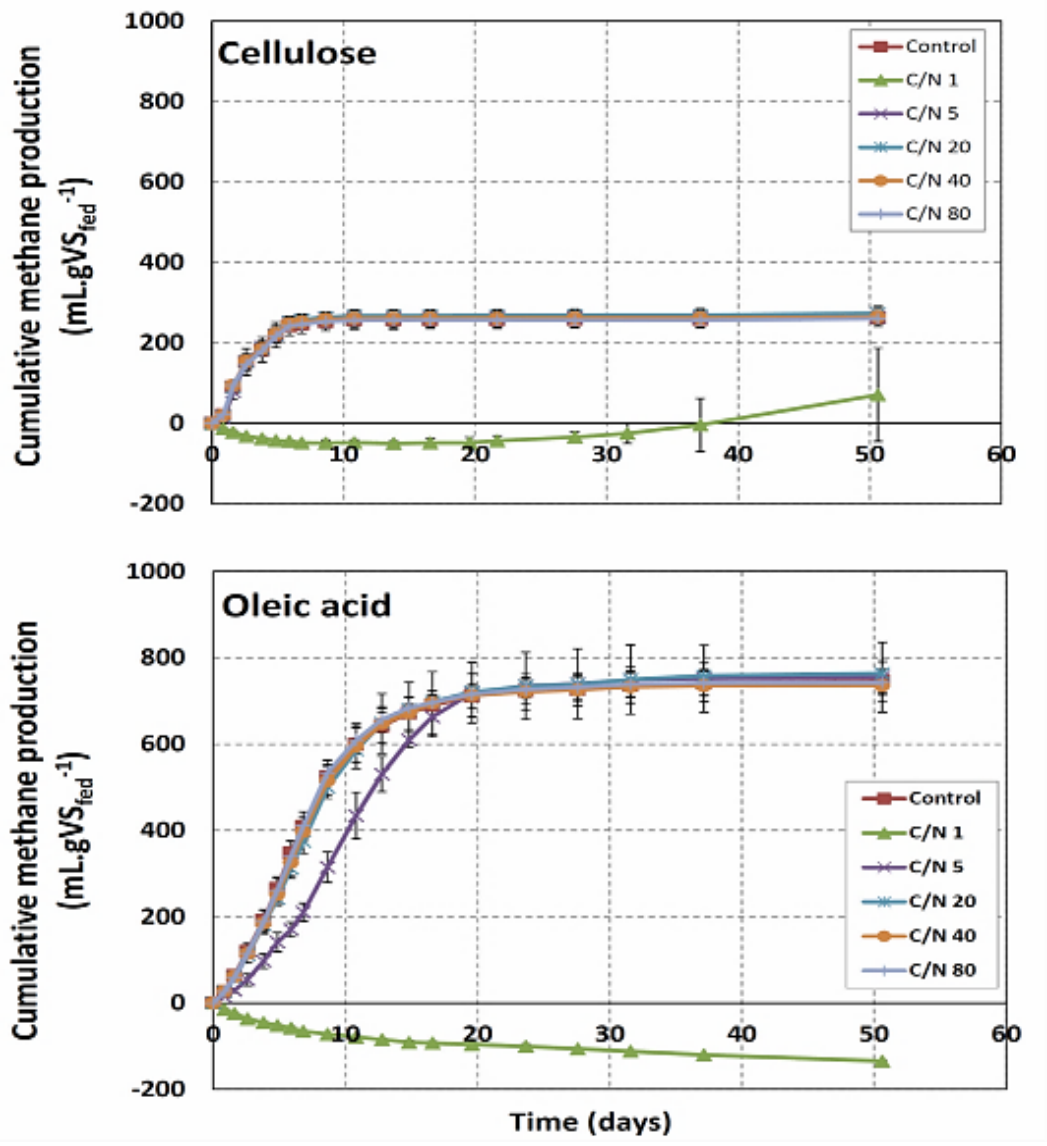

Figure 6: Cumulative methane produced during batch testing at various indicated C/N ratios, for the said carbon substrates at 37°C. The data are average values for triplicate tests and error bars are 95% confidence intervals.

Figure 7 shows methane production data for experiment two. For cellulose, there was no significant difference in net methane production when added TAN was at or below 2.0 $g.L^{-1}$, corresponding to total TAN of 3.3 $g.L^{-1}$. This is seen by 95% confidence intervals overlapping in Figure 7. For oleic acid, there was no significant difference in net methane production from added substrate when added TAN was at or below 1.0 $g.L^{-1}$, corresponding to total TAN of 2.3 $g.L^{-1}$. However, at an added TAN of 1.5 $g.L^{-1}$ (2.8 $g.L^{-1}$ total TAN), a significant delay was observed in the onset of methane production with oleic acid, and this effect worsened at the higher added TAN of 3 $g.L^{-1}$.

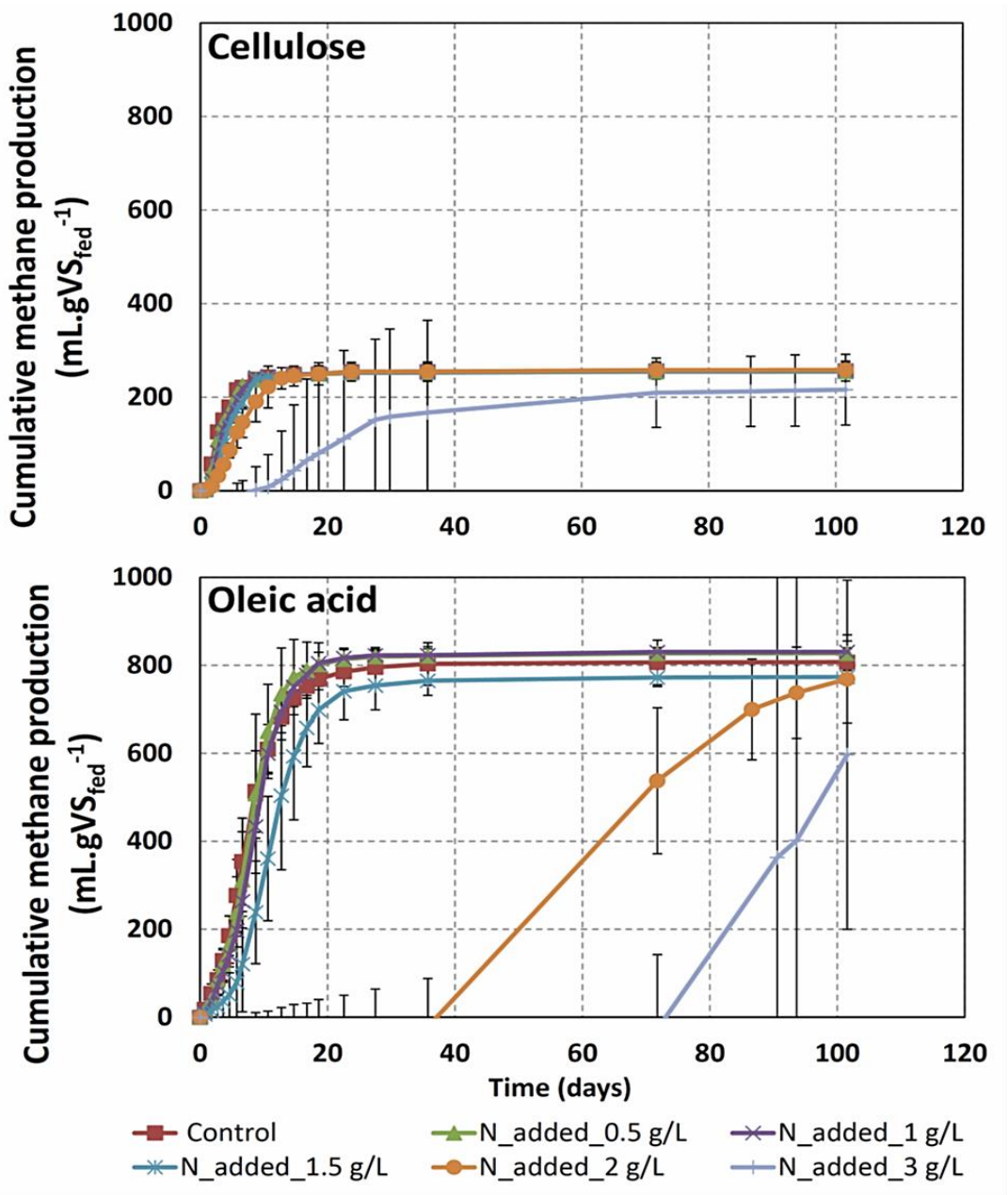

Figure 7: Cumulative methane produced during batch testing at various indicated total ammonia concentrations (added), and for the said carbon substrates, at 37°C. The data are average values for triplicate tests and error bars are 95% confidence intervals.

It was interesting to note that, when compared to cellulose, oleic acid was more inhibited by added TAN. This was possibly due to synergistic inhibition by ammonia together with the oleic acid substrate itself. These results also suggested that inoculum used in the tests had different ammonia tolerances depending on the carbon substrate being digested (i.e. cellulose or oleic acid). This shows that waste mixture macro-composition should be considered during AcoD mixture selection. This was implied in past studies where C/N ratios have been investigated by mixing one substrate with low C/N ratio (e.g. pig manure) with another substrate with high C/N ratio (e.g. energy crops). In the general trend of past studies, optimum C/N ratios are identified as the mixture with higher methane yield, typically C/N between 10-30 (Mata-Alvarez et al., 2011). However, to the best of our knowledge, no previous studies have systematically studied the impact of macro-composition of base substrate and co-substrate in the mixture. Therefore it is probably not surprising that available literature studies vary widely in terms of suggested "optimum" C/N ratios. For example, the maximum dose of some co-substrates such as glycerol and FOG is limited by secondary inhibitory mechanisms, whilst the deficiency of alkalinity or essential nutrients can limit the dosage of energy crops and paper waste (Passos et al., 2018).

5.1.4 Summary

The experiments described in this section showed that macro-composition (in terms of carbon substrate type) is also important for selection of AcoD waste substrate mixtures. C/N ratio by itself was found to be an inadequate predictor of anticipated digestion performance. Methane production was near-identical over a wide range of C/N ratios (5-80 for cellulose; 20-80 for oleic acid). Negative impacts occurred at low C/N ratios. At C/N ratios of 5, only methane production from oleic acid was inhibited, although inhibition impact was minor. At C/N ratios of 1, methane production from both cellulose and oleic acid were severely inhibited, attributed to high TAN concentrations and thus ammonia inhibition. However, threshold ammonia concentrations were different for the two different carbon sources. Specifically, when cellulose was the carbon source, TAN inhibition occurred at 3.3 $g.L^{-1}$ TAN, whereas when oleic acid was the carbon source, severe inhibition already occurred at 2.8 $g.L^{-1}$ TAN.

The results demonstrated that digestion would likely be reasonable, provided that adequate amounts of essential nutrients are available and do not exceed inhibition thresholds. Also, safe organic loading limits should not be exceeded by the addition of co-substrate to a CAP or digester. The differences in observed behaviour for cellulose and oleic acid highlighted the importance of substrate mixture macro-composition in determining digestion performance. Following on from the test work in this section, targeted experiments described in the next section sought to determine organic loading limits with different substrate macro-compositions.

5.2 Impact of Co-digestion Substrate Type on Organic Loading Capacity

5.2.1 Background

It is difficult to estimate the organic loading limits for individual anaerobic co-digestion applications prior to testing. This is because organic loading limits for co-substrates can vary with composition of the co-substrate and with the composition of the base substrate, leading to interactions that influence general health and resilience of the digestion system. For example, in the case of glycerol (a widely studied AcoD co-substrate), the loading threshold is said to be an additional 1% w/w (wet basis) with sewage sludge as base substrate (Fountoulakis et al., 2010; Jensen et al., 2014), an additional 4% w/w with pig manure as base substrate (Astals et al., 2012), and an additional 10% w/w with cattle slurry as base substrate (Robra et al., 2010). These differences can be due to:

1) differences in macro-composition (fat vs protein vs carbohydrates) of the base substrate vs the co-substrate, leading to compositional effects such as alkalinity (synergistic) or chemical inhibition (antagonistic) (Mata-Alvarez et al., 2014); and/or
2) capacity of the digestion system to adapt and tolerate increased organic loading for particular substrate types (in terms of macro-composition) (Jensen et al., 2014); and/or
3) relative digestion rates and yields of the base substrate vs the co-substrate as affected by macro-composition, leading to a balance or imbalance of upstream and downstream biological reactions (Regueiro et al., 2016).

There is a clear need for systematic studies on organic loading limits to inform co-substrate selection for AcoD. This requires a careful consideration of co-substrate effects in terms of macro-composition (carbohydrates, proteins and lipids). Accordingly, specific research objectives of the present work were:

1) To determine co-digestion dosage limits for a real and complex base substrate, being co-digested with model co-substrates representing ideal macro-composition of carbohydrates, proteins or lipids; and
2) To clarify digestion failure conditions and recovery time (if possible) to regain process stability after overdose/failure.

To achieve these objectives, a set of experiments were conducted, as part of Mike Meng's PhD project, using well-controlled continuous anaerobic digesters fed with sewage sludge as well characterised complex base substrate, co-digested with model co-substrates (cellulose, gelatine, and oleic acid) added at various increasing loading rates up to the point of overload/failure.

5.2.2 Methods

5.2.2.1 Continuous digesters

Primary Sludge and Waste Activated Sludge were sourced separately from a municipal WWTP in South East Queensland. Fresh sludge was collected fortnightly and, prior to collection, was thickened by centrifugation at the sewage treatment plant. The thickened primary sludge was sieved using 4mm stainless steel mesh with 4mm aperture (BS 410/ 1986, Endecotts Ltd London, Ser no: 944406) to remove coarse solids that could clog the feeding pumps/lines of the continuous digesters. A mixture was then prepared with primary sludge and waste activated sludge at a 1:1 VS ratio, and the concentration of the final mixture was adjusted to 40 g tCOD.L^{-1} by dilution with deionized water. This mixture was the base substrate for operation of the digesters. Fresh feed was prepared three times per week.

Four continuous bench scale anaerobic digesters (working volume of 5 L) were constructed and commissioned for the tests (Figure 8). The digesters were completely mixed during operating, using overhead motorised stirrer units. Temperature was maintained using a heating/cooling jacket with water circulated to and from a heated/cooled water bath. These digesters were inoculated with the same digester sludge as was used as inoculum in the batch tests of Section 5.1. For an initial establishment period (Day 0 – Day 80), the digesters were fed with the base substrate described above.

Figure 8: Four continuous bench-scale digesters constructed and operated to test organic loading limits for a complex base substrate co-digested with model co-substrates

After the initial establishment period and over the remainder of the experiment, the test digesters (R2, R3, R4) were instead fed with base substrate plus a model co-substrate (i.e. AcoD) added at a nominal percentage increase in organic loading (Table 6), whilst the control digester (R1) continued to be only fed with base substrate. R2 was fed with base substrate and α-cellulose as co-substrate, being a model complex carbohydrate. R3 was fed with base substrate and gelatin as co-substrate, being a model protein. R4 was fed with base substrate and oleic acid as co-substrate, being a model lipid. The co-substrates were added to the feed bottle together with base substrate, which was then connected to the digesters for feeding. Through the course of the experiment, the co-substrate loading was progressively increased step-wise, and held at a particular loading for a respective operational period to identify loading limits (Table 6).

Table 6: Operating periods for the continuous bench-scale digesters examining co-digestion loading limitations based on co-substrate macro-composition

Period	Time	Approximate base substrate organic loading	Target increase in R2-R4 organic loading by addition of co-substrate	Operating Notes
1	Days 0–80	2.2 gCOD.L^{-1}.d^{-1}	0	All digesters operating at 37°C and HRT of 18 days
2	Days 80–291	2.2 gCOD.L^{-1}.d^{-1}	50%	All digesters operating at 37°C and HRT of 18 days
3	Days 280–491	2.2 gCOD.L^{-1}.d^{-1}	100%	All digesters operating at 37°C and HRT of 18 days
4	Days 491-636	2.2 gCOD.L^{-1}.d^{-1}	125%	All digesters operating at 37°C and HRT of 18 days

Throughout the digester operation, biogas volume being produced over time was measured with a tipping-bucket flow meter. Biogas samples from the headspace of each digester were collected three times per week to measure composition (hydrogen, methane, and carbon dioxide) using GC (Section 4.1). During these sampling events, digester feed and effluent samples were also collected and analysed for pH, TS, VS, tCOD, sCOD, TKN and TKP, TAN and VFAs, as described Section 4.1. pH was only monitored and was NOT adjusted. VS destruction and COD removal was calculated using Equations 1 and 2, where *t* is experimental sampling time, and *t*-1 is the time at the previous sampling event:

$$VS_{destruction,(t)} = \frac{VS_{feed,(t-1)} - VS_{effluent,(t)}}{VS_{feed,(t-1)}} \times 100\% \quad (1)$$

$$COD_{removal,(t)} = \frac{COD_{feed,(t-1)} - COD_{effluent,(t)}}{COD_{feed,(t-1)}} \times 100\% \quad (2)$$

5.2.2.2 Batch digestion testing

Separate aliquots of digestate were collected from each of the four digesters, typically over a week during each of the respective operational periods (i.e. with changed organic loading in R2-R4). This digestate was stored in a refrigerator and when an adequate amount was collected, the composite was placed in an incubator at 37°C for a day prior to use. This digestate was subsequently used as inoculum in separate batch digestion experiments. These experiments tested the capacity of each inoculum to digest each of the model co-substrates, namely α-cellulose, gelatin, and oleic acid. These tests followed a near-identical method as described in Section 5.1, except that the collected inocula were first pre-diluted to a common 15 g $COD.L^{-1}$ with deionized water, and was then added together with the model substrate at a ratio of 1 part COD as substrate to 2 parts COD as inoculum. Since hydrolysis was expected to be rate-limiting in this experiment, net methane production from added substrate was measured as described in Section 4.3, and normalised to the amount of substrate COD initially added to each test bottle.

5.2.3 Results and Discussion

5.2.3.1 Continuous digester operation

Figure 9 presents a time trend of OLR for each of the four continuous digesters. The OLR of R1 (control digester) of 2.29 ± 0.04 g $COD.L^{-1}.d^{-1}$ was moderate compared to typical OLRs of mesophilic completely mixed digesters (Pastor et al., 2013; Razaviarani et al., 2013). The targeted dose increases of 50%, 100% and 125% in loading to R2-R4 resulted in OLRs of 2.95-3.35 g $COD.L^{-1}.d^{-1}$, 3.90-4.36 g $COD.L^{-1}.d^{-1}$ and 4.16-4.5 g $COD.L^{-1}.d^{-1}$, respectively. These increased OLRs were moderate to high as compared to typical loadings of mesophilic completely mixed digesters (Pastor et al., 2013; Razaviarani et al., 2013).

Figure 9 also presents time trend methane production data and calculated VS destruction for each of the four digesters, and Table 7 gives average values (±95% confidence intervals) for measurements and calculations during each operational period. During the initial 80 days start-up period, methane production and volatile solids destruction was statistically identical for the four digesters. When organic loading rate in R2-R4 was increased, methane production increased as expected, and was significantly higher than that of R1. A significantly higher VS destruction in R2-R4 in Period 2-4 (Table 7) as compared to the control, is due to the addition of the highly biodegradable model co-substrates to R2-R4. However, the residual TS in the effluent of R2 and R4 increased substantially with the additional organic load of the co-substrate. This is important because, whilst VFAs remained low in R2 and R4 (Figure 10) throughout the experimental period, the increase in residual solids in the effluent suggested that R2 and R4 had inadequate digestion capacity for the applied organic loading. This has obvious impacts for real applications, because an increase in residual solids can increase post treatment and disposal costs.

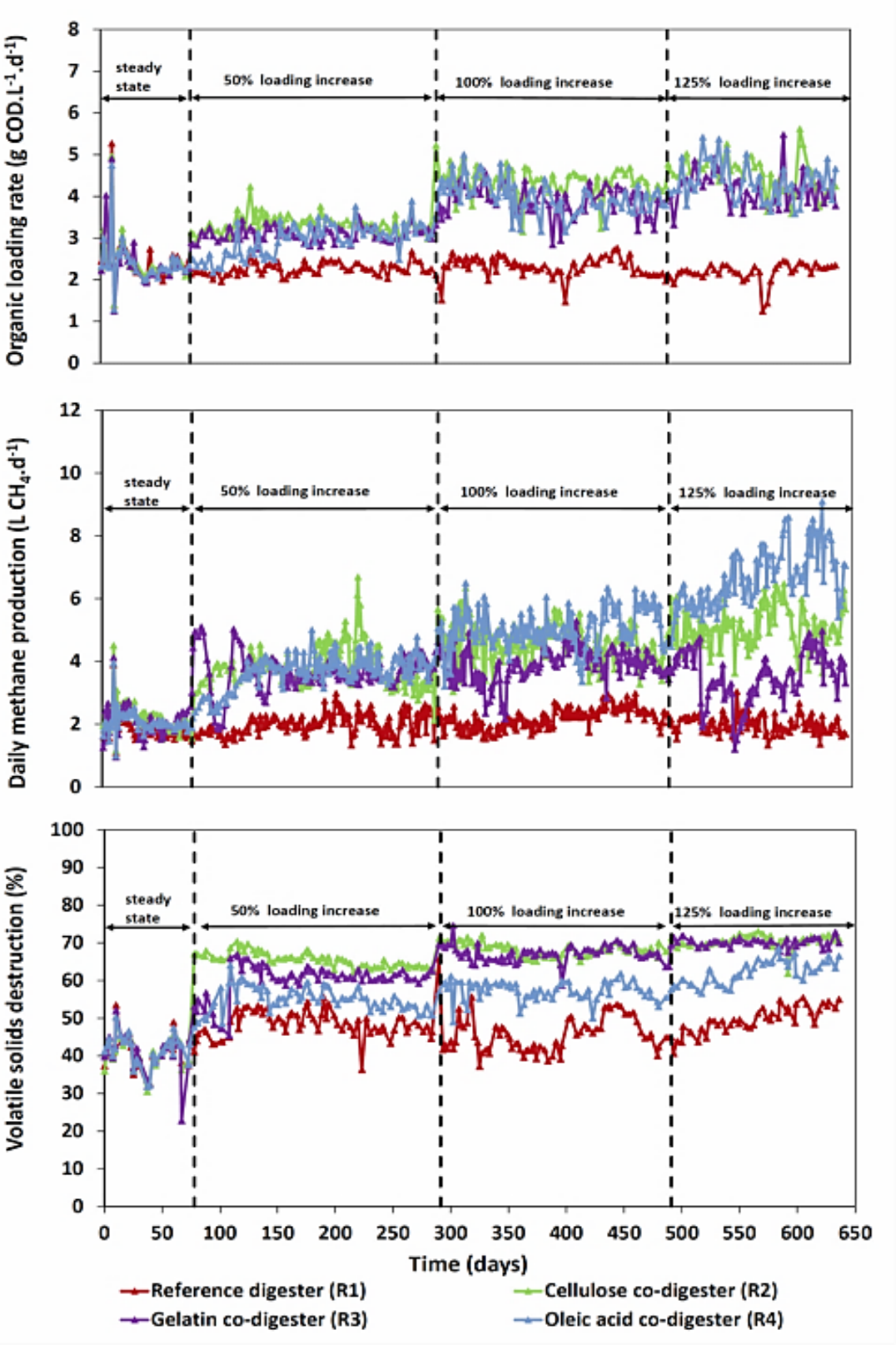

Figure 9: Loading and performance measures for four continuous digesters operated to test organic loading limits for a complex base substrate co-digested with model co-substrates

Table 7: Performance summary of the four continuous digesters operated to test organic loading limits for sewage sludge as complex base substrate being co-digested with model co-substrates

Organic loading rate (g $COD.L^{-1}.d^{-1}$)				
Period	R1 (control)	R2 (cellulose)	R3 (gelatin)	R4 (oleic acid)
1	2.29 ± 0.04	2.50 ± 0.21	2.46 ± 0.24	2.43 ± 0.20
2		3.35 ± 0.05	3.07 ± 0.04	2.95 ± 0.09
3		4.36 ± 0.08	3.90 ± 0.10	4.00 ± 0.10
4		4.51 ± 0.15	4.16 ± 0.14	4.37 ± 0.16
Methane Production ($L.d^{-1}$)				
Period	R1 (control)	R2 (cellulose)	R3 (gelatin)	R4 (oleic acid)
1	2.05 ± 0.03	2.25 ± 0.12	2.15 ± 0.15	2.08 ± 0.09
2		3.89 ± 0.11	3.63 ± 0.09	3.71 ± 0.08
3		4.60 ± 0.10	3.92 ± 0.09	5.00 ± 0.09
4		5.09 ± 0.12	3.42 ± 0.16	6.80 ± 0.17
Effluent Solids Concentration ($g.L^{-1}$)				
Period	R1 (control)	R2 (cellulose)	R3 (gelatin)	R4 (oleic acid)
1	18.57 ± 0.2	19.38 ± 0.54	19.39 ± 0.56	19.27 ± 0.5
2		19.67 ± 0.41	19.97 ± 0.32	18.75 ± 0.37
3		24.34 ± 0.24	23.48 ± 0.29	21.99 ± 0.25
4		24.46 ± 0.49	24.62 ± 0.32	21.15 ± 0.48
Volatile solids destruction (%)				
Period	R1 (control)	R2 (cellulose)	R3 (gelatin)	R4 (oleic acid)
1	46.8 ± 0.68	42.1 ± 2.3	41.5 ± 2.1	41.9 ± 1.5
2		65.7 ± 0.5	60.4 ± 1.1	55.2 ± 0.8
3		68.3 ± 0.4	67.2 ± 0.5	57.3 ± 0.6
4		70.7 ± 0.6	70.1 ± 0.4	62.7 ± 1.1

During period 2, the protein digester (R3) showed deterioration in performance between day 90 and day 100, but eventually recovered after day 150, likely due to ammonia release from the gelatin digestion resulting in ammonia inhibition (Figure 10). TAN concentration continued to increase when load to R3 was further increased, and further exacerbated ammonia inhibition. This led to generally unstable methane production from R3 (Figure 9). The ammonia inhibition caused a progressive build-up in VFAs in R3 (Figure 10), showing that ammonia inhibition also affected downstream biological processes (i.e. methanogenesis). It was very interesting to note that following a step increase in organic loading to R3 and after an initial recovery period, daily methane production by R3 somewhat converged towards that of R2 and R4. This indicated the microbial community in R3 was adapting to some extent to new higher ammonia loads. Following the second and third increase in organic loading to R3, VFAs continued to accumulate indicating that digestion failure was imminent.

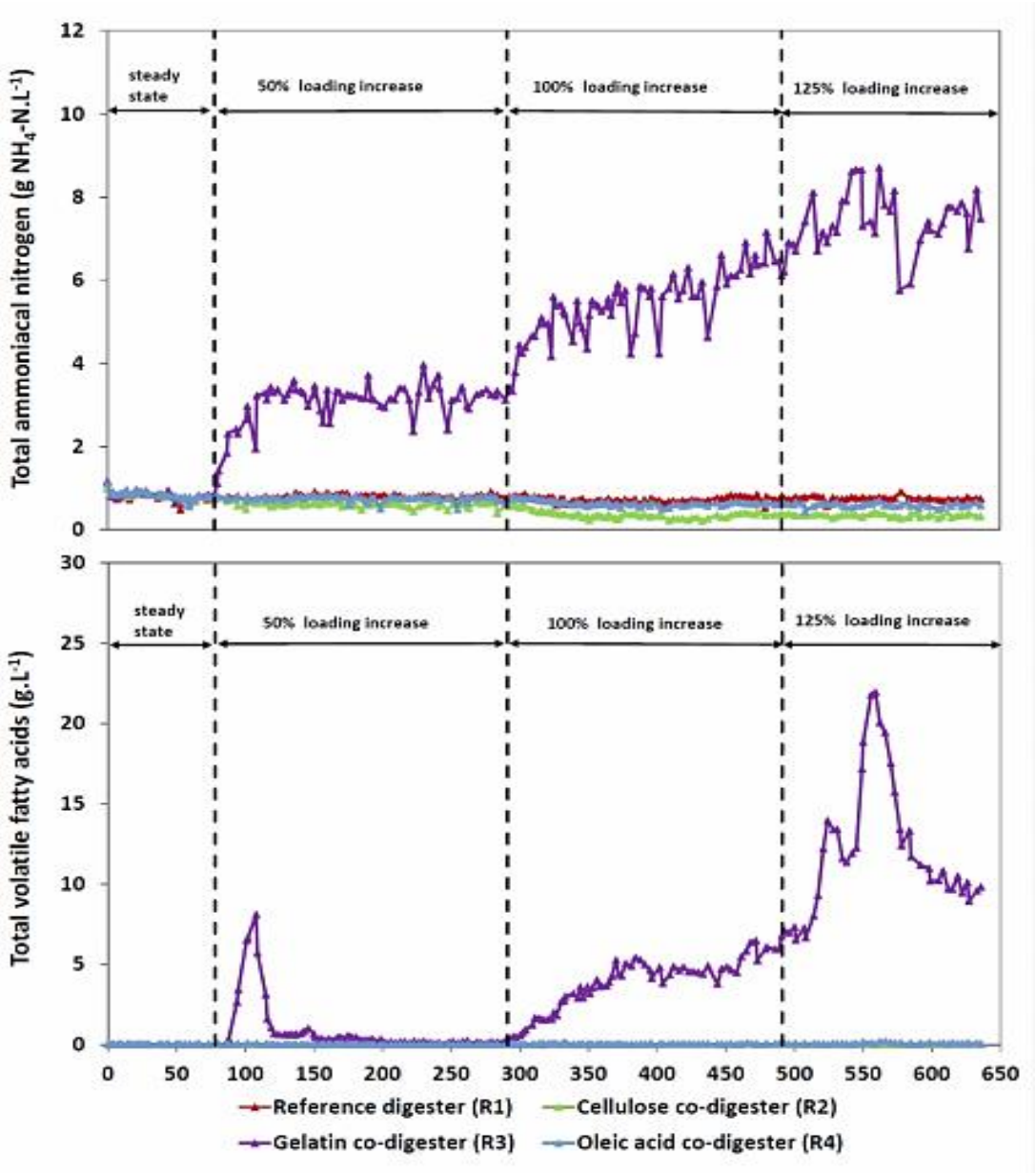

Figure 10: Stability measures for four continuous digesters operated to test organic loading limits for a complex base substrate co-digested with model co-substrates

5.2.3.2 Batch Capacity Testing

Figure 11, Figure 12 and Figure 13 show measured data from batch digestion tests performed with digestate from R2-R4 as inoculum, for cellulose, gelatin and oleic acid as carbon substrates, respectively. In each case the results for control tests using R1 (control digester) inoculum are also presented. Any differences between the R2-R4 results and the R1 results show an impact of either the inoculum background/microbial health and/or an impact of carbon substrate being batch digested.

The batch cellulose digestion results with inoculum from the cellulose digester R2 (Figure 11A) showed a near comparable capacity to degrade cellulose, despite increases in organic load to this continuous digester. The batch cellulose digestion results with inoculum from the gelatin digester R3 (Figure 11B) showed clear signs of inhibition, with a slowing in methane production. However, this inhibition was recoverable, with the final yield of cellulose being the same between the R3 tests and the R1 control tests (Figure 11B). The batch cellulose digestion results with inoculum from the oleic digester R4 (Figure 11C) were similar to that of the R2 inoculum tested on cellulose, i.e. no notable signs of changes in digestion capacity despite the increases in organic loading to R4 during the continuous digester operation. Overall these results indicated that co-digestion of sewage sludge as base substrate and cellulose or oleic acid as co-substrates, does not increase microbial capacity to digest cellulose, and that the ammonia inhibition from gelatin co-digestion has sustained negative impacts on subsequent batch digestion performance.

The batch gelatin digestion results with inoculum from the cellulose digester R2 (Figure 12A) showed near comparable capacity to degrade gelatin, despite the increases in organic load to this continuous digester. These results suggested that loading of gelatin as carbon substrate in the batch tests was not excessive, i.e. did not cause notable ammonia inhibition. Similar results were obtained for gelatin digestion using inoculum from the oleic acid digester R4 (Figure 12C).

The batch gelatin digestion results with inoculum from the gelatin digester R3 (Figure 12B) showed signs of inhibition, likely due to background ammonia carried over with the inoculum from R3. However, interestingly, the capacity to degrade gelatin was greater for inoculum from the 125% load increase period than for inoculum from the 100% load increase period (Figure 12C), indicating that capacity to degrade gelatin was increased by the 125% loading increase to gelatin digester R3.

The batch digestion of oleic acid with inoculum from the cellulose digester R2 showed a decrease in digestion capacity with increasing loading to this continuous digester (Figure 13A). In contrast, The batch digestion of oleic acid using inoculum from the oleic acid digester R4 showed near-comparable digestion capacity, despite the increases in oleic acid loading to this continuous digester (Figure 13C). These results may indicate that adaptations that occurred in R2 (the cellulose digester) decreased the microbial capacity to digest oleic acid. The batch digestion of oleic acid with inoculum from the gelatin digester R3 (Figure 13B) showed a strong deterioration in performance compared to the control R1 inoculum, likely due to combined effects of inhibition by oleic acid used as carbon substrate as well as high background ammonia carried over with the inoculum from R3. These results align with findings reported in Section 5.1.

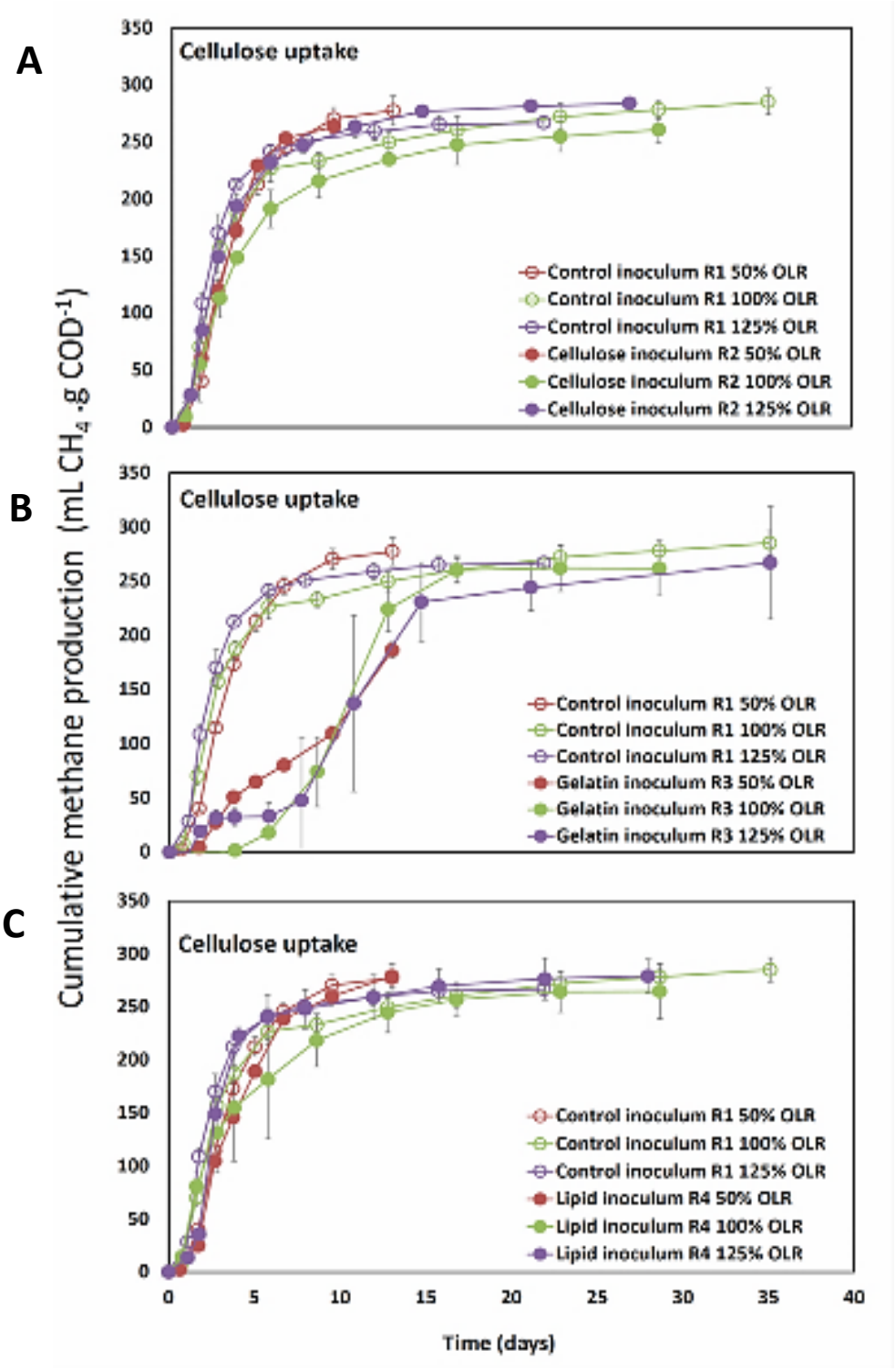

Figure 11: Methane production for batch cellulose digestion tests inoculated with digestate from four continuous digesters testing organic loading limits for a complex base substrate co-digested with model co-substrates

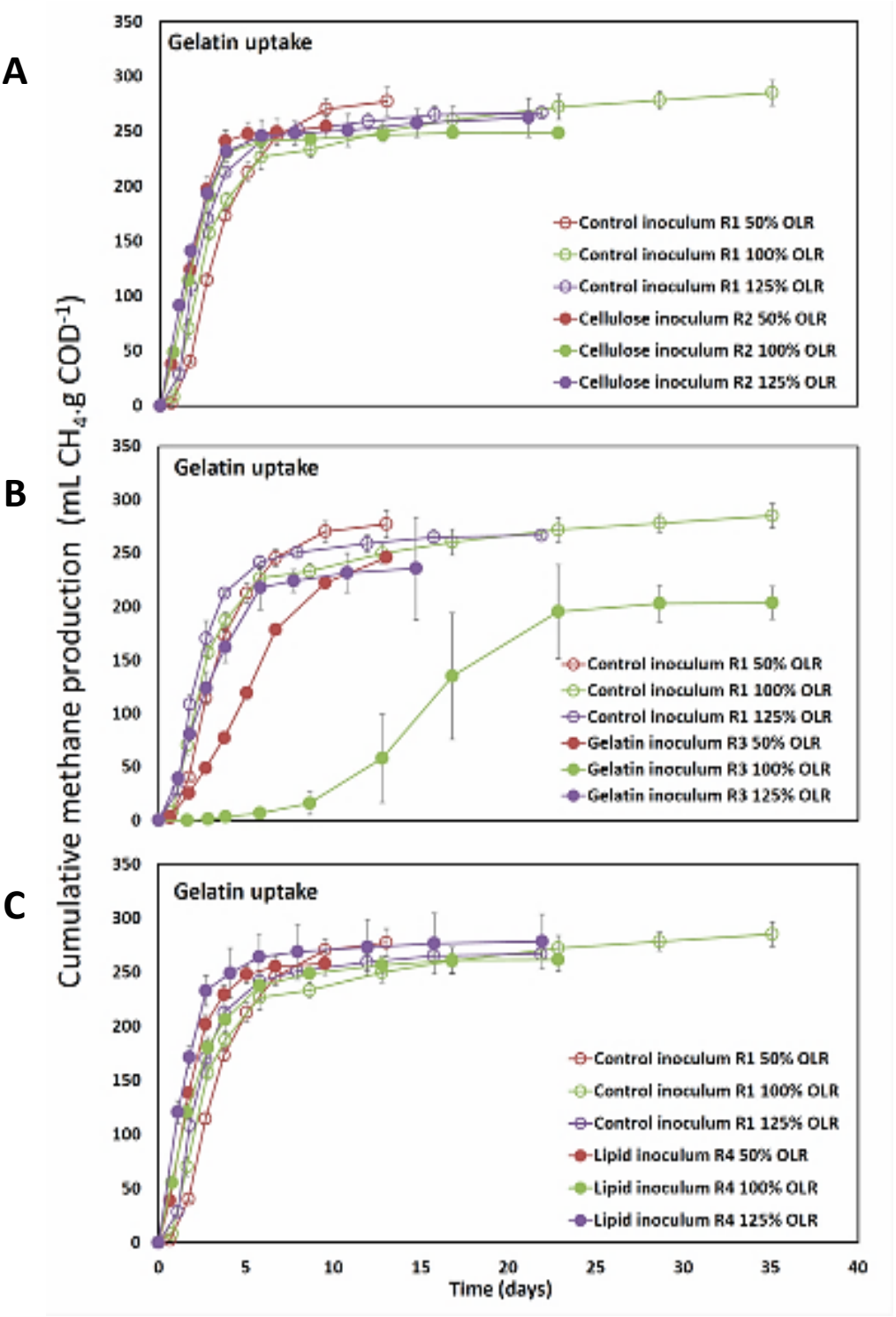

Figure 12: Methane production for batch gelatin digestion tests inoculated with digestate from four continuous digesters testing organic loading limits for a complex base substrate co-digested with model co-substrates

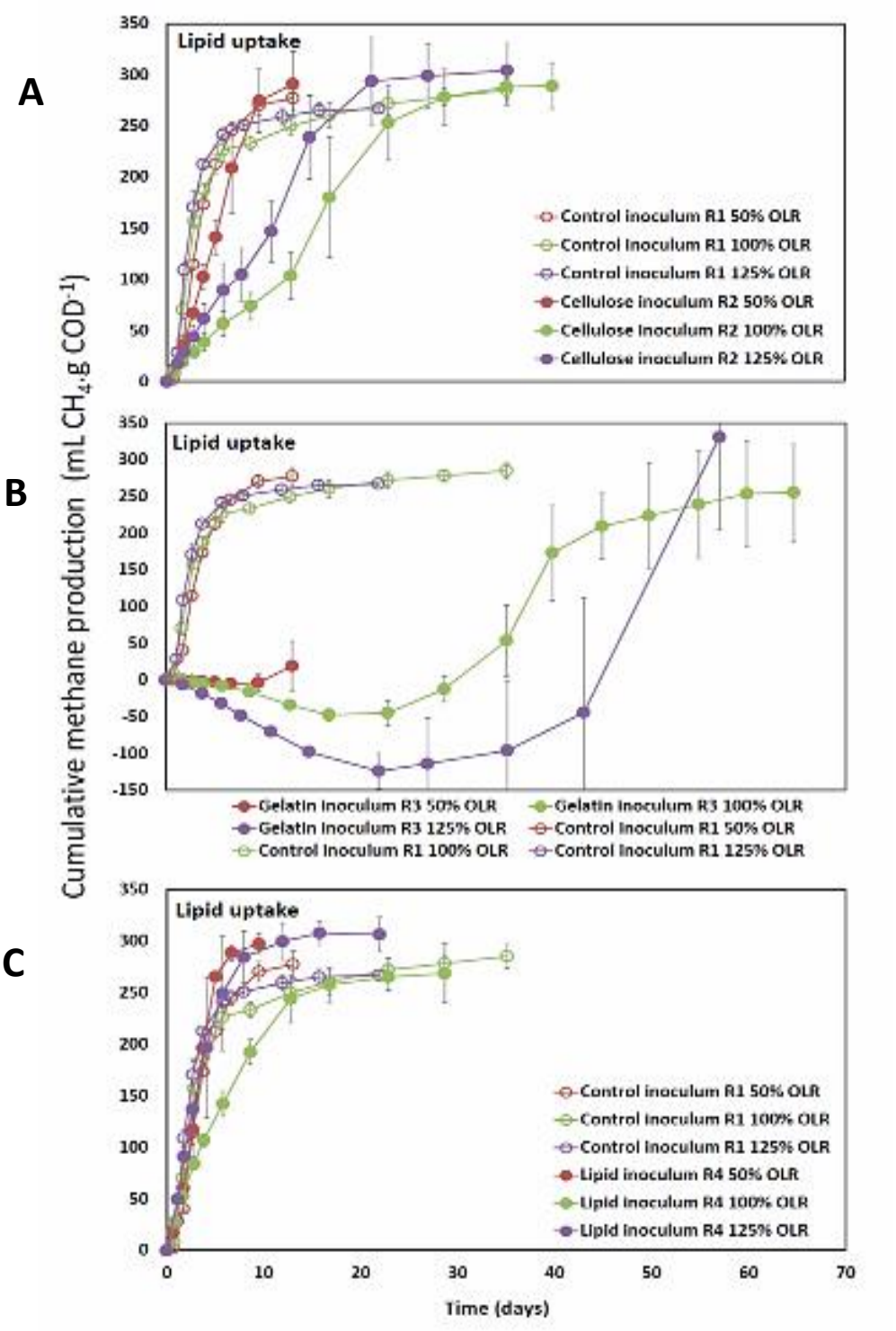

Figure 13: Methane production for batch oleic acid digestion tests inoculated with digestate from four continuous digesters testing organic loading limits for a complex base substrate co-digested with model co-substrates. Note the change of scale on the vertical axes.

5.2.4 Summary

The experiments described in this section used well-controlled continuous anaerobic digesters fed with sewage sludge as well-characterised complex base substrate and increased loads of a model co-substrate (cellulose as carbohydrate, gelatine as protein, or oleic acid as fat/lipid). The tests assessed the impacts of co-substrate macro-composition on digestion performance in the continuous digesters as well as on the subsequent batch digestion capacity of acclimated/developed microbial communities collected from the continuous digesters.

The results showed that fat is a preferred co-substrate, provided that treatment times are long enough for digestion, followed by carbohydrates as a strong candidate, and lastly by proteins due to high risk of ammonia inhibition. Ammonia and oleic acid did appear to show an antagonistic effect on digestion performance by jointly inhibiting the digestion of oleic acid, and this may be important to consider with the AcoD of high protein – high fat mixtures.

The tests noted that microbial community balance is not well suited to high protein feeds, with substantial organic acids accumulation as a key process risk. Interestingly, the tests showed that co-substrate macro-composition had significant long-term impacts on microbial community development. In particular, protein based co-digestion seemed to significantly weaken the microbial community in the continuous digesters, as noted from deterioration in digester performance, and from a deterioration in performance of the separate batch capacity tests. Fat (oleic acid) co-digestion seemed to improve parts of the community without harming others. Carbohydrate (cellulose) co-digestion in the continuous digesters seemed to weaken the community's ability to subsequently digest lipids in the batch capacity tests, but this effect should be further investigated in future studies.

Overall, there was a clear substrate macro-composition impact on co-digestion capacity, and this behaviour could not be anticipated from C/N ratio, in agreement with the results from Section 3.

5.3 Impact of Psychrophilic Temperature on Co-digestion Performance

5.3.1 Introduction

Temperature has a significant impact on the anaerobic digestion process, being a strong determinant of microbial consortia, microbial growth rates, decay rates, substrate affinity and metabolic pathways (Batstone et al., 2002; De Vrieze et al., 2015; Kosaka et al., 2008). The rates of particular metabolic steps may also be affected differently by temperature, and this has the potential to alleviate or exacerbate imbalances in upstream and downstream biological reactions (Figure 1). Generally, treatment at thermophilic conditions (50-70°C) results in faster digestion, whilst treatment at psychrophilic temperatures (up to 15°C) results in slower digestion (Lettinga et al., 2001; Lin et al., 2016). Previous temperature studies have mostly compared processes at two temperatures, usually mesophilic (35°C) and thermophilic (55°C) (Ghasimi et al., 2015; Guo et al., 2014; Li et al., 2015b). A small minority of studies compare mesophilic with psychrophilic temperatures (e.g. 10°C or 20°C) (Bialek et al., 2013). A very small number of studies compared a wide range of temperatures incorporating psychrophilic, mesophilic and thermophilic temperatures, but these have mostly used inoculum from digesters fed with model substrates rather than with complex organic wastes relevant to AcoD (Lin et al., 2016; Van Lier et al., 1997). Accordingly, specific research objectives of the present work were to:

1. Investigate how operating temperature influences relative biological rates within the AD activity profile for a complex yet well-defined AD substrate; and
2. Examine the temperature-dependency of AD and potential bottleneck biological reactions in AcoD with complex substrate mixtures.

This research was carried out under the PhD project of Katie Macintosh.

5.3.2 Methods

5.3.2.1 Continuous Anaerobic Digester Experiments

Primary Sludge and Waste Activated Sludge were sourced from the same site and treated in the same way as described in Section 5.2.2. Based on measured VS (Section 4.1), the thickened primary sludge and thickened waste activated sludge were mixed together at a 1:1 VS ratio. Deionized water was then added to dilute the final mixture to a total VS content of 30 g $VS.L^{-1}$.

Pig manure was manually collected bi-monthly as fresh scrapings from sow sheds at a specialised piggery in South East Queensland (Skerman & Collman, 2012). Weekly, 5L feed batches were created by firstly blending the collected pig manure with deionized water in a Breville Kinetix Control blender (BBL605BS) using the pulse function until a homogenous slurry was formed. The pig manure slurry was then screened using the same 4mm stainless steel sieve as used for primary sludge, to remove course solids that could clog the feeding pumps/lines of the continuous digesters. The resultant filtered pig manure slurry was then characterised for VS and finally diluted with deionized water to a total VS content of 30 g $VS.L^{-1}$.

Four lab-scale continuous digesters were constructed and operated (Figure 14) to prepare temperature-adapted inocula for further testing (Section 5.3.2.2). The digesters were completely mixed during operation using overhead motorised stirrer units. Temperature was maintained using a heating/cooling jacket with water circulated to and from a heated/cooled water bath. Two of the digesters were fed with the prepared pig manure slurry and the other two were fed with the prepared sewage sludge mixture.

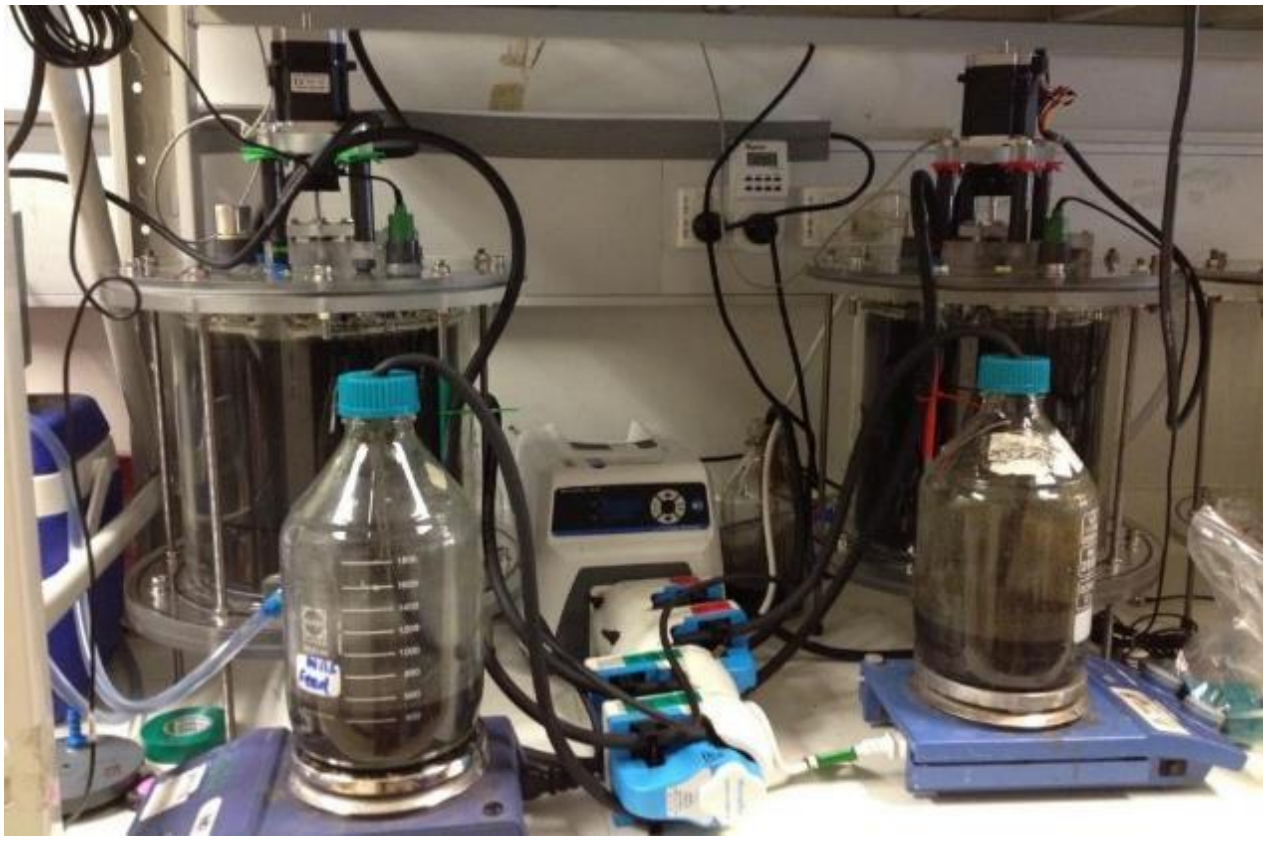

Figure 14: Continuous digesters used to prepare temperature-acclimated microbial inocula for separate batch activity testing of the effect of temperature on anaerobic digestion process rates

Table 8 summarises operating conditions for the four digesters. All four digesters were started at an equal OLR of 1.5 g $COD.L^{-1}.d^{-1}$ and 37°C, and were held at this temperature for an initial establishment period. The OLR was then reduced to 1.2 g $COD.L^{-1}.d^{-1}$ for a subsequent holding period at 37°C to monitor and confirm that steady state operation had been achieved. The temperature of one of the two pig manure digesters and one of the two sewage sludge digesters was then adjusted to 25°C for a subsequent operational period, whilst the other two digesters were kept at 37°C as control digesters for statistical comparison. The temperature of the two test digesters was then further decreased to 15°C for an additional operational period, whilst again maintaining the control digesters at 37°C. However, the pig manure digester at 15°C experienced significant instability as identified by accumulating VFAs (see below), so a decision was made to decrease the OLR to all four digesters to 1 g $COD.L^{-1}.d^{-1}$ for the final operational period. This allowed the pig manure digester at 15°C to partially recover. HRT during this final operating period simultaneously increased from 20-24 days to 30 days, because the concentration of the feed was the same as before.

Table 8: Operating strategies for continuous bench-scale digesters examining impact of temperature on AD performance and functionality

Phase	Time (Day)	Organic Loading (g COD.L^{-1}.d^{-1})	Control Digesters Temperature (°C)	Test Digesters Temperature (°C)	Operating Notes
1	0 – 60	1.5	37	37°C	All digesters operating at HRT of 20-24 days
2	61 – 222	1.2	37°C	37°C	All digesters operating at HRT of 20-24 days
3	222 – 371	1.2	37°C	25°C	All digesters operating at HRT of 20-24 days
4	371 - 453	1.2	37°C	15°C	All digesters operating at HRT of 20-24 days
5	453 - 600	1.0	37°C	15°C	All digesters operating at HRT of 30 days

Throughout each operational period, biogas samples were collected 2-3 times per week from the headspace of each digester to measure gas composition via GC-TCD (Section 4.1) and a biogas volume produced over time was measured with a counter meter. Samples of digester feed and effluent were also periodically collected and analysed for pH, TS, VS, tCOD, sCOD, TKN and TKP, TAN and VFAs, as also described in Section 4.1. pH of the digesters liquid contents were measured and recorded using a calibrated Hanna pH sensor (HI2910B/5) and meter/transmitter HI 8614LN. The pH probes were screwed into the digester lid. VS destruction and COD removal were calculated using Equations 1 and 2 in Section 5.2.2, and were used along with biogas production profiles to assess digestion performance.

5.3.2.2 Process Capacity Batch Testing

Digestate outflow from the digesters were collected daily and stored in the refrigerator until an adequate amount had been collected (typically 10 days). This digestate was then used as temperature-acclimated inocula in separate batch activity testing. The batch activity tests investigated the impact of temperature on various biological rate processes relevant to AcoD (Figure 1). Eight individual batch activity assays were performed in triplicate in 160 mL serum bottles (100-120 mL working volume), in two independent time replicates, at each temperature condition. The inocula used in the tests were pre-diluted to achieve an approximate biomass concentration of 10g VS L^{-1} and all the tests were performed at an inoculum to substrate ratio of 5±1 on a VS basis to ensure conditions of excess biomass. Table 9 below presents the model substrates that were tested and the measurements that were performed in each case. A monod-kinetics style (Equation 3) rate parameter (k_m, monod maximum specific uptake rate, g COD g VS^{-1} d^{-1}) was determined for each respective biological reaction.

$$r_i = -k_{m,i}\frac{S_i}{K_{s,i}+S_i} \qquad (3)$$

It is important to note that in most cases the concentration of the reagent was measured directly, thus providing a direct measure of biological uptake. Whilst typical healthy AD of all respective substrates would ultimately progress to the formation of methane, methane production was only used to

determine the rate of methanogenesis (Table 9). In Equation 3, r_i is the biological uptake rate for substrate i, given in g COD g VS^{-1} d^{-1}, $K_{S,i}$ is the half saturation constant g COD L^{-1}, and Si is the substrate concentration given in g COD L^{-1}. The value of k_m was determined in each case as the slope of a linear regression fit (Analysis Toolpak in Microsoft Excel 2016) applied to measured specific substrate concentration ($g\ COD_{substrate} \cdot g^{-1}\ VS_{inoculum}$, y-axis) over time (x-axis) for subsets of data over which the uptake rate was approximately constant.

Table 9: Details of batch activity tests performed to examine the effect of temperature on AD performance and functionality

Specific Activity	Model Substrate Tested	Concentration tested		Sample Type Collected	Analysis performed
		($gVS.L^{-1}$)	($gCOD.L^{-1}$)		
Hydrolytic - Carbohydrate	Cellulose	2	2.4	L/G	$sCOD/CH_4$
Hydrolytic - Protein	Gelatine	2	3.2	L/G	$sTAN/CH_4$
Hydrolytic - Lipid	Oleic Acid	2	5.8	L/G	VFA/CH_4
Acidogenic – Glucose	Glucose	2	2.1	L	Glucose
Acidogenic - Glycerol	Glycerol	2	2.4	L	Glycerol
Acetogenic – Propionic	Sodium Propionate	2	2.8	L	Propionate
Acetogenic – Butyric	Sodium Butyrate	2	2.4	L	Butyrate
Methanogenesis - Acetoclastic	Sodium Acetate	2	1.8	G	CH_4
Methanogenesis - Hydrogenotrophic	Sodium Formate	2	0.4	G	CH_4

5.3.2.3 Assessment of Relative Biological Process Kinetics and Identification of Biological Process Bottlenecks

The values of k_m were used in a simple kinetic model of a completely mixed tank digester (Equation 4), to determine the digestion time *t* required to convert 80% of the biodegradable in the respective substrate. This was also done for pig manure and sewage sludge fed as base substrates to the continuous digesters (Section 5.2.1), to compare with the degradation rates of the respective model substrates (Section 5.2.2).

$$0.8 = 1 - \frac{1}{1 - k_m t_{80}} \qquad (4)$$

In Equation 4, t_{80} is the digestion time required to convert 80% of the degradable fraction for a particular substrate and k_m is as above.

5.3.3 Results and Discussion

5.3.3.1 Continuous Digester Performance

Figure 15 presents time trend data for the continuous digesters fed with pig manure. Figure 16 presents time trend data for the continuous digesters fed with sewage sludge. Table 10 and Table 11 provide summary performance measures; giving average values for all measurements in each respective operating period together with 95% confidence intervals.

Table 10: Performance summary of continuous digesters treating pig manure for a temperature study

Operating Period		Methane Production ($mL.L^{-1}.d^{-1}$)		VS destruction (%)		Effluent sCOD ($mg.L^{-1}$)	
		P1 (control)	P2 (test)	P1	P2	P1	P2
1	Start-up	317 ± 82	313 ± 125	61 ± 13	50±22	2331 ± 688	2250 ± 579
2	37°C	436 ± 89	404 ± 21	52 ± 5	51 ± 5	1633 ± 119	1640 ± 121
3	25°C	451 ± 108	359 ± 68	54 ± 4	47 ± 5	1536 ± 82	1570 ± 186
4	15°C	370 ± 125	289 ± 82	55 ± 7	40±10	1530 ± 212	2318 ± 540
5	15°C Lower OLR	333 ± 133	273 ± 82	57 ± 8	38 ± 8	1499 ± 157	2098 ± 303

Throughout the experiment, VS concentrations in the feed and effluent of the control digesters (P1 and S1) and their VS removal extents were reasonably consistent over time. During Phase 1 and 2, when all the digesters were operating at 37°C, VS removal and methane production were similar in the test and control digesters. These observations indicated that digestion performance was reasonable and stable. When temperature of the test digesters was decreased to 25°C, there was a small but notable decrease in VS removal and COD removal by the pig manure test digester at 25°C (Table 10), whilst the sewage sludge test digester at 25°C showed similar performance to the parallel sewage sludge control digester at 37°C (Table 11). For reference, a decrease in VS removal causes an increase in VS concentration in the effluent. Due to large inherent variability in measurements, methane production of the test digesters at 25°C was statistically indistinguishable from that of the control digesters at 37°C.

Table 11: Performance summary of continuous digesters treating sewage sludge for a temperature study

Operating Period		Methane Production ($mL.L^{-1}.d^{-1}$)		VS destruction (%)		Effluent sCOD ($mg.L^{-1}$)	
		S1 (control)	S2 (test)	S1	S2	S1	S2
1	Start-up	156 ± 133	237 ± 99	45 ± 9	47 ± 7	797 ± 216	756 ± 274
2	37°C	218 ± 114	259 ± 55	45 ± 5	47 ± 5	674 ± 111	656 ± 94
3	25°C	264 ± 46	278 ± 54	48 ± 5	43 ± 6	650 ± 66	643 ± 86
4	15°C	310 ± 91	233 ± 94	45 ± 3	34 ± 7	579 ± 72	963 ± 212
5	15°C Lower OLR	225 ± 71	185 ± 75	48 ± 4	40 ± 6	607 ± 102	821 ± 144

When temperature of the test digesters was decreased to 15°C, VS removal, COD removal and methane production notably decreased. When OLR was decreased in Phase 5, COD removal by the sewage sludge test digester at 15°C appeared to partially converge with that of the parallel sewage sludge control digester at 37°C (Figure 16).

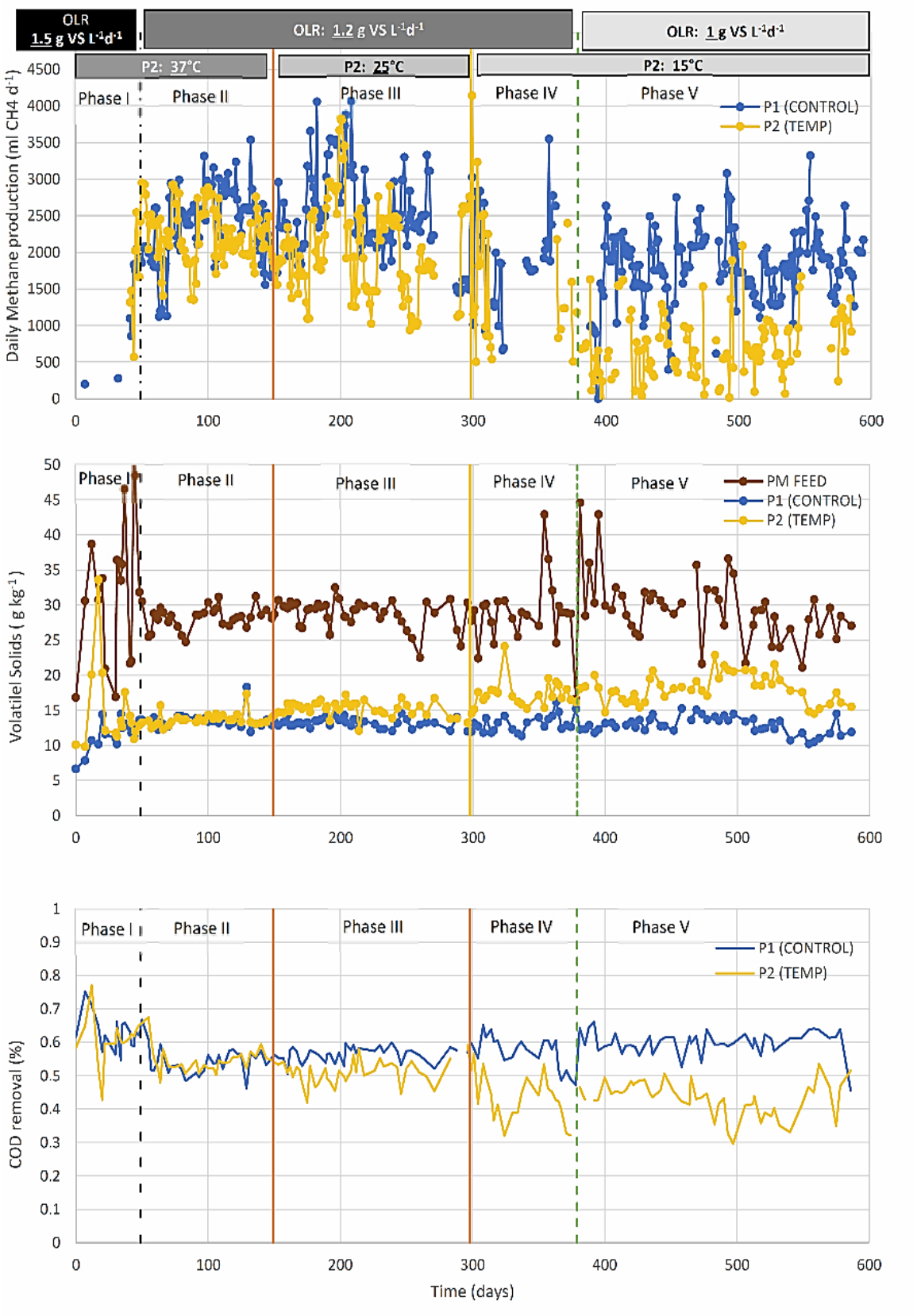

Figure 15: Performance time trends for continuous digesters fed with pig manure. These digesters were operated to prepare temperature-acclimated microbial inocula for batch activity testing.

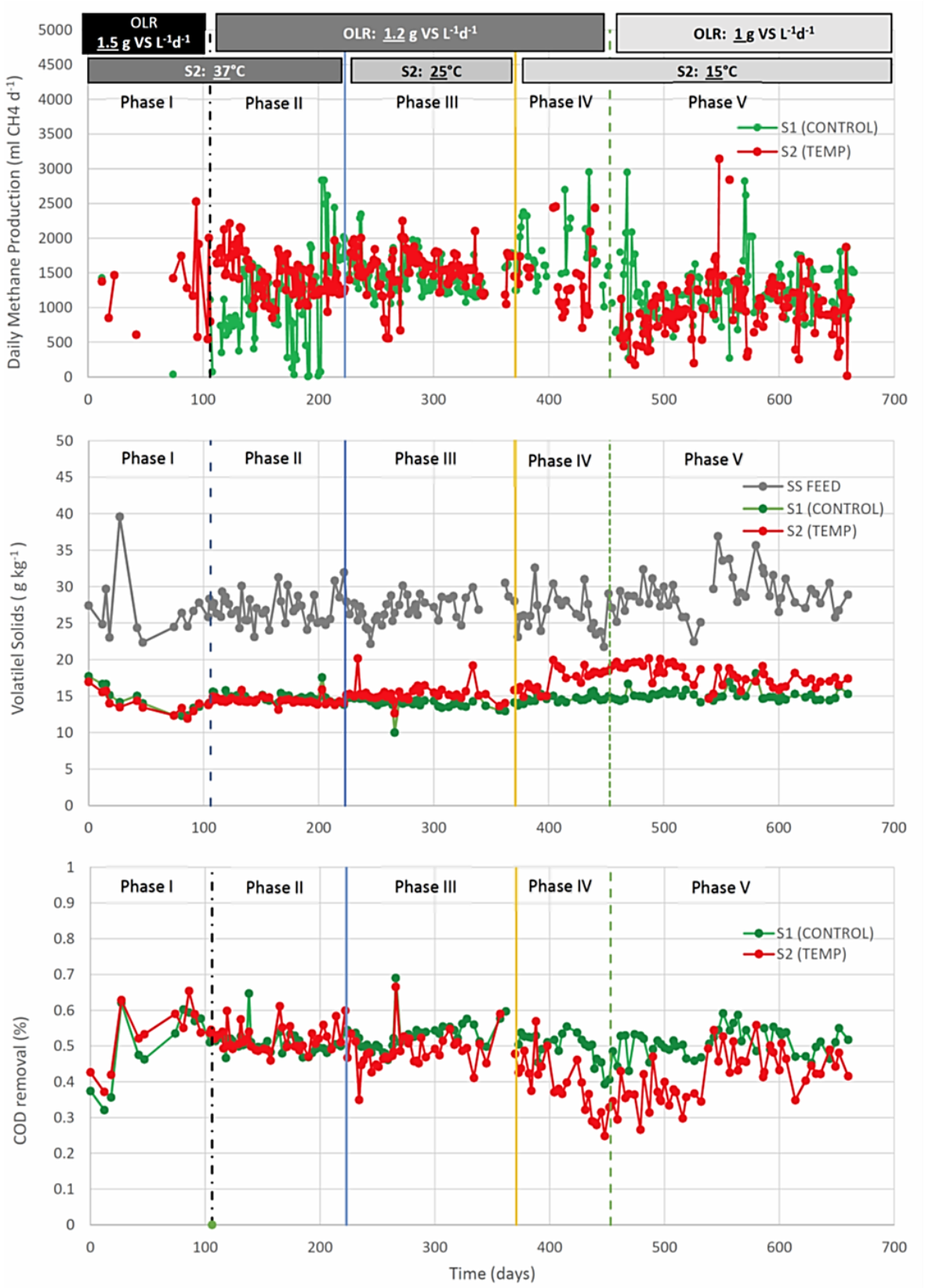

Figure 16: Performance time trends for continuous digesters fed with sewage sludge. These digesters were operated to prepare temperature-acclimated microbial inocula for separate batch activity testing.

5.3.3.2 Co-digestion Process Capacity and Risk

Figure 17 and Figure 18 present estimated monod maximum uptake rates (k_m) for the various metabolic steps in AcoD of complex substrates. As expected, the various metabolic rates generally decreased with decreasing temperature. The decrease in metabolic activity was not linear, with the decrease in rates being more severe between 25°C and 15°C than between 37°C and 25°C. The results also showed that the rates of different reactions were decreased to different extents with a temperature drop from 37 to 15°C. For example, with a decrease in temperature, the rate of protein hydrolysis decreased to a greater extent than the rate of carbohydrate hydrolysis. This agrees with findings of prior studies (Ge et al., 2011; Ho et al., 2014; Van Lier et al., 1996). The effect of temperature also differed to some extent between the two base substrate types (pig manure vs sewage sludge). With inoculum from the pig manure digester, there was a significant decrease in carbohydrate hydrolysis rate from 37 to 25°C, but hydrolysis rate again increased when temperature was further decreased to 15°C, possibly due to hydrolytic capacity developed over time in the continuous pig manure digester.

Of key concern was the observation that rates of downstream processes of acetogenesis and methanogenesis were substantially slower when temperature was decreased from 37 to 15°C, whilst the rate of carbohydrate hydrolysis was less affected. This could exacerbate imbalances between upstream (hydrolysis, acidogenesis) and downstream (acetogenesis and methanogenesis) biological processes in the case of AcoD with high carbohydrate loads.

In general, the low k_m values for lipid showed that this substrate is a key process bottleneck in AcoD of lipid-rich waste mixtures. This would be especially important when digestion temperature is low, as with unheated covered ponds during cooler months. The slowing down of lipid hydrolysis could cause an accumulation of lipids as non-degraded residue and can negatively affect digestate quality. This highlights the importance of allowing adequate treatment times in covered pond designs for lipid-rich AcoD mixtures.

Because activities associated with lipid hydrolysis and propionate degradation were the slowest and most affected by a decrease in temperature, digestion with highly particulate and complex feedstocks would likely be controlled by lipid and carbohydrate loading, with propionate degradation being the potential bottleneck for the overall process. In fact, the continuous digester fed with pig manure as feedstock, showed propionate build-up as evidence of stress, when the temperature of the digester was decreased to 15°C (data not shown).

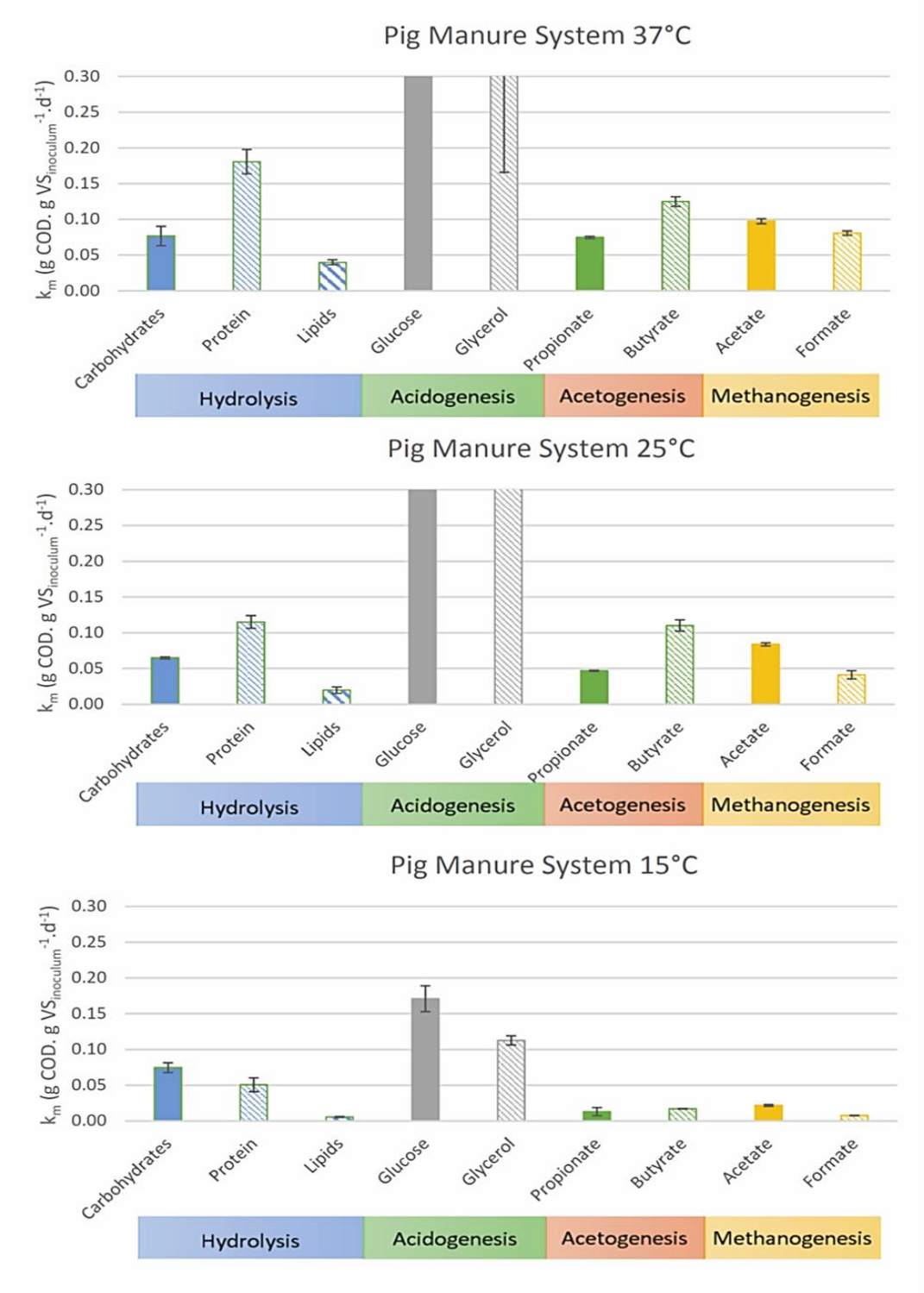

Figure 17: Values of monod maximum specific uptake rates determined from batch activity testing using temperature adapted inoculum sourced from bench-scale continuous digesters treating piggery manure. The groups of metabolic processes to which each reaction/reagent uptake belongs is also shown along the horizontal axes. Error bars are standard deviations on replicate estimates.

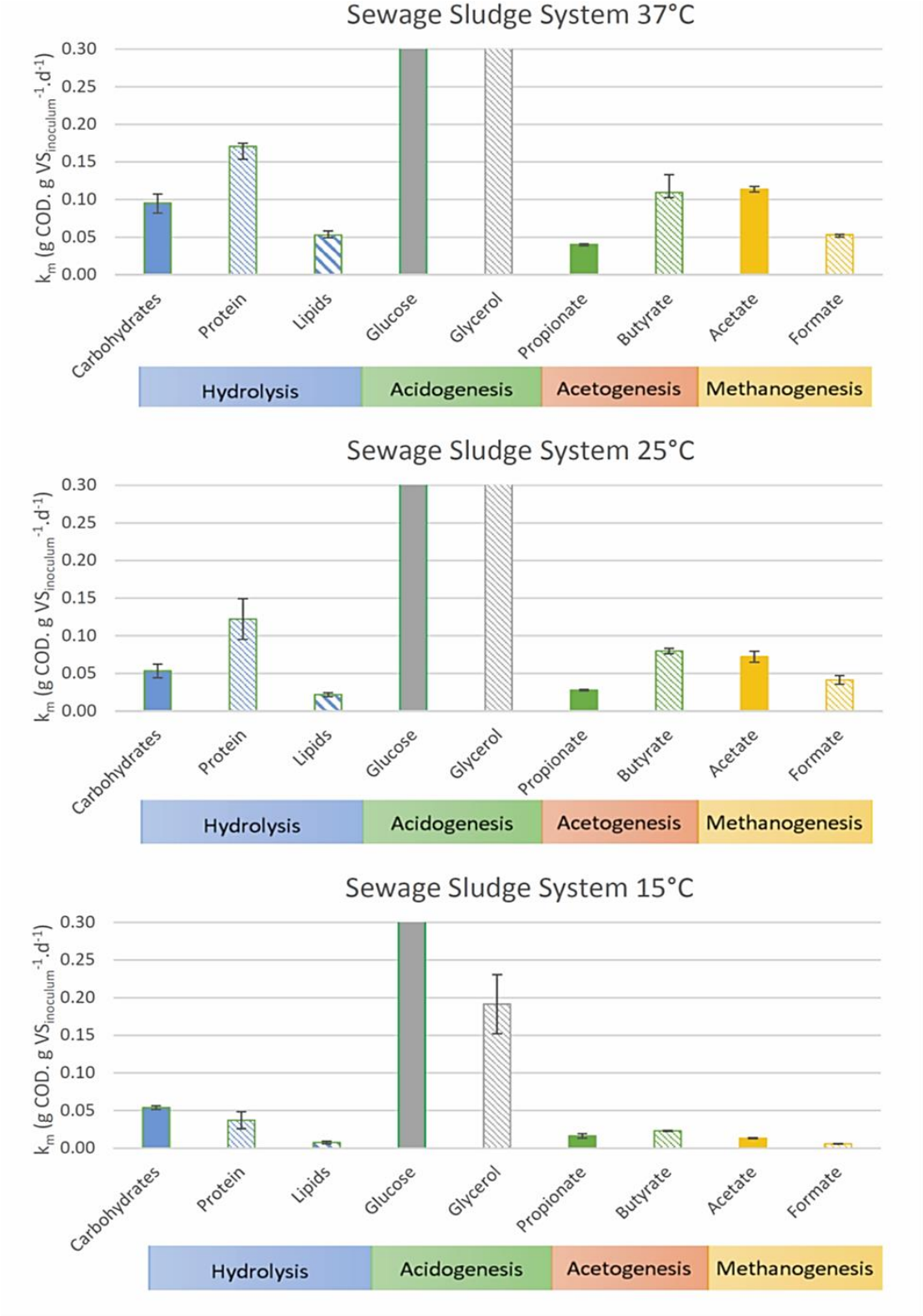

Figure 18: Values of monod maximum specific uptake rates determined from batch activity testing using temperature adapted inoculum sourced from bench-scale continuous digesters treating sewage sludge. The groups of metabolic processes to which each reaction/reagent uptake belongs is also shown along the horizontal axes. Error bars are standard deviations on replicate estimates.

5.3.3.3 Process Rates Comparison and Potential AcoD Bottlenecks

The relative rates of upstream hydrolytic and fermentation process, as compared to downstream metabolic process rates such as acetogenesis and methanogenesis, can provide an insight into potential biological reaction bottlenecks of an AcoD process.

To illustrate potential process bottlenecks, Figure 19 and Figure 20 summarise relevant kinetic information together with yield information for the various biological processes of importance to AcoD. The horizontal axes on the plots in Figure 19 and Figure 20 show treatment times required for various substrates to be digested to 80% of their respective biodegradabilities in a continuous mixed tank digester. The time taken to achieve 80% of the biodegradability of the base substrate (pig manure or sewage sludge) is also shown as a vertical dashed black line. The practical meaning for this kinetic information is that:

1. a line for a co-substrate that lies to the right of the base-substrate line shows that the co-substrate will take longer to degrade than the base substrate, and therefore requires longer treatment times to fully digest; and
2. a line for a co-substrate that lies to the left of the base-substrate line shows that the co-substrate will degrade faster than the base substrate, and therefore requires shorter treatment times to fully digest, provided that organic loading limits are not exceeded and that chemical inhibition is not occurring.

The vertical axes on the plots in Figure 19 and Figure 20 compare anticipated yields for the respective co-substrates, in the case where these are treated in a digester designed to achieve 80% of the biodegradability of the base-substrate (i.e. pig manure or sewage sludge). So, substrates that degrade slower than the base substrate will show less than 80% of its respective biodegradability, and substrates that degrade faster will show greater than 80% of its respective biodegradability.

In Figure 19 and Figure 20, a shift of the base substrate line (dashed vertical lines) towards the right with decreasing temperature, clearly shows that digestion rate of the base substrate slows down with decreasing temperature. With unheated covered ponds, this highlights the importance of retaining the base substrate for extended periods, such as by solids settling and retention within the digestion system, to allow enough time for adequate digestion.

Figure 19 and Figure 20 clearly show that lipid hydrolysis takes the longest to digest, with the lines for oleic acid lying far to the right of the base substrate lines (dashed vertical black lines). This difference becomes even more noticeable with decreasing temperature, because of a further slowing down of lipid hydrolysis. Again, these results highlight the process risk with waste mixtures rich in lipids, especially under ambient temperature conditions such as in unheated covered ponds.

The shift of the propionate uptake line further towards the right with decreasing temperature (i.e. slower propionate degradation) highlights the risk of imbalance with propionate accumulation. This is especially noted because at 15°C, the line for cellulose (carbohydrate) and gelatin (protein) lie far towards the left of the base substrate line (dashed vertical black line) and so these will degrade quicker than the base substrate whilst acidogenic reaction rates remain high. Again, this shows that propionate degradation could be a potential bottleneck for AcoD, limiting the digestion capacity for particulate and complex feedstocks.

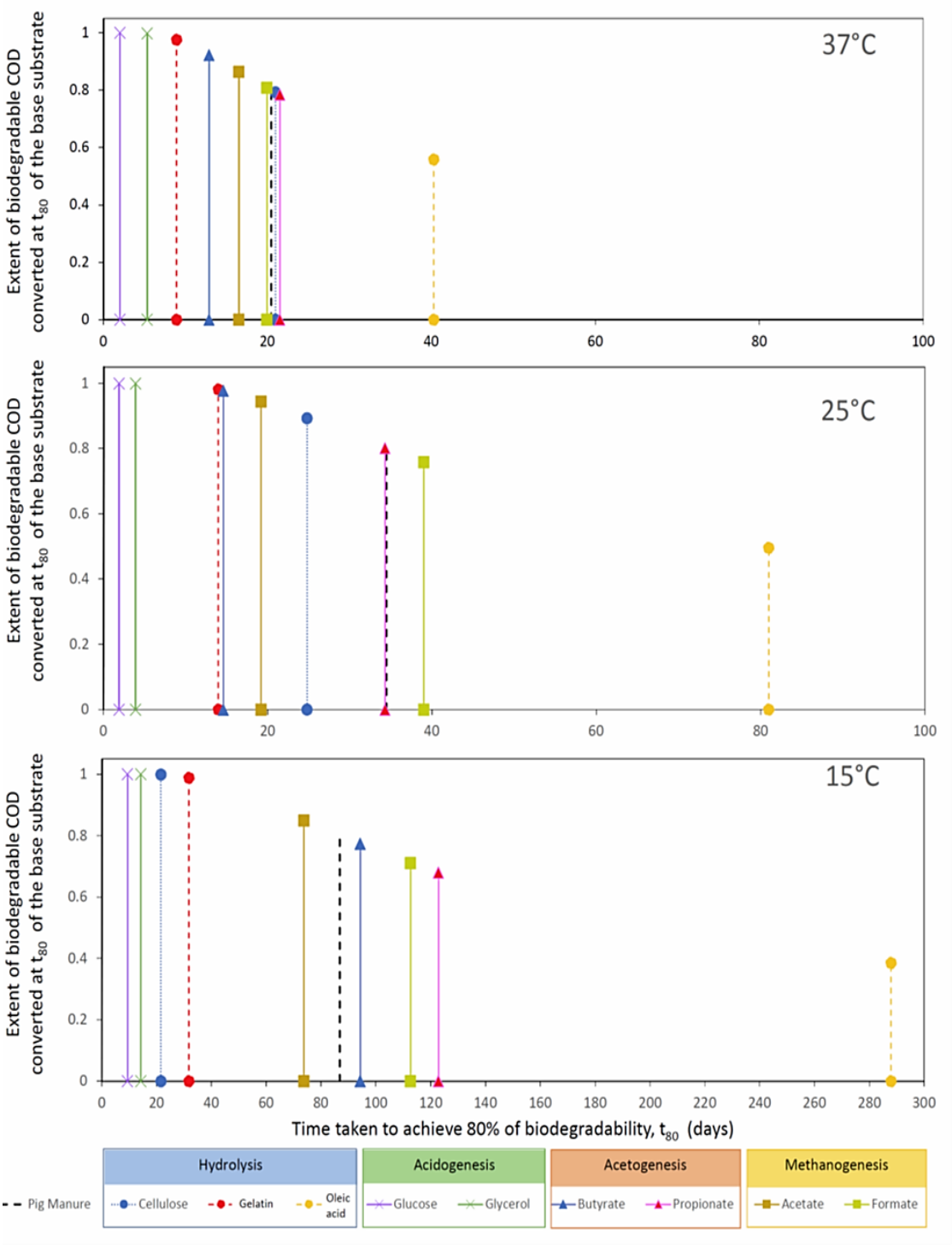

Figure 19: Summary plots of kinetic (horizontal axes) and yield (vertical axes) characteristics for various model co-substrates digested by temperature adapted inoculum from the bench-scale digesters treating pig manure. The vertical black dashed lines give kinetic and yield characteristics of pig manure as base substrate. Note the change of scale on the horizontal axes for 15°C.

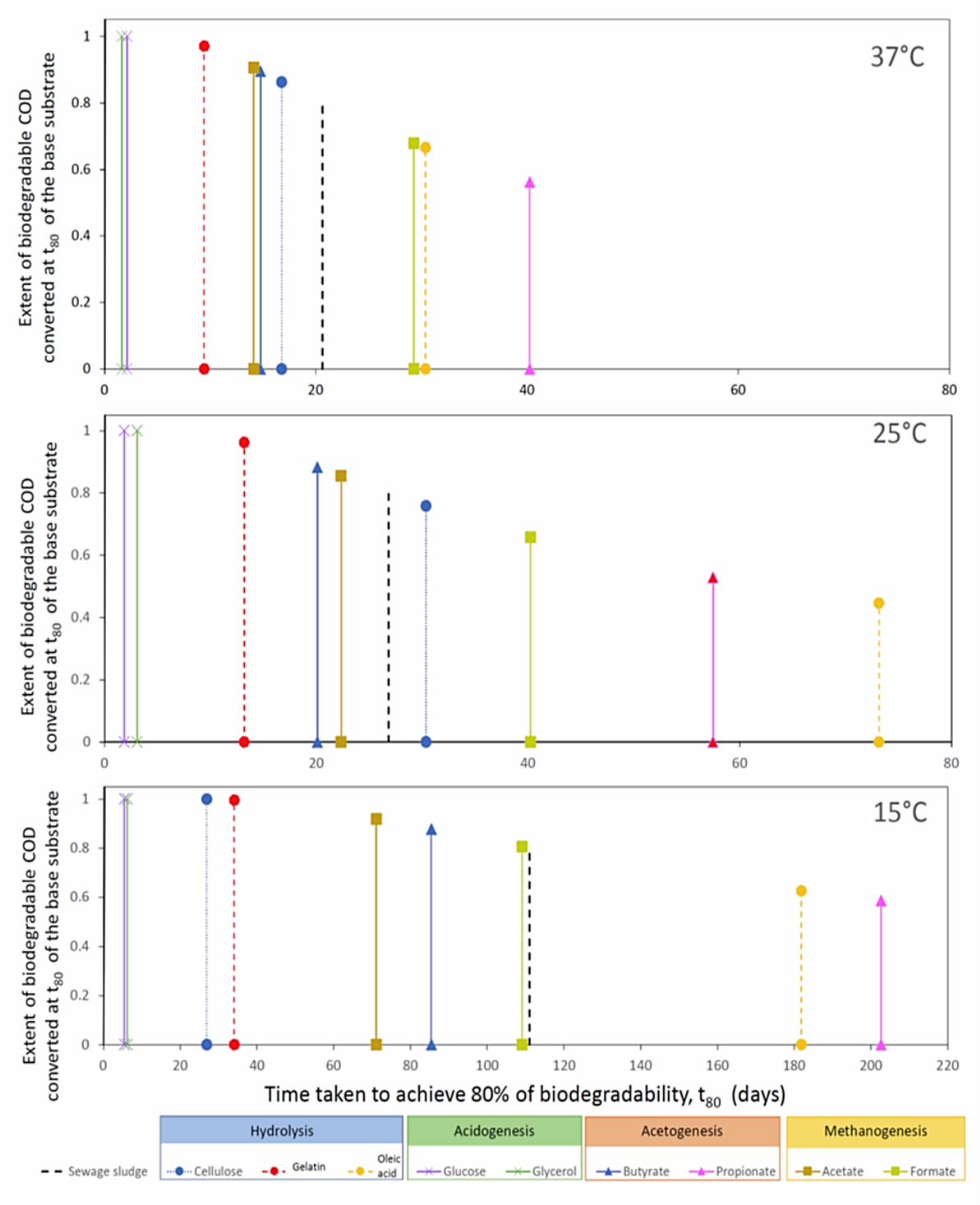

Figure 20: Summary plots of kinetic estimates (horizontal axes) and yield estimates (vertical axes) for various model co-substrates digested by temperature adapted inoculum from the bench-scale digesters treating sewage sludge. The vertical black dashed lines give kinetic and yield characteristics of sewage sludge as base substrate. Note the change of scale on the horizontal axes for 15°C.

5.3.4 Summary

The results reported in this section provided detailed fundamental knowledge of the influence of temperature on AD systems operating in the mesophilic/psychrophilic range (15-37°C). This temperature range was selected, because it is most applicable to the seasonal variations experienced by ambient anaerobic systems operating in the Australian climate. The main focus of the study was to investigate how operating temperature influences relative process rates of the four key metabolic steps and how this contributes to overall AD performance. Four continuous well-mixed digesters were operated at controlled temperatures in the 15-37°C range and fed with a complex base substrate, namely pig manure or sewage sludge. These continuous digesters provided temperature adapted microbial communities on which further activity and capacity testing could be performed to determine complete microbial activity profiles for AcoD. This helped to identify potential biological process bottlenecks for AcoD of complex mixtures in ambient anaerobic systems.

A key process risk in AcoD comes from overload inhibition. Overload inhibition occurs when upstream processes (acid production) occur faster than downstream processes (acid consumption) and results in an unstable accumulation of organic acids. The impact of overload inhibition is more extreme at higher loading rates and more extreme at lower temperatures. The risks of overload inhibition can be managed to an extent by limiting organic loading and selecting appropriate co-digestion mixtures.

When considering how temperature impacts overload risk, all microbial processes were slower at 25°C compared to 37°C, and slower again at 15°C compared to 25°C. This result shows reduced capacity for methane production during Autumn-Winter months and as a result co-substrate loading should be reduced. Most importantly, results showed that the activities for downstream biological steps slowed to a greater extent than the upstream acid producing steps, causing a greater potential for imbalance and overload at cooler temperatures. Highly particulate feedstocks with high protein concentrations are most at risk, carbohydrate based substrates have lower risk at warmer temperatures with increasing risk at cooler temperatures. Lipid based substrates were an exception where acid production remained slower than acid consumption even at cooler temperatures, limiting overload risk. The outcomes are consistent with results elsewhere in this report ranking lipid substrates as lowest risk (as long as treatment times are adequate to ensure adequate extents of lipid digestion) and ranking protein substrates as highest risk.

In terms of practical application, maximum co-substrate loads could occur during Spring-Summer when the covered pond/process operates at warmer temperatures. During Spring-Summer, there would also be more flexibility to include protein, carbohydrate or lipid based waste mixtures, however lower protein concentrations would generally be recommended. Co-substrate loads should be reduced during Autumn-Winter when the covered pond/process operates at cooler temperatures. During cooler months, protein based wastes and soluble sugar based wastes should be avoided or at least minimised. Complex cellulose wastes may be applied, but at 50% lower loading than Spring-Summer. Lipid based wastes are expected to have the lowest risk, but could influence digestate quality if treatment times are inadequate.

6 PROJECT OUTCOMES: APPLIED ANAEROBIC CO-DIGESTION

6.1 Examination of Novel Co-substrates

6.1.1 Background

Australian industries already operate a number of technologies to recover energy from waste, there are >100 full-scale installations in Australia representing >$100 million in infrastructure. This infrastructure includes a broad range of technology configuration, designed to treat different organic wastes with different properties. However, much of this existing infrastructure is currently underutilised. This is significant as Australia generates over 14 million tonnes of organic waste per year and approximately 50% is currently disposed to landfill. Anaerobic co-digestion (AcoD) of high-energy substrates together with sewage sludge, piggery manure or combined slaughterhouse effluent can substantially increase biogas production and hence leverage existing anaerobic processes and infrastructure (Mata-Alvarez et al., 2011). Currently, co-substrates are available from a broad range of industries that generate organic wastes. However the physical, chemical and biological properties of these co-substrates can be very different and are therefore suited to different AD technologies and different waste mixtures. This section examines co-substrates from a broad range of industries to assess suitability for co-digestion in different applications.

6.1.2 Summary of Organic Waste Samples Assessed

Figure 21 provides an overview of the organic waste samples examined during the project distributed by the industry of origin. In total, thirty (30) samples have been selected from a range of industries to provide different characteristics for assessment. The co-substrates have been categorised into 6 source industries, as shown in Figure 21 and Table 12.

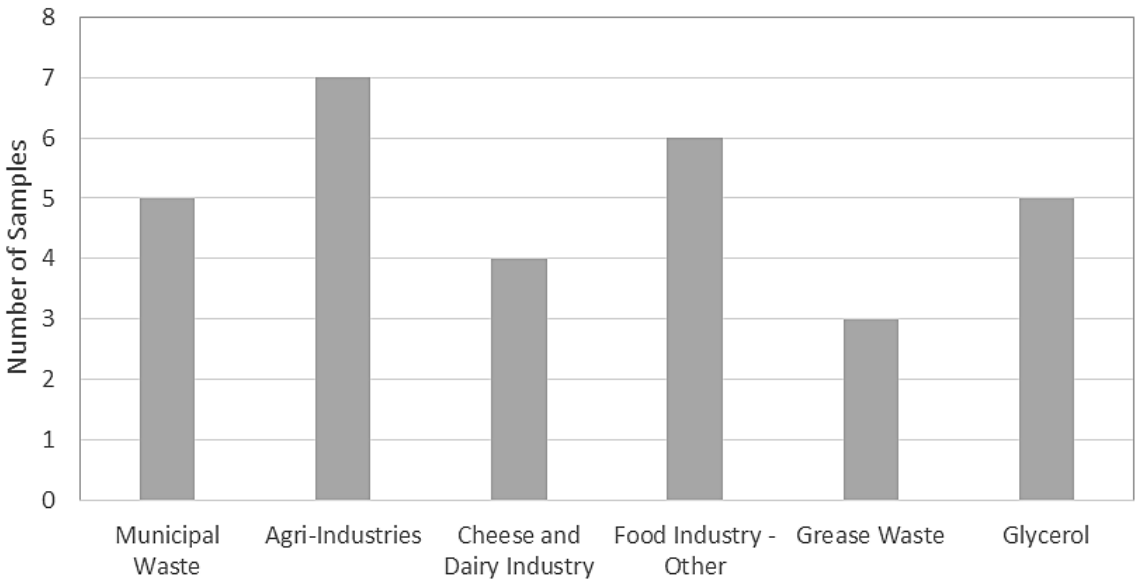

Figure 21: Summary of Organic Waste Samples distributed by the industry of origin.

Table 12: Description and Classification of all Co-substrates assessed during project

	Sample CODE	Industry	Description
1	SS1	Municipal Sludge	Mixed Sewage Sludge (primary sludge and waste activated sludge
2	MSW1	Municipal Solids	Source separated Organic Fraction of Municipal Waste
3	MSW2	Municipal Solids	Source separated Organic Fraction of Municipal Waste
4	SGW1	Municipal Solids	Solid Green Waste – leaf, branch and grass waste
5	SFW1	Municipal Solids	Solid Food Waste – supermarket food waste, separated and macerated
6	PM1	Agri-industry	Screened Pig Manure
7	RMP1	Agri-industry	Screened and Dewatered Paunch Solid Waste from Red Meat Processing (RMP) Plant 1
8	RMP2	Agri-industry	Waste Activated Sludge from wastewater treatment at RMP Plant 1
9	RMP3	Agri-industry	Dissolved Air Flotation Sludge from primary treatment at RMP Plant 1
10	RMP4	Agri-industry	Screened and Dewatered Red Solids from primary treatment at RMP Plant 1
11	RMP5	Agri-industry	Screened and Dewatered Paunch Waste from RMP Plant 2
12	ALG1	Agri-industry	Raw Algae
13	CD1	Cheese and Dairy	Dairy Sample collected 6/7/2017
14	CD2	Cheese and Dairy	Dairy Sample collected 21/7/2017
15	CD3	Cheese and Dairy	Cheese Whey collected 17/8/2017
16	CD4	Cheese and Dairy	Ricotta Whey collected 17/8/2017
17	FIO1	Food - Other	Beverage Processing Waste
18	FIO2	Food - Other	Wastewater from honey packaging facility
19	FIO3	Food - Other	Macerated waste from food/salad packaging plant
20	FIO4	Food - Other	Spreadwaste/Food Processing Trade Waste
21	FIO5	Food - Other	Dissolved Air Flotation from food processing plant
22	FIO6	Food - Other	RTD AM – DAF Sample collected 16/1/2018.
23	FOG1	Grease Trap	Grease Waste (FOG)
24	FOG2	Grease Trap	Grease Waste (FOG) SP1
25	FOG3	Grease Trap	Grease Waste (FOG) SP2
26	GLY1	Glycerine	A120 – ICI Glycerine collected 21/12/2017
27	GLY2	Glycerine	A120 – IR Glycerine collected 21/12/2017
28	GLY3	Glycerine	A150 – ICI Glycerine collected 21/12/2017
29	GLY4	Glycerine	S290 – ICI Glycerine collected 21/12/2017
30	GLY5	Glycerine	Glycerol (GLY)

6.1.3 Substrate Compositions

The physical and chemical compositions of anaerobic digestion co-substrates were assessed and compared based on 4 key areas:

(a) Solids Concentrations (TS or Dry Matter) – solids concentration is linked to i) organic loading potential, where materials with higher solids can potentially achieve higher loading rates, ii) materials handling requirements, where materials with higher solids may be more difficult handle, transport and/or mix within the digester and iii) technology suitability, where co-substrates with very low solids are generally suited to different digester technologies compared to co-substrates with very low solids. The fractionation between VS and ash also provides an indication of inert material entering the digestion process. Ash will not contribute to AD and will either accumulate within the process or exit as residual solids. Therefore co-substrates with very low ash content would be generally preferred.

(b) Chemical Oxygen Demand (COD) – Chemical oxygen demand represents the energy potential of a substrate. Anaerobic co-digestion is generally applied to existing AD infrastructure, where the application digester volumes would be fixed. Adding co-substrates will generally increase the total volume of waste being treated in the digester and this decreases retention time. Co-substrates with very high COD can be used to significantly increase organic loading to a digester with only minor changes to volume loading and retention time. However, dosing of these substrates must be managed carefully to limit the risk of organic overload. The fractionation between soluble COD and particulate COD provides a qualitative indication of degradable fraction and speed of degradation. Co-substrates with high soluble fractions are more likely to have high degradability and rapid digestion kinetics.

(c) Nitrogen Concentration – Depending on the application, nitrogen represents a significant cost associated with importing wastes for co-digestion (i.e. where nitrogen is mobilised and must be removed prior to discharge of the centrate) or a value-add opportunity where nitrogen increases the fertilizer value of the centrate and/or solid digestate.

(d) Phosphorous Concentration – Depending on the application, phosphorous can represent a significant cost associated with importing wastes for co-digestion (i.e. where phosphorous is mobilised and must be removed prior to discharge of the digestate) or can be a value-add opportunity where phosphorous increases the fertilizer value of the centrate and/or solid digestate.

A summary of dry matter (solids concentrations) and the fractionation between VS and ash for all co-substrates is shown in Figure 22. Similarly, a summary of COD and the fractionation between soluble COD and particulate COD for all co-substrates is shown in Figure 23. These figures show that all wastes, with the exception of CD1, FIO4 and FIO6, contain higher concentrations of both solids and COD compared to sewage sludge and pig manure (as key base substrates). Therefore, all of these wastes will have stronger impact of organic loading rate and a lesser impact of volume loading. Glycerine wastes were very high strength with COD values more than 10× larger than sewage sludge, therefore Glycerine wastes could be used to substantially increasing organic loading with little impact on volume. The Glycerine wastes had very high soluble fractions, suggesting that these wastes may degrade rapidly and to a high extent. The high soluble fraction of the Glycerine wastes also suggests that the wastes are liquid and readily transportable, thus reducing materials handling challenges.

Macerated solid food waste and macerated organic fraction of MSW were also concentrated sources of organics at greater than 5× sewage sludge, however these wastes contained a much lower soluble fraction, and materials handling may be more challenging. Generally Cheese and Dairy wastes and Other Food Industry wastes (such as beverage processing, honey packaging and Spreadwaste/Food processing) were moderately concentrated. These wastes generally contained high soluble fractions, suggesting high biodegradability and good materials handling characteristics, however the lower strength of these wastes suggests that limited increases in OLR can be achieved without exceeding the volume loading limits of existing digesters. The low strength may also increase the relative transport costs to import these co-substrates to site.

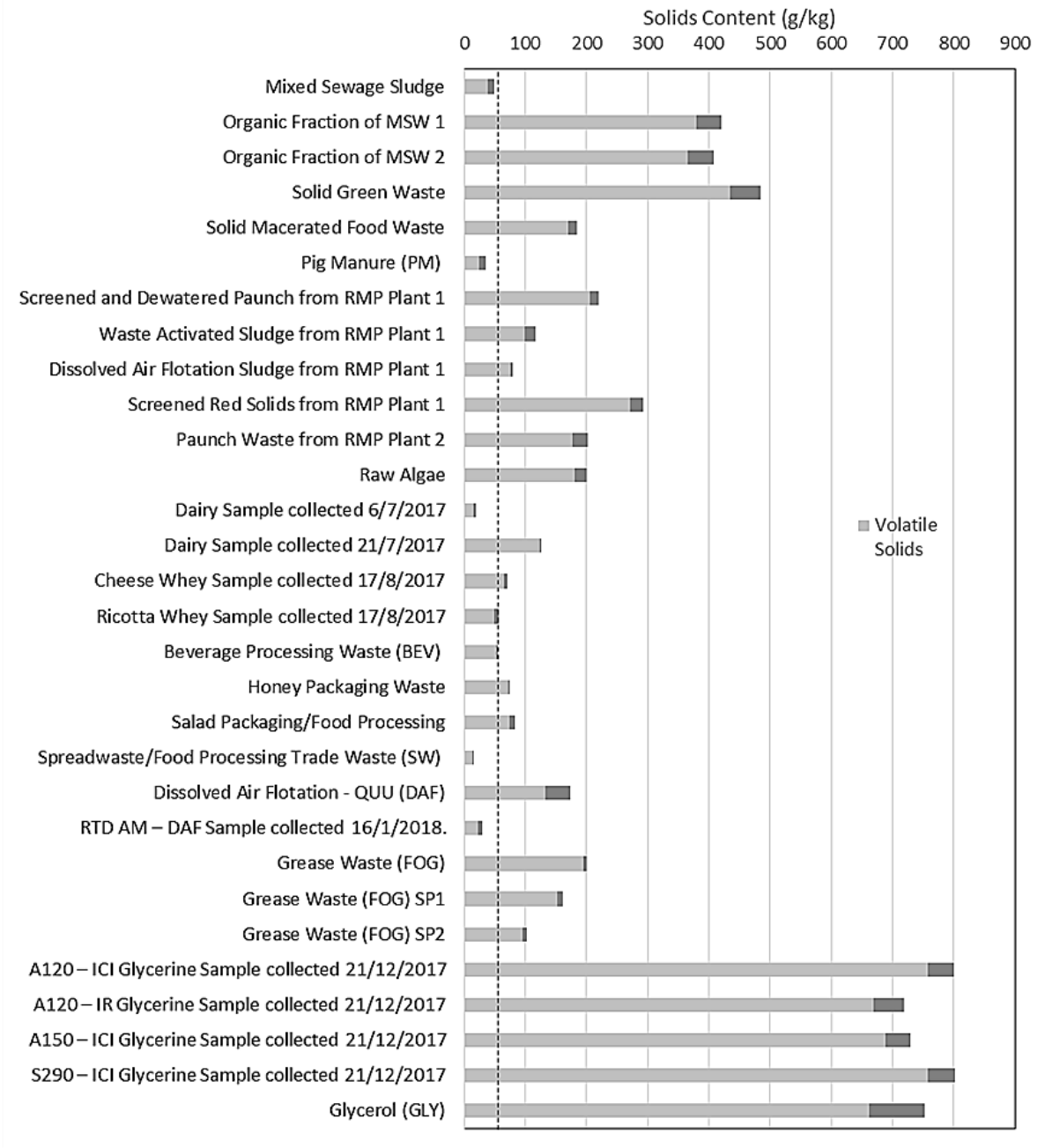

Figure 22: Solids content of co-substrates assessed during project in comparison to mixed sewage sludge. Each bar represents total solids and is split to show volatile solids and ash fractions.

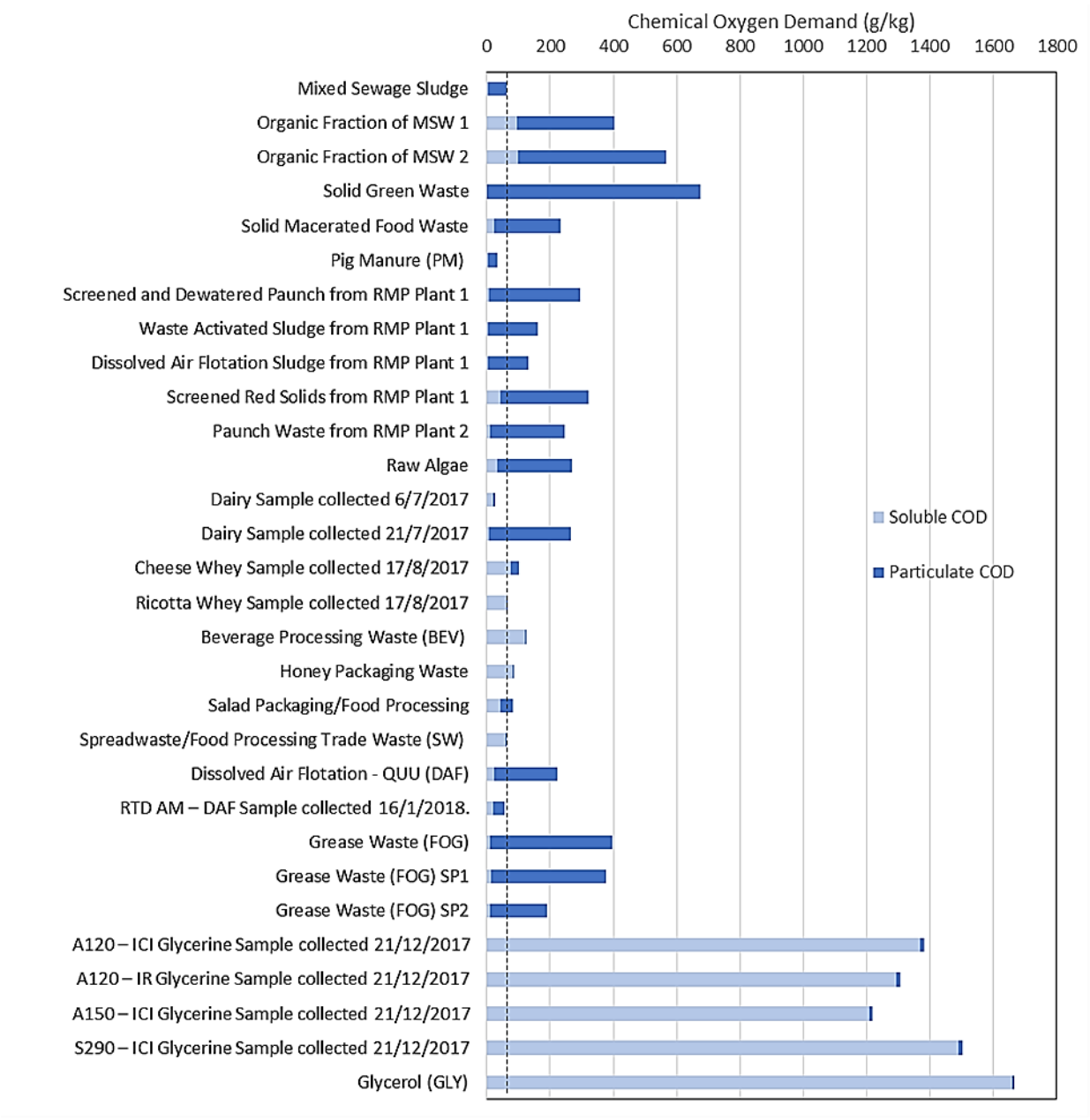

Figure 23: Chemical oxygen demand content of co-substrates assessed during project in comparison to mixed sewage sludge. Each bar represents total COD and is split to show soluble and particulate fractions.

Nitrogen concentrations and phosphorous concentrations for all co-substrates are summarised in Figure 24 and Figure 25 respectively. In the case of AcoD at municipal WWTPs, co-substrates of inherently low nitrogen and phosphorus contents would be preferred. With increasingly stringent regulations for nutrient discharge limits (e.g. lower effluent nitrogen and phosphorus concentrations), costs associated with power consumption for nitrification and adding external carbon sources for de-nitrification are on the rise. This means that co-digesting a waste of high nitrogen content can lead to nitrogen-rich supernatant returning to the mainstream wastewater treatment chain for biological nitrogen removal. The same applies to high-phosphorus co-substrates, which can result in increased costs for coagulants to remove phosphorus. Limitations around nitrogen and phosphorous may not apply in rural applications, such as agricultural industries, where wastewater is treated to remove organics prior to land irrigation where nutrients have fertilizer value and there is sufficient land available.

Glycerine wastes, fat oil and grease wastes and some food industry wastes are better suited to applications aiming to limit the import of nutrients. Agricultural wastes are a source of nitrogen, while macerated organic fraction of MSW (MSW1 and MSW2), solid green waste (SGW1) and macerated solid food waste (SFW1) are good sources of both nitrogen and phosphorous. Algae is also a rich source of both nitrogen and phosphorous, however the nutrient requirements for algal growth need to considered for locally produced algae.

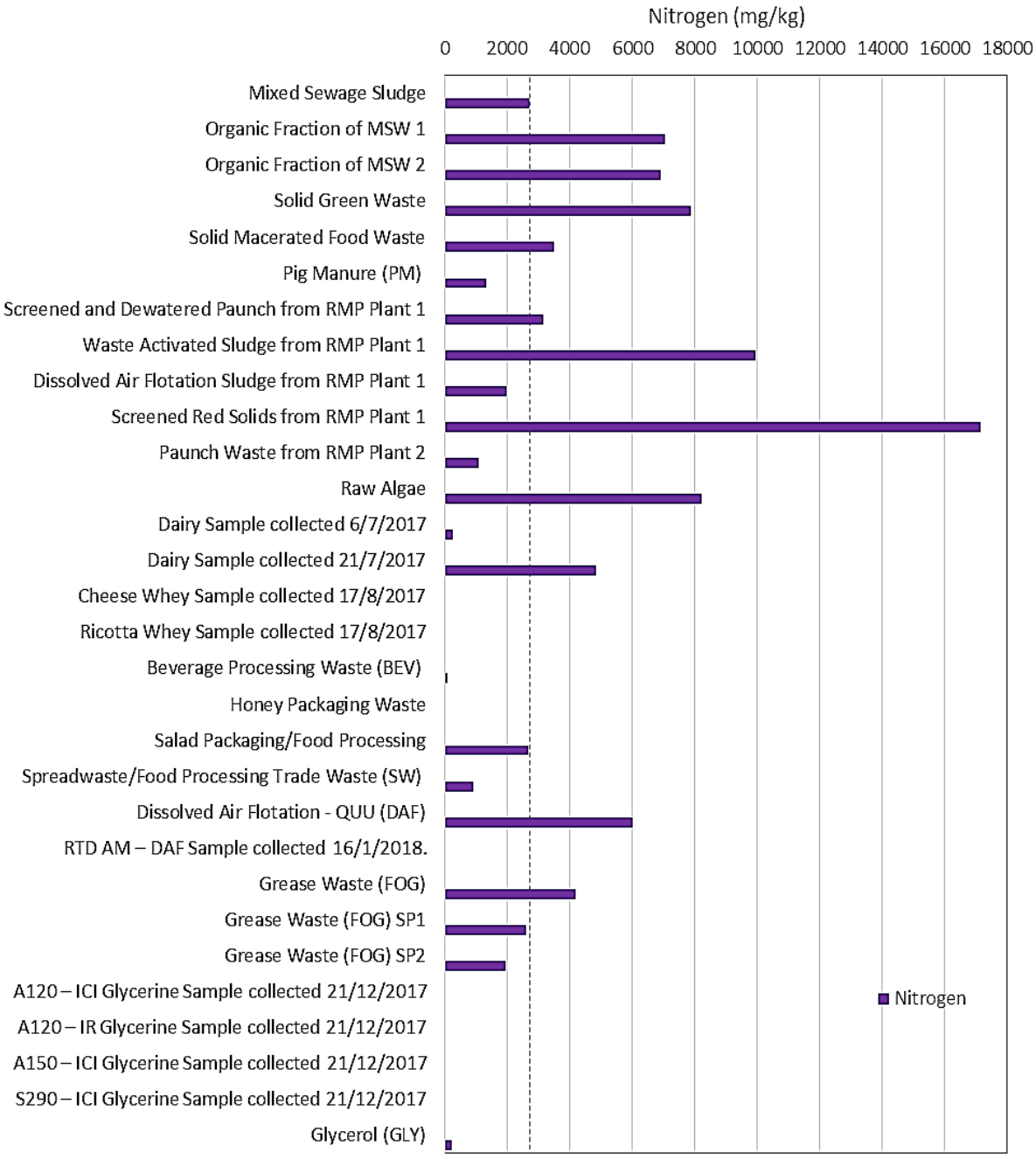

Figure 24: Nitrogen content of co-substrates assessed during project in comparison to mixed sewage sludge.

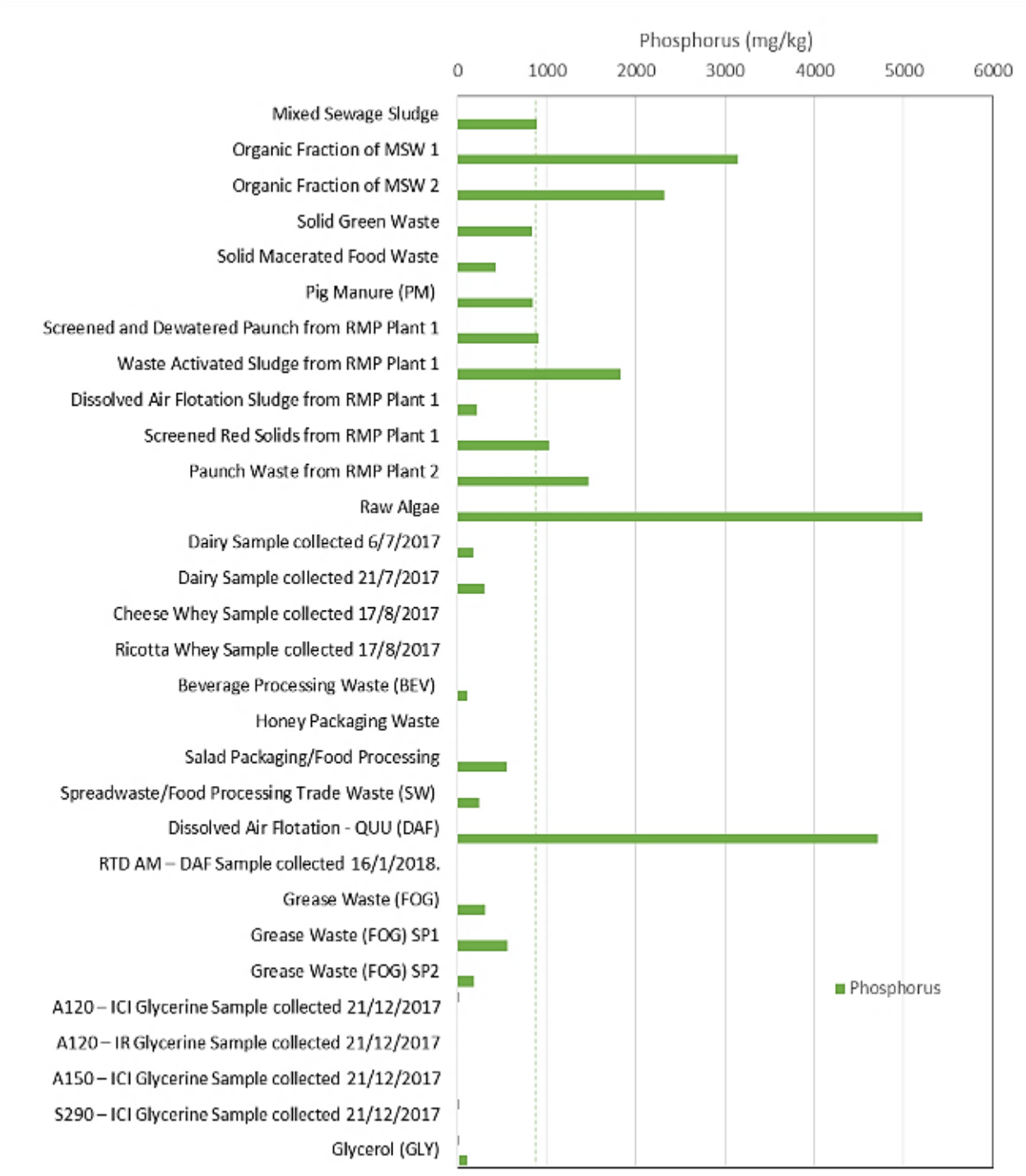

Figure 25: Phosphorous content of co-substrates assessed during project in comparison to mixed sewage sludge.

6.1.4 Substrate Methane Potential and Degradability

Biological characteristics of potential co-substrates were assessed based on:

(a) Biochemical methane potential - representing the final methane yield that can be achieved through anaerobic digestion of the trade waste under ideal conditions. Expressed at L CH_4.kg VS^{-1} added.
(b) Degradable fraction (f_d) – representing the fraction of organic material in the waste that can be converted to methane under ideal conditions. Degradable fraction is typically based on the fraction of COD converted to methane, but may be used to estimate volatile solids destruction, nitrogen mobilisation and phosphorus mobilisation during AD.
(c) Apparent Hydrolysis rate constant (k_{hyd}) – representing the speed of anaerobic digestion at mesophilic conditions. The hydrolysis rate constant is used to determine digester sizing to enable complete conversion of a waste, or to estimate the extent of conversion based on an existing digester size.

Biochemical methane potential (B_0) and degradable fractions of all co-substrates assessed during the project (and compared to mixed sewage sludge) are shown in Figure 26 and Figure 27, respectively. The methane potentials of the different trade wastes were highly variable ranging from 130 L CH_4.kg VS^{-1} added up to 850 L CH_4.kg VS^{-1} added. In general, fat oil and grease wastes and glycerol wastes all had very high methane potentials and very high degradable fractions. This is consistent with the high theoretical methane potential expected for lipid based wastes. The very high degradable fractions for these wastes suggests that biogas revenue can be increased significantly with little or no impact on residual solids production. Cheese and Dairy wastes and Other Food Industry wastes generally had moderate methane potentials, however the degradable fraction of these wastes was generally high, with minimal impact of residual solids expected.

Municipal wastes (MSW1, MSW2 and SGW1) and several agricultural wastes exhibited moderate to low methane potential and lower degradable fractions. The lower methane potential is consistent with lower theoretical methane expected for carbohydrate based wastes, particularly complex lignocellulosic wastes. Poor degradable fractions, such as a degradability 0.22 for Solid Green Waste (SGW1), 0.43 for Waste Activated Sludge (RMP2) and 0.4 for paunch solids (RMP5), would result in poor biogas production and large increases in residual solids for subsequent disposal.

A summary of hydrolysis rate constant (k_{hyd}) of co-substrates assessed during project and compared to mixed sewage sludge is shown in Figure 28. The speed of co-substrate degradation is an important factor, especially for hydrolysis controlled AD systems. For example, WWTP AD digesters are normally designed for a minimum HRT based on specific requirements for the stabilisation of sewage sludge in these processes. Therefore, it is vital to select a co-substrate with a degradation speed that matches or exceeds sewage sludge in order to ensure sufficient destruction within the process and acceptable biosolids quality. Otherwise, AcoD of slowly degraded co-substrate relative to the main substrate is likely to have a portion of un-degraded organic materials that incur extra costs to treat. In general, FOG wastes, Glycerine wastes and most food industry wastes degrade rapidly in comparison to sewage sludge and are therefore suitable for digesters designed to treat sludge. Solid Green waste and almost all agricultural wastes degraded slowly in comparison to sludge. These wastes would not be suitable for sludge digesters or high rate processes, but may still be suitable for long retention time covered pond systems if the material is able to settle and be captured within the process.

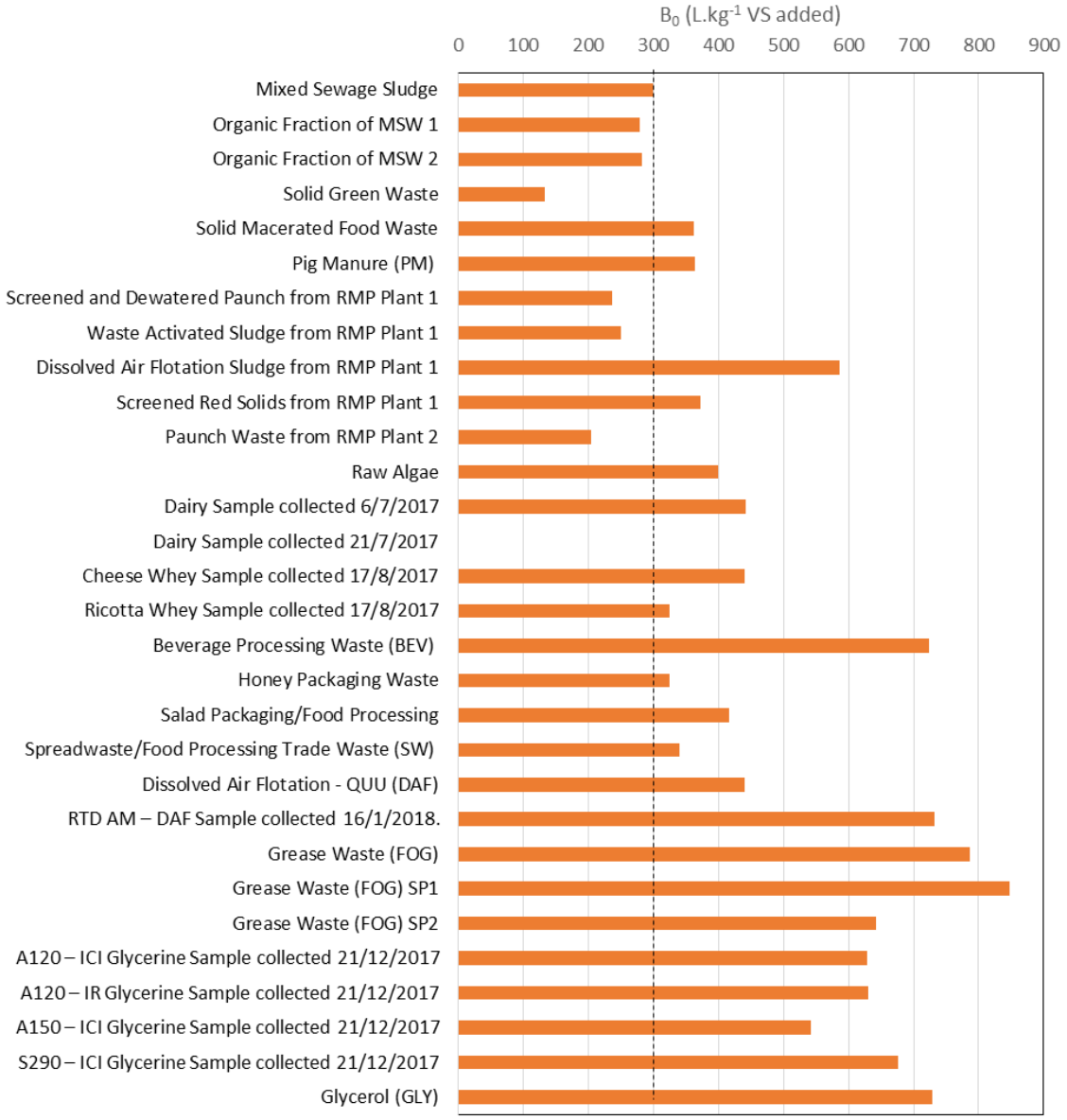

Figure 26: Summary of biochemical methane potential (B_0) of co-substrates assessed during project and compared to mixed sewage sludge.

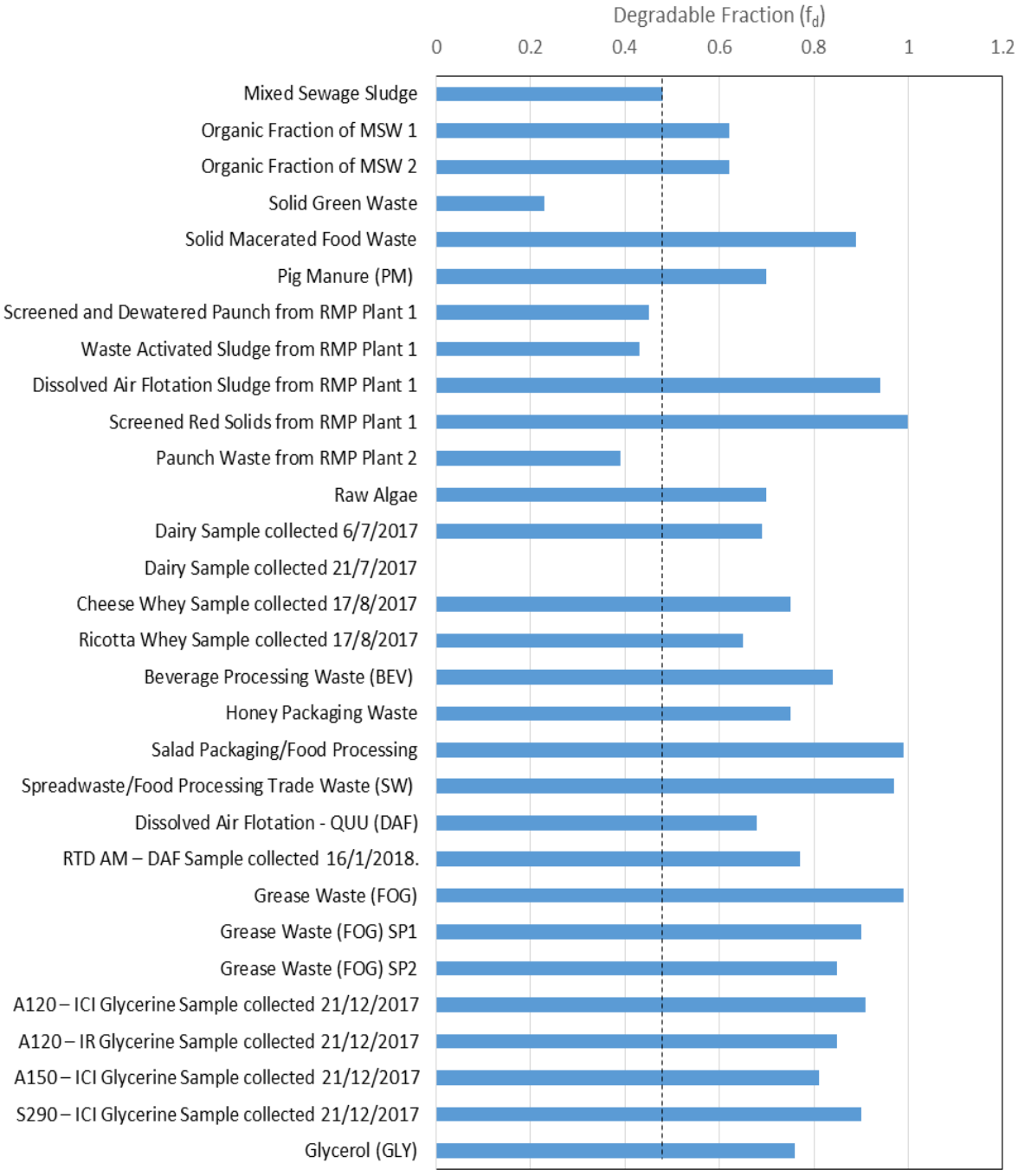

Figure 27: Summary of degradable fraction (f_d) of co-substrates assessed during project and compared to mixed sewage sludge.

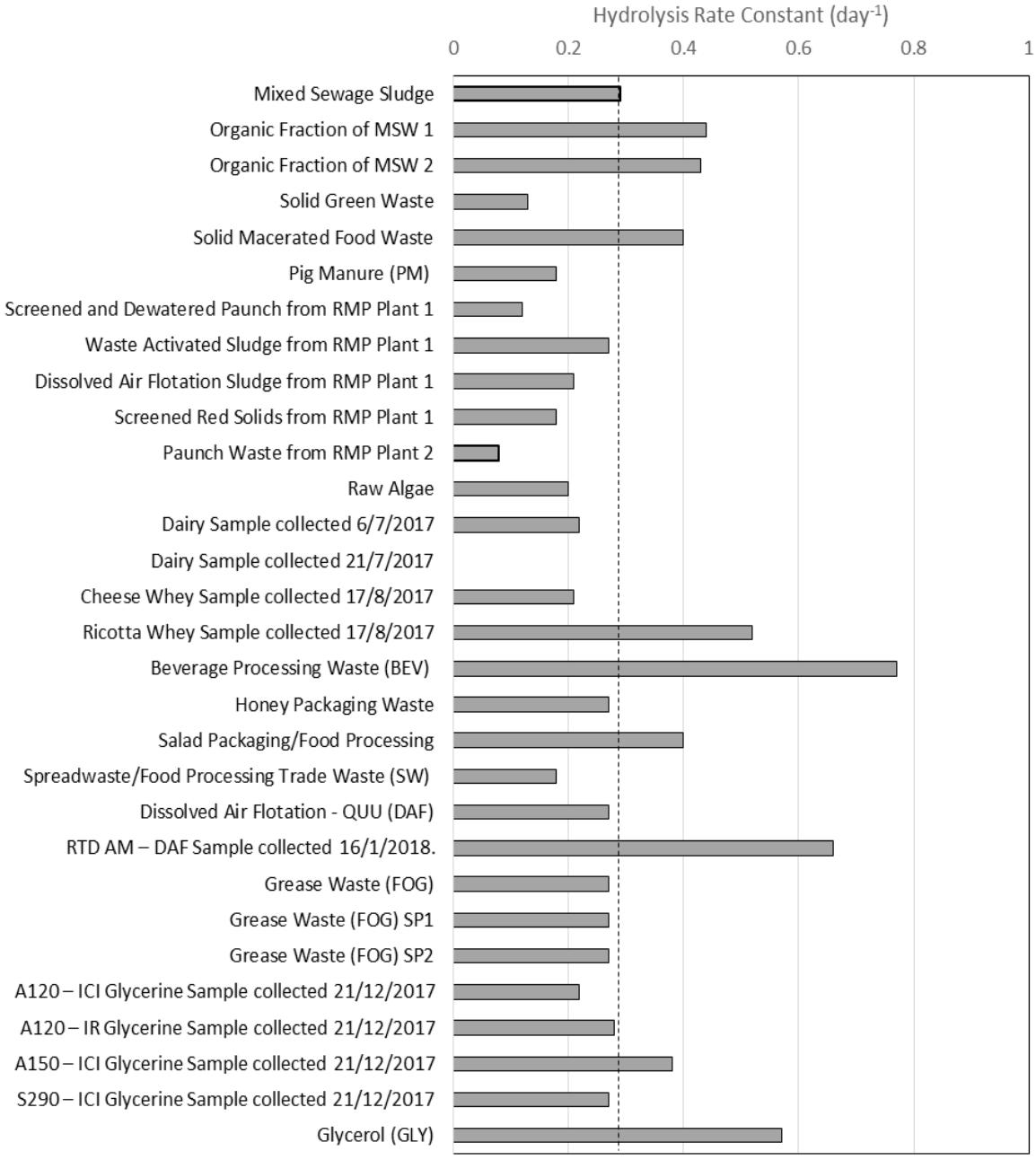

Figure 28: Summary of hydrolysis rate constant (k_{hyd}) of co-substrates assessed during project and compared to mixed sewage sludge.

6.1.5 Substrate Ranking and Conclusions

6.1.5.1 In vessel Anaerobic Digestion at Municipal WWTP

This section presents a risk assessment and ranking of co-substrates assessed in this project for application to a conventional mixed liquor digester treating mixed sewage sludge as a base substrate. In this scenario, the digester is well mixed with a conventional solids retention time (SRT) of 20-25 days.

Ranking of co-digestion substrates was based on a combination of 4 parameters: inhibition risk, whether complete degradation would be expected within the infrastructure (pass/fail based hydrolysis rate of co-substrate relative to base substrate), the organic loading potential of the substrate (COD of co-substrate relative to base substrate) and economic position. Tradewaste charges are application specific and were not considered in this analysis. The economic assessment considered 4 primary factors:

- Revenue from electricity, estimated at $0.20 per kWh
- Additional biosolids production and disposal cost, estimated at $60 per tonne of wet biosolid (20% cake solids)
- Additional nitrogen mobilisation (to centrate) and removal cost, estimated at $2 per kg
- Additional phosphorus mobilisation (to centrate) and removal cost, estimated at $2 per kg

Rankings are presented in Table 13. Co-substrates are initially classed as “suitable” or “not suitable” and are then ranked based on economic position. Co-substrates are deemed “not suitable” if the inhibition risk cannot be managed, if the substrates will not degrade sufficiently within the digester or if the net economic position is negative. Substrates classed as not suitable are shaded in red.

Glycerine samples and Fat, Oil and Grease wastes are the highest ranked co-substrates for application to mixed liquor digesters at municipal WWTP, this is based on very high space loading, fast degradation and good biological performance (B_0 and f_d). Glycerol and FOG addition needs to be controlled carefully to manage inhibition risk, however this management was achieved effectively within the BMP testing and should be present minimal risk at full-scale. Waste glycerol can contain a relatively high potassium concentration and the impact on downstream processing has not been considered.

Macerated food waste and other food industry wastes (FIO1, FIO2 and FIO3) are also strong candidates for co-digestion at municipal WWTP based on a very fast hydrolysis rate, low impact on residual solids content and low inhibition risk. However, FIO1, FIO2 and FIO3 are relatively dilute compared to the GLY and FOG samples and therefore requires much higher volume loading to achieve similar improvements in methane production. The dilute nature of FIO1, FIO2 and FIO3 will limit the increases in organic loading that can be obtained and therefore the ultimate biogas production.

Agricultural samples rank poorly and largely unsuitable for co-digestion in municipal WWTP infrastructure, this was mostly due to the expected presence of non-degradable VS and mineral solids. Both would likely result in increased costs associated with residual solids handling and poor economics. Agricultural samples also tend to degrade slowly and require longer treatment times than sewage sludge.

Table 13: Rankings and risk assessment of co-digestion substrates for application to a Continuous Stirred Tank Reactor (CSTR) treating mixed sewage sludge

	Risk Assessment			Economics				
	Inhibition Risk	Degradation Speed	Space Loading	Electricity $/T	Residual solids $/T	N $/T	P $/T	Total $/T
GLY4	Moderate	Good	Extreme	338.8	-36.1	0.00	0.00	302.67
GLY5	Moderate	Good	Extreme	318.6	-74.7	-0.35	-0.14	243.40
GLY2	Moderate	Pass	Extreme	278.7	-45.1	0.00	0.00	233.60
FOG1	Moderate	Pass	Very high	207.1	-2.1	-8.32	-0.61	196.06
GLY3	Moderate	Good	Extreme	247.3	-51.6	0.00	0.00	195.77
FOG2	Moderate	Pass	Very high	85.2	-7.6	-4.69	-1.01	71.98
FOG3	Moderate	Pass	High	40.8	-6.1	-3.32	-0.32	31.02
SFW1	Low	Good	Very high	40.8	-10.1	-6.23	-0.75	23.71
FIO1	Low	Good	Moderate	25.3	-2.6	-0.16	-0.17	22.40
FIO3	Low	Good	Moderate	20.6	-2.5	-5.29	-1.09	11.78
FIO2	Low	Pass	Moderate	15.5	-5.5	-0.01	0.00	10.07
FIO6	Low	Good	Poor	10.7	-3.6	0.00	0.00	7.14
CD4	Low	Good	Moderate	10.8	-7.3	0.00	0.00	3.46
MSW2	Low	Good	Very high	68.5	-54.3	-8.56	-2.88	2.72
MSW1	Low	Good	Very high	70.2	-55.5	-8.74	-3.89	2.02
GLY1	Moderate	Fail	Extreme	314.8	-33.3	0.00	0.00	281.45
RMP4	Low	Fail	Very high	66.8	-6.3	-34.26	-2.04	24.17
RMP3	Low	Fail	Moderate	29.7	-2.3	-3.71	-0.41	23.25
CD3	Low	Fail	Moderate	19.0	-6.5	0.00	0.00	12.48
ALG1	Low	Fail	High	47.6	-22.2	-11.52	-7.28	6.60
CD1	Low	Fail	Poor	4.7	-2.6	-0.37	-0.24	1.48
FIO4	Low	Fail	Moderate	3.1	-0.8	-1.78	-0.47	0.04
FIO5	Low	Pass	High	38.5	-25.0	-8.20	-6.39	-1.08
PM1	Low	Fail	Poor	5.9	-5.2	-1.89	-1.18	-2.39
SS1	Low		N/A	7.5	-9.4	-2.62	-0.86	-5.34
RMP1	Low	Fail	High	32.3	-38.3	-2.85	-0.82	-9.71
RMP2	Low	Pass	Moderate	16.5	-22.1	-8.55	-1.57	-15.72
RMP5	Low	Fail	High	24.0	-40.0	-0.85	-1.14	-17.94
SGW1	Moderate	Fail	Very High	38.4	-115.2	-3.62	-0.38	-80.78
CD2	Moderate	Fail	High	N/A	N/A	N/A	N/A	N/A

The substrate row in grey is the base substrate for this specific application.

6.1.5.2 Lagoon Based Anaerobic Digestion at Municipal WWTP

This section presents a risk assessment and ranking of co-substrates assessed in this project for application to a high rate anaerobic lagoon treating mixed sewage sludge as a base substrate. In this scenario, the digester has a HRT of 2 days. The digester is not well mixed and relies on the settling an accumulation of solids to achieve full conversion to methane.

Ranking of co-digestion substrates was based on a combination of 4 parameters: inhibition risk, the volume of inert solids that would accumulate in the lagoon, the organic loading potential of the substrate (COD of co-substrate relative to base substrate) and economic position. Tradewaste charges are application specific and were not considered in this analysis. The economic assessment considered 4 primary factors:

- Revenue from electricity, estimated at $0.20 per kWh
- Additional biosolids production and disposal cost, estimated at $20 per T of wet biosolid (20% cake solids)
- Additional nitrogen mobilisation (to centrate) and removal cost, estimated at $2 per kg
- Additional phosphorus mobilisation (to centrate) and removal cost, estimated at $2 per kg

Rankings are presented in Table 14. Co-substrates are initially classed as “suitable” or “not suitable” and are then ranked based on economic position. Co-substrates are deemed “not suitable” if the inhibition risk cannot be managed or if the net economic position is negative. Substrates classed as not suitable are shaded in red. Degradation speed is not a key criteria in lagoon based digestion due to very long retention times of settled material.

Based on the criteria in Table 14, glycerine samples and Fat, Oil and Grease wastes are the highest ranked co-substrates for application to lagoons at municipal WWTP, this is based on very high space loading, fast degradation and good biological performance (B_0 and f_d). However, there are areas of concern. Glycerol and FOG are predominantly liquid wastes and the settling behaviour has not been assessed. It is not clear if the Glycerol and FOG wastes would be retained in a high rate lagoon for sufficient time to allow complete digestion. This is considered a minor risk due to the very high dilution rates and therefore low concentrations of substrate to be consumed.

Organic fraction of MSW (MSW1, MSW2) and some agricultural co-substrates (RMP3, RMP4) are strong candidates for co-digestion in WWTP lagoons. These wastes have high space loading allowing for significant increases in organic loading. These wastes also contain high solid fractions which will promote settling in lagoon based treatment. The high degradable fractions suggest that the material will completely degrade within the lagoons with very little impact on lagoon sludge levels. However, not all agricultural solid wastes are well suited to lagoon treatment. RMP1, RMP2 and RMP5 are poor candidates for lagoon treatment due to poor degradable fractions. These wastes are expected to accumulate in lagoons allowing very long treatment time, however the high fraction of non-degradable solids would lead to rapid sludge accumulation in the lagoon and frequent desludging operations.

Food industry wastes (FIO1, FIO2 and FIO3) are moderate candidates for co-digestion at municipal WWTP based on a very fast hydrolysis rate, low impact on residual solids content and low inhibition risk. However, FIO1, FIO2 and FIO3 are predominantly soluble wastes. The settling behaviour has not been assessed and may impact suitability for high rate lagoons.

Table 14: Rankings and risk assessment of co-digestion substrates for application to a high rate anaerobic lagoon treating mixed sewage sludge

	Risk Assessment			Economic Assessment				
	Inhibition	Residual Solids	Space loading	Electricity	Residual solids	N	P	Total
				$/T	$/T	$/T	$/T	$/T
GLY4	Moderate	Very Good	Extreme	338.8	-12.0	0.00	0.00	326.73
GLY1	Moderate	Very Good	Extreme	314.8	-11.1	0.00	0.00	303.68
GLY5	Moderate	Good	Extreme	318.6	-24.9	-0.35	-0.14	293.22
GLY2	Moderate	Very Good	Extreme	278.7	-15.0	0.00	0.00	263.64
GLY3	Moderate	Very Good	Extreme	247.3	-17.2	0.00	0.00	230.15
FOG1	Moderate	Very Good	Very high	207.1	-0.7	-8.32	-0.61	197.45
FOG2	Moderate	Very Good	Very high	85.2	-2.5	-4.69	-1.01	77.02
MSW1	Low	Good	Very high	70.2	-18.5	-8.74	-3.89	39.02
MSW2	Low	Good	Very high	68.5	-18.1	-8.56	-2.88	38.94
FOG3	Moderate	Very Good	High	40.8	-2.0	-3.32	-0.32	35.10
SFW1	Low	Very Good	High	40.8	-3.4	-6.23	-0.75	30.45
RMP4	Low	Very Good	Very high	66.8	-2.1	-34.26	-2.04	28.37
RMP3	Low	Very Good	Moderate	29.7	-0.8	-3.71	-0.41	24.81
FIO1	Low	Very Good	Moderate	25.3	-0.9	-0.16	-0.17	24.13
ALG1	Low	Good	High	47.6	-7.4	-11.52	-7.28	21.40
CD3	Low	Good	Moderate	19.0	-2.2	0.00	0.00	16.82
FIO5	Low	Moderate	High	38.5	-8.3	-8.20	-6.39	15.57
FIO2	Low	Good	Moderate	15.5	-1.8	-0.01	0.00	13.70
FIO3	Low	Very Good	Moderate	20.6	-0.8	-5.29	-1.09	13.43
FIO6	Low	Good	Poor	10.7	-1.2	0.00	0.00	9.54
CD4	Low	Moderate	Moderate	10.8	-2.4	0.00	0.00	8.33
CD1	Low	Moderate	Moderate	4.7	-0.9	-0.37	-0.24	3.19
PM1	Low	Good	Poor	5.9	-1.7	-1.89	-1.18	1.10
SS1	Low	N/A	N/A	7.5	-3.1	-2.62	-0.86	0.92
FIO4	Low	Very Good	Moderate	3.1	-0.3	-1.78	-0.47	0.56
RMP1	Low	Poor	High	32.3	-12.8	-2.85	-0.82	15.85
RMP5	Low	Poor	High	24.0	-13.3	-0.85	-1.14	8.69
RMP2	Low	Poor	Moderate	16.5	-7.4	-8.55	-1.57	-1.00
SGW1	Moderate	Poor	Extreme	38.4	-38.4	-3.62	-0.38	-3.97
CD2	Moderate	Poor	High	N/A	N/A	N/A	N/A	N/A

The substrate row in grey is the base substrate for this specific application.

6.1.5.3 In vessel Anaerobic Digestion at Read Meat Processing Facility

This section presents a risk assessment and ranking of co-substrates assessed in this project for application to a conventional mixed liquor digester treating solid paunch waste as a base substrate. In this scenario, the digester is well mixed with a conventional SRT of 30 days.

Ranking of co-digestion substrates was based on a combination of 4 parameters: inhibition risk, whether complete degradation would be expected within the infrastructure (pass/fail based hydrolysis rate of co-substrate relative to base substrate), the organic loading potential of the substrate (COD of co-substrate relative to base substrate) and economic position. Tradewaste charges are application specific and were not considered in this analysis. The economic assessment considered 4 primary factors:

- Revenue from heat generation, estimated at $10 per GJ
- Additional biosolids production and disposal cost, estimated at $20 per T of wet biosolid (20% cake solids)
- There is no direct cost associated with additional nitrogen mobilisation (to centrate).
- There is no direct cost associated with additional phosphorous mobilisation (to centrate).

Rankings are presented in Table 15. Co-substrates are initially classed as “suitable” or “not suitable” and are then ranked based on economic position. Co-substrates are deemed “not suitable” if the inhibition risk cannot be managed, if the substrates will not degrade sufficiently within the digester or if the net economic position is negative. Substrates classed as not suitable are shaded in red.

Glycerine samples and Fat, Oil and Grease wastes are the highest ranked co-substrates for application to mixed liquor digesters at RMP, this is based on very high space loading, fast degradation and good biological performance (B_0 and f_d). Additionally, there are examples of positive substrate interactions when solid paunch waste and FOG are co-treated, where lipid-degrading organisms present in paunch can improve fat biodegradability, thus resulting in a synergistic effect (Astals et al., 2014). As previously stated, glycerol and FOG addition needs to be controlled carefully to manage inhibition risk, however this management was achieved effectively within the BMP testing and should be present minimal risk at full-scale. Waste glycerol can contain a relatively high potassium concentration and the impact on downstream processing has not been considered.

Organic fraction of MSW (MSW1, MSW2), macerated food waste (SFW1) and some cheese and dairy wastes (CD3) and some food industry wastes (FIO1, FIO2) are also strong candidates for co-digestion at RMP based on low inhibition risk and low contribution to residual solids. FIO1, FIO2 and CD3 are relatively dilute waste streams, however this may be desirable in a red meat processing digester to reduce overall solids content and improve materials handling.

Table 15: Rankings and risk assessment of co-digestion substrates for application to an in-vessel digester treating screened and dewatered paunch solid waste

	Risk Assessment			Economic Assessment				
	Inhibition	Degradation Speed	Space loading	Electricity	Residual solids	N	P	Total
				$/T	$/T	$/T	$/T	$/T
GLY4	Moderate	Good	Very High	174.2	-12.0	0.00	0.00	162.19
GLY1	Moderate	Good	High	161.9	-11.1	0.00	0.00	150.78
GLY5	Moderate	Good	Very High	163.9	-24.9	-0.35	-0.14	138.46
GLY2	Moderate	Good	High	143.3	-15.0	0.00	0.00	128.29
GLY3	Moderate	Good	High	127.2	-17.2	0.00	0.00	110.01
FOG1	Moderate	Good	Moderate	106.5	-0.7	-8.32	-0.61	96.87
FOG2	Moderate	Good	Moderate	43.8	-2.5	-4.69	-1.01	35.62
FOG3	Moderate	Good	Moderate	21.0	-2.0	-3.32	-0.32	15.29
FIO1	Low	Good	Poor	13.0	-0.9	-0.16	-0.17	11.83
SFW1	Low	Good	Poor	21.0	-3.4	-6.23	-0.75	10.63
RMP3	Low	Good	Poor	15.3	-0.8	-3.71	-0.41	10.38
CD3	Low	Good	Poor	9.8	-2.2	0.00	0.00	7.60
FIO2	Low	Good	Poor	8.0	-1.8	-0.01	0.00	6.16
MSW2	Low	Good	Moderate	35.2	-18.1	-8.56	-2.88	5.68
MSW1	Low	Good	Moderate	36.1	-18.5	-8.74	-3.89	4.94
FIO6	Low	Good	Poor	5.5	-1.2	0.00	0.00	4.32
FIO3	Low	Good	Poor	10.6	-0.8	-5.29	-1.09	3.41
CD4	Low	Good	Poor	5.5	-2.4	0.00	0.00	3.10
CD1	Low	Good	Poor	2.4	-0.9	-0.37	-0.24	0.92
RMP1	Low	N/A	N/A	16.6	-12.8	-2.85	-0.82	0.16
CD2	Moderate	Good	Poor	0.0	0.0	0.00	0.00	0.00
FIO4	Low	Good	Poor	1.6	-0.3	-1.78	-0.47	-0.93
ALG1	Low	Good	Poor	24.5	-7.4	-11.52	-7.28	-1.72
PM1	Low	Good	Poor	3.0	-1.7	-1.89	-1.18	-1.77
SS1	Low	Good	Poor	3.9	-3.1	-2.62	-0.86	-2.73
RMP5	Low	Poor	Poor	12.3	-13.3	-0.85	-1.14	-2.97
FIO5	Low	Good	Poor	19.8	-8.3	-8.20	-6.39	-3.12
RMP4	Low	Good	Moderate	34.3	-2.1	-34.26	-2.04	-4.06
RMP2	Low	Good	Poor	8.5	-7.4	-8.55	-1.57	-9.00
SGW1	Moderate	Pass	Moderate	19.8	-38.4	-3.62	-0.38	-22.64

The substrate row in grey is the base substrate for this specific application.

6.1.5.4 Covered Pond Based Anaerobic Digestion at Piggery

This section presents a risk assessment and ranking of co-substrates assessed in this project for application to a long retention time covered pond treating screened pig manure as a base substrate. In this scenario, the digester has a HRT of greater than 30 days and is not well mixed.

Ranking of co-digestion substrates was based on a combination of 4 parameters: inhibition risk, the volume of inert solids that would accumulate in the covered pond, the organic loading potential of the substrate (COD of co-substrate relative to base substrate) and economic position. Trade-waste charges are application specific and were not considered in this analysis. The economic assessment considered 4 primary factors:

- Revenue from electricity, estimated at $0.20 per kWh
- Additional sludge production and disposal cost, estimated at $20 per T of wet solid (20% cake solids)
- No additional direct cost was affiliated with additional nitrogen mobilisation. Nitrogen fertilizer value potentially $1/kg, but case and location specific.
- No additional direct cost was affiliated with additional phosphorous mobilisation. Phosphorus fertilizer value potentially $1.4/kg, but case and location specific (US$130 per tonne of 32% P_2O_5 rock).

Rankings are presented in Table 16. Co-substrates are initially classed as “suitable” or “not suitable” and are then ranked based on economic position. Co-substrates are deemed “not suitable” if the inhibition risk cannot be managed or if the net economic position is negative. Substrates classed as not suitable are shaded in red. Degradation speed is not a key criteria in covered pond based digestion due to very long retention times of settled material.

Glycerine samples and Fat, Oil and Grease wastes are the highest ranked co-substrates for application to piggery lagoons, this is based on very high space loading, fast degradation and good biological performance (B_0 and f_d). The settling behaviour of Glycerol and FOG does not impact suitability for treatment in conventional piggery covered ponds, due to the much longer HRT and therefore lower washout risk. Glycerol and FOG addition needs to be controlled carefully to manage inhibition risk, however this management was achieved effectively within the BMP testing and should be present minimal risk at full-scale.

As with WWTP covered ponds, Organic fraction of MSW (MSW1, MSW2), macerated food waste (SFW1) and some agricultural co-substrates (RMP3, RMP4, ALG1) are strong candidates for co-digestion in piggery covered ponds. These wastes have high space loading allowing for significant increases in organic loading. These wastes also contain high solid fractions which will promote settling in covered pond based treatment. The high degradable fractions suggest that the material will completely degrade within the covered ponds with very little impact on covered pond sludge levels. However, not all agricultural solid wastes are well suited to covered pond n treatment. RMP1, RMP2 and RMP5 are poor candidates for covered pond treatment due to poor degradable fractions. These wastes are expected to accumulate in covered ponds allowing very long treatment time, however the high fraction of non-degradable solids would lead to rapid sludge accumulation in the covered pond and frequent desludging operations.

Food industry wastes (FIO1, FIO2 and FIO3) and dairy wastes (CD3 and CD4) are moderate candidates for co-digestion at municipal WWTP based on low impact on residual solids content and low inhibition

risk. While the settling behaviour of these wastes has not been assessed, they are expected to fully degrade in piggery covered ponds with long HRT.

Table 16: Rankings and risk assessment of co-digestion substrates for application to a covered pond with long hydraulic retention time treating screened pig manure

	Risk Assessment			Economic Assessment				
	Inhibition	Residual Solids	Space loading	Electricity $/T	Residual solids $/T	N $/T	P $/T	Total* $/T
GLY4	Moderate	Very Good	Extreme	338.8	-12.0	0.00	0.00	326.73
GLY1	Moderate	Very Good	Extreme	314.8	-11.1	0.00	0.00	303.68
GLY5	Moderate	Good	Extreme	318.6	-24.9	0.17	0.10	293.71
GLY2	Moderate	Very Good	Extreme	278.7	-15.0	0.00	0.00	263.64
GLY3	Moderate	Very Good	Extreme	247.3	-17.2	0.00	0.00	230.15
FOG1	Moderate	Very Good	Extreme	207.1	-0.7	4.16	0.43	206.38
FOG2	Moderate	Very Good	Extreme	85.2	-2.5	2.35	0.71	82.72
RMP4	Low	Very Good	Very High	66.8	-2.1	17.13	1.43	64.67
MSW1	Low	Good	Extreme	70.2	-18.5	4.37	2.73	51.66
MSW2	Low	Good	Extreme	68.5	-18.1	4.28	2.01	50.37
ALG1	Low	Good	Very High	47.6	-7.4	5.76	5.10	40.20
FOG3	Moderate	Very Good	Very High	40.8	-2.0	1.66	0.22	38.75
SFW1	Low	Very Good	Very High	40.8	-3.4	3.12	0.52	37.43
FIO5	Low	Moderate	Very High	38.5	-8.3	4.10	4.47	30.16
RMP3	Low	Very Good	High	29.7	-0.8	1.85	0.29	28.92
FIO1	Low	Very Good	High	25.3	-0.9	0.08	0.12	24.47
FIO3	Low	Very Good	Moderate	20.6	-0.8	2.64	0.76	19.80
CD3	Low	Good	High	19.0	-2.2	0.00	0.00	16.82
FIO2	Low	Good	Moderate	15.5	-1.8	0.01	0.00	13.72
FIO6	Low	Good	Moderate	10.7	-1.2	0.00	0.00	9.54
CD4	Low	Moderate	Moderate	10.8	-2.4	0.00	0.00	8.33
PM1		N/A	N/A	5.9	-1.7	0.94	0.83	4.17
CD1	Low	Moderate	Poor	4.7	-0.9	0.19	0.17	3.80
FIO4	Low	Very Good	Moderate	3.1	-0.3	0.89	0.33	2.81
RMP1	Low	Poor	Very High	32.3	-12.8	1.42	0.58	19.52
RMP5	Low	Poor	Very High	24.0	-13.3	0.43	0.80	10.69
RMP2	Low	Poor	High	16.5	-7.4	4.27	1.10	9.12
SS1	Low	Poor	Moderate	7.5	-3.1	1.31	0.60	4.40
SGW1	Moderate	Poor	Extreme	38.4	-38.4	1.81	0.27	0.04
CD2	Moderate		Very High	N/A	N/A	N/A	N/A	N/A

*Potential revenue from N and P fertilizer is case specific and has not been included in totalised economic assessments. The substrate row in grey is the base substrate for this specific application.

6.1.6 Summary

This section examined co-substrates from a broad range of industries to assess the suitability for co-digestion in municipal, red meat or piggery applications. In total, 30 co-substrates were assessed across municipal, agricultural, dairy, food, grease and glycerol industries. Risk assessments and rankings were conducted based on physical, chemical and biological properties of each co-substrate. To be suitable for co-digestion, wastes needed a low or manageable inhibition risk (based on successful AD at lab

scale). In general, wastes with high COD were preferred to promote increased organic loading without impacting HRT and treatment times. Additional criteria including speed of degradation, residual solids impact and nutrient content were also considered when relevant.

Glycerol and FOG wastes were the highest ranked co-substrates in all scenarios due to very high space loading, fast degradation and good biological performance (B_0 and f_d). Glycerol and FOG addition needs to be controlled carefully to manage inhibition risk, however this management was achieved effectively within the BMP testing and should be present minimal risk at full-scale. The settling behaviour of FOG and GLY was not assessed and may impact AcoD suitability, however this would only apply to high rate lagoons with short HRT.

Macerated food waste (SFW1) and other food industry wastes (FIO1, FIO2 and FIO3) were generally strong candidates for co-digestion due to fast hydrolysis rates, low impact on residual solids content and low inhibition risk. However, FIO1, FIO2 and FIO3 are relatively dilute compared to the GLY and FOG samples and therefore biogas production is likely to be limited by volume loading rather than organic loading when using these substrates.

Agricultural samples achieved mixed rankings and were most impacted by application. Agricultural samples such as DAF sludge or Red Screenings are suitable for covered pond based technologies and will degraded completely if able to settle in covered ponds. However, other agricultural samples, such as paunch, WAS and pig manure are poorly ranked as imported co-substrates in all applications. This is because these wastes degrade relatively slowly, and can have a large impact on residual solids. This behaviour requires large infrastructure when using mixed liquor digesters or frequent desludging when using covered pond infrastructure, and only makes sense if such wastes are the base substrates processed on the sites that generate them. Importantly, while analysis suggests that agricultural wastes are poor candidates for co-substrates in AcoD, the analysis also shows that AD of these wastes as base substrates could be significantly improved through co-treatment with high energy co-substrates.

6.2 Full-scale Validation Studies

6.2.1 Trial 1 Co-digestion of Sewage Sludge and Beverage Waste

6.2.1.1 Substrate selection – Hydrolysed Sewage Sludge and Beverage Processing Waste (FIO1)

The substrate used in the first full-scale validation study will be Beverage Processing Waste from a plant located in South East Queensland. The Beverage Waste is a combined process effluent and composition will vary based on the production schedule of the host plant. The waste (shown in Figure 29) is a sugar and alcohol based substrate that is most closely aligned to cellulose assessed in Section 5.2, however degradation rates of FIO1 are much faster due to the simpler and soluble substrate structure. The COD to nitrogen ratio of Beverage waste is very high at >1000, therefore the N content is much lower than concentrations expected to cause ammonia inhibition. The very high COD/N ratio may result in nitrogen limitations, where the N concentration is not enough to support biological growth, if beverage waste is treated using mono-digestion, however this is not likely to present an issue in co-digestion where additional substrates also contribute nitrogen.

Beverage processing waste, designated FIO1 was ranked 9/30 when assessing co-substrates for addition to mixed liquor in-vessel anaerobic digesters at a municipal WWTP (Section 6.1.5.1, Table 13). FIO1 was selected due to local availability near the WWTP. In addition to local availability, 2 additional criteria were applied in the selection process:

1. The WWTP used in the trial was risk adverse and therefore FIO1 was preferred over higher ranked GLY and FOG co-substrates due to a lower risk of overload inhibition.
2. Solids handling is a significant cost and bottleneck at the WWTP used in the trial, therefore FIO1 was preferred over food waste due to a lower contribution to residual solids.

Figure 29: Sample of Beverage Waste to be used in co-digestion trials (FIO1). The waste has a very high soluble fraction with very small contribution to residual solids.

Example compositions of the Beverage Waste measured during previous batch testing is summarised in Table 17. The COD/VS ratio (~2) is much higher than the ratio typically expected for a sugar based waste (1.07), this is likely due to alcohols in the waste which contain high COD, but volatile during drying and therefore do not contribute to VS. Ethanol was measured at 33 $g.L^{-1}$ for the waste. The COD to nitrogen ratio was very high at >1000, the low N content could become a factor limiting microbial growth in long term mono-digestion, however there is more than sufficient nitrogen in sewage sludge to enable co-digestion with nutrient supplements. Actually, the very high COD/N ratio indicates potential to significantly improve biogas production without adding to the nitrogen load of downstream processes.

Table 17: Initial characterization of Beverage Processing Waste (BPW) used in laboratory analysis

Characteristic	Units	FIO1 2014	FIO1 2015
pH		2.90	2.97
tCOD	$g.L^{-1}$	119.6	129.0
sCOD	$g.L^{-1}$	126.3	123.7
TS	$g.L^{-1}$	53.2	74.3
VS	$g.L^{-1}$	52.8	73.6
VS/TS	$g.L^{-1}$	0.99	1.0
COD/VS	ratio	2.27	1.8
FOG	$g.L^{-1}$	<0.1	<0.1
VFA	$mg.L^{-1}$	1250	2,820
Ethanol	$mg.L^{-1}$		31,200
Partial Alkalinity (pH 5.7)	mg $CaCO_3.L^{-1}$	0	0
Total Alkalinity (pH 4.3)	mg $CaCO_3.L^{-1}$	0	0
α (alkalinity ratio)	dimensionless	N/A	N/A
Conductivity	$mS.cm^{-1}$	0.42	0.73
TKN	$mg.L^{-1}$	94.4	106
TAN	$mg.L^{-1}$	30.2	0
Total phosphorus (TP)	$mg.L^{-1}$	104.0	127
Phosphate (PO_4)	mg $P.L^{-1}$	91.9	105

6.2.1.2 Configuration of Municipal Wastewater Trade Waste Facility

The configuration of the Trade Waste facility utilised during Study 1 is shown in Figure 30. Trade Waste batches can be mixed or separated into different storage tanks. Storage tanks can be added to the digester feed separately or together. The trade waste is combined with WAS after thermal hydrolysis pre-treatment and added to all operating digesters. Trade waste cannot be fed to digesters separately.

Trade Waste shipments (20,000 L) may be received 1-5 times per week and generally on an ad hoc basis. Trade Waste can be added to the digesters rapidly (i.e. within a few hours) or slowly (i.e. over 1-2 days). The 6 month analytical program assessed the impacts on both rapid and slow dosing on digester stability and biogas production.

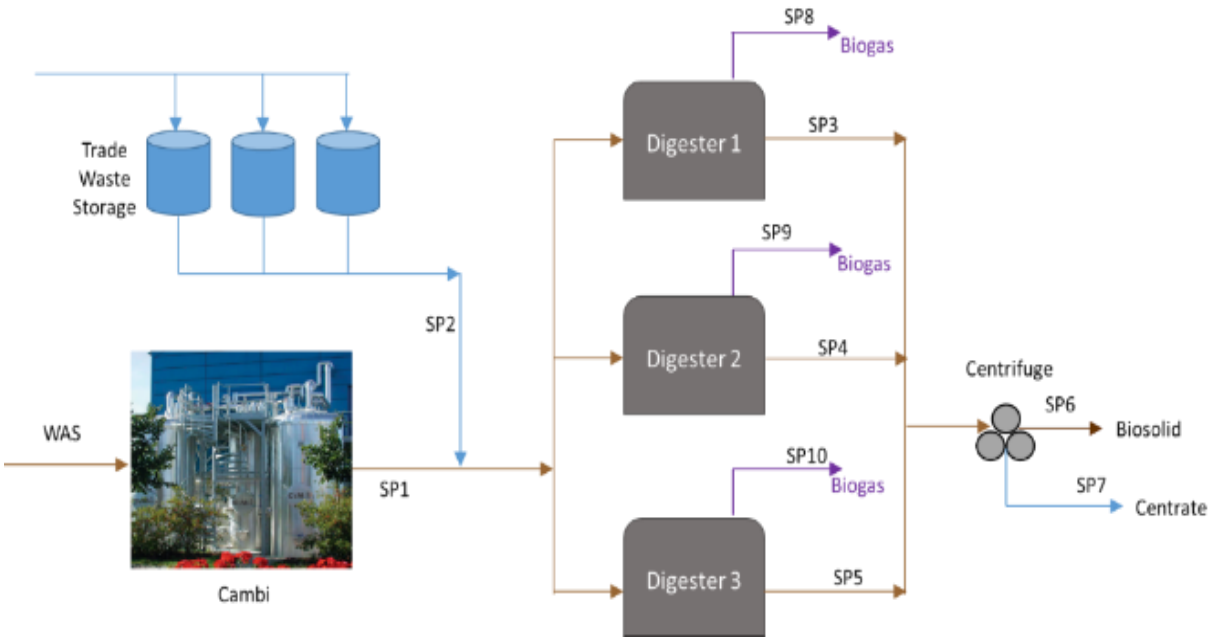

Figure 30: Configuration of Trade Waste Dosing Facility at South East QLD Municipal WWTP and Location of Sample Points.

6.2.1.3 Predicted Performance of Full-scale Process under co-substrate dosing

The performance of a full-scale digester treating THP sludge and FIO1 co-substrate at the South East QLD Municipal WWTP was estimated by implementing a first order CSTR model using degradability parameters from batch testing. The working volume of each digester at the WWTP is 2260 m^3. When Cambi is operated at full capacity, the digesters at the WWTP are fed approximately 122.5 $m^3.d^{-1}$ THP sludge at a concentration of 8% (TS) corresponding to a baseline SRT of 18.5 days and an organic loading rate of 5.04 $kgCOD.m^{-3}.d^{-1}$. Co-substrate is added in addition to the base sludge load, this has the impact of increasing organic load (and therefore increasing methane potential) and increasing volumetric load (and therefore reducing treatment time). A summary of full-scale digester performance when Cambi THP is operating at full capacity is shown in Table 18. For reference, the shaded squares identify the impact of FIO1 loading at 15 $t.d^{-1}$ – this is the maximum volumetric loading rate based on batch testing. Batch testing identified that FIO1 loading can be applied as a shock load once per day without impacting process stability, however distributed loading is recommended to minimise washout of substrate, and reduce soluble COD in the digestate (especially at the start of co-digestion). Higher loadings may be possible subject to acclimatisation of the digester microbial community.

Table 18: Performance of a full-scale digester treating THP sludge and CCA Waste based on a single digester at the South East QLD WWTP undertaking Trial 1 (Cambi THP at full capacity) and predicted using a CSTR model.

Co-substrate Feed Volume ($t.d^{-1}$)	Volume Load %	SRT (days)	Change COD Load (%)	Representative OLR ($kg\ COD.m^{-3}.d^{-1}$)	Predicted Methane ($m^3\ CH_4.d^{-1}$)	Effluent Volume ($m^3.d^{-1}$)	Residual Solids ($kg.d^{-1}$)
0.0	0.00	18.4	0.00	5.04	1661	123	6761
2.0	1.63	18.1	2.29	5.16	1734	125	6802
4.0	3.27	17.9	4.55	5.27	1806	127	6843
6.0	4.90	17.6	6.82	5.38	1877	129	6884
8.0	6.53	17.3	9.09	5.50	1949	131	6926
10.0	8.16	17.0	11.4	5.61	2020	133	6969
15.0	12.2	16.4	17.0	5.90	2196	138	7077
20.0	16.3	15.8	22.7	6.18	2370	143	7189
30.0	24.48	14.8	34.0	6.76	2711	153	7422

If the Cambi THP was operating at full capacity and a CCA loading rate of 15 $t.d^{-1}$ was applied to a single digester at a South East Queensland WWTP, the volume loading would be 12% and this would correspond to an increase in organic load of 17% and an increase in methane potential of 32%. The higher relative increase in methane potential (compared to organic load) is due to a higher relative degradability of FIO1 waste compared to the THP sludge at the WWTP. If 15 $t.d^{-1}$ of FIO1 waste was added to a single digester at the WWTP (and Cambi was operating at full capacity) the resulting SRT would be 16.4 days.

6.2.1.4 Results from Trial 1

Full-scale Trial 1 was limited by ongoing issues with the control system used for co-substrate dosing. Due to these issues, automated dosing was not achieved within the project, however the system was operated manually for several shorter study periods. Control system issues are not an inherent difficulty with co-digestion processes, however issues in this project highlight the potential for difficulties when modifying existing or older infrastructure. Example results from manual co-substrate dosing are shown in Figure 31. The WWTP used in the trial is a centralised facility that receives sewage sludge from multiple local plants, for this reason the volume of sludge treated varies and the baseline methane production is therefore also variable. Co-substrate dosing was intermittent and adhoc and represented a small fraction of the feed volume and organic load added to the digester, therefore changes in methane production were limited and only short term (until co-substrate dose was used up). Generally, the volume load of co-substrate was 5-10% of the total feed and biogas production increased by 15-20% in the short term. These results are consistent with data from laboratory testing.

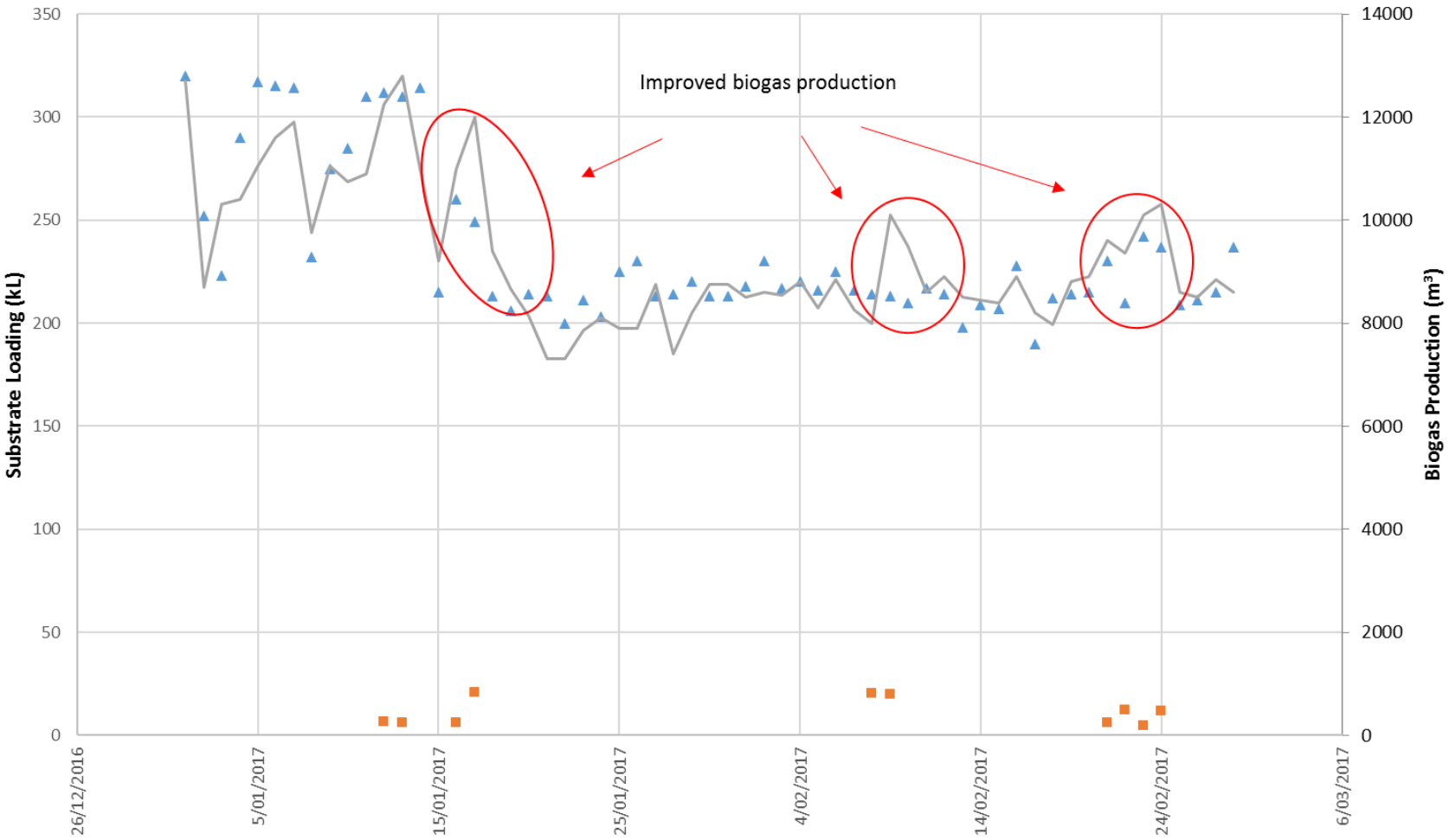

Figure 31: Results from intermittent full-scale co-digestion of sewage sludge and beverage waste. Blue marker shows the volume of hydrolysed sewage sludge added to the process, orange marker shows the beverage waste added. Grey line shows biogas production recorded in the trial.

6.2.2 Trial 2 Co-digestion of Pig Manure and Solid Cattle Paunch

6.2.2.1 Substrate selection – Pig Manure and Solid Cattle Paunch

Trial 2 included co-digestion of screened pig manure and dewatered cattle paunch. The dewatered cattle paunch was similar to RMP1 and RMP5 investigated during laboratory testing. Paunch Waste is stomach contents from animal processing and is dewatered prior to disposal. The Paunch Waste has as very high solids fraction with fibres in the range of 1-10 mm in length. The waste was dewatered prior to collection, but did not require pre-treatment or mechanical processing prior to the experiments. Paunch Waste is shown in Figure 32, a summary of the composition of Paunch Waste and Pig Manure used in laboratory experiments and model predictions is shown in Table 19. Paunch waste represents a solid lignocellulose waste similar to cellulose assessed in Section 5.2, however degradation rates and methane yields are lower due to the more complex substrate structure. Paunch also contains proteins and fats in variable amounts (Astals et al., 2014). The COD to nitrogen ratio of Paunch waste is very high at approximately 250, therefore the N content is much lower than concentrations expected to cause biological inhibition.

Mixed liquor digesters to be used in Trial 2 are not typically used at Australian piggeries and were not directly assessed in Section 6.1.5, instead co-substrate assessments at piggeries were based on anaerobic covered ponds. Solid paunch waste, designated RMP1 and RMP2 was ranked 25/30 and 26/30 when assessing co-substrate addition for covered pond based anaerobic digestion at a piggery (Section 6.1.5.4, Table 16). The poor rankings were due to the high portion of non-degradable solids in paunch which would result in rapid sludge accumulation in a covered pond. Therefore, paunch solid waste was classed as unsuitable for covered pond treatment. The mixed liquor digesters used in Trial 2 are better equipped to handle non-degradable solids and are therefore better suited to paunch co-digestion. Paunch waste was ranked 17/30 based on economics, and may be ranked higher where nitrogen and phosphorous have fertilizer value. Despite the moderate ranking, solid paunch waste was used in the trial due to local availability.

Figure 32: RMP5 Example of Screened and Dewatered Paunch Waste from RMP plant 2.

Table 19: Composition of Pig Manure and Cattle Paunch used in laboratory analysis and modelling

Characteristic	Units	Pig Manure	Cattle Paunch
tCOD	$g.L^{-1}$	67.4	295
TS	$g.L^{-1}$	69.2	221
VS	$g.L^{-1}$	49.0	205
VS/TS	ratio	0.71	0.93
COD/VS	ratio	1.38	1.44
TKN	$mg.L^{-1}$	2696	3160
TP	$mg.L^{-1}$	1684	920

6.2.2.2 Configuration of Trade Waste Facility

The configuration of the Trade Waste facility utilised during Trial 2 is shown in Figure 33. The digesters process 120,000 L of screened piggery manure each day and receive 10-30 tonnes of dewatered paunch. Paunch is generally dosed throughout the day from a holding area.

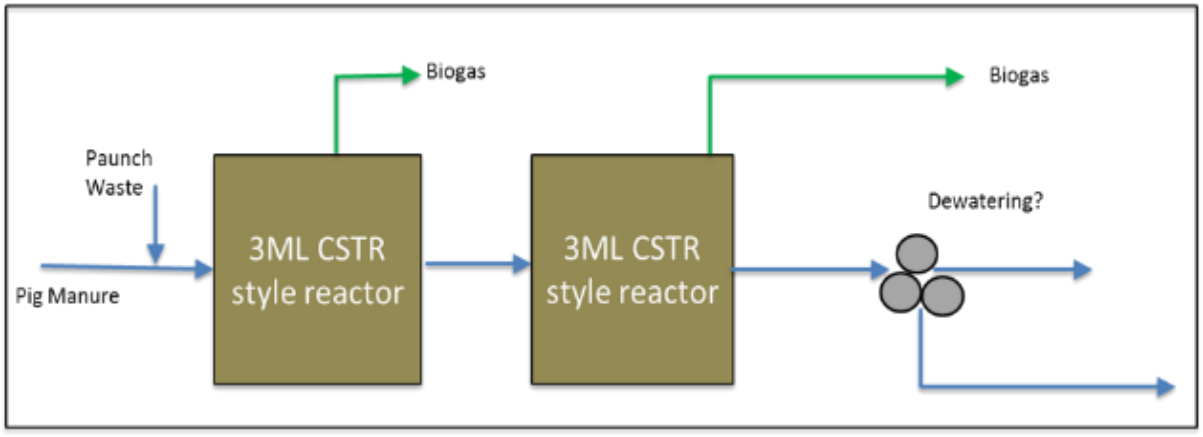

Figure 33: Configuration of Trade Waste Dosing Facility at Piggery A.

6.2.2.3 Predicted Performance of Full-scale Process under co-substrate dosing

The performance of a full-scale digester treating pig manure and screened cattle paunch, represented by PM1 and RMP1 respectively, was estimated by implementing a first order CSTR model using degradability parameters from batch testing. The working volume for the 2 stage digester is 6000 m^3 (2 × 3000m^3 configuration). When Piggery A is operated at full capacity, the digesters are fed pig manure at approximately 3900 $kg.d^{-1}$ VS. This loading rate corresponds to flow of 170 $m^3.d^{-1}$ pig effluent at a concentration of 7% (TS) corresponding to a baseline SRT of 35 days and an organic loading rate of 2.8 $kgCOD.m^{-3}.d^{-1}$ to the first digester. Co-substrate is added in addition to the base sludge load, this has the impact of increasing organic load (and therefore increasing methane potential) and increasing volumetric load (and therefore reducing treatment time). A summary of predicted full-scale digester performance is shown in Table 20.

Piggery A typically operates at a Paunch loading rate of 20 to 25 wet tonnes per day, corresponding to 4,000 to 5,000 $kg.d^{-1}$ of paunch VS. Based on the typical co-substrate loading at Piggery A, methane

production of 2340 – 2550 $m^3.d^{-1}$ is predicted from the CSTR co-digestion model (shaded rows in Table 20).

Table 20: Performance of a full-scale digester treating pig manure and dewatered paunch waste based on general substrate characteristics and predicted using a CSTR model.

Co-substrate Feed Volume (wet $t.d^{-1}$)	Volume Load %	SRT (days)	Change COD Load (%)	Predicted Methane m^3 $CH_4.day^{-1}$	Predicted Power $kWh.day^{-1}$*	Effluent Volume ($m^3.d^{-1}$)	Residual Solids ($kg.day^{-1}$)
0	0.00	35.29	0.00	1462	4833	170	7995
5	2.94	34.29	17.38	1683	5563	175	8698
10	5.88	33.33	34.76	1903	6290	180	9406
15	8.82	32.43	52.15	2120	7008	185	10117
20	11.76	31.58	69.53	2335	7718	190	10832
25	14.71	30.77	86.91	2549	8426	195	11550
30	17.65	30	104.29	2760	9123	200	12273

*Based on 34MJ.m^{-3} CH_4 and electrical efficiency of 35%

6.2.2.4 Results from Trial 2

Example results from Trial 2 are shown in Figure 34. There are large uncertainties associated with the data shown in Figure 34, this is because i) feed volumes were measured on a wet weight basis and converted to volatile solids based on average compositions, however compositions are known to vary; and ii) Biogas volumes are not metered directly and are calculated based on electrical energy production and operating time of the onsite flare. However, despite the uncertainty, the methane production volumes from the full-scale co-digestion plant are generally similar to the values predicted in Table 20. During Trial 2, the co-substrate addition was more consistent than Trial 1 and represented additional feed at 60-100% on a total solids basis.

Results confirm that methane production from the full-scale co-digestion process is much higher than methane production that could be achieved through mono-digestion of pig manure at Piggery A, improvements in methane production ranged from 80% to 100% depending on the mass of paunch added. Importantly, the full-scale process showed a considerable decline in methane production when Paunch waste was not added to the process (decline in gas production 10/11/2017 to 14/11/2017). The results from Trial 2 are consistent with laboratory results in Section 5.2, which concluded that organic loading and methane production could be improved by 100% or more in continuous digesters using a cellulose/carbohydrate based co-substrate. Methane yields achieved from paunch co-substrate addition were estimated at 200 to 250 $L.kgVS^{-1}$ added, this yield is consistent with laboratory testing in Section 6.1 and demonstrate complete recovery of available methane, this further confirms successful co-digestion during the trial.

Therefore, results from Full-scale Trial 2 validate the CSTR model predictions.

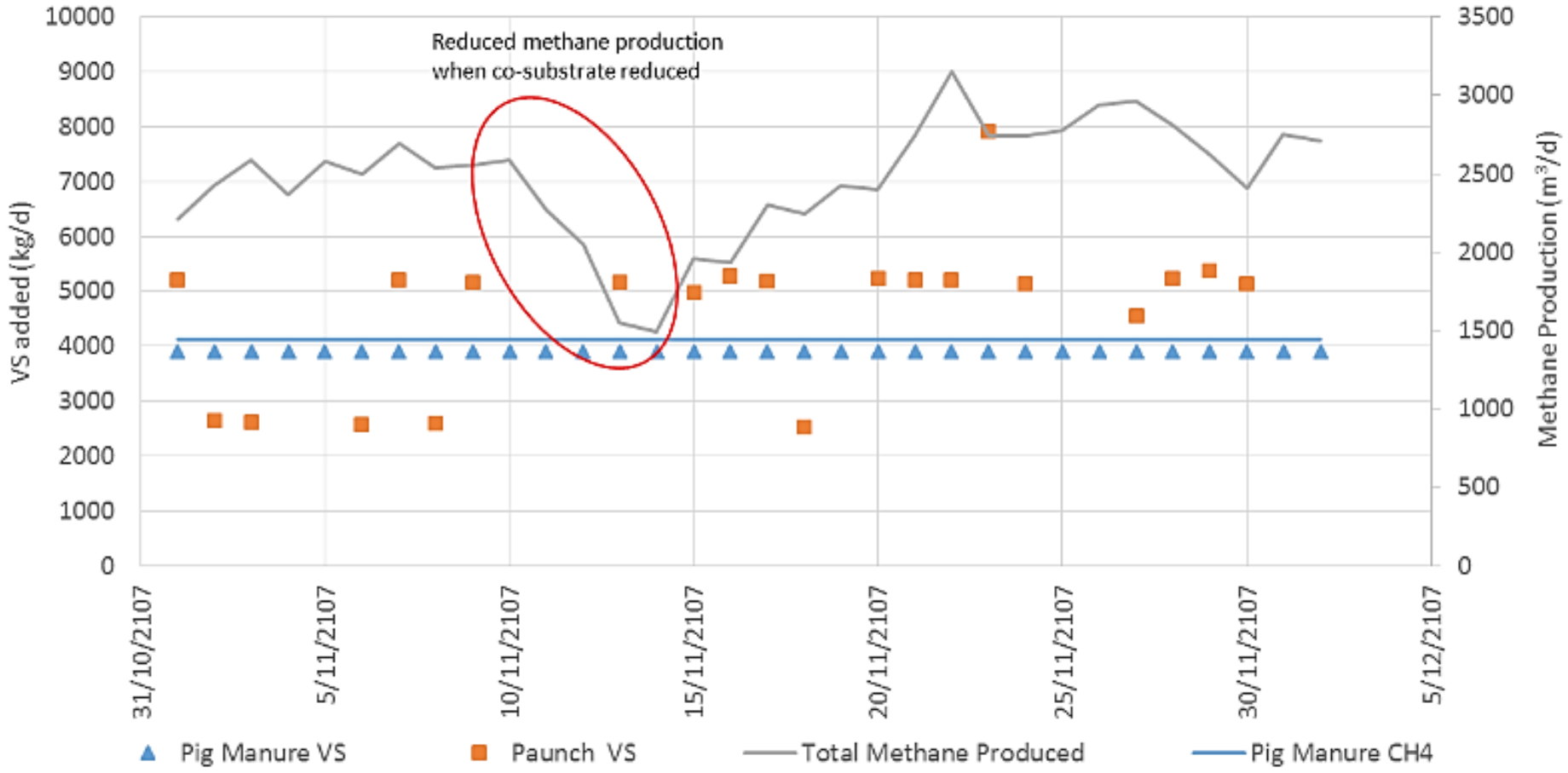

Figure 34: Results from full-scale co-digestion of pig manure and solid cattle paunch. Blue marker shows the pig manure added to the process (based on average data), orange marker shows the paunch added to the process based on daily measurements. Grey line shows methane production recorded in the trial (based on electricity generation), the blue line shows the methane production predicted if co-digestion was not applied and only pig manure was treated.

7 CONCLUSIONS/RECOMMENDATIONS

Project "Enhanced Energy Recovery in Australian Industry through Anaerobic Co-digestion" is a collaboration between the Pork CRC, Australian Meat Processor Corporation, Queensland Urban Utilities, Melbourne Water Corporation and The University of Queensland. The primary focus of the project is to improve the economics of biogas projects by maximizing renewable energy recovery (and revenue) from anaerobic digestion infrastructure. The project structure includes detailed fundamental analysis leading to development of a co-digestion manual (Sub-project 1), and validation of outcomes through full-scale co-digestion trials (Sub-project 2). Key outcomes are:

7.1 Impact of C/N ratio of Co-digestion Performance:

Experiments were conducted to assess the impact of carbon to nitrogen (C/N) ratio on anaerobic digestion performance (carbon representing methane potential and N representing nutrients for microbial growth); C/N ratios in the range of 1 to 80 were tested and represent the range of many common AD substrates (sewage sludge, manures, agri-industry residues and many food wastes). Experiments were repeated with cellulose and oleic acid to determine whether the type of carbon source impacts the finding. The results showed that:

- Methane production was near-identical over a wide range of C/N ratios (5-80 for cellulose; 20-80 for oleic acid). Therefore, C/N ratio is not critical as long as N is sufficient to enable microbial growth, but not high enough to cause ammonia inhibition.
- Negative impacts occurred at low C/N ratios. Inhibition at low C/N ratios was attributed to TAN concentrations, however threshold concentrations were different for different carbon sources. When cellulose was the carbon source, TAN inhibition occurred at 3.3 $g.L^{-1}$ TAN. When Oleic acid was the carbon source, minor TAN inhibition occurred at 2.3 $g.L^{-1}$ TAN with severe inhibition at 2.8 $g.L^{-1}$ TAN.
- Co-digestion of lipid-rich substrates may increase process failure risks at sites with high background concentrations of ammonia (i.e. piggeries, or sewage treatment plants with thermal hydrolysis pre-treatment);
- The results demonstrate that digestion is likely to be reasonable, provided that adequate amounts of essential nutrients are available (but do not exceed inhibition thresholds) and given that organic loading limits are not exceeded by addition of the AcoD substrate mixtures to the CAP or digester. The differences in observed behaviour for cellulose and oleic acid did however highlight the importance of macro-composition of AcoD in determining the digestion performance.
- Following on from the test work in this section, targeted experiments described in the next section below sought to determine organic loading limits for different carbon substrates (in terms of macro-composition), being co-digested with a complex base substrate.

7.2 Impact of Substrate Macro-Composition on Co-digestion Performance:

The macro-composition of organic waste is the fractionation between carbohydrates, protein and lipids. Macro-composition is expected in impact co-digestion performance as carbohydrates, proteins and lipids degrade through different metabolic pathways and often require different microbial consortia. The macro-composition also impacts methane potential (and therefore biogas revenue), process kinetics (influencing treatment times and overload risks) and inhibition risk. Therefore, a set of continuous bench-scale digesters were operated to assess the impact of co-substrate macro-composition on AcoD loading potential and process performance. The digesters initially operated with sewage sludge at a base organic load of 2.2 $gCOD.L^{-1}.d^{-1}$ and were then converted to co-digestion processes where either cellulose, protein or lipid was added to sewage sludge to increase loading rates up to 4.5 $gCOD.L^{-1}.d^{-1}$.

The results showed that fat is a preferred co-substrate provided that treatment times are long enough for the fat digestion, followed by carbohydrates as a strong candidate, and lastly by proteins due to high risk of ammonia inhibition. Ammonia and oleic acid did appear to show an antagonistic effect on digestion performance by jointly inhibiting the digestion of oleic acid, and this may be important to consider with the AcoD of high protein – high fat mixtures.

Microbial community balance is not well suited to high protein feeds, with substantial accumulation of organic acids as a key process risk. Co-substrate macro-composition had significant long-term impacts on microbial community development in the continuous digesters. In particular, protein based co-digestion seemed to significantly weaken the microbial community, as noted from deterioration in digester performance, and from a deterioration in performance of the separate batch capacity tests. Fat co-digestion seemed to improve parts of the community without harming others. Carbohydrate (cellulose) co-digestion seemed to weaken the community's ability to digest fat/lipids, but the extent and overall impact of this effect was not determined.

When designing co-substrate mixtures:

- When operating a mixed in vessel anaerobic digester at a conventional loading rate of 2-3 $gCOD.L^{-1}.d^{-1}$, fat/lipid co-substrates can be applied at 1 kg fat per m^3 digester volume per day with no negative impact. This represents a fat OLR of 2.7-3 $gCOD.L^{-1}.d^{-1}$. Under these conditions, methane production is expected to increase by 100-150% (compared to baseline performance).
- When operating a mixed in vessel anaerobic digester at a conventional loading rate of 2-3 $gCOD.L^{-1}.d^{-1}$, carbohydrate co-substrates can be applied at 2.5 kg carbohydrate per m^3 digester volume per day with no negative impact. This represents a carbohydrate OLR of 2.5-2.7 $gCOD.L^{-1}.d^{-1}$. Under these conditions, methane production is expected to increase by 100-125% (compared to baseline performance). Higher loading may be achieved, but will result in higher concentrations of undigested residues for disposal. If OLR is applied as soluble sugar, organic acid content must be monitored.
- When operating a mixed in vessel anaerobic digester at a conventional loading rate of 2-3 $gCOD.L^{-1}.d^{-1}$, protein co-substrates can be applied to increase OLR. However negative impacts are expected. Methane production may improve up to 100%, however protein co-substrates result in high TAN concentrations which destabilise methane production and lead to accumulation of organic acids. Process instability occurs at protein loading of 1 kg protein per m^3 digester volume per day (above based substrate load).

Importantly, the results showed clear substrate macro-composition impact on co-digestion capacity, which cannot be adequately described by C/N ratio. From an economic perspective, co-digestion needs

to balance improvements in biogas revenue with increased solids disposal costs and increased nutrient management costs. Methane production increased with increasing co-substrate loading, however the results are not linear. Maximum benefit is achieved at lower co-substrate dosing. As co-substrate loading increases, volumetric loading to the process also increases and digester retention times decrease. Retention times >20 days need to be maintained to allow sufficient time to convert many co-substrates to methane, for complex lignocellulosic substrates (i.e. crop residues, spent bedding, paunch solids) longer treatment times are required.

7.3 Impact of Temperature on Co-digestion Performance:

Many biogas processes operate at ambient temperature and without temperatures adjustment. Covered anaerobic lagoons/covered ponds are an example of processes that operate at ambient temperature conditions, generally with the range of 10-40°C depending on local and season. Individual covered pond can show seasonal temperature variations of 20°C. Detailed analysis was conducted on the impact of temperature on anaerobic digestion. This work included operation of long term experiments treating pig manure at 37, 25 and 15°C, this temperature range was selected, because it is most applicable to the seasonal variations experienced by ambient anaerobic systems operating in the Australian climate. The main focus of the study was to investigate how operating temperature influences relative process rates of the four key metabolic steps and how this contributes to overall AD performance. Four continuous well-mixed digesters were operated at controlled temperatures in the 15-37°C range and fed with a complex base substrate, namely pig manure or sewage sludge. These continuous digesters provided temperature adapted microbial communities on which further activity and capacity testing could be performed to determine complete microbial activity profiles for AcoD. This helped to identify potential biological process bottlenecks for AcoD of complex mixtures in ambient anaerobic systems. Key findings include:

- Pig manure and sewage sludge systems have different microbial community structures as well as different metabolic activity profiles.
- Activity profiles generally show that upstream processes (hydrolysis-acid producing) are slower than downstream processes (methane production-acid consuming) under warmer conditions. This prevents uncontrolled accumulation of organic acids and maintains process balance.
- When temperature decreased, all metabolic processes decreased. Activities associated with lipid hydrolysis and propionate degradation were most affected by the temperature reduction. In highly particulate feedstocks, digestion is controlled by lipid and carbohydrate loading and degradation of the intermdediate propionate will become a potential bottleneck for the process at cooler temperatures.
- The reduction in metabolic activity as temperature decreases is not linear with temperature. The reduction in metabolic activity being more severe between 25°C and 15°C compared to 37°C and 25°C.

The capacity for methane production during Autumn-Winter months is lower than summer months. As a result co-substrate loading should be reduced. Most importantly, results showed that the activities for downstream biological steps slowed to a greater extent than the upstream acid producing steps, causing a greater potential for imbalance and overload at cooler temperatures. Highly particulate feedstocks with high protein concentrations are most at risk, carbohydrate based substrates have lower risk at warmer temperatures with increasing risk at cooler temperatures. Lipid based substrates

were an exception where acid production remained slower than acid consumption even at cooler temperatures, limiting overload risk. The outcomes are consistent with results elsewhere in this report ranking lipid substrates as lowest risk and protein substrates as highest risk.

In terms of practical application, maximum co-substrate loads will occur during Spring-Summer when the covered pond/process operates at warmer temperatures. During Spring-Summer, there is more flexibility to include protein, carbohydrate or lipid based waste mixtures, however lower protein concentrations are recommended. Co-substrate loads should be reduced during Autumn-Winter when the covered pond/process operates at cooler temperatures. During cooler months, protein based wastes and soluble sugar based wastes should be avoided or at least minimised. Complex cellulose wastes may be applied, but at 50% lower loading than Spring-Summer. Lipid based wastes are expected to have the lowest risk.

7.4 Examination of Novel Co-digestion Mixtures:

Australian industries already operate a number of technologies to recover energy from waste. There is a broad range of biogas infrastructure available at municipal WWTP, red meat processing facilities and pig production facilities, much of this existing infrastructure is currently underutilised. This project examined 30 wastes from across municipal, agricultural, dairy, food, grease and glycerol industries as possible co-substrates. Risk assessments and rankings were conducted based on physical, chemical and biological properties of each waste. To be suitable for co-digestion, wastes needed a low or manageable inhibition risk (based on successful AD at lab scale). In general, wastes with high COD were preferred to promote increased organic loading without impacting HRT and treatment times. Additional criteria including speed of degradation, residual solids impact and nutrient content were also considered when relevant.

Glycerol and FOG wastes were the highest ranked co-substrates in most scenarios due to very high space loading, fast degradation and good biological performance (B_0 and f_d). Glycerol and FOG addition needs to be controlled carefully to prevent process overload and failure, however these risks can be managed effectively. The settling behaviour of FOG and GLY was not assessed and may impact AcoD suitability in high rate lagoons with short HRT.

Macerated food waste (SFW1) and other food industry wastes (FIO1, FIO2 and FIO3) were generally strong candidates for co-digestion due to fast hydrolysis rates, low impact on residual solids content and low inhibition risk. However, these wastes are relatively dilute and volume loading constraints will limit biogas enhancement when using these substrates.

Agricultural samples achieved mixed rankings and were most impacted by application. Energy dense agricultural samples such as Lipid rich DAF sludge or Protein and Lipid Rich Animal Screenings are suitable for covered pond based technologies and will degraded completely if able to settle in covered ponds. However, lower energy agricultural samples, such as paunch, WAS and pig manure are poorly ranked due to slow reaction times and significant non-degradable fractions that increase residual solids for disposal. Importantly, the analysis also shows that AD of these lower ranked Ag wastes could be significantly improved through co-treatment with high energy substrates.

7.5 Co-digestion Prediction Tools and Full-scale Validation:

The project supported 2 full-scale co-digestion trials. Trial 1 was conducted at a municipal WWTP co-digesting beverage processing wastewater and sewage sludge after pre-treatment. During Trial 1, Co-substrate dosing was intermittent and adhoc and represented a small fraction of the feed volume and organic load added to the digester, therefore changes in methane production were limited and only short term (until co-substrate dose was used up). Generally, the volume load of co-substrate was 5-10% of the total feed and biogas production increased by 15-20% in the short term. This results are consistent with data from laboratory testing.

Trial 2 was conducted at a pig production facility co-digesting pig manure and dewatered cattle paunch in a 2-stage CSTR process. Cattle paunch was ranked poorly in co-substrate assessments, however Trial 2 was conducted in a rural area where co-substrate options were limited. Cattle paunch was used due to local availability rather than ranking. During Trial 2, the co-substrate addition was more consistent than Trial 1 and represented additional feed at 60-100% on a total solids basis. Improvements in methane production ranged from 80% to 100% depending on the mass of paunch added. The results from Trial 2 are consistent with laboratory results in Section 5.2, which concluded that organic loading and methane production could be improved by 100% or more in continuous digesters using a cellulose/carbohydrate based co-substrate. Methane yields achieved from paunch co-substrate addition were estimated at 200 to 250 $L.kgVS^{-1}$ added, this yield is consistent with laboratory testing in Section 6.1 and demonstrate complete recovery of available methane, this further confirms successful co-digestion during the trial.

Finally, the project developed a co-digestion tool, implemented in Microsoft Excel, to predict the performance of full-scale co-digestion processes. The tool is based around first-order continuous stir tank reactor (CSTR) models that incorporate biological and kinetic parameters from laboratory testing. An example of the tool is described in Appendix 2:

- The tool interface is based on a flowchart of an anaerobic digester. The tool allows users to set base operational conditions for the process.
- Users are able to select co-substrate mixtures from a range of industries, using dropdown menus. Users are not required to enter specific compositions or biological characteristics for the co-substrates. All required parameters are pre-loaded based on laboratory testing.
- Up to 4 different co-substrate can be added at a time.
- Users are able to add the daily loading rate of each co-substrate based on tons or m^3 of the material. Loading rates of co-substrates would generally be determined by material availability.
- The tool provides colour coded feedback when co-substrate loading exceeds capacity limits for the process. For example, if the OLR exceed 5 $gCOD.L^{-1}.d^{-1}$ or the solids retention time falls below 20 days.
- The tool shows key process parameters including organic loading rate, process retention time, methane production, solids residues and centrate properties.
- Predictions from the tool should be considered as general predictions and may not represent al processes.

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9 APPENDIX 1 - DETAILED ASSESSMENT AND DATA OF CO-SUBSTRATES TESTED

9.1 Co-substrate Sample List

Table 21: Description and Classification of all Co-substrates assessed during project

	Sample CODE	Industry	Description
1	SS1	Municipal Sludge	Mixed Sewage Sludge (primary sludge and waste activated sludge
2	MSW1	Municipal Solids	Source separated Organic Fraction of Municipal Waste, supplied by MW
3	MSW2	Municipal Solids	Source separated Organic Fraction of Municipal Waste, supplied by MW
4	SGW1	Municipal Solids	Solid Green Waste – leaf, branch and grass waste, supplied by QUU
5	SFW1	Municipal Solids	Solid Food Waste – supermarket food waste, separated and macerated
6	PM1	Agri-industry	Screened Pig Manure
7	RMP1	Agri-industry	Screened and Dewatered Paunch Solid Waste from RMP plant 1
8	RMP2	Agri-industry	Waste Activated Sludge from wastewater treatment at RMP plant 1
9	RMP3	Agri-industry	Dissolved Air Flotation Sludge from primary treatment at RMP plant 1
10	RMP4	Agri-industry	Screened Red Solids from primary treatment at RMP plant 1
11	RMP5	Agri-industry	Dewatered Paunch Waste from RMP plant 2, supplied by QUU
12	ALG1	Agri-industry	Raw Algae
13	CD1	Cheese and Dairy	Dairy Sample supplied by MW and collected 6/7/2017
14	CD2	Cheese and Dairy	Dairy Sample supplied by MW and collected 21/7/2017
15	CD3	Cheese and Dairy	Cheese Whey supplied by MW and collected 17/8/2017
16	CD4	Cheese and Dairy	Ricotta Whey supplied by MW and collected 17/8/2017
17	FIO1	Food - Other	Beverage Processing Waste supplied by QUU
18	FIO2	Food - Other	Wastewater from honey packaging facility, supplied by QUU
19	FIO3	Food - Other	Macerated waste from food/salad packaging plant, supplied by QUU
20	FIO4	Food - Other	Spreadwaste/Food Processing Trade Waste, supplied by MW
21	FIO5	Food - Other	Dissolved Air Flotation from food processing plant, supplied by QUU
22	FIO6	Food - Other	RTD AM – DAF Sample collected 16/1/2018.
23	FOG1	Grease Trap	Grease Waste (FOG), supplied by QUU
24	FOG2	Grease Trap	Grease Waste (FOG) SP1, supplied by QUU
25	FOG3	Grease Trap	Grease Waste (FOG) SP2, supplied by QUU
26	GLY1	Glycerine	A120 – ICI Glycerine supplied by MW and collected 21/12/2017
27	GLY2	Glycerine	A120 – IR Glycerine supplied by MW and collected 21/12/2017
28	GLY3	Glycerine	A150 – ICI Glycerine supplied by MW and collected 21/12/2017
29	GLY4	Glycerine	S290 – ICI Glycerine supplied by MW and collected 21/12/2017
30	GLY5	Glycerine	Glycerol (GLY), supplied by QUU

9.2 Co-substrate Example Images

9.2.1 Municipal Waste Samples

Figure 35: SGW1 – Example of Solid Green Waste – leaf, branch and grass waste, supplied by QUU

Figure 36: SFW1 – Example of Food waste used in BMP testing, separated prior to maceration (extracted from Cleanaway presentation to QUU).

9.2.2 Agri-Industry Waste Samples

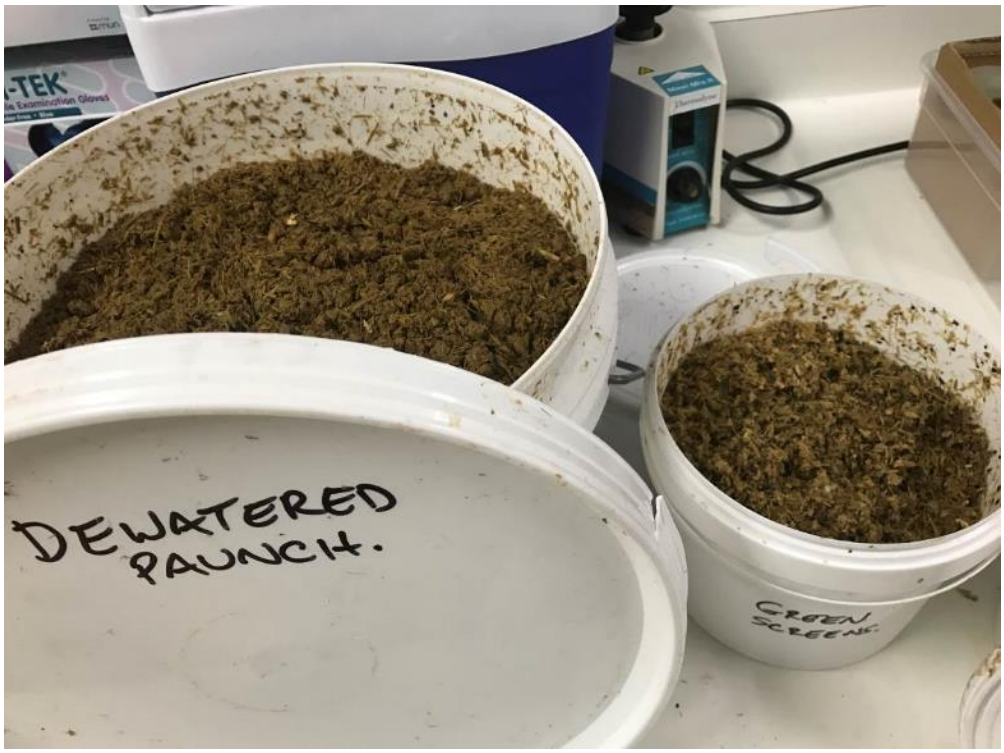

Figure 37 RMP1 – Example of Screened and Dewatered Paunch Solid Waste from RMP plant 1

Figure 38 RMP2 – Example of thickened waste activated sludge from RMP plant 1

Figure 39 RMP3 – Example of Dissolved Air Flotation Sludge from primary treatment at RMP plant 1

Figure 40 RMP4 – Example of Screened and Dewatered Red Solids from primary treatment at RMP plant 1

Figure 41: RMP5 Example of Screened and Dewatered Paunch Waste from RMP plant 2, supplied by QUU.

9.2.3 Cheese and Dairy Samples

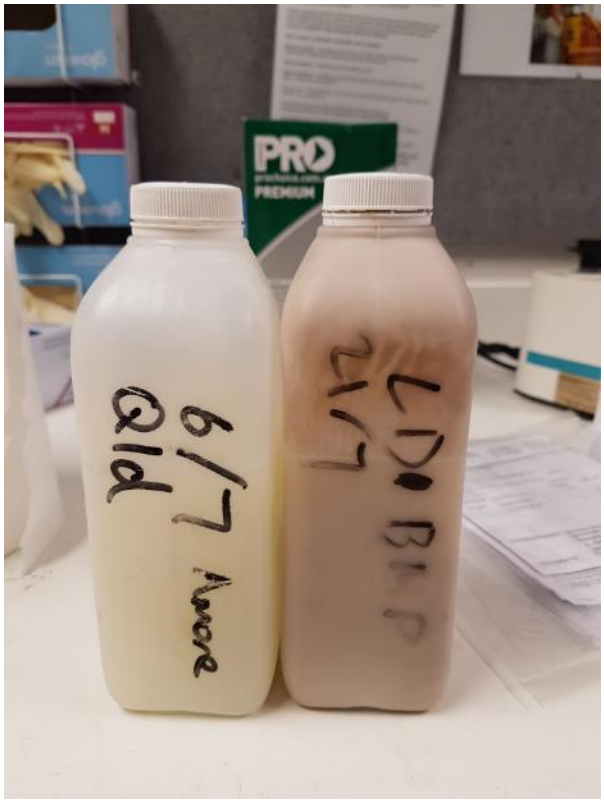

Figure 42 CD1 and CD2 – Examples of Dairy Waste samples supplied by Melbourne Water

Figure 43 CD3 and CD4 – Examples of Cheese Whey and Ricotta Whey supplied by Melbourne Water

9.2.4 Food Industry – Other Samples

Figure 44: FIO1- Sample of Beverage Processing Waste supplied by QUU.

Figure 45: FIO2- Sample of wastewater from a honey processing plant, compared to honey. Waste was supplied by QUU.

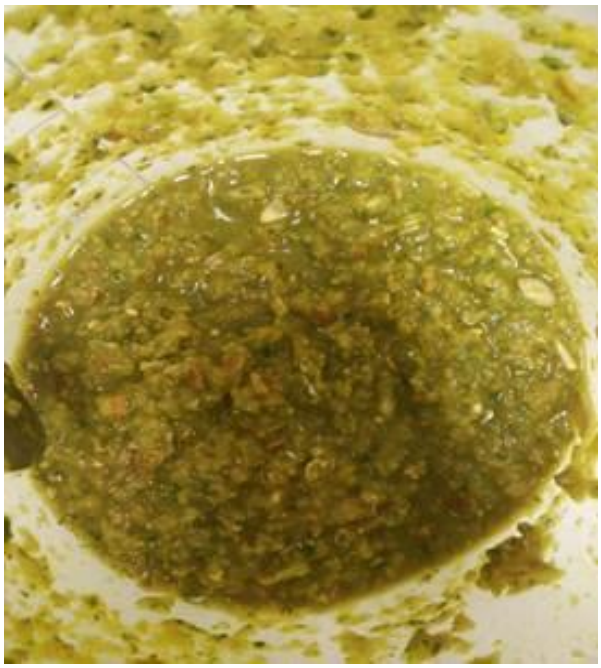

Figure 46: FIO3 – Sample of macerated waste from food/salad packaging plant, supplied by QUU

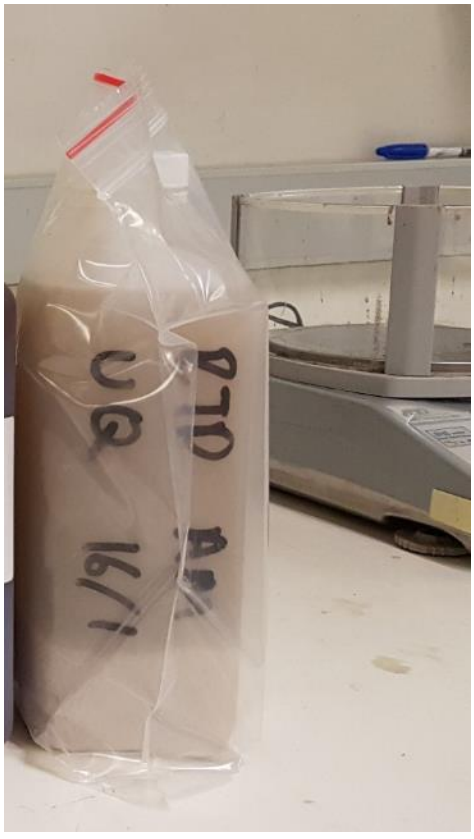

Figure 47 FIO6 – RTD AM DAF sample supplied by Melbourne Water for Testing

9.2.5 Grease Trap Samples

No images available.

9.2.6 Waste Glycerol Samples

Figure 48 GLY1 to GLY4 – samples of glycerine trade wastes supplied by Melbourne Water for Testing

Figure 49: GLY5 - Sample of GLY Waste supplied by QUU for co-digestion trials.

9.3 Detailed Chemical Characterisation of Co-Substrates

9.3.1 Municipal Waste Samples

Table 22 Concentration of organics, solids and key nutrients in Trade Waste Samples from Municipal Industries

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	FOG	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	ratio	$g.L^{-1}$	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
-	Digested SS	7.86	28.06	0.48	24.88	17.3	0.7	<0.1	6000	2102.3	1195.4	524.3	133.2	210	419
SS1	Mixed SS	5.43	63.01	2.6	49.58	38.09	0.77	0.3	5800	2726.9	134.2	894.1	438.1	286	428
MSW1	MSW1		402	95	420	379	0.9			7050	13	3140	5	4494	970
MSW2	MSW2		567	97	408	366	0.9			6900	14	2320	6	4411	978
SGW1	Green Waste	7.7	675.3	N/A	483.9	434	0.9	<0.1	3750	7878	5.9	828.4	121.9	6900	953
SFW1	Solid Food Waste	6.9	230		185	170	0.92			3500		420		5900	900

9.3.2 Agri-Industry Waste Samples

Table 23 Concentration of organics, solids and key nutrients in Waste Samples from Agricultural Industries

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	FOG	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$g.kg^{-1}$	$g.kg^{-1}$	$g.kg^{-1}$	$g.kg^{-1}$	ratio	$g.L^{-1}$	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
PM1	Piggery Manure		33.7	4.8	34.6	24.5	0.7			1348		842			
RMP1	Paunch Solids		295.5	5.9	220.5	206.1	0.9		3094	3164	150	916	618.5		
RMP2	WAS		160.8	3.1	116.4	99.7	0.9		4694	9937	285.5	1828.3333	355		
RMP3	DAF sludge		131.2	3.9	79.9	76.7	1		955	1972	114.75	217	105.5		
RMP4	Red Solids		321	43.3	292.5	271.5	0.9			17130	188	1019.5	252		
RMP1	Paunch Solids	7.9	247.1	N/A	202.6	178.3	0.88	<0.1	12250	1090	146.1	1466.8	776.4	533.2	241.3
ALG1	Raw Algae		270		200	180				24000					

9.3.3 Cheese and Dairy Samples

Table 24 Concentration of organics, solids and key nutrients in Waste Samples from Cheese and Dairy Industries

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	COD/VS	FOG	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$g.kg^{-1}$	$g.kg^{-1}$	$g.kg^{-1}$	$g.kg^{-1}$	ratio	ratio	$g.L^{-1}$	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
CD1	Dairy		26.7	20.2	19.5	15.9	0.82	1.68			271	45.2	177	145.5		
CD2	Dairy		265.5	5.4	126.1	123.4	0.98	2.15			4846	2.4	306	39.4		
CD3	Cheese_Whey		101.1	74.8	70.7	65.3	0.92	1.55								
CD4	Ricotta_whey		66.2	62	56.9	50.1	0.88	1.32								

9.3.4 Food Industry – Other Samples

Table 25 Concentration of organics, solids and key nutrients in Waste Samples from other Food Industries

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	FOG	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	ratio	$g.L^{-1}$	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
FIO1	Beverage Processing	2.9	120.0	126	53.2	53.0	0.99	<0.1	0	94.4	30.2	104	92	94.4	95.3
FIO2	Honey Packaging	3.9	82.2	81.7	72.4	72.3	1	<0.1	0	8.4	<0.2	1	<0.2	14	15.7
FIO3	Food Processing	4.2	91.4	43.7	82.5	75.0	0.91		0	2670	310	550	102	2776	174
FIO4	Spread Waste	4.6	62.8	57.87	15.8	13.6	0.86	0	249	918	69.5	243	144.8	606	109
FIO5	DAF sludge	4.9	223.9	N/A	173.2	132.1	0.76	8.6	2350	6030	7	4700	285	908	804
FIO6	RTD DAF		55.4		29.1	22.2	0.76								

9.3.5 Grease Trap Samples

Table 26 Concentration of organics, solids and key nutrients in Waste Samples from Fat, Oil and Grease Processing Facilities

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	FOG	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	ratio	$g.L^{-1}$	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
FOG1	Grease Trap	4.37	398	11	201	195	0.97	117.3	N/A	4200	25.4	308	11.9	103	590
FOG2	Grease Trap - SP1	4.42	376	12	163	156	0.96		0	2901	59.2	391	21.6	114	430
FOG3	Grease Trap - SP2	4.31	223	10	111	105	0.96		0	1994	39.7	193	12.6	114	323

1.1.1 Waste Glycerol Samples

Table 27 Concentration of organics, solids and key nutrients in Waste Glycerol Samples

Code	Waste	pH	TCOD	SCOD	TS	VS	VS/TS	Total Alk.	TKN	NH_3	TP	PO_4	K	S
			$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	ratio	mg $CaCO_3.L^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
GLY1	A120-ICI		1382		800.3	757	0.95							
GLY2	A120-IR		1305.6		718.5	667.6	0.93							
GLY3	A150		1219.9		729.7	687.8	0.94							
GLY4	S290		1505.4		802.5	757.9	0.94							
GLY5	GLY	9.1	1666	1659	752.2	662	0.88	38025	228	0	91.3	92	35623	21.2

9.4 Degradability Assessment of Co-digestion Candidates from Agri-Industries

9.4.1 Municipal Waste Samples

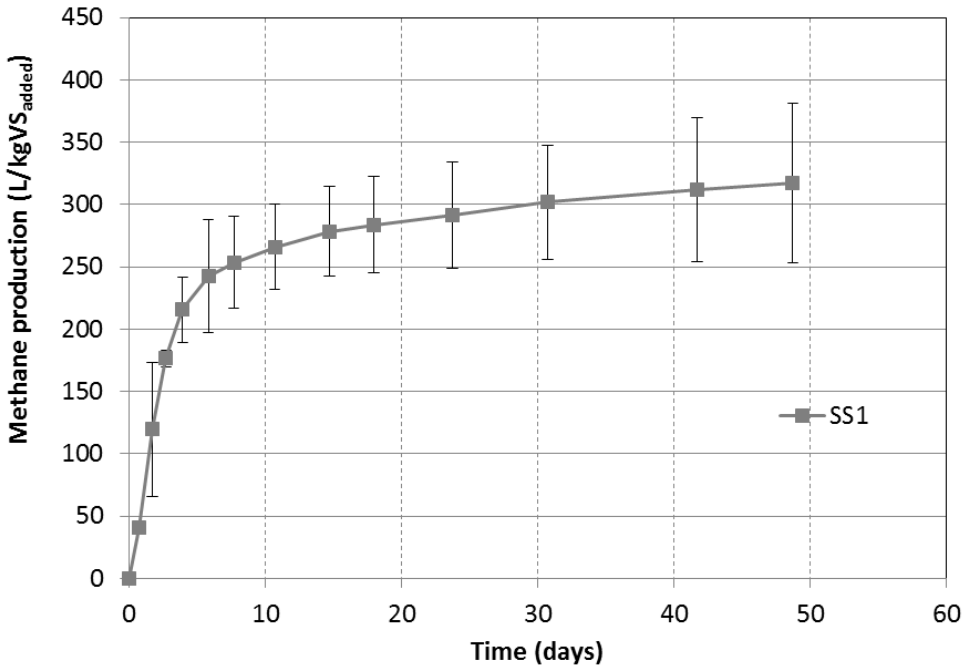

Figure 50: Methane production from SS1 - Mixed Sewage Sludge (primary sludge and waste activated sludge) supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

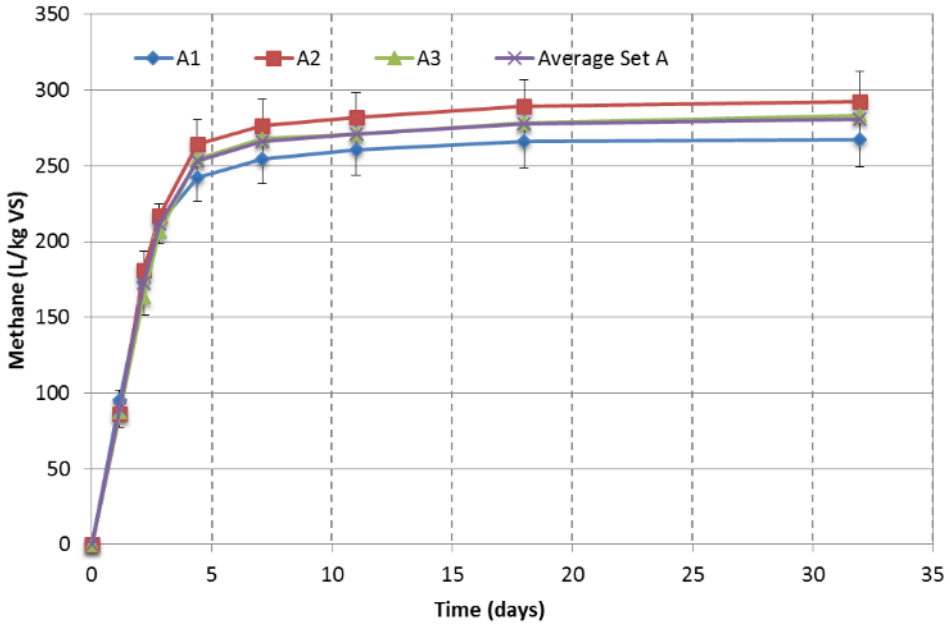

Figure 51: Methane production from MSW1 – Source Separated OFMSW supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

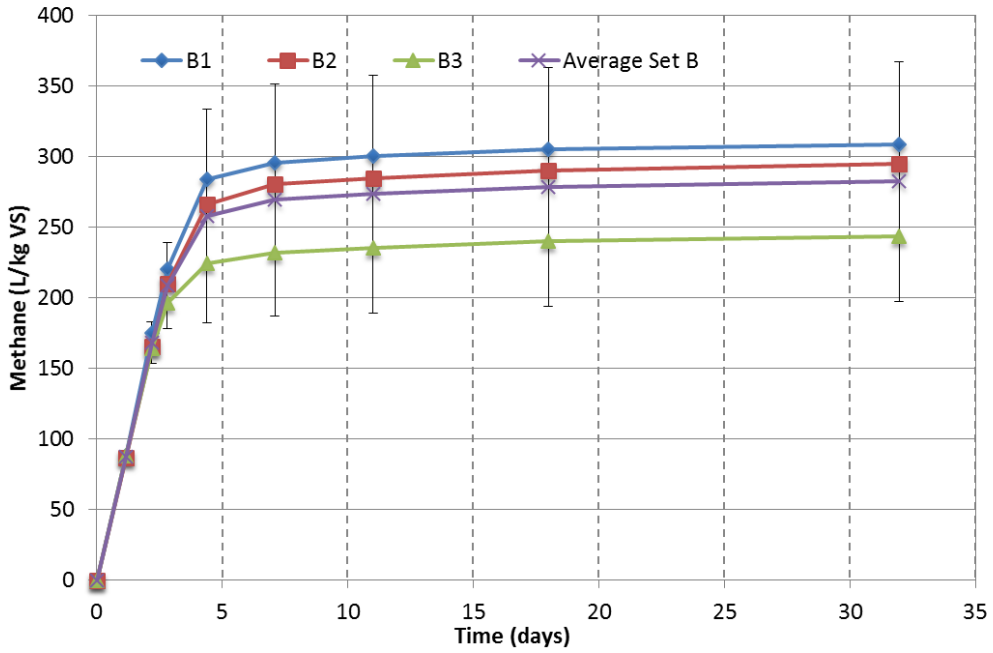

Figure 52: Methane production from MSW2 – Source Separated OFMSW supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

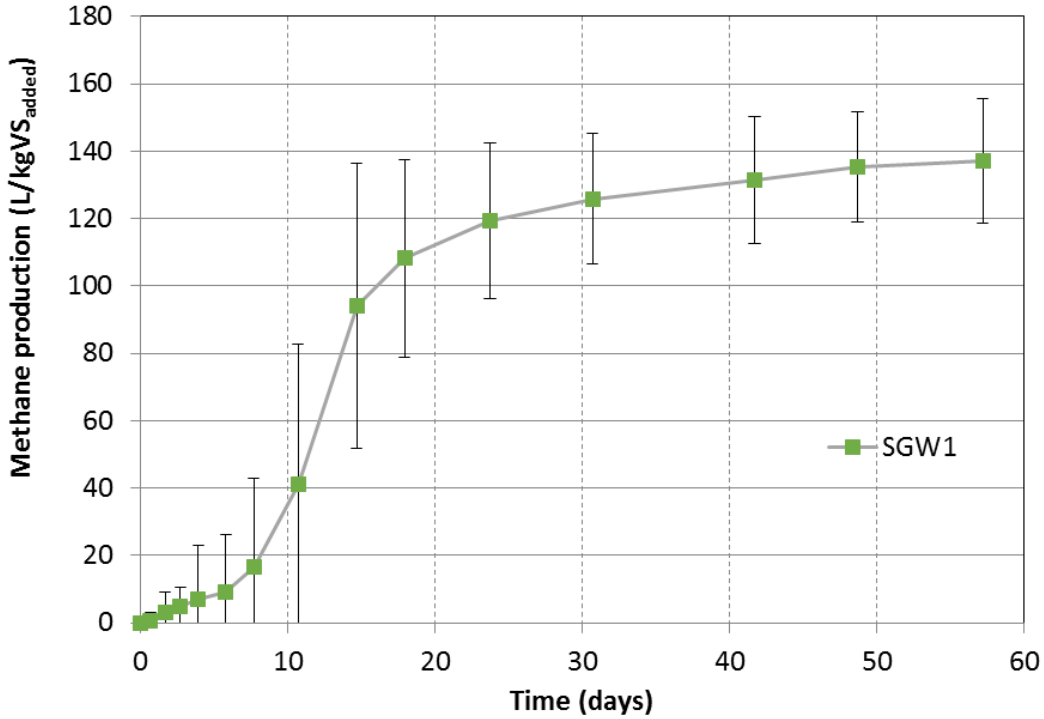

Figure 53: Methane production from SGW1 - Solid Green Waste – leaf, branch and grass waste, supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm).

9.4.2 Agricultural-Industry Waste Samples

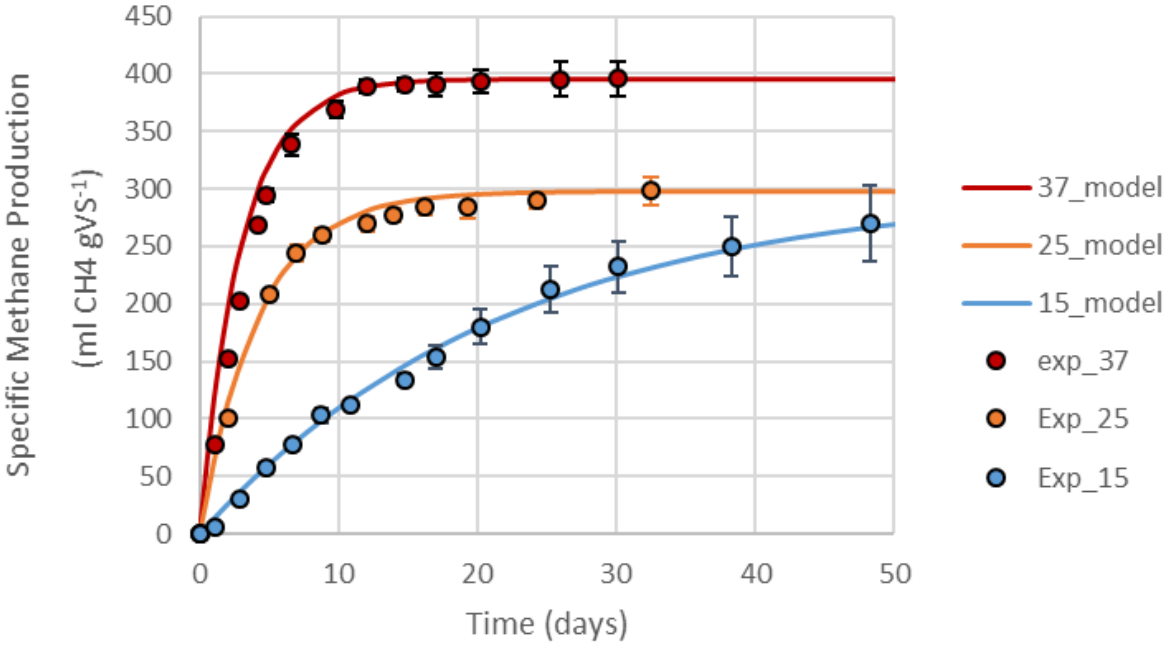

Figure 54: Methane production from PM1 - Screened Pig Manure degraded at 37°C (normalised to 25°C and 1 atm)

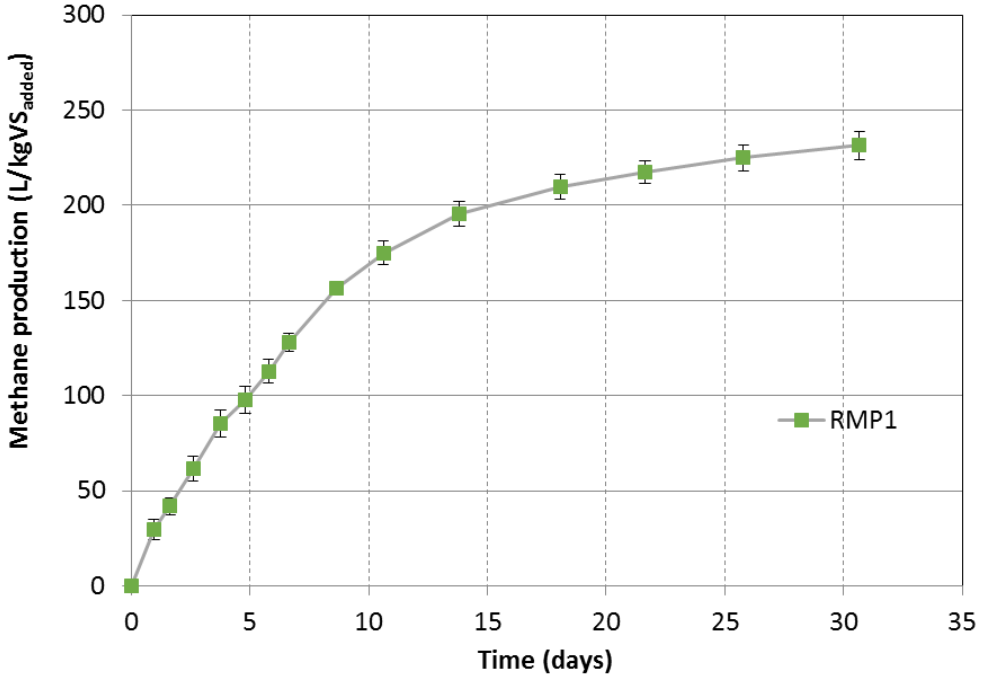

Figure 55: Methane production from RMP1 - Screened and Dewatered Paunch Waste from RMP plant 1 and degraded at 37°C (normalised to 25°C and 1 atm)

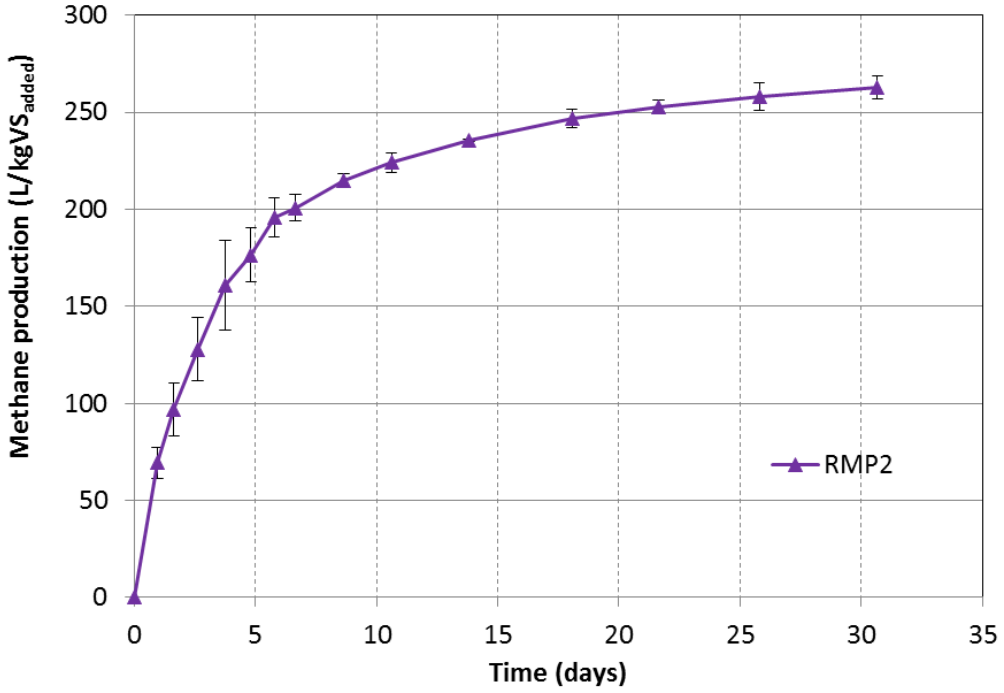

Figure 56: Methane production from RMP2 - Dewatered WAS from wastewater treatment at RMP plant 1 and degraded at 37°C (normalised to 25°C and 1 atm)

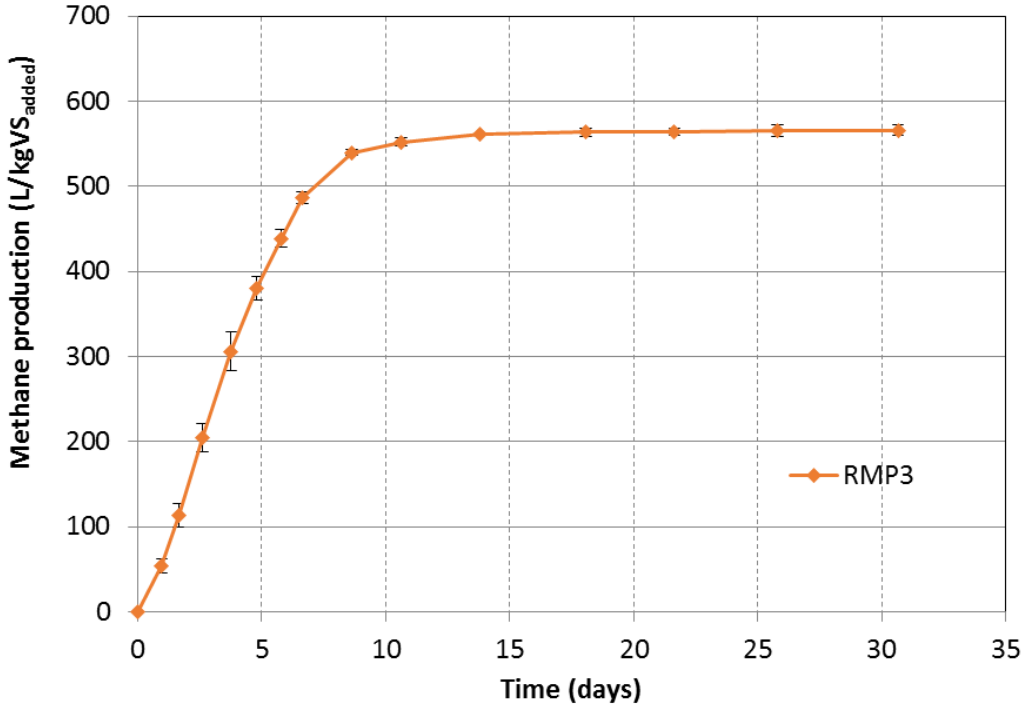

Figure 57: Methane production from RMP3 – DAF Sludge from Primary Treatment at RMP plant 1 and degraded at 37°C (normalised to 25°C and 1 atm)

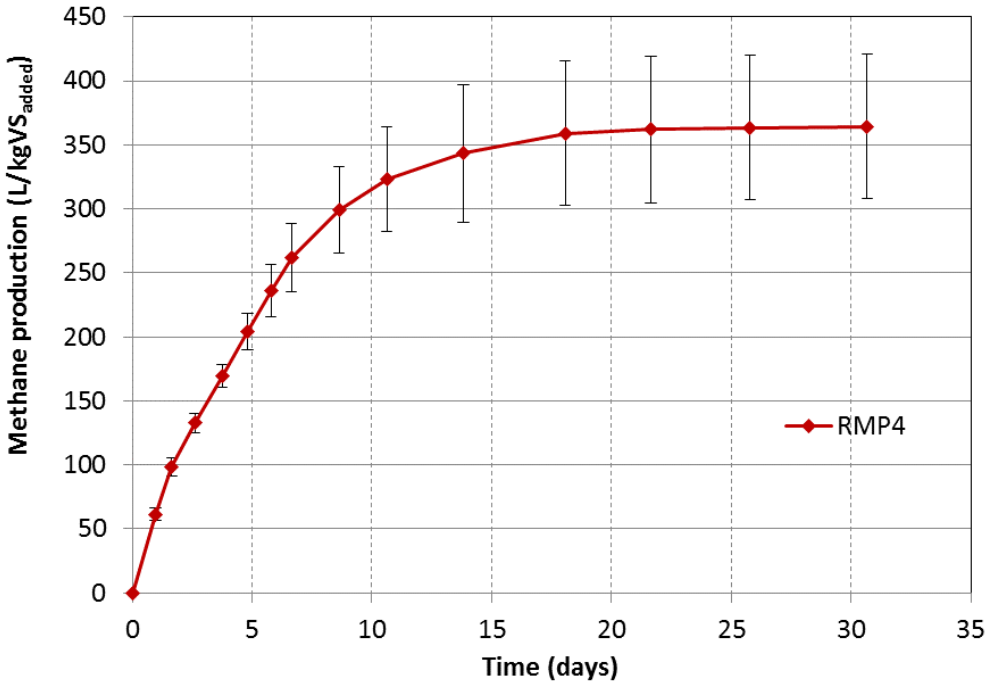

Figure 58: Methane production from RMP4 – Red Screenings from Primary Treatment at RMP plant 1 and degraded at 37°C (normalised to 25°C and 1 atm)

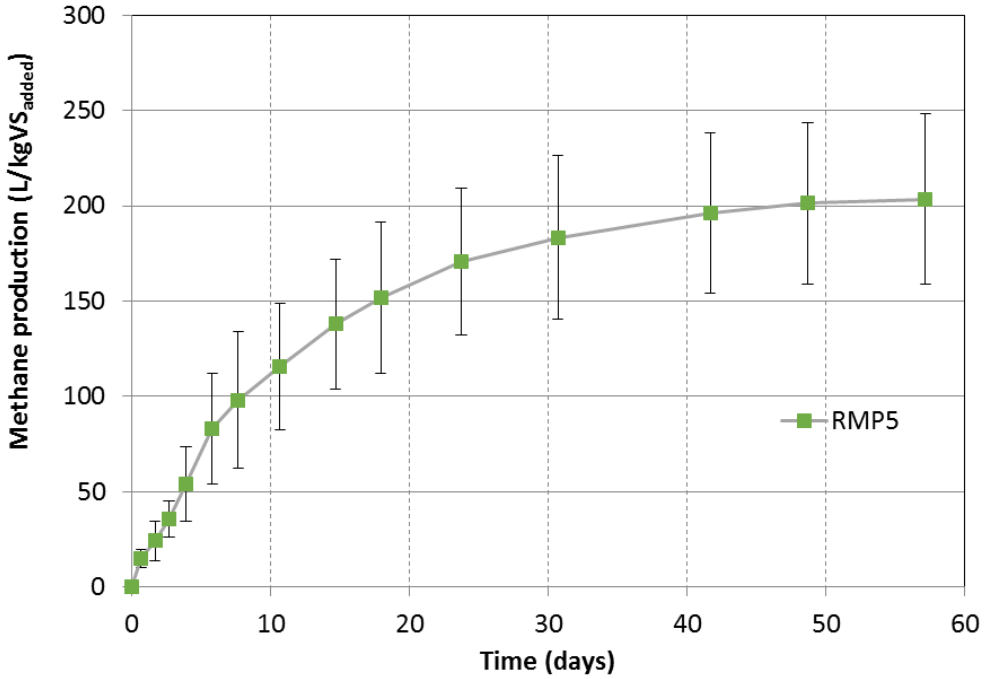

Figure 59: Methane production from RMP5 - Screened and Dewatered Paunch Waste from RMP2, supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

9.4.3 Cheese and Dairy Samples

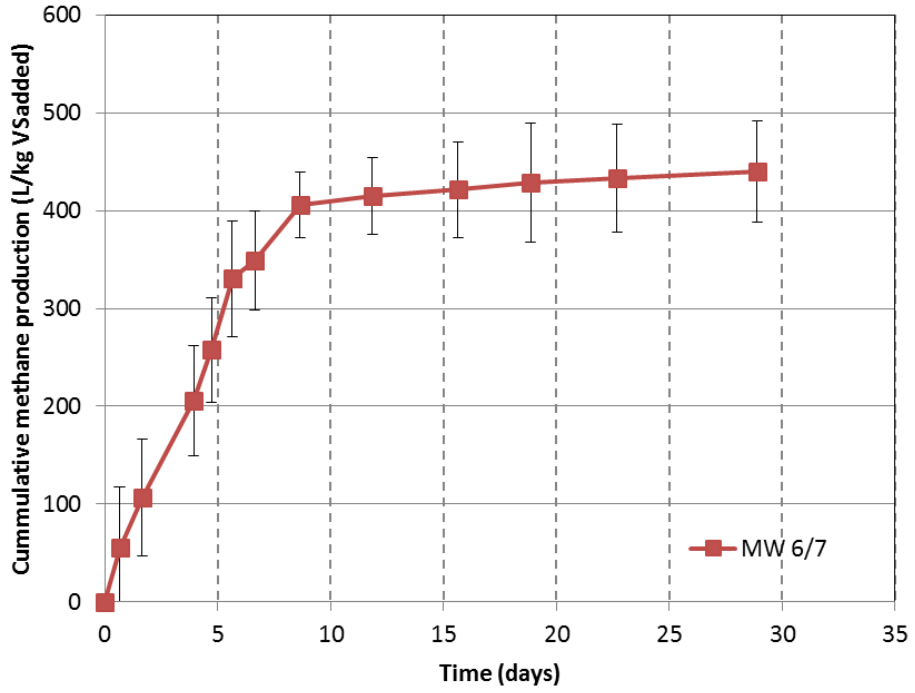

Figure 60: Methane production from CD1 - Dairy 6/7 supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

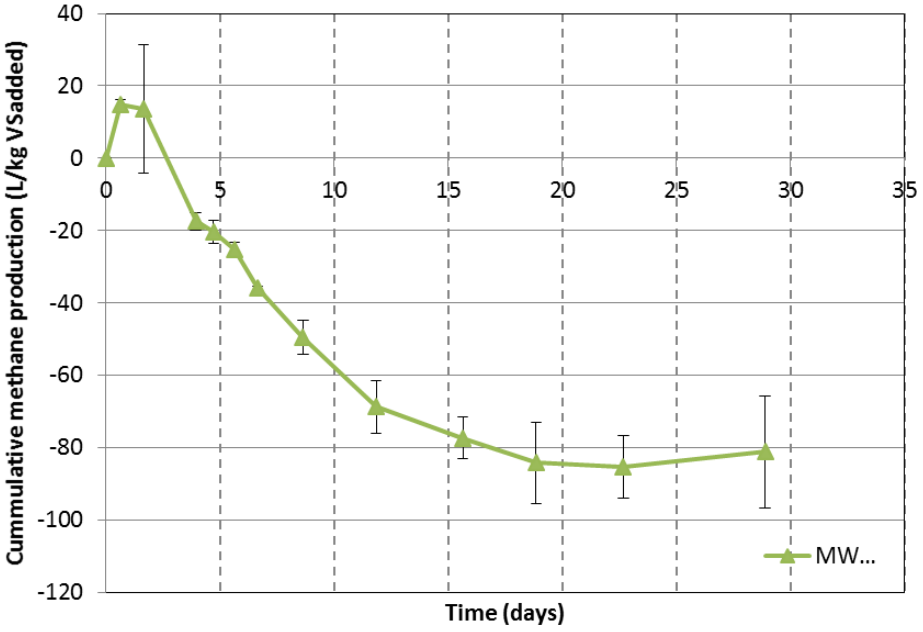

Figure 61: Methane production from CD2 - Dairy 21/7 supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

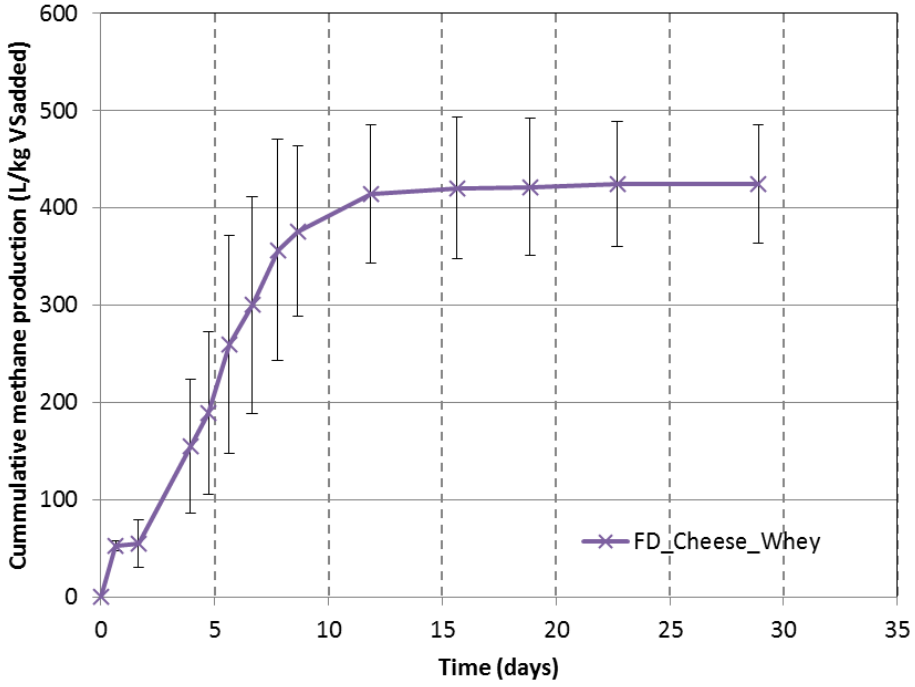

Figure 62: Methane production from CD3 – Cheese Whey supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

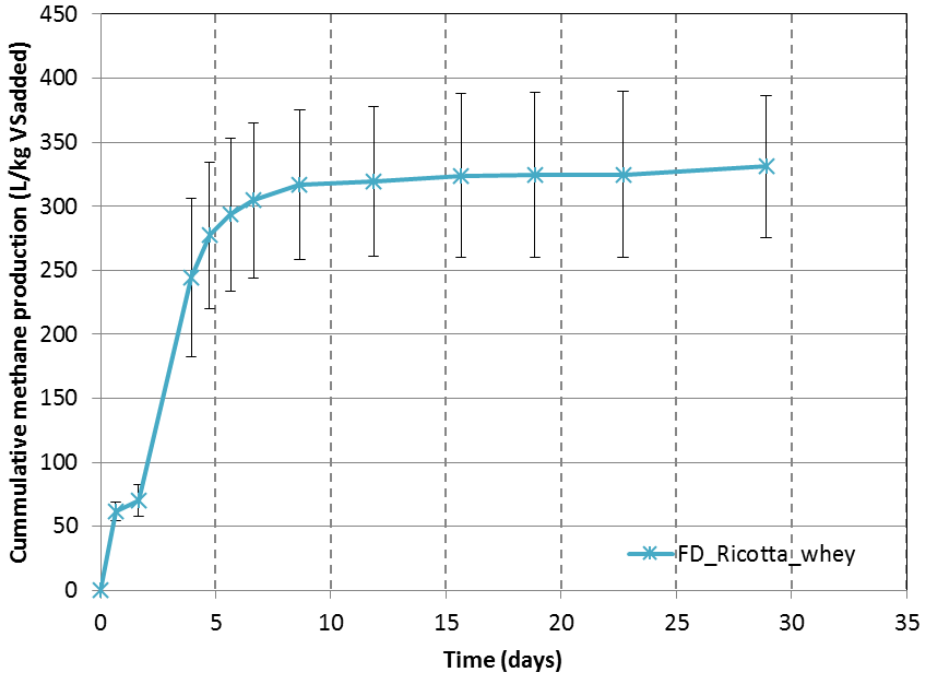

Figure 63: Methane production from CD4 – Ricotta Whey supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

9.4.4 Food Industry – Other Samples

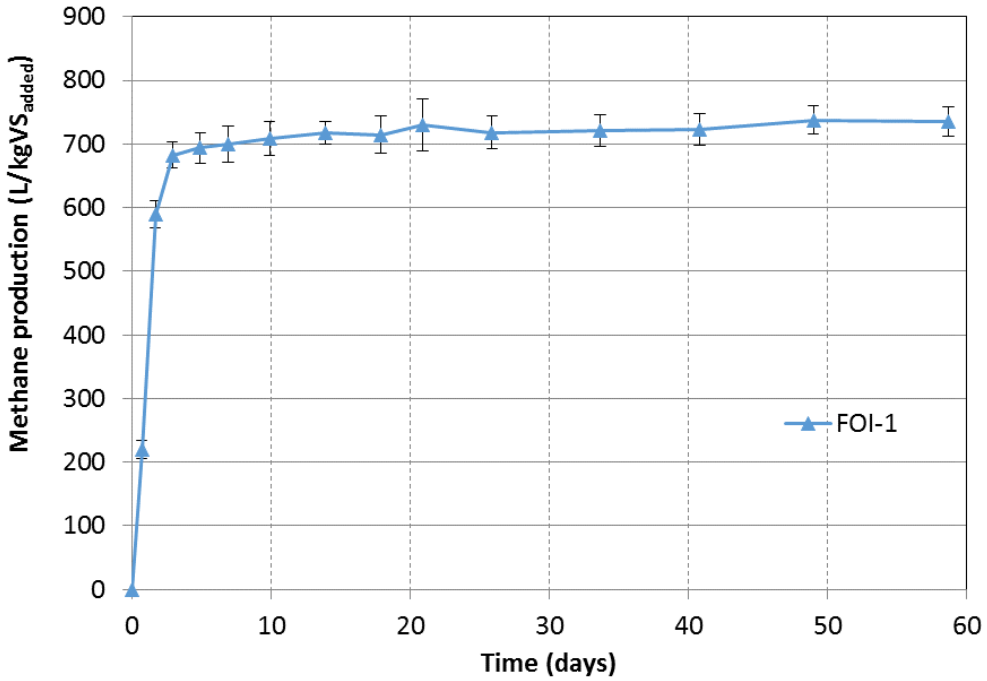

Figure 64: Methane production from FOI1 – Beverage Processing Waste supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

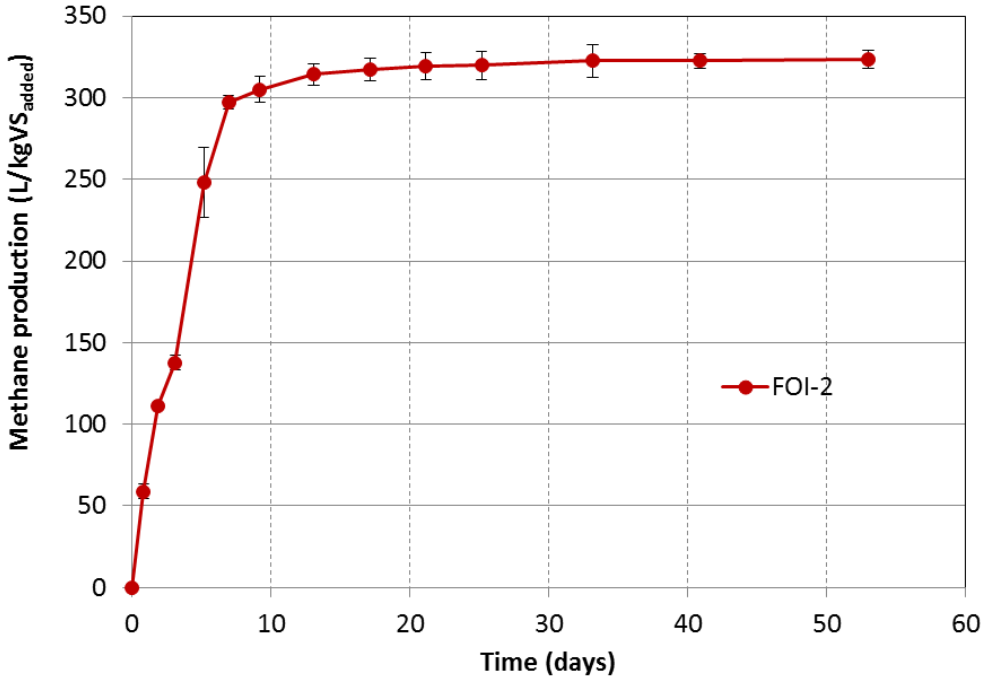

Figure 65: Methane production from FOI2 – Wastewater from honey packaging facility, supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

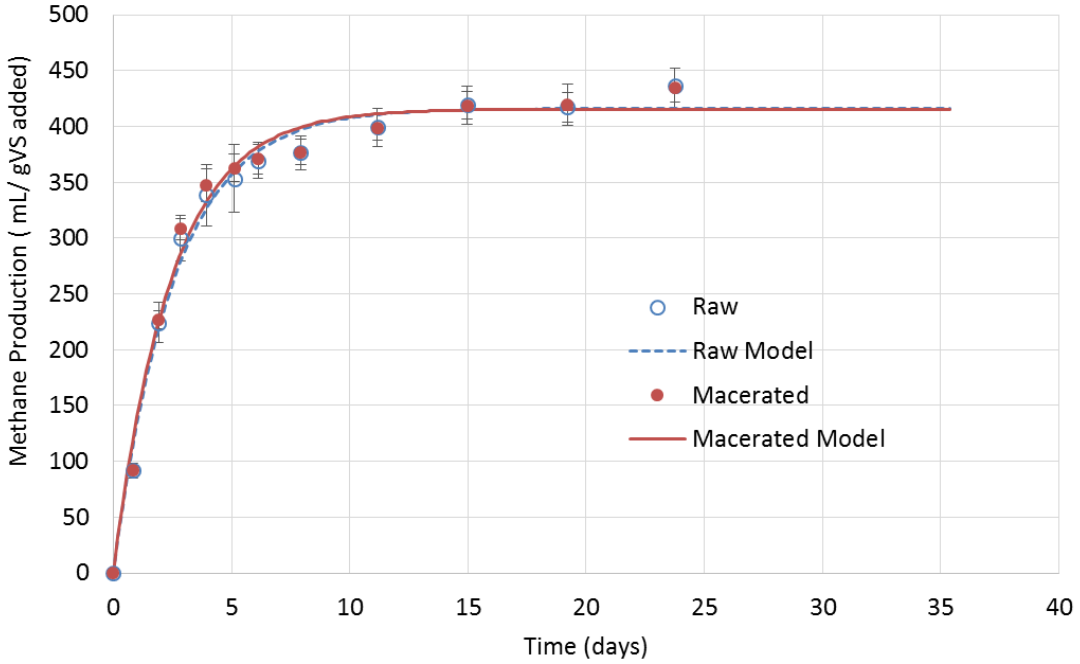

Figure 66: Methane production from FOI3 – Macerated waste from food/salad packaging plant, supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

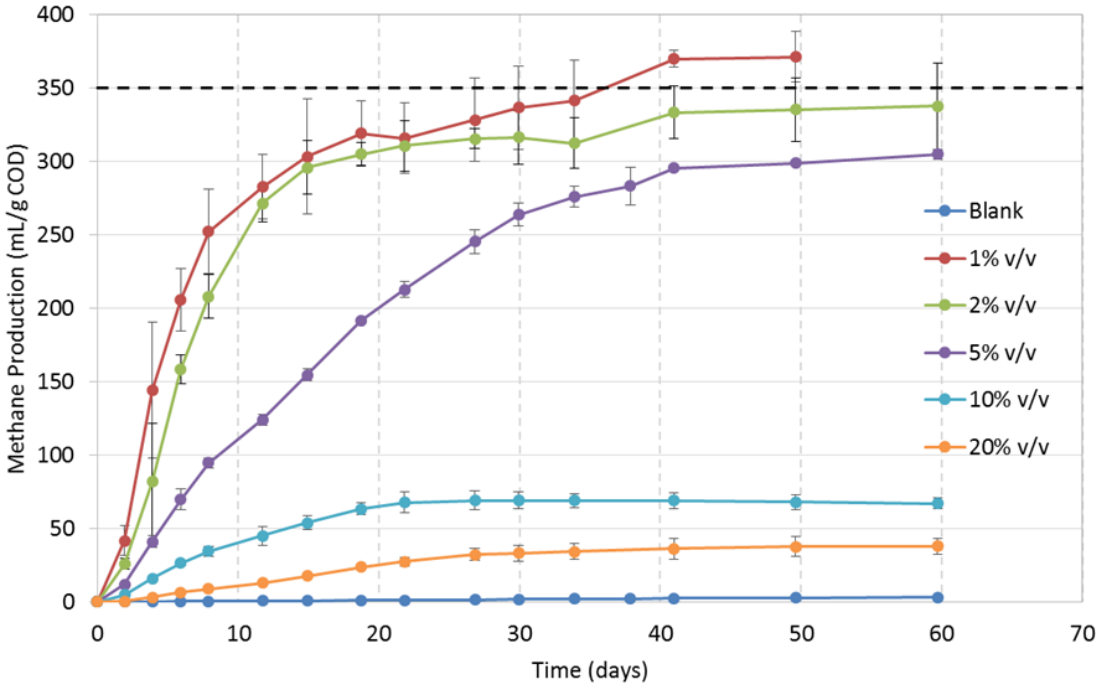

Figure 67: Methane production from FOI4 – Spreadwaste/Food Processing Trade Waste, supplied by MW and degraded at 20°C (normalised to 25°C and 1 atm)

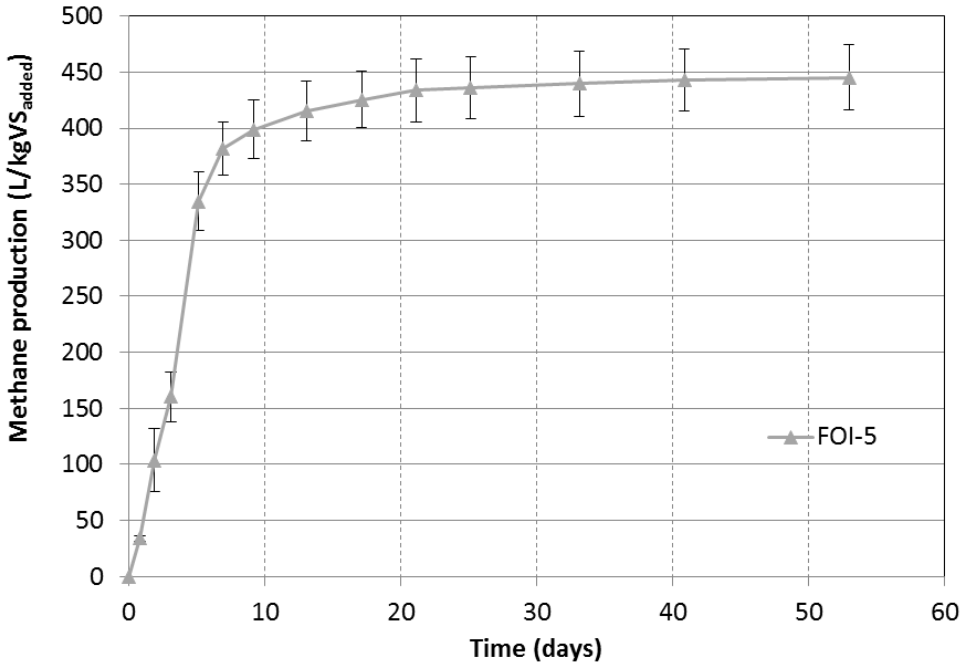

Figure 68: Methane production from FOI5 – DAF from food processing plant, supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

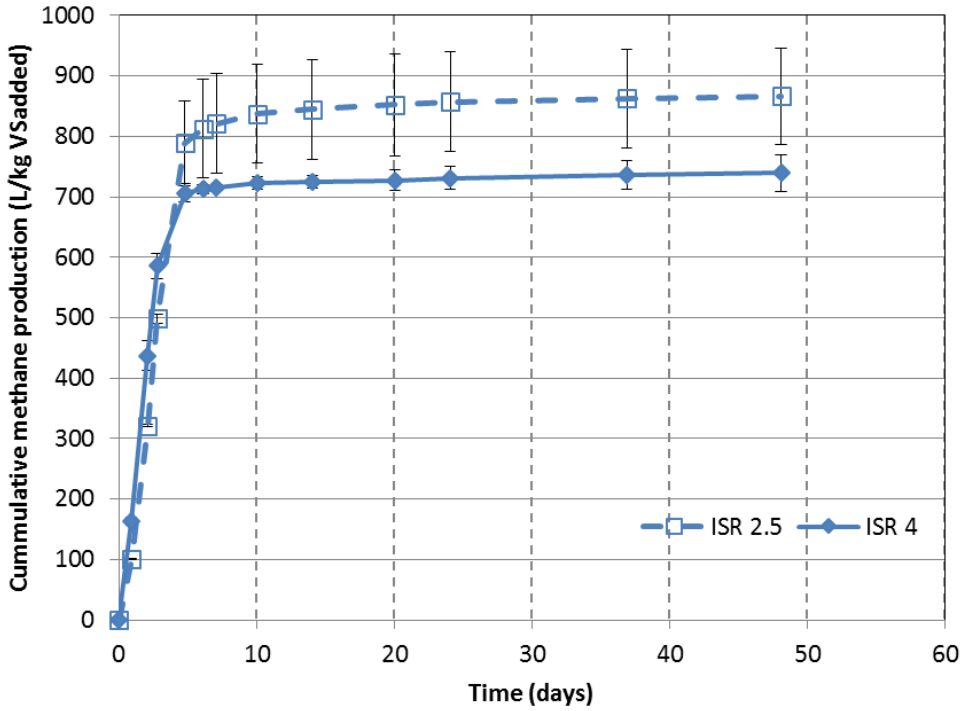

Figure 69: Methane production from FOI6 – DAF RTD AM supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

9.4.5 Grease Trap Samples

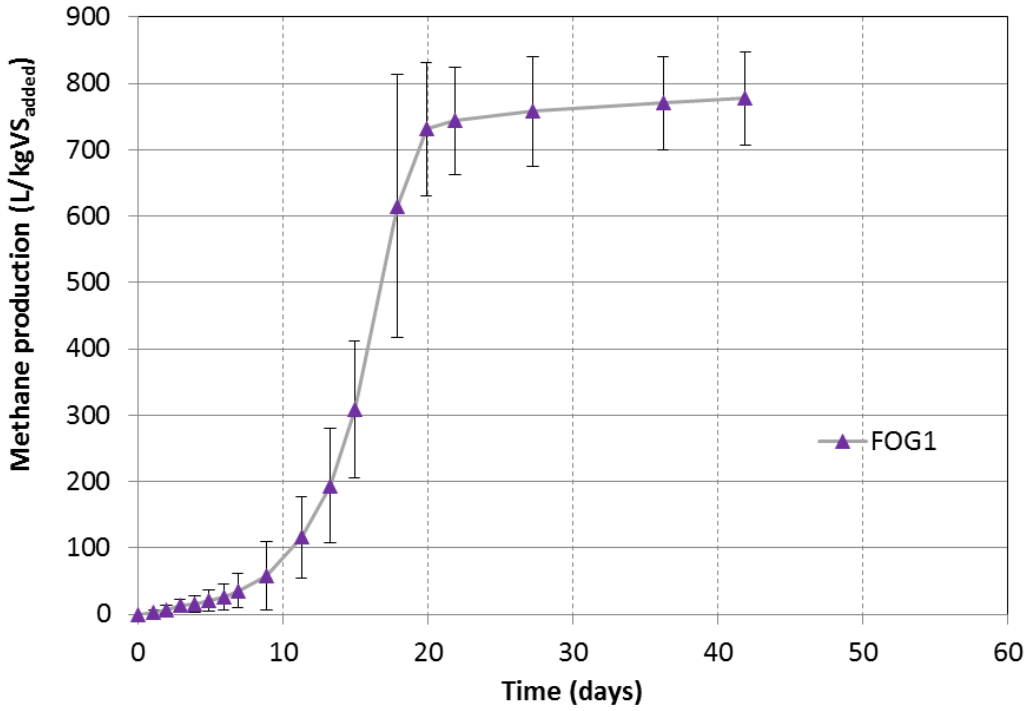

Figure 70: Methane production from FOG1 - Grease Waste supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

9.4.6 Waste Glycerol Samples

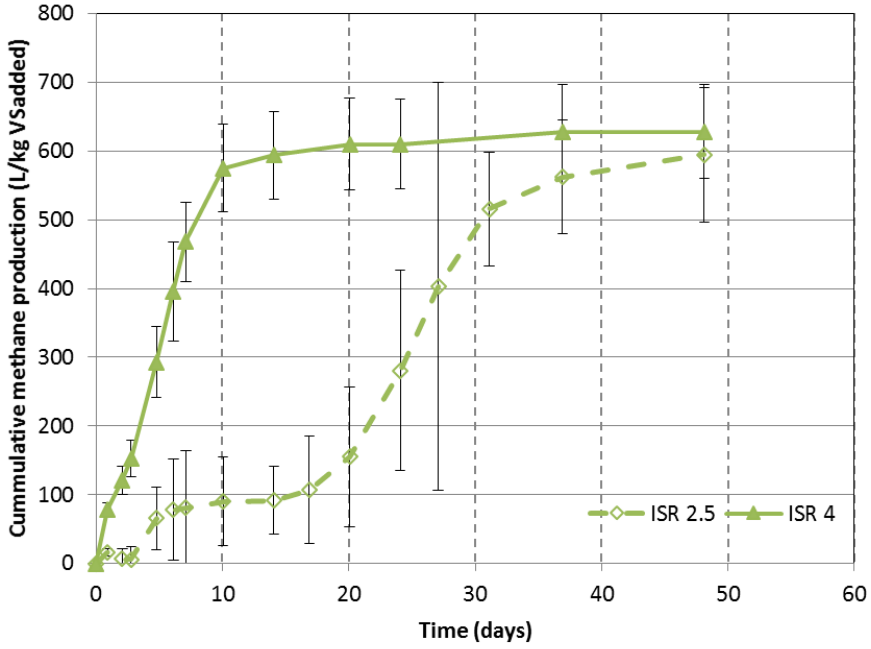

Figure 71: Methane production from GLY1 - A120 - ICI supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

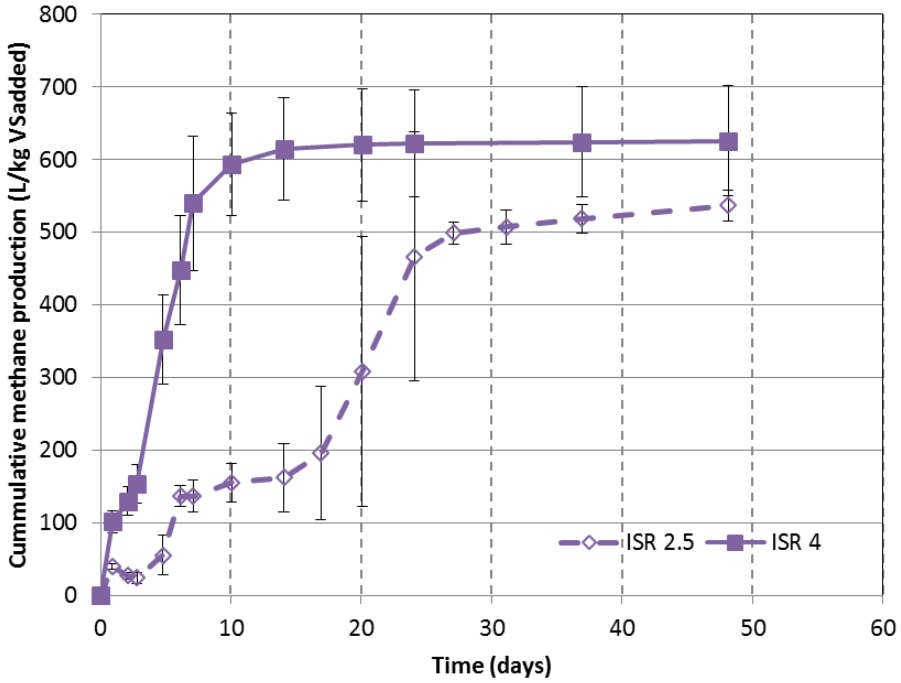

Figure 72: Methane production from GLY2 - A120 - IR supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

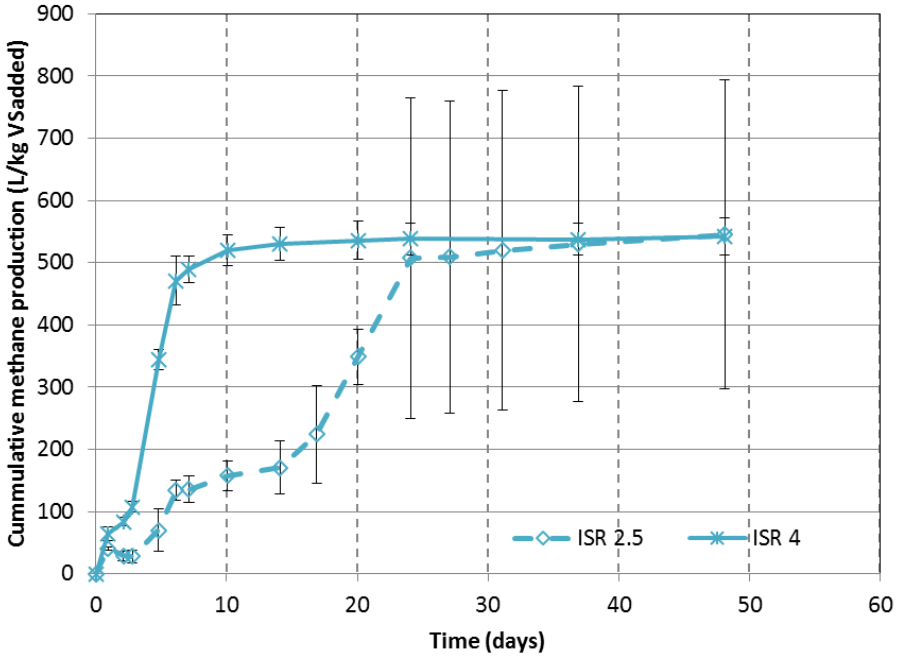

Figure 73: Methane production from GLY3 - A150 supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

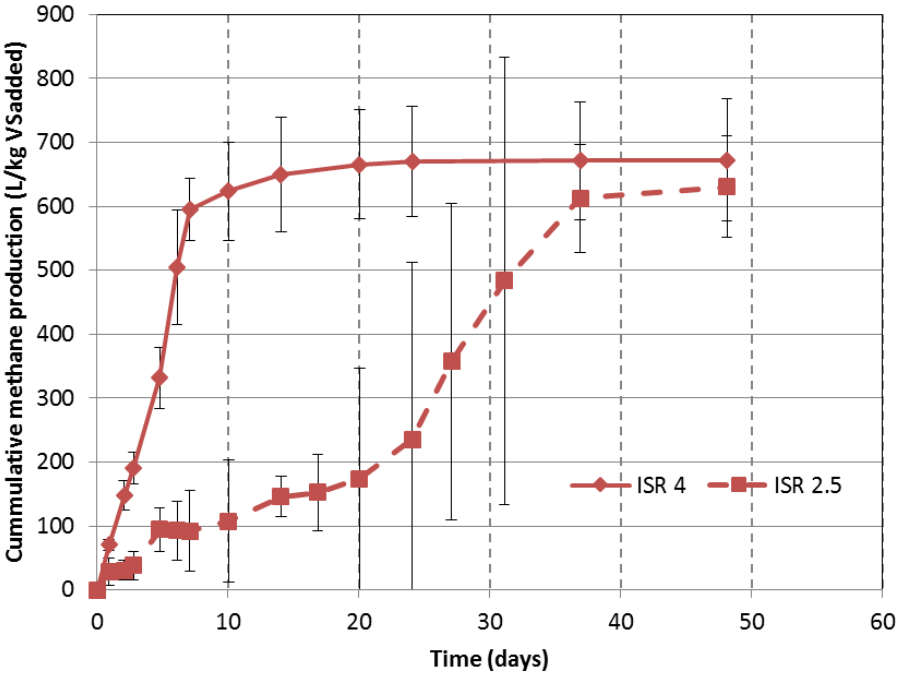

Figure 74: Methane production from GLY4 – S290 supplied by Melbourne Water and degraded at 37°C (normalised to 25°C and 1 atm)

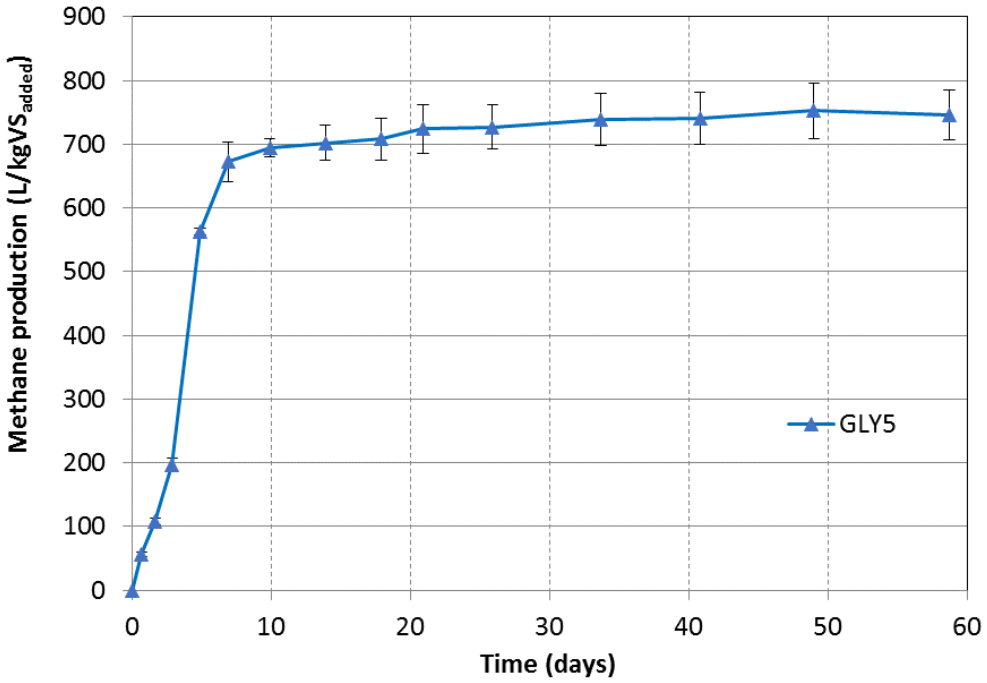

Figure 75: Methane production from GLY5 – Waste glycerol supplied by QUU and degraded at 37°C (normalised to 25°C and 1 atm)

9.4.7 Summary of all Kinetic Parameters

Table 28: Kinetic parameters fitted to BMP tests using individual trade wastes

Code	Description	B_0	f_d	K_{hyd}	CH_4 per wet T
		$L.kg^{-1}$ VS		day^{-1}	$m^3.T^{-1}$
SS1	Mixed Sewage Sludge (primary sludge and WAS)	299	0.48	0.29	11.4
MSW1	Organic Fraction of Municipal Waste, supplied by MW	280	0.62	0.44	106.1
MSW2	Organic Fraction of Municipal Waste, supplied by MW	283	0.62	0.43	103.6
SGW1	Green Waste (leaf, branch, grass), supplied by QUU	134	0.23	0.13	58.2
SFW1	Supermarket food waste, separated and macerated	363	0.89	0.4[a]	61.7
PM1	Screened Pig Manure	365	0.7	0.18	8.9
RMP1	Paunch Solid Waste from RMP plant 1	237	0.45	0.12	48.9
RMP2	Waste Activated Sludge from RMP plant 1	250	0.43	0.27	24.9
RMP3	Dissolved Air Flotation Sludge from RMP plant 1	586	0.94	0.21	44.9
RMP4	Red Solids from primary treatment at RMP plant 1	372	1	0.18	101.0
RMP5	Paunch Waste from RMP plant 2, supplied by QUU	204	0.39	0.08	36.3
ALG1	Raw Algae	400	0.7	0.2	72.0
CD1	Dairy Sample supplied by MW 6/7/2017	443	0.69	0.22	7.0
CD2	Dairy Sample supplied by MW 21/7/2017	N/A	N/A	N/A	N/A
CD3	Cheese Whey supplied by MW 17/8/2017	440	0.75	0.21	28.7
CD4	Ricotta Whey supplied by MW 17/8/2017	325	0.65	0.52	16.3
FIO1	Beverage Processing Waste supplied by QUU	723	0.84	0.77	38.3
FIO2	Honey packaging facility, supplied by QUU	325	0.75	0.27	23.5
FIO3	Food/salad packaging plant, supplied by QUU	416	0.99	0.4	31.2
FIO4	Spread waste/Food Processing, supplied by MW	341	0.97	0.18[b]	4.6
FIO5	DAF from food processing plant, supplied by QUU	441	0.68	0.27	58.2
FIO6	RTD AM – DAF Sample collected 16/1/2018.	732	0.77	0.66	16.3
FOG1	Grease Waste (FOG), supplied by QUU	787	0.99	0.27	153.5
FOG2	Grease Waste (FOG) SP1, supplied by QUU	848.25	0.9	0.27	128.9
FOG3	Grease Waste (FOG) SP2, supplied by QUU	642.6354	0.85	0.27	61.7
GLY1	A120 – ICI Glycerine supplied by MW 21/12/2017	629	0.91	0.22	476.2
GLY2	A120 – IR Glycerine supplied by MW 21/12/2017	631	0.85	0.28	421.5
GLY3	A150 – ICI Glycerine supplied by MW 21/12/2017	543	0.81	0.38	374.1
GLY4	S290 – ICI Glycerine supplied by MW 21/12/2017	676	0.9	0.27	512.4
GLY5	Glycerol (GLY), supplied by QUU	728	0.76	0.57	481.9

[a] BMP test conducted by Cleanaway

[b] BMP test conducted at 20°C

10 APPENDIX 2 – CSTR MODEL: LP EXAMPLE

This section describes a CSTR model, implemented in Microsoft Excel, to predict the performance of full-scale digesters. The tool is illustrated using a worked example based on the Luggage Point WWTP in South East Queensland. The working volume of each digester at Luggage Point is 4950 m^3. The volume of substrate added the digester was a model input, with 212m^3/d sludge as the default base load. Co-substrate can be added to this volume load, or used to replace sludge as defined by the user. The solids retention time in the digester is then defined by Equation A-1.

$$SRT\,(day) = \frac{4950 m^3}{Feed\,(m^3/d)} \qquad \text{(Equation A-1)}$$

Each substrate added to the digester is split into 3 fractions i) degradable VS, non-degradable VS and mineral solids (MS), using chemical characterisations and results from batch methane potential tests. Volatile solids destruction of each substrate added to the digester ($VS_{destroyed,sb}$) is calculated using a first-order model (Equation A-2); where $VS_{0,sb}$ is the mass flowrate of degradable VS added to the reactor (t/d) for a particular substrate, k_{hyd} is the first-order hydrolysis rate coefficient for that substrate and t is the reactor SRT (days).

$$VS_{destroyed,sb} = VS_{0,sb} - VS_{0,sb}\left(\frac{1}{1 - k_{hyd,sb} t}\right) \qquad \text{(Equation A-2)}$$

The methane production predicted from the digester is estimated by first converting the VS destroyed (calculated in Equation A-2) to COD removed – using the COD/VS ratio of the substrate. The COD destroyed is then converted to methane equivalents using the value of 380 L CH_4 $kgCOD^{-1}$ as the theoretical conversion of methane to COD at 298 K and 101.3 kPa. The method is presented in Equation A-3.

$$B_t = 380 \times VS_{destroyed,sb} \times \left(\frac{COD_{sb}}{VS_{sb}}\right) \qquad \text{(Equation A-3)}$$

A summary of kinetic inputs used in the CSTR model is presented in Figure 76, while a demonstration of substrate loading and fractionation is shown in Figure 77.

Kinetic Inputs					Substrate Characterisation			
	Source	khyd d-1	fd %	Bo CH4/gVS added	COD g/L	TS %	VS %	
Base Substrate:	Mixed SS	0.30	0.48	274.7	50.00	4.00	3.32	
Co-Substrate 1:	None	0.10	0.00	0.0	-	-	-	
Co-Substrate 1:	CCA	0.77	0.84	725.5	120.00	5.32	5.28	
Co-Substrate 2:	Glycerol	0.57	0.76	723.4	1,660.00	75.22	66.27	
Co-Substrate 3:	Paunch	0.08	0.39	205.3	247.00	20.26	17.83	
Co-Substrate 4:	Green Waste	0.13	0.23	135.9	675.00	48.39	43.40	
Co-Substrate 5:	Honey	0.27	0.75	325.0	82.20	7.24	7.23	
Co-Substrate 6:	DAF Sludge	0.27	0.68	441.0	224.00	17.30	13.20	
Co-Substrate 7:	Grease Trap	0.27	0.99	780.0	398.00	20.10	19.50	

Figure 76: Summary of kinetic inputs and substrate characterisations implemented in the CSTR model

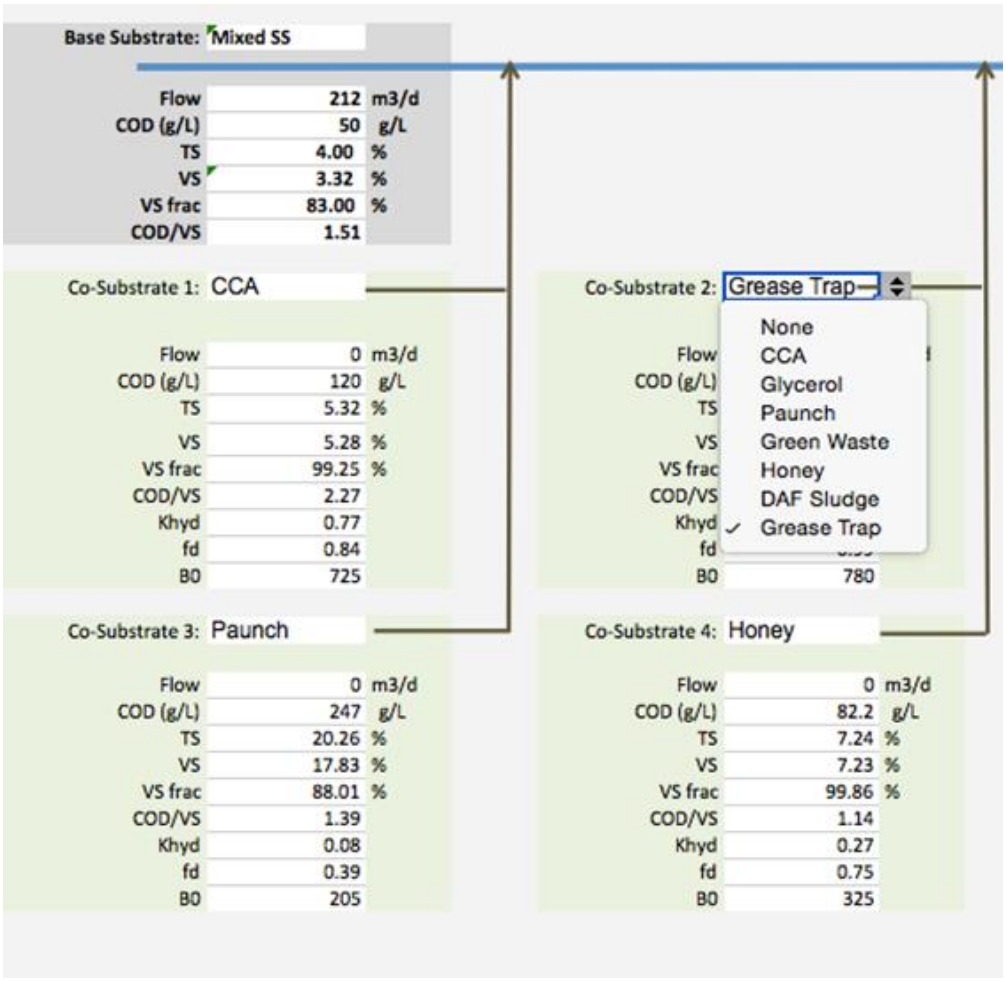

Figure 77: Demonstration of substrate loading to the digester (user defined) and fractionation into degradable VS (X_{sb}), non-degradable VS (X_i) and mineral solids (MSS). Degradable VS shown separately for each substrate, while remaining fractions are grouped.

A demonstration of the digester effluent and the fractionation between biosolids cake and dewaterting centrate is presented in Figure 78. In this example, a very high solids capture of 95% is assumed, however this capture rate can be user defined. Cake solids is estimated using full-scale dewatering data from the host plant, and lab-scale dewaterability tests. The nitrogen and phosphorus fractionation is based on the extent of solids destruction in the process.

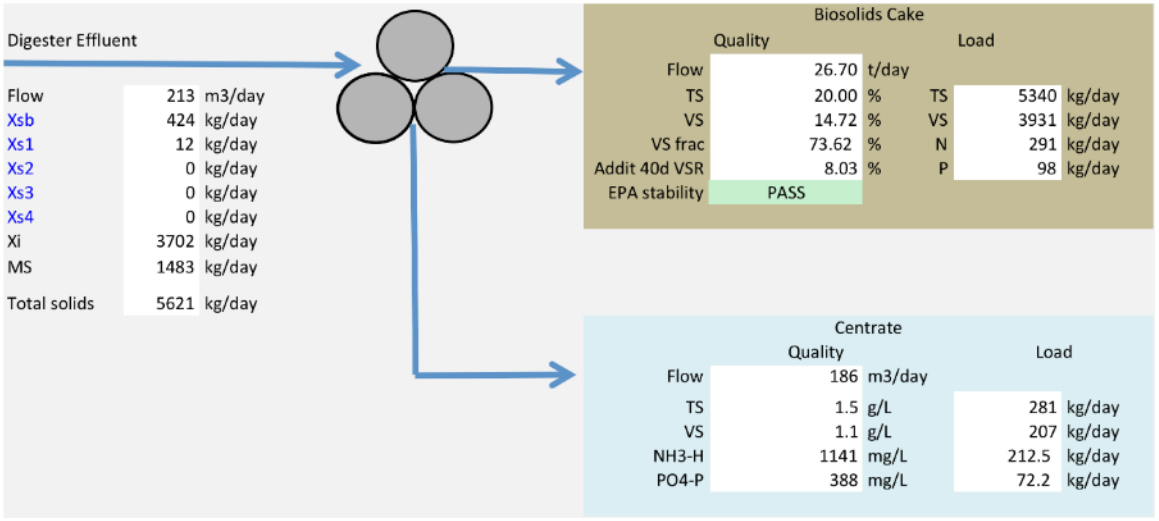

Figure 78: Demonstration of digester effluent and fractionation into dewatered biosolid (solid organic mulch) and centrate.

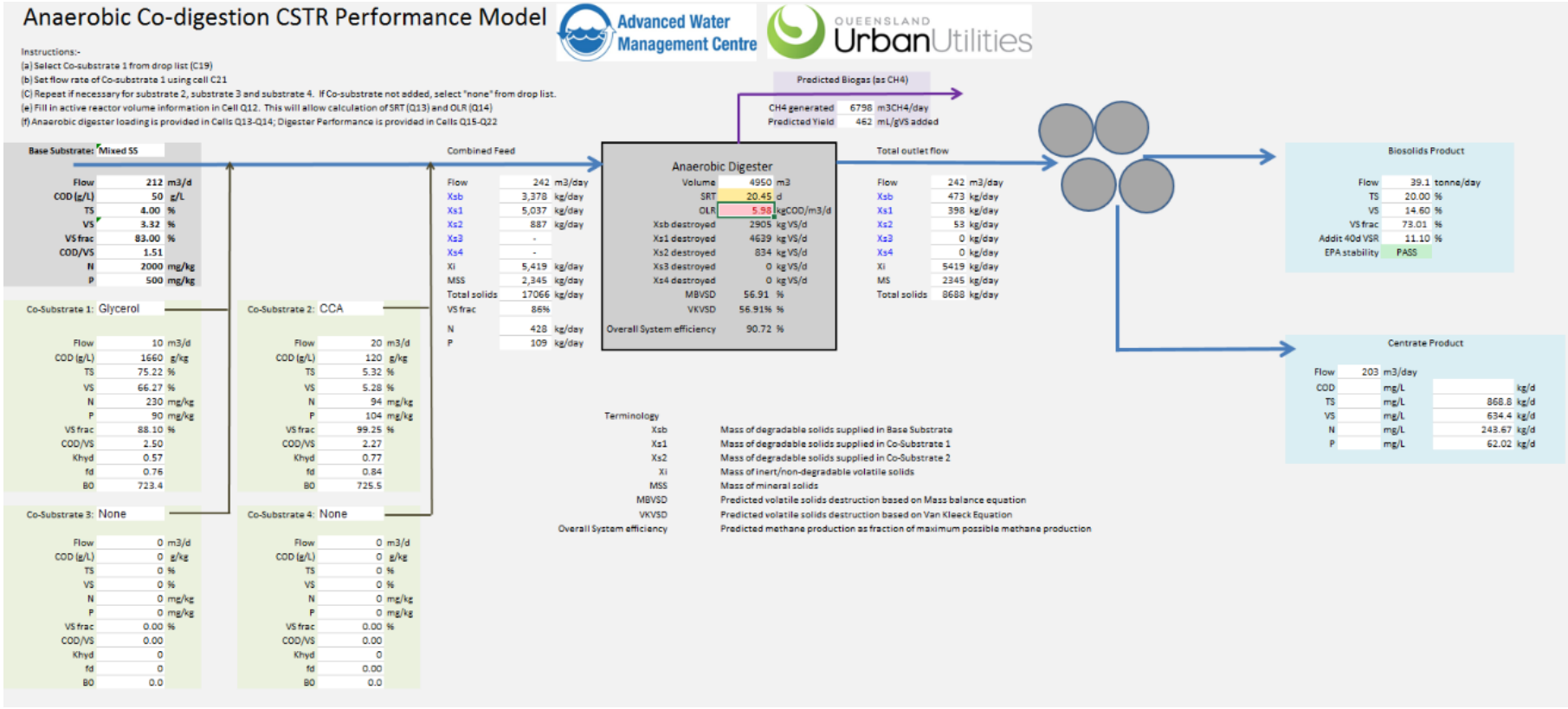

Figure 79: Example of CSTR co-digestion model implemented in Microsoft Excel