UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING AND THE ENVIRONMENT

CO-DIGESTION OF CATTLE SLURRY AND FOOD WASTE

By

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Thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

FACULTY OF ENGINEERING AND THE ENVIRONMENT
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Anaerobic co-digestion of a low energy substrate with one of a higher potential can enhance biogas production, lead to improved process stability, and make the system more energetically and economically viable. The aim of this research was to evaluate the potential of co-digestion of food waste and cattle slurry in mesophilic conditions under a variety of different conditions. One of the batches of cattle slurry used was taken from a farm using gypsum plasterboard as animal bedding. The high sulphate content proved to be detrimental to the digestion process and led to further experiments to determine process tolerance to both substrate sulphate concentration and digester soluble sulphide concentration.

The majority of the work was carried out in laboratory-scale digesters with a working volume of 4 L. They were operated with semi-continuous feeding over a minimum of three hydraulic retention times (HRT) and at mesophilic temperature (35 $\pm$ 2 ^{o}C). A number of operating regimes were tested which included varying the wet-weight ratio of cattle slurry and food waste from 3 : 1 to 6 : 1 and the organic loading rate (OLR) from 3 to 5 g volatile solids (VS) L^{-1} day^{-1}. The lower OLR of 3 g VS L^{-1} day^{-1} was shown to be optimal in terms of specific methane production (SMP) and gave values of 0.332 and 0.239 L g^{-1} VS. These were higher than the SMP values obtained at OLRs of 4 and 5 g VS L^{-1} day^{-1} at the same ratios. The volumetric methane production (VMP) for co-digestion was consistently higher than for cattle slurry mono-digestion and increased significantly with increasing OLR at both CS : FW ratios tested. Economic considerations may mean that this increased VMP is more significant than a marginal reduction in SMP, as higher values could increase farm incomes and reduce capital expenditure and payback periods. The methane (CH_4) content of the biogas ranged from 60.3 – 61.0 % and 61.6 – 62.2 % for ratios of 3 : 1 and 6 : 1 respectively. The requirement for trace element addition was considered with the conclusion that the cattle slurry could provide all of the nutrients required for stable digestion, whereas the mono-digestion of food waste was deficient in some key elements which resulted in process instability.

Mono-digestion of cattle slurry showed a variable performance which could be related to the source and condition of the feedstock material. Where the SMP and VMP of the cattle slurry was low the effect of adding the co-digestate brought the combined gas production to a higher and more consistent value, which is an important consideration in the selection, design and operation of gas utilisation equipment and the overall economic viability of the digestion plant. A reasonably accurate prediction of SMP values in co-digestion trials

could be derived from BMP data using a first-order pseudo-parallel model to derive kinetic coefficients. This was most accurate when applied to systems operating at a longer HRT where the effect of daily removal of a proportion of the digestate was not significant. Comparison of values derived from this approach with those based on the SMP of mono-digestion controls on a pro rata VS basis was able to provide additional insights into possible mechanisms for any reduction in gas productivity.

The cattle slurry collected from a farm using gypsum as bedding had a sulphate concentration of 6876 mg L^{-1} and sulphur content of 2.79% of total solids (TS). Mono-digestion of this material failed and less than 35% methane was detected in the biogas with the SMP below 0.03 L g^{-1} VS. Dissolved sulphides in the digestate reached 500 mg L^{-1} which exceeded the toxicity limit of 200 mg L^{-1}. When co-digested with food waste at an OLR ranging from 3 to 5 g VS L^{-1} day^{-1} volumetric biogas production (VBP) showed a significant drop and in less than 3 HRT, all the digesters failed. The SMP fell below 0.03 L g^{-1} VS, the biogas CH_4 was < 35%, intermediate alkalinity (IA) to partial alkalinity (PA) ratio rose to > 1.5 and volatile fatty acids (VFA) concentration were > 10000 mg L^{-1}.

The final part of the study digested low sulphate cattle slurry spiked with calcium sulphate ($CaSO_4.2H_2O$) to equivalent added sulphate concentrations ranging from 0 to 7000 mg SO_4 L^{-1}. As the amount of sulphate increased in the cattle slurry feedstock, the SMP dropped gradually from 0.143 L g^{-1} VS for control to 0.055 L g^{-1} VS in digesters fed at a substrate sulphate concentration of 7000 mg SO_4 L^{-1}. The biogas hydrogen sulphide concentration increased from 499 ppmv detected in the control to 39441 ppmv in digesters with 7000 mg SO_4 L^{-1}. Digesters with spiked sulphate concentrations of more than 4000 mg SO_4 L^{-1}, gave sulphide concentrations above the suggested inhibition threshold and showed elevated concentrations of acetic and propionic acid. At sulphate substrate concentrations > 7000 mg L^{-1} there was a rapid and progressive increase in total and individual VFA species leading to process failure.

In general it was concluded that positive benefits could be gained from the co-digestion of food waste and cattle slurry although a precautionary principle should be adopted until an assessment of the variability and composition of the slurry had been carried out.

Keywords: anaerobic digestion, cattle slurry, food waste, gypsum, sulphate

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DECLARATION OF AUTHORSHIP

I, Jethro Henry Adam declare that the thesis entitled **CO-DIGESTION OF CATTLE SLURRY AND FOOD WASTE** and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published:
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Signed: ……

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ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
CH_4	Methane
CHP	Combined Heat Power
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
COSHH	Control of Substances Hazardous to Health
CS	Cattle Slurry
CSTR	Continuously Stirred Tank Reactor
DI	Deionised Water
FW	Food Waste
GC	Gas Chromatograph
GHG	Greenhouse Gas
H_2S	Hydrogen Sulphide
HRT	Hydraulic Retention Time
IA	Intermediate Alkalinity
LCFA	Long Chain Fatty Acid
M	Molarity
NH_3	Ammonia
OLR	Organic Loading Rate
PA	Partial or Bicarbonate Alkalinity
ppmv	Parts per million volume
SMP	Specific Methane Production
SRB	Sulphate-Reducing Bacteria
STP	Standard Temperature and Pressure
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TE	Trace Element
ThCV	Theoretical Calorific Value
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solid
UK	United Kingdon
VFA	Volatile Fatty Acids
VMP	Volumetric Methane Production
VS	Volatile Solids
WW	Wet Weight

1 INTRODUCTION

1.1 Overview

We live in a world where all our activities produce residual materials, which we have no further use for and wish to get rid of. In the United Kingdom (UK), a total of 27.3 million tonnes of household waste were generated in 2016, of which an estimated 353,000 tonnes was separately-collected food waste from households, up from 307,000 tonnes in 2015 (DEFRA, 2018). Around 7.1 million tonnes of food waste is generated from households every year, with a further 3.1 million tonnes from the commercial and industrial sector (WRAP, 2018). This represents a lost value of over £20 billion per year. Food waste not only damages the pocket, but is harmful to the environment. WRAP (2018) reported that food waste is responsible for the equivalent of 25 million tonnes of carbon dioxide emissions yearly. Landfilling of this material generates methane, which can accelerate global warming. DEFRA (2010) estimated that 41% of UK's methane emissions originated from landfills.

Apart from food waste, air pollution from methane gas associated with livestock manure management is also a concern since 72% of UK land is use by the agriculture sector (DEFRA, 2017). Smith and Williams (2016) reported that of 83 million tonnes of UK annual livestock manure production during the housing period, 67 million tonnes (80%) was cattle manure. This figure does not include the excreta deposited directly on fields by grazing livestock. Livestock manure management is one of the major agricultural sources of methane emissions. Besides methane, ammonia emissions are also an environmental nuisance related to livestock manure. Agriculture is the main source of ammonia emissions in the UK and comprised 88% of the total in 2016 (DEFRA, 2018), while dairy cattle management is one of the main contributing factors in increased ammonia emissions since the mid 2000s (Guthrie et al., 2018).

Traditionally, the manure and slurry of housed cattle are collected and stored in storage facilities for substantial periods of time. According to the Farm Practices Survey 2017, only 5.5% of the livestock farms surveyed are already processing their waste by anaerobic digestion (AD) (DEFRA and National Statistics, 2017). This is disappointing because by using AD to treat the cattle slurry, potentially 978 to 1776 kg CO_2 eq $year^{-1}$ of greenhouse

gas (GHG) emissions per livestock unit could be saved (Marañón et al., 2011). The savings could help UK to achieve at least 80% reduction of GHG emissions by 2050, and a cut in emissions of at least 34% by 2020 against the 1990 baseline, as stipulated by the Climate Change Act 2008.

Anaerobic digestion could convert the methane produced by the cattle slurry into biogas that can be used to generate power for on-farm consumption or to provide extra income for the farmers. An Agri-Food and Biosciences Institute (AFDI) AD plant in Hillsborough, operated from January 2009 to April 2011, managed to produce 15.2 m^3 of biogas with a gross energy value of 85 kWh per tonne of cattle slurry (Frost and Gilkinson, 2011). The biogas was utilised through a biogas boiler and a combined heat power (CHP) unit, with overall energy efficiencies of 87% and 78% respectively. Apart from the production of renewable fuel, AD of dairy manure can also improve the manure fertilizer quality and reduce odours, pathogens and greenhouse gas emissions, as reviewed by Rico et al. (2011). Unfortunately, the relatively low biogas potential makes the mono-digestion of cattle slurry alone unattractive (Lukehurst and Bywater, 2015).

Studies by Cornell et al. (2011) showed that different batches of cattle slurry gave different biogas yields. The specific methane yields for mono-digestion of cattle slurry collected in the winter and the summer were 0.069 L g^{-1} VS day^{-1} and 0.176 L g^{-1} VS day^{-1} respectively, which suggested that diets of the cattle play an important role in the slurry biogas production. Amon et al. (2007) found that manure from cows with medium milk yield that were fed a well-balanced diet produced the highest methane yield when digested anaerobically, and those from cows fed with high grass silage produced the lowest methane. This showed that biogas can be influenced by the content of the cattle slurry – in this case lignin present in the slurry due to the grass feeding had reduced the methane yield. Apart from these internal factors, external factors such as floor condition, collecting areas and dilution, as studied by Vedrenne et al. (2008), also influenced the methane production. The floor conditions considered only concerned whether the floor was solid or slatted, however, and did not mention the type of bedding used.

The animal bedding used in farms is also important not only because it provides comfort for the cow, making it healthier and more productive (Endres, 2012); but also as the usage of natural materials (such as pine bark, soil, sawdust, wood shavings) could be an effective means of conserving the N and S in the manure, and therefore reducing the emissions of

ammonia (NH_3) and hydrogen sulphide (H_2S) (Luo, 2004). In other cases, mineral products such as sand are used and when the current study started in 2011, some UK farms were using gypsum (calcium sulphate dihydrate, $CaSO_4.2H_2O$) and gypsum-based products such as recycled plasterboard as animal bedding. Although gypsum has some good characteristics as animal bedding, its use can lead to the production of H_2S. Some deaths have occurred due to H_2S poising from slurry pits in the UK, including the high profile case of Nevin Spence in County Down (BBC, 2012). In 2012 in the UK, the Environment Agency and the Scottish Environmental Protection Agency issued Position Statements which excluded the use of reprocessed gypsum in animal bedding (Environment Agency, 2012; SEPA, 2012); a similar statement was issued for Northern Ireland in 2016 (DAERA, 2016). Gypsum from other sources can still be utilised (Crook et al., 2017), however, and in many other countries the use of recycled plasterboard is not yet banned.

As for food waste, previous studies have shown that it is highly desirable as a sole feedstock for AD due to the high biodegradability and methane yield, and hence the high energy potential (Zhang et al., 2007; Forster-Carneiro et al., 2008; El-Mashad and Zhang, 2010). The digestion process may be hindered, however, by the presence of various inhibitors such as ammonia and volatile fatty acids (VFA). In addition, a lack of trace elements in the feedstock could lead to failure of the process (Banks and Zhang, 2010). Co-digestion with other substrates is one option that has been proven to be successful in overcoming these problems (Zhang et al., 2011; Zhang et al., 2013). Co-digestion can utilize the nutrients and microbial diversities in the various feedstocks to optimise the digestion process, and the properties of cattle slurry make it a very suitable substrate for co-digestion with food waste (Zhang et al., 2012a). Banks, Salter, et al. (2011) carried out a study on the feasibility of centralised pre-processing and pasteurisation of source-separated domestic food waste followed by transport to farms for anaerobic co-digestion with dairy cattle slurry. The study was based on balancing the nutrient demand at a farm level, and used data obtained from laboratory experimental studies of biogas yield. The available data covered a range of food waste : cattle slurry ratios, but was not sufficiently wide to reflect the overall availability of these two important AD feedstocks in the UK.

1.2 Aims

The aims of the current research were to quantify the potential of co-digestion of food waste and cattle slurry in mesophilic conditions as a means to improve biogas yields, and to compare the co-digestion of both substrates with anaerobic digestion of their single substrates. In addition, the consequences of using cattle slurry with high sulphate content (due to the use of gypsum plasterboard as animal bedding) were also considered.

1.3 Objectives

In order to achieve the aims, the following objectives were outlined:

a) determine the characteristics of typical examples of both feedstocks;
b) determine the specific methane production of both feedstocks using biochemical methane potential (BMP) tests;
c) investigate the performance of the co-digestion of cattle slurry and food waste in terms of biogas production and stability at different organic loading rates, at a wet-weight ratio of cattle slurry to food waste of 3 : 1, to provide a baseline for comparison with other studies;
d) increase the proportion of cattle slurry to be co-digested with food waste to reflect the actual wet-weight ratio of both feedstocks in the UK (6 : 1) and compare the performance with that in the previous study;
e) investigate the potential issues of using cattle slurry with high sulphate content (collected from a farm using gypsum plasterboard as a bedding material) in the co-digestion reactors and evaluate the potential problems that might occur; and
f) propose and evaluate control measures for any problems found in (e).

2 LITERATURE REVIEW

2.1 Anaerobic Digestion

2.1.1 Overview of anaerobic digestion

Anaerobic digestion (AD) is the microbiologically-mediated process in which organic matter is degraded in an oxygen-free environment. There are two end products of the process – energy-rich biogas and stabilised digestate. The biogas is principally a mixture of methane (CH_4) and carbon dioxide (CO_2) with traces of other contaminant gases, and can be combusted to generate heat and electricity, used directly as vehicle fuel or upgraded and injected into the gas grid. The digestate contains valuable nutrients (e.g. nitrogen, phosphorus and potassium) for plants and can be utilised as fertiliser and soil conditioner.

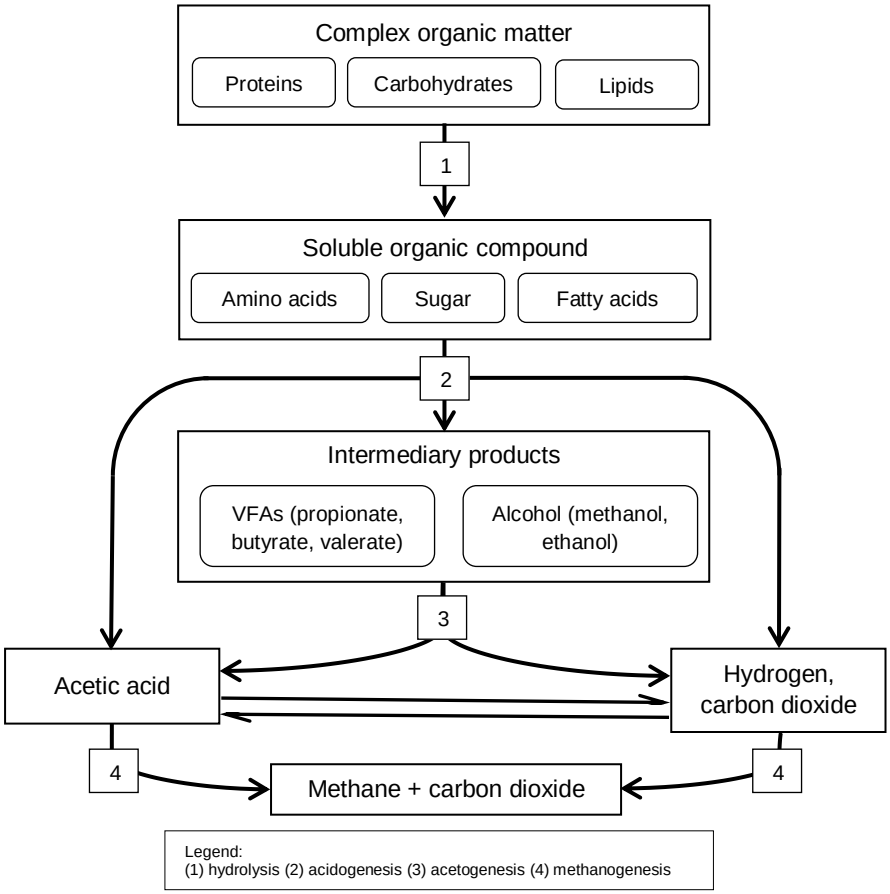


Figure 2.1 Anaerobic digestion pathway

The AD process is generally classified into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as shown in Figure 2.1. Each stage is facilitated by different groups of microorganisms (Forster-Carneiro et al., 2008), hence different operating conditions (pH, temperature, hydrogen concentration, etc.) are optimal for each stage (Mata-Alvarez, 2003).

2.1.1.1 Hydrolysis

Hydrolysis is the process in which complex polymers such as carbohydrates, proteins, and lipids are transformed by hydrolytic microorganisms into monomers such as sugars, amino acids and fatty acids. This first step in the AD process is catalysed by enzymes (protease, amylase, cellulase, hemi-cellulase, pectinase, glucosidase, etc.) secreted by the microorganisms (such as *Bacteroides* and *Clostridium*) and makes the substrates more readily available for the next stages (Khanal, 2008; Yang et al., 2010; Quinones et al., 2012). Christ et al. (2000) as cited by Forster-Carneiro et al. (2008) consider this step as the most rate limiting stage. Arantes and Saddler (2011) suggested that this was due to the physical structure of cellulosic substrates that limited the accessibility of the cellulose chains to the enzymes. The optimum pH for most hydrolytic microorganisms is between 5 – 7, while temperature optima can range from 30 – 60 ^{o}C (Azman et al., 2015).

2.1.1.2 Acidogenesis

Acidogenic or fermentative microorganisms are responsible for degrading the soluble compounds generated in the first stage, to produce volatile fatty acids (VFA) such as acetic acid / acetate, propionic, butyric, and valeric acids, carbon dioxide and hydrogen. Apart from this, some alcohols (ethanol and methanol) and ammonia are also produced (Ahring, 2003; Garcia-Heras, 2003). These intermediary products (VFAs and alcohols) will be transformed in a later step as they are still too large and unusable for methane production. Acidogenesis is generally the fastest step in AD and the optimal conditions are similar to those for hydrolysis.

2.1.1.3 Acetogenesis

The next stage is acetogenesis where VFAs and alcohols are further digested by acetogenic microorganisms to form acetate, carbon dioxide, and hydrogen. Acetogens are

a diverse group able to tolerate a relatively wide range of pH and temperature conditions (Yoon, 2015). In this stage, it is important to note that the process can be inhibited by hydrogen gas, resulting in accumulation of VFAs. Zinder (1988) as cited by Khanal (2008) reported that the hydrogen partial pressure has to be below 10^{-3} atm for acetogenesis to be regarded as thermodynamically feasible. Thus, the hydrogen concentration, measured as partial pressure in an anaerobic digester, can be an indicator of its health (Mata-Alvarez, 2003).

2.1.1.4 Methanogenesis

The final stage in which biogas is formed from acetate, hydrogen and carbon dioxide is methanogenesis. Methane is normally the main product which typically accounts for approximately 65% and about 35% is carbon dioxide (Mata-Alvarez, 2003). However, the percentage of gasses depends on substrate. There are two major pathways by which methanogenesis occurs – acetoclastic and hydrogenotrophic. Acetoclastic methanogens convert acetate to methane, while hydrogenotrophic methanogens produce methane via the reduction of carbon dioxide by hydrogen (Table 2.1).

Table 2.1 Methanogenic reactions in the AD of organic matter

Process	**Reaction (pH 7, 1 atm pressure, and all reactants and products at 1 M)**	**Free Energy, ΔG^0 (kJ)**
Acetoclastic	$CH_3COOH \rightarrow CH_4 + CO_2$	– 31.0
Hydrogenotrophic	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	– 135.6

Source: Adapted from O'Flaherty et al. (2010)

Under normal conditions, almost 65 – 70% of methane produced is derived from acetate (Gavala et al., 2003) since acetate is the major end product of the acidogenesis step (Yu and Schanbacher, 2010). The two important genera of methanogens that are responsible for the production of methane via the acetoclastic pathway are *Methanosarcina* and *Methanosaeta* (Griffin et al., 1998). Their importance comes primarily due to their tolerance for environmental factors such as nutrients and temperature (Palmisano and Barlaz, 1996). Both genera have different doubling times on acetate, of 1 – 2 days and 4 – 9 days for *Methanosarcina* and *Methanosaeta* respectively. Thus, at shorter retention time (higher substrate concentrations), the later are more likely to wash out from the

system and *Methanosarcina* would be the predominant genera. At higher retention time, however, *Methanosaeta* is the prevailing genera in methane production (Khanal, 2008).

In hydrogenotrophic methanogenesis, methane is produced by utilising hydrogen to convert carbon dioxide. Griffin et al. (1998) found that *Methanobacteriaceae* were the most abundant hydrogenotrophic methanogens. Although only about one third of methane is typically produced by this path, it has a significant role to keep a low hydrogen pressure which is important for acetogenesis as mentioned previously (Gavala et al., 2003). Banks et al. (2012) have shown that in mesophilic conditions at high ammonia concentrations the hydrogenotrophic pathway can dominate.

Methanogenesis is very sensitive to changes in the environment such as temperature, pH and organic loading rate, and is inhibited by a number of organic and inorganic compounds (Gavala et al., 2003). A study by El-Mashad et al. (2004) on cow manure in continuously stirred tank reactors (CSTRs) showed that the maximum specific methanogenic activity was affected more severely by imposed upward temperature fluctuations compared to downward fluctuations. The optimum pH for methanogenic activity ranges from pH 7.0 – 7.2, and activity may decrease if the pH is lower than 6.3 or higher than 7.8 (Bitton, 1994; van Haandel and Lettinga, 1994). Results from Lay et al. (1996) supported this optimal pH range in a study on high solids (90 – 96%) sludge in a mesophilic batch digester, which functioned over a pH range of 6.6 – 7.8 with an optimum pH of 6.8, and failed at pH below 6.1 or higher than 8.3. A suitable organic loading rate is vital in the AD process as overloading could result in low biogas production. The accumulation of VFAs due to overloading could decrease the pH, which would disturb the methanogenic population. Salminen and Rintala (2002) in a study on solid poultry slaughterhouse waste reported that AD performed well at a loading of up to 0.8 kg VS m^{-3} with specific methane yield from 0.52 – 0.55 m^3 kg^{-1} VS, while at a higher loading of 1.0 – 2.1 kg VS m^{-3} there was accumulation of VFAs (up to 17.6 g L^{-1}) and decreasing specific methane yields as low as 0.07 m^3 kg^{-1} VS.

2.1.2 Inhibition in anaerobic digestion

One of the main purposes of AD as an engineering process is to produce biogas which is a source of renewable energy. Several substrates and products of anaerobic respiration can inhibit the methanogens in the system, however, hence leading to low methane yield

and process instability. Thus, in order to optimise AD performance, it is essential to understand and control the inhibitory factors.

2.1.2.1 Ammonia

Ammonia is produced during the anaerobic digestion of nitrogenous materials such as amino acids and proteins. Ammonium ion (NH_4^+) and free ammonia or un-ionised ammonia (NH_3) are the two main forms of reduced nitrogen that are toxic to methanogens. However, NH_4^+ is also the nutrient source of nitrogen for microorganisms in AD (Gerardi, 2003), while NH_3 is more toxic since it can easily diffuse into the cell membrane (Ahring, 2003 and Chen et al., 2008).

The amount of both forms of reduced nitrogen in the system is pH dependent. At pH 9.25, the forms are relatively equal in the equilibrium state as shown in Equation 2.1 (Khanal, 2008).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad \text{Equation 2.1}$$

With increasing pH, NH_3 increases as does ammonia toxicity, while at neutral pH, NH_3 only accounts for 0.5% of the total ammonia nitrogen (TAN). It is essential to keep the system close to neutral pH, as concentrations of NH_3 as low as 100 mg N L^{-1} can be enough to have an inhibitory effect on the methanogens (Khanal, 2008). Khanal (2008) also reported that at higher pH, TAN concentrations of more than 1500 mg N L^{-1} could have an adverse effect on the system while concentrations of more than 3000 mg N L^{-1} could cause digester failure. Sawayama et al. in 2004 as quoted by Khalid et al. (2011) reported that in the fluidized-bed anaerobic digester, decreases of 10% and 50% of methanogenic activity were detected at ammonia concentrations of 1670 – 3720 and 4090 – 5550 mg NH_4-N L^{-1}, respectively. It was also reported that no methanogenic activity was detected at an ammonia concentration of 5880 – 6000 mg NH_4-N L^{-1}. Sung and Liu (2003) in a thermophilic CSTR fed with soluble non-fat dry milk found that TAN concentrations of 4920 and 5770 mg N L^{-1} caused a 39% and 64% reduction in specific methane activity, respectively. Earlier reports by McCarty and McKinney (1961) also noted that NH_3 was the main culprit in ammonia inhibition (a concentration of 150 mg N L^{-1} was completely inhibitory), with toxicity thresholds being dependent on pH and temperature.

During mesophilic and thermophilic digestion of cattle manure by Hashimoto (1986), ammonia inhibition began at 2500 mg N L^{-1} for digesters that were not acclimated to high ammonia concentrations, but for acclimated thermophilic digestion, signs of stress started at 4000 mg N L^{-1}. Angelidaki and Ahring (1992) agreed that ammonia concentrations of 4000 mg N L^{-1} or more inhibited thermophilic digestion of cattle manure. In their batch study, a higher reduction in specific methanogenic activity was reported in ammonia-inhibited reactors (6000 mg L^{-1}) fed with acetate (73%), compared to only 52% reduction in reactors using hydrogen as substrate. These results showed that acetoclastic methanogens have higher sensitivity to ammonia compared to hydrogenotrophic methanogens.

Angelidaki and Ahring (1992) showed that it was possible to run stable AD of cattle manure with ammonia concentrations of more than 4000 mg N L^{-1} but with a methane yield approximately 25% lower than in the non-inhibited reactors and with higher VFA concentrations. Some recovery remedies have also been suggested for AD after ammonia inhibition. For thermophilic AD of swine manure at TAN 6000 mg N L^{-1} with a low methane productivity of 67 mL CH_4 g^{-1} VS, the addition of 1.5% (w/w) activated carbon, 10% (w/w) glauconite or 1.5% (w/w) activated carbon and 10% (w/w) glauconite, increased the methane yield to 126 mL CH_4 g^{-1} VS, 90 mL CH_4 g^{-1} VS, and 195 mL CH_4 g^{-1} VS respectively (Hansen et al., 1999). In thermophilic batch AD of cattle manure as studied by Nielsen and Angelidaki (2008) with ammonium chloride (NH_4Cl) addition to induce ammonia inhibition, four recovery strategies were introduced – no dilution, dilution with 50% water, dilution with 50% digested manure, and dilution with 50% fresh manure. The highest methane production during the recovery period was achieved by the dilution with fresh cattle manure, corresponding to 420% of the control value. They also studied the same strategies in CSTR and found that dilution with fresh cattle manure gave the shortest recovery period (5 – 6 days), and gave additional methane production during the recovery periods compared to the other options. However, the process was unstable due to the high organic loading rate, as indicated by higher VFAs and lower pH.

2.1.2.2 Sulphide

Sulphide is the major source of sulphur for methanogens (Gerardi, 2003). It is an essential micronutrient, but if the concentration is too high, it can cause toxicity. Sulphide often results from the reduction of sulphate by sulphate-reducing bacteria (SRB). The dissolved

sulphide is then transformed into gaseous form as hydrogen sulphide (H_2S), which has an unpleasant odour in concentrations higher than 0.2 ppmv and is toxic at concentrations above 300 ppmv (Tchobanoglous et al., 2006). In anaerobic digestion systems, SRB compete with methanogens for the same substrates and hence reduce the methane production. This subject is discussed in more detail in Section 2.4.

2.1.2.3 Light metal ions

Chen et al. (2008) listed aluminium (Al), sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) as light metal ions that are essential for microbial growth but toxic at high concentrations in anaerobic digestion. These metals occur in the AD system from two main sources – discharge from industrial sources and addition of chemicals such as alkali used for pH adjustment. The concentrations of these salts that could cause inhibitory effects are shown in Table 2.2.

Table 2.2 Light metals ions toxicity thresholds

Cations	**Inhibitory concentrations (mg L^{-1})**	**Reference**
Al^{3+}	2500	Jackson-Mos and Duncan (1991)
Mg^{2+}	400	Schmidt and Ahring (1993)
K^{+}	400	Kugelman and McCarty (1964)
Na^{2+}	3500 – 5500	McCarty (1964)
Ca^{2+}	700	Jackson-Mos et al. (1989)

Most of the salt ions inhibit methanogenic activity, although some are also inhibitory to acetogenic microorganisms. For example, a study by Cabirol et al. (2003) as quoted by Chen et al. (2008) showed that the specific activities of methanogens and acetogens were reduced by 50% and 77% respectively after 59 days of exposure to 1000 mg L^{-1} aluminium hydroxide ($Al(OH)_3$). Meanwhile, excess calcium could cause calcium carbonate to precipitate and this may adversely affect the biomass and reduce the specific methanogenic activity (van Langerak et al., 1998).

2.1.2.4 Heavy metals

Sludge originating from industrial sources and fed to the anaerobic digester is one source of heavy metals in the system. A trace amount of some of these metals is beneficial to the

growth and metabolism of anaerobic microorganisms. However, because heavy metals are not biodegradable and can accumulate to toxic concentrations (Sterritt and Lester, 1980 as quoted by Chen et al., 2008), they may disrupt the enzyme function of the microorganisms and are often regarded as the cause of many anaerobic process failures (Mignone, 2013).

A review by Chen et al. (2008) showed that there are wide variations in reported toxic concentrations for heavy metals. This was in part because the toxicity correlated better to the metal's free ionic concentration than the total concentration. They also reported that variations were due to the physico-chemical form of the metal, substrate differences, microbial genetics, and environmental factors. Mignone (2013) listed the concentrations of soluble heavy metals that could inhibit the AD process, as summarised in Table 2.3. Many authors, however, are reluctant to specify potentially toxic concentrations because of the multiple factors affecting metal bioavailability, and instead prefer to rank relative toxicity (Chen et al., 2008).

Table 2.3 Soluble heavy metal concentrations inhibitory to the AD process

Heavy metal	**Soluble concentrations (mg L^{-1})**
Arsenic	0.5 – 1.0
Cadmium	0.02
Chromium	1.5
Copper	0.5 – 1.0
Nickel	2.0
Zinc	0.5 – 1.0

Heavy metals are toxic when they are in soluble, free form (Chen et al., 2008). The metal cations are adsorbed onto the negatively charged membrane and later absorbed by the microbial cell, where they inactivate the enzymatic systems (Gerardi, 2003). When the heavy metals are not in free form they are not toxic, and combining metal ions could thus control the toxicity. Oleszkiewicz and Sharma (1990) as quoted by Chen (2008) suggested that heavy metals can be controlled by precipitation, sorption, and chelation by organic and inorganic ligands. In his review, Lewis (2010) reported that metal sulphide precipitation has been studied extensively; hence sulphide has been widely used as a precipitating agent for heavy metals. Lewis (2010) also listed the advantages of using sulphide precipitation, which are: lower solubility of metal sulphide precipitates, potential for selective metal removal, fast reaction rates, better settling properties, and potential for

reuse of sulphide precipitates. It is suggested by Khanal (2008) that for every 1.0 mg of heavy metal, approximately 0.5 mg of sulphide is needed for precipitation. Care has to be taken not to overdose the sulphide; however, as Anderson et al. (1982) as quoted by Jin et al. (1998) suggested that sulphide itself is one of the most threatening inhibitors to methanogens.

2.1.2.5 Short-chain fatty acids

As mentioned earlier in section 2.1.1, complex organic matter is hydrolysed and fermented into low molecular weight compounds, including VFAs such as acetic, propionic, butyric, isobutyric, valeric, isovaleric, and hexanoic acids. Excess production and accumulation of acetate can happen when methanogenesis is disrupted by toxicity (ammonia, sulphide, heavy metals, etc.), or from shocks in environmental factors (such as pH and temperature), or due to lack of nutrients. Higher production of hydrogen during the process increases the hydrogen partial pressure and inhibits propionic acid degrading bacteria causing propionic acid to accumulate. The imbalance can sometimes be corrected, however, by adding alkaline buffering material (Mignone, 2013) or using a two-phase anaerobic digester system (Gerardi, 2003).

2.1.2.6 Long-chain fatty acids

Lipids such as fats, oils and grease originate from many sources including wastewater and sludges from municipalities, restaurants, slaughterhouses, and dairy processing. When hydrolysed these produced long-chain fatty acids (LCFAs) (Khanal, 2008). The acids can interfere with the activity of the microorganisms once they are adsorbed into the cell even at very low concentrations (Khanal, 2008). Examples of LCFAs are caprylic, capric, lauric, myristic, and oleic acids. Koster and Cramer (1987) conducted a study on inhibition of methanogenesis from acetate in granular sludge using batch anaerobic toxicity assays. Their results showed that caprylic acid was only slightly inhibitory, while the most complete inhibitor was lauric acid, followed by oleic acid. In order to overcome the inhibitory effect of LCFAs on thermophilic manure reactors, Palatsi et al. (2009) showed that the two best strategies were increasing the biomass/LCFA ratio by adding inoculum to the reactor; and adding adsorbents (such as powder bentonite and fibres) which help to bind the lipids or LCFAs on their surface and minimise adsorption into the microbial cells.

2.1.3 Trace elements in anaerobic digestion

One of the requirements for a stable anaerobic process is the presence of trace elements. Speece (1983) reported that trace metals such as iron (Fe), cobalt (Co), nickel (Ni), molybdenum (Mo), tungsten (W), and selenium (Se) were essential for methanogens. They are needed in very low concentrations but the lack of them could lead to poor performance and instability in the digesters, as reported by Demirel and Scherer (2011). A review by Thanh et al. (2016) noted that while lack of trace elements can cause process instability and low methane production, overdosing may have toxic effects on the microorganisms.

Generally, trace elements have a vital effect on the growth and performance of anaerobic microorganism as the cofactors for the microbial enzyme system. For example, Kayhanian and Rich (1995) noted that cobalt was used in the carbon monoxide dehydrogenase (CODH) enzyme which is essential in acetogenic activity. The lack of Ni, Co, Mo, W and manganese (Mn) greatly affected the activity ratio of the dominant methanogens *Methanosarcina* and *Methanoculleus* (Wintsche et al., 2016).

A study by Karlsson et al. (2012) illustrated that the addition of Fe, Co, and Ni increased methane yields and helped acetoclastic methanogens to overcome ammonia inhibition. They also found increases in the population of *Methanosarcinales*, which explained the lower VFA concentrations and higher degradation rates. Similarly, Ariunbaatar et al. (2016) in their batch experiments on the biomethane potential of synthetic food waste found that specific biomethane potential were increased to 481.3 mL CH_4 g^{-1} VS (an increment of 14%) when Fe, Co and Ni were added.

In a study on supplementation of trace elements in anaerobic digestion of food waste by Banks et al. (2012), the addition of Se, Co, and Ni to a digester with VFA of 10,000 mg L^{-1} managed to reduce the concentration to less than 500 mg L^{-1}. It was also found that Se was essential for propionate oxidation and syntrophic hydrogenotrophic methanogenesis in food waste digestion at high ammonia concentrations. The study established critical Se and Co concentrations as 0.16 and 0.22 mg kg^{-1} fresh matter feed at moderate loading, which improved the digestion stability and hence the energy yield. Yirong et al. (2014, 2017) found, however, that this trace elements strategy did not work at high ammonia concentrations in thermophilic conditions.

While there is growing evidence for the importance and role of certain trace elements in anaerobic digestion, supplementation is still largely empirical and further research is needed to identify the effect of key factors such as bioavailability (Choong et al., 2016). In anaerobic digestion of slaughterhouse waste under mesophilic conditions (Ortner et al., 2015), methane production reduced to 35% when trace elements were not added. The highest methane yields were recorded when 11.4 mg L^{-1}, 25.4 mg L^{-1} and 4.8 mg L^{-1} of Ni, Co and Mo respectively were present in the digester, of which 62% of Ni and Co, and 68% of Mo were bioavailable for microbial uptake. The authors also concluded that the amount of bioavailable trace elements directly affected the performance of the process.

Co-digestion of substrates is one strategy that has been applied to utilise the existing trace elements contained in a feedstock and this is discussed further in section 2.3.

2.2 Mono-Digestion

2.2.1 Food waste

The EU Landfill directive requires the diversion of biodegradable waste, including food waste, from landfill. As noted above, AD provides a sustainable alternative technology as it produces biogas that can be used to generate heat and electricity. Food waste is a highly desirable feedstock for AD (Forster-Carneiro et al., 2008; Zhang et al., 2011; Zhang et al., 2007) because of its relatively high biodegradability and methane potential. It has also high moisture content which makes it less suitable for thermal technologies, again making AD an attractive option.

El-Mashad and Zhang (2010) carried out a study under mesophilic conditions using food waste collected in San Francisco (total solids (TS) and volatile solids (VS) ratio of 0.86) as the sole feedstock in a batch digester. The results after 30 days showed that at an initial organic loading of 2 g VS L^{-1}, methane yield was 353 L kg^{-1} VS which was 54% of the biogas production with VS reduction of 82%.

A biochemical methane potential (BMP) test using Korean mixed food waste at an initial loading of 2 g VS L^{-1} was conducted by Cho et al. (1995), and gave a high methane yield of 472 L kg^{-1} VS. The biodegradability based on stoichiometric methane yield was recorded as 0.86. In Japan and the United State, batch studies on food waste by Kawai et

al. (2014) and Ebner et al. (2016) also found high methane yields of 435 and 496 L kg^{-1} VS, respectively.

A study of food waste digestion in Norway by Zamanzadeh et al. (2016) was carried out in both mesophilic (37ºC) and thermophilic (55ºC) conditions. The digesters used were 10-L continuously stirred tank reactors with a working volume of 6 L. Both sets of experiments were fed with the same batch of pre-treated (milled to pass a 10 mm sieve and pasteurised at 70ºC for an hour) food waste at an OLR of 3 kg VS m^{-3} day^{-1} and hydraulic retention time (HRT) of 20 days. The specific methane yield for the mesophilic digester was 480 L kg^{-1}, which was 7% higher than under thermophilic condition (448 L kg^{-1} VS). The high degradability of the pre-treated food waste used in this study could be one reason why the methane yield was higher than obtained by other studies (Banks et al., 2010; Zhang et al., 2011; and Dearman and Bentham, 2007).

Banks, Chesshire, et al. (2011) monitored the digestion of source-segregated domestic food waste in a demonstration-scale AD plant in the UK. The digester was a continuously mixed tank with a volume of 900 m^3 and was operated at a temperature of 42 ºC. The average VS : TS ratio was around 0.88 and the digester was fed at an average OLR of 2.5 kg VS m^{-3} day^{-1} with an average hydraulic retention time (HRT) of 80 days. The results showed that the specific methane yield was 402 L kg^{-1} VS. An overall energy balance for the process gave a total potentially recoverable energy of 405 kWh $tonne^{-1}$ wet weight.

The studies discussed above and a number of recent reviews (Capson-Tojo et al, 2016; Zhang et al., 2014; Bong et al., 2018; Ren et al., 2018) have shown that although there are differences in feedstock characteristics, digester design, and operating conditions used, the AD of food waste can provide a high methane yield, as shown in Table 2.4.

Table 2.4 Review on AD of food waste as sole feedstock

TS (% WW)	VS (% WW)	Reactor type	Temp (°C)	Methane yield (L kg^{-1} VS)	Reference
28.0	24.1	Batch	35	353	El-Mashad and Zhang (2010)
n.a.	n.a.	BMP	35	472	Cho et al. (1995)
n.a.	n.a.	Batch	37	435	Kawai et al. (2014)
n.a.	n.a.	BMP	37	496	Ebner et al. (2016)
27.7 – 27.8	24.3 – 24.4	CSTR	42	402	Banks et al. (2010)
17.8	16.1	CSTR	35	480	Zamanzadeh et al. (2016)
17.8	16.1	CSTR	55	448	Zamanzadeh et al. (2016)
23.9	21.6	CSTR	37	460	Yirong et al. (2017)
18.1	17.1	Semi-continuous	37	396	Zhang et al. (2011)
n.a.	n.a.	Sequential batch	37	229	Dearman and Bentham (2007)

Although the methane yield and hence the energy potential is high, AD of food waste as a sole feedstock has its drawbacks. Food waste as compared to other feedstocks is rich in proteins and this could lead to high ammonia concentrations which may become inhibitory (Banks and Zhang, 2010; Hartmann and Ahring, 2005; Shi et al., 2017). Accumulation of VFAs was also found to be inhibitory to the process (Ike et al., 2010; Yu et al., 2018). VFA accumulation and the associated decrease in pH could result in unstable AD or even process failure (Banks et al., 2008; Banks, Chesshire, et al., 2011; Park et al., 2008; Zhang et al., 2012a).

Banks and Zhang (2010) studied the digestion of food waste as a mono-substrate in a 75 L continuously mixed digester at 36 $\pm$ 1°C, with an OLR of 2 kg VS m^{-3} day^{-1}, and a nominal solids retention time (SRT) of 30 days. They found that although biogas production had reached a steady state by 3 retention times, the concentration of total ammonia exceeded 4000 mg L^{-1} by day 150 (5 retention times) and continued to increase. The VFA concentration indicated signs of stress by 6 retention times and increased up to 5 times higher than the stable level of about 200 mg L^{-1}. They suggested that the mono-

digestion of food waste alone led to increased VFA, probably due to deficiency of some trace elements needed for microbial metabolism, combined with increasing ammonia concentrations. Studies by Zhang et al. (2011) and Voelklein et al. (2017) also supported these findings. In a long-term study of AD of Korean food waste by Zhang et al. (2011), the addition of trace elements (a solution containing Co, Mo, Ni and Fe) to the system gave stable methane production compared to operation without trace elements addition. High methane yields of 396 L kg^{-1} VS and VS destruction of 75.6% with no significant VFA accumulation were recorded. In the study by Voelklein et al. (2017), both single and two-stage reactor performance showed instability (low methane yield and high VFA accumulation) after exceeding a threshold OLR of 2 g VS L^{-1} day^{-1}. The addition of trace elements (Co, Fe, Mo, Ni and Se) managed to stabilise the system, however, and restored the methane yield back to the levels before nutrient deficiencies were detected.

A study on a full scale AD plant treating food waste in Kyoto was conducted by Ike et al. (2010). The food waste was first mixed and stored in a storage tank before being digested in a gas-stirred type tank operated at 30 ^{o}C for the first 15 days and increased to 54 ^{o}C until the end of the study. They found that VFAs (acetate, propionate, butyrate, and valerate) had already been produced at high concentrations in the storage tank and were degraded in the digestion tank. However, on days 110 – 120, the propionate concentration increased to over 200 mg L^{-1}. Consequently, a drop in the biogas production was noted. The cessation of feeding around days 120 – 130 decreased the propionate concentration maybe due to washout and/or conversion to acetate by propionate degrading syntrophs.

2.2.2 Cattle slurry

Anaerobic digestion of cattle slurry is a technology that offers many potential benefits (Lukehurst and Bywater, 2015). Apart from the production of biogas, it also effectively removes pathogens, reduces odour, promotes the uptake of fertilisers from digestate, and may reduce the potential GHG emissions (Ward et al., 2008; Marañón et al., 2011; Bywater, 2010; Amon et al., 2006). Amon et al. (2006) in a study on cattle slurry management in dairy farms in Austria reported that AD technology was a very effective means to manage GHG emissions and found reductions of 59%.

The high buffering capacity and continuous and steady supply of cattle slurry make it a suitable feedstock for AD (Luste and Luostarinen, 2011). Moreover, it often contains trace

elements such as Co, Ni, Mo and Fe that are important for maintaining enzyme activities of anaerobic microbial consortia, in sufficient amounts to ensure the stability of the process (Zhang et al., 2011). Table 2.5 shows some of the trace elements contained in some samples of cattle slurry and dairy manure as reported by other researchers. As shown, the concentrations vary from sample to sample as the elements in manure mostly originated directly from the animals' diet, while some are indirectly derived during the collection process (Bolan et al., 2004).

From Table 2.5 it can be seen that cattle manure contains sufficient essential trace elements to make co-digestion a promising strategy for stable anaerobic digestion of materials such as food waste, which are lacking in some of these elements according to several studies (Choong et al., 2016)). Copper, nickel and zinc, identified in Table 2.3 as toxic to AD when in soluble form, are also typically present in cattle slurry at relatively high concentrations but their bioavailability may be limited. The fact that cattle slurry has been widely digested both in mono-digestion and as a co-substrate, as discussed in sections 2.2.2 and 2.3 below, confirms that in practice there is no intrinsic reason why its metals content should be inhibitory to anaerobic digestion.

Table 2.5 Trace element requirement for AD of food waste and trace element concentrations in cattle slurry / dairy manure

Trace Elements	**TE Required (mg L^{-1})**		**TE in Cattle Manure (mg kg^{-1})**		
	Banks et al. (2012)	VALORGAS (2013)	Sager (2007)	McBride and Spiers (2001)	Raven and Loeppert (1997)
Aluminium	0.1	–	–	–	–
Boron	0.1	–	–	8.1	–
Cobalt	1	0.35	2.1	2.5	3.57
Copper	0.1	–	51	139	–
Iron	5	–	–	–	–
Manganese	1	–	180	–	357
Nickel	1	1	6.3	8	8.7
Zinc	0.2	–	164	191	164
Molybdenum	0.2	–	3.5	2.5	–
Selenium	0.2	0.2	0.59	3	0.48
Tungsten	0.2	–	–	–	–

The disadvantage of mono-digestion of cattle slurry is the low biogas and methane production. The low biogas yield is due to the efficient digestive system of the cattle. Most of the biodegradable carbon has been digested in the rumen and gut (Amon et al.,

2007) hence the lower biogas potential of the manure. Due to the rumen fermentation, cattle slurry has a C : N ratio of 11 – 14 (Umetsu et al., 2006) which is relatively low compared to the optimum C : N ratio of between 20 – 30 required to ensure a sufficient nitrogen supply for cell production and the degradation of the carbon present (Fricke et al., 2007). Excessive nitrogen (low C : N ratio) could lead to instability in AD since high concentrations of ammonia are toxic to methanogens as discussed earlier.

Another reason for the low biogas potential is the high content of lignin in cattle manure which originates from the animal's diet (Triolo et al., 2013). Lignin is non-degradable in anaerobic environments due to its refractory nature and resistance to microbial attack, which limits its convertibility (Burke, 2001; Tsapekos et al., 2015). This is the reason why only between 40 – 50% of the lignin-rich organic materials will be converted to biogas in AD. Only after some pre-treatment to degrade the lignin or make it solubilise, such as oxygen assisted wet-explosion pre-treatment as conducted by Ahring et al. (2015), could the methane yield be increased to 4.5 times that of the untreated feedlot manure.

As reported by various researchers, the methane yield from AD of cattle slurry as sole feedstock is relatively low and ranges from 125 – 310 L kg^{-1} VS added (Table 2.6).

Table 2.6 Review on AD of cattle slurry as sole feedstock

Reactor type	**Temperature (ºC)**	**Methane yield (L kg^{-1} VS)**	**Reference**
Batch	35	241 – 302	El-Mashad and Zhang (2010)
CSTR	35	190 – 310	Karim et al. (2005)
Batch	35	210 – 280	Luste and Luostarinen (2011)
Batch	38	125 – 166	Amon et al. (2007)
Batch	38	282	Himanshu et al. (2018)
CSTR	37	206 – 223	Rico et al. (2011)
CSTR	37	160	Frost and Gilkinson (2011)
Plug-flow	30	240	Arikan et al. (2018)
CSTR	35	279	Li et al. (2016)
CSTR	35	204	Lehtomäki et al. (2007)
Batch	37	147	Vivekanand et al. (2018)
CSTR	35	151 – 155	Moset et al. (2015)
CSTR	50	176 – 185	Moset et al. (2015)
CSTR	53	210	Tsapekos et al. (2016)

One explanation for the variety in the reported methane yields was the animals' diets and milk yields as investigated by Amon et al. (2007). This study found that manure from dairy cows with medium milk yield and fed a well-balanced diet gave the highest methane yield. It was also found that a higher feeding intensity and milk yield caused an increase in lignin content and hence reduction in the methane production.

In a batch study conducted by Luste and Luostarinen (2011) to look at ultrasound pre-treated and hygienised dairy cattle slurry, the pre-treatments of the cattle slurry did significantly increase the methane yield. The biochemical methane potentials (BMP) obtained after ultrasound pre-treatment and hygienisation were 250 and 280 m^3 $CH_4 tonne^{-1}$ VS added respectively, compared to the BMP of raw cattle slurry which was 210 m^3 CH_4 $tonne^{-1}$ VS added. As reported by Tsapekos et al. (2016), in continuous digesters, thermal alkaline pre-treatment of digested manure fibres at various NaOH concentrations and temperatures notably enhanced the biogas production, where treatment with 6% NaOH at 55ºC was the most efficient pre-treatment method recording a 26% increment in methane production.

Experiments conducted by El-Mashad and Zhang (2010) showed that screening of dairy manure affected the biogas yield. The fine fraction of screened manure has the highest methane yield at an average of 302 L kg^{-1} VS. The difference was quite significant if compared to the coarse fraction and unscreened manure, which had average values of 228 and 241 L kg^{-1} VS respectively. It was believed that the higher rate in fine fraction maybe due to the smaller particle sizes and the presence of larger quantities of easily biodegradable organics.

Rico et al. (2011) studied the performance of a dairy manure biogas plant in Cantabria, Spain. The plant used a continuously stirred-tank reactor (CSTR) digester to digest the screened liquid fraction of dairy manure which was separated using a screw press separator. It was found that with HRT as short as 10 days, the methane production was highest at 223 L kg^{-1} VS added. The performance of another pilot plant was also studied by Frost and Gilkinson (2011). The plant which was in Hillsborough, UK was also using a CSTR digester but the cow slurry used was only macerated to a nominal particle size of 12 mm. The plant produced an average of 160 L CH_4 kg^{-1} VS, while the electricity and heat produced by the combined heat and power (CHP) unit were 1.51 kWh m^{-3} biogas and 2.8351 kWh m^{-3} biogas respectively.

Besides low methane yield, another problem related to cattle manure digestion is the production of hydrogen sulphide (H_2S). Although water-saturated biogas from dairy-manure digesters contains less than 10000 ppmv of sulphur impurities of which the majority are H_2S (Pellerin et al., 1987 as quoted by Zicari, 2003), H_2S concentrations can vary from 600 to over 7000 ppmv. This indicates that specific characteristics of digestion systems such as environmental conditions, animal feeds, water, and addition of other organic materials may influence the concentration of H_2S in the biogas generated (Fiesinger, 2006; Andriamanohiarisoamanana, 2017). H_2S can damage tanks, pipes and machines due to its corrosive behaviour, and cause health hazards due to it toxicity. Human exposure to more than 100 ppmv (parts per million by volume) of H_2S will instantly cause lung damage, respiratory failure, and unconsciousness, while further exposure to around 800 ppmv could cause complete failure of the nervous system and result in sudden death (Sakirkin et al. 2013).

2.3 Co-Digestion: Cattle Slurry and Food Waste

The previous sections have discussed the advantages and disadvantages of AD of food waste and cattle slurry as sole feedstocks. One of the commonly used methods to rectify the disadvantages is by co-digestion with other substrates.

Sharing the same equipment for digestion of two or more substrates can not only give significant economic advantages (Mata-Alvarez et al., 2000), but most importantly can improve the biogas yields. Cattle slurry is known as having low methane yield and Angelidaki and Ellegaard (2003) as cited by Zhang et al. (2011) stated that in order for the process to be economically viable, the methane yield should be higher than 20 m^3 CH_4 m^{-3} substrate. As shown in Table 2.7, co-digestion with other substrates has improved the methane yields of cattle slurry.

Co-digestion could also improve the C : N ratio. Too high C : N ratios or inadequate nitrogen will upset the metabolic balance of the microorganisms and cause incomplete carbon utilisation and hence low biogas yield. Meanwhile, too low C : N ratios will cause ammonia accumulation and increases the pH value, which is toxic to methanogens. It has been suggested that the C : N ratio should be in the optimal range of 20 – 30 (Liu et al., 2010). As different feedstocks have different C : N ratios, it was recommended to co-

digest feedstocks with low C : N ratios (such as manures) with higher C : N ratios feedstocks (such as food waste, municipal solid waste and crops) to achieve the ideal ratio (Ward et al., 2008; Dai et al., 2016) for a more stable process. In addition, the supplementation of trace elements from one co-substrate to make up for those that are lacking in other could also improve the digestion (Zhang et al., 2011).

Because of the potential benefits resulting from co-digestion, there have been numerous studies looking at the possibilities, and optimising co-digestions of two or more substrates. Table 2.7 illustrates some of the many investigations that have been conducted on co-digestion of cattle slurry and food waste with other substrates, and on the smaller number of studies on digestion of these two wastes as co-substrates.

Cattle slurry/manure has been digested with other substrates such as industrial sludge, municipal waste, energy crops, crop residues and slaughterhouse waste to improve its performance. Batch studies conducted by Capela et al. (2008) demonstrated that it was possible to co-digest the organic fraction of municipal organic waste (OFMSW), cattle slurry (CS) and industrial sludge (IS) together. In this study CS was the co-substrate and although it was shown that less CS in the system would produce higher methane yields, the methane yield by co-digestion was much higher than for the digestion of CS alone (15 – 30 L CH_4 kg^{-1} VS). Results from batch studies by Vivekanand et al. (2018) also supported these findings. Higher methane yields were produced when less cow manure (CM) was co-digested with whey, with 11 – 80% more than the methane yields obtained from CM alone.

The same trend was also illustrated by a study in Bolivia conducted by Alvarez and Lidén (2008) with co-digestion of fruit and vegetable waste (FVW), solid cattle and swine slaughterhouse waste (SCSSW) and solid cattle and swine manure (SCSM). In their study, which was carried out using 2-L reactors with semi-continuous mixing, the co-digested mixtures between all three components gave better methane yields compared to digestion of sole feedstock, or a mixture of just two substrates, with the highest methane yield recorded at 350 L CH_4 kg^{-1} VS obtained from the mixture of 17% SCSSW, 17% SCSM, and 67% FVW (based on VS).

In the study of cow manure with energy crops and crop residues by Lehtomäki et al. (2007) in laboratory scale continuously stirred tank reactors (CSTRs), the highest specific methane yields found were 268, 229 and 213 L CH_4 kg^{-1} VS in co-digestion of cow

manure with grass, sugar beet tops, and straw respectively. The figures were achieved by adding 30% (based on VS) of crops, and were 16 – 65% more than obtained from mono-digestion of cow manure. However, increasing the amount of crops further to 40% decreased the methane yields. This was because the addition of 30% of crops in the feedstock has balanced the C : N ratio to approximately 15 – 25, which is within the optimal range for anaerobic digestion, while a further increment in the proportion of crops will exceed the optimal value.

Tsapekos et al. (2018) performed both batch and CSTRs at thermophilic condition for co-digestion of source separated municipal organic waste (SSMOW), pre-treated using a biopulper, and cattle manure. In the batch study, different proportions of substrates were used and it was found that the highest methane yield of 382 L CH_4 kg^{-1} VS was obtained using a mixture of 20% of cattle slurry and 80% of SSMOW on a VS basis. The results from the batch study were used in linear, quadratic and cubic models and it was predicted that 90% SSMOW in the feedstock mixture would give the highest methane production. Hence, a CSTR study was conducted using this substrate proportion, which gave a methane yield of 437 L CH_4 kg^{-1} VS. Compared to the results of BMP assays at the same substrate proportion, these methane yields were relatively high (96% of the expected value), showing the efficiency of the system. The high performance of the co-digestion was due to the fact that the SSMOW contained a large amount of lipids and soluble carbohydrates and that the pre-treatment boosted biomethanation. It was also aided by the ability of the cattle manure to provide buffering capacity and avoid inhibition resulting from the accumulation of intermediate products (Tsapekos et al., 2018).

In the three examples of co-digestion of food waste with other substrates apart from cattle slurry given in Table 2.7, anaerobic digestion of food waste as the only feedstock (control) failed, as indicated by very low biogas production and accumulation of VFAs. By introducing fruit and vegetable waste, card packaging, and piggery wastewater into the system as co-substrates, however, the performance of the digestions as studied by Lin et al. (2011), Banks and Zhang (2010) and Zhang et al. (2011) respectively, were all stabilised.

Lin et al. (2011) suggested the optimal mix ratio of food waste and fruit and vegetable waste (FVW) based on VS was 1 : 1 to give the best performance, in which the methane

production was 490 L kg^{-1} VS, and VS and soluble chemical oxygen demand (sCOD) removal efficiencies were recorded at 74.9% and 96.1% respectively.

Banks and Zhang (2010) noted that by combining food waste (high lipid and protein content which could cause inhibitory effects due to accumulation of VFAs and / or ammonia) with card packaging (high fibre / carbohydrate but low nitrogen content), a more stable digestion with a better C : N ratio could be achieved. Their study in laboratory-scale 4-L semi-continuous reactors showed specific methane yields of 320 L kg^{-1} VS for a mixture of food waste and card packaging on a 53 : 47% VS basis, with total ammonia nitrogen (TAN) stabilised at around 1 g N L^{-1} and total VFA concentrations of less than 100 mg L^{-1}.

In a semi-continuous anaerobic digestion study by Zhang et al. (2011), it was noted that food waste was deficient in essential trace elements. This was supported by the higher methane yield, low residual sCOD and lack of VFAs accumulation when food waste was co-digested with the solid fraction of piggery wastewater compared to co-digestions of food waste with whole piggery wastewater and liquid fraction of piggery wastewater, and digestion of food waste alone. Analysis confirmed that the solid fraction contained more trace elements.

Table 2.7 Previous studies on co-digestion of cattle slurry and food waste

Feedstocks	Reactor type	Proportion of substrate	Methane yield (L CH_4 kg^{-1} VS)	VS removal	Reference
Organic fraction of municipal solid waste (OFMSW) + cattle slurry CS) + industrial sludge (IS)	Batch (35 °C)	OFMSW : CM : IS (w/w) 1 : 1 : 2 2 : 2 : 1 6 : 1 : 1 18 : 1 : 1	 87 116 250 245	 32% 35% 57% 59%	Capela et al. (2008)
Cow manure (CM) + energy crops – sugar beet tops (ST), grass silage (GS), oat straw (OS)	CSTR (35 °C)	CM + ST: varies 10 – 40% ST (VS) CM + GS: varies 10 – 40% GS (VS) CM + OS: varies 10 – 40% OS (VS)	 149 – 229 143 – 268 145 – 213	 28 – 49% 41 – 53% 27 – 43%	Lehtomäki et al. (2007)
Solid cattle and swine manure (SCSM) + solid cattle and swine slaughterhouse waste (SCSSW) + fruit and vegetable waste (FVW)	CSTR (35 °C)	varied % of VS but wet-weight maintained at 4%	270 – 350	52.4 – 67.4%	Alvarez and Lidén (2008)
Cow manure (CM) + whey (W)	Batch (37 °C)	CM : W (VS basis) 85 : 15 75 :25 50 : 50 25 : 75 15 : 85	 164 177 219 259 266	 n.a. n.a. n.a. n.a. n.a.	Vivekanand et al. (2018)

Feedstocks	Reactor type	Proportion of substrate	Methane yield (L CH_4 kg^{-1} VS)	VS removal	Reference
Source separated municipal organic waste (SSMOW) + cattle manure (CM)		SSMOW : CM (VS basis)			Tsapekos et al. (2018)
	Batch (54 °C)	80 : 20	382	n.a.	
	CSTR (54 °C)	90 : 10	437	n.a.	
FW + FVW	CSTR (35 °C)	FW : FVW (VS basis)			Lin et al. (2011)
		1 : 2	440	71.7%	
		1 : 1	490	74.9%	
		2 : 1	490	79.3%	
FW + card packaging (CP)	Semi-continuous (35 °C)	FW : CP (VS basis) 53 : 47	320	n.a.	Banks and Zhang (2010)
FW + piggery wastewater (PW)	Semi-continuous (35 °C)	FW : PW (based on contribution OLR on COD)			Zhang et al. (2011)
		93 : 7	358	n.a.	
		83 : 17	388	n.a.	
FW + dairy manure (DM)	Batch (35 °C)	FW : DM (VS basis)			El-Mashad and Zhang (2010)
		32 : 68	282	60%	
		48 : 52	311	68%	
CM + FW	CSTR (35 °C)	CM : FW (TS basis)			Neves et al. (2009)
		1 : 1	210 – 260	64 – 67%	
FW + CS	Semi-continuous (36 °C)	FW : CS (VS basis)			Banks and Zhang (2010)
		1 : 4	OLR 2 : 220	n.a.	
		2 : 3	OLR 2 : 260	n.a.	
			OLR 3 : 230	n.a.	
		3 : 2	OLR 3 & 4: not mentioned but > 300 (from the graph)	n.a.	

Feedstocks	Reactor type	Proportion of substrate	Methane yield (L CH_4 kg^{-1} VS)	VS removal	Reference
FW + cattle manure (CM)	Batch (35 ºC)	FW : CM (VS basis) 8.0 : 4.0 8.0 : 2.7 8.0 : 2.0	 388 352 343	 n.a. n.a. n.a.	Zhang et al. (2013)
FW + DM	CSTR (36 ºC)	FW : DM 1 : 1	OLR 1 : 530 (fine) 470 (medium) 460 (course) OLR 2 : 630 (fine) 560 (medium) 470 (course) OLR 3 : 510 (fine) 470 (medium) 470 (course)	 n.a n..a n.a n..a n.a n..a n.a n..a n.a	Agyeman and Tao (2014)
Dairy cow slurry (DCS) + municipal food waste (MFW)	CSTR (37 ºC)	DCS : MFW (w/w) 100 : 0 86 : 14 75.5 : 24.5 67.8 : 32.2	 OLR 1.83 : 218 OLR 2.99 : 358 OLR 4.03 : 402 OLR 5.04 : 445	 28.2% 46.7% 52.6% 55.2%	Morken et al. (2018)
Kitchen waste (KW) + chicken fat (CF) + DM	Semi-continuous (35 ºC)	KW : CF : DM (VS basis) 1 : 1 : 3	 328	 74%	Ye et al. (2015)

The main focus of the current research is on the co-digestion of cattle slurry and food waste. Some previous studies that have been conducted on the co-digestion of these two substrates are listed on Table 2.7.

In one of these studies, El-Mashad and Zhang (2010) studied the co-digestion of unscreened dairy manure and food waste in batch reactors. Based on the availability of both substrates, two mixtures were used with the food waste and cattle manure in proportions of 32% : 68% and 48% : 52% on a VS basis. The average methane yields after 30 days were 282 L kg^{-1} VS and 311 L kg^{-1} VS with the mixture with more portion of food waste giving the higher methane yield. These values were higher than for digestion of dairy manure alone (241 L kg^{-1} VS). Although the digestion of food waste alone gave higher biogas production, it was considered that co-digestion of manure and food waste reduced the accumulation of intermediates during the initial period. The methane yields obtained from co-digestion in this study were similar to those in the CSTR study by Neves et al. (2009) in which the ratio of 1 : 1 based on TS gave methane yields of 210 – 260 L kg^{-1} VS added.

Banks and Zhang (2010) studied the co-digestion of food waste and cattle slurry alongside mono-digestion of food waste. The digestion of food waste alone was not stable as could be seen from the accumulation of VFA which was attributed to the decreasing concentration of essential trace elements, and high total ammonia nitrogen (TAN) concentration. By adding cattle slurry as a co-substrate, which contributes essential trace elements to the system, the performance of the digesters was stabilised with lower TAN concentrations and the total VFA dropped to less than 200 mg L^{-1}. When co-digestion first started with OLR 2 kg VS m^{-3} day^{-1} and 80% cattle slurry on a VS basis, the methane yield was recorded at 220 L CH_4 kg^{-1} VS. Decreasing the amount of cattle slurry in the mixture increased the methane yield. A mixture with 40% cattle slurry and 60% food waste gave a specific biogas production of 520 L kg^{-1} VS which was better than that produced by digestion of food waste alone due to failure of the mono-digestion.

Co-digestion of dairy cow slurry and municipal food waste in mesophilic conditions was studied by Morken et al. (2018). Four digesters were fed daily with cattle slurry and different amounts of food waste at 0%, 14%, 24.5% and 32.2% of wet-weight were used. Increasing the amount of food waste while keeping the supply of cattle slurry constant decreased the HRT and increased the OLR. The control digester with OLR of 1.83 g VS

L^{-1} day^{-1} and HRT of 25.9 days had the lowest specific methane yield of 210 L CH_4 kg^{-1} VS. Increasing the percentage of food waste in the substrate increased the OLR and also the specific methane yield, due to the high energy potential and degradability of the food waste compared to cattle slurry. The highest specific methane yield of 445 L CH_4 kg^{-1} VS and degradation of 55.2% were achieved when 32.2% (wet-weight) of food waste was co-digested with cattle slurry at an OLR of 5.04 kg VS m^{-3} day^{-1}. The study also found that the relationship between a kinetic constant and the OLR was linear and gave an estimated methane yield of 301 L CH_4 kg^{-1} VS.

The effects of food waste particle size were tested in co-digestion of food waste and dairy manure at different OLRs. The experiment was conducted at 36 ^oC in bench-scale, semi-continuous digesters where the feedstock used was combination of food waste and dairy manure at a VS ratio of 1. The food waste used was categorised into three grades – fine, medium and coarse, based on the aperture diameters (2.5, 4 and 8 mm respectively) of the cutting plates used for grinding the food waste. The digesters were also operated at three different OLRs of 1, 2, and 3 g VS L^{-1} day^{-1}. Specific methane yields obtained from this study (460 – 630 L CH_4 kg^{-1} VS) were relatively higher than other studies on co-digestion of food waste and cattle slurry (210 – 445 L CH_4 kg^{-1} VS). It was suggested that this could have due to the long solids retention times associated with 'dry' digestion (without water addition) and the higher lipid content of the feedstock in this study. The results of this study also indicated that specific methane yield in the co-digestion of food waste and dairy manure could be increased by 9 – 34% by reducing the food waste particle size from 8 to 2.5 mm. Izumi et al. (2010) and Palmoski and Muller (2000) as quoted by Agyeman and Tao (2014) stated that the larger specific area due to the smaller particle size led to enhanced hydrolysis, and hence higher biogas production. In terms of OLR, biogas production rates increased with increasing OLR; it was suggested that co-digestion of food waste and dairy manure could be loaded up to 3 g VS L^{-1} day^{-1} without ammonia inhibition. It was also concluded that the specific methane yield was highest at OLR of 2 g VS L^{-1} day^{-1} regardless of particle size.

2.4 Inhibition by Sulphide

Sulphide is required by methanogens, and Scherer and Salim (1981) as quoted by Speece (1983) reported that the optimal sulphide concentration for methanogenic growth is 1 –

25 mg L^{-1}. At higher concentrations, however, it becomes toxic. Speece (1983) reported the toxicity threshold to be above 100 – 150 mg L^{-1} of un-ionised H_2S; meanwhile Parkin et al. (1983) as quoted by Karhadkar et al. (1987) reported that 50 mg L^{-1} could cause sulphide toxicity to unacclimated methanogens.

2.4.1 Sulphate reducing bacteria (SRB) versus methanogens

In anaerobic digestion, sulphate in the influent wastewater or feedstock is reduced to sulphide by sulphate reducing bacteria (SRB). The reduction of sulphate by SRB is carried out using the same substrates (such as acetate and H_2) as those used by methanogens, thus causing competition which can lead to a reduction in methane yields. Some studies have shown that SRB can out-compete methanogens for the substrates (Gupta et al., 1994; Schonheit et al., 1982; Omil et al., 1998).

The competition between SRB with both hydrogenotrophic and acetoclastic methanogens over mutual substrates has been studied by many authors. A study by Harada et al. (1994) on synthetic low strength wastewater in laboratory-scale UASB reactors at same influent strength (COD at 500 mg L^{-1}) but different sulphate levels (30, 150 and 600 mg SO_4^{2-} L^{-1}), showed that methane production decreased when the level of sulphate was higher. They also found that the specific methanogenic activities of hydrogen-utilising methanogens (H-SMAs) decreased with increasing sulphate concentration, which indicates that SRB prevailed over methanogens in scavenging hydrogen. Van Houten et al. (1995) studied the microbiological aspects of biological sulphate reduction in gas-lift reactors using hydrogen as the energy source. Their finding revealed that SRB of *Desulfovibrio* species were the most dominant microorganism in the system.

Hydrogen utilising sulphate reducing bacteria (H-SRB) out compete hydrogenotrophic methanogens (H-methanogens) mainly due to kinetic and thermodynamic advantages over H-methanogens. Utilising hydrogen by H-SRB (reduction of sulphate to sulphide) yields more energy than the process of methanogenesis, hence the growth of SRB is more favourable than methanogens (Karhadkar, 1986), as indicated by equations 2.2 and 2.3.

H-SRB: $4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$ $\Delta G = -154$ kJ Equation 2.2

H-methanogens: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ $\Delta G = -135$ kJ Equation 2.3

As for acetate, the literature shows contradictory outcomes from the competition between acetate utilising SRB (A-SRB) and acetoclastic methanogens (A-methanogens). One of the parameters that governs this is the sulphate concentration. Omil et al. (1998) used UASB reactors to study this competition with acetate as sole substrate and at different COD : sulphate ratios. When the feeding at a COD : sulphate ratio of 2, after 200 days of operation and throughout the steady state, acetate was removed approximately 77% and 23% by A-MPB and A-SRB respectively. However, at a higher concentration of sulphate (COD : sulphate ratio lower than 0.67), SRB were the dominant species after prolonged reactor operation.

In high-rate anaerobic reactors, methanogens may out compete SRB (Isa et al., 1986). Using reactors with reticulated polyurethane sponges as a carrier matrix and with acetate as a substrate, at the rate of 10 g COD removed per L of reactor per day, SRB scavenged only 10 – 20% of the total electron flow. The study concluded that the ability of methanogens to outcompete SRB was due to the high substrate concentration and the preferential colonisation of the carrier matrix by the methanogens.

Another factor that affects the competition is pH and temperature. At pH values above 7.7, acetate degradation via sulphate reduction was favourable, while below pH 6.9, methanogenesis was favoured (Visser et al., 1996). With respect to temperature, a study by Shin et al. (1996) found that methanogens were more affected by temperature shocks than were sulphate reducers. This study used UASB reactors treating tannery wastewater: when shock increases and decreases in temperature between 25 and 35 ^{o}C were applied, the proportion of COD reduction by SRB increased from 15% to 80%, while methane production decreased. This indicated that methanogens are more sensitive than SRB to sudden temperature changes.

2.4.2 Sulphide toxicity

Sulphides are produced in an anaerobic environment by the reduction of sulphates present in the feedstock and by the degradation of proteins. The dissolved sulphides formed can be transferred to the biogas in the form of hydrogen sulphide (H_2S).

It is known that un-ionised sulphide or free sulphide is the most toxic form of sulphide (Speece, 1996; Khanal, 2008; Chen et al., 2008) since it can diffuse into the cell membrane. Once across the cell wall, it modifies native proteins and interferes with the

numbers of coenzyme sulphide linkages and the assimilatory metabolism of sulphur (Vogels et al., 1988 as quoted by Chen et al., 2008).

The form in which dissolved H_2S is found varies with pH. At acid to neutral pH (pH 6.4 – 7.2), the sulphide toxicity is due to free sulphide, and at basic pH (pH 7.8 – 8.0), it is caused by total sulphide. As free sulphide is the most toxic form, inhibition increases when pH falls (Khanal, 2008).

In a batch assay study on the effect of sulphide addition on reactor performances, Karhadkar et al. (1987) found that 50% of inhibition occurred when total sulphide level were observed at 100 – 224 mg L^{-1} (sulphide addition of 500 mg L^{-1}). As the relationships between reactors performance and sulphide added (and observed) were not clear, they concluded that, as H_2S concentration in the gas was proportional to the added sulphide while the performances were inversely proportional, the inhibition was thus directly proportional to the H_2S concentration. Studies by Koster et al. (1986) and Oleszkiewicz et al. (1989) agreed with this at pH ranges 6.4 – 7.2 and 6.5 – 7.4 respectively. Koster et al. (1986) observed that 50% of inhibition was detected at an H_2S concentration of 246 – 252 mg L^{-1} (total sulphide of 357 – 810 mg L^{-1}) and 125 mg L^{-1} of H_2S (total sulphide of 320 mg L^{-1}) caused 90% COD removal using acetate as substrate (Oleszkiewicz et al., 1989).

2.5 Sulphide Removal

Sulphate is a chemically inert, non-volatile and non-toxic compound and hence does not pose any threat to the environment. The matter that needs to be looked into is the production of sulphide (especially H_2S). H_2S is quite soluble in water (Speece, 1996), and due to its properties can cause problems such as corrosion, release of obnoxious odours, safety hazards to workers in gaseous form (Nielsen et al., 2005). Apart from that, it can reduce the methane yield and consequently less energy can be recovered from the anaerobic digestion process.

Khanal (2008) classified sulphide removal techniques into physical (stripping), chemical (adsorption, precipitation, and oxidation) and biological, and combinations of all three. Cirne et al. (2008) identified approaches to sulphide emission control as adding selective inhibitors of sulphidogenic bacteria (such as molybdate, divalent transition metals, nitrite,

and antibiotics); raising the pH to reduce free sulphide but increase ionised sulphide; oxidising sulphide by adding oxygen or nitrate; and adding sulphide scavengers (such as metal ions to precipitate sulphide). A large number of studies have focused on biological methods of sulphide removal (Syed et al., 2006; Krayzelova et al., 2015), some of which are in use in full-scale AD plants (Kobayashi et al., 2012), but the simplicity of chemical approaches makes them attractive for on-farm use and this work therefore focuses on chemical methods.

Table 2.8 summarises the results from some studies by previous researchers on sulphide control in anaerobic digestion using iron salts. Some previous studies have managed to reduce the sulphide concentrations in wastewater using iron dosing (Firer et al., 2008; Zhang et al., 2013; Padival et al., 1995; Nielsen et al., 2005; Ganigue et al., 2011; Zhang et al., 2009; Wang and Banks, 2007). Dosing with iron salts has become a conventional sulphide control strategy because iron ions (ferrous or Fe(II) (Fe^{2+}) and ferric or Fe(III) (Fe^{3+})) precipitate dissolved sulphide and H_2S effectively, easy dosing technique, and the precipitates has no toxic effect on microbial activity (Speece, 2008). However, a study by Utgikar et al. (2002) suggested that metal sulphide precipitate needs to be removed to protect SRB from its inhibitory impact. The drawback of iron dosing is that it does not prevent the initial sulphide formation and thus still gives a reduction in biogas and methane production (Smith and Carliel-Marquet, 2008 and Zhang et al., 2009).

Both Fe(II) and Fe(III) can be used in sulphide control. Fe(II) removes sulphide and precipitates as ferrous sulphide (FeS), whereas Fe(III) can remove sulphide by oxidising it chemically to elemental sulphur while being reduced to Fe(II), which can subsequently produce FeS as shown in Equation 2.4 (Speece, 2008).

$$2Fe^{3+} + S^{2-} \rightarrow 2Fe^{2+} + S^{0}$$

$$Fe^{2+} + HS^{-} \rightarrow FeS \downarrow + H^{+} \qquad \text{Equation 2.4}$$

It cannot be firmly said which iron salt works better. In controlling H_2S from anaerobic swine manure, Barber and McQuitty (1977) compared ferrous and ferric ion dosages in both laboratory and bench-scale trials. They concluded that ferrous ion performed better as 100% of sulphides were eliminated compared to only 72% of reduction using ferric ion. In contrast, Tomar and Abdullah (1994) compared both iron salts to control the generation of H_2S in wastewater, and concluded that Fe(III) salt solution was slightly

more effective than Fe(II) salt solution. They detected that only 8 g of ferric salt was required per g of dissolved sulphide at pH 7.6 for complete removal of sulphide from the wastewater, compared to 10 g for ferrous salt.

In a study by Erdirencelebi and Kucukhemek (2018) in a full-scale AD plant at a municipal wastewater treatment works in Turkey, ferric chloride ($FeCl_3$) was applied at a range of 24 – 105 mg L^{-1} and in some of the strategies, sodium hydroxide (NaOH) solution was also added in. The initial H_2S in the biogas in the gas and desulphurisation outlets reached 8070 µg L^{-1} and 6050 µg L^{-1} respectively. It was obtained that when dosed with 43 – 50 mg L^{-1} $FeCl_3$, H_2S were reduced to half (4000 – 4300 µg L^{-1}) of the initial concentrations. The strategy by adding additional alkali solution together with $FeCl_3$ dosing however did not show any significant effect.

On the other hand, Padival et al. (1995) in their study using iron salts to control dissolved sulphide in Los Angeles trunk sewers found that a combination of ferric / ferrous salts was more effective than either salt alone. A dosage of 16 mg L^{-1} Fe and a blend ratio of 1.9 : 1 (ferric : ferrous) produced maximum sulphide control with an average dissolved sulphide reduction of 95%.

In a study by Firer et al. (2008) to control sulphide in municipal raw wastewater, ferrous, ferric and combination of both salts were used. They found out that in order to reduce sulphide to 0.1 mg L^{-1}, a minimal molar ratio Fe : S of 1.3 : 1 should be applied when ferrous salts were used. Meanwhile if ferric salts or a mixture of ferrous and ferric salts (at a ratio of 2 : 1) were used, a minimal ratio Fe : S of 0.9 : 1 was required.

Apart from using chemical containing iron salts, iron-rich sludge can also being used to control sulphide as studied by Sun et al. (2015). The use of iron rich drinking water treatment sludge had managed to decrease the sulphide concentration from 15.5 – 19.8 mg L^{-1} to below 0.7 – 2.3 mg L^{-1} at a Fe : S molar ratio of 1 : 1. The sludge was high in ferric due to the usage of ferric chloride as coagulant in the water treatment process.

Based on the results in Table 2.8, ferric chloride appears to be an effective choice with a well-established history in the anaerobic digestion industry.

Table 2.8 Previous studies on sulphide control using iron salts

Chemicals	Molar ratio Fe : S	Study conditions	Initial sulphide (mg L^{-1})	Average elimination of sulphide	Reference
$FeCl_3.6H_2O$	0.6 : 1	– lab scale – anaerobic sewer biofilms – real sewage – 1.0 L	3.0	93.3%	Zhang et al. (2009)
Ferrous (Fe^{2+}) solution	1.3 : 1	– lab scale – add 0.3 – 0.315 mL sulphide stock solution (from $Na_2S_xH_2O$)	5.0	98%	Firer et al. (2008)
Ferric (Fe^{3+}) solution	0.9 : 1	– lab scale – add 0.3 – 0.315 mL sulphide stock solution (from $Na_2S_xH_2O$)	5.0	98%	Firer et al. (2008)
Fe^{3+} & Fe^{2+} solution at ratio 2 : 1	0.9 : 1	– lab scale – add 0.3 – 0.315 mL sulphide stock solution (from $Na_2S_xH_2O$)	5.0	98%	Firer et al. (2008)
$FeSO_4.7H_2O$	1.7 : 1	– lab scale – wastewater sample	18 – 25	95 – 97%	Tomar and Abdullah (1994)
$FeClSO_4$	1.2 : 1	– lab scale – wastewater sample	18 – 25	88 – 98%	Tomar and Abdullah (1994)
$FeCl_3$ & $FeCl_2$ at ratio 1.9 : 1		– full scale – 75,000 m^3 day^{-1}	6.4	97%	Padival et al. (1995)
$FeCl_3$	0.54 : 1 0.95 : 1	– lab scale – anaerobic fluidized membrane bioreactor (AFMBR)	n.a.	90% 95%	Lee et al. (2016)

Chemicals	Molar ratio Fe : S	Study conditions	Initial sulphide (mg L^{-1})	Average elimination of sulphide	Reference
Iron-rich sludge	1 : 1	– lab scale – domestic sewage	15.5 – 19.8	88 – 96%	Sun et al. (2015)
$FePO_4$	0.84 : 1 3.5 : 1	– lab scale – continuously mixed – 55°C – add with Na_2SO_4	2400	85% 98%	McFarland and Jewell (1989)

2.6 Hydrogen Sulphide from Gypsum Bedding in Dairy Farms

Gypsum, which is composed of calcium sulphate dihydrate ($CaSO_4.2H_2O$), has been widely used as livestock bedding. It is a popular choice not only because it is affordable but most importantly as it provides comfort to the animal through increased moisture adsorption and low bacteria growth in a pH-neutral material (Kour, 2017). A number of fatalities related to manure storage facilities in dairy farms have raised awareness on the danger of H_2S produced on the farms. Studies have shown that the presence of gypsum in manure could boost the generation of toxic H_2S gas (Crook et al., 2017; Hile, 2015; Fabian-Wheeler et al., 2017). Gypsum provides a source of sulphate and under anaerobic conditions could be converted to H_2S by sulphate reducing bacteria.

Crook et al. (2017) conducted a study to assess the influence of gypsum in cattle slurry in releasing H_2S. Slurry was placed in closed-system tubs fitted with mechanical stirrers to simulate the environment of a slurry tank or lagoon. In some experimental set-ups, gypsum powder was added to slurry at concentrations of 1% weight per volume, and one set contained farm-added slurry (slurry from a farm with gypsum bedding). The results showed that adding gypsum to the slurry significantly enhanced the production of H_2S by around 5 times compared to the unamended slurry. Maximum H_2S concentrations were recorded at 1618 ppmv and 1772 ppmv for amended slurry and farm-added slurry respectively. These concentrations were well beyond the UK Short-term Workplace Exposure Limit of 10 ppmv (HSE, 2011). These findings were also included in the report by Health and Safety Executive (HSE) in 2015. HSE (2015) concluded that even unamended slurry could produce sufficient H_2S to create a hazard, though if gypsum residues enter the slurry it could further increase the concentrations of H_2S produced. It is therefore vital to consider this in the risk management of dairy farms.

A similar study was done by Hile (2016) looking into the production of H_2S in manure storage of 19 dairy farms in Pennsylvania, USA. The farms were grouped into 3 categories based on their beddings: non-gypsum, gypsum and gypsum with treatment (additive to reduce H_2S emission levels). Results from the study showed that cumulative H_2S concentrations after 60 minutes of manure agitation ranged from 66 – 263 ppmv for non-gypsum farms, 203 – 13262 ppmv for gypsum farms and 61 – 3645 ppmv for gypsum with treatment farms (except for 1 farm with 21076 ppmv). The study also concluded that

gypsum bedding contributed to the elevation of H_2S concentrations during manure agitation, and addition of additive did not significantly reduce the H_2S emissions.

An on-farm study was also conducted by Fabian-Wheeler et al. (2017) to monitor the production of H_2S and exposure to the farm operators. The dairy bedding management of the ten farms was divided into three categories – traditional organic bedding, gypsum bedding, and gypsum bedding with an additive believed to reduce H_2S formation. Data collected from farms using gypsum bedding, with or without additive, showed elevated H_2S concentrations during manure agitation. The H_2S concentrations could reach as high as 500 ppm, compared to less than 20 ppm from traditional bedding farms. Farms with gypsum bedding were also found to record unacceptable H_2S concentrations of more than 20 ppm during agitation, even at a distance of 10 m away from the manure storage. It was suggested that during this event, operators should avoid close proximity to the storage structure.

In the UK, the use of gypsum bedding is now banned in accordance with guidelines from the relevant regulatory agencies (Environment Agency 2012; SEPA 2012, DAERA, 2016). In the United States of America, gypsum still can be used as bedding with or without additive (PennState Extension, 2018; USA Gypsum, 2018). Risks and hazards associated with gypsum bedding at dairy farms are not neglected, however, as there are guidelines and safety considerations need to be observed (PennState Extension, 2015; USDA, 2012; USDA, 2016). Meanwhile in Australia where livestock and their products are one of the country's main exports, bedding materials in farms are made of organic materials and sawdust has been used extensively (McCarthy and Banhazi, 2016; Banney et al., 2009; FarmOnline, 2018). Little information is available for other jurisdictions but although the use of gypsum bedding is not traditional in many locations it has also not been specifically banned in all cases.

While the current work focuses on the UK it is important to remember that dairy farming is widespread around the globe. Estimates from the United Nations Food and Agriculture Organisation put the number of dairy cattle worldwide at over 278 million in 2017 (FAO, 2018) with a global distribution as shown in Figure 2.3.

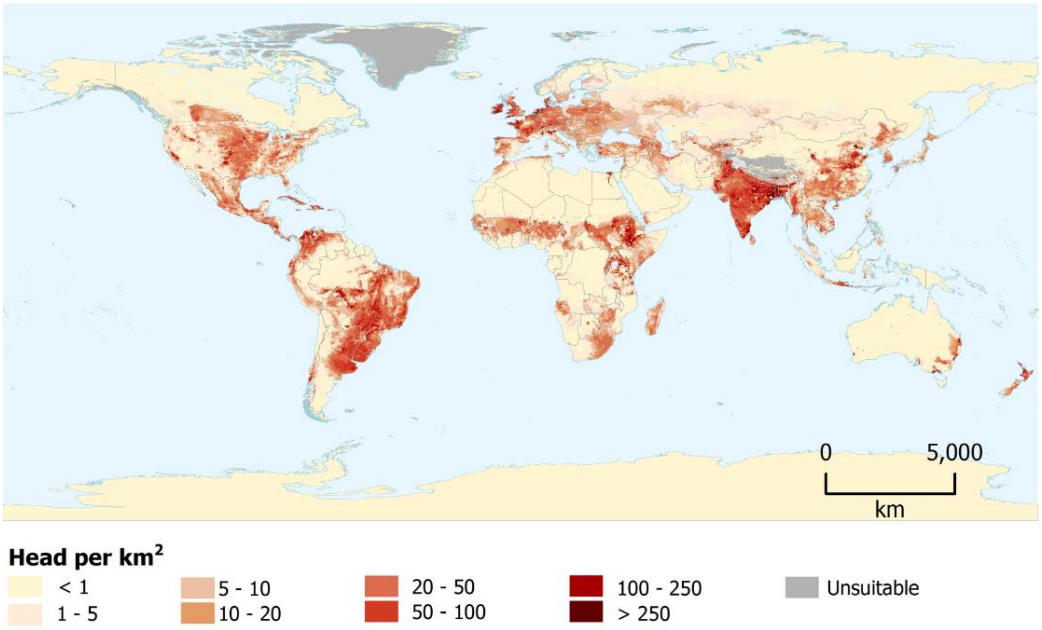


Figure 2.2 Estimated global distribution of cattle. Source Robinson et al. (2014).

2.7 Summary of Key Issues from Literature Review

A considerable amount of work has been conducted on the digestion of both cattle slurry and food waste as mono-substrates, reflecting the importance of these two organic waste streams in terms of tonnage quantities, energy potential and environmental impacts and benefits (Banks, Salter, et al., 2011).

A wide range of studies has also been carried out on co-digestion of cattle slurry. These have been driven mainly by the desire to increase the economic viability of the process through increasing the biogas production, although also by other factors such as better nutrient management and improved process stability (Zhang et al., 2012a). Many of the existing studies, however, have focused on maximising the methane yield through selection of co-digestion substrates, rather than at ratios that might match the availability of existing feedstocks.

There are still relatively few studies on the co-digestion of cattle slurry with source separated food waste, despite growing popularity of food waste collection schemes. While food waste appears to be a fairly consistent product (Banks et al., 2018), it is also known that the properties of cattle slurry can vary considerably from study to study, reflecting differences in the animals' diet, housing and other living conditions as well as

seasonal factors (Amon et al., 2007). Further studies with cattle slurry from different sources are therefore needed to add to the existing database on performance and see if patterns of results can be identified. A number of studies have focused on relatively low OLR of 1 – 3 kg VS L^{-1} day^{-1} and/or have identified these as maximising the specific methane productivity, but it is also useful to consider the effect of higher loading rates on methane productivity and energy recovery potential.

There is thus a clear need for more data on mono-digestion and co-digestion of these feedstocks to support studies such as that of Banks, Salter, et al. (2011) which aim to provide a basis for rational assessment of options under different scenarios of feedstock availability, operating regime and requirements for process economic viability.

The literature review also identified some key factors that can cause inhibition of the anaerobic digestion process or affect its operational stability. The importance of ammonia concentrations, particularly in food waste digestion, is well-established (Yirong et al., 2017), and the significance of trace elements in these conditions is also well known (Zhang and Jahng, 2012; Banks et al, 2012). In practice, understanding the bioavailability of both trace elements and heavy metals in the complex environment of an anaerobic digester may be challenging (Chen et al., 2008). There is therefore scope for experimental studies comparing co-digestion with mono-digestion of food waste and cattle slurry without regular trace element supplementation at least in first instance.

Amongst the known inhibitors of anaerobic digestion, sulphates and sulphides are of particular interest in cattle slurry digestion and co-digestion: although use of gypsum for animal bedding is now forbidden in the UK (Crook et al., 2017; Environment Agency, 2012) in other jurisdictions it is not, or the situation is unclear. The biochemistry of sulphur in anarobic digestion systems is also complex (Muyzer and Stams, 2008; Dar et al., 2008), and the current work was not originally intended to focus on this topic. Experimental trials where sulphates and other inhibitors are present or suspected of being present can add to our understanding of the behaviour of these systems, however: in particular, of the balance between substrate competition and sulphide toxicity, and the response to simple chemical methods for control of hydrogen sulphide production.

Based on the above, the current work aimed to gather data on the comparative performance of co-digestion of cattle slurry and food waste under a range of conditions, that would extend our understanding of their behaviour and could be used to support

future theoretical and modelling studies. This led to the overall research aim and objectives given in Chapter 1, and to the specific scientific and technical objectives of the experimental trials reported in the following sections.

3 METHODOLOGY

For this study, the experiments were conducted in the Environmental Laboratory in University of Southampton. Some of the following sections are based on standard methods used in this laboratory, and are therefore similar to those in other theses produced by this group.

3.1 Analytical Methods

3.1.1 General

Reagents

Except where otherwise stated all chemicals used were of laboratory grade and obtained from Fisher Scientific (Loughborough, UK).

Water

Solutions and standards were prepared using ultra-pure deionised (DI) water obtained from a Barnstead Nanopure ultrapure water purification system (Thermo Scientific, UK).

Laboratory practice

All laboratory operations were carried out using good laboratory practice, and having first carried out the appropriate risk assessments and, where necessary, COSSH assessments. All equipment, laboratory apparatus, and analytical instruments were operated in accordance with the manufacturer's instructions. All glassware was washed using washing detergent followed by rinsing with tap water and deionised water. The glassware used for the acid digestion was soaked in a 10% nitric acid bath for a 24 hour period after which the glassware was rinsed with ultrapure water (Milli-Q).

3.1.2 Gravimetric analysis

3.1.2.1 Total solids (TS) and volatile solids

TS and VS determination was based on Standard Method 2540 G (APHA, 2005). After thorough agitation, approximately 10 g of sample was transferred into a weighed crucible by pipetting (digestate samples) or spatula (substrate samples). Samples were weighed to an accuracy of ± 0.001 g (Sartorius LC6215 balance, Sartorius AG, Gottingen Germany) and placed in an oven (LTE Scientific Ltd., Oldham UK / Heraeus Function Line series, UK) for drying overnight at 105 ± 1 ºC. After drying the samples were transferred to a desiccator to cool for at least 40 minutes. Samples were then weighed again with the same balance, transferred to a muffle furnace (Carbolite Furnace 201, Carbolite, UK) and heated to 550 ± 10 ºC for two hours. After this ashing step, samples were again cooled in a desiccator for at least one hour before weighing a third time.

After all analyses, crucibles were washed with detergent, rinsed with deionised water, and stored in an oven at 105 ± 1 ºC until required for the next analysis. Crucibles were transferred from the oven to a desiccator for cooling to room temperature before each analysis. Total and volatile solids were calculated according to the following equations:

$$\%\,\mathrm{TS} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \qquad \text{Equation 3.1}$$

$$\%\,\mathrm{VS\ (on\ a\ wet\ weigh\ t\ basis)} = \frac{W_3 - W_4}{W_2 - W_1} \times 100 \qquad \text{Equation 3.2}$$

$$\%\,\mathrm{VS\ (on\ a\ TS\ basis)} = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \qquad \text{Equation 3.3}$$

where:

W_1 = weight of empty crucible (g)
W_2 = weight of crucible containing fresh sample (g)
W_3 = weight of crucible and sample after drying at 105 ºC (g)
W_4 = weight of crucible and sample after heating to 550 ºC (g)

3.1.3 Chemical and electrochemical analysis

3.1.3.1 pH

pH was measured using a Jenway 3010 meter (Bibby Scientific Ltd, UK) with a combination glass electrode, calibrated in buffers at pH 4, 7 and 9.2. The pH meter was temperature compensated and had a sensitivity of ± 0.01 pH unit and accuracy of 0.01 ± 0.005 pH units. Buffer solutions used for calibration were prepared from buffer tablets (Fisher Scientific, UK) according to the supplier's instructions. During measurements, the sample was stirred to ensure homogeneity. In addition, the pH probe was rinsed with DI water in between measurements and placed into a mild acid solution to avoid cross-contamination. Digestate samples were measured immediately after sampling to prevent changes in pH due to the loss of dissolved CO_2.

3.1.3.2 Alkalinity

Alkalinity was measured by titration based on Standard Method 2320B (APHA, 2005). Digestate was sieved to obtain a homogenous sample and 2 – 5 g of this was added to 40 mL of DI water. Titration was done using a Schott Titroline Easy automatic digital titration burette system (Schott, Mainz, Germany), with the samples being magnetically stirred while the titration was carried out. A 0.25 N H_2SO_4 titrant was used to determine endpoints of pH 5.7 and 4.3, allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA) (Ripley et al., 1986). PA is a measurement of bicarbonate buffering while IA is attributed to the buffering capacity of Volatile Fatty Acids (VFA).

The pH probe was calibrated before titration using buffers as described before and washed with DI water between subsequent samples to avoid cross contamination. Alkalinity was calculated according to the following equations:

$$TA = \frac{(V_{4.3}+V_{5.7}) \text{ x N x } 50}{W_s} \quad \text{Equation 3.4}$$

$$PA = \frac{V_{5.7} \text{ x N x } 50}{W_s} \quad \text{Equation 3.5}$$

$$IA = \frac{V_{4.3} \text{ x N x } 50}{W_s} \quad \text{Equation 3.6}$$

where:

TA = total alkalinity (g $CaCO_3$ kg^{-1} wet weight (WW))
PA = partial or bicarbonate alkalinity (g $CaCO_3$ kg^{-1} WW)
IA = intermediate or volatile fatty acid alkalinity (g $CaCO_3$ kg^{-1} WW)
Ws = weight of sample (g)
$V_{subscript}$ = volume of titrant required to reach the pH value indicated in the subscript (mL)
N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

3.1.3.3 Total ammonia nitrogen

Total ammonia nitrogen (TAN) analysis was based on Standard Method 4500-NH3 B and C (APHA, 2005). A sample aliquot of between 2 – 3 g was weighed (i201, My Weigh Europe, Huckelhoven Germany) into a digestion tube and 50 mL of DI water added. Blanks (50 mL DI water) and standards (containing 10 mL of 1000 mg L^{-1} NH_4Cl with 40 mL DI water) were also prepared in digestion tubes. 5 mL of 10 M sodium hydroxide (NaOH) was added to each digestion tube to raise the pH above 9.5 and the samples were distilled using either a Foss Tecator Kjeltec system 1002 distillation unit (Foss Tecator A-B, Hoganas, Sweden) or a Büchi K-350 Distillation Unit (Büchi, UK). Erlenmeyer flasks previously filled with 25 mL of boric acid as an indicator were used to collect the distillate and progress of the distillation was indicated by a colour change from purple to green. The distillate was titrated manually with 0.25N H_2SO_4 using a digital titration system (Schott Titroline, Gerhardt UK Ltd) until an endpoint was reached as indicated by a colour change to purple at which point the volume of titrant added was recorded. Standards and blanks were distilled in the same way. The TAN concentration was calculated according to the following equation:

$$\mathrm{TAN} = \frac{(\mathrm{A}-\mathrm{B})\ \mathrm{x}\ 14.0\ \mathrm{x}\ \mathrm{N}}{\mathrm{W_s}} \qquad \text{Equation 3.7}$$

where:

TAN = total ammonia nitrogen (g N kg^{-1} WW)
A = volume of titrant used to titrate the sample (mL)
B = volume of titrant used to titrate the blank (mL)
N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant
Ws = weight of sample (g)

3.1.3.4 Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) analysis was carried out on duplicate samples alongside blanks and controls as follows: 3 – 5 g (weighed to ± 1 mg) of sample was placed in a glass digestion tube. Two Kjeltab Cu 3.5 catalyst tablets (Copper Kjeltabs, 3.5 g, FOSS) were added to facilitate acid digestion by lowering the activation energy of the reaction. 12 mL of low nitrogen concentrated H_2SO_4 was added carefully to each digestion tube and agitated gently to ensure that the entire sample was completely exposed to acid. The digestion tubes were then placed into the heating block with exhaust system using either a Foss Tecator 1007 Digestion System 6 (Foss Analytical, Hoganas Sweden) or a Büchi K-435 Digestion Unit (Büchi, UK) for approximately two hours until the solution colour became a clear blue-green. Both systems operated at 420 ± 5 ^{o}C and once the reaction was completed the tubes were cooled to around 50 ^{o}C and 40 mL of deionised water slowly added to the digestion tube to prevent later crystallisation on further cooling. Samples, blanks and standards were then distilled and titrated as for total ammonia nitrogen using a BÜCHI Distillation Unit K-350 with NaOH addition, followed by collection of the distillate in boric acid indicator and titration with 0.25 N H_2SO_4. The TKN concentration was calculated according to the following equation:

$$\mathrm{TKN} = \frac{(\mathrm{A}-\mathrm{B})\ \mathrm{x}\ 14.0\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 1000}{\mathrm{W_s}} \qquad \text{Equation 3.8}$$

where:

TKN = Total Kjeldahl Nitrogen (mg N kg^{-1} WW)
A = volume of titrant used to titrate the sample (mL)
B = volume of titrant used to titrate the blank (mL)
N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant
W_s = wet weight of sample (g)

3.1.3.5 Gas chromatographic determination of volatile fatty acids (VFA)

The method used was based on SCA (1979): Determination of Volatile Fatty Acids in Sewage sludge (1979). Samples were prepared for analysis by centrifugation at 14,000 rpm (micro-centrifuge, various manufacturers) for 15 minutes. 0.9 mL of the supernatant was transferred by pipette to vials with 0.1 mL formic acid to give a final concentration of 10% formic acid. Where dilution was necessary, deionised water was used and formic

acid was added to give a concentration of 10% of the total volume for analysis. If the samples at this point were turbid they were centrifuged again at 14,000 rpm to obtain a clearer supernatant. The supernatant after acidification and centrifugation was transferred into the vials and loaded onto the GC auto-sampler ready for the VFA measurement.

A standard solution containing acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids, at three dilutions to give individual acid concentrations of 50, 250 and 500 mg L^{-1} respectively, was used for calibration and also loaded onto the GC.

Quantification of the VFA was by a Shimazdu GC-2010 gas chromatograph (Shimadzu, Milton Keynes, UK), using a flame ionisation detector and a capillary column type SGE BP-21. The carrier gas was helium at a flow of 190.8 mL min^{-1} and a split ratio of 100 to give a flow rate of 1.86 mL min^{-1} in the column and a 3.0 mL min^{-1} purge. The GC oven temperature was programmed to increase from 60 to 210 ^{o}C in 15 minutes with a final hold time of 3 minutes. The temperatures of injector and detector were 200 and 250 ^{o}C, respectively.

3.1.3.6 Soluble sulphate

Two methods of sulphate analysis were attempted. The first was a turbidimetric method based on Standard Method 4500E (APHA, 2005). A stock standard solution (100 mg L^{-1}) was prepared by dissolving 147.9 mg of anhydrous Na_2SO_4 to 1000 mL volumetric flask and making to volume with deionised water. Sulphate standards were prepared by diluting the initial stock solution : 0, 10, 25, 50, 75 and 90 mL which corresponds to the contents of 0, 2, 5, 10, 15 and 18 mg SO_4^{2-}. Both standards and samples were treated in the same manner. In a 100 mL volumetric flask, 5 mL of conditioning reagent (mixture of 25 mL glycerol, 15 mL concentrated HCl, 50 ml 95% isopropyl alcohol, 37.5 g NaCl and made up to final volume to 250 mL using deionised water) was added to the sample (or standard) and made up the volume using deionised water. The mixture was stirred for 60 seconds using magnetic stirrer. A spoonful (capacity of 0.2 – 0.3 mL) of barium chloride ($BaCl_2$) crystals was added while stirring. Both standards and the sample were measured as absorption at a wavelength of 420 nm using a spectrophotometer (Cecil 3000 series, Cecil Instruments Ltd., UK).

Sulphate was also analysed using a Metrohm ion chromatograph 882 with autosampler and 6.1006.100 Metrosep Anion Dual 2 column. A solution of 2 mmol L^{-1} $NaHCO_3$ and 1.5 mmol L^{-1} $NaCO_3$ with 2% v/v acetonytryl eluent was used for cleaning and the mobile phase of operation. This was filtered through 0.45 μm filter and sonicated for 30 minutes before use to remove particulates and CO_2. Each run was conducted with a residence time of 17 minutes. 0.1 M sulphuric acid was used to clean the suppressor apparatus after each injection to maintain the baseline. Milli-Q water was replenished each day of use, and new reagents were used for each run. Standards were used at concentrations between 0.01 – 10 mg L^{-1}. Samples were filtered through a 0.2 μm Whatmann GFF before analysis.

3.1.3.7 Soluble sulphide

Soluble sulphide was initially measured using Standard Method 4500-$S2^-$ D (APHA, 2005). This methylene blue method was found to be unreliable due to the strong colour present in samples derived from cattle slurry, and was replaced by an ion selective electrode method.

For the ion selective electrode method, standards were made up in an alkaline anti-oxidant reagent (AAR) prepared in 1 L of de-gassed deionised water to which was added 80 g of sodium hydroxide, 35 g of ascorbic acid and 67 g of ethylene diamine tetraacetic acid disodium (EDTA-Na_2). This was stored in tightly capped brown glass bottle. A 2 M zinc acetate solution was made up by adding 300 mL of de-gassed water to 220 g of zinc acetate dihydrate ($Zn(C_2H_3O_2)_2.2H_2O$) and making up to 0.5 L in a volumetric flask with de-gassed water. A stock standard sulphide solution (133 mg L^{-1}) was prepared by adding 100 mg of sodium sulphide nanohydrate ($Na_2S.9H_2O$) to a 100 mL volumetric flask and making up to volume with de-gassed water. The de-gassed water was prepared earlier by bubbling it with nitrogen gas for few minutes. Sulphide standards were prepared as shown in Table 3.1 to give a concentration range between 0.013 and 13 mg L^{-1}.

The samples were prepared by adding 40 mL AAR, 75 μL of zinc acetate solution and 50.0 mL of sample to a 100 mL volumetric flask and making up to the mark with AAR. Both the standards and the sample were measured with a sulphide ion selective electrode following the manufacturer's instructions for use of the electrode (JENWAY 3345 Ion Meter, Double Junction Reference Electrode and 1225 Sulphide Instructions).

Table 3.1 Soluble sulphide standards

Cal ID	Dilution	AAR (mL)	Sulphide solution ID	Sulphide solution (mL)	Zinc Acetate (µL)	Final concentration (mg L^{-1})
Cal. 4	1:10	45	Stock sol. (133 mg/l)	10.0	75	13.0
Cal. 3	1:100	50	Stock sol. (133 mg/l)	1.0	75	1.3
Cal. 2	1:1000	45	Cal. 3 (1.3 mg/l)	10.0	70	0.13
Cal. 1	1:10000	50	Cal. 3 (1.3 mg/l)	1.0	75	0.013

3.1.3.8 Trace elements

Approximately 1 – 3 g of fresh sample or 0.5 – 1.0 g of dried sample was added to the digestion tube, with blanks prepared in parallel. 15 mL of 35 – 36% w/v HCl (hydrochloric acid) was added, then after around 5 minutes 5 mL of 70% w/v HNO_3 (nitric acid) was added, and the tubes were gently agitated. The tubes were placed into the digestion block (Gerhardt Kjeldatherm), connected to the condenser system and left for 24 hours prior to heating. The acid digestion involved gradually increasing the temperature first to 100 ^oC and then to the final temperature of 180 ^oC which was maintained for about 2 hours ± 10 min. After cooling, the mixtures were filtered (Filter paper No. 1 Qualitative 11 cm, Whatman, UK) into a 50 mL volumetric flask. Any remaining residue in the tube was washed out with 5 mL of warm 12.5% v/v HNO_3 and transferred to the 50 mL flask, with up to 5 washes being performed. The volume was then made up to 50 mL with HNO_3 (12.5% v/v). The filtrate was then transferred into a PET bottle and sent for analysis by ICP-MS (Severn Trent Services, Coventry, UK).

3.1.3.9 Elemental composition

Carbon, hydrogen, nitrogen and sulphur contents of samples were determined using a FlashEA 1112 Elemental Analyser (ThermoFinnigan, Italy), with the oxygen content calculated by difference. Samples were air dried and milled to ensure homogeneity. Sub-samples of approximately 2 – 3 mg were weighed into standard weight tin disks using a five decimal place analytical scale (Radwig, XA110/X, Poland). These were placed in a combustion/reduction reactor held at 900°C then flash combusted in a gas flow temporarily enriched with oxygen resulting in a temperature greater than 1700 ^oC and the release of N_xO_x, CO_2, H_2O and SO_2 (depending on the composition of the sample). The

gas mixture was then analysed by GC with the different components were measured by appropriate detectors. The working conditions of the elemental analyser were as described in the manufacturer's technical literature and method sheets. Standards used in this analysis were atropine, nicotinamide and birch leaf.

3.1.4 Gas production and composition

3.1.4.1 Gas volume

Gas production throughout the semi-continuous digestion trials was measured using gas counters. The gas counters operated by the alternate filling and discharging of a calibrated cell which logged each discharge via a labjack (LabJack Ltd.) computer interface (Walker et al, 2009). The calibration of each gas counter was checked once a week by attaching a 10-L gas-impermeable sampling bag (Tedlar SKC 232, SKC Ltd, Blandford Forum, UK) to the gas vent of gas counter. Gas bag volumes were measured using a weight-type water displacement gasometer (Walker et al. 2009). The measurement procedure was as follows: the initial height of solution in the gasometer (h_1) was recorded before the collected gas was introduced into the column through the top valve. After the bag was empty, the final height (h_2) and the weight of water (m) were recorded, as well as the temperature (T) and pressure (P) in the room. All gas volumes reported are corrected to standard temperature and pressure of 0°C, 101.325 kPa as described by Walker et al. (2009) according to the following equations:

Height Gasometer Governing Equation Equation 3.9

$$V_{stp} = \frac{T_{stp}A}{T_{atm}p_{stp}}\left(\left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g\left(h_{t2} - h_{c2}\right)\right)h_{c2} - \left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g\left(h_{t1} - h_{c1}\right)\right)h_{c1}\right)$$

Weight Gasometer Governing Equation Equation 3.10

$$V_{stp} = \frac{T_{stp}A}{T_{atm}p_{stp}}\left[\left(\left(P_{atn} - p_{H_2O}(T_{atm}) + \rho_b g\left(H - h_1 - \frac{m_b}{A\rho_b}\right)\right)\left(h_1 + \frac{m_b}{A\rho_b}\right)\right) - \left(p_{atn} - p_{H_2O}(T_{atm}) + \rho_b g\left(H - h_1\right)\right)h_1\right]$$

where:

V = dry gas volume (i.e. not including calculated water vapour content) (m^3)
P = pressure (Pa)
T = temperature (K)
H = total height of column (m)
h = distance to liquid surface from a datum (m)

A = cross-sectional area of gasometer (m^2)
m_b = mass of barrier solution (kg)
ρ = density of barrier solution (kg m^{-3})
g = gravitational acceleration (m s^{-2})
$^{1, 2, stp, atm, b, t, c}$ subscripts refer to condition 1 (before addition of gas to column), condition 2 (after gas addition to column), standard temperature and pressure, atmospheric, barrier solution, collection trough and column respectively.

The weight method was used as this is considered to be more accurate, with the height method used only to check for any gross measurement or data recording errors.

3.1.4.2 Gas composition

The gas produced during anaerobic digestion of wastes contains methane and carbon dioxide (CO_2) as its major components with minor quantities of hydrogen (H_2) and hydrogen sulphide (H_2S).

Methane and carbon dioxide

Biogas composition was quantified using a Varian Star 3400 CX gas chromatograph (Varian Ltd, Oxford, UK). The GC was fitted with a Hayesep C column and used either argon or helium as the carrier gas at a flow of 50 mL min^{-1} with a thermal conductivity detector. The biogas composition was compared with a standard gas containing 65 % CH_4 and 35% CO_2 (v/v) for calibration. A sample of 10 mL was taken from a Tedlar bag used for sample collection and was injected into a gas sampling loop.

Hydrogen sulphide

This was measured using a H_2S-AE sensor supplied by Alphasense Ltd (Essex, UK) running on an Alphasense digital transmitter board with signal conditioning using the Alphasense Digital Transmitter Interface v.1.0.2 and associated software. The gas flow over the sensor was maintained at 500 mL min^{-1} during calibration and normal operation. Calibration was performed at a zero calibration point using clean air and a span calibration point using the calibration gas, which was a mixture of H_2S and CO_2 made by SIP Analytical Ltd (composition: 404 ppmv H_2S, 35.19 % CO_2, balance – methane). Samples were run from gas collected in gas sampling bags, with gas passed over the sensor until a stable reading was obtained. A dilution of the gas sample with air was carried out if the sample was too concentrated. The output was recorded and compared to the standard

curve to give a direct reading in ppmv H_2S. No other specific quality assurance measures were conducted but results were compared with previous data to identify apparent inconsistencies or outliers.

3.2 Materials

3.2.1 Laboratory-scale digesters

The individual digesters used had a total volume of 5 L and were operated at a working volume of 4 L. A schematic drawing of a pair of digesters is shown in Figure 3.1 while Figure 3.2 shows the photographs of the laboratory-scale digesters used in the research. The digesters were constructed in PVC with a top flange to which a top plate was secured using stainless steel bolts and wing nuts. A gas tight seal between the top plate and the digester flange was maintained using a closed pore neoprene gasket. The top plate was fitted with a gas outlet connector and a feed port sealed with a rubber bung. On the top plate a DC motor was mounted which was coupled to the digester stirrer through a draught tube water gas seal, the draught tube itself being secured in a gas tight compression seal. Digestate was removed from the digester via a 15 mm diameter outlet port at the base of the digester. The contents of the digesters were continuously stirred at 40 rpm by means of an asymmetric stirrer. Temperature was maintained at 35 ± 0.5 ^{o}C; by water circulating through an external heating coil that surrounded the digesters. When assembled, and before filling, each digester was tested for gas leaks by applying a positive pressure to the digester and submerging in water to ensure there was no gas escape when all ports were sealed. The digesters were connected to gas counters, which continuously measured gas production throughout the digestion period. The operation of the gas counters and their calibration is described in section 3.1.4.1.

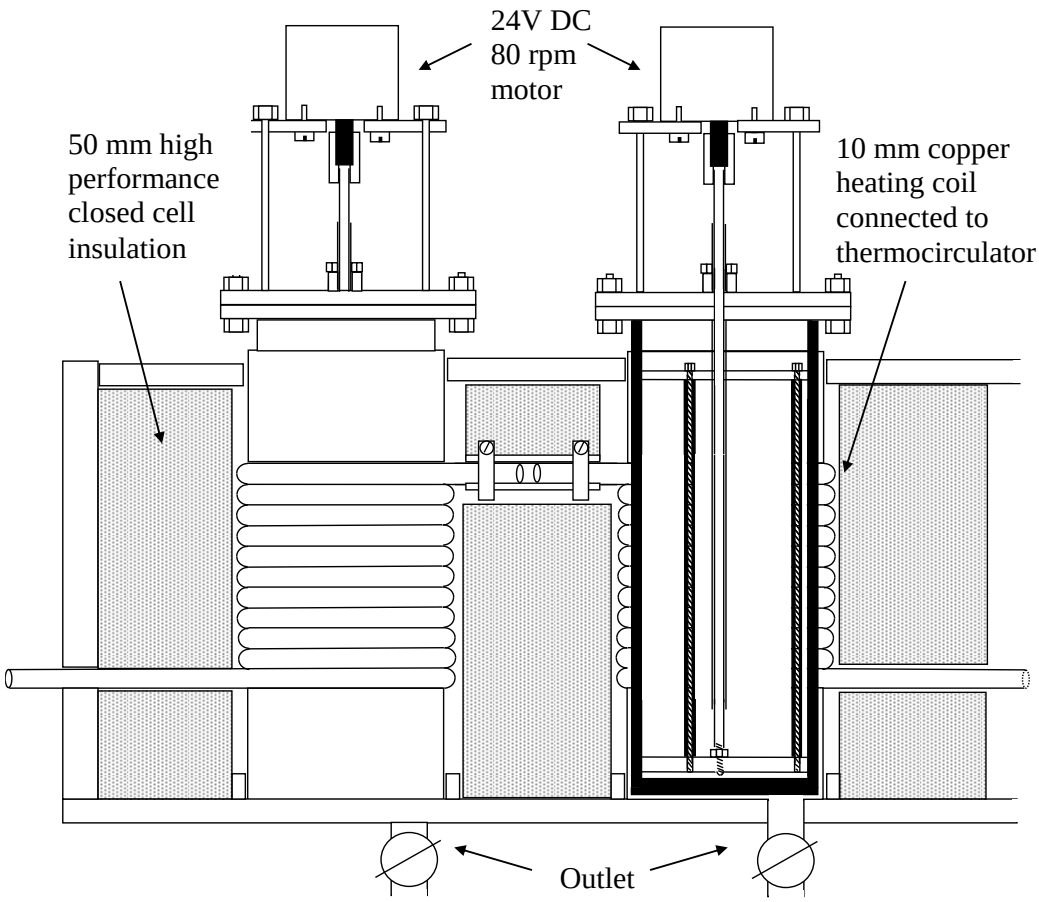
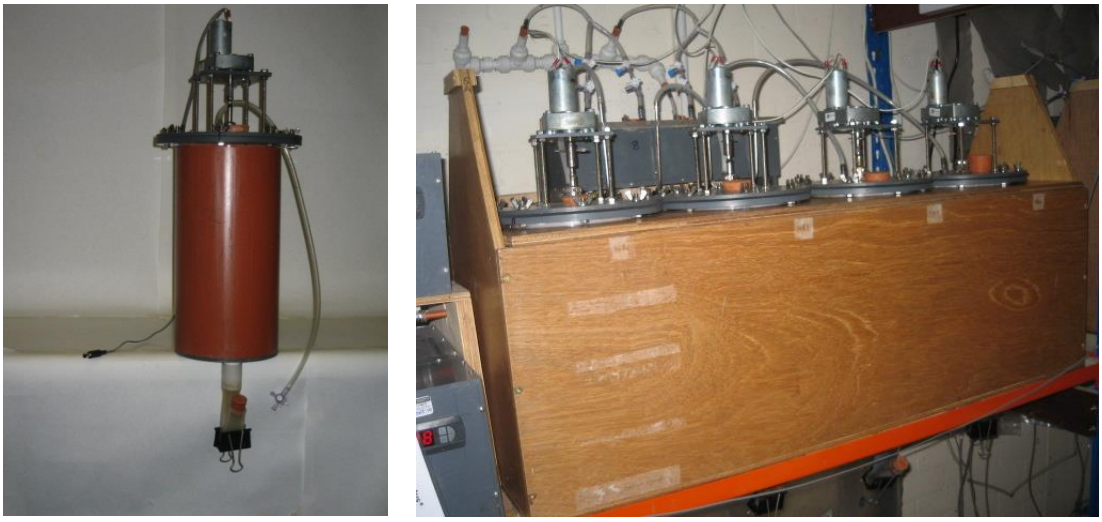


Figure 3.1 Schematic diagram of 5-L CSTR digester



(a) 5-L digester (b) Set of 5-L digesters in temperature controlled box

Figure 3.2 Laboratory-scale digesters used in the research

3.2.2 Inoculum

Inoculum used for the studies was obtained from a mesophilic anaerobic digestion plant treating municipal wastewater biosolids (Millbrook wastewater treatment plant (WWTP), Southampton, operated by Southern Water PLC). Digestate collected was used as inoculum within 48 hours of collection.

3.2.3 Feedstock: Food waste

The batches of food waste used came from digestion plant in Ludlow (Biocycle Ltd, Ludlow, Shropshire), and from the waste transfer station in Otterbourne, Hampshire (Veolia Environmental Services). A representative sample of collected food waste was first manually sorted out to eliminate non-food contamination such as plastic bags, paper and garden waste, together with large bones and seeds. Figure 3.3 shows the sorting and grinding in the food waste preparation. Any large items of food waste were manually chopped to reduce their size, then the material was ground using a commercial garbage grinder (S52/010 Waste Disposer, Imperial Machine Company Limited), mixed thoroughly and placed in 4-L plastic boxes then frozen at -20 ^{o}C. When needed, the frozen food waste was thawed overnight at room temperature, then kept in a refrigerator at 4 ^{o}C.

Figure 3.3 Food waste processing

3.2.4 Feedstock: Cattle slurry

The cattle slurry used in this study was collected from dairy farms in Wrexham, North Wales, Blandford Forum, Dorset and Stockbridge, Hampshire (Figure 3.4). In the period when the slurry was collected, the farm in Dorset was using gypsum for bedding. Slurry was pumped either directly from the disposal truck or from the collection pit to ensure the sample was as representative as possible. Before homogenisation, the collected material was placed in a 120-L drum for several hours to allow unwanted materials such as stones and wood chips to settle at the bottom. The contents of the drum apart from these materials were then passed through a garbage grinder (S52/010 Waste Disposer, Imperial Machine Company Limited), to prevent the passage of any remaining small stones or wood chips, reduce the size of any oversize materials (such as straw) that might cause blockages in the digester, and ensure the homogeneity of the feedstock. After passing through the grinder the material was placed in 5-L plastic containers and stored in the freezer at -20 ^{o}C. Before being used, the frozen cattle slurry was first thawed overnight at ambient temperature and shaken thoroughly; and then stored at 4 ^{o}C in the fridge and used over a short period.

For the co-digestion, feedstock with a mixture of cattle slurry and food waste was prepared daily by adding both feedstocks based on the required ratio (wet weight). The combined feedstock was mixed thoroughly before use to ensure a homogeneous mixture.

Figure 3.4 Unloading of cattle slurry at Stockbridge farm

3.2.5 Trace element solution

Two trace element (TE) solutions were used, one composed of cations and the other oxyanions as shown in Table 3.2, which were prepared and stored separately to prevent precipitation. The solutions were based on a modified TE recipe developed by University of Southampton (VALORGAS, 2013). When needed, the two TE solutions were added weekly either at a rate of 1 mL of each solution for every 1 L of digestate removed, or based on the amount of feedstock added, to give a steady state minimum concentration of TE in the digester.

Table 3.2 Concentration of trace elements in stock solution

Trace element	**Compound used**	**Element concentration in the working condition (after dilution by 1000 times) (mg L^{-1})**	**Compound concentration in stock solution (mg L^{-1})**
Cation			
Aluminium (Al)	$AlCl_3.6H_2O$	0.1	1.790
Boron (B)	H_3BO_3	0.1	1.144
Cobalt (Co)	$CoCl_2.6H_2O$	1.0	8.076
Copper (Cu)	$CuCl_2.2H_2O$	0.1	0.536
Iron (Fe)	$FeCl_2.4H_2O$	5.0	35.6
Manganese (Mn)	$MnCl_2.4H_2O$	1.0	7.204
Nickel (Ni)	$NiCl_2.6H_2O$	1.0	8.100
Zinc (Zn)	$ZnCl_2$	0.2	0.834
Oxyanion			
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24}.2H_2O$	0.2	0.736
Selenium (Se)	Na_2SeO_3	0.2	0.876
Tungsten (W)	$Na_2WO_4.2H_2O$	0.2	0.718

3.3 Biochemical Methane Potential (BMP) Test

The BMP test was performed in 2-L digesters at a working volume of 1.5 L, similar to those shown in section 3.2.1 except for the size and the fact that there was no outlet port in the base of the digester. The digesters were maintained at 35 ^oC in a temperature-controlled water bath at 35 ^oC with each gas outlet connected by PVC tubing to liquid displacement gasometers. The gasometers themselves were sealed acrylic cylinders with graduated markings and were partially immersed in a trough filled with a barrier solution of acidified saline (75% saturated solution of sodium chloride (NaCl) at pH 2, designed to minimise the solubility of CH_4). When the barrier solution in the tubes was displaced by biogas the trough was maintained at a constant head by means of a fixed height

overflow. The volume of biogas collected was corrected to a STP of 0°C and 101.325 kPa (Walker et al., 2009). Biogas samples were taken from the gas collection cylinder via a 3-way valve and a syringe and analysed for gas composition (section 3.1.4.2) whenever the collection tube was refilled with barrier solution. The gas collection tubes were refilled when required but never less frequently than every 7 days. This was done using a vacuum pump connected to the 3-way valve at the top of each cylinder. Figure 3.5 shows the BMP apparatus used in the research.

(a) Stirred digesters (b) Gasometers

Figure 3.5 BMP apparatus

Before starting the test, the BMP apparatus was checked for leaks by filling the trough in which the gasometers were mounted with the acidified saline solution, pulling it up into the gasometer and making sure that the level did not drop over a 48-hour period. On the day of test, fresh inoculum and substrate were collected (section 3.2.2 – 3.2.4). The inoculum was sieved before use to remove large particles such as grit. Blank control digesters were filled with 1000 g inoculum whereas the test digesters were filled with a mixture of inoculum and substrate in a ratio of about 5 : 1 on a VS basis. Positive controls (cellulose powder from Sigma-Aldrich, Dorset) were also run alongside the test digesters. The test was run for a duration of at least 28 days. Temperature, pressure and level of barrier solution in the gasometers was recorded manually during working hours every 1 – 2 hours in the first week, twice a day in the second and third week and once a day over the remaining period of the test.

The BMP for a given substrate was obtained by calculating the cumulative STP volume of methane produced from each test digester; subtracting the average cumulative STP

methane production from the inoculum-only controls; and dividing the result by the weight of substrate volatile solids added to each test digester. The average value for all test digesters in L CH_4 g^{-1} VS was taken as the final BMP value.

3.4 Anaerobic Digestion Trials

3.4.1 Digester operation and calculations

The digesters were operated in a semi-continuous mode, i.e. fed daily with a specific amount of feedstock and with digestate removed to maintain a constant volume in the digesters. The organic loading rate (OLR) was determined according to Equation 3.11.

$$\mathrm{OLR} = \frac{mVS_{substrate}}{V_{reactor}} \qquad \text{Equation 3.11}$$

where:

OLR = organic loading rate (g VS L^{-1} day^{-1})
m = mass of substrate daily added to the reactor (g day^{-1})
$VS_{substrate}$ = volatile solid content of feedstock (% WW)
$V_{reactor}$ = volume of reactor (L)

The Hydraulic Retention Time (HRT) of the digester is expressed in Equation 3.12.

$$\mathrm{HRT} = \frac{V_{reactor}}{Q} \qquad \text{Equation 3.12}$$

where:

HRT = hydraulic retention time (days)
$V_{reactor}$ = volume of reactor (L)
Q = daily flow of material (substrate added and digestate removed) through the reactor (L day^{-1})

Quantities of substrate and digestate were measured on a weight basis, but for ease of calculation it was assumed that both the substrate and digestate had a specific gravity of 1.0. Therefore, 1 g of substrate and digestate was considered to be equivalent to 1 mL.

Digestion performance was monitored in terms of specific and volumetric biogas and methane production which were calculated using Equations 3.13 – 3.16.

$$\text{VBP} = \frac{V_{biogas}}{V_{reactor}} \qquad \text{Equation 3.13}$$

$$\text{SBP} = \frac{V_{biogas}}{OLR \times V_{reactor}} \qquad \text{Equation 3.14}$$

where:

VBP = volumetric biogas production (L L^{-1} day^{-1})
SBP = specific biogas production (L g^{-1} VS)
V_{biogas} = volume of biogas produced daily (L day^{-1})
OLR = organic loading rate (g VS $L^{-1}day^{-1}$)
$V_{reactor}$ = volume of reactor (L)

$$\text{VMP} = \frac{V_{CH_4}}{V_{reactor}} \qquad \text{Equation 3.15}$$

$$\text{SMP} = \frac{V_{CH_4}}{OLR \times V_{reactor}} \qquad \text{Equation 3.16}$$

where:

VMP = volumetric biogas production (L L^{-1} day^{-1})
SMP = specific methane production (L CH_4 g^{-1} VS)
V_{CH4} = volume of methane produced daily (L day^{-1})
OLR = organic loading rate (g VS L^{-1} day^{-1})
$V_{reactor}$ = volume of reactor (L)

Feedstock solids content was measured at regular intervals during the trials. Minor natural variations meant that the average OLR applied during the trials sometimes varied slightly from planned values based on the initially assumed solids content. This also leads to small differences in HRT, and may affect the direct comparability of VBP and VMP values, but not of SBP and SMP. For clarity the OLR in each trial is referred to by its original nominal value.

The destruction of volatile solids was calculated as in Equation 3.17.

$$VS_{destruction} = \frac{Feed\ (g) x\ VS_{feed} - Digestate\ (g) x\ VS_{digestate}}{Feed\ (g) x\ VS_{feed}} \qquad \text{Equation 3.17}$$

where:

Feed = weekly wet weight of feed added to digester (g WW)
VS_{feed} = VS content of feed (%WW)

Feed = weekly wet weight of feed added to digester (g WW)
VS_{feed} = VS content of feed (%WW)

3.5 Sulphide Calculations

3.5.1 Total soluble sulphide - calculated

Soluble H_2S was calculated using Henry's Law based on the measured headspace H_2S concentration. The un-ionised fraction of H_2S was determined according to Standard Method 4500-S^{2-} H (APHA, 2005), with the total soluble sulphide fraction presumed to consist of H_2S and HS^- due to the operational pH range within the digesters.

3.5.2 Sulphate removal

The percentage removal of sulphate in the form of sulphide achieved in the digestion process was estimated using Equation 3.18.

$$\%S_{rem} = \frac{100\ x\ S(out)}{S(in)} \qquad \text{Equation 3.18}$$

Where:

$\%S_{rem}$ = percentage sulphate removal
$S_{(in)}$ = mass of sulphur entering the digester as sulphate (g)
$S_{(out)}$ = calculated mass of sulphur exiting the reactor as sulphide in both the aqueous ($H_2S_{(aq)}$ and HS^-) and gas (H_2S) phases

3.6 Summary of Experimental Work

The experimental work carried out included characterisation of feedstock materials and batch, semi-continuous anaerobic digestion trials and a flask trial. The operating conditions for each trial are summarised in Table 3.3. Methodologies specific to the individual experiments are given in Chapter 4. Note that trial C2 is presented out of chronological sequence in order to provide a more logical grouping of the trials dealing primarily with co-digestion and those dealing with aspects of sulphate content.

Table 3.3. Summary of experimental trials

Trial	From (day)	To (day)	Duration (days)	Objective
C1	0	220	220	To quantify gas production and assess process performance and stability of co-digestion of cattle slurry and food waste at a wet-weight ratio of 3:1 and target OLR of 3, 4 and 5 g VS L^{-1} day^{-1}
C2	544	866	322	To assess co-digestion performance at a cattle slurry to food waste ratio of 6 : 1 on a wet weight basis, at the same OLR as in trial C1
S1	243	351	108	To assess co-digestion performance of high-sulphate cattle slurry and food waste at different organic loading rates at wet-weight cattle slurry to food waste ratio of 6 : 1
Flask trial	354	369	15	To determine the optimum dose of $FeCl_3$ to control dissolved sulphide from digestion of high sulphate cattle slurry
S2	348	543	195	To assess the co-digestion performance of high-sulphate cattle slurry and food waste at a wet-weight ratio of 6 : 1 with daily $FeCl_3$ addition to control digestate sulphide concentration
S3	788	1183	320	To determine sulphate concentrations causing could cause reduction in specific methane productivity and the onset of instability in cattle slurry digestion

4 DIGESTION TRIALS

This section reports the results of the anaerobic digestion trials of food waste and cattle slurry in CSTR under mesophilic conditions (35 $\pm$ 2 ^{o}C). Unless noted, each trial lasted for at least 3 HRT. The effects of cattle slurry to food waste ratio, sulphate content, ferric chloride addition and trace element supplementation on the performance and stability of AD were investigated. Performance and stability during the trials were monitored through biogas production and composition, pH, total VFA, total alkalinity, total ammonia nitrogen (TAN), total Kjeldahl nitrogen (TKN), dissolved sulphide, total and volatile solids, and VS destruction.

4.1 Characteristics of Cattle Slurry and Food Waste

The batches of cattle slurry used were obtained from Wrexham, North Wales (referred as CS1), Blandford Forum, Dorset (CS2) and Stockbridge, Hampshire (CS3). CS1 was from an organic farm and was used in trials C1 and C2. CS2 was collected from a farm using gypsum as bedding for cattle, and was used in trials S1 and S2. CS3 was collected from a farm without gypsum bedding and was used in trial S3. Table 4.1 presents the characteristics of each cattle slurry used.

From Table 4.1, it can be seen that the batches of cattle slurry were quite different in physical characteristics. CS2 and CS3 had higher TS and VS contents than CS1. Although theoretically higher VS may mean a higher potentially digestible organic content, CS2 had a distinct bad odour. It was suspected that this was due to hydrogen sulphide (H_2S), which can indicate an inferior quality of feedstock for use in anaerobic digestion. CS2 also had a significantly higher sulphur content of 2.75% of TS compared to 0.47 and 0.49% for CS1 and CS3 respectively.

On the other hand, all the cattle slurry batches had relatively low carbon contents (ranging from 36.5 – 41.5 % on a TS basis) and high ash content. This could be due in part to the efficiency of the rumen digestion process that has converted most of the biodegradable carbon. This gave low C : N ratios of 12.87, 12.04 and 15.23 respectively, which are close to the range of 11 – 14 quoted by Umetsu et al. (2006) for cattle slurry.

Trace elements concentrations were only determined for CS1 and CS2. Both batches of cattle slurry had relatively high concentrations of trace elements such as cobalt, copper, iron, manganese, molybdenum, nickel, zinc, and selenium. In comparison with the results from studies by Sager (2007), McBride and Spiers (2001), and Raven and Loeppert (1997) presented in Table 2.5, the values are similar, with some concentrations a little higher (Mo, Ni) or lower (Co, Mn, Zn). In terms of trace element requirements, both batches of cattle slurry should provide sufficient trace elements for a stable anaerobic process (Banks et al., 2012).

Table 4.1 Feedstock characteristics

Parameters	**Unit**	**Cattle Slurry**			**Food Waste**	
		CS1	**CS2**	**CS3**	**FW1**	**FW2**
Total solids	%	8.44	15.03	16.59	24.30	23.09
Volatile solids	%	5.70	10.45	12.00	22.90	20.39
Total Kjeldahl Nitrogen	mg kg^{-1} WW	3029	3902	2734	7503	6693
Total Nitrogen	mg kg^{-1} TS	2.90	3.00	2.40	4.22	4.00
Total Carbon	mg kg^{-1} TS	37.33	36.05	36.54	52.32	52.05
C : N	–	12.87	12.04	15.23	12.39	13.02
Sulphate	mg SO_4 kg^{-1} ww	–	6876	289	–	320
Trace elements						
Cobalt (Co)	mg kg^{-1} TS	1.76	1.26	–	0.10	0.15
Copper	mg kg^{-1} TS	64.60	55.70	–	5.85	5.20
Iron (Fe)	mg kg^{-1} TS	1330.9	1202.3	–	88.9	125.7
Manganese (Mn)	mg kg^{-1} TS	94.8	150.4	–	92.1	86.9
Molybdenum (Mo)	mg kg^{-1} TS	5.24	4.27	–	0.37	0.33
Nickel (Ni)	mg kg^{-1} TS	19.5	9.0	–	0.73	0.62
Zinc (Zn)	mg kg^{-1} TS	123.1	131.5	–	35.7	18.9
Selenium (Se)	mg kg^{-1} TS	0.24	0.79	–	0.17	0.17
Elemental composition						
N	%TS	2.18	2.23	2.45	3.98	3.61
C	%TS	38.99	36.53	41.51	59.00	59.08
H	%TS	4.67	4.44	4.97	6.74	7.18
S	%TS	0.48	2.79	0.52	0.34	0.30
O	%TS	25.76	23.66	25.52	29.95	29.83
Calculated parameters based on elemental composition						
TMP [a]	L CH_4 g^{-1} VS	0.540	0.522	0.562	0.609	0.624
Biogas methane [a]	% CH_4 (v/v)	53.5	53.3	54.3	55.3	56.6
Theoretical CV [b]	MJ kg^{-1} VS	21.9	22.0	22.6	24.4	24.8

[a] Based on Buswell equation (Symons and Buswell, 1933); [b] based on Dulong equation (IFRF, 2017)

The characteristics of the two batches of food waste used in this study are also shown in Table 4.1. Both batches had high VS values of 22.90 and 20.39% respectively and higher carbon contents than the cattle slurries, at 52.32 and 52.05 mg kg^{-1} of total solids. These values suggest they had the potential for a high specific biogas production. The C : N ratios of 12.39 and 13.02 were relatively low compared to the 37.0 reported by Forster-Carneiro (2008) but similar to the 13.8 found by Zhang et al. (2012a) for this type of material.

Both batches of food waste had similar trace element concentrations, and values were also close to those previously reported for food wastes from similar sources (Yirong et al., 2014). Compared to the values for the cattle slurry, however, the food waste batches had relatively low trace elements contents. These lower concentrations may cause problems in terms of biogas production in mono-digestion of food waste where sufficient amounts of trace elements are needed by the microorganisms. In particular the concentrations of Co, Mo, Ni and Se in the food waste were below the recommended values in Table 2.5 when expressed on a wet weight basis. Anaerobic digestion of the food waste in this study is therefore likely to need either a supplement of trace elements stock solution or co-digestion with cattle slurry that provides adequate quantities of the required trace elements.

The results of the elemental composition analyses were used to calculate the theoretical methane production (TMP) using the Buswell equation (Symons and Buswell, 1933), and the theoretical calorific value (CV) was calculated using the Dulong equation according to the method in Combustion File 24 (IFRF, 2017). The Buswell equation provides the maximum theoretical methane potential if the substrate is completely mineralised, and this and the theoretical CV provide a useful baseline for assessment of the efficiency of energy recovery from a substrate in the form of methane. Different batches of feedstock showed reasonably similar characteristics, with the food waste having a higher energy production potential as expected.

In general, the properties of the two feedstocks were characteristic of those reported elsewhere in the literature, and especially to those of materials obtained from similar UK sources (Cornell, 2011; Yirong et al., 2014).

4.2 Biochemical Methane Potential (BMP) Testing

4.2.1 Objective for BMP testing

The biochemical methane potential (BMP) assay is used to determine the ultimate biogas or methane yield of substrates during their anaerobic decomposition. In this research, the BMP of food waste and cattle slurry used were determined to characterise the materials and provide a baseline for comparison with the semi-continuous digestion trials.

4.2.2 Methodology for BMP testing

Two sets of BMP assays were carried out as described in section 3.3. The first test was carried out on two batches of food waste (FW1 and FW2) and two batches of cattle slurry (CS1 and CS2), and ran for 37 days. The second test on the third batch of cattle slurry (CS3) ran for 45 days.

4.2.3 Results and discussion for BMP testing

The first test was done in triplicate but due to problems with some of the digesters and collection cylinders, only two readings were used for each sample and for the cellulose control. In the second test, triplicate samples and a single positive control were used.

Figure 4.1 shows the cumulative methane production of the blank controls and the specific methane yield of the positive controls in tests 1 and 2. In all cases the values show a smooth curve without disturbances or discontinuities. Methane production from the blanks in test 1 was slightly lower than in test 2 on the equivalent days: this could be due in part to the fact that test 1 started later in the day, so that the inoculum had a little more time to consume any residual feed. The average specific methane production from the positive controls in test 1 was 0.408 L CH_4 g^{-1} VS added, very close to the theoretical value of 0.415 L CH_4 g^{-1} VS added. Unfortunately in test 2 a different type of cellulose was used as the control, which is known to have a lower specific methane yield of around 0.32 L CH_4 g^{-1} VS added (unpublished data, University of Southampton). The measured value for this cellulose was 0.315 L CH_4 g^{-1} VS added. In both cases, therefore, these control values indicated that the inoculum was healthy and thus gave confidence in the overall validity of the test results.

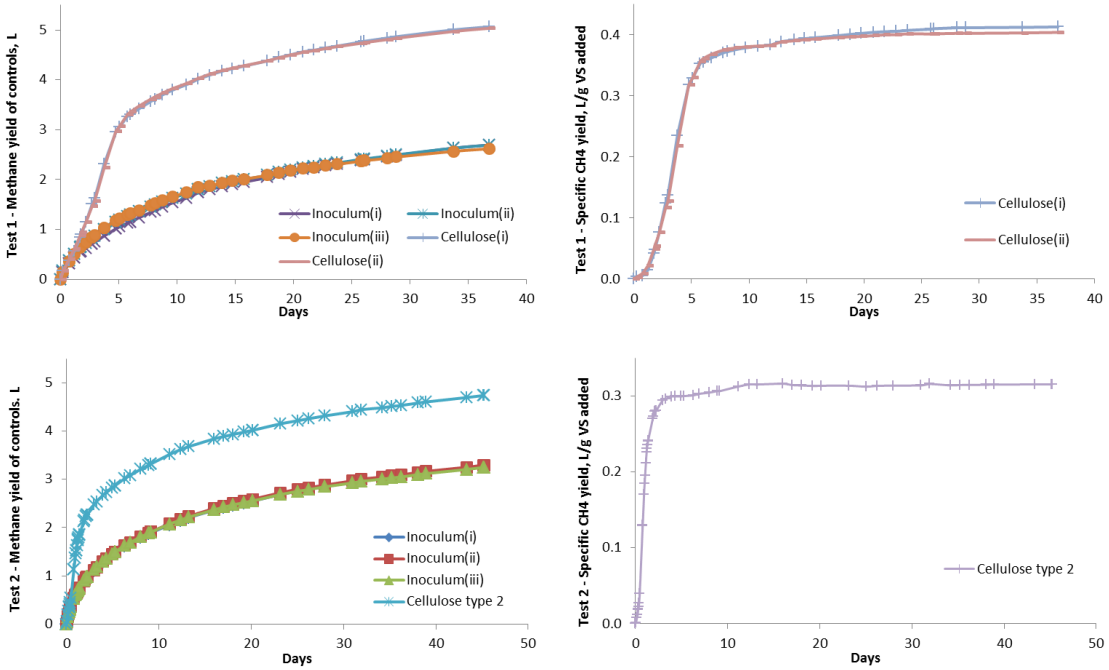


Figure 4.1 Methane production from control reactors in BMP test 1 and 2

Table 4.2 and Figure 4.2 show the results for specific methane production for the feedstocks for a period of 37 days (for FW1, FW2, CS1, and CS2) and 45 days (for CS3). The average BMP values for the food waste were 0.459 and 0.470 L CH_4 g^{-1} VS for batches FW1 and FW2, respectively. The results obtained were close to the range of 0.467 – 0.529 L g^{-1} VS quoted by Browne and Murphy (2013), and were very similar to the values of 0.456 and 0.471 L CH_4 g^{-1} VS found by Zhang et al. (2012a) and Yirong et al. (2014) for source segregated domestic food waste obtained from the same collection scheme. Minor variations between replicates, batches and food wastes from different sources are probably due to the slightly heterogeneous nature of the material, and to variations in the composition of food waste with time and source even in bulk samples. Browne and Murphy (2013) also stated that operating temperature, bioreactor design, and loading rate can significantly affect the results.

Table 4.2 Specific biogas and methane productions

Feedstock	Samples	Biogas (L g^{-1} VS)	Methane (L g^{-1} VS)
Food waste FW1	FW1(i)	0.724	0.463
	$FW1(ii)_i$	0.705	0.455
	$FW1_{average}$	**0.714**	**0.459**
	BMP/TMP	–	75.4%
Food waste FW2	FW2(i)	0.664	0.478
	FW2(ii)	0.632	0.462
	$FW2_{average}$	**0.648**	**0.470**
	BMP/TMP	–	75.3%
Cattle slurry CS1	CS1(i)	0.291	0.187
	CS1(ii)	0.305	0.200
	$CS1_{average}$	**0.298**	**0.193**
	BMP/TMP	–	35.7%
Cattle slurry CS2	CS2(i)	0.291	0.167
	CS2(ii)	0.312	0.177
	$CS2_{average}$	**0.302**	**0.172**
	BMP/TMP	–	33.0%
Cattle slurry CS3	CS3(i)	0.234	0.165
	CS3(ii)	0.324	0.220
	CS3(iii)	0.277	0.195
	$CS3_{average}$	**0.278**	**0.193**
	BMP/TMP	–	34.3%

From the graphs in Figure 4.2, it can be seen that the food waste gave a rapid early methane production, with FW1 reaching over 95% of its final methane potential after 8 days and FW2 after 12 days.

As expected, the measured BMP value of the cattle slurry was much lower than that of the food waste with average values of 0.193, 0.172 and 0.193 L g^{-1} VS for batches CS1, CS2 and CS3 respectively. These low values are very typical of the material (Luste and Luostarinen, 2011), although there is a considerable range in the literature. They are below the values of 0.267 L g^{-1} VS reported by Zhang et al. (2012a) and 0.242 L g^{-1} VS by Labatut et al. (2011). The values are higher, however, than those obtained by Cornell et al. (2011) and Amon et al. (2007) of 0.134 and 0.126 – 0.166 L g^{-1} VS respectively.

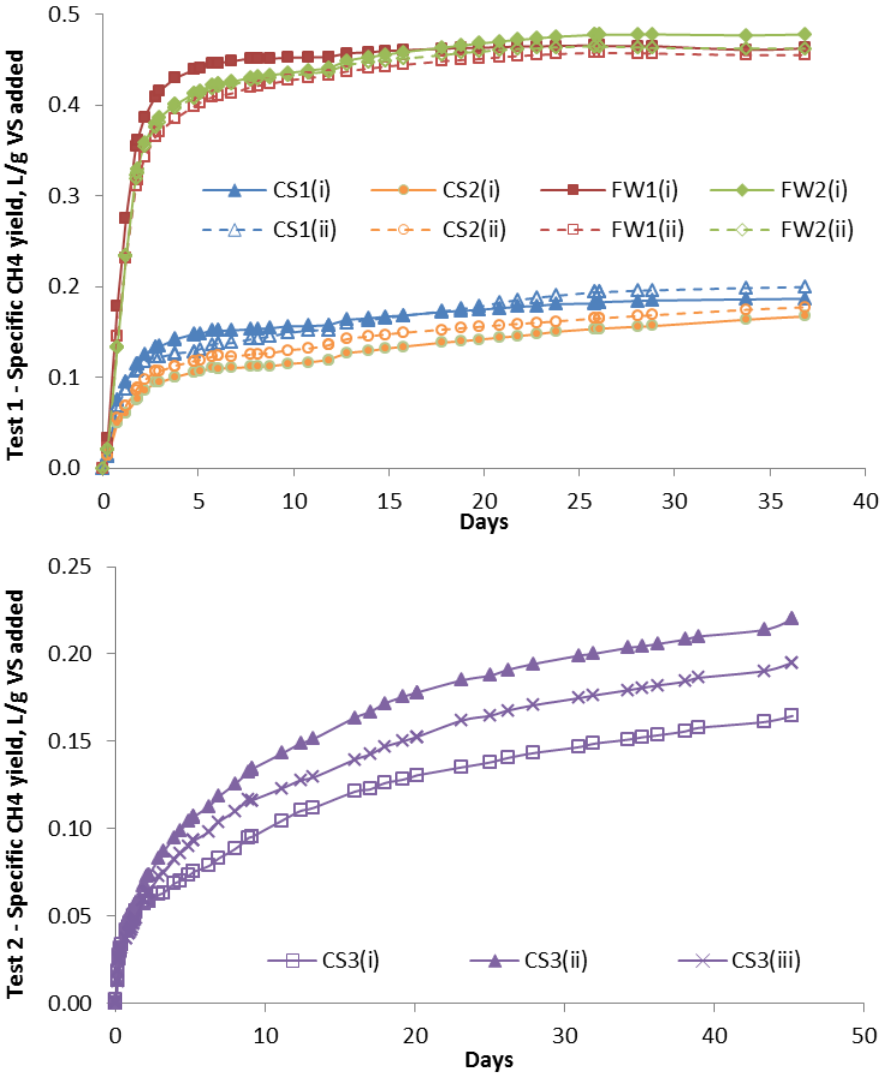


Figure 4.2 Cumulative net specific CH_4 production of the cattle slurry and food waste feedstocks

Gas production from the cattle slurries occurred much more slowly than from the food waste, requiring 23, 28 and 40 days to produce 95% of the final BMP values for CS1, CS2 and CS3 respectively. Agreement between replicates in batches CS1 and CS2 was good, but in CS3 there was a much wider scatter: the reason for this is not known, but may indicate inadequate homogenisation.

The wide range of BMP values reported in the literature is due in part to the variable nature of the material and of dairy cattle operations, which involves different animal breeds, ages, diets, and management practices. Although the cattle slurries used in the experiments came from dairy farms, they were different in nature: CS1 and CS3 were obtained from an organic farm while CS2 came from a farm which used gypsum for

bedding. Luste and Luostarinen (2011) also reported that pre-treatment (hygienisation and ultrasound) of cattle slurry can increase BMP values compared to untreated ones.

Table 4.2 also shows the BMP as a proportion of the TMP. It can be seen that each type of substrate are relatively consistent, and the value again confirm the much higher degradability of the food waste.

Kinetic parameters

The BMP data was fitted using both a simple first-order model (Model 1, Equation 4.1) and a pseudo-parallel first-order model (Model 2, Equation 4.2) to provide further information on the substrate characteristics and behaviour.

$$Y = Y_m\left(1 - e^{-kt}\right) \qquad \text{Equation 4.1}$$

where:

Y = cumulative methane yield at time t
Y_m= ultimate methane yield
k= first order rate constant

$$Y = Y_m\left(1 - Pe^{-k_1 t} - (1 - P)e^{-k_2 t}\right) \qquad \text{Equation 4.2}$$

where:

k_1 = first order rate constant for the proportion of readily degradable material
k_2 = first order rate constant for the proportion of less readily degradable material
P = proportion of readily degradable material

The results of modelling and the kinetic constants are shown in Figure 4.3 and Figure 4.4 and summarised in Table 4.3. Full details of values obtained are shown in Appendix A.

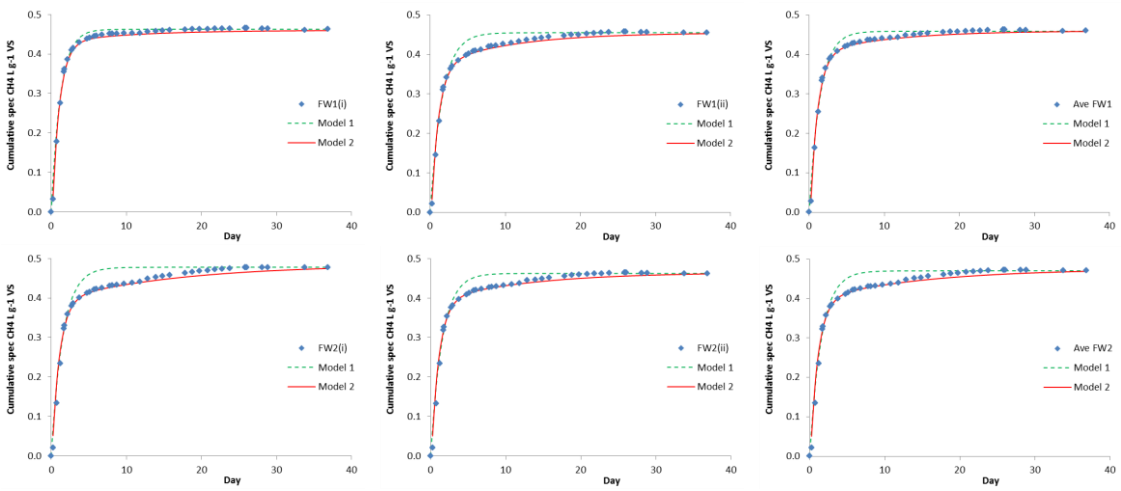


Figure 4.3 Real and modelled results for cumulative net specific CH_4 production of FW in BMP test 1

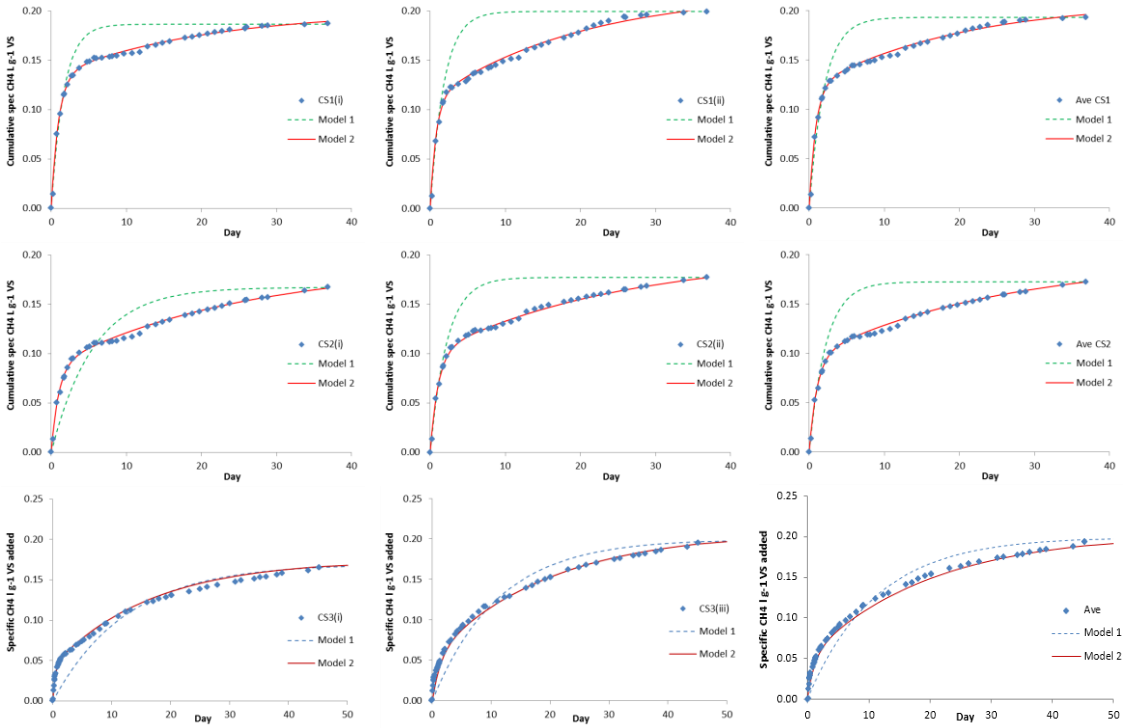


Figure 4.4 Real and modelled results for cumulative CH_4 production of CS in BMP test 1 and 2

Table 4.3 Coefficient and parameters values for Model 2 using average BMP data

Parameter	**FW1**	**FW2**	**CS1**	**CS2**	**CS3**
Methane yield (L g^{-1} VS)	0.460	0.475	0.210	0.195	0.205
Proportion of readily degradable fraction (P)	0.87	0.82	0.58	0.49	0.25
Degradation rate for the readily degradable fraction (k_1)	1.00	0.96	1.15	0.96	0.85
Degradation rate for the slowly degradable fraction (k_2)	0.10	0.07	0.05	0.04	0.05
Coefficient of determination (R^2)	0.9981	0.9951	0.9959	0.9972	0.9990

As can be seen from Figure 4.3 and 4.4, Model 2 gave a much better fit than Model 1, especially for cattle slurry: in all cases R^2 values for the experimental and modelled data were > 0.99. Modelling of the two batches of food waste gave the best result when a small lag of 0.15 days (for FW1) and 0.10 days (for FW2) was introduced at the beginning of the run, whereas this was not needed for the cattle slurry samples. This type of short lag may represent the time required for the inoculum to come into contact with the substrate and hydrolytic organisms to attach to the surface: it may reflect the moisture content of the sample, since lags of 1 day are common for positive cellulose controls in powdered form. Other reasons may include the need to produce inducible enzymes substrates with unfamiliar chemical components. An apparent lag may occur when biogas production is inhibited by an initial excess of VFA due to an inadequate inoculum substrate ratio (Walker et al., 2010; Holliger et al., 2016)); but these latter two generally involve a longer timescale and were not thought to be relevant in this case. The samples in test 1 would probably also have benefitted from a slightly longer run, especially CS2 which still appeared to have a small net gas production.

As expected food waste had a greater proportion of readily degradable material (*P*) when compared to cattle slurry. Coefficients for the two food waste batches were in reasonable agreement (Table 4.3), although FW1 had slightly higher values both for *P* for and the degradation coefficients k_1 and k_2. The coefficients for cattle slurry samples showed a wider range of properties between the different types, although the degradation rate for the less readily degradable material (k_2) was similar and lower than that for food waste in all cases. In general the final BMP values estimated from the modelling agreed well with the measured values for food waste, and were slightly higher for cattle slurry: these

modelled values can be used for comparison with the substrate performance in semi-continuous digestion.

4.3 Mono Digestion of Cattle Slurry and Food Waste

This section describes the results for digestion of cattle slurry and food waste as single substrates under mesophilic conditions (35 $\pm$ 2 ^{o}C), throughout the experimental period. Where relevant the results are also presented in the following sections when they acted as controls in co-digestion experiments.

4.3.1 Objective for mono-digestion of feedstocks

The primary purpose of these trials was to operate as controls for co-digestion experiments and to provide baseline data on process performance and stability when cattle slurry and food waste were mono digested.

4.3.2 Methodology for mono-digestion of feedstocks

Two pairs of 5-L CSTR digesters were inoculated with digestate from Millbrook WWTP and initially fed on food waste (batch FW1, Table 4.1) at a loading of 1 g VS L^{-1} day^{-1}. Beginning on day 38, food waste feeding was discontinued for one pair of digesters and replaced with cattle slurry (batch CS1, Table 4.1), while the other pair continued to receive FW1. Subsequent changes in OLR and other operating parameters are described in the following sections. Note that in this section, day numbers are given consecutively from the start of operation i.e. from day 0 of the first trial.

4.3.3 Results and discussion for mono-digestion of feedstocks

4.3.3.1 Mono-digestion of cattle slurry

During trial C1 feeding of the cattle slurry digesters on CS1 continued at 1 g VS L^{-1} day^{-1} until day 54, after which the OLR was incrementally raised to 1.7 g VS L^{-1} day^{-1} (HRT 33 days) by day 74. Operation continued under these conditions until day 220, at which point the OLR was raised in a step increase to 3 g VS L^{-1} day^{-1} (HRT 18 days) and maintained at this value until day 243.

In this period the digesters ran smoothly and without any major issues for more than 4 HRT. The average volumetric biogas production (VBP) and specific methane production (SMP) over the last 90 days of operation at 1.7 g VS L^{-1} day^{-1} were 0.500 L L^{-1} day^{-1} and 0.194 L g^{-1} VS respectively (Figure 4.5). As expected the specific methane yield was not particularly high, but was within the range of values shown in Table 2.6. There was an initial peak in VFA around day 60, which is believed to be associated with adaptation of this inoculum to a change in substrate: a similar phenomenon has been seen on many previous occasions (e.g. Climenhaga and Banks, 2008; Zhang et al., 2012a; Zhang and Banks, 2012; Roberts et al., 2016; Yirong et al., 2017). Apart from this, the monitoring parameters shown in Figure 4.6 indicated that the mono-digestion of CS1 ran in a stable manner, with average values for pH and IA/PA ratio of 7.76 and 0.32 respectively over the last 90 days at OLR 1.7 g VS L^{-1} day^{-1}.

After day 221 when the OLR was raised to 3 g VS L^{-1} day^{-1} there was an increase in VBP (Figure 4.5) but no major disturbance in stability parameters (Figure 4.6), confirming that performance was stable and robust enough to withstand the impact of this sharp change.

Trial S1 began on day 243 when the feedstock was changed to a new batch of cattle slurry (CS2, Table 4.1). As digestion continued, both VBP and SMP dropped quite sharply. Feeding was ceased on day 283 as VBP and SMP values were below 0.20 L L^{-1} day^{-1} and 0.03 L CH_4 g^{-1} VS respectively. During this period, the biogas methane content was less than 40% v/v and other monitoring parameters (as shown in Figure 4.6) showed signs of instability, with the IA : PA ratio reaching 0.8 and total VFA of up to 9.9 g L^{-1}.

Trial S2 used the same cattle slurry feedstock (CS2, Table 4.1) as trial S1 to see whether the same problems were repeated. Starting from day 348, the digesters were re-seeded with fresh inoculum and were initially fed on food waste FW1 at an OLR of just below 1 g VS L^{-1} day^{-1}. On day 373 the feedstock was changed to CS2 at an OLR of 1 g VS L^{-1} day^{-1}, which was gradually increased to 3 g VS L^{-1} day^{-1} (HRT 35 days) by day 404. After about 1 HRT (around day 432 – 438) of feeding with cattle slurry CS2 at the target OLR the VBP and SMP peaked at 0.23 L L^{-1} day^{-1} and 0.04 L g^{-1} VS respectively, before dropping as the digestion again failed. At this point, VBP and SMP values were below 0.10 L L^{-1} day^{-1} and 0.01 L g^{-1} VS respectively, with a methane content of less than 15%. Other monitoring parameters (Figure 4.6) also showed instability, with an IA : PA ratio of 1.25 and total VFA at 15 g L^{-1}. Feeding was stopped on day 529. The failure of these

two trials was attributed to the feedstock itself. Cattle slurry CS2 was taken from a farm using gypsum as bedding, and hence had high sulphate content of 6876 mg SO_4 L^{-1} and a sulphur content of 2.79% S on a TS basis compared to only 0.48% for CS1 (Table 4.1).

Trial C2 started on day 544 with fresh inoculum. The digesters were initially fed on food waste (batch FW2) at an OLR of 2 g VS L^{-1} day^{-1} (HRT 105 days). Feeding with cattle slurry (batch CS1) started on day 606 at an OLR of 2 g VS L^{-1} day^{-1} (HRT 28 days). From day 620 the OLR was gradually increased at a rate of about 0.03 g VS L^{-1} day^{-1} until it reached 3 g VS L^{-1} day^{-1} (HRT 19 days) on day 648. Operation continued until day 710 (3.5 HRT) when the OLR was reduced to 2 g VS L^{-1} day^{-1} (HRT 28 days), in response to the unexpectedly low specific and volumetric gas production observed at 3 g VS L^{-1} day^{-1} (Figure 4.5). On day 764, feedstock was switched to cattle slurry CS3 at the same OLR of 2 g VS L^{-1} day^{-1} (HRT 41 days) and operation continued until day 1184 (12.1 HRT in total, 1.9 with CS1 and 10.2 with CS3). Trials S3a and b started on days 788 and 863 respectively, but there was no change in the operation of the cattle slurry mono-digestion controls during this period. Gas production (Figure 4.5) and other monitoring parameters (Figure 4.6) indicated that mono-digestion of cattle slurry batch CS3 ran in a stable manner, with the pH and IA/PA ratio averaging 7.68 and 0.55 respectively and total VFA generally below 50 mg L^{-1}.

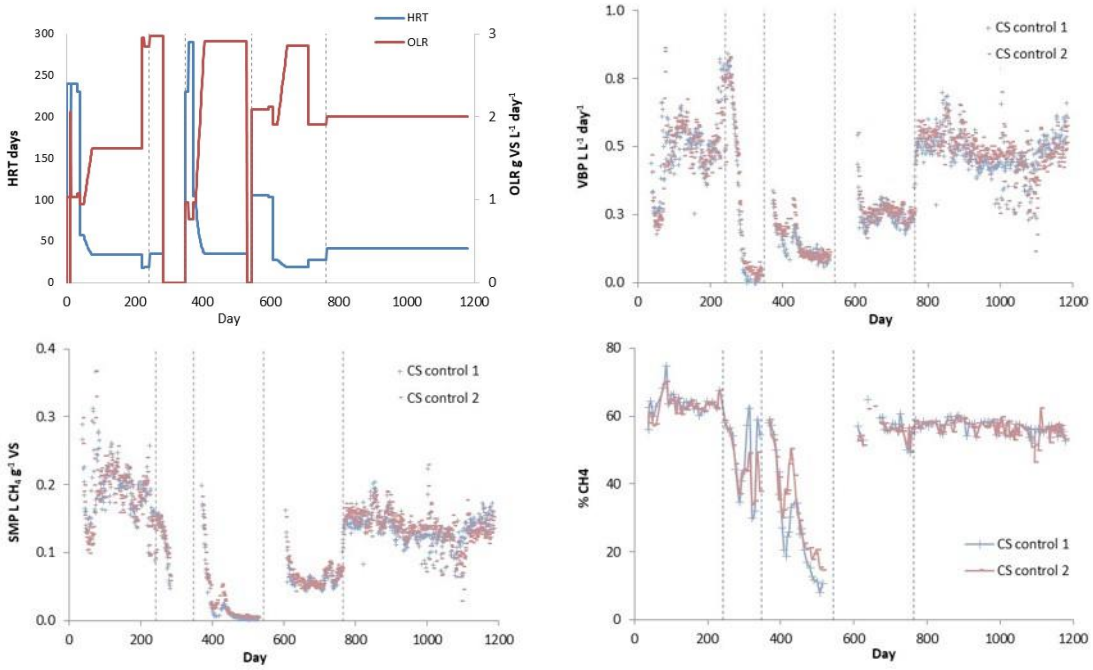


Figure 4.5 OLR, HRT, VBP, SMP and biogas methane content during mono-digestion of cattle slurry. Vertical dotted lines indicate change of CS batch (days 243 and 764) and re-seeding with new inoculum (days 348 and 544).

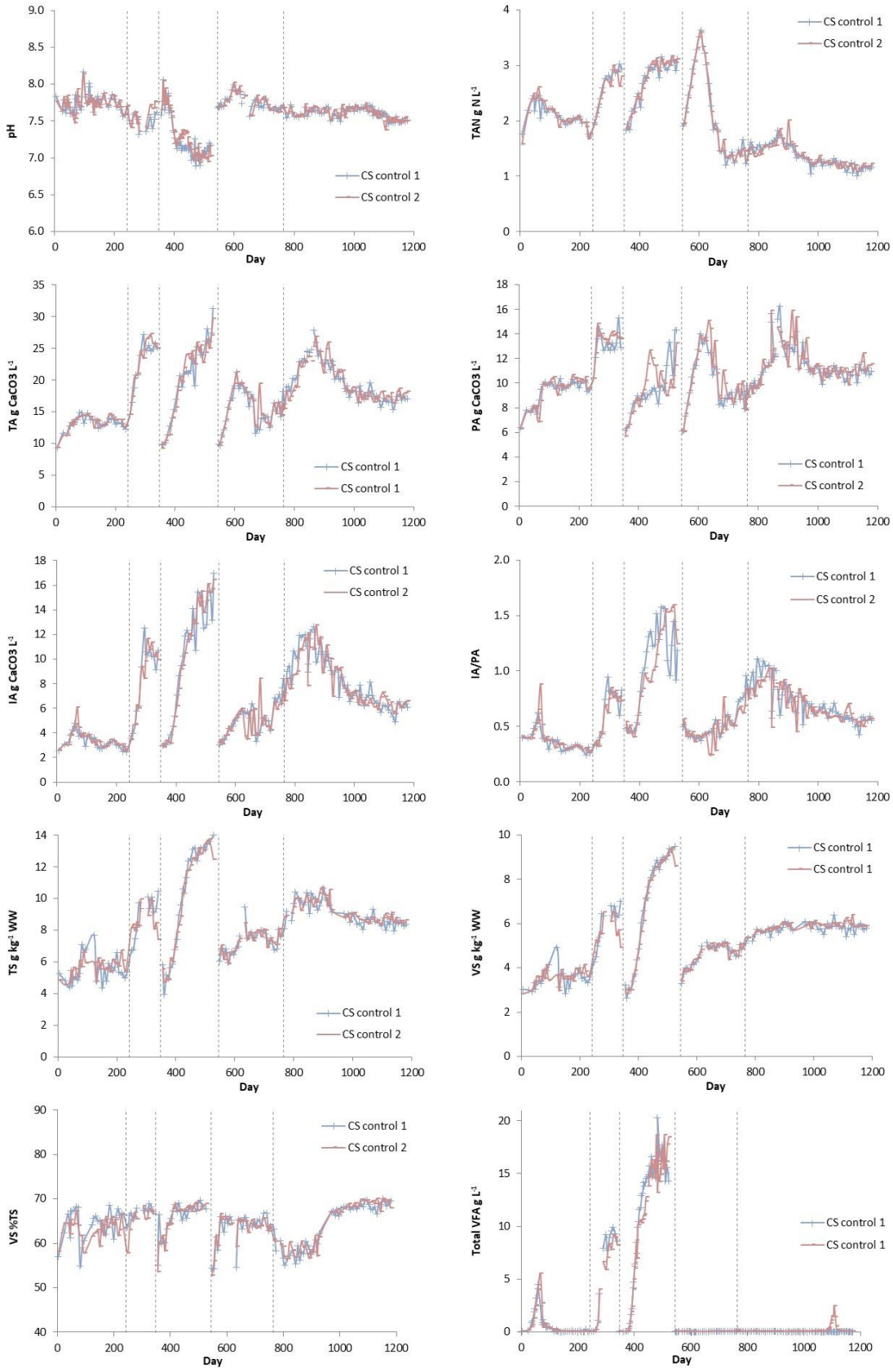


Figure 4.6 Monitoring parameters during mono-digestion of cattle slurry. Vertical dotted lines indicate change of CS batch (days 243 and 764) and re-seeding with new inoculum (days 348 and 544).

Average values for gas production parameters during stable periods of semi-continuous digestion are summarised in Table 4.4 for each cattle slurry feedstock. The SMP values for CS1 in trial C1 and for CS3 are similar to values reported elsewhere (Table 2.6), although CS1 is relatively high and also represents 100% of the BMP value. The low values for CS2 and for CS1 in trial C2 are discussed in more detail in sections 4.4.2, 4.4.3 and 4.4.5 below.

Table 4.4 Average gas production values for CS feedstocks in semi-continuous digestion

Parameter	**Feedstock**	**CS1**	**CS2**	**CS1**	**CS1** [a]	**CS3**
	Ave. from days	**121 – 209**	**505 – 523**	**676 – 710**	**711 – 763**	**1154 – 1183**
	In trial	**C1**	**S2**	**C2**	**C2**	**S3a**
Nominal OLR	g VS L^{-1} day^{-1}	1.7	3	3	2	2
HRT	days	33	35	18	28	41
SBP	L g^{-1} VS	0.308	0.032	0.094	0.121	0.260
SMP	L CH_4 g^{-1} VS	0.194	0.004	0.053	0.069	0.144
VBP	L L^{-1} day^{-1}	0.500	0.093	0.268	0.230	0.522
VMP	L CH_4 L^{-1} day^{-1}	0.314	0.012	0.151	0.125	0.288
CH_4 content	% v/v	62.8	12.6	56.6	54.7	55.1
SMP/BMP	%	100.4%	2.3%	27.5%	35.5%	74.4%
SMP/TMP	%	35.9%	0.8%	9.8%	12.7%	25.5%

[a] Note: operating period at this OLR < 3 HRT but data included for comparison

4.3.3.2 Mono-digestion of food waste

During the mono-digestion of food waste, the digesters were fed on two batches of food waste: FW1 from day 1 to day 367 and FW2 from day 368 onwards (Table 4.1).

The initial loading on the FW digesters of 1 g VS L^{-1} day^{-1} was gradually increased from day 39 onwards until it reached the target OLR of 3 g VS L^{-1} day^{-1} (HRT 76 days) on day 56. Daily feeding continued at this loading until day 500, with the daily volume of feed being adjusted slightly on days 244 and 346 to improve alignment with the target OLR.

After acclimatisation to the increasing OLR, gas production in the digesters continued smoothly until around day 500 (5.8 HRT). VBP and SMP showed some periodic fluctuations (Figure 4.7) but averaged around 2.4 L L^{-1} day^{-1} and 0.50 L CH_4 g^{-1} VS respectively for FW1 and 2.3 L L^{-1} day^{-1} and 0.47 L CH_4 g^{-1} VS for FW2. Figure 4.8 shows other monitoring parameters during the mono-digestion. An initial VFA peak was seen at a similar time to that in the cattle slurry mono-digestion, and was attributed to the

same cause. TAN, total and partial alkalinity and solids content all increased gradually and appeared to be stabilising after 2 HRT. The pH was around 7.8 and the IA/PA ratio was 0.25, indicating stable operation.

At around day 280 there was a small peak in VFA concentration and in IA/PA ratio. At this point TAN concentration had reached around 5 g N kg^{-1} WW, above the limiting value of 4 g N kg^{-1} WW suggested by Angelidaki and Ahring (1994) for stable mesophilic digestion. The VFA peak was consumed, but from around day 340 total VFA concentrations started to rise again, reaching 10 g L^{-1} by day 500 before falling to around 6 g L^{-1} over the next 40 days. This was accompanied by a corresponding peak in SMP as some of the accumulated VFA were converted. The high alkalinity was sufficient to buffer the VFA, resulting in a pH between 7.93 – 8.26.

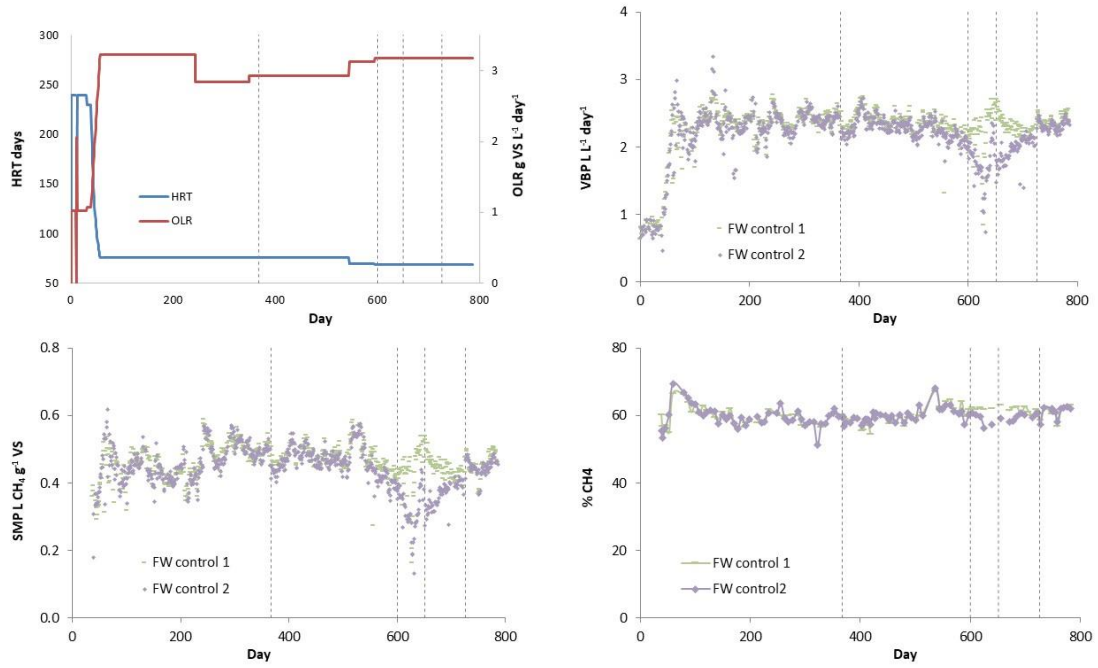


Figure 4.7 OLR, HRT, VBP, SMP and biogas methane content during mono-digestion of food waste. Vertical dotted lines indicate change from FW1 to FW2 (day 368), TE addition (day 599), foaming-related digestate loss from control digester 2 (day 650) and re-seeding with fresh FW digestate (day 727).

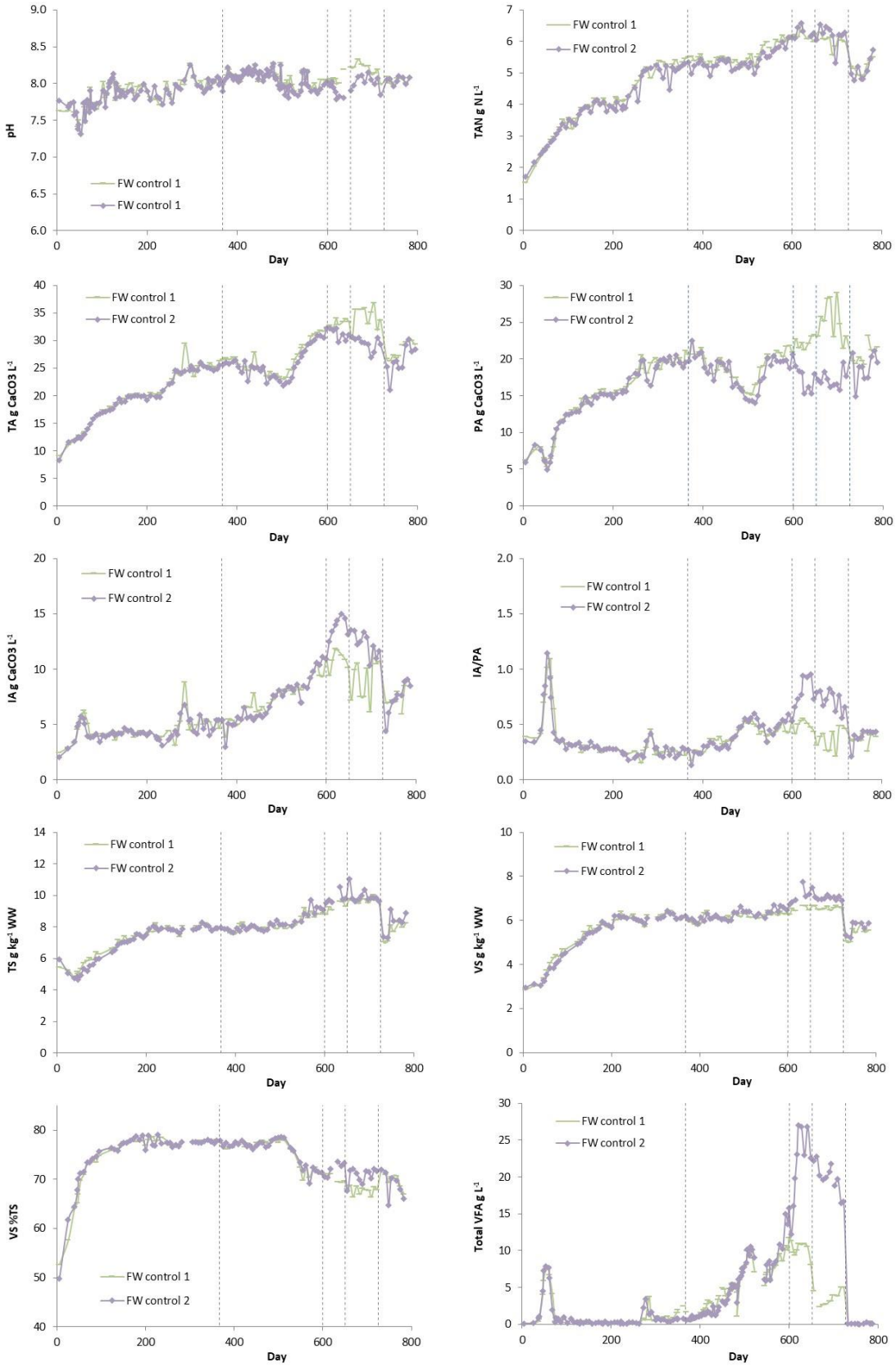


Figure 4.8 Monitoring parameters during mono-digestion of food waste. Vertical dotted lines indicate change from FW1 to FW2 (day 368), TE addition (day 599), foaming-related digestate loss from control digester 2 (day 650) and re-seeding with fresh FW digestate (day 727).

From day 550 onwards, however, total VFA concentrations began to rise again, reaching 11.7 and 15.8 g L^{-1} in FW control digesters 1 and 2 respectively by day 599. On day 600, both digesters were given a one-off dose of cobalt, nickel and selenium at 10 times the normal supplementation (section 3.2.5), followed by two further additions of trace elements on a weekly basis at a rate of 1 mL of TE solution for every 1 L of digestate removed, based on Banks et al. (2012).

Although the digesters were replicates, the effects of this TE dosing were different in each. FW control digester 1 continued to show stable gas production, with a small peak in VBP and SMP around day 650 associated with a reduction in accumulated VFA (Figure 4.7 and 4.8). TAN and alkalinity concentrations remained high at around 6 g N kg^{-1} WW and 30 g $CaCO_3$ kg^{-1} WW respectively. In FW control digester 2, foam appeared and this caused the VBP and SMP to drop. The VFA concentration increased to more than 20 g L^{-1}. Foam was removed from FW control digester 2 on day 640 in the attempt to recover the process, which later produced a slight increment in the gas production. On day 651, however, the foaming became worse and a small amount of digestate was lost from the digester as foam blocked the gas outlet causing a pressure build-up. After this incident, the digester seemed to recover: the VBP and SMP increased gradually and matched the gas production of the other replicate after around 70 days.

Figure 4.9 shows VFA profiles for the two digesters. The pattern of a rise in acetic acid concentrations which then falls and is replaced by an increase in propionic acid is highly characteristic of this mode of stress due to high ammonia concentrations without TE supplementation: the profiles below closely resemble those found by Zhang et al. (2012a) in mono-digestion of source separated food waste.

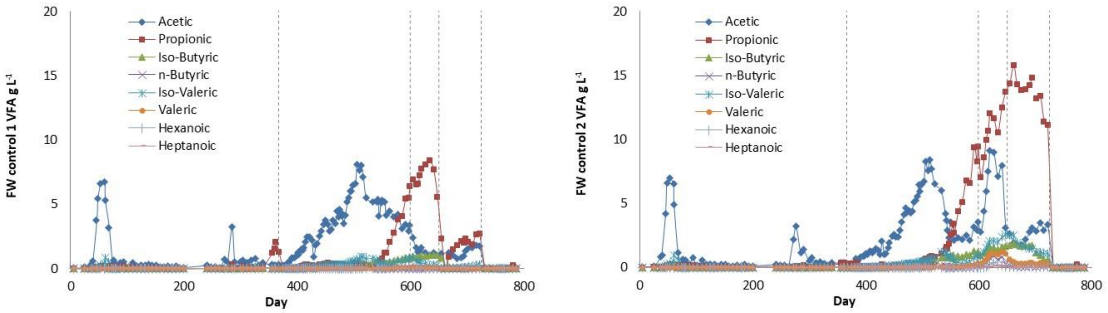


Figure 4.9 VFA profiles during mono-digestion of food waste. Vertical dotted lines indicate TE addition (day 599), foaming-related digestate loss from F-2 (day 650) and re-seeding with fresh FW digestate (day 727).

On day 727 both digesters were reseeded with digestate from a laboratory-scale digester fed on a similar food waste and that had been regularly supplemented with TE solution. Feeding on FW2 was continued at an OLR of 3 g VS L^{-1} day^{-1} (HRT 69 days) with weekly TE supplementation until day 787. VFA concentrations remained low (Figure 4.9) and all other monitoring parameters showed signs of stabilising. After day 787 these digesters were no longer used as FW mono-digestion controls.

Table 4.5 summarises the average gas production parameters for food wastes FW1 and FW2 in semi-continuous digestion during stable operating periods. Specific and volumetric production was high but similar to other values reported in the literature (Capson-Tojo et al., 2016). SMP values were almost equal to the respective BMP in each case, reflecting the long natural HRT for this feedstock when it is digested without the addition of water or other low-solids co-substrates. The SMP achieved also made up a high proportion of the TMP confirming that this material is well suited to single phase digestion.

Table 4.5 Average gas production values for FW feedstocks in semi-continuous digestion

Parameter	**Feedstock**	**FW1**	**FW2**	**FW2**
	Ave from days	**307 – 366**	**450 – 480**	**758 – 787**
Nominal OLR	g VS L^{-1} day^{-1}	3	3	3
HRT	days	76	76	69
SBP	L g^{-1} VS	0.843	0.798	0.754
SMP	L CH_4 g^{-1} VS	0.498	0.468	0.469
VBP	L L^{-1} day^{-1}	2.42	2.33	2.39
VMP	L CH_4 L^{-1} day^{-1}	1.43	1.37	1.49
CH_4 content	% v/v	59.0	58.6	62.3
SMP/BMP	%	108.5%	99.6%	99.9%
SMP/TMP	%	81.7%	75.0%	75.2%

The above findings were similar to those of Banks et al. (2008) in their study of mesophilic digestion of source segregated food waste in a pilot-scale CSTR digester. After 35 weeks of operation at an initial OLR of 4 g VS L^{-1} day^{-1} without trace element addition VFA accumulated to 27.4 g L^{-1}. This did not appear to have a negative effect on gas production: the biogas yield was around 0.63 m^3 kg^{-1} VS at a methane concentration of 58%. This was attributed to the high ammonia and alkalinity of 5.2 g N kg^{-1} WW and 13.9 g $CaCO_3$ kg^{-1} WW respectively. To prevent further VFA accumulation the feedstock was diluted by replacing a proportion of the digestate recycled for pumping and feedstock maceration with water, bringing the ammonia concentration down to a safe level of around 3 g N L^{-1}.

The addition of a specific combination of TE is known to help resolve the problems caused by high concentrations of ammonia and the accumulation of VFA. This was confirmed by Yirong et al. (2014) who compared food waste digestion under mesophilic conditions with and without TE supplementation. In that study, an SMP of 0.45 L g^{-1} VS was observed in the TE supplemented digesters for a period of up to 158 days despite the high concentrations of ammonia and alkalinity (4.2 g N kg^{-1} WW and 15 - 20 g $CaCO_3$ kg^{-1} WW respectively). In the digesters without TE supplementation, high concentrations of ammonia and alkalinity caused the SMP to decrease from day 70 and from day 140 it fell rapidly with an IA/PA ratio above 1. The above results confirmed that trace elements must be present at the correct concentration and not in excess or deficit for stable food waste digestion.

These findings together with previous work and the results of the literature review confirm that, even though mono-digestion of food waste is possible with correct trace element supplementation, co-digestion with cattle slurry offers potential advantages through dilution of the naturally high ammonia concentration and addition of a proportion of the trace element needs. Conversely food waste has the potential to improve the C/N ratio of cattle slurry and the volumetric energy production, which can vary significantly from batch to batch, while having relatively little impact on system capacity retention times (Banks et al., 2011b). The following sections describe the results of experimental work to investigate energy yield and digestion stability in a variety of co-digestion scenarios.

4.4 Co-Digestion of Cattle Slurry and Food Waste

Cattle slurry and food waste were co-digested in mesophilic conditions (35 ± 2 ºC) at different wet-weight ratios and OLRs in order to obtain an understanding of digestion stability and energy production potential.

4.4.1 Trial C1: Co-digestion of cattle slurry to food waste at wet-weight ratio of 3 : 1

4.4.1.1 Objective of trial C1

The purpose of this trial was to quantify the increase in volumetric gas production and to assess the effect on the process performance and stability when cattle slurry is digested with food waste at wet-weight ratio of 3:1 at target OLR of 3, 4 and 5 g VS L^{-1} day^{-1}.

The CS : FW ratio of 3 : 1 was chosen based on previous work by Zhang et al. (2012a) who successfully trialled co-digestion of cattle slurry and food waste at 5 : 1 and 2.2 : 1 on a wet weight basis (40 : 60 and 60 : 40 on a VS basis), and by Banks, Chesshire, et al. (2011) who considered a CS : FW scenario of 2.9 : 1 for Hampshire, UK. The trial was conducted at three different loading rates to determine the effect of OLR on the process performance and stability at a fixed CS : FW ratio. Combined CS : FW loading rates of 3, 4 and 5 g VS L^{-1} day^{-1} were chosen to cover a typical operating range for full-scale digestion plant.

4.4.1.2 Methodology for trial C1

Ten 5-L CSTR digesters with 4 L operating volume were inoculated with digestate from Millbrook WWTP. The planned steady-state operating conditions are summarised in Table 4.6.

Table 4.6 Planned operating conditions of trial C1

Digester	Feeding (g wet-weight day^{-1})			OLR (g VS L^{-1} day^{-1})			HRT (days)
	CS [a]	FW [b]	Total	CS	FW	Total	
3-1 & 3-2	90	30	120	1.3	1.7	3.0	33
4-1 & 4-2	120	40	160	1.7	2.3	4.0	25
5-1 & 5-2	150	50	200	2.1	2.9	5.0	20
C-1 & C-2	120	0	120	1.7	0.0	1.7	33
F-1 & F-2	0	52	52	0.0	3.0	3.0	76

[a] Feedstock cattle slurry CS1, [b] Feedstock food waste FW1

Throughout this trial the feedstocks used were food waste batch FW1 and cattle slurry batch CS1 (Table 4.1). All of the digesters were initially fed with food waste at a loading of 1.0 g VS L^{-1} day^{-1}. Beginning on day 38, when gas production had stabilised, cattle slurry was added to the feed of three pairs of digesters at a proportion of three times the food waste weight, resulting in an increase in OLR to 1.7 g VS L^{-1} day^{-1}. From day 47 onwards, the quantity of mixed food waste and cattle slurry feed was gradually increased until OLRs of 3, 4 and 5 g VS L^{-1} day^{-1} were reached on days 58, 69 and 79 respectively. In another pair of digesters (food waste controls) the food waste OLR was gradually increased to 3 g VS L^{-1} day^{-1} by day 57. In the remaining pair of digesters, feeding of food waste was discontinued and the feedstock was changed to cattle slurry CS1 (cattle slurry controls), at the original loading rate of 1 g VS L^{-1} day^{-1}. From day 50 the OLR in the cattle slurry controls was then increased steadily until it reached the target OLR of 1.7 g VS L^{-1} day^{-1} on day 75. Once each digester had reached the desired OLR, feeding continued for 3.5 HRT.

4.4.1.3 Results and discussion for trial C1

Figure 4.10 shows the OLR, HRT and different feed types applied during the trial. Minor discrepancies were due to a missed feed on day 10 which was made up on day 11; and a one-day cessation of feed on day 61 to allow consumption of accumulated VFA (see below for further details).

Performance and monitoring parameters for the CSTRs leading up to and at the targeted OLR are presented in Figures 4.11 to 4.14, while Table 4.7 summarises the average values for key parameters at steady state. Throughout this section, the co-digestion performance is compared to the CSTRs of cattle slurry and food waste alone (of day 1 to day 210) which were presented in section 4.1.5 and are shown in Table 4.7.

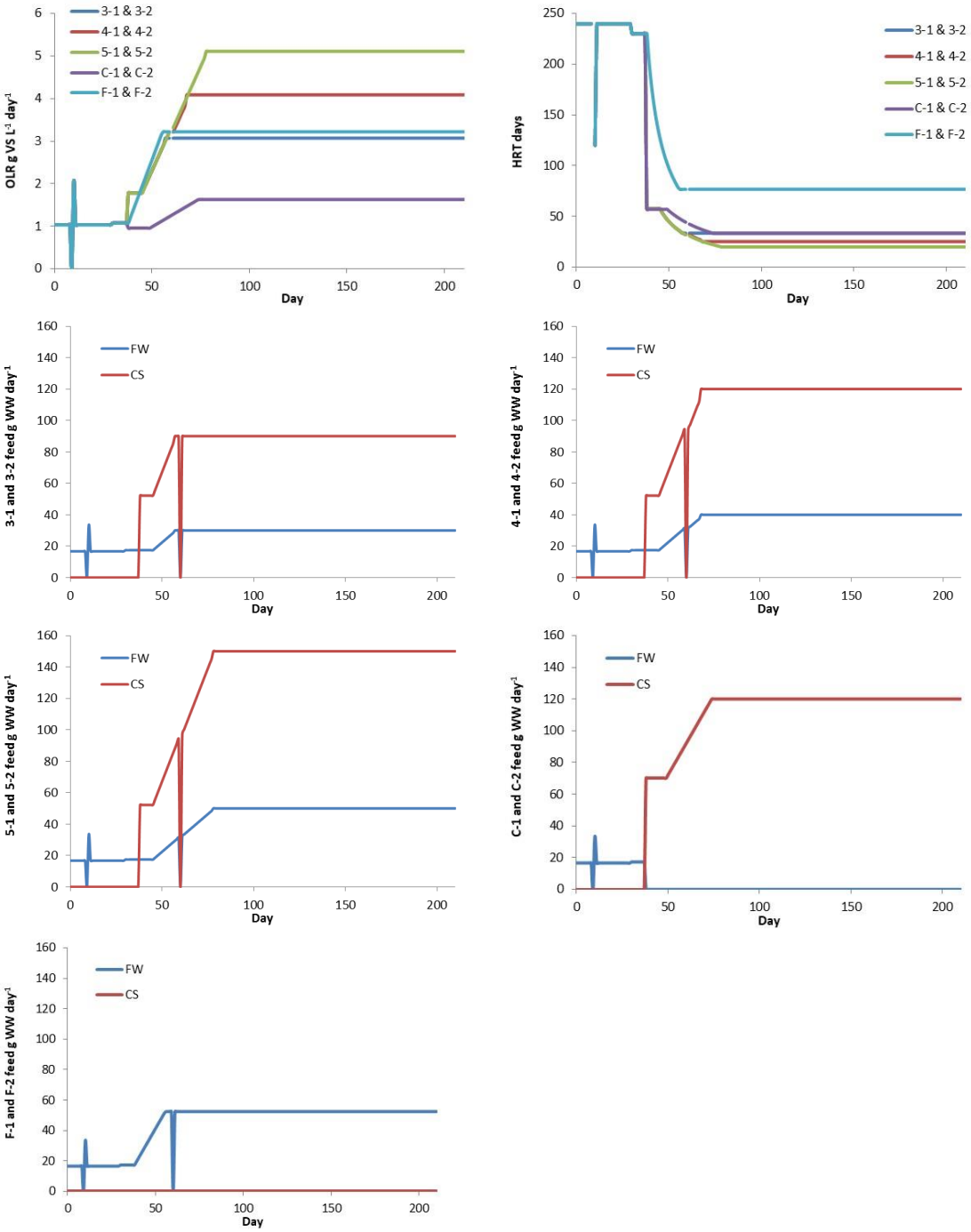


Figure 4.10 OLR, HRT and feed types applied during FW and CS co-digestion at 3 : 1 wet weight ratio (trial C1)

Table 4.7 Steady-state values for key parameters in trial C1 (average for last 90 days of operation)

Parameter	**Unit**	**Co-digestion**									**Control**					
											Cattle slurry			**Food waste**		
Nominal OLR [a]	g VS L^{-1} day^{-1}	3			4			5			1.7			3		
SBP	L g^{-1} VS	0.550	±	0.016	0.542	±	0.002	0.531	±	0.007	0.308	±	0.000	0.759	±	0.005
SMP	L CH_4 g^{-1} VS	0.332	±	0.009	0.330	±	0.002	0.324	±	0.004	0.194	±	0.001	0.448	±	0.002
VBP	L L^{-1} day^{-1}	1.686	±	0.048	2.215	±	0.009	2.715	±	0.038	0.500	±	0.000	2.450	±	0.016
VMP	L L^{-1} day^{-1}	1.018	±	0.029	1.349	±	0.010	1.658	±	0.020	0.314	±	0.001	1.447	±	0.006
CH_4 content	% v/v	60.3	±	0.0	60.9	±	0.2	61.0	±	0.2	62.7	±	0.1	59.0	±	0.0
Digestate TS	%WW	6.50	±	0.02	6.67	±	0.04	7.00	±	0.02	5.66	±	0.05	7.20	±	0.05
Digestate VS	%WW	4.47	±	0.01	4.65	±	0.05	4.84	±	0.01	3.62	±	0.00	5.57	±	0.03
VS destruction	%VS	58.2	±	0.1	59.7	±	0.3	59.9	±	0.1	37.4	±	1.3	90.5	±	0.0
pH	–	7.78	±	0.02	7.72	±	0.01	7.69	±	0.00	7.76	±	0.01	7.92	±	0.01
TA	g $CaCO_3$ kg^{-1} WW	16.8	±	0.0	16.0	±	0.3	15.3	±	0.1	13.4	±	0.1	19.5	±	0.1
PA	g $CaCO_3$ kg^{-1} WW	12.2	±	0.1	11.6	±	0.3	11.2	±	0.1	9.9	±	0.0	14.8	±	0.2
IA	g $CaCO_3$ kg^{-1} WW	4.1	±	0.1	3.9	±	0.0	3.6	±	0.0	3.1	±	0.0	4.2	±	0.0
IA/PA ratio	–	0.33	±	0.01	0.33	±	0.01	0.32	±	0.00	0.32	±	0.00	0.29	±	0.01
TAN	g N kg^{-1} WW	2.46	±	0.01	2.34	±	0.02	2.29	±	0.02	2.01	±	0.01	3.97	±	0.05
TKN [b]	g N kg^{-1} WW	4.49	±	0.02	4.42	±	0.01	4.40	±	0.03	3.03	±	0.01	7.50	±	3.13
Total VFA	g L^{-1}	0.17	±	0.01	0.14	±	0.01	0.20	±	0.00	0.05	±	0.00	0.23	±	0.00

[a] Feedstock cattle slurry CS1, food waste FW1, [b] Measured at end of run

Gas production. Gas production throughout the trial is shown in Figure 4.11. During the start-up period of 38 days, all the digesters were producing biogas at a roughly constant rate of about 0.8 L L^{-1} day^{-1}. As the OLR gradually increased in the food waste control digesters, volumetric biogas production (VBP) also increased accordingly. The same pattern can also be seen for the co-digestion digesters, but with lower VBP values. In the cattle slurry control digesters, gas production decreased to about half on the cessation of food waste addition. This was expected as cattle slurry has low biogas potential.

As can be seen in Figure 4.11, the volumetric biogas production for digesters fed on a mixture of food waste and cattle slurry was more or less stable, with average values of 1.69, 2.22, and 2.72 L L^{-1} day^{-1} for at OLR of 3, 4 and 5 g VS L^{-1} day^{-1} respectively (Table 4.7). The average VBP of the controls was 0.50 and 2.45 L L^{-1} day^{-1} for cattle slurry and food waste respectively. The VBP and VMP for co-digestion at OLR 5 g VS L^{-1} day^{-1} were thus higher than for food waste alone.

Throughout the trial, the biogas methane content ranged from 58 – 63% (Figure 4.11). Specific methane production (SMP) in the control digesters was 0.448 and 0.194 L CH_4 g^{-1} VS for food waste and cattle slurry respectively. These values were about the same as the corresponding BMP values of 0.459 and 0.193 L CH_4 g^{-1} VS. In the co-digestion digesters, increasing the OLR appeared to have only a small effect on the average SMP values, which were 0.332, 0.330, and 0.324 L CH_4 g^{-1} VS for OLR of 3, 4, and 5 g VS L^{-1} day^{-1} respectively.

VMP and SMP for co-digestions of cattle slurry and food waste were much higher than for cattle slurry alone, due to the contribution from the readily degradable and high-BMP food waste component. As shown in Table 4.8, at a wet weight ratio of 3 : 1, the ratio of VS in the feed is approximately 40% : 60% from cattle slurry and food waste, respectively. If the SMP values for the control reactors are multiplied in these proportions, the estimated SMP value for co-digestion is 0.349 L CH_4 g^{-1} VS, slightly higher than the actual value of 0.332 L CH_4 g^{-1} VS found at OLR 3 g VS L^{-1} day^{-1}. This small difference may be due to the reduction in HRT for the food waste component during co-digestion.

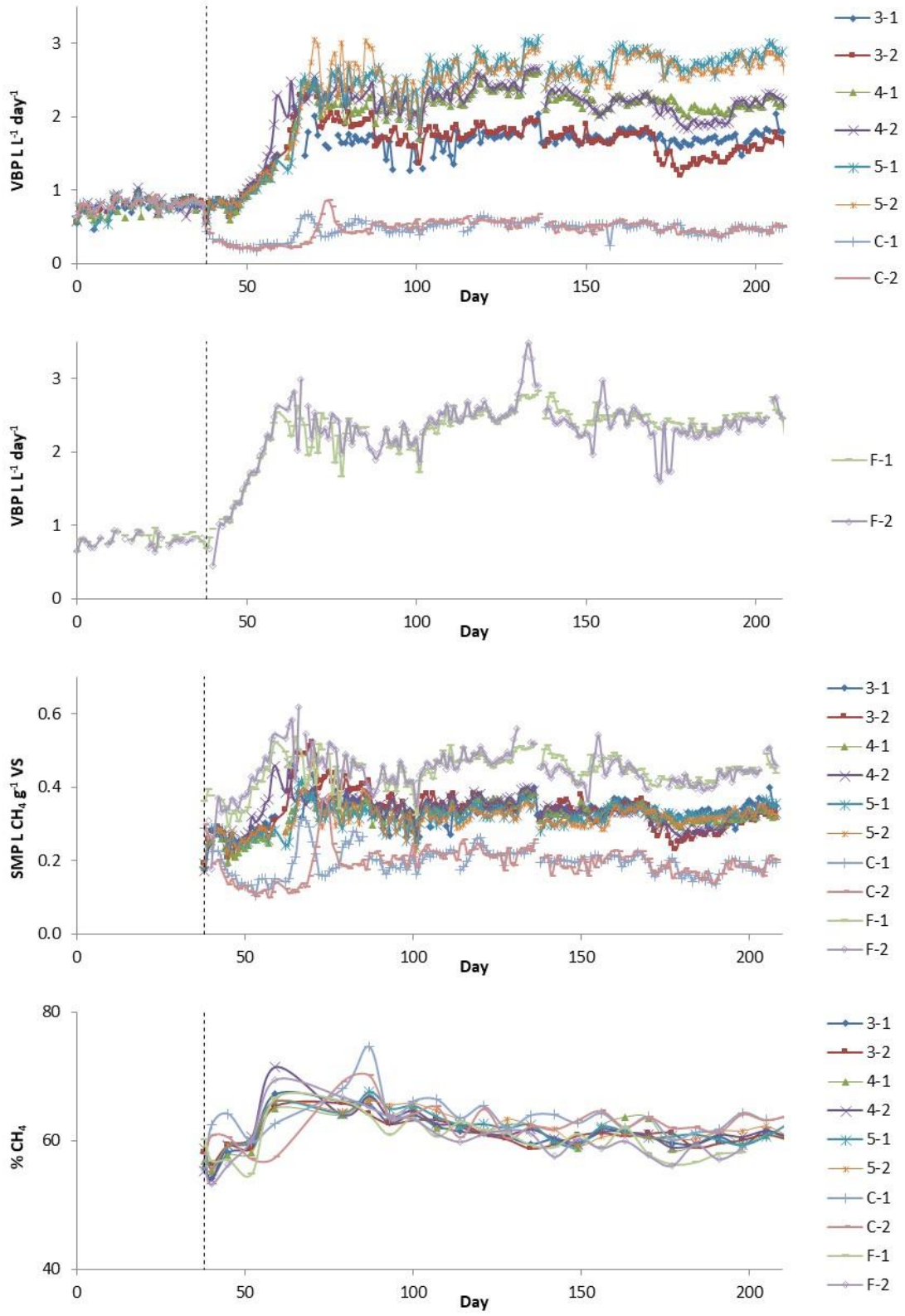


Figure 4.11 Gas production (VBP, SMP and % CH_4) during co-digestion trial C1. Vertical dotted line indicates start of feeding on CS (day 38)

Table 4.9 shows the predicted SMP and VMP values for the co-digestions based on the values obtained in the controls multiplied by the relative proportions of each type of feed in each case. It can be seen that the actual measured VMPs are similar to but slightly lower than the predicted values, indicating that little or no synergy is occurring between the two feedstocks. Predicted VMP values based on the SMP and the VMP of the controls show very good agreement, supporting the accuracy of the experimental data.

Table 4.8 Ratio of VS in CS and FW feedstocks

	WW ratio	**VS %WW**	**Ratio of VS added**	**SMP control L CH_4 g^{-1} VS**	**VMP control L CH_4 L^{-1} day^{-1}**
FW [a]	1	24.64	0.603	0.447	1.443
CS [b]	3	5.42	0.397	0.203	0.329

[a] Feedstock food waste FW1 [b] Feedstock cattle slurry CS1

Table 4.9 Actual and predicted values of SMP and VMP for co-digestion trial C1

Parameter	**Unit**	**CS/FW**	**CS/FW**	**CS/FW**	**CS**	**FW**
Nominal OLR	g VS L^{-1} day^{-1}	3	4	5	1.7	3
FW addition	g WW day^{-1}	30.0	40.0	50.0	0.0	52.4
FW addition	g VS L^{-1} day^{-1}	1.85	2.46	3.08	0.00	3.23
CS addition	g WW day^{-1}	90	120	150	120	0
CS addition	g VS L^{-1} day^{-1}	1.22	1.62	2.03	1.62	0.00
HRT	days	33.3	25.0	20.0	33.3	76.34
SMP	L CH_4 g^{-1} VS	0.332	0.330	0.324	0.194	0.448
VMP	L CH_4 L^{-1} day^{-1}	1.018	1.349	1.658	0.314	1.447
Predicted SMP[a]	L CH_4 g^{-1} VS	0.347	0.347	0.347	0.194	0.448
Predicted SMP[b]	L CH_4 g^{-1} VS	0.353	0.348	0.343	0.193	0.460
Actual/Predicted SMP[a]	%	95.6%	95.0%	93.4%	-	-
Actual/Predicted SMP [b]	%	94.1%	94.9%	94.5%	100.3%	106.1%
Predicted VMP[a]	L CH_4 L^{-1} day^{-1}	1.065	1.420	1.775	0.315	1.447
Predicted VMP[c]	L CH_4 L^{-1} day^{-1}	1.064	1.418	1.773	-	-
Actual/Predicted VMP[a]	%	95.6%	95.0%	93.4%	99.7%	99.9%
Actual/Predicted VMP[b]	%	95.7%	95.1%	93.5%	-	-

[a] based on feedstock ratios and experimental SMP values; [b] based on feedstock ratios and BMP kinetic coefficients; [c] based on feedstock ratios and experimental VMP values

Figure 4.12 shows the change in SMP and VMP for different amounts of added food waste and cattle slurry. Incrementing the OLR of either the food waste or the cattle slurry led to an increase in VMP in the co-digestion reactors but to a plateau in the SMP, probably due to the reduction in HRT from 33 to 20 days.

Some further support for this explanation was provided by modelling using the BMP kinetic coefficients. The Model 2 coefficients derived in section 4.2.3 were used to predict the methane produced by each fraction of the feedstock at the given HRT. The predicted values were very close to the actual SMP, and also confirmed that increasing the OLR in co-digestion reactors would be expected to produce only small reduction in SMP (see Appendix A Table A3 for tabulated calculation). This behaviour agrees with the studies by Linke (1997) on cattle slurry and pig waste slurries, as quoted by Karim et al. (2005). A shorter retention time may become insufficient for efficient degradation, which may be indicated by the presence of more undegraded matter in the digestate (Lehtomäki et al., 2007).

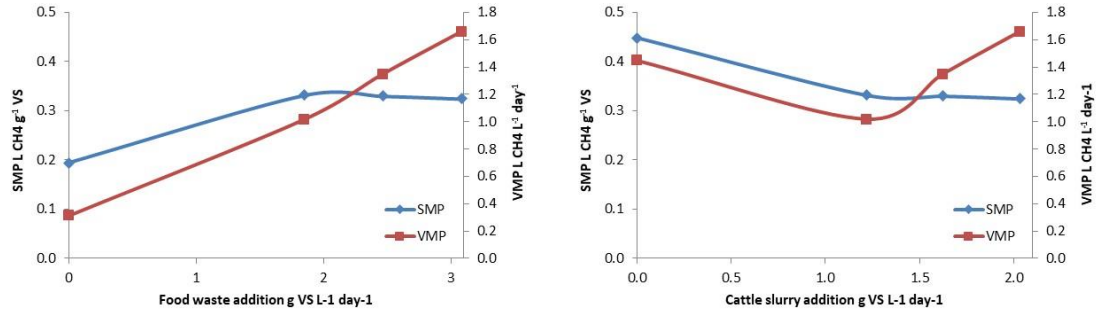


Figure 4.12 Average SMP and VMP at different FW and CS addition rates in trial C1

Other monitoring parameters. Figure 4.13 shows the results for digestion stability parameters throughout the trial. pH fell slightly in all digesters from day 38 to 53, but then recovered and stabilised at around 7.7 – 7.8 in the co-digestion reactors and cattle slurry controls, and at 7.9 in the food waste controls.

TAN concentrations for all reactors increased until day 38, reflecting the TKN content of the incoming food waste feedstock. After day 38 TAN concentrations in the co-digestion and cattle slurry control digesters fell slightly. The cattle slurry controls had the lowest final concentration at around 2.01 g N kg^{-1} WW, while the co-digestion digesters stabilised at 2.3 – 2.5 g N kg^{-1} WW, with the lower OLR showing a slightly higher TAN concentration (Table 4.7). No signs of inhibition by ammonia nitrogen were seen in these digesters, as the concentrations were lower than the limit of 4.0 g N kg^{-1} WW in mesophilic digestion suggested by Angelidaki and Ahring (1994). The food waste controls, however, stabilised at an average TAN concentration of 3.97 g N kg^{-1} WW, very close to the toxicity threshold; but there was no sign of inhibitory effect as the biogas production was still stable at around 2.45 L L^{-1} day^{-1}.

The addition of food waste significantly increased the TKN concentration compared to digestion of cattle slurry alone, from 3.03 g N kg^{-1} WW to around 4.4 – 4.5 g N kg^{-1} WW. With the increase of OLR, the TKN concentrations decreased, which were the same trend as total ammonia.

Total alkalinity started at around 9 g $CaCO_3$ kg^{-1} WW and increased during the first part of the trial. The co-digestion digesters stabilised at 15.3 – 16.8 g $CaCO_3$ kg^{-1} WW (Table 4.7) and showed the same pattern as pH and TAN with slightly higher values at lower OLRs; while the cattle slurry controls had lower TA values at around 13.4 g $CaCO_3$ kg^{-1} WW. In the food waste controls TA values were much higher, at around 19.5 g $CaCO_3$ kg^{-1} WW. Buffering capacity is proportional to concentration of bicarbonate, which food waste released more (in form of ammonium bicarbonate) due to degradation of high nitrogen matter such as proteins.

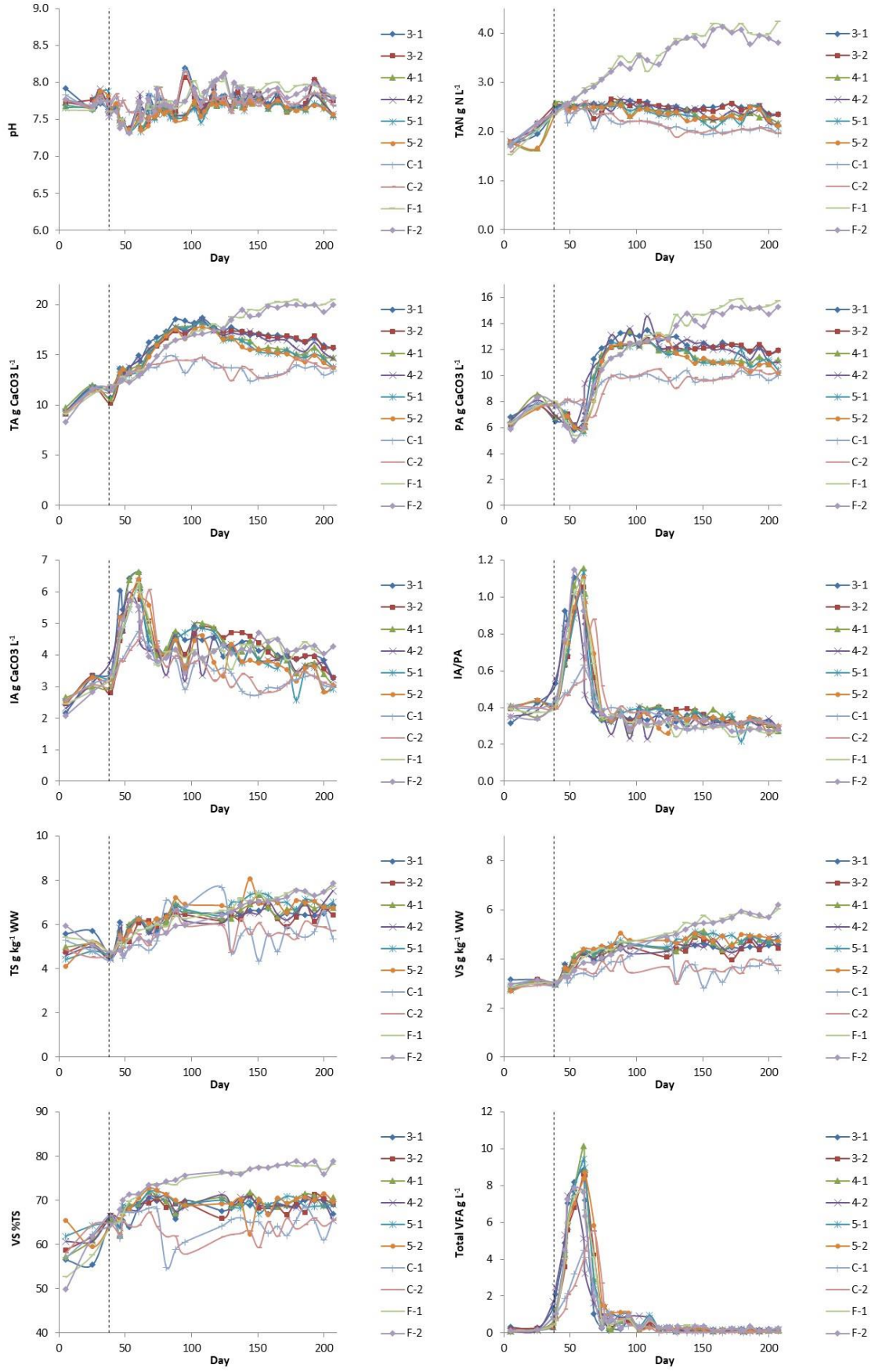


Figure 4.13 Monitoring parameters during co-digestion trial C1: pH, TAN, TA, PA, IA, IA/PA, TS, VS and VFA. Vertical dotted line indicates start of feeding on CS (day 38)

Figure 4.14 shows the relationship between OLR and TAN, TA and VS desctruction. It indicates that an increase in OLR corresponds to a decrease in TAN and in alkalinity, as TAN is produced by the degradation of proteins and other organic nitrogen compounds and alkalinity is related to TAN concentration. The relationships appeared to be linear between 3 – 5 g VS L^{-1} day^{-1}, with TAN and alkalinity falling by 0.0867 g N kg^{-1} WW and 0.7352 g $CaCO_3$ kg^{-1} WW per g VS L^{-1} day^{-1}, respectively. The decrease in TAN may be linked to an increase in nitrogen uptake associated with the higher biomass content and shorter HRT at higher OLR (Lindorfer et al., 2012). VS destruction, however, did not show a very clear relationship with OLR.

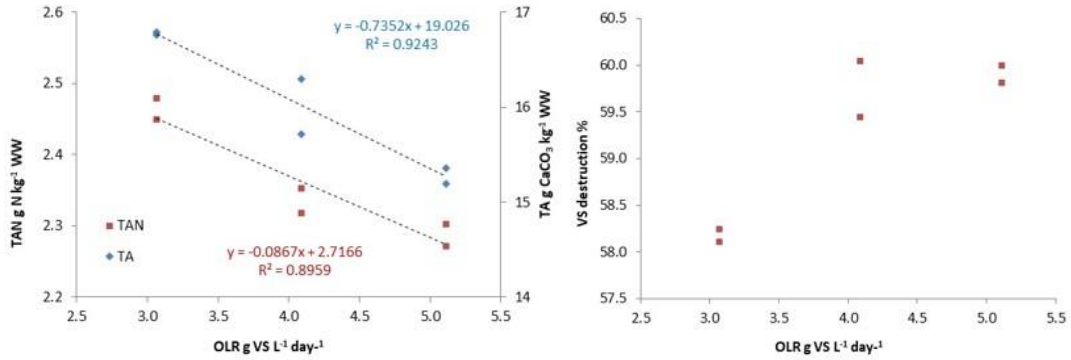


Figure 4.14 Relation between OLR and TAN, TA and VS destruction in co-digestion trial C1

The ratio of intermediate to partial alkalinity (IA : PA ratio) stabilised after around 2 HRT at ~0.3 (Table 4.7), a value suggested by Ripley et al. (1986) as indicating stable operation.

VFAs are another parameter indicating the performance of anaerobic digestion as the concentrations show the metabolic status of the process (Habiba et al., 2009 cited by Lin et al., 2011). The higher concentrations of total VFAs between days 25 – 88 of the trial (as shown in Figure 4.13) indicated higher acidogenesis activity as compared to methanogenesis. All of the co-digestion digesters and the food waste controls reached their peak VFA concentrations at around day 60, with values of 7.5 – 10 g L^{-1}. In the cattle slurry control digesters, the rate of VFA accumulation was lower and the peaks were smaller at 4.4 – 5.5 g L^{-1}. As noted in section 4.3.3 similar behaviour has frequently been seen during acclimatisation of this inoculum to new substrates.

During the period of rapid VFA accumulation, the pH dropped and the IA/PA ratio increased (Figure 4.13), indicating the onset of instability. Despite the accumulation of VFAs, however, any inhibition was negligible as the gas production and methane yields continued to increase with the incremental increases in OLR. This may be because the pH of the digesters was still in the optimal range for methanogenesis.

A one-off dose of trace elements was also added to the digesters on day 46, based on previous observations that this can assist with acclimatisation (Yirong et al., 2014 and unpublished Southampton data). Additionally, feeding was suspended on day 60 to allow conversion of the accumulated VFAs. The accumulated VFA mainly consisted of acetic acid, with small peaks in iso-valeric acid appearing briefly in some digesters (Figure 4.15). From around day 60 VFA concentrations fell rapidly, decreasing by half within one week. After about 2 HRT, VFA concentrations stabilised at less than 0.3 g L^{-1} in the co-digestion digesters, and at about 0.05 and 0.23 g L^{-1}in the cattle slurry and food waste controls respectively.

As mentioned earlier, the stabilised pH values revealed that for co-digestion digesters, with digesters fed with lower OLR showed slightly higher pH and the SMP were also modestly higher, as presented in Table 4.7. This was coherent with findings by Alvarez and Lidén (2008) which suggested it were due to overloading which reduced the methanogenic activity. The higher value for cattle slurry controls indicated depletion of VFAs in the digestate, while for food waste controls, the high ammonia concentration was the reason.

Conclusions. Cattle slurry and food waste combined at ratio of 3 : 1 on a wet weight basis was a feasible feedstock for co-digestion in mesophilic conditions. It was observed in the study that the VBP and SMP for the co-digestions at the three OLR tested of 3, 4, and 5 g VS L^{-1} day^{-1} were higher than those for mono-digestion of cattle slurry. The lower OLR of 3 g VS L^{-1} day^{-1} was optimal in terms of SMP as it gave a higher value (0.332 L CH_4 g^{-1} VS) compared to the higher OLRs. It was also noted that the VBP and VMP for the co-digestion at 5 g VS L^{-1} day^{-1} exceeded the production by food waste alone. The results also showed that the co-digestion was a stable process. It indicated that cattle slurry contributed sufficient trace elements (as presented in section 4.1.3) needed for the stability of the process.

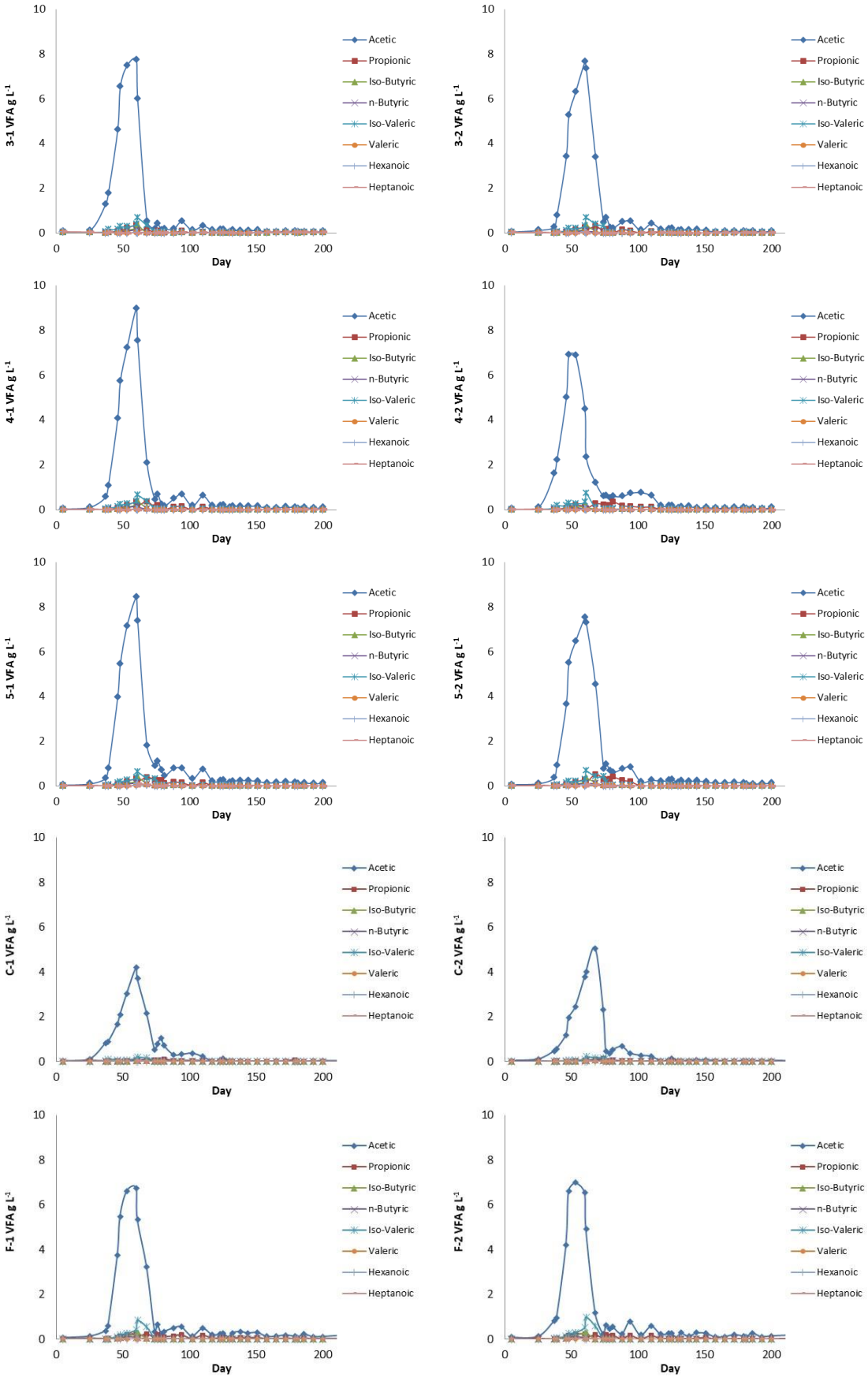


Figure 4.15 VFA profiles for digester during co-digestion trial C1

4.4.2 Trial C2: Co-digestion of cattle slurry to food waste at wet-weight ratio of 6 : 1

4.4.2.1 Objective of trial C2

The objective of this trial was to examine the performance of the co-digestion digesters if the proportion of cattle slurry in the process were increased, while maintaining the loading rates as in trial C1. The co-digestion investigated had a cattle slurry to food waste ratio of 6 : 1 on a wet weight basis. This ratio was chosen to reflect the overall availability of these two AD feedstocks in the UK.

4.4.2.2 Methodology for trial C2

Like trial C1, this trial was also conducted in duplicate with co-digestion of cattle slurry and food waste at OLR of 3, 4, and 5 g VS L^{-1} day^{-1} respectively, but at a wet-weight ratio of 6 : 1.

The feedstocks used during this trial were cattle slurry batch CS1 and food waste batch FW2 (Table 4.1). At the beginning of the trial all digesters apart from the food waste controls were freshly inoculated with digestate from Millbrook WWTP, Southampton. These digesters were initially fed with food waste at an OLR of 2 g VS L^{-1} day^{-1}. Starting from day 62, the feedstock to three pairs of these digesters was switched to a mixture of cattle slurry CS1 and food waste at wet-weight ratio of 6 : 1 while maintaining the OLR at 2 g VS L^{-1} day^{-1}. From day 77, the loading rate in the digesters was increased steadily until the target OLRs of 3, 4 and 5 g VS L^{-1} day^{-1} were achieved on days 80, 84 and 87 respectively. Feeding then continued until day 178, corresponding to at least 3.5 HRT in these conditions.

In the cattle slurry controls, after day 62 the initial food waste feed was replaced with cattle slurry CS1 at an OLR of 2 g VS L^{-1} day^{-1}. Between day 77 and day 105 the OLR was gradually increased to 3 g VS L^{-1} day^{-1}. This loading was maintained until day 166 (3.5 HRT). From day 167 the OLR was reduced to 2 g VS L^{-1} day^{-1}, with a corresponding increase in HRT to 28 days, in response to lower than expected gas production. Feeding of the food waste controls continued at an OLR of 3 g VS L^{-1} day^{-1} throughout the trial.

Table 4.10 shows the planned operating conditions for the trial.

Table 4.10 Planned operating conditions of trial C2

Digester	Feeding (g WW day^{-1})			OLR (g VS L^{-1} day^{-1})			HRT (days)
	CS [a]	FW [b]	Total	CS [a]	FW [b]	Total	
3-1 & 3-2	133.2	22.2	155.4	1.85	1.15	3.0	26
4-1 & 4-2	177.6	29.6	207.2	2.46	1.54	4.0	19
5-1 & 5-2	222.0	37.0	259.0	3.08	1.92	5.0	15
C-1 & C-2	216.5	0	216.5	3.0	0.0	3.0	18
F-1 & F-2	0	58.0	58.0	0.0	3.0	3.0	69

[a] Feedstock cattle slurry CS1, [b] Feedstock food waste FW1 to day 19, FW2 from day 20

4.4.2.3 Results and discussion for trial C2

For the purposes of comparison with the mono-digestion results in section 4.33, day 0 in the current trial corresponds to day 544 in the overall operating period. During this period the food waste controls were experiencing VFA accumulation. This is described in section 4.3.3.2, and the reasons are therefore not discussed in detail here, but results for these digesters are included in graphs and tables where useful for comparative purposes.

Figure 4.16 shows the OLR, HRT and different feed types applied during the trial. There were no significant disturbances or changes in the feeding regime, apart from the reduction in the OLR on the cattle slurry control digesters to 2 g VS L^{-1} day^{-1} from day 167.

Values for key parameters at the steady state stage (average values for last 90 days of the trial) are shown in Table 4.11.

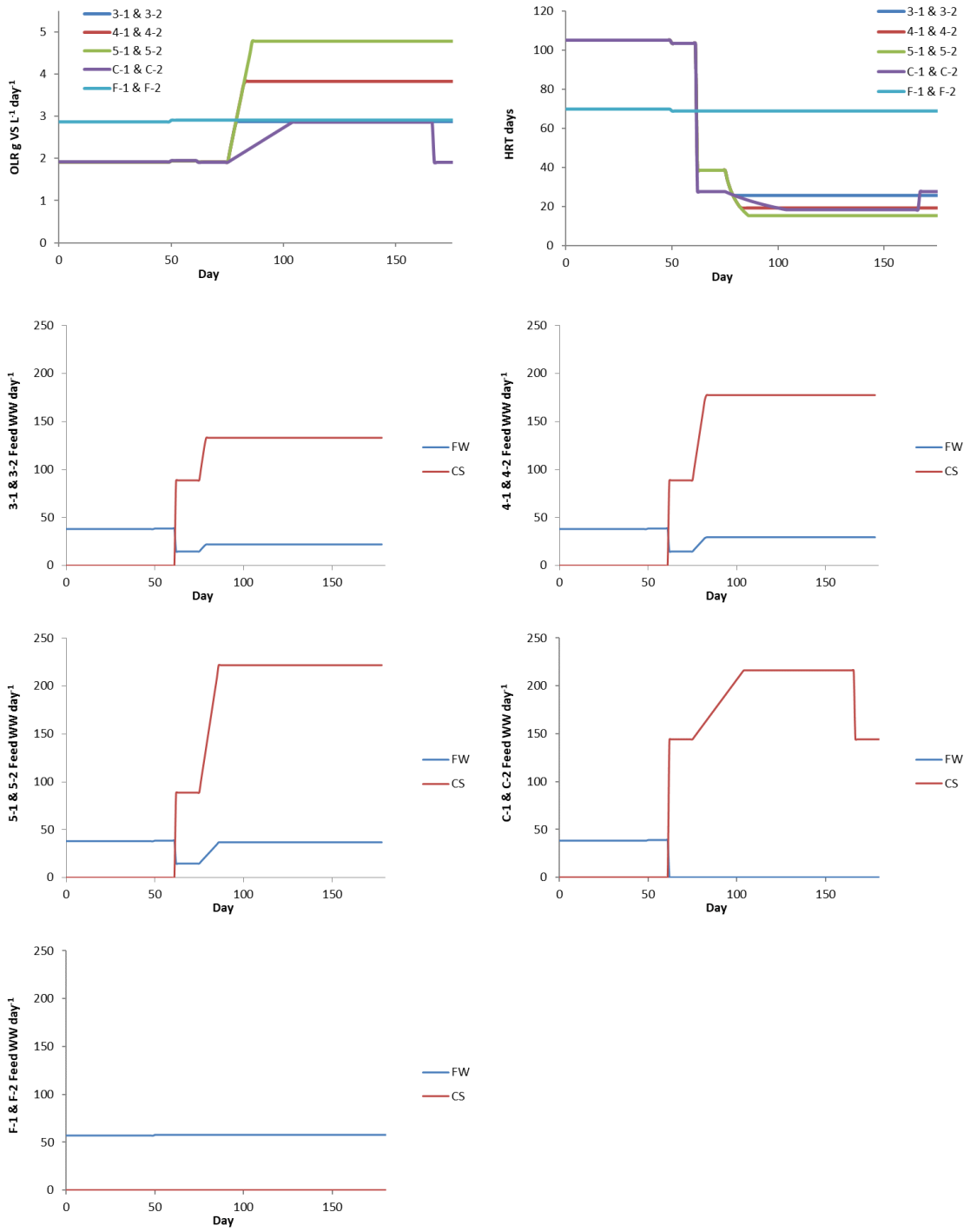


Figure 4.16 OLR, HRT and feed types applied during co-digestion trial C2 with FW and CS at 6 : 1 wet weight ratio

Table 4.11 Steady-state values for key parameters in trial C2 (average for last 90 days of operation)

Parameter	Unit	Co-digestion									Control					
											Cattle slurry			Food waste		
OLR [a]	g VS L^{-1} day^{-1}	3			4			5			3			3		
SBP	L g^{-1} VS	0.388	±	0.020	0.359	±	0.002	0.346	±	0.000	0.094	±	0.001	0.747	±	0.025
SMP	L CH_4 g^{-1} VS	0.239	±	0.014	0.221	±	0.002	0.215	±	0.000	0.053	±	0.000	0.458	±	0.016
VBP	L L^{-1} day^{-1}	1.114	±	0.057	1.375	±	0.009	1.654	±	0.001	0.267	±	0.003	2.195	±	0.052
VMP	L CH_4 L^{-1} day^{-1}	0.687	±	0.039	0.849	±	0.007	1.033	±	0.001	0.152	±	0.001	1.298	±	0.082
CH_4 content	% v/v	61.6	±	0.4	61.7	±	0.1	62.2	±	0.3	56.6	±	0.2	60.7	±	0.9
Digestate TS	%WW	8.18	±	0.11	8.16	±	0.09	8.41	±	0.15	7.64	±	0.02	9.85	±	0.11
Digestate VS	%WW	5.20	±	0.02	5.35	±	0.05	5.51	±	0.07	4.91	±	0.03	6.81	±	0.23
pH	–	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
TA	g $CaCO_3$ kg^{-1} WW	7.75	±	0.01	7.75	±	0.02	7.75	±	0.00	7.75	±	0.02	8.12	±	0.07
PA	g $CaCO_3$ kg^{-1} WW	17.9	±	0.6	16.7	±	0.1	16.3	±	0.1	14.0	±	0.6	31.7	±	2.7
IA	g $CaCO_3$ kg^{-1} WW	11.5	±	0.2	10.6	±	0.0	10.3	±	0.0	9.0	±	0.3	21.0	±	3.9
IA/PA ratio	–	6.4	±	0.4	6.1	±	0.1	5.9	±	0.1	5.0	±	0.3	10.7	±	1.2
TAN	g N kg^{-1} WW	0.56	±	0.03	0.57	±	0.00	0.58	±	0.01	0.55	±	0.01	0.55	±	0.16
TKN [b]	g N kg^{-1} WW	1.97	±	0.00	1.81	±	0.01	1.76	±	0.01	1.37	±	0.03	6.02	±	0.07
Total VFA	g L^{-1}	3.06	±	0.15	3.34	±	0.08	3.69	±	0.09	2.72	±	0.19	10.52	±	0.15

[a] Feedstock cattle slurry CS1, food waste FW1 to day 19 and FW2 from day 20, [b] Measured at end of run

Gas production. During the first 61 days the digesters were fed with food waste only at OLR of 2 g VS L^{-1} day^{-1}, hence the high VBP of about 1.60 L L^{-1} day^{-1}. Once cattle slurry was added, the VBP dropped to around 0.81 L L^{-1} day^{-1} for the co-digestion digesters , before increasing in response to the rise in OLR from day 77 (Figure 4.17).

Steady state values for gas production are shown in Table 4.11. The SMP for mono-digestion of FW2 was 0.458 L CH_4 g^{-1} VS, around 5% higher than the value of 0.448 L CH_4 g^{-1} VS obtained in trial C1 with FW1. The actual operating OLR and HRT in the two trials were similar, at 3.2 and 2.9 g VS L^{-1} day^{-1} and 69 and 76 days respectively for C1 and C2: modelling using the BMP coefficients suggests that this difference in HRT would have no significant effect on SMP, so the difference simply reflects their different BMP values (Table 4.2). The SMP of the CS was only around 0.05 L CH_4 g^{-1} VS, however, well below the value of 0.19 L CH_4 g^{-1} VS in trial C1. The reason for this is not clear, although it may possibly have been due to a difference in storage conditions after collection from site but prior to processing: this is discussed in more detail below. Reduction of the OLR to 2 g VS L^{-1} day from day 167, with a corresponding increase in HRT from 18 to 28 days, did produce some recovery in SMP (see Table 4.4), but still not to the values observed in trial C1.

Table 4.11 and Figure 4.17 clearly show that the co-digestion reactors have higher VBP and SMP compared to cattle slurry digestion alone. The co-digestion at the lowest OLR of 3 g VS L^{-1} day^{-1} had an average SMP of 0.239 L g^{-1} VS. Increasing the OLR to 4 and 5 g VS L^{-1} day^{-1} increased the VBP but led to a slight decrease in SBP and SMP (Figure 4.16 and 4.17). From Figure 4.18, it can be seen that food waste addition up to 1.15 g VS L^{-1} day^{-1} (OLR of co-digestion of 3 g VS L^{-1} day^{-1}, with wet-weight ratio of cattle slurry to food waste of 6 : 1) increased the SMP but further addition did not give any significant effect, as seen from the plateau line.

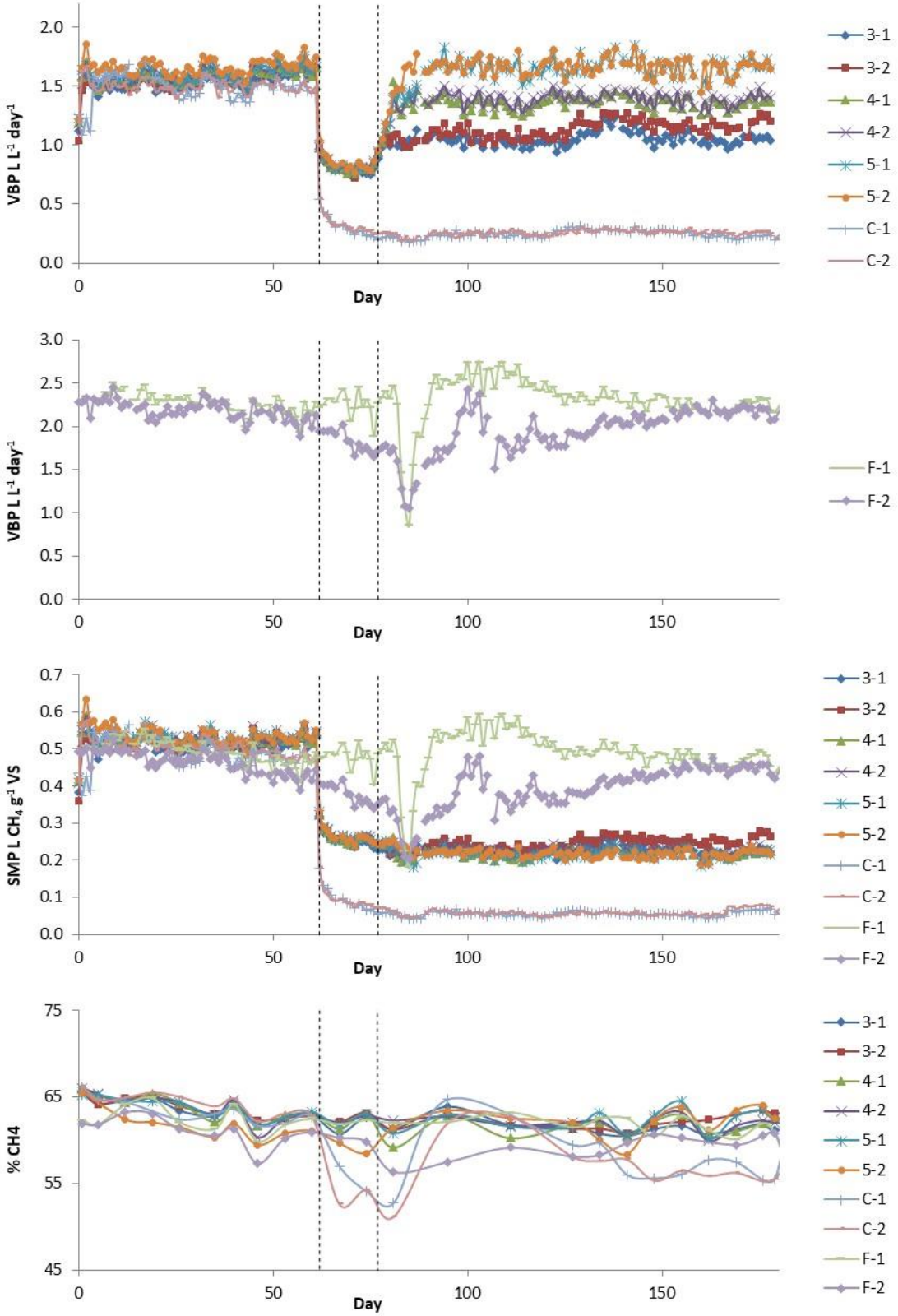


Figure 4.17 Gas production (VBP, SMP and % CH4) during co-digestion trial C2. Vertical dotted lines indicate start of co-digestion from day 62 and OLR increment from day 77.

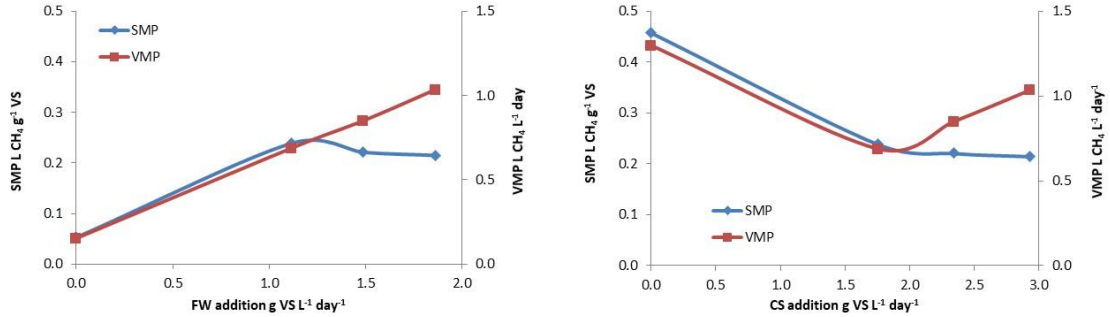


Figure 4.18 Average SMP and VMP for different FW and CS additions in trial C2

At a wet weight ratio of 6 : 1 the feed VS ratio is approximately 0.6 : 0.4 from cattle slurry and food waste, respectively. If the SMP values for the control reactors are multiplied in proportion to the VS added, the predicted SMP value for co-digestion is 0.210 L CH_4 g^{-1} VS, which is lower than the actual values of 0.239, 0.221 and 0.215 L CH_4 g^{-1} VS for OLR of 3, 4 and 5 g VS L^{-1} day^{-1} respectively. Table 4.12 shows the predicted SMP and VMP values for the co-digestions based on the values obtained in the controls. It can be seen that the actual measured values are higher than the predicted values by up to 16%, indicating either that synergy is occurring between the two feedstocks or that inhibition is being relieved, e.g. through dilution of a toxic or inhibitory component in the cattle slurry. On the other hand, the measured SMP values are all lower than the predicted SMP based on BMP kinetic coefficients: details of this modelling are presented in Appendix A Table A4. Some of this difference may be due to the relatively short retention times used in this trial. Although modelling based on BMP coefficients considers the effect of HRT it does not take into account the removal of a proportion of the digester contents each day, which leads to lower SMP values in semi-continuous operation, especially at shorter HRT. The difference between predicted and actual SMP is large enough, however, to indicate some loss of methane potential in CS1 compared to the original value. Taken together, these results may support the idea of a reduction in degradability of the slurry combined with dilution of an inhibitory component in the co-digestion. Predicted VMP values based on the SMP and VMP of the controls show good agreement, supporting the accuracy of the experimental data.

Table 4.12 Actual and predicted values of SMP and VMP for co-digestion trial C2

Parameter	**Unit**	**CS/FW**	**CS/FW**	**CS/FW**	**CS**	**FW**
Nominal OLR	g VS L^{-1} day^{-1}	3	4	5	3	3
FW addition	g WW day^{-1}	22.2	29.6	37.0	0.0	58.0
FW addition	g VS L^{-1} day^{-1}	1.12	1.49	1.86	0.00	2.91
CS addition	g WW day^{-1}	133.2	177.6	222.0	216.5	0.0
CS addition	g VS L^{-1} day^{-1}	1.76	2.34	2.93	2.86	0.00
HRT	days	26	19	15	18	69
SMP	L CH_4 g^{-1} VS	0.239	0.221	0.215	0.053	0.458
VMP	L CH_4 L^{-1} day^{-1}	0.687	0.849	1.033	0.152	1.298
Predicted SMP[a]	L CH_4 g^{-1} VS	0.210	0.210	0.210	0.053	0.458
Predicted SMP [b]	L CH_4 g^{-1} VS	0.293	0.284	0.277	0.175	0.474
Actual/Predicted SMP[a]	%	113.5%	105.1%	102.2%	-	-
Actual/Predicted SMP [b]	%	81.6%	77.9%	77.7%	30.4%	96.6%
Predicted VMP[a]	L CH_4 L^{-1} day^{-1}	0.604	0.806	1.007	0.152	1.334
Predicted VMP[c]	L CH_4 L^{-1} day^{-1}	0.590	0.787	0.984	-	-
Actual/Predicted VMP[a]	%	113.6%	105.3%	102.5%	99.9%	97.3%
Actual/Predicted VMP[c]	%	116.3%	107.8%	105.0%	-	-

[a] based on feedstock ratios and experimental SMP values; [b] based on feedstock ratios and BMP kinetic coefficients; [c] based on feedstock ratios and experimental VMP values

An extensive review of the preparation and storage conditions of the CS1 cattle slurry was carried out, but could not definitely confirm the reason for the lower than expected SMP in trial C2. The CS1 cattle slurry used was collected in a single batch of around 400 kg. The most likely explanation is that this material was processed in two halves, with an extended interval between them. If storage during this period was at ambient temperature some VS degradation might have occurred, together with an increase in the concentration of potentially inhibitory compounds such as sulphide from the breakdown of proteins and sulphates, or heavy metals from solubilisation under acidic conditions. This is supported in part by the VS and VS/TS contents of the cattle slurry feedstock. The average VS contents of CS1 in this trial was 5.27 % WW compared to 5.42% in trial C1, while the VS/TS ratios were 59% and 71% respectively, again suggesting that some degradation of the slurry may have occurred before the start of trial C2. In the absence of definitive information, partial degradation of CS1 leading to the presence of some inhibitory component remains the most likely explanation. Actual gas production values showed good consistency throughout trial C2, however, indicating that feedstocks compared used were homogeneous and consistent in this period, and the results thus still provide an insight into the benefits of co-digestion.

Other monitoring parameters. Figure 4.19 shows the results for digestion stability parameters throughout the trial. After the introduction of cattle slurry and the increase in OLR on day 77, the pH in all of the co-digestion reactors fell until around day 114, then recovered to the same value as in the cattle slurry controls. TAN concentrations fell smoothly reflecting the change in feedstock. TA and PA values showed a small decrease, with a corresponding slight rise in the IA/PA ratio to around 0.6. This was slightly higher than the value of around 0.33 in trial C1 and may have been linked to the presence of a suspected inhibitory component in the cattle slurry fraction. A study by Foresti (1994) as quoted by Chernicharo (2007) suggested, however, that process stability is possible for IA/PA ratios above 0.3. VFA concentrations remained very low in the reactors at OLR 3 and 4 g VS L^{-1} day^{-1}, while at 5 g VS L^{-1} day^{-1} there was a small increase from day 118 indicating slightly higher stress at the higher OLR. This was insufficient to affect pH or alkalinity parameters, however, and the VFA concentrations appeared to decline by the end of the run. The VFA present consisted primarily of acetic acid (Figure 4.20). In general the behaviour of monitoring parameters for the co-digestion reactors was similar to that in the cattle slurry controls, as might be expected at the 6:1 feed ratio.

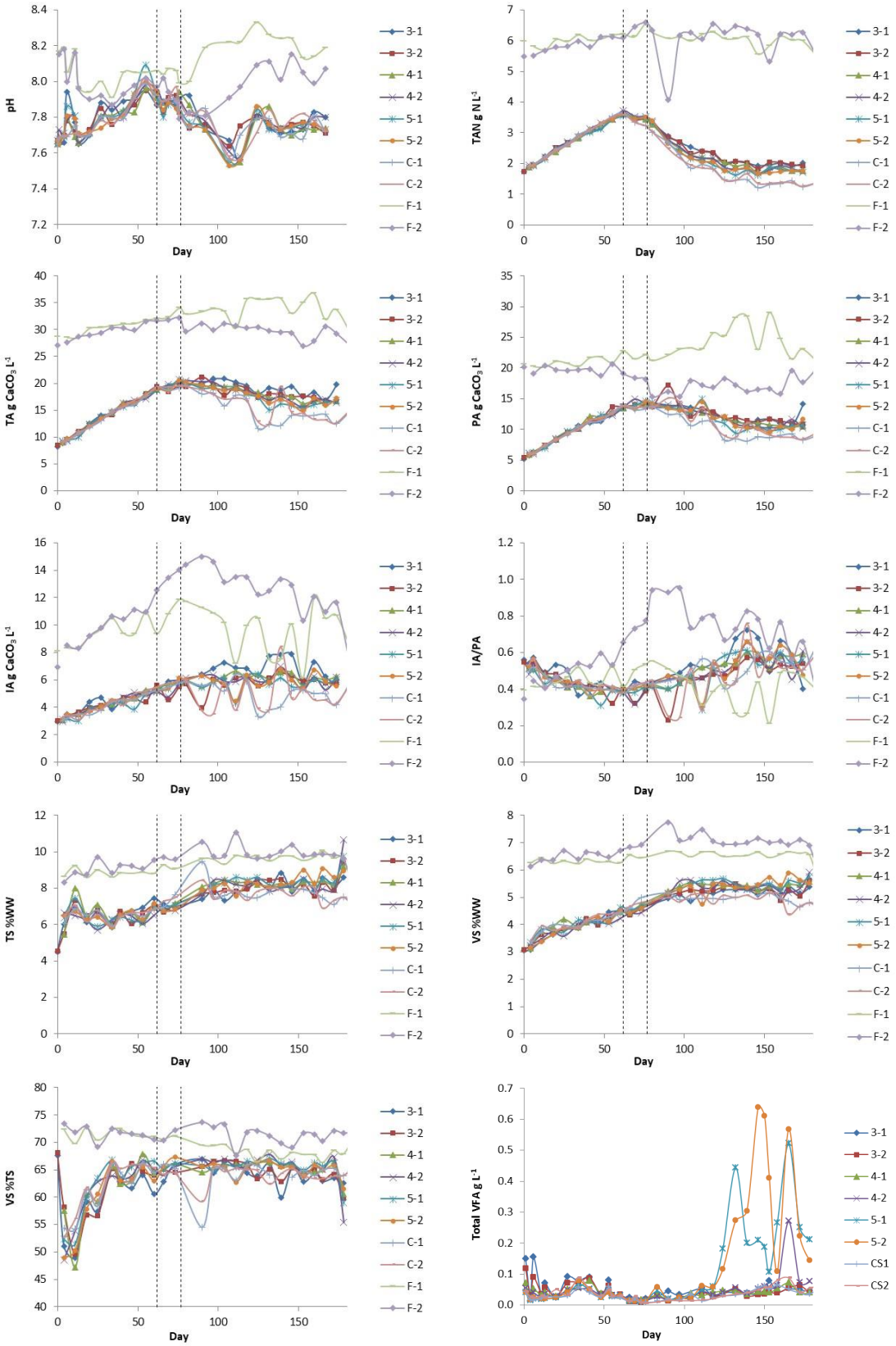


Figure 4.19 Monitoring parameters during trial C2. Vertical dotted lines indicate changes in feeding. Note F-1 and F-2 omitted from total VFA.

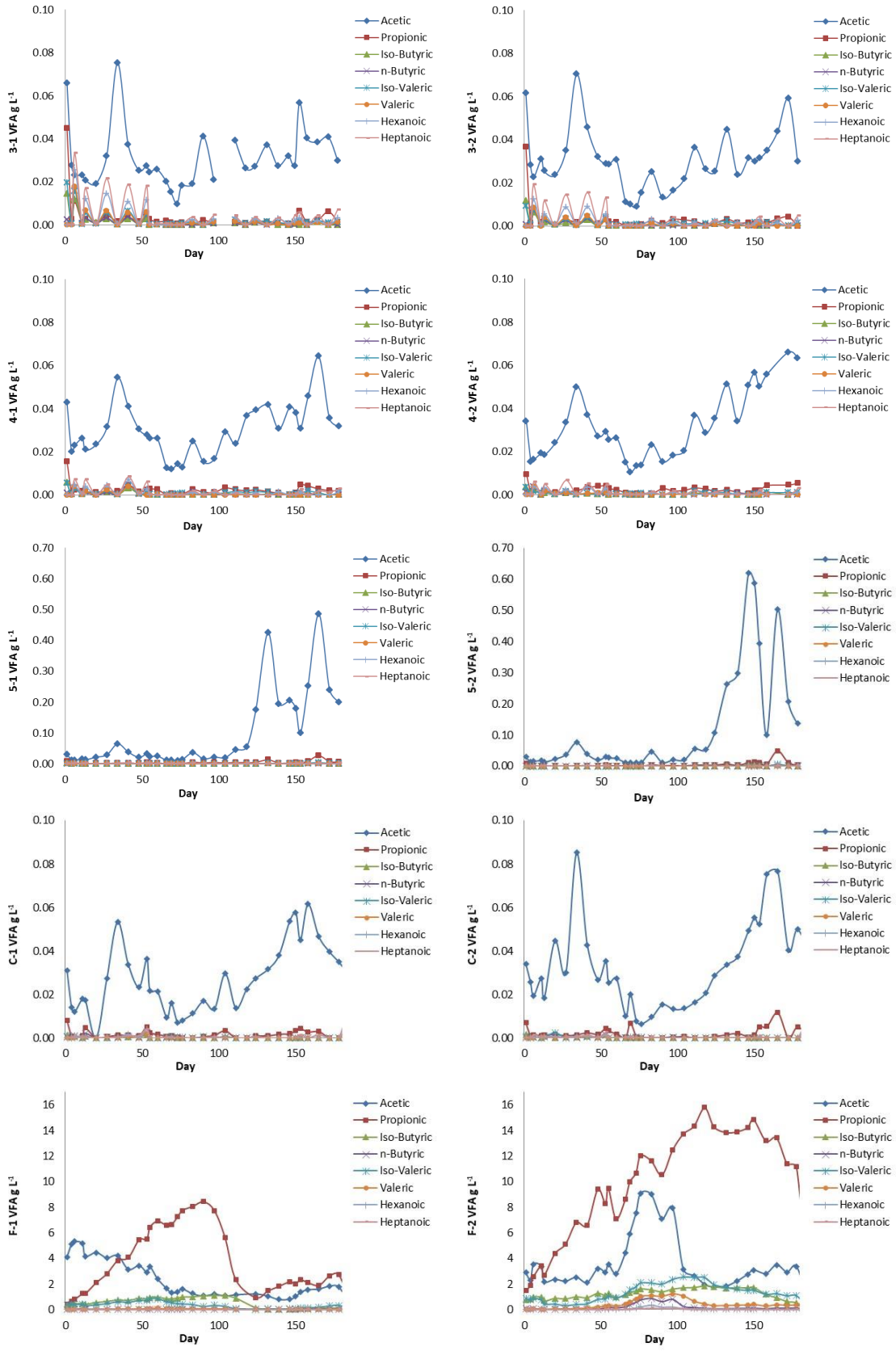


Figure 4.20 VFA profiles during trial C2. Note different scales on y-axes for digesters 5-1 & 5-2 and F-1 & F-2.

The average VS destruction for the co-digestion reactors was 43.8%, 42.3%, and 37.2% for the OLR of 3, 4, and 5 g VS L^{-1} day^{-1} respectively but only 18.5 % for the cattle slurry control, accounting at least in part for the low specific methane production. If some degradation of the cattle slurry had occurred before this trial, it would also be likely to contain a higher proportion of more recalcitrant solids. As for the co-digestion digesters, increasing OLR decreased the VS destruction (Figure 4.21) with a reduction of 3.4% of VS destruction per g VS L^{-1} day^{-1}. This could be due to the reduction in HRT, and related to the decline in specific methane production. TAN and TA showed a similar relationship to OLR as in trial C1.

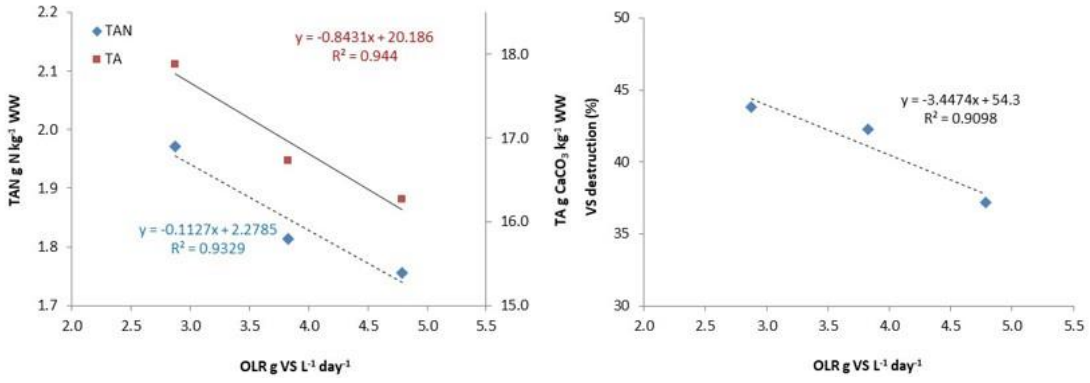


Figure 4.21 Correlation between OLR and TAN, TA and VS destruction in trial C2

Conclusions. The trial showed that successful co-digestion of cattle slurry and food waste at wet-weight ratio of 6 : 1 was possible. As expected, the SBP and SMP were lower compared to results at a wet-weight ratio of 3 : 1. The extent of this difference, however, was affected by the fall in gas production from CS1 in this trial as well as by the shorter HRT and reduced proportion of food waste. In both trials, the optimal loading for the co-digestion was 3 g VS L^{-1} day^{-1}, giving a SMP of 0.332 L g^{-1} VS and 0.239 L g^{-1} VS at ratios of 3 : 1 and 6 : 1 respectively. The lower SMP in the latter trial was due to the higher proportion of cattle slurry in the co-digestion which has a lower readily degradable organic fraction due to rumen digestion by the cattle (Amon et al., 2007) and to the high lignin found in cattle slurry due to their diets. Increasing the proportion of cattle slurry in the co-digestion system led to a rise in the IA/PA ratio from around 0.33 to around 0.56 when the cattle slurry to food waste ratio was increased from 3 to 6, possibly indicating a reduction in stability. The results confirmed previous reports that the properties of cattle slurry can vary considerably from source to source and season to season; or even as in this case within one batch, probably due to difference in initial storage conditions. Co-

digestion with food waste thus becomes an attractive option because of its ability to smooth out these variations and provide a baseline methane productivity, helping to improve the economic viability of the investment in an AD plant.

4.4.3 Trial S1: Co-digestion of cattle slurry with food waste using high-sulphate cattle slurry without any control measures

This study was intended to form part of the work in trials C1 and C2 on co-digestion of cattle slurry and food waste at a range of wet-weight ratios. This trial was conducted at a wet-weight ratio of cattle slurry to food waste of 6 : 1. A new batch of cattle slurry was used, however, which was obtained from a farm using gypsum material as animal bedding. Due to the high sulphate concentration in the cattle slurry, problems with high dissolved sulphide occurred during the trial, and the focus of the subsequent research was adjusted to tackle this issue.

4.4.3.1 Objective of trial S1

The original objective of this trial was to investigate the performance of the co-digestion of cattle slurry and food waste at different organic loading rates at a wet-weight ratio of cattle slurry to food waste of 6 : 1.

4.4.3.2 Methodology for trial S1

This trial was a continuation and extension of trial C1 described in section 4.2.1, with increment of the wet-weight ratio of cattle slurry to food waste from 3 : 1 to 6 : 1. Eight 5-L digesters were used. One pair of digesters was fed with cattle slurry only at an OLR of 3 g VS L^{-1} day^{-1}, and the remaining three pairs were used in co-digestion of cattle slurry and food waste at a wet-weight ratio of 6 : 1 and at OLRs of 3, 4, and 5 g VS L^{-1} day^{-1} respectively. The digesters were fed with the same batch of food waste as in trial C1 (batch FW1, Table 4.1), but with a different batch of cattle slurry (batch CS2, Table 4.1, VS of 10.45%) which was collected from a farm using gypsum bedding. As the trial was a continuation of trial C1, all of the digesters were already at the target OLRs and there was no start-up period. The operational details of the trial are given in Table 4.13. The trial was run for 100 days; however, feeding of digesters 5-1 & 5-2 and of C-1 & C-2 was discontinued on day 36 and 40 respectively.

Table 4.13 Operating conditions of trial S1

Digester	Feeding (g wet-weight day^{-1})			OLR (g VS L^{-1} day^{-1})			HRT (days)
	CS [a]	FW [b]	Total	CS [a]	FW [b]	Total	
3-1 & 3-2	85.0	14.2	99.2	2.2	0.8	3.0	40
4-1 & 4-2	113.4	18.9	132.3	3.0	1.0	4.0	30
5-1 & 5-2	141.7	23.6	165.3	3.7	1.3	5.0	24
C-1 & C-2	114.8	0.0	114.8	3.0	0.0	3.0	35
F-1 & F-2	0	52.4	52.4	0	2.9	2.9	76

[a] Feedstock cattle slurry CS2, [b] Feedstock food waste FW2

4.4.3.3 Results and discussion for trial S1

Initial VBP for the co-digestion digesters averaged 1.30, 1.74 and 1.91 L L^{-1} day^{-1} for OLR of 3, 4 , and 5 g VS L^{-1} day^{-1} respectively, while the cattle slurry control had an average of 0.84 L L^{-1} day^{-1}. As seen in Figure 4.22, however, after 1 HRT the VBP began to drop. Feeding of reactors with OLR 5 g VS L^{-1} day^{-1} and cattle slurry controls ceased at day 36 and 40 after they reached low average VBP productions of 0.24 L L^{-1} day^{-1} (SMP of 0.03 L g^{-1} VS day^{-1}) and the percentage of methane fell below 40%.

From Figure 4.22 and Figure 4.23, it can be seen that by the end of the trial on day 99, biogas production in all of the digesters fed with cattle slurry (both co-digestion at OLR of 3, 4, and 5 g VS L^{-1} day^{-1} and cattle slurry controls) had failed. The time taken to reach SMP of below 0.05 L g^{-1} VS day^{-1} varied, however, depending on the amount of cattle slurry added to the digester. Digesters 5-1 & 5-2 which had the highest cattle slurry fed (of 3.7 g VS L^{-1} day^{-1}) took only 28 days to plateau at a low SMP while digesters 4-1 & 4-2 (cattle slurry OLR of 3.0 g VS L^{-1} day^{-1}) and 3-1 & 3-2 (cattle slurry OLR of 2.2 g VS L^{-1} day^{-1}) took about 55 days and 70 days to reach a plateau respectively. In terms of gas composition, the percentage of methane dropped significantly from an initial value above 60% to below 35% at the 'failed' condition. It was also suspected that hydrogen sulphide (H_2S) was being produced, based on the detection of a foul odour of rotten egg from all of the reactors. Dissolved sulphide analysis on the digestate in the cattle slurry control reactor gave a concentration of 500 mg L^{-1}, which was well above the limit of toxicity of 200 mg L^{-1} suggested by Lawrence and McCarty (1965).

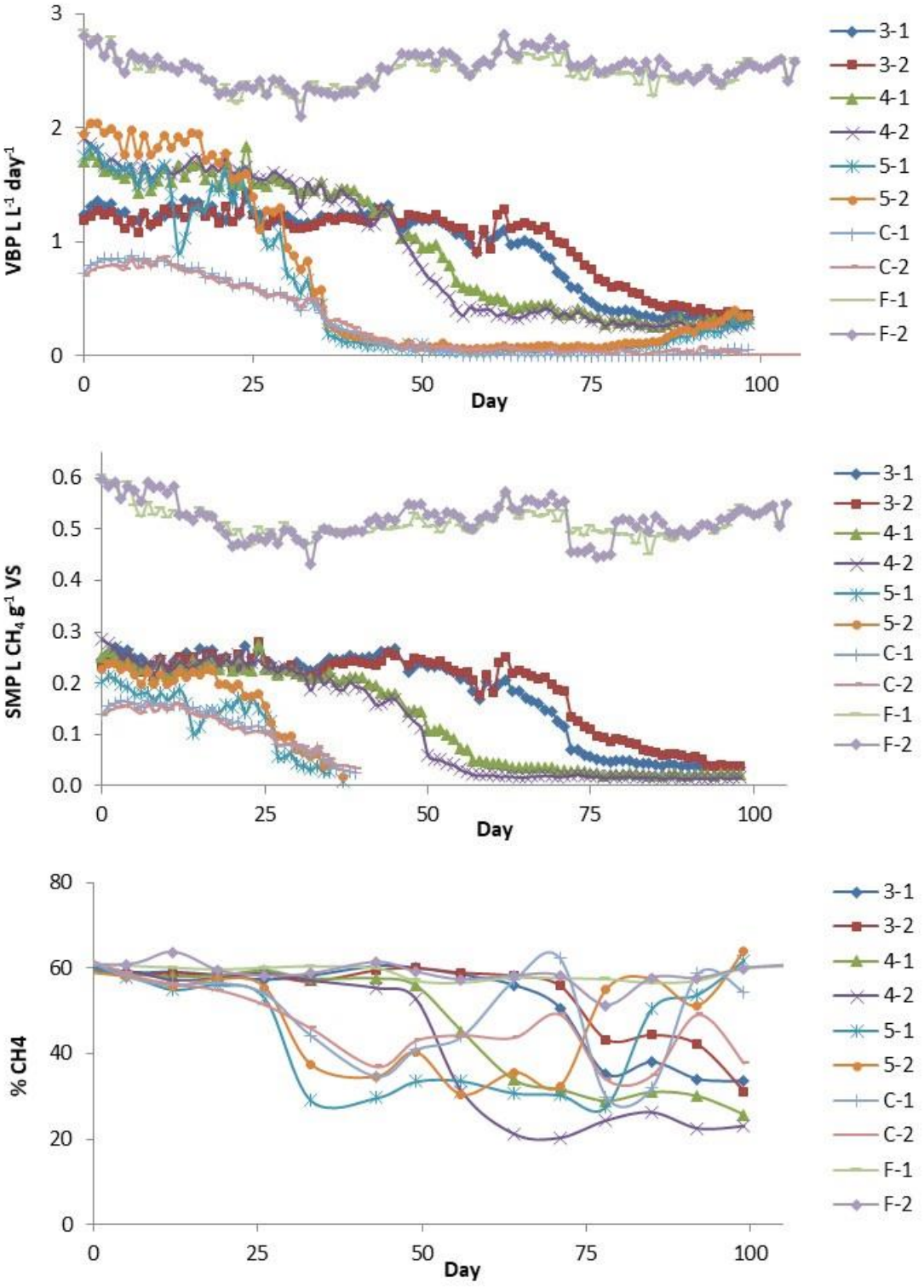


Figure 4.22 Gas production (VBP, SMP and % CH_4) for trial S1

The failure of the digestion was also reflected in the high IA : PA ratio of more than 1.5 and in an increase of VFA concentration from around 0.05 g L^{-1} at the initial stage to more than 10 g L^{-1} at the failure stage (as shown in Figure 4.23). The accumulated VFA mainly

consisted of acetic acid, but significant increases in propionic acid and n-butyric acid started to appear in some digesters (Figure 4.24). In digesters 5-1 & 5-2 accumulation of propionic and longer-chain acids stopped from around day 57 after the cessation of feeding and acetic acid concentrations fell. This led to a fall in total VFA concentration which was matched by an increase in VMP and a rise in pH (Figure 4.23), indicating some recovery from inhibition. The cattle slurry controls showed less sign of recovery, although there was an increase in pH.

Studies by Omil et al. (1998), Cappenberg (1974), and Muyzer et al. (2008) mentioned that although SRB and methanogens compete for the same substrates, the completion was in favour of SRB. Apart from the primary stages of methanogenic inhibition, Anderson et al. (1982) as quoted by Koster et al. (1986) reported that the cause was also due to the decline of methanogen population due to direct inhibition of cells function by dissolved sulphide.

These results clearly showed inhibition of the methanogens, and perhaps also of other populations. Since the suspected cause was sulphide toxicity, in the following experiment a flask test was carried out to determine whether addition of $FeCl_3$ could control the digestate sulphide concentration to a level not considered toxic.

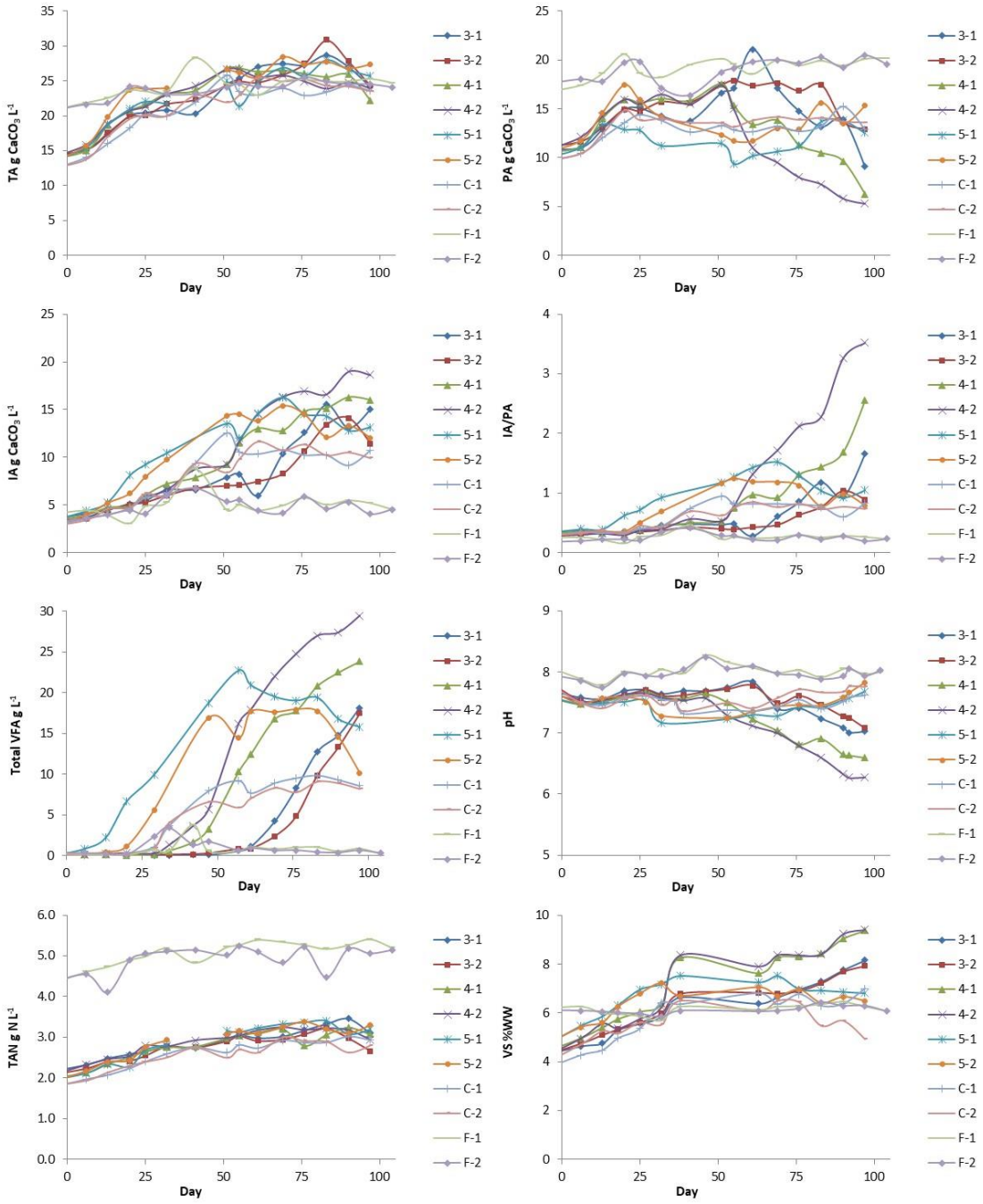


Figure 4.23 Monitoring parameters during trial C2: pH, TAN, TA, PA, IA, IA/PA, VFA and VS

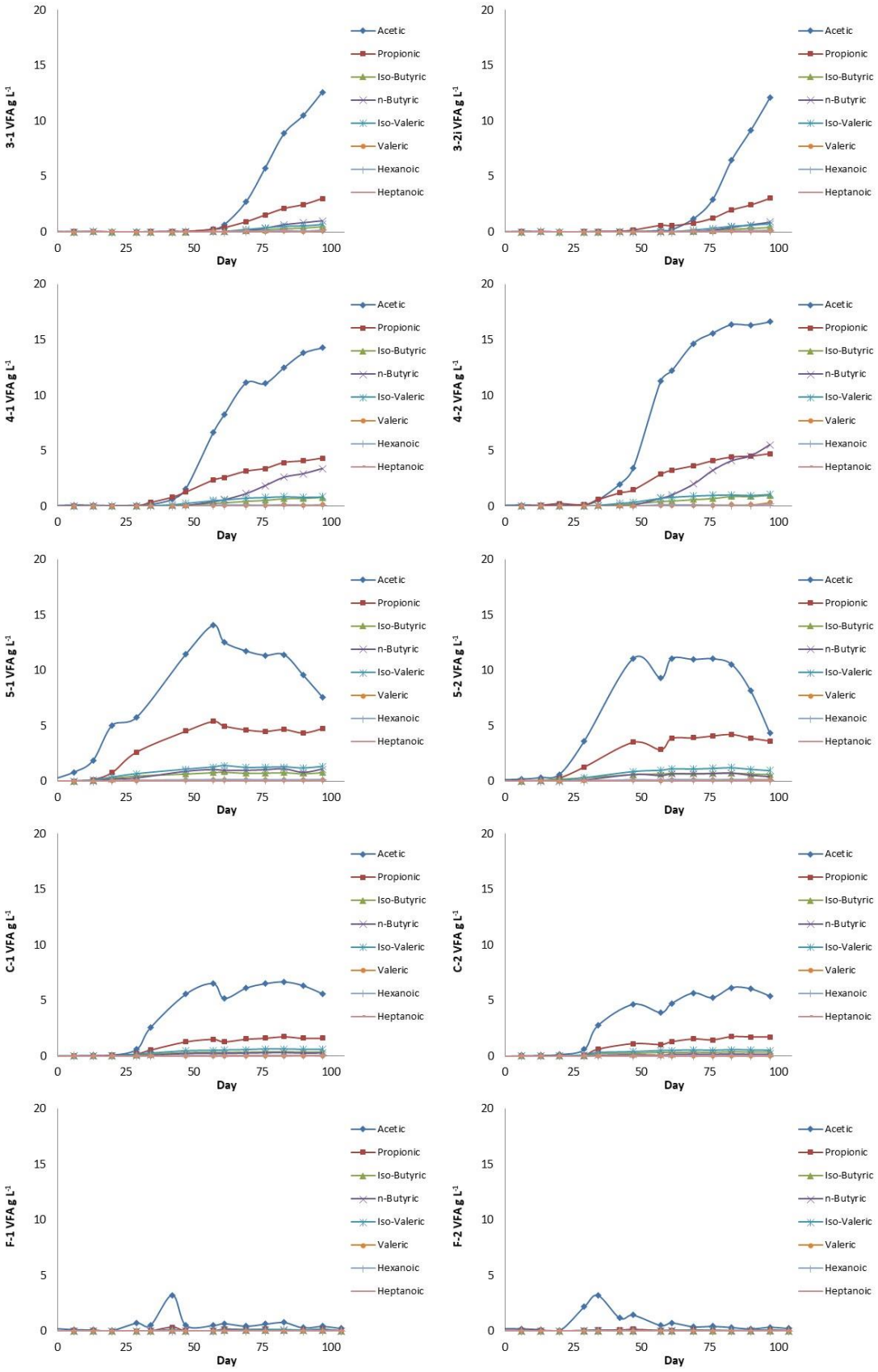


Figure 4.24 VFA profiles during trial S1

4.4.4 Flask Test: Effect of $FeCl_3$ Addition

In order to determine an appropriate $FeCl_3$ dose for control of dissolved sulphide in the digestate, a flask test was conducted.

4.4.4.1 Objective of flask test

The objective of this flask test was to determine the optimum dose of ferric chloride ($FeCl_3$) for controlling dissolved sulphide in the digestate from anaerobic digestion of the high sulphate cattle slurry. Ferric chloride was chosen based on the results of the literature review, since it appears to be an effective option with a well-established history of use in the anaerobic digestion industry.

4.4.4.2 Methodology for flask test

Six 250 mL conical flasks were filled with 100 mL of digestate from digester S1-CSii in trial S1, which had been fed on cattle slurry CS2 without food waste addition. The digestate was taken over several days from day 40 of operation onwards, and was stored in a fridge for a further 30 days before use on order to allow biological activity to decline. A $FeCl_3$ solution was made up by dissolving 50 g of $FeCl_3.6H_2O$ in 20 mL of DI water to give a theoretical concentration of 516.5 g Fe L^{-1}. This was added to five of the flasks in volumes of 20, 50, 100, 150, and 200 µL respectively. The remaining flask acted as a control without $FeCl_3$ addition.

4.4.4.3 Results and discussion for flask test

The initial concentration of dissolved sulphide in the digestate was determined using the methylene blue method (section 3.1.3.7) and found to be around 500 mg S L^{-1}, although there were some concerns over the accuracy and reproducibility of the measurements.

Table 4.14 shows the results from the flask test. Adding 0.5 mL of $FeCl_3$ solution to 1 L of digestate appeared to be optimal, giving the largest reduction in dissolved sulphide of 43% compared to the initial value, while still requiring a low chemical input. The removal of sulphide through precipitation as FeS was much lower at $FeCl_3$ concentrations below this, and slightly lower at concentrations from 1.0 to 1.5 mL $FeCl_3$ L^{-1}. At 2 mL $FeCl_3$ L^{-1} addition sulphide removal was negligible, with the final concentration equal to that in the control. Measurements were conducted 4 times on two consecutive days and in all but

one case showed a similar pattern of removal, although as there were issues with both calibration and repeatability. The one exception showed higher concentrations of dissolved sulphide (1 – 3 g S L^{-1}) and an increase in removal with increasing $FeCl_3$ dosage. According to Pomeroy and Bowlus (1994) as quoted by Speece (2008), if too much $FeCl_3$ is added, the process will not be optimal because the effectiveness of iron salts reduces as the pH drops. Unfortunately pH was not measured in the experiment.

Table 4.14 Effect of $FeCl_3$ addition

Sample	**$FeCl_3$ added (mL L^{-1})**	**Fe : S molar ratio**	**Final sulphide concentration (mg L^{-1})**	**Reduction in sulphide (%)**
1	0.0 (control)	0	500	0
2	0.2	0.12	409	18
3	0.5	0.30	284	43
4	1.0	0.59	348	30
5	1.5	0.89	348	31
6	2.0	1.19	501	0

From the test results, the optimum molar ratio of Fe to S obtained was 0.30. This was lower than the ratio of 0.9 obtained by Firer et al. (2008) and of 0.6 found by Zhang et al. (2009). The limitations of the analytical method used mean the values obtained were regarded as indicative rather than fully quantitative, but based on these results it was decided to replicate the previous trial S1 using this rate of $FeCl_3$ addition.

4.4.5 Trial S2: Operation of CSTR with high sulphate found in cattle slurry using $FeCl_3$ control strategy

This trial was conducted in order to determine whether addition of $FeCl_3$ at the concentration identified in the flask testing could allow stable co-digestion of food waste and high-sulphate cattle slurry.

4.4.5.1 Objective of trial S2

The objective of this trial was to assess the performance of co-digestion of high-sulphate cattle slurry and food waste at a wet-weight ratio of 6 : 1 with daily addition of $FeCl_3$ to control the digestate sulphide concentration.

4.4.5.2 Methodology for trial S2

The set-up of this trial was similar to that of trial S1 as described in section 4.3.1. Three sets of duplicate CSTR digesters were used for the co-digestion of cattle slurry and food waste (wet-weight ratio of 6 : 1) at OLR of 3, 4, and 5 g VS L^{-1} day^{-1} respectively. Two more sets of duplicates acted as cattle slurry and food waste controls respectively at OLR 3 g VS L^{-1} day^{-1}. At the start of the trial all of the digesters receiving cattle slurry were re-seeded with fresh inoculum from Millbrook WWTW: only the FW controls continued from the previous experiment without re-seeding. For the first 23 days, the digesters were fed only with food waste. On day 24, cattle slurry was introduced and feeding was gradually increased, reaching the target OLR at day 56 for the cattle slurry control and at days 59, 66 and 73 for co-digestion digesters at OLR of 3, 4, and 5 g VS L^{-1} day^{-1} respectively. The cattle slurry used was from the farm using gypsum as bedding (batch CS2 in Table 4.1), hence problems with sulphide were anticipated as the digestion had failed in trial S1 (see section 4.3.1). The food waste used was batch FW2 (Table 4.1).

In this trial $FeCl_3$ solution was added to the digesters in an attempt to reduce the soluble sulphide concentration. The amount of $FeCl_3$ added was based on results of the flask test in section 4.3.2, and was around 260 mg Fe L^{-1} of cattle slurry feedstock. Addition of $FeCl_3$ started at day 25 once feeding with cattle slurry was started. Table 4.15 shows the operational details of trial S2. Minor differences from trial S1 in the daily wet weights of material added reflect small variations in the TS and VS content, which were monitored regularly throughout the relevant operating periods.

Table 4.15 Operating conditions for trial S2

Digester	**Feeding ($g_{wet\text{-}weight}$ day^{-1})**			**Target OLR (g VS L^{-1} day^{-1})**			**HRT (days)**	**Daily $FeCl_3$ added (µL)**
	CS [a]	**FW** [b]	**Total**	**CS**	**FW**	**Total**		
3-1 & 3-2	82.8	13.8	96.6	2.2	0.8	3.0	40	58
4-1 & 4-2	110.4	18.4	128.8	3.0	1.0	4.0	30	77
5-1 & 5-2	138.6	23.1	161.7	3.7	1.3	5.0	24	97
C-1 & C2	115.5	0.0	115.5	3.0	0.0	3.0	35	81
F-1 & F-2	0.0	52.4	52.4	0.0	3.0	3.0	76	0

[a] Feedstock cattle slurry CS2, [b] Feedstock food waste FW2

4.4.5.3 Results and discussion for trial S2

Figure 4.25 shows the OLR, HRT and different feed types applied during the trial. It can be seen that the initial increases in OLR were implemented without problems, and there were no unplanned disturbances in feeding until near the end of the trial. Feeding of digesters 5-1 & 5-2 was stopped on day 160 after 87 days (3.6 HRT) at the target OLR; of 4-1 & 4-2 on day 176 (108 days, 3.6 HRT); and of C-1 & C-2 on day 177 (121 days, 3.5 HRT). Feeding of digesters 3-1 & 3-2 continued to the end of the trial on day 180 (122 days, 3.1 HRT), and F-1 & F-2 continued to run as a control throughout the period, corresponding to days 348 – 543 in section 4.3.3 on mono-digestion.

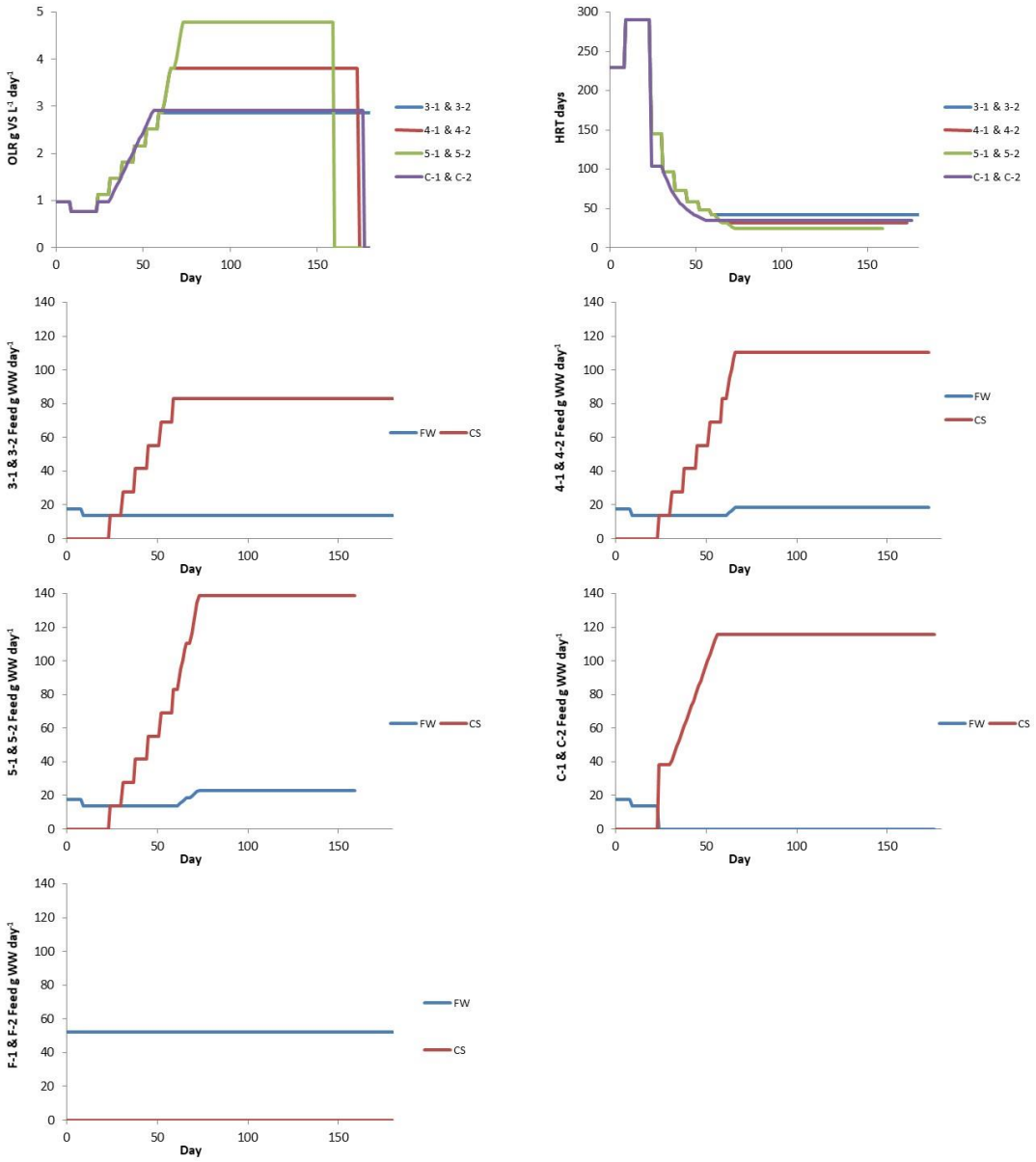


Figure 4.25 OLR, HRT and feed types applied during FW and CS co-digestion at 6 : 1 wet weight ratio with batch 2 cattle slurry in trial S2

Results for the biogas production, biogas methane content and specific methane yield for the CSTRs in this trial leading up to and at the targeted OLR are shown in Figure 4.26. VBP in C-1 & C-2, the cattle slurry controls, fell rapidly until around day 45 (Figure 4.26a). One of the control digesters showed a slight increase in VBP until around day 80, but by day 120 VBP had stabilised at around 0.10 – 0.11 L L^{-1} day^{-1}. Biogas methane content followed a similar trend (Figure 4.26d), but continued to fall from day 100 reaching 10 – 15% by the end of the trial; as a result the SMP in these digesters dropped to around 0.005 L CH_4 g^{-1} VS added in the last 20 days of the trial. In contrast the FW controls F-1 & F-2 showed relatively stable VBP, SMP and biogas methane content throughout.

In the co-digestion digesters, VBP production also fell until around day 45 then increased for the next 15 – 25 days (Figure 4.26a). Gas production then fluctuated in all digesters until day 100 – 120, with pairs of digesters at the same OLR showing similar patterns of peaks and troughs but with a lag of 10 or more days between the duplicates in some cases. Yirong et al. (2014) described this type of variation, in which duplicate reactors show similar behaviour starting at slightly different times, in a study of food waste digestion and attributed it to a form of the Anna Karenina principle. This essentially states that stable systems are similar as they require a range of factors to work well, while unstable ones show differences as they may be affected by minor perturbations (Moore, 2001; Zaneveld et al., 2017). In all of the digesters, however, gas production declined steadily until the end of the trial. Biogas methane concentrations showed a similar pattern of decline followed by recovery then fluctuation until day 100 – 130 (Figure 4.26d). By the end of the trial the methane content in the digesters at lower OLR appeared to have stabilised at around 15 – 30%, slightly above the content in the digesters at higher OLR and in the cattle slurry controls. After day 130 the SMP fell gradually, remaining slightly above that in the cattle slurry controls (Figure 4.26c), and slightly higher in the digesters at lower OLR, but ended the run at less than 0.02 L CH_4 g^{-1} VS in all digesters.

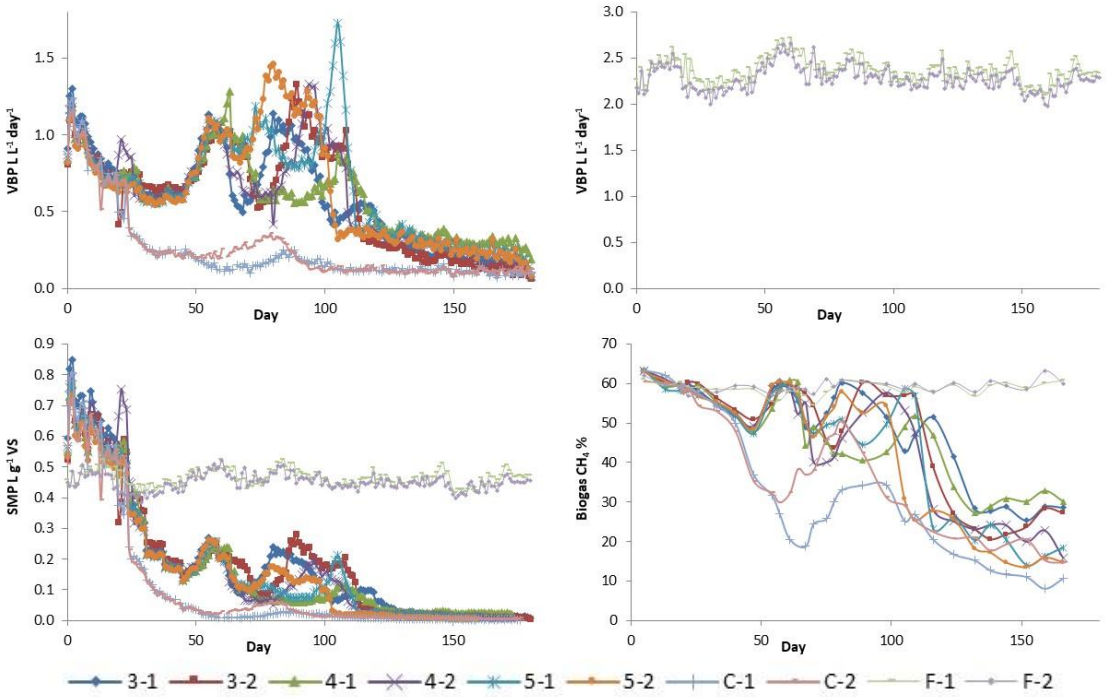


Figure 4.26 VBP, SMP and biogas methane content for trial S2

Table 4.16 shows the predicted SMP value for the co-digestions based on the values obtained in the controls. Expected values for the SMP for the co-digestion reactors were estimated based on the SMP from the food waste and cattle slurry controls at the end of the run (averages for last 20 days). The food waste control was taken from day 349 to day 528 (the period during which trial S2 was conducted). The SMP of FW and CS respectively was 0.460 and 0.005 L CH_4 g^{-1} VS. The daily wet weights of feed were as shown in Table 4.16. The expected SMP values, ignoring the effects of HRT, were therefore 0.127 L CH_4 g^{-1} VS, compared with actual average values for the last 20 days of the run of 0.013, 0.017 and 0.009 L CH_4 g^{-1} VS respectively. These and equivalent results based on modelling using the BMP coefficients are shown in Table 4.16 and Appendix A Table A5. It therefore appeared that methane production was inhibited, and/or that COD even from the food waste component was being diverted to another route e.g. into H_2S production.

Table 4.16 Actual and predicted values of SMP and VMP for co-digestion trial S2

Parameter	**Unit**	**CS/FW**	**CS/FW**	**CS/FW**	**CS**	**FW**
Nominal OLR	g VS L^{-1} day^{-1}	3	4	5	3	3
FW addition	g WW day^{-1}	13.8	18.4	23.1	0	52.4
FW addition	g VS L^{-1} day^{-1}	0.77	1.03	1.29	0.00	2.93
CS addition	g WW day^{-1}	82.8	110.4	136.8	115.5	0
CS addition	g VS L^{-1} day^{-1}	2.09	2.78	3.45	2.91	0.00
HRT	days	41	31	25	35	76
SMP	L CH_4 g^{-1} VS	0.013	0.017	0.009	0.005	0.460
Predicted SMP[a]	L CH_4 g^{-1} VS	0.127	0.127	0.129	-	-
Predicted SMP [b]	L CH_4 g^{-1} VS	0.255	0.247	0.240	0.170	0.475
Actual/Predicted SMP[a]	%	10.3%	13.7%	7.2%	-	-
Actual/Predicted SMP[b]	%	5.1%	7.1%	3.8%	2.7%	96.9%

[a] based on feedstock ratios and experimental SMP values; [b] based on feedstock ratios and BMP kinetic coefficients

The instability (or 'failure') of the digesters fed with cattle slurry was also shown in the results for total VFA, IA : PA ratio and pH as shown in Figure 4.27.

For digesters receiving cattle slurry feed, total VFA concentrations started to increase from around day 27. This rise began slightly earlier in digesters that were also receiving food waste. After day 50 these digesters showed some recovery, suggesting that this difference could have been due in part to transient VFA peaks associated with a change in feedstock (section 4.3.3.1 and 4.3.3.2).

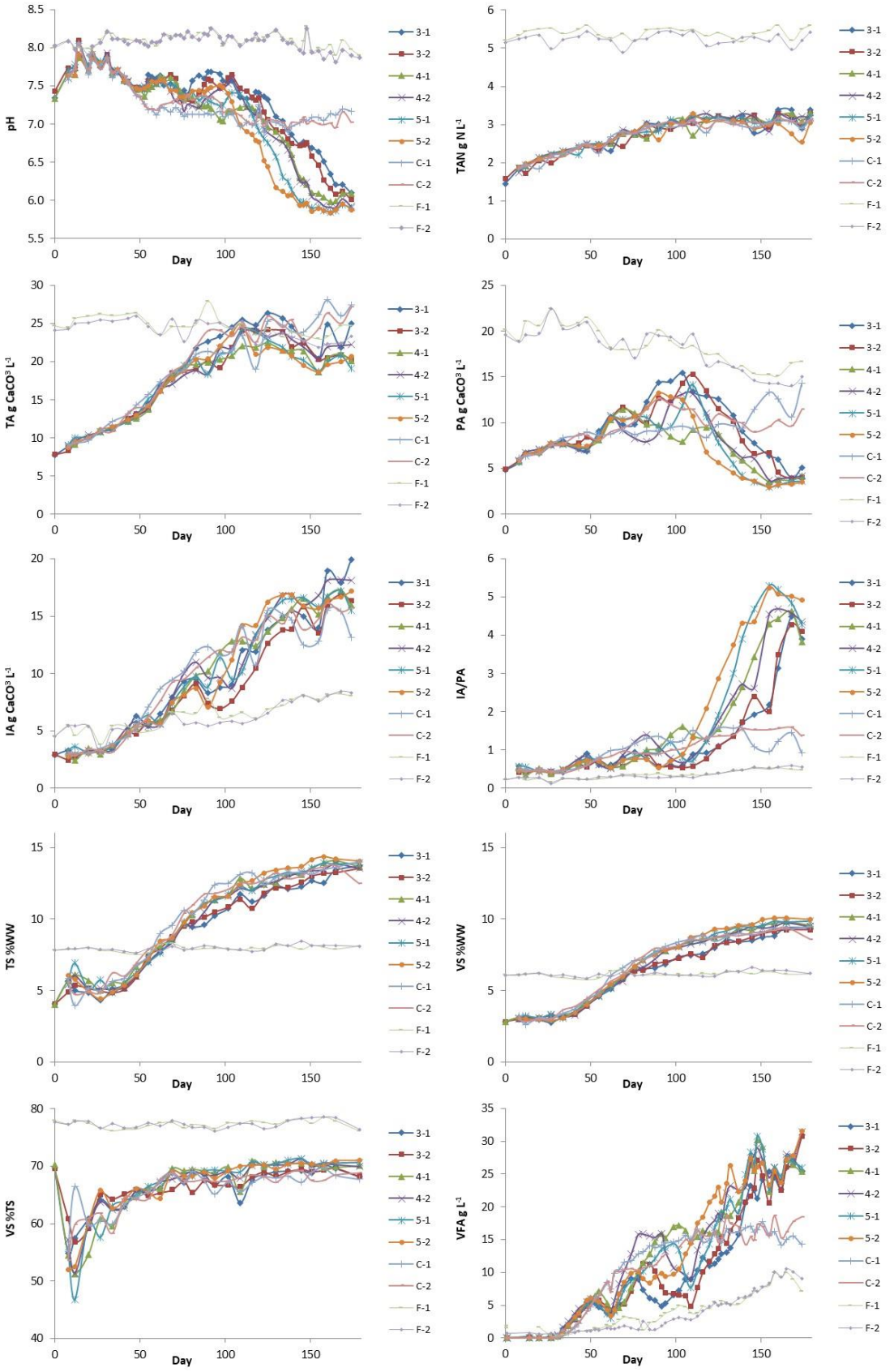


Figure 4.27 Monitoring parameters during trial S2: pH, TAN, TA, PA, IA, IA/PA, TS, VS and total VFA

From day 62 onwards, however, despite occasional recovery and some differences in the timeline for duplicate digesters, total VFA concentrations for the co-digestion digesters showed a strong upward trend. By the end of the trial average values were 25.8, 26.8, and 26.9 g L^{-1} for OLR of 3, 4 and 5 g VS L^{-1} day^{-1} respectively. In contrast, total VFA in the cattle slurry control reactors stabilised at around 15 g L^{-1}. pH values mirrored the VFA concentrations, showing some fluctuation between days 70 – 100 but then starting to decline in all co-digestion digesters in order of OLR. TA, PA and IA in these digesters increased until somewhere between days 70 – 100. From this time onwards PA began to fall, leading to a rise in the IA/PA ratio. This rise began in reverse order of OLR, and average IA/PA values reached 3.90, 4.42, and 4.89 at OLR 3, 4 and 5 g VS L^{-1} day^{-1} respectively, indicating that shorter HRT and greater loading were having an effect. TAN rose in all of the digesters receiving cattle slurry, stabilising at around 3 g N L^{-1}. TS and VS also increased, and by the end of the run there were indications of small differences between reactors, with those at higher OLR showing a higher VS content. Calculated VS destruction rates at the end of the trial fluctuated considerably but appeared lower than in trial C2.

The FW controls also showed some VFA accumulation to around 10 g L^{-1} during the period of this trial, as discussed in section 4.3.3.2. This was believed to be due to a lack of trace element supplementation, however, and was thus not directly relevant to the performance of the other digesters since, unlike them, the FW controls had not been re-seeded with fresh inoculum at the start of the trial. All other parameters remained relatively stable in the FW digesters, perhaps indicating that the higher TAN concentration provided sufficient buffering to prevent a fall in pH.

Figure 4.28 shows the VFA profiles for each digester. In the co-digestion reactors acetic acid accumulation started soon after the introduction of the cattle slurry feed, with propionic acid appearing about 30 days later. The propionic acid may indicate inhibition of propionate-degrading organisms, with periods of stability representing a balance between production and washout. Other longer-chain VFAs also appeared, initially at concentrations of around 1 g L^{-1}, but after day 100 n-butyric acid concentrations rose in all digesters indicating some blockage in the metabolic pathway to acetogenesis.

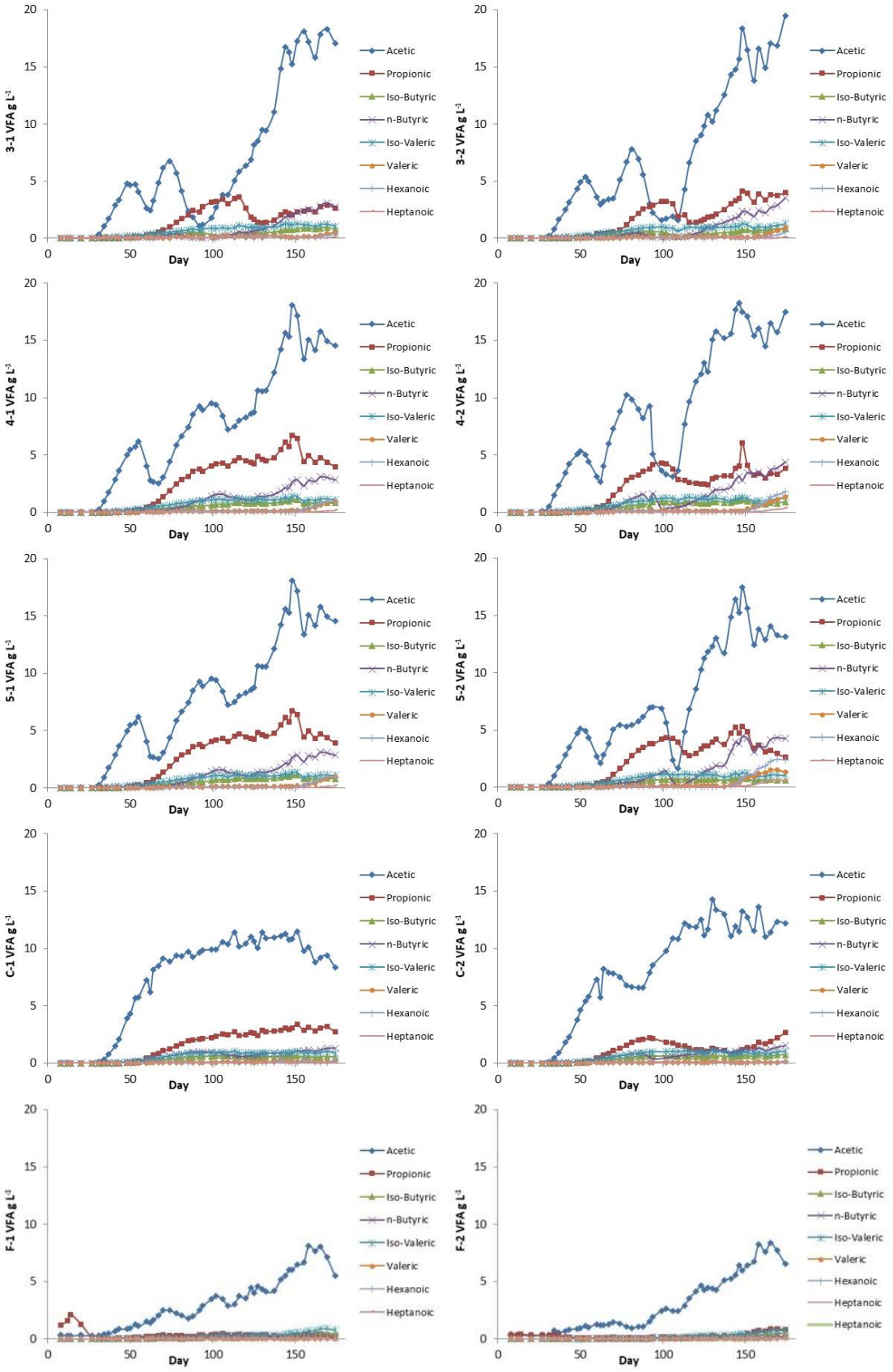


Figure 4.28 VFA profiles during trial S2

As the VFA concentrations increased the biogas methane content fell: by the end of the trial the biogas consisted almost entirely of CO_2 indicating near-complete inhibition of methanogenesis with gas production almost entirely from acidogenesis. The sequence of events is shown in Figure 4.29 where it can be clearly seen that as the methanogenic pathway becomes blocked there is a sharp decline in biogas methane content linked to a rise in VFA concentration, followed some days later by a fall in pH. In the cattle slurry controls the VFA accumulation was lower and the pH remained around 7, but the biogas methane content still fell. This suggests that inhibition of methanogenesis was still occurring but the feedstock has a lower VFA production potential, allowing total and individual VFA concentrations to stabilise as shown. The combination of food waste with cattle slurry thus had a further destabilising influence in these conditions, but methanogenesis could not be sustained with the cattle slurry feedstock alone.

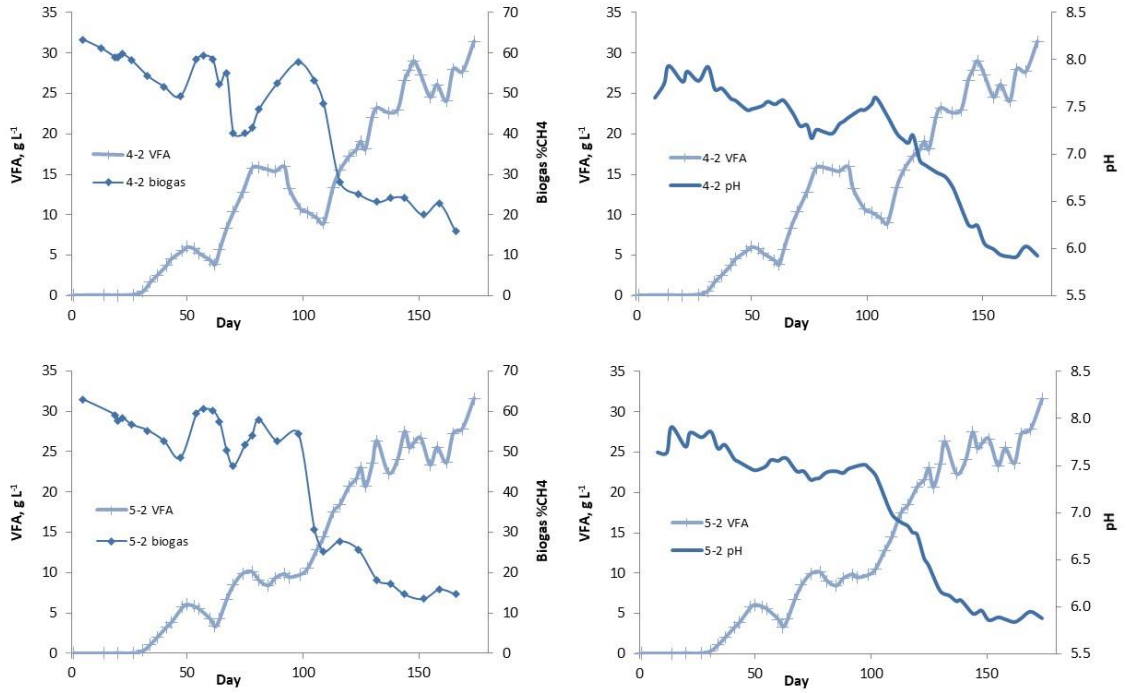


Figure 4.29 Total VFA concentration, biogas methane content and pH for selected digesters at OLR 4 and 5 g VS L^{-1} day^{-1} in trial S2

As mentioned earlier, $FeCl_3$ solution was added to the digesters in an attempt to control the dissolved sulphide content. Dissolved sulphide analyses were conducted on five occasions after the start-up period, between day 72 and day 131. Table 4.17 shows the average results for pairs of digesters.

Table 4.17 Average dissolved sulphide concentrations for duplicate digesters in trial S2

Day	Dissolved sulphide concentration (mg S L^{-1})			
OLR (g VS L^{-1} day^{-1})	3	4	5	CS control
72	47.5	63.0	53.8	62.8
85	30.3	69.0	79.2	71.3
113	60.8	62.9	63.8	63.3
119	62.4	62.8	42.6	62.4
131	55.8	53.3	41.3	62.1

Based on these results, it seems that addition of $FeCl_3$ was able to reduce the dissolved sulphide to a concentration that is not considered toxic. In samples taken from trial S1, when there was no sulphide control, the dissolved sulphide concentration in the digestate was measured as 500 mg S L^{-1} which is well above the toxic level of 200 mg S L^{-1} suggested by Lawrence and McCarty (1965). Although the dissolved sulphide concentration was reduced in this trial, and the onset instability was later than in trial S1 without $FeCl_3$ addition, the digestion still failed. One reason may be that the amount of $FeCl_3$ added for removal of soluble sulphide by precipitation as FeS was not sufficient. Although the flask test suggested a Fe : S molar ratio of 0.3 was effective, this was lower than the minimum ratio of 0.6 suggested by Zhang et al. (2009). Scherer and Sahm (1981) as reported by Speece (1983) stipulated the optimal sulphide concentration for methanogenic growth to be 1 – 25 mg S L^{-1}. On the other hand, Parkin et al. (1983) stated that concentrations above 50 mg S L^{-1} may cause sulphide toxicity to unacclimated methanogens. Erdirencelebi and Kucukhemek (2018) found that dosing at 24 – 50 mg $FeCl_3$ L^{-1} gave a significant reduction in H_2S in the biogas from sewage sludge digestion, but the proportion of primary sludge with its higher proteinaceous content had a strong effect on performance.

Another possible reason could be if the FeS precipitate settled at the bottom of the digesters, and was not completely removed when digestate was wasted: in this case it could have surrounded the surface of methanogenic cells and affected their function, as hypothesised by Utgikar et al. (2002). Speece (2008) stated, however, that the precipitate has no toxic effect on microbial activity.

The flask test in Section 4.3.2 was conducted as a batch experiment, but the situation is more complex in a semi-continuous trial where the daily addition of high-sulphate cattle

slurry provides a source of sulphide from the activities of SRB. In the flask experiment the digestate had been stored to reduce its biological activity. Any hydrogen sulphide present in the headspace would have been released, reducing the concentration of both HS^- and dissolved H_2S in the digestate. Sulphate reduction and oxidation are also complex processes with many potential pathways (Muyzer and Stams, 2008), and a range of physico-chemical changes may have occurred during the digestate storage period.

H_2S is considered the more toxic form of dissolved hydrogen sulphide (Chen et al., 2008) and is dominant at lower pH. Thus while the decline in biogas methane content in all of the semi-continuous co-digestion reactors preceded the drop in pH (Figure 4.29), once the pH started to fall this would have accelerated inhibition of the mixed microbial community and accumulation of VFA.

While the most likely cause of digestion failure appeared to be sulphide toxicity, it was also possible that some other toxicant was present in the batch of cattle slurry used. For this reason the following experiment investigated both the effect of spiking low-sulphate cattle slurry with sulphate, and the co-digestion performance of high-sulphate cattle slurry with different proportions of food waste as a co-substrate.

4.4.6 Trial S3: Operation of CSTR with plain and gypsum-spiked cattle slurry

In trials S1 and S2 feeding with high sulphate cattle slurry (batch CS2, sulphate concentration 6876 mg SO_4 L^{-1}, Table 4.1) had caused elevated concentrations of sulphide in the reactors. It was believed that sulphide toxicity had inhibited methanogenesis, hence causing process failure as observed in the previous sections. It was possible, however, that some other component in CS2 was causing or contributing to the observed inhibition. Trial S3 investigated the behaviour of digesters fed on cattle slurry with high sulphate content. One set of digesters were fed with low sulphate cattle slurry (batch CS3, 289 mg SO_4 L^{-1}, Table 4.1) spiked with gypsum to give a range of sulphate concentrations. Another pair of digesters was fed on the high-sulphate cattle slurry from batch CS2 at different CS : FW ratios. Co-digestion with food waste effectively acts as a form of dilution, and comparison of the results with those from the spiked trial could help to indicate whether the sulphate content alone was sufficient to account for the observed inhibition, or whether some other factor was at work.

4.4.6.1 Objectives of trial S3

The overall objective of this trial was to determine the concentrations of sulphate that could cause reduction in specific methane productivity and the onset of instability in cattle slurry digestion. This was investigated in two sub-experiments.

Trial S3a involved controlled addition of calcium sulphate in the form of gypsum to a low-sulphate cattle slurry with the objective of determining the limiting sulphate concentration.

In trial S3b one pair of digesters was fed at different CS : FW ratios using high-sulphate cattle slurry from batch CS2. The objective was to determine the 'safe' dilution for CS2 for comparison with results from addition of known amounts of sulphate.

4.4.6.2 Methodology for trial S3a - sulphate addition to low-sulphate cattle slurry

Eight 5-L CSTR digesters with working volumes of 4 L were used in this part of the trial. Six of the digesters (R1 – R6) were initially inoculated with Millbrook digestate and fed on food waste (batch FW2, Table 4.1) for 5 days, then acclimated to cattle slurry (batch CS3, Table 4.1) at an OLR of 2 g VS L^{-1} day^{-1} and operated for a period of around 2.6 HRT to ensure reproducible and representative conditions. The other two digesters (R7&8) had been used as controls in the preceding experiments and had previously been fed on cattle slurry only (batch CS1, low sulphate) for 258 days. These were fed on CS3 at an OLR of 2 g VS L^{-1} day^{-1} to provide comparative baseline values. The cattle slurry used (batch CS3) was from a single source and where necessary its solids content was adjusted by addition of small amounts of tap water to maintain a VS content of 8.26%, corresponding to a HRT of around 41 days at the applied OLR.

On the days specified in Table 4.18 the digesters were spiked with calcium sulphate ($CaSO_4.2H_2O$) to give equivalent added sulphate concentrations in the digestate of 0, 1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg SO_4 L^{-1}; these concentrations were selected with the aim of covering a range up to the measured sulphate concentration in CS3, and if possible of identifying a specific threshold for the onset of inhibition as opposed to the effects of substrate competition. The input concentration was then maintained by addition of equivalent amounts of sulphate with the feed. The digesters

were operated as four sets of duplicates, with one set acting as a control (fed only with CS3) throughout the experimental period.

Table 4.18 Operating conditions for trial S3a

	SO_4 added (mg SO_4 L^{-1})						
Phase	**I**	**II**	**III**	**IV**	**V**	**V1**	**VIII**
Day	**0 – 111**	**112 – 218**	**219 – 226**	**227 – 232**	**233 – 239**	**240 – 281**	**282 – 321**
R1&2	0	1000	3000	3000	4000	4000	4000
R3&4	0	2000	3000	4000	4000	5000	6000
R5&6	0	3000	4000	4000	5000	6000	7000
R7&8	0	0	0	0	0	0	0

Biogas production, pH, alkalinity, total ammonia nitrogen and total VFA concentrations in all digesters were monitored throughout the experimental period. Additional parameters such as hydrogen sulphide gas and dissolved sulphide content were also monitored and are discussed in section 4.5.4.6 with the results from trial S3b.

4.4.6.3 Results and discussion for controlled sulphate addition (trial S3a, R1 – R8)

Operating parameters. Figure 4.30 shows the weight of cattle slurry CS3 added, the applied OLR, HRT and sulphate addition. The OLR in R1&2, R3&4 and R5&6 was briefly increased to 3 g VS L^{-1} day^{-1} from day 32 – 49 but was then reduced again to the target value of 2 g VS L^{-1} day^{-1}. There were a number of issues with gas counters, in particular for R7&8, leading to some low apparent gas production values for these digesters between day 120 – 240. On day 138 the feed for R7&8 may have been accidentally switched with that used for R9&10 in trial S3b. Around day 214 there was a temperature drop in R7&8 due to a thermostat failure. No other major disturbances occurred.

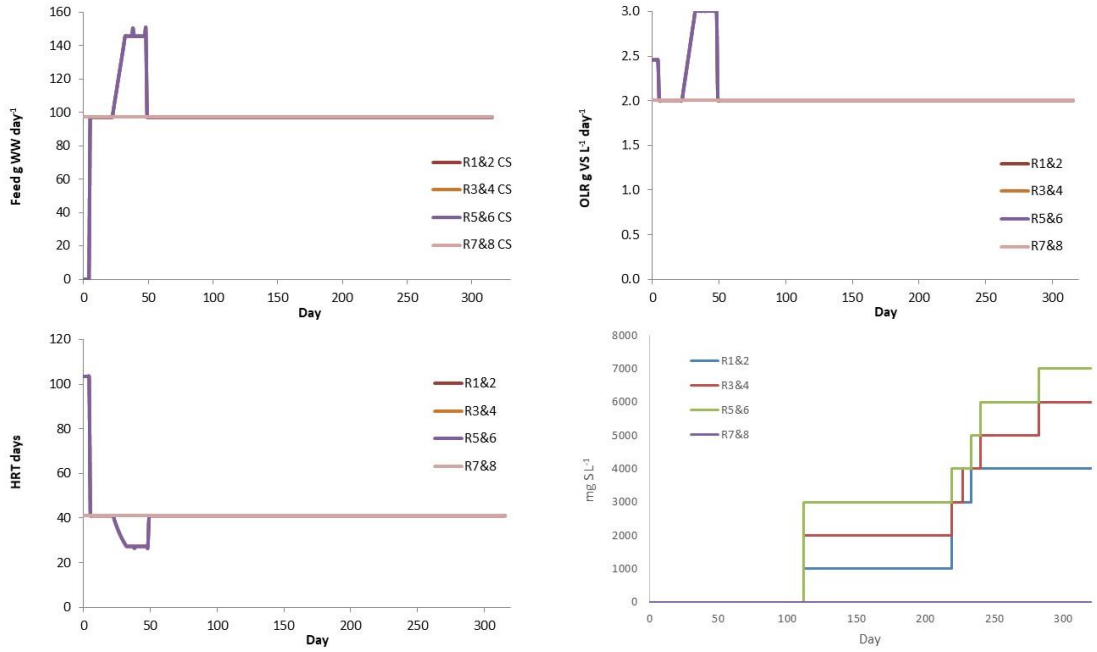


Figure 4.30 Wet weight of feed, OLR, HRT and added sulphate in digester contents and feed during mono-digestion of cattle slurry CS3 with sulphate addition (trial S3a)

Biogas production. Figures 4.30 and 4.31 show biogas production and composition for each pair of digesters during the trial. Average values for these and other parameters at the end of each major increment in sulphate addition are shown in Table 4.18. From day 0 – 75 digesters R1-6 showed a similar pattern of successive falls and rises in VBP and SMP followed by a degree of stabilisation: these peaks and troughs were attributed to the changes in feedstock to food waste FW2 and then to cattle slurry CS3 on day 5, followed by adaptation of the fresh inoculum to this new feed. The response in the control reactors R7&8 to the change in feedstock from CS1 to CS3 was much less marked.

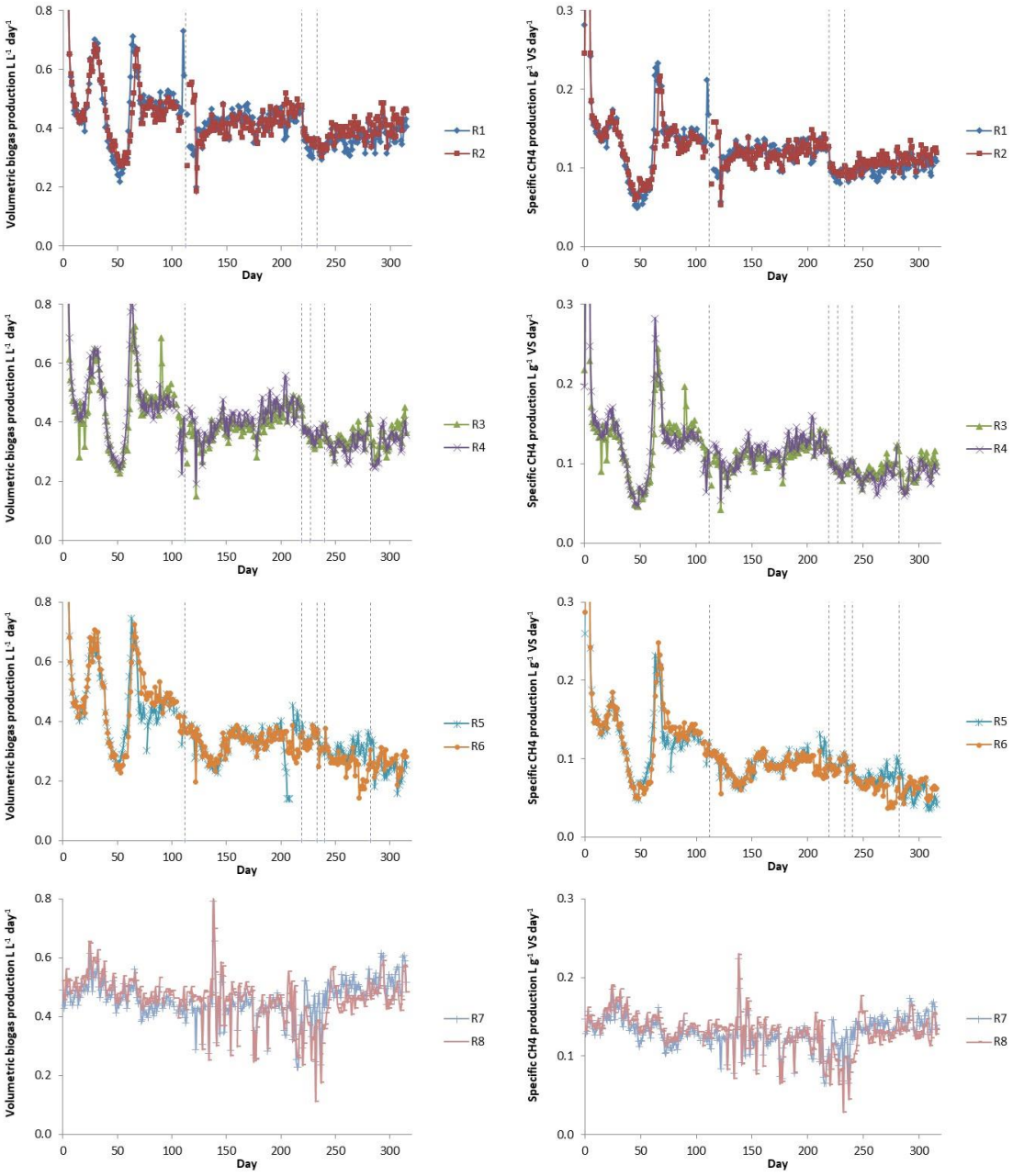


Figure 4.31 Volumetric biogas production and specific methane production during mono-digestion of cattle slurry CS3 with sulphate addition (trial S3a). Vertical dotted lines indicate changes in sulphate addition (Table 4.18).

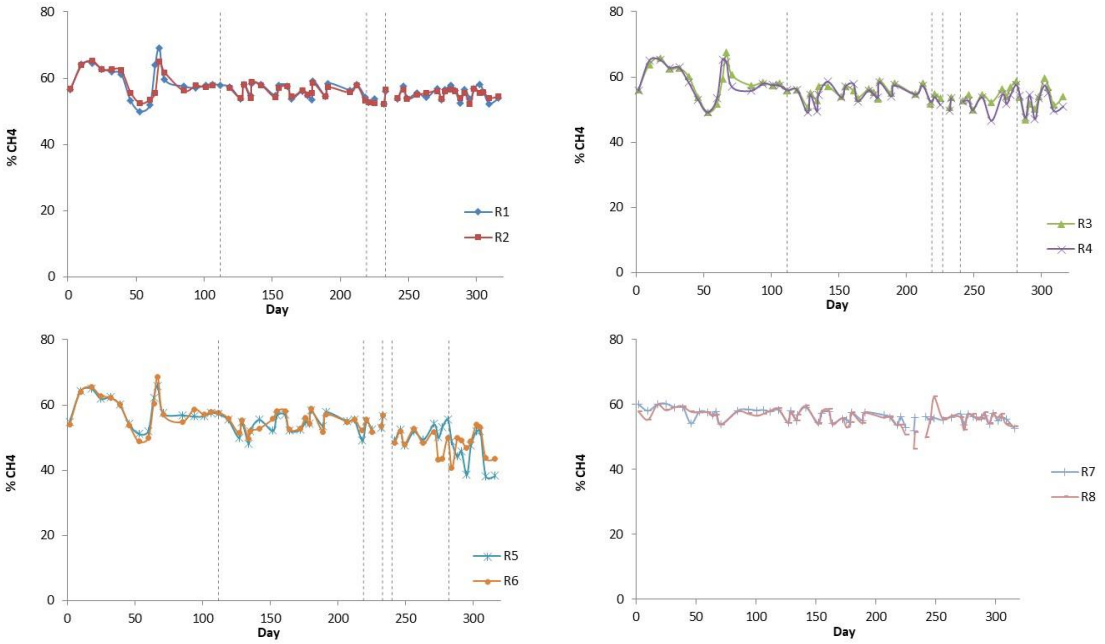


Figure 4.32 Biogas methane content during mono-digestion of cattle slurry CS3 with sulphate addition (trial S3a). Vertical dotted lines indicate changes in sulphate addition (Table 4.18).

After the initial sulphate dose on day 112 there was some disturbance in SMP and VBP. R1&2 with sulphate addition of 1000 mg SO_4 L^{-1} showed the least disturbance and the most rapid recovery. R3&4 at 2000 mg SO_4 L^{-1} recovered more slowly, and in R5&6 at 3000 mg SO_4 L^{-1} there was partial recovery but the SMP was still lower at the end of this phase on day 219 (Table 4.18). The peak in SMP and VBP in R7&8 on day 138 is probably associated with mis-feeding, while some low values around that time are likely due to gas counter issues. The effect of the temperature drop in R7&8 around day 214 can be clearly seen in a fall in both biogas production and methane content, with recovery occurring slowly over the next 40 days.

When the sulphate additions were increased to 4000, 5000, 6000 and 7000 mg SO_4 L^{-1} there were further reductions in SMP and VBP, as well as some variation between duplicate digesters. At 4000 mg SO_4 L^{-1} the SMP in R1 showed stronger recovery that R2, with values at the end of the run approaching those before sulphate addition. When operating at 5000 mg SO_4 L^{-1} between days 240 – 281, SMP and VBP in R5 were more stable than in R6; while R3 in particular showed some recovery in gas production when operated at 6000 mg SO_4 L^{-1}. Figure 4.33 shows the average values for SMP and biogas methane content in the final 20 or 30 days in each digester, when conditions were approximately stable. While there is some scatter, the results indicate that sulphate

addition up to 2000 mg SO_4 L^{-1} had little or no effect on SMP, while at 4000 mg SO_4 L^{-1} and above there was a clear fall in both SMP and methane content. The falls corresponded approximately to a loss of around 0.017 L CH_4 g^{-1} and 2.87 % CH_4 per g of SO_4 added (Figure 4.33c and d). Examination of Figure 4.33a and b suggests, however, that some acclimatisation to higher sulphate concentrations may also have been taking place, as values for the same added sulphate concentration obtained from day 300 – 321 (♦) are generally higher than those from day 264 – 282 (●).

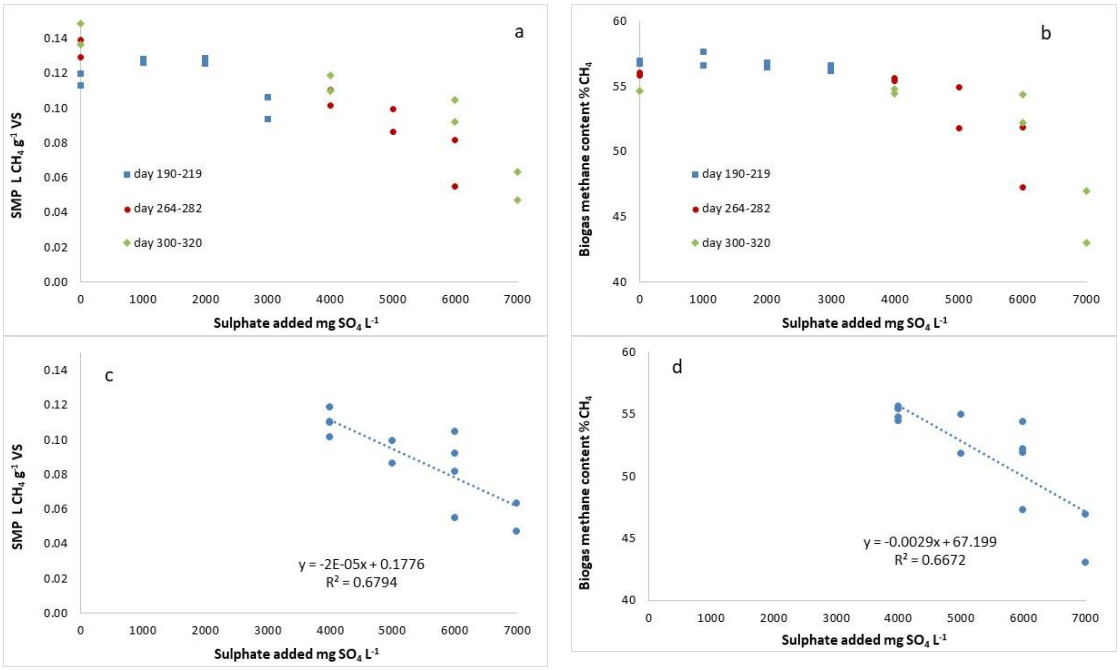


Figure 4.33 SMP and biogas methane content versus sulphate addition (trial S3a)

The average VBP of the control digesters for the last 20 days of the run was 0.523 L L^{-1} day^{-1} and the SMP was 0.143 L g^{-1} VS, or about 74% of the BMP value of 0.193 L g^{-1} VS for CS3 (Table 4.2). When 1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg SO_4 L^{-1} were spiked into the cattle slurry the average SMP at the end of each period was 0.127, 0.127, 0.100, 0.114, 0.093, 0.099 and 0.055 L g^{-1} VS respectively (Table 4.19).

Figure 4.34 shows monitoring parameters for the digesters during the trial. Total VFA concentrations in R1 – R6 increased sharply between day 40 – 70 reflecting the change in feedstock. Similar behaviour was also seen in trial C1 (Figure 4.13), although the accompanying reduction in SMP was more marked in the current trial. VFA concentrations then stabilised below 200 mg L^{-1} until the start of sulphate addition at day 112. pH in R1 – R6 dipped to 7.2 around the time of the VFA increase, but then recovered

to stabilise at around 7.6 in all digesters. After some fluctuations associated with the change in feedstock and the initial VFA peak, TAN declined gradually from around day 44 onwards, reflecting the properties of the CS3 feed, and settled between 1.5 – 2.0 g N L^{-1} by day 112. Alkalinity values rose in R1 – R6 and fell in R7&8 until around day 72, then stabilised at similar values in all digesters. The IA/PA ratio in R1 – R6 rose around day 40, reflecting the peak in VFA, but stabilised at around 0.65 by day 112. Solids content in R1 – R6 initially increased as the digesters adapted to the new feedstock. TS and VS were not measured between day 65 – 107 but by day 112 had stabilised at similar values in all digesters. These results indicated that the digesters were acclimated and in a stable operation prior to sulphate addition.

Around two weeks after the first incremental sulphate addition on day 112 total VFA started to increase in R1 – R6: this delay probably represented the time needed for an increase in the population of SRB leading to potential sulphide inhibition. The VFA peak was higher in the digesters with higher sulphate concentrations, took longer to decline and left slightly elevated VFA concentrations of around 0.1, 0.16 and 0.27 g L^{-1} in digesters at 1000, 2000 and 3000 mg SO_4 L^{-1} respectively. Profiles for individual VFA species are discussed in more detail below. As expected, VFA concentrations in R7&8 remained low. In the reactors with sulphate addition pH initially rose slightly, possibly reflecting an increase in consumption of H^+ for reduction of the initial sulphate spike, but by day 219 has returned to around 7.6. TA and IA remained fairly stable, but in the reactors with sulphate addition there was a slight upward trend in PA, leading to corresponding slight falls in IA/PA ratio. TS and VS content also rose slightly in proportion to sulphate addition, while TAN decreased slightly. These results indicate that while there was some response to sulphate additions of 1000 – 3000 mg SO_4 L^{-1}, with the slight elevations in VFA indicating minor inhibition of methanogenesis, stable operation at these concentrations was still possible.

When added sulphate concentrations were increased from day 219 onwards, total VFA concentrations rose. In R1&2 at 4000 mg SO_4 L^{-1} total VFA appeared to have stabilised by the end of the run at values below 0.5 mg L^{-1}. The other digesters showed some discrepancies between members of each pair, but at 6000 mg SO_4 L^{-1} total VFA rose to between 1.0 – 1.5 g L-1 then appeared to stabilise, while at 7000 mg SO_4 L^{-1} there were signs of progressive accumulation with total VFA reaching 2.5 g L^{-1} by the end of the run. pH fell slightly in response to these changes, but remained above 7.5 in R1 – R4 and

above 7.3 in R5 – R6. Despite the rise in VFA, a further increase in PA in the digesters with sulphate addition meant that the IA/PA ratio remained low in all digesters. TAN also stabilised at a slightly higher value in R1 – R6 than in R7&8. The solids content in R1 – R6 and the control reactors R7&8 continued to show some divergence, with higher values for both TS and VS in the digesters with higher sulphate addition.

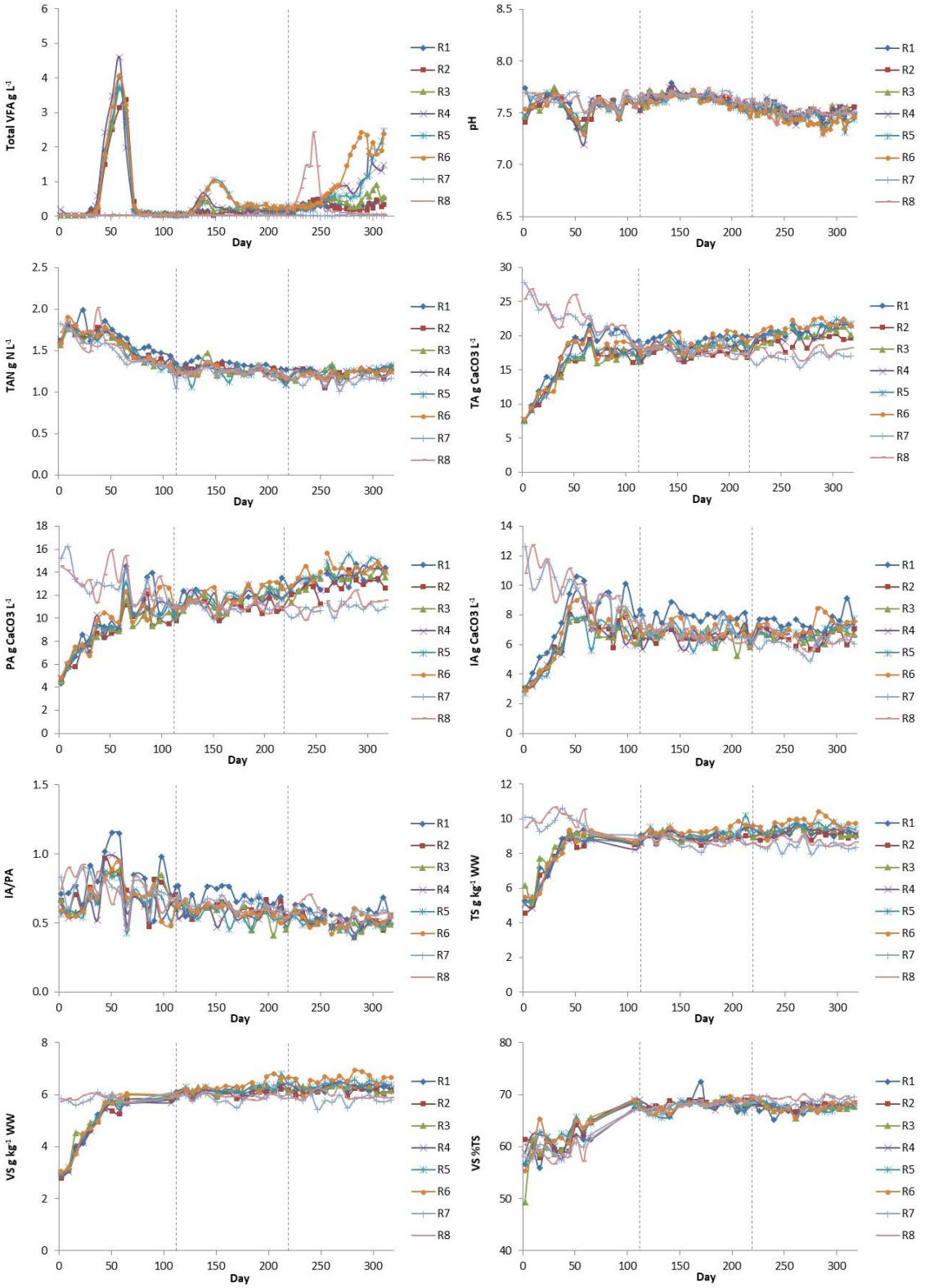


Figure 4.34 Monitoring parameters during mono-digestion of cattle slurry CS3 with sulphate addition (trial S3a). Vertical dotted lines indicate first and second increments in sulphate addition on days 112 and 219, respectively (Table 4.18).

Figure 4.35 shows VFA profiles in the digesters during the trial. It can be seen that the initial response to the change in feedstock was similar in R1 – R6, with a peak consisting mainly of acetic acid at a concentration of up to 4.5 g L^{-1}. In R1&2 there appeared to be only a minor response to sulphate addition at 1000 mg SO_4 L^{-1}, with a very slight rise in acetic acid concentrations from around day 150 to day 219 when the next sulphate increment was introduced. From day 233 – 321 when the digesters were operated at 4000 mg SO_4 L^{-1} sulphate addition, slightly elevated concentrations of acetic and propionic acid were seen of up to 0.37 and 0.26 g L^{-1} respectively, possibly indicating minor inhibition of methanogenesis.

In R3&4, starting from around 12 days after the introduction of sulphate at 4000 mg SO_4 L^{-1} on day 112, a small peak of up to 0.36 g L^{-1} in acetic acid was seen, followed by one of up to 0.27 g L^{-1} in propionic acid. Both peaks declined over the next 30 – 40 days but acetic concentrations remained slightly raised. From day 240 – 282 at 5000 mg SO_4 L^{-1} acetic acid rose to around 0.45 g L^{-1} in both digesters, while propionic acid also appeared in R4 at up to 0.32 g L^{-1}. When the sulphate addition was further increased to 6000 mg SO_4 L^{-1} acetic acid concentrations rose in both digesters, reaching over 1 g L^{-1} in R6, accompanied by propionic acid at 0.4 g L^{-1} and traces of other VFA.

In R5&6 the initial peaks in acid production after sulphate addition at 3000 mg SO_4 L^{-1} were more marked than at lower sulphate loadings, reaching up to 0.74 and 0.31 g L^{-1} of acetic and propionic respectively. These peaks declined over the next 40 days, however, leaving only slightly elevated concentrations of both acids. When the sulphate addition was increased to 6000 mg SO_4 L^{-1} acetic acid concentrations started to climb, particularly in R6, but stabilised at around 0.45 g L^{-1} in R5. A further increase to 7000 mg SO_4 L^{-1} led to a rapid accumulation of acetic acid in R5 with fluctuating values in R6 and the appearance of iso-butyric and iso-valeric acids in both digesters at individual acid concentrations of up to 0.29 g L^{-1} by the end of the run.

In contrast, in R7 without sulphate addition concentrations of all VFA remained below 0.05 g L^{-1}. In R8 there was a transient peak consisting primarily of acetic acid from day 217 – 275, perhaps due to the temperature shock: but by the end of the run all VFA species in both R7&8 were below 0.05 g L^{-1}.

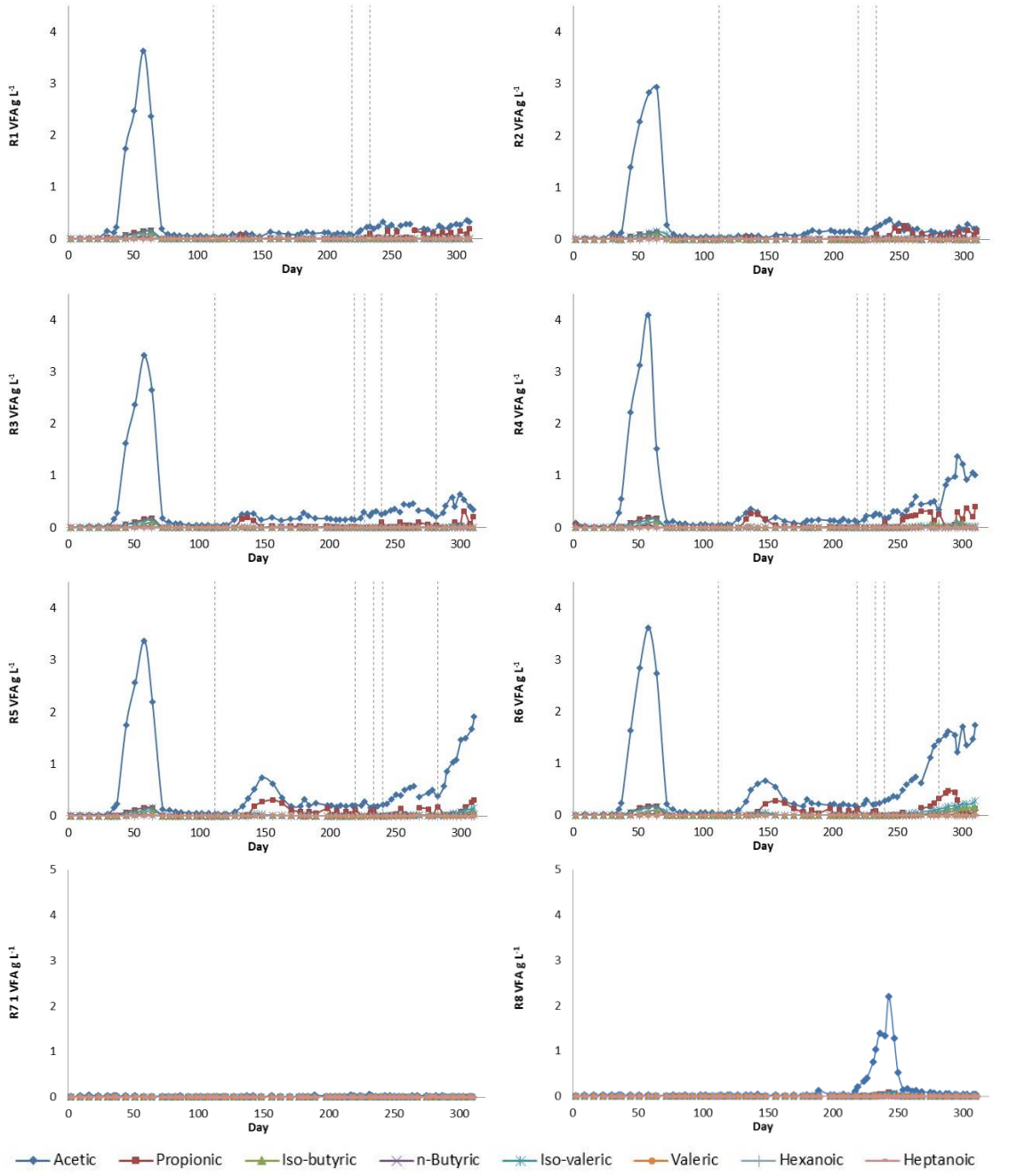


Figure 4.35 VFA profiles during experimental run with sulphate addition (trial S3a)

Figure 4.36 shows average values for selected monitoring parameters in the final 20 or 30 days at each sulphate loading, plotted against added sulphate concentration. Alkalinity showed a clear upward trend with increasing sulphate addition while pH showed a general downward trend, apart from a higher value at 6000 mg SO_4 L^{-1} at the end of the trial. The increase in alkalinity is likely associated with the sulphate reduction process in which hydrogen ions are consumed. This normally also leads to an increase in pH, but may have been countered by the rise in VFA concentrations, apart from at 6000 mg SO_4 L^{-1} where

the VFA accumulation was lower than at 7000 mg SO_4 L^{-1} (Figure 4.35) but the H_2S production almost as high (see section 4.4.6.6 below). VS content also increased with sulphate addition, but showed the effect of sampling period: this could have reflected acclimatisation as the difference decreased over time. The results for total VFA concentration reflected those for SMP and biogas methane content (Figure 4.33) as there appeared to be little or no increase in VFA at added sulphate concentrations of less than 4000 mg SO_4 L^{-1}.

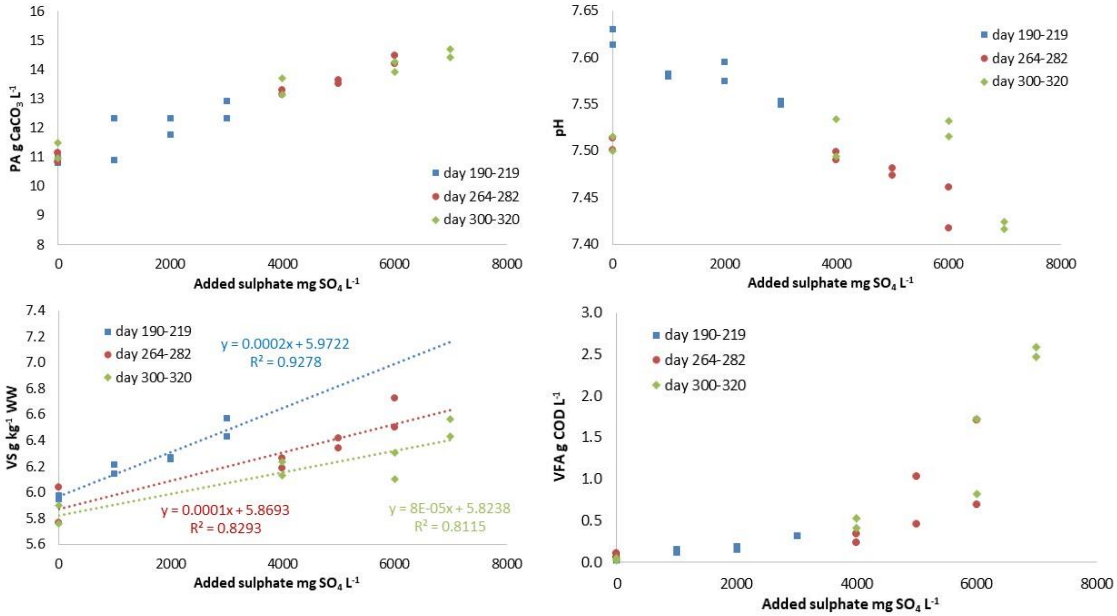


Figure 4.36 pH, PA, VS and total VFA versus sulphate addition in trial S3a

Table 4.19 Average values for key parameters in trial S3a

Parameter	**Unit**	**SO_4 added (mg SO_4 L^{-1})**							
		0 [a]	**1000** [b]	**2000** [b]	**3000** [b]	**4000** [a]	**5000** [c]	**6000** [a]	**7000** [a]
SBP	L g^{-1} VS	0.261	0.223	0.224	0.162	0.209	0.166	0.185	0.122
SMP	L g^{-1} VS	0.143	0.127	0.127	0.100	0.114	0.093	0.099	0.055
VBP	L L^{-1} day^{-1}	0.523	0.447	0.450	0.336	0.420	0.350	0.370	0.244
VMP	L L^{-1} day^{-1}	0.286	0.255	0.255	0.183	0.229	0.178	0.197	0.111
CH_4 content	% v/v	54.7	57.1	56.7	56.4	54.6	53.4	53.3	45.0
H_2S content	ppmv	449	3558	4087	5600	13491	21846	26149	39441
TS	%WW	8.45	9.02	9.15	9.45	9.08	9.40	9.16	9.57
VS	%WW	5.83	6.18	6.26	6.50	6.18	6.38	6.20	6.50
pH	–	7.51	7.58	7.59	7.55	7.51	7.48	7.52	7.42
TA	g $CaCO_3$ kg^{-1} WW	17.6	18.7	18.6	19.5	20.7	20.0	21.2	21.9
PA	g $CaCO_3$ kg^{-1} WW	11.2	11.6	12.1	12.6	13.4	13.6	14.1	14.6
IA	g $CaCO_3$ kg^{-1} WW	6.4	7.1	6.5	6.9	7.3	6.5	7.1	7.4
IA/PA ratio	–	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5
TAN	g N kg^{-1} WW	1.73	1.25	1.20	1.20	1.24	1.24	1.28	1.27
Total VFA	g L^{-1}	0.04	0.14	0.18	0.32	0.47	0.74	1.27	2.53

[a] Average for day 300 – 320
[b] Average for day 190 – 219
[c] Average for day 264 – 282

Taken together the above results suggest that the digesters responded well to the introduction of sulphate at 1000, 2000 and 3000 mg SO_4 L^{-1}; the rise in VFA concentrations indicated a proportionally greater degree of shock at the higher addition but they were able to recover to stable performance with only a small loss in SMP and mildly elevated VFA concentrations indicating the onset of inhibition of methanogenesis.

At sulphate additions above 4000 there was a clear decline in SMP and biogas methane content, suggesting both competition with SRB for substrate and an increasing degree of inhibition. At 7000 mg SO_4 L^{-1} rapid acetic acid accumulation started to occur in one digester and other VFA species began to accumulate, suggesting operation at this sulphate content is at best likely to be sensitive to any shock changes and may show progressive failure. The appearance of VFA suggest sulphide inhibition as well as competition, while signs of increase in volatile solids content also indicate the onset of inhibition of hydrolysis. The significance of these results in terms of sulphide inhibition is discussed in section 4.5.4.6 below in conjunction with the results from trial S3b.

4.4.6.4 Methodology for trial S3b – co-digestion of high-sulphate cattle slurry with food waste

Two 5-L CSTR digesters with working volumes of 4 L were used in this part of the trial. Before the start of the trial these two digesters had acted as controls in trial C2 and had been fed on food waste only (batch FW2, Table 4.1) for 244 days. On day 0 the feedstocks were switched to mixtures of cattle slurry (batch CS2, Table 4.1) and FW2 at ratios of 1 : 1 and 2 : 1 on a wet weight basis in R9 and R10 respectively. Between days 176 and 184 the feedstock CS : FW ratios were incrementally raised to 3 : 1 and 4 : 1 in R9 and R10, respectively. The corresponding sulphate concentrations based on the feedstock characteristics in Table 4.1 are shown in Table 4.20. The target OLR was maintained at close to 3 g VS L^{-1} day^{-1} throughout, with the HRT allowed to vary, giving values of 60, 52, 47 and 45 days at CS : FW ratios of 1 : 1, 2 : 1, 3 : 1 and 4 : 1 respectively. Feeding was stopped on day 330 after 3 HRT, and monitoring of the digesters continued until day 396. Note: This trial was run in the same period as trial S3a but began earlier, so that day 75 in trial S3b corresponds to the same calendar date as day 0 in trial S3a.

Table 4.20 Calculated feedstock sulphate content at different CS : FW ratios

Parameter	Unit	R9	R10	R9	R10
CS : FW ratio	WW basis	1	2	3	4
FW2 sulphate [a]	mg SO_4 kg^{-1} WW	398	398	398	398
FW2 daily feed	g WW day^{-1}	33.4	25.8	21.1	17.8
CS2 sulphate [a]	mg SO_4 kg^{-1} WW	6876	6876	6876	6876
CS2 daily feed	g WW day^{-1}	33.4	51.6	63.3	71.2
SO_4 from CS	mg SO_4 kg^{-1} WW	3438	4584	5157	5501
Total SO_4	mg SO_4 kg^{-1} WW	3637	4717	5257	5580

[a] Based on values in Table 4.2

4.4.6.5 Results and discussion for co-digestion of FW2 and CS2 (trial S3b, R9&10)

Operating parameters for R9 and R10 are shown in Figure 4.37. As can be seen, the CS : FW ratios were maintained at the intended values until feeding stopped on day 330 (Figure 4.37a). The amount of food waste added was slightly reduced from day 46 (Figure 4.37b) leading to a small increase in HRT (Figure 4.37c) in order to adjust the organic loading rate to the desired value (Figure 4.37d).

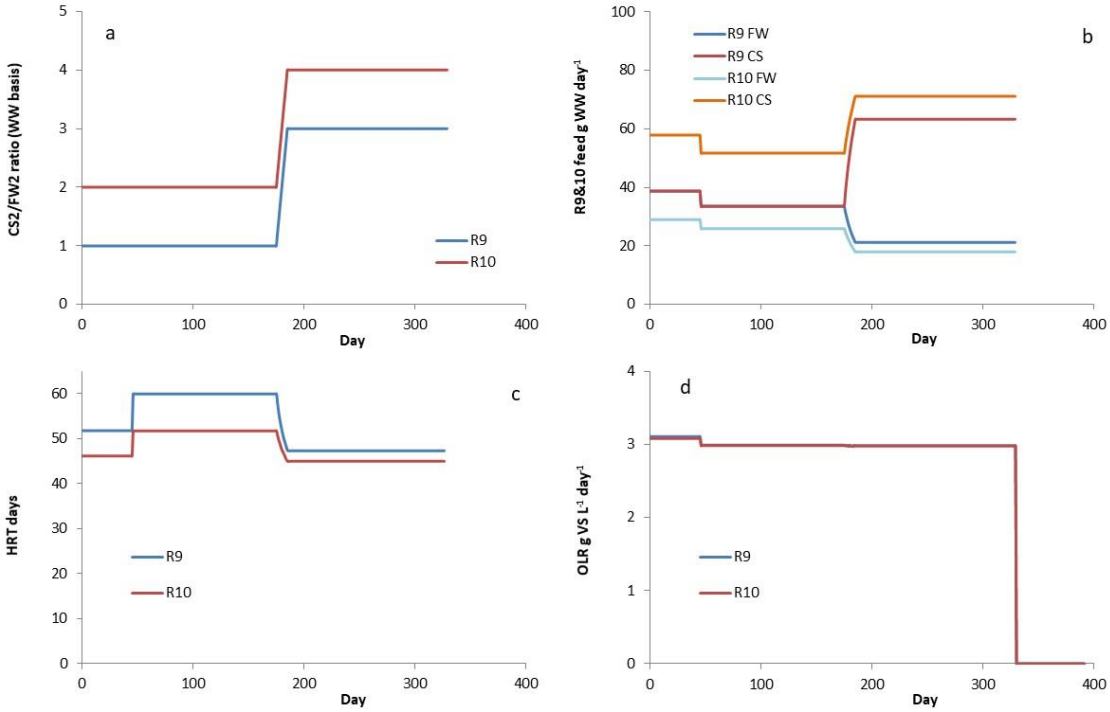


Figure 4.37 CS ; FW ratio, wet weight of feed, HRT and OLR during co-digestion of cattle slurry CS2 and FW2 (trial S3b)

Figure 4.38 shows monitoring parameters for R9 and R10 during the trial. Average values for the end of apparently stable operating periods at different CS : FW ratios are presented in Table 4.21. At CS : FW ratios of 1 : 1 and 2 : 1 (up to day 176) operation remained stable. TAN concentrations fell in both reactors, reflecting the change from the previous pure FW feedstock, with TAN slightly lower in R10 than R9 due to the higher proportion of CS in the mixed feed. pH showed minor fluctuations, and was lower in R10 than in R9 for much of this period, but stabilised at around 8 in both digesters. There was some fluctuation in alkalinity values, but IA/PA ratios remained below 0.7 and settled at around 0.4. The solids content rose slightly with the new feedstock, stabilising at around 11.0% TS and 7.4% VS on a wet weight basis in both digesters. Total VFA concentrations rose quite rapidly following the change in feedstock, peaking at around 3.4 g L^{-1} in R9 and 5.7 g L^{-1} in R10 on day 49, then gradually reduced in both digesters to around 2 g L^{-1} by day 177. Based on these results, at the applied CS : FW ratios of 1 : 1 and 2 : 1 both digesters appeared to be operating well.

After the incremental increase in feedstock CS : FW ratios to 3 : 1 and 4 : 1, stable operation continued for a further ~70 days, corresponding to around 1.5 HRT. On day 254, however, there was a sudden peak in total VFA concentrations to around 15 mg L^{-1}, indicating some disturbance to methanogenesis. The reason for this is uncertain, although the fact that it occurred simultaneously in both digesters may indicate some external cause. There was, however, no known temperature shock, mis-feeding or other incident in this period. By day 273 total VFA had fallen to 7.5 g L^{-1} in R9 and 5.1 g L^{-1} in R10, but over the following 30 days total VFA concentrations rose continually. This was reflected in a fall in PA, a rise in IA and a sharp increase in the IA/PA ratio and a fall in pH. From day 280 onwards the VS and TS concentrations also rose, indicating inhibition of hydrolysis.

On day 303 a one-off dose of $FeCl_3$ (based on a Fe : S molar ratio of 0.6) was added to R9 and R10 with a one-off addition of 8 mL of TE solution (Table 3.2) to raise the digestate TE concentration. From day 304 onwards $FeCl_3$ and TE solution were added daily at 0.550 g and 31.6 μL to R9 and at 0.661 g and 35.6 μL to R10, respectively, to maintain digester concentrations. Total VFA continued to rise, however, accompanied by a fall in total and partial alkalinity. Feeding was stopped on day 330 when the pH fell below 6.5, but monitoring of digestion parameters continued over the following weeks and the digesters showed signs of recovery with degradation of accumulated VFA and VS. Over the next 50 days VFA concentrations fell to < 100 mg L^{-1}, pH rose to around 8

and the IA/PA ratio returned to 0.4 – 0.5, indicating that there was no irreversible disturbance to the microbial community and the inhibition experienced was temporary.

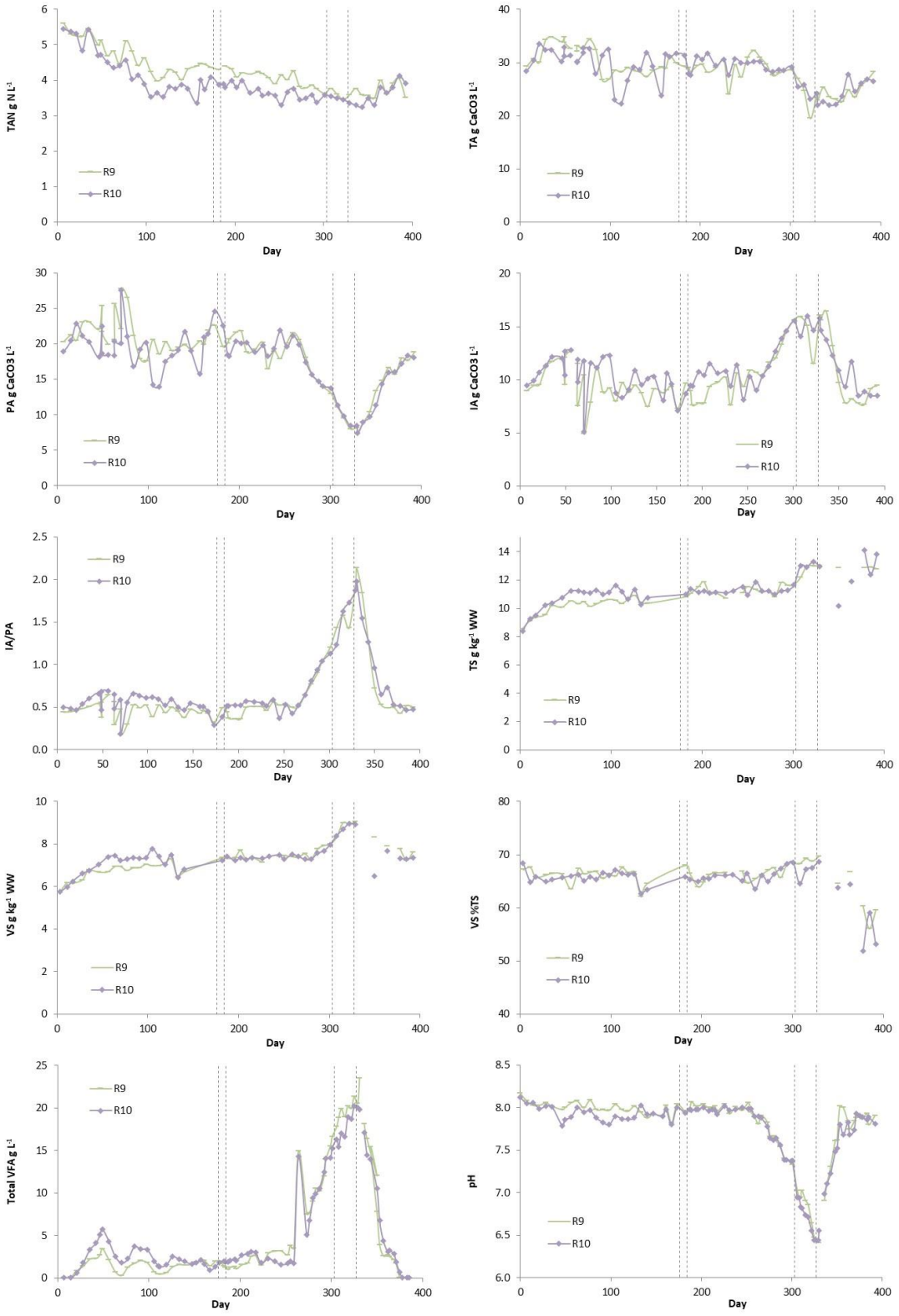


Figure 4.38 Monitoring parameters during trial S3b with variable CS : FW ratios. Vertical dotted lines indicate changes in ratio (day 176 – 185), $FeCl_3$ and TE addition (day 303 onwards) and end of feeding (day 330)

Table 4.21 Average values for key parameters in trial S3b during stable or pseudo-stable operating periods

Parameter	Unit	R9 [a]	R10 [a]	R9 [b]	R10 [b]
CS : FW	–	1 : 1	2 : 1	3 : 1	4 : 1
SBP	L g^{-1} VS	0.649	0.541	0.446	0.429
SMP	L g^{-1} VS	0.388	0.315	0.261	0.253
VBP	L L^{-1} day^{-1}	1.94	1.61	1.33	1.28
VMP	L L^{-1} day^{-1}	1.16	0.94	0.78	0.75
CH_4 content	% v/v	59.8	58.3	58.5	58.9
H_2S content	ppmv	6930	10997	15238	11403
pH	–	7.92	7.90	8.00	7.99
TA	g $CaCO_3$ kg^{-1} WW	29.54	29.50	29.27	30.19
PA	g $CaCO_3$ kg^{-1} WW	20.84	20.36	19.00	20.26
IA	g $CaCO_3$ kg^{-1} WW	8.70	9.13	10.27	9.93
IA/PA ratio	–	0.42	0.46	0.54	0.50
TAN	g N kg^{-1} WW	4.40	3.79	4.04	3.50
Total VFA	g L^{-1}	1.81	1.60	3.02	1.75

[a] Average day 147 – 176; [b] Average day 235 – 254

VFA profiles throughout the trial are shown in Figure 4.39. Acetic acid concentrations fluctuated in both digesters but were slightly higher in R10 up to day 176. Other than this there were no clear differences between the two digesters at different CS : FW ratios before or after the change in feedstock ratio. The peak on day 264 consisted primarily of acetic acid, at 10.4 and 11.0 g L^{-1} in R9 and R10 respectively, but there were also increases in propionic acid and other species. Addition of TE and $FeCl_3$ on day 303 did not appear to either accelerate or slow the rate of VFA accumulation in R10, although it coincided with a reduction in the rate of acetic accumulation in R9 and a fall in propionic acid concentrations. Accumulated acetic and n-butyric acid was consumed rapidly after feeding stopped, but propionic acid took longer to decline.

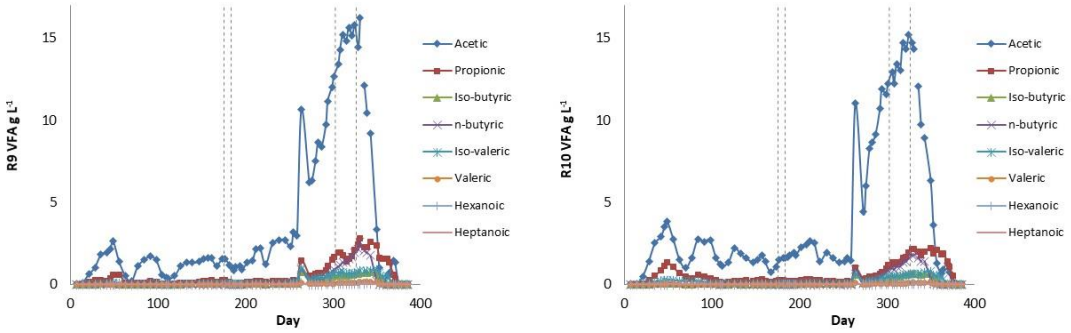


Figure 4.39 VFA profiles during trial S3b with variable CS : FW ratios. Vertical dotted lines indicate changes in ratio, $FeCl_3$ and TE addition and end of feeding

Figure 4.40 shows the VBP, SMP and biogas methane content for R9 and R10. At CS : FW ratios of 1 : 1 and 2 : 1 gas production parameters were relatively stable, with the higher biogas yield in R9 attributable in part to the higher proportion of FW and the longer HRT.

After the change in CS : FW ratio there was a step decrease in volumetric and specific gas production, with similar values seen in both digesters (Table 4.21). At this point the digesters appeared to be still operating quite stably, though with reduced SMP. Around day 264, however, after the peak in VFA, gas production began to fall and did not stabilise until around day 300. Biogas methane content also fell in this period. On day 330 when feeding was stopped, SMP had fallen to around 0.05 L g^{-1} VS with a biogas methane content of only 20%.

Although cattle slurry is normally considered slow to degrade, the cumulative gas production curves in Figure 4.2 show that both CS2 and FW2 achieved a high proportion of their final BMP values by day 30 of the BMP test. They might therefore be expected to achieve something close to the BMP value at the HRT used in this trial. Table 4.22 shows the predicted SMP based on BMP values for FW2 and CS2: the actual SMP at CS : FW ratio 1 : 1 is very close to the predicted value, indicating that there is little loss of methane in these conditions. The SMP is progressively lower at the higher CS : FW ratios even before the decline in gas production, suggesting that competition and inhibition maybe beginning to take effect. Figure 4.40d shows a strong linear relationship between volumetric and specific gas production and sulphate concentration from the CS component. Details of the BMP modelling are given in Appendix A in Table A6.

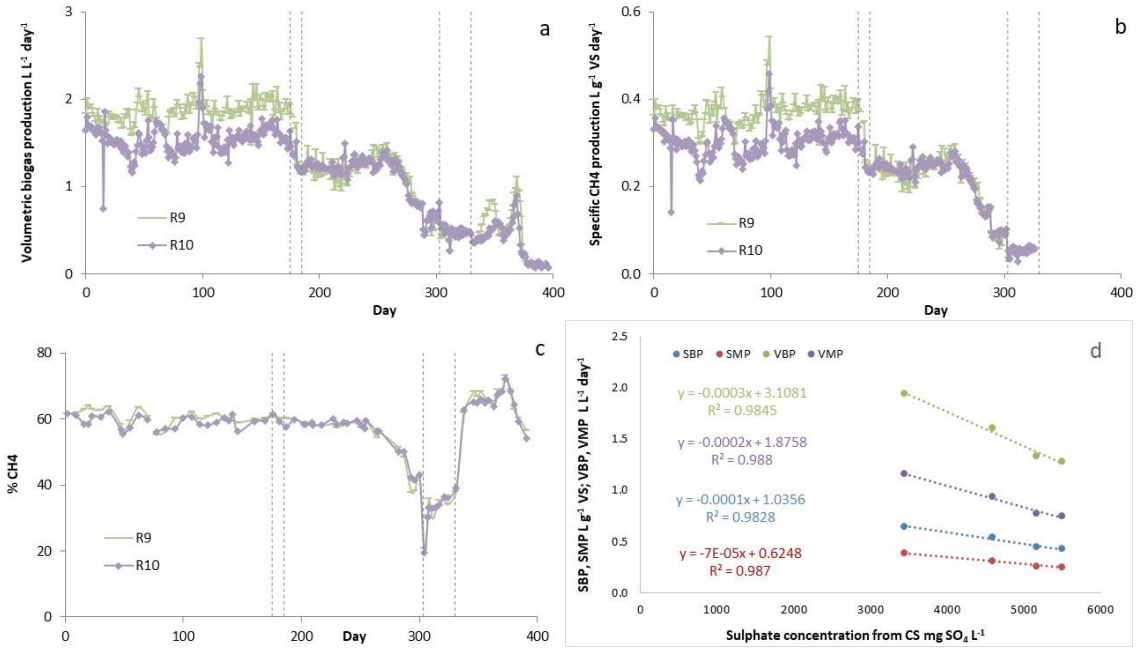


Figure 4.40 Gas production results during trial S3b with variable CS : FW ratios. Vertical dotted lines indicate changes in ratio, FeCl and TE addition and end of feeding

Table 4.22 Actual and predicted values of SMP and VMP for co-digestion trial S3b

Parameter	**Unit**	**R9**	**R10**	**R9**	**R10**
CS : FW ratio	WW basis	1	2	3	4
FW addition	g WW day^{-1}	33.4	25.8	21.1	17.8
FW addition	g VS L^{-1} day^{-1}	2.13	1.64	1.34	1.13
CS addition	g WW day^{-1}	33.4	51.6	63.3	71.2
CS addition	g VS L^{-1} day^{-1}	0.87	1.34	1.64	1.84
OLR	g VS L^{-1} day^{-1}	3.0	3.0	3.0	3.0
HRT	days	60	52	47	45
SMP	L CH_4 g^{-1} VS	0.388	0.315	0.261	0.253
VMP	L CH_4 L^{-1} day-^{1}	1.16	0.94	0.78	0.75
Predicted SMP[a]	L CH_4 g^{-1} VS	0.375	0.323	0.290	0.267
Predicted SMP[b]	L CH_4 g^{-1} VS	0.390	0.342	0.311	0.290
Actual/ Predicted SMP[a]	%	103.5%	97.5%	90.0%	94.6%
Actual/ Predicted SMP[b]	%	99.5%	92.0%	83.8%	87.2%

[a] based on feedstock ratios and experimental SMP values; [b] based on feedstock ratios and BMP kinetic coefficients

The above results indicate that stable operation is possible at CS : FW ratios of 1 : 1 and 2 : 1, corresponding to sulphate concentrations from the CS component of 3.4 and 4.5 g SO_4 L^{-1}, or 3.6 and 4.7 g SO_4 L^{-1} taking into account the food waste component. At the higher CS : FW ratios of 3 : 1 and 4 : 1 stable operation appeared to be possible to around day 264 when rapid VFA accumulation was seen. Until then there was a strong linear

relation between sulphate addition and SMP, suggesting that substrate competition from SRB was responsible for much of the loss in methane productivity up to that point.

4.4.6.6 Sulphide results for trials S3a and S3b

Sulphide measurements were taken during trials S3a and S3b. The most reliable values were considered to be from headspace gas measurements using the H_2S-AE sensor, taken from day 74 onwards in trial S3a and day 149 onwards in trial S3b. The methylene blue method was attempted for soluble sulphide determination, but was ineffective due to interference from the strong colour present in samples derived from cattle slurry. The ion selective electrode appeared to give more consistent results, but values were generally lower than those obtained by calculation, particularly for the CS-only digesters in trial S3a. The measurements were also time-consuming and not highly replicable for spiked samples, despite showing good repeatability with standards. It was therefore decided to use the measured headspace H_2S concentrations and use calculated values for dissolved H_2S and HS^-.

Figure 4.41 shows measured and calculated sulphide values for trials S3a and S3b. Once the initial peak in response to a step increase in sulphate in trial S3a had dissipated, the measured headspace H_2S concentrations at added sulphate concentrations of 1000, 2000 and 3000 mg SO_4 L^{-1} were considerably lower between days 176 – 212 than at any of the CS : FW ratios tested in trial S3b (Figure 4.41a and b). The actual volumes of gaseous H_2S generated (Figure 4.41c and d) were consistently lower in trial S3a than in trial S3b up to the point when feeding in trial S3b ceased on day 300. This reflects both the lower H_2S concentrations in trial S3a and the smaller volumes of biogas generated by a CS-only feedstock at OLR 2 g VS L^{-1} day^{-1} compared to the FW : CS feed at OLR 3 g VS L^{-1} day^{-1} as used in trial S3b.

Figures 4.41e and f show the proportion of dissolved hydrogen sulphide present in the more toxic form of H_2S. Up to the point at which VFA accumulation began in trial S3b around day 264, this proportion was lower in trial S3b than in trial S3a. This was due to the respective pH values, which averaged around 7.5 in the CS only digesters R1 – R6 in trial S3a and around 8.0 in the FW : CS digesters R9&10 until the rise in VFA concentrations in trial S3b. In both cases, however, the proportion as H_2S was low, at < 20% in the CS digesters in trial S3a and < 6% in the FW : CS digesters in trial S3b before

VFA accumulation occurred. The actual concentration of dissolved H_2S in each set of digesters in both trials mirrors the headspace H_2S concentration in each case, and is shown in Figure 4.41a and b. The concentration of dissolved HS^- is shown in Figures 4.41g and h. As this made up the majority of the dissolved hydrogen sulphide, the profile is similar to that for total dissolved H_2S as shown in Figures 4.41i and j.

Comparison of data from the two trials helps to explain several features of their performance. The H_2S data sets were not complete or replicated enough to permit proper statistical analysis of relationships with all other monitoring parameters, but examples for some average values in relatively stable periods are shown in Table 4.23.

Until day 219 of trial S3a, added sulphate concentrations in R1&2 and R3&4 were lower than the sulphate values in trial S3b. The concentration of 3000 mg SO_4 L^{-1} in R5&6 was only slightly below that in R9, however, which was around 3438 mg SO_4 L^{-1} at the FW : CS content of 1 : 1. Total VFA concentrations were slightly elevated in both cases, but the average total VFA in R5&6 between days 189 – 219 was 0.27 g L^{-1}, which was considerably less than the average of 1.88 g L^{-1} in R9 between days 152 – 175. This is likely due to differences in the total dissolved sulphide concentrations in these periods, which averaged around 0.13 g S L^{-1} in R5&6 compared to 0.41 g S L^{-1} in R9 (Figure 4.41i and j).

After sulphate addition was increased in trial S3a (day 219 onwards), the headspace H_2S and dissolved H_2S content in R1&2 at 4000 mg SO_4 L^{-1} (Figure 4.41a) was similar to that in R10 (Figure 4.41b) when it was working at a FW : CS ratio of 2 : 1 and 4584 mg SO_4 L^{-1} (up to day 175). In these same periods, however, the total VFA concentrations in R1&2 were around five times lower than in R10, and again this is reflected in a difference in total dissolved sulphide concentrations in each case (Figure 4.41i and j).

On the other hand, similar concentrations of dissolved HS^- and total dissolved sulphide occurred in R5&6 at the end of trial S3a (days 249 to 298) and in R10 from day 217 – 264 in trial S3b (Figure 4.41i and j). In these periods the average total dissolved sulphide concentration in R5&6 and R10 was around 0.67 g S L^{-1} and the average total VFA concentrations were 1.6, 2.1 and 1.9 g L^{-1} in R5, R6 and R10 respectively. The two trials thus appeared to show similar behaviour in terms of VFA accumulation when total dissolved sulphide concentrations were the same. A wide range of total soluble sulphide concentrations have been reported as inhibitory due to differences in environmental

factors and composition of the microbial consortium, as well as occasional confusion between inhibition and substrate competition (Chen et al., 2008). O'Flaherty et al. (1998) noted that total dissolved sulphide concentrations were more critical than dissolved H_2S at pH values above 7.2: the results from this study confirm the importance of dissolved sulphide in these conditions, and also indicate that failure of digestion of cattle slurry CS2 in trials S1 and S2 was likely due to the high sulphate concentrations in the undiluted feedstock.

After the start of sulphate addition in trial S3a there was a transient peak in headspace H_2S concentration around day 135 (Figure 4.41a): this probably represented the response to the initial step increase in sulphate concentration. In R5&6 this led to total soluble sulphide concentrations of around 0.8 g S L^{-1} (Figure 4.41i), followed in the next few days by VFA peaks of up to 1 g L^{-1}. Similar or slightly higher total sulphide concentrations in R9 and R10 around day 191 – 210 (Figure 4.41j) were associated with VFA concentrations of 1 – 2 g L^{-1}. In trial S3b the VFA consisted almost entirely of acetic acid (Figure 4.35) whereas in trial S3a the transient peaks included some propionic acid (Figure 4.39). Rinzema et al. (1988) reported that propionate degradation could be inhibited at dissolved H_2S concentrations above 100 mg L^{-1}. The dissolved H_2S concentration in trial S3a was above that in trial S3b at this point (Table 4.23), peaking at around 0.12 g S L^{-1}, which may explain this difference.

The soluble sulphide speciation may also provide a possible explanation for the rapid onset of VFA accumulation leading to temporary digestion failure in trial S3b. As noted above, this could have been due to an unknown external factor. It is interesting to observe, however, that the first sign of a change is a drop in pH between day 252 and 259 from around 8.0 to 7.9 in digesters R9&10. Although small, this could result in an increase of almost 50% in the proportion of dissolved sulphide present as the more toxic H_2S. This in turn could increase inhibition of methanogenesis, causing a rise in VFA accumulation and a further small reduction in pH; and thus leading by small incremental steps to a downward spiral. As can be seen from Figures 4.38 and 4.41d this did fact occur: there was a sharp increase from 6 % to around 35 % in the proportion of dissolved H_2S by day 303 and a rise in concentration to 0.12 g S L^{-1}. The rapid rate of change may also have been a disruptive factor. Unfortunately some of the monitoring data are too sparse to be certain which factor if any initiated the failure, and how it progressed on a daily basis. This observation raises the possibility, however, that while co-digestion of the high-

sulphate cattle slurry with food waste offers some protection through dilution of the sulphate content and through increasing the pH to favour the less toxic form of HS^-, it can also contribute to the risk of sudden failure due to the greater potential for a rapid shift in pH and therefore sulphide equilibrium once the buffering capacity of the system is overcome. Examples of food waste digesters running for long periods at high pH and low IA/PA ratios despite significant VFA concentrations have been reported in the literature (e.g. Banks et al., 2012; Zhang et al., 2012a) and are also known to occur in industry (Pers. Com., Prof Charles Banks).

$FeCl_3$ was added from day 303 onwards in trial S3b in an attempt to reduce the headspace H_2S concentration, but also led to a further fall in pH (Figure 4.38) which may have added to the inhibition both directly and through a further shift in equilibrium: as can be seen in Figure 4.41f, the proportion present as H_2S had increased to over 65% on day 330 when feeding was stopped.

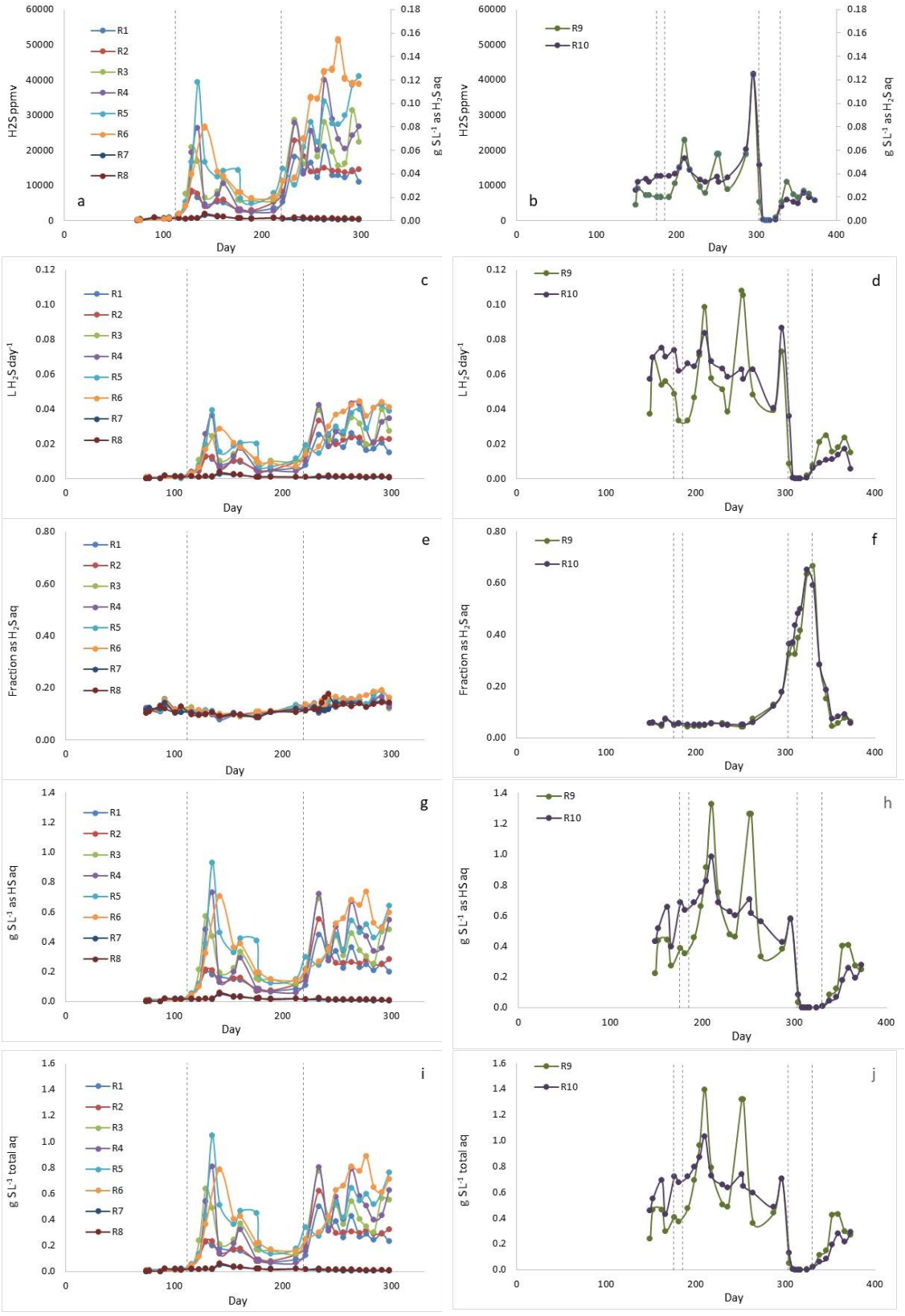


Figure 4.41 Hydrogen sulphide parameters in digestion trials S3a and S3b

Table 4.23 Values for sulphate, pH, sulphide and VFA concentrations in trials S3a and S3b

	Day	SO_4	pH	Headspace H_2S	Fraction as H_2S	Dissolved H_2S	Dissolved HS^-	Total dissolved	VFA
		mg L^{-1}	-	ppmv	%/100	g S L^{-1}	g S L^{-1}	g S L^{-1}	g L^{-1}
R5	189 – 219	3.0	7.58	5117	0.12	0.02	0.11	0.13	0.27
R6	189 – 219	3.0	7.58	6083	0.12	0.02	0.13	0.15	0.27
R9	152 – 175	3.4	7.94	7523	0.06	0.02	0.39	0.41	1.88
R1	249 – 298	4.0	7.50	14138	0.14	0.04	0.26	0.30	0.30
R2	249 – 298	4.0	7.50	14204	0.14	0.04	0.26	0.30	0.28
R10	152 – 175	4.5	7.92	11567	0.06	0.03	0.56	0.60	1.63
R5	291 – 310	7.0	7.39	39787	0.17	0.12	0.57	0.69	1.64
R6	291 – 310	7.0	7.38	39096	0.18	0.12	0.54	0.66	2.11
R10	230 – 260	5.5	7.99	11403	0.05	0.03	0.64	0.67	1.87
R5	135 – 142	3.0	7.65	27976	0.10	0.08	0.69	0.78	1.07 [a]
R6	135 – 142	3.0	7.66	26654	0.10	0.08	0.71	0.79	1.02 [a]
R9	191 – 210	5.1	8.03	13823	0.05	0.04	0.84	0.88	1.19
R10	191 – 210	5.5	7.96	14682	0.05	0.04	0.81	0.86	2.16

[a] Value on day 148 of trial S3a

For dates on which sufficient data were available, sulphate removal rates were estimated based on a mass balance of sulphur entering as SO_4 in the feed and leaving as sulphide in the liquid or gaseous phases, according the the calculate values shown in Figure 4.41. The results are shown in Figure 4.42. There was considerable fluctuation in calculated removal rates on a day-to-day basis, but values between 40 – 60% were achieved during more stable periods in in trial S3a and around 30% in trial S3b.

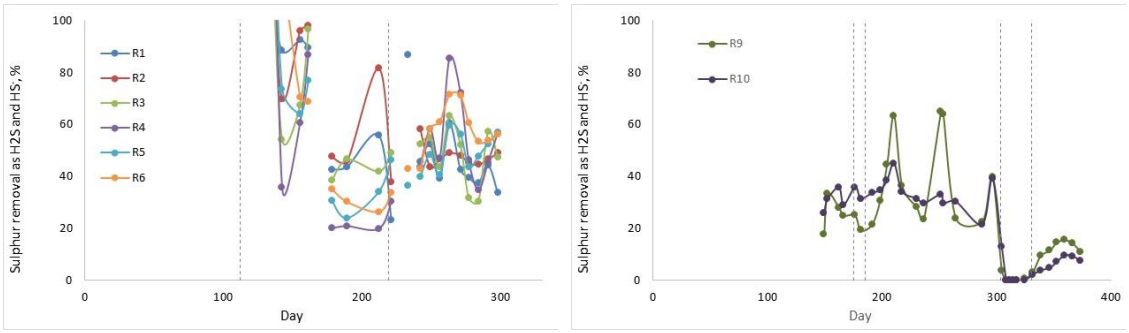


Figure 4.42 Calculated removal rates for sulphate in digestate and gaseous phases in trials S3a and S3b

The loss of SMP at different sulphate loadings was estimated as shown in Tables 4.24 and 4.25, based on a conversion factor of 0.35 L CH_4 g^{-1} COD. The stoichiometric requirement for sulphate removal is theoretically 0.67 g COD g^{-1} SO_4, although in practice higher values are commonly found especially with carbohydrate-rich feedstocks (Hoeks et al.,

1984). The calculated COD/SO_4 ratio was between 2.0 – 3.5 for trial S3b indicating that the 'missing' SMP was more than sufficient to account for the 30% of sulphate removal achieved. A small amount of COD was also lost as VFA but based on the values in Table 4.23 this only represented about 15% of the loss in SMP.

Table 4.24 COD and sulphate removal in trial S3a

Parameter	**Unit**	**SO4 added (mg SO_4 L^{-1})**						
		1000	**2000**	**3000**	**4000**	**5000**	**6000**	**7000**
Loss of SMP [a]	L CH_4 g^{-1} VS	0.015	0.016	0.043	0.028	0.050	0.044	0.087
	g COD g-1 VS	0.043	0.044	0.122	0.081	0.142	0.126	0.249
Feed SO_4 conc	g SO_4 g^{-1}VS	0.010	0.019	0.029	0.039	0.048	0.058	0.068
COD : SO_4 ratio	g COD g^{-1} SO4	4.50	2.30	4.21	2.10	2.93	2.18	3.69

[a] Calculated from Table 4.19

Table 4.25 COD and sulphate removal in trial S3b

Parameter	**Unit**	**R9**	**R10**	**R9**	**R10**
CS : FW ratio	WW basis	1	2	3	4
Loss of SMP [a]	L CH_4 g^{-1} VS	-0.004	0.022	0.045	0.033
	g COD g^{-1} VS	–	0.062	0.130	0.094
Feed sulphate content	g SO_4 kg^{-1} WW	3.4	4.6	5.2	5.5
Feed VS content	g VS kg^{-1} WW	179	154	141	134
Feed sulphate content	g SO_4 g^{-1} VS	0.019	0.030	0.036	0.041
COD : SO_4 ratio	g COD g^{-1} SO4	–	2.07	3.56	2.28
Digestate VFA content	g COD L^{-1}	1.81	1.60	3.02	1.75
Digestate removal	mL day^{-1}	66.8	77.4	84.4	89
COD lost as VFA	g COD/day	0.12	0.12	0.26	0.16
as a proportion of SMP		–	16.8%	16.5%	14.0%

[a] Calculated from predicted and actual values in Table 4.22

Conclusions. The results from the above trials, in conjunction with those from trial S1 and S2, gave a clear indication of the limitations on digestion of high-sulphate cattle slurry. In trial S1 and S2 it proved impossible to achieve stable digestion at a sulphate concentration of around 6.9 g SO_4 L^{-1}. This was supported by the results from trial S3a, in which digestion with low-suphate cattle slurry spiked to give an added sulphate concentration of 7 g SO_4 L^{-1} showed progressive VFA accumulation. At added sulphate concentrations between 4 – 6 g SO_4 L^{-1} stable digestion was possible but signs of inhibition of hydrolysis were observed in the rise in VS content. At 3 g SO_4 L^{-1} or less there was little sign of inhibition apart from very slight elevations in VFA content, but the effects of competition by SRB were evident in reduced SMP. These results were based

on spiking of low-sulphate cattle slurry with SO_4 and were backed up by similar findings from co-digestion of high sulphate cattle slurry and food waste at different ratios. The equilibrium between dissolved HS^- and the more toxic H_2S is highly dependent on pH. The two trials showed comparable responses in terms of VFA accumulation when similar concentrations of HS^- or total dissolved sulphide were seen, as this is the dominant form in the pH range at which digestion occurred. In the case of co-digestion with food waste, the higher TAN concentration raises the pH and provides additional buffering. If the digester buffering is overcome by build-up of VFA, however, or there is a sudden pH shift for some other reason, this can result in rapid progressive failure as the fall in pH shifts the H_2S equilibrium, which in turn increases the inhibitory conditions.

4.5 Discussion

This section summarises and compares some results from the individual trials from the viewpoint of energy production and digestion stability.

Table 4.26 shows the SMP and VMP in trials C1 and C2 expressed as a percentage of the values for the cattle slurry controls. The same information is also provided for the stable and pseudo-stable periods in trial S3a, with results expressed as a percentage of the cattle slurry controls in trials C1 (CS1) and S2 (CS2) as mono-digestion controls were not run during this trial. Values shown are based on the actual OLR calculated from the average feedstock VS during the trial, rather than the nominal OLR values.

It can be seen that in each case the SMP and VMP for co-digestion is considerably higher than for the cattle slurry controls. The SMP for the control substrates varies, but the driving force for methane production in co-digestion is clearly the food waste.

Table 4.26 Increase of SMP and VMP of co-digestion compared to cattle slurry controls

Trial	Parameter	Unit					
C1	CS1/FW1	w/w	3	3	3	-	0
	OLR	g VS L^{-1} day^{-1}	3.1	4.1	5.1	1.6	3.2
	SMP	L CH_4 g^{-1} VS	0.332	0.330	0.324	0.194	0.449
	SMP incr[a]	%	171%	170%	167%	-	-
	VMP	L CH_4 L^{-1} day^{-1}	1.018	1.349	1.657	0.314	1.447
	VMP incr[a]	%	324%	430%	528%	-	-
C2	CS1/FW2	w/w	6	6	6	-	0
	OLR	g VS L^{-1} day^{-1}	2.9	3.8	4.8	2.9	2.9
	SMP	L CH_4 g^{-1} VS	0.239	0.221	0.215	0.053	0.458
	SMP incr[a]	%	449%	415%	404%	-	-
	VMP	L CH_4 L^{-1} day^{-1}	0.687	0.849	1.033	0.152	1.298
	VMP incr[a]	%	452%	559%	680%	-	-
S3b[a]	CS2/FW2	w/w	1	2	3	4	-
	OLR	g VS L^{-1} day^{-1}	3.0	3.0	3.0	3.0	-
	SMP	L CH_4 g^{-1} VS	0.388	0.315	0.261	0.253	-
	SMP incr[c]	%	200%	162%	135%	130%	-
	SMP incr[d]	%	8313%	6739%	5584%	5411%	-
	VMP	L CH_4 L^{-1} day^{-1}	1.163	0.939	0.778	0.753	-
	VMP incr[c]	%	370%	299%	248%	240%	-
	VMP incr[d]	%	8549%	6903%	5724%	5537%	-

[a] based on ratio of co-digestion SMP to SMP of cattle slurry controls; [b] during pseudo-stable operation as defined in Table 4.20; [c] based on ratio of co-digestion SMP to CS1 cattle slurry controls in trial C1; [d] based on ratio of co-digestion SMP to CS2 cattle slurry controls in trial S2

Banks, Salter, et al. (2011) looked at co-digestion of food waste and cattle slurry in proportions based on balancing the nutrient demand of farms in Hampshire. The county of Hampshire has a high human population and a relatively small dairy farming sector: unusually for the UK it also has three incinerators for municipal solid wastes, making centralised pasteurisation of food waste using the waste heat from combustion a particularly attractive option. The ratio of cattle slurry and food waste production can vary considerably from area to area, however. In the current research, trials were run at CS : FW ratios of 3 : 1 and 6 : 1 on a wet weight basis to confirm and extend the results of earlier studies. The ratio of 6 : 1 was based on an estimate of the overall tonnages of these materials in the UK, but these values may change. Based on the contributions of food waste and cattle slurry to the methane yield in co-digestion, however, and the relative volumes of these materials, the most sensible option will always be to transport the food waste to the cattle slurry digester, especially if the digestate can be applied to land locally as a means of promoting closed-loop nutrient recovery.

Table 4.27 shows the calorific value of the methane production in each trial expressed as a proportion of the theoretical calorific value (ThCV) of the feedstocks. The proportion of theoretical CV recovered as methane for FW1 and FW2 as mono-substrates was 73 and 74% respectively: this is close to the value of 79% obtained by Yirong et al. (2017) in mesophilic digestion of a similar food waste. The cattle slurry controls showed greater variability, as expected, at 31% and 10% for CS1 in trials C1 and C2. The equivalent values for CS2 and CS3 controls in trials S2 and S3a were less than 1% and 25%, respectively. Despite this, the co-digestion mixtures still show good recovery with only a moderate decline at higher OLR. Anaerobic digestion is often cited as the most appropriate technology for 'wet' organic wastes and biomass since, unlike thermal processing, it can recover a high proportion of the energy potential in the form of methane. The results obtained support this view.

Table 4.27 SMP as a proportion of theoretical calorific value of feedstocks

Trial	**Parameter**	**Unit**					
C1	CS1/FW1	w/w	3	3	3	-	0
	OLR	g VS L^{-1} day^{-1}	3.1	4.1	5.1	1.6	3.2
	FW addition	g VS L^{-1} day^{-1}	1.85	2.46	3.08	0.00	3.23
	CS addition	g VS L^{-1} day^{-1}	1.22	1.62	2.03	1.62	0.00
	SMP	L CH_4 g^{-1} VS	0.332	0.330	0.324	0.194	0.449
	SMP	MJ kg^{-1} VS	13.2	13.1	12.9	7.7	17.9
	ThCV [a]	MJ kg^{-1} VS	23.4	23.4	23.4	21.9	24.4
	SMP/ThCV	%	57%	56%	55%	35%	73%
C2	CS1/FW2	w/w	6	6	6	-	0
	OLR	g VS L^{-1} day^{-1}	2.9	3.8	4.8	2.9	2.9
	FW addition	g VS L^{-1} day^{-1}	1.12	1.49	1.86	0.00	2.91
	CS addition	g VS L^{-1} day^{-1}	1.76	2.34	2.93	2.86	0.00
	SMP	L CH_4 g^{-1} VS	0.239	0.221	0.215	0.053	0.458
	SMP	MJ kg^{-1} VS	9.5	8.8	8.6	2.1	18.2
	ThCV [a]	MJ kg^{-1} VS	23.0	23.0	23.0	21.9	24.8
	SMP/ThCV	%	41%	38%	37%	10%	74%
S3b [b]	CS2/FW2	w/w	1	2	3	4	-
	OLR	g VS L^{-1} day^{-1}	2.99	2.98	2.98	2.98	-
	FW addition	g VS L^{-1} day^{-1}	2.13	1.64	1.34	1.13	-
	CS addition	g VS L^{-1} day^{-1}	0.87	1.34	1.64	1.84	-
	SMP	L CH_4 g^{-1} VS	0.39	0.31	0.26	0.25	-
	SMP	MJ kg^{-1} VS	15.5	12.5	10.4	10.1	-
	ThCV [a]	MJ kg^{-1} VS	24.0	23.6	23.3	23.1	-
	SMP/ThCV	%	64%	53%	45%	44%	-

[a] calculated pro rata from CV in Table 4.1 and feedstock VS input, at 39.84 MJ m^{-3} CH_4; [b] during pseudo-stable operation as defined in Table 4.20; [b] based on trial C1 CS controls

Estimation of methane production using the kinetic coefficients from BMP testing provided useful insights into the co-digestion trials. Table 4.28 shows the actual SMP

values in each trial and the predicted BMP based on modelling coefficients. This information is also provided in Tables 4.12, 4.16 and 4.22, but is presented again here for ease of comparison. As noted earlier, agreement between actual and predicted values was generally good, reflecting the relatively long HRT in these trials. As the HRT reduces, the ratio of actual to predicted SMP falls: this probably mainly reflects the effect of daily digestate removal rather than over-loading, as the OLR used are still quite moderate. Taken together, these results support the view that co-digestion of food waste and cattle slurry considerably increases the feedstock methane potential without reducing the combined HRT to a point where there are major losses in SMP.

Table 4.28 Actual SMP versus predicted values based on BMP kinetic coefficients

Trial	Parameter	Unit					
C1	CS1/FW1	w/w	3	3	3	-	0
	OLR	g VS L^{-1} day^{-1}	3.1	4.1	5.1	1.6	3.2
	HRT	days	33.3	25.0	20.0	33.3	76.3
	Predicted SMP [b]	L CH_4 g^{-1} VS	0.347	0.347	0.347	0.194	0.449
	Actual/Predicted SMP [b]	%	96%	95%	93%	-	-
C2	CS1/FW2	w/w	6	6	6	-	0
	OLR	g VS L^{-1} day^{-1}	2.9	3.8	4.8	2.9	2.9
	HRT	days	25.7	19.3	15.4	18.5	69.0
	Predicted SMP [b]	L CH_4 g^{-1} VS	0.210	0.210	0.210	0.053	0.458
	Actual/Predicted SMP [b]	%	113%	105%	102%	-	-
S3b [a]	CS2/FW2	w/w	1	2	3	4	-
	OLR	g VS L^{-1} day^{-1}	3	2.98	2.98	2.97	-
	HRT	days	59.9	51.7	47.4	44.9	-
	Predicted SMP [b]	L CH_4 g^{-1} VS	0.375	0.323	0.290	0.267	-
	Actual/Predicted SMP [b]	%	104%	97%	90%	95%	-

[a] during pseudo-stable operation as defined in Table 4.20; [b] based on feedstock ratios and experimental SMP values

BMP measurements and analyses are also useful as the shape of the gas production curves and the coefficients obtained from it allow assessment of the likely importance of HRT in semi-continuous operation, while the kinetic coefficients indicate the proportion of readily degradable material and the degree of recalcitrance of the more and less degradable fractions. The modelling approach adopted in this work has been used for various substrates (Rao et al., 2000; Roberts et al., 2019; Mushtaq et al., 2019) and these results provide additional comparative data.

The results confirm that the energy potential of cattle slurry from different sources can vary considerably. The reason for the difference in the SMP of cattle slurry CS1 in trials C1 and C2 is uncertain. It may have been due to storage, but farmers do store cattle slurry for extended periods when land access is restricted. The laboratory trials in this research were carried out with specific batches of feedstock each with relatively homogeneous characteristics. In large-scale systems where cattle slurry is collected and fed to the digester on a regular basis there may be more day-to-day variation, in some cases potentially reducing the risk of build-up of inhibitory components.

The results for trial S3b in Tables 4.26, 4.27 and 4.28 clearly show the effects of dilution of the high sulphate cattle slurry. Under stable steady-state conditions at CS : FW ratios of 1 : 1 and 2 : 1 there were significant improvements in SMP and VMP compared to mono-digestion of cattle slurry, with good recovery of the theoretical CV as methane and little or no sign of inhibition of methane production. While use of gypsum bedding is now banned in the UK, other toxicants could be expected to show similar behaviour. These results again support the benefits of co-digestion of cattle slurry with food waste in providing a more stable baseline with positive energy production. The data obtained in the current work can be used to extend future studies and provide a basis for assessment of economic viability of co-digestion schemes.

5 CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

This research aimed to quantify the potential of co-digestion of food waste and cattle slurry in mesophilic conditions as a means of increasing on-farm biogas yields, in particular methane production, in comparison with anaerobic digestion of cattle slurry as a single substrate. This would improve the process economics and thus uptake of the technology, with associated social and environmental benefits. The effects of using cattle slurry with a high sulphate content were not part of the original work plan, but were also considered since one source of cattle slurry used came from a farm using gypsum bedding.

The following main conclusions can be drawn from the results of this research:

- Cattle slurry has relatively high concentrations of trace elements compared to food waste, which were sufficient to provide the requirement for stable anaerobic digestion and biogas production. When food waste was digested with cattle slurry at CS : FW ratios of 1: 1, 2 : 1, 3 : 1 or 6 : 1 on a wet weight basis, no additional trace elements were needed for stable operation. Mono-digestion of food waste without trace element supplementation was demonstrated to lead to VFA accumulation, once again supporting the results of previous studies.
- BMP tests showed that the food waste batches tested had higher SBP and SMP compared to the cattle slurry batches. SBP of food waste was 0.648 – 0.714 L biogas g^{-1} VS compared to 0.278 – 0.302 L biogas g^{-1} VS for cattle slurry; and SMP of food waste 0.459 – 0.470 L CH_4 g^{-1} VS compared to 0.172 – 0.193 L CH_4 g^{-1} VS for cattle slurry. These results confirmed the view that co-digestion of cattle slurry with food waste could increase the biogas and methane yield compared to cattle slurry alone, giving the potential for uplift in farm incomes.
- In semi-continuous mono-digestion of cattle slurry, different batches had widely different gas production potentials, ranging from 0.20 – 0.55 L L^{-1} day^{-1} for VBP and 0.004 – 0.203 L g^{-1} VS for SMP. The lowest values in each case were from a farm using gypsum bedding, and were attributed to the high sulphate concentration leading to sulphide toxicity.

- For cattle slurry without gypsum bedding, co-digestion with food waste at CS : FW wet-weight ratios of 3 : 1 and 6 : 1 was feasible in mesophilic conditions. Compared to the baseline for mono-digestion of cattle slurry, all co-digestion conditions showed significant increases in SMP. At both ratios, the lower OLR of 3 g VS L^{-1} day^{-1} was optimal in terms of SMP as it gave higher values (of 0.332 and 0.239 L g^{-1} VS for ratios of 3 : 1 and 6 : 1 respectively) than those at OLR of 4 and 5 g VS L^{-1} day^{-1} at the same ratios.
- VMP for co-digestion was also consistently higher than for cattle slurry mono-digestion and increased significantly with increasing OLR at both CS : FW ratios tested. Although the SMP was marginally higher at lower OLR, in practice economic considerations mean that this increase in VMP is more significant, as higher values could increase farm incomes and reduce capital expenditure and payback periods.
- The results indicated that even where cattle slurry quality is poor, with low SMP and VMP, co-digestion with food waste can bring the combined gas production up to higher and more consistent values. The higher volumes and relative stability of gas production are likely to be important factors in the design and performance of downstream gas utilisation equipment, such as combined heat and power (CHP) or gas upgrading systems, and in the overall economic viability of the digestion plant.
- Cattle slurry collected from the farm using gypsum as bedding for the cattle was not a good feedstock either as single feed or for co-digestion with food waste. Mono-digestion failed and only less than 35% methane was detected in the biogas and the SMP was below 0.03 L g^{-1} VS. Dissolved sulphides in the digestate were up to 500 mg L^{-1} which exceeds the toxicity limit of 200 mg L^{-1}. Since this study was carried out, the use of gypsum bedding has been prohibited in the UK; but where this practice continues it will severely limit the practicality of using this material as an anaerobic digestion feedstock. Stable co-digestion of cattle slurry containing 6.8 g SO_4 L^{-1} was possible at CS : FW ratios of 1 : 1 or 2 : 1.
- BMP data was used to derive kinetic coefficients based on a first-order pseudo-parallel model. This allowed reasonably accurate prediction of SMP values in co-digestion trials, especially at longer HRT where the effect of daily removal of a proportion of the digestate is not significant. Comparison of values derived from this approach with those based on the SMP of mono-digestion controls on a pro rata basis

was able to provide additional insights into possible mechanisms for any reduction in gas productivity.

- An attempt to reduce sulphate-induced toxicity in the gypsum-laden cattle slurry by addition of ferric chloride was unsuccessful, probably as a result of too low a dosage. The results did indicate, however, that soluble sulphide concentrations were reduced and the onset of failure was delayed.
- In trials where calcium sulphate ($CaSO_4.2H_2O$) was deliberately spiked into a cattle slurry feedstock, the SMP dropped from 0.143 L g^{-1} VS for controls with no sulphate addition to 0.055 L g^{-1} VS at a concentration of 7000 mg SO_4 L^{-1}. Hydrogen sulphide in the gas phase increased from 449 ppmv for the control to 39441 ppmv in digesters with 7000 mg SO_4 L^{-1}. Elevated concentrations of acetic and propionic acid were seen at 4000 mg SO_4 L^{-1} indicating the onset of mild inhibition. At 7000 mg SO_4 L^{-1} rapid and progressive accumulation of VFA was observed indicating full inhibition of methanogenesis.
- Comparison of the results from sulphate spiking of cattle slurry with those from co-digestion of gypsum-containing cattle slurry showed some differences in terms of the onset of VFA accumulation in relation to SO_4 concentration. When total dissolved sulphide concentrations were considered, however, the behaviour of the two was similar.
- Higher sulphide concentrations may be tolerated in co-digestion of sulphate-bearing cattle slurry with food waste, due to the increase in pH compared to cattle slurry mono-digestion. If VFA accumulation occurs to the point where there is a fall in pH, however, the proportion of dissolved sulphide present as the more toxic form of H_2S will increase, making failure more rapid and recovery more challenging.

5.2 Areas for Further Research

The following areas would benefit from further research:

- One major factor making interpretation of some of the results of this work more challenging was the variation in properties between batches of cattle slurry from different sources, or even from the same source. An in-depth study is required of the characteristics and properties of different batches of cattle slurry, relating these to the origin of the material and to variables such as diets, seasons, and housing and slurry

storage conditions on the farm. The results should then be linked to differences in the biogas production potential, to provide a basis for prediction of the material's performance as a substrate for mono- and co-digestion.

- The work should be linked to the further development of AD modelling tools that can be used to predict the overall energy balance of the system. The models could then be used to assess scenarios such as different co-substrates at varying ratios and loadings. This would provide a wider picture on how different operating conditions affect the system as a whole as a basis for economic assessment.
- More details studies of bioavailability of trace elements and heavy metals would allow rationalisation of dosing strategies and avoidance of any risk of toxicity to the digestion process.
- It would be useful to conduct more detailed evaluation of the microbiology of cattle slurry digestion and co-digestion to determine the dominant groups of organisms under different steady state conditions. Possible techniques include gene sequencing and Fluorescent In Situ Hybridisation (FISH) technique coupled with isotope labelling for metabolic pathway identification. This work could also extend to looking at the interactions of methanogen and sulphate-reducing communities.

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Appendix A: Full results for values from kinetic modelling of BMP test

Table A1 Kinetic coefficients and values in test 1

Parameter		Model 1			Model 2		
FW1		*FW1(i)*	*FW1(ii)*	*Ave FW1*	*FW1(i)*	*FW1(ii)*	*Ave FW1*
Y_m	L g^{-1}VS	0.463	0.455	0.459	0.460	0.455	0.460
P	-	-	-	-	0.93	0.81	0.87
k_1	day^{-1}	0.82	0.64	0.71	1.02	0.99	1.00
k_2	day^{-1}	-	-	-	0.10	0.10	0.10
Lag	day	-	-	-	0.15	0.15	0.15
R^2	-	0.9926	0.9815	0.9880	0.9983	0.9978	0.9981
FW2		*FW2(i)*	*FW2(ii)*	*Ave FW2*	*FW2(i)*	*FW2(ii)*	*Ave FW2*
Y_m	L g^{-1}VS	0.478	0.462	0.470	0.485	0.465	0.475
P	-	-	-	-	0.81	0.84	0.82
k_1	day^{-1}	0.65	0.66	0.65	0.97	0.96	0.96
k_2	day^{-1}	-	-	-	0.06	0.08	0.07
Lag	day	-	-	-	0.10	0.10	0.100
R^2	-	0.9740	0.9808	0.9776	0.9947	0.9954	0.9951
CS1		*CS1(i)*	*CS1(ii)*	*Ave CS1*	*CS1(i)*	*CS1(ii)*	*Ave CS1*
Y_m	L g^{-1}VS	0.187	0.200	0.193	0.200	0.220	0.210
P	-	-	-	-	0.67	0.50	0.58
k_1	day^{-1}	0.60	0.55	0.50	1.03	1.28	1.15
k_2	day^{-1}	-	-	-	0.05	0.05	0.05
Lag	day	-	-	-	-	-	-
R^2	-	0.9343	0.8341	0.8951	0.9961	0.9947	0.9959
CS2		*CS2(i)*	*CS2(ii)*	*Ave CS2*	*CS2(i)*	*CS2(ii)*	*Ave CS2*
Y_m	L g^{-1}VS	0.167	0.177	0.172	0.190	0.200	0.195
P	-	-	-	-	0.46	0.50	0.49
k_1	day^{-1}	0.18	0.45	0.45	1.00	0.96	0.96
k_2	day^{-1}	-	-	-	0.04	0.04	0.04
Lag	day	-	-	-	-	-	-
R^2	-	0.9092	0.8908	0.8750	0.9961	0.9976	0.9972

Table A2 Kinetic coefficients and values in test 2 (units as in Table A1 above)

Parameter	Model 1				Model 2			
CS3	*CS3(i)*	*CS3(ii)*	*CS3(iii)*	*Ave*	*CS3(i)*	*CS3(ii)*	*CS3(iii)*	*Ave*
Y_m	0.170	0.220	0.200	0.200	0.175	0.230	0.210	0.205
P	-	-	-	-	0.25	0.24	0.26	0.25
k_1	0.08	0.10	0.09	0.09	2.50	0.91	0.71	0.85
k_2	-	-	-	-	0.060	0.060	0.050	0.050
Lag	-	-	-	-	-	-	-	-
R^2	0.9863	0.9926	0.9916	0.9910	0.9995	0.9993	0.9990	0.9990

Optimum values for constants were obtained by varying P, k_1 and k_2 in sequence to provide the maximum correlation coefficient (R^2) between experimental and model data.

Tables A3 – A6 show the predicted SMP values in trials C1, C2, S2 and S3b respectively. The predicted SMP for a given feedstock is calculated by using the BMP Model 2 kinetic coefficients to estimate the methane production in a BMP test after a number of days corresponding to the HRT in semi-continuous digestion. The combined SMP is then calculated by adding the SMP for each feedstock, on a pro rata basis in proportion to the amount of VS contributed. This approach takes into account the effect of HRT, but not of daily removal of part of the digester contents as occurs in semi-continuous operation. The agreement between predicted and actual values is thus expected to be closer at longer HRT, when a smaller proportion of digester contents is removed each day.

Table A3 Modelling SMP in semi-continuous digestion in trial C1 using BMP Model 2 kinetic coefficients

HRT	Modelled CH_4 production		OLR		SMP		
	FW1	CS1	FW	CS	Predicted	Actual	Actual/ Predicted
Days	L g^{-1} VS	L g^{-1} VS	g VS L^{-1} day^{-1}	g VS L^{-1} day^{-1}	L g^{-1} VS	L g^{-1} VS	%
33	0.458	0.193	1.85	1.22	0.353	0.332	94.1%
25	0.455	0.185	2.46	1.62	0.348	0.330	94.9%
20	0.452	0.178	3.08	2.03	0.343	0.324	94.5%
33	0.458	0.193	0.00	1.62	0.193	0.194	100.3%
76	0.460	0.208	3.23	0.00	0.460	0.488	106.1%

Table A4 Modelling SMP in semi-continuous digestion in trial C2 using BMP Model 2 kinetic coefficients

HRT	Modelled CH_4 production		OLR		SMP		
	FW1	CS1	FW	CS	Predicted	Actual	Actual/ Predicted
Days	L g^{-1} VS	L g^{-1} VS	g VS L^{-1} day^{-1}	g VS L^{-1} day^{-1}	L g^{-1} VS	L g^{-1} VS	%
26	0.461	0.186	1.12	1.76	0.293	0.239	81.7%
19	0.453	0.176	1.49	2.34	0.284	0.221	77.9%
15	0.446	0.169	1.86	2.93	0.277	0.215	77.7%
18	0.451	0.175	0.00	2.86	0.175	0.053	30.3%
69	0.474	0.207	2.91	0.00	0.474	0.458	96.6%

Table A5 Modelling SMP in semi-continuous digestion in trial S2 using BMP Model 2 kinetic coefficients

HRT	Modelled CH_4 production		OLR		SMP		
	FW1	CS1	FW	CS	Predicted	Actual	Actual/ Predicted
Days	L g^{-1} VS	L g^{-1} VS	g VS L^{-1} day^{-1}	g VS L^{-1} day^{-1}	L g^{-1} VS	L g^{-1} VS	%
41	0.470	0.176	0.77	2.09	0.255	0.013	5.1%
31	0.465	0.166	1.03	2.78	0.247	0.017	7.1%
25	0.460	0.158	1.29	3.45	0.240	0.009	3.8%
35	0.467	0.170	0.00	2.91	0.170	0.005	2.7%
76	0.475	0.190	2.93	0.00	0.475	0.460	96.9%

Table A6 Modelling SMP in semi-continuous digestion in trial S3b using BMP Model 2 kinetic coefficients

HRT	Modelled CH_4 production		OLR		SMP		
	FW1	CS1	FW	CS	Predicted	Actual	Actual/ Predicted
Days	L g^{-1} VS	L g^{-1} VS	g VS L^{-1} day^{-1}	g VS L^{-1} day^{-1}	L g^{-1} VS	L g^{-1} VS	%
60	0.474	0.197	2.13	0.87	0.390	0.388	99.5%
52	0.473	0.193	1.64	1.34	0.342	0.315	92.0%
47	0.472	0.191	1.34	1.64	0.311	0.261	83.8%
45	0.471	0.189	1.13	1.84	0.290	0.253	87.2%