Anaerobic co-digestion of municipal wastewater sludge with organic wastes

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School of Civil, Mining and Environmental Engineering
Faculty of Engineering and Information Sciences

ANAEROBIC CO-DIGESTION OF MUNICIPAL
WASTEWATER SLUDGE WITH ORGANIC WASTES

A thesis submitted in the fulfilment of the requirements for
the award of the degree of

Doctor of Philosophy

from

University of Wollongong

by

Richard John Wickham

April 2019
DECLARATION

This declaration is to certify that the work presented in this thesis was completed by the author, unless specified otherwise, and that no part of it has been submitted in a thesis to any other university or institution.

This thesis was prepared in the compilation style format and is based on the published journal articles listed in Thesis Related Publications. The citation and status of each journal article are also shown at the beginning of each chapter. All related journal articles were conducted and written during the author’s candidature. Despite a certain level of repetition between chapters in introductory material and analytic methods, each single chapter was substantially different in focus and content within the scope of the thesis theme.

Richard John Wickham

April 2019
Dedication

This thesis is dedicated to my loving partner Tabea, who makes every day so much brighter. Thank you for your love and support, without you I would not have made it here.
THESIS RELATED PUBLICATIONS

Peer-reviewed Journal Articles


Conference Presentation

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Special thanks must go to Sydney Water for allowing me to be involved in the project. I would also like to express my gratitude towards the University of Wollongong for their financial support throughout my studies.
ABSTRACT

In municipal wastewater treatment plants, anaerobic digestion is applied for the treatment of sewage sludge. Digestion of sewage sludge produces a high level of alkalinity for buffering against pH variation; however, biogas production is limited due to the low organic content of the substrate. Anaerobic co-digestion is the simultaneous treatment of two or more organic waste streams such as sewage sludge and food waste. This process allows for the stabilisation of solid organic materials, whilst simultaneously providing a renewable source of energy in the form of biogas. Anaerobic co-digestion extends the scope of conventional anaerobic digestion by allowing for the treatment of substrates that are unsuitable for mono-digestion. Substrates rich in inhibitory contaminants (such as sulphides, heavy metals or excess ammonia), readily degradable organics or deficient in buffering capacity can be paired with sewage sludge for stable operation and enhanced biogas production. Prior examples of co-substrates paired with sewage sludge include food waste, the organic fraction of municipal waste and slaughterhouse wastes. Ideal co-digestion pairings can also instigate a synergistic effect whereby degradation of both substrates is enhanced through the optimisation of reactor stoichiometry and biomass balance.

Despite the lauded benefits of co-digestion, its uptake by water utilities is still limited. Factors restricting full-scale co-digestion include shortage of suitable co-substrates, along with the uncertainty on the impacts on reactor performance, biogas quality and biosolids production. Uncertainty associated with these factors derives largely from the deficit of pilot scale studies that can accurately represent full-scale application. This thesis focuses on screening and providing detailed evaluation of new potential co-substrates. Promising substrate pairings were subsequently demonstrated in long term, pilot-scale studies which evaluated the impact of co-digestion on downstream processes. The presented data provides an invaluable reference for full scale application of co-digestion, providing clarification on its potential impacts on factors such as biosolids quality, odour and dewaterability. The potential use of a promising pre-treatment technology in conjunction with co-digestion was also studied.

Candidate organic wastes were screened using biomethane potential testing to determine their suitability for co-digestion with sewage sludge. These organic wastes dairy, soft drink, bakery and fat oil and grease and paper manufacturing wastes. Co-substrates were evaluated in terms of their ultimate biomethane production and their impact on biomass stability. The
co-digestion of each co-substrate was demonstrated across a range of concentrations in order to provide preliminary figures for the ideal mixing ratios. Results were compared to the control mono-digestion results using sewage sludge. Each of the co-substrates was found to be richer in organic matter than sewage sludge and significantly increased biomethane production in each digestion bottle than the control. Overloading was observed higher concentrations in several co-substrates due to the accumulation of organic acids. Differentiation between solid and liquid co-substrates was made in the results in terms of their impact on biosolids. Solid co-substrates had a greater tendency to increase the level of organic matter at the conclusion of the experiment. Liquid co-substrates on the other hand were seen to instigate a synergistic effect in terms of the removal of organic matter.

Further biomethane potential testing was conducted in order to elucidate any synergistic/antagonistic effects between sewage sludge and two organic wastes: simulated food waste and paper pulp. Synergisms were evaluated in terms of their impact on biosolids quality, biodegradability and biomethane kinetics. Simulations based on the modified Gompertz model were employed, with specific focus on process kinetics along with a chemical oxygen demand (COD) balance. Each of the methodologies to evaluate synergistic/antagonistic effects of co-digestion suggested pairing of the sewage sludge with either paper pulp or food waste positively impacted on digestion performance. Shortening of the lag phases were observed during co-digestion relative to mono-digestion of paper and food waste. Co-digestion was also seen to significantly improve the hydrolysis rate in paper waste digestion, leading to greater removal rates of organic matter in the substrate relative to during mono-digestion. In the case of food waste co-digestion with sewage sludge, synergisms in terms of the hydrolysis rate were limited. On the other hand, the effect of synergistic metabolism during food waste co-digestion on the conversion of COD was estimated to be 32% in this study. The COD balance suggested that synergistic effects improved biomethane production during co-digestion between sewage sludge with both paper pulp and food waste, with a greater impact observed in the latter.

Following the positive results from co-substrate screening, sewage sludge co-digestion one of the more promising co-substrates, soft drink waste, was studied in greater detail. Co-digestion was evaluated across a range of mixing ratios using a series of identical semi-continuous laboratory-scale reactors, one of which was used as a mono-digestion control reactor for reference. Beyond the screening study, this experiment considered impacts of soft drink co-digestion on biomethane composition, biosolids odour and reactor stability in an
ongoing system. Stable co-digestion of was demonstrated up to a mixing ratio of 20% (v/v) soft drink addition beyond which organic overloading and total reactor inhibition was observed. At the 20% mixing ratio biomethane production was nearly triple that of the control reactor. The OLR increase of 171% corresponding to 20% (v/v) BW in the feed was the optimum co-digestion ratio, and resulted in an biogas production increase of 191%. Whilst no synergistic effects were observed during co-digestion when employing a COD balance methodology, biosolids odour was unaffected and its organic content increased only slightly despite a significantly higher loading rate. Methane content in the biogas was seen to improve during transitional periods as soft drink content in the feed was increased from 10 to 20%.

To accurately represent full-scale application of co-digestion and address the uncertainties surrounding its impacts on downstream processes, a series of pilot scale experiments were conducted using both new and promising co-substrates. Two parallel anaerobic digesters were used to compare sewage sludge mono-digestion with co-digestion. Expanding on previous studies concerning soft drink waste, a series of beverage wastes were studied including juice, beer and wine waste. The study provided stable and consistent data that can be accurately scaled up to represent full-scale co-digestion. The findings suggest that increases in the biogas production due to co-substrate addition were approximately proportionate with the increases in the organic loading rate. Wine waste was the exception for this as it instigated antagonistic effects, considerably reducing biomethane production. Excluding wine waste, addition of the co-substrates had no destabilising effects on the reactors, whilst causing only slight increases in organics in the digestate. COD was found to be a more appropriate parameter than volatile solids for measuring organic content in beverage wastes, as the latter fails to account for volatile acids or ethanol.

The second pilot scale study concerned the co-digestion of two food wastes produced from the hospitality sector. Whilst being one of the most prevalent co-digestion pairings in the literature, there are limited large scale studies on food waste and sewage sludge digestion and those that exist afford insufficient focus on biosolids quality. Consequently the focus of this study was to provide sufficient data to facilitate full-scale application of food waste and sewage sludge co-digestion. Further emphasis in the study was in elucidating the impact of the avoidable/unavoidable fractions of food waste. Avoidable food waste concerns the edible components of food waste produced through wastage or spoilage, whilst the unavoidable fraction is the inedible organic matter that is inherent to food preparation, such as fruit skins.
or animal bones. As society embraces more sustainable practices, the fraction of avoidable food waste is expected to decline, however the effects of this on food waste co-digestion and biomethane yields are not presently understood. Consequently, two commercially produced food wastes representative of these two categories were evaluated in terms of their biodegradability. Whilst both wastes were found to be suitable co-substrates in terms of biomethane production, the avoidable fraction of food waste exhibited a notably higher specific methane yield. Addition of the avoidable and unavoidable food wastes resulted in 76 and 56% increase in organic loading rate and 104 and 64% increase in biomethane production relative to the control, respectively. Neither food waste caused any destabilisation of the reactors, whilst the unavoidable food waste increased the alkalinity production in the reactor. Food waste addition was seen to increase the volume of biosolids produced.

As co-digestion can lead to increased biosolids production from the higher organic loading rate, pre-treatment methods are regularly used in conjunction with co-digestion to offset this effect. Despite the number of studies evaluating existing pre-treatment technology, such techniques are rarely applied in full scale. The reason for this is the excessive chemical or energy costs required to achieve significant improvements in biodegradability. One proposed technique that offers lower chemical costs with significant improvements to biodegradability is free ammonia (FA) pre-treatment. This involves dosing with ammonia/ammonium (which can be sourced from onsite) and pH adjustment. This technique has thus far only been demonstrated with waste activated and primary sludge mono-digestion. Thus this technique was applied during co-digestion to determine its potential benefits. The co-digestion mix selected was sewage sludge with food waste addition, which was observed previously to increase organic content of the digestate. Results suggested that FA pre-treatment can significantly increase biodegradability of the co-digestion blend. Methane yield was increased by 30% relative to the control co-digestion reactor during the application of FA pre-treatment. Based on prior studies, the improvement to food waste biodegradability was even greater than in primary sludge.
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<td>AD</td>
<td>Anaerobic digestion</td>
</tr>
<tr>
<td>VFAs</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulphur-reducing bacteria</td>
</tr>
<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
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<tr>
<td>tCOD/COD</td>
<td>(Total) chemical oxygen demand</td>
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<td>Beverage waste</td>
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<tr>
<td>SCADA</td>
<td>Supervisory control and data acquisition</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
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CHAPTER 1: Introduction

1.1. Anaerobic digestion

Anaerobic digestion (AD) is a relatively mature technology. AD has been widely utilized for the management of organic wastes including municipal wastewater sludge, biomass and manure from the agro-industry throughout the past century. Despite an extensive research and application history, persisting focus within the field has facilitated further application development along with the emergence of new concepts and techniques. The driving force behind this development in recent years has been the societal realignment towards the promotion of sustainable practices. AD presents a particularly enticing avenue for development towards environmental sustainability, through its potential to generate renewable energy in the form of biogas. Thus, a plethora of emergent papers have been reported over the past decade focused primarily on the production and utilization of biogas through AD.

AD uses a consortium of microorganisms to biodegrade organic matter under anaerobic conditions to stabilise waste materials and produce biogas (Raposo et al., 2011). The diverse community of microorganisms facilitate AD through several stages, which involve the breakdown of complex organic compounds and ultimately the production of a gas by-product comprised of 60% CH$_4$ and 40% CO$_2$, along with other trace gasses (e.g., H$_2$S). The degradation of organic materials ultimately leads to a more stable and hygienic biosolids product and more efficient sludge dewatering.

AD can be used to breakdown of biodegradable organic matter, with the exclusion of woody materials, due to the inability of anaerobic bacteria to decompose lignin (Chen et al., 2008). Early application of AD has been associated with the conversion of organic materials such as animal manure for biogas production for street lighting. But one of its most essential and enduring applications is for the treatment of wastewater sludge. The digestion of both primary and waste activated sludge from wastewater treatment has become ingrained practices within the industry. Considering the proportionality of sludge disposal costs with respect to overall operating expenditures, which can be up to 50% (Appels et al., 2008a), the reduction of these volumes is an essential requirement within the practice of wastewater treatment. Additionally, the process promotes the dewaterability of the sludge, thus furthering the cost reduction value of AD in the industry. These economic benefits, coupled with the
treatment necessities with regards to stabilization and pathogen destruction, ensure the persistent relevance of AD processes.

The treatment of wastewater through conventional AD involves its application to either primary or waste activated sludge. The rationale behind this limited application relates to the organic concentration requirements for AD. Whilst increasing the dilution can be a means to reduce the impact of inhibitors (Chen et al., 2008), digesters require a high organic concentration in order to operate efficiently and digesting raw wastewater rather than sludge would significantly increase the reactor volume requirements. A higher organic content promotes an increased potential rate of degradation and subsequently the biogas production and volatile solid (VS) reduction (Gomez et al., 2006). However, this increase only occurs up to the concentration at which inhibition effects outweigh the benefits of increased organic availability.

Both primary and waste activated sludge can be efficiently anaerobically digested on their own but biogas production is low. Figure 1.1 describes the different waste streams within the primary and secondary stages of conventional wastewater treatment (i.e. raw wastewater, primary and waste activated sludge, biosolids and biogas). Primary sludge is composed of settled solids generated from the initial separation of settleable solids prior to aerobic treatment. Waste activated sludge is from the secondary aerobic treatment process. The aerobic treatment process similarly involves the use of microorganisms for degrading dissolved organic substances but in the presence of oxygen (i.e., aerobic conditions). Thus, primary and wastewater activated sludge can differ in their composition and characteristics.
1.1.1. **Process requirements**

Fundamentally, AD involves the subjection of organic matter to anaerobic microorganisms in an oxygen free environment. The maintenance of a relatively neutral pH and temperature range is an essential requirement for AD operation (Raposo et al., 2011). Subsequent to this innate process simplicity, AD does not require complex infrastructure. The provision of a stable anaerobic environment within a digestion tank is sufficient to facilitate the AD process when a suitable organic substrate is introduced. However, the optimization of the process to maximize both the treatment potential and the by-product parameters is complex. This complexity derives from the presence of four distinct yet interrelated biological degradation mechanisms simultaneously occurring within the system. Meanwhile, each microorganism group has a set of environmental ideal conditions. Several substances have been found to be inhibitory to differing species used in AD, including ammonia, sulphides, certain trace elements and organic compounds (Chen et al., 2008). However, a major research gap in the field of AD relates to the understanding of the complex mechanisms by which inhibition occurs. There is a significant deficit in the conditional understanding of AD requirements, with only a few emergent rules in process assessment widely adopted in the field (Madsen et al., 2011). Difficulties arise from a lack of framework in AD testing, resulting in widespread inconsistency in results (Mata-Alvarez et al., 2014).

1.2. **Biological degradation during anaerobic digestion**

1.2.1. **Primary degradation mechanisms**

The degradation of organics in the AD process can largely be separated into four stages. Each of these steps is carried out by distinct species of microorganisms, largely categorized through their primary method of energy attainment. Figure 1.2 describes the primary degradation pathways involved in the production of both methane and carbon dioxide.
The initial stage of degradation, namely hydrolysis, involves the solubilisation of complex organic particles. It is performed by fermentative and hydrolytic bacteria species. The kinetics of hydrolysis are largely driven by the composition of the organics, which can be categorized into carbohydrates, lipids and proteins, along with the particulate matter content (Khanal, 2008). Consequently, hydrolysis is commonly regarded as the rate limiting stage for the AD of many feedstocks (Raposo et al., 2011). The longest hydrolysis time is associated with lipids, whilst the most rapid solubilisation involves the breakdown of carbohydrates (Esposito et al., 2012). As a result, substrates containing higher lipid concentrations restrict the overall degradation rate, due to the symbiotic dependency of the microbial community. The further determinant of hydrolytic kinetics; the particulate content, simply involves the limitation of active surface area exposed to the microorganisms. Higher particulate size and concentration inevitably restrict the solubilisation of organics in this manner, which can be a considerable determinant in the rate of hydrolysis (Ebenezer et al., 2015). The breakdown of the complex organics ultimately involves the transformation to simple soluble substances, such as amino acids, sugars, fatty acids and glycerine (Khanal, 2008), depending on the
composition of the feedstocks. These intermediate substances serve as the feed products for the secondary stage of biodegradation.

The simple soluble organics formed during the hydrolysis stage are transformed into volatile fatty acids (VFAs) by a secondary subset of microorganisms, in a process known as acidogenesis. These acids are characterized by the presence more than 2 carbon atoms in their molecular arrangement. The responsible microorganisms are characterized as acidogens, whose functioning has been shown to be sensitive to factors such as temperature, hydraulic retention time, feed ammonia concentration and pH (Yu and Fang, 2003, Demirel and Yenigun, 2004, Lu et al., 2008). The kinetics of the acidogenesis stage is typically dependent upon the hydrolysis stage. As acidogenesis is rarely the rate limiting stage of AD, optimization generally relates to the extent of this process rather than kinetics. In fact, an overly rapid acidogenesis phase (acidification) leads to the accumulation of VFAs, thus souring of the reactor.

The acetogenesis phase of AD is dependent upon the substrates produced from multiple degradation stages. The formation of acetic acid, hydrogen gas and carbon dioxide from the fermentation products of the acidogenesis phase are performed by an acetogen subspecies known as hydrogen-producing acetogenic bacteria (Khanal, 2008). An alternate acetogenic pathway involves the transformation of hydrogen gas and carbon dioxide, produced in both the acidogenic and acetogenic stages, into acetic acid. This transformation function is performed by a second acetogenic species, categorized as homoacetogens or CO₂-reducing acetogens (Ragsdale and Pierce, 2008). The final acetogenic pathway involves acetic acid formation via the transformation of certain simple soluble organics produced during the hydrolytic stage of degradation. The primary inhibition mechanism concerning acetogenesis acknowledged in prior studies relates to the impact of excessive hydrogen partial pressure. A pressure above $10^{-3}$ atm. was found to be inhibitory to acetic acid formation (Ahring and Westermann, 1988). Whilst the acetogenesis and acidogenesis stages rarely act as the rate limiting phases for AD, they are influential in the composition of the final gas, with particular reference to the formation of H₂S and other sulphurous compounds. Intermediate acid products from the acidogenesis stage are used not only by acetogens, but also sulphur reducing bacteria, which are involved in the formation of H₂S (Moon et al., 2015).

Methanogenesis involves the formation of methane via two primary pathways. The first biological mechanism concerns the transformation of hydrogen and carbon dioxide, known as hydrogenotrophic or carbon-dioxide-reducing methanogenesis. This pathway accounts for up
to 28% of methane formation in AD (Khanal, 2008). Alternatively acetotrophic methanogenesis converts acetate into methane, via the dominant methane formation pathway. Whilst secondary pathways exist with regards to methane formation, these are rarely effectual to overall production. Methanogens are regarded as particularly sensitive to several chemicals and conditions (Zhao et al., 2010). Inhibition of methanogens also occurs due to competition for organic nutrients with other species’ as well as through reactor conditions, in particular the pH level. Due to the acidification processes present in prior stages, the pH sensitivity furthers the need to balance the biological processes.

1.2.2. Secondary degradation mechanisms

Whilst the primary mechanisms are responsible for the majority of the degradation of organics; the diversity of microbes in AD leads to a greater range of processes. These secondary processes are rarely relevant in methane production rate computations. Instead the production of biogas contaminants or odorous pollutants is of particular concern. The most prevalent secondary process studied in literature concerns the transformation of sulphate into sulphide; which is performed by sulphur reducing bacteria (SRBs) (Chen et al., 2008). \( \text{H}_2\text{S} \) is biosynthesized during the acidogenesis, acetogenesis and methanogenesis stages (Lar and Xiujin, 2009, Moon et al., 2015). This process is largely responsible for the production of hydrogen sulphide gas; as dissolved sulphides can partition from reactor sludge in the form of \( \text{H}_2\text{S} \) (Dhar et al., 2011). SRBs compete with both methanogens and acetogens for intermediate products as carbon sources while reducing sulphate to sulphide. Further related pathways involving the formation of odorants, particularly VOSC’s have also been allotted considerable attention in prior studies. Methanethiol, along with \( \text{H}_2\text{S} \), are formed through the biodegradation of sulphur containing amino acids, whilst subsequent transformations see the conversion of these compounds into dimethyl sulphide and dimethyl disulphide (Higgins, 2003). Compounds like dimethyl sulphide and dimethyl disulphide have two of the lowest odour thresholds, at around 0.6-40 and 0.1-3.6 ppb respectively (Smet, 1998). The sulphur cycling involved in these pathways was found to be interrelated with the activity of methanogenic bacteria, as certain species were found to be capable of degradation of methanethiol, dimethyl sulphide and dimethyl disulphide, whilst methanogenic inhibition saw increased VOSC production (Higgins, 2003).
1.3. Co-digestion

Whilst AD can generally achieve effective solids reduction, the reuse of biogas for energy purposes has often been limited in the wastewater and municipal solid waste industries. In Australia, biogas is still a largely underutilised resource due to a range of unfavourable economic and policy factors (Edwards et al., 2015a). The maintenance cost of co-generators in Australia is largely invariable and quite high. Thus, small scale energy recovery systems tend to be economically infeasible. In addition, rebates for renewable energy production in Australia are only available to large scale producers (Edwards et al., 2015b). As a result, there is a critical scale of biogas production above which biogas utilisation can be economically feasible. This critical threshold can be overcome through the use of co-digestion of the sewage sludge with concentrated organic wastes (Silvestre et al., 2015, Fersi et al., 2014). Sewage sludge is ideal for use as the base substrate in co-digestion due to its low concentrations of inhibitors and high alkalinity (Mata-Alvarez et al., 2014).

The practice of co-digestion involves the amalgamation of multiple substrates in the formation of the feed solution for AD. The rationale driving its widespread utilization focuses on the optimization of the anaerobic environment with respect to the desired outcomes of the digestion process. This partly involves the targeted augmentation of the stoichiometry of the feed so as to maximize the resultant extent of degradation. Increasing the effectiveness of digestion with respect to volatile solid reduction and biogas production generally involves the prevention of conditions that are inhibitory to AD processes. Furthermore, increasing the organic loading enhances the potential biogas production. Domestically, reactors typically are fed at well below the organic loading capacity. Subsequently there is considerable potential to greatly increase the biogas production through the introduction of concentrated organic co-substrates. The benefits of co-digestion extend beyond biogas maximization, particularly with regards to environmental concerns. The practice of co-digestion allows for the utilisation of organic wastes that would otherwise be landfilled for their bio-methane potential. This extends AD benefits to a wider range of applications and facilitates a significant decrease in the total volume of solids entering landfills (Murto et al., 2004). The benefits can be further leveraged if the biosolids produced are utilized within the agricultural industry, thus enabling nutrient recovery along with divergence from landfill reliance.
1.4. Research objectives

The experimental research presented within this thesis represents the culmination of the joint efforts from the University of Wollongong, Sydney Water and funding allotted by the Australian Research Council (ARC). The findings of the studies have direct applications within the municipal wastewater treatment industry.

The overarching aim of this thesis is to evaluate the feasibility of adopting co-digestion in the municipal wastewater industry, with particular emphasis on the issues and challenges within an Australian context. Specific objectives of this thesis work are to:

1. Improve the existing array of potential co-substrates by demonstrating the stable co-digestion of sewage sludge with new potential organic wastes.
2. Determine the ideal mixing and loading rates and evaluate the potential for synergistic effects for the digestion of new and existing substrates with sewage sludge.
3. Demonstrate the long term stable digestion of sewage sludge with various organic wastes within a high volume, pilot scale study.
4. Evaluate the feasibility of emerging pre-treatment technology in conjunction with anaerobic co-digestion.

1.5. Thesis outline

This thesis is comprised of nine interrelated chapters, which can be categorised into two primary research components (Figure 1.3). Chapters 1 and 2 provide the context to this research by supplying background information on AD and critical review of the literature. The first component concerns the biomethane potential screening stage, providing preliminary testing and evaluation of methodology (Chapters 3 – 4). Biomethane potential evaluation offers an essential screening tool in anaerobic digestion, due to its lower resource requirement and subsequently its potential to assess a greater range of substrates compared laboratory or pilot scale experimentation. Chapter 3 lays the foundation for subsequent research by screening a range of new potential co-substrates for pairing with sewage sludge. Substrates that proved most promising within the biomethane potential screening were evaluated in subsequent chapters. In Chapter 4, comparative assessment was applied to the existing methods of synergistic effect evaluation. This strategy involved the application of linear modelling and COD balance techniques, along with the comparison of synergistic organic removal and methane yields.
The second core component of this is the laboratory and pilot-scale demonstration of co-digestion, providing detailed evaluation of substrates from prior chapters (Chapters 5 – 7). The increased scale of the experiments allows for co-digestion pairings to be studied in greater detail and facilitates the application of a greater range of analytical techniques. Evaluation of the dewaterability and residual odour of biosolids was only feasible during laboratory and pilot scale analysis. Chapters 5 and 6 concern the anaerobic co-digestion of beverage waste with sewage sludge. Chapter 5 concerns solely the digestion of soft drink waste at laboratory-scale, whilst Chapter 6 provides pilot scale evaluation of a range of beverage wastes including soft drink waste. Evaluation of the identical substrate in three different scales allows for the direct comparison of methodologies and provides data for the scalability of the experimental findings. Chapter 7 concerns the pilot scale demonstration of food waste and sewage sludge co-digestion and elucidating the impact of the fraction of avoidable/unavoidable food waste on the specific biomethane yield. An additional study, described in Chapter 8 was conducted into the application of free ammonia pre-treatment in conjunction with co-digestion. Conclusions and recommendations for further research are presented in Chapter 9.
Figure 1.3. Thesis outline.
CHAPTER 2: Literature review

A diverse range of literature exists pertaining to the technology of anaerobic digestion and associated techniques such as co-digestion. Whilst specific areas of the literature are mature, research gaps, such as the research objectives highlighted in Section 1.4, still persist. This chapter reviews the current state of the literature concerning the field of anaerobic digestion and more specifically co-digestion.

2.1. Current trends and issues in anaerobic digestion

Several trends in scientific publications have surfaced in recent years with regards to AD. Development and increased availability of analytical technology has seen a broader range of techniques applied in the field. For example the emergence of more diverse analytical technologies over the past few decades has seen more focus attributed to elucidating the complex biological conversions that drive the process (Xu et al., 2018). Furthermore technologies that exploit spectroscopic and electrochemical principles are increasingly employed in developing AD understanding (Madsen et al., 2011). Developments in modelling software in recent years have driven a surge of studies combining experimental and modelling components. Further trends involve the increased application of pre-treatment techniques both to typical AD substrates such as waste activated sludge, or to less degradable lignocellulos wastes.

The most prominent and enduring trend in the literature of the past decade has undoubtedly been the focus on the by-products generated. The formation of biogas, along with the stabilization of the residual sludge, presents operators with two potentially reusable products for the purposes of energy recovery or agricultural applications. Figure 2.1 describes the potential management options presently available to wastewater treatment operators. The most sustainable practices, with respect to environmental and economic factors, involve the utilisation of both biosolids and biogas resources for land applications and power generation.
Recent years have seen increasing interest in the potential reuse options for the digested solids produced from AD. The focus on the potential to utilize this substance as a resource, rather than a form of waste, derives largely from economic factors, whilst aligning practices with societal sustainability demands. Reuse of the digestate allows the elimination of disposal costs, whilst representing an increased efficiency of the utilization of existing resources. To date, the most common reuse option for the biosolids produced from AD relates to land application as soil conditioners (Wang et al., 2008). The high nutrient content of digestate makes it an ideal replacement for inorganic fertilizers (Tambone et al., 2010). The potential to offset demand for artificial fertilizer is gaining notable relevance in recent times, as the developing scarcity of phosphorous obtainable for mass agricultural utilization is being increasingly regarded with considerable concern (Cordell et al., 2009, Calvo et al., 2017). The suitability of this reuse option is furthered by the low pathogen survival rate in the digestate of AD (Weiland, 2010), justifying their use as fertilizers and soil conditioners.
Despite the considerable potential of land application options for AD biosolids, their application has often been restricted by several persistent issues, primarily including concern around the presence of odours, pathogens and harmful contaminants. National standards and agricultural perspectives vary largely across an international perspective; and subsequently so does the rate of reuse. In Australia, around two thirds of biosolids are reused in agricultural applications, whilst New Zealand, a country with a similarly prominent agricultural sector, only employs about 30% of its generated solids (Gapes and Andrews, 2015). Public concerns relate mostly to malodours emitted from biosolids. The most prominent odours can largely be attributed to the emission of volatile organic sulphur-containing compounds (VOSC’s) and inorganic sulphur compounds in biosolids (Gruchlik et al., 2013). VOSC’s commonly present in biosolids that are concerning with regards to odour include methanethiol and dimethyl sulphide, whilst the prominent inorganic sulphur compound is hydrogen sulphide (Higgins et al., 2008, Carrera-Chapela et al., 2014). Higher concentrations of VOSC’s in biosolids have been associated with increased residual biological activity, which is in turn reflective of the effectiveness of AD treatment in VS removal (Forbes et al., 2007). Exploitation of this phenomenon, along with other techniques toward the optimization of biosolids quality is required to overcome the issue of biosolids odour.

2.1.2. **Biogas utilisation**

There has been an increasing focus on the generation of biogas in recent years. Biogas generation presents an enticing secondary benefit to the treatment of organic wastes as a renewable energy resource (Esposito et al., 2012). The potential for energy recovery presented by anaerobic biogas production has been widely acknowledged throughout the literature (Holm-Nielsen et al., 2009). However, the current rate of biogas utilisation marks the resource as a largely under-utilized energy product. In Australia, over 20% of domestic biogas-generating wastewater treatment plants do not recover energy from biogas and do not have a biogas utilisation strategy (Edwards et al., 2015b). Generally, biogas recovered that is not utilized is flared off. Biogas flaring is necessary to reduce the impact on the earth’s climate, following that methane has a global warming potential 23 times that of carbon dioxide (Myhre et al., 2013). Whilst biogas flaring is effective in impact reduction, much greater benefits are can be obtained through energy recovery.

The primary onsite options involve the recovery of energy in the form of heat, electricity, or a combination of both. Cogeneration involves the use of combined heat and power systems for energy recovery from wastewater biogas (Appels et al., 2008b). Such systems are
considered the most appropriate due to their capacity to generate electricity along with offsetting waste water treatment plant (WWTP) thermal energy requirements. However, Fersi et al. (2014) demonstrated through a case study that AD of sewage sludge for the sole purpose of energy generation is not economically viable. But this discounts the value of treatment involved. In Silvestre et al. (2015) it was found in a study of 5 different WWTP’s in Catalonia, depending on the plant configuration and infrastructure quality, between 39 and 76% of their total energy requirements could be offset through the cogeneration of AD biogas. Nowak et al. (2015) described two Austrian WWTPs that achieved a positive net energy balance between 6-10% through biogas utilization using combined heat and power systems.

The benefits of biogas utilization can be related to both environmental and economic factors. The capacity to generate reliable energy onsite from existing resources represents a clear economic benefit in itself. Nevertheless, the considerable drive towards biogas utilization has related largely to mitigation of climate change impacts. The co-digestion of different materials has the potential to reduce dependency on other energy sources, whilst reducing the impact of emissions from other sectors (Holm-Nielsen et al., 2009). This is particularly the case for the livestock sector, as methane generated from the natural decomposition of manure can be captured and utilized, rather than simply emitted (Clemens et al., 2006).

Despite the fervent optimism surrounding the reuse of biogas, considerable issues still restrict the global realization of its potential. The limitation of biogas reuse derives largely from the economics involved in installation and maintenance of the systems. The restriction is most prevalent for the recovery of electricity, due to the higher maintenance requirements compared to that of heat recovery systems. Particularly within the context of Australian wastewater AD, energy recovery is limited due to the presence of a high fixed labour maintenance cost, which is considerable irrespective of the size of the system. In order for an energy recovery system to become economically viable, it must provide economic benefits offsetting these costs along with other monetary requirements from installation costs and component replacement. This situation leads to a conceptual break-even threshold in terms of the minimum required biogas production, as the economic benefits of reuse increase somewhat proportionately with biogas volume. Thus methods are required to increase biogas production from the AD of sewage sludge in order to overcome this threshold.
A ubiquitous and significant obstacle to biogas utilisation relates to concentrations of hydrogen sulphide (H$_2$S). Depending on the feed composition, H$_2$S concentrations can be between 10 – 2000 ppmv (Cosoli et al., 2008). Avoidance of H$_2$S generation would already be ideal due to the odour and adverse health impacts attributed to the gas. This issue also enters considerations of the potential land use applications for biosolids, which are plagued by concerns surrounding odorous emissions. Furthermore, H$_2$S has the potential to cause considerable damage to equipment employed for the utilization of biogas; causing the deterioration of lubrication oil and the corrosion of combustion engine components and piping (Weiland, 2010). In conjunction with the issue of maintenance costs, H$_2$S contamination has seen to considerably hamper the drive for biogas reuse.

Another immediately observable hindrance to wider spread utilization of biogas relates to the low content of methane in biogas. Observations of methane purity have ranged significantly in previous AD studies, generally between 48% - 65%, but even as high as 70% in some cases (Rasi et al., 2007, Nasir et al., 2012, Amon et al., 2007). However, the figure of around 60% methane has long since been widely adopted as a representative average concentration (Chynoweth et al., 2001). The majority of the remaining 40% is made up of carbon dioxide, along with traces of other compounds. The low methane percentage in biogas is a major issue for its utilisation, particularly concerning offsite systems. In order to qualify as a fuel-grade bio-methane resource, methane concentration must exceed 98%; meanwhile removal options are typically expensive (Grande and Rodrigues, 2007). Whilst biogas composition issues can be addressed in post-digestion treatment, researchers are still pressing for more appropriate solutions with respect to effectiveness and efficiency.

One technique that has been increasingly employed towards the improvement of biomethane production is known as anaerobic co-digestion.

### 2.2. Anaerobic co-digestion

Towards the optimisation of biogas production and biosolids removal, the practice of co-digestion has been widely studied in the literature, making up half of AD publications from 2011 – 2013 (Mata-Alvarez et al., 2014). Co-digestion involves the amalgamation of multiple substrates in the formation of the feed solution for AD. The rationale driving its widespread utilization focuses on the optimization of the anaerobic environment. This partly involves the targeted augmentation of the stoichiometry of the feed so as to maximize the resultant extent of degradation. Increasing the effectiveness of digestion with respect to volatile solid
reduction and biogas production generally involves the prevention of conditions that are inhibitory to AD processes. The benefits of these practices extend beyond biogas maximization, particularly with regards to environmental concerns. Co-digestion expands the scope of substrates that are appropriate for AD treatment, through the likely prevention of inhibition. This allows the extension of AD benefits to a wider range of applications and helps divert organic wastes away from landfilling (Murto et al., 2004). The benefits can be further enhanced by utilising produced biosolids within the agricultural industry, which allows for nutrient recovery.

Anaerobic co-digestion research throughout the past decade has centred on the maximization of biogas production. This is essential in order to overcome the biogas production threshold for cogeneration’s economic viability, where energy benefits must outweigh the costs of maintaining the system. In the aforementioned process of cogeneration, the economic viability of the process hinges largely upon the maximization of energy recovery (Silvestre et al., 2015, Fersi et al., 2014). The increased potential biogas generation offered through co-digestion derives from two primary mechanisms. The first means of biogas optimization relates to the potential to manipulate the nature and concentration of organics present within the feed. The increasing of the concentration of organics, typically represented through the VS content, logically imbues the feed with a higher digestible potential, which clearly correlates with the achievable biogas production. Existing literature is rich in studies targeted at the manipulation of the organic content of AD feed solutions (Li et al., 2015, Fernandez et al., 2005, Gomez et al., 2006). The common theme that resonates throughout this segment of the literature describes the increased biogas production potential at higher organic loading rates. However, as is the case with the digestion of single substrate feeds, inhibition has been observed at higher concentrations; thus an optimal organic content is presented by any proposed feed blend.

Optimization of the organic content of the AD feeds can extend beyond maximization of VS concentrations. Differing substrates have been seen to possess contrasting ratios of methane potential to total VS concentrations. Luostarinen et al. (2009) observed that the difference in methane production potential with respect to VS concentrations between sewage sludge and grease trap waste was considerable, recording levels of 263 and 918 L CH₄/kg VS added respectively. The differences in methane potentials of volatile solids complicate the process of AD biogas production optimization and places significant emphasis on the selection of the differing substrates in co-digestion. Additionally factors concerning the
kinetics of the biological processes are seen to vary considerably based on the organic composition of feed stocks.

The secondary mechanism by which co-digestion practices facilitate higher biogas production relates to the aforementioned potential abatement of inhibitory conditions. Efficiency reduction in the functioning of the microbial community of AD logically impedes the degradation of organics, and thus the formation of biogas. Therefore the prevention of inhibitory conditions is a necessity in biogas-targeted process optimization. The prevention through the adoption of co-digestion involves either the dilution of the inhibitors of one of the substrates, or the exploitation of synergisms between different substances that result in mutual reduction in compound/ion toxicity (Chen et al., 2008). The higher concentrated substrates investigated in literature generally have a greater tendency to promote inhibitory conditions. Thus, the co-digestion of substrates of highly differing compositions, particularly in terms of organic content, can often facilitate improved microbial activity in AD.

2.2.1. **Substrates for anaerobic co-digestion**

The most detailed segment of existing literature is probably related to the evaluation of potential substrates. Due to the aforementioned applicability of AD, an array of singular substrates and organic composites has been evaluated. Screening of new potential substrates remains vital to the uptake and expansion of full scale co-digestion. The focus of substrate screening concerns the effect on the degradation process, with respect to factors such as total biogas production and solids reduction. Beyond directly identifying useful substrates the collective aspiration of researchers is for these studies to identify the properties of an ideal substrate solution, thus producing a selection criterion for initial evaluation of feed solutions. However the diversity of variables, inconsistency of methodologies along with the variability of substrate composition impedes the final realisation of this aspiration (Holliger et al., 2016). Certain emergent rules have surfaced describing requirements for AD. The most prevalent feed stock requirement concerns the carbon-nitrogen ratio, with additional focus on the relative concentrations of phosphorus and sulphur (Weiland, 2010). Beyond these fundamental stoichiometric ratios, there is little consensus for any exacting concentrations, as the complexity of the biological processes, exacerbated by the synergistic interrelation of substances, prevent the isolation of ideal concentrations. Therefore, further optimization of feed stocks becomes particularly situational, based on the composition of the base substrate targeted.
Whilst there is inevitably an amount of variation in substrate composition from differing sources and areas, certain traits can be expected based on the nature of the organic feedstocks. When considering specific substrates, the materials are assessed for a diverse range of factors such as sulphur concentration, organic type, lignin content or the presence of toxic compounds. Based on this characterisation of substrates, they can be further categorised based on their potential function during co-digestion and their suitable pairings. Co-digestion substrates can generally be separated into two categories based on their intended function; either for the boosting of biogas production or the stabilization of the total feed blend. Substrates rich in degradable organic matter generally possess characteristics that are ill-suited to mono-digestion (Mata-Alvarez et al., 2014). Thus, the balancing of these inhibitory qualities is required, either through contaminant dilution, stoichiometric manipulation or the optimization of factors such as alkalinity. This requirement regularly sees the pairing of high methane producing substrates with robust stabilisers (Figure 2.2).

![Figure 2.2. Optimisation of co-digestion through the selection of complimentary substrates.](image)

One of the most common substrates for AD is sewage sludge, representing one of the most common substrate studied in existing co-digestion papers (Mata-Alvarez et al., 2011). The popularity of the substrate owes itself to the availability and associated solid reduction requirements. However it possesses qualities that make it an ideal substrate for use in co-digestion. The stability of the substrate allows its use in dilution of inhibitory substances, whilst a high alkalinity concentration makes sewage sludge useful in conjunction with substrates highly concentrated with rapidly degradable organics (Mata-Alvarez et al., 2014). Table 2.1 shows several of the co-substrates that have been paired with sewage sludge. Several other substrates possess similar stabilising traits and therefore are quite similar in their applications. Substrates such as animal manure, waste activated sludge and algal sludge
all possess low organics and high buffering capacities due to ammonia concentrations and subsequently are suited to similar co-substrate pairings (Wang, 2012, Yen and Brune, 2007). The high ammonia nitrogen in these substrates can be attributed to the high protein concentrations present in each (Esposito et al., 2012). Waste activated sludge divergence from sewage sludge characteristics largely relates its requirements for long hydraulic retention times for AD (Wang et al., 2014). This owes itself to the processes carried out within the aerobic digestion stage, causing the limitation of the hydrolytic rate of waste activated sludge. Significant microbial immobilization is required in order to generate usable organic matter for digestion. Alternatively, animal waste is typically plagued with excessively high ammonia concentrations, which becomes inhibitory to AD processes without dilution (Esposito et al., 2012).

To boost biogas production in stabilising substrates, carbon rich substrate pairings are required. Common carbon sources for AD can include cellulosic wastes such as from the paper or textile industries (Esposito et al., 2012). Example studies pairing cellulose substrates with organic deficient substrates are common in the literature. It was found in Yen and Brune (2007) that an optimum biogas yield for a combination of algal sludge and paper waste occurred where 60% of VS is from the paper substrate; where a 180% increase in methane production occurred from the sole digestion of algae. Mshandete et al. (2004) demonstrated methane yield increase by 59-94% using a combination of 67% sisal fibre pulp with 33% fish waste, above that of yields from digestion of the singular substrates. Low toxicity in these wastes simplifies inhibition consideration in their application, thus organic stoichiometry optimization coupled with logistical and biosolids volume concerns govern their application. It is noted that paper wastes have been observed having higher concentrations of sulphate along with the characteristic high COD levels (Wei et al., 2007). These wastes represent a largely unseparated potion of municipal solid wastes internationally, currently being directed to landfill (Esposito et al., 2012). Consequently, there is considerable potential for an increased involvement in co-digestive practices.

A notable carbon-rich substrate used in co-digestion is the organic fraction of municipal solid waste. The considerable availability and logistical favourability of the organic fraction of municipal wastes has attracted significant attention to the use of substrate for C/N balancing in co-digestion. The effectiveness of the substrate’s use with nitrogen rich substrates has been demonstrated in numerous studies, particularly with sewage sludge; which represents the most common co-digestive blend in literature (Mata-Alvarez et al.,
In Sosnowski et al. (2008) it was shown that the stronger kinetics of methane production for the organic fraction of municipal solids could be maintained through co-digestion with DS. Applicability of the organic fraction of municipal waste in co-digestion is furthered by its capacity to prevent acid accumulation when digesting lipid rich wastes (Fernandez et al., 2005).

In some instances, the separation of the fruit and vegetable fraction of solid municipal waste has been conducted for use as a co-substrate. Fruit and vegetable wastes are characterized by a high concentration of easily degradable VS, which invariably leads to rapid hydrolysis and the accumulation of VFA’s at higher organic loading rates (Wang et al., 2014). Expectantly, the fruit and vegetable fraction has been observed as a successful co-substrate for high buffering base substrates such as sewage sludge or animal manure (Gomez et al., 2006, Zhang et al., 2013). However, a common concern surrounding the utilization of fruit and vegetable wastes in AD relates to the potential generation of H₂S. Feed stocks containing food waste have been observed to have higher concentrations of sulphate, which ultimately leads to higher H₂S concentrations in the produced biogas (Moestedt et al., 2013). Food wastes also lead to higher phosphorous concentrations, which concentrate in the centrate. When this occurs in WWTP’s struvite precipitation can often be observed at disadvantageous locations in the treatment cycle.

Energy crops are another common substrate for co-digestion, one that has been conferred with perhaps the most fervent optimism. Such substrates that are commonly considered can include maize, sunflower and rapeseed residues (Cuetos et al., 2011). The defining parameter for the crops is their exceptional net energy yield per hectare, which reflects positively on their biogas recovery prospects (Weiland, 2010). Energy crops also possess the high C/N ratio required for co-digestion with protein rich substrates. However, a considerable obstacle to their use in AD is the concentrations of lignin found in energy crops (Cuetos et al., 2011). In nature, the breakdown of lignin is achieved through enzymes or microbial based chemical reactions which require aerobic conditions (Ahring et al., 2015). Thus considerable pre-treatment practices are required to increase the bioavailability of organic material to achieve optimal AD outcomes. Despite this, the promotion of this substrate persists in co-digestion studies, due to the favourable availability and logistics, along with synergistic factors with respect to biosolids. Wang et al. (2008) suggested that an ideal reuse option for AD biosolids was for cultivating energy crops to circumvent legislative restrictions on land applications in
certain countries. This potential for nutrient cycling further promotes the involvement of energy crops in co-digestive practices.

With regards to maximum theoretical gas yield, lipid rich substrates exceed those containing primarily other organic types (Esposito et al., 2012). These substrates are categorized as fat, oils and grease wastes, and can be sourced from slaughterhouses, food processing and municipal solid waste (Rasit et al., 2015). But despite their high methane potential, digestion of fats oils and greases suffer from slow production rates as well as lipid based inhibition. Sun et al. (2014) found that inhibition occurred beyond a VS concentration of 65% lipids.

Table 2.1. Summary of biomethane potential reported during co-digestion of sewage sludge with organic wastes

<table>
<thead>
<tr>
<th>Co-substrate</th>
<th>Biogas yield (m³/kg VS)</th>
<th>Observed Implications of Co-substrate</th>
<th>Substrate Availability</th>
<th>Scale</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Poultry Industrial Waste</td>
<td>0.81</td>
<td>No significant impacts observed</td>
<td>High</td>
<td>Full Scale</td>
<td>(Budych-Gorzna et al., 2016)</td>
</tr>
<tr>
<td>Used Oil</td>
<td>~1.2*</td>
<td>No significant impacts observed</td>
<td>High</td>
<td>Pilot Plant</td>
<td>(Pastor et al., 2013)</td>
</tr>
<tr>
<td>Microalgae</td>
<td>0.2 – 0.38</td>
<td>No tipping fees, substrate production cost</td>
<td>Not a waste product</td>
<td>Lab Scale</td>
<td>(Caporgno et al., 2015)</td>
</tr>
<tr>
<td>Fruit and Vegetable Waste</td>
<td>0.4 – 0.61</td>
<td>No significant impacts observed</td>
<td>Very High</td>
<td>Lab Scale</td>
<td>(Gomez et al., 2006)</td>
</tr>
<tr>
<td>Meat Processing Waste</td>
<td>0.918</td>
<td>No significant impacts observed</td>
<td>Moderate</td>
<td>Lab Scale</td>
<td>(Luostarinen et al., 2009)</td>
</tr>
<tr>
<td>Fat Oil and Grease</td>
<td>0.598</td>
<td>Other results suggest higher biogas potential and synergetic digestion are possible</td>
<td>High</td>
<td>Lab Scale</td>
<td>(Wan et al., 2011)</td>
</tr>
<tr>
<td>Domestic kitchen waste</td>
<td>~0.36*</td>
<td>No significant impacts observed</td>
<td>Very High</td>
<td>Full Scale</td>
<td>(Zupancic et al., 2008)</td>
</tr>
</tbody>
</table>

* Assuming 60% methane concentration

2.3. Implications of substrate characteristics on reactor performance

Appropriate co-substrate selection is the most effective and cost efficient means of managing downstream conditions. Reactor management and post treatment processes are typically less effective or significantly more expensive. Concentrations of inhibitory
compounds, stoichiometry and organic contents are all factors with considerable influence on the final outcomes of the digestion process. Following the selection of substrates, decisions regarding the reactor conditions and post treatment requirements are also necessary in order to negate the negative implications of differing substrate compositions.

2.3.1. The role of carbon/nitrogen stoichiometry in co-digestion

The ratio between carbon and nitrogen in the reactor feed can be considered the primary factor in co-substrate selection. The prevalence of C/N stoichiometry in AD considerations is reflective of the influence of ammonia concentrations in the processes. High nitrogen contents, when associated with low relative carbon concentrations lead to high levels of ammonia formation within the reactor. Ammonia is required as a nutrient for methanogen activity, whilst being the by-product of protein and amino acid degradation in earlier stages (Eldem et al., 2004b). Additionally, ammonia provides buffering capacity required to reduce the impact on pH from VFA accumulation (Prochazka et al., 2012). Ammonia (NH$_3$) is produced through degradation of nitrogen rich proteins and increases bicarbonate by forming an ammonium salt with bicarbonate (Georgacakis et al., 1982). The formation of Ammonium Carbonate is summarised by the following equation:

\[
\text{NH}_3 + H_2O \leftrightarrow OH^- + \text{NH}_4^+ \\
\text{NH}_4^+ + OH^- \leftrightarrow \text{NH}_4\text{HCO}_3
\]

It is noted that nitrogen is an important nutrient utilized by microorganisms responsible for digestion. Differing optimal values have been identified throughout the numerous studies on the topic; however they generally fall within a fairly consistent range. For a blend of dairy manure, chicken manure and wheat straw; a stable C/N range of 25:1 to 30:1 has been observed, with an optimal blend occurring at 27.2:1 (Wang, 2012). For a blend of algal sludge and paper mill waste; one study found the ideal range also occurred within this range of 25:1 to 30:1 C/N ratios (Yen and Brune, 2007). It is generally acknowledged that a C/N ratio should fall somewhere between 15:1 and 30:1, whilst a carbon-nitrogen-phosphorus-sulphur mix of 600:15:5:1 is considered sufficient (Weiland, 2010).

Insufficient or excessive ammonia concentrations can lead to pH levels which become inhibitory to the biological processes of AD. Inhibition based on pH is especially complex
due to the interdependency of differing pH-dependent toxicity of substances. The extent of inhibition can range from a declining methane production to a total cessation of methanogenic activity, depending on ammonia concentrations and the inhibition pathways that are relevant. Eldem et al. (2004b) observed that non-ammonia-based pH inhibition was categorized as “direct” pH inhibition; being associated with interactions of substances such as ionized and unionized VFA’s, along with hydroxide or hydrogen ions. Under the conditions associated with insufficient ammonia concentrations, the expected pH decline leads to a shift in the composition of total ammonia nitrogen towards a decreased free ammonia concentration (Chen et al., 2008), along with inhibition based on the non-ammonia pathways (Figure 2.3).

Figure 2.3. Inhibition due to low ammonia concentrations.

Ammonia nitrogen exists within AD reactors in two basic forms; the unionized free ammonia, or ammonium ($\text{NH}_4^+$) (Prochazka et al., 2012). Free ammonia is universally acknowledged as the more toxic of the two compounds (Ho and Ho, 2012). However the mechanism by which ammonia inhibition occurs is still not fully understood, with proposed theories covering intracellular pH changes, specific enzyme inhibition, proton imbalances and other possibilities (Chen et al., 2008). Muller et al. (2006) noted that ammonia inhibition of bacteria requires its presence within the cell, following on from observations that inhibition affected bacteria with less permeable cell wall structures. Subsequently it has been asserted that the increased permeability of free ammonia, relative to ionized ammonium, is responsible for the compound’s higher toxicity. Maintaining a low pH condition within an AD reactor can reduce free ammonia concentration.

Inhibition based on ammonia levels involves the interaction between the concentrations of not only ammonia, but also that of the VFAs and subsequently the pH. Thus the critical total
ammonia nitrogen concentration is largely situational. Ammonia inhibition has been observed above concentrations of about 1400-1700 mg/L of ammonia nitrogen in certain substrates, with sharp declines in methanogenic activity at around 2000 mg/L N for a pH range of 7 - 7.5 (Liu et al., 2012). However, considerable variation in the onset of ammonia inhibition has been observed in other papers. Eldem et al. (2004b) observed inhibition at around 1500 - 2000 mg/L for a pH of 7.8, whilst in a different substrate with a pH of 8.4 inhibition occurred at a concentration above 1000 mg/L. This study aligns with the scientific consensus stating that ammonia sensitivity increases with pH (Eldem et al., 2004a). This phenomenon derives from the aforementioned influence of pH on the total ammonia nitrogen composition. Higher pH levels lead to increased proportionality of free ammonia relative to ammonium. Subsequently, this instigates the exceedance of critical free ammonia levels in feedstocks with high total ammonia nitrogen contents (Figure 2.4). These instances of excessive or insufficient pH levels can be initiated by disruption to the balance between the methanogenic and acidogenic biochemical processes (Rajagopal et al., 2013). It must also be noted that ammonia toxicity is not solely dependent upon pH and free ammonia concentrations. The presence of other ions can potentially produce a synergistic scenario whereby toxicity is reduced. This is the case for concentrations of Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ (Chen et al., 2008).

Figure 2.4. Inhibition due to high ammonia concentrations

The prevention of Ammonia and pH inhibition can occur in several different ways. As previously established, considerable focus is allotted to the generation of C/N balanced feeds for co-digestion. A sufficient C/N balance is usually a reasonable indicator for the prevention of pH and ammonia inhibition. Beyond this the artificial maintenance of pH levels to prevent
inhibitory levels and free ammonia accumulation can be applied in AD. Kabdash et al. (2008) evaluated several combinations of treatment options for their potential to reduce ammonia concentrations, including acidic air stripping, struvite precipitation, dilution and activated sludge processes. Each option displayed a potential for application in pre-treatment of feed stocks, depending on the situational requirements.

2.3.2. **Impacts of readily degradable organic concentrations in co-digestion**

High concentration of organic carbon within AD feedstocks is expected to increases methane yield. However, excessive concentration of easily degradable content can lead to inhibition of biological processes through the rapid hydrolysis of these organics. This inhibitory mechanism is invariably linked to the aforementioned C/N ratios, whereby the rapid degradation of such organics can lead to the accumulation of VFA’s. This occurs when the formation rate of the VFA’s exceeds their rate of consumption. As previously described, when coupled with a high C/N ratio, the associated pH drop can cause inhibition of the microorganisms, particularly the methanogens. The nitrogen concentration requirements are dependent upon not only the organic loading rate, but also the composition of the organics (Prochazka et al., 2012). This demonstrates the potential shortcomings of the reliance on C/N figures for feed composition management, as the rate of hydrolysis and subsequently the formation of VFAs are the determinant factor for buffering requirements, not simply the carbon concentrations.

2.3.3. **Implications of sulphur content in co-digestion**

The presence of sulphur within the feedstock to AD has several important implications both on reactor processes and downstream product quality. The in-reactor issues relate to the inhibition of methanogens indirectly caused by the presence of sulphur containing compounds. The most simplistic form of inhibition relates to the added competition methanogens face for certain nutrients. Under certain environmental conditions, SRB can outcompete methanogens for acetate and hydrogen (Lovley and Klug, 1983). Additionally, SRB acting as hydrogen consumers in the syntrophic oxidation of fatty acids cause considerable limitation on total methane production (Mizuno et al., 1998). The eventuation of substantial methanogen inhibition requires a sufficient sulphate concentration as well as a relative acetate/hydrogen scarcity within the reactor. Increasing sulphur concentrations in reactor feeds logically lead to higher competitive inhibition at low levels; however, higher
concentrations of sulphides as well as SRB by-products have been demonstrated to be inhibitory to SRB. It was found in (McCartney and Oleszkiewicz, 1991) that SRB inhibition was more extensive than that of methanogens with respect to increases in total sulphide and unionized sulphide/gaseous H$_2$S concentrations.

The alternative means of sulphur-based inhibition relates to the formation of H$_2$S within the reactor (Figure 2.5). As mentioned earlier, the reduction of sulphate to sulphide by SRBs allows the partitioning of sulphide into the headspace gas region of the reactor in the form of H$_2$S. Hydrogen sulphide gas is considered the primary sulphur-attributed cause of inhibition (Mizuno et al., 1998), whilst methanogens were found to be the most sensitive microorganism towards sulphide toxicity (O'Flaherty et al., 1999). In the current literature, a complete understanding of the mechanism driving sulphide based inhibition remains elusive; however a measure of a consistency exists in theories relating to the permeability of cell membranes to H$_2$S gas, and the subsequent interference in enzymatic processes (Chen et al., 2008). The toxicity of sulphide towards methanogens or other microbial groups has proven inconsistent, thus impairing the validity of any theoretical toxic concentration. The source of this inconsistency can partly be attributed to an interrelation with pH levels, whereby toxicity is increased at higher pH levels (O'Flaherty et al., 1999). An additional influential parameter is the COD/sulphate ratio, which can influence the proportion of sulphate reduction by SRB’s (Wei et al., 2007). The ultimate implication of inhibition via sulphide toxicity or SRB competition is a reduction to the extent and kinetics of biodegradation.
Ultimately the avoidance of excessive sulphur concentrations is ideal through the selection of alternative substrates. Whilst mechanisms exist to restrict the impact of SRB in the reactor, the implications of sulphurous feeds on downstream processes are unavoidable.

2.4. Synergistic effect

In co-digestion the term “synergistic effect” describes any improvements in the reactor performance due to the complementary characteristics of the substrates. Synergisms between substrates have been reported between several substrate pairings and may relate to the dilution of inhibitors, improvement of stoichiometry or the beneficial evolution of the biocommunity (Labatut et al., 2011, Ebner et al., 2016, Aichinger et al., 2015). Macias-Corrал et al. (2008) attributed an increased methane yield and biosolids reduction during the co-digestion of cow manure and the organic fraction of municipal waste to the balancing of reactor stoichiometry. Vivekanand et al. (2018) explained an improved degradation of manure during fish ensilage addition through the priming of the microbial community. This priming phenomenon has been observed when digesting poorly degradable wastes with labile substrates (Insam and Markt, 2016).
Several methods have been proposed in the literature to detect and quantify synergistic effects. Labatut et al. (2011) evaluated the synergistic effect by comparing the weighted specific methane yields of the substrates during biomethane potential evaluation. Aichinger et al. (2015) employed a COD balance approach using the theoretical COD of methane to directly compare input COD with changes in specific methane yields during batch co-digestion. It must be noted that these techniques on their own only consider increases in biodegradability and don't account for improvements in process kinetics.

To account for kinetics, a range of mathematical models have been adopted during co-digestion (Xie et al., 2016). One of the most commonly reported approach involves modifications upon the anaerobic digestion model 1 (ADM1). Simple models such as the first order, dual pool and Gompertz model have also been used extensively in the literature to estimate the hydrolysis rate constant (Dennehy et al., 2016). Ebner et al. (2016) considered the effect of synergisms on process kinetics by comparing the weighted hydrolysis constant of the individual substrates with that observed during co-digestion.

Despite the volume of papers focusing on the synergistic effect of co-digestion, uncertainty persists as to whether the synergistic effect increases solely the process kinetics or also the biodegradability. During biomethane potential analysis of mixtures of both pure and waste sourced carbohydrates, protein and lipids, Astals et al. (2014) observed that the synergistic effects were primarily related to process kinetics, however the pairing of paunch and lipids was seen to improve the overall degradability of the lipids. Such findings suggest the mechanisms behind synergistic increases in process kinetics differ from those driving improvements in overall biodegradability. Koch et al. (2016) further suggested that biomethane potential examination is unsuitable for evaluating the synergistic effect due to the brevity of the experiments and continuous laboratory or pilot scale testing would be required.

2.5. Implications on downstream processes

2.5.1. Nutrient accumulation

In a typical WWTP configuration, the centrate from AD is returned to the plant influent. The centrate is rich in nutrients such as nitrogen and phosphorus and can subsequently increase the loading on biological systems and can adversely impact final effluent quality (Holloway et al., 2007). Following AD, 15 – 20% of the digester’s nitrogen loading is retained in the centrate (Fux et al., 2002). Wild et al. (1997) reported that phosphorous retention in the centrate is largely influenced by operating conditions and feed characteristics.
like hardness, total phosphorous and polyphosphate concentrations. However expected levels generally fall below 10%.

Co-digestion introduces increased nutrient loads, thus influencing accumulation in a full scale plant. As noted earlier, ideal co-digestion of sewage sludge involves the pairing with high a C/N co-substrate. Thus the introduction of co-digestion is not likely to be the largest contributor to nitrogen centrate concentrations. Concentrations of phosphorous on the other hand can be greatly influenced by co-digestion, depending on the selection of co-substrate. Notably, food waste possesses considerable phosphor levels (Wickham et al., 2016). Excessive concentrations of nitrogen and phosphorous collectively can lead to struvite precipitation, damaging plant infrastructure (Sabbag et al., 2015). Consequently, management strategies are required to cope with the increased nutrient accumulation that co-digestion can cause.

A range of nutrient reduction strategies have been proposed, often focusing on nutrient recovery for fertilizing purposes as well as centrate treatment. Nutrient separation in phosphorous rich waters typically relies on precipitation in the form of struvite. Fattah et al. (2008) investigated the feasibility of a fluidized reactor to crystalize and recover struvite from a nutrient rich wastewater, achieving removal of over 90% phosphorous yet only 4% of nitrogen. Whilst the practice has not become widespread, phosphorous separation and recovery from a fluidized reactor has been demonstrated at full scale (Ueno and Fujii, 2001). Forward osmosis-based centrate treatment has also proved capable of high nutrient separation of both phosphorous and nitrogen at a water recovery of 70% (Holloway et al., 2007).

Nitrogen rich centrate streams have a range of physiochemical treatment options, including air stripping, ammonia recovery and breakpoint chlorination, along with biological treatments (Constantine, 2006). Conventional management in full scale plants involves nitrification/denitrification, which requires high carbon input and energy costs due to aeration requirements (Fux et al., 2002). Differing treatments seek to reduce the high requirements whilst retaining high removal efficiencies.

2.5.2. **Biogas quality and upgrading requirements**

Biogas quality parameters generally refer to the concentrations of H₂S and CO₂. Co-digestion allows for little optimisation of CO₂ concentrations in biogas. However, the H₂S levels in biogas can be significantly impacted through the introduction of certain substrates.
The aforementioned formation of H$_2$S and subsequent contamination of AD-produced biogas is especially inhibitory to its utilization as a renewable energy source. During biogas utilization, H$_2$S can be transformed into sulphuric acid and sulphur dioxide (Abatzoglou and Boivin, 2009) The associated corrosiveness imparts significant damage to piping and motors, and thus the formation of the gas is highly disadvantageous (Ryckebosch et al., 2011). H$_2$S itself is highly reactive to metals, exacerbated by certain parameters, including temperature, pressure and the presence of water (Nghiem et al., 2014a). Considering the high capital costs associated with efficient systems for biogas energy conversion, the presence of high concentrations of H$_2$S make the process of cogeneration completely infeasible economically.

Subsequent to the adverse implications of H$_2$S contamination within biogas, several techniques both during and after the digestion process have been employed to reduce or remove the concentrations. Techniques have also been explored to alter AD conditions in order to limit H$_2$S production in the reactor. The prominent methods investigated in the removal of H$_2$S are compared in Table 2.2.
Table 2.2. Summary of performance of desulphurization techniques

<table>
<thead>
<tr>
<th>H₂S Removal/Reduced Production Method</th>
<th>H₂S in biogas (ppmv)</th>
<th>Additional implications of removal technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-Oxygen Injection</td>
<td>20 – 100</td>
<td>Potential over-oxygenation, biosolids odour may be impacted.</td>
<td>(Schomaker et al., 2000)</td>
</tr>
<tr>
<td>Activated Carbon</td>
<td>≤ 1</td>
<td>Regeneration requirements, H₂SO₄ formation.</td>
<td>(Lau et al., 2015)</td>
</tr>
<tr>
<td>FeCl₃ Dosing</td>
<td>≥ 100</td>
<td>Potential FeS accumulation.</td>
<td>(Lar and Xiujin, 2009)</td>
</tr>
<tr>
<td>Thermophilic AD</td>
<td>~ 0</td>
<td>Increased energy requirements, reduced reactor stability.</td>
<td>(Ahammad et al., 2008)</td>
</tr>
<tr>
<td>Biotrickling filter</td>
<td>&gt; 0</td>
<td>Removal efficiency can be easily disrupted.</td>
<td>(Singh and Mandal, 2012, Montebello et al., 2013)</td>
</tr>
<tr>
<td>Iron Sponge (Iron Oxide/Hydroxide)</td>
<td>≥ 65</td>
<td>Higher operating costs and excessive heat generation.</td>
<td>(Cherosky and Li, 2013)</td>
</tr>
<tr>
<td>Chemical Absorption</td>
<td>~ 0</td>
<td>Higher chemical costs</td>
<td>(Tippayawong and Thanompongchart, 2010)</td>
</tr>
</tbody>
</table>

Reactor conditions can be amended to alter the microbial competition in order to suppress the activities of SRB. Methanogens have been observed to be capable of outcompeting SRB’s in thermophilic conditions, however the reasoning is thus far undetermined (Chen et al., 2008). Ahammad et al. (2008) found that the operation of a reactor at 55 °C could prevent the formation of H₂S completely during the treatment of a mixture of cow rumen fluid and cow dung. The shortcomings of thermophilic digestion relate to the increased energy costs along with the decreased system stability.

Another operation-based H₂S abatement method involves the chemical precipitation of sulphur. Lar and Xiujin (2009) evaluated a ferric chloride precipitator for the digestion of a mixture of dairy manure and seeding sludge from a wastewater treatment plant. They reported that FeCl₃ was capable of removing all H₂S at a dose of 2% FeCl₃ and above to a residual H₂S concentration of below 15 ppmv. However these results are not reflective of the overarching themes in prior studies, with expected removal efficiencies generally exceeding 100 ppm, whilst also presenting concerns regarding iron sulphide accumulation in the reactor (Schomaker et al., 2000). Iron salts are considered useful in reducing high concentrations, but
incapable of achieving fuel grade biogas, whilst also requiring considerable ongoing chemical costs (Ryckebosch et al., 2011).

Micro-oxygen injection involves the introduction of a limited supply of oxygen into an aerobic reactor in order to prevent the formation of H$_2$S. The basic setup is described in Figure 2.6 Error! Reference source not found.. Anaerobic conditions must still be maintained, but the altered conditions potentially allow species such as *Thiomicrospira* and *Thiobacillus* to oxidize sulphide present in the reactor (Diaz et al., 2011). H$_2$S reductions in biogas to levels between 20–100 ppmv have been reported (Schomaker et al., 2000). However, the primary issues inhibiting the adoption of this system relate to the risks of overdosing of oxygen with regards to biogas quality, system safety and digestion inhibition (Ryckebosch et al., 2011). In response, steps have been made in determining optimum dosing rates. Duangmanee (2009) reported that the O$_2$/S$^2-$ molar ratio was an important parameter, whereby a ratio in excess of 1.0 resulted in excess sulphate formation, a maximum elemental sulphur formation occurred between a ratio of 0.6 – 1.0, and thiosulphate formation required a ratio of 0.3 – 0.7. Whilst the complexity of parameter measurement is restrictive to the ideal maintenance of a simplistic operational design, an alternate approach was proposed in Nghiem et al. (2014a). This study demonstrated that the oxidation-reduction potential could be used to dictate oxygen dosing adjustments, whilst achieving a minimal H$_2$S concentration of 30 ppm.

![Image](image_url)

Figure 2.6. Micro-oxygen Injection for H$_2$S Reduction.
Concerns regarding H$_2$S concentrations in biogas can also be addressed in post-digestion processes. Current desulphurization for biogas commonly involves the use of biological oxidative processes (Weiland, 2010). Such techniques are advantageous due to their minimal ecological impacts and lower energy requirements, along with the potential recovery of elemental sulphur (Shou et al., 2015). These systems utilize sulphur oxidizing bacteria to transform sulphide into sulphate and elemental sulphur. The basic counter-current setup described in Figure 2.7, shows the biogas flows through a biological filter in the opposite direction to the liquid nutrients; which are generally recirculated. The systems can operate in both aerobic and anoxic conditions, with the latter capable of using nitrate as the electron donor (Soreanu et al., 2008). Montebello et al. (2012) found that both configurations were capable of treating H$_2$S concentrations between 20-2000 ppm. Removal efficiencies can be observed as high as 100% when operated under optimal conditions, but parameters such as biogas loading rate, pH, aeration rate, trickling velocity and H$_2$S concentrations can all regularly impact removal efficiencies (Singh and Mandal, 2012, Montebello et al., 2013). Further issues limiting the utilization extent of biological H$_2$S systems relate to the sensitivity of bacteria to sudden changes in temperature or biogas H$_2$S concentrations (Krischan et al., 2012). However Fortuny et al. (2008) observed that even after sudden increases in biogas H$_2$S concentrations from 1500 to 1600 ppm, a biotrickling system could reduce concentrations down to 500 ppm. This is a borderline quality for restrictions in combined heat and energy combustion engine use, whilst also representing a significant reduction from the 100% removal efficiency prior to the feed gas change.
Adsorption based removal options rely on selective reactivity of the H$_2$S within biogas. Typically ferric oxide or hydroxide are used as the reactant in iron sponge removal techniques, where organic wastes such as wood chips act as the supportive medium (Ryckebosch et al., 2011). Considerable focus has been allocated to the effectiveness of differing organic media, but existing commercial products generally achieve the highest H$_2$S removals but can be particularly expensive. (Cherosky and Li, 2013) found that coated yard waste could achieve comparable removal to commercial grade products, with a removal efficiency only 2% lower than the product, which itself could remove H$_2$S down to concentrations of around 65 ppm. An issue with this technique however is the considerable heat is produced, which can strip the sponge of its reactivity and also cause the system to reach combustible temperatures (Ravishanker and Hills, 1984).

Activated carbon represents an alternative absorbent which offers higher removal efficiency and lower operating temperatures. Figure 2.8 shows the basic arrangement of a pressure swing activated carbon unit, where the non-methane compounds are adsorbed within the activated carbon structure, and are removed during regeneration in the off-gas though temperature induced desorption. The two carbon filters are alternated in adsorption and desorption in order to maintain the adsorption potential of the active system. Oxygen injection can be involved, particularly in biological filter arrangements, as it is necessary for the oxidation of sulphide to elemental sulphur (Ho, 2012).
However the mechanism by which this occurs has been widely debated (Chen et al., 2010). This is infeasible when fuel-grade quality is required, and subsequently the impregnation with potassium iodide is necessary, as the associated reactions are not oxygen dependent (Petersson and Wellinger, 2009). Activated carbon units are rarely used in an unmodified state, rather they are typically impregnated with chemicals such as sodium hydroxide, potassium hydroxide or sodium carbonate in order to improve the rate of oxidation of sulphur and subsequently their removal from biogas (Sitthikhankaew et al., 2011, Bandosz, 2002). In Phooratsamee et al. (2014), the effect of impregnation on activated carbon from palm oil shells was evaluated, with the lowest achieved and control concentrations of H$_2$S in the treated biogas measuring between 22 – 29 ppmv and 19 – 22 ppmv respectively. However, much higher removal efficiencies are achievable with regular regeneration or replacement of activated carbon units, to levels below 1 ppmv (Lau et al., 2015). The shortcomings of the technology relate primarily to the associated of regeneration and replacement of carbon units, along with the potential reduction of methane purity (Ryckebosch et al., 2011). Additionally, the formation of sulphuric acid as a by-product of the involved reactions is an unfavourable consequence (Yuan and Bandosz, 2007).

Figure 2.8. Activated carbon H$_2$S absorption unit.
Absorption-based H₂S removal can make use of liquid absorbers through physical or chemical based processes. In Tippayawong and Thanompongchart (2010), H₂S was reduced below the detection limit in a packed column reactor using the solvents sodium hydroxide, calcium hydroxide and mono-ethanolamine. Whilst this technique is effective, the chemicals used are generally non-regenerative and thus are continually exhausted, with the associated costs becoming prohibitive to commercial utilization. In Krischan et al. (2012) the concurrent absorption of CO₂ was identified as the primary reactant with chemicals, and subsequently an arrangement for a selective oxidative scrubbing system was formulated and evaluated targeting H₂S removal. The findings showed that at both lab and pilot scale the system could achieve high separation efficiency for H₂S, at around 97%, whilst minimizing CO₂ absorption and consequently the chemical requirements.

Whilst there is a considerable range of treatment options, ideally the maintenance of suitable biogas quality can be achieved through the appropriate selection of substrates.

2.5.3. **Biosolids quantity and quality**

The implications of co-digestion on biosolids largely concern the biosolids volume, dewaterability and odour. Increases in biosolid volume have significant impacts on the economic prospects of AD processes. The introduction of additional organics through co-digestion can potentially lead to an increase in the total biosolid volume. The extent to which biosolids volumes are affected is in part determined by the degradability of the co-substrates added. However, co-digestion has also been observed in certain cases to improve the degradation of VS in the overall blend from that of mono-digestion of the base substrate (Molinuevo-Salces et al., 2013, Ara et al., 2015, Liu and Buchanan, 2011). Synergetic effects can potentially be attributed to the optimizations of organic stoichiometry of the feeds or the impacts on microbiological communities. This is not always the case, as the addition of co-substrates may produce negligible or adverse impacts on the digestion of the base substrate. Jensen et al. (2014) demonstrated a decrease in VS and COD removal using a blend of pig manure and crude glycerol. This was attributed largely to the limitation of the biomass’ hydrolysis of the pig manure, favouring the glycerol co-substrate. Co-substrates rich in easily degradable organics may be susceptible to this phenomenon, particularly in digesters with less diverse microbial communities. In alternate studies digesting glycerol with sewage sludge, negligible or even synergistic impacts on digestion were witnessed (Nghiem et al., 2014c, Jensen et al., 2014). The comparison of results demonstrates the importance of
balancing both the co-digestion blend and the substrate pairings in the optimization of biosolids processing. Increases in biosolids volume from co-digestion can also result from the inhibition of overall degradation, which would be accompanied by a decrease in biogas production.

Following on from the cost issues of biosolids management and disposal, maintaining a high dewaterability is essential to the economic favourability of co-digestion. The impact of co-digestion on the dewaterability represents an underexplored topic within the literature, with few dedicated studies assigning it significant focus (Jensen et al., 2014). In the limited studies mentioning the impact on dewaterability, the results show dependency on a range of factors. Concentration of co-substrate represents a key factor in the resultant dewaterability of the digestate. Wang et al. (2013) found that for the co-digestion of waste activated sludge with microalgae, an algal concentration of 4 and 11% produced an improved dewaterability of that from mono-digestion, whilst higher concentrations at 40 and 100% proved detrimental to digestate dewaterability. Co-digestion’s influence on dewaterability is dependent on the feed characteristics. Jensen et al. (2014) found that the addition of glycerol to sewage sludge had no impact on the dewaterability. In other studies, co-digestion of fat, oil and grease waste with sewage sludge resulted in improved dewaterability along with reductions in biosolids volume (Long et al., 2012). But unfortunately the shortage of studies in this area limits the conclusions that can be collectively drawn surrounding the impacts and mechanisms by which dewaterability is impacted on by co-digestion. However it can be concluded that the primary determinate factors for conventional AD dewaterability remain prevalent. The forms of pre-treatment, substrate particle size and ultimately the extent of degradation are highly influential in optimizing the dewaterability of co-digestion digestate (Agyeman and Tao, 2014).

The formation of odorous compounds in biosolids represents a pressing issue regarding its reuse. As stated earlier, the responsible compounds for odours are primarily the VOSC’s formed after the AD process. Few studies focus on biosolid odour potential from anaerobic digestion, but with regards to the impacts of co-digestion, this represents an unexplored area of research. In the case of mono-digestion, kinetic models for H₂S production have been extensively explored, however research into the formation rates of the other odorous compounds have been limited (Du and Parker, 2012). Within traditional AD of sewage sludge, the compounds that lead to the formation of VOSCs have been identified, notably the amino acids cysteine and methionine (Higgins et al., 2006). However the implications of the
differing feed compositions offered through co-digestion have yet to be determined. Certain commonly considered co-substrates contain considerable sulphur concentrations, and thus have the potential to significantly impact the odour profile of the biosolids.

Further potential implications of co-digestion on biosolids may relate to their reusability as fertilizers and in particular the concentrations of contaminants present following digestion and dewatering. The differing compositions of potential co-substrates raises considerable concerns to the agricultural reuse prospects of co-digestion biosolids. Whilst certain organic wastes may help produce favourable nutrients for land reuse applications (Demirel et al., 2013), different sources may negatively impact upon contaminant concentrations. Bonetta et al. (2014) found that the co-digestion of the organic fraction of municipal solid waste with a blend of sewage and manure sludge yielded digestate with high concentrations of copper, nickel and zinc, whilst also containing unacceptable levels of certain pathogens. However the accumulation of the heavy metals is highly predictable due to the simplicity of the involved mass balances. Nitrogen content is also an important factor affecting the reusability of the digestate, where high percentages of total Kjeldahl nitrogen (TKN) present as $\text{NH}_4^+$ are associated with improved fertilizing propensity (Astals et al., 2012). Along with the extent of nitrogen fixation, the solubilisation of phosphorus further impacts on the digestate reusability (Owamah et al., 2014). Whilst these factors are important for the improvement of digestion practice sustainability, process design for digestate quality rarely occurs. Given the large number of desired outcomes from the process, coupled with the limited international biosolid reuse, considerable shortcomings exist in this area of the literature.

2.6. Co-digestion pre-treatment

One prevailing trend in the literature has seen the application of pre-treatment technologies to substrates used for co-digestion (Tyagi et al., 2018). Such studies are aimed at improving the degradability of the substrates with lower specific methane yields, frequently concerning lignocellulos wastes (Mata-Alvarez et al., 2014). Improvements in degradability increase the reduction and stabilisation of biosolids as well as optimising the resource recovery in the form of biomethane.

A range of pre-treatment options have been evaluated in conjunction with co-digestion typically categorised into biological, chemical and physical processes. Pre-treatment effectiveness is typically evaluated in terms of the increase in biomethane yield. However, improvements in digestion performance must be balanced against the ongoing energy or
chemical costs of pre-treatment techniques (Mata-Alvarez et al., 2014). Excessive installation, operational and environmental costs have thus far proved inhibitory to widespread uptake of pre-treatment technology (Wei et al., 2017b).

Performance of pre-treatment techniques varies greatly between substrates, based on the initial biodegradability of the substrates as well as the suitability of the pre-treatment (Table 2.3). Indeed, greater improvements in methane yield have been observed when applied to substrates with slowly degradable organics or those rich in lignocellulosic material (Habashi et al., 2018, Deng et al., 2017).
Table 2.3. Summary of pre-treatment techniques applied to co-digestion feedstocks.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Substrates</th>
<th>Improvement in yield</th>
<th>Pre-treatment dosage/ energy usage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-aeration pre-treatment</td>
<td>Brown water and food waste</td>
<td>10 – 21 %</td>
<td>37.5 mL-O₂/L/d</td>
<td>(Lim and Wang, 2013)</td>
</tr>
<tr>
<td>Thermal pre-treatment</td>
<td>Sewage sludge and food waste</td>
<td>34 % in sludge</td>
<td>90 °C for 90 min</td>
<td>(Mirmasoumi et al., 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 % in food waste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrodynamic heat cavitation</td>
<td>Waste activated sludge and oily wastewater</td>
<td>43 %</td>
<td>4.0 W.h/L</td>
<td>(Habashi et al., 2018)</td>
</tr>
<tr>
<td>Thermo-chemical pre-treatment</td>
<td>Fat, oil and grease and primary sludge</td>
<td>37.5 %</td>
<td>pH adjustment to 10 at 55 °C</td>
<td>(Li et al., 2013)</td>
</tr>
<tr>
<td>Biological pre-treatment</td>
<td>Rice straw and soybean straw</td>
<td>807 %</td>
<td>214.1 NmL/g TS</td>
<td>(Deng et al., 2017)</td>
</tr>
<tr>
<td>(Trichoderma reesei RUT C30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonic pre-treatment</td>
<td>Sewage sludge and olive and grape pomices</td>
<td>10 – 15 %</td>
<td>10000 kJ/kg TS</td>
<td>(Aylin Alagöz et al., 2018)</td>
</tr>
<tr>
<td>Microwave pre-treatment</td>
<td>Sewage sludge and olive and grape pomices</td>
<td>30 – 44 %</td>
<td>87000kJ/kg TS</td>
<td>(Aylin Alagöz et al., 2018)</td>
</tr>
<tr>
<td>Particle size reduction</td>
<td>Food waste and sewage sludge</td>
<td>9 – 34 %</td>
<td>Particle size reduction from 8 – 2.5 %</td>
<td>(Agyeman and Tao, 2014)</td>
</tr>
</tbody>
</table>

2.6.1. Free ammonia pre-treatment

A promising pre-treatment technique offering both lower operational costs and considerable improvements in biodegradability is free ammonia (FA) pre-treatment. The technique exploits the ability of FA to diffuse between cell membranes, resulting in chemical imbalances and subsequently cell immobilisation and lysis (Wang et al., 2018a). Substrates are dosed with NH₄ and temporarily subjected to alkaline conditions in order to increase the FA concentration whilst limiting addition of total NH₄/NH₃. Wei et al. (2017a) observed improvements in the biomethane potential and hydrolysis rate constant of 22 and 140% respectively relative to the untreated sludge. This enhancement of the degradation kinetics suggests the pre-treatment technology may be particularly effective in improving less hydrolysable substrates. Conversely, when applying FA pre-treatment to primary sludge Wei et al. (2017b) reported a decreased hydrolysis rate, despite improved solubilisation and final methane yield relative to the untreated waste.
Whilst similar improvements in methane yield are offered by alternate pre-treatment technologies, the additional benefit of FA pre-treatment is its favourable economic prospects (Wang et al., 2018b). Indeed Wang (2017) proposed a design of recycling ammonia within WWTPs in order to achieve nitrogen removal and improved biomethane production during anaerobic digestion.

Despite the optimism and promising results presented in existing studies, FA pre-treatment remains in its infancy. A lack of understanding persists with respect to the mechanisms behind improvements in the biodegradability of substrates (Wang et al., 2018a). The technique has yet to be demonstrated within a continuous system, only having been applied during batch testing. Furthermore, FA pre-treatment has only been applied during sewage sludge digestion, nor has it been applied during co-digestion.

2.7. Summary

Anaerobic co-digestion in municipal wastewater treatment is a promising practice to improve sustainability and utilisation of resources. Co-digestion allows for the treatment of substrates that are unsuitable for conventional mono-digestion. Anaerobic co-digestion utilises the spare organic loading capacity of existing digesters at wastewater treatment plants without requiring significant modification of existing infrastructure. As sewage sludge possesses significant buffering capacity yet low organic content, it may be suitable for co-digestion with countless potential co-substrates. Furthermore the potential for synergistic effects between subjects offers greater methane yields and degradation kinetics.

Despite the fervent optimism surround anaerobic co-digestion, persistent knowledge gaps restrict uptake of the practice. Lack of available co-substrates can hinder full scale co-digestion in rural regions, thus the identification of further potential co-substrates is essential. Indeed, several high volume waste streams have yet to be tested in anaerobic co-digestion. Additional shortcomings relate to uncertainty in the nature of the synergistic effect. Conflicting views arise in whether synergistic effects solely influence biodegradability, process kinetics or both. Meanwhile it has not been differentiated if separate mechanisms are behind the two forms of synergisms. Furthermore there is a persistent deficit of pilot scale research, whilst laboratory experiments tend to provide overly favourable results through the elimination of variables. Laboratory-scale papers also tend to focus overly on the volume of biogas produced during co-digestion, without sufficiently considering its impacts on downstream processes. Factors such as biosolids quality and odour are insufficiently covered
in the literature, as is the composition of biogas, particularly in terms of the hydrogen sulphide content. Further research is also required to elucidate the impact of different pre-treatments when used in conjunction with co-digestion. In the literature most pre-treatments applied during co-digestion involve thermal based processes. Free ammonia pre-treatment has demonstrated promising results and favourable economics, yet has only been applied during mono-digestion of sewage sludge and has not been demonstrated in a continuous system or in conjunction with co-digestion.

The identified gaps within the literature form the basis on which this study was developed. These objectives of the research were addressed by methodologies derived from prior research, whilst also introducing techniques that were previously absent from the field anaerobic co-digestion.
CHAPTER 3: Biomethane potential evaluation of the co-digestion of sewage sludge with solid and liquid organic wastes

Corresponding publication:


3.1. Introduction

Anaerobic digestion is an essential process in wastewater treatment, involving the use of microorganisms to break down organic material in the absence of oxygen (Tchobanoglous and Burton, 1991). Traditional anaerobic digestion applications focus on the stabilisation and volume reduction of sewage sludge produced in primary and secondary treatment of municipal wastewater. However, evolving social values and economic considerations have prompted an objective scope expansion. This additional scope includes the utilization of the biogas which is a product of the anaerobic digestion process for beneficial use.

Biogas represents a renewable energy resource for the industry (Esposito et al., 2012). It composes of about 60% CH₄, 40% CO₂, and a few trace gases such as H₂S and water vapour (Chynoweth et al., 2001, Tchobanoglous and Burton, 1991). Biogas can be readily converted to electrical and thermal energy via a co-generator, typically for onsite consumption (Shen et al., 2015, Tchobanoglous and Burton, 1991).

In Australia, biogas is still a largely underutilised resource due to a range of unfavourable economic and policy factors (Edwards et al., 2015a). Energy production from biogas does not qualify for a feed-in-tariff in all states in Australia with the exception of Victoria, where systems smaller than 100 KW are eligible to receive 0.068 AUD$/kWh (Edwards et al., 2015b). The maintenance cost of co-generation in Australia is high regardless of their size. Thus, small scale energy recovery systems tend to be economically infeasible. In Australia, rebates for renewable energy production from biogas of 0.038 AUD$/kWh are only available to large scale producers through the Large-scale Generation Certificates (LGCs) scheme under the Renewable Energy Target policy (Edwards et al., 2015b). As a result, there is a
critical scale of biogas production above which biogas utilisation can be economically feasible. This critical threshold can be overcome through the use of co-digestion of the sewage sludge with concentrated organic wastes (Silvestre et al., 2015, Fersi et al., 2014). Sewage sludge is ideal for use as the base substrate in co-digestion due to its low concentrations of inhibitors and high alkalinity (Mata-Alvarez et al., 2014). In addition, anaerobic digestion facilities are readily available at most wastewater treatment plants.

Co-digestion offers several benefits over traditional mono-digestion when applied (Pavan et al., 2007, Wang et al., 2013, Mata-Alvarez et al., 2014). Beyond the improvements to biogas production, co-digestion facilitates the optimisation of digester stoichiometry, which can positively influence digestion performance with respect to sludge degradation. In other words, by adding a carbon rich organic waste co-substrate to wastewater sludge (which usually has a low C:N ratio), an optimum C:N ratio for anaerobic digestion can be obtained.

The economic viability of co-digestion can be significantly enhanced through the contribution of supplementary revenue from gate fees (i.e. commercial charges for waste disposal). In Australia, once the generation capacity reaches 1 MW, there can be additional revenue from LGCs as noted above. Co-digestion substantially improves the sustainability of waste management practices (Kim and Kim, 2010). In particular, co-digestion allows the diversion of solid organic wastes from landfill, thus limiting greenhouse gas emission while facilitating energy recovery through biogas production (Holm-Nielsen et al., 2009).

Despite the active attempts to optimize co-digestion, there remain several technological challenges associated with its implementation (Mata-Alvarez et al., 2014, Koch et al., 2015b, Haider et al., 2015, Giuliano et al., 2013). Uncertainty related to the potential implications of co-digestion on biosolids (dewatered digested sludge) quality and volume are considerable due to the proportionality of their disposal costs, which account for a significant proportion of overall wastewater treatment expenditures (Appels et al., 2008b). Poor co-substrate selection and excessive co-digestion can also instigate digester inhibition, often through the introduction of inhibitory substances and overloading of organic ratios. Additionally, the presence of sulphur can facilitate the formation of H$_2$S (Dewil et al., 2009, Park et al., 2014). High H$_2$S concentration in biogas can damage combustion engine components and piping (Weiland, 2010). Excessive phosphorous in AD can cause struvite precipitation on pipelines, valves and other plant infrastructure (Sabbag et al., 2015).
This study aims to screen seven carbon rich organic wastes with regards to their potential use as co-substrates for further biogas production. Bio-methane potential (BMP) assessment and co-substrate characterisation are conducted for comparative analysis of organic wastes with varying compositions. Data obtained from this study will be used to design a pilot scale study to assess the anaerobic digestion of these organic rich wastes and wastewater sludge.

3.2. Materials and methods

3.2.1. Wastewater sludge and co-substrates

Sludge from a full scale anaerobic digester at the Wollongong wastewater treatment plant (WWTP) was used as the inoculum and sludge co-substrate. The organic co-substrates were categorized into either solid (or slurry) and free-flowing (solids free) liquid materials. All organic co-substrates were collected fresh and were stored at 4 °C for less than three days prior to BMP evaluation.

The solid organic wastes included municipal food waste from Randwick city council in Sydney Australia (denoted as RW-FW), commercial food waste from a commercial waste collector (denoted as PM-FW), paper pulp reject (denoted as PW), and untreated waste from a bakery (denoted as UBW). Food waste (RW-FW) from the local council was macerated into slurry without any water addition. Food waste from the commercial waste collector (PM-FW) was macerated with water as part of their collection process. These two types of food waste were both sampled on two separate occasions to assess their temporal variability. Paper pulp reject was cellulose in powder form from a paper mill in New South Wales, Australia. Untreated bakery waste was from a large bread making factory in Sydney Australia and was in the form of thickened slurry.

In addition to the solid organic wastes, dehydrated Ulva macroalgae powder from Venus Shell Systems (Australia) was also evaluated for comparison purposes as it has been a widely used substrate for anaerobic digestion as noted in several recent reviews (Montingelli et al., 2015, McKennedy and Sherlock, 2015). These algae are not a waste product but are abundant in coastal area in Australia. The chemical composition of dehydrated Ulva macroalgae has been systematically described elsewhere (Yaich et al., 2011). Briefly, it contains approximately 54.9% carbohydrate, 10.0% uronic acid, 8.5% protein, and 7.9% lipid. The ash content of Ulva macroalgae is about 19.6% (Yaich et al., 2011). It is noteworthy that the lignin (non-degradable) fraction in the carbohydrate of Ulva macroalgae is very low (about 1%) (Montingelli et al., 2015).
The liquid organic wastes included non-alcoholic beverage reject (denoted as BJ), pre-treated organic waste from the same bakery as mentioned above (denoted as TBW), fat-oil-grease (FOG) from a commercial waste collector, and waste from an industrial dairy processor (denoted as DW).

3.2.2. **Biomethane potential experimental equipment**

The co-digestion of sludge and organic co-substrate was evaluated using a customised BMP system (Nghiem et al., 2014c). The BMP system included an array of 1 L fermentation glass bottles (Wiltronics Research Pty Ltd) and a gas collection gallery (Figure 3.1). The fermentation bottles were submerged in a water bath (Model SWB20D, Ratek Instrument Pty Ltd) to maintain a constant temperature of 35.0±0.1 °C. Each bottle setup comprised of a rubber stopper, a water-filled S-shaped airlock, and a valve. Biogas from the bottle could flow through the airlock into the gas collector via flexible plastic tubing. The gas collector was an inverted plastic measuring cylinder (1000 mL), which was initially filled with, and partially submerged in, a 1M NaOH solution.

![Figure 3.1. Bio-methane potential experimental equipment: (a) Schematic diagram (b) Photograph](image)

3.2.3. **Experimental protocol**

Prior to all BMP experiment, fermentation bottles were flushed with pure N₂ for 5 minutes before filling with 750 mL of organic co-substrate and inoculum (section 2.1). A set of BMP experiments using partially digested sludge as the only substrate was also conducted.
as a reference. After filling with the substrate, the bottle was flushed again with N₂ and immediately sealed with the rubber stopper. They were then placed into the shaking water bath and the valve was opened to allow biogas to enter the gas collection gallery.

To measure the volume of CH₄ generated from the BMP bottle, the cylinder was first filled with 1 M NaOH solution, and was inverted and then partially submerged into a container also containing 1 M NaOH. Biogas from the fermentation bottle was introduced into the submerged part of the cylinder, thus allowing the NaOH solution to absorb CO₂ and H₂S from the biogas. The remaining CH₄ gas displaced the NaOH solution inside cylinder and the CH₄ gas volume generated was recorded daily. The experiment was terminated when less than 5 mL/day of CH₄ was produced. CH₄ production was expressed at the standard temperature and pressure condition.

All BMP experiments were conducted in duplicate. With the exception of the algae, all co-substrates were co-digested with sludge in concentrations of 5, 10 and 15% by weight. Algae and sewage sludge co-digestion was carried out at mixing ratios of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 3, 4.5, 6, 7.5 and 9%.

3.3. Analytical methods

A range of parameters were measured for the co-substrates, sludge and sludge/co-digestion mixtures before and after the BMP experiment. Total chemical oxygen demand (COD) was measured using a Hatch DRB200 COD Reactor and Hatch DR3900 spectrophotometer (program number 435 COD HR) following the US-EPA Standard Method 5220 with a dilution factor of 10. Total solids (TS), volatile solids (VS), pH, conductivity and alkalinity were conducted within 3 days of collecting the samples. Samples were preserved at 4 °C. Further details of these analyses are available elsewhere (Yang et al., 2016). Total sulphur and total phosphorous were analysed within 24 hours by Sydney Water’s NATA accredited West Ryde Analytical Laboratory.

3.3.1. VS reduction calculation

The removal efficiencies used in digestion performance evaluation for all co-substrates were calculated using the following equation:

\[
\text{Reduction} = 100 \times \left( 1 - \frac{C_{\text{Co End}} - C_{\text{End}}}{C_{\text{Co Init}} - C_{\text{End}}} \right)
\]  
(Equation 3.1)
Where $C_{\text{CoEnd}}$ is the concentration of the co-digested sample at the end of the BMP test; $C_{\text{Colm}}$ is the concentration of the co-digested sample at the beginning of the test; and $C_{\text{IEnd}}$ is the post-digestion concentration of the inoculum. A reduction of 100% indicates that the co-substrate is expected to contribute no residuals of this parameter. Greater than 100% removal demonstrates a synergetic digestion of the co-substrate and sewage sludge, whereby the presence of the co-substrate positively impacts on the digestion performance in the sludge.

3.4. Results and discussion

3.4.1. Co-substrate characteristics

The primary characteristics of the wastewater sludge from Wollongong WWTP, individual individual co-substrates are collated in
Table 3.1. A clear distinction between solid and liquid co-substrates was the significantly higher TS and VS contents in the former. An exception to this was the commercial food waste (PM FW-2) sample, which could be due to water dilution as noted in section 3.2.1. The implication of the higher solids content is a greater propensity to contribute to biosolids production in the downstream processes. Further notable characteristics concern the concentrations of sulphur and phosphorus measured in the food waste co-substrates compared with the wastewater sludge.
Table 3.1. Key properties of sludge and co-substrates.

<table>
<thead>
<tr>
<th></th>
<th>Sludge</th>
<th>Solid organic waste</th>
<th>Liquid organic waste</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RW-FW 1</td>
<td>RW-FW 2</td>
<td>PM-FW 1</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>16</td>
<td>135.7</td>
<td>194.4</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>15</td>
<td>102.1</td>
<td>121.3</td>
</tr>
<tr>
<td>COD (g/L)</td>
<td>20.1</td>
<td>179.6</td>
<td>296.8</td>
</tr>
<tr>
<td>S (mg/L)</td>
<td>285.5</td>
<td>NA</td>
<td>3450</td>
</tr>
<tr>
<td>P (mg/L)</td>
<td>657.5</td>
<td>3660</td>
<td>3710</td>
</tr>
</tbody>
</table>

RW-FW 1 = Randwick Food Waste 1; RW-FW 2 = Randwick Food Waste 2; Please complete the rest to explain your abbreviations in this table.
Co-substrate selection also fringes upon sourcing factors. With the exception of the algae, all other co-substrates are essentially waste materials. As a result, there can be significant temporal and spatial variation in their properties. Indeed, notable variation can be observed in the composition of the municipal (RW-FW) and commercial (PM-FW) food waste samples between the two sample occasions (}
Table 3.1).

3.4.2. Co-digestion with algae

It is noteworthy that the algae used in this study are not a waste material. Given their consistency in carbohydrate and lipid content (section 3.2.1), they were used as a reference organic material. The algae co-substrate was mixed with the wastewater sludge on a mass fraction percentage (dry waste of algae over total weight of the substrate) over a range of concentrations from 0.25 – 9% (wt/wt).

Figure 3.2 shows the cumulative methane production increased as the algae fraction increased to 6% (wt/wt). Above the optimum point the introduction of additional co-substrate was inhibitive to overall methane production. The trend is further demonstrated in Figure 3.3, which shows a sharp decline in production beyond the optimum 6% (wt/wt) algae concentration. The inhibition of the anaerobic system was attributed to organic overloading. A high carbon/nitrogen stoichiometric ratio resulted in excessive production and thus build-up of volatile fatty acids. Fatty acid accumulation leads to pH decrease, subsequently inhibiting microbiological function (Prochazka et al., 2012). Within the algae fraction of 6% or below, the addition of the co-substrate did not cause an excessive build-up of volatile fatty acids and there was sufficient time for the produced acids to be digested.
Figure 3.2. Cumulative methane production from a combination of algae and sewage sludge as a function of time.

Figure 3.3. Cumulative methane production plotted against algae fraction (error bars are standard deviations from duplicate experiments).
The removals of TS and VS were found to be approximately 59% and 75% respectively for the algae co-substrate samples. These results indicate that the use of algae as a co-substrate would lead to additional biosolids production. The methane potential of the algae co-substrate was approximately 139 L CH₄/kg of co-substrate.

3.4.3. Co-digestion with solid wastes

All organic waste co-substrates increased the methane yield above that of only wastewater sludge. However, organic overloading was observed for municipal food waste (RW-FW) at both sampling occasions when the co-digestion ratio was 10 and 15% (wt/wt) (Figure 3.4a). Indeed, biogas production was substantially lower when the co-digestion ratio was 5% (Figure 3.4a). Anaerobic digestion inhibition was also observed with untreated bakery waste (UBW) at the co-digestion ratio of 10 and 15% (data not shown). Similar to the results from algae (section 3.3.2), the observed inhibition at high municipal food waste (RW-FW) and untreated bakery waste (UBW) co-digestion ratios was attributed to the build-up of volatile organic acids, evidenced by a low pH (less than 5) of the substrate at the end of the experiments of all BMP bottles with poor methane production (Li et al., 2015).

Temporal variability of VS and COD of the municipal food waste (RW-FW) was observed between the two sampling occasions. As can be seen in Table 1, variations in VS and COD values of the two municipal food waste (RW-FW) samples were 20 and 65%, respectively. The co-digestion ratio of 5% (wt/wt) was suitable for both occasions. Temporal variation in VS and COD content (10 and 90%, respectively) could also be seen with the two commercial food waste (PM-FW) samples. Nevertheless, the two commercial food waste (PM-FW) samples did not display any inhibition even at the co-digestion ratio of 15% (wt/wt). Whilst the dilution conducted prior to collection (section 3.2.1) proved effective in reducing the inhibition potential, at the same co-digestion ratio the maximum achieved biogas production was lower than that of municipal food waste (RW-FW). Both RW-FW and PM-FW are food waste materials. In other words, the original co-substrate of PM-FW prior to dilution would be expected to be similar in composition to that of RW-FW, and thus, they would result in similar methane productions. Thus, higher co-digestion ratio between PM-FW and sludge would be required to validate the effectiveness of dilution of this co-substrate.

The BMP results from the co-digestion of paper waste (Figure 3.4b) show a continual increase in biogas production as the co-digestion ratio increased. It is possible that the rate of paper waste hydrolysis (that is responsible for the
production of volatile fatty acid) is slow. Thus, a high co-digestion ratio of paper waste and sludge did not result in volatile fatty acid accumulation in the system. It is also noteworthy that the benefit of adding additional concentrations beyond 5% was negligible.

![Graph showing cumulative CH₄ production](image)

**Figure 3.4.** Cumulative methane production plotted against time for co-digestion of council food waste and paper waste as co-substrates

The removal efficiencies for TS, VS and COD were evaluated for the different co-substrates. All solid wastes show a tendency for incomplete removal of these parameters (Table 3.2), indicating that these waste materials may result in additional sludge production and may negatively affect sludge stabilization targets. The only exception was RW-FW 2 (council food waste), for which high removal efficiencies for TS and VS were observed. Paper waste also displayed some positive results in terms of the removal of both VS and COD. However, a lower TS removal indicates that paper waste might also result in additional sludge production.

The additional methane yields were calculated based on the best BMP results of these co-substrates (Table 3.2). As expected, all solid waste materials evaluated in this study produce less methane than dehydrated algae (139 L CH₄/kg of algae) with the exception of UBW.

**Table 3.2. Performance of solid waste co-digestion with sewage sludge.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PW</th>
<th>RW-FW 1</th>
<th>RW-FW 2</th>
<th>PM-FW 1</th>
<th>UBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄ (L/kg substrate)</td>
<td>35</td>
<td>73.1 ± 0.19</td>
<td>127.1 ± 8.0</td>
<td>30.9 ± 5.7</td>
<td>184 ± 2.8</td>
</tr>
<tr>
<td>TS Removal (%)</td>
<td>96</td>
<td>85.8</td>
<td>104.4</td>
<td>68.5</td>
<td>89.0</td>
</tr>
<tr>
<td>VS Removal (%)</td>
<td>102</td>
<td>88.1</td>
<td>207.6</td>
<td>68.0</td>
<td>94.8</td>
</tr>
</tbody>
</table>
### 3.4.4. Co-digestion with liquid wastes

All liquid wastes displayed highly reproducible BMP results. This high level of reproducibility is consistent with several previous studies (Koch et al., 2015a, Angelidaki et al., 2009c). The results confirm the validity of BMP as a screening tool for co-substrate evaluation.

Organic overloading was observed with beverage reject at co-digestion ratio of 10% (wt/wt) (Figure 3.5a). The inhibition of beverage reject waste beyond a co-substrate concentration of 10% was attributed to the rapidly degradable organics in the substrate. The sugar content of non-alcoholic beverage reject can be quickly converted into organic acids, which in turn impact upon the digester pH. This premise could be demonstrated through a more systematic co-digestion evaluation using a semi-continuous anaerobic digester. Each of the other co-substrates showed a nearly proportionate increase in biogas production with regards to co-substrate concentration.

![Cumulative methane production plotted against time for co-digestion of beverage reject and fat oil and grease wastes as co-substrates](image)

**Figure 3.5.** Cumulative methane production plotted against time for co-digestion of beverage reject and fat oil and grease wastes as co-substrates.
The digestion performance in terms of VS and COD removals when co-digesting with liquid wastes was generally much higher compared to solid wastes (section 3.3.3). In fact, not only did the addition of co-substrate result in no additional VS and COD residual, synergistic removal of COD, VS and TS were also observed with all liquid co-substrates with the exception of FOG. In other words, the observed COD, VS, and TS removals of above 100% were attributed to the synergistic effect of liquid waste co-digestion. The lower digestion performance involving FOG is likely related to its higher lipid content. Co-digestion of lipid rich wastes complicates considerations with issues such as lipid floatation, long chain fatty acid accumulation, pre-treatment requirements and lower degradation rates (Wan et al., 2011, Li et al., 2013). The synergistic removal efficiencies observed in the co-digestion of the other wastes signifies a potential reduction in sludge production and improvement in the final biosolids stability.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BJ</th>
<th>TBW</th>
<th>DW</th>
<th>FOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{CH}_4) ((\text{L/L substrate}))</td>
<td>26 ± 1.16</td>
<td>27 ± 5.6</td>
<td>94 ± 40.4</td>
<td>47 ± 2.8</td>
</tr>
<tr>
<td>COD Removal (%)</td>
<td>141</td>
<td>119</td>
<td>102</td>
<td>108</td>
</tr>
<tr>
<td>VS Removal (%)</td>
<td>116</td>
<td>219</td>
<td>218</td>
<td>72</td>
</tr>
<tr>
<td>TS Removal (%)</td>
<td>133.9</td>
<td>366.2</td>
<td>119.3</td>
<td>84.2</td>
</tr>
</tbody>
</table>

3.5. Conclusions

In this study, algae and seven organic waste materials were evaluated as potential co-substrates for anaerobic digestion with sewage sludge for their bio-methane potential and likely influence on digested sludge quality in term of TS, VS and COD. All co-substrates increased the bio-methane yield by three to six times compared with conventional anaerobic digestion of sewage sludge. While solid/slurry co-substrates resulted in notable more methane gas production, they are associated with a higher risk of organic overloading. The maximum co-digestion ratios were identified for most solid/slurry co-substrates including algae (6% wt/wt), undiluted food waste (5% wt/wt), untreated bakery waste (5%), and diluted commercial food waste (10% wt/wt). On the other hand, the maximum co-digestions ratio of beverage reject and sewage sludge was 10% (wt/wt). Elevated concentrations of sulphur and phosphorous were observed in all food waste co-substrates from both municipal and commercial sources. In addition, with bakery waste being the only exception, the co-digestion of all other solid co-substrates resulted in additional VS and COD residuals in digested sludge. By contrast, most liquid co-substrates evaluated here showed a notable
synergistic effect, which enhanced the removals of TS, VS and COD during anaerobic digestion.
CHAPTER 4: Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes

Corresponding publication:


4.1. Foreword

The following Chapter was contributed to, in part, by Sihaung Xie, as reflected in the authorship of the publication. Sihuang Xie’s contribution amounted to the kinetics modelling of the experiment. With respect to the authoring of the publication on which this Chapter is based, Section 4.4, Section 4.5.3 were written be Sihuang Xie, whilst his partial contribution to Section 4.2 and Section 4.3.3 is noted. The entire experimental component, the production of all tables/figures and the composing of all remaining sections of this Chapter were carried out by the thesis author (R. Wickham).

4.2. Introduction

Concern over the disposal of organic wastes from domestic, industrial and agricultural sources together with the need to reduce green-house gas emissions have been a major driver for further development of anaerobic digestion technology (Edwards et al., 2015a). Anaerobic digestion has been widely used by wastewater treatment plants (WWTPs) to stabilize sewage sludge prior to land application or disposal and at the same time produce biogas (which is a renewable fuel) to offset some of the energy input to the treatment process (Tyagi and Lo, 2013). During anaerobic treatment, nitrogen and phosphorus are liberated into the liquid phase in the form of ammonia and phosphate (Yilmazel and Demirer, 2013) thus, anaerobic digestion can also be an excellent platform for nutrient recovery (Xie et al., 2013).

A recent and notable trend in the development of anaerobic digestion technology is to co-digest two or more substrates together (Xie et al., 2016). Co-digestion can overcome several inherent problems associated with single substrate digestion such as the lack of micronutrients, imbalanced C/N ratio, and unfavourable (i.e. too high or too low) organic
loading rates (Mata-Alvarez et al., 2011). In the context of the water industry, the existing spare capacity of anaerobic digestion infrastructure at wastewater treatment plants allows for anaerobic co-digestion of sewage sludge with organic waste to generate supplementary revenue via gate fees or service charges, whilst producing electricity and heat (Edwards et al., 2015a). In addition, co-digestion can also help to defer capital investment for additional waste management facilities (Nghiem et al., 2014c). Indeed, rapidly increasing landfill levies worldwide along with the possibility for nutrient recovery present considerable potential driving forces for further adoption of co-digestion (Yong et al., 2015). Although successful co-digestion of sewage sludge and various organic wastes such as food waste (Koch et al., 2016, Ratanatamskul et al., 2015, Tuyet et al., 2016), fat oil and grease (Martínez et al., 2012), crude glycerol (Nghiem et al., 2014c, Silvestre et al., 2015), have been reported in many recent studies, several key aspects of the anaerobic co-digestion process remain poorly understood. In particular, little is known about the synergistic effect of co-digestion on anaerobic performance and the associated mechanisms responsible for such effect (Mata-Alvarez et al., 2014)

Co-digestion can enhance anaerobic degradation of each individual substrate (Mata-Alvarez et al., 2011). In other words, co-substrate addition can result in synergistic effects, which result in either a boost in specific methane yield of the individual substrate in the mixture or an increase in biogas production kinetics, differing from the additive effect where an increase in methane production is simply due to a higher mass of available biodegradable organic matter per unit volume from co-substrate addition. There have been some evidence that co-digestion can also result in some antagonistic effects (Silvestre et al., 2014). In some cases, no obvious effects of co-digestion compared to mono-digestion have also been reported (Silvestre et al., 2015). It is widely hypothesized that co-digestion can improve the process performance mainly because of (i) a more balanced C:N ratio and sufficient macro and micronutrients (Wang et al., 2012), (ii) a high buffering capacity (Xie et al., 2011), and (iii) a higher readily biodegradable organic fraction (Astals et al., 2014). These factors attributed to the synergistic effects are associated inherently with co-substrate properties and composition. For example, sludge with a low C/N ratio can be co-digested with waste paper with a high carbon content to achieve an optimum C/N ratio of 20–25 (Yen and Brune, 2007)

The reported synergistic effects vary in the literature (Pagés-Díaz et al., 2014, Aichinger et al., 2015, Astals et al., 2014). In other words, such effects can be reflected as increased
methane yields, accelerated biodegradation processes or a combination of both. Pagés-Díaz et al. (2014) investigated optimal mixture composition between cattle slaughterhouse wastes, municipal solid waste, manure and various crops, and assessed the synergistic effect solely by specific methane production rate. Aichinger et al. (2015) interpreted the synergistic effect as an increased hydrolysis rate constant rather than an increased specific biogas yield for a mixture of raw sludge and co-substrates over the specific biogas yield for individual substrates. Similarly, Astals et al. (2014) identified the synergetic effect during anaerobic co-digestion of pure and slaughterhouse carbohydrate, protein, and lipid substrates as an improvement of process kinetics, rather than an increase in ultimate biodegradability. As the rate limiting step in anaerobic co-digestion is the hydrolysis of complex polymeric substances such as extracellular polymeric substances in sewage sludge, it is important to evaluate the impact of synergistic effects during anaerobic co-digestion on both the specific methane yields and the process kinetics.

Diversified approaches have been implemented to analyze the synergistic effects in the previous studies. Yun et al. (2015) defined the synergistic effects as an increased methane yield from waste activated sludge, and analyzed the synergistic effects during anaerobic co-digestion with food waste assuming a full conversion of food waste (1 g COD = 350 mL methane). Ebner et al. (2016) used a co-digestion performance index calculated as the ratio of the bio-methane potential of the co-digestion blend to the weighted average based upon VS content of the individual substrate bio-methane potentials. However, both studies have not quantified the extent of such effect. Aichinger et al. (2015) employed a COD balance approach to quantify the extent of synergistic effects. It is noteworthy that whey was chosen to be the model co-substrate corresponding to a full conversion rate, thus enabling a simplified quantitative analysis of the extent of synergistic effects from raw sludge (Aichinger et al., 2015). Nevertheless, in most of these studies, kinetics modelling has not been applied to further elucidate the impact of co-substrates addition on improving the anaerobic co-digestion process kinetics.

This study aims to systematically elucidate synergistic effects during anaerobic co-digestion of primary sludge with organic wastes by applying a BMP assay based kinetics modelling approach together with COD balance calculation. The specific objectives of this study are: (i) to assess the process stability, (ii) to quantify synergistic or antagonistic effects of co-digesting primary sludge and organic waste on specific methane yields and VS
removals based on COD balance, and (iii) to examine whether the reaction kinetics can be associated with the synergistic effect.

4.3. Materials and methods

4.3.1. Primary sludge and co-substrates

Digested sludge from the Wollongong WWTP was used as the inoculum. Raw primary sludge was also from the same plant. Primary sludge was stored at 4 °C for less than three days prior to BMP evaluation. The organic co-substrates include food waste and paper pulp reject (denoted as FW and PPR respectively). Dog food from Optimum (lamb and Rice) composing mainly of carbohydrates, protein, and lipids was used to represent food waste. Paper pulp reject was primarily cellulose in powder form from a paper mill in New South Wales, Australia. Key properties of inoculum, sludge and co-substrates was shown in Table 4.1

Table 4.1. Key properties of inoculum, sludge and co-substrates (mean ± standard deviations of two replicates).

<table>
<thead>
<tr>
<th></th>
<th>Inoculum</th>
<th>Primary sludge</th>
<th>Food waste</th>
<th>Paper pulp reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>2.4 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>17.4 ± 0.4</td>
<td>47.3 ± 0.6</td>
</tr>
<tr>
<td>VS (%)</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>16.1 ± 0.2</td>
<td>36.3 ± 0.5</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>58.0</td>
<td>84.6</td>
<td>92.7</td>
<td>76.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.50 ± 0.01</td>
<td>6.02 ± 0.01</td>
<td>6.45 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>COD (mg/kg fresh weight)</td>
<td>11250 ± 950</td>
<td>20250 ± 1350</td>
<td>333300 ± 13850</td>
<td>531250 ± 29750</td>
</tr>
<tr>
<td>COD/V S ratio</td>
<td>0.8</td>
<td>1.84</td>
<td>2.07</td>
<td>1.46</td>
</tr>
</tbody>
</table>

4.3.2. Biochemical methane potential (BMP) assay

4.3.2.1. BMP experimental equipment

BMP assay was performed according to the protocol described by Angelidaki et al. (2009a). The BMP system previously used by Nghiem et al. (2014b) was modified for this study. The BMP system included an array of 12 fermentation glass reactors (Wiltronics Research Pty Ltd) and a gas collection gallery. The glass reactor consisted of a rubber stopper, a water-filled S-shaped airlock with a valve, and a syringe for collecting liquid samples. The fermentation glass reactor (1 L in volume) was submerged in a water bath (Model SWB20D, Ratek Instrument Pty Ltd) to maintain a constant temperature of 35.0 ± 0.1
°C. The gas collector was an inverted plastic measuring cylinder (1 L), which was initially filled with and partially submerged in a NaOH solution (1 M).

4.3.2.2. Experimental protocol

Prior to the BMP experiment, all fermentation reactors were flushed with pure N2 and subsequently filled with 750 mL of organic substrates and inoculum (Table 4.2). Co-substrate and primary sludge were added to the reactor on a 1:1 VS basis. Inoculum and tap water were then added to obtain 750 mL of substrate volume in total. Reactor 1 and 2 served as controls with the addition of inoculum and tap water to obtain the residual biogas production from the inoculum alone. After loading with the substrate, the reactors were flushed with N2 for 5 min and immediately sealed with the rubber stopper. The reactors were then placed into the water bath, and the valve was opened to allow biogas to enter the gas collection gallery. All reactors were manually mixed once a day. Since the inoculum can provide all necessary micronutrients, no supplemental nutrients were added to the mixture. All BMP experiments were conducted in duplicate.

Table 4.2 Experimental design for BMP assay

<table>
<thead>
<tr>
<th>Reactor No.</th>
<th>Feed composition</th>
<th>Respective volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>Tap water:Inoculum</td>
<td>300:450</td>
</tr>
<tr>
<td>3, 4</td>
<td>Primary Sludge:Inoculum</td>
<td>300:450</td>
</tr>
<tr>
<td>5, 6</td>
<td>Diluted Food Waste:Inoculum</td>
<td>300:450</td>
</tr>
<tr>
<td>7, 8</td>
<td>Diluted Paper Pulp Reject:Inoculum</td>
<td>300:450</td>
</tr>
<tr>
<td>9, 10</td>
<td>Diluted Food Waste:Primary Sludge:Inoculum</td>
<td>150:150:450</td>
</tr>
<tr>
<td>11, 12</td>
<td>Diluted Paper Pulp Reject:Primary Sludge:Inoculum</td>
<td>150:150:450</td>
</tr>
</tbody>
</table>

4.3.3. Analytical methods

Liquid sample was taken from reactor once every 3-4 days using a 5-mL syringe. After immediate pH measurement, the sample was then centrifuged at 3900 rpm for 10 min and then at 18,000 rpm for 20 min at 4 °C. The supernatant was obtained for soluble COD measurement using a Hach DBR200 COD Reactor and a Hach DR/2000 spectrophotometer (program number 430 COD LR) according to the US-EPA Standard Method 5220. For analysis of volatile fatty acids (VFAs) and ammonia-N, the supernatants were further filtered through 0.45 μm cellulose filter paper. TS, VS, alkalinity and VFAs were measured according to the guidelines given by the standard methods 2540G, 2320B and 5560C.
respectively (APHA, 1998). The ammonium-N concentrations in the liquid samples were measured by injection analysis (Lachat instruments, Milwaukee, USA). CH₄ production for each reactor was measured daily through the displacement of water. The method for measuring the volume of CH₄ generate from BMP reactors was detailed in Wickham et al. (2016). CH₄ was expressed at the standard temperature and pressure condition.

The methane potential of each substrate was evaluated based on the specific methane yield, which is defined as the cumulative methane produced subtracting the inoculum contribution of the control after anaerobic degradation over the total mass of VS initially added (i.e. mL CH₄/g VS added). The results reported are expressed as the average of duplicate samples.

4.4. Kinetics modelling

4.4.1. Bio-methane production

The methane production is simulated with the modified Gompertz model as described by Lay et al. (1998):

\[ M = P \exp \left\{ - \exp \left[ \frac{e^{R_{\text{max}}(\lambda-t)}}{P} \right] + 1 \right\} \]

Eq. 1

where \( M \) is the cumulative methane yield (mL); \( P \) is the methane production potential (mL); \( R_{\text{max}} \) is the maximum methane production rate (mL/d); \( \lambda \) is the lag phase (d); \( e \) is Euler's number (≈2.71828); and \( t \) is time (d).

4.4.2. Hydrolysis process

Hydrolysis rate constants can be determined by using the cumulative biogas yield as an indirect method. The expression is shown in Eq. 2 (Angelidaki et al., 2009b):

\[ \ln \frac{P-M}{P} = -k_h t \]

Eq. 2

The value of the apparent first order hydrolysis constant \( (k_h) \) can be determined from a linear regression of \( \ln [(P-M)/P] \) against time. Data was analysed by means of statistical analyses (one way ANOVA and post-hoc least significance difference analysis) and non-linear regression modelling using software IBM SPSS statistics 23.0 (IBM, USA). \( P \)-values less than 0.05 is considered to be statistically significant.
4.5. Results and discussion

4.5.1. Process stability

A key aspect to assess anaerobic co-digestion is process stability, which is largely governed by several parameters, including pH and concentrations of intermediate products (e.g., VFAs and ammonium-N). A slightly low pH was observed in the first two days corresponding to the maximum VFAs concentrations in the same time period in all reactors (Figure 4.1). Afterwards, the pH value gradually increased and was stable around pH 8 towards the end of the experiments in all reactors. Soluble COD reached the maximum value in Day 2 then decreased to a stable level after Day 6, in accordance with methane production (section 3.2.1). The maximum soluble COD concentrations (2900 – 3400 mg/L) were higher in co-digestion reactors than those in mono-digestion reactors, possibly due to higher hydrolytic activities in co-digestion reactors (data not shown). It is noteworthy that stabilized digestate still contains a high residual soluble COD (1000 – 2000 mg/L), which is recalcitrant to biodegradation.
Figure 4.1. pH and total VFA concentration during mono-digestion and co-digestion of primary sludge and organic waste (PS, FW and PPR denote primary sludge, food waste, and paper pulp reject, respectively).

An accumulation of VFAs indicates an onset of the inhibition for methanogenesis processes. Nevertheless, microbial communities, particularly the indigenous community of methanogenic archaea, can withstand high VFAs concentrations in anaerobic co-digestion systems with a good buffering capacity (Franke-Whittle et al., 2014). In this study, low VFAs concentration (< 600 mg/L) and basic pH throughout the experiments suggest a low acidification risk that can cause system instability (Figure 1). Less than 25% of soluble COD was comprised of VFAs in all reactors, indicating a rapid conversion of organic acids to biomethane. Subsequently, it can be surmised that methanogenesis is not a rate-limiting step in this study.

As a major inhibitory intermediate in anaerobic digestion, NH$_4$-N can cause severe reduction in methane production at above threshold concentrations. Nevertheless, in this study, ammonium-N concentrations were in the range of 480 to 830 mg/L (data not shown), which is much lower than the reported value (1700 to 14000 mg/L) that could cause inhibition (Chen et al., 2008, Sung and Liu, 2003). It is noteworthy that the organic loading rate used in this study is quite low. Due to the high digestibility of some substrates such as food waste, co-digestion at a higher organic loading rate may result in NH$_4$-N inhibition (Xu and Li, 2012).

4.5.2. Process performance

4.5.2.1. Cumulative methane production

At the same loading rate, the cumulative methane productions from the co-digestion of food waste and primary sludge (ca. 2500 mL) as well as paper pulp reject and primary sludge (ca. 1230 mL) were higher than those from mono-digestion (Figure 4.2). These cumulative methane productions correspond to specific methane yields of 799 and 368 mL/g VS added, respectively (Figure 4.2). During both mono-digestion of food waste and co-digestion of primary sludge with food waste or paper pulp reject, the methane production rate was relatively high in the first 3 days (>280 mL/d). In contrast, during mono-digestion of paper pulp reject or primary sludge, the initial methane production rate was much lower (<80 mL/d). The methane production reduced to a negligible level within 2 weeks in both mono-digestion and co-digestion reactors. This was probably due to the low organic loading rate applied for all mono and co-digestion reactors (0.4 kg/m$^3$.d) in this study and higher
hydrolytic and methanogenic activities within the reactors at a high inoculum to substrate VS ratio (1.9:1). Mono-digestion of paper pulp reject showed a prolonged hydrolysis period for approximately a week before an obvious increase in cumulative methane production; while it has been observed that co-digestion of paper pulp reject with primary sludge accelerated the biogas production process by shortening the rate-limiting step, hydrolysis, to less than 4 days.

![Figure 4.2. Cumulative methane production from mono-digestion and co-digestion of sewage sludge and organic waste](image)

4.5.2.2. VS and COD removals

The total COD and VS removal efficiencies used in process performance for all co-substrates can be determined according to Eq. 3 (Wickham et al., 2016):

\[
\text{Reduction} = 100 \times \left(1 - \frac{C_{\text{CoEnd}} - C_{\text{IEnd}}}{C_{\text{Colni}} - C_{\text{IEnd}}} \right) \quad \text{Eq. 3}
\]

Where \( C_{\text{CoEnd}} \) is the parameter concentration of the co-digested sample at the end of the BMP test; \( C_{\text{Colni}} \) is the co-digested sample concentration prior to co-digestion; and \( C_{\text{IEnd}} \) is the post-digestion concentration of the inoculum. Removal efficiencies greater than 100% suggest synergistic effects exist between primary sludge and the co-substrate during the digestion.
The VS removal in the mono-digestion of paper pulp reject was the lowest at 60%. The ineffective hydrolysis of VS in digestion of the paper pulp reject correlates with the low production of biogas. Conversely, the co-digestion of primary sludge with either food waste or paper pulp reject demonstrated removal efficiencies exceeding 100% for COD and VS. The maximum COD removal rate was observed in the co-digestion of food waste with primary sludge (117.8%), meanwhile a maximum removal of VS was demonstrated by the co-digestion of paper pulp reject with primary sludge (140.0%). The high removal efficiencies during co-digestion relative to the mono-digestion of co-substrates further supports the synergisms in the substrate pairings. Particularly for paper pulp reject co-digestion, these synergisms likely derived from the optimization of basic organic nutrient compositions, such as the C/N ratio (Yen and Brune, 2007). However, other sources of synergisms must also be considered, such as an increased hydrolytic extracellular protease activity (Yun et al., 2015).

4.5.2.3. **COD balance to identify synergistic effects**

The COD balance approach has been employed to distinguish and quantify the extent of synergistic effects in this study. The COD balance is expressed in Eq. 4.

\[
\text{COD}_{\text{Ino}} + \text{COD}_{\text{PS}} + \text{COD}_{\text{OW}} = \text{COD}_{\text{Met}} + \text{COD}_{\text{Res}} \\
\text{Eq. 4}
\]

Where the input COD includes COD in inoculum (\text{COD}_{\text{Ino}}), primary sludge (\text{COD}_{\text{PS}}) and organic waste (\text{COD}_{\text{OW}}); and the output COD includes COD in the produced methane (\text{COD}_{\text{Met}}) and residue (\text{COD}_{\text{Res}}).

COD in the form of methane can be further expressed in Eq. 5 to calculate the extent of synergistic effects.

\[
\text{COD}_{\text{Met}} = \text{COD}_{\text{Met,Ino}} + \text{COD}_{\text{Met,PS}} + \text{COD}_{\text{Met,OW}} + \text{COD}_{\text{Met,Syn}} \\
\text{Eq. 5}
\]

Where the amount of COD in the form of methane produced during anaerobic co-digestion (\text{COD}_{\text{Met}}) is equal to the sum of the amount of COD in the form of methane produced from mono-digestion (i.e. inoculum (\text{COD}_{\text{Met,Ino}}), primary sludge (\text{COD}_{\text{Met,PS}}), organic waste (\text{COD}_{\text{Met,OW}})) and due to the synergistic effects (\text{COD}_{\text{Met,Syn}}).

The inoculum alone exhibited a relatively low residual specific methane yield (127 mL/g VS) after 14 days of experiment duration. The specific methane yield of the inoculum and its corresponding COD conversion rate were then allocated identically to the COD balance.
calculation for the subsequent co-digestion experiments. Biogas production from inoculum accounts for 19.9 – 21.8% of the input COD (Figure 4.3). The stabilized inoculum contained a high residual COD content, most likely consisting of recalcitrant dissolved organic matter and residual solids (Xie et al., 2015).

Figure 4.3. Substrate mixture and corresponding gas potential during co-digestion of primary sludge and food waste (A) or paper pulp reject (B) expressed as COD balance.
Mono-digestion of primary sludge presented an ultimate specific methane yield of 159 mL/g VS (Table 4.3). The COD balance calculation showed that a 25% conversion rate was achieved for methane production from mono-digestion of primary sludge. The extent of degradation for primary sludge during anaerobic digestion is much lower than that for food waste (90%), in which ultimate specific methane yield of 652 mL/g VS was obtained. Assuming the same partial conversion from input COD of inoculum and food waste to methane, the additional biogas production accounted for 32% of input COD during the co-digestion of primary sludge and food waste, which exceeded the input COD of primary sludge (26%). Since the COD conversion rate for primary sludge cannot exceed 100%, the effect of synergistic metabolism is demonstrated and estimated to be 32% in this study (Figure 3A). It is probable that the synergistic metabolism resulted in a higher extent of degradation and methane yield for both substrates (primary sludge and food waste). It is noteworthy that an additional methane production may have originated from the inoculum, as the sum of COD_PS and COD_OW (COD fraction: 56%) is less than COD_Met (COD fraction: 64.9%). This indicates a further solid reduction in digested sludge (inoculum) can be achieved during anaerobic co-digestion due to the synergistic effects (Figure 3A).

Table 4.3. Performance of organic waste mono-digestion and co-digestion with primary sludge (mean ± standard deviation of two replicates).

<table>
<thead>
<tr>
<th></th>
<th>Mono-digestion</th>
<th>Co-digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS</td>
<td>FW</td>
</tr>
<tr>
<td>Lag phase, λ (day)</td>
<td>0.91 ± 0.12</td>
<td>0.46 ± 0.09</td>
</tr>
<tr>
<td>$R_{\text{max}}$ (mL CH$_4$/day)</td>
<td>266 ± 36</td>
<td>807 ± 66</td>
</tr>
<tr>
<td>$P$ (mL)</td>
<td>527 ± 10</td>
<td>2153 ± 39</td>
</tr>
<tr>
<td>Ultimate specific methane yield (mL/g VS added)$^1$</td>
<td>159 ± 3</td>
<td>652 ± 12</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>Total COD removal rate (%)</td>
<td>75.8 ± 3.5</td>
<td>93.4 ± 4.3</td>
</tr>
<tr>
<td>VS removal rate (%)</td>
<td>83.7 ± 1.6</td>
<td>87.1 ± 2.5</td>
</tr>
</tbody>
</table>

The COD balance calculation showed a conversion rate of 31% from mono-digestion of paper pulp reject, which was slightly higher than that from primary sludge. During anaerobic co-digestion of primary sludge with paper pulp reject, an additional methane production (equivalent to 18.7% of COD fraction) was observed, which can be attributed to the
synergistic effects (Figure 3B). Since residue COD represents 45.3% of output COD fraction, the synergistic effect of co-digesting primary sludge with paper pulp reject was not as significant as that of food waste (Figure 4.3B). Compared with the marginal increase in methane yield for co-digestion of paper pulp reject, the significant synergistic interactions during co-digestion of food waste can be attributed to the additional provision of nutrients and micro/trace elements from different substrates, as the catalytic centers of the enzymes involved in methanogenic pathways are mostly micronutrient dependent (Pagés-Díaz et al., 2014, Facchin et al., 2013). It is probable that the synergistic metabolism during co-digestion of paper pulp reject and primary sludge resulted in higher specific methane yield from both substrates.

4.5.3. **Kinetics modelling**

4.5.3.1. **Bio-methane production**

The ultimate methane production potential \( P \) was obtained according to simulation results based on the modified Gompertz model. \( P \) in mono-digestion reactors (527 – 2153 mL) were much lower than that in the corresponding co-digestion reactors (1217 – 2638 mL) despite the same organic loading (Table 4.3). Compared with the ultimate specific methane yield in the mono-digestion of primary sludge, more than a 5-fold increase was observed in specific methane yield during co-digestion of food waste and primary sludge, providing support to the potential for deployment of co-digestion using co-substrates of similar properties in anaerobic digesters with spare capacity within wastewater treatment plants. The daily maximum methane production rates are 266 and 49 mL/day during mono-digestion of primary sludge and paper pulp reject, respectively; in contrast, it is 807 mL/day during mono-digestion of food waste, indicating a high proportion of readily degradable fraction in food waste for methane production (Labatut et al., 2011).

The duration of the lag phase is an important factor in evaluating the anaerobic digestion process. Methane production started after the commencement of the BMP assay for all reactors with less than 1 day of lag phase, likely due to the presence of VFAs. There is a consistent decrease in lag phase for co-digestion of food waste and paper pulp reject with primary sludge, in comparison with mono-digestion of each substrate (Table 4.3). Therefore, in practice, co-digestion of primary sludge with food waste or paper pulp reject can increase the efficiency of anaerobic digestion by reducing the effective biogas production period (Xie et al., 2011).
4.5.3.2. Hydrolysis process

The first order hydrolysis constant can be used to identify whether co-digestion can improve hydrolysis kinetics and the subsequent biogas production rates. The hydrolysis rate constant, $k_h$, is different for each substrate and co-substrates, and varies with culturing conditions. For instance, Hu and Yu (2005) estimated $k_h$ to be 0.94 d$^{-1}$ when rumen microorganisms with high cellulosylytic activity were applied in batch digestion of corn stover at 40 °C. In contrast, a much lower $k_h$ value of 0.11 d$^{-1}$ can be obtained during co-digestion of primary sludge and thickened excess activated sludge at 1:1 ratio on a volume basis (Sosnowski et al., 2008).

The first order rate constants obtained from indirect simulation (Eq. 2) using methane production rates are presented in Table 4.4. In this study, mono-digestion of paper pulp reject exhibited the lowest $k_h$ (0.18 d$^{-1}$) due to its composition, mainly consisting of lignocellulosic materials. In contrast, the primary sludge used in this study has a rather high hydrolysis rate with a $k_h$ value of 0.68 d$^{-1}$. When co-digesting with paper pulp reject, primary sludge improved the hydrolysis kinetics of the mixture of the two substrates, yielding a $k_h$ value of 0.63 d$^{-1}$. This co-digestion $k_h$ value is close to that of only primary sludge itself and significantly higher than that of paper pulp reject. The simulation results are consistent with the shorter lag phase and higher maximum methane production rate during co-digestion of paper pulp reject and primary sludge (Section 3.3.1).

Table 4.4. Hydrolysis process for mono and co-digestion of primary sludge with organic waste.

<table>
<thead>
<tr>
<th>Mono-digestion</th>
<th>Co-digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS$^1$</td>
</tr>
<tr>
<td>$k_h$ (d$^{-1}$)</td>
<td>0.68</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.95</td>
</tr>
<tr>
<td>$P$-value</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The synergistic effect for co-digestion of food waste and primary sludge was largely attributed to a greater extent of degradation and a higher specific methane yield (Section 3.3.2). Nevertheless, co-digestion of food waste and primary sludge did not increase the apparent hydrolysis rate. Thus, it is probable that the synergistic effects may be associated with the improved kinetics in acidification and methanogenesis stages. In other words, the synergistic effect during co-digestion of primary sludge and food waste was mainly attributed.
to the increased methane yields and VS removals, rather than the accelerated hydrolysis process.

It is noteworthy that the specific substrate properties and composition can affect the durations of the BMP tests. For example, the paper pulp reject is mostly cellulose in powder form, thus, resulting in a faster hydrolysis (Bayr and Rintala, 2012). In addition, a recommended highest inoculum to substrate ratio (2:1) was adopted in the BMP assay, also resulting in a higher reaction rate. These factors lead to the shorter durations of the BMP tests in this study.

4.6. Conclusion

Synergistic effects from the co-digestion of primary sludge and food waste as well as paper pulp reject was observed using BMP assay, VS and COD removal assessment, and COD balance. Co-digestion between primary sludge and either food waste or paper pulp reject increased the specific methane yields. The synergistic effect was further elucidated using kinetics modelling. Kinetics modelling revealed that a high first order rate constant may be attributed to the initial high biodegradable fraction. This study shows that co-digestion of primary sludge with food waste led to an increase in the specific methane yields rather than the reaction kinetics. On the other hand, co-digestion of primary sludge with paper pulp reject enhanced the reaction kinetics with a moderate increase in the specific methane yields. It is recommended that the synergistic effect of co-digestion be further verified using a continuous reactor.
CHAPTER 5: Anaerobic digestion of soft drink beverage waste and sewage sludge

Corresponding publication:


5.1. Introduction

Anaerobic digestion is an integral component of municipal wastewater treatment, providing the efficient stabilisation and volume minimisation of sewage sludge through biological degradation (Wan et al., 2011, Sawatdeenarunat et al., 2016). In most wastewater treatment plants (WWTPs), sewage sludge is currently digested on its own. In recent years, concern about climate change and energy security has renewed the interest in anaerobic digestion as a platform for renewable energy production from organic wastes and sewage sludge (Berkessa et al., 2018, Tuyet et al., 2016, Li et al., 2017, Dennehy et al., 2018, Chu et al., 2015, Nguyen et al., 2017). Indeed, research activities in anaerobic co-digestion have gained significant momentum over the past decade (Luostarinen et al., 2009, Nghiem et al., 2017). Several water utilities around the world have begun to explore the possibility of co-digesting organic waste with sewage sludge using the spare digestion capacity at existing WWTPs (Tampio et al., 2016, Nghiem et al., 2017).

Anaerobic co-digestion involves the pairing of two or more organic wastes with complementary characteristics (Xie et al., 2016). In the context of a WWTP, sewage sludge is rich in nutrients (i.e. nitrogen and phosphorus) and contains all necessary micronutrients for the anaerobic process. On the other hand, organic wastes are a source of carbon for methane production but are often deficient in nutrients including micronutrients. Co-digestion can also benefit the anaerobic digestion process through the dilution of inhibitory substances that may originate from either sewage sludge or organic waste co-substrates (Mata-Alvarez et al., 2011). Furthermore, co-digestion presents an array of environmental and economic benefits. These include the diversion of putrescible wastes from landfill or incineration, the increase in the generation and feasibility of onsite renewable energy production and the added revenue offered through charging gate fees (Nghiem et al., 2017).
The selection for suitable co-substrate pairing and the optimisation of mixing ratios and organic loading rates (OLRs) are paramount to the widespread adoption of the practice. The prevailing substrate selection parameter for AD co-digestion in literature concerns the total organic carbon to total nitrogen (C/N) ratio of the feed solution, with the ideal ratio generally accepted to fall in the range of 15:1 and 30:1 (Weiland, 2010). Because substrates generally do not possess an ideal C/N ratio, co-digestion can mutually improve overall performance and stability. In general, sewage sludge has a low C/N ratio. Despite a somewhat limited biomethane potential, sewage sludge can provide a high buffering capacity and all the necessary micronutrients for the anaerobic digestion process (Mata-Alvarez et al., 2014). Consequently, sewage sludge is arguably the most prevalent co-substrate in the current anaerobic digestion literature (Mata-Alvarez et al., 2011). In addition, due to the low carbon content in sewage sludge, the digesters at most WWTPs are operated at a low OLR.

The C/N ratio is not the only parameter that is important in regulating anaerobic co-digestion performance. Excessive inclusion of a carbon rich substrate (that can be rapidly hydrolysed into volatile fatty acids (VFAs), which are an intermediate product) can destabilise the anaerobic digestion process. Indeed, severe accumulation of these acids can cause the acidification of the reactor and subsequent inhibition of further digestion, i.e. methanogenesis. Furthermore, whilst it is attractive to utilise the spare digestion capacity in WWTPs for anaerobic co-digestion to enhance biogas production, there are some concerns regarding potential inhibition or negative implications for biosolids (solid fraction of digested sludge after dewatering) properties due to co-substrate addition. Thus the identification and demonstration of suitable co-substrates for sewage sludge co-digestion remain essential for the widespread uptake of the practice. Inhibition can lead to the reduction and even collapse of the biodegradation process. The primary cause of instability is the imbalance between methanogenic and acidogenic functional microbial groups, resulting from their variable requirements and growth kinetics (Chen et al., 2008). From an operational perspective, inhibition in co-digestion is seen to derive from both substrate selection and the mixing ratio adopted. These parameters can be represented through the OLR, which is subsequently the primary comparative measure for the operational ranges of different substrates.

The potential impact of co-digestion of sewage sludge upon biosolids quality and volume can restricts the implementation of the practice at full scale WWTPs. Biosolids management accounts for as much as 50% of the operational cost at some WWTPs (Semblante et al., 2014). Whilst in Europe biosolids are generally incinerated, in countries such as Australia
and the USA, biosolids volume and quality are of higher importance as they are most commonly used for land application (Nghiem et al., 2017). In addition, some co-substrates may contain contaminants such as heavy metals and persistent organic chemicals. The occurrence of these contaminants in biosolids at a high concentration can render them unsuitable for land applications (Bonetta et al., 2014, Demirel et al., 2013). At the same time, there are a range of organic wastes in the urban environment that are both abundant and benign, making them highly attractive as a co-substrate for anaerobic co-digestion.

Beverage waste is a major source of organic substrate in metropolitan areas and a potential candidate for co-digestion with sewage sludge. It includes soft drink, alcoholic beverage, pre-mixed drink, and juice. Beverage waste such as soft drink consists primarily of water along with approximately 10-12% w/v dissolved carbon, mostly in the form of sugar (Isla et al., 2013). The volume of beverage waste produced annually is enormous. About 4.5 million m$^3$/year of beverage waste is produced in Argentina. In the UK, it is estimated that 200,000 million m$^3$ of beverage waste was produced in 2012 (Isla et al., 2013, Quested et al., 2013). Disposal of beverage production waste typically involves dilution into municipal wastewater streams, onsite treatment, or land spreading. These all constitute a loss in the potential recoverable energy and may result in environmental pollution. Despite the significant volume of beverage waste and its potential as a co-substrate for biogas production, the co-digestion of beverage waste has yet to be demonstrated in the current literature.

This work focuses on the optimisation of the co-digestion ratio between beverage waste and sewage sludge and the overall OLR in terms of biomethane production and system stability. The study further seeks to determine the likely type of inhibition associated with excessive concentrations of the co-substrate. Particular emphasis is directed toward elucidating the impact of co-digestion on the digestate quality in terms of biosolids odour potential.

5.2. Materials and methods

5.2.1. Co-digestion Substrates

Anaerobically digested sludge was obtained from the Wollongong wastewater treatment plant (WWTP) in New South Wales (NSW) Australia and used as the inoculum. Primary sludge was obtained from the same plant every fortnight. A mixture of carbonated soft drinks was obtained from a commercial waste collector in NSW Australia. These soft drinks did not meet market requirements (e.g. out of date, damaged packaging, and contamination) and thus
had to be destroyed and disposed. Diet and sugar free soft drinks were excluded from this study. The primary sludge and beverage waste were stored at 4 °C in the dark. Any unused portion of these substrates was discarded after two weeks of storage.

5.2.2. Experimental systems

Three identical anaerobic digesters were operated in parallel in this study. Each digester consisted of a 28 L stainless steel conical shape reactor, a peristaltic hose pump (DULCO® Flex from Prominent Fluid Controls, Australia), a biogas counter (Ritter Company™, MilliGascounter), a thermal probe and a gas trap for biogas sampling. Biogas production data reported was adjusted to standard temperature and pressure conditions. A temperature control unit (Neslab RTE 7, Thermo Fisher Scientific, Newington, USA) was used to maintain the reactor temperature at 35 ± 1 °C. This was achieved by circulating hot water from the temperature control unit through a rubber tube that was firmly wrapped around the reactor. The reactor and pipeline were encased in polystyrene foam for insulation. The peristaltic hose pump was continuously operated to circulate the digestate at 60 L/h for mixing. Further details of these anaerobic digesters are available elsewhere (Yang et al., 2017).

5.2.3. Experimental protocol

The working volume of each reactor was set at 20 L. At the beginning of this study, all three reactors were seeded with digestate from the Wollongong WWTP and were flushed with N₂ gas for 5 min. Unless otherwise stated, the hydraulic retention time (HRT) was set at 20 days. Each day, 1 L of digestate was removed from the digester and then 1 L of co-substrate (either primary sludge or a combination of primary sludge and beverage waste) was fed into the digester via the peristaltic pump.

Table 5.1. Operating conditions of the three anaerobic digesters over the 3 experiment stages.

<table>
<thead>
<tr>
<th></th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Reactor 1</td>
<td>Reactor 2</td>
<td>Reactor 3</td>
</tr>
<tr>
<td>OLR (kg COD/m³/d)</td>
<td>1.16</td>
<td>1.16</td>
<td>1.16</td>
</tr>
<tr>
<td>Beverage waste (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Duration (d)</td>
<td>52</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>OLR (kg COD/m³/d)</td>
<td>3.03</td>
<td>2.08</td>
<td>1.16</td>
</tr>
<tr>
<td>Beverage waste (%)</td>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Duration (d)</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>OLR (kg COD/m³/d)</td>
<td>3.80</td>
<td>3.88</td>
<td>1.16</td>
</tr>
<tr>
<td>Beverage waste (%)</td>
<td>30</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
The experiment was conducted over three stages (Table 5.1). In Stage 1, all three reactors were fed with primary sludge for 7 weeks to establish the baseline conditions. In the subsequent stages, Reactor 3 was used as the control system (same operating condition as in Stage 1) while Reactors 1 and 2 were used to evaluate the co-digestion of sewage sludge and beverage waste. In Stage 2, in addition to primary sludge, beverage waste was also fed into Reactors 1 and 2 at 20 and 10% (vol/vol) of the total feed, respectively. In Stage 3, the portion of beverage waste fed into Reactors 1 and 2 was increased further to 30 and 20% (vol/vol) respectively. It is noted that the HRT of Reactor 2 was shortened to 15 d to achieve a similar OLR in both reactors.

5.2.4. Analytical methods

TS, VS, alkalinity, total COD and soluble COD, pH and total organic acids (TOA) of the digestate were measured weekly. The primary sludge feed and beverage waste were also characterised on a weekly basis. COD measurements were conducted using a Hatch DRB200 COD Reactor and Hatch DR3900 spectrophotometer (program number 435 COD HR) following the US-EPA Standard Method 5220. Samples were diluted with milli Q water on the basis of mass prior to COD measurement, thus concentrations are reported in the units mg/kg. Biogas composition analysis was conducted on a weekly basis by a portable gas analyser (GA5000 Gas Analyser, Geotechnical Instruments (UK) Ltd., England) using the gas trap to store the required 1 L gas sample prior to measurement. The details of these analytical techniques are available elsewhere (Yang et al., 2016, Nghiem et al., 2014c).

Odour measurement was conducted based on an incubation technique previously reported by Glindemann et al. (2006) This method allows for the monitoring of hydrogen sulphite and six other sulphur bearing odour compounds. In brief, digestate was dewatered by laboratory centrifuge using the method previously developed by To et al. (2016). Then, 25 g of biosolids cake was collected into a 500 mL PET bottle. The bottle was sealed using a rubber cap and incubated at 28±1°C. The head space was extracted using a syringe at a specific time interval for Gas Chromatography – Mass Spectrometry analysis. The results are reported as volumetric concentration in the incubation bottle headspace.

<table>
<thead>
<tr>
<th>HRT (d)</th>
<th>20</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (d)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
5.3. Results and discussion

5.3.1. Substrate characteristics

Key properties of the inoculum, primary sludge and beverage waste are summarised in Table 5.2. Beverage waste contained a significantly higher organic fraction mostly in the form of dissolved sugars than the primary sludge. On the other hand, VS of the beverage waste is only marginally higher than that of primary sludge (Table 5.2). Given the high carbon content and easily degradable organics (owing to the high sugar content) of beverage waste, its co-digestion with nutrient rich, high buffering capacity primary sludge can provide complementary benefits to both co-substrates. Co-digestion is necessary to achieve a balance between organic carbon and nutrients to prevent pH-derived inhibition based on the rapid formation of intermediate products, specifically volatile fatty acids.

Table 5.2. Characteristics of the inoculum, primary sludge feed, and beverage waste (mean ± standard deviation of at least 5 samples).

<table>
<thead>
<tr>
<th></th>
<th>Inoculum</th>
<th>Primary sludge</th>
<th>Beverage waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>0.85</td>
<td>1.7 ± 0.2</td>
<td>4.73 ± 0.5</td>
</tr>
<tr>
<td>VS (%)</td>
<td>0.56</td>
<td>1.3 ± 0.1</td>
<td>4.59 ± 0.5</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>65</td>
<td>78 ± 1</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
<td>6.1 ± 0.5</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>COD (mg/kg fresh weight)</td>
<td>8300</td>
<td>22300 ± 1750</td>
<td>204000 ± 27000</td>
</tr>
</tbody>
</table>

5.3.2. Process performance

5.3.2.1. Biomethane production

In Stage 1, the three reactors were preconditioned under the same operating parameters. As expected, biomethane production from all three reactors was almost identical during this stage (Figure 5.1 A). In Stage 2, stable biomethane production was observed during the co-digestion of sewage sludge with beverage waste co-digestion ratios of 10 and 20%, corresponding to an increase in OLR of 86 and 171% and in overall biogas production of 89 and 191% (compared to the control reactor).

During the early phase of Stage 2, an elevated methane content of approximately 70% was observed in the biogas produced from Reactors 1 and 2 compared to the baseline value of 60% from Reactor 3 (Figure 5.1 B). However, at the end of Stage 2, the methane content in biogas from Reactors 1 and 2 returned to the baseline. The brevity of this increase in methane content suggests the change may result from the transitory condition of co-digestion with carbohydrate rich co-substrates. A permanent increase in methane content was observed by
Jang et al. (2016) during the co-digestion of food waste and waste activated sludge, corresponding to higher food waste mixing ratios. However, it is noted that the initial methane content recorded in previous feeding conditions in Jang et al. (2016) was unusually low (i.e. only 50%) and their observations concern the co-digestion of different co-substrates. The disparity between our findings and that from Jang et al. (2016) highlights the need for further research to ascertain the potential effect of co-digestion on methane content.

In addition to the overall biomethane production, co-digestion may also affect the specific methane yield. Over the duration of the experiment the methane yield of the sewage sludge was 284 mL CH\(_4\)/g COD added, suggesting a highly degradable sludge substrate compared to typical values in literature, often in the range of 188 to 214 mL CH\(_4\)/g COD (Astals et al., 2013). It is noted that in Stage 2, the specific methane yield of the control (Reactor 3) decreased slightly from 300 to 275 mL/g COD added. On the other hand, the specific methane yield of Reactors 1 and 2 were stable despite the addition of beverage waste (Figure 5.2). This is consistent with the findings by Razaviarani et al. (2013), who observed a slight increase in the specific methane yield when co-digesting sewage sludge with glycerine. Further results suggest the increase was likely due to the higher digestible fraction of beverage waste relative to sewage sludge, rather than due to any synergistic effect.

Inhibition of both co-digestion reactors was observed during Stage 3 at an OLR of ~3.8-3.9 kg COD/m\(^3\)/d. During Stage 3, both the volume and methane content of the biogas declined sharply in Reactors 1 and 2. When applying mono-digestion of a similar soft-drink wastewater, Redzwan and Banks (2007) observed complete inhibition of methanogenic processes at a loading rate of 1.33 kg COD/m\(^3\) in batch experiments, which was attributed to alkalinity loss (from 2300 mg/L to 1000 mg/L) and accumulation of volatile fatty acids, which reached a concentration of 1500 mg/L. Whilst this inhibitory OLR value was obtained in batch experiments and therefore is not directly comparable, it demonstrates the risk of overloading when co-digesting with organic rich co-substrates. Furthermore, the stable operation demonstrated at a much higher OLR in this study suggests that co-digestion of the substrate with sewage sludge can improve digestion stability.
Figure 5.1. Biomethane Production: (A) Daily biomethane production at different experimental stages and (B) Methane content in biogas during different experimental stages (temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1).
5.3.2.2. Digestate quality

The removal efficiency of both VS and COD was constant throughout Stages 1 and 2 of the experiment, indicating that co-digestion does not significantly impact on the performance of anaerobic digestion (Figure 5.3). Nevertheless, a small increase in VS and COD in the digestate from Reactors 1 and 2 during Stage 2 could be observed. At steady-state condition in Stage 2, the average VS content in the digestate from Reactor 1 and 2 by 21% and 27% respectively, compared to Stage 1. Similarly, the average total COD in the digestate from Reactor 1 and 2 also increased by 18% and 23% compared to Stage 1. This is a small increase compared to the increase in OLR of 171 and 86% in Reactor 1 and 2, respectively. Indeed, this small increase in VS and COD in the digestate is expected and can be attributed to a high OLR value. A greater biomass would be required to degrade the increased concentrations of organic matter in the reactor, which would correspond to higher concentrations of VS and COD in the biosolids. During Stage 2 the ratio of VS/TS in the
digestate was relatively constant in Reactors 1, 2 and 3, with ratios of 63, 65 and 62% respectively.

Figure 5.3. Biosolids stabilisation: (A) Volatile removal efficiency and (B) total COD removal efficiency with corresponding OLR value (temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1).

Soft drinks only contain sugar and flavours, thus as expected, their co-digestion with sewage sludge does not result in any notable impact on biosolids odour. Similar to the VS and COD removal data, under a stable condition (Stage 2), the odour potential of biosolids samples from all three Reactors was almost identical (Figure 5.4). It is also noteworthy that among the seven sulphur bearing odour compounds (namely H₂S, CH₃S, C₂H₆S, C₃H₈S₂, CS₂, (CH₃)₂S, COS) from biosolids monitored in this study, only hydrogen sulphide (H₂S) was prevalent in all samples. In most instances, the concentration of H₂S in the head space of biosolids samples from Reactor 3 (mono digestion) was slightly higher than that from Reactor 1 and 2 (co-digestion). The decreased H₂S production is likely due to lower concentrations of reducible sulphur in the beverage wastes relevant to the sewage sludge.
5.3.2.3. Impact of co-digestion on specific methane yield

The co-digestion of beverage waste with primary sludge was evaluated using a COD balance approach, previously adopted in Aichinger et al. (2015). A COD balance was used to represent the digestion performance of each of the reactors during Stage 2 (Figure 5.5). The specific biomethane yields and subsequent COD consumption were determined for the monodigestion of each substrate. Reactor 3 demonstrated a biomethane yield of 275 mL/g COD added, which was used to represent sewage sludge COD consumption. Meanwhile a yield of 321 mL/g COD was adopted for beverage waste, derived from previous biomethane potential evaluation of the substrate (Wickham et al., 2016). Based on this data, the conversion of COD into biomethane in each substrate precisely matched their performance during monodigestion. Results in Figure 6 show that beverage waste was fully digested. In other words, beverage waste addition did not result in any discernible increase in the COD content of the final digestate. On the other hand, data in Figure 5.5 cannot be used to confirm the synergistic effect of sewage sludge and beverage waste co-digestion. The lack of observable synergism is not unexpected as substrates rich in rapidly degradable organic matter have been observed to produce little to no synergetic effects during co-digestion (Jensen et al., 2014).
Figure 5.4. Odour generation over 15 days from biosolids produced during Stage 2, sampled on (A) day 64, (B) day 71 (C), day 78 and (D) day 85 (Reactor 1: co-digestion with 20% (v/v) beverage waste, Reactor 2: co-digestion with 10% (v/v) beverage waste and Reactor 3: mono-digestion of primary sludge) temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1.
Figure 5.5. Average substrate mixture and corresponding gas production represented as COD balance during Stage 2 for (A) Reactor 1 (co-digestion with 20% (v/v) beverage waste), (B) Reactor 2 (co-digestion with 10% (v/v) beverage waste) and (C) Reactor 2 (mono-digestion of primary sludge) Temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1.
5.3.2.4. Process stability

Process stability was evaluated through the measurement of soluble COD, alkalinity, TOA, and pH. Stable co-digestion was observed in each of the parameters throughout Stages 1 and 2 (Figure 5.6). A slight decline in alkalinity in Reactors 1 and 2 relative to the control was observed. However, this was not accompanied by any accumulation of TOAs or significant drop in pH, indicating the degradation of BW generates less alkalinity than that of sewage sludge. A sharp decline in the stability of Reactors 1 and 2 occurred with the commencement of Stage 3. The higher OLR values adopted in these reactors instigated the rapid accumulation of COD in the form of organic acids, leading to the consumption of alkalinity and sharp decline in pH. Indeed, in Stage 3, the profiles of soluble COD and TOA in Reactors 1 and 2 closely resemble each other (Figure 5.6). The progression of inhibition aligns well with previous findings, as carbohydrate rich co-substrates are known to pose risks in the accumulation of intermediaries such as volatile fatty acids (Astals et al., 2014). It is noteworthy that a similar rate of inhibition was observed in both in Reactors 1 and 2. In Stage 3, these reactors have similar OLR value but different HRT (20 vs 15 days). These results suggest that inhibition was intrinsically due to a high OLR value rather than the sudden variation in organic loading.
Figure 5.6. Basic Stability Parameters: Soluble COD, Alkalinity, TOA and pH (Temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1).

5.4. Conclusions

Soft drink beverage waste (BW) was evaluated for anaerobic co-digestion with sewage sludge for the first time. Biogas production increase was proportional to the increase in organic loading rate (OLR) from BW addition. The OLR increase of 171% corresponding to 20%(v/v) BW in the feed was the optimum co-digestion ratio, and resulted in an biogas production increase of 191%. Under this optimum condition, co-digestion with BW did not result in any significant impact on digestate quality and biogas composition. Furthermore, the biosolids odour in the form of H₂S was seen to marginally improve during co-digestion, likely due to lower sulphur concentrations in the beverage waste relative to primary sludge. The results suggest that sewage sludge can support about 2 kg COD/m³/d OLR increase from a carbon rich co-substrate. The use of a COD balance approach suggested no synergistic
effect was present and that the degradation of the substrates was identical during mono-
digestion and co-digestion.
CHAPTER 6: Pilot Evaluation of Anaerobic Co-digestion between Beverage Waste and Sewage Sludge

**Corresponding Publication:**


**6.1. Introduction**

Anaerobic digestion involves several biological transformation steps in which microorganisms break down biodegradable materials in the absence of oxygen to produce biogas and stable solid residues. Biogas (specifically the biomethane) can be utilised to generate electricity and heat, while solid residues can be beneficially reused for land applications. As a mature technology, anaerobic digestion has been deployed for a range of applications including the treatment of sewage sludge, landfill leachate, food waste, livestock manure, and agriculture residues (Jeong et al., 2019, Begum et al., 2018, Tuyet et al., 2016, Xie et al., 2017a, Yang et al., 2017). In recent years, a new approach known as co-digestion has been explored and applied to cater for a wider range of organic wastes (Xie et al., 2018).

Co-digestion refers to the utilisation of two or more compatible organic substrates in the anaerobic digestion process(Xie et al., 2017a, Jabeen et al., 2015, Ratanatamskul and Manpetch, 2016, Liu et al., 2016). Successful implementation of co-digestion at wastewater treatment plants (WWTPs) can increase biomethane production for subsequent energy generation and, in addition, divert organic waste away from landfill (Xie et al., 2018). Improvements in anaerobic digestion performance can occur due to the balancing of the key stoichiometric ratios, the provision of sufficient micronutrients and the dilution of inhibitors (Mata-Alvarez et al., 2014).

Co-digestion within municipal WWTPs is an attractive option as existing anaerobic digestion infrastructure can be utilised without significant capital investment (Nghiem et al., 2017). Sewage sludge usually has low organic carbon content but can produce a high alkaline buffering capacity to maintain stable anaerobic digestion operation. Thus, co-substrates adopted for co-digestion with sewage sludge are typically carbonaceous and higher in organic content (Koch et al., 2016).
Adopting co-digestion allows WWTPs to increase biomethane production for electricity generation via gas combustion to offset their energy consumption and reduce the carbon footprint of wastewater treatment (Silvestre et al., 2015). The addition in biomethane production can be particularly important for small WWTPs where the current biomethane output is not sufficient to justify for electricity production due to the high capital and ongoing maintenance cost of the co-generation engine. In the United States for example, small plants with wastewater influents less than 18.9 ML/d are considered to have insufficient biomethane production for economically feasible electricity generation (Shen et al., 2015). The biomethane production threshold can vary from country to country, depending on operating factors, electricity prices, and energy regulations and policies (Fersi et al., 2014, Edwards et al., 2015a). Nevertheless, it is well established that co-digestion can increase biomethane production to above the economic threshold thus facilitating biomethane utilisation at small WWTPs. In addition to biomethane utilisation for electricity generation, co-digestion at WWTPs can also generate a new source of income to the plant through gate fees or commercial charge for accepting organic wastes (Nghiem et al., 2017).

Whilst co-digestion has been intensively investigated at laboratory-scale (Siddique and Wahid, 2018), there remain several challenges in full-scale implementation (Xie et al., 2018). These challenges cover several aspects including energy pricing structure, regulatory uncertainty regarding gate fee, and lack of operational experience (Pfluger et al., 2019). In particular, Pfluger et al., (2019) highlighted the lack of pilot and full-scale experience as an underlying factor contributing to the reluctance of wastewater treatment facilities in the state of Colorado (USA) to change their sludge digestion practices and adopt co-digestion. Indeed, the literature is still dominated by laboratory-scale and batch-test studies which are well controlled and thus can provide an idealistic performance scenario but are not capable to simulating realistic co-digestion operation (Xie et al., 2018). Overcoming these obstacles requires, among other steps, the identification and demonstrated operation of alternate substrates.

A major source of organic substrate from metropolitan areas and can potentially be co-digested with sewage sludge is beverage waste. The annual production of beverage is enormous and due to various reasons (e.g. damaged packaging, use by date expiration, or contamination) a faction of this beverage ends up as waste materials. As an example, Isla et al., (2013) estimated that 4.5 million m$^3$ of soft drink beverage waste is generated each year in Argentina. Beverage waste has a large content of readily digestible organic carbon, thus, it
has to be processed prior to disposal. Current disposal method of beverage waste typically involves dilution into municipal wastewater streams, onsite treatment, and land spreading.

This study aims to evaluate the co-digestion of four types of beverage wastes (namely beer, wine, soft drink and fruit juice) with primary sewage sludge in a research anaerobic plant that can closely resemble full scale operation. These four types of beverage waste are acquirable from commercial waste collectors in sufficient quantity for anaerobic co-digestion. The suitability of beverage waste for co-digesting with primary sludge was investigated in terms of methane production, stabilisation of organic matter and reactor stability.

6.2. Materials and methods

6.2.1. Pilot plant design

Figure 6.1. (A) Schematic diagram of the research anaerobic plant; and (B) a panoramic photograph of the plant inside a 24 ft shipping container.
A research anaerobic plant was installed at the Shellharbour WWTP for this study. The plant was housed in a 24 ft shipping container and could be operated automatically via a supervisory control and data acquisition (SCADA) system. It could also be controlled remotely via a secured internet connection. The research plant contained two identical anaerobic digestion systems (Figure 6.1), each system consisted of a 1000 L anaerobic conical stainless-steel reactor, a recirculation pump for mixing, and biogas storage. Both digesters were equipped with a biogas a thermal mass flow meter (model SLA5800, Brooks Instrument, USA) for continuous monitoring and recording of biogas production via the SCADA system. Biogas production data reported in this study were adjusted to standard temperature and pressure conditions. The reactors and all pipework were wrapped with insulating foam to prevent heat loss. Heating was supplied by circulating heated water through a water jacket encasing the bottom of the reactor.

6.2.2. Co-digestion substrates

Four beverage wastes - namely beer, wine, soft drink and juice - were obtained from a commercial waste collector in NSW and used as co-substrate in this study. They were stored at 4 °C in a mobile cool room until utilised. Each of these wastes was a mixture of expired, contaminated or damaged beverages deemed unsatisfactory with respect to market standards and thus must be destroyed and disposed. The beer waste comprised of both beer and pre-mixed alcoholic beverage. Wine waste contained mostly red wine that was not suitable for consumption. The soft drink was a combination of sugar-rich carbonated drinks (e.g. Coca Cola, Sprite, and Pepsi). Whilst the juice waste was a mixture of several kinds of fruit juice including orange, apple, mango, and berry. Primary sludge was obtained directly from the Shellharbour WWTP and used as the primary substrate in this study.

6.2.3. Experimental protocol

The two anaerobic digestion systems (denoted as A and B) of the research plant were operated in parallel. They were initially seeded with 200 L of digested sludge from a full scale anaerobic digester. Feeding was conducted in a semi-continuous regime, which involved four discharging/feeding cycles of 7.5 L per day at a feeding rate of 1 L/min. Both systems were operated at a hydraulic retention time of 20 days, corresponding to an active sludge volume of 600 L. Temperature inside the digester was maintained at 35 ± 1 °C throughout the experiment by the SCADA system. The external recirculation pumps were operated continuously at 25 L/min to achieve 60 volume turn overs per day.
The experiment program was comprised of seven key operating phases (Table 6.1). In each phase, digester A was used for co-digestion while digester B was operated in monodigestion mode for comparison. In other words, the feed to digester A consisted of a mixture of sewage sludge and beverage waste while digester B was fed with only sewage sludge. Apart from the difference in the feed, all operating conditions of digester A and B were identical.

Each experimental phase was operated until all key performance indicators (e.g. biogas production and VS removal) were stable and the digesters had reached steady state conditions. One exception was during the co-digestion between wine waste and primary sludge, in which the experiment was terminated due to poor performance of the co-digestion system. Immediately after terminating the co-digestion experiment with wine waste, for consistency both digesters were reseeded with digested sludge from a full scale anaerobic digester. Co-digestion was resumed when both digesters have shown similar and satisfactory performance in terms of biogas production and VS removal.

Table 6.1: Organic loading rate during co-digestion of various co-substrates in comparison to the control digester.

<table>
<thead>
<tr>
<th>Phase</th>
<th>No of days</th>
<th>Reactor</th>
<th>Substrates</th>
<th>Co-substrate content (v/v%)</th>
<th>OLR (kg COD/m³/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>A</td>
<td>PS + Beer Waste</td>
<td>10</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.77</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>A</td>
<td>PS + Wine Waste</td>
<td>10</td>
<td>3.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>2.20</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>A</td>
<td>PS + Soft drink</td>
<td>10</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.48</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>A</td>
<td>PS + Juice waste</td>
<td>10</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.80</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>A</td>
<td>PS + Juice Waste</td>
<td>20</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.72</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>A</td>
<td>PS + Juice Waste</td>
<td>30</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.28</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>A</td>
<td>PS + Juice Waste</td>
<td>40</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>PS</td>
<td>0</td>
<td>1.49</td>
</tr>
</tbody>
</table>

6.2.4. Analytical techniques

Sampling of digestate and primary sludge was conducted three times a week. pH was immediately measured using a portable pH probe (Thermo Scientific, Australia). Total solids (TS) and volatile solids (VS) measurement was also conducted immediately after sample collection. Total COD (tCOD) was measured using a Hatch DRB200 COD Reactor and
Hatch DR3900 spectrophotometer (program number 435 COD HR) in compliance with the US-EPA Standard Method 5220. Sludge samples were centrifuged for 10 min at 3750 rpm (Allegra X-12R centrifuge, Beckman Coulter, Australia) and filtered (1 μm filter paper, Filtech, Australia) to obtain the supernatant. Subsequent testing of the supernatant included total organic acids (TOA), alkalinity and soluble COD (sCOD) in accordance with standard methodology. TOA and alkalinity were measured weekly. The biogas content was analysed using a portable gas analyser (GA5000 gas analyser, Geotechnical Instruments Ltd., UK) three times a week (Nghiem et al., 2014c).

6.3. Results and discussion

6.3.1. Substrate characteristics

The beverage wastes in this study possessed considerably higher organic contents than that of primary sludge (Table 6.2). All four types of beverage wastes are rich in COD, in the range from 151 g/L (beer waste) to 206 g/L (wine waste), which is approximately 4 to 6 times higher than the primary sludge. Organics in beverage waste are mostly in the form of solubilised carbohydrates, thus they are expected to be more rapidly degradable than primary sludge. Indeed, these beverage wastes had been hydrolysed and were significantly more acidic than primary sludge. Their pH was in the range of 2.61 (soft drink waste) and 3.62 (juice waste).

Table 6.2: Key properties of primary sludge, and individual types of beverage waste in this study.

<table>
<thead>
<tr>
<th></th>
<th>Primary Sludge</th>
<th>Beer</th>
<th>Wine</th>
<th>Soft drink</th>
<th>Juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.06</td>
<td>3.13</td>
<td>3.44</td>
<td>2.61</td>
<td>3.62</td>
</tr>
<tr>
<td>Total COD (g/L)</td>
<td>36.4</td>
<td>151</td>
<td>206</td>
<td>128</td>
<td>126</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>20.9</td>
<td>53.2</td>
<td>60.3</td>
<td>82.1</td>
<td>47.0</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>25.8</td>
<td>59.3</td>
<td>63.5</td>
<td>90.5</td>
<td>65.3</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>80.9</td>
<td>89.7</td>
<td>95.0</td>
<td>90.7</td>
<td>72.0</td>
</tr>
</tbody>
</table>

The high acidity these beverage waste co-substrates indicates that they are unsuitable for mono-digestion. Rapidly degradable substrates have a greater tendency to cause volatile acid accumulation. The initial acidity of the substrates also exacerbates the likelihood of process inhibition due to extreme acidity. The pairing of beverage wastes and primary sludge for co-digestion is justified as the latter possesses the necessary buffering capacity, macronutrients and micronutrients to balance carbon rich substrates (Mata-Alvarez et al., 2014, Nghiem et al., 2017). The organic rich, carbonaceous beverage wastes could improve the carbon/nitrogen balance and biomethane potential of sewage sludge. The beverage wastes have comparable
primary characteristics in terms of pH, TS, VS and tCOD. Consequently, rapid transition between these co-substrates is feasible.

Of a particular note, tCOD appears to be a better indicator for organic content of the sample than VS for determining the increase in organic load rate from the addition of beverage waste (Table 6.3). Whilst tCOD and VS can both be used to evaluate the organic content of substrates, they are not interchangeable. tCOD represents the oxygen required to oxidise both organic and inorganic substances in either dissolved or suspended form. By contrast, VS is the mass of solids (including dissolved solids) that can evaporate at 510 °C. Since beverage wastes may contain volatile organic substances (e.g. ethanol) that cannot be accounted for as solid materials, VS measurement may not represent the correct organic content of the sample. Indeed, Table 3 shows that OLR increase based on VS measurement is substantially lower than that based on tCOD measurement for beer, wine, and juice waste. Soft drink is the only exception in Table 6.3. This is because the key carbohydrates in soft drink, sucrose and fructose have high boiling points (186 °C and 440 °C). Thus they can be fully accounted for by VS measurement. This finding has important practical implications as most WWTPs rely solely on VS measurement (which requires less equipment and consumables than tCOD measurement) for determining the organic loading rate.

Table 6.3: OLR increase in percentage (%) determined by COD and VS at 10% v/v substrate addition.

<table>
<thead>
<tr>
<th></th>
<th>Beer</th>
<th>Wine</th>
<th>Soft drink</th>
<th>Juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in tCOD loading rate (%)</td>
<td>33</td>
<td>37</td>
<td>33</td>
<td>25</td>
</tr>
<tr>
<td>Increase in VS loading rate (%)</td>
<td>14</td>
<td>11</td>
<td>37</td>
<td>17</td>
</tr>
</tbody>
</table>

6.3.2. **Biogas production**

Co-digestion of beverage wastes with primary sludge was not observed to adversely affect the composition of biogas, with the exception of the wine substrate (Table 6.4). Methane content was within the range of 60 – 70% throughout the experiment. A slight increase in the methane content of the co-digestion reactor was observed during the soft drink addition phase, reaching as high as 74.2% before returning to typical values. The same phenomenon was noted in prior studies and is seen to be a transitional state in the co-digestion of soft drink waste (Wickham et al., 2018). In most cases, the co-digestion reactor showed a slightly lower H₂S concentration than the control reactor, explicable through the absence of sulphur and greater volumetric biogas potential of the beverage wastes, which instigated a dilution effect.
in terms of H$_2$S content. The inverse to this trend was observed in the wine waste co-digestion phase. A higher H$_2$S production can be explained by the use of sulphite in wine production as a preservative.

Table 6.4: Average biogas composition for each operational condition (operating condition of phase 1 to 7 are as described in Table 6.1; mean ± standard deviation of at least 20 readings).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Reactor A – Co-digestion</th>
<th>Reactor B – Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH$_4$ (%)</td>
<td>CO$_2$ (%)</td>
</tr>
<tr>
<td>1</td>
<td>64.7 ± 2.6</td>
<td>35.3 ± 2.6</td>
</tr>
<tr>
<td>2</td>
<td>60.6 ± 6.1</td>
<td>39.4 ± 6.1</td>
</tr>
<tr>
<td>3</td>
<td>68.9 ± 4.8</td>
<td>31.1 ± 4.8</td>
</tr>
<tr>
<td>4</td>
<td>62.9 ± 1.6</td>
<td>37.1 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>63.3 ± 2.3</td>
<td>36.6 ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>61.1 ± 1.3</td>
<td>38.9 ± 1.3</td>
</tr>
<tr>
<td>7</td>
<td>61.7 ± 1.1</td>
<td>38.3 ± 2.2</td>
</tr>
</tbody>
</table>

Biomethane production and specific methane yield were derived from biogas flow rate and tCOD content data. Considerable variation in the methane yield of the control reactor suggests significant temporal variation in the degradability of primary sludge throughout the experiment (Table 6.5). In fact, variation in tCOD content of the primary sludge was a major factor influencing the methane yield of the substrates. This observation indicates that reactor performance during the mono-digestion of sewage sludge cannot be extrapolated as a reference point during co-digestion research and that the inclusion of a control reactor is essential for a systematic comparison.

With the exception of wine waste, all other types of beverage waste in this study showed significantly improved biomethane production compared to the mono-digestion of sewage. Consequently juice, soft drink and beer wastes present appropriate co-substrates for co-digestion in municipal wastewater treatment. Co-digesting soft drink with sewage sludge at 10% (v/v) led to 33% increase in OLR in terms of tCOD and 41% increase in biomethane production relative to the control reactor. Methane production increased with higher mixing ratios of juice waste. Co-digesting juice waste with sewage sludge at 40% (v/v) resulted in 128% increase in OLR in terms of tCOD and 137% increase in biomethane production compared to the control reactor.

The organic content in beverage waste is mostly solubilised carbohydrates. As a result, the increase in biomethane production was driven primarily by the increase in OLR from beverage waste addition. In fact, the proportionate increase in methane production was
always higher than the increase in OLR from co-substrate addition for all types of beverage waste in this study (with the exception of wine waste) (Table 6.5). These results indicate that beverage waste was more readily biodegradable than primary sludge. The specific methane yield of the co-digestion digester and control digester can be calculated from data in Table 6.5. By comparing the co-digestion and control digester at 10% v/v co-substrate addition, the specific methane yields of beer, soft drink and juice were calculated to be 146, 263 and 260 L/kg COD added respectively. The yields for soft drink and juice are high but are still lower than the theoretical specific methane yield of 350 L/kg COD added for complete COD conversion. Thus, there is a possible repercussion that although readily degradable, beverage waste addition may incur COD residue in the digested sludge. This issue will be further delineated in the next section.

Table 6.5: Biomethane production from anaerobic co-digestion between sludge and different beverage wastes (mean ± standard deviation of at least 20 readings).

<table>
<thead>
<tr>
<th>Phase</th>
<th>OLR increase in tCOD (%)</th>
<th>Daily methane production (L)</th>
<th>Methane production increase (%)</th>
<th>Specific methane yield (L/kg COD added)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Co-digestion</td>
<td>Control</td>
<td>Co-digestion</td>
</tr>
<tr>
<td>1</td>
<td>33</td>
<td>190 ± 75</td>
<td>137 ± 64</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>144 ± 75</td>
<td>175 ± 75</td>
<td>-18</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>280 ± 76</td>
<td>199 ± 61</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>218 ± 87</td>
<td>133 ± 72</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>241 ± 59</td>
<td>84 ± 32</td>
<td>187</td>
</tr>
<tr>
<td>6</td>
<td>116</td>
<td>342 ± 70</td>
<td>157 ± 33</td>
<td>118</td>
</tr>
<tr>
<td>7</td>
<td>128</td>
<td>398 ± 71</td>
<td>168 ± 24</td>
<td>137</td>
</tr>
</tbody>
</table>

6.3.3. **Digestate quality**

With the exception of wine waste, co-digestion involving all other types of beverage waste did not result in any observable impact on the quality of digestate (Figure 6.2). Despite a higher OLR during co-digestion, tCOD and VS contents in the digestate from the co-digestion reactor were similar to those in the control reactor. In contrast to the concern about organic residue from the co-substrate noted in section 3.2, results in Figure 4 show insignificant contribution from undigested beverage waste possibly because of the small fraction of beverage co-substrate of only 10 to 40% in this study.

The only observable difference in tCOD and VS content in the digestate from the co-digestion and control reactors was when inhibition caused by wine waste (phase 2 of the study). In phase 2, when wine waste was used as the co-substrate, tCOD and VS in the digestate of the co-digestion reactor were 22.7 and 14.4% higher than those from the control
reactor, respectively. The discrepancy between control and co-digestion reactors was more pronounced when comparing the reactors in terms of soluble COD (Figure 6.2).

The changeover between co-substrates did not instigate any observable impact on digestate quality. Rather, the bio-community of the co-digestion reactor proved capable of rapidly shifting between the differing beverage wastes without any lag phases. This has important implications for full-scale application of beverage waste co-digestion as operators are likely to be required to swap between substrates. This derives from factors such as the seasonality of co-substrate supply and limitations in on-site storage capacity. The observed interchangeability of the beverage wastes is likely due from the similarities in their composition.

![Graph](image)

**Figure 6.2. Concentration of organics in the digestate of beverage wastes and sewage sludge co-digestion and mono-digestion.**

6.3.4. Process stability

Notwithstanding the disturbance during wine addition, the co-digestion process was relatively stable in terms of alkalinity, TOA and pH throughout this study (Figure 6.3). Co-digestion with beer and juice wastes was stable across all experimental phases. Alkalinity,
TOA and pH profiles of the co-digestion reactor were identical to that of the control across each of the conditions.

Soft drink waste addition initially resulted in a slight increase in the TOA content in the digestate; however, this period immediately followed a recovery stage (Figure 6.3). The variation in the co-substrate content in feed constituted a significant increase in organic loading, resulting in a limited and short lasted increase in TOA but with no impact on other stability parameters. Furthermore the TOA matched that of the mono-digestion reactor by the end of the soft drink co-digestion condition.

The pH profile highlights two major periods of stability decline in the reactors. The first of which, occurring during the first stage of beer co-digestion between days 15-25, can be attributed to a malfunction in the heating equipment. Overheating of both reactors led to the inhibition of the bio-community. With methanogens being the most susceptible to heat, the issue resulted in the destabilisation of the digestion stages. Insufficient methanogenesis relative to acidogenesis activity caused the rapid accumulation of organic acids, consumption of alkalinity and decline in pH.

The second instance of reactor destabilisation occurred during the wine waste co-digestion phase. Whilst the slight accumulation of organic acids and the subsequent pH decline were observed in the co-digestion reactor, the OLR value of the co-digestion reactor was 3.01 kg COD/m$^3$/d which had not exceeded the threshold value for anaerobic digestion. In other words, high OLR value was not the main cause of inhibition when wine waste was used as a co-substrate. Indeed, stable operation was observed in this study at comparable or even higher OLR values when other types of beverage waste (e.g. juice waste) were used (Figure 6.3). The inhibition may have originated from the high concentration of sulphites or polyphenols used in wine manufacture (Da Ros et al., 2014). Following this period of inhibition the co-digestion reactor was reseeded with digested sludge then fed with primary sludge until biomethane production matched that of the control. The extended duration of this recovery time suggests lingering inhibitory compounds continued to hamper reactor digestion until sufficiently diluted.
Figure 6.3. The evolution of stability parameters during the co-digestion of beverage wastes and sewage sludge and mono-digestion.

6.4. Conclusions

With wine waste being the only exception, all other types of beverage waste investigated in this study were suitable for co-digestion. The methane content in biogas from all co-digestion experiments was stable in the range of 60 to 65%. In addition, co-digestion with organic rich substrates resulted in a lower H$_2$S content in biogas, possibly due to the dilution effect. The increase in OLR due to beverage waste co-substrate addition was converted to a proportional increase in biogas production. Co-digestion involving soft drink, beer and juice did not result in any observable impact on the quality of digestate in terms of total COD and VS. In addition, the interchanging of co-substrates did not show any discernible impact on digester performance. Results from this study suggest that total chemical oxygen demand (COD) is a better indicator than volatile solids (VS) for determining the organic loading rate (OLR) during co-digestion. Due to significant temporal variation in the organic content of the sludge, results from this study also show the need for a control experiment for systematic evaluation of co-digestion performance.
CHAPTER 7: Anaerobic co-digestion between food waste and sewage sludge: A pilot study

Corresponding Publication:


7.1. Introduction

In recent years, there has been a considerable increase in the generation of food waste, with the Food and Agriculture Organisation of the United Nations forecasting as much as 2.2 billion tonnes to be generated annually by 2025 (Mehariya et al., 2018, Morales-Polo et al., 2018). Managing these food wastes presents a considerable challenge in modern society, with food waste production leading to excessive landfilling and generation of greenhouse gasses during decomposition (Darlington et al., 2009).

Food waste is any food and inedible parts of food, removed from the food supply chain for disposal. It can be further classified as avoidable and unavoidable food waste. The avoidable fraction refers to food appropriate for human consumption being discarded because it has been kept beyond the expiry date or left to spoil. Unavoidable food waste refers to inedible materials such as fruit peels, vegetable skins, and animal skin. These are nonetheless organic materials (Papargyropoulou et al., 2014). Recent efforts in progressive nations to prevent avoidable food waste are increasing the fraction of unavoidable food waste significantly. In Spain, up to half of all food waste was unavoidable while in Sweden unavoidable food waste accounts for around 35% household waste (Garcia-Herrero et al., 2018, Bernstad Saraiva Schott and Andersson, 2015). Being inherent to food production, unavoidable food waste requires suitable treatment to reduce its environmental impact (Bernstad Saraiva Schott et al., 2013).

Due to the typically high organic content of the waste, one of the prevailing methods proposed is to treat unavoidable food waste using anaerobic digestion. This technique involves the biodegradation of organic matter and subsequent production of biogas, a by-product rich in methane which is suitable for co-generation. However, with the term food waste encompassing a broad range of organic wastes, composition of food wastes varies considerably from source to source, not all of which are suitable for anaerobic digestion.
Whilst certain studies have demonstrated the stable anaerobic digestion of food waste, many of the wastes tend to cause organic overloading through the accumulation of volatile fatty acids (Mehariya et al., 2018, Zhang et al., 2007, Ariunbaatar et al., 2015). Consequently, food waste is often co-digested with substrates such as primary sludge (PS) or manure, which possess sufficient buffering capacity to neutralise the organic acids produced in food waste degradation (Koch et al., 2016).

The concept of food waste/PS co-digestion is ideal due to the availability of existing infrastructure used in sludge digestion. Anaerobic digestion is a mainstay of traditional municipal waste treatment. However due to the lower organic content of sludge, anaerobic digesters are typically under-utilised with respect to the potential organic loading rate (OLR) (Nghiem et al., 2017, Fonoll et al., 2015). Co-digesting PS with an organic-rich food waste allows for the employment of this excess reactor capacity, facilitating greater production of biogas and the diversion of food waste from less sustainable waste management strategies. Greater biomethane production improves the financial viability of onsite co-generation of electricity/thermal energy, as sufficient biomethane production is required to offset capital investment and upkeep costs to justify the instillation of combined heat and power generators.

A further benefit of co-digestion is the potential for a synergistic effect between the two substrates. This effect describes the improved reactor performance resulting from the blending of two or more differing substrates. Synergistic effects can either involve the improvement of process kinetics or the degradability of substrates and in turn their methane yield (Astals et al., 2014). Synergisms can result from the optimisation of macro and micro-nutrient stoichiometry, the dilution of inhibitory compounds or optimisation of moisture profiles (Mata-Alvarez et al., 2014). With respect to the feedstock stoichiometry, historically the C/N ratio is afforded the most attention in literature (Weiland, 2010). PS typically possesses a low C/N ratio, from which its high buffering capacity is derived. However, due to the aforementioned source-based variability of food waste, such substrates range from carbon-rich to nitrogen-rich derived from their carbohydrate, protein and lipid profiles. Consequently identifying typical food waste sources which produce ideal substrates is imperative to the improvement of predictive analytics.

The following study seeks to elucidate the impact of co-digesting PS with food wastes in a pilot scale anaerobic system. Particular focus was also afforded to the impact upon biosolids and biogas quantity/quality. Such information is vital to the commercial uptake of the practice, as a deficit of pilot-scale studies in food waste/PS co-digestion persists, whilst
lab-scale and batch testing results have a tendency to provide overly favourable results (Ratanatamskul et al., 2015). The study also seeks to compare the biodegradability of the avoidable and unavoidable fractions of food waste. Existing studies concerning the co-digestion of food waste predominantly involve mixed wastes, whilst comparison of the avoidable and unavoidable food waste digestion has only been conducted in batch testing. Consequently two differing wastes, representative of these categories of food waste, were evaluated.

7.2. Materials and methods

7.2.1. Pilot plant design

A mobile anaerobic research plant was constructed within a 24-foot shipping container and installed in the Shellharbour wastewater treatment plant (Figure 7.1). Operation and monitoring of the plant was conducted via the supervisory control and data acquisition (SCADA) system, whilst allowing remote access. The research plant included two 1,000 L conical stainless-steel reactors, each fitted with a recirculation/mixing pump and biogas storage tank. Continuous biogas production measurement in each reactor was carried out by thermal mass flow meters (model SLA5800, Brooks Instrument, USA) and recorded with the SCADA software. Biogas data was expressed at the standard temperature and pressure condition. Insulation foam encased all pipework and reactors to prevent heat loss, whilst the recirculation of heated water through reactor water jackets supplied heating to the systems.
Figure 7.1. (A) Research anaerobic plant schematic and (B) Photograph of the anaerobic system

7.2.2. Co-digestion substrates

Primary sludge was obtained onsite from the Shellharbour WWTP. Food waste was sourced from two different locations denoted as FW1 and FW2. FW1 was from the university cafeteria at the University of Wollongong. This food waste largely consisted of left over meals (e.g. potato chips, salad, and pasta). FW2 was from a series of upmarket restaurants in Barangaroo. This food waste was primarily comprised of materials discarded during the process of cooking and meal preparation. Based on summaries from the sources, FW1 is expected to consist largely of avoidable food waste, whilst FW2 was primarily unavoidable waste. Bio-degradable cutlery and packaging were used in both locations to ensure that the produced food waste is compatible for composting. Due to their differing sources FW1 is expected to contain higher lipid and starch-based carbohydrate fractions than FW2, which is likely to be richer in protein. Prior to collection both food wastes were diluted with water and
macerated to produce a slurry consistency. The food wastes were obtained on a monthly basis and were stored at 4 °C in a mobile cool room on-site.

7.2.3. Experimental protocol

During the experiment reactors A and B were operated in parallel. Feeding was controlled automatically by the SCADA software and consisted of 4 feeding/discharging cycles per day. An active sludge volume of 600 L with a 400 L headspace was maintained in the system and manually monitored through an external sight glass. The reactors were operated in mesophilic conditions at 35 ± 1 °C throughout the experiment.

The experiment consisted of three major operating stages described in Table 7.1. During each condition reactor B was operated as a PS mono-digestion control. Reactor A on the other hand demonstrated the co-digestion of two food wastes with PS. Both co-substrates were co-digested at 10% (v/v) food waste addition for a minimum of 2 HRT (40 days). Furthermore, a conditioning period of mono-digestion of PS in Reactor A was conducted between the co-digestion of the two different food wastes in order to allow gas production and reactor conditions to reach parity in the two reactors.

Table 7.1 Organic loading rate during co-digestion of food wastes in comparison to the control digester

<table>
<thead>
<tr>
<th>Co-substrate</th>
<th>No. of days</th>
<th>Reactor</th>
<th>HRT</th>
<th>Co-substrate content (v/v %)</th>
<th>OLR (kg COD/m³/d)</th>
<th>OLR (kg VS/m³/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW1</td>
<td>49</td>
<td>A</td>
<td>20</td>
<td>10</td>
<td>1.43</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>20</td>
<td>0</td>
<td>0.81</td>
<td>0.56</td>
</tr>
<tr>
<td>Conditioning</td>
<td>26</td>
<td>A</td>
<td>20</td>
<td>0</td>
<td>0.90</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>20</td>
<td>0</td>
<td>0.90</td>
<td>0.51</td>
</tr>
<tr>
<td>FW2</td>
<td>60</td>
<td>A</td>
<td>20</td>
<td>10</td>
<td>1.40</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>20</td>
<td>0</td>
<td>0.90</td>
<td>0.45</td>
</tr>
</tbody>
</table>

7.2.4. Analytical techniques

Digestate and PS were sampled three times a week and stored at 4 °C. Samples were initially tested for pH using a portable pH probe (Thermo Scientific, Australia), total solids (TS) and volatile solids (VS), along with total COD (tCOD) measurement using a Hatch DRB200 COD Reactor and Hatch DR3900 spectrophotometer (program number 435 COD HR) in compliance with the US-EPA Standard Method 5220. Samples were centrifuged for 10 min at 3750 rpm (Allegra X-12R centrifuge, Beckman Coulter, Australia) and filtered (1 μm filter paper, Filtech, Australia) to obtain the supernatant. The total organic acids (TOA), alkalinity and soluble COD (sCOD) of the supernatants were tested in accordance with
standard methodology. Biogas production was measured and controlled every 5 min by a thermal mass flow meter (model SLA5800, Brooks Instrument, USA) managed by the SCADA system. The biogas content was analysed using a portable gas analyser (GA5000 gas analyser, Geotechnical Instruments Ltd., UK) three times a week.

7.3. Results and discussion

7.3.1. Substrate characteristics

Both food wastes exhibited significantly higher organic contents than that of the PS in terms of total COD (tCOD) and VS (Table 7.2). Due to water addition and maceration, the food wastes consisted of a liquid fraction and a solid fraction. Consequently, the co-substrates were very heterogeneous. Furthermore, the food wastes were acidic, suggesting partial hydrolysis and acidogenesis preceded sampling. Due to the high organic content and low pH of the food wastes, pairing with PS is ideal due to its high buffering capacity and limited biomethane potential.

Both food wastes exhibited significantly higher VS/TS ratios than in PS, indicating the higher fraction of degradable matter in these substrates. These high VS/TS ratios suggest that the food wastes would have a lower contribution to biosolids volume in the form of inorganic solids. This is favourable in full scale applications in Australia as biosolids management is the single largest operating cost for WWTPs (Appels et al., 2008b).

High temporal variability in the organic content in the tCOD of both food wastes was observed over the experiment. This has implications for full scale application, where consistency in the loading rate is highly desirable. Potential implications of variable loading rates include underperformance of reactors, decline in stability and the accumulation of organic matter in the digestate.

It must be noted that the variation in the VS was significantly lower than from the tCOD in the food wastes. tCOD is a more inclusive parameter for organic content, in that it accounts for volatile compounds such as volatile fatty acids. By contrast, VS accounts only for organics which volatilise at temperatures between 110 – 550 °C. However, the excessive variation in the tCOD of the food wastes is indicative of the lack of reliability in the parameter. Because solid and semi-solid wastes exhibit lower homogeneity compared to liquid substrates, tCOD methodology involves insufficient sample size to accurately represent the average organic content in the food wastes.
Table 7.2 Key parameters of primary sludge and food wastes used in this study (mean ± standard deviation of at least 5 readings).

<table>
<thead>
<tr>
<th></th>
<th>Primary Sludge</th>
<th>FW1</th>
<th>FW2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>2.0 ± 0.4</td>
<td>3.84 ± 0.74</td>
<td>11.8</td>
</tr>
<tr>
<td>VS (%)</td>
<td>1.7 ± 0.3</td>
<td>3.61 ± 0.63</td>
<td>10.7</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>83</td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>Total COD (g/L)</td>
<td>30 ± 4</td>
<td>136 ± 89</td>
<td>214 ± 95</td>
</tr>
<tr>
<td>Soluble COD (g/L)</td>
<td>6.1 ± 0.7</td>
<td>44 ± 31</td>
<td>75 ± 35</td>
</tr>
<tr>
<td>pH</td>
<td>5.8 ± 0.5</td>
<td>3.4 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
</tbody>
</table>

7.3.2. Biomethane production

The addition of both food wastes to the reactors was seen to have no significant impact upon biogas composition (Table 7.3). The consistency of the H₂S profiles of the two reactors suggests the sulphur content of both food wastes was comparable to that of the PS. This assumption is in agreement with prior findings, as sulphur levels in food wastes reported in literature tend to be similar to those reported in PS (Zhang et al., 2007, Latha et al., 2019, Dewil et al., 2008). The absence of any increases in H₂S production is favourable as the trace gas is both odorous and damaging to combustion generators.

Table 7.3 Average biogas composition for each operational condition (mean ± standard deviation of over 20 readings)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Reactor A – Co-digestion</th>
<th>Reactor B - Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH₄ (%)</td>
<td>CO₂ (%)</td>
</tr>
<tr>
<td>FW1</td>
<td>65.7 ± 0.7</td>
<td>34.3 ± 0.7</td>
</tr>
<tr>
<td>FW2</td>
<td>68.2 ± 1.1</td>
<td>31.8 ± 1.1</td>
</tr>
</tbody>
</table>

The addition of both food wastes significantly improved biomethane production relative to the mono-digestion of PS (Table 7.4). This can largely be attributed to the higher loading supplied by the organic-rich food wastes, particularly in the case of the FW2. The benefit of co-digestion of FW1 was seen to increase over time during the feeding condition. Whilst the initial improvement could be due to the acclimation of the biomass to the higher loading rate, the continued improvement suggests a potential synergistic effect resulting from the balancing of reactor stoichiometry.

The benefit of co-digesting FW2 with PS was lower than what was observed in FW1, despite having a higher VS and comparable tCOD content. Addition of FW2 was seen to increase biomethane production relative to the control by 65 ± 21 L, constituting a 64% increase in biomethane production. This is compared to 98 ± 35 L during FW1 addition,
which was a 105 % increase on the control reactor. These findings suggest FW2 is a less suitable co-substrate pairing for PS. This could be due either to a lower proportion of easily degradable organic matter, or a less favourable stoichiometric profile, which imparts no synergistic effect. Despite this, highly stable digestion was observed throughout the stage, providing a consistent improvement to biomethane production.

Table 7.4. Biomethane production from co-digesting PS with different food wastes (mean ± standard deviation of over 40 readings)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Average Methane Production (CH₄/d)</th>
<th>Increase in CH₄ production (%)</th>
<th>Increase in OLR (as COD) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactor A</td>
<td>Reactor B</td>
<td></td>
</tr>
<tr>
<td>FW1</td>
<td>190 ± 49</td>
<td>93 ± 49</td>
<td>104</td>
</tr>
<tr>
<td>FW2</td>
<td>167 ± 41</td>
<td>102 ± 25</td>
<td>64</td>
</tr>
</tbody>
</table>

7.3.3. Specific methane yield of FW

Specific methane yields based on tCOD addition from PS, FW1 and FW2 were obtained to evaluate the biodegradability of each substrate (Figure 7.2). It is not possible to conduct mono-digestion of the food wastes; thus their specific methane yields were calculated by comparing the yields from the mono and co-digestion reactors. These specific methane yields are however based on the assumption that co-digestion instigated no synergistic effect.

Both co-substrates exhibited significantly higher methane yields than PS, signifying that the substrates are more readily biodegradable. These observations suggest the food wastes are highly suitable substrates for co-digestion when targeting increased biomethane production, as their ability to increase the loading rate is compounded by a higher methane yield. Furthermore, the high biodegradability suggests food waste addition should not significantly impact digestate quality.

The specific methane yield observed in FW1 was 19.4% higher than that of FW2. Considering the similar organic contents and identical prior substrate handling, the notably different specific methane yields demonstrate the variability of food waste based on the source. Without further compositional analysis, the disparity in biodegradability of the two food wastes cannot be conclusively explained. However, the lower methane yield observed in the FW2, which is representative of unavoidable food waste, supports findings in Bernstad Saraiva Schott et al. (2013), where the unavoidable fraction of household food waste was found to have a lower specific methane yield than previously observed in the avoidable fraction. An alternate contributing factor to the higher yield of FW1 could be the occurrence
of synergistic effects between FW1 and PS, however without specific methane yields during mono-digestion this cannot be confirmed or quantified.

![Graph showing specific methane yields of FW1, FW2, and primary sludge](image)

**Figure 7.2.** Specific methane yields of FW1, FW2 and primary sludge (food waste yields were calculated from yields in both reactors and assuming no synergistic effect in primary sludge degradability)

### 7.3.4. Digestate quality

Digestate quality was assessed in terms of the VS and tCOD content (Figure 7.3). The divergence between the two reactors in terms of their organic profiles was limited considering the substantially higher OLRs in the co-digestion system. During the addition of FW2, VS was consistently slightly higher in the co-digestion reactor. In the case of FW1 addition, co-digestion resulted in no observable increase of additional VS in the digestate relative to the control reactor. This would corroborate the substantially higher methane yield observed during FW1 co-digestion compared to FW2. On the other hand, the tCOD profile suggests there was considerable increase of organic material in the digestate. The erraticism of the tCOD profile however raises concerns over the reliability of this observation.
The temporal variability of the food wastes was seen to have little impact on the digestate quality. Rather, inconsistency in the sewage sludge was the defining factor governing the accumulation of VS and COD in the digestate. Indeed, notable variance in the specific methane yield of the control reactor was observed over the experiment. The limited impact of co-digestion on digestate quality assuaged concerns raised by the temporal variability observed in the food wastes.

![Graph](image)

**Figure 7.3.** Organic content in the digestate from the co-digestion and mono-digestion reactor in terms of (a) VS; and (b) tCOD.

### 7.3.5. Reactor stability

The impact of co-digestion on reactor stability was assessed in terms of the impact on pH, TOA, alkalinity and soluble COD (Figure 7.4). No instances of reactor destabilisation were observed throughout the experiment. Indeed, during FW1 addition there was no observable disparity between the co-digestion and control reactors across each of the stability parameters.

Limited variance between reactors was observed during the addition of FW2. A slight accumulation of additional soluble COD was observed, however this was not accompanied by any increase in TOA. Rather a significant increase in alkalinity was observed in the co-
digestion reactor relative to the control. The greater production of alkalinity is likely indicative of a larger nitrogen content in the FW2, leading to higher accumulation of NH$_4$ in the reactor. Whilst composition testing is required to confirm this assumption, a higher nitrogen content would provide further explanation for the significantly lower degradability of FW2 during co-digestion, as PS is best suited for pairing with a high C/N co-substrate (Koch et al., 2016). The difference between the two food wastes highlights the variability of the substrate properties based on the source.
Figure 7.4. The evolution of stability parameters during the co-digestion of food wastes and PS and PS mono-digestion in terms of (a) soluble COD, (b) alkalinity, (c) pH and (d) TOA.
7.4. Conclusions

Co-digestion of primary sludge and two food waste was evaluated using a pilot scale anaerobic digestion plant. FW1 was primarily avoidable food waste. FW2 was largely unavoidable food waste and was therefore predominantly composed of the inedible food waste fraction. The average organic contents in the two food wastes were similar and both exhibited considerable temporal variability. Addition of the FW1 and FW2 at 10 v/v resulted in 76 and 56% increase in organic loading rate and 104 and 64% increase in biomethane production relative to the control, respectively. Co-digestion had no impact on biogas quality in terms of CH₄ and H₂S content. The specific methane yield calculation suggests that both types of food waste were more readily biodegradable than PS. The difference in specific methane yields suggests that the avoidable fraction of food waste is more readily degradable than the unavoidable component. Increased tCOD and VS was observed in the digestate during co-digestion. The pH and TOA profiles between the co-digestion and control reactors showed little difference. However, FW2 addition caused the concentration of alkalinity and soluble COD in the digestate.
CHAPTER 8: Evaluation of free ammonia pre-treatment during the anaerobic co-digestion of food waste and sewage sludge in a semi-continuous system

8.1. Introduction

With the current shift towards a circular economy, a stronger emphasis has been placed on the optimal utilisation of resources (Tyagi et al., 2018). Within the municipal wastewater treatment industry, anaerobic co-digestion has been increasingly applied towards these ends. Co-digestion utilises the latent chemical energy in organic wastes, and through the bioconversion process of anaerobic digestion, to produce biogas which is a renewable fuel. Biogas is an energy-rich by-product of the degradation of organic matter under anaerobic conditions, primarily composed of methane (~65% v/v) and carbon dioxide (~35% v/v) (Weiland, 2010). In contrast to traditional anaerobic digestion, co-digestion involves the pairing of two or more organic substrates with complementary characteristics. This blending allows for the optimisation of stoichiometry and the dilution of inhibitory compounds, in turn allowing for the digestion of substrates unsuitable for mono-digestion (Mata-Alvarez et al., 2014). Such substrates can therefore be diverted from landfilling, composting or incineration, which each offer inferior energy recovery compared to anaerobic digestion (Uçkun Kiran et al., 2014).

In municipal wastewater treatment plants (WWTPs), anaerobic digestion is used in the treatment of primary and secondary sludge. Primary sludge degradation produces a high level of alkalinity, whilst only producing limited biogas volume. Consequently, when co-digested, primary sludge is generally paired with carbonaceous, organic-rich co-substrates. This can lead to higher stability and biogas production in the reactors, in turn improving the economic viability of on-site co-generation from combined heat and power generators. Commercially produced organic wastes such as food waste tend to possess higher organic contents ideal for digestion with primary sludge. Ideal pairing of substrates in co-digestion has been observed to instigate a beneficial synergistic effect. This refers to where co-digestion is seen to mutually benefit the degradation of both substrates. Consequently, the biosolids quality and volume minimisation, along with the methane yield are improved. Synergistic effects have been observed previously during the co-digestion of primary sludge with food wastes from canteen meal wastage and spoilage (Koch et al., 2016, Wang et al., 2015).
In WWTPs, up to 40% of operation costs relate to the handling and disposal of biosolids. As a result, an increasing number of studies have been conducted into pre-treatment techniques in order to improve degradability of substrates and further decrease the volume of biosolids produced (Tyagi et al., 2018). Pre-treatments have often been used in conjunction with waste activated sludge in order to improve the degradability of the substrate. However, in recent years these techniques have been increasingly applied during co-digestion in order to limit the greater production of biosolids and to improve resource utilisation. Whilst co-digestion can aid the removal of volatile solids, the increased organic loading inevitably leads to a higher total volume of biosolids. Thus, pre-treatments can be seen to help offset the negative impacts of co-digestion on biosolids production.

Free ammonia (FA) pre-treatment is a promising technique, which has been observed to enhance the degradability of both primary and waste activated sludge. This technique involves the dosing of ammonia/ammonium into the substrate along with pH adjustment to basic conditions to increase FA proportion. Excessive FA concentrations are theorised to rupture cell membranes, thus enhancing the degradability of the substrate. Wang (2017) proposed an approach to sustainably source the ammonium from the anaerobic digester supernatant (centrate), which would substantially improve the financial viability of the technique. Alternatively, ammonia could be sourced via co-digestion with ammonia/ammonium rich substrates. However, the effectiveness of FA pre-treatment has yet to be verified with substrates other than sewage sludge, nor has it been used in conjunction with co-digestion. Furthermore, FA pre-treatment has to-date only been evaluated in biomethane potential batch testing, not in a continuous system. Consequently, the impacts of ammonia accumulation have not been verified. Excessive concentrations of FA are known to cause inhibition of methanogenic bacteria.

This study aims to elucidate the effects of pairing anaerobic co-digestion with FA pre-treatment under a semi-continuous feeding regime. Primary sludge and food waste were selected for the anaerobic co-digestion experiment. A key focus of the study was to determine whether FA pre-treatment had any benefit on food waste degradability. Further attention was also directed towards the effects of ammonia accumulation. The study further serves as a proof of concept for subsequent studies in pairing sewage sludge with ammonia-rich co-substrates in order to reduce chemical requirements of FA pre-treatment.
8.2. Materials and methods

8.2.1. Co-digestion substrates

Anaerobically digested sludge and primary sludge were sampled onsite from the Shellharbour wastewater treatment plant in New South Wales Australia. Food waste was obtained from a PulpMaster™ unit on the University of Wollongong campus which is used to process food waste for commercial waste collection. The raw food waste was diluted and macerated by the PulpMaster™ system in order to produce a slurry consistency. After sampling the food waste was further blended and passed through a 5 mm plastic filter in order to remove large particles. Primary sludge was sampled on a one to two week basis, whilst food waste was sampled monthly. After collection, the substrates were immediately refrigerated at 4°C.

8.2.2. Experiment systems

![Laboratory scale anaerobic reactor schematic](image1)
![Setup photograph](image2)

Figure 8.1. (A) Laboratory scale anaerobic reactor schematic and (B) Setup photograph
The experimental setup consisted of three 28 L stainless steel reactors operated in parallel (Figure 8.1). Each reactor was fitted with a peristaltic hose pump (DULCO® Flex from Prominent Fluid Controls, Australia), which was operated continuously at 60 L/h to provide digestate circulation. Temperature in each system was maintained at 35 ± 1 °C using a thermal control unit (Neslab RTE 7, Thermo Fisher Scientific, Newington, USA), which circulated heated water through rubber tubing encasing the reactor. The reactor and tubing were further insulated in a polystyrene foam layer. Biogas production volume was measured using a gas counter (Riter Company™, MilliGascounter), whilst a custom-built gas trap was fitted for biogas sampling.

8.2.3. Experimental protocol

Each reactor was operated with a working volume of 20 L and a hydraulic retention time (HRT) of 20 days. The reactors were initially seeded with digestate from the Shellharbour WWTP after which 1 L of digestate was sampled and replaced with 1 L of feed each day via the peristaltic pump circulation line. The experiment consisted of four operating conditions described in Table 8.1, each spanning at least 40 days (2 HRT). During Stage 1 each of the reactors was operated in primary sludge mono-digestion. Subsequent stages involved the co-digestion of primary sludge and food waste in reactors 1 and 2, up to the target concentration of 20 % (v/v). During stages 2 and 3, Reactor 1 feed received alkaline pre-treatment, whilst FA pre-treatment was applied during stage 4. No pre-treatment was employed in Reactor 2, whilst the co-digestion ratio was identical to Reactor 1. Reactor 3 was used as a control to assess the effect of co-digestion on reactor performance during stages 1 – 2 and was discontinued during the pre-treatment assessment stage.
### Table 8.1. Experimental conditions (mean ± standard deviation of over 40 readings).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Days</th>
<th>Reactor</th>
<th>Feed</th>
<th>Pre-treatment</th>
<th>Feed ratio (v/v %)</th>
<th>OLR (kg VS/m(^3)/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 – 46</td>
<td>1</td>
<td>Primary sludge</td>
<td>No</td>
<td>N/A</td>
<td>1131 ± 41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Primary sludge</td>
<td>No</td>
<td>N/A</td>
<td>1131 ± 41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Primary sludge</td>
<td>No</td>
<td>N/A</td>
<td>1131 ± 41</td>
</tr>
<tr>
<td>2</td>
<td>47 – 104</td>
<td>1</td>
<td>Food waste/ primary sludge</td>
<td>Alkaline</td>
<td>10</td>
<td>1171 ± 155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Food waste/ primary sludge</td>
<td>No</td>
<td>10</td>
<td>1171 ± 155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Primary sludge</td>
<td>No</td>
<td>N/A</td>
<td>1022 ± 155</td>
</tr>
<tr>
<td>3</td>
<td>105 – 145</td>
<td>1</td>
<td>Food waste/ primary sludge</td>
<td>Alkaline</td>
<td>20</td>
<td>1146 ± 178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Food waste/ primary sludge</td>
<td>No</td>
<td>20</td>
<td>1146 ± 178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Primary sludge</td>
<td>No</td>
<td>N/A</td>
<td>950 ± 197</td>
</tr>
<tr>
<td>4</td>
<td>146 – 186</td>
<td>1</td>
<td>Food waste/ primary sludge</td>
<td>FA</td>
<td>20</td>
<td>853 ± 106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Food waste/ primary sludge</td>
<td>No</td>
<td>20</td>
<td>853 ± 106</td>
</tr>
</tbody>
</table>

FA pre-treatment involved the dosing of ammonium chloride and adjustment of pH to alkaline conditions. After 1 day storage the pH was readjusted to the initial conditions. The ammonium chloride dosing was carried out at a concentration of 500 NH\(_4\)-N with pH adjustment up to 10 ± 0.1, corresponding to the ideal conditions identified in Wei et al. (2017b). pH adjustment was conducted using HCl and NaOH stock solutions. To verify that the FA concentration is responsible for any impacts on reactor performance, alkaline pre-treatment was also evaluated. This involved identical adjustment of pH to 10 ± 0.1, without additional NH\(_4\) dosing.

8.2.4. **Analytical techniques**

Analysis of digestate was conducted weekly during steady state conditions and every third day during adaptive reactor states. pH of samples was immediately tested using a portable pH probe (Thermo Scientific, Australia), followed by total solids (TS) and volatile solids (VS) testing, along with total COD (tCOD) measurement using a Hach DRB200 COD Reactor and Hach DR3900 spectrophotometer (program number 435 COD HR) in compliance with the US-EPA Standard Method 5220. Samples were centrifuged for 10 min at 3750 rpm (Allegra X-12R centrifuge, Beckman Coulter, Australia) and filtered (1 μm filter paper, Filtech, Australia) to obtain the supernatant. Subsequent testing of the supernatant included alkalinity,
total ammonia nitrogen and soluble COD (sCOD) in accordance with standard methodology. Supernatant samples were stored for a maximum of 2 weeks at 4°C. Biogas production was automatically measured by a gas counter (Ritter Company™ MilliGascounter) and recorded daily. The biogas content was analysed weekly using a portable gas analyser (GA5000 gas analyser, Geotechnical Instruments Ltd., UK). Methane production was expressed at the standard temperature and pressure condition.

8.3. **Results and discussion**

8.3.1. **Substrate characteristics**

The basic characteristics of the primary sludge and food waste substrates were monitored throughout the experiment (Table 8.2). The high concentrations of VS and COD in the food waste validate its selection as a co-substrate to increase biomethane production. Meanwhile the low pH in the food waste is indicative of the partial digestion of the substrate prior to sampling. The substrate acidity coupled with its high organic content make it unsuitable for mono-digestion.

Considerable temporal variation in both the food waste and primary sludge substrates was observed during the experiment. Ideally substrates present minimal temporal variability, which allows operators to easily maintain a consistent loading rate. Thus, the high variability may be problematic, potentially incurring declines in the stability or digestate quality in the reactor. Primary sludge also exhibited considerable temporal variability. During the mono-digestion and 10% co-digestion stages, the organic content present in the primary sludge was considerably higher than during latter stages, which is evident in the OLR’s in Table 8.1. This variation in primary sludge can be attributed largely to seasonal weather patterns.

Table 8.2. Characteristics of digested sludge, primary sludge, and food waste (mean ± standard deviation of over 20 readings).

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>tCOD (g/L)</th>
<th>sCOD (g/L)</th>
<th>TS (g/L)</th>
<th>VS (g/L)</th>
<th>VS/TS (%)</th>
<th>NH$_3$-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digested sludge</td>
<td>7.2</td>
<td>11.6</td>
<td>2.3</td>
<td>7.47</td>
<td>4.91</td>
<td>65</td>
<td>640</td>
</tr>
<tr>
<td>Primary sludge</td>
<td>5.6 ± 0.3</td>
<td>32.1 ± 8</td>
<td>6.4 ± 2</td>
<td>22.9 ± 5</td>
<td>18.5 ± 7</td>
<td>85.5 ± 4</td>
<td>NA</td>
</tr>
<tr>
<td>Food waste</td>
<td>3.4 ± 0.1</td>
<td>103.5 ± 39</td>
<td>27.5 ± 11</td>
<td>39.7 ± 13</td>
<td>36 ± 11</td>
<td>91.0 ± 5</td>
<td>88 ± 15</td>
</tr>
</tbody>
</table>
8.3.2. **Biogas production**

![Graph showing specific methane yield during co-digestion](image)

Figure 8.2. Specific methane yield during co-digestion of food waste and primary sludge with and without pre-treatment and primary sludge mono-digestion.

Co-digestion was seen to significantly improve the specific methane yield and overall methane production in Reactors 1 and 2 (Figure 8.2). Whilst in steady-state conditions in Stage 2 Reactor 2 produced an average of 40% more methane per gram of VS added relative to the control reactor. Whilst a higher degradability is expected in the food waste than the primary sludge, the magnitude of this effect suggests there may be a synergistic effect, whereby the complementary characteristics of the two substrates enhances the overall degradation. Synergistic co-digestion has been recorded previously in the literature with pairings of food waste and sewage sludge (Mirmasoumi et al., 2018, Xie et al., 2017b, Koch et al., 2016).

A minimal level of variation between the specific methane yield profiles was observed between Reactors 1 and 2 during Stages 2 and 3 of the experiment. The sole difference in conditions between the reactors in these stages was the application of alkaline pre-treatment.
to Reactor 1. With no observable improvement in Reactor 1, the impact of the pre-treatment can be discounted. The ineffectiveness of alkaline pre-treatment on primary sludge degradability is consistent with prior findings.

With the application of FA pre-treatment, a notable increase in the methane yield of Reactor 1 was observed. The average methane yield in Reactor 1 rose by approximately 30% relative to Reactor 2. These findings provide additional evidence to the efficacy of FA pre-treatment. Furthermore the observed improvement in methane yield was considerably higher than that in Wei et al. (2017b) during the application of FA-pre-treatment to primary sludge mono-digestion. This discrepancy between the improvements in the studies suggests FA pre-treatment may have a larger impact on food waste digestion than that of primary sludge. Zhang et al. (2017) reported a similar finding using biological co-pre-treatment, whereby the effectiveness of the technique was greater during food waste and sludge co-digestion than in sludge mono-digestion, resulting in a 10.1% increase in volatile solids removal. The greater efficacy is likely related to the degradability and high concentration of non-solubilised organics in food waste relative to primary sludge. It is theorised that FA-pre-treatment improves degradation through aiding the disintegration and hydrolysis stages of anaerobic digestion, meanwhile primary sludge tends to be an easily hydrolysed and highly degradable substrate (Wang et al., 2018b, Wang et al., 2017). Whilst the addition of food waste improved the methane yield through a clear synergistic effect, the substrate was also observed to contain a high concentration of larger particles and increased levels of non-solubilised organic material, which would be consistent with a lower hydrolysis rate.
8.3.3. Digestate quality

Figure 8.3. Digestate quality during co-digestion of food waste and primary sludge with and without pre-treatment and primary sludge mono-digestion.

Considerable variation in the stabilisation of organic matter was observed throughout the experiment (Figure 8.3). The addition of food waste and the increased primary sludge organic content instigated the accumulation of VS in the digestate during stage 2 (food waste addition at 10% v/v). This stabilised in Reactors 1 and 2 at around 9 g/L during stage 3. A strong correlation between these two reactors during stages 2-3 further supports the hypothesis that alkaline pre-treatment has no impact on reactor performance. On the other hand, FA pre-treatment was seen to improve digestate quality in terms of VS content. The improvement in VS removal reduced the impact of food waste addition in terms of digestate stabilisation. However, as the control reactor was not operated during this stage, the extent of the offset cannot be calculated. Furthermore the impact of food waste addition on the sampled digestate VS was limited during the first 3 stages of the experiment. This may be indicative of insufficient reactor mixing, as the increase in loading could be expected to increase reactor VS.
8.3.4. Reactor stability

Figure 8.4. The evolution of stability parameters during co-digestion of food waste and primary sludge with and without pre-treatment and primary sludge mono-digestion.
Each of the reactors demonstrated stable digestion with respect to the pH, alkalinity and soluble COD throughout the experiment (Figure 8.4). Co-digestion of the tested food waste and primary sludge was seen to cause no decrease in pH or depletion of alkalinity, suggesting there is no risk of organic overloading up to 20% substrate addition. An initial increase in the concentration of sCOD was observed in Reactor 1 when subjected to FA pre-treatment. This follows on from prior findings reporting greater disintegration and an increased the hydrolysis rate constant during digestion (Wei et al., 2017a). The increased solubilisation led to the slight accumulation of sCOD, which stabilised once the bio-community acclimated to the new feed regime.

Accumulation of total ammonia nitrogen was observed in Reactor 1 whilst it underwent FA pre-treatment. A peak concentration of 968 mg/L NH$_3$-N was reached after 6 weeks of NH$_4$ addition. This is significantly lower than inhibitory levels reported in literature (Chen et al., 2008), however stable concentrations in the digestate were not achieved. Consequently, longer term continuous study would be required to evaluate the risk of methanogenic inhibition. Alternatively the intermittent application of FA pre-treatment could be adopted but would limit the attainable benefits.

### 8.4. Conclusions

The application of FA pre-treatment during the co-digestion of primary sludge and food waste was evaluated in a semi-continuous lab scale system. During co-digestion at 10% food waste addition, the methane yield was 40% higher in the co-digestion reactors relative to the mono-digestion control. The magnitude of improvement in the yield suggests there was a synergistic effect between food waste and primary sludge, however this cannot be confirmed without demonstrating mono-digestion of food waste. Alkaline pre-treatment did not show any observable impact on biodegradability of primary sludge and food waste. Conversely, FA pre-treatment increased the methane yield by 30% during 20% food waste addition relative to the co-digestion reactor with without pre-treatment. These findings, in conjunction with prior studies, suggest that FA pre-treatment is more effective in improving the degradability of food waste than of primary sludge. Accumulation of NH$_3$-N was observed during the 40 day (2 HRT) application period of the pre-treatment but did not reach the inhibitory level.
CHAPTER 9: Conclusions and recommendations for future work

9.1. Conclusions

Biomethane potential screening identified several potential co-substrates for anaerobic digestion with sewage sludge. For all co-substrates, at maximum stable co-digestion ratios the biomethane production was three to six times higher than during sewage sludge mono-digestion. Solid and slurry wastes caused greater improvements to gas production due to the higher loading rate, but were also more likely to increase volatile solids and chemical oxygen demand in the biosolids. The solid paper mill waste, food wastes and bakery waste produced 35, 30.9 – 127.1 and 184 L CH$_4$/kg of substrate added respectively. On the other hand, liquid wastes tended to instigate synergistic removal of organics, whereby degradation of the sewage sludge was enhanced due to the presence of the co-substrates. Methane production based on substrate addition were lower for the liquid wastes, with yields of 26, 94 NS 47 L CH$_4$/kg of beverage waste, dairy waste and fat oil and grease respectively. Preliminary maximum digestion ratios were obtained for most of the co-substrates, facilitating further investigation. Elevated concentrations of sulphur and phosphorous were observed in all food wastes regardless of their source, suggesting these substrates may increase hydrogen sulphide levels in biogas.

Detailed evaluation of the synergistic effects using three approaches was conducted between sewage sludge and two separate organic wastes: simulated food waste and paper pulp. Both co-digestion pairings resulted in increased methane yields compared to mono-digestion when applying a chemical oxygen demand (COD) balance. A greater impact was observed in the food waste co-digestion mix rather than that with paper pulp. The effect of synergistic metabolism during food waste co-digestion on the conversion of COD was estimated to be 32% in this study, compared to 18.7% in paper waste co-digestion. The reverse was true with respect to the kinetics, as paper pulp was hydrolysed significantly faster during co-digestion. During mono-digestion hydrolysis rate of paper and primary sludge were 0.18 d$^{-1}$ and 0.68 d$^{-1}$ respectively, whilst the hydrolysis rate during co-digestion was 0.63 d$^{-1}$. 


Meanwhile, co-digestion between sewage sludge and food waste had little impact on the hydrolysis rate. This suggests the mechanics driving synergisms in biodegradability differ from those causing improved kinetics. Consequently, both approaches are required to comprehensively evaluate the synergistic effect during anaerobic co-digestion.

Co-digestion of beverage waste with sewage sludge was evaluated for the first time in a semi-continuous system. The specific methane yield during co-digestion was similar to that during the mono-digestion of sewage sludge. This suggests the absence of any antagonistic/synergistic effects, whilst the slight increase in yield was attributed to the more degradable co-substrate. The maximum stable co-digestion mixing ratio of 20% co-substrate addition resulted in 191% increase in biomethane production, corresponding to a 171% increase in the OLR, without significant impacts on digestate quality. The results suggest sewage sludge can support approximately 2 kg COD/m³/d additional OLR of a carbon rich co-substrate. Beyond this level organic overloading and significant reactor stability decline were observed. An increase in the methane content of approximately 70% in biogas was observed during a transitional digestion state following an increase in the co-substrate content of the feed.

Co-digestion of several potential beverage wastes with sewage sludge was evaluated in a long term, high volume pilot-scale study. Whilst wine reject instigated antagonistic effects, beer, soft drink and juice wastes all proved suitable co-substrates for co-digestion with sewage sludge. Soft drink waste was confirmed to temporarily increase the methane content in biogas before returning to normal levels, whilst the methane content remained relatively stable during addition of all other co-substrates. Increases in the loading rate in terms of COD resulted in proportionate increase in biomethane production for most beverage wastes. Methane yields calculated for beer waste, soft drink waste and juice waste during co-digestion at 10% mixing ratio were 146, 263 and 260 L CH₄/kg COD added. Despite concerns based on the higher loading rate, co-digestion did not significantly impact upon digestate quality. Furthermore the rapid interchanging of substrates had no discernible impacts on reactor performance. Results also suggest that COD is a superior indicator of the organic loading rate compared to volatile solids during co-digestion with beverage wastes. Significant temporal variation in the biodegradability of sewage sludge also demonstrates the need for ongoing control reactor for systematic co-digestion evaluation.

Pilot scale anaerobic co-digestion of two different commercial food wastes with sewage sludge was evaluated in a long term study. One food waste was primarily avoidable food
waste, whilst the second was representative of the unavoidable fraction. The organic contents of the two wastes were similar, allowing direct comparison of the degradability of the two components of food waste. The avoidable fraction of food waste exhibited a higher specific methane yield than that of the unavoidable fraction, however both significantly increased the total methane production relative to the sewage sludge mono-digestion control reactor. Addition of the avoidable food waste and the unavoidable food waste at 10% v/v resulted in 76 and 56% increase in organic loading rate and 104 and 64% increase in biomethane production relative to the control, respectively. Addition of both food wastes resulted in increased COD in the digestate, whilst no impact was observed in the biogas quality in terms of methane or hydrogen sulphide content. Furthermore co-digestion of neither food waste with sewage sludge resulted in any decline in stability, whilst addition of the unavoidable component resulted in increased alkalinity production.

Free ammonia pre-treatment was applied for the first time in conjunction with co-digestion and in a continuous system. The substrates selected were food waste and sewage sludge. Co-digestion resulted in a 40% increase in the specific methane yield compared to the mono-digestion control reactor. The magnitude of the increase suggests a possible synergistic effect, however this could not be conclusively proven. Alkaline pre-treatment had no impact on biodegradability. On the other hand, FA pre-treatment significantly increased the specific methane yield of the reactor by as much as 30% during the food waste mixing ratio of 20% (v/v) when comparing the co-digestion reactors with and without FA pre-treatment. In conjunction with findings in prior studies, results suggest the pre-treatment may have a greater impact on food waste than on sewage sludge. Pre-treatment inevitably resulted in the accumulation of ammonia nitrogen in the anaerobic reactor, however concentrations did not reach inhibitory concentrations.

The overarching aim of this thesis is to evaluate the feasibility of adopting co-digestion in the municipal wastewater industry, with particular emphasis on the issues and challenges within an Australian context. This study produced significant findings towards this objective, whilst addressing targeted literature gaps which are seen to restrict the uptake of co-digestion in full-scale practice. The study provided screening of new potential co-substrates, the most promising of which was beverage/soft drink waste. Determination of ideal co-digestion mixing ratios was attained through batch and laboratory-scale research, whilst validation was provided through large-scale pilot testing. Long term stable pilot-scale demonstration of anaerobic co-digestion was achieved, providing directly applicable data for full-scale
adoption the practice. Meanwhile the pairing FA pre-treatment was used in conjunction with co-digestion for the first time, providing validation for future application of this technique.

9.2. Recommendations for future work

The findings in this study present a potential for subsequent research involving the use of different wastewater sludge sources. This thesis focuses on the co-digestion of organic wastes with primary sludge, however conventional wastewater treatment involves the anaerobic digestion of a mixture of primary and secondary sludge. The rationale behind the selection of primary sludge as the key substrate related to the replicability of its degradation. This consistency of primary sludge digestion allowed for more conclusive evidence of synergisms to be obtained. Subsequent research is required to elucidate the impact of alternative forms of sewage sludge on the synergistic effect with the demonstrated co-substrates.

Continued research into the impacts on biosolids quality could further co-digestion uptake in nations such as Australia or North America, where land application of biosolids is common. Whilst this study addressed the impacts of co-digestion on biosolids odour, stabilisation and quantity, factors such as the dewaterability, residual biological activity and pathogen destruction require elucidation. Adverse impacts on these parameters can increase operating costs and lower the effective nutrient recovery of anaerobic digestion processes. Lower dewaterability affects the water content in biosolids and therefore the transport volumes/costs. Furthermore the classifications for biosolids require greater pathogen destruction to qualify for direct land application. Meanwhile residual biological activity can result in long term odour production and in turn reduce the desirability of biosolids application.

Further research is also required to develop comprehensive predictive analytics for digestion performance based on substrate selection. In the literature co-substrate pairings and mixing ratios are generally based on both the organic loading rate and C/N ratio. However the stoichiometric ratio fails to account for factors such as the fraction of readily degradable organics. Indeed advisory C/N ratios put forward in literature are broad and vary between studies. The relative concentrations of lipids, proteins and carbohydrates may represent a more encompassing predictive ratio. Indeed experimentation to determine the ideal organic ratios using pure sources of proteins, lipids and carbohydrates has been presented, however with limited scope and without consideration over the role of micro-nutrients. Determining the characteristics of an ideal anaerobic digestion feed would open up the possibility for
operators to mix different organic wastes to maximise digestion performance when logistically feasible.
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