Operational and Economic Perspectives of Pig Manure and Food Waste Anaerobic Co-digestion

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DECLARATION

I hereby certify that all of the following work is my own.

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ABSTRACT

On-farm anaerobic co-digestion of pig manure (PM) and food waste (FW) is practiced at commercial scale across the world. However, there is a paucity of information regarding how to optimise such co-digestion systems in terms of methane yields, process control, enteric indicator organism removal and digestate disposal. In addition, no analysis of this concept in an Irish economic and regulatory context has been undertaken. In order to identify the most suitable operating conditions for the anaerobic co-digestion of PM and FW, evaluate the viability of using simplified mathematical tools for process simulation, and assess the economic feasibility of on-farm PM/FW co-digestion on Irish pig farms, experiments at laboratory scale and meso-scale were carried out.

In the batch scale experiment, the synergistic effects of co-digesting FW and PM were quantified**.** Co-digestion of PM and FW had synergistic effects on specific methane yields (SMYs) and digestion kinetics. In lab-scale semi-continuous experiments, varying digester feedstock composition from 85 %/15 % to 40 %/60 % PM/FW (volatile solids basis) did not significantly affect digestate biosafety or dewaterability. Decreasing hydraulic retention time (HRT) from 41 to 21 days did not significantly increase the concentrations of the pathogenic indicator microorganisms in digestate. However reducing HRT below 21 days has a significant negative effect on pathogenic indicator microorganisms reduction rates. Decreasing HRT resulted in an increase in the relative abundance of syntrophic acetate oxidising bacteria such as *Synergistetes,* indicating that hydrogenotrophic methanogenesis may be a key methanogenic pathway at low HRTs.

A meso-scale reactor was operated in order to validate a rudimentarily calibrated mathematical model which simulated the co-digestion of PM and FW. The Anaerobic Digestion Model No. 1 provided a somewhat accurate simulation of the system, however more complex parameter optimisation was required to improve model accuracy.

An economic model was developed which assessed the financial viability of on-farm biogas plants in Ireland. FW availability was the key factor in determining plant viability. Due to the currently limited amount of FW available for anaerobic digestion, smaller on-farm co-digestion plants were found to be most financially viable as such sites had an increased likelihood of securing sufficient FW.

***Keywords*:** ADM1, Biogas, biosafety, DNA sequencing, Monte Carlo, on-farm digestion, pathogenic indicator microorganisms, stochastic modelling.

CHAPTER 1

Introduction

1.1 Background

In Ireland, the agricultural sector (in particular livestock) plays a major role in the economy; the agri-food sector comprises 10% of the country's exports and 7.7 % of national employment (Teagasc, 2016). This sector also contributes 32 % to national greenhouse gas (GHG) emissions (EPA Ireland, 2015). The average EU contribution of the agricultural sector to national GHG emission is 9 %, therefore in this context the contribution of Irish agriculture to national GHG emissions is high (EPA Ireland, 2011). As Ireland aims to reduce national GHG emissions to 30 % of 2005 levels by 2030, there is increasing pressure on the agricultural sector to reduce or mitigate GHG emissions (even as the sector is expanding) (Irish Department of Agriculture Food and the Marine, 2016a).

Utilising byproducts generated by the agri-food sector (manure and other organic wastes) as feedstocks for on-farm or near farm anaerobic digestion (AD) has been undertaken in many European countries (Denmark, Germany, the Netherlands and the UK in particular) as a means to generate renewable energy and mitigate greenhouse gas emissions from the agricultural sector (Irish Department of Agriculture Food and the Marine, 2016b). The technology is not widely used in Ireland (Irish Department of Agriculture Food and the Marine, 2016c). There are two main reasons for this. Firstly the pasture-based nature of the dairy and cattle farming in Ireland means that year round collection of manure for biogas feedstock is not possible (O'Shea et al., 2016). Additionally due to the pasture-based system, and the low population density in agricultural areas, there is rarely a near-farm or on-farm heat demand to meet when biogas is utilised via combined heat and power (CHP) units to generate heat and electricity (Goulding & Power, 2013). This factor limits biogas plants ability to meet minimum energy efficiency standards to qualify for the renewable energy feed in tariff (REFIT) from which plants derive the majority of their income.

The pig industry in Ireland does not operate in a pasture based system and therefore is not as affected by these issues; farms generate and collect manure all year round,

and the pig houses have a heat demand which needs to be met (Nolan et al., 2012). Therefore pig farms may be the ideal locations from which on-farm biogas plants can be developed in Ireland.

Biogas plants which operate with a feedstock comprised solely of manure are rare. On-farm biogas plants typically operate by co-digesting manure with energy crops and/or organic wastes, due to the higher methane yields (and therefore revenues) which can be realised compared to mono-digestion of manure (Goulding & Power, 2013). The EU Landfill Directive regulating the diversion of organic wastes from landfills has led to a major increase in the amount of biodegradable municipal waste, food wastes (FW) in particular, being collected in Ireland over the past 6 years (EPA Ireland, 2016b). The increasing availability of this feedstock provides an opportunity for the development of on-pig farm co-digestion plants.

1.2 Objectives

While the on-farm anaerobic co-digestion of pig manure (PM) and FW is practiced at commercial scale across the world, no analysis of the concept in an Irish economic and regulatory context has been undertaken. Further to this, there is a paucity of information regarding how to optimise and control such co-digestion systems in terms of methane yields, process stability, enteric indicator organism removal and digestate disposal. This study will address these research gaps using a mix of laboratory-scale and meso-scale experiments combined with established physical, chemical and microbiological techniques, and advanced techniques such as high-throughput DNA sequencing, financial modelling and mathematical modelling. The structure of the research project carried out is presented in Figure 1-1.

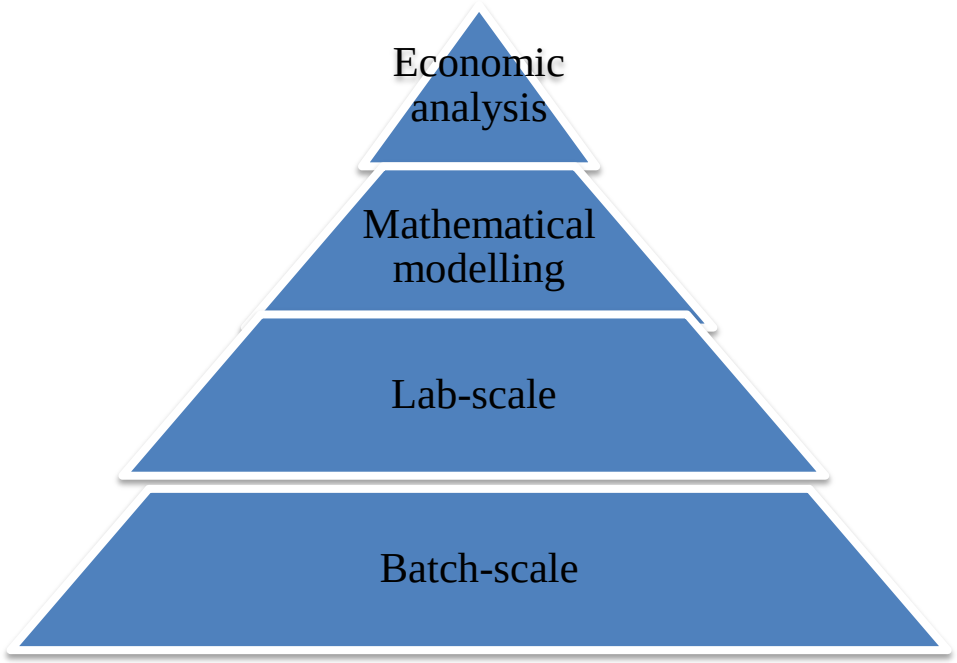


Figure 1-1 Overview of research plan structure

The specific objectives of this study were as follows;

1. *Assess and quantify synergistic or antagonistic effects of co-digesting FW and PM on methane yields and reaction kinetics using batch scale experiments.*

Batch experimentation provides fundamental data on maximizing methane yields, substrate degradability and causes of synergy between substrates. This information is useful in digester design, and was crucial in the semi-continuous and meso-scale experimentation, digester modelling and financial analysis undertaken in the study. In addition such an experimental design provides an opportunity to investigate methodological factors such as the effect of high volatile fatty acid (VFA) concentrations on synergistic effects, and the optimal models for defining parameters such as hydrolysis rate.

2. *Assess the effect of varying substrate composition (PM/FW mixing ratio) and digester hydraulic retention time (HRT) on; methane yields and process stability; digestate dewaterability; digestate biosafety; and microbial community dynamics.*

Semi continuous experiments are undertaken to simulate full scale systems. In doing so, optimal digester operating conditions can be determined in terms of a wide number of critical factors such as methane yields, process stability (chemical and microbial) and digestate quality. Such information is useful in digester

optimization and control, both for full scale systems and in operating the meso-scale digester used in this study.

3. *Assess whether a rudimentary calibration of the International Water Association (IWA) Anaerobic Digestion Model No. 1 (ADM1) can result in an accurate simulation of a PM/FW co-digestion system.*

Mathematical models have the potential to play a major role in digester control and optimisation, particularly if they become more straightforward to calibrate and deploy. The mathematical model used to simulate reactor performance was calibrated using data generated from the batch scale experiments and validated using data from semi-continuous meso- scale experiments.

4. *Assess the financial viability of on-farm PM/FW co-digestion plants in Ireland.*

Data on the economic viability of on-farm PM/FW co-digestion is essential to Irish farmers, developers and State bodies interested in the development of an indigenous biogas industry. Data generated from preceding experiments were used to develop a financial model which assessed the viability of on-farm co-digestion of PM and FW, both now and in the future.

Addressing these four distinct items provides important information for the design, operation, control and planning of on-farm PM and FW co-digestion systems.

1.2.1 Contribution to knowledge

The specific contributions made to science in this thesis are;

- Identification and quantification of the synergistic effects of PM and FW co-digestion on specific methane yield (SMY) and reaction kinetics.
- Identification of the effect of high substrate VFA concentrations on the observation of synergistic effects of co-digestion on SMY, and on the suitability of a range of kinetic models.
- Detailed statistical analysis of the effect of PM/FW co-digestion, and its interaction with digester HRT, on digester microbial community structure, digestate dewaterability, and digestate enteric indicator organism concentrations.
- Identification of minimum HRTs required for effective enteric indicator organism removal during PM/FW co-digestion, and identification of

potential biomarker microbial populations for digester instability at low HRTs.

- Assessment of the suitability of a rudimentarily calibrated iteration of the ADM1 model for accurate simulation of a PM/FW co-digestion system.
- Development of a stochastic financial modelling methodology for the assessment of the economic viability of on-farm PM/FW co-digestion in Ireland.

1.3 Procedures

This PhD research consisted of laboratory-scale and meso-scale experiments, and desk-based research.

In the laboratory-scale research, the SMYs of PM, FW and mixtures thereof were measured in batch experiments undertaken in 0.5 L conical flasks incubated on an orbital shaker at 37 ^{o}C. The cumulative methane generation curve from each mixture was simulated using a range of kinetic models.

Semi-continuous co-digestion of PM was undertaken in three 10 L stainless steel jacketed continuously stirred tank reactors (CSTRs) operating at mesophilic temperatures. The effects of various operating conditions (changes in substrate mixing ratio and HRT) on digester operation, digestate quality and microbial communities within the digester were assessed.

The data generated from the batch experiments were used to calibrate a mechanistic model of the anaerobic digestion system, which was then validated using data generated from a meso-scale digester (360 L effective volume). The farm based meso-scale digester was operated for a period of 120 days under varying organic loading rates (OLRs) and PM and FW mixing ratios. Methane yields and system stability were monitored. Data generated from both lab-scale and meso-scale experiments, along with data provided by regulators, engineering firms and biogas plant operators were used to develop a financial model which assessed the viability of on-farm co-digestion of PM and FW in Ireland. Deterministic and stochastic modelling was undertaken in order to assess the financial viability under current market conditions and assess financial viability considering potential variation in market conditions in future.

1.4 Structure of Thesis

This dissertation is comprised of 8 chapters:

Chapter 1 is the introduction. The background to the research, main objectives and research procedures are presented.

Chapter 2 reviews the literature relevant to the aims and objectives of this thesis and the research methods used. Topics included in this review are; current PM and FW management practices in Ireland, fundamentals of anaerobic digestion, co-digestion at batch and semi-continuous scale, digestate dewaterability, enteric indicator organism removal during anaerobic digestion, mathematical modelling of anaerobic digestion, 16s rRNA profiling and its role in analysing anaerobic digestion systems, and financial modelling of biogas plants.

Chapter 3 describes the batch-scale co-digestion experiment undertaken in order to identify optimal PM/FW mixing ratios and to quantify synergistic effects of co-digestion.

Chapter 4 describes the semi-continuous co-digestion experiment undertaken to assess how varying HRT from 41 days to 21 days, and varying PM/FW mixing ratio from 85 % PM to 40 % PM (VS basis), affected methane yields, process stability, digestate biosafety and dewaterability, and microbial community dynamics.

Chapter 5 describes the semi-continuous co-digestion experiment undertaken to assess how decreasing HRT from 21 days to 10.5 days affected digester stability, digestate biosafety and microbial community dynamics.

Chapter 6 describes the meso-scale experiment undertaken to validate a mathematical model which simulated the co-digestion of PM and FW.

Chapter 7 describes a study of the economic viability of on-farm co-digestion of PM and FW in Ireland.

Chapter 8 summarises the results obtained from the lab-scale, meso-scale and desk-based studies, and highlights the significance of the findings made. It also makes recommendations for future research work.

CHAPTER 2

Literature Review

2.1 Introduction

Pork is the most widely consumed meat in the world (Philippe & Nicks, 2015). Due to the rapid economic growth in populous countries such as China and India over the past 20 years, the demand for pork is expected to increase by up to 40 % by 2050 compared with 2010 levels (FAO, 2011). The size of the total global swine herd has doubled since the 1970s (FAO, 2016), and is expected to increase by a further 25 % by 2030 (FAO, 2013). Livestock based agriculture contributes between 8 and 11 % of the total global anthropogenic GHG emissions (O'Mara, 2011; Smith et al., 2008), with pigs accounting for 13 % of this (Philippe & Nicks, 2015). Therefore the pig industry is a significant contributor to global GHG emissions.

As highlighted in detail by Steinfeld et al. (2006), the reduction and mitigation of GHGs from the livestock sector is essential in order to reduce the impact of climate change. To that end, national and international emission targets stipulated by the Paris Agreement are driving the need to reduce GHG emissions on a national level.

2.1.1 Addressing GHG emissions from Irish agriculture

The abolishment of the EU common agricultural policy in 2014 has resulted in significant expansion of the Irish agricultural sector, due to the expansion of the dairy industry in particular, where a 50 % increase in milk production is expected by 2020 (relative to 2007-2009 levels) (Finnegan et al., 2017). In addition to this, the government's Harvest 2020 policy has set targets of increased output from all agricultural sectors by 2020. For example the policy seeks to increase the output value of the pig, beef and sheep sectors by 50 %, 20 % and 20 %, respectively, relative to 2007-2009 levels (Committee, 2010).

As highlighted in Chapter 1, the relatively high contribution of agriculture to national GHG emissions (33.3 % in 2014 (Irish Department of Agriculture Food and the Marine, 2016b)) means that reducing emissions from the sector, despite the major growth projected, will be key in meeting binding EU targets on national

GHG emission reductions (requiring Ireland to reduce emissions by 30 % of 2005 levels by 2030)(EPA Ireland, 2016c).

The Irish Department of Agriculture, Food and the Marine suggests that the expansion of the Irish livestock industry will not necessarily result in a commensurate increase in GHG emissions from the agricultural sector as measured by IPCC National Greenhouse Gas Emission Inventories (Irish Department of Agriculture Food and the Marine, 2016a). Despite major growth in the sector in the past 26 years, GHG emissions from agriculture are currently lower than that of 1990 levels (Irish Department of Agriculture Food and the Marine, 2016a).This has been attributed to the high level of innovation and development which has occurred over the same period, with improvements in manure management, fertilizer application, and soil carbon management occurring. The Department of Agriculture, Food and the Marine suggests that internationally established, cost-effective solutions can play a role in further mitigating GHG emissions from the expanding agricultural sector (Irish Department of Agriculture Food and the Marine, 2016a).

As an internationally established technology, the use of on-farm biogas plants may play a key role in maximising the sustainability of the Irish agricultural sector, in this time of significant expansion. As discussed in Chapter 1, pig farms have considerably greater potential as sites for on-farm biogas plants than the cattle and dairy sector. As such, on-farm biogas plants in the pig sector are best placed to play a strategic role in mitigating GHG emissions from the Irish agricultural sector. It should be noted that due to the EU Animal By-products regulations (Regulation (EC) 1069/2009), biogas plants co-digesting FW and PM cannot be placed directly on farm, but rather must be located in an adjacent fenced site with entrances and exits separate to the farm. However, in this dissertation the term on-farm will be used to describe such a scenario.

2.2 Pig manure management

PM can be managed in a number of different ways (Figure 2-1), depending on the pig farming system employed, as well as site specific environmental requirements.

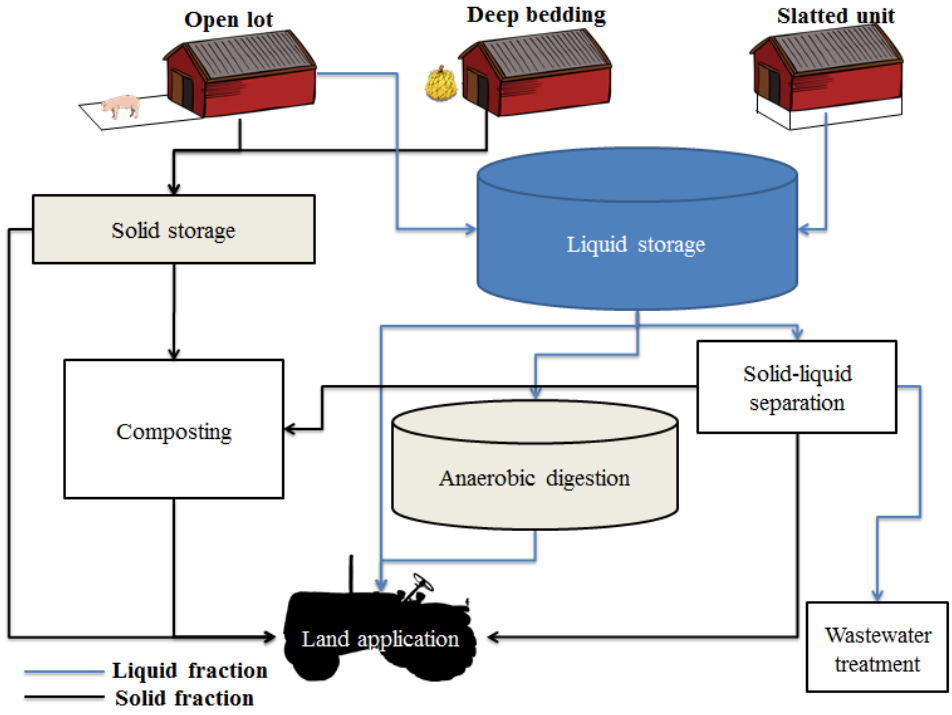


Figure 2-1 Illustration of collection and management options for piggery wastes

Managing PM in liquid form is the most prevalent PM management method in the EU. Liquid PM is typically collected from pits beneath slatted floors on which the pigs are housed (Burton, 2007). The manure contained in these pits is periodically emptied into long term storage pits or tanks. Regulations such as the Nitrates Directive (1991/676/EEC) and the Water Framework Directive (2000/60/EC) are in place to reduce the impact of land application of manure on water courses and to ensure efficient nutrient cycling on farms. Such regulations stipulate that manure can only be spread on land during spring/summer times, typically when drier weather is likely (McKenna et al., 2013). As such, manure may be in storage for between 1 and 10 months (Burton & Turner, 2003). Subsequent to storage, manure is applied to land to realise its fertilizer value. In some cases manure may undergo dewatering processes to generate a liquid and solid fraction (Deng et al., 2014; Wnetrzak et al., 2013). Both fractions are typically then applied to land, however they may also undergo further treatment (Dinuccio et al., 2008). In the case of the solid fraction, composting is commonly used, while the liquid fraction may be treated by various wastewater treatment processes, for instance aerobic wastewater

treatment for removal of nutrients from liquid manure. It should be noted that the use of aerobic wastewater treatment processes is restricted to farms located in areas where land application of PM poses a threat to sensitive waterways. In recent years the use of straw-based deep litter and open lot systems has increased in prevalence. Such systems are perceived to be an improvement on traditional systems in terms of animal welfare (Philippe & Nicks, 2015). In deep litter systems, PM and bedding materials become mixed. This results in a solid manure/bedding mixture. This solid mixture is periodically removed from the pig housing unit and stored in piles prior to land application. These piles are typically housed in sheds. In some instance the solid piles will be constructed to allow for passive or active composting to occur during storage. Dry anaerobic digestion may also be used to treat these solid piles. In any case, the ultimate disposal route for this solid mixture is land application (Tait et al., 2009). For open lot systems, solid waste is typically collected in a separate manner to any liquid runoff from the site. The treatment applied to the resulting liquid fraction is dependent on site specific conditions, while the solid fractions are typically land applied or composted (Chynoweth et al., 1998).

In Ireland the collection of liquid manure followed by a period of storage and land application is by far the most common manure treatment and disposal route. The use of separation technology is rare, and manure is stored typically for between 4-6 months. As of 2013 there were approximately 1.5 million pigs in Ireland (Teagasc, 2013) and approximately 3 million tonnes of pig manure is produced each year (Xie, 2012).

2.3 Food waste management and disposal

Up until the introduction of the EU Landfill directive (Council Directive 1999/31/EC) in 1999, the vast majority of food waste generated in Ireland was not separated, but combined with general refuse and ultimately landfilled. However the Landfill directive requires Ireland to reduce the amount of biodegradable waste going to landfill. Over the past 10 years, source separated food waste collection has been introduced in the majority of urban areas (EPA Ireland, 2014). Waste water treatment plants and other industries which produce organic wastes were disincentivised from landfilling through the roll out of a landfill levy. The final target of reducing the amount of biodegradable waste landfilled to 35 % of 1990 levels was achieved in 2016. This has resulted in an increase in the demand for solid organic waste treatment methods over the last 15 years (EPA Ireland, 2016b).

The EPA (EPA Ireland, 2016a) found that 300,000 tonnes of waste was received by composting and AD plants in 2015, with 194,000 tonnes comprised of source segregated municipal FW. 20 % of all waste received by AD and compositing facilities (which, in addition to municipal FW, was comprised of wastewater treatment sludge and commercial organic wastes) was treated in one of the 6 AD facilities licenced to accept municipal waste (EPA Ireland, 2016a). This illustrates the limited size of the Irish AD market currently. However, desk based studies have found that over 624,000 t of FW could be source separated and made available for treatment in Ireland each year (O'Shea et al., 2016). There is consequently significant potential for the expansion of the organic waste treatment market (of which AD facilities are a part), provided separation and collection rates are improved in future.

2.4 Anaerobic digestion for greenhouse gas emission mitigation

Anaerobic digestion may mitigate GHG emissions from agriculture. It may achieve this by generating biogas which replaces fossil fuels, by reducing demand for chemical fertilizers (the production of chemical fertilisers generates significant quantities of GHGs) and by reducing emissions from subsequent manure handling, storage and land application (Amon et al., 2006). As an example, Table 2-1 illustrates that AD can significantly reduce GHG emissions from PM management.

Table 2-1 Greenhouse gas (GHG) mitigation potential of anaerobic digestion of pig manure (PM) via continuously stirred tank reactor (CSTR) systems

Total kg $CO_{2\ eq}$/t PM mitigated	% GHG mitigated - biogas utilisation via CHP*	% GHG mitigated -reduced chemical fertilizer use	% GHG mitigated - lower emissions in storage and land application	Location	Reference
87.7-125.6	40-60	28- 33	12-27	Finland	Kaparaju and Rintala (2011)
20	100	-.	-	Desk-Based	Prapaspongsa et al. (2010)
45.3	34.6	65.4	-	Ireland	Xie (2012)
68.3	11 (electricity only)	14- 20	60-85	Australia	Maraseni and Maroulis (2008)
16†	-	-	-	Desk-Based	De Vries et al. (2012)

† This figure describes the net effect anaerobic mono-digestion has on PM management, not breakdown of the contribution each mitigation factor makes to GHG mitigation potential anaerobic digestion provides.* Combined heat and power unit.

CH_4 utilisation is the most widely reported factor contributing to the GHG mitigation potential of AD in PM management. Kaparaju and Rintala (2011) found that employing AD on pig farms could lead to between 87.7 and 125.6 kg $CO_{2\ eq}$/t PM being mitigated through the use of biogas generated, reduced chemical fertiliser use and lower emission during manure storage and land application. The wide variation in measured values can be attributed to the variation in season

during which these measurements were taken, emphasising the strong impact regional climatic variation can have on GHG emissions. This study seems to be an outlier when compared with the work of Prapaspongsa et al. (2010) (who assessed the GHG mitigation potential of energy generation only), Xie (2012) and De Vries et al. (2012), all who found that the AD of liquid PM can result in a mitigation of between 15 and 20 kg $CO_{2\ eq}$/t PM when biogas utilisation and reduced emissions from manure storage were considered. Maraseni and Maroulis (2008) reported a considerably lower mitigation value of 7.5 kg $CO_{2\ eq}$/t in their study of an Australian pig farm however their study did not consider the effect the heat generated from the combustion (via CHP) of the biogas generated would have on GHG emissions (i.e. displacing fossil fuels).

GHG emissions from the storage of digestate have been found to be half that of untreated PM (Amon et al., 2006). This is due to AD systems removing between 40 % to 80 % of the volatile solids (VS) in PM (Hansen et al., 1998). In addition, digestate generates lower N_2O emissions (thus overall GHG emissions) during land application than untreated manures. The primary mechanism responsible for the decrease in N_2O emissions from the land application of digestate is that the reduced VS content in the manure due to AD results in decreased microbial activity and therefore a reduction in both the rate of nitrification and denitrification in soils (Montes et al., 2013; Thomsen et al., 2010).

Due to the mineralisation of amino acids, digestate tends to have a higher ammonium-N concentration than that in raw PM. A review by Möller and Müller (2012) on the topic, found that N availability in digestate is increased by 10-25 % relative to untreated manure. This results in a more efficient fertilizer, thereby reducing the amount of chemical fertilizer required by farmers. The specific improvement in N use efficiency between PM and digestate determines the amount of chemical fertilizer use avoided. Production of such chemical fertilizer results in GHG emissions. As illustrated in Table 2-1, studies have found that between 9 and 20 kg $CO_{2\ eq}$/t PM may be avoided through replacement of chemical fertilisers as a result of AD.

2.5 Fundamentals of anaerobic digestion

2.5.1 Biochemical pathways

In the broadest terms, AD is a process achieved by the syntrophic interactions between two domains; bacteria and archaea. Bacteria are responsible for disintegration, hydrolysis, acidogenesis and acetogenesis, while archaea are responsible for methanogenesis.

2.5.1.1 Hydrolysis and acidogenesis

The first step of anaerobic digestion is the hydrolysis of carbohydrates, proteins and lipids into sugars (monosaccharides), amino acids and long chain fatty acids (LCFAs) (Batstone et al., 2015). As Figure 2-2 illustrates, sugars and amino acids are further converted into volatile fatty acids (VFAs) in the acidogenesis phase (Batstone et al., 2002). These first 2 stages of AD are typically regarded as robust and capable of operating successfully under a wide range of reactor and substrate conditions (Xie et al., 2016). This is due to the high level of functional redundancy present in hydrolytic and acidogenic bacterial populations (De Vrieze et al., 2016).

2.5.1.2 Acetogenesis, syntrophic acetate oxidation and homoacetogenesis

In the acetogenic phase the LCFAs generated from hydrolysis are converted directly to acetate and hydrogen via β oxidation, while VFAs generated from the acidogenic stage are, in the case of valerate and butyrate, oxidised to propionate, acetate and hydrogen (and a small amount of formate) (Batstone et al., 2002). In the case of propionate, acetogenesis proceeds directly to acetate and hydrogen production as illustrated by Equation 2-1.

2-1 $$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$$

The resulting pools of hydrogen and acetate from acetogenesis may be converted to one another via syntrophic acetate oxidation (SAO), and homoacetogenesis. SAO can be described as per Equation 2-2 while homoacetogenesis can be described as per Equation 2-3.

2-2 $$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$$

2-3 $$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$

The importance of SAO and homoacetogenesis is dependent on reactor conditions and substrate types. For example, high ammonia concentrations (common in

farm-based biogas plants) typically promotes syntrophic acetate oxidation (Werner et al., 2014).

In terms of thermodynamics, homoacetogenesis is less thermodynamically favourable than methanogenesis, while SAO is only thermodynamically possible when hydrogen partial pressures are maintained at a low concentration ($1x10^{-3}$ mBar) by hydrogenotrophic methanogenesis (Demirel & Scherer, 2008).

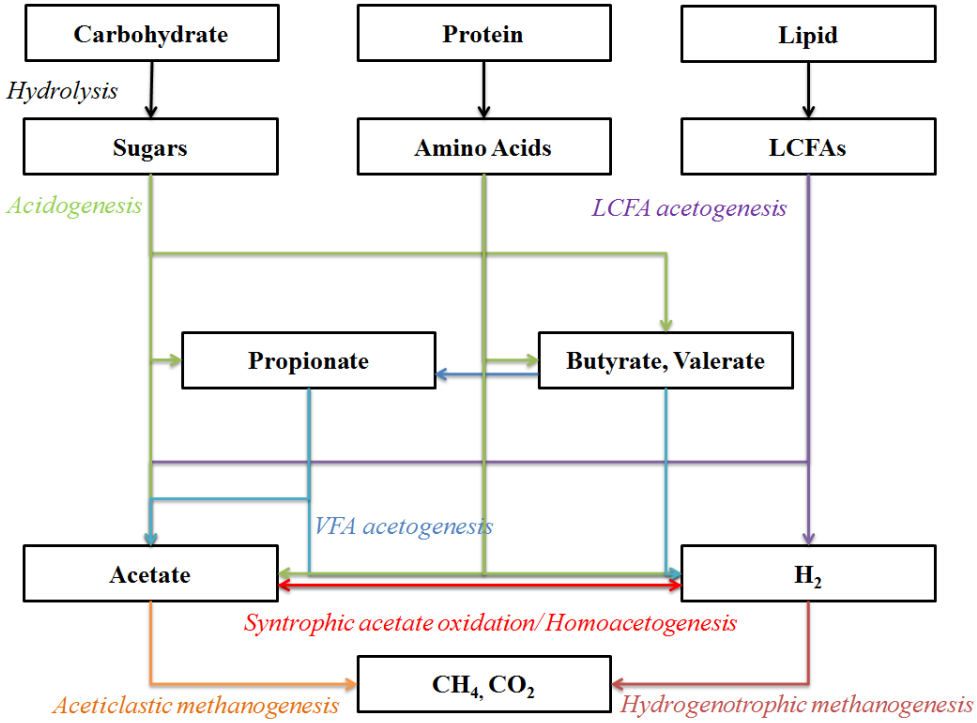


Figure 2-2 Overview of the anaerobic digestion process. Amended from Batstone et al. (2002)

2.5.1.3 Methanogenesis

Methanogenesis is undertaken by archaeal population. Slower growing than bacterial populations, methanogenic populations are typically cited as the most sensitive microbial consortia in AD systems, in particular to changes in reactor pH (Xie et al., 2016). The reason for this is that due to the low diversity of methanogenic archaea, low functional redundancy is expected (Carballa et al., 2015).

Acetate and hydrogen may be converted to methane by 2 distinct pathways. Hydrogenotrophic methanogenesis generates methane by utilising hydrogen or

formate as an electron donor (Demirel & Scherer, 2008), and reducing CO_2 to methane and water in the following manner (Batstone et al., 2002);

2-4 $$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

Aceticlastic methanogenesis proceeds by oxidising acetate to produce methane and CO_2 in the following manner (Batstone et al., 2002);

2-5 $$CH_3COOH \rightarrow CH_4 + CO_2$$

While aceticlastic methanogenesis is typically cited as the dominant methanogenic pathway in the majority of anaerobic digestion systems (Batstone et al., 2002), hydrogenotrophic methanogenesis has been shown to play a major role in agricultural-based biogas plants (Alsouleman et al., 2016) where due to the addition of ammonium rich manures, SAO becomes a significant biochemical pathway (increasing the production of hydrogen). Maintaining low hydrogen partial pressures is crucial for maintaining a stable AD system; in order for acetogenesis to be thermodynamically possible, hydrogen partial pressures must remain low (Demirel & Scherer, 2008). Therefore hydrogenotrophic methanogenesis plays a more significant role in maintaining process stability, as it maintains low H_2 partial pressures, allowing the more dominant acetoacetic methanogenic pathway to proceed (De Vrieze et al., 2015).

2.5.2 Temperature effects

One of the main factors which affect AD reactors is temperature. As Figure 2-3 illustrates, while growth rates increase with temperature, there are three distinct temperature ranges at which methanogen growth rates peak and decline, as different methanogenic populations predominate (Lettinga et al., 2001).

While the application of psychrophilic AD to wastewater treatment is an area of significant research interest (Collins et al., 2006; Mao et al., 2015; Petropoulos et al., 2016; Rajagopal et al., 2017), it is not commonly applied on a commercial scale in Europe. The mesophilic range is the most common studied and industrially applied temperature range, with thermophilic less common.

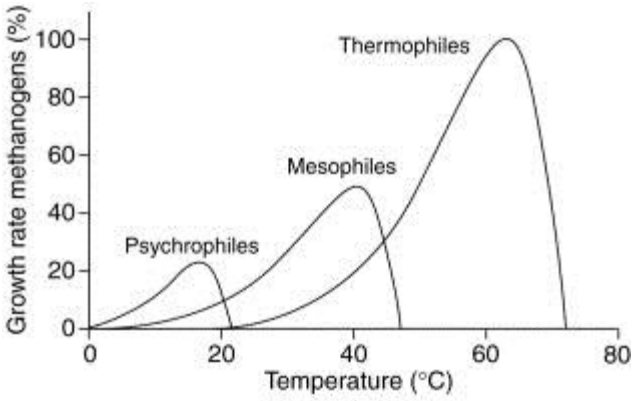


Figure 2-3 Methanogenic growth rates vs temperature (Lettinga et al., 2001)

The rate of the AD process is significantly higher at thermophilic temperatures than mesophilic temperatures (Xie et al., 2016). This in turn results in thermophilic AD typically having higher organic substrate destruction rates than mesophilic AD (Angelidaki & Ellegaard, 2003). However higher temperatures also make VFA oxidation and hydrogen generation more energetically favourable (Xie et al., 2016). Hydrogenotrophic methanogenesis is the primary hydrogen utilisation pathway (due to the resulting high hydrogen partial pressures) and is therefore key to stable operation at thermophilic temperatures (Schink, 1997). Due to the high rate of hydrolysis and acidification, VFA accumulation and subsequent process inhibition is a greater risk at thermophilic conditions than mesophilic conditions. This allied to the fact that higher temperatures typically results in high free ammonia concentrations, makes thermophilic AD systems more challenging to control than mesophilic digesters (Hagos et al., 2016). Nevertheless there are many full scale AD plants operating at thermophilic temperatures due to the more rapid process kinetics which in turn allow for higher loading rates and higher volumetric methane yields than at mesophilic temperatures (Cavinato et al., 2010). Angelidaki and Ellegaard (2003) suggest that the negative reputation thermophilic AD has in terms of process instability can be attributed to historically poor start up regimes and lack of availability of thermophilic biomass for the establishment of digesters.

While mesophilic AD cannot operate at the rates of thermophilic systems, due to the stability associated with operating at such temperatures it remains the most common temperature range used in commercial AD plants, and in studies of AD systems (Hagos et al., 2016). Mesophilic anaerobic digestion is generally dominated by the aceticlastic methanogenic pathway (Schink, 1997).

2.5.3 Inhibitors

The AD process can be inhibited, partially or completely by the presence of specific compounds above a certain concentration (Chen et al., 2008). The most commonly observed inhibition factors are ammonia/ammonium, sulfur and LCFAs. While other compounds, such as chlorinated organic compounds, heavy metals and high salt concentrations (Chen et al., 2008) have been found to inhibit AD systems, they are only an issue in niche situations.

2.5.3.1 Volatile fatty acids

As key intermediary products in AD, VFAs are present in all AD systems. However accumulation of VFAs can result in a decrease in the system pH (Chen et al., 2008), and ultimately, process failure. VFA accumulation is typically caused by inhibition of the syntrophic or methanogenic populations (Siegert & Banks, 2005). In addition to causing inhibition by reducing system pH, unionised VFAs can cause direct toxicity to digester archaea and bacteria by penetrating cell walls and affecting cell pH (Puchajda & Oleszkiewicz, 2006).

2.5.3.2 Hydrogen

As mentioned previously, high hydrogen partial pressure (which would be present in systems where SAO predominates) can have significant negative effects on digester operation; specifically high concentrations of hydrogen can make aceticlastic methanogenesis and the oxidation of VFAs thermodynamically not favourable, increasing the potential for VFA accumulation (Batstone et al., 2002). Such conditions may be avoided by ensuring digester inoculum suitable for the substrate being treated. For example sourcing inoculum from a manure-based digester when treating manure will ensure that a large, diverse and stable SAO and hydrogenotrophic methanogenic population is present, thereby ensuring that hydrogen production and removal is in sync (Vanwonterghem et al., 2014). Operating at lower OLRs may also ensure low hydrogen partial pressures.

2.5.3.3 Ammonia

High concentrations of ammonium can cause inhibition of AD systems, specifically inhibition of methanogens resulting in VFA accumulation. VFA accumulation in turn results in a drop in pH which can result in complete process failure (Chen et al., 2008), or simply an inhibited steady state, in which the system remains stable but with depressed methane yields (Hansen et al., 1998; Yenigün & Demirel, 2013). While ionised ammonium (NH_4-N) is thought to play a role, the

main cause of ammonia inhibition is free ammonia nitrogen (Rajagopal et al., 2013), as it may pass through cell membrane and directly affect cells, resulting in proton imbalance (Sung & Liu, 2003). Free ammonia nitrogen (FAN) concentration is calculated based on the measured pH and NH_4-N concentration at a given temperature using the method described by Anthonisen et al. (1976):

2-6 $$\mathbf{NH_3} = \frac{\mathbf{NH_4^+ \times 10^{pH}}}{\mathbf{10^{pH} + e^{6344/(273+t)}}}$$

By definition, FAN concentrations are higher at higher temperatures and at higher pH. The reported inhibitory threshold concentrations for both FAN (337-1450 mg/L) and total ammonium (2800-11000 mg/L) varies widely (Hansen et al., 1998; Yenigün & Demirel, 2013). This is due to the widely varying feedstocks, temperatures, reactor configurations and, crucially, inocula used in these experiments (Yenigün & Demirel, 2013). Ammonia inhibition is reversible; microbial populations may acclimatise to high ammonia concentrations provided total ammonia nitrogen concentrations do not exceed critical inhibitory concentrations (between 6,700 mg/L to 18,300 mg/L) (Yenigün & Demirel, 2013). Ammonia inhibition may be avoided by ensuring ammonia concentrations within digester influent remain stable, and a suitable (ammonia acclimated) inoculum is used during digester start up. Co-digestion can also be used to mitigate against ammonia inhibition. The addition of carbon rich substrates (such as FW) to ammonia rich substrates (such as PM) can reduce concentrations of ammonia in reactors and alter carbon to nitrogen (C/N) ratios so they are within the range recommended to ensure stable AD (20 to 30) (Dai et al., 2016).

2.5.3.4 Sulfur

Reduced sulfur compounds are common in AD systems treating organic and industrial wastes (Chen et al., 2008). They are present in AD systems normally in reduced forms as S_2^- and HS^- (or its associated form H_2S) (Tanaka & Lee, 1997). Microbial groups which reduce sulfur compounds (such as sulfate reducing bacteria (SRBs)) utilise VFAs as a carbon source and electron donor in the process (Batstone et al., 2002). Hydrogen may also be used as an electron donor. In doing so they compete with syntrophic bacteria, aceticlastic methanogens and hydrogenotrophic methanogens (Batstone et al., 2002; Chen et al., 2008). SRBs limit the growth of methanogens and suppress methane production. In addition to this, H_2S is directly toxic to syntrophic bacteria and methanogens (Chen et al., 2008) at concentrations of 0.003 M- 0.005 M total sulfur. Aside from removal of

sulfur from substrates prior to AD, one way of alleviating sulfur inhibition is the selective inhibition of SRBs by molybdate (Mo) addition (Tanaka & Lee, 1997). Molybdate as a selective SRB inhibitors has been widely studied (Chen et al., 2008; Patidar & Tare, 2005). A typical effective dosing rate of 3 mM (or 288 mg/L) of Mo has been suggested (Patidar & Tare, 2005), in order to inhibit SRBs, thereby boosting methane yields. However the high cost associated with Mo, and the fact that such high Mo concentrations in digestate would prohibit the land application, means that this is not a viable solution of alleviation of sulfate inhibition.

2.5.3.5 Long chain fatty acids

Lipids are very common constituents to substrates which undergo anaerobic digestion. As illustrated in Figure 2-2, these lipids are hydrolysed into LCFAs. LCFAs have been reported to be inhibitory to the methanogens and bacteria involved in AD and result in VFA accumulation (Xie et al., 2016). LCFAs inhibit methanogens by disrupting cell transport; they adsorb onto the archaea cell walls/membranes disrupting extracellular transport (Chen et al., 2008). They also can be adsorbed onto biomass and increase the buoyancy, resulting in increased biomass washout (Chen et al., 2008). Biomass can be readily adapted to the presence of LCFAs, and therefore stable digestion of high concentrations of LCFAs is possible, provided a suitably acclimated biomass is used during digester start-up, and shock loads of LCFAs are avoided (Batstone et al., 2002; Chen et al., 2008).

2.5.4 Reactor configurations

AD may be carried out in one of two broad categories; wet digestion (where total solids (TS) concentrations are below 15 %-20 %), and dry digestion (where TS concentrations exceed 20 % (Stolze et al., 2015)). Wet AD is the most common mode for undertaking AD, while dry anaerobic systems remain niche (Kwak et al., 2013).

2.5.4.1 Dry anaerobic digestion

The application of dry AD to farm-based biogas generation is somewhat limited considering most farm-based biogas plants operate utilising liquid manure (typically in conjunction with additional feedstocks) (Massé et al., 2011). However as the popularity of solid liquid separation on farms increases (Deng et al., 2014), and with the increase in prevalence of deep bedding animal systems (Tait et al.,

2009) a greater proportion of solid organic farm waste will be generated. Dry digestion may play a role in managing this waste.

Dry AD is typically undertaken in batch mode; substrate is mixed with an inoculum and placed under anaerobic conditions for a fixed period of time (Forster-Carneiro et al., 2007), while the biogas is collected. The proposed (Huang et al., 2016) advantages of dry AD over wet AD systems are:

- Lower footprint
- Lower energy demand
- Lower digestate volumes so lower disposal costs

Dry AD systems have disadvantages such as long digestion times (due to limitations in mass transport in such systems), and the presence of high VFA and ammonia concentrations potentially leading to system failure (Li et al., 2011).

Wet AD can be undertaken in several ways. For agricultural based systems the most common AD systems are anaerobic lagoons, plug flow reactors and continuously stirred tank reactors (CSTRs).

2.5.4.2 Anaerobic lagoons

Anaerobic lagoons are perhaps the simplest type of AD reactor. They are typically used to treat manures with a TS content of <3 % (Ogejo et al., 2009). An overview of an advanced anaerobic lagoon is presented in Figure 2-4. Substrate (such as manure) is fed into a covered (sometimes uncovered) lagoon. There are a large number of potential hydraulic designs (most common being the bottom fed design in Figure 2-4). The residence time of substrates in such lagoons is in the order of weeks to months and is dependent on ambient temperature (Pandey et al., 2016). Any biogas generated may be collected. Anaerobic lagoons are most commonly used to provide primary treatment to agricultural wastes and industrial waste waters. Provided sufficient land is readily available at low cost, this can be a low cost means of reducing the organic load of wastes while generating biogas. It does have significant disadvantages, even aside from its large footprint (Ogejo et al., 2009). As temperature is uncontrolled this technology can only be utilised effectively in warm climate (Wall et al., 2000). In addition solids can accumulate within such systems, limiting the hydraulic capacity (Batstone et al., 2015) and requiring periodic system downtime.

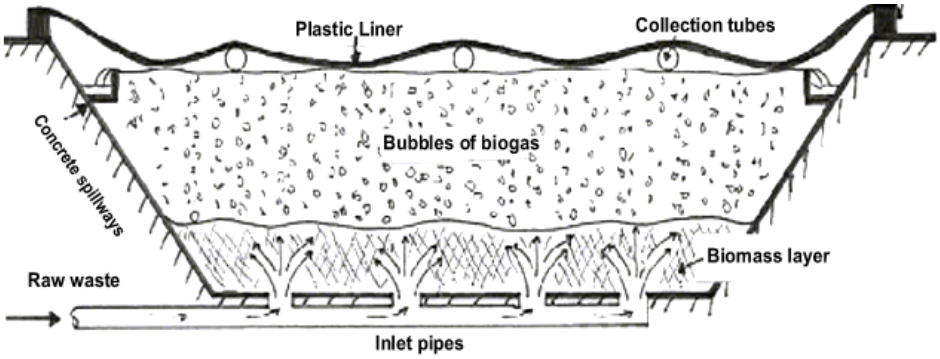


Figure 2-4 High rate anaerobic lagoon design (Wall et al., 2000) © WIOA, 2000

2.5.4.3 Plug flow anaerobic digesters

Plug flow AD systems are another relatively low cost means of undertaking on-farm anaerobic digestion (Metcalf et al., 1991). These are commonly used for treating high solids manure (TS of 8 % - 12 %) (Batstone et al., 2015). Plug flow reactors are typically comprised of long narrow tanks. As illustrated in Figure 2-5, substrate moves horizontally along the tank, coming in contact with anaerobic biomass as it does so. As it moves through the system the available carbon is converted to biogas and collected (Ogejo et al., 2009). It typically takes >20 days for fresh substrate to pass through the system (Batstone et al., 2015).

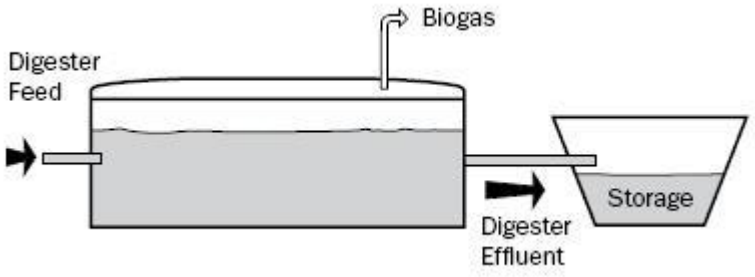


Figure 2-5 Schematic of a plug flow anaerobic digestion system (Ogejo et al., 2009)

While the design and operation of such systems is simple, it is largely limited to feedstocks with a TS content >8 % and less than 20 % (Ogejo et al., 2009).

2.5.4.4 Completely stirred tank reactors

By far the most common technology used for on-farm biogas systems is the CSTR. It has the capacity to treat substrate with TS contents up to 15 % (Metcalf et al., 1991), and therefore is highly versatile. As Figure 2-6 illustrates it is comprised of a tank (or tanks in series) containing active anaerobic biomass which is fed with

substrate (continuously or semi-continuously) and completely stirred. Stirring is essential to ensure the particulates in the substrate remain in suspension (Metcalf et al., 1991). Mixing can be achieved using mechanical agitators (such as is illustrated in Figure 2-6), or by recirculation of the collected biogas (Metcalf et al., 1991). The tanks are heated to the desired temperature (mesophilic or thermophilic) and biogas is collected. In single stage CSTRs, all AD processes occur in a single reactor. Single tank CSTR systems are straightforward to design and operate, and are widely used for on-farm AD (Ward et al., 2008).

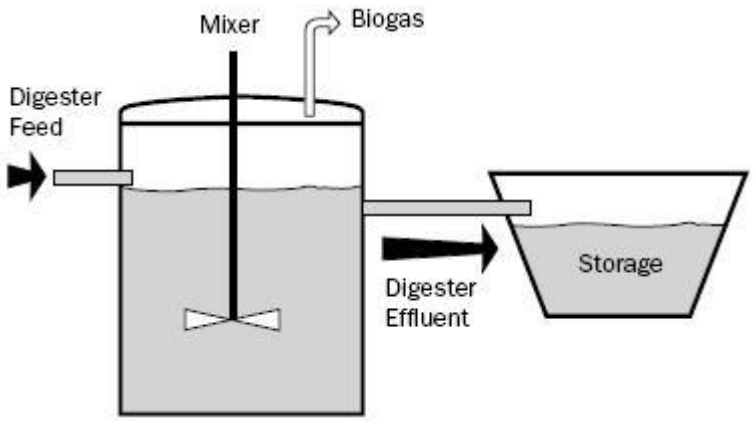


Figure 2-6 Schematic of a single stage continuously stirred tank reactor (CSTR) digester (Ogejo et al., 2009)

Two stage CSTRs may also be used. These are comprised of two tanks. In the first tank hydrolysis/acidogenesis/acetogenesis are the predominant processes, and in the second (typically larger) tank, methanogenic processes dominate. The separation of these biochemical stages is desired in order to optimise substrate solubilisation, and subsequent methanation. As the optimal conditions for the growth of hydrolytic, acidogenic and acetogenic bacteria occur at a pH between 5 and 7, and as they have more rapid growth rates than methanogenic organisms, the first reactor may operate at higher hydraulic rates and low pH (Aslanzadeh et al., 2014). The second reactor can then be steadily fed with the acetate rich substrate produced in the first reactor, while operating at a higher pH and lower hydraulic rates. Overall this allows 2 stage systems to treat higher volumes of substrate over time (in a stable manner) compared to a single stage digester of a similar size (Aslanzadeh et al., 2014).

2.5.4.4.1 Design and optimisation of CSTR systems

Perhaps the most critical design and operational consideration for CSTR reactors is the HRT. Also referred to as dilution rate, HRT is the theoretical average residence time of substrate in the CSTR (Metcalf et al., 1991). HRT is calculated by dividing digester tank size by flow rate. Longer HRTs result in a greater level of substrate utilisation and higher methane yields per unit of substrate added. Longer HRTs also result in a more stable digestion system, as all microbial populations (methanogens in particular) can develop into stable mature populations as biomass washout rates are low (Krakat et al., 2010). Additionally longer HRTs result in greater dilution, so if inhibitory compounds occur, they are diluted into low concentrations, reducing the risk of system instability. However, by definition, long HRTs require either low flow rates or necessitates larger tank sizes. While shorter HRTs can reduce the need for large tanks, or increase substrate throughput, it may result in a process prone to instability due to biomass loss (Ziganshin et al., 2016). As substrates are anaerobically degraded at different rates, the optimal HRT/digester tank size is dependent on the substrate treated. Therefore determining the rate of degradation of substrates is critical in design and operation of CSTRs. This is discussed in detail in Section 2.6.3. HRTs used in biogas systems vary widely, but are typically between 30 - 50 days for manure-based biogas plants (Chynoweth et al., 1998).

Another key operational parameter for CSTR systems is the organic loading rate (OLR). This is the rate of addition of the organic fraction of substrate per unit time (Metcalf et al., 1991). It is therefore a function of HRT and organic composition of substrate. It is typically expressed in terms of g VS/L/d (Nasir et al., 2012a). For substrates with a low VS content such as PM, OLR is intrinsically linked with and limited by HRT. Increased HRT reduces OLR, while decreased HRT increases OLR. For substrates with high TS and VS content (such as FW), it is often desirable to decouple OLR from the HRT of the CSTR system by diluting substrate (either with water or via co-digestion (Atandi & Rahman, 2012)). This is done in order both to ensure digester TS content remains below 15 % (the limit for CSTRs), and to ensure that the system does not become organically overloaded (Zhang et al., 2012). When OLR is too high, hydrolysis/acidogenic/acetogenic processes decouples from the slower growing methanogens, resulting in VFA accumulation (Metcalf et al., 1991). In order to optimise the performance of a CSTR, OLR should be maximised while ensuring process stability can be maintained. Identifying such an OLR can be challenging considering maintaining process

stability in CSTR systems is also dependent on HRT, digester TS content, digester pH and the buffering capacity of the system.

2.6 Analysing and optimising anaerobic digestion

Characterising and analysing the AD of substrates can be done in a variety of methods. Predominately however, AD experiments are carried out in two ways; batch tests which identify intrinsic anaerobic degradation properties of substrates and combinations thereof, and continuous or semi-continuous experiments where simulation of full-scale conditions is undertaken. Such experiments can allow for the determination of the optimal conditions required for the AD of specific substrates, and provide insight into the physical, chemical and microbial interactions occurring during the AD process.

2.6.1 Batch anaerobic digestion

Batch tests are typically carried out in order to quantify the biomethane production potential (BMP) and hydrolysis rates (also denoted as k_H) of substrates or mixes of substrates (Angelidaki et al., 2009). Note BMP can also be referred to as the specific methane yield (SMY), particularly when referring to mixtures of substrates. The BMP is the amount of methane that may be generated per g of organic matter in the substrate (either volatile solids (VS) or chemical oxygen demand (COD)) (Hagos et al., 2016).

Batch tests are typically undertaken by combining a known quantity of substrate with fresh biomass (taken from an operating anaerobic digester) in a sealed reactor, placing the reactor in an incubator (typically set at mesophilic temperatures) and monitoring the resulting biogas flow and biogas composition over a set period of time.

As hydrolysis is typically the rate limiting step in AD of particulate matter (Brulé et al., 2014), the rate of hydrolysis can be determined by measuring the rate of methane emission from the batch AD of a substrates. The cumulative methane yield from a batch test normally follows a first-order pattern; the majority of methane release in the initial stages of digestion, with increasingly smaller volumes released as the experiment continues. Therefore it is common to fit a simple first-order model to determine hydrolysis rate and the BMP. Equation 2-7 describes the first-order model;

2-7 $$\boldsymbol{M(t) = M_m(1 - e^{-k_H t})}$$

where M_m is the theoretical BMP (in mL CH_4/g of VS or COD basis) at time ∞, M(t) is the measured specific methane yield (mL /g of VS or COD) at time t (days)

and k_H is the hydrolysis rate. A typical cumulative methane yield and first-order model thereof are presented in Figure 2-7. It should be noted that, in some instances when hydrolysis is not the rate limiting step in the AD system, or when substrates exhibits two or more distinct periods of methane generation, other mathematical models may be used to identify the kinetic parameters of the substrates (Brulé et al., 2014; Dennehy et al., 2016). This is discussed in detail in Section 2.10.1.

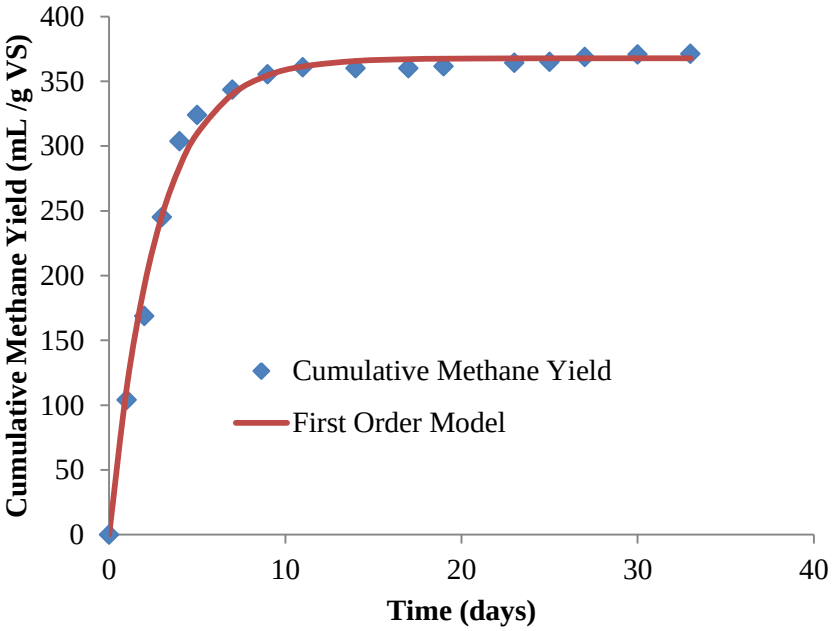


Figure 2-7 Cumulative specific methane yield and first-order simulation of the same from batch AD of FW (Dennehy et al., 2015a)

2.6.2 Application of batch AD results

It has become standard practise to undertake batch tests when assessing the suitability of substrates for AD, and when assessing the effects of co-digesting substrates (Raposo et al., 2011). As BMP provides a measure of the maximum amount of methane generated per unit of substrate organic matter (typically VS), it is in effect a measurement of the substrate degradability. It is increasingly common in the literature to express BMP in terms of substrate degradability (Fd) (Batstone et al., 2015). Due to its application in substrate characterisation in continuous models, it is perhaps the most useful output of batch experiments. Batstone and Rodríguez (2015) presented the method for deriving the term Fd;

2-8 $$\boldsymbol{Fd = \frac{M_m}{\left(\frac{COD}{VS} * 350\right)}}$$

Where COD is the substrate COD concentration in g, VS is the substrate VS concentration *in g. 1 g COD can theoretically be converted to 350 mL CH_4. Therefore multiplying the COD/VS ratio by 350 will result in the theoretical maximum possible specific methane yield being calculated. Fd is calculated by dividing the BMP measured experimentally by this value. This term is used to determine the amount of inert COD in a substrate, which is crucial in mathematical modelling of continuous and semi-continuous anaerobic systems (Batstone et al., 2009). This will be discussed in detail in Section 2.10.2

The hydrolysis rates and BMP data generated from batch tests are also useful when designing continuous AD systems; understanding the rate of substrate degradation allows for the determination of optimal HRT (more specifically identifying how HRT affects methane yields), and therefore digester tank size. The following equation illustrates that the rate of substrate degradation (Fd) at a given HRT can be determined (Batstone, 2014):

2-9 $$\boldsymbol{Fd\ (HRT) = Fd * (1 - \frac{1}{HRT * k_H + 1})}$$

Where HRT is the hydraulic retention time in days and Fd (HRT) is the substrate degradation rate at a specified HRT. With a known BMP, hydrolysis rate and COD/VS ratio, the substrate utilisation (and therefore methane yield and VS or COD removal rate) at a given HRT can be determined.

2.6.2.1 BMP: a standard method?

While BMP tests are an established method of investigation, a widely used standard method has yet to have been embraced by the research community (Angelidaki et al., 2009; Hagos et al., 2016). This is an issue as measured BMPs and hydrolysis rates can be affected by the experimental design (Raposo et al., 2011). Angelidaki et al. (2009), during their proposed standard protocol for BMP tests, and Raposo et al. (2011), in their inter-laboratory BMP calibration study, reviewed key parameters that must be considered when undertaking batch ADs. Selected key parameters in batch test experimental design, and their recommended values, are listed in Table 2-2. Despite these guidelines, there is wide variation in experimental conditions used in the literature (Bond et al., 2012; Mata-Alvarez et al., 2014), and therefore inter-study comparisons of BMPs and hydrolysis rates should be undertaken with caution.

Table 2-2 Key parameters in batch anaerobic digestion experiment design and recommended guidelines/values.

Parameter	Recommendations from Angelidaki et al. (2009)
Reactor volume	0.1-2 L
Inoculum activity	> 0.1 g CH_4-COD/g VS/d
Degassing of inoculum	2-5 days
Inoculum to substrate ratio	>2 (VS basis)
Substrate characterisation	Alteration of VS loading rates to account for VFA presence is suggested
pH control	Neutralised
Nutrient/trace element supplementation	Required unless inoculum and/or substrate shown to contain all necessary elements

The high variation in inoculum to substrate ratios is a factor which has been noted by several authors (Cheng & Zhong, 2014; Dechrugsa et al., 2013; Dennehy et al., 2015b; Fabbri et al., 2014). Inoculum to substrate ratio's as low as 0.16 have been used in some studies (Zhang et al., 2014b). Due to the high ammonia content of manure, selection of an ammonia acclimated inoculum, and selection of a sufficiently high inoculum to substrate ratio is crucial in the co-digestion of PM and FW (González-Fernández & García-Encina, 2009; Hidalgo & Martín-Marroquín, 2014). This is clearly illustrated by Dennehy et al. (2015b). Incorrect selection of inoculum to substrate ratio can lead to the reactor failure thereby compromising the ability of experiments to observe and quantify the effects of co-digestion. This occurred in the study of kitchen waste AD completed by Tian et al. (2015), where due to a low inoculum to substrate ratio, digesters treating kitchen waste alone failed due to a build-up of VFAs and pH drop.

2.6.3 Semi-continuous anaerobic digestion

Undertaking lab scale AD using CSTR-type semi-continuous systems is crucial in scaling up and validating batch scale-based measurement of methane yields and VS removal at given HRTs. Observations with respect to optimal digester HRT/OLR for biogas generation and substrate utilisation made in semi-continuous mode are clearly more directly applicable to and comparable to the full scale commercial AD systems (Batstone et al., 2009) than batch tests. However in addition to assessing the maximum methane yields realisable in semi-continuous CSTR operation, such studies can also assess how varying process parameters such as OLR, HRT, and temperature affect process stability (changes in pH, alkalinity, VFA

concentrations), digestate quality (indicator organism content, dewaterability; see Sections 2.8 and 2.9) and microbial communities (see Section 2.11).

2.7 Anaerobic digestion of pig manure and food waste

Table 2-3 presents the BMPs of a range of substrates commonly used in farm-based AD plants. Undertaking AD of manures alone is not common (Mata-Alvarez et al., 2014). As it is typically highly dilute, the low energy yield possible from manure AD limits its economic feasibility. It is more common for PM to be co-digested with other, more energy dense, substrates.

Table 2-3 Biomethane potentials (BMPs) of selected common substrates for farm-based biogas plants

Substrate	BMP (mL/g VS)	Reference
Pig manure	99-440	(Browne et al., 2013; Chae et al., 2008; Kafle & Chen, 2016; Nasir et al., 2012a)
Cattle manure	100-370	(Browne et al., 2013; Kafle & Chen, 2016; Nasir et al., 2012a)
Food waste	99-644	(Browne et al., 2014; Cabbai et al., 2013; Lisboa & Lansing, 2013; Uçkun Kiran et al., 2014; Zhang et al., 2014a)
Maize	270-300	(Bond et al., 2012)
Grass silage	107	(Wall et al., 2013)
Abattoir waste	165-403	(Browne et al., 2013)

2.7.1 Food waste and anaerobic digestion in Ireland

Energy crops such as maize and grass silage have been common co-substrate for manures in farm-based AD plants (Mata-Alvarez et al., 2014). However growing concern about the environmental and social sustainability of utilising energy crops has seen a growth in the use of organic wastes as co-substrates (Uçkun Kiran et al., 2014). O'Shea et al. (2016) found that, aside from manure, the largest volumes of organic waste available in Ireland is household food waste (FW), which is either source separated and collected, or combined in general refuse.

As discussed in Section 2.3, there has been a major increase in the amount of biodegradable municipal waste, FW in particular, being separately collected in

Ireland over the past 6 years (EPA Ireland, 2016b). Increased availability of FW provides an opportunity for the development of on-pig farm co-digestion plants.

As Table 2-3 illustrates, FW has a high BMP and therefore co-digesting such a substrate along with PM would increase the energy yields from on-farm anaerobic digesters.

2.7.2 Substrate characteristics

2.7.2.1 Pig manure

Varying in composition depending on season, PM management technique, and pig growth stage (Zhang et al., 2014b), PM is typically characterised by high nitrogen and carbon contents and a low but variable total solids (TS) content (Table 2-4). While variable, its composition is typically dominated by cellulosic carbohydrates, some protein and a low amount of lipids (Kafle & Chen, 2016). As pigs are monogastric animals, their manure typically has a higher proportion of biodegradable carbon than that of ruminant manure e.g. cattle (Amon et al., 2007). As a result, PM has been shown to generate higher BMPs than cattle or dairy manure (Nasir et al., 2012a) (see Table 2-3).

However, the high lignin and cellulose and low lipid and protein contents typical of PM result in low substrate degradability, and low hydrolysis rates (Nasir et al., 2012a), which limits the rate of methane generation (Brulé et al., 2014). This allied to the low TS content typical of PM limits the methane yield on a wet weight basis (Nasir et al., 2012a).

The high alkalinity and therefore buffering capacity inherent to it means that the AD of PM is typified by high stability. Changes in pH are buffered and any VFAs in the system remain in their ionized form, thereby reducing the potential for VFA inhibition (Chen et al., 2008). In addition to this, PM is typically found to be high in trace metals essential to stable AD; Zn, Cu, Fe, Co and Ni in particular (Demirel & Scherer, 2011; Nordell et al., 2016).

The slightly basic pH and high ammonium-N content of PM means that ammonia inhibition can occur (Yenigün & Demirel, 2013). While in theory a limitation of PM as a substrate for AD, the fact that this inhibition is reversible, and digester biomass can acclimate to high ammonia and ammonium concentrations, means that in manure-based AD systems complete ammonia inhibition is rare (particularly when digestion occurs at mesophilic temperatures) (Rajagopal et al., 2013).

Inhibition may occur when a biomass unacclimated to high ammonia concentrations is used during the process, or when a shock load of high ammonia is applied to a system (Yenigün & Demirel, 2013).

Table 2-4 Macro physical-chemical characteristics of PM (adapted from Dennehy et al., 2016; Kafle & Chen, 2016; Xie, 2012 **)** and FW (Browne et al., 2014; Browne et al., 2013; Dennehy et al., 2016; Uçkun Kiran et al., 2014; Xie et al., 2017; Zhang et al., 2014a)

Parameters	Pig manure	Food waste
Total solids (% fresh weight)	0.78-9.95	19-35
Volatile solids (% fresh weight)	0.30-8.16	17-30
Total Kjeldahl nitrogen (mg/L) [a]	1217-6698	>500
Alkalinity (mg/L HCO_3^-) [a]	1020-12000	>1000
NH_4^+-N (mg/L) [a]	540-5875	>500
Total COD (mg/L) [a]	7138-174300	150000-511000
Total volatile fatty acid (mg/L acetic acid equivalents [HAceq])	4900-12000	200-15350
Carbohydrate (% of dry weight)	64-80	62-90
Protein (% of dry weight)	14-27	4-21
Lipid (% of dry weight)	6-9	6-24
pH	7.01-7.91	3.8-6.0

[a] *Values reported for FW presented in mg/ kg.*

2.7.2.2 Food waste

Food waste composition varies depending on season and location of collection (urban or rural) (Browne et al., 2014), but is characterised by an acidic pH, high TS and high VS/TS ratio, high carbon content (in terms of COD) and low nitrogen and alkalinity content. While carbohydrate content is typically higher than protein and fat content, this can vary significantly. As illustrated by Table 2-3, FW has a high BMP. This allied with its high TS content means it can generate high methane yields on a wet weight basis. However as a substrate for AD, FW has limitations.

Due to its high COD concentration, high protein and lipid contents, and the low cellulosic nature of its carbohydrate content, FW hydrolyses rapidly. Due to the low alkalinity in the system, changes in pH are not buffered. Therefore the rapid hydrolysis can result in a drop in pH due to the generation of VFAs, which in turn can inhibit methanogens and result in complete process failure (Chen et al., 2008). The low N concentration in FW (and consequently high C/N ratio) also results in an unstable system. In order to provide sufficient N to sustain anaerobic microbial populations, a C/N ratio of between 20 and 30 is recommended (Zhang et al., 2007). C/N ratios of FW are often higher than this and therefore can result in unstable microbial populations. In addition to these two issues, FW is typically deficient in a wide range of trace metals necessary for stable AD systems (Fe, Co, Ni, Zn, Se, Cu and Mo) (Feng et al., 2010; Zhang & Jahng, 2012).

Due to these factors, the mono-digestion of FW is technically challenging and, if undertaken, requires trace element supplementation and is limited to operating at low organic loading rates.

2.7.3 Pig manure and food waste co-digestion

Co-digestion is the process of combining two or more substrates during AD. It is typically undertaken to increase methane yields or improve process stability (Hartmann & Ahring, 2005). It has been the focus of a large amount of research over the past 10 years as the effects, both synergistic and antagonistic, of combining a wide array of substrates were investigated (Mata-Alvarez et al., 2014).

Mata-Alvarez et al. (2014) found that of all the papers published on the topic of anaerobic co-digestion up to 2013, 50 % had been published between 2012 and 2013, with 75 % of all publications found dating from 2009 onwards. This illustrates the rapid rise in academic interest in this area. The analysis also revealed that, due to their plentiful supply and high alkalinity, nitrogen and trace metal content, manures were found to be the most commonly analysed primary substrate. Trace metals found in PM in particular could result in increased production of key co-factors and enzymes, low concentrations of which may be limiting factors in FW mono-digestion. In terms of co-digestion studies with PM, agro-industrial wastes (seasonally produced wastes from agriculture and industry such as fruit processing or dairy production by-products) were the most common co-substrates studied (the subject of 54 % of papers). FW was found to be the co-substrate used in just 8 % of PM co-digestion studies.

As discussed in Section 2.7.2, the mono-digestion of FW and the mono-digestion of PM have limitations. However co-digestion of FW with PM allows for stable digestion (due to high alkalinity, improved C/N ratios and trace metals provided by PM), and higher methane yields (due to the high energy potential of FW) (Hartmann & Ahring, 2005). The co-digestion of PM and FW is an established practise, with many biogas plants in Germany, Denmark and the UK in particular operating with such a substrate mixture (Mata-Alvarez et al., 2014). However, as the study by Mata-Alvarez et al. (2014) illustrates, there are fewer studies on the co-digestion of these two substrates compared with other substrate combinations (such as agri-food by-products and glycerol).

Research undertaken on the topic of co-digestion of PM and FW can be categorised into batch studies and semi-continuous studies.

2.7.3.1 Batch co-digestion of pig manures and food waste

There are many batch studies assessing the BMP and hydrolysis rate of FW and PM (Banks et al., 2011; Browne et al., 2014; Cabbai et al., 2013; Fisgativa et al., 2016; Forster-Carneiro et al., 2008; Lisboa & Lansing, 2013; Tampio et al., 2014; Zhang et al., 2014a). While Table 2-3 illustrates that the range at which FW BMPs have been observed is wide, it typically achieves BMPs > 400 mL CH_4/g VS (Meng et al., 2014; Nasir et al., 2012b; Zhang et al., 2014a). Hydrolysis rates for FW are typically found to be between 0.3 and 0.5 d^{-1} (Pagés Díaz et al., 2011; Vavilin et al., 2008; Zhang et al., 2014a; Zhang et al., 2007). While some studies have observed that the batch mono-digestion of FW failed (Tian et al., 2015; Ye et al., 2013), this was due to insufficient inoculum or trace element addition to the reactor. Compared to FW, batch studies of PM have found that it typically generates lower BMPs (99 to 440 mL CH_4/g VS; see Table 2-3) and lower hydrolysis rates of between 0.01 and 0.15d^{-1} (Vavilin et al., 2008; Zhang et al., 2014b). Unlike FW, the emission profile of manures is regularly characterised by 2 or more distinct periods of methane emissions (Brulé et al., 2014; Dennehy et al., 2015b; Zhang et al., 2014b). This has been attributed to distinct pools of rapidly and slowly biodegradable substrates in the manure, and can result in challenges in estimating the hydrolysis rate with the use of the first-order model (Brulé et al., 2014; Esposito et al., 2012). Other models may be used, and will be described in Section 2.10.1.

Undertaking batch tests for co-digestion along with mono-digestion of the substrates of interest can allow for the identification of how co-digestion affects the SMY and the hydrolysis rate of the system; in particular it allows for the identification of whether any synergistic or antagonistic effects may occur.

Table 2-5 presents the results from a number of batch co-digestion studies of FW and PM, and similar substrate mixes (e.g. FW with cattle manure, PM with agro-industrial wastes etc.).

Table 2-5 Selected relevant mesophilic batch co-digestion studies which measured the specific methane yield (SMY) of pig manure (PM), food waste (FW) and similar mixtures of same. nd denotes not determined. ns denotes not specified.

Substrates	Optimal mixing ratio for SMY (VS basis)	SMY at optimal mix	k_H at optimal mix (d^{-1})	Synergy observed?	Notes	Ref.
Apple waste, PM	33 % apple waste	249 mL CH_4/g COD	0.032	For biogas yield	No comprehensive analysis of optimal mixing ratio	(Kafle & Kim, 2013)
PM, FW, dairy manure	ns	ns	nd	For SMY; 30 % increase	Detailed methodology for quantification of observed synergy reported	(Adelard et al., 2014)
FW, dairy manure	55 % FW	413 mL CH_4/g VS	nd	nd	No analysis of optimal mixing ratio undertaken.	(Lisboa & Lansing, 2013)
PM, 4 different agri. by-products	50 % pepper	343 mL CH_4/g VS	nd	nd	By-products analysed were tomato, pepper, persimmon and peach	(Ferrer et al., 2014)
Olive mills waste, PM	40 % olive mill waste	277 mL CH_4/g COD	nd	nd	As mono-digestion of olive mill waste failed, identification of synergy not possible	(Kougias et al., 2014)
PM, fruit and vegetable waste	100 % fruit and vegetable waste	c. 360 mL CH_4/g VS	nd	No	2 factoral central composite design where mixing ratio and inoculum to substrate ratio were analysed	(Molinuevo-Salces et al., 2010)
PM, winery wastewater	85 % winery wastewater	348 mL CH_4/g VS	nd	For SMY	2 factoral central composite design where mixing ratio and inoculum to substrate ratio were analysed	(Riano et al., 2011)
PM, FW	50 % FW	409.5 mL CH_4/g VS	nd	For SMY	FW mono-digestion failed due to VFA accumulation, biomodal emission pattern observed during co-digestion	(Tian et al., 2015)
Piggery waste, FW leachate	33 % FW leachate	310 mL CH_4/g VS	nd	For SMY	3 factoral central composite design where mixing ratio, alkalinity and salinity concentrations were analysed	(Han et al., 2012)
PM, rice straw	c. 50 % rice straw	c. 225 mL CH_4/g VS	nd	For SMY	4 factoral central composite design experiment	(Wang et al., 2013b)
FW, cattle manure	33 % FW	388 mL CH_4/g VS	nd	For SMY	Loading rates of mono-digestion and co-digestion batches were not equal, which affected the optimal SMY observed	(Zhang et al., 2013)
FW, sewage sludge	100 % FW	c. 440 mL CH_4/g VS	0.1-0.42	For k_H; see note	Found positive effect on k_H when FW was added < 35 % (VS basis), and negative thereafter	(Koch et al., 2015)
PM, waste vegetable oils	89 % vegetable oils	500-600 mL CH_4/g VS	nd	nd	In addition to co-digestion, assessed the effect of two different inocula	(Hidalgo & Martín-Marroquín, 2014)
FW, sewage sludge	50 % FW	368 mL CH_4/g VS	0.67	For Fd	Did not assess optimal mixing ratio, just a single mixing ratio analysed	(Xie et al., 2017)
PM, sewage sludge	33 % sewage sludge	315.8 mL CH_4/g VS	0.094	For SMY and k_H		(Zhang et al., 2014c)
PM, paper sludge	75 % paper waste (wet weight basis)	nd	<0.03	For k_H	Expressed all methane yields in terms of COD to CH_4 conversion efficiency	(Parameswaran & Rittmann, 2012)

Of the studies which co-digested manure and a FW-type substrate in Table 2-5, the optimal manure to FW ratio was found to be between 33 % FW and 100 % FW, highlighting the positive effect FW addition has on SMY.

A wide range of maximum SMYs were found by the studies presented in Table 2-5, due to the wide variation in co-substrates used. From studies where FW or FW-type substrates were mixed with manures the SMYs ranged from 360 mL CH_4/g VS to 410 mL CH_4/g VS . Higher yields were achieved by Hidalgo and Martín-Marroquín (2014) when PM was co-digested with vegetable oils and Koch et al. (2015) when FW was co-digested with sewage sludge (a substrate which typically has a higher SMY than manure).

Experimental design is crucial in identifying the optimal substrate mixing ratios and identifying the occurrence of any synergy between substrates. Some studies presented in Table 2-5 undertook preliminary assessment of the sustainability of co-digestion by undertaking co-digestion at a single mixing ratio (Kafle & Kim, 2013; Lisboa & Lansing, 2013; Xie et al., 2017). While such studies can generally highlight the positive effects of co-digestion on methane yields and process stability, they do not provide information on the optimal conditions for co-digestion.

Studies in which this was an objective designed experiments whereby mixing ratios were varied across a set range. This allowed for the identification of how the AD process is affected as the proportions of substrates comprising the feedstock were varied (Ferrer et al., 2014; Hidalgo & Martín-Marroquín, 2014; Koch et al., 2015; Parameswaran & Rittmann, 2012; Tian et al., 2015; Zhang et al., 2013; Zhang et al., 2014c). This therefore allowed for the identification of optimal mixing ratios, but could also be used to readily identify synergistic or antagonistic effects.

Several studies have undertaken a more complex experimental design to identify optimal substrate mixing conditions, in addition to assessing the effects of varying one or more other independent variables (Adelard et al., 2014; Han et al., 2012; Molinuevo-Salces et al., 2010; Riano et al., 2011; Wang et al., 2013b). A central composite design (CCD) methodology is a multi-order factoral experimental design methodology, where the relationship between two or more independent variables and responses are assessed. The method results in a number of mathematically determined mixtures of substrates being analysed, the results of which are then used to generate a polynomial regression model describing the

relationship of the two or more independent variables to response variables (Riano et al., 2011). It is a common methodology in co-digestion experiments as it can determine the effects of the substrate mixing ratio and other factors (inoculum to substrate ratio is the most commonly co-analysed independent variable), in a single experiment. Table 2-5 illustrates that most of the co-digestion studies presented observed some level of synergy when co-digesting substrates. Synergetic effects in terms of SMY were most commonly observed, however few studies analysed the kinetics of digestion, and therefore only a couple of studies identified that co-digestion had synergistic effects on this.

Few studies were designed in such a way that synergistic effects could be quantified. Adelard et al. (2014), in a study of the co-digestion of FW, PM and cattle manure, provided detailed methodology for quantifying synergistic responses in SMY to co-digestion. The relative synergistic effect was quantified over the duration of the study using the following equation-

2-10 $$\Delta B_{CH4}(t) = \frac{(B_{CH4\,mix})}{\sum(x_i B_{CH4\,i}(t))} - 1$$

where $\Delta B_{CH4}(t)$ is the relative change in the SMY of the substrate mix, $B_{CH4mix,}$ compared to the SMY of the substrate mixture determined by proportionally summing the BMPs of each substrate measured in mono-digestion (B_{CH4i}) at mixing ratio x_i at digestion time t. This equation therefore quantifies the increase or decrease in SMY observed during co-digestion, compared to the SMY expected based on the BMPs of the substrates mixed, and the mixing ratio used. Subsequent work by some of the same authors, using similar methodology assessing the effects of co-digesting 9 different substrates found that, in 85 % of cases, co-digestion resulted in synergistic effects on SMY (Poulsen et al., 2016). However of the literature surveyed, there has not yet been an attempt to identify and quantify the synergistic SMY effects of PM and FW co-digestion.

Further to this, the effect of PM and FW co-digestion on digestion kinetics has also not been well explored. Of the studies presented in Table 2-5, few report the effect of co-digestion on the hydrolysis rate. Of those that do, none combine FW type substrates with manures, aside from Kafle and Kim (2013) who combined PM with apple waste, but did not identify the optimal mixing ratio or attempt to identify or quantify synergy. Table 2-5 also illustrates that the majority of co-digestion systems involving a solid type substrate such as FW, and a more liquid substrate like PM express all results in terms of mL CH_4 /g VS. While this expression is more

versatile than expression in terms of COD (particularly in the context of manures as the total solids content can vary so widely), it merits further attention during co-digestion studies where perceived synergism may be explained by the presence of soluble substrates. This is of particular concern during the digestion of manures due to the high (>1 g/L) initial VFA concentrations (Xie et al., 2011).

Co-digestion of FW and PM at batch scale has been undertaken previously. However much more research is required in relation to identifying and quantifying the synergistic effects on SMY and the hydrolysis rate. In addition, assessing the effects of the high VFA content of PM on the observed synergy merits investigation, considering the wide prevalence of expressing results in terms of mL CH_4 /g VS.

2.7.3.2 Semi-continuous anaerobic co-digestion of pig manures and food waste

Experimentation in a semi-continuous or continuous mode allows for the validation of observations about optimal mixing ratio and HRT (based on hydrolysis rate) from batch experiments and identification of optimal OLR in terms of methane yields. It can also allow for the assessment of how co-digestion can affect digestates - a key consideration when operating at commercial scale.

Table 2-6 summarises the findings of relevant CSTR-type semi-continuous co-digestion studies in terms of optimal operating conditions for methane generation. Due to the variability in operating conditions, inter-study comparisons of optimal conditions for methane generation are challenging. Nevertheless, some general observations may be made as to the operational parameters used in semi-continuous studies of FW and manure co-digestion.

The PM/FW mixing ratios used in the studies treating manure and FW presented in Table 2-6 vary from 32 % FW to 90 % FW. The reason for this wide variation in mixing ratios studied is due to both the combination of loading rate (i.e. whether substrates are diluted) used and the characteristics (VS content in particular) of the substrates. Hartmann and Ahring (2005) highlight that in CSTR-type reactors, the maximum addition rate of FW to manure in a co-digestion system is limited by the TS content of the feedstock. Therefore operating CSTR systems when feedstock TS content exceeds 15 % is challenging. While addition of FW to PM in VS mixing ratios of 70-90 % may be the theoretical optimal mixing ratio for methane generation, it is typically limited to mixing ratios of between 50 to 60 % (VS basis).

The SMYs measured at the conditions found to result in the highest volumetric methane yield in each study also varied significantly. While this can be partly attributed to differences in the nature of manures and FW used, it can also be attributed to the variation in the operational approach taken by different studies in terms of temperature, OLR and, in particular, HRT. As mentioned in Section 2.6.3, decreasing HRT results in decreased substrate utilisation and lower SMYs.

Table 2-6 Summary of results of selected semi-continuous continuously stirred tank reactor anaerobic co-digestion studies. Ns denotes not stated.

Substrates	SMY[a] (mL CH_4/g VS)[b]	Mixing ratio (VS basis)[b]	OLR[c] (kg VS/ m^3/d)[b]	HRT[d] (d)[b]	Duration operated[e](d)	Temperature	Notes	Ref.
PM, Apple waste	190	33 % apple waste	1.6	30	24	Mesophilic	Did not vary HRT	(Kafle & Kim, 2013)
PM, FW, slaughterhouse waste	682	45 % PM,10 % FW	2.6	36	108	Mesophilic	OLR and HRT not varied	(Murto et al., 2004)
PM, winery wastewater	107[b]	40 % wine waste	0.8[f]	12	60	Mesophilic	OLR and HRT not varied	(Riano et al., 2011)
FW, cattle manure	306	60 % FW	4	30	35	Mesophilic	OLR stepwise increase, HRT maintained stable	(Zhang et al., 2012)
PM, FW	390-440	50 % FW	4	14-18	>300	Thermophilic	Compared co-digestion with mono-digestion of FW with recirculation of supernatant.	(Hartmann & Ahring, 2005)
Piggery wastewater, FW	388	83 % FW	4.36	20	45	Mesophilic		(Zhang et al., 2011)
Dairy manure, FW	Failure	54 % FW	8.25	20	60	Mesophilic	Mixing ratio OLR and HRT not varied	(Yamashiro et al., 2013)
Dairy manure, FW	170	54 % FW	8.25	20	60	Thermophilic	Mixing ratio OLR and HRT not varied	(Yamashiro et al., 2013)
Dairy manure, cheese whey	158	26 % whey	5.9	8.7	~Ns	Mesophilic		(Rico et al., 2015)
PM, fish waste	250	70 % fish waste	1.5	30	55	Mesophilic		(Regueiro et al., 2012)
PM, sugar beet	260	Ns	11.2	6	18	Mesophilic	Identification of maximising VMY, while maintaining reactor stability was aim of research	(Aboudi et al., 2015)
FW, dairy manure	450-550	50 % FW	3	54	55	Mesophilic	Identified optimal OLR for SMY at 2 kg VS /m^3/d	(Agyeman & Tao, 2014)
FW, cattle manure	450	68 % FW	5.01	21	28	Mesophilic		(Callaghan et al., 2002)
FW, garden waste, sewage sludge	430	65 % FW	5	15	40	Thermophilic	HRT15 days identified as optimal considering both yield and SMY	(Fitamo et al., 2016)
FW, cattle manure	297	50 % FW	4	25	900	Mesophilic	Increasing FW in substrate led to failure from high TS content	(Usack & Angenent, 2015)
PM, FW	285	60% FW	0.6	15	45	Mesophilic	PM/FW mixing ratio not varied	(Molinuevo-Salces et al., 2012)

[a]Specific methane yield. [b]At the highest measured volumetric methane yield (VMY) achieved. [c]Organic loading rate. [d]Hydraulic retention time. [e] At optimal VMY conditions. [f]COD basis.

In general, the highest SMYs (>400 mL CH_4/g VS) identified for FW co-digestion were found at thermophilic conditions (Fitamo et al., 2016; Hartmann & Ahring, 2005), with studies identifying that the optimal HRT and OLR lies between 15-20 days and 4-5 kg VS/m^3/d respectively. Such HRTs at thermophilic conditions would be typical in commercial scale digesters (Moset et al., 2014). In terms of mesophilic studies, SMYs were typically found to be lower (100-400 mL CH_4/g VS), with the optimal HRT and OLR in terms of the volumetric methane yield (VMY) found to be longer (>20 days) and similar to those found in thermophilic studies, respectively. Considering the more rapid rate of substrate utilisation typical of thermophilic temperatures (Moset et al., 2014), it makes sense that longer HRTs are necessary in mesophilic digestion. It is important to note that decreasing HRT leads to an increase in OLR which, up to a critical point at which slow growing methanogens are washed out of the system, will lead to an increase in VMY. However as mentioned in Section 2.6.3, decreasing HRT has a negative effect on substrate utilisation resulting in lower SMY and a greater VS content in the digestate.

Just a single study directly compared the operation of a manure and FW co-digestion system at mesophilic and thermophilic conditions. Yamashiro et al. (2013) found that the co-digestion of concentrated FW and cattle manure failed at mesophilic temperatures, but generated an SMY of 170 mL CH_4/g VS at thermophilic temperatures. Digester failure at mesophilic temperatures was attributed to the high (8 kg VS/m^3/d) OLR used in the study. Thermophilic digestion can operate with a higher OLR and higher SMY than mesophilic digestion, however, as mentioned in Section 2.5.2, may be more susceptible to system disturbance and upset conditions.

Two studies specifically assessed the optimal conditions for semi-continuous co-digestion of FW-type substrates and PM. Hartmann and Ahring (2005) did identify optimal conditions for semi-continuous co-digestion, but did so at thermophilic temperatures, while Molinuevo-Salces et al. (2012) studied vegetable processing waste rather than domestic FW, and studied rather low OLRs (0.4 and 0.6 kg VS /m^3/d). There has yet to be studies which assess the effects of varying OLR and HRT in the anaerobic co-digestion of PM and FW at mesophilic temperatures.

However in terms of optimising biogas process, while maximising methane yields is crucial, other factors need to be considered. Of particular importance is the effect of digester operating conditions on digestate quality; be it physical (affecting dewatering, transport and disposal costs) or biosafety-related (which can have regulatory consequences which can affect disposal).

2.8 Anaerobic co-digestion and digestate dewaterability

It is increasingly common for farmers in Europe to employ solid liquid separation techniques when managing PM (Bortone, 2009). As of 2003, the use of separation technologies varied somewhat across Europe, with Greece (90% of PM), Italy (40% of PM), and Spain (10% of slurries) being the countries where it is the most prevalent (Burton & Turner, 2003). Separation of the solid and liquid fractions of PM may allow, in some instances, farmers to reduce the cost associated with managing PM. Due to its high nitrogen content, liquid PM should not be applied to sites which are approaching 170 kg ha /year nitrogen limit imposed by the EU Nitrates Directives (91/676/EEC). Additionally in areas with a high soil P, the P content of unseparated PM can limit its land spreading (Hansen et al., 2006). Therefore farmers may be required to travel considerable distances to dispose of PM (Moller et al., 2000). This increases the overall cost associated with PM management (Deng et al., 2014; Nolan et al., 2012). By employing separation, the nutrient rich (particularly P) solid fraction of PM may be spread further from the farm on lands which have a P requirement. The liquid fraction can be spread near the farm, without risk of breaching P limits. Thus, in some instances, it may reduce costs associated with PM management. In a similar manner, the practice of dewatering digestate from agricultural-based biogas plants is becoming more common, and therefore the effect of AD, co-digestion in particular, on digestate dewaterability merits attention.

Dewaterability can be measured in a number of ways, the two most common methods being Specific Resistance to Filtration (SRF) and Capillary Suction Time (CST). SRF describes the resistance to filtration caused by the deposition of solids on the filtration device. It is obtained by filtering a known volume of sample through a filter, recording the volume of filtrate generated over time, and using the following formula (Pollice et al., 2007):

2-11 $$\boldsymbol{SRF = \left(\frac{2PaA^2}{\mu w}\right) b}$$

where SRF is the specific resistance to filtration (m/kg), Pa is the vacuum pressure applied in Pa, A is the filter surface area of the filter (m^2), μ is the dynamic viscosity of the filtrate (Pa/s), w is the weight of dried solids per unit of filtrate (kg/m) and b is the slope of the line generated from plotting the filtration time over the filtration volume (t/V) versus the filtrate volume. CST is a measure of the rate water is separated from solids. It is quantified by measuring the time required for a

given sample to travel a specific radial distance, via capillary suction, from a reservoir of sample placed in the middle of chromatographic (or filter) paper, outward (Sawalha & Scholz, 2007). While both are useful measure of dewaterability, CST is more focused on the volume of water removed per unit time, and therefore is more important when deciding the hydraulic capacity of a digestate handing step, while specific resistance is more focused on the volume of digestate that can be dewatered per unit time.

The effect of AD on digestate dewaterability has been the focus of some research (Habiba et al., 2009); however, much of this work focused on sewage sludge as a primary substrate. Nevertheless some of the studies undertaken can provide useful information on how digester operation affects digestate dewaterability. The overall effect of AD on dewaterability has been found to be dependent on digester operating conditions (Houghton & Stephenson, 2002; Lawler et al., 1986).

Some authors found that as longer HRTs resulted in more complete digestion, a uniform destruction of particles of all sizes and therefore improved dewaterability (relative to undigested substrate) (Habiba et al., 2009). Other studies found that shorter HRTs improved dewaterability (compared with untreated substrate) by generating a digestate comprised of a greater proportion of larger particles (Lü et al., 2015). An increase in proportion of smaller particles in digestate has been shown to cause SRF to rise, leading to a decrease in the dewaterability of digestate, as smaller particles easily clog filters (Houghton & Stephenson, 2002).

In terms of the effects of co-digestion on digestate dewaterability, Agyeman and Tao (2014) found that the addition of FW as a co-substrate to cattle manure AD resulted in improved dewaterability when HRT was controlled. Habiba et al. (2009) found the addition of fruit and vegetable waste to the digestion of activated sludge reduced the SRF despite a slight increase in the OLR when fruit and vegetable waste was added. While these studies suggest that co-digestion improves digestate dewaterability, there has yet to be a study in which the effect of varying substrate mixing ratio on dewatering is assessed (under controlled HRT and OLR conditions).

2.9 Anaerobic co-digestion and pathogen removal

A key measure of digestate quality is the concentration of faecal indicator organisms (Orzi et al., 2015). Many industry standards (IRBEA, 2012) as well as national and EU regulations (EU Commission, 2011) on land application of digestate require *E. Coli*, *Enterococcus* and *Salmonella* concentrations in digestate to be below specific limits. Both the Animal By-Products regulation (EU Commission Regulation (EU) No 142/2011) and the draft Irish industry standard for digestate quality (IRBEA, 2012) require that digestate must be negative for *Salmonella* in every 25g tested and have less than 1000 CFU/mL *E. coli* or *Enterococcus* in order to be spread on land.

E. coli and *Enterococcus* are the most common indicator organisms, as they are a general indicator of the presence of enteric pathogenic bacteria and viruses (Lund et al., 1996; Sahlström, 2003). *Salmonella*, as a pathogenic faecal bacterium, is a more specific indicator of the presence of pathogenic organisms (McCarthy et al., 2011), and for that reason its presence is subject to much lower regulatory limits than *E. coli* and *Enterococcus.*

Ensuring that digestates meet these standard is crucial to the operation of biogas plants. The removal of faecal indicator organisms via AD is a mature area of research, with the majority of studies focused on the removal of such organisms from wastewater treatment sludge (Sahlström, 2003).

AD achieves pathogen removal in a number of ways. Primarily pathogen inactivation is a function of time and temperature (Elmerdahl Olsen & Errebo Larsen, 1987; Lund et al., 1996); longer detention times and higher digester temperatures result in greater reductions in measured indicator organisms. Sahlström (2003) highlights that the decimation times for indicator organisms in thermophilic conditions is typically hours, while under mesophilic conditions is days. Smith et al. (2005) illustrated that for mesophilic AD, temperature is not the mechanism for pathogen inactivation, rather microbial competition and substrate limitation are the likely causes of pathogen inactivation. Digester pH, VFA and ammonia concentrations also play a role in pathogen inactivation (Orzi et al., 2015; Sahlström, 2003). In particular, pH <7 combined with high VFA concentrations (resulting in high free VFA concentrations) results in greater reduction in pathogen concentrations than neutral conditions (Sahlström, 2003), while pH >7 combined with high ammonium concentrations (leading to high free ammonia concentration)

results in greater reduction in pathogen concentrations than neutral conditions (Orzi et al., 2015).

Despite the growth in numbers of biogas plants undertaking anaerobic co-digestion of manures and FW over the past 10 years (Mata-Alvarez et al., 2014), few studies have assessed the effects of co-digestion (and the mixing ratios used) on faecal indicator organism concentrations. The current regulatory environment within the EU is largely responsible for the paucity of studies on the effect of co-digestion on pathogen removal. The Animal By-products Regulation (as applied in Ireland) stipulates that feedstock entering or digestate leaving biogas plants treating animal by-products (such as FW) must be either reduced in particle size to below 12 mm, and then pasteurised at 70°C for a period of 60 minutes, or reduced in size to 400 mm or less and held for 48 hours at 60°C twice. This treatment must reduce pathogenic indicator organisms to below the regulatory limit in place for land application. Therefore, the AD process *per-se* is not relied upon to achieve a specific level of pathogen removal. However, the effect of varying feedstock composition and HRT (and consequently OLR) on the digestate pathogen content merits investigation in instances where such regulations are not in place. It is also important to assess whether changes in a digestate treatment regime would be required to ensure the land application standards are met when changes are made to digester HRT or feedstock composition.

2.10 Mathematical modelling and anaerobic digestion

Over the past 10 years, the development of mathematical modelling (in particular the uptake of a single framework for modelling AD systems) has allowed for improved design and control of AD systems (Arnell et al., 2016). Understanding mathematical models of AD systems not only allows for the correct application of such models (thereby providing a greater level of control when managing AD systems) but can provide insight into the physical and biochemical processes occurring during AD.

2.10.1 Batch scale modelling/hydrolysis models

As mentioned in Section 2.6.1, provided that no inhibitory effects occur during digestion, cumulative methane generation profiles recorded in batch tests typically follow a first-order accumulation pattern. In the majority of biogas plants hydrolysis is the rate limiting step (Brulé et al., 2014). Therefore cumulative methane generation profiles can be used to determine the rate of hydrolysis and theoretical methane yields (and combined with COD/VS ratios, the Fd) by utilising first-order models. This in turn can be used both in more complex continuous models of AD systems to define hydrolysis rates and the inert fraction of COD present in substrates (see Section 2.10.2), and in more basic models to estimate the methane yield of a substrate at a given HRT in a continuous system (Batstone & Rodríguez, 2015) (See Section 2.6.1).

First-order models are the most common means of modelling batch scale AD, but they do not satisfactorily model methane emission profiles in every case. Such a case is where methane emissions from a substrate or mix of substrates occur in two distinct periods (biomodal emission pattern). Such bimodal emission patterns are typical of co-digestion systems where one substrate has an inherently higher hydrolysis rate than another, or in any AD system where pools of readily degradable COD (simple carbohydrates for example) are present alongside more slowly degradable substrates (such as fibres) (Brulé et al., 2014). While a simple first-order model can approximate the lumped hydrolysis rates of such substrates and substrate mixtures, several studies have proposed more complex models which allow for the identification of the hydrolysis rates of both the slowly (lignin, cellulose and hemi cellulose) and rapidly (sugars, amino acids VFAs) degradable components of the substrate (Brulé et al., 2014; Esposito et al., 2011b; García-Gen et al., 2015; Nielfa et al., 2015), and in one case, even the estimation of VFA utilisation rate (Brulé et al., 2014). Not only can such models provide a better fit to

cumulative methane curves from batch experiments, they can be incorporated into more complex models of continuous anaerobic systems to improve the accuracy of the simulation of hydrolysis processes (Esposito et al., 2011b; García-Gen et al., 2015). It should be noted however the first-order models which account for bimodal peaks have been criticised as secondary emission peaks may be attributed to delays in methanogenesis rather than distinct substrate pools (Brulé et al., 2014).

Non-first-order models have also been used to model batch AD. The most common of these is the sigmoidal Gompertz function which was originally used for modelling growth curves (Lay et al., 1998):

2-12 $$\boldsymbol{M(t) = M_m \cdot exp\left\{-exp\left[\frac{R_{max}\, e}{M_m}\lambda - t + 1\right]\right\}}$$

where R_{max} is the maximum methane production rate (mL/d), λ is the lag phase (d) and e is exp(1) = 2.7183. The Gompertz model is useful as it can account for lag phases during the digestion of specific substrates.

2.10.2 Modelling of continuous and semi-continuous anaerobic digestion

Attempts to simulate full scale anaerobic systems have been undertaken since the 1970s (Donoso-Bravo et al., 2011). While initially rather simple, models have grown in complexity as more detailed knowledge of the AD process and increased computing power became available (Donoso-Bravo et al., 2011). By the late 1990s several complex mechanistic models of AD systems had been developed (Angelidaki et al., 1999; Batstone et al., 2000; Siegrist et al., 1993; Vavilin et al., 1994). These models not only accounted for methane and biogas generation, but many of chemical speciation and bioconversion processes. As such models were similar conceptually (albeit different in application) (Batstone et al., 2015), the development of a model which combined the approach of these previous models into a generic framework was needed. The International Water Association Task Group for Mathematical Modelling of Anaerobic Digestion Processes addressed this need, and in 2002, the Anaerobic Digestion Model No. 1 (ADM1) was published (Batstone et al., 2002). It has since become the framework upon which the vast majority of mechanistic AD models are based.

2.10.2.1 The IWA's Anaerobic Digestion Model No. 1 (ADM1)

ADM1 is the modelling framework developed to provide a standard approach to the modelling of anaerobic systems (Batstone et al., 2002). While most often used to simulate AD processes in wastewater treatment systems, its use in modelling

multi-substrate biogas plants is increasing. ADM1 is a complex mechanistic model developed in order to simulate the fundamental processes occurring in AD systems. It models the interactions of 24 chemical species with 19 bioconversion processes. Figure 2-8 illustrates the biochemical framework associated with the original iteration of the model.

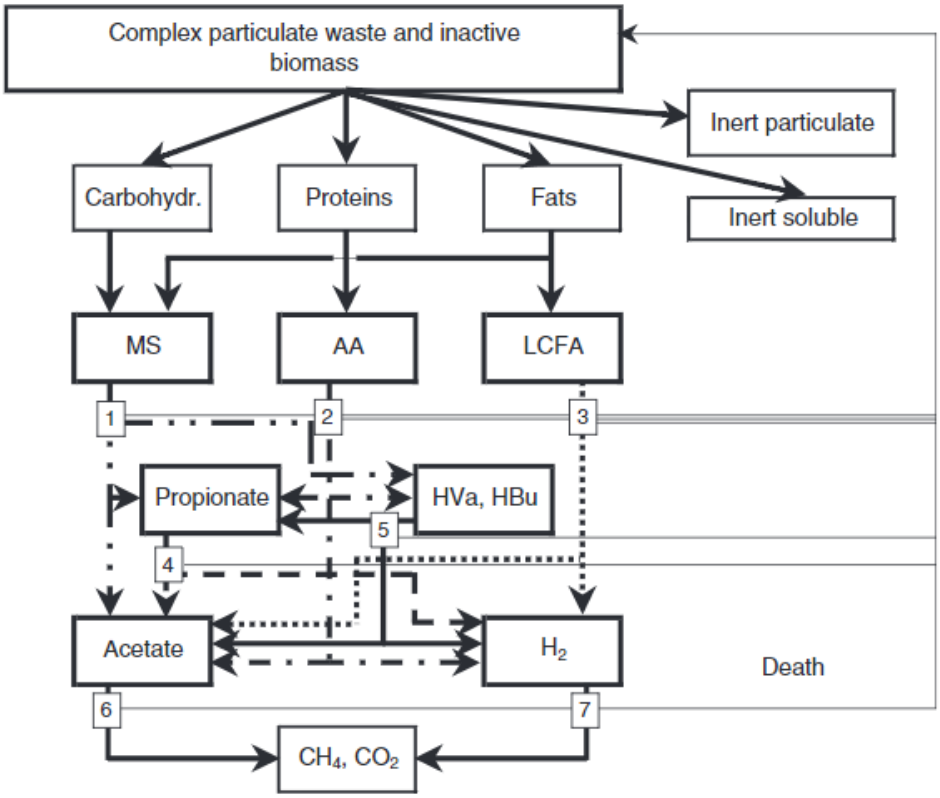


Figure 2-8 Structure of the ADM1 model taken from Batstone et al. (2002)[1]

The first biochemical step in ADM1 is disintegration of the substrate into inert fractions, carbohydrates, proteins and lipids. This is assumed to be a first-order reaction, with an associated rate constant. In recent years several studies by the original authors of the ADM1 model have not included this step in the model (Batstone et al., 2015), as it is now accepted that hydrolysis occurs before, during

[1] where MS is monosaccharide; AA, amino acids; LCFA, long chain fatty acids; HVa, valeric acids; HBu, butyric acids. Numbers denoting the biochemical processes: (1) acidogenesis from sugars, (2) acidogenesis from amino acids, (3) acetogenesis from LCFA, (4) acetogenesis from propionate,(5) acetogenesis from butyrate and valerate, (6) aceticlastic methanogenesis, and (7) hydrogenotrophic methanogenesis.

and after disintegration, and therefore, in modelling terms, disintegration is not significant and is generally omitted.

As mentioned previously, hydrolysis is the rate limiting step in the majority of anaerobic systems (Batstone et al., 2009). In ADM1, the hydrolysis of carbohydrates, proteins and lipids to glucose, amino acids and LCFAs respectively is considered separately. Hydrolysis is assumed to be a first-order reaction, and therefore distinct hydrolysis rates are required for carbohydrates, proteins and lipids. As the rate limiting step, using the correct hydrolysis rates is crucial in accurately modelling anaerobic systems. The hydrolysis rates of carbohydrates, proteins and lipids are dependent on the substrates which they are part of, and vary widely (Batstone et al., 2002). Identifying the specific hydrolysis rates of these fractions for each substrate entering an anaerobic system is onerous. This has resulted in more straightforward means of characterising the rate of hydrolysis. The hydrolysis rates of carbohydrates, proteins and lipids are often combined into a general hydrolysis rate of a substrate (Garcia-Gen et al., 2013). This can be determined by optimising the model to fit laboratory data from continuously operating reactors (Batstone et al., 2009) and by using hydrolysis rates determined by batch tests. While use of hydrolysis rates from batch experiments for ADM1 calibration has been reported (García-Gen et al., 2015; Souza et al., 2013), batch tests have been shown to underestimate the hydrolysis rate constants observed in full scale plants, and their application to ADM1 has been questioned (Batstone et al., 2009).

As Figure 2-8 illustrates, following hydrolysis, monosaccharide, amino acids and LCFAs are converted, by monosaccharide utilising bacteria, amino acid utilising bacteria and LCFA utilising bacteria, to VFAs (valerate, butyrate, propionate, acetate) and hydrogen. Unlike hydrolysis all these biochemical interactions follow Monod-type kinetics. Monod type kinetic can be described by the following equation:

2-13 $$\mu = \frac{\mu_m S}{(K_s + S)}$$

where S is substrate concentration (kg COD/m^3), μ is specific growth rate (day^{-1}), μ_m is maximum specific growth rate (day^{-1}) and K_s is the half saturation constant (kg COD/m^3). In this way the ADM1 model factors in biomass growth (i.e. the growth of active bacterial populations). Biomass decay is assumed to be a

first-order process, with dead biomass recirculating in the system as particulate matter.

The VFAs resulting from monosaccharide and amino acid acidogenesis are converted to acetate (via butyrate, valerate and propionate degrading bacteria). Aceticlastic methanogenesis and hydrogenotrophic methanogenesis occur through the utilisation of the available acetate and hydrogen (via acetate degrading and hydrogen degrading groups respectively). Aside from these organic fractions, the biochemical pathways also account for the uptake and production of inorganic carbon and inorganic nitrogen. These factors are crucial in simulating digester alkalinity and NH_4^+ concentrations. Finally, in terms of biochemical interactions, the model accounts for pH inhibition for all microbial groups, hydrogen inhibition on acetogenic bacteria, and ammonia inhibition an aceticlastic methanogens. In terms of physical chemical interactions, the model addresses liquid-liquid interactions (in particular concentrations of ions, cations and disassociation of acids), and liquid gas interactions.

Accurate characterisation of the substrates entering the digester is essential for precise simulation of the reactor operation. While up to 28 state variables describing the feedstock may be defined to describe the feedstock characteristics the most critical and the most commonly defined parameters are the fractionation of COD into carbohydrate, protein, lipid and inerts (derived from Fd), the concentrations of individual VFA species, inorganic carbon, inorganic nitrogen and pH. As the majority of the model output, the methane yield in particular, is dependent on the biologically available COD, determining this input is crucial. The inert fraction of COD used in many ADM1 studies is determined by undertaking batch tests (Arnell et al., 2016; Batstone et al., 2009; Jurado et al., 2016; Souza et al., 2013). This illustrates the importance of batch studies in defining Fd of substrate or mixes of substrates (which in turn is used to define the inert fraction of COD in a substrate as descried in Section 2.6).

2.10.2.2 ADM1 and co-digestion

The original iteration of the ADM1 model, while comprehensive, has been intended to be a generalised approach to modelling AD systems. Indeed, as evidenced from the changes in approach most authors have made to disintegration and hydrolysis steps in the last 15 years, it has the capacity to change as the knowledge which underpins it changes. In addition, the model framework allows

for the incorporation of processes not included in the original form. Over the past 15 years the model has been extended to simulate the anaerobic digestion when glycerol, alcohols (Astals et al., 2011), high salt concentrations (Hierholtzer & Akunna, 2012) and high solid concentrations (Liotta et al., 2015; Poggio et al., 2016) occur.

Several different approaches can be made to apply ADM1 to co-digestion. The most straightforward is, after characterising the co-substrates, to calculate the reactor influent concentrations based on the mixing ratio of substrates, and subsequently to use combined hydrolysis rates to model the process. This requires few changes to the original model, however as it cannot reflect dynamic changes in hydrolysis rates due to changes in the substrate mixing ratio, it can be imprecise. An advantage is, as such an approach has a single combined feedstock, synergistic changes in Fd due to co-digestion (measured in batch tests) can be accounted for by modifying the combined inert fraction of the influent, thereby improving the precision of the simulation when co-digestion has synergistic effects on SMY. No study has yet undertaken this approach despite the major impact synergism has been shown to have on methane yields (increasing SMYs by up to 65% according to Poulsen et al. (2016)). Due to the inability of this approach to reflect changes in substrate hydrolysis rates, several authors were prompted to propose extensions to the model to improve the accuracy of ADM1 for co-digestion modelling.

The most common approach to applying ADM1 to co-digestion is individual characterisation of substrates, and modification of the model to account for individual disintegration/hydrolysis processes occurring on each substrate (Arnell et al., 2016; Zaher et al., 2009). This allows for dynamic changes in disintegration/hydrolysis rates as substrate mixing ratios change, thereby improving the precision of modelling such scenarios. Such an approach however does not consider the effects of synergistic (or indeed antagonistic) effects co-digestion may have on SMYs (Fd values). Several authors have proposed similar hydrolysis models to this effect (Esposito et al., 2011a; Zaher et al., 2009), as shown in Figure 2-9.

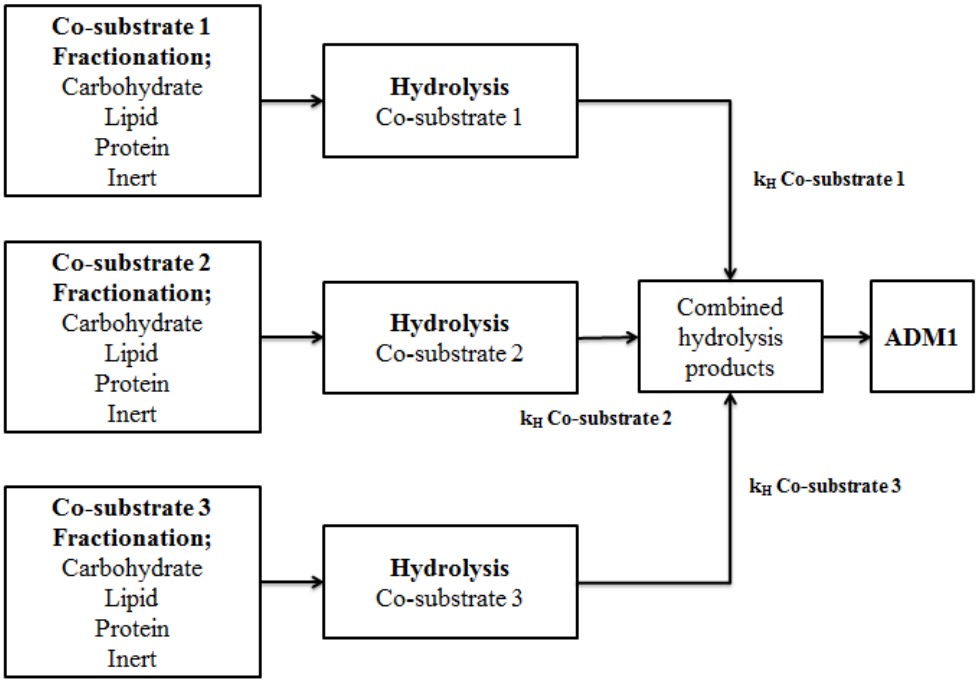


Figure 2-9 Model layout of ADM1 extension for co-digestion suggested by Zaher et al. (2009). k_H denotes hydrolysis rate.

Another approach to co-digestion is to utilise batch tests to identify the hydrolysis (or disintegration) rates of rapidly and slowly degradable pools of COD in the substrate (as described in Section 2.10.1) and, when applying ADM1, characterise the disintegration or hydrolysis of each substrate into these two fractions (García-Gen et al., 2015; Hidaka et al., 2015; Mottet et al., 2013). García-Gen et al. (2015) proposed such an approach, in which separated co-substrate disintegration processes are followed by a combined carbohydrate, lipid, protein and (newly proposed) fibre (i.e. slowly degrading carbohydrates) hydrolysis process. Esposito et al. (2011b) took a similar approach but instead of combined carbohydrate, protein and lipid fractions, they suggest disintegration products should be divided into readily and slowly degradable sub-fractions of carbohydrate, protein and lipid (see Figure 2-10).

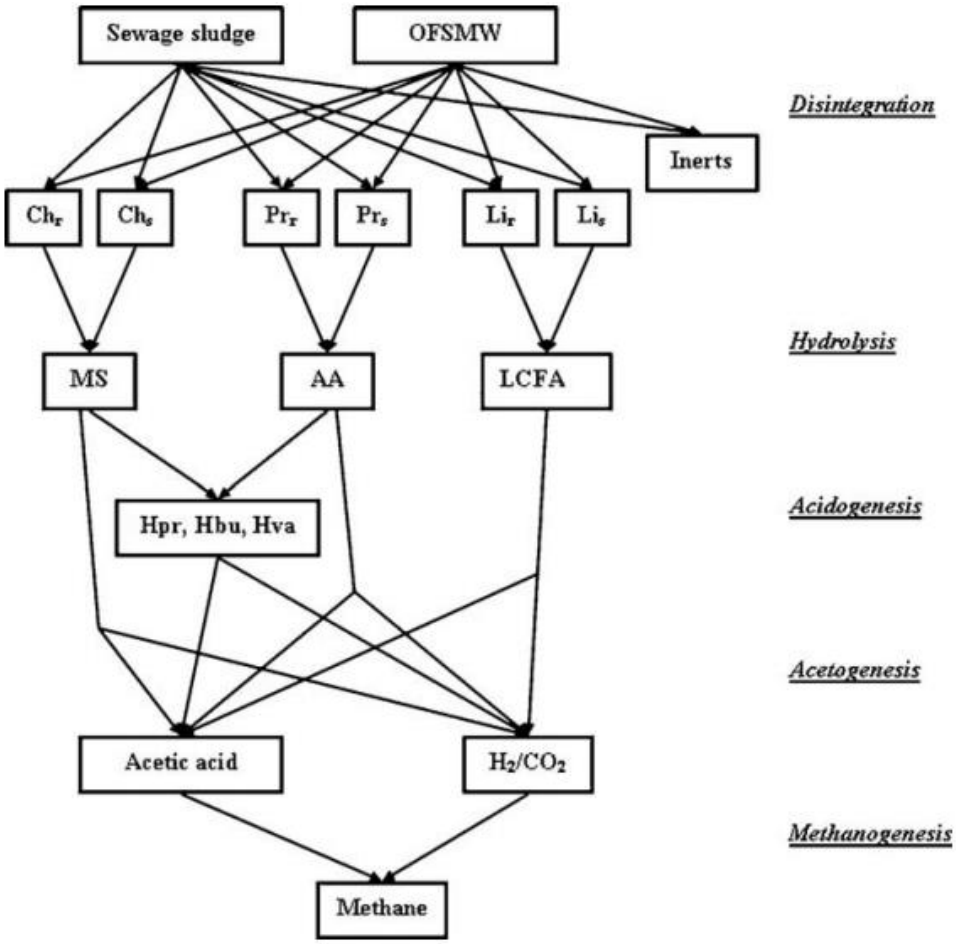


Figure 2-10 Overview of hydrolysis model proposed by Esposito et al. (2011b). Ch_r, Pr_r and Li_r are the rapidly hydrolysable carbohydrate, protein and lipid fractions, respectively. Ch_s, Pr_s and Li_s are the slowly hydrolysable carbohydrate, protein and lipid fractions, respectively. MS is monosaccharides, AA is amino acids and LCFA are long chain fatty acids. Hpr, Hbu and Hva denote propionate, butyrate and valerate. Copyright © 2017 Elsevier B.V

2.10.2.3 Other mathematical approaches to modelling AD systems

Mechanistic models such as ADM1 are not the only type of AD model proposed. Black box models and artificial neural networks, which predict methane yields and other process parameters based on trends determined by large volumes of previous reactor performance data have been proposed (Yusof et al., 2014). Without requiring information on the highly complex mechanisms occurring in the AD system, such models can predict AD performance, provided data of sufficient quality is used to calibrate the algorithms involved (Yu et al., 2013). Its application to AD is still rare. While it has the potential to provide accurate simulation of several outputs from established systems, the fact that large volumes of data are required to "train" the model and that it cannot provide information useful for

digester design and process scale up, limits its use to large well established full scale plants (Yu et al., 2013).

2.11 Microbial ecology and anaerobic digestion

High throughput DNA sequencing has the potential to provide vast amounts of information about the composition and function of microbial communities. This in turn allows for a deeper understanding of how diverse and complex microbial systems, such as AD systems, operate.

2.11.1 High-throughput sequencing and development of "-omic" approaches to biosystems

The advent of high throughput molecular DNA techniques means that the limitations of traditional polymerase chain reaction (PCR)-based analysis (such as low resolution when looking at metagenomes of complex biomes) are no longer an issue (Tang et al., 2015). A highly detailed analysis of the DNA profile of specific microbiomes is possible i.e. the metagenome of specific environments can now be mapped. Further to this, due to the ability to analyse a far greater amount of genetic material during analysis, assessment of the microbiome RNA profile (metatranscriptomics) is possible (Langille et al., 2013). The data generated from metagenomic and metatranscriptomic profiling of specific microbiomes when combined with further analytical methods (such as tandem mass spectrometry) can be used to associate expressed proteins (metaproteomics (Abram et al., 2011)) and metabolites (metabolomics) with microbiome function and structure (Bremges et al., 2015). In summary, characterisation of all the "-omics" highlighted previously allows microbiome DNA potential, gene expression and cell function for a specific microbiome to be understood (Vanwonterghem et al., 2014).

Figure 2-11 provides an overview of the components which comprise the meta-genetic approach to characterising microbiomes.

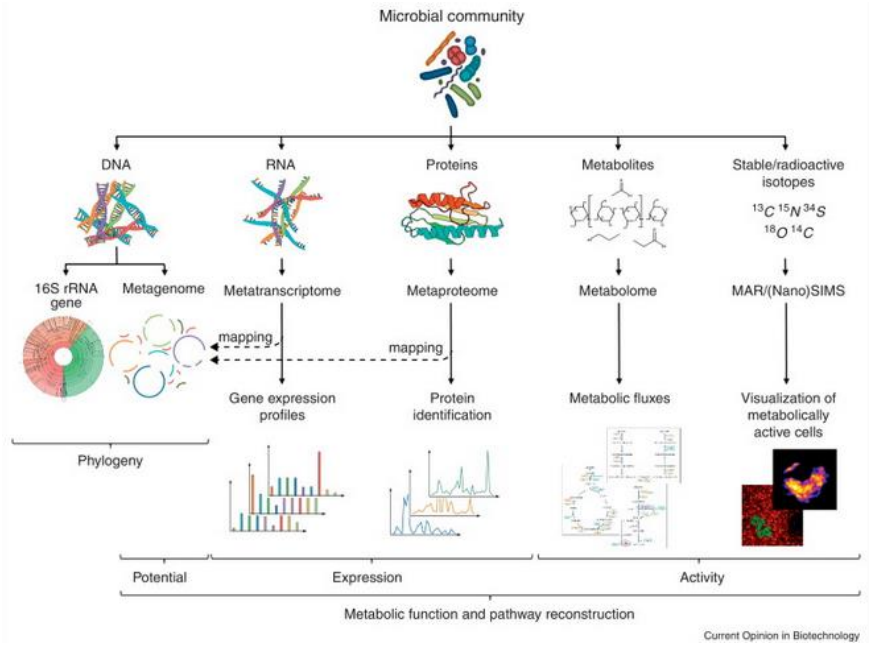


Figure 2-11 Overview of components which comprise and characterise a microbiome. The specific application of the information provided by each component is also highlighted. Taken from Vanwonterghem et al. (2014). Copyright © 2017 Elsevier B.V

2.11.2 16s rRNA profiling

Metagenomics, wherein all available DNA within a microbiome is sequenced, can provide a detailed overview of the phylogeny of the entire microbiome, whilst also providing information on functional genes contained within the DNA assessed (which in turn can be used to assess microbiome function under different environmental conditions) (Campanaro et al., 2016). One weakness in the 16s rRNA profiling approach to analysis of microbial populations is that it says nothing of activity of specific microorganisms or the rate at which functional genes contained within the metagenome is expressed (Zakrzewski et al., 2012). Therefore it is challenging to definitively link specific environmental conditions with specific shifts in population e.g.

1. Does the high abundance of specific species indicate such species as highly active players in a biochemical process, or are they inactive?
2. Are there low abundant species operating with high activity in a system?
3. Is an apparently resistant species truly resistant to a specific change in conditions, or has it undergone a metabolic shift to compensate for changing conditions?

Despite this, observations of relative changes in reactor metagenomes can provide useful insights into AD systems.

2.11.3 Application of molecular microbiology to AD

16s rRNA profiling is increasingly being used in the field of AD in order to provide a detailed overview of the phylogeny of the AD biomass. In particular, the effect of varying reactor operating conditions on microbiome phylogeny has been assessed, with attempts made to link changes in population dynamics to specific reactor conditions (limitations of 16s rRNA profiling aside) (Tang et al., 2015). As the process of AD inherently involves syntrophic relationships, analysis for the AD biome as a whole is appropriate.

DNA analysis of the microbiome of anaerobic digesters has been taking place over many years, using PCR-based techniques such as denaturing gradient gel electrophoresis (DGGE), real time qPCR and fluorescent in-situ hybridisation (FISH) techniques. However the limited capacity at which such technology could operate meant that, in many cases, coverage of the microbiome was somewhat limited and that many low abundant species could not be identified (Vanwonterghem et al., 2014). The species richness and diversity observed from the use of such techniques was limited in terms of resolution. Additionally it was challenging to identify specific sequences associated with specific system functions for a whole microbiome using these techniques and so assessment of both phylogeny and microbiome function was limited. The highly syntrophic nature of AD systems has meant that very few of the key organisms involved in AD could be cultured. This, allied with the low resolution of conventional DNA analysis, has meant that a large amount of organisms comprising the AD microbiome may not have been observed.

Applying high throughput molecular techniques to AD systems has furthered the current understanding of how all component communities in the AD biome are structured and how they interact and overlap with each other. From an engineering and management point of view, such advances pave the way for the development of methods to optimise the process (e.g. changing operating conditions to favour specific organisms, biome functions and metabolic pathways which generate higher methane yields) and systems which improve process control (e.g. provide early warning to operators of potentially upset conditions) (Tang et al., 2015). In addition, the analysis of the AD microbiome may present opportunities to identify

hitherto unknown enzymes and organisms which may be “bioprospected” for uses within the AD sector (such as identifying enzymes which increase hydrolysis rates and using them to optimise operation of full scale reactors (Gerhardt et al., 2007)) and in other disciplines.

Phylogenetic analysis has been the primary application of high throughput DNA techniques to AD thus far (Vanwonterghem et al., 2014). Conventional methods of making inferences about the function of specific organisms by relating known functions of related culturable species, or by statistically relating shifts in populations to changes in operating conditions using multivariate statistics have been undertaken in AD studies to explain changes in reactor conditions (Carballa et al., 2015).

2.11.4 Phylogenetic composition of AD reactors and the effect of operating conditions

As mentioned in Section 2.5, AD is a process achieved by the syntrophic interactions between two domains; bacteria and archaea. Phenotyping of AD systems reveal that bacterial populations are typically more abundant and diverse than archaeal populations, with bacteria typically 10 times more abundant than archaea (Carballa et al., 2015; Sundberg et al., 2013). However metaproteomics and metatranscriptomic analysis typically indicate (through the expression of key enzymes and cDNA reads) that methanogenic archaea are active to a far higher degree than bacterial populations (Vanwonterghem et al., 2014; Zakrzewski et al., 2012). The general metabolic pathways associated with AD have been well established (see Section 2.5). However, the application of 16s rRNA profiling (and in future metatranscriptomics and metaproteomics) to AD has allowed for the identification of the effects of specific process conditions (substrate characteristics, temperature, inoculum etc.) on specific bacterial and archaeal populations and metabolic pathways which generate biogas.

2.11.4.1 Archaea

Due to the relatively low diversity of methanogenic archaea, low functional redundancy is expected in this segment of the AD community (Sundberg et al., 2013; Zakrzewski et al., 2012). Studies have indicated that a small number of highly active organisms are responsible for methanogenesis (Carballa et al., 2015; Vanwonterghem et al., 2014). This lack of functional redundancy may explain the

observed dependence of archaeal populations (and the associated metabolic pathways) on feedstock composition, temperature and reactor loading rates.

At mesophilic temperature (and in the absence of significant ammonia or VFA concentrations), aceticlastic methanogens predominate. This results in *Methanosaeta* being the dominant genus observed in studies of sewage sludge-based AD systems operating at relatively low OLRs (Carballa et al., 2015; Razaviarani & Buchanan, 2015; Sundberg et al., 2013) .

In reactors where high VFA or ammonia concentrations occur, hydrogenotrophic methanogenesis plays a greater role (De Vrieze et al., 2016; Stolze et al., 2015; Sundberg et al., 2013). In a study using shotgun pyrosequencing to assess syntrophic acetate oxidation (SAO), Werner et al. (2014) noted that before the addition of ammonia, 5 % of acetate was metabolised by SAO, while at an ammonia addition rate of 5.5 g/L N up to 25 % of acetate was metabolised by SAO, suggesting a partial shift from aceticlastic to hydrogenotrophic methanogenesis as ammonia concentrations increased. Therefore, co-digestion plants treating substrates such as manure, slaughterhouse waste and the organic fraction of municipal solid waste are typically dominated by hydrogenotrophic methanogens such as *Methanoculleus*, and facultative aceticlastic or hydrogenotrophic methanogens such as *Methanosarcina* (Razaviarani & Buchanan, 2015).

Razaviarani and Buchanan (2015) noted a major increase in sequence abundance of all archaea when OLR was increased, suggesting that methanogenic populations may be limited by substrate availability in digesters operating at low OLRs. The link between archaeal population shifts and varying OLR is an area which may merit further attention, as understanding such interactions may allow for optimisation of biogas yields from biogas plants while maintaining process stability.

2.11.4.2 Bacteria

The main bacterial phyla associated with AD are *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Chloroflexi*, with *Firmicutes* and *Bacteroidetes* being typically the most dominant (Carballa et al., 2015; Stolze et al., 2015). The dominance of each species varies significantly between studies. Vanwonterghem et al. (2014) and De Vrieze et al. (2016) highlighted that bacterial populations in anaerobic digesters are dynamic, even with stable operating conditions. This can make it challenging to associate specific population shifts to specific reactor conditions. This along with

the functional redundancy typical of fermentative bacterial populations (De Vrieze et al., 2016) may explain why studies have found that while significant differences in methanogenic populations occurred due to changes in total solids concentration and temperature range respectively, no significant changes in bacterial populations were observed (Franke-Whittle et al., 2014; Stolze et al., 2015).

Nevertheless some studies did link differences in reactor conditions with differences in bacterial communities. Sundberg et al. (2013) found that *Firmicutes* was the dominant phyla in co-digestion plants, with the *Clostridia* genus the most abundant. They postulated that in addition to high VFA and ammonia concentrations, this may be due to the hygienisation of feedstock prior to co-digestion. De Francisci et al. (2015) noted distinct shifts in bacterial populations (and increase in diversity) as organic overloading of systems was undertaken using carbohydrates, proteins and lipids. This suggests that when substrate composition changes dramatically, bacterial populations shift (De Francisci et al., 2015).

2.11.5 Development of biomarkers for AD process stability

By utilising the information provided by studies which varied reactor operating conditions and associated changes in reactor conditions with changes in the microbiome, some authors have attempted to develop a system which categorises the stability of anaerobic digesters based on its phylogenetic profile (Carballa et al., 2015). Such systems can use phylogenetic indicators (also referred to as biomarkers, which utilise the presence or absence of specific organisms), or numerical tools (which take into account changes in diversity, evenness etc.) as a means of assessing reactor stability. For either approach, statistical correlation between upset conditions and population change is required. Some studies have been undertaken to identify biomarkers for reactor stability.

Carballa et al. (2015) found that an early indicator for unstable reactor conditions may be rapid shift in methanogenic population from aceticlastic methanogens (e.g. *Methanosaeta*) to hydrogenotrophic methanogens (beyond the typical relative abundance ranges associated with the specific reactor), and a reduction in the evenness of bacterial populations. Overall however, the authors suggest a diverse bacterial population (not necessarily stable in composition) and a stable archaeal population are indicative of a stable reactor.

De Vrieze et al. (2016) suggests that the presence of *Methanosaeta* in systems with low ammonia is a biomarker for process stability, but for systems with high

ammonia, *Methanosarcina* has been suggested as a biomarker for stable operation. They also suggest that the presence of *Syntrophomonas* in high relative abundance may be a biomarker for potential LCFA inhibition.

Poirier et al. (2016a) suggests that a reduction in the relative abundance of syntrophic/hydrogenotrophic methanogens *Methanosarcina* and *Syntrophomonadaceae* and an increase in the relative abundance of *Methanoculleus* and *Synergistaceae* may act as a biomarker for phenol inhibition. In a similar study assessing the effects of ammonia inhibition on digester microbiomes, Poirier et al. (2016b) found that the presence of archaea *Methanoculleus* and *Methanosarcina* and the bacteria *Treponema* were markers for digester stability at elevated ammonium concentrations.

Further work is required to validate proposed biomarkers for stability and instability. Indeed there exists more scope to develop biomarkers for instability caused by reactor configurations (such as methanogen washout at low HRTs) or the presence of specific toxic compounds (sulfide, trace metals etc.).

2.12 Anaerobic co-digestion and economic analyses

Considering the currently limited number of on-farm (and indeed off farm) biogas plants in Ireland, undertaking economic analysis of novel on-farm co-digestion concepts is crucial in defining the economic potential of such plants. A challenge when undertaking such analyses is identifying accurate capital expenditure (CAPEX), and operational expenditure (OPEX) associated with on-farm biogas plants; specific CAPEX and OPEX of plants can vary significantly due to site or country specific conditions. Table 2-7 illustrates the variation in CAPEX and OPEX (reported in a €/MW of installed electricity generating capacity (MWe) basis) reported by a range of European studies.

Table 2-7 Capital (CAPEX) and operational (OPEX) expenses used in selected studies analysing the economic viability of co-digestion systems in European countries

Capacity (kWe)	CAPEX (€/kWe)	OPEX (€/kWe/year)	Substrates	Location	Source
50	€5,700	ns	Manure	Italy	(Agostini et al., 2016)
66	€8,437	€827	Grass silage, PM	Ireland	(Nolan et al., 2012)
67	€4,151	€1,644	Energy crops, manure	Germany	(Blokhina et al., 2011)
73	€4,100	€1,662	Energy crops, manure	Germany	(Blokhina et al., 2011)
75	€7,504	€577	Maize silage, manure	Germany	(Blumenstein et al., 2016)
90	€3,913	€1,620	Energy crops, manure	Germany	(Blokhina et al., 2011)
100	€4,515	€124	Maize silage, manure	Austria	(Walla & Schneeberger, 2008)
153	€3,403	€1,495	Energy crops, manure	Germany	(Blokhina et al., 2011)
206	€3,063	€1,489	Energy crops, manure	Germany	(Blokhina et al., 2011)
250	€4,866	€406	Maize silage, manure	Germany	(Blumenstein et al., 2016)
250	€3,906	€ 93	Maize silage, manure	Austria	(Walla & Schneeberger, 2008)
250	€4,449	€1,383	Olive mill waste, PM	Spain	(Orive et al., 2016)
500	€ 3,703	€79	Maize silage, manure	Austria	(Walla & Schneeberger, 2008)
500	€3,681	€382	Maize silage, manure	Germany	(Blumenstein et al., 2016)
500	€5,020	€180	Maize silage, wheat	Germany	(Balussou et al., 2012)
750	€6,763	€974	Sewage sludge, FW	Germany	(Balussou et al., 2012)
1000	€4,000	ns	Maize, manure	Italy	(Agostini et al., 2016)

Table 2-7 illustrates that while there is some evidence economies of scale (for CAPEX in particular) when studies assessing the viability of co-digestion of

similar substrates are compared, substrates type treated has a major effect on the CAPEX and OPEX, as evidenced by the higher CAPEX and OPEX associated with sewage sludge and FW co-digestion (Balussou et al., 2012), and olive mill waste and PM co-digestion (Orive et al., 2016). This is of particular significance in biogas plants where FW and manure are co-digested. Compared with biogas plants co-digesting manure and energy crops, such plants have significantly higher CAPEX due to the need for additional infrastructure (such as additional civil works for site entrances and exits, depacking systems, macerators and digestate pasteurisers), and higher OPEX due to higher digestate disposal costs, energy costs and regulatory compliance costs (Balussou et al., 2012). Location also appears to have an effect, with plants of similar size treating similar substrates in Germany (Blokhina et al., 2011) and Ireland (Nolan et al., 2012), and Austria (Walla & Schneeberger, 2008) and Germany (Blumenstein et al., 2016) having significantly different CAPEX and OPEX. Table 2-7 also illustrates that there have not been a large number of studies assessing the CAPEX, OPEX or economic viability of FW and manure-based co-digestion systems.

2.12.1 Approaches to economic modelling

Economic modelling of biogas plants is typically undertaken by estimating the CAPEX, OPEX and revenues associated with a plant and then integrating these data in an economic model. The economic model can then be used to assess the potential financial viability of a plant throughout its lifetime. Several metrics may be used to assess the financial viability of a project. Perhaps the most well-known, simplest and most widely used is return on investment (RoI). RoI is calculated below.

2-14 $$\boldsymbol{RoI} = \frac{(\boldsymbol{Total\ net\ revenue + CAPEX\ salvage\ value}) - \boldsymbol{Total\ CAPEX}}{\boldsymbol{Total\ CAPEX}}$$

RoI can rapidly determine the overall return of investment in a project, accounting for both net revenues (i.e. profit) as well as the residual costs associated with the assets purchased with initial CAPEX after depreciation is considered. While useful, such a metric has limitations; it does not account for the time dependant (due to inflation) value of the capital investment or the revenues generated and provides no indication on the timeline required to return the capital investment (Barnett & Jawadi, 2012).

The net present value (NPV) of an investment provides the value of the investment, accounting for the CAPEX and the time dependant value of the capital investment and the revenues (the discount rate) at any point during the lifespan of a project/investment. It is a widely used (Agostini et al., 2016; Balussou et al., 2012; Orive et al., 2016) measure, in particular for estimating net cash flows from a given investment. Note that, as a measure of cash flow, depreciation is not factored into the calculation. NPV is defined below.

2-15 $$\boldsymbol{NPV = -C_0 + \sum_{t=1}^{n} \frac{R_t - C_t^{O\&M}}{(1+r)^t}}$$

C_0 is the initial investment, R_t is the revenue in time period t, $C_t^{O\&M}$ is the operating cost in time period t, r is the discount rate (%), and t is the time period from 0 to n (years). One drawback of the NPV is that it does not provide a clear representation of the profitability of a project in terms of initial investment made. When comparing the viability and profitability of projects of different scale, NPV is of limited use (Orive et al., 2016). Instead, the internal rate of return (IRR) is often used to compare the profitability of different projects. IRR is the return on investment required for the project to overcome the reduction in value of the capital invested in the project i.e. the discount rate at which NPV after n years becomes zero (Agostini et al., 2016). It is defined below.

2-16 $$\boldsymbol{0 = -C_0 + \sum_{t=1}^{n} \frac{R_t - C_t^{O\&M}}{(1+IRR)^t}}$$

where IRR is the financial internal rate of return. A project is only financially viable if the IRR is higher than the discount rate.

Finally, it is often useful to express the viability of an investment in terms of the time required to pay back the capital investment. This is referred to as the payback period (Balussou et al., 2012). The payback period is the time required for the NPV to reach zero.

2.12.1.1 Deterministic economic models

The majority of studies which assessed the viability of biogas plants use either generalised conceptual scenarios or specific currently operating plants (De Clercq et al., 2017) to develop deterministic financial models. Such models utilise data, typically provided by governmental agencies, operating biogas plants and/or engineering firms, to assess project viability based on fixed revenue, CAPEX and OPEX values. Such studies are typically undertaken to assess how economic

viability of a plant is affected by use of specific co-substrates (sugar beet (Boldrin et al., 2016), energy crops (Balussou et al., 2012; Nolan et al., 2012), olive mill waste (Orive et al., 2016)), specific digester sizes (Walla & Schneeberger, 2008) or specific biogas utilisation regimes (Blokhina et al., 2011). Such an approach can be useful in illustrating the net effect of changing key operating parameters. However due to the rigid nature of deterministic models, this approach cannot account for the uncertainty associated with specific model inputs i.e. the values of some model inputs may be subject to significant change in future which may strongly affect the project viability.

2.12.1.2 Stochastic economic models

Understanding which variables have the greatest impact on the economic viability of a project, and accounting for uncertainty in model inputs is crucial, particularly when key inputs are vulnerable to changes in external markets. Stochastic modelling is an approach which can account for variation of the inputs to economic models. Stochastic models can account for the potential variation in key model inputs across estimated or known probability distributions. They can therefore allow for identification of the most sensitive system inputs, as well as providing an assessment of the overall risk associated with a proposed plant (De Clercq et al., 2017; Hertz & Thomas, 1983; Van Groenendaal & Kleijnen, 1997).

In relation to PM and FW co-digestion, the structure and dynamics of the FW collection, separation and disposal market can have a major effect on project viability. Regional and temporal differences in the amounts of FW source separated, collected and available for treatment via AD, the gate fees possible for receipt of FW wastes and the cost charged by farmers for ultimate disposal of digestate generated in the co-digestion process can have a major effect on project viability. Accounting for these potential variations allows for a more dynamic and realistic analysis of the economic viability of on-farm biogas plants.

Stochastic modelling can be undertaken using Monte Carlo simulation; selected model inputs are randomly varied across a specific predefined probability distribution a set number of times (typically > 5,000 (De Clercq et al., 2017)) and the effect of this on the model output (NPV, IRR, RoI etc.) are recorded. The resulting data can be analysed to identify the sensitivity of the model output to changes in the input factor which was varied (Agostini et al., 2016). It can also provide information on the input values required for a model to be economically

viable, and can be used to estimate the likelihood of a project to make a return on investment (De Clercq et al., 2017; Hertz & Thomas, 1983).

This approach has been applied to a small number of biogas-based studies. De Clercq et al. (2017) used Monte Carlo simulation to assess how sensitive the economic viability of a Chinese biogas plant treating a range of biowaste was to changes in market conditions and operational efficiency, and to quantify the likelihood of the facility to return a profit. They found that based on current and projected future market conditions, the project faced an 85 % likelihood of failure. In a study to determine whether to generate biogas or produce butanol in a biorefinery treating sugarcane biomass, Mariano et al. (2013) similarly undertook Monte Carlo simulation. They found that the production of alcohols and acetone were less sensitive to changes in market conditions and likely to generate higher IRRs than biogas production.

2.12.2 Economic modelling of biogas plants in Ireland

Some analysis of the potential for biogas plants in Ireland has been undertaken. Most studies have focused on biogas utilisation pathways (Goulding & Power, 2013; Murphy et al., 2004) and quantifying the energy potential of available substrates (Murphy & Power, 2009; O'Shea et al., 2016).

Goulding and Power (2013) compared the economic viability of utilising biogas to generate heat and electricity via CHP with upgrading biogas to biomethane from small to medium scale biogas plants co-digesting manure and energy crops. To compare the 2 utilisation pathways, they expressed the profits from each on a €/ha of land used for growing of energy crops. They found that, despite CAPEX being more than double that of a CHP system, the generation of biomethane for transport fuel was found to be most economically favourable on a €/ha basis on smaller scale plants in particular. This study also found that for CHP systems to be viable under the scenarios studied 8 to 11 farms would need to be in partnership to generate sufficient feedstock, and a source to utilise the heat generated must be present. This illustrates the challenges facing the viability of crop fed, farm-based biogas plants; the need to secure stable sufficient feedstock, and the need to meet a nearby demand for heat. Murphy and Power (2009) undertook a similar analysis, however assessed a range of differed energy crop substrates for on-farm biogas plants. No specific study has been undertaken to assess the viability of undertaking on-farm co-digestion of PM and FW.

Few studies have undertaken in depth analysis of the concept of on-farm biogas plants. Nolan et al. (2012) assessed the viability of on-farm co-digestion of grass silage and PM, and subsequent utilisation via CHP for a single specific plant using a deterministic financial model. They found that on-farm co-digestion (pig manure and grass silage) was not feasible on an average farm but suggested that such systems may be viable on larger farms. This study did not assess the effect of changes in market conditions (changes to silage costs, energy costs and digestate disposal for example), or attempt to identify the optimal plant size for on-farm biogas plants.

2.13 Summary

This review illustrated that while the topic of anaerobic co-digestion is a rapidly maturing research area, there are some significant gaps in the literature in terms of PM and FW co-digestion.

In particular, there has been no comprehensive assessment on the effect of PM and FW co-digestion in terms of both methane yields and reaction kinetics, and little assessment of the suitability of established mathematical models for simulating batch PM/FW anaerobic co-digestion.

No semi-continuous experiments have been carried out which identify the optimal HRT and OLR for PM and FW co-digestion at mesophilic temperatures, and there have been no significant studies of the effect of co-digestion on the digestate enteric indicator organism content or on digestate dewaterability.

The development of extensions for the ADM1 model's application to co-digestion has thus far focused on the provision for separately modelling the hydrolysis of constituent substrates. Its use thus far has also relied on complex mathematical optimisation which requires (in addition to data on hydrolysis rates and substrate degradability generated from batch tests) large amounts of digester operating data to be fully calibrated. There is a need to investigate whether the application of this tool can be streamlined in order to increase its suitability for widespread adoption.

The development of low cost high throughput DNA sequencing may revolutionise the way biogas plants are monitored and optimised. These techniques provide information which can explain observed changes in reactor performance during experimentation. In doing so, biomarkers for system stability/instability can be identified. Applying high throughput DNA sequencing to samples gathered under

controlled experimental conditions can therefore result in significant contributions to the development of biomarkers.

Economic modelling of biogas systems has generally been undertaken in a deterministic manner thus far. Analysis of plant viability, either currently operating plants or hypothetical scenarios, often fail to assess the sensitivity of model inputs to future changes in market conditions. Stochastic modelling via Monte Carlo simulations can provide information of the sensitivity of biogas plant viability to changes in specific model inputs. Analysis of the economic viability of on-farm co-digestion systems in Ireland has been limited thus far to high level identification of the most plentiful substrates on a national level, and rudimentary deterministic analysis of specific plant designs.

CHAPTER 3

Synergism and effect of high initial volatile fatty acid concentrations during batch food waste and pig manure anaerobic co-digestion

The following chapter is comprised of a study previously published as; Dennehy C, Lawlor PG, Croize T, Jiang Y, Morrison L, Gardiner GE, X Zhan *Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion*. Waste Management 2016, 56, 173.

3.1 Introduction

This batch study aimed to identify optimal conditions for the anaerobic co-digestion of PM and FW. The specific objectives of this study were:

1. To assess and quantify synergistic or antagonistic effects of co-digesting FW and PM on SMY, substrate degradability (Fd) and reaction kinetics.
2. To assess the effect of initial VFA concentrations on observed synergy.
3. To identify the most suitable mathematical models for modelling batch anaerobic co-digestion where high initial VFA concentrations are found.

3.2 Materials and Methods

3.2.1 Substrates

The PM used in this study was taken from the manure storage tanks of the finishing unit of a local pig farm in Galway, Ireland. PM was stored overnight at 10 °C prior to use. FW was collected from 5 residences, combined and subsampled as per the method described by Browne et al. (2014). After subsampling, the FW was blended with a food processor (Russell Hobbs 500W 18087 Blender). The inoculum used in this study was sourced from a semi-continuous digester operating in the laboratory for 6 months at 39 °C with a feedstock comprising of 60 % FW and 40 % PM (on a VS basis). The inoculum was stored for a period of 3 days at ambient temperature (20 °C), prior to use. Characteristics of the PM, FW and inoculum used in this experiment are presented in Table 3-1.

Table 3-1 Characteristics of pig manure, food waste and inoculum used in the study. Values reported are the average and standard deviation of 3 measurements.

Parameter	Pig Manure	Food Waste	Inoculum
pH	7.05 ± 0.1	4.24 ± 0.1	7.85 ± 0.1
Total COD (g/L)[a]	80.9 ± 8.3	511.5 ± 10.6	32.3 ± 0.4
Soluble COD (g/L)[a]	16.5 ± 0.14	160.0 ± 80.0	3.1 ± 0.23
Total solids (% fresh weight)	7.81 ± 0.1	39.94 ± 0.8	3.75 ± 0.03
Volatile solids (% fresh weight)	5.61 ± 0.3	29.45 ± 0.5	2.56 ± 0.1
NH_4-N (mg/L)[b]	4,622 ± 84	2,966 ± 77	4,307 ± 72
Alkalinity (mg/L HCO_3^-)[b]	3,898 ± 36	864 ± 11	2,234 ± 60
Volatile fatty acids (g/L acetic acid equivalents (HAc_{eq})[a]	11.96 ± 0.4	15.35 ± 0.8	0.975 ± 0.2
Acetic	6 ± 0.1	4.4 ± 0.3	0.18 ± 0.2
Propionic	2.265 ± 0.1	2.9 ± 0.2	<0.1
Isobutyric	0.525 ± 0.1	8.05 ± 1	<0.1
Butyric	1.525 ± 0.2	<0.1	0.8 ± 0.2
Isovaleric	0.885 ± 0.2	<0.1	<0.1
Valeric	0.765 ± 0.1	<0.1	<0.1

[a] *Values reported for FW presented in g/kg.* [b] *Values reported for FW presented in mg/kg.*

As presented in Table 3-1, the PM used in this study had high NH_4-N and VFA concentrations. The FW had low pH and low bicarbonate alkalinity. The NH_4-N concentration of the inoculum used in this study suggests that the biomass had been acclimated to high ammonia concentrations and therefore the anaerobic digestion of the PM in this study was unlikely to be affected by ammonia inhibition. The high bicarbonate alkalinity content of PM indicates that it had a high buffering capacity. This buffering capacity can help to maintain a neutral pH and consequently prevent VFA inhibition which may occur during the batch anaerobic digestion of FW (Zhang et al., 2014a).

3.2.2 Experimental Design

Six PM to FW mixing ratios were tested in triplicate; 1/0, 4/1, 3/2, 2/3, 1/4 and 0/1. An inoculum to substrate ratio (VS basis) of 3 was used in this experiment. 5.2 g of substrate VS was added to each reactor. A blank sample comprising of inoculum only was analysed in triplicate in order to correct samples for the methane yield generated by inoculum only. Deionised water was added to digesters to give a 500 mL working volume to each.

Each reactor was made from a 500 mL conical flask and a butyl-rubber stopper. Each stopper had two ports; one for liquid sampling, and the other for biogas sampling. Biogas was collected in 1 L ALTEF gas sampling bags (Restek Corporation, USA). Directly prior to the initiation of the experiment, each reactor was flushed with N_2 and sealed. Digesters were then incubated at 37°C on an orbital shaker operating at 50 rpm. Biogas volumes were measured daily in the first ten days of the experiment, and subsequently every three days. This experimental set-up is illustrated in Figure 3-1.

Figure 3-1 Batch test incubator, shaker and digesters

TS, VS, alkalinity, Total COD and soluble COD of the substrates and inoculum were measured at the beginning of the experiment. For FW samples COD was measured via serial dilution. Liquid samples were withdrawn from digesters every three to five days via a 5 mL syringe. The amount of volatile solids withdrawn from the digesters was included when calculating SMY as the experiment proceeded. Samples were centrifuged (Model 2-15, Sigma, Germany) at 14500 rotations per minute (rpm) (1175 g) for 15 min. The supernatants were then filtered through 0.45 µm cellulose nitrate membrane filter paper (Sarstedt Germany).

Following this, soluble COD, alkalinity, NH_4-N and VFA concentrations were measured.

3.2.3 Analytical Methods

TS, VS, alkalinity, total COD and soluble COD were measured according to Standard Methods (APHA, 1998). NH_4-N concentrations were measured using a nutrient analyser (Konelab, Thermo Clinical Labsystems, Vantaa, Finland). VFA concentrations were measured via high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) using a UV index detector. Separation was achieved using a 0.1 % H_2SO_4 mobile phase at a flow rate of 0.6 mL/min and an Aminex HPX-87H column (Bio-Rad, USA). The column and detector temperatures were 65 °C and 40 °C respectively. The HPLC was calibrated using a 1 mM VFA mix containing acetic, propionic, isobutyric, butyric, isovaleric and valeric acids (Sigma-Aldrich, USA).

Prior to metals analysis, samples were digested using concentrated HNO_3 and 30 % H_2O_2 via the method described by USEPA (1996). Filtered samples were acidified to 1 % using nitric acid (HNO_3) (Trace Metal Grade 67-69 %, Fisher, UK) and metal concentrations (Cd, Pb, Al, Co, Cr, Cu, Fe Mn, Mo, Ni, Pb, Se, Ti, Zn) were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; ELAN DRCe, Perkin Elmer, Waltham, USA) in a class 1000 (ISO class 6) cleanroom (Ratcliff et al., 2016).

The biogas volume was measured using the water displacement method and converted to standard temperature and pressure. The methane content of biogas was analysed via gas chromatography (7809A, Agilent Technology, USA) installed with a thermal conductivity detector and a 45-60 mesh matrix molecular sieve 5A column (Sigma-Aldrich, USA). The N_2 carrier gas had a flow rate of 30 mL/min. The temperature at the inlet, oven and detector was 100, 60 and 200 °C, respectively.

VS removal was calculated as described by Browne et al. (2014);

3-1 $$\boldsymbol{VS\ Removed} = \left(\mathbf{1} - \frac{\boldsymbol{VS_f - VS_{fb}}}{\boldsymbol{VS_i - VS_{ib}}}\right) \times \mathbf{100}\%$$

Where: VS_i, the amount of total input VS (g); VS_f, the amount of total VS at the end of the digestion (g); VS_{ib}, the amount of VS (g) in the inoculum (blank) at the beginning of the experiment; and VS_{fb}, the amount of VS (g) in the inoculum (blank) at the end of the experiment.

3.2.4 Reaction Kinetics

Three different kinetic models were compared in order to determine the most suitable one for describing the kinetics of batch co-digestion of FW and PM; (1) first-order (Equation 2-7), (2) Gompertz (Equation 2-12) and (3) dual pooled first-order. The dual pooled first-order equation (Equation 3-2) is a model suitable for simulating the batch digestion of substrates whose digestion has two distinct methane generation periods (Brulé et al., 2014):

3-2 $$\boldsymbol{M(t) = M_m\left(1 - \alpha * e^{-k_f t} - (1 - \alpha) * e^{-k_L t}\right)}$$

where k_f is the rate constant for rapidly degradable substrate, k_L is the rate constant for slowly degradable substrate, and α is the ratio of rapidly degradable substrate to total degradable substrate.

Analysis of the precision of fit of each kinetic model to the experimental data was determined by the r^2 value and the root mean square prediction error (rMSPE) as described by El-Mashad (2013):

3-3 $$\boldsymbol{rMSPE = \sqrt{\sum_{n=1}^{n} \frac{(Pv_i - Mv_i)^2}{n}}}$$

where, Mv_i is measured methane volume, Pv_i is predicted methane volume and n is number of data points.

The parameters (k_H and M_m for example) for each model were estimated by minimizing an objective function (the model residual sum of squares (J)) using the generalised reduction gradient (GRG) non-linear solver function in Excel 2010 (Microsoft, USA). In order to illustrate the uncertainty associated with the model estimated parameters, a parameter surface searching method as described by Jensen et al. (2011) was used. Specifically, as an optimal parameter set exists where J (the model residual sum of squares) is at its lowest (J_{min}). The area wherein J_{min} will occur (known as the parameter surface) given a specific probability (J crit; in this instance 95 %) can be described using the F distribution (assuming residuals are normally distributed) and the following equation.

3-4 $$\boldsymbol{J_{crit} = J_{opt}\left(1 + \frac{p}{N_{data} - p} F_{\alpha,p,Ndata-p}\right)}$$

where p is the number of parameters, N_{data} is the number of data points, and $F_{\alpha, p, Ndata-p}$ is the F distribution value. This equation can therefore generate a surface

area bracketed by 95 % confidence intervals, inside which the true parameter values are at 95 % likely to lie.

Fd was determined from the M_m simulated and the COD/VS ratios as described by Equation 2-8.

All statistical analyses (one Way ANOVA and post-hoc least significance difference analysis) and non-linear regression modelling were undertaken using SPSSv22.0 (IBM, USA).

3.3 Results and Discussion

3.3.1 Methane Yields, VS Removal and Synergistic Effects

Figure 3-2 presents the cumulative and daily methane yields from this experiment. The majority of the methane was generated from all samples in the first five days of the experiment. Food waste mono-digestion resulted in a significantly higher average daily methane yield than PM mono-digestion. However the highest observed average daily methane yields were generated by substrate mixtures with a PM/FW mixing ratio of 2/3 and 1/4. This suggests that combining PM and FW increased the overall rate of methane generation compared with mono-digestion of PM and FW. As the confidence intervals associated with substrate mixtures 2/3, 1/4 and 0/1 overlap, the observed average daily methane yields may not be significantly different. This further suggests that the co-digestion of PM and FW had synergistic effects on digestion kinetics to some extent.

The measured SMY of FW (516 ± 33 mL CH_4/g VS) was higher than that of PM (260 ± 13 mL CH_4/g VS). These SMY values are in general agreement with SMYs measured in other studies (Tian et al., 2015; Zhang et al., 2011). Figure 3-2 illustrates that no inhibition was observed during the digestion of any of the treatments. Previous studies have reported VFA accumulation and inhibition when undertaking batch digestion of FW, with Tian et al. (2015) observing a lag phase of 35 days due to VFA accumulation. Ammonia inhibition has been observed during batch digestion of PM, with Dennehy et al. (2015a) observing lag phases of 35 days due to ammonia inhibition. The reason that no inhibition was observed in this study was that the inoculum used had been acclimated to the PM and FW substrates and that batch tests were undertaken at a suitable inoculum to substrate ratio.

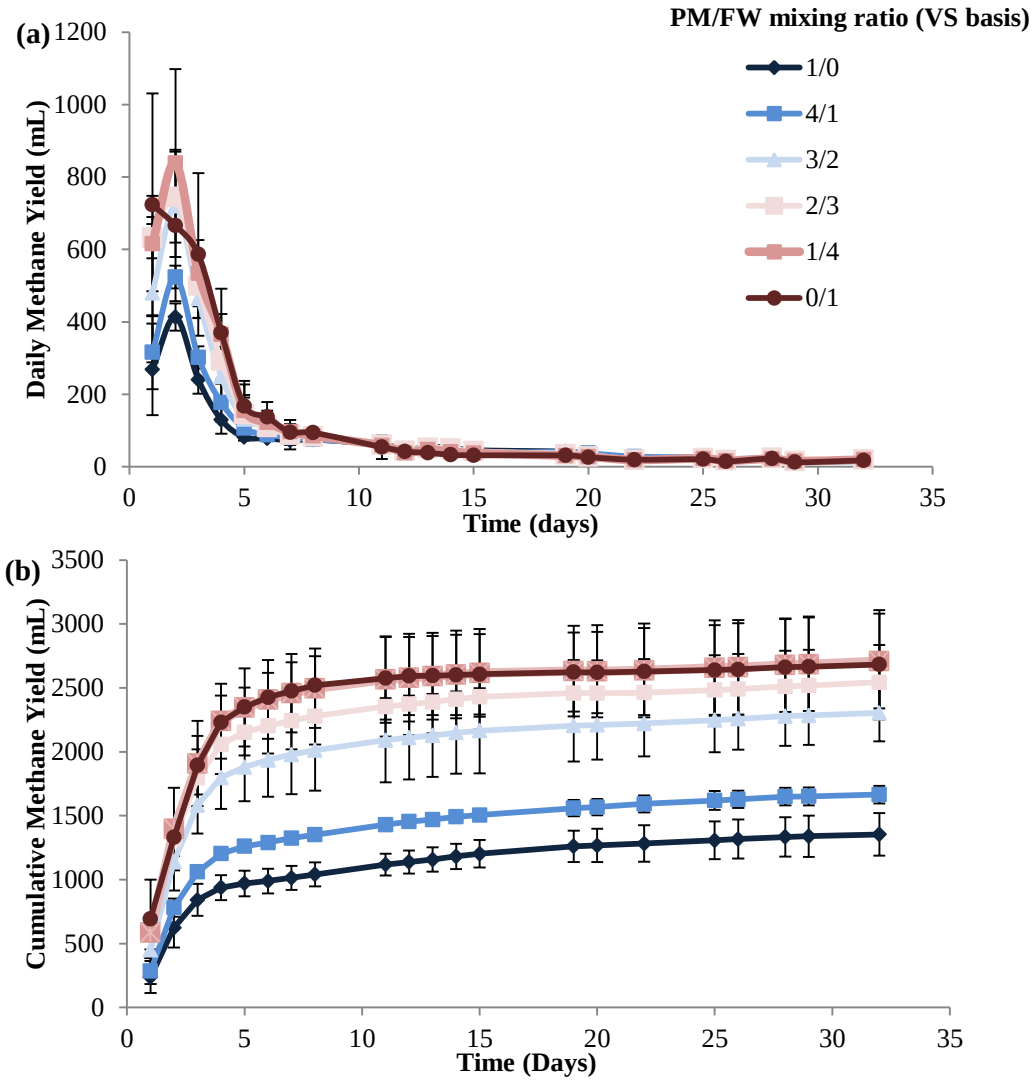


Figure 3-2 Average daily methane yield (a) and cumulative methane yield (b) measured from three replicates of the co-digestion of PM and FW mixing ratios (VS basis) of 1/0, 4/1, 3/2, 2/3, 1/4 and 0/1. Error bars represent 95% confidence intervals.

Table 3-2 presents the final SMY and VS removal measured in each substrate mixture analysed.

Table 3-2 Average and standard deviation of the specific methane yield and % VS removed during the co-digestion of PM and FW mixing ratios (VS basis) of 1/0, 4/1, 3/2, 2/3, 1/4 and 0/1 in triplicate.

PM/FW ratio	1/0	4/1	3/2	2/3	1/4	0/1
SMY (mL CH_4/g VS)	260 ±13[a]	320 ±5[b]	443 ±17[c]	489 ±23[d]	521±29[d]	516 ±33[d]
VS Removal (%)	53.9 ±5.9[a]	53.6 ±4.9[a,b]	63.2 ±2.4[b,c]	68.2 ±0.9[c]	71.4 ±2.8[d]	75.0 ±3.2[d]

Means in a row without a common superscript are significantly different ($P < 0.05$). Means separation was performed from least significance difference analysis.

Changing the substrate mixing ratio, while maintaining an equal VS loading in each treatment, had a significant effect on SMY. Increasing the proportion of FW in the feedstock led to an increase in SMY up to the PM/FW ratio of 2/3 ($p < 0.05$). However, SMYs at PM/FW ratios of 2/3, 1/4 and 0/1 were not significantly different ($p > 0.05$). The highest measured SMY in this study was obtained at a PM/FW mixing ratio of 1/4. These results are indicative of synergy between the substrates, as PM had an inherently lower SMY than FW, but the addition of PM did not negatively affect measured SMY. VS removal significantly increased (Table 3-2; $p < 0.01$) in proportion to the PM/FW ratio but no synergistic effect was observed, indicating the synergistic effects on SMY observed cannot be attributed to improved VS removal.

The magnitude of the synergy observed during this digestion was calculated using a method adapted from that of Adelard et al. (2014):

$$\Delta_{p,r,t} = \frac{(SMY_{m,r,t} - SMY_{p,r,t})}{SMY_{p,r,t}} \cdot 100 \quad \textbf{3-5}$$

where t is time (day), r is PM/FW mixing ratio, $\Delta_{p,r,t}$ is the change (in percentage) in SMY attributable to apparent substrate synergism or antagonism at PM/FW mixing ratio r and at time t, $SMY_{m,r,t}$ is the measured SMY at PM/FW mixing ratio r and at time t, and $SMY_{p,r,t}$ is the predicted SMY at PM/FW mixing ratio r and at time t, calculated with Equation 3-6:

$$SMY_{p,r,t} = (SMY_{m,FW,t} * \frac{r}{r+1}) + (SMY_{m,PM,t} * \frac{1}{r+1}) \quad \textbf{3-6}$$

Where $SMY_{m,FW,t}$ is the measured SMY of FW at time t, and $SMY_{m,PM,t}$ is the measured SMY of PM at time t.

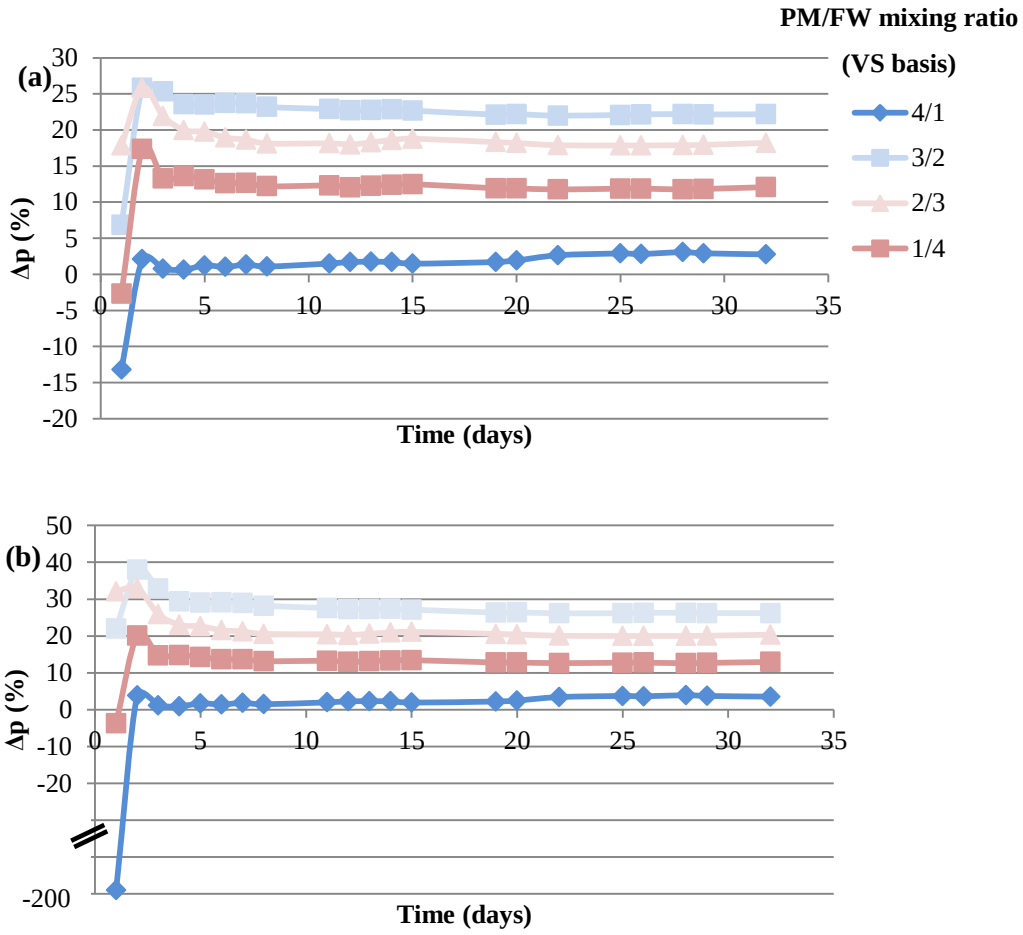


Figure 3-3 Δp of PM/FW mixing ratio's (VS basis) 4/1,3/2, 2/3 and 1/4, as calculated by Equation 3-5 and Equation 3-6 on a VS basis (a), and a VS + VFA basis (b).

As shown in Figure 3-3(a), apparent synergy was observed in all PM/FW mixtures. The pattern of Δp was similar in all substrate mixtures; there was an apparent increase between day 1 and day 2 followed by a gradual reduction for the remainder of the experiment. An exception was that for the mixture with a PM/FW ratio of 4/1 where Δp had a gradual slight increase from day 9 onwards.

The Δp pattern in the mix with a PM/FW ratio of 4/1 is different as synergy was observed in the latter stage of digestion after an initial period of antagonism. A similar lag time prior to increases in Δp was observed in samples 3/2, 2/3 and 1/4. This can be explained by examining the kinetics of digestion. The maximum daily methane yield of FW mono- digestion occurred from day 1 onward while the maximum daily methane yield was observed on day 2 for all other PM/FW mixtures. Therefore as the daily methane emission rate of the mixtures were higher than that of FW mono digestion on day 2, and as FW mono-digestion is a key input to determining the predicted SMY for each mixture, a rapid increase in the amount

of synergy measured occurred. The highest amount of synergy occurred at the period of maximum methane emission from substrate mixtures (day 2).

The high initial VFA concentrations of PM affect the SMY values when expressed on a VS basis (in terms of mL CH_4/g VS). The initial VFA concentration, which would be rapidly converted to CH_4, was not accounted for as part of VS and therefore the calculated SMY (in mL CH_4/g VS) may be overestimated by between 5.2 % (FW alone) and 21.3 % (PM alone). The expression of SMY on a VS basis is the predominant means of expressing SMY in the literature (Raposo et al., 2011) even in putrescent samples such as manure and food wastes (Angelidaki et al., 2009). However the effect of initial VFA loading is crucial when assessing the cause of apparent synergy occurring from mixing two substrates when mixing is undertaken on a VS basis (as was the case in this study).

In order to identify whether initial VFA concentrations had any effects on observed synergies, the SMY and Δp of all PM/FW mixtures were recalculated. It was assumed that all initial VFAs present were converted to methane. The volume of methane generated was calculated by converting HAc_{eq} to COD concentrations (with COD having a theoretical methane yield of 350 N mL CH_4/g COD) (Batstone et al., 2002), resulting in a theoretical yield of 373 N mL CH_4/g HAc_{eq}. This was then subtracted from the cumulative methane yield of each sample before the SMY and Δp of all PM/FW mixtures were recalculated. Results from this recalculation are presented in Table 3-3 and Figure 3-3(b).

Table 3-3 Effect of initial volatile fatty acid (VFA) concentration on Δp of PM and FW mixing ratio's (VS basis) 4/1,3/2, 2/3 and 1/4

Mixing Ratio	SMY (mL CH_4/g VS)	Δp	SMY Without Initial VFA (mL CH_4/g VS)	Δp Without Initial VFA
1/0	260	n/a	215	n/a
4/1	320	3	271	3.5
3/2	443	22	386	26
2/3	489	18	438	20
1/4	521	12	481	13
0/1	516	n/a	491	n/a

Figure 3-3(b) illustrates that the temporal pattern in Δp when initial VFA's methane contribution is removed is similar to the pattern found when it was

calculated from mL CH_4/g VS. One clear difference is the drop in Δp in the mixture of 4/1 at the beginning of the digestion. This can be explained by the negative adjusted SMY when VFAs were accounted for at this point, which may be a sign of antagonistic effects of the co-digestion on initial VFA utilization. While the magnitude of this drop appears large, Δp is a measure of relative difference, and the absolute difference between predicted and observed SMY was small (8.6 mL of CH_4) by the end of the digestion. Table 3-3 and Figure 3-3 illustrate that the synergy observed during the co-digestion of PM and FW is not simply explained by the addition of VFA from PM. Further to this, the differences between the Δp values presented in Table 3-3 suggest that the high initial VFA concentration of PM may slightly mask synergistic effects on the conversion of VS and non VFA COD to CH_4. Synergies between PM and FW have been demonstrated previously (Tian et al., 2015). Adelard et al. (2014) found a pattern of synergy somewhat similar to that observed in this study (higher methane yields in the first week of digestion) when undertaking analysis of the co-digestion of cow manure, pig manure and food waste. Previous studies have suggested that this synergy may be due to the addition of PM improving the C/N ratio, providing additional alkalinity and supplying trace elements to the microbial biomass which are not present in FW (for example Fe, Mo, Ni) (Mata-Alvarez et al., 2014).

The PM used in this study had a markedly different trace metals concentrations than the FW used (Table 3-4). The digestion of FW has previously been shown to improve when Se, Co, Ni and Fe are added (Banks et al., 2012; Nordell et al., 2016; Zhang et al., 2011). These elements are crucial to enable enzyme activity of anaerobic microorganisms, particularly in high ammonia environments where hydrogenotrophic methanogenesis plays a major role (Banks et al., 2012). Co and Se have previously been found to be essential in the production of enzymes required for hydrogenotrophic methanogenesis, while Ni and Fe have been found to play a role in increasing the activity of these enzymes (Westerholm et al., 2015). Fe, Cu, Mn and Zn concentrations were all 30 % higher in the substrate mixtures with a PM/FW ratio of 1/0 than mixtures with a PM/FW ratio of 0/1. This may have caused the synergy observed.

Table 3-4 Trace metal contents (ppb) of PM, FW, inoculum (ino.) and all treatments analysed.

				PM/FW ratio					
Metal	PM	FW	Ino.	1/0	4/1	3/2	2/3	1/4	0/1
Al	428	69	525	507	492	476	461	446	430
Co	14	>1	12	12	12	11	11	10	9
Cr	11	3	10	10	9	9	9	8	8
Cu	581	14	506	520	498	477	455	434	412
Fe	3636	127	3006	3124	2989	2855	2721	2586	2452
Mn	1616	75	1275	1338	1279	1219	1160	1100	1040
Mo	24	1	19	20	19	18	17	16	15
Ni	14	9	22	21	20	20	19	19	18
Pb	105	2	9	27	23	19	15	12	8
Se	5	>1	5	5	5	5	4	4	4
Ti	135	19	119	122	117	112	107	102	97
Zn	2660	38	2045	2160	2061	1962	1863	1765	1666

On the other hand, when the concentrations of trace metals are excessive they can have antagonistic effects on the anaerobic digestion process. Hickey et al. (1989) found that Zn and Cu were particularly toxic to methanogens.

Lin (1992) found that the degradation of acetic acid, butyric acid and isobutyric acid was inhibited in the presence of Zn and Cu concentrations of > 16 and >7 mg/L, respectively. The concentrations of Zn (1.67 - 2.16 mg/L) and Cu (412 - 520 µg/L) in this study were far below these levels.

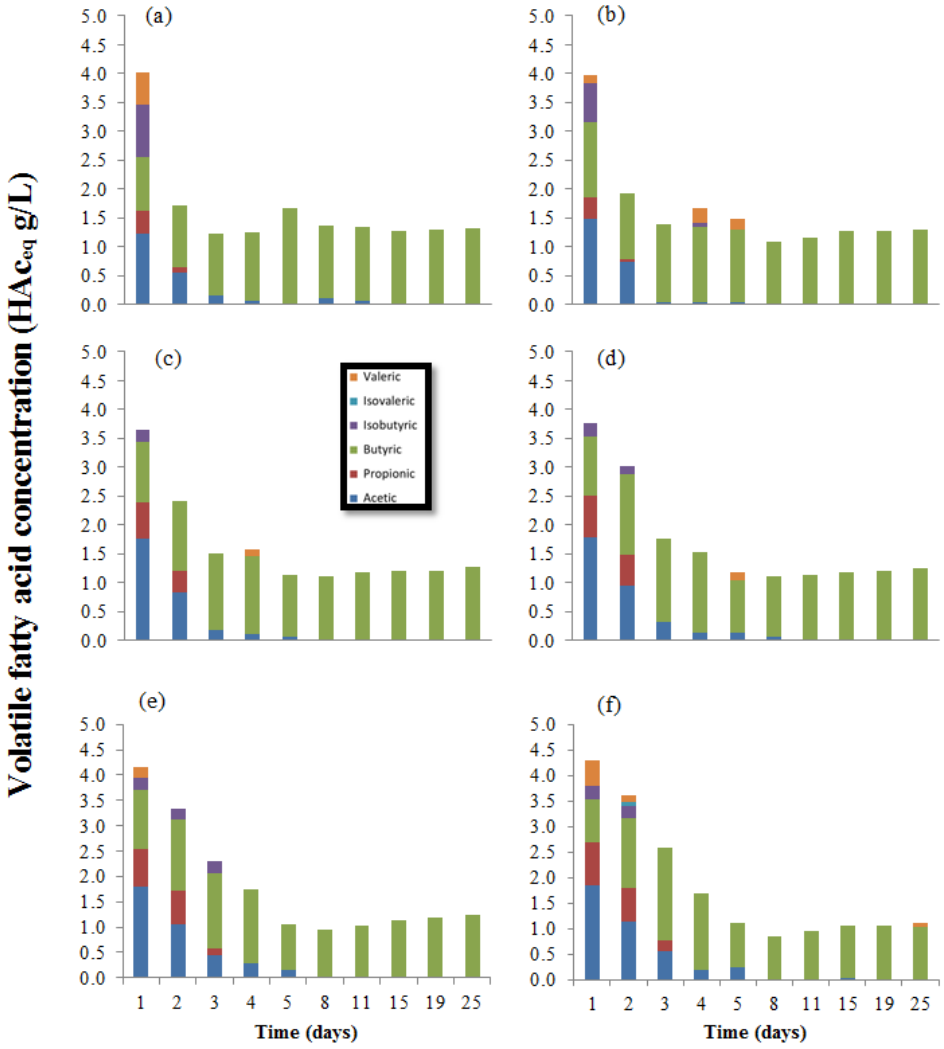


Figure 3-4 VFA degradation trends for PM/FW mixtures 1/0 (a), 4/1 (b), 3/2 (c), 2/3 (d), 1/4 (e) and 0/1 (f).

Semi-continuous digestion of FW alone would be more unstable than illustrated in this batch study because the inoculum provided sufficient trace elements and alkalinity. Co-digestion of FW with PM may therefore have more pronounced synergic effects in a semi-continuous study, than in a batch study.

3.3.2 Reaction Kinetics

3.3.2.1 Model fit to cumulative methane generation curves

Provided that no inhibitory effects occur during digestion, cumulative methane generation profiles typically follow a first-order accumulation pattern. In the majority of biogas plants hydrolysis is the rate limiting step (Brulé et al., 2014).

Therefore cumulative methane generation profiles can be used to determine the rate of hydrolysis by utilizing first-order models.

When high concentrations of VFAs occur in the feedstocks the hydrolysis rate cannot be accurately determined from methane yields. Initial VFA concentrations in excess of 1 g/L are common in manures (pig manure in particular) (Xie et al., 2011) and in this study, initial VFA concentrations in the substrate mixtures ranged from 0.67 to 1.5 g/L. Table 3-5 presents the model's output for all mixtures analysed in this study.

Table 3-5 Initial volatile fatty acid (VFA) concentrations, experimentally determined cumulative methane yields (CMY), root mean squared percentage error (rMSPE), coefficient of determination (r^2) and outputs from first-order modelling of all samples analysed

PM/FW mixing ratio	**1/0**	**4/1**	**3/2**	**2/3**	**1/4**	**0/1**
Initial VFA content (g/L)	1.509	1.341	1.173	1.005	0.837	0.668
Experimental CMY (mL)	1354	1665	2305	2544	2710	2684
First-order						
k_H	0.28	0.31	0.36	0.39	0.39	0.40
M_m	1265	1572	2214	2460	2649	2642
rMSPE	66.79	69.95	75.84	67.25	75.20	59.23
r^2	0.963	0.973	0.984	0.989	0.989	0.993
Gompertz						
M_m	1241	1540	2174	2423	2609	2608
R_{max}	205	310	546	642	723	692
λ	0.70	0.67	0.56	0.45	0.50	0.47
rMSPE	117.61	114.21	109.46	109.80	90.00	94.11
r^2	0.936	0.955	0.978	0.985	0.990	0.994
Dual Pooled						
M_m	1685	2256	2781	2977	3034	3536
α	0.57	0.59	0.74	0.79	0.86	0.74
k_F	0.455	0.415	0.409	0.426	0.402	0.399
k_L	0.026	0.015	0.012	0.011	0.005	0.001
rMSPE	16.55	17.43	12.23	14.54	22.56	16.31
r^2	0.989	0.989	0.989	0.992	0.990	0.993

Figure 3-2 illustrates that for all substrate mixtures, and mixtures with PM/FW ratios of 1/0, 4/1 and 3/2 in particular, there were two clear phases of methane generation; an initial period of rapid release in the first 5 days which was due to the

conversion of the initial VFA concentration and rapidly hydrolysable substrate to methane, followed by a gradual decomposition of the remaining organic matter in the remainder of the experiment. Such a pattern has also been observed in the anaerobic digestion of PM in previous studies (Zhang et al., 2014b).

The relatively high rMSPE, for samples with high PM content in particular, indicates that the first-order model does not fit the data precisely. This is linked with the high initial VFA concentrations in substrate mixtures. As the majority of methane was generated in the first 5 days of digestion, the model does not simulate the subsequent period of slower methane generation with precision. Previous studies have found that the k_H of PM is typically around 0.1 d^{-1} (Vavilin et al., 2008). The high initial VFA concentration may explain why k_H determined by the first-order model from PM in this study was higher (0.28 d^{-1}) than these values. k_H values for FW are typically found to lie between 0.3 and 0.5 d^{-1} (Pagés Díaz et al., 2011; Vavilin et al., 2008). As the k_H calculated for mono-digestion of FW was close to this range, and as rMSPE decrease with decreasing initial VFA (and decreasing PM in substrate), there is evidence that the first-order model is increasingly more precise for modelling cumulative methane emissions as initial VFA concentrations decreased.

Compared with the first-order model, the Gompertz model provides more precise fit (in term of r^2) to the methane emission profiles of substrate mixtures with PM/FW mixing ratio of 0/1 and 1/4, but an increasingly less precise fit in terms of r^2 and rMSPE to the methane emission profiles of substrate mixtures comprised of greater amounts of PM (Table 3-5). The rMSPE values indicate that the Gompertz model provides the least precise fit of all models analysed for samples where PM was the primary substrate. This can be explained by the fact that this model assumes a slow initial phase, followed by an exponential phase. Therefore the initial rapid release of methane caused by initial VFA concentrations in mixtures high in PM cannot be accounted for by this model.

The first-order and Gompertz models cannot account for the two distinct phases of methane accumulation observed in this study, and therefore cannot fully simulate the digestion. The development of a model which accounts for two distinct methane generation periods, referred to as a dual pooled first-order equation has led to proposed changes to the ADM 1 framework (Batstone et al., 2002) to describe the different solubilisation rates of particulate matter (Garcia-Gen et al.,

2013). Applying this model to the data presented in this paper resulted in the determination of a rate describing the rapidly degradable substrate (which would include VFAs in addition to rapidly degradable VS) and a rate describing the remainder of the degradable substrate. As Table 3-5 illustrates, the rMSPE values, show the dual pooled model provides a more accurate simulation of the methane generation profiles observed.

The α values show an increase as the proportion of FW in the substrate mixtures increased. This is indicative of a more rapid digestion. This can be explained by FW being a more rapidly degradable substrate than PM (Zhang et al., 2014a). The substrate mix with a PM/FW ratio of 1/4 has a greater α than the mix with the 0/1 ratio. This suggests that co-digestion of FW and PM had synergistic effects on reaction kinetics. The k_F and k_L values decreased slightly as the proportion of FW increased. As there was a greater concentration of VFA in PM, the higher k_F in mixtures which contained a greater proportion of PM may be explained by the rapid conversion of VFA to methane. Further to this point, the k_L of PM encapsulates the rate of methane generation of a greater proportion of substrate than the k_L of FW. Thus it models the rate of a greater amount of material readily converted to methane than the k_L of FW (as the k_F of FW models the majority of the material readily converted to methane).

3.3.2.2 Accuracy of parameter estimation

While the dual pooled model provided a more accurate simulation of the cumulative methane yield observed, it did not provide a more accurate estimation of M_m and the kinetic parameters, compared to the simple first-order model. This is illustrated in Figure 3-5, where the simulated parameters and parameter surface areas (graphed area within which k_H and M_m are 95 % likely to be present) from the first-order model, and from the dual pooled first-order model are presented.

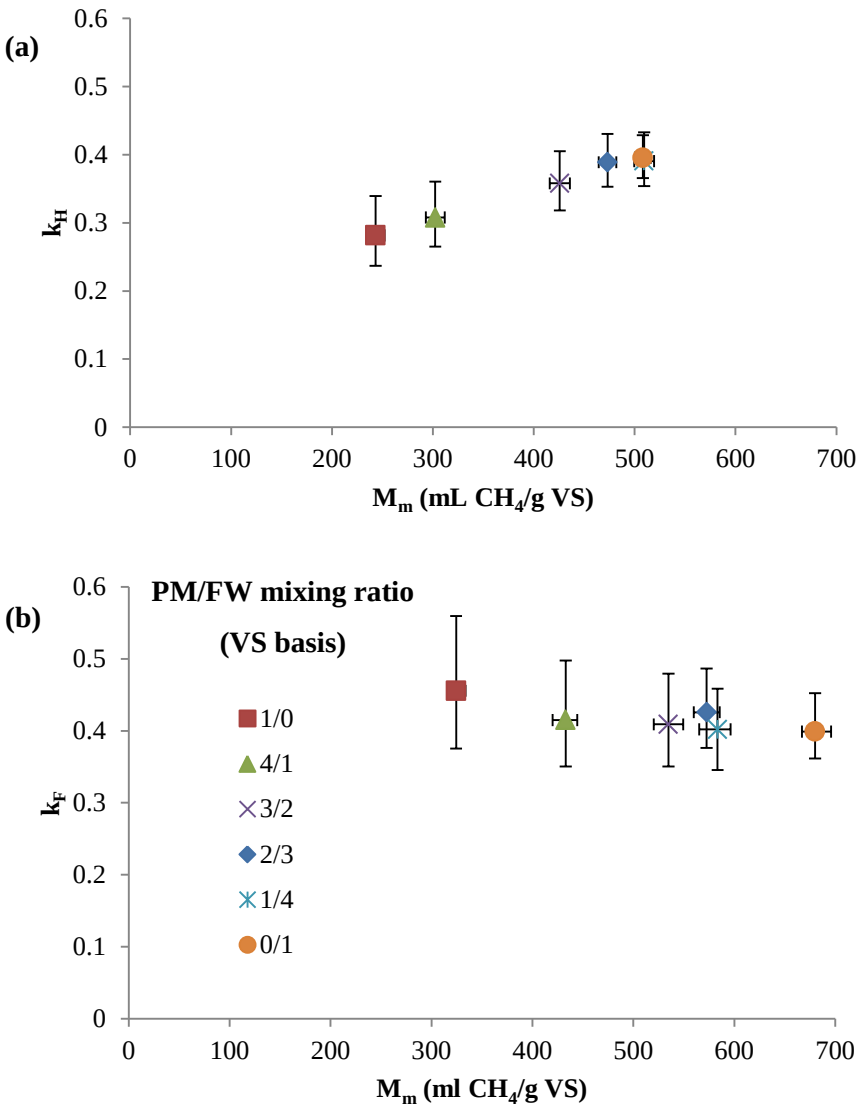


Figure 3-5 Parameter surface areas for hydrolysis rate (k_H) and theoretical maximum methane yield (M_m) from the first-order model (a), and for the hydrolysis rate of the rapidly hydrolysable substrate pool (k_F) and M_m from the dual pooled first-order model (b). Error bars represent 95 % confidence interval.

The larger critical parameter surfaces observed for the dual pool first-order kinetic constants is due to the larger number of parameters required to be estimated (5 compared with 2), while the number of data points remained the same. Therefore the 95 % confidence intervals around the simulated values are larger. Despite more precise fitting to cumulative methane generation curves, dual pool first-order models were not as accurate as estimating critical substrate kinetic properties compared with a simple first-order model.

3.3.2.3 Trends in substrate degradability (Fd)

By utilizing the COD:VS ratio measured in each substrate mixture, and the M_m values estimated by first-order simulations, substrate degradability (Fd) of each substrate mixture was determined. Fd is a measure of COD to CH_4 conversion (Batstone et al., 2015). As such, it is a useful parameter to monitor when analysing the synergistic effects of co-digestion. Figure 3-6 illustrates that the trend in Fd values as the proportion of FW in the substrate increased is indicative of synergy. Fd did not increase linearly as FW composition increased; the highest calculated Fd values were found when PM/FW mixing ratios (VS basis) were between 2/3 and 1/4. This strongly supports the findings presented in Section 3.3.1, and provides evidence to suggest that the synergistic effects on SMY could be attributed to improved COD utilization (considering the synergistic nature of the Fd values measured, and that no synergy was observed on VS removal rates).

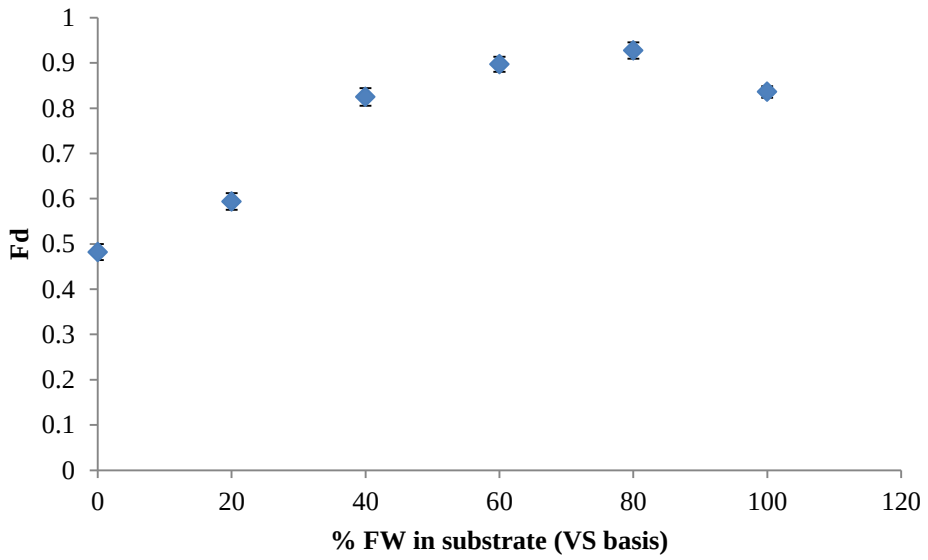


Figure 3-6 Trends in substrate degradability (Fd) as substrate composition was varied from 0 % FW to 100 % FW on a VS basis. Error bars represent 95 % confidence interval.

3.4 Summary

Co-digestion of PM and FW had synergistic effects on SMY, Fd and digestion kinetics, with the highest SMYs occurring at PM/FW mixing ratio (VS basis) of 1/4. The highest level of synergy (a 22 % to 26 % increase in SMY) was observed a PM/FW mixing ratio of 3/2.

Initial VFA concentrations in PM did not explain the observed synergy. Rather, the presence of VFAs masked a slightly higher level of synergy occurring from the conversion of VS and non VFA COD to methane. The synergy may be due to the higher concentration of trace metals in PM compared to FW. Butyric acid was only slightly degraded in all substrate mixtures.

The high initial VFA concentrations in PM also resulted in a dual pooled first-order model providing the most precise fit for the data, however it was found to be less accurate for parameter estimation than the first-order model.

These results will allow for the maximizing of methane yields when undertaking PM/FW co-digestion, which will be further tested in semi-continuous digesters (Chapters 4 and 5). This in turn may increase the GHG mitigation potential and commercial viability of AD systems co-digesting FW and PM. Finally the parameters estimated in this study can be used to improve the accuracy of the models designed to simulate the co-digestion of PM and FW in both batch and continuous mode. This will be described in Chapter 6.

CHAPTER 4

Anaerobic co-digestion of pig manure and food waste; effects of operating conditions on digestate biosafety and dewaterability, and microbial community dynamics

The contents of this chapter are currently under review for publication in journal of Biomass and Bioenergy.

4.1 Introduction

CSTRs are the most common AD system utilized. As such undertaking lab scale AD using CSTR-type semi-continuous systems is crucial in scaling up and validating batch scale-based calculations of methane yields and VS removal at given HRTs. Observations with respect to optimal digester HRT/OLR for biogas generation and substrate utilization made in semi-continuous mode are clearly more directly applicable to and comparable to the full scale commercial AD systems (Batstone et al., 2009) than batch trials. In addition to assessing the maximum methane yields achievable in semi-continuous CSTR operation, such studies can also assess how varying process parameters such as PM/FW mixing ratio and OLR/HRT, interact with each other and affect process stability.

Combining molecular analysis with chemical, physical and culture-based methods allows for an in-depth assessment of the effects that changes in HRT and substrate composition might have on anaerobic digester performance and stability.

Therefore, the aim of this semi-continuous study was to assess the effect of varying substrate composition (PM/FW ratio) and HRT on; methane yields; digestate dewaterability; digestate biosafety; and microbial community dynamics.

4.2 Materials and Methods

4.2.1 Experimental Design

Three 10 L reactors (R1, R2, and R3) with a working volume of 7.5 L were used as anaerobic digesters. The experimental apparatus is presented in Figure 4-1. The reactors were operated at 39°C for the duration of the experiment. The temperature

was maintained by water jackets and a thermostatically controlled water bath from which water was circulated to the reactor jackets via a peristaltic pump. Mixing was undertaken for a period of 1 hour per day prior to digestate removal and feeding by mechanical stirrers at 60 rpm. After the inoculum was added, the reactors were sealed and the contents were flushed with N_2. Reactors were fed with substrate every weekday. Daily OLR was adjusted to account for the lack of feeding at weekends in order to ensure that OLR was maintained at a correct level. The feedstock mixture was prepared daily.

The reactors were subject to 4 operation phases; Phase I, a start-up phase, and three operational phases – Phase II at a HRT of 41 days, Phase III at a HRT of 29 days and Phase IV at a HRT of 21 days. Therefore the organic loading rate (OLR) was progressively increased from 1 kg VS/m^3/day (Phase II) to 1.5 kg VS/m^3/day (Phase III) and to 3 kg VS/m^3/day (Phase IV). As reducing HRT resulted in increases in OLR, it is not possible to definitively conclude that the effects observed in this study were due to either factor, rather a combination of both. However, this remains a useful observation, as such a relationship between OLR and HRT exist in most on-farm biogas plants.

Figure 4-1 The semi-continuously stirred reactors used during the study

During the start-up phase (Phase I), all three reactors were fed with the same feedstock (85 % PM and 15 % FW on a VS basis). This was done in order to ensure that the operating conditions in three reactors were identical prior to varying the substrate composition. During Phase II the substrate composition of R2 and R3

were altered so that the proportion of PM in the feedstock mixture of each reactor (on a VS basis) was 85 % in R1, 62.5 % in R2 and 40 % in R3, with the remaining portion comprised of FW. In order to ensure uniform OLR and HRT for each mixture, the feedstock mixture for each reactor was made up to the required volume with deionised water. OLR and HRT were coupled in order to ensure differences observed between substrate compositions were not confounded by differences in OLR.

The PM/FW mixing ratio range was chosen as higher FW ratios would result in a semi-solid feedstock. On-farm biogas plants are usually CSTRs and therefore operate with a typical feedstock TS concentration of 15 %. This limits the mixing ratio between manure and solid co-substrates such as FW. Therefore, this study aimed to focus on PM/FW mixing ratios likely to be used at farm-scale.

4.2.2 Substrates and inoculum

The PM used in this study was taken in batches every 4 weeks from the manure storage tanks of a pig farm in Co. Galway, Ireland. It was stored at 11°C (in order to simulate typical manure storage temperatures in Ireland) prior to use during the subsequent 4 weeks. FW was collected weekly from 5 residences, combined and subsampled as described by Browne et al. (2014). After subsampling, the FW was blended using a food processor (Russell Hobbs 500W 18087 Blender) and stored at 4°C until use (for a maximum of 5 days). Unfrozen PM and FW samples were used during the feeding of the reactors in order to ensure that, microbially, the feedstocks were representative of feedstock entering full scale digesters. Every new PM and FW samples were analysed for pH, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS), NH4-N, alkalinity and volatile fatty acids (VFA). The inoculum used in this study was taken from an anaerobic digester treating cattle manure. The characteristics of the PM, FW and inoculum used in this experiment are presented in Table 4-1.

Table 4-1 Characteristics of pig manure, food waste and inoculum used (mean of 5 samples ± standard deviation)

Parameter	Pig Manure	Food Waste	Inoculum
pH	7.52 ± 0.5	5.57 ± 0.3	7.62 ±0.1
Total COD (g/L)[a]	73.7 ± 7.7	516.7 ± 15.3	40.0 ± 0.2
Soluble COD (g/L)[a]	27.8 ± 4.4	201.2 ± 10.8	8.4 ± 0.1
Total solids (% fresh weight)	7.3 ± 4.3	21.4± 6.7	4.3 ± 0.02
Volatile solids (% fresh weight)	5.61 ± 3.2	18.68 ± 5.39	2.96 ± 0.01
NH_4-N (g/L)[a]	4.8 ± 0.9	1.8 ± 0.1	1.5 ± 0.1
Alkalinity (g/L)[a]	6.9 ± 1.8	0.2 ± 0.1	5.1 ± 0.1
Total volatile fatty acids (g/L acetic acid equivalents (HAc_{eq})[a]	21.1 ± 3.56	2.31 ± 1.01	7.84 ± 0.27
Acetic (g/L HAc_{eq})	9.8 ± 3.4	0.66 ± 0.2	<0.1
Propionic (g/L HAc_{eq})	3.3 ± 0.4	0.44 ± 0.3	<0.1
Isobutyric (g/L HAc_{eq})	1.3 ± 0.3	1.21 ± 0.6	<0.1
Butyric (g/L HAc_{eq})	2.5 ± 0.4	<0.1	<0.1
Isovaleric (g/L HAc_{eq})	2.2 ± 0.4	<0.1	<0.1
Valeric (g/L HAc_{eq})	2.0 ± 0.1	<0.1	<0.1

[a] *Values reported for FW presented in g/kg.*

4.2.3 Analytical methods

Biogas volumes were measured daily via mass flow meters (OMEGA, USA). The methane content of the biogas was analysed daily using gas chromatography (7809A, Agilent Technology, USA) as described in Chapter 3.

The digestate from the reactors was sampled on the same day each week in order to ensure that measurements were not confounded by the week day feeding regime effects. pH was measured immediately using a pH meter (WTW, Germany). In order to measure alkalinity, NH_4-N and VFA concentrations in the digestate, samples were centrifuged (Model 2-15, Sigma, Germany) at 14500 rpm for 15 min. The supernatants were then filtered through 0.45 µm cellulose nitrate membrane filter paper (Sarstedt, Germany). TS, VS, alkalinity, NH_4-N, TCOD and SCOD of

the substrates and inoculum were measured, as described in Chapter 3, according to Standard Methods (APHA, 1998). Free ammonia nitrogen (FAN) concentration was calculated based on the measured pH and NH_4-N concentration using the method described by Anthonisen et al. (1976) (see Equation 2-6). VFA concentrations were measured via high performance liquid chromatography (HPLC; Agilent 1200, Agilent Technology, USA) as described in Chapter 3.

In each operational phase, after the duration of a full HRT, total coliforms, *E. coli*, and *Enterococcus* were enumerated in the digestate and feedstock (after FW was added to PM) for each reactor. Each sample underwent 10-fold serial dilution with the maximum recovery diluent (MRD, Oxoid, UK) as required and was pour-plated in duplicate. The following media and incubation conditions were used; McConkey agar (Oxoid, UK) at 37°C for 24 hours for total coliforms, chromoCult tryptone bile X-glucuronide (CTBX) agar (Merck, USA) at 37°C for 24 hours for *E. coli* and kanamycin azide aesculin (KAA) agar (Applichem, Germany) at 45°C for 18 hours for *Enterococcus*. After incubation, all colonies were counted, averaged and expressed in colony forming units per g of sample (CFU/g). In each case, the enteric indicator counts of feedstocks were expressed as the average of the indicator bacteria counts obtained from the feedstock analysed at each period (i.e. n=3).

In order to assess the effect that anaerobic digestion had on dewaterability, the digestate and feedstock samples were analysed for particle size distribution (PSD) and specific resistance to filtration (SRF) in each operational phase (Phase II – Phase IV), after the duration of a full HRT. PSD was determined according to the light scattering method described by APHA (1998) using a Mastersizer 2000 (Malvern Instruments Ltd., UK), with each sample analysed in triplicate. Prior to PSD analysis all samples were screened for particles > 2 mm. In order to quantify shifts in PSD and transform for statistical analysis, distributions were expressed as the % mass of the sample comprised of particles > 500 µm, of particles < 500 µm but > 100 µm, and of particles < 100 µm. SRF analysis was undertaken as described by Pollice et al. (2007). Specifically 15 cm^3 of sample was filtered through Whatman No. 2 filter paper, the volume of filtrate over time was recorded, and SRF was determined by applying Equation 2-11. Each SRF analysis was carried out in triplicate.

4.2.4 16S rRNA gene sequencing

In order to assess potential changes in bacterial and archaeal communities within each digester as HRT and PM/FW ratio changed, 5 cm^3 of digestate was sampled from each reactor during each operational phase, after the duration of a full turnover of HRT. Samples were immediately snap-frozen in liquid N_2 and stored at -80°C. Each sample was crushed to a fine powder under liquid N_2 using a pestle and mortar, and refrozen at -80°C. DNA was extracted from 600 mg of each sample using the repeated bead beating and column purification extraction process (Yu & Morrison, 2004). The quality of extracted DNA was assessed on a 1% agarose gel. Quantification of DNA was achieved by heating each sample at 52°C for 2 min, mixing and analysing in triplicate on a Nanodrop 1000 spectrophotometer.

Modified 16S Illumina adapter fusion primers were used to generate amplicon libraries. The primers were CaporasoNexF 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG[GTGCCAGCMGCCGCGGTAA]3' and CaporasoNexR5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG[GGACTACHVGGGTWTCTAAT]3'. The primer sequence outside the square brackets are partial Illumina adapters. The primer sequences inside the square brackets bind to the hypervariable (V4) region of the 16S rRNA gene in bacteria and archaea and are derived from the 16S binding sites of primers previously described by Caporaso et al. (2012). PCR was conducted using 20 ng of digestate DNA as a template and Kapa HiFi Hotstart ReadyMix (Kapa Biosystems, UK) according to the manufacturer's instructions. Thermocycling conditions were: one cycle of 95°C for 3 minutes, then 26 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, followed by one cycle of 72°C for 5 minutes. QIAquick PCR Purification Kits (Qiagen, UK) were used to purify libraries. The purity and quantity of PCR products were measured via Nanodrop 1000. Two unique 8 bp indices were then added (one index at the 5' end of the amplicon and the other at the 3' end) to each amplicon in a second round of PCR using primers from the Illumina Nextera XT indexing kit. PCR was performed with 5 µL of each amplicon as a template and Kapa HiFi Hotstart ReadyMix (Kapa Biosystems, UK). PCR conditions for this second round of PCR were: one cycle of 95°C for 3 minutes, then 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, followed by one cycle of 72°C for 5 minutes. Indexed libraries were then purified, pooled, gel purified, spiked and denatured as described previously (McCabe et al., 2015). Sequencing was performed on the Illumina MiSeq sequencer using 500

cycle MiSeq reagent kits (version 2) (San Diego, USA). Sequence quality control, pre-processing, amplicon sequencing and data analysis was carried out as previously described (McCabe et al., 2015). Specifically, an in-house perl script was used to carry out demultiplexing of sequence reads. Trim Galore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to trim and filter sequence adaptor contamination from raw sequences. Read pairs were then merged into a single sequence. An in-house perl script was used to carry out a size selection of 254 bp ±20 bp. All reads were combined into a single data set for analysis via QIIME(Caporaso et al., 2010).

OTU identification was undertaken using open reference calling method within QIIME, using a combination of *de novo* and reference based methods(DeSantis et al., 2006). Sequences were clustered into individual OTUs using a default similarity level of 97%, with a single representative sequence from each clustered OTU was used to align to the Greengenes database (version: gg_13_5) (DeSantis et al., 2006). A default AN RDP Classifier (Wang et al., 2007) was used to classify the taxonomy of each OTU, using a minimum confidence cut-off of 0.8. Any OTUs with < 100 sequences across all samples were excluded from analysis.

4.2.5 Statistical Analysis

For statistical analysis, 9 dependent variables (volumetric methane yield [VMY], SMY, total coliforms, *Enterococcus*, *E. coli*, proportion of particle mass > 500 µm, proportion of particle mass < 100 µm, SRF and VS) and two independent variables (proportion of PM comprising feedstock on a VS basis [% PM] and HRT) were considered. 3 values of each % PM and HRT were chosen, as described in Section 4.2.1. (i.e. 85%, 62.5%, 40% and 41, 29 and 21, respectively). At each one of the 9 combinations of these two independent variables (X_1, X_2) one observation was made on each of the dependent variables ($Y_1, Y_2 \ldots, Y_9$). The following (multivariate) linear regression model was proposed to describe this, relating the *j*th random dependant variable Y_j to X_1 and X_2.

4-1 $$\boldsymbol{Y_j = \beta_{0\,j} + \beta_{1\,j} X_1 + \beta_{2\,j} X_2 + \epsilon_j,\ j = 1, \ldots, 9}$$

This model supposes a univariate linear model relating each one of the 9 responses to the two independent variables. Given the 9 values $X_{i\,1}$ and $X_{i\,2}$, i =1,2,...,9, chosen for the input variables X_1 and X_2, we then have the model

4-2 $$Y_{j\,i} = \beta_{0\,i} + \beta_{1\,i}X_{j\,1} + \beta_{2\,i}X_{j\,2} + \epsilon_i, \quad j = 1, 2, \dots, 9;\ i = 1, \dots, 9$$

where $Y_{j\,i}$ is the jth random observation that will be taken on the ith response variable. It assumes that random errors of each measured dependent variable (ϵ_i) are independent and normally distributed, that data set for each dependent variables are independent and have common co-variance.

For each of the two input variables %PM and HRT, multivariate tests were conducted to see if there was statistical evidence (with a p-value of 0.05) to conclude that this input variable affects had an effect on at least one of the response variables. Univariate tests followed to determine the effect of each of the two input variables on each of the 9 response variables.

Since many statistical tests were performed, and due to the lack of replicate it was desirable to tightly control the global level of significance. It was decided to control the level of significance in each individual univariate test performed at the value 0.005. Note that a conservative Bonferroni method would then ensure that if a total of k statistical tests are performed, the global error rate will not exceed 0.005k. While this method has limited power in this experiment (due to lack of replicates), it is a useful means of statistically quantifying the strength of the interaction between each independent and dependant variable.

The values used for the dependent variables in this model were averages of the values measured after 1 HRT turnover under each condition (provided chemical and physical parameters indicated that a steady state was reached), in order to ensure such values were representative of the conditions after changes in substrate composition and HRT.

Due to the typically non-normal nature of the microbial relative abundance data, these results were excluded from the multivariate multiple liner regression. Instead, relative abundances of OTUs were correlated with HRT, % PM, alkalinity, pH, free ammonia nitrogen, acetic and butyric acids (no other VFAs correlated significantly) via Spearmans Rank. Correlations were deemed significant with a 95 % confidence interval (p-value < 0.05).

All statistical analysis was carried out using the statistical software package SPSS v22.0 (IBM, USA).

4.3 Results and Discussion

4.3.1 Methane yields

Figure 4-2 presents the weekly average SMYs and VMYs measured for each reactor throughout the experiment. Due to the feeding cycle (no feeding at weekends), methane yields were determined on a weekly basis as this accounted for the confounding effects of increasing daily yields on weekdays followed by a drop in yields at weekends. During the start-up phase (Phase I), when operating conditions were the same across all reactors, the VMY of each reactor was similar.

Adjusting the proportion of PM in the substrate mixture of R2 and R3 to 62.5 % and 40 % respectively after the start -up Phase resulted in R3 generating the highest VMY throughout Phases II – IV. In addition, a general trend of increasing the proportion of FW in the feedstock resulted in increased VMY and SMY. This observation agrees with Chapter 3 where increased proportions of FW in the feedstock generated higher SMYs and VS removal rates.

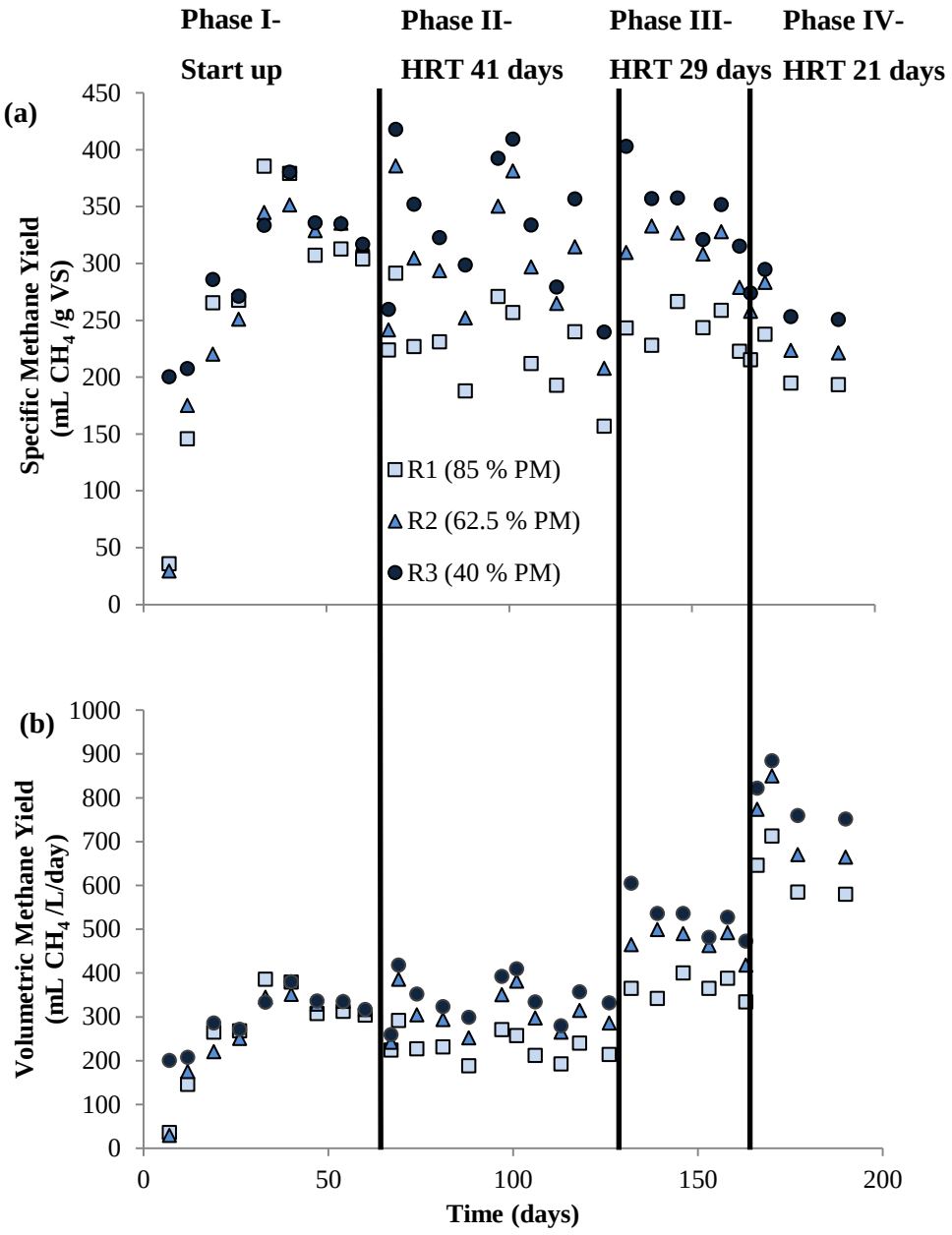


Figure 4-2 Weekly average specific methane yields (SMY) (a) and volumetric methane yields (VMY) (b) of R1, R2 and R3 throughout the experiment

The variation in SMYs observed during the early stages of Phase II can be attributed to the lower OLR, which meant that any slight changes in daily methane yield (due to slight variation in fresh FW and, in particular, PM composition) resulted in a large change in SMY. This somewhat masked the effects of reducing HRT from 41 to 29 days on SMY. In fact, the highest average SMYs measured (after each phase operated for 1 HRT) for R1, R2 and R3 were 240, 303 and 333 mL CH_4 /g VS, respectively, and occurred in Phase III. While this suggests that a

decrease in HRT from 41 to 29 days had positive effect on SMYs, the change was slight (between 0.7 % - 5 % increase). In all reactors, SMYs decreased when the HRT was reduced to 21 days (Phase IV). Compared to the SMYs measured at each mixing ratio in the batch study in Chapter 3, these values were between 25 % and 31 % lower. This may be due to a combination of differences in PM and FW composition, system losses and differences in reactor conditions (higher temperature, intermittent stirring and semi-continuous feeding regimes).

The highest average VMYs measured (after each phase operated for 1 HRT) for R1, R2 and R3 were 579, 664 and 751 mL CH_4 /L/day in Phase IV, respectively. This can be explained by the highest OLR occurring at this Phase. Analysis of digestate VS and VFA concentrations (Figure 4-3) revealed that decreasing the HRT from 29 to 21 days appeared to cause an increase in VS and VFA concentrations. This, along with the reduction in SMY observed, is evidence of a decrease in the methane conversion efficiency as HRT was reduced to 21 days and OLR increased to 3 kg VS/m^3/day.

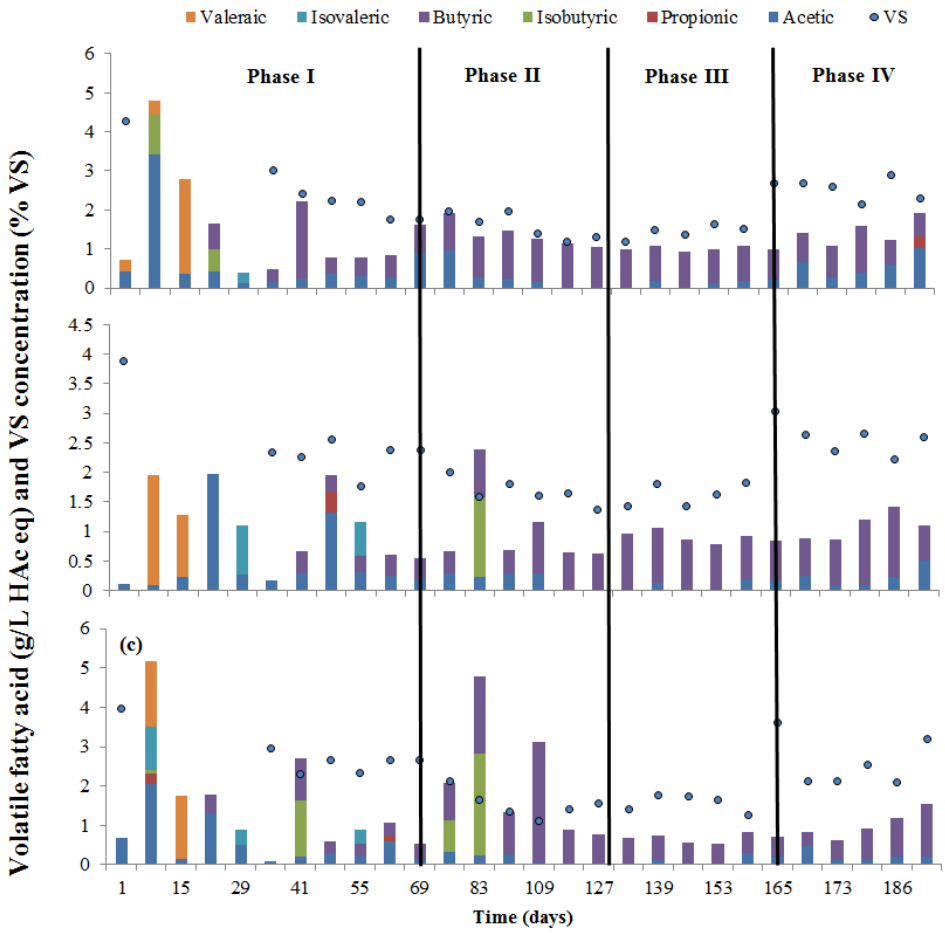


Figure 4-3 Weekly volatile solids (VS) and volatile fatty acid concentrations (VFA) of the digestate from R1 (a), R2 (b) and R3 (c) throughout the experiment

4.3.2 Digestate dewaterability

Figure 4-4 (a) presents the average SRF values measured in the digestate from each reactor during each phase and Figure 4-4 (b) presents the proportion of particles > 500 µm in the digestate (derived from PSD analysis) from each reactor during each phase. The latter is presented, as it clearly illustrates the shift in digestate composition. There was no clear relationship between the reactor substrate composition (PM/FW mixing ratio) and digestate SRF or PSD. Decreasing HRT/ increasing OLR clearly resulted in a decrease in SRF, resulting in a more readily dewaterable digestate.

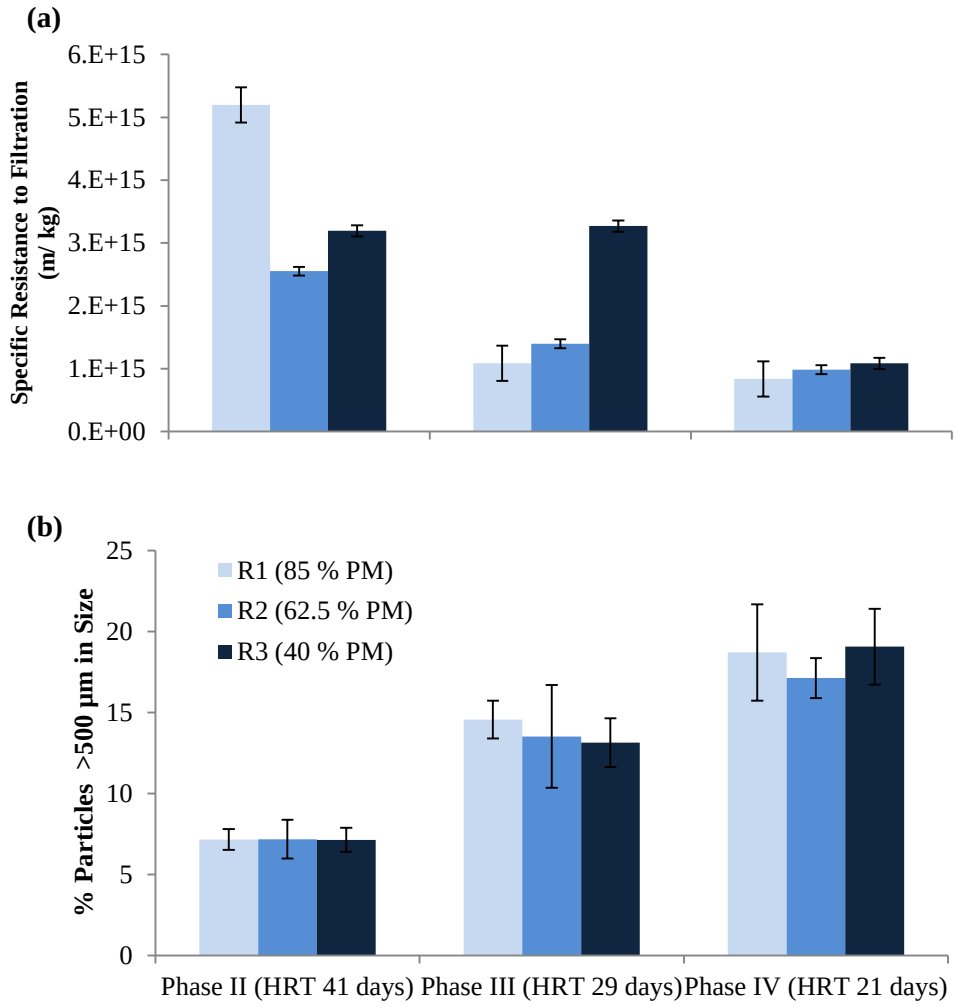


Figure 4-4 Average Specific Resistance to Filtration (SRF) (a) and proportion of particles > 500 µm in size (b) measured in the digestate from R1, R2 and R3 during each operational phase of the experiment. Error bars represent standard deviation of 3 replicates.

An increase in proportion of smaller particles in digestate has been shown to cause SRF to rise, leading to a decrease in the dewaterability of digestate, as smaller particles easily clog filters (Houghton & Stephenson, 2002). A shift in the particle size distribution occurred in all reactors as the HRT decreased. Compared to the digestate of Phase II, digestate in Phase III had a higher proportion (84 to 104 % higher) of particles in the > 500 µm range. Compared to digestate from Phase III, digestate from Phase IV was comprised of a higher (between 27 to 57 % higher) proportion of particles in the > 500 µm range. A decrease in the proportion of particles <100 µm also occurred as HRT decreased. This can be seen from the complete PSD analysis presented in Figure 4-5.

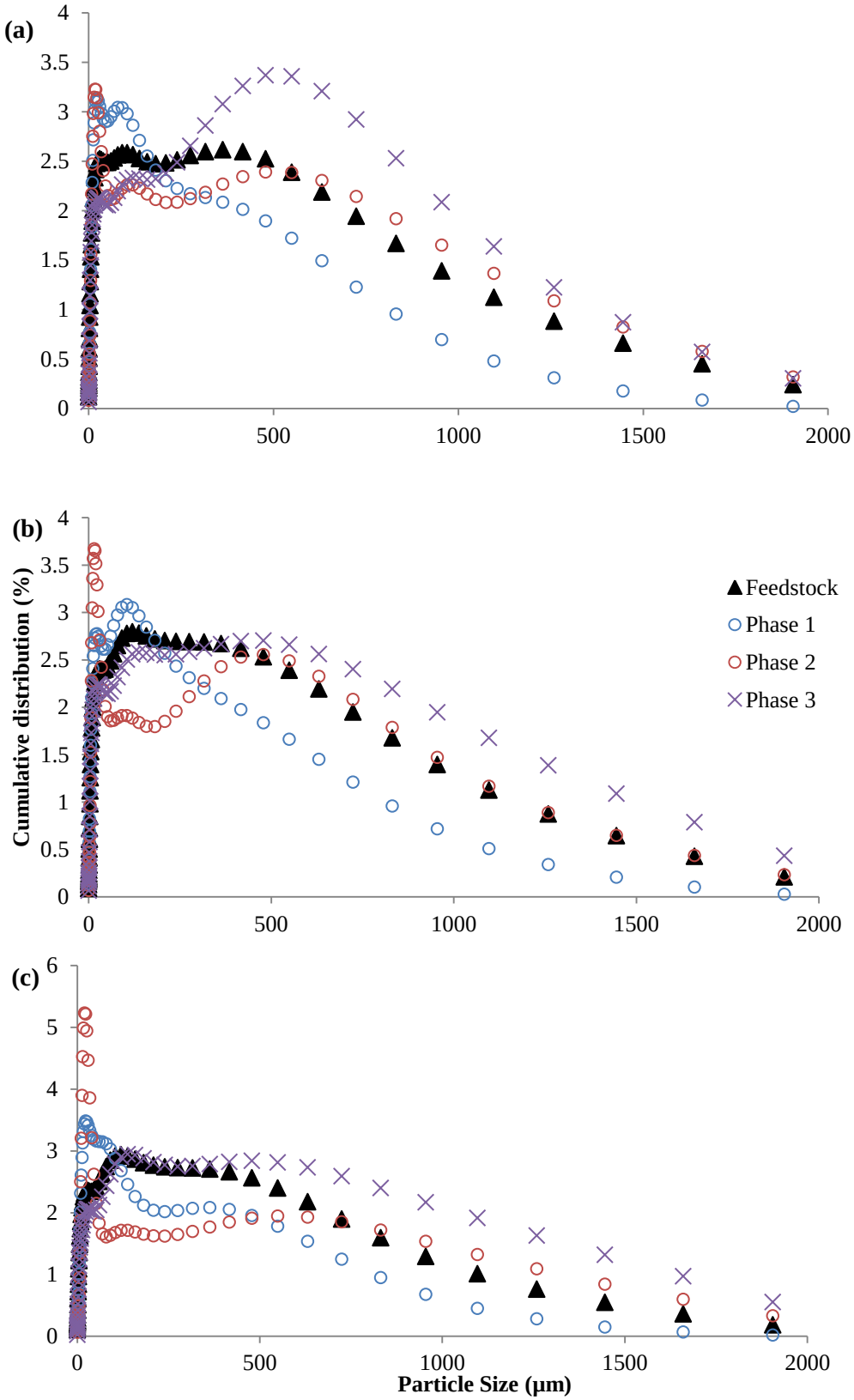


Figure 4-5 Particle size distribution of feedstock and digestate from R1 (a), R2 (b) and R3 (c) during Phase II, Phase III and Phase IV of the experiment

This shift in the particle size was attributed to the digestion process failing to hydrolyse a larger proportion of larger particles as the HRT decreased. The proportion of larger particles being converted to smaller particles decreased as the HRT decreased (and OLR increased), leading to an increase in dewaterability.

4.3.3 Indicator organism removal

Figure 4-6 presents the total coliform, *E. coli* and *Enterococcus* counts found in the digestate from each of the reactors at the end of each phase of the experiment.

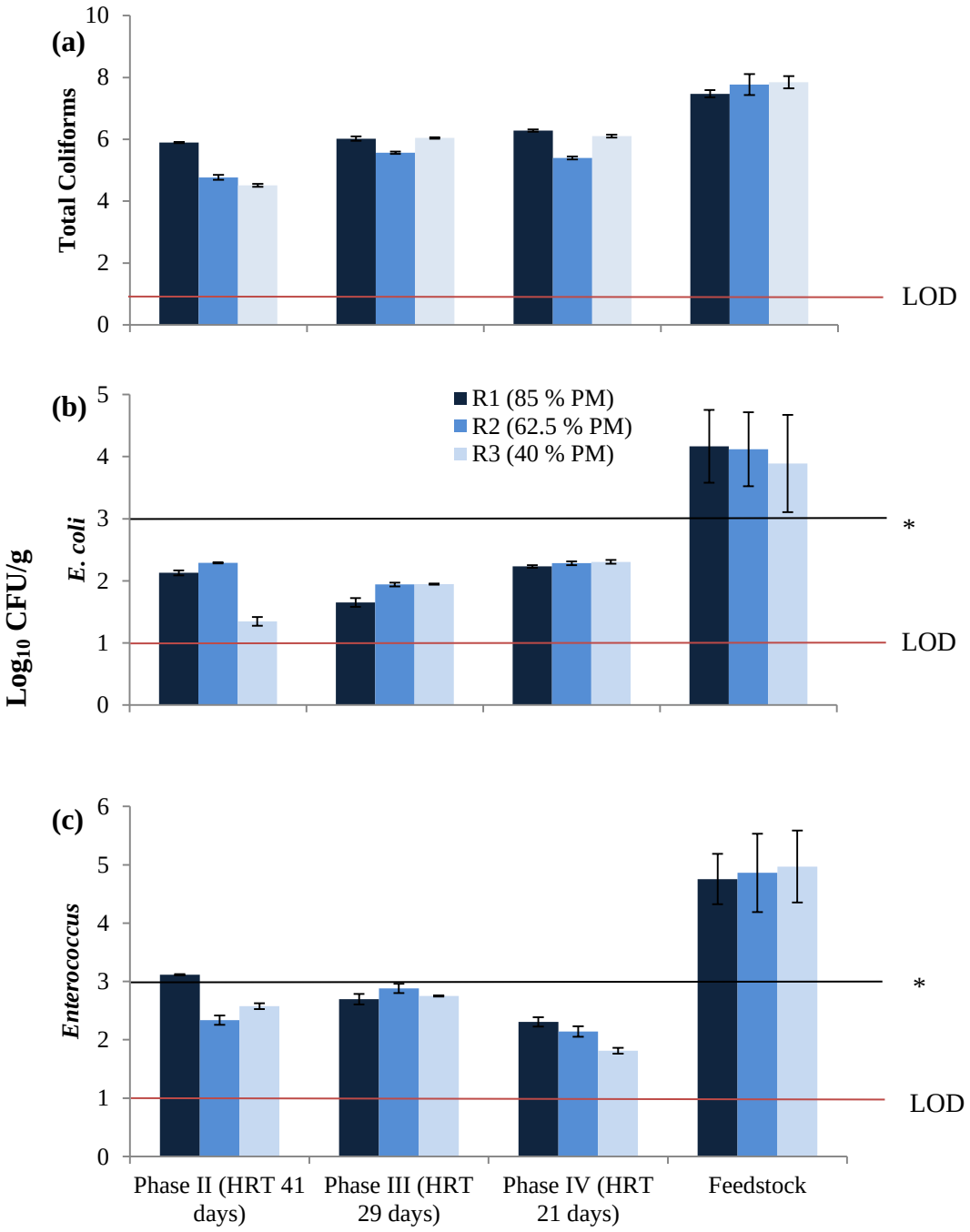


Figure 4-6 Average counts (Log_{10} CFU/g) of total coliforms (a), *E. coli* (b) and *Enterococcus* (c) in the digestate from R1, R2, R3 and feedstock during each operational phase of the experiment. Error bars represent standard deviation of 3 analytical replicates, and in the case of feedstock, the standard deviation of the 3 feedstock samples analysed. * EU animal by-products regulation limit of < 1000 CFU/g for *E. coli* or *Enterococcus* in digestate prior to land application. LOD represents limit of detection

Figure 4-6 illustrates that the feedstock composition (PM/FW mixing ratio) had no clear effect on the observed concentrations of any of the enteric indicator bacteria in the digestate. The lower total coliform, *Enterococcus* and *E. coli* counts found in R3 than compared to R1 in Phase II suggest that increasing FW addition reduced digestate enteric indicator organism content when a longer HRT was used. However the trend in counts of *E. coli* and *Enterococcus* from the digestate from R2 did not support this supposition. Additionally, no consistent trend between reactors was observed as the HRT was decreased. The respective total coliform, *E. coli* and *Enterococcus* counts in the fresh feedstock of each reactor were not found to be significantly different (p-values from one-way ANOVA of 0.598, 0.624 and 0.968, respectively) despite increasing the proportion of FW in the feedstock resulting in increased average total coliforms and *Enterococcus* but reduced *E. coli* counts. The lack of a consistent trend in digestate and feedstock indicator organism counts may be explained by the mixing ratio range used, which varied from 40 % PM/60 % FW to 85 % PM/15 % FW. However, this was on a VS basis, which resulted in mixing on a wet weight basis occurring within the range of 94 % PM/6 % FW and 71 % PM/29 % FW. Because of this, for the most part, the magnitude of differences in counts of the enteric indicator bacteria between substrate mixing ratios was small. This is noteworthy as co-digesting FW and PM in farm-scale anaerobic digesters, which typically involves the use of CSTRs, would likely operate within the mixture range used here. Overall, these data illustrate that biosafety of the digestate would not be impacted much by varying the PM/FW mixing ratio in the range studied (assuming an equal OLR/HRT).

Decreasing HRT did not appear to have any clear effects on the concentrations of total coliforms. While decreasing the HRT from 29 (Phase III) to 21 days (Phase IV) appeared to increase *E. coli* concentrations slightly, the increases were not significant (see Section 3.4), and counts remained well below regulatory limits. On the other hand, the decrease in *Enterococcus* observed, albeit slight, was a positive finding in terms of digestate management. While pathogen inactivation is a function of time and temperature (Elmerdahl Olsen & Errebo Larsen, 1987), differences in pathogen inactivation in reactors with HRTs > 20 days may be slight. This is in agreement with a study which found that the HRT of commercially operating mesophilic AD plants treating sewage sludge did not clearly affect faecal coliforms concentrations (which were between 2 and 4 $\log_{10}$ MPN (most probable number)/g) (Watanabe et al., 1997). The relatively low OLRs and high HRTs

investigated and the high level of stability observed throughout this present study could explain why no differences were observed. It is possible that the mechanisms responsible for pathogen inactivation (high free ammonia nitrogen, high volatile fatty acids, competition from other microbial species and resource limitations (Orzi et al., 2015)) were capable of achieving a high level of sanitation within 20 days, and that any of the slight changes in these properties due to HRT variations did not have an observable effect on indicator organism counts. Furthermore, the increases in VFA concentrations observed as the HRT was decreased from 29 to 21 days (due to the increase in OLR) may have played a role in mitigating and masking any negative impacts that the shorter retention time would have had on enteric indicator bacteria. In summary, it can be concluded that decreasing the HRT from 41 to 21 days (resulting in an increase in OLR from 1 to 3 kg VS/m/d) would have the advantage of increasing VMY while not leading to a major increase in digestate enteric indicator bacteria counts.

4.3.4 Multivariate multiple linear regression analysis

Multivariate multiple linear regression analysis was undertaken to assess and quantify the significance of the effects of varying HRT and substrate composition on the physical, chemical and microbiological dependent variables analysed. Table 4-2 and Table 4-3 provide the model output from this Analysis. The multivariate test (Table 4-2) found that both HRT (p-value of 0.015) and the proportion of PM comprising the total feedstock on a VS basis (% PM) (p-value of 0.037) significantly affected at least one of the dependant variables.

Table 4-2 Multiple multivariate linear regression- multivariate analysis

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	12850.280	6.000	1.000	.007
	Wilks' Lambda	.000	12850.280	6.000	1.000	.007
	Hotelling's Trace	77101.679	12850.280	6.000	1.000	.007
	Roy's Largest Root	77101.679	12850.280	6.000	1.000	.007
PM_Percent	Pillai's Trace	1.000	412.505	6.000	1.000	.038
	Wilks' Lambda	.000	412.505	6.000	1.000	.038
	Hotelling's Trace	2475.031	412.505	6.000	1.000	.038
	Roy's Largest Root	2475.031	412.505	6.000	1.000	.038
HRT	Pillai's Trace	1.000	2783.670	6.000	1.000	.015
	Wilks' Lambda	.000	2783.670	6.000	1.000	.015
	Hotelling's Trace	16702.018	2783.670	6.000	1.000	.015
	Roy's Largest Root	16702.018	2783.670	6.000	1.000	.015

For the univariate tests (Table 4-3) the dependent variables were deemed to be significantly affected by changes in HRT and the % PM at a p-value of < 0.005. This significance-value for the univariate analysis was chosen to reduce the global error.

Table 4-3 Multiple multivariate linear regression- univariate analyses

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected	VMY	237892.211	2	118946.105	78.645	.000
Model	Total Coliforms	1.639	2	.820	3.411	.102
	Enterococci	.593	2	.296	2.514	.161
	E. coli	.176	2	.088	.754	.510
	Prop_500um	188.488	2	94.244	186.302	.000
	Prop_100um	312.450	2	156.225	7.177	.026
	SRF	.492	2	.246	9.308	.014
	VS	3.955	2	1.978	23.715	.001
	SMY	15974.084	2	7987.042	6.581	.031
Intercept	VMY	514504.857	1	514504.857	340.180	.000
	Total Coliforms	13.588	1	13.588	56.547	.000
	Enterococci	.536	1	.536	4.546	.077
	E. coli	1.810	1	1.810	15.482	.008
	Prop_500um	297.458	1	297.458	588.017	.000
	Prop_100um	552.082	1	552.082	25.363	.002
	SRF	72.971	1	72.971	2759.818	.000
	VS	8.523	1	8.523	102.207	.000
	SMY	31197.330	1	31197.330	25.705	.002
PM_Percent	VMY	29228.134	1	29228.134	19.325	.005
	Total Coliforms	.383	1	.383	1.592	.254
	Enterococci	.159	1	.159	1.347	.290
	E. coli	.028	1	.028	.240	.642
	Prop_500um	.188	1	.188	.371	.565
	Prop_100um	6.910	1	6.910	.317	.594
	SRF	.025	1	.025	.929	.372
	VS	.131	1	.131	1.571	.257
	SMY	10907.726	1	10907.726	8.987	.024
HRT	VMY	208664.077	1	208664.077	137.965	.000
	Total Coliforms	1.257	1	1.257	5.229	.062
	Enterococci	.434	1	.434	3.681	.103
	E. coli	.148	1	.148	1.268	.303
	Prop_500um	188.300	1	188.300	372.233	.000
	Prop_100um	305.540	1	305.540	14.036	.010
	SRF	.468	1	.468	17.687	.006
	VS	3.824	1	3.824	45.860	.001
	SMY	5066.358	1	5066.358	4.174	.087

The statistical analysis indicates that varying the substrate composition between PM/FW ratios of 85 %/15 % and 40 %/60 % had the most significant effect on VMY. Changes in SMY ($p=0.024$) were observed as the proportion of FW in the feedstock increased but was not deemed significant. The p-values found for the biosafety and dewatering parameters were not suggestive of any interaction. This indicates that increasing the proportion of FW in the feedstock mixture within the range studied (when OLR and HRT were maintained as equal) can significantly increase VMY without negatively affecting indicator organism removal or digestate dewaterability.

In terms of reducing HRT, the analysis found that decreasing HRT significantly increased VMY, and led to an increase in the proportion of particles > 500 µm in the digestate. P-values for decreases in SRF (0.006) were suggestive of an interaction, but were not deemed statistically significant. The increase in digestate particle size may explain the observed increase in digestate dewaterability as measured by decreases in SRF. This, allied to the significantly increased VS concentration in the digestate, suggests that decreasing the HRT (while increasing OLR) would result in a reduction in the methane conversion efficiency. Additionally, this analysis indicates that decreasing HRT from 41 to 21 days would not have a significant effect on digestate indictor organism content.

It should be noted that increasing the proportion of FW in the digester feedstock would allow higher OLRs to be achieved, as FW had a far higher VS content than PM. This study kept VS loading at each mixing ratio the same. Therefore, while this study found that increasing the proportion of FW in feedstock from 15 % to 60 % can significantly increase VMY without significantly impacting other digestate parameters, it did not assess the effects of increased OLRs achievable from co-digestion which may negatively affect digestate quality.

4.3.5 Changes in reactor microbial communities

Rarefaction curves presented in Appendix C Figure C1 reveal that sequencing was undertaken at sufficient depth. Figure 4-7 presents the relative abundance of different bacterial and archaeal taxa within digestate samples taken from each reactor at the end of each operational phase of the experiment at phylum and genus level. The correlation matrix for selected OTUs and selected abiotic factors can be found in Table 4-4.

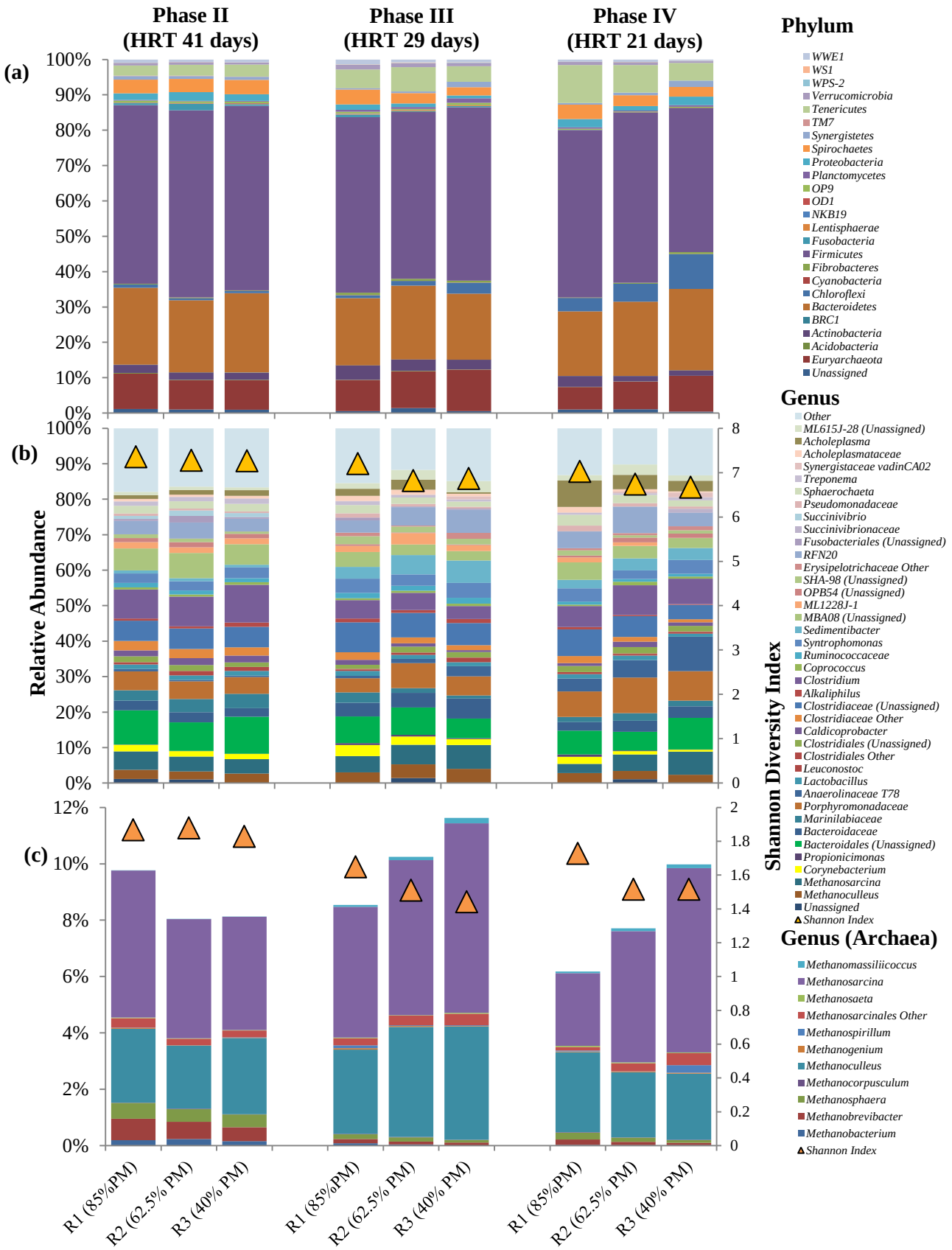


Figure 4-7 Relative abundance in the digestate of each reactor from each Phase II of (a) communities at phylum level, (b) communities at genus level including Shannon diversity index (represented by ▲) and (c) archaeal communities at genus level including Shannon diversity index (represented by ▲). OTUs < 1% in relative abundance grouped as "other". OTUs unclassified grouped as "unassigned".

Figure 4-7 illustrates that there were no major shifts in digester microbial community composition throughout the experiment. This supports the observation that varying HRT (from 41 days to 21 days) and substrate mixing ratio did not significantly affect digester stability in terms of chemical composition and indicator organism removal. Nevertheless some trends were observed.

4.3.5.1 Changes in diversity and PCoA analysis

Decreasing HRT had a slight but clear effect on microbial communities, with the microbial diversity within samples decreasing (as indicated by decreases in the Shannon diversity index; Figure 4-7(b) and (c)) and principle component analysis (PCoA) showing unclustering of samples as HRT decreased (Figure 4-8).

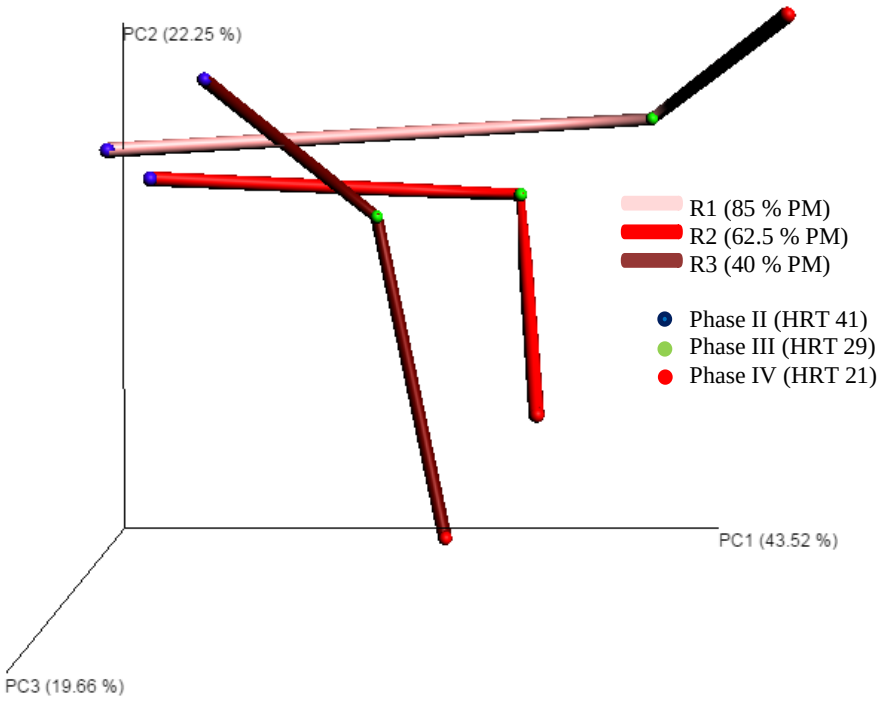


Figure 4-8 PCoA of samples analysed in this study. Data point colour denotes Phase of the experiment, while trend lines denote each reactor

On the other hand, the effects of substrate composition only became evident as HRT decreased. This can be seen from the Shannon diversity index data (Figure 4-7(b) and (c)), with trends due to substrate composition (i.e. increasing diversity as PM/FW mixing ratio increased) becoming more obvious as HRT was reduced. The PCoA plot also supports this, with a greater divergence among the reactors as HRT decreased.

Decreasing HRT resulted in a slight decrease in both overall community diversity (Figure 4-7(b)) and archaeal diversity (Figure 4-7(c)) as measured by the Shannon index, while reducing % PM in the feedstock also resulted in a lower Shannon index, in particular at the lower HRTs. Reductions in diversity may be indicative of a lower level of functional redundancy within the microbial population. Reduction in functional redundancy of methanogens in particular would increase the risk of instability as loss of methanogenic functions can lead to VFA build up, pH increase and total process failure (Li et al., 2014). While the magnitude of the differences observed was small, this analysis suggests that microbial populations had a greater potential for instability as HRTs decreased from 41 to 21 days and at lower PM/FW mixing ratios.

4.3.5.2 Changes in relative abundance

4.3.5.2.1 Hydrolytic bacteria

At the phylum level, decreasing HRT resulted in an increase in the relative abundance of *Chloroflexi* and *Tenericutes*. At the genus level, it is clear that the increase in these two phyla can be explained by increases in the relative abundance of the genera *Anaerolinaceae T78* and *Acholeplasma*, respectively. The increase in the relative abundance of these carbohydrate and amino acid degrading acidogens (Stolze et al., 2015; Yamada & Sekiguchi, 2009) may reflect their ability to exploit the higher level of substrate availability which occurred as HRT decreased (and OLR increased).

Sphaerochaeta (hydrolytic bacteria associated with the breakdown of cellulosic materials (Rui et al., 2015)), *Corynebacterium* (a diverse genera of acidogens (Neuner et al., 2013)) and unclassified *Pseudomonadaceae* (a highly diverse family containing species found in both anaerobic digesters and compost piles (Palleroni, 1981)) all positively correlated with increases in % PM comprising the substrate (Table 4-4). The correlation of these OTUs with % PM comprising the substrate may be associated with the high cellulosic fiber content of PM compared with the more rapidly hydrolysable FW (Nasir et al., 2012a; Uçkun Kiran et al., 2014). The presence of larger quantities of cellulose would provide a niche for bacteria more suited to the hydrolysis of cellulose (such as *Sphaerochaeta*) than in FW where a greater proportion of the VS was comprised of protein, fat and sugars (Uçkun Kiran et al., 2014).

Table 4-4 Correlation Matrix of and relevant abiotic factors and selected OTUs significantly correlated with HRT or % PM. * Correlation is significant at the 0.05 level (2-tailed).**Correlation is significant at the 0.01 level (2-tailed). Red signifies a positive correlation, blue signifies a negative correlation, white signifies no correlation.

	%PM	HRT	Alkalinity	FAN	pH	Acetic Acid	Butyric	VS	Corynebacterium	Porphyromonadaceae	Anaerolineae T78	Clostridiaceae (Unclassified)	Fusobacteriales	Pseudomonadaceae (Unclassified)	Sphaerochaeta	Treponema	Synergistaceae vadinCA02	Acholeplasma
HRT																		
Alkalinity	*																	
FAN	*		**															
pH			**	**														
Acetic Acid		**																
Butyric Acid																		
VS		*	**	**	**													
Corynebacterium	*																	
Porphyromonadaceae		*																
Anaerolineae T78		**				*												
Clostridiaceae (Unclassified)		**	*		**			**		*	**							
Fusobacteriales		**								**	**	**						
Pseudomonadaceae (Unclassified)	*	*				*												
Sphaerochaeta	*		*	*														
Treponema		**				**	*			*	*	*						
Synergistaceae vadinCA02		*	*	*	**			**			**	**	*					
Acholeplasma		*											*	*				
Methanobacteriaceae		*	**	*	**			**			**	**	*				**	

4.3.5.2.2 Acetogenic/syntrophic bacteria

Shifts in acetogenic populations were also observed. The genus *Treponema* of the *Spirochaetaceae* family was significantly positively correlated with HRT ($p<0.05$). *Treponema* is a presumptive homoacetogen, that consumes H_2 and CO_2 to generate acetate (Wang et al., 2013a), and has been reported as a key member of the bacterial communities of biogas plants (Stolze et al., 2015), where it is suggested to work synergistically with aceticlastic methanogens. There was a significant negative correlation between *Treponema* and the *Synergistetes* genus *VadinCA02*. *Synergistetes* are syntrophic acetate oxidizing bacteria (SAOBs) (Treu et al., 2016). The trend for an increase in an SAOB such as *VadinCA02* and decrease in the relative abundance of the acetate producer *Treponema* is suggestive of a shift in methanogenic activity further towards hydrogenotrophic methanogenesis as HRT decreased.

4.3.5.2.3 Archaea

Figure 4-7(c) illustrates that decreasing HRT from 41 to 29 days appeared to lead to an initial increase in overall methanogen relative abundance, while the subsequent decrease in HRT to 21 days led to a decrease in overall methanogen relative abundance. The facultative hydrogenotrophic and methylotrophic *Methanosphaera* (Hoedt et al., 2016), *Methanobacterium* (Bleicher et al., 1989), and *Methanobrevibacter* (Samuel et al., 2007) genera decreased in relative abundance as HRT decreased. The parent family *Methanobacteriaceae* correlated significantly positively with HRT (Table 4-4). This in turn resulted in a reduction in the archaeal community diversity (as measured by the Shannon diversity index) as HRT decreased.

The PM/FW mixing ratio did not correlate significantly with any archaeal family or genus. Nevertheless some trends can be seen; methanogenic populations were less abundant but more diverse as the PM/FW mixing ratio increased when HRT was reduced to 29 and 21 days. The overall increase in the relative abundance of *Methanosarcina* as PM/FW mixing ratio increased may have been caused by the higher NH_4-N content occurring in sample with higher PM content. Sun et al. (2014) found that *Methanosarcina* was present in significantly higher abundance in reactors with low ammonia concentrations, than in reactors where high ammonia concentrations occurred.

4.4 Summary

This study showed that varying digester feedstock composition from 85 %/15 % PM/FW to 40 %/60 % PM/FW increased SMYs but did not significantly affect digestate biosafety or dewaterability. Decreasing HRT from 41 days to 21 days (thereby increasing OLR from 1 to 3 kg VS/m^3/day) reduced the methane conversion efficiency (decreased SMY) and improved digestate dewaterability but did not significantly increase the enteric indicator organism content of the digestate.

The observation that changing these conditions did not greatly affect digester stability or function was supported by the fact that the microbial communities were only slight affected as PM/FW mixing ratio and HRT were varied. *Sphaerochaeta* increased slightly in abundance as PM/FW ratio increased, possibly in response to higher concentrations of cellulosic material found in PM. Decreasing HRT resulted in a slight increase in the relative abundance of SAOBs such as *Synergistetes*, indicating that SAO and hydrogenotrophic methanogenesis may play a greater role in system function at lower HRTs.

CHAPTER 5

Process stability and microbial community composition in pig manure and food waste anaerobic co-digesters operated at low HRTs

The following chapter is comprised of a study previously published as; Dennehy, C., Lawlor, P.G., Gardiner, G.E., Jiang, Y., Cormican, P., McCabe, M.S., Zhan, X. 2017. *Process stability and microbial community composition in pig manure and food waste anaerobic co-digesters operated at low HRTs*. Frontiers of Environmental Science & Engineering, 11(3), 4.

5.1 Introduction

As highlighted in Chapter 3 and Chapter 4, PM is characterised by its low hydrolysis rates and low BMP. Chapter 3 and 4 illustrate that co-digesting FW with PM results in higher substrate hydrolysis rates and higher SMYs, allowing for digester operation at higher organic loading rates (OLR).

In the case of plants designed for operation with feedstocks with lower hydrolysis rates (such as PM) additional capacity exists for HRT to be reduced and OLR to be increased when a rapidly hydrolysable substrate, such as FW, is added. This can increase digester throughput and maximize volumetric methane yields (VMYs). Most commercial mesophilic biogas plants operate with HRTs within the range of 20-50 days, however plants operating with a HRT as low as 10 days have been reported (Sundberg et al., 2013).

Chapter 4 illustrates that operating semi continuous PM/FW co-digestion systems at a HRT of 21 days can increase VMYs without negatively affecting digestate quality (in terms of dewaterability or indicator organism content). While analysis of microbial communities suggested that operating at lower HRTs should increase the potential for reactor instability, no unstable conditions were observed.

Analysing the co-digestion of PM and FW at HRTs lower than 21 days merits attention as it may identify threshold levels above which the process can function

stably while generating digestate which meets quality standards (in terms of enteric indictor organism removal).

The aim of this study was therefore to identify how CSTR-type reactors were affected when HRTs were reduced from 21 days to 10.5 days, in terms of both process stability and digestate quality.

The specific objectives of this study were to assess how reducing HRT from 21 days to 10.5 days would affect

(i) specific and volumetric methane yields (SMY and VMY);
(ii) reactor operation stability;
(iii) digestate enteric indicator organism content and post-methane production potential; and
(iv) digester microbial community composition.

5.2 Materials and Methods

5.2.1 Substrates and Inoculum

In a similar manner to Chapter 4, the PM used in this study was taken from beneath the slatted unit of a local pig farm in Galway, Ireland. PM was sampled every 4 weeks and, upon entering the laboratory, it was stored at 11°C (the average annual temperature in Ireland) until use. It was essential to ensure a fresh, microbially representative PM was used in the experiment so as to assess the enteric indicator organism removal efficacy of the reactors. The PM VS content was standardised to 4.35 % (the average VS content of Irish pig manure (McCutcheon, 1997) in order to ensure organic loading rates (OLR) were kept constant within each HRT condition.

In a similar manner to Chapter 4, the FW used was sampled weekly from the brown bins of 5 local residences. Upon arrival to the laboratory, the FW samples were combined and subsampled using the method described by Browne et al. (2014). After subsampling, the FW was blended via a food processor (Russell Hobbs 500W 18087 Blender) to a particle size of < 20 mm.

At this point the FW was placed in autoclaved bags and underwent sanitization at 121°C for 15 min. via laboratory autoclave (LTE Scientific, UK). This was undertaken in order to simulate the operation of small farm-scale biogas plants, where the EU Animal By-Products Regulation (EU Commission Regulation (EU) No 142/2011) requires the sanitization of any non-farm sourced Category 3 animal

by-products entering the anaerobic digester. After autoclaving, the FW was stored at -20°C and defrosted as required. The inoculum used during digester start-up was sourced from the three 10 L laboratory-scale anaerobic digesters operated during Chapter 4.

The PM and autoclaved FW samples were analysed for pH, COD, soluble COD, TS, VS, NH_4-N, alkalinity and VFA. NH_3 concentrations were calculated using pH and NH_4-N measurements as per Anthonisen et al. (1976) (see Equation 2-6). The average characteristics of the PM, FW and inoculum used in this experiment are presented in Table 5-1.

Table 5-1 Characteristics of pig manure, food waste and inoculum used in this experiment

Parameter	PM[a]	FW[a]	Inoculum[a]
pH	7.29 ± 0.1	5.26 ± 0.1	7.85 ±0.1
Total COD (g/L)[b]	48.2 ± 4.1	431 ± 11.7	59 ± 1
Soluble COD (g/L)[b]	18.5 ± 2.5	58.5 ± 2.3	5.5 ± 0.7
Total solids (% fresh weight)	5.8 ± 0.5	25.8± 7.2	3.81 ± 0.07
Volatile solids (% fresh weight)	4.35 ± 0.3	20.3 ± 5.7	2.69 ± 0.04
NH_4-N (g/L)[b]	2.6 ± 0.6	0.69 ± 0.6	2.4 ± 0.3
Alkalinity (g/L)[b]	2.3 ± 0.7	0.17 ± 0.3	5.3 ± 0.1
Total volatile fatty acids (g/L acetic acid equivalents (HAc_{eq}) [b]	8.3 ± 3.4	7.8 ± 2.3	2.1
Acetic (g/L HAc_{eq})	3.0 ± 1.2	1.1 ± 0.9	0.32
Propionic (g/L HAc_{eq})	1.4 ± 0.3	< 1	< 1
Isobutyric (g/L HAc_{eq})	0.3 ± 0.1	6.7 ± 1.4	1.73
Butyric (g/L HAc_{eq})	1.7 ± 1.0	< 1	< 1
Isovaleric (g/L HAc_{eq})	0.74 ± 0.1	< 1	< 1
Valeric (g/L HAc_{eq})	1.1 ± 0.8	< 1	< 1

[a]Average of 4 measurements ± standard deviation.[b] Values reported for FW presented in g /kg.

5.2.2 Experimental Design

The same experimental apparatus used in Chapter 4 was used in this study, however the working volume of the three 10 L reactors (R1, R2, and R3) was reduced to 3.75 L (in order to facilitate the measurement of higher volumes of biogas generated using the mass flow meters specified in Chapter 4). Reactor

temperature was increased to 42°C, in order to simulate the operating temperature most commonly used in biogas plants treating manure and FW in Ireland (Cormack, 2016; McEniry, 2016). The reactors were operated in triplicate with the same feeding and mixing regime as specified in Chapter 4. After the inoculum was added, the reactors were sealed and the contents were flushed with N_2. The digesters were then operated in triplicate.

This experiment consisted of 3 phases; a start-up phase consisting of a period operating at a HRT of 21 days (from Day 0 to 28), a transitional phase operating at a HRT of 15 days (Day 29 to 54) and a phase where HRT was reduced to 10.5 (from Day 55 to 85). The corresponding organic loading rate (OLR) at these periods was 3.1, 5.1 and 7.25 kg VS/m^3/day, respectively.

As the inoculum used in this study was taken from the digesters operating in Chapter 4, it was highly acclimated to the substrates being used in this experiment. Therefore a shorter start-up period (1.3 turnovers of HRT) could be justified. This is supported by both the observed digester stability in terms of VS removal, methane yields and VFA concentrations (Section 5.3.1), and the DNA sequencing data which illustrates the similarities between the microbiome of the inoculum and the digestate generated after 28 days (Figure 5-1).

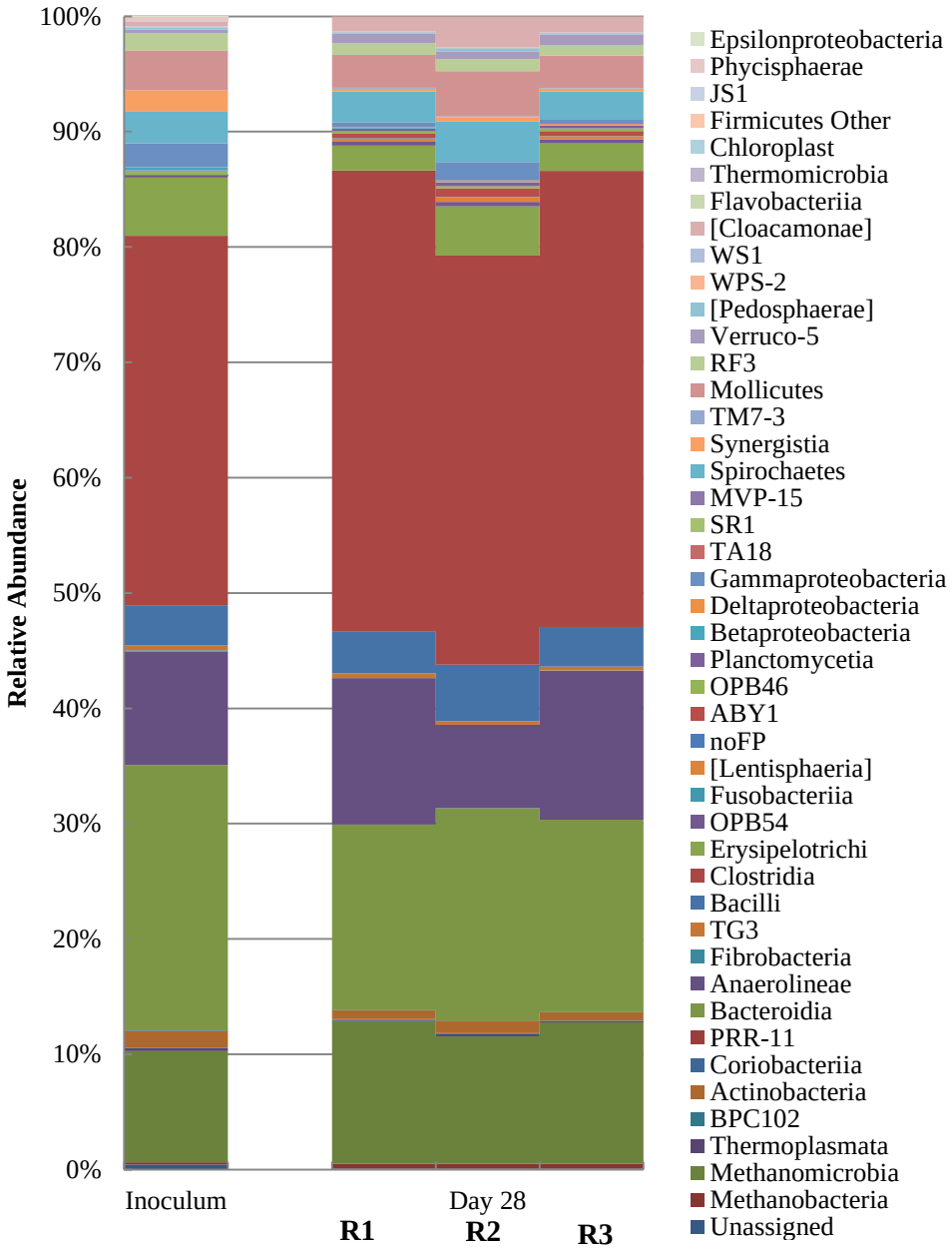


Figure 5-1 Comparison of relative abundance of bacterial classes within the inoculum used for digester start-up and digestate sampled from each replicate reactor on Day 28

The decrease in HRT from 15 to 10.5 days after 1.67 turnovers of HRT was undertaken once SMY, VMY, digestate VS content and VFA concentrations appeared to have stabilised. Similarly the conclusion of the experiment on Day 85 was chosen as VMY, SMY and VFA concentrations appeared to have stabilized after 2.8 turnovers of HRT. While this did not guarantee that reactor conditions had returned to a pseudo steady state, the stability of VFA concentrations and pH indicated that the methanogenic and hydrolytic microbial populations within the reactor were somewhat in balance.

The feed to each reactor was comprised of 55 % FW and 45 % PM on a VS basis. This mixing ratio was chosen as it maximized the proportion of FW entering the system (thereby maximizing methane yields) while ensuring that TS concentrations remained below 10 % in the reactor (thereby avoiding issues of reactor clogging and insufficient mixing). The addition of feedstock was undertaken every weekday, with the amount added and removed adjusted to ensure that the target HRT was achieved on a weekly basis.

5.2.3 Analytical methods

Biogas volume and composition was measured as described in Chapter 4.

Digestate was sampled and analysed on a weekly basis on the same day (in order to ensure that data points were not confounded by weekday feeding regime effects) until Day 50, after which samples were taken every 2-4 days. pH was immediately measured via a pH meter (WTW, Germany). This digestate was analysed for alkalinity, NH_4-N and VFA concentrationsas described in Chapter 4. TS, VS, alkalinity, Total COD and soluble COD of the substrates and inoculum were measured as described in Chapter 4.

The post methane production potential (PMP) of the digestate generated by each reactor was analysed on samples taken on Days 25, 53 and 70, when the HRT was 21, 15 and 10.5 days respectively. Digestate was placed in a 0.5 L conical flask with a butyl-rubber stopper and incubated at 11°C, in order to simulate methane emissions expected from digestate storage in Ireland. The flask content was purged with N_2 for 5 minutes prior to stoppering. Biogas was collected in 1 L ALTEF gas sampling bags (Restek Corporation, USA). After 50 days of incubation, provided that weekly biogas generation rates were negligible, the volume of biogas collected in the gas bag was measured via glass gas syringe, and the biogas methane content was measured via gas chromatography.

In order to assess potential changes in enteric indicator organism counts in the digestate as HRT was decreased, digestate samples were taken on Day 28 when HRT was 21 days, Day 47 when HRT was 15 days, and Days 72 and 82 when HRT was 10.5 days. Feedstock from Days 28 and 82 were also sampled. These samples were immediately analysed for the presence of *E. coli* and *Enterococcus* as described in Chapter 4. All digestate and feedstock samples outlined above (25 g) were also analysed for the presence of *Salmonella* according to the International

Organisation for Standardisation (ISO) 6579:2007 (Amendment 1: Annex D) method (International Organization for Standardization, 2007).

5.2.4 High throughput 16S rRNA gene sequencing

Five cm^3 of digestate was sampled from each reactor on Day 28 when the HRT was 21 days, Day 47 when the HRT was 15 days and twice when the HRT was 10.5 days (Days 72 and 84). Samples were immediately snap-frozen in liquid N_2 and stored at -80°C. DNA was extracted, purified and underwent PCR as described in Chapter 4. Subsequently the samples were sequenced and resulting data underwent bioinformatic analysis as described in Chapter 4.

Quantitative PCR of bacterial DNA was undertaken on the DNA extracted as described above in order to assess the effects of reducing HRT on total bacterial DNA concentrations. This was performed using the Roche 480 Lightcycler platform in a manner similar to that described by Fouhy et al. (2012). A calibration curve using 10^9 to 10^2 copies 16S rRNA /µl was established. Values were then converted to copies 16S rRNA /g. The settings used to quantify total bacterial numbers were: 95°C for 3 minutes followed by 45 cycles of 95°C for 10 s, 60°C for 20s and 72°C for 1 s followed by melting curve analysis of 95°C for 5 s, 65°C for 1 min, and 97°C continuously and a final cooling at 40°C for 10 s. Each samples contained 7.2 µl of PCR-grade water, 0.4 µl of the forward primer F1 (5′-ACTCCTACGGGAGGCAGCAG) , 0.4 µl of the reverse primer R1 (5′-ATTACCGCGGCTGCTGG), 2 µl of a 1 in 10 dilution of extracted DNA, and 10 µl of SYBR green (Roche Diagnostics, West Sussex, United Kingdom). Samples, negative controls (where PCR-grade water was used instead of DNA) and standards were run in triplicate.

5.2.5 Statistical analysis

In order to assess the effect of HRT on methane yields and VS removal from day 14 onwards, the weekly averages of volumetric methane yield (VMY), specific methane yield (SMY) and % VS removed measured at each HRT were compared via repeated measures ANOVA. In order to compare the PMP, *E. coli* and *Enterococcus* counts (log transformed) measured at each sampling point, one-way ANOVA followed by the Bonferroni method was used to identify significant differences. For DNA sequencing data, Kruskal Wallis one-way ANOVA was used to assess changes of OTU relative abundance due to HRT.

All statistical analyses were carried out using SPSS v22.0 (IBM, USA).

5.3 Results and discussion

5.3.1 Reactor performance

Figure 5-2(a) illustrates that SMYs significantly decreased (p=0.014) and VMYs significantly increased (p< 0.000) as HRT decreased (and OLR increased). The SMY measurements mirrored closely the VS removal trends observed (Figure 5-2 (c)); a decrease of VS was observed as HRT was decreased (p< 0.000). This, allied with the increase in PMP values observed as HRT was decreased, indicates that a reduction in the level of substrate utilization occurred as HRT was reduced from 21 to 10.5 days. Longer HRTs and lower OLRs result in a greater level of substrate utilization, and therefore this was expected. (Noike et al., 1985).

From Day 64, when VMYs peaked at 1728 mL CH_4/L/d (after HRT was reduced to 10.5 days) until Day 78, SMY and VMY values decreased markedly. After this period VMY, SMY and VFA concentrations appeared to stabilize. While the average % VS removed continued to decrease between Day 78 and 82, the change was not significant (p= 0.4562).

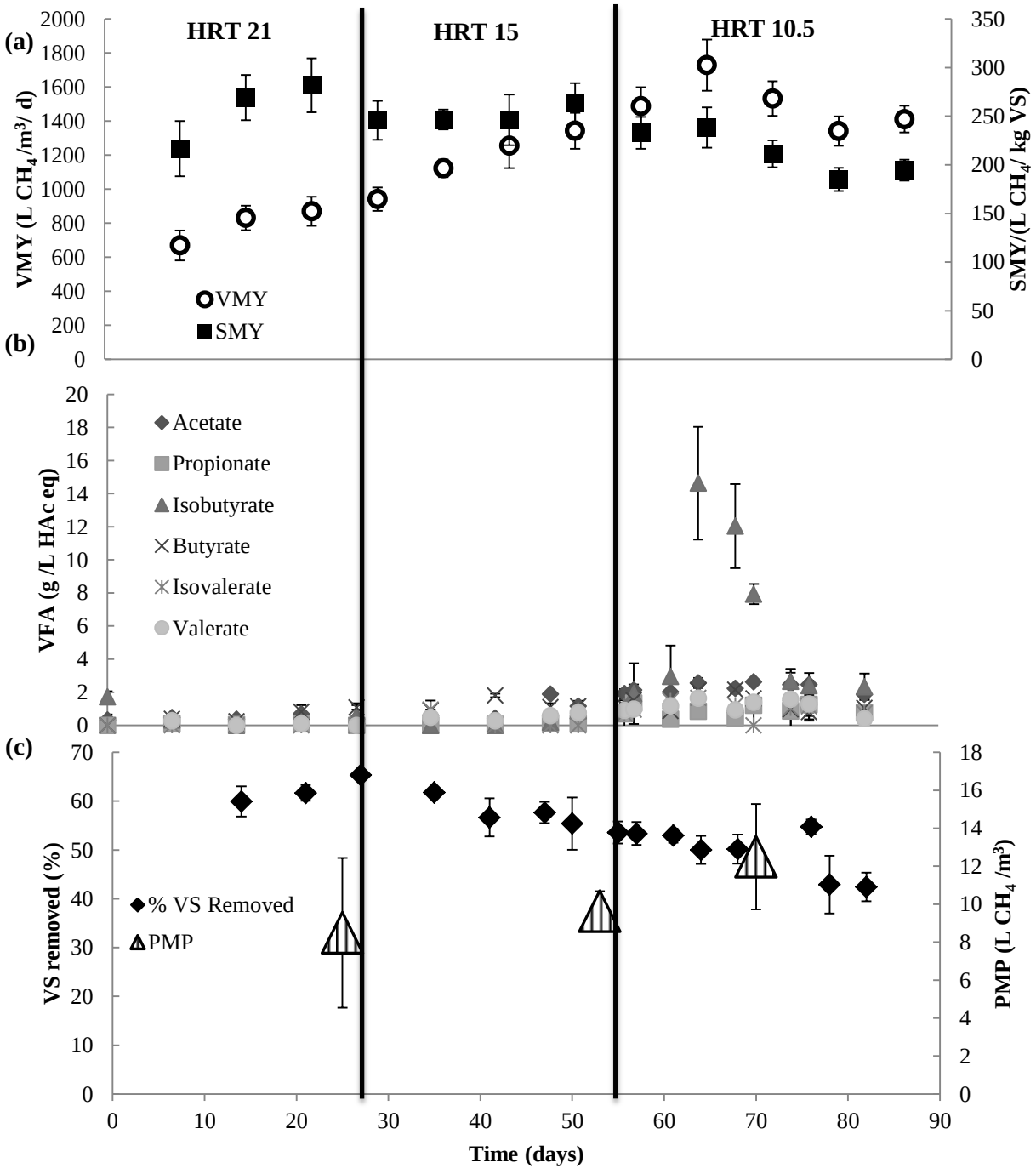


Figure 5-2 Average specific methane yield (SMY), volumetric methane yield (VMY) (a), volatile fatty acid (VFA) concentrations (b), and post methane production potential (PMP) and % VS removed (c), measured throughout the experiment as hydraulic retention time (HRT) was reduced. Values are the mean of three replicates with error bars representing standard deviations.

The accumulation of isobutyric acid in the reactors from Day 61 to 64 (Figure 5-2(b)) indicates that the decoupling of acetogenesis and hydrolysis may have been responsible for the drop in VMY and SMY observed from Day 64 onwards. The reason that SMYs did not drop extensively despite very high VFA concentrations was due to the high pH and high buffering capacity in the system (see Figure 5-3). This meant that VFA accumulation did not result in a pH drop below 7, and therefore did not result in complete process failure. The work of Franke-Whittle et al. (2014) shows that, particularly for highly buffered systems, there is no general threshold for VFA inhibition, as each system, depending on pH and buffering capacity (and the presence of salts and other anions) is vastly different. Nevertheless, pH and alkalinity did decrease due to this increase in VFA. These were signs of process instability. Isobutyric acid concentrations decreased from Day 64 and appeared to stabilize from Day 75 onwards. Assessment of any changes that occurred in the digester microbial community as HRT was reduced to 10.5 days may identify the specific reasons for the increase and subsequent decrease in isobutyric acid concentrations.

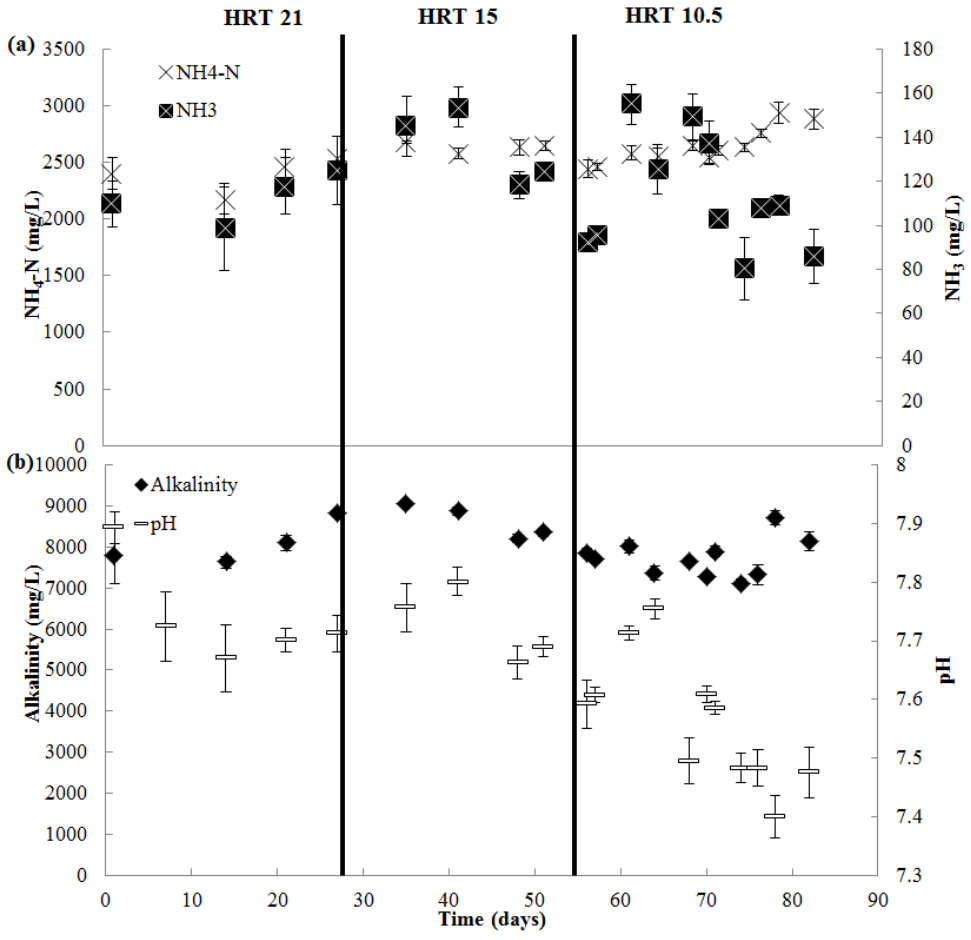


Figure 5-3 Average NH_4-N and NH_3 (a), pH and alkalinity (b) concentrations in each reactor as HRT was decreased. Values are the mean of three replicates with error bars representing standard deviations

5.3.2 Microbial community analysis

Rarefaction curves presented in Appendix C Figure C2 reveal that sequencing was undertaken at sufficient depth. Results of the 16S rRNA gene sequencing of the digestate samples taken throughout this experiment revealed that the minimum number of sequence reads per sample was 81000, resulting in an average of 583 ± 10 OTUs per sample.

5.3.2.1 Sample clustering and microbial diversity indices

The PCoA plot presented in Figure 5-4 illustrates that changing HRT had an effect on microbial communities in the digester, with samples from each respective HRT clustering together. PC1 could explain 67.42 % of the variation observed in microbial communities. While the two samples taken at the HRT of 10.5 days (on Days 72 and 84) clustered to some extent, there are some indications of differences between the two sample points.

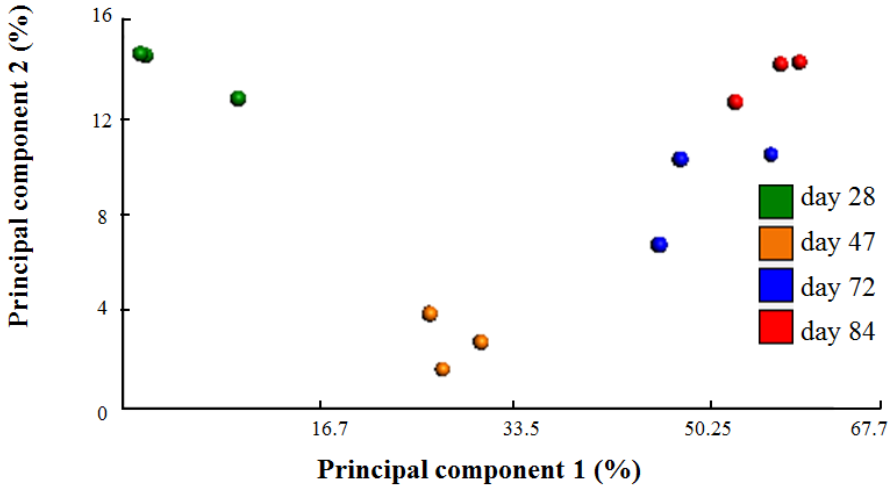


Figure 5-4 Principal component analysis (PCoA) plot of samples taken from the reactors throughout the experiment.

Changes in the number of observed species, Chao 1 species richness and the Shannon index were slight throughout the experiment (Figure 5-5). In general, a small increase in these richness and diversity indices was observed between day 28

and 47, followed by a decrease on day 84. This may be indicative of a period of ecological succession as HRT was decreased to 15 (Day 47) and 10.5 days (Day 72), with new species progressively displacing established species and exploiting new ecological niches created by the reduction in HRT until a climax community was established, at which point species diversity decreased.

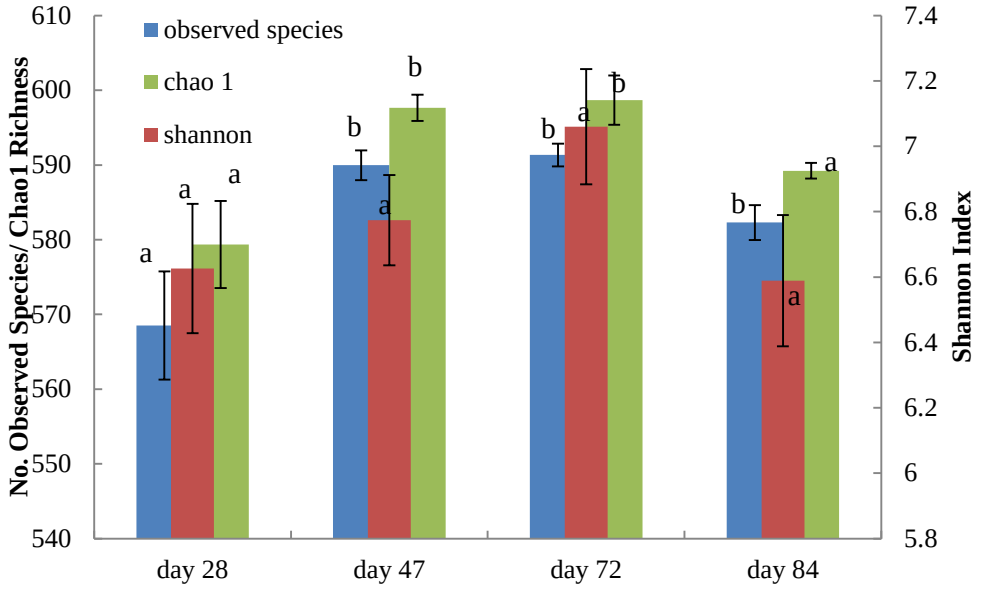


Figure 5-5 Shannon index, Chao 1 species richness and number of observed species measured at each sampling point. Values are the mean of three replicates with error bars representing standard deviations. Within each time point, bars sharing a common letter are not significantly different ($p>0.05$), as measured by the Bonferroni method

5.3.2.2 Changes in bacterial relative abundance

Quantitative PCR of bacterial DNA found on average 1.28×10^{10} bacterial DNA copies per g of digestate and this did not change significantly as HRT was reduced from 21 to 10.5 days (Figure 5-6). Similarly, Maspolim et al. (2015) found that decreasing HRT from 30 to 12 days had no clear effect on the concentration of bacterial and methanogen 16S rRNA reads.

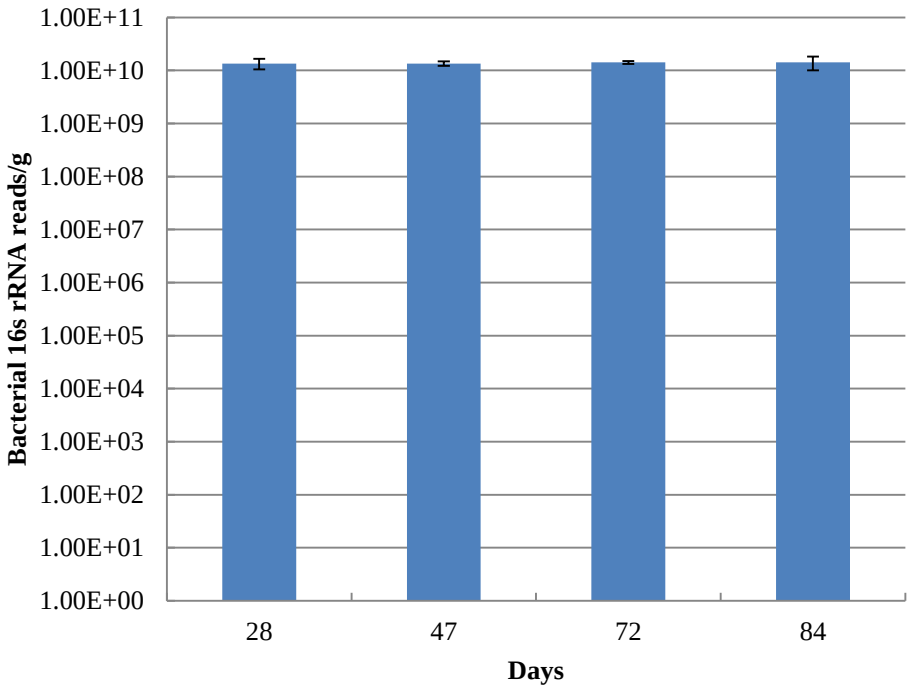


Figure 5-6 Bacterial DNA reads per g of digestate as measured by qPCR at each sampling point. Values are the mean of three replicates with error bars representing standard deviations

Figure 5-7 presents the taxonomic breakdown of the bacterial communities observed within each of the three reactors at each sampling point. Bacteria comprised between 87 % and 93 % of the observed microbial community within the digesters. While De Vrieze et al. (2016) highlights that dynamic changes in digester microbial populations occur even when there are no changes in digester operating conditions, a response to the progressive reduction in HRT was seen for several phyla, families and genera in the present study.

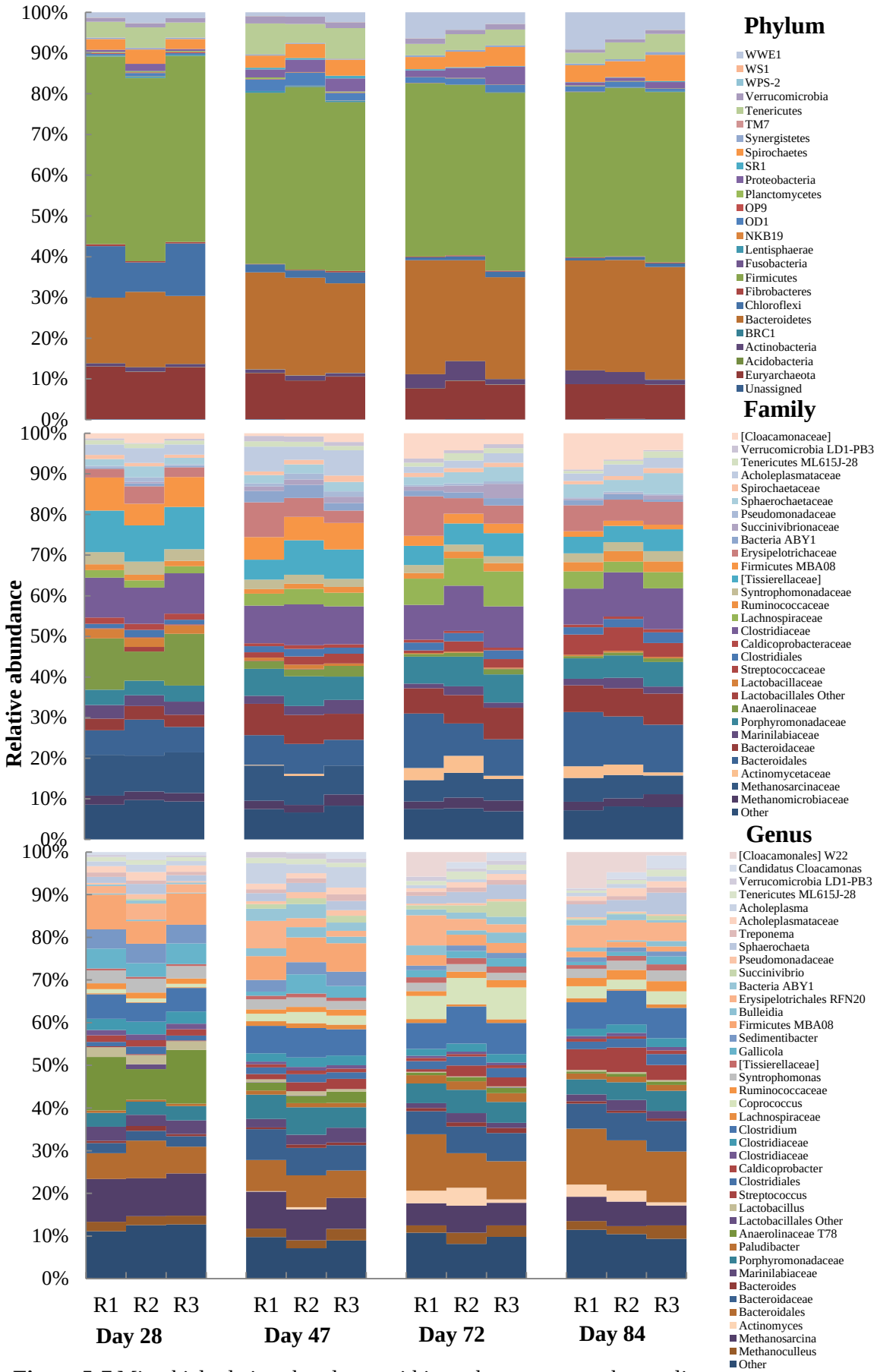


Figure 5-7 Microbial relative abundance within each reactor at each sampling point at phylum, family and genus level. Genera and families with relative abundance <1 % at any point during the experiment were grouped in the "Other" category.

A decrease in *Firmicutes* (the most abundant phylum; p= 0.037) and an increase in *Bacteroidetes* (second most abundant phylum; p= 0.009) relative abundance was observed as the HRT was decreased. Previous studies have suggested that a decrease in the ratio of *Firmicutes* to *Bacteroidetes* (both of which are associated with hydrolytic and acidogenic stages of anaerobic digestion) ratio can be an indicator of reactor instability (De Vrieze et al., 2014). Figure 5-8 illustrates that the ratio of *Firmicutes* to *Bacteroidetes* progressively decreased as HRT was decreased; from 2.7 on day 28 to 1.5 on day 84. It is noteworthy that the ratio of *Firmicutes* to *Bacteroidetes* continued to decrease even as the concentrations of isobutyric acid decreased, suggesting that stable condition had not been re-established by day 84.

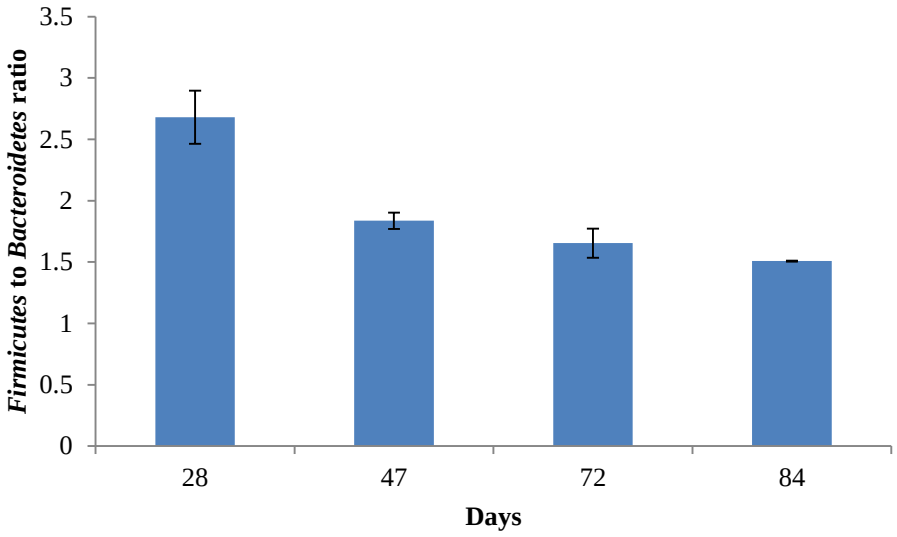


Figure 5-8 Change in *Firmicutes* to *Bacteroidetes* ratio as the experiment progressed. Error bars denote standard deviation of the ratio

Chloroflexi decreased in relative abundance as HRT decreased, while *WWE1* and *Actinobacteria* increased in relative abundance, particularly as HRT was decreased to 10.5 days. At family and genus level, it is clear that the reduction in *Chloroflexi* at phylum level can be attributed largely to a significant (p=0.009) reduction in genus *T78* of the family *Anaerolineae* as HRT was reduced. *Anaerolineae* are associated with degradation of carbohydrates and amino acids (Yamada & Sekiguchi, 2009), and were observed to increase in abundance as HRT decreased in Chapter 4. *T78* is uncultured, but it has previously been found in anaerobic digesters treating wastewater treatment sludge (Riviere et al., 2009). While *T78* decreased in relative abundance, *Actinomyces*, of the phylum *Actinobacteria*,

increased. *Actinomyces* is a fermentative bacterium that has been suggested to be a cause of foaming in anaerobic digesters treating sewage sludge (Ganidi et al., 2009). Therefore, its presence may be a sign of potential reactor instability.

An increase in the relative abundance of the genus *Coprococcus* (phylum *Firmicutes)* was observed as HRT decreased ($p= 0.015$), in particular at day 72 (when isobutyric acid concentrations increased). *Coprococcus* has been found to degrade carbohydrates and amino acids into a range of VFA's (butyric, propionic, acetic and formic acids) (Holdeman & Moore, 1974). An increase in the relative abundance of *Paludibacter*¸ a genus which has been found to produce propionate and acetate (Qiu et al., 2014), was also observed as HRT was decreased to 10.5 days. These observations, along with the increase in the relative abundance of *Actinomyces* are indicative of a shift in acidogenic populations. It is possible that the shift in the acidogenic population may have indirectly caused the increase in isobutyric acid observed between days 61 and 74 by (i) producing isobutyric acid directly; (ii) producing isobutyric acid indirectly via an intermediate organism isomerising the butyric acid produced; or by (iii) producing formate. Formate has been shown to disrupt syntrophic degradation of butyric and isobutyric acids, by inhibiting the reversible isomerisation of isobutyric acid to butyric acid, when formate is present at concentrations above 1mM (Wu et al., 1996). This would result in isobutyric acid accumulation, as isomerisation to butyric acid is an intermediate step in syntrophic isobutyric acid degradation (Wu et al., 1994).

The reduction in HRT, and the accumulation of isobutyric acid, appeared to positively affect syntrophic VFA oxidizers, such as genus *W22* and *Candidatus Cloacanomas* of family *Cloacamonaceae* (which are responsible for the increase in relative abundance observed for the candidate phylum *WWE1*). *W22* tended to increase as HRT decreased ($p= 0.09$), particularly as isobutyric acid began to accumulate. These genera have been commonly found in anaerobic digesters, and while *W22* is uncultured, the *Cloacamonaceae* family has been found to ferment amino acids and syntrophically oxidize butyric and propionic acid (Hagen et al., 2014) into H_2, CO_2 and acetate. Therefore, they may have played a key role in the observed reduction in isobutyric acid concentrations between days 64 and 76. The increase in relative abundance of the phylogenetically similar *Spirochaetes* (a phylum containing several species identified as SAOBs (Lee et al., 2013)) as HRT decreased suggests that an overall increase in the activity of the syntrophic VFA oxidation pathway occurred as HRT was decreased.

5.3.2.3 Changes in Archaeal relative abundance

As Figure 5-9 illustrates, Archaea comprised between 13 % and 7 % of the microbial community observed across all data points. Reducing HRT resulted in a reduction in the overall relative abundance of Archaea. In Chapter 4, it was observed that decreasing HRT from 29 to 21 days resulted in a decrease in the relative abundance of methanogens. Together these sets of data suggest that decreasing HRT below 29 days results in a decrease in archaeal relative abundance.

In contrast to the bacterial populations, the composition of the archaeal community was remarkably stable throughout the experiment. Due to their specialised function in terms of methanogenesis, less functional redundancy is likely to be present and stability is maintained by resilience of a small number of established organisms rather than shifts to alternative genera (De Vrieze et al., 2016) particularly when the methanogenic pathways remain unchanged.

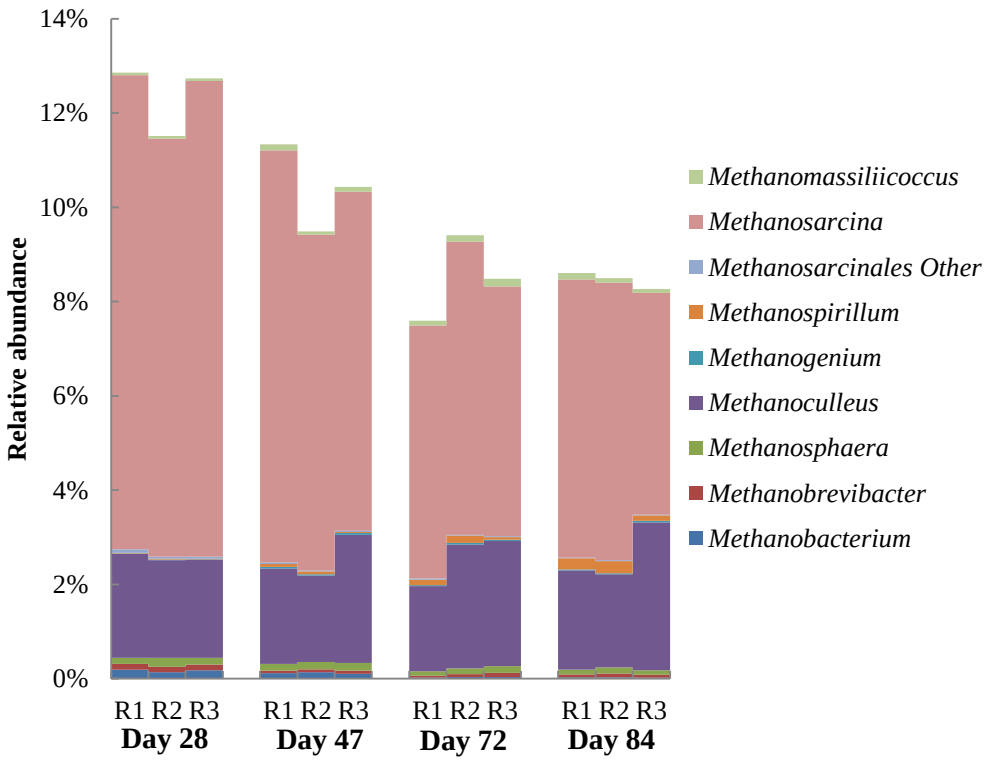


Figure 5-9 Relative abundance of Archaea within each reactor at each sampling point at genus level.

Figure 5-9 illustrates that hydrogenotrophic methanogens dominated the archaeal populations observed. The facultative hydrogenotroph, *Methanosarcina*, was the dominant genus observed throughout the experiment. It is very commonly found in biogas plants and, as it can utilize hydrogenotrophic, aceticlastic and

methylotrophic pathways for methanogenesis, it can operate over a wide temperature range (mesophilic and thermophilic) and in the presence of high concentrations of FAN (De Vrieze et al., 2015). The significant reduction in *Methanosarcina* relative abundance (p= 0.009) as HRT decreased was the cause of the overall decrease in archaeal relative abundance as HRT decreased. This may be due to the reduced niche for facultative aceticlastic methanogenesis (due to the apparent increased role of hydrogenotrophic methanogenesis) when HRT was reduced to 10.5 days. Sun et al. (2014) showed that while *Methanosarcina* was found at a relatively high abundance in reactors where SAOB activity was high, it was generally less dominant than in reactors where aceticlastic methanogenesis prevailed.

Methanoculleus which was the 2nd most abundant methanogen, was not significantly affected by the decrease in HRT (p= 0.868). It is an obligate hydrogenotroph and is very commonly found in digesters treating nitrogen-rich substrates, such as manures (Stolze et al., 2015). The fact that the relative abundance of *Methanoculleus* remained stable as HRT decreased indicates that *Methanoculleus* growth rates were sufficiently rapid to be unaffected by HRTs as low as 10.5 days.

Allied with the observations from Chapter 4 of increased relative abundance of SAOBs as HRT decreased, the results of this study suggests that decreasing HRT should increase the importance of hydrogenotrophic methanogenesis as a methanogenic pathway.

5.3.3 Digestate biosafety

Figure 5-10 presents the average counts of enteric indicator organisms found in the digestate and feedstock during this experiment. As FW was autoclaved prior to being fed to the digesters, the enteric indicator organisms found in the feedstock were attributed to the PM. One-way ANOVA of counts vs HRT revealed increases in the counts of both *E. coli* (p= 0.002) and *Enterococcus* (P < 0.001) in the digestate as HRT was decreased.

The Bonferroni procedure was performed in order to assess the significance of the changes observed between data points. For *E. coli*, this analysis indicates that while decreasing HRT resulted in significant increases in counts as HRT was decreased from 21 to 15 days, the counts observed at HRT of 15 and the digestate sample take on day 84 (when HRT was 10.5 days) were not significantly different from

each other. Further to this, the counts measured from digestate sampled on day 72, during VFA accumulation, were not significantly different from the feedstock count. For *Enterococcus*, Bonferroni analysis found that while decreasing HRT from 21 days to 15 days significantly increased *Enterococcus* counts, the counts found at the HRT of 15 days were similar to those found at a HRT of 10.5 days and in the feedstock. *Enterococcus* counts measured on Day 72 were significantly lower than those found in the feedstock ($p= 0.009$), despite there being no significant difference between counts at Day 82 and those in the feedstock. Increases in VFA combined with a drop in pH have been found to reduce the survival rates of indicator organisms in anaerobic digesters (Sahlström, 2003), and therefore the spike in VFA concentrations and concurrent drop in pH observed on Day 72 may explain this drop in *Enterococcus* survival.

The effect of increasing HRT from 11 to 25 days on *E. coli* counts has been studied previously (Chen et al., 2012), with regulatory acceptable levels of *E.coli* removal being achieved at a HRT of 18 days. In the present study anaerobic digestion did not achieve a sufficiently high level of *Enterococcus* removal at HRTs < 21 days, or of *E. coli* removal at HRTs < 15 days. From an operational perspective, the high *Enterococcus* counts present in the digestate at HRTs of < 21 days means that the digestate would not meet the standards set out in the EU animal by-products regulations (< 1000 CFU/g for *E.coli* or *Enterococcus*) and would therefore require further treatment before disposal. Each digestate sample was also tested for the presence of *Salmonella*, with no *Salmonella* being found in any of the digesters at any point throughout the experiment.

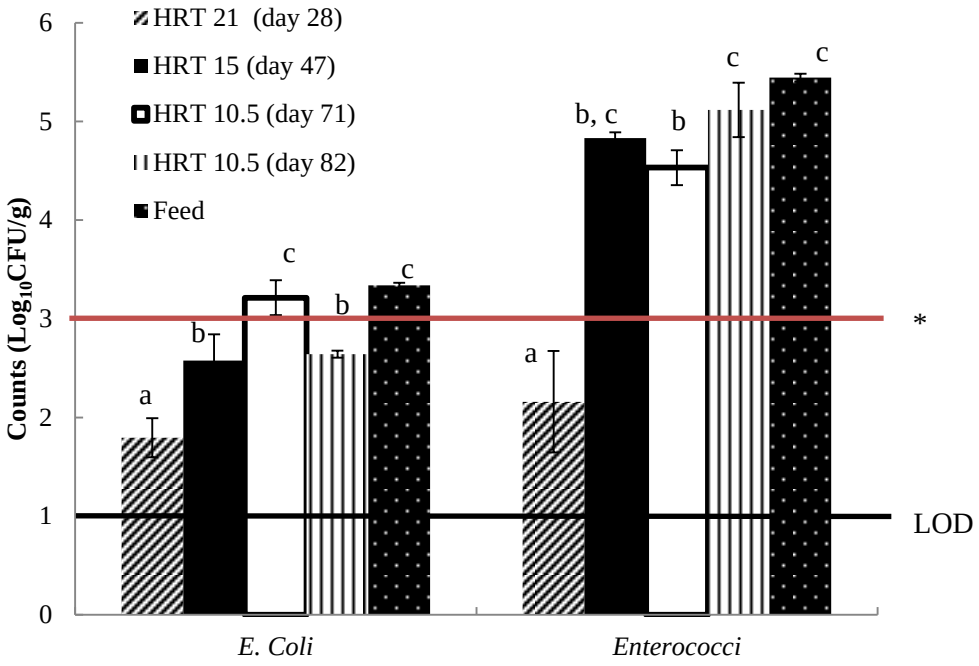


Figure 5-10 *E. coli* and *Enterococcus* counts in digestate throughout the experiment, as well as in the feed on Days 28 and 82. Values are the mean of three replicates with error bars representing standard deviations. Bars representing the count for the same bacteria and for the same sample type sharing a common letter are not significantly different (P>0.05), as measured by the Bonferroni method. *represents the EU animal by-products regulation limit of < 1000 CFU/mL for *E. coli* or Enterococcus in digestate prior to land application. LOD represents limit of detection.

5.4 Summary

Decreasing HRT to 10.5 days resulted in a drop in SMYs and VMYs and a rapid increase in isobutyric acid concentrations. This increase in isobutyric acid may have been caused by the shift in relative abundance of acidogenic bacteria.

The increase in the relative abundance of the family *Cloacamonaceae* may have played a role in the subsequent reduction in isobutyric acid concentrations, as it oxidised non-acetate VFAs to acetate, CO_2 and H_2. This, along with the increase in the relative abundance of other syntrophic VFA oxidizers such as *Spiorchatetes* suggests that syntrophic VFA oxidation plays an increasingly important role when CSTRs are operated at low HRTs.

Combined with the results from Chapter 4, these sets of data suggest that decreasing HRT below 29 days results in a decrease in archaeal relative abundance and an increase the importance of hydrogenotrophic methanogenesis as a methanogenic pathway.

Reducing HRT below 21 days compromised the ability of the anaerobic digestion system to reduce the concentrations of *Enterococcus*. Reducing HRT below 15 days compromised the ability of the anaerobic digestion system to reduce the concentrations of *E.coli.*

CHAPTER 6

The application of the Anaerobic Digestion Model No. 1 to the simulation of the meso-scale co-digestion of pig manure and food waste using a simple calibration protocol

This chapter details the results of the operation of a meso-scale co-digestion system, and the calibration and validation of a model to simulate the CSTR-based mesophilic co-digestion of PM and FW.

6.1 Introduction

Mathematical models such as ADM1 have the potential to be important tools in digester design, control and operation. Their use in the industry thus far has been limited due to the detailed substrate characterisation required, and requirement for detailed complex model calibration (Kleerebezem & Van Loosdrecht, 2006).

Optimisation of model fit by modifying key model rate constants (such as substrate utilization rates and inhibition factors) is typically carried out using complex mathematical methods such as minimizing cost functions (Dochain & Vanrolleghem, 2001; Garcia-Gen et al., 2013), genetic algorithms (Wichern et al., 2009) and particle swarm optimization (Bai et al., 2017) in order to fit the model to a calibrating data set. Such optimization may improve the model precision, however can result in an over calibrated model which does not provide accurate simulation of system dynamics (Donoso-Bravo et al., 2011). In addition, applying such complex numerical methods may be challenging to model end users i.e. biogas plant operators.

This study assessed the accuracy and precision of a rudimentarily calibrated ADM1 model when simulating the operation of a meso-scale digester co-digesting PM and FW.

6.2 Materials and Methods

6.2.1 Digester Set-up

A 400 L meso-scale completely stirred tank reactor, with an effective volume of 360 L, was used in this experiment. It was located on the Teagasc Moorepark Research Centre, Fermoy, Co. Cork. It was operated at 42°C through a thermostatically controlled water jacket. An image of the experimental set-up is presented in Figure 6-1.

Figure 6-1 Meso-scale reactor used in this experiment

The feedstock was comprised of PM and FW. The PM/FW mixing ratio was varied from 0 % FW/100 % PM to 55 % FW/ 45 % PM (on a volatile solids (VS) basis), throughout the experiment (see Figure 6-2). This maximum mixing ratio was selected in order to maximise methane yields through the addition of FW, while maintaining feedstock TS concentrations below 15 % so as to avoid any pipe blockages in the system. Agitation was undertaken for one hour prior to and one hour after digestate withdrawal and feeding using a paddle agitator set at 100 rpm using a speed regulator (Hitachi SJ200, Japan). At the same time, a vortex chopper pump (Arven S.R.L., Italy) was activated in order to break up any solid agglomerations within the reactor. The HRT was set at 25.2 days throughout the experiment. Digestate withdrawal and feedstock addition was undertaken each weekday using PLC (Rockwell Automation, USA) controlled pneumatic valves. The reported OLR (Figure 6-2) was adjusted to account for the lack of feeding at weekends.

PM was taken directly from the slatted unit in which pigs were housed, and stored in a 1 m^3 container. This container was refilled with manure as required (every 20-50 days; see Table 6-1). The FW used in this study was collected from the onsite canteen daily. It was subsampled as described by Browne et al. (2014), macerated to a particle size of 2 mm, and frozen in order to minimize FW putrefaction during storage. Due to local regulatory stipulations based on the EU Animal By-product Regulations, batches of FW were autoclaved at 121 °C for 15 minutes every 2-3 days. This autoclaved FW was then used as digester feedstock. The inoculum used to start-up of this reactor was taken from a commercial-scale biogas plant treating a combination of cattle manure, chicken manure and FW at mesophilic conditions (42 °C).

6.2.2 Experimental Design

The digester was operated for a period of 120 days. The PM/FW mixing ratio was varied during this period in order to ensure that the ADM1 model could simulate the effects of changing feedstock composition. Figure 6-2 illustrates the PM/FW mixing ratio and the OLR used throughout the experiment.

Digester pH, biogas volume and biogas composition (% CH_4 and % CO_2) were determined daily, with digestate analysed for TS and VS content twice per week. Twice per week, digestate was subsampled and frozen for subsequent alkalinity, ammonia and VFA concentration analysis. In addition to this, each new batch of PM and FW were analysed for TS and VS concentrations, subsampled and frozen for subsequent analysis of carbohydrate, lipid and protein contents, alkalinity, ammonia, VFA, total COD and soluble COD. PM and FW samples from day 0 were frozen, stored and subsequently analysed for trace metals. Table 6-1 presents the chemical properties of the inoculum and PM used in this study, as well as the average composition of the FW used during the experiment. Average FW values are presented as, unlike PM of which different batches were fed on a monthly basis, FW composition varied on a day-to-day basis. Therefore presentation of average values provides a better representation of the composition over longer periods.

Table 6-1 Chemical properties of inoculum, PM and average FW composition. These compositions were used as inputs for the ADM1 model. [a] average values of 12 samples. [b] average of 3 samples

Sample	Inoculum	FW day 0-120	PM day 0-53	PM day 54-79	PM day 70-101	PM day 102-120
Total solids (%)	4.0	30± 4.2[a]	3.4	5.5	4.0	3.5
Volatile solids (%)	3.0	27± 4.7[a]	2.6	4.4	3.0	2.0
Total COD (g/L)	37.1	210.7± 56[a]	56.0	78.2	43.3	41.8
Soluble COD (g/L)	9.5	87.8± 4.2[a]	10.7	14.0	13.3	11.5
Alkalinity (HCO_3^- g/L)	6.0	0.8± 0.4[a]	5.5	6.4	5.9	12.0
NH_4-N (g/L)	2.6	0.2± 0.09[a]	2.5	2.8	2.7	3.6
pH	7.9	5.4± 0.02[a]	8.3	7.8	8.0	8.3
Acetic acid (HAc_{eq} g/L)	0.0	1.0± 0.35[a]	2.8	3.2	1.8	1.3
Propionic acid (HAc_{eq} g/L)	0.0	1.1± 1.41[a]	2.7	0.8	0.0	1.0
Isobutyric acid (HAc_{eq} g/L)	0.0	0.0± 0.07[a]	0.4	0.0	0.0	0.0
Butyric acid (HAc_{eq} g/L)	0.0	0.6± 0.2[a]	0.0	0.0	0.0	0.3
Isovaleric acid (HAc_{eq} g/L)	0.0	0.0± 0.0[a]	0.0	0.0	0.0	0.0
Valeric acid (HAc_{eq} g/L)	0.0	0.0± 0.0[a]	0.0	0.0	0.0	0.0
% Carbohydrate	0.65	0.55± 0.09[b]	0.79	0.75	-	0.75
% Protein	0.25	0.15± 0.14[b]	0.14	0.17	-	0.15
% Lipid	0.1	0.29± 0.05[b]	0.07	0.08	-	0.1
Al (mg/L)	-	8.66	11.73	-	-	-
Cr (mg/L)	-	0.24	0.35	-	-	-
Mn (mg/L)	-	1.01	17.06	-	-	-
Fe (mg/L)	-	5.49	65.81	-	-	-
Co (mg/L)	-	0.00	0.08	-	-	-
Ni (mg/L)	-	0.08	0.33	-	-	-
Cu (mg/L)	-	0.38	7.75	-	-	-
Zn (mg/L)	-	2.28	32.34	-	-	-
Se (mg/L)	-	0.06	0.20	-	-	-
Mo (mg/L)	-	0.12	0.68	-	-	-
Cd (mg/L)	-	0.01	0.02	-	-	-

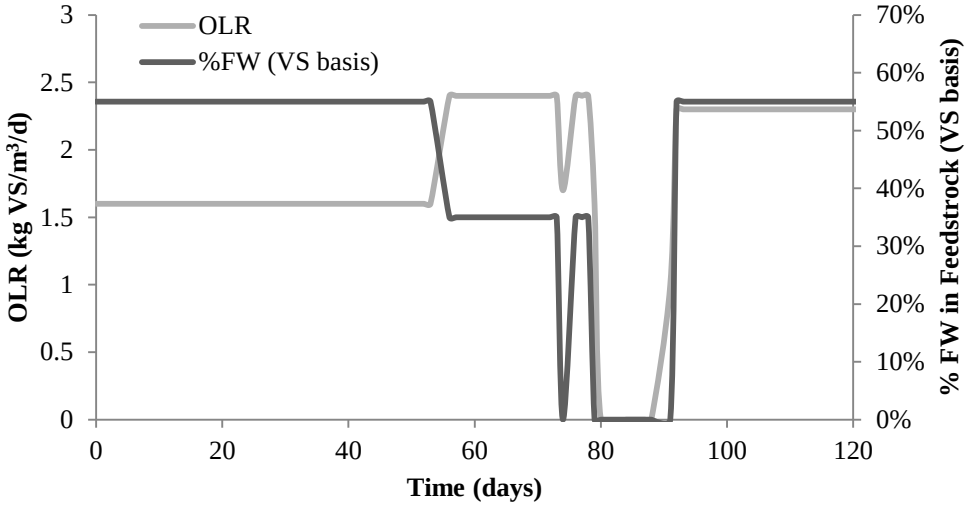


Figure 6-2 Digester OLR and % FW mixing ratio (remainder comprised of PM) throughout the experiment.

6.2.3 Analytical Methods

The biogas volume was measured daily via mass flow meter (OMEGA, USA). Biogas composition (% CH_4, % CO_2 and H_2S concentration) was determined using a portable biogas analyser (GA2000 BIOGAS, GeoTech, UK). pH was recorded daily using an online pH probe (Hamiltion electro-chemical sensors, Esslab, UK) . TS, VS, total COD concentrations were measured via Standard Methods (APHA, 1998). Subsequent to this samples were centrifuged (Model 2-15, Sigma, Germany) at 14500 rpm (1175 g) for 15 min and filtered through 0.45µm syringe filter (Sarstedt, Germany). COD concentrations were measured via Standard Methods (APHA, 1998). Alkalinity and ammonia concentrations were measured via nutrient analyser (Konelab, Thermo Clinical Labsystems, Vantaa, Finland) and VFA concentrations were measured via high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) as described in Chapter 3. Crude protein was determined by the Dumas method (Jung et al., 2003), while crude fat was determined by acid hydrolysis method (Sukhija & Palmquist, 1988). The total % carbohydrate was equal to the remaining fraction of the total solids after protein and fat had been determined.

6.2.4 Mathematical Modelling

In order to simulate the co-digestion of PM and FW, the IWA's ADM1 was used (Batstone et al., 2002). It was executed via Matlab/Simulink (Rosen et al., 2006). A variable step (ode15) solver was used. A dynamic algebraic equation (DAE) model with algebraic solutions of both pH and H_2 was applied. As suggested by Batstone et al. (2015), the disintegration step was neglected in the model. The hydrolysis

rates of carbohydrates (k_{hyd_CH}), proteins (k_{hyd_PR}) and lipids (k_{hyd_LI}) were made equal i.e. a single rate of substrate hydrolysis was used, rather than discrete hydrolysis rates for each carbohydrate, protein and lipid fraction. The model was calibrated using the hydrolysis rate determined by the batch co-digestion study of PM and FW undertaken in Chapter 3. At the PM/FW mixing ratio of 60 % FW (VS basis) the hydrolysis rate was determined to be 0.39 d^{-1}.

Despite studies suggesting batch tests underestimate hydrolysis rates which occur at full scale continuous operation (Batstone et al., 2009), as the values reported in Chapter 3 are similar to the hydrolysis rates suggested in ADM1(Batstone et al., 2002) for manure and organic waste digestion, it was deemed acceptable for the purposes of this experiment. All other kinetic constants were set to the default values for liquid high rate mesophilic digestion (Rosen et al., 2006), as reported and programmed by Rosen et al. (2006). While this model cannot dynamically change hydrolysis rates in response to changes in PM/FW mixing ratio (unlike proposed extensions to the model (Zaher et al., 2009)), it does allow for accounting for the significant synergistic effect PM/FW mixing ratio has on the inert fraction of carbon in the combined feedstock.

The batch study (Chapter 3) was also used to identify the fraction of inert COD present in PM, FW and various PM/FW mixing ratios (using the method described by Batstone et al. (2003)). While unautoclaved FW was used in Chapter 3, previous studies have shown that autoclaving FW does not significantly affect the measured BMPs of FW (Tampio et al., 2014), and therefore the data is suitable for providing a rudimentary calibration of the model.

Characterisation of individual substrates was undertaken as per Arnell et al. (2016) using the carbohydrate, protein, lipid, total COD, soluble COD, VFA, alkalinity, NH_4-N and pH values measured for each substrate throughout the experiment. Table 6-2 summarises the calculations and conversions undertaken to generate model inputs used in this study. All remaining model inputs (the inputs designated to represent active microbial biomass (X_{su}, X_{aa}, X_{fa}, X_{c4}, X_{pro}, X_{ac}, X_{h2})) were set to zero.

Specifically, the soluble and particulate COD (total COD minus soluble COD) were partitioned into the necessary model input terms by firstly determining the inert fraction of COD in the combined substrates. Chapter 3's results indicate that co-digesting FW and PM can result in synergistic effects which can increase the

fraction of degradable COD in the mixture (Fd) by up to 20 %, but the synergy is dependent on the mixing ratio of PM and FW. Therefore the Fd values presented in Chapter 3 for PM/FW mixtures were used in this study. From the Fd value at the specific mixing ratio used, the inert particulate COD (X_I) and inert soluble COD (S_I) were calculated (Arnell et al., 2016).

The soluble COD fractions (monosaccharides, S_{su}; amino acids, S_{aa}; and long chain fatty acids, S_{fa}) were determined based on the fractions of carbohydrates, protein and lipids (respectively) measured in the substrate and the amount of soluble COD remaining when the inert COD (S_I) and VFAs were accounted for (Garcia-Gen et al., 2013). In a similar manner, the particulate COD fractions (carbohydrates, X_{ch}; protein, X_{pr}; and lipids, X_{li}) were determined based on the fraction of carbohydrate, protein and lipids (respectively) measured in the substrate and the amount of particulate COD remaining when the inert COD (X_I) was accounted for (see Table 6-2). In terms of inorganic fractions, inorganic carbon (S_{IC}) was approximated from total alkalinity, while inorganic nitrogen (S_{IN}) was assumed to be equal to the NH_4-N concentrations (Hierholtzer & Akunna, 2012). The cation (S_{cat}) and anion (S_{an}) concentrations were determined as per Poggio et al. (2016) by letting either S_{cat} or S_{an} concentration to be zero and calculating the corresponding anion or cation concentrations from the total charge balance determined from the equation below.

6-1 $$S_{CAT}+S_{NH4}+S_H-S_{AN}-\frac{S_{va}}{208}-\frac{S_{bu}}{160}-\frac{S_{pro}}{112}-\frac{S_{ac}}{64}-S_{HCO3}-S_{OH}=0$$

Whichever S_{cat} or S_{an} concentration was calculated as being positive was the used in the model, with the other charge then set to 0. FW and PM were characterised separately, with the specific concentration of each model input (except for S_I and X_I) calculated based on the wet weight mixing ratio of the substrates and the composition of each substrate. As the PM/FW mixing ratio has synergistic effects on combined substrates Fd, the Fd used for calculating the S_I and X_I of PM and FW separately was taken from the Fd value determined at the mixing ratio simulated (rather than using the Fd measured for PM and FW when digested alone). Due to the composition of FW used varying from day to day, average FW compositions were used to provide a more general approximation of FW composition for the model input. The PM composition used as a model input was altered as the composition of the PM fed to the digester changed (see Table 6-1).

Table 6-2 Summary of calculation of model inputs used for execution of ADM 1 model (Arnell et al., 2016)

Model Input	Description	Calculation Method
S_{su}	monosaccharides (kg COD/m^3)	% Carb.*(Soluble COD-($\sum$ Sva,Sbu,Spro,Sac)-Si)
S_{aa}	amino acids (kg COD/m^3)	% Protein*(Soluble COD-($\sum$ Sva,Sbu,Spro,Sac)-Si)
S_{fa}	long chain fatty acid (kg COD/m^3)	% Lipids*(Soluble COD-($\sum$ Sva,Sbu,Spro,Sac)-Si)
S_{va}	total valerate (kg COD/m^3)	$\left(\frac{\text{Total valerate (g/L HAc eq)}}{60}\right)$*64
S_{bu}	total butyrate (kg COD/m^3)	$\left(\frac{\text{Total butyrate (g/L HAc eq)}}{60}\right)$*64
S_{pro}	total propionate (kg COD/m^3)	$\left(\frac{\text{Total propionate (g/L HAc eq)}}{60}\right)$*64
S_{ac}	total acetate (kg COD/m^3)	$\left(\frac{\text{Total acetate (g/L HAc eq)}}{60}\right)$*64
S_{IC}	inorganic carbon (kmole C/m^3)	Alkalinity (g/L HCO_3^-) *61
S_{IN}	inorganic nitrogen (kmole N/m^3)	NH_4N (g/L N) *14
S_I	soluble inerts (kg COD/m^3)	(1-Fd)*Soluble COD
X_{ch}	carbohydrates (kg COD/m^3)	% Carbohydrates*((Total COD-Soluble COD)-Xi)
X_{pr}	proteins (kg COD/m^3)	% Proteins*((Total COD-Soluble COD)-Xi)
X_{li}	lipids (kg COD/m^3)	% Lipids*((Total COD-Soluble COD)-Xi)
X_I	particulate inerts (kg COD/m^3)	(1-Fd)*(Total COD-Soluble COD)
S_{cat}	cations (base) (kmole/m^3)	Calculated as per Poggio et al. (2016)
S_{an}	anions (acid) (kmole/m^3)	Calculated as per (Poggio et al. (2016))

Initial conditions were determined in a similar manner, using the total COD, soluble COD, VFA alkalinity, NH_4-N and pH of the inoculum. However instead of

distribution of the non-inert particulate COD to carbohydrate, proteins and lipids, it was equally distributed among the inputs designated to represent active microbial biomass (X_{su}, X_{aa}, X_{fa}, X_{c4}, X_{pro}, X_{ac}, X_{h2}).

The model was validated using data generated from the validation phase of the meso-scale experiment, and further validated using data generated from a lab-scale experiment presented in Chapter 5. The data from Chapter 5 was converted into model inputs in a similar manner as the meso-scale data, however as no characterisation of substrate carbohydrate, lipid and protein contents was undertaken, the carbohydrate, lipid and protein content of PM and FW were assumed to be equal to the average values measured for the PM and FW samples measured in the meso-scale experiment.

The mean absolute percentage error (MAPE) was calculated for each parameter in order to assess model fit to the empirical data. Calculation of MAPE is undertaken using the following formula (Tofallis, 2015);

6-2 $$\boldsymbol{MAPE = \frac{100}{n}\sum_{t=1}^{n}\left|\frac{A_t - S_t}{A_t}\right|}$$

Where n is the number of measured data points in the parameter set, A_t is the measured parameter value and S_t is the simulated parameter value. MAPE is a useful measure of model accuracy as it presents the differences between the simulation and measured values (i.e. the error) in the context of the absolute values of the parameters.

In addition, the precision of the model's simulation of each parameter was assessed by calculating using the Nash-Sutcliffe model efficiency coefficient, described by Koch et al. (2010) as;

6-3 $$\boldsymbol{E = 1 - \frac{\sum_{i=1}^{n}|X_m - X_s|}{\sum_{i=1}^{n}|\bar{X}_m - X_s|}}$$

where X_m and X_s are measured and simulated values respectively, and $\bar{X}_m$ is the average of all measurements.

6.3 Results

Figure 6-2 illustrates that OLR (1.6 kg VS/m^3/d) and feedstock composition (55 % FW on a VS basis), were relatively stable from day 0 to day 57. From day 57, OLR was increased to 2.4 kg VS/m^3/d, and the feedstock composition altered (to 35 % FW), due to changes in the PM composition (higher VS content). From day 79 to

91, all feeding was stopped in order to assess the accuracy of the model when simulating physical and chemical effects of extended reactor detention time. From day 92 feeding was restarted, with the OLR at 2.3 kg VS/m^3/d and feedstock composition at 55 % FW. The changes in OLR and PM/FW mixing ratio were undertaken in order to ensure the model could simulate the effects of changes to influent substrate composition. Figure 6-3 illustrates the model simulation and the measured digester pH, biogas flow, alkalinity, biogas composition and VFA concentrations while the OLR and % FW in the feedstock varied. MAPE was used to assess model fit, while Nash-Sutcliffe coefficients were used to assess the precision of the calibrated model to the data measured. The output of this analysis is presented in Table 6-3

Table 6-3 Nash-Sutcliffe coefficients and MAPE values for the model fit from meso-scale pH, NH_4-N, alkalinity biogas flow, % CH_4 in biogas, acetic acid, propionic acid and butyric acid concentrations.

	Nash-Sutcliffe coefficient	MAPE
pH	-0.337	1.47 %
NH_4-N	-0.133	14.43 %
Alkalinity	-0.228	7.78 %
Biogas flow	-0.139	23.28 %
% CH_4 in biogas	-0.551	8.38 %
Acetic acid	-0.349	53.18 %
Propionic acid	-0.013	66.02 %
Butyric acid	-0.156	88.48 %

6.3.1 Digester and model performance

6.3.1.1 % CH_4 and pH

Aside from a reduction between day 57 and 79 (when OLR increased), pH values (Figure 6-3 (d)) fluctuated around 7.5 throughout the study. Similarly, % CH_4 in the biogas (Figure 6-3 (c)) was not greatly affected by changes in OLR or substrate composition, remaining approximately 65% throughout the phase. However the weekly feeding regime (5 days feeding, 2 days no feed) clearly had a great impact on both pH and biogas CH_4 content, with values for both reaching a maximum at the beginning of the week (after 2 days without feeding), and then declining after 5 days of consecutive days of feed by as much as 0.3 pH units and 20 % respectively. The calibrated model appeared to provide a generally accurate simulation of the measured pH and % CH_4 values, and was able to account for changes in both OLR

and substrate mixing ratio. This is reflected in the relatively low MAPE values presented in Table 6-3. However it failed to simulate the magnitude of the effect of the feeding regime on observed values, indicating the model may overestimate the pH buffering capacity present in the system. The negative Nash-Sutcliffe coefficients calculated suggest the model does not fit the data with precision. In particular such values indicate that the ADM1 output for pH and % CH_4 provided a less precise simulation than the mean of the measured data.

The buffering capacity of the system calculated by the model is determined by the charge balance. This in turn is determined from by the balance of the concentrations of pH, NH_4-N, alkalinity and anions, and pOH, VFAs and other cations. As total anions and cation concentrations were not empirically determined in this study, but approximately determined by Equation 6-1, as per Poggio et al. (2016), they may not reflect accurately the true system change balance. Further to this, any inaccuracy in the simulation of VFA, alkalinity etc. will negatively impact the simulation of pH in the system. This ultimately may have contributed to the model not simulating the weekly fluctuations in pH and % CH_4 observed.

6.3.1.2 Biogas flow

The pattern of biogas flow appeared to be somewhat accurately simulated by the calibrated model (Figure 6-3 (f)), even as OLR and substrate mixing ratio varied. The increase in OLR at day 56, and the cessation of feeding on day 90 had clear effects on the biogas flow, as observed experimentally and in the model simulation. The biogas flow data also illustrates that the 5 day on, 2 day off feeding regime resulted in a characteristic biogas flow pattern; the lowest in the beginning of the week and the highest after 5 days of feeding.

However the MAPE of biogas flow was calculated as 23.28 % (higher than all other parameters excluding VFAs), and the Nash-Sutcliffe coefficient of -0.138 indicates the simulation lacked precision. The higher MAPE may be associated with the model not simulating the highest biogas flows measured each week between day 25 and 58, and not simulating the lowest values measured weekly from days 63 to 74 and from days 105 to 119. In a similar manner to pH and % CH_4 simulation, the modelled values could not simulate the weekly fluctuations due to the feeding regimes with a high degree of accuracy. The reason for this may be related to the simulation of VFAs. VFA concentrations affect the level of VFA inhibition simulated by the model, and also affects pH (by changing the charge

balance), which in turn affect % CH_4 and biogas flow rates (the model pH inhibition function means that as pH deviates from 7, methanogens become partially inhibited (Batstone et al., 2002)).

6.3.1.3 VFAs

The model output generally simulated the trends measured in acetic acid concentrations well (Figure 6-3 (e)); a gradual decrease in concentrations from day 0 to day 53, followed by an increase in concentrations as OLRs increased from day 54 to 73. Concentrations varied between 3 g/L and 5 g/L HAc_{eq}. Table 6-3 illustrates that the MAPE for acetic acid was high (53.18 %), and the Nash-Sutcliffe coefficient calculated was -0.349. These values indicate that despite the model providing a simulation which tracked the general trends in acetic acid concentration, the precision of the model fit was low.

Figure 6-3 (e) also illustrates that the model did not accurately simulate the changes in propionic and butyric acids observed during this experiment, with high MAPE values of 66.29 % and 88.48 % calculated for each, respectively. The cause of the low accuracy of VFA simulation may be the default kinetic parameters used in the Rosen et al. (2006) programmed ADM1 model, which do not accurately simulate either the acidogenic or acetogenic reaction pathways which occurred when PM and FW were co-digested. The substrate uptake rates for propionate and butyrate degraders may be overestimated, resulting in a simulated immediate utilization of these VFAs rather than a slight level of accumulation, as observed in the data. For example, the rate of propionic acid or butyric acid uptake is governed by the following Monod type kinetic equation:

6-4 $$k_m \frac{S_{bu}}{K_s+S_{bu}} X_{c4} \frac{1}{1+S_{va}/S_{bu}} I$$

Where k_m is the maximum substrate uptake rate (kgCOD/m^3S/kgCOD/m^3X/day), K_s is the Monod half saturation constant (kgCOD/m^3), S_{bu} is the butyric acid concentration (kgCOD/m^3), X_{c4} is the concentration of butyric and valeric acid utilizing bacteria(kgCOD/m^3), S_{va} is the valeric acid concentration (kgCOD/m^3) and I is the inhibition factor for the process (based on H_2 and pH concentrations). If the value for k_m is overestimated then the concertation of butyric acid observed in the system decreases as it is rapidly utilised, rather than having a higher steady state concentration in the reactor.

The pathways of acidogenesis simulated by the model may not have reflected the speciation of the products of sugar and amino acid acidogenesis particular to PM and FW co-digestion (Batstone et al., 2002). The model factor which determines this is $f_{product,\ substrate}$, the yield of product from substrate. For example the biochemical rate coefficient for the yield of butyric acid from amino acid is determined by the following equation(Batstone et al., 2002):

6-5 $$(1 - Y_{aa})f_{bu,aa}$$

Where Y_{aa} is the yield of biomass from amino acid uptake (kgCOD/m^{3}S/kgCOD/m^{3}X/day) and $f_{bu,\ aa}$ is the yield of butyric acid from amino acid (kgCOD/kgCOD). Calibrating the product yield factor $f_{product,\ substrate}$ for all products of hydrolysis would clearly improve the accuracy of the model, not only when simulating VFAs, but when simulating all subsequent process outputs. Both batch and semi-continuous lab-scale studies (Chapter 3, 4 and 5) have illustrated that non-acetate VFAs were a key intermediates in methane production from PM and FW. Optimisation of $f_{product,\ substrate}$ in the ADM1 model would be required to reflect this.

6.3.1.4 NH_4-N

From day 0 to day 80 NH_4-N concentrations appeared to be accurately simulated (Figure 6-3 (a)). This is reflected in the low MAPE value 14.43 %. This low valve was calculated in spite of NH_4-N concentrations being overestimated by approximately 30 % from day 95 onwards. The cause of the overestimation in concentrations from day 85 onwards is unclear but may be due to changes in substrate protein or ammonium concentrations were not identified during substrate characterisation carried out during this period (day 85 to 119).

6.3.1.5 Alkalinity

Alkalinity increased in concentration from 11038 mg/L on day 25 to 14000 mg/L on day 119 (Figure 6-3 (b)). The calibrated model provided an accurate simulation of the concentrations throughout the experiment, as evidenced by the low MAPE value (7.78 %) presented in Table 6-3. However, between day 56 and 80, the measured and simulated alkalinity concentrations diverged, with measured concentration increasing and simulated concentrations decreasing. This coincided with the occurrence of a new batch of PM in the experiment and therefore the differences between modelled and measured values may have been due to

non-representative measurement of the inorganic carbon content (alkalinity) of the PM batch used in this period (see Table 6-1).

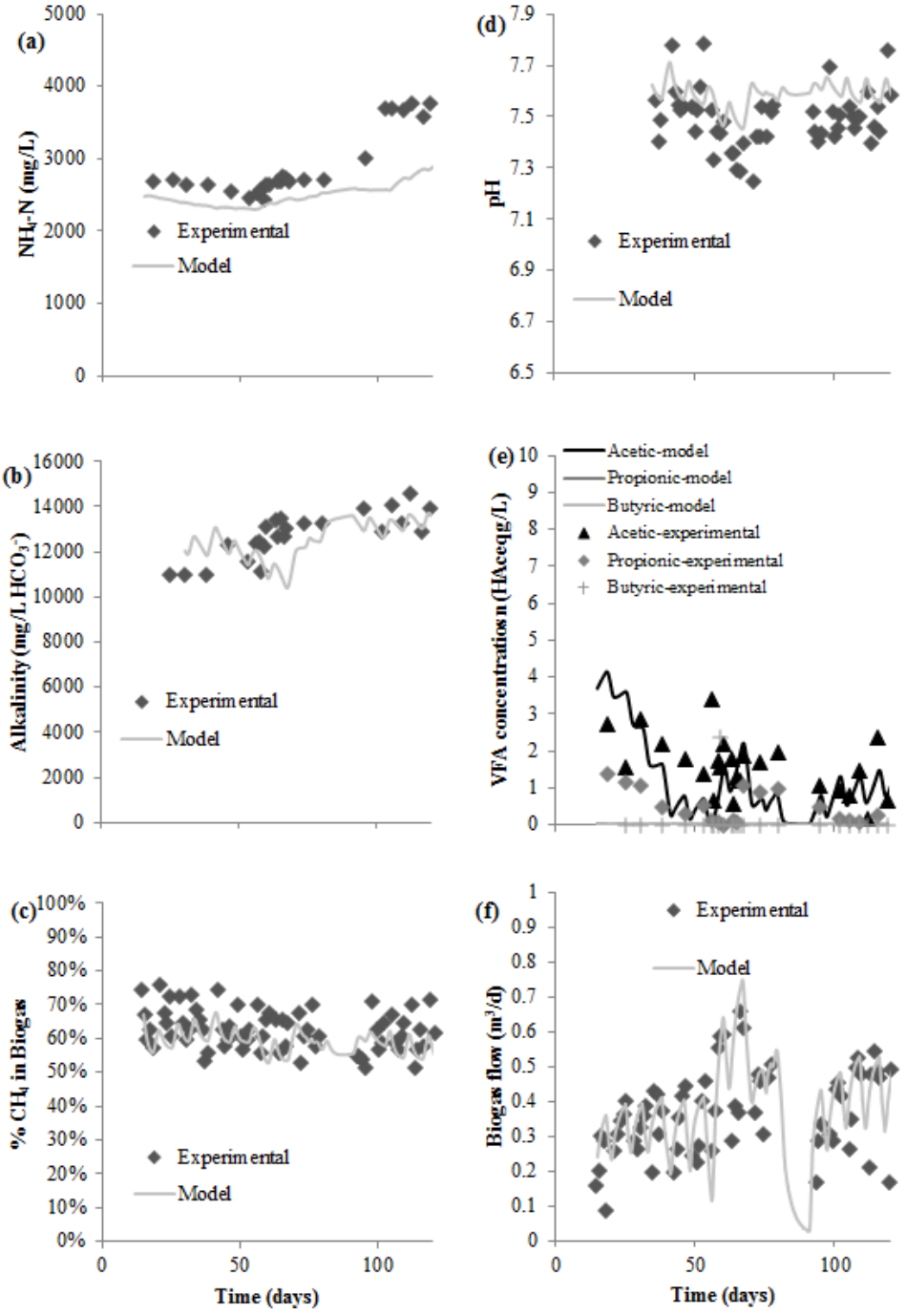


Figure 6-3 Measured and ADM1-simulated NH_4-N (a), pH (b), Alkalinity (c), VFAs (d), % CH_4 in biogas (e) and biogas flow (f) from meso-scale PM and FW co-digestion

6.3.2 Further model validation

Compared to this study, Poggio et al. (2016) achieved a similar level of model accuracy when a similar, simply calibrated iteration of the ADM1 model was used to simulate the digestion of a range of solid wastes.

However, several deficiencies in the ADM1 simulation have been identified. The VFA concentrations were not accurately simulated by the model, which in turn may have resulted in the inaccurate simulation of the weekly fluctuations in pH, % CH_4 and biogas flow. Therefore the model was further validated using the data generated from the lab-scale co-digestion described in Chapter 5. This is presented in Figure 6-4. Table 6-4 presents the Nash-Sutcliffe and MAPE values calculated for the model fit of this data set.

Table 6-4 Nash-Sutcliffe coefficients and MAPE values for the model fit on data from Chapter 5 and for pH, NH_4-N, alkalinity biogas flow, % CH_4 in biogas, acetic acid, propionic acid and butyric acid concentrations.

	Nash-Sutcliffe coefficient	MAPE
pH	-1.941	3.61 %
NH_4-N	-0.526	7.11 %
Alkalinity	-0.574	10.99 %
Biogas flow	-0.035	42.64 %
% CH_4 in biogas	-3.230	18.64 %
Acetic acid	-0.609	62.25 %
Propionic acid	-0.055	152.32 %
Butyric acids	-0.116	104.41 %

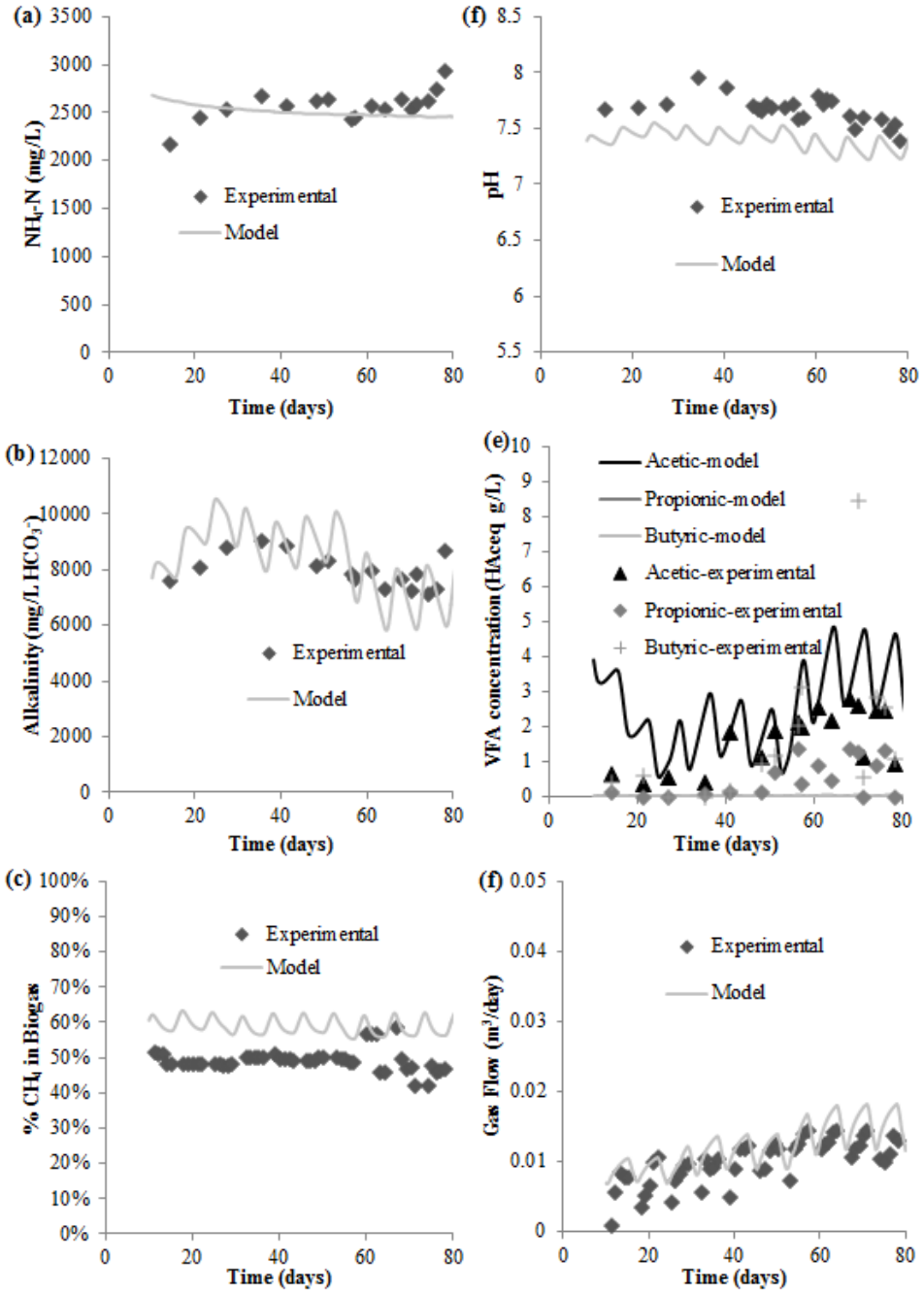


Figure 6-4 Validation of ADM1 model used in experiment with data from Chapter 5. Measured and ADM1-simulated NH_4-N (a), pH (b), Alkalinity (c), VFAs (d), % CH_4 in biogas (e) and biogas flow (f).

Figure 6-4 illustrates that, again, the calibrated model appeared to provide an accurate approximation of most of the measured parameters. The MAPE values and Nash-Sutcliffe values presented in Table 6-4 illustrate that similar trends in terms of model fit to each parameter occur; pH, % CH_4, alkalinity and ammonia

generate MAPEs of >20 %, while biogas flow, despite apparent satisfactory fit from Figure 6-4 (f) generated an MAPE of 64.42 %. VFAs again were poorly simulated in terms of MAPEs. This supports the reasoning that the lower accuracy observed for these parameters was likely due to inherent model factors (i.e. the use default substrate utilization and inhibition rates).

It should be noted that despite the low MAPE values, % CH_4 in biogas was overestimated by between 5 % and 10 % throughout the simulation, while pH was underestimated by between 0.2 and 0.55 pH units throughout. Further to this, the simulation of biogas flow and VFAs from the data from Chapter 5 generated MAPEs which were far lower than those measured from meso-scale simulation.

The reason for the lower degree of accuracy of the model when applied to the data generated in Chapter 5 may be due to the less detailed substrate characterisation data available in this instance. The carbohydrate, lipid and protein concentrations of the PM and FW used were assumed to be equal to the average values measured for the PM and FW samples used in the meso-scale digester. Therefore determination of subsequent hydrolysis, acidogenic and acetogenic products would not be precise, and would affect simulated VFA and biogas values, but also NH_4-N, alkalinity, pH and % CH_4 values.

6.4 Summary

This study illustrates that ADM1 model, even when calibrated in a rudimentary manner, can provide a generally accurate simulation of reactor performance. However, a low level of precision was achieved particularly when VFAs were simulated, which can limit it efficacy in predicting process stability. Therefore, a greater level of system calibration is merited when modelling systems which are highly buffered, and treat a diverse substrate composition (such as PM/FW co-digestion).

In particular, this study highlights the importance in calibrating the rates and product yields of acidogenesis in order to accurately simulate VFA concentrations and speciation.

CHAPTER 7

Stochastic modelling of the economic viability of on-farm co-digestion of pig manure and food waste in Ireland

This chapter details the results of financial modelling undertaken to assess the economic viability of on-farm co-digestion of FW and PM in Ireland. The contents of this chapter are currently under review for publication in the scientific journal Applied Energy.

7.1 Introduction

Several studies have assessed the financial viability of agriculture-based biogas plants. Some of these utilise generalised conceptual scenarios to develop deterministic financial models which identify the potential viability of utilizing specific co-substrates (sugar beet (Boldrin et al., 2016), energy crops (Agostini et al., 2016), olive mill waste (Orive et al., 2016)), specific digester sizes (Walla & Schneeberger, 2008) or specific biogas utilization regimes (Blokhina et al., 2011). Other studies have modelled the financial viability of specific plants (De Clercq et al., 2017), thereby providing guidance for improved operation and design of similar plants. Few of these studies have assessed the potential effects of changes in key market variables on viability of biogas plants. Such analysis is crucial when considering novel biogas plant concepts. The use of stochastic models which can account for the potential variation in key model inputs across estimated or known probability distributions can allow for identification of the most sensitive system inputs, as well as providing an assessment of the overall risk associated with a proposed plant (Hertz & Thomas, 1983; Van Groenendaal & Kleijnen, 1997). In an Irish context few studies have undertaken an in-depth analysis of the concept of on-farm biogas plants (Nolan et al., 2012), and no stochastic analysis of the viability of on-farm biogas plants has been undertaken.

The objectives of this study were to assess the financial viability of on-farm biogas plants co-digesting FW and PM. In particular, the study aimed to

1. Identify and quantify the key revenue streams, CAPEX and OPEX costs associated with mono- and co-digestion.

2. Assess the current financial viability of PM/FW co-digestion and PM mono-digestion plants using a deterministic model.
3. Present a methodology which can assess the sensitivity of overall profitability of co-digestion plants to changes in key revenue streams and operational expenses using stochastic modelling.

7.2 Materials and Methods

7.2.1 Description of Scenarios

Six scenarios were used to assess the effect of farm size and either mono- or co-digestion on project feasibility. The scenarios comprised three hypothetical farm sizes with digester tank volume based on the utilisation of the PM generated and assuming operation of the digester at a HRT of 50 days (1500 m^3, 7500 m^3 and 15,000 m^3 respectively). This corresponded to three farm sizes of 521, 2607 and 5214 sows. This is illustrated in Table 7-1. These three scales were chosen in order to represent a wide spectrum of potential farm sizes in Ireland, from average (Nolan et al., 2012), large and large co-located farms. Each farm was then assumed to either operate with a biogas plant treating manure only (mono-digestion; scenarios m1, m2 and m3), or treating manure along with source segregated FW (co-digestion; scenarios c1, c2 and c3). All scenarios utilized biogas via CHP generation.

Table 7-1 Digester capacity, farm size and manure volumes available for scenarios c1, c2, c3, m1, m2 and m3.

Scenario	c1	c2	c3	m1	m2	m3	Comments
Digester Size (m^3)	1500	7500	15000	1500	7500	15000	
Farm Size (no. of sows)	521	2607	5214	521	2607	5214	
Annual Manure Available (t)	10950	54750	109500	10950	54750	109500	21 m^3/sow and progeny/year (McCutcheon, 1997)

The most commonly used digester configuration for on-farm biogas plants in Ireland was applied to this study (McEniry, 2016); mesophilic digestion (at 42 ºC)

comprised of two tanks in series, each with a HRT of 25 days, resulting in an effective HRT of 50 days. Such reactors are typically limited to operating with a feedstock comprised of 15 % to 20 % total solids (Dong et al., 2010). This limits the amount of FW which can be co-digested with manure to approximately 30 % on a fresh weight basis. The digestate storage was comprised of lagoons with 6 months storage capacity. For the mono-digestion scenario it was assumed that no substrate reception, feedstock maceration facilities or additional civil works such as separate biogas plant entrances were required, with manure being pumped directly from beneath the pig unit to the digester equalisation tanks. Additionally, no digestate pasteurization facilities were included in the mono-digestion scenario. Figure 7-1 schematically illustrates the differences between the mono-and co-digestion configurations.

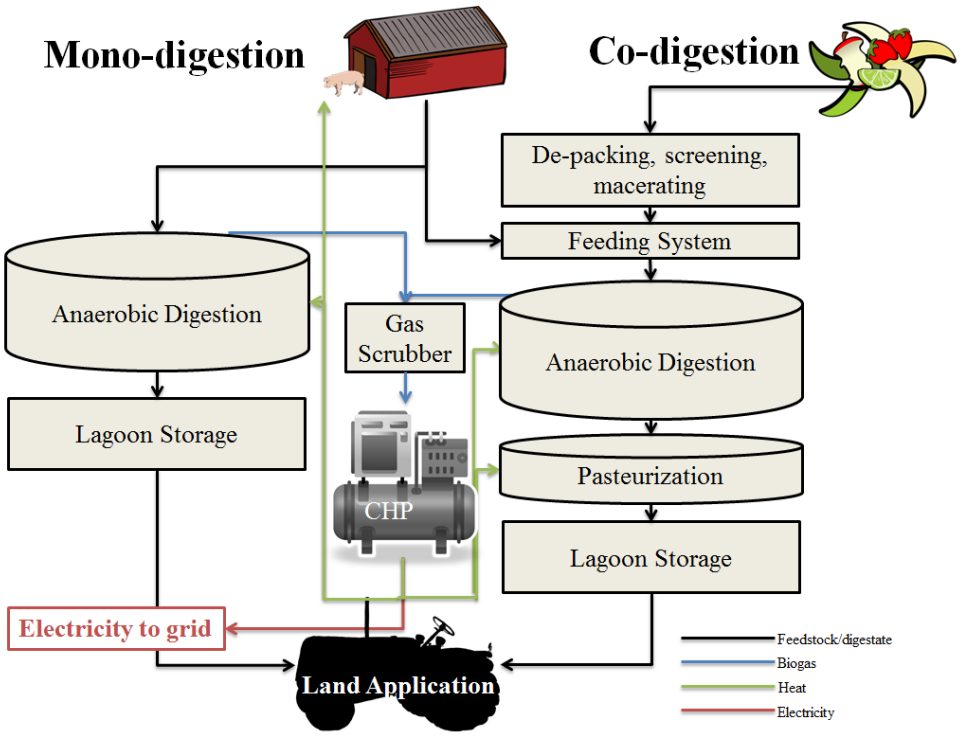


Figure 7-1 Schematic of material and energy flows of mono- and co-digestion scenarios of pig manure

7.2.2 Financial Model

In order to assess the feasibility of each scenario, a financial model was developed and executed using Microsoft Excel 2010. A detailed overview of the deterministic model for each scenario analysed (m1, m2, m3, c1, c2, c3) can be found in Appendix D.

7.2.2.1 Expenditure

The CAPEX and OPEX costs associated with each scenario were calculated based on the costs associated with biogas plants currently in operation and in development in Ireland, provided by operators and engineering firms (Cormack, 2016; Lenehan, 2016; McEniry, 2016; Nolan et al., 2012). Details of CAPEX and OPEX assumptions and calculations can be found in Table 7-4 and Table 7-5, respectively.

In terms of CAPEX, digester construction, lagoon construction and additional site works were calculated on m^3 of reactor tank size basis. Additional site civil works refers to site clearance, roads, utilities and any ancillary building works. CAPEX for feedstock reception and pre-treatment, as well as additional pasteurization infrastructure required for co-digestion were calculated on a per tonne of co-substrate treated per day basis. The CAPEX associated with the CHP unit was calculated on a per kW of installed electrical capacity basis. The remainder of the subtotal CAPEX costs (grid connections, pipeworks, heat recovery) were deemed to be fixed and were not varied according to plant size. Finally an additional 5 % for development costs (costs associated with engaging development consultants and undertaking administration), 4 % for insurance during the build, 10 % for contingency and 7 % for engineering costs (additional onsite engineering) were included in the final total CAPEX figures.

In terms of annual OPEX, plant operation and maintenance (O&M; including maintenance of pumps, pipes, macerators, feedstock and digestate storage, biogas storage and control systems), digester maintenance (specifically digester tank and mixing system) and site labour were calculated on a m^3 of reactor volume basis. Plant O&M and labour costs for mono-digestion systems were assumed to be half of that associated with co-digestion due to the simpler plant design and lack of feedstock reception area, macerator and pasteurization maintenance. Electricity costs were calculated per tonne of feedstock treated. Again due to the simpler plant design, electricity costs for mono-digestion were assumed to be half of the value associated with co-digestion (McEniry, 2016). The cost of CHP maintenance was calculated based on the annual hours of operation, the metric commonly used to determine the price of service contracts offered by CHP providers (McEniry, 2016). Due to the geographic and temporal variability associated with digestate disposal costs, this variable underwent stochastic analysis. The baseline (i.e. most likely value under current market conditions) disposal estimate presented in Figure 7-5 is

taken from estimates of typical manure spreading costs on Irish farms transporting manure <10 km from farm of €4/ t, increasing to €7/ t for every tonne above 5,000 t generated per annum, thereby accounting for increased disposal distances and costs (McCutcheon & Lynch, 2008).

Note that the OPEX figures used account for reinvestment costs associated with CHP units, pasteurizers and feedstock reception equipment. In addition to the operation and maintenance of each plant, the gross OPEX accounted for straight-line depreciation (a linear decrease in initial value) of CAPEX over its 15 year lifespan to a salvage value of 10 %, interest on the loan for CAPEX (6 % of principle over a 15 year period, as per Nolan et al. (2012)) and insurance of 0.5 % on the initial CAPEX (see Appendix D).

7.2.2.2 Revenue

This model calculated the net energy yield from the biogas plant (and CHP unit) in terms of both electricity and heat. It then calculated the revenue generated from electricity sales, the use of heat to displace other heat sources on farms and gate fees (for co-digestion scenarios). Table 7-6 presents an overview of how baseline (i.e. most likely scenario under current market conditions) revenues were calculated.

7.2.2.2.1 Methane yields, electricity generation and heat utilization

The gross energy yield was determined, in part, by the SMY of PM and mixtures of PM and FW. The SMYs and PM and FW TS/VS compositions used were taken from Chapter 3. As illustrated in Chapter 3, co-digesting FW and PM can result in synergistic effects which can increase SMYs by up to 20% compared with mono-digestion of each substrate alone, but was dependent on the mixing ratio of PM and FW. These synergistic effects were accounted for as follows; the mixing ratio between PM and FW in the digester was calculated by assuming that the annual amount of FW received was fed evenly throughout the year to the digester, with the remainder of the feed comprised of PM. Based on this mixing ratio, the SMY was taken from Chapter 3. A 10 % safety factor was applied in order to account for the fact that the SMYs measured in batch scale trials are typically higher than the SMYs realised in full scale operation (due to leaks or variability in substrate composition etc.). The effect of HRT was accounted for by deriving the theoretical biomethane potential and the fraction of degradable substrate from the

SMY and hydrolysis rate data provided in Chapter 3. This derivation is presented in Table 7-2.

Table 7-2 Calculation of specific methane yields of pig manure and food waste

PM/FW mixing ratio (w/w basis)	100.0 %	95.5 %	88.7 %	77.8 %	56.8 %	0.0 %
Experimental SMY	243.2	302.4	425.8	473.1	509.4	508.1
Theoretical BMP[a]	504.5	509.2	516.1	527.5	549.2	607.9
Theoretical Fd[a]	0.48	0.59	0.82	0.90	0.93	0.84
Fd at HRT 50[c]	0.45	0.56	0.78	0.85	0.88	0.80
SMY at HRT 50 days	227.1	283.9	403.3	450.0	484.6	483.7
SMY-10% safety factor	204.4	255.5	362.9	405.0	436.1	435.3

[a]As calculated in Chapter 3[b]Estimated based on the theoretical Fd and the first-order hydrolysis rate constant as described in Equation 2-9

It was assumed that the plant would have 10 % downtime throughout the year (Nolan et al., 2012). The CHP unit was assumed to operate with 40 % electrical efficiency and 45 % thermal efficiency (Nolan et al., 2012) (see Appendix D).

In Ireland, the renewable energy feed-in tariff (REFIT) is the mechanism by which renewable energy generation is promoted. For biogas plants with an installed electric generating capacity ≤ 0.5 MWe, the REFIT is €0.15/kWh, and for plants with a capacity > 0.5 MWe, the REFIT is €0.13 /kWh. The REFIT has been the subject of ongoing lobbying and discussion since its inception, with critics highlighting the disparity in price offered to biogas plant operators compared to the equivalent rate offered to UK biogas plants (up to €0.27 /kWh) (Nolan et al., 2012). It was therefore the subject of stochastic modelling.

Pig farms in Ireland require a heat source to provide heat to pig houses (particularly farrowing rooms and post-weaning accommodation). This demand has been estimated to be ~728 kWh /sow /year (McCutcheon, 2012). Such heat demand can be met via onsite biogas generation and utilization. Doing so would provide a means of using the heat generated by CHP co-generation as well as generating revenue by replacing oil fired boilers often used to provide heat on pig farms. The net heat available for use by farms was determined by calculating and subtracting the digester and pasteurisation heating requirement from the total heat generated. The heat requirement was calculated as described by Nolan et al. (2012). In

particular, the daily parasitic heat demand of the digester was determined from Equation 7-1;

7-1 $$\boldsymbol{Q = M * C * \Delta T_1 + H}$$

where *Q* is the daily parasitic heat demand (kJ /day), *M* is the daily mass of substrate treated (kg/day), *C* is specific heat of substrate (assumed to be equal to that of water = 4.18 kJ kg /°C), ΔT_1 is the temperature difference between the substrate (42 °C) and the ambient temperature (taken as 9 °C in the present study) and *H* is the heat loss through the digester surface (kJ /day). *H* was determined using Equation 7-2;

7-2 $$\boldsymbol{H = U * A * \Delta T_2 * 86.4}$$

where U is heat transfer coefficient of the insulation material (rockwool, 0.34 J /s m^2 °C), *A* is the surface area of the digester (m^2) and ΔT_2 is the difference between the internal (42 °C) and external (9 °C) temperatures. The same method was used to determine the parasitic heat requirement for pasteurisation of treated substrate, which required heating of digestate from 42 °C to 70 °C.

Subsequent to this the amount of revenue generated from displacing oil purchase was calculated by selecting an oil price of €0.5 /L (Nolan et al., 2012) which is similar to the current market price of heating oil in Ireland, and calculating the volume of oil that would have been required assuming a boiler with 80 % thermal efficiency was used (with the gross energy of heating oil given a value of 10 kWh/L) (Nolan et al., 2012).

It should be noted that due to the typically rural location of pig farms it was assumed that, aside from on-farm use, there was no demand for the use of heat in a local district heating network. As the heat generated was to be used on the farms where the biogas plant was co-located only, the costs associated with heat distribution in all scenarios were assumed to be equal (i.e. no increase in distance of district heating network required as scale increased).

7.2.2.2.2 Food waste and gate fees

The amount of FW received by co-digestion plants on an annual and indeed monthly basis can vary significantly. In 2015, 255000 t of source segregated FW (comprised of municipal waste from households and commercial premises, and food and beverage manufacturing) was accepted for treatment by compost and AD facilities (EPA Ireland, 2016a). 20 % of this was treated by AD (51000 t), with the

remainder treated via composting (EPA Ireland, 2016a). There are currently 6 biogas plants and 15 composting plants in operation in Ireland (Irish Department of Agriculture Food and the Marine, 2016c). Therefore, on average each biogas plant treats 8500 t of FW per year (however, this would be supplemented with wastewater treatment sludge, manure and other animal byproducts (EPA Ireland, 2016a)). As FW is a finite resource we assume that 8500 t of FW per annum is available for co-digestion in scenarios c2 and c3 and this was used to define the baseline methane yields presented in Table 7-6. A value of 3000 t was used for scenario c1 as, rather than being limited by the availability of FW, the amount taken in by such a scenario is limited by the potential TS of the combined PM/FW feedstock; this must remain below c. 15 % for the correct function of the reactor configuration used. Therefore 3000 t/year was determined to be the most likely FW amount collected in scenario c1. It should be noted that, while the baseline scenarios for c2 and c3 are set at the average amount received by Irish co-digestion plants currently (8500 t), each plant has scope to significantly increase the amount it can treat, depending on availability of FW. The effect of this on the value proposition of each scenario will be quantified through stochastic analysis.

In order to discourage landfilling of organic waste in particular (thereby meeting national targets set out in the EU Landfill directive), Ireland has a landfill levy in place which means that disposal of FW via landfill currently costs €75 /t. Therefore, AD plants receiving source segregated FW normally charge gate fees for treating FW. Due to the competitive nature of the Irish waste disposal industry, the value of gate fees may vary significantly. Again, average gate fees provided by plant operators (Cormack, 2016; McEniry, 2016; Nolan et al., 2012) and values cited by other European studies (Nghiem et al., 2017) were used to define the baseline revenues presented in Table 7-6. An average gate fee for FW of €30 /t was assumed.

7.2.3 Financial Metrics

In order to assess the viability of each scenario, a set of financial indicators were used. In order to account for the payback of CAPEX, the cash flow based on OPEX, revenue, and the future value of current capital and cash flow (in consideration of the discount rate), the NPV of the biogas plant over a 15 year lifespan was calculated as described by Equation 2-15. The internal rate of return (IRR) is the return on investment (ROI) required for the project to overcome the reduction in value of the capital invested in the project i.e. the discount rate at which NPV after

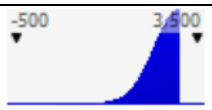
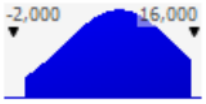
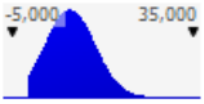
n years becomes zero, as described by Equation 2-16. The payback period is the time required for the NPV to reach zero. In this study the discount rate was set at 6 %, as suggested by Agostini et al. (2016). The IRR was calculated using the Solver function on Excel 2010 (Microsoft, USA). RoI was also used as a further financial metric calculated using Equation 2-14.

7.2.4 Monte Carlo simulation

While the deterministic model can assess the viability of each scenario under current market conditions, stochastic analysis completed can assess the viability of each scenario when the range in which each input can vary under future market conditions is considered.

In this study the effect of changes to four key independent variables on the 15 year NPV of the three co-digestion scenarios was assessed; REFIT, gate fees, annual amount of FW received, and digestate disposal costs. This was undertaken by varying each parameter across a range of values and then tracking its effects on 15 year NPV. Monte Carlo simulation was undertaken as described by De Clercq et al. (2017) using @Risk 7 software (Palisade, USA). Each input parameter was varied randomly across a specific range, with a defined distribution, 10000 times. Table 7-3 illustrates the distributions used.

Table 7-3 Distributions used for Monte Carlo simulation of co-digestion scenarios c1, c2 and c3

Scenario	Input	Graph	Min	Mean	Max
c1	Annual Food Waste Required (t)		0	2601	3000
	REFIT (€/KWh)		-∞	0.15	+∞
	Average FW Gate Fee (€/t)		-∞	30	+∞
	Digestate disposal costs (€/t)		-∞	4	+∞
c2	Annual Food Waste Required (t)		0	8049	15000
	REFIT (€/KWh)		-∞	0.13	+∞
	Average FW Gate Fee (€/t)		-∞	30	+∞
	Digestate disposal costs (€/t)		-∞	4	+∞
c3	Annual Food Waste Required (t)		0	8992	30000
	REFIT (€/KWh)		-∞	0.13	+∞
	Average FW Gate Fee (€/t)		-∞	30	+∞
	Digestate disposal costs (€/t)		-∞	4	+∞

A normal distribution with a mean of €0.15 /kWh for c1 and €0.13 /kWh for c2 and c3, (each with standard deviation of €0.03 /kWh) was chosen to simulate potential changes in REFIT in each scenario.

Gate fees and digestate disposal costs were also normally distributed, with means and standard deviations of €30 /t and €10 /t, and €4 /t and €1.5 /t, respectively. These ranges were selected to reflect the range across which these values were deemed likely to vary (i.e. worst and best case scenarios) (McEniry, 2016).

A normal distribution with a mean of 3000 t, and standard deviation of 500 t was used to simulate potential FW availability values for scenario c1. However, the distribution was truncated between 0 and 3000 t, due to the limited capacity of scenario c1 to receive FW. The standard deviation was chosen in order to reflect the strong likelihood that FW availability would not deviate significantly from 3000 t.

Normal distributions with a mean and standard deviation of 8500 and 5000 were chosen to reflect the potential changes in FW availability possible for scenarios c2 and c3. Distributions were truncated in order to reflect the maximum capacity of each plant (due to influent TS increasing above 15 %) and the minimum amount possible to be treated (0). Therefore the range in which values were simulated was between 0 and 15000 t for scenario c2 and between 0 and 30000 t for c3.

The impacts of changing these four parameters (all at the same time) within the ranges specified on the 15 year NPV were then tracked, and the resulting data were used to model how changes in each parameter may affect 15 year NPV.

7.3 Results

7.3.1 Capital and baseline operational costs

The CAPEX and baseline (i.e. most likely under current market conditions) OPEX figures used in this study for co-digestion systems (between €4,797 and €6,964 /kWe for CAPEX and between €724 and €1,021 /kWe/yr for OPEX) are similar to values previously published for manure-based co-digestion plants on a €/kWe basis (€3,680-€7,504 /kWe for CAPEX and €79-€1,421/ kWe/yr for OPEX (Blumenstein et al., 2016; Orive et al., 2016; Walla & Schneeberger, 2008)). Comparable costs

for manure mono-digestion systems were not found in the literature, presumably due to the low economic viability of such systems.

As Table 7-4 and Table 7-5 illustrate, co-digestion plants have slightly higher capital and operational costs when compared with mono-digestion systems. This is due to the ancillary feedstock infrastructure (fenced site, access roads, reception areas, macerators, feeding systems, and pasteurizers), stipulated by the EU Animal By -products Regulation (EC, 1069/2009), which must be complied with when FW is anaerobically digested.

These regulations require any biogas plants treating animal by-products to macerate feedstocks to a particle size of 20 mm and to heat all digestate to 70 ºC for 60 minutes prior to disposal via land application. In addition to these prescriptive treatment methods, the regulations require detailed record keeping and sampling, which contributed to the increased operational and capital costs.

A key, highly variable, factor in determining feasibility of biogas plants, in particular co-digestion plants, is the cost of digestate disposal. Table 7-5 illustrates that this is a major component of the OPEX of each digestion scenario. Digestate from biogas plants, particularly biogas plants treating manure and waste, is often not a preferred agricultural fertilizer due to the increased regulation and compliance required when land spreading (spreading limited to non-food crop land and requires lag time before stocking of land with livestock) , and a fee is therefore typically charged by farmers for its disposal. In addition to this, finding an available local land bank (i.e. an area where digestate can be applied so that N and P application, does not exceed plant growth requirements i.e. EU Nitrates Directive (91/676/EEC)) can be a major issue for larger biogas plants. Disposal at greater distances from the biogas plant has an associated cost. Indeed, in some areas where there is no suitable land for manure or digestate spreading (such as Brittany, France), manure may undergo solid liquid separation, followed by wastewater treatment of the liquid fraction, and composting (and subsequent long distance transport) of the solid fraction as a means of manure disposal (Béline et al., 2008). However, as such extreme situations do not occur in Ireland, due to its low relative animal density, it is assumed that all digestate can be transported off farm, in liquid form, and land spread.

Table 7-4 Calculated capital costs of biogas plants for each mono-digestion (m1, m2, m3) and co-digestion scenario (c1, c2, c3)

Scenario	c1	c2	c3	m1	m2	m3	Unit	Reference
CAPEX								
Digester construction (€)	300,000	1,500,000	3,000,000	300,000	1,500,000	3,000,000	€200/m^3 reactor	(Lenehan, 2016; McEniry, 2016; Nolan et al., 2012)
Digestate storage lagoons (€)	46,800	234,000	468,000	46,800	234,000	468,000	€31/m^3 reactor	(McEniry, 2016)
Additional site civil works (€)	207,000	1,035,000	2,070,000	57,000	285,000	570,000	€138/m^3 reactor co-digestion, €38/m^3 reactor mono-digestion	(Lenehan, 2016; McEniry, 2016; Nolan et al., 2012)
Feedstock reception building and infrastructure (€)	10,555	29,906	29,906	-	-	-	€1,284/t co-substrate treated/d	(McEniry, 2016)
Pasteurizer and pasteurizer heat exchangers (€)	17,466	49,486	49,486	-	-	-	€2,125/t co-substrate treated/d	(McEniry, 2016)
Manure pipework and pumps (€)	8,000	8,000	8,000	8,000	8,000	8,000		(Lenehan, 2016; McEniry, 2016; Nolan et al., 2012)
CHP Unit (€)	161,644	540,424	540,424	31,399	156,994	313,987	€600/kWe	(Lenehan, 2016; McEniry, 2016; Nolan et al., 2012)
Grid connection (€)	229,007	229,007	229,007	229,007	229,007	229,007		(McEniry, 2016; Nolan et al., 2012)
Heat recovery and distribution (€)	45,000	45,000	45,000	45,000	45,000	45,000		(McEniry, 2016)
Subtotal	1,025,521	3,671,067	6,618,310	717,206	2,458,001	4,633,994		
Development costs (€)	51,276	183,533	330,915	35,860	122,900	231,700	5% of subtotal	(McEniry, 2016)
Insurance (€)	41,021	146,843	264,732	28,688	98,320	185,360	4% of subtotal	(McEniry, 2016)
Contingency (€)	102,552	367,107	661,831	71,721	245,800	463,399	10% of subtotal	(McEniry, 2016)
Engineering (€)	71,786	256,975	463,282	50,204	172,060	324,380	7% of subtotal	(McEniry, 2016)
Total (€)	1,292,156	4,625,545	8,339,070	903,679	3,097,081	5,838,833		

Table 7-5 Calculated baseline operational costs of biogas plant for mono-digestion (m1, m2, m3) and co-digestion scenarios (c1, c2, c3)

Scenario	c1	c2	c3	m1	m2	m3	Unit		Reference
Baseline OPEX							*Co-digestion*	*Mono-digestion*	
Plant O+M (€/year)	6,000	30,000	60,000	3,000	15,000	30,000	€4/m^3 reactor /year	€2 /m^3 reactor /year	Extrapolated (McEniry, 2016)
Digester maintenance (€/year)	4,500	22,500	45,000	4,500	22,500	45,000	€3/m^3 reactor/year		Extrapolated (McEniry, 2016)
CHP maintenance (€/year)	63,072	63,072	63,072	63,072	63,072	63,072	€8/hour of operation[a]		(McEniry, 2016)
Labour (€/year)	15,300	76,500	153,000	7,200	36,000	72,000	€10/m^3 reactor /year[b]	€5/m^3 reactor /year [c]	(McEniry, 2016)
Electricity (€/year)	11,498	57,488	114,975	8,870	44,348	88,695	7 kWh/m^3 feedstock[d]	5.4 kWh/m^3 feedstock[d]	(McEniry, 2016; Nolan et al., 2012)
Admin (€/year)	35,000	35,000	35,000	10,000	10,000	10,000			Extrapolated (McEniry, 2016; Nolan et al., 2012)
Digestate disposal (€/year)	61,650	368,250	751,500	61,650	368,250	751,500	€4/t up to 5kt, €7/t thereafter		Based on (McEniry, 2016)
Total-Ex. , depreciation, insurance and interest (€/year)	*197,020*	*652,810*	*1,222,547*	158,292	559,170	1,060,267			

[a] Service contract rate [b] Assuming 0.34 labour units/1000m^3 capacity; 1 labour unit = 1 FT employee with €30,000 annual salary. [c] Assuming 0.16 labour units/1000m^3 capacity.[d] electricity cost of €0.15/KWh

7.3.2 Revenue

Table 7-6 presents an overview of the baseline revenue generated from electrical sales, oil for heat replacement and gate fees, using the most realistic assumptions on annual FW received and gate fees, for the biogas systems of each scenario. From the baseline calculation, it is clear that the majority (>70 %) of the revenue from co-digestion systems and all of the revenue from mono-digestion systems is generated by electricity sales. Electrical revenue is a function both of the amount of FW received by the plant annually and the REFIT.

By contributing between 14 % and 20 % of annual revenues, gate fees are also a significant revenue stream for co-digestion plants (aside from the additional methane generated by the FW provided). However, due to the competitive nature of the waste treatment market, gate fees can be highly variable (in a similar manner to availability of FW).

Table 7-6 Baseline revenue from electricity, heat and gate fees for mono-digestion (m1, m2, m3) and co-digestion scenario (c1, c2, c3)

Scenario	c1	c2	c3	m1	m2	m3	Comments
Annual FW treated (t)	3000	8500	8500	-	-	-	Average annual FW received by Irish biogas plants (Cormack, 2016). FW properties taken from Chapter 3
Annual PM treated (t)	7950	46250	101000	10950	54750	109500	PM properties taken from Chapter 3
% FW in substrate mix (wet weight)	27.4	15.5	7.76	0	0	0	
Methane Yield (m^3 CH_4/t)	59.9	40.0	26.6	11.6	11.6	11.6	Based on SMYs at each PM/FW mixing ratio measured in Chapter 3
Annual gross energy yield (MWh)	5900	19725	26222	1146.1	5730	11461	Conversion from annual methane generated (assuming 10% downtime annually), converted assuming 10 kWh/m^3 CH_4 (Nolan et al., 2012)
Annual gross heat generated (MWh)	2655	8876	11800	516	2578	5157	Assuming biogas utilization via CHP with 45% thermal efficiency (Nolan et al., 2012)
Heat demand of digester (MWh/a)	472	2361	4722	472	2360	4721	Calculated using Equations 7-1 and 7-2 for heating from 10ºC to 42ºC
Heat demand of pasteurization (MWh/a)	357	1784	3569	-	-	-	Calculated using Equations 7-1 and 7-2 for heating from for heating from 42ºC to 70ºC
Net annual heat generated (MWh)	1826	4731	3509	43.55	218	435.5	See Appendix D
Annual electricity generated(MWh)	2360	7890	10489	458	2292	4584	Assuming biogas utilization via CHP with 40% electrical efficiency (Nolan et al., 2012)
MWe plant size	0.27	0.90	1.19	0.05	0.26	0.52	See Appendix D
Revenue							
Revenue from Heat Replacing Oil boiler (€/a)	22,329	111,647	206,439	2,562[a]	12,809[a]	25,618[a]	Based on 728 kWh/sow/year heat demand to be met (McCutcheon, 2012), heat value of oil (10 kWh/L), oil boiler efficiency (0.8) and oil cost (0.5 €/L)
Annual Gross Revenue from REFIT (€)	354,001	1,025,725	1,363,567	68,763	343,816	595,947	€0.15 for plant ≤0.5 MWe, €0.13 for plant >0.5
Revenue from Gate Feeds (€)	90,000	255,000	255,000	-	-	-	Average €30/t gate fees (Cormack, 2016)
Total Annual Revenue (€)	466,331	1,392,372	1,825,007	71,325	356,625	621,565	

[a]This meets a portion of the heating requirement, but does not displace all oil use on pig unit.

7.3.3 Baseline economic analysis

Using the CAPEX and baseline conditions (i.e. most likely under current market conditions) presented in Table 7-4, Table 7-5 and Table 7-6, the RoI, NPV, 15 year IRR and payback period for each scenario were calculated. Figure 7-2 clearly illustrates that, despite lower CAPEX and OPEX, the lower energy yield and less diverse income stream (absence of gate fees) prevent mono-digestion plants from being viable at each farm scale simulated in this study. Indeed, the revenues generated by such scenarios would fail to keep pace with either the discount rate or interest on the loan for the initial CAPEX (both 6 %).

Scenarios c1 and c2 appeared to be viable with RoI's of 126 % and 11 %, IRRs of 20 % and 9 %, and payback periods of 6 and 10 years, respectively. Scenario c3 was not viable using the baseline (RoI of -169 %). This can be largely attributed to the high CAPEX, depreciation and OPEX associated with a digester of this size (see Table 7-4 and Table 7-5), and the limited energy yields and gate fees (see Table 7-6) due to constraints on the availability of FW for treatment. This illustrates that the current limitations in available FW in Ireland restrict the scale of on-farm AD systems. It should be noted, however, that FW availability can vary significantly, depending on location, and potential development of long term links with waste management and food processing facilities. It may also vary in future if higher targets for source segregated FW collection are agreed upon (EPA Ireland, 2016b).

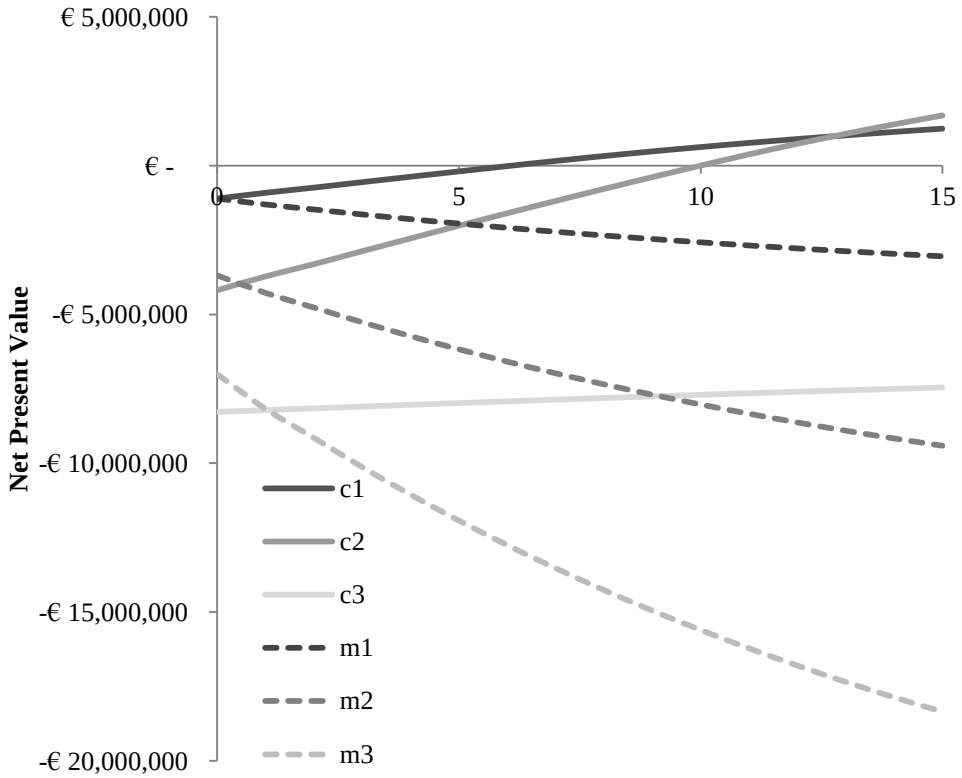


Figure 7-2 Net present values of for each mono-digestion (m1, m2, m3) and co-digestion scenario (c1, c2, c3) under baseline conditions.

7.3.4 Monte Carlo simulation

The returns projected for scenarios c1, c2 and c3 in Figure 7-2 are pursuant to all the input factors remaining stable throughout the 15 year project lifespan. As previously discussed, several of the key inputs to this model are either prone to variation (amount of FW available, gate fees, digestate disposal costs), or may change in the near future due to governmental policy changes (REFIT). Therefore an understanding of how sensitive each co-digestion scenario is to changes in these parameters is required in order to assess the robustness of financial projections. Therefore, Monte Carlo simulation of the 15 year NPV as the REFIT, amount of FW available, gate fees and digestate disposal costs vary was undertaken. Each of the inputs was varied 10000 times across a normally distributed (truncated normal distribution for FW availability) range of possible values (see Section 7.2.4). The model inputs specified are presented in Table 7-3.

Figure 7-3 illustrates the relationship between 15 year NPV and changes in the value of the input parameters, where input percentile is a given simulated input value between lowest and highest simulated input value given the specified distribution, and with all other input factors held at their mean simulated values.

Steeper lines therefore represent a great impact on the 15 year NPV. Figure 7-3 illustrates that REFIT, gate fees and FW availability had a linear positive relationship with 15 year NPV, while digestate disposal costs had a linear negative relationship.

Figure 7-4 illustrates how the 15 year NPV changed as each individual input value was varied within the distribution by one standard deviation away from the overall simulated mean towards the maximum and minimum values. It therefore illustrates the sensitivity of NPV to each input. It should be noted that while the mean simulated values for gate fees, digestate disposal costs and REFIT were similar to the values used in the baseline current condition estimates presented in Section 7.3.3, the values for FW availability differed. This was due to the truncation of the normal distribution for this parameter which was done to account for the maximum receiving capacity for each plant (and to ensure that no negative values were simulated). Therefore, the mean simulated FW availability for scenarios c1 (2610 t), c2 (8474 t) and c3 (8984 t) differed by –13.3 %, -0.3 % and +5.0 % from the baseline value, respectively. In particular, this resulted in the mean simulated 15 year NPV presented in Figure 7-4 (a) for scenario c1 being lower (€619,210) than the baseline scenario 15 year NPV for c1 presented in Figure 7-2 (€1,688,801).

From the slope of the graph presented in Figure 7-3(a), and the values presented in Figure 7-3(b), it appears that the 15 year NPVs for scenario c1 were most sensitive to changes in REFIT and FW availability. Overall, however, the 15 year NPVs for scenario c1 did not appear to be particularly sensitive to changes in the inputs. When all other factors were maintained at the simulated mean values, positive 15 year NPVs were simulated provided REFIT was greater than the 15th percentile of the input distribution (i.e. €0.12 /kWh or greater). Similarly, only when FW availability was below the 15th percentile of the input distribution (2300 t) (when all other factors are maintained at the simulated mean values) did 15 year NPVs become positive. In other words, this scenario would remain profitable provided FW availability remained above 2300 t and all other factors remained at the baseline values. Figure 7-4(a) illustrates that the 15 year NPV did not appear to be strongly affected by changes in digestate disposal costs or gate fees; variation of these values by one standard deviation above and below the mean simulated value did not result in major changes in 15 year NPV. The reason for this robustness in viability is primarily due to the increased probability of being able to secure

enough FW to generate profit, while having low CAPEX and OPEX (relative to larger plants). Figure 7-3(a) also illustrated that the potential 15 year NPV of scenario c1 is limited to ~€2 million. The reason for this is, due to its low capacity, it has little potential to increase revenue generation if additional FW becomes available.

For systems similar to scenario c1, REFIT is crucial. Government intervention to maintain the REFIT at its current level (or indeed increasing it) would have a significant positive impact on the viability of biogas plants of this size, and may promote further development of on-farm biogas plants. Nolan et al. (2012) previously highlighted the role that increases in REFIT may have in improving the viability of on-farm co-digestion of PM and grass silage. The considerably higher REFIT (or equivalent) possible in countries such as the UK (up to €0.27 /kWh (Nolan et al., 2012)) and Germany (€0.26 /kWh (Blokhina et al., 2011)) has encouraged widespread development of smaller scale on-farm co-digestion systems in those countries.

Figure 7-3(b) and (c) and Figure 7-4(b) and (c) illustrate that for scenarios c2 and c3, FW availability is the factor which would most affect 15 year NPV. This is due to the fact that it is the major revenue driver, as it increases both plant energy output and gate fees.

Despite the increased scale, the probability distribution (mean of 8500 t, standard deviation of 5000 t) of FW availability for scenarios c3 and c2 were similar (albeit with c2 truncated). This means that, despite c3 having double the FW treatment capacity of c2, the probability of securing FW in greater amounts than c2 was low (i.e. the 90^{th} percentile input value of c3 was 15000 t, while the value for c2 was c 13000 t). The similar mean simulated FW availability of scenarios c2 and c3 means that, with scale resulting in increased CAPEX and OPEX for c3, the mean simulated 15 year NPV for scenario c2 was far higher (Figure 7-4b), and the amount of FW required to generate positive 15 year NPVs lower than for scenario c3.

Figure 7-3 illustrates that, of all scenarios analysed, scenario c2 offers the highest potential 15 year NPVs. This is due to its potential to generate higher revenues than scenario c1, whilst having lower CAPEX and OPEX (and similar amounts of FW available) compared to scenario c3. While scenario c2 has the greatest potential to

generate the highest 15 year NPVs (and RoIs), it requires 6000 t of FW per annum to ensure profitability, which is considerably higher than the 2300 t required to ensure profitability in scenario c1. Scenario c3 would require >10500 t of FW to ensure profitability. This illustrates that, as scale increases, the sensitivity of cash flow to FW availability increases.

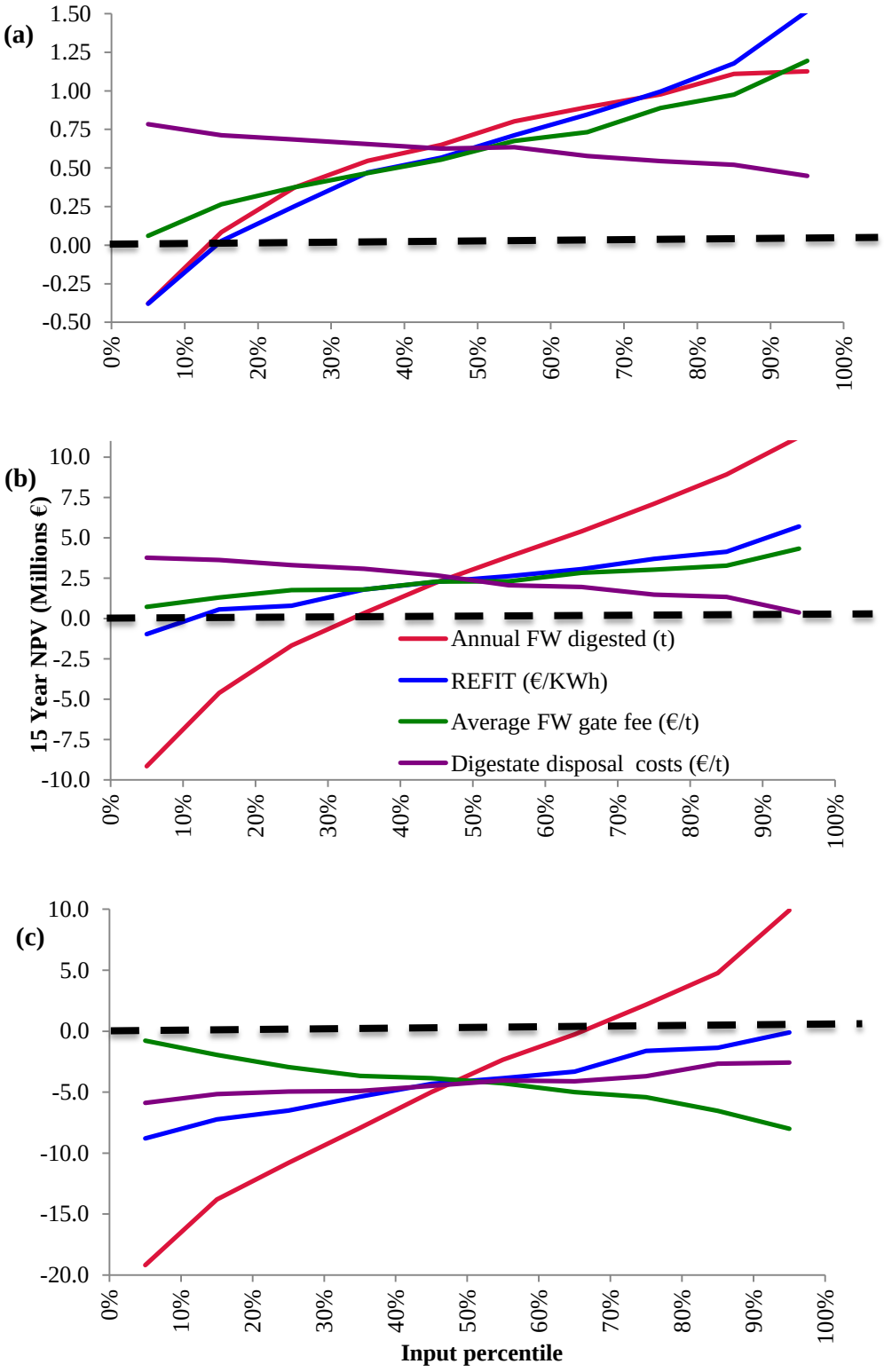


Figure 7-3 Change in simulated mean 15 year net present values as input values were randomly varied across the range specified for each input for scenarios c1 (a), c2 (b) and c3 (c)

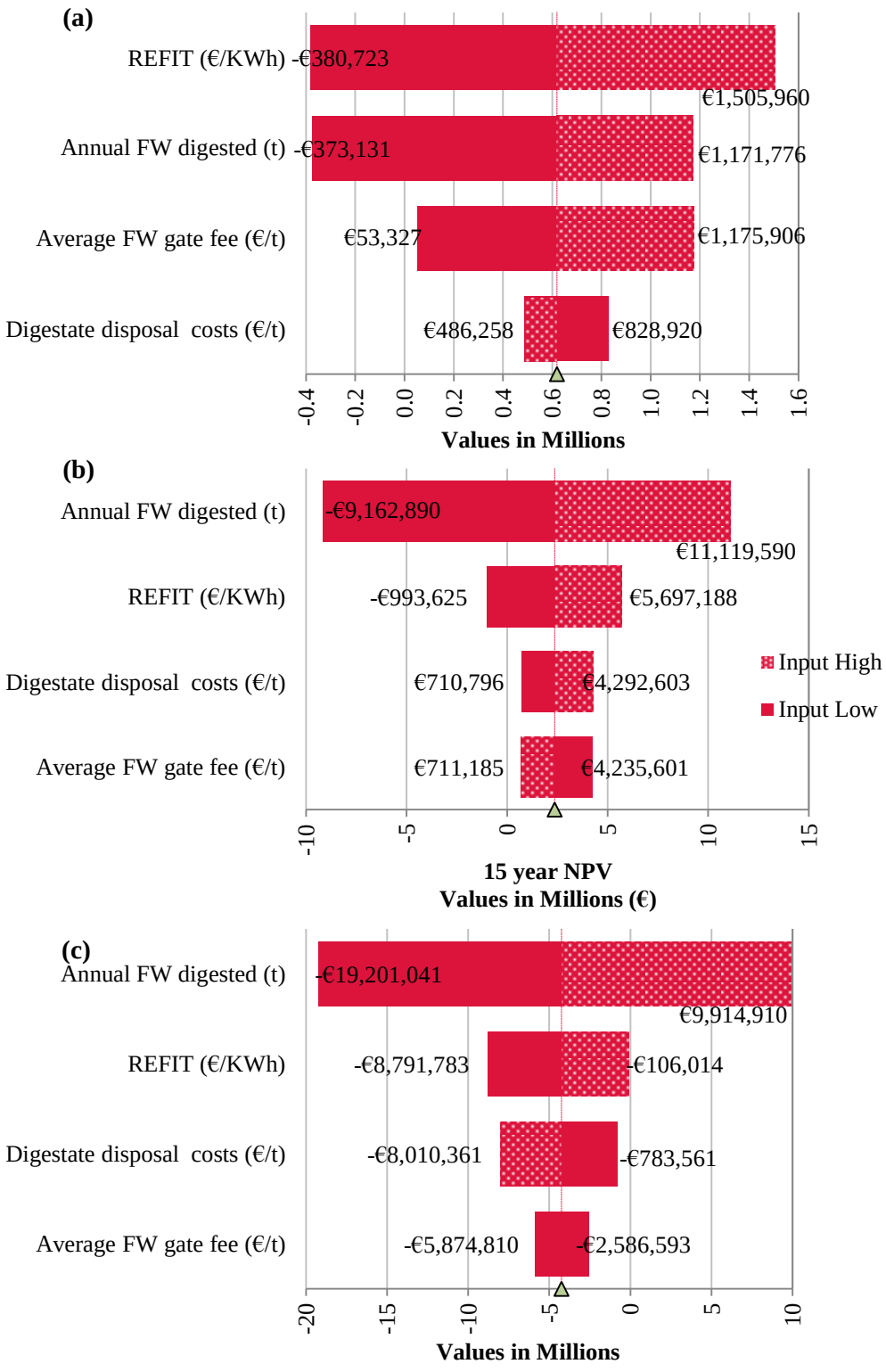


Figure 7-4 Tornado graph of change in mean simulated 15 year net present values as input values were varied by one standard deviation towards the maximum and minimum values of the range used in scenarios c1 (a), c2 (b) and c3 (c). Δ represents the mean simulated 15 year net present value.

Sourcing a reliable high volume supply of FW is therefore critical. There are two primary sources of FW in Ireland; the source segregated municipal FW collected by waste management companies, and food processing waste generated by various factories. Typically waste management companies which do not have an in-house organic waste disposal route prefer short term (1-2 year) agreements with compost/biogas facilities, in order to ensure that the lowest market price for disposal is secured regularly (Siebert, 2014). As transport is a significant cost associated with FW disposal, ensuring that biogas plants are located near densely populated areas where FW is generated would provide a market advantage, and would minimize the risk of not securing enough FW to ensure profitability. However, as most Irish pig farms, particularly large farms such as those represented by the c2 and c3 scenarios presented here, are located in rural areas (O'Shea et al., 2016), relying on municipal FW may be challenging and therefore put downward pressure on gate fee receipts.

While food processing companies also look for the lowest price for disposal, a local and reliable disposal route with the operational flexibility to handle varying volumes of waste is desirable (Cormack, 2016). Therefore, locating biogas plants near food processing plants (many of which are located in rural areas) may play a key role in ensuring a stable, long term supply of FW, thereby reducing the financial risk. The fact that all scenarios are not particularly sensitive to reductions in gate fees would suggest that, provided digesters have sufficient capacity, they may be able to outcompete disposal options such as composting, which is significantly commercially dependent on gate fees, and in doing so securing a greater FW volume.

Due to constraints on the amount of FW available for treatment currently, biogas systems need to be designed to match the amounts of FW available. However, in a survey of the potential feedstocks available for AD in Ireland, O'Shea et al. (2016) found that the potential amount of FW available in Ireland exceeds 624000 t per annum. Therefore, there is significant potential to grow the amount of FW available for treatment, thereby increasing the viability of on-farm co-digestion in Ireland. Improving the penetration of source waste segregation, while engaging in further public education in relation to waste separation, would increase the annual volumes of FW which could potentially be available for AD plants.

7.4 Summary

This study revealed that mono-digestion systems have lower CAPEX and OPEX than co-digestion systems. Seventy percent of revenues from co-digestion systems and all of the revenue from mono-digestion systems are generated by electricity sales, while 14 % - 20 % of annual revenues from co-digestion plants come from gate fees.

Despite having lower OPEX and CAPEX than co-digestion, mono-digestion of PM is not financially viable at all three farm sizes considered. Using baseline (current market) conditions, an integrated pig farm of 521 sows co-digesting 3000 t of FW per annum was found to be financially viable with an IRR of 20 % and 126 % RoI. Co-digesting manure from a pig farm of 2607 sows and 8500 t of FW per annum was found to be financially viable with an IRR of 9 % and 11 % RoI. Larger farms of 5214 sows co-digesting 8500 t of FW per annum were found to be unviable.

Monte Carlo simulation was undertaken to assess the sensitivity of overall profitability of co-digestion plants to changes in key revenue streams and operational expenses. Due to the high likelihood of accessing sufficient FW, cash flows from scenario c1 were found to be least sensitive to any future changes in FW availability, gate fees, digestate disposal costs and REFIT. Due to its potential to treat greater amounts of FW than scenario c1, whilst requiring a lower amount of FW availability to remain profitable relative to scenario c3, scenario c2 was found to have the highest revenue-generating potential under optimal market conditions.

This study has found that FW availability limits the scale of on-farm biogas plants co-digesting FW and PM in Ireland to farms of > 2500 sows, and that farm-based biogas plant capacity should be determined by the availability of the co-substrate which drives methane production (and revenue generation), rather than manure availability.

CHAPTER 8

Conclusions and Recommendations

The following chapter details the main conclusions from the experiments carried out as part of this study and makes recommendations for future research directions.

8.1 Conclusions

This study was carried out in order to identify the most suitable operating conditions for the anaerobic co-digestion of PM and FW, evaluate the viability of using simplified mathematical tools for process simulation, and assess the economic feasibility of on-farm PM/FW co-digestion on Irish pig farms.

Experiments at batch scale (Chapter 3) were carried out to identify optimal PM/FW mixing ratio, approximate optimal operating conditions in terms of methane yield and to identify parameters critical to modelling of PM and FW co-digestion. The results of this experiment provided some guidelines as to the suitable operating conditions for semi-continuous laboratory scale experiments (Chapters 4 and 5).

Semi-continuous experiments provided more detailed data on optimization of PM/FW co-digestion in terms of process stability, digestate quality and methane yields. In addition to identifying suitable operating conditions, lab-scale experiments provided data crucial to the calibration and validation of a mathematical model simulating meso-scale digester operation carried out in (Chapter 6).

Finally, using data generated from lab-scale experimentation , an economic model for assessing the financial viability of on-farm biogas plants in Ireland was developed (Chapter 7).

8.1.1 Summation of findings

8.1.1.1 Batch anaerobic co-digestion of PM and FW

In the batch scale experiment, the synergistic effects of co-digesting FW and PM on SMY**s** were quantified, the effect of initial VFA concentrations on observed synergy was examined, and the suitability of several mathematical models for

simulating batch anaerobic co-digestion of FW and PM were assessed. The co-digestion of PM and FW had synergistic effects on SMY, Fd and digestion kinetics, with the highest SMYs occurring at PM/FW mixing ratio (VS basis) of 1/4. The highest level of synergy (a 22 % to 26 % increase in SMY) was observed at a PM/FW mixing ratio of 3/2.

Initial VFA concentrations in PM did not explain the observed synergy. Rather, the presence of VFAs masked a slightly higher level of synergy occurring from the conversion of VS and non-VFA COD to methane. The high initial VFA concentrations in PM also resulted in a dual pooled first-order model providing the most precise fit for the data, however due to the large number of parameters required to be fit, it was found to be less accurate for parameter estimation than the first-order model.

8.1.1.2 Semi-continuous anaerobic co-digestion of PM and FW

In lab-scale semi-continuous experiments, the effects of varying PM/FW ratio and HRT on methane yields, process stability, digestate dewaterability, digestate biosafety and microbial community dynamics were examined. Subsequently the effect of undertaking PM/FW co-digestion at very low HRTs (from 21 days to 10.5 days) on methane yields, digester stability, microbial communities and digester enteric indicator organism content were examined. Chapter 4 illustrated that varying digester feedstock composition from 85 %/15 % PM/FW to 40 %/60 % PM/FW (VS basis) increased SMYs but did not significantly affect digestate biosafety or dewaterability. Decreasing HRT from 41 days to 21 days (thereby increasing OLR from 1 to 3 kg VS/m^3/day) reduced the methane conversion efficiency (decreased SMY and VS removal) and improved digestate dewaterability but did not significantly increase the enteric indicator organism content of the digestate.

The observation that changing these conditions did not greatly affect digester stability or function was supported by the fact that the microbial communities were only slightly affected as PM/FW mixing ratio and HRT were varied. Decreasing HRT resulted in a slight increase in the relative abundance of SAOBs such as *Synergistetes*, indicating that hydrogenotrophic methanogenesis may play a greater role at lower HRTs.

This was further supported by the findings of Chapter 5. It was found that decreasing HRT below 21 days resulted in an increase in the relative abundance of SAOBs such as (*Spiorchatetes* and *Cloacamonaceae*). Chapter 5 also showed that decreasing HRT to 10.5 days resulted in a drop in SMYs and VMYs and a rapid increase in isobutyric acid concentrations. Reducing HRT below 21 days also compromised the ability of the anaerobic digestion system to reduce the concentrations of *Enterococcus* in digestate to acceptable levels.

8.1.1.3 Mathematical modelling of meso-scale anaerobic co-digestion of PM and FW

The meso-scale reactor was operated in order to validate a mathematical model calibrated to simulate the co-digestion of PM and FW. Chapter 6 illustrated that ADM1 model, even when calibrated in a rudimentary manner, can provide a generally accurate simulation of reactor performance. However, a low level of precision was achieved particularly when VFAs were simulated, which can limit its efficacy in predicting process stability. Therefore, a greater level of system calibration is merited when modelling systems which are highly buffered, and which treat substrates which significantly vary in chemical composition (such as PM/FW co-digestion).

8.1.1.4 Financial viability of on-farm anaerobic co-digestion of PM and FW in Ireland

Utilizing data generated from batch and semi-continuous experiments, as well as data collated from planned and currently operating biogas plants, an economic model was developed. This was used to assess the financial viability of on-farm biogas plants in Ireland. Despite lower OPEX and CAPEX than co-digestion, mono-digestion of PM was found to not be financially viable in Ireland. Using baseline (current market) conditions, a pig farm of 521 sows co-digesting 3000 t of FW per annum (scenario c1) was found to be financially viable with an IRR of 20 % and 126 % RoI. Co-digesting manure from a pig farm of 2607 sows (scenario c2) and 8500 t of FW per annum was found to be financially viable with an IRR of 9 % and 11 % RoI. Larger farms of 5214 sows (scenario c3) co-digesting 8500 t of FW per annum were found to be unviable.

Monte Carlo simulation revealed that, due to the high likelihood of accessing sufficient FW, net revenues from scenario c1 are least sensitive to any future changes in FW availability, gate fees, digestate disposal costs and REFIT. Due to

its potential to treat greater amount of FW than scenario c1, whilst requiring a lower amount of FW availability to remain profitable relative to scenario c3, scenario c2 appears to have the highest revenue generating potential under optimal market conditions.

8.1.2 Significance of findings

8.1.2.1 Identification of optimal operating conditions for PM/FW co-digestion

The detailed study of co-digestion of PM and FW at batch scale yielded information on the kinetics and methane yields achievable from co-digestion. Such findings have a wide variety of applications in research and in industry, from calculation of potential revenues from co-digestion of these substrates (based on methane yields) and identification of optimal digester size during biogas plant design, to simulating PM/FW co-digestion where the Fd and k_H values measured in the experiment are key model inputs.

The semi-continuous lab scale experiments provided valuable information on optimal digester operating conditions. The information that varying PM/FW mixing ratio has no significant effect on downstream processes (dewatering, digestate enteric indicator organism content prior to land application) and that reducing HRT below 21 days compromises enteric indicator organism removal efficacy will be useful to plant operators and researchers interested in the feasible operating range (in terms of HRT) of CSTR reactors when co-digesting these substrates.

8.1.2.2 Improved methodology for batch test data analysis

The batch experiment highlights a methodology for quantifying the synergistic effects of co-digestion which has been rarely used in co-digestion studies up to this point. Applying this methodology clearly illustrated the benefits of PM and FW co-digestion. The illustration of the minimal effect high initial VFA concentrations have on observed synergy is also valuable to researchers working in this field, as it addresses the effects of this potentially confounding factor.

The kinetic analysis illustrated advantages and limitations of using more complex kinetic models to describe batch anaerobic digestion. This is an important finding as the application of complex alternatives to the first-order kinetic model is becoming more common place. Highlighting the uncertainty associated with

parameters simulated by such models may result in improved experimental design (higher numbers of replicates) when such models are applied to parameter estimation in future.

8.1.2.3 Identification of trends in microbial dynamics due to HRT reduction

The semi-continuous lab scale experiments provided valuable information on potential microbial biomarkers for process instability. The identification of shifts in microbial community organisation towards SAO and hydrogenotrophic methanogenesis as HRT was decreased, in particular the correlation of non-acetate VFA producing bacteria (such as *Coprococcus*) with system instability as HRT was reduced to 10.5 days, may be useful in the development of biomarkers.

8.1.2.4 Highlighting the potential for simple ADM1 calibration

By illustrating the accuracy of digestion models not numerically optimised, chapter 6 shows that the ADM1 model calibrated just with substrate data can be used to provide process simulation without needing months of operational data, thereby simplifying its calibration and increasing its potential use by plant operators. However the chapter also illustrates the trade- off between model accuracy and ease of calibration. Studies of biogas process modelling with a focus on end-user accessibility are rare. This chapter makes a modest but important contribution to developing this aspect of anaerobic digestion modelling.

8.1.2.5 Methodology for stochastic modelling of biogas plant viability

The stochastic economic modelling methodology presented in Chapter 7 addresses a gap in the literature in relation to assessing the impact of highly variable inputs to static financial models.

8.1.2.6 Financial model of the viability of on-farm anaerobic co-digestion of PM and FW in Ireland

The economic research provides crucial information to Irish farmers, developers and State bodies interested in the development of an indigenous biogas industry. Illustrating the effect of limitations caused by FW availability challenges previously widespread hypotheses that FW co-digestion was the most immediately viable substrate around which on-farm biogas plants may be established.

8.2 Recommendations for future research

Based on the research carried out in the course of this study, several recommendations for future research directions are made.

Investigation of the viability of novel and advanced AD technologies on-farms is required. Chapter 3 illustrated that the highest SMY from PM/FW co-digestion can be achieved at a mixing ratio of 1/4 (VS basis). However such a mixing ratio cannot be applied in CSTR reactors without dilution of substrates (due to the high TS nature of FW). Therefore investigation of the potential for dry anaerobic co-digestion systems, which may be able to operate at the optimal substrate mixing ratio, is merited. Further to this, while mesophilic single stage CSTRs are the most commonly used AD technology on farms in Europe, the higher potential methane yields, loading rates, and, in relation to thermophilic digestion, improved pathogen removal rates mean that the potential economic and technical improvements provided by more advanced AD technologies merit investigation. Two-stage AD, thermophilic AD, temperature phased anaerobic digestion (TPAD) and the myriad of AD pre-treatment technologies all have potential to displace established mesophilic single stage CSTR-type technology particularly in large scale AD projects, despite the added complexity (TPAD and two-stage AD) and perceived risk of instability (thermophilic AD).

Further research is required to develop biomarkers for biogas plant control and optimization. The development of high-throughput DNA sequencing has the potential to revolutionise the management of AD systems. Instead of using changes in pH, alkalinity, methane yield etc. to assess reactor stability, developing biomarkers which are representative of a highly active and efficient AD system may allow for improved biogas plant management. Identifying present or absent consortia and then alerting operating conditions in order to promote specific genera or species may result in higher methane yields and more stable digestion systems. While the work present in Chapter 4 and 5 provide information useful to the development of biomarkers, much more research must be undertaken to develop a robust set of biomarkers. Microbial surveying studies of many commercial and experimental biogas plants will be required to validate proposed biomarkers.

Development of user-friendly software based tools to simplify the application of the ADM1 for plant operators and designers would be a significant development. Chapter 6 illustrated that while a rudimentary calibration of the ADM1 model allows for a somewhat accurate simulation of AD processes, familiarity with

advanced data and programming software is require to run the model. In addition further numerical optimization is required to maximise model accuracy. The development of simple software which, once substrate and biogas plant properties are inputted, can run the ADM1 model (and potentially optimise model fit), may pave the way for more widespread uptake of this simulation tool by plant operators and plant designers.

Further stochastic modelling of the economic viability of on-farm biogas plants is required. While Chapter 7 illustrated the viability of on-farm co-digestion of PM and FW using co-generation, other similar on-farm biogas plant concepts merit analysis. For example, considering the current limitations in terms of FW availability in Ireland, analysis of scenarios where FW can be imported internationally, or biogas plant feedstocks could be diversified through the co-digestion of grass silage in addition to FW and manure, would provide further information on the extent of the viability of on-farm biogas in Ireland. In addition, while no biomethane projects currently exist in Ireland, assessment of the economic viability of biogas upgrading and grid injection or use as transport fuel from on-farm biogas plants would be valuable.

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Appendix A -Publications and presentations

Publications

Dennehy, C., Lawlor, P.G., Croize, T., Jiang, Y., Morrison, L., Gardiner, G.E., Zhan, X. 2016. Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion. *Waste Management*, 56 (October), 173-80.

Dennehy, C., Lawlor, P.G., Gardiner, G.E., Jiang, Y., Cormican, P., McCabe, M.S., Zhan, X. 2017. Process stability and microbial community composition in pig manure and food waste anaerobic co-digesters operated at low HRTs. *Frontiers of Environmental Science & Engineering*, 11(3), 4.

Dennehy, C., Lawlor, P.G., Gardiner, G.E., Jiang, Y., Xie., S., Nghiem L. D., Zhan, X. 2017. Greenhouse gas emissions from different pig manure management techniques: a critical analysis. *Frontiers of Environmental Science & Engineering*, In Press

Dennehy, C., Lawlor, P.G., Cormican, P., McCabe, M.S., Sheahan, J., Jiang, Y., Zhan, X., Gardiner, G.E. 2017. Anaerobic co-digestion of pig manure and food waste; effects of operating conditions on digestate biosafety and dewaterability, and microbial community dynamics. *Biomass and Bioenergy*, Under Review

Dennehy, C., Lawlor, P.G., Gardiner, G.E., Jiang, Y., Shalloo, L., Zhan, X. 2016. Stochastic modelling of the economic viability of on-farm co-digestion of pig manure and food waste in Ireland. *Applied Energy*, Under Review

Presentations

Xie, S., Lawlor, P., **Dennehy C.**, Zhan X. "Greenhouse gas mitigation in pig manure management using anaerobic digestion" **Irish Agricultural Research Forum 2014,** Tullamore, Ireland

Dennehy, C., Lawlor, P., Gardiner, G., Zhan X. “Effect of pig manure to food waste ratio on methane production, dewaterability and pathogen content in batch anaerobic co-digestion of pig manure and food waste” **4th Annual Conference of the Ireland Chinese Association of Environment, Resources & Energy (ICAERE),** Trinity College Dublin, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Specific methane yields and reaction kinetics of co-digestion of stored pig manure and food waste" **Biogas Science 2014,** Vienna, Austria

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "On-Farm Anaerobic Co-digestion; Biomethane Potential of Pig Manure and Food Waste" **Irish Pig Farmers Conference 2014,** Tipperary, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Greenhouse Gas Emissions and Mitigation Potential of Anaerobic Digestion on Irish Pig Farms" **Irish Pig Farmers Conference 2014,** Tipperary, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Methane yields and assessment of pathogen removal via co-digestion of pig manure and food waste" **Environ 2015,** Sligo Institute of Technology, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Kinetics of anaerobic co-digestion of pig manure and food waste" **Irish Agricultural Research Forum 2015,** Tullamore, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Anaerobic Co-digestion of Pig Manure and Food Waste: Effects on Dewaterability and Biosafety" **5th Annual Conference of the Ireland Chinese Association of Environment, Resources & Energy 2015,** National University of Ireland, Galway

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Maximizing specific methane yields in co-digestion of pig manure and food waste" **International Water Association 14th World Congress on Anaerobic Digestion,** Vina Del Mar, Chile

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Green Farm: On-Farm Anaerobic Co-digestion of Pig Manure and Food Waste" **Teagasc Pig Farmers' Research Dissemination Day 2015,** Cavan, Ireland

Dennehy, C., Lawlor, P., Jiang, Y., Gardiner, G., Zhan X. "Effects of co-digestion of pig manure and food waste on digestate stability and methane yields" **Livestock Waste 2016,** Galway, Ireland

Dennehy, C., Lawlor, P., Gardiner, G., Jiang, Y., Shalloo, L., Zhan X. "Economic viability of farm-based co-digestion of pig manure and food waste" **Green Farm project research dissemination workshop,** Grange, Co. Meath, Ireland

Appendix B - Glossary of terms

ADM1: anaerobic digestion model number 1

BMP: biomethane production potential

CAPEX: capital expenditure

CHP: combined Heat and Power

CMY: cumulative methane yield

COD: chemical oxygen demand

CSTR: continuously stirred tank reactor

FW: food waste

GHG: greenhouse gas

HRT: hydraulic retention time

IRR: internal rate of returnAD: anaerobic digestion

LCFA: long chain fatty acid

NH_4N: ammonium-nitrogen

NPV: net present value

OLR: organic loading rate

OPEX: operational expenditure

PCoA: principle component analysis

PM: pig manure

PSD: particle size distribution

REFIT: renewable energy feed in tariff

RoI: return on investment

SAO: syntrophic acetate oxidation

SMY: specific methane yield

SRF: specific resistance to filtration

TS: total solid

VFA: volatile fatty acids

VS: volatile solid

Appendix C- Chapter 4 and 5 rarefaction curves

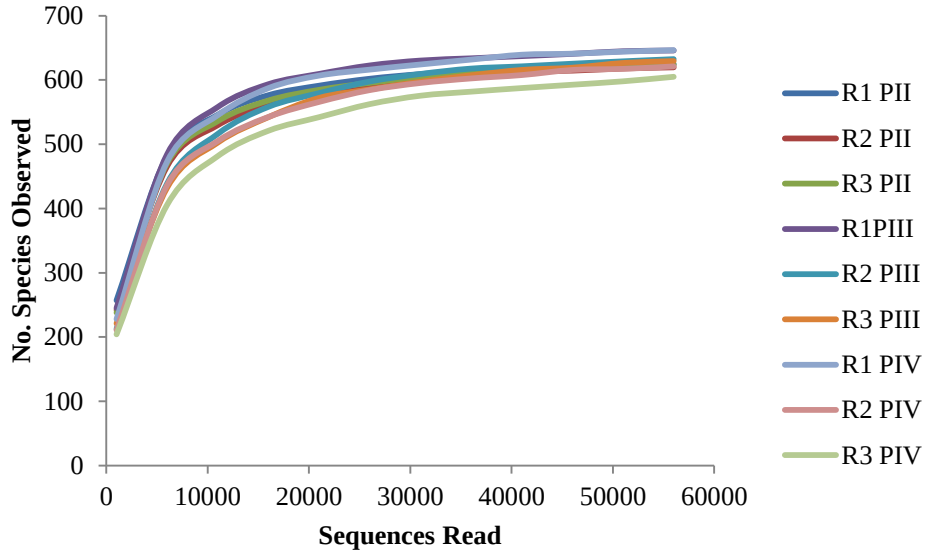

Figure B1 Rarefaction curves for each sample that underwent 16S rRNA sequencing in Chapter 4. PII= Phase 2, PII= Phase 3, PIV= Phase 4.

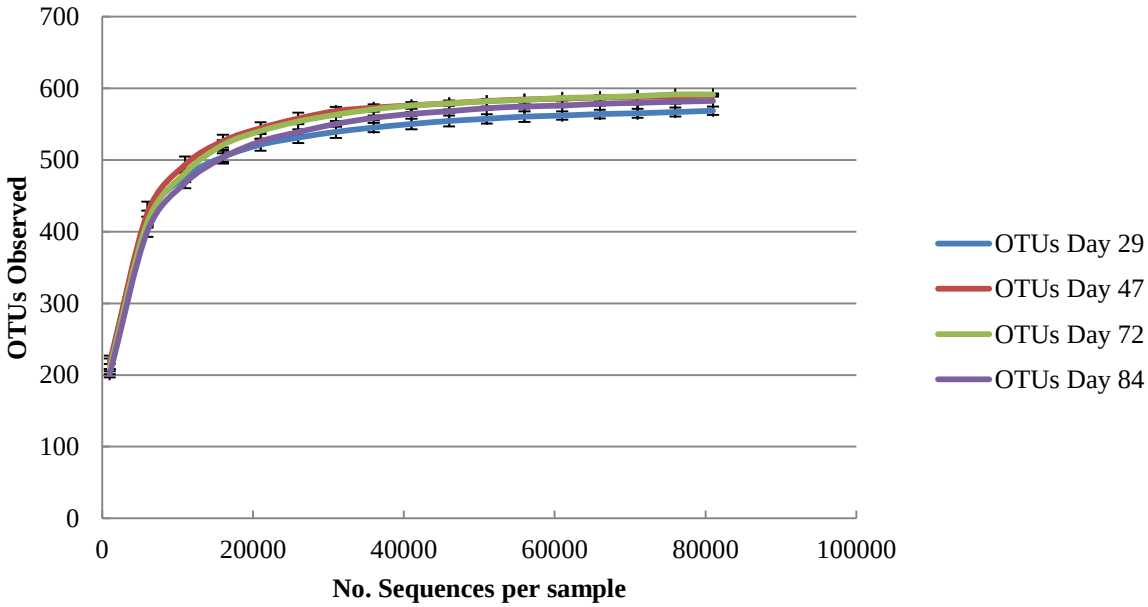

Figure B2 Rarefaction curves of samples underwent 16S rRNA sequencing in Chapter 5. Error bars denote standard deviation.

Appendix D - Chapter 7 detailed calculations

Table C1 Deterministic model framework for the calculation of the economic viability on scenario m1, as described in Chapter 7

Parameter	value
Digester Size (m^3)	1500
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	100%
%PM (rest % FW) mixing ratio (WW)	100%
TS content (%)	7.2%
VS content (%)	5.6%
Methane yield (m^3 CH_4/tVS)	192.33
Methane yield (m^3 CH_4/t)	11.6
Gross energy potential of substrate (kWh/t)	116.3
OLR (kg VS/m^3/d)	1.1
PM required (t/d)	30.0
FW required (t/d)	0.0
Annual FW required (t)	0
Farm size	521.4
Gas producing days per year (days)	328.5
Annual methane yield	114605.3
Annual gross energy yield (MWh)	1146.1
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	516
Heat requirement of digester system (MWh/a)	472
Heat requirement of pasteurization system (MWh/a)	0
Net annual heat (MWh)	44
Annual electricity (MWh)	458
MW plant size	0.052331178
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	380
Annual heat demand from pig unit provided by CHP (MWh/a)	43.54988992
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	5124
	€
Revenue from heat replacing oil boiler (€/a)	2,562
Revenue from electricity	
REFIT (€/kWh)	0.15
	€
Annual gross revenue from REFIT	68,763
Revenue from gate fees	
Average FW gate fee (€/t)	20

	€
Revenue from gate feeds (€)	-
Expenditure	
Capital costs	
	€
Total capital costs (€)	903,679
Operating costs	
	€
Plant O+M	3,000
	€
Digester maintenance	4,500
	€
CHP maintenance	63,072
	€
Labour	7,200
	€
Electricity	8,870
	€
Admin	10,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
	€
Annual digestate disposal cost (EUR)	61650
	€
OPEX (exc. insurance and interest)	158,291.50
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized over	€
15 years)	54,221
	€
Insurance	4,518
	€
Depreciation	54,221
	€
Total annual OPEX (€/a)	271,251

Table C2 Deterministic model framework for the calculation of the economic viability on scenario m2, as described in Chapter 7

Parameter	value
Digester Size (m^3)	7500
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	100%
%PM (rest % FW) mixing ratio (WW)	100%
TS content (%)	7.2%
VS content (%)	5.6%
Methane yield (m^3 CH_4/tVS)	192.33
Methane yield (m^3 CH_4/t)	11.6
Gross energy potential of substrate (kWh/t)	116.3
OLR (kg VS/m^3/d)	1.1
PM required (t/d)	150.0
FW required (t/d)	0.0
Annual FW required (t)	0
Farm size	2607.1
Gas producing days per year (days)	328.5
Annual methane yield	573026.4
Annual gross energy yield (MWh)	5730.3
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	2579
Heat requirement of digester system (MWh/a)	2360
Heat requirement of pasteurization system (MWh/a)	0
Net annual heat (MWh)	218
Annual electricity (MWh)	2292
MW plant size	0.261655889
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	1898
Annual heat demand from pig unit provided by CHP (MWh/a)	217.7494496
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	25618
	€
Revenue from heat replacing oil boiler (€/a)	12,809
Revenue from electricity	
REFIT (€/kWh)	0.15
	€
Annual gross revenue from REFIT	343,816

Revenue from gate fees	
Average FW gate fee (€/t)	20
	€
Revenue from gate feeds (€)	-
Expenditure	
Capital costs	
	€
Total capital costs (€)	3,097,081
Operating costs	
	€
Plant O+M	15,000
	€
Digester maintenance	22,500
	€
CHP maintenance	63,072
	€
Labour	36,000
	€
Electricity	44,348
	€
Admin	10,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
Annual digestate disposal cost (EUR)	368250
	€
OPEX (exc. insurance and interest)	559,170
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized	€
over 15 years)	185,825
	€
Insurance	15,485
	€
Depreciation	185,825
	€
Total annual OPEX (€/a)	946,305

Table C3 Deterministic model framework for the calculation of the economic viability on scenario m3, as described in Chapter 7

Parameter	value
Digester Size (m^3)	15000
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	100%
%PM (rest % FW) mixing ratio (WW)	100%
TS content (%)	7.2%
VS content (%)	5.6%
Methane yield (m^3 CH_4/tVS)	192.33
Methane yield (m^3 CH_4/t)	11.6
Gross energy potential of substrate (kWh/t)	116.3
OLR (kgVS/m^3/d)	1.1
PM required (t/d)	300.0
FW required (t/d)	0.0
Annual FW required (t)	0
Farm size	5214.3
Gas producing days per year (days)	328.5
Annual methane yield	1146052.8
Annual gross energy yield (MWh)	11460.5
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	5157
Heat requirement of digester system (MWh/a)	4722
Heat requirement of pasteurization system (MWh/a)	0
Net annual heat (MWh)	435
Annual electricity (MWh)	4584
MW plant size	0.523311778
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	3796
Annual heat demand from pig unit provided by CHP (MWh/a)	435.4988992
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	51235
	€
Revenue from heat replacing oil boiler (€/a)	25,618
Revenue from electricity	
REFIT (€/kWh)	0.13
	€
Annual gross revenue from REFIT	595,947
Revenue from gate fees	

Average FW gate fee (€/t)	20
	€
Revenue from gate feeds (€)	-
Expenditure	
Capital costs	
	€
Total capital costs (€)	5,838,833
Operating costs	
	€
Plant O+M	30,000
	€
Digester maintenance	45,000
	€
CHP maintenance	63,072
	€
Labour	72,000
	€
Electricity	88,695
	€
Admin	10,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
Annual digestate disposal cost (EUR)	751500
	€
OPEX (exc. insurance and interest)	1,060,267
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized	€
over 15 years)	350,330
	€
Insurance	29,194
	€
Depreciation	350,330
	€
Total annual OPEX (€/a)	1,790,121

Table C4 Deterministic model framework for the calculation of the economic viability on scenario c1, as described in Chapter 7

Parameter	value
Digester Size (m^3)	1500
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	34%
%PM (rest % FW) mixing ratio (WW)	72.60%
TS content (%)	16.2%
VS content (%)	12.1%
Methane yield (m^3 CH_4/tVS)	413.3524943
Methane yield (m^3 CH_4/t)	59.9
Gross energy potential of substrate (kWh/t)	598.7
OLR (kgVS/m^3/d)	2.4
PM required (t/d)	21.8
FW required (t/d)	8.2
Annual FW required (t)	3000
Farm size	378.6
Gas producing days per year (days)	328.5
Annual methane yield	590002.0
Annual gross energy yield (MWh)	5900.0
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	2655
Heat requirement of digester system (MWh/a)	472
Heat requirement of pasteurization system (MWh/a)	357
Net annual heat (MWh)	1826
Annual electricity (MWh)	2360
MW plant size	0.269407324
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	380
Annual heat demand from pig unit provided by CHP (MWh/a)	379.6
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	44659
	€
Revenue from heat replacing oil boiler (€/a)	22,329
Revenue from electricity	
REFIT (€/kWh)	0.15
	€
Annual gross revenue from REFIT	354,001
Revenue from gate fees	

Average FW gate fee (€/t)	30
	€
Revenue from gate feeds (€)	90,000
Expenditure	
Capital costs	
	€
Total capital costs (€)	1,292,095
Operating costs	
	€
Plant O+M	6,000
	€
Digester maintenance	4,500
	€
CHP maintenance	63,072
	€
Labour	15,300
	€
Electricity	11,498
	€
Admin	35,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
Annual digestate disposal cost (EUR)	61650
	€
OPEX (exc. insurance and interest)	197,020
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized	€
over 15 years)	77,526
	€
Insurance	6,460
	€
Depreciation	77,526
	€
Total annual OPEX (€/a)	358,531

Table C5 Deterministic model framework for the calculation of the economic viability on scenario c2, as described in Chapter 7

Parameter	value
Digester Size (m^3)	7500
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	51%
%PM (rest % FW) mixing ratio (WW)	84.5%
TS content (%)	12.3%
VS content (%)	9.3%
Methane yield (m^3 CH_4/tVS)	377.1028495
Methane yield (m^3 CH_4/t)	40.0
Gross energy potential of substrate (kWh/t)	400.3
OLR (kgVS/m^3/d)	1.9
PM required (t/d)	126.7
FW required (t/d)	23.3
Annual FW required (t)	8500
Farm size	2202.4
Gas producing days per year (days)	328.5
Annual methane yield	1972548.3
Annual gross energy yield (MWh)	19725.5
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	8876
Heat requirement of digester system (MWh/a)	2361
Heat requirement of pasteurization system (MWh/a)	1784
Net annual heat (MWh)	4731
Annual electricity (MWh)	7890
MW plant size	0.900706978
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	1898
Annual heat demand from pig unit provided by CHP (MWh/a)	1898
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	223294
	€
Revenue from heat replacing oil boiler (€/a)	111,647
Revenue from electricity	
REFIT (€/kWh)	0.13
	€
Annual gross revenue from REFIT	1,025,725
Revenue from gate fees	

Average FW gate fee (€/t)	30
	€
Revenue from gate feeds (€)	255,000
Expenditure	
Capital costs	
	€
Total capital costs (€)	4,625,237
Operating costs	
	€
Plant O+M	30,000
	€
Digester maintenance	22,500
	€
CHP maintenance	63,072
	€
Labour	76,500
	€
Electricity	57,488
	€
Admin	35,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
Annual digestate disposal cost (EUR)	368250
	€
OPEX (exc. insurance and interest)	652,810
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized	€
over 15 years)	277,514
	€
Insurance	23,126
	€
Depreciation	277,514
	€
Total annual OPEX (€/a)	1,230,964

Table C6 Deterministic model framework for the calculation of the economic viability on scenario c3, as described in Chapter 7

Parameter	value
Digester Size (m^3)	15000
HRT (days)	50
% PM (rest % FW) mixing ratio (VS)	69%
%PM (rest % FW) mixing ratio (WW)	92.24%
TS content (%)	9.7%
VS content (%)	7.5%
Methane yield (m^3 CH_4/tVS)	321.8758695
Methane yield (m^3 CH_4/t)	26.6
Gross energy potential of substrate (kWh/t)	266.1
OLR (kgVS/m^3/d)	1.5
PM required (t/d)	276.7
FW required (t/d)	23.3
Annual FW required (t)	8500
Farm size	4809.5
Gas producing days per year (days)	328.5
Annual methane yield	2622244.8
Annual gross energy yield (MWh)	26222.4
CHP heat conversion efficiency	45%
CHP electrical conversion efficiency	40%
Annual heat (MWh)	11800
Heat requirement of digester system (MWh/a)	4722
Heat requirement of pasteurization system (MWh/a)	3569
Net annual heat (MWh)	3509
Annual electricity (MWh)	10489
MW plant size	1.19737207
Revenue	
Revenue from heat	
Heat demand from pig unit (kWh/Sow)	728
Annual heat demand from pig unit (MWh/a)	3796
Annual heat demand from pig unit provided by CHP (MWh/a)	3509.466469
Gross energy of heating oil (kWh/L Oil)	10
Oil boiler efficiency	85%
Net heat from oil boiler (kWh/L)	8.5
Cost of oil (€/L)	0.5
Annual oil use for heat (L)	412878
	€
Revenue from heat replacing oil boiler (€/a)	206,439
Revenue from electricity	
REFIT (€/kWh)	0.13
	€
Annual gross revenue from REFIT	1,363,567
Revenue from gate fees	

Average FW gate fee (€/t)	30
	€
Revenue from gate feeds (€)	255,000
Expenditure	
Capital costs	
	€
Total capital costs (€)	8,338,456
Operating costs	
	€
Plant O+M	60,000
	€
Digester maintenance	45,000
	€
CHP maintenance	63,072
	€
Labour	153,000
	€
Electricity	114,975
	€
Admin	35,000
	€
Digestate disposal up to 15000t (EUR)	4
	€
Digestate disposal > 15000t (EUR)	7
Annual digestate disposal cost (EUR)	751500
	€
OPEX (exc. insurance and interest)	1,222,547
Insurance (% of CAPEX)	0.5%
Loan period (years)	15
Interest rate	6%
Annual interest (6% of principle amortized	€
over 15 years)	500,307
	€
Insurance	41,692
	€
Depreciation	500,307
	€
Total annual OPEX (€/a)	2,264,854