Optimising wastewater anaerobic digestion through small-scale screening studies

Kimberley Barnes

PhD

University of York

Department of Biology

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Abstract

Both now, and in the future, a warming climate and a clearer understanding of our ecological impact will require us to take a closer look at how we generate electricity and treat our waste. Anaerobic digestion as part of the treatment of wastewater, is an area of research where both of these concerns overlap. During anaerobic digestion, organic material is degraded into biogas, a methane rich product that can be burnt to generate heat and electricity, and a nutrient rich digestate that can be used as organic fertilizer. Not only can anaerobic digestion help to produce green energy and recycle nutrients, but also reduces the tonnage of organic material that would otherwise be sent to landfill or incineration. A better understanding and optimization of such a valuable process is essential.

Traditionally experimental anaerobic digestion is done under conditions that do not accurately reflect real world processes. These can include either singularly or in combination: batch rather than continuous feeding, the use of synthetic feedstock material, shorter experimental run times due to increased labour and <1L digesters. A fleet of 60 semi-continuous anaerobic digestors was built and trialled to better simulate process scale anaerobic digestion of sewages sludges at lab-scale. The digesters were able to run continuously and fed real world feedstocks collected from a local wastewater treatment plant hourly for months at a time.

Biologically triplicate digesters showed high consistency in producing biogas and reducing the organic load of the digestate in initial trials, and stability under varying real-world feedstocks. The digesters also showed stability while trialling the integration of a new industrial waste stream into anaerobic digestion, as well as the scalability of the data up to full scale integration.

Finally, reduction of hydraulic retention time and increase of feeding rates had no impact on biogas yields or reduction of organic solids. The digesters were run for 94 days with a retention time of 8.9 days with no measurable instability or reduction in biogas yields compared to digesters with a 14 day retention time.

These data show how the use of System 60 is able to assist in the derisking and making of commercially relevant decisions at smaller scale than pilot scale, by more accurate simulation of a full scale anaerobic digestion plant.

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List of Abbreviations

AD – anaerobic digestion

ABR - anaerobic baffled reactor

C/N - carbon to nitrogen ratio

C_5H_{12} - pentane

CH_4 – methane

CAES - compressed air energy storage

CO - carbon monoxide

CO_2 - carbon dioxide

COD - chemical oxygen demand

CSTR - continuously stirred tank reactor

DS - dry solids

FS - Fixed solids

GFM - gas flow meter

GHG - greenhouse gas

H_2 - hydrogen

H_sS - hydrogen sulphide

HRT - hydraulic retention time

IPA - isopropanol

KOH - potassium hydroxide

LCA - Life cycle analysis

LCFA - long chain fatty acid

LNG/CNG - liquefied/ compressed natural gas

MCR - methyl-coenzyme M reductase

N_2 - nitrogen

N_2O - nitrogen dioxide

NaOH - sodium hydroxide

NDCs - nationally determined contributions

OLR - organic loading rate

P2G - power to gas

PF - plug flow

PHA - polyhydroxyalkanoate

PHB - poly-3-hydroxybutyrate

PHES - Pumped hydroelectricity storage

PLC - programmable logic controller

RSD - relative standard deviation

SAS – secondary activated sludge

SCFA - short chain fatty acid

SS-AD - solid-state AD

THP - thermal hydrolysis pre-treatment

UASB - up-flow anaerobic sludge blanket

VFA - volatile fatty acid

VS - volatile solids

YW - Yorkshire Water

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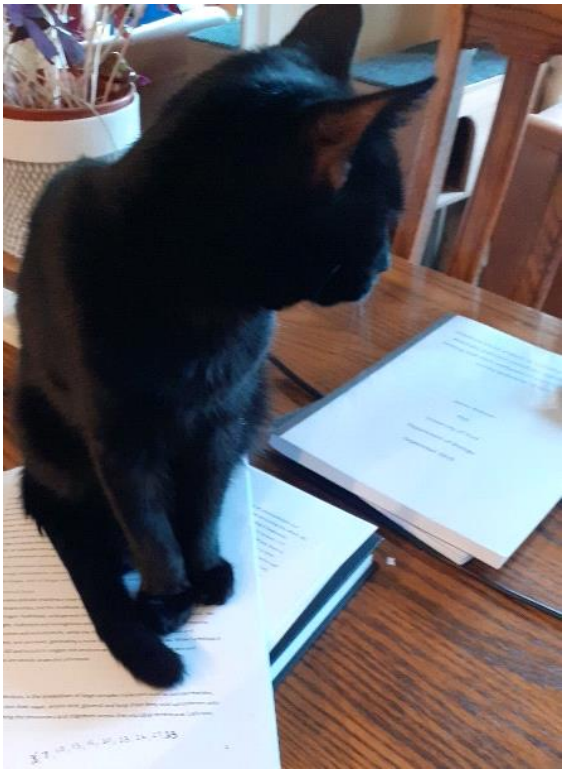


Authors declaration

I declare that this thesis has been composed solely by myself, except where stated otherwise by reference or acknowledgement.

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Chapter 1: Introduction

1.1 What is anaerobic digestion?

In the simplest terms anaerobic digestion (AD) is the breakdown of organic material, in the absence of oxygen, into biogas, using a broad spectrum of bacterial species and a limited, but specialised collection of archaeal species. AD occurs around the globe naturally in any conditions that contain organic material and a lack of oxygen, or other higher energy electron acceptors as reduction of carbon is not particularly energetically favourable, such as fresh and saline sediments[1,2] and ruminant animals. The biogas primarily consists of methane (CH_4) and carbon dioxide (CO_2), with small amounts of trace gases including nitrogen (N_2), hydrogen (H_2) and hydrogen sulphide (H_sS). AD is an essential part of nutrient recycling in anoxic environments and the global carbon cycle.

AD is also used in industry and agriculture to reduce microbial load, stabilise nutrients and reduce tonnage of waste before end of life in landfill or incineration. Anaerobic treatment has lower capital costs, operational costs and energy consumption than aerobic treatments, as well as the added bonus of CH_4 production as a biproduct. $1m^3$ of 75% CH_4 biogas is able to generate 1.4 kWh of electricity[3].

1.1.1 Chemical Steps

AD is a complex process involving a large network of microbes, intermediate metabolites, and symbiotic relationships, but the multitude of metabolic processes can be broadly grouped into 4 successive stages: hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis[4]. The first two stages, hydrolysis and acidogenesis are broad, encompassing a large variety of starting products, enzymes, and end products, while the third and fourth stages, acetogenesis and methanogenesis, are more specific, generating a more limited range of end products[5]. While hydrolysis is not specific to AD and occurs in aerobic environments, acidogenesis, acetogenesis and methanogenesis are strictly anaerobic processes.

1.1.1.1 Hydrolysis

The first stage, hydrolysis, is the breakdown of large complex molecules such as polysaccharides, proteins and lipids into their sugar, amino acid, glycerol, and long chain fatty acid (LCFA) components with the aim of transporting the monomers and oligomers across the microbial membrane. Cellulase, cellobiase, xylanase, amylase, protease, and lipase are a few of the various hydrolytic enzymes secreted by bacteria to this aim[6,7].

Plant biomass, which can make up a large portion of municipal or agricultural waste, typically contains a high percentage of lignocellulosic material which consists of lignin, cellulose, and hemicellulose[8]. These insoluble polymers are large and chemically inert, requiring specialised hydrolases to be secreted to allow hydrolysis into smaller sugars, and as such lignocellulosic materials alone typically result in slow degradation and low biogas yields[8–10]. Hemicelluloses are a large class of polysaccharides consisting of many different branched sugars including xylans and glucomannans assembled from sugar monomers such as glucose, xylose, mannose, galactose, and arabinose[8]. Cellulose is a linear sugar consisting of hundreds to thousands of glucose molecules[8]. In themselves hemicelluloses and cellulose are easy to digest, however they form large, cross-linked networks with lignin, which is water insoluble and chemically inert[8]. The difficulty in breaking down lignocellulosic material, and that the rate of hydrolysis determines substrate availability for subsequent steps, means that hydrolysis can be considered one of the rate-limiting steps of AD[7,10]. The rate of digestion of high lignocellulosic materials is often greatly improved by co-digestion with another nutrient-rich material such as animal manure, with greater N/C ratio and higher levels of trace metals[10]. In comparison, hydrolysis of proteins is much simpler, with proteases readily cleaving protein molecules into amino acids and ammonia[6].

1.1.1.2 Acidogenesis (fermentation)

The second stage, acidogenesis, is the fermentation of the small molecules released during the previous step, hydrolysis. Sugars, amino acids and glycerol enter into fermentative pathways producing CO_2, H_2 and a variety of small carbon compounds including alcohols, aldehydes, ammonia and volatile fatty acids (VFAs)[7,9]. Under aerobic conditions, these molecules would typically enter respiration and acidogenesis is often the fastest step of the four[7]. Acidogenesis can be split broadly into homo-fermentation, where only a single end product is produced such as acetate, or hetero-fermentation, where multiple co-products are produced[9]. Acidogenesis can occur via both hydrogenation or dehydrogenation depending on the microbial metabolism[7].

1.1.1.3 Acetogenesis

Acetogenesis, considered the third stage in AD, is the obligately anaerobic generation of acetate from mono- or small chain poly-carbon molecules and H_2, from acidogenesis and/or hydrolysis stages via the Wood-Ljungdahl pathway[11]. Acetogenesis can also include the β-oxidation of longer chain fatty acids (LCFA), produced from the hydrolysis of lipids, into the shorter chain fatty acid (SCFA) acetate, outcompeting fermentative pathways[7]. Acetogenesis can take a wide range of organic

compounds, such as sugars, and inorganic compounds such as H_2 and CO as electron donors, and a wide range of carbon acceptors such as formate, methanol or methyl groups from methoxylated aromatic compounds resulting in a single product, acetate[11]. In addition to providing substrates for methanogenesis, acetogenesis also removes inhibitory products. The build-up of H_2 from acidogenesis inhibits further fermentation by creating an unfavourable thermodynamic equilibrium[11].

The Wood-Ljungdahl pathway is split into the Eastern (Methyl) and Western (Carbonyl) branches. The Eastern branch is important in one-carbon metabolism, while the Western branch is only used in carbon fixation[11]. When utilising CO_2 as a growth substrate, the first step in reduction of CO_2 and conversion into acetate is the two-electron reduction of CO_2 into formate, part of the Eastern branch of the pathway. Next the formate is combined with H_4folate, forming 10-formyl-H_4folate, which is then dehydrated and reduced into 5,10-methylene-H_4folate. The last step in the Eastern branch removes one methyl group from 5,10-methylene-H_4folate that is then used in the biosynthesis of acetate, with one carbon from the Western branch of the Wood-Ljungdahl pathway[11]. The Western branch of the Wood-Ljungdahl pathway fixes one molecule of CO_2 that is reduced to CO by CODH, and becomes the carbonyl group of acetyl-CoA[11]. Acetate is then cleaved, and CoA regenerated.

1.1.1.4 Methanogenesis

The fourth stage of AD is methanogenesis, representing the final step in the reduction of carbon and the formation of biogas, a mixture of CH_4 and CO_2. Acetate, alcohols, H_2, and CO_2 produced during the previous stages of AD serve as substrates[7]. Methanogenesis is not a thermodynamically favourable process, and will only take place in the absence of alternate electron acceptors such as oxygen, nitrates or sulphates[12]. There are three routes of methanogenesis, split according to their terminal electron acceptor. Although different classes, the last step in CH_4 production is always the same. Methyl-coenzyme M reductase complex McrABCDG is utilized in the conversion of methyl-coenzyme M into CH_4 and a heterodisulphide of coenzyme M and coenzyme B[13].

Acetoclastic methanogenesis produces a combination of CH_4 and CO_2 utilising acetate as a substrate[14]. Acetoclastic methanogenesis has the lowest free energy change of any of the methanogenic reactions at only -36 kJ/mol, leading to the likely use of multiple ion gradients for the production of ATP in an attempt to conserve as much energy as possible[12]. Acetoclastic methanogenesis generates approximately two-thirds to three quarters of the global biogenic CH_4, with the other third to quarter being produced by hydrogenotrophic methanogenesis[5,15].

Hydrogenotrophic methanogenesis produces CH_4 and H_2O, using CO_2 as an electron acceptor and H_2 as an electron donor, although sometimes formate is also used. Hydrogenotrophic methanogenesis has a much higher free energy change than acetoclastic methanogenesis at -131 kJ/mol, however at the low H_2 partial pressures observed in AD, the energy produced is even lower allowing for the synthesis of only a fraction of an ATP[12].

Methylotropic methanogenesis, a newly discovered collection of pathways, utilises methyl groups as electron acceptors to produce CH_4. Methyl groups used have been shown to include methanol, methylated-amines (mono-, di-, and trimethylamine), methylated-sulphides (dimethylsulfide), and methoxylated aromatic compounds, each using a slightly different pathway of enzymes and substrate specific methyltransferases, instead of CO_2 or acetate[1,16]. Currently this is considered a single class of methanogenesis and only a small portion of known methanogens have been shown to singularly use a combination of H_2 and methyl-compounds[17]. Due to its use of methylated-sulphides as a substrate, methylotrophic methanogenesis is able to function under high sulphate concentrations that would typically favour sulphate reducing bacteria and H_2S production[1]. As such it is thought to dominate in coastal sediments and other hypersaline marine environments[1].

Although acetoclastic and hydrogenotrophic are currently thought to represent the predominant pathways of methanogenesis, increasing evidence points towards the high probability that the relative impact of environmental methylotrophic methanogenesis is likely to have been underestimated[17].

1.1.2 The microbial community

In 1995 The Institute for Genomic Research (TIGR), J. Craig Venter's research institute, released the first two complete bacterial genomes, both human pathogens[18,19]. The first sequenced genome of an archaea, a methanogen found in sea floor sediment, followed shortly after in 1996[20]. By 1997 the first complete genome of a methanogen from sewage sludge was published[21]. This marked the beginning in what would become an explosion in microbial sequencing, starting with highly studied cultured representatives and leading to the metagenomic sequencing of unculturable microbes from environmental samples[22]. In fact some of the first Archaea to be sequenced came from environmental samples, from environments so extreme that the diversity was low enough to reconstruct complete genomes [23–25]. J. Craig Venter later made some of the first leaps into environmental metagenomic sequencing with his paper "Environmental Genome Shotgun Sequencing of the Sargasso Sea"[26]. This sequencing has led to greater understanding of the complex microbial communities that underpin many of the essential nutrient cycles, of which AD is one.

Despite its use for hundreds of years in treating waste, and the explosion in sequencing technology, very little is known about the microbial community that facilitates AD.

AD consists of a broad ecological microbial community, much of which is so far unculturable, and from all 3 domains of life. Both 16S rRNA amplicon and metagenomic sequencing have had a large impact in identifying and predicting novel species and have emphasised the complexity of the microbial community within anaerobic digesters [27–32]. The complex, competitive and symbiotic relationships between functionally diverse microorganisms is essential for a balanced AD community. Consisting of prototrophs, which are more metabolically flexible and able to both use and synthesise metabolites, and auxotrophs, requiring symbiotic growth with other microorganisms to gain essential metabolites for growth, they can harm or help one another by competing for resources or by cross feeding[33]. AD communities also contain a large amount of functional redundancy and population diversity among the hydrolytic, acidogenic, and acetogenic bacteria and methanogenic archaea, which increases species richness and complicates understanding of the microbial metabolic community, but is required to ensure the stability of the AD process[5,7]. This resilience helps to maintain stable biogas yield even under unstable conditions and the heterogeneous feedstocks found in industrial waste AD[7]. Study of microbial communities of 90 full-scale digesters at municipal wastewater treatment plants from five countries show that ecological diversity is more highly correlated to the heterogeneity of the organic feed instead of their geographic locations[7].

1.1.2.1 Bacteria

Bacteria make up the bulk (95%) of the microbial community in AD and are largely responsible for hydrolysis, fermentation and acetogenesis[28,34]. The genetic pathways for hydrolysis, fermentation and acetogenesis are metabolic rather than phylogenetic traits, and are widely spread throughout the bacterial phyla with some genera such as *Clostridium* having representatives in all 3 bacterial stages of AD[7]. Under 16S rRNA amplicon sequencing of full scale digesters, the phyla Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, Bacteroidetes and Spirochetes make up the largest populations, however bacterial diversity strongly correlates with physical conditions within the digester [27–31,35]. For example, sequencing of 21 full scale sewage sludge and co-digestion digesters in Sweden found the relative abundance of Firmicutes sequences was higher in the co-digestion digesters than in the sewage sludge digesters (69% vs. 25%), but sequences belonging to Bacteroidetes were at similar levels for both the sewage sludge (15%) and the co-digestion digesters (14%)[28].

Hydolysers

Members of the phyla Firmicutes and Bacteroidetes have been repeatedly identified to be the main hydrolytic cellulose degraders in AD across multiple studies, in particular the genera *Clostridium*, a member of Firmicutes [7,10,27,31]. Although at phylum level the microbial community of AD appears highly conserved, the environmental conditions have a large impact on abundance and genera present. In the rumen of cattle and digesters fed with dried hay or straw, genera including *Clostridium* (Firmicutes), *Bacteroides* (Bacteroidetes), *Succinivibrio* (Proteobacteria), *Prevotella* (Bacteroidetes) and *Ruminococcus* (Firmicutes) are ubiquitously detected[10]. In monodigestion of avicel (partially depolymerized cellulose) and glucose, the relative abundance of *Clostridiaceae* (Firmicutes) was 37-67%[36], whereas during AD of ryegrass Bacteroidia (Bacteroidetes), Betaproteobacteria (Proteobacteria), Clostridia (Firmicutes), Gammaproteobacteria (Proteobacteria), and Negativicutes (Firmicutes) made up to 93% of the total operational taxonomic units (OTUs), although this resulted in the acidification of the digesters[7].

Hydrolytic microorganisms secrete various hydrolytic enzymes, such as cellulase, cellobiase, xylanase, amylase, protease, and lipase[7]. Members of the Firmicutes phylum seem to be the main degraders of cellulose while the Bacteroidetes phylum expressed a high number of sugar transporters and seemed to specialize in the digestion of other polysaccharides[31]. Both phyla are able to secrete enzymes extracellularly or produce stable enzyme complexes tightly attached to the cell[27].

Fermenters

Metagenomic studies provide evidence for members of Bacteroidetes, Firmicutes and Proteobacteria to likely be key fermentative bacteria[9,31]. Proteobacteria, such as *Gluconacetobacter*, *Acetobacter* and *Acidipropionibacterium*, contains some of the most important acetate fermenters in biotechnology. Members of *Clostridium* (Firmicutes) have been researched for their production of butyric acid[9,37]. Heterofermentation of amino acids is a metabolism only found in two phyla: Firmicutes and Synergistetes[38].

Acetogens

While most acetogens are members of the phylum Firmicutes, the phyla Spirochaetota, Proteobacteria, Bacteroidetes, Chloroflexota and Thermotogae also contain known acetogens[11,27,30].

Acetogens, or also known as homoacetogens as they only generate acetate, are obligate anaerobic bacteria able to utilize a number of carbon sources and terminal electron acceptors other than CO_2 including fumarate and nitrate[11].

LCFAs are also oxidized via β-oxidation to acetate by acetogenic bacteria belonging to *Clostridiaceae* (Firmicutes), *Syntrophomonadaceae* (Firmicutes), *Syntrophaceae* (Thermodesulfobacteriota), *Enterobacteriaceae* (Proteobacteria), and Bacteroidia (Bacteroidetes)[7]. As a consequence of this pathway, the population of acetogens is significantly higher in digesters fed a high concentration of lipids, where they can account for more than half of the total microbial population[7]. An increase in the abundance of the acetogenic LCFA oxidizers *Sporosarcina*, and *Syntrophomonas* (both Firmicutes) was prominent during the co-digestion of lipidic waste[39].

1.1.2.2 Archaea

Archaea, or more specifically methanogens, make up a much smaller portion of the microbial community (5%) but are solely responsible for methanogenesis[34]. While the bacterial fraction of AD usually consists of a wide range of phyla, methanogens are a highly specialised and small group of 7 orders within a single phylum, Euryarchaeota[13]. Although single celled in nature like Bacteria, and previously thought of as a subset of the bacterial kingdom, in 1977 Carl R. Woese cemented their place as the third domain of life based on sequencing the 16S subunit of ribosomal RNA gene[40,41]. Initially only thought to inhabit extreme ecological niches, such as hydrothermal vents, they are now known to be organisms of universal importance and often significant contributors to microbial biomass in environments such as soil, wetlands and lakes to name but a few[15]. Archaea are important contributors to both the nitrogen cycle and carbon cycle[15].

Methanogens tend to have a highly specialised and restricted metabolism revolving around methanogenesis. Until recently it was agreed they were split across 6 orders, Methanococcales, Methanopyrales, Methanobacteriales, Methanosarcinales, Methanomicrobiales and Methanocellales[13]. A 7th putative order, Methanomassiliicoccales, is the latest in Euryarchaeota to be proposed[17,42]. However novel putative methanogens have also been placed in the phyla *Candidatus* Bathyarchaeota, *Candidatus* Methanomethyliaceae and *Candidatus* Thermoplasmata[1].

In general the cultured representatives of 5 of the 7 orders, Methanococcales, Methanopyrales, Methanobacteriales, Methanomicrobiales, and Methanocellales, primarily utilize hydrogenotrophic methanogenesis[13]. The cultured members of Methanosarcinales are able to use a broader spectrum of substrates, capable of utilizing hydrogenotrophic, acetoclastic and methylotrophic

methanogenesis[13]. However members of *Methanosarcina* (Methanosarcinales), *Methanoculleus* (Methanomicrobiales), *Methanobacterium* (Methanobacteriales), *Methanosaeta* (Methanosarcinales), *Methanomicrobia* (Methanomicrobiales), *Methanobrevibacter* (Methanobacteriales), and *Methanosphaera* (Methanobacteriales) have been found with both hydrogenotrophic and acetoclastic capabilities[7]. Methylotrophic methanogenesis via methanol is also present in a few species belonging to the Methanobacteriales[13]. Regardless of their chosen methanogenesis pathway, the last step for all methanogens is performed by the same enzymatic complex, and consists of the conversion of methyl-coenzymeM(methyl-S-CoM) into CH_4, by the methyl-coenzyme M reductase (MCR)[13].

Typically in metagenome studies bacterial sequence represent at least 95% of the community, however metaproteome studies find that 20-30% of the identified proteins were archaeal, indicating methanogens are disproportionally active within AD[31].

1.1.2.3 Eukarya

The study of AD largely focusses on the single cell microbial aspect of the community: the bacteria and archaeal fraction[5]. Very little is known about the Eukarya within AD in comparison to the Bacterial and Archaeal fraction, with only 15% of 18S rRNA clones showing >97% sequence identity to known Eukaryotes[34,43]. Bacterial diversity in AD is generally the highest, however surprisingly Eukaryotic diversity has been found to be higher than Archaeal diversity under some conditions[5]. Quantitative real-time PCR and 18S rRNA sequencing of 4 Japanese WWT anaerobic digestors found that 0.1-1.4% of microbial rRNA could be attributed to Eukarya[34]. Of this fraction, approximately 42% were attributed to Fungi, approximately 29% to Animalia, 13% to Protista and 9% to Plantae[5].

Lignocellulosic biomass is challenging to degrade bacterially, however a wide range of fungi have been found to have high fibrolytic potential that could be exploited in AD. Fungi are able to enzymatically degrade lignocellulosic material in a similar manner to bacterial degradation, but also mechanically via their hyphae – long, branching, thread-like structures[43]. DNA sequencing of multiple full-scale anaerobic digesters digesting various animal wastes have identified the presence of anaerobic fungi of the phyla Ascomycota, Basidiomycota and Mucoromycotina, however transcriptional cellulolytic activity was less prevalent[43]. Abundance of fungi in AD is highly linked to feedstock, more so than bacterial or archaeal abundance which are highly influenced by temperature or OLR, as feedstocks high in lignocellulosic material may offer better living conditions[43].

1.1.3 Operational parameters

The performance of AD and the microbial community varies considerably based on physiochemical factors such as organic loading rate (OLR), dry solids (DS), volatile solids (VS) and trace elements and operational conditions such as hydraulic retention time (HRT), temperature and pH, and changes in any of these parameters can result in microbial community shifts[4,7]. Even variations such as the size of digester and therefore pressure within the vessel can affect the relative abundance of microorganisms within the microbial community[7]. However, feedstock is one of the main drivers for the structure of the microbial community within AD. Even digesters dealing with wastewater treatment all have differing communities.

1.1.3.1 Temperature

Temperature is a highly influential parameter on the performance and stability of an anaerobic digester and the process is very sensitive to fluctuations[7,44]. This can manifest itself in altering of the microbial community or metabolic activity of organisms, or on physiochemical ways such as settling characteristics and gas transfer rate[44], and a slow transition period of operating temperature may be required to overcome the imbalance[7]. Digestion can typically be split into mesophilic (25–40 °C) and thermophilic (>45°C) temperature ranges. Thermophilic digestion can increase digester performance by increasing the solubility of organic compounds, increasing chemical and biochemical reaction rates, lowering the solubility of biogas, lowering liquid viscosity, increasing pathogen kill and reducing odours[45,46]. In comparison, lower energy input and greater digester stability are considered the advantages of mesophilic digestion, especially as operating at higher temperatures does not typically result in higher CH_4 yields [44,46,47].

Conversion of mesophilic temperatures to thermophilic temperatures can lead to significant decline in the abundance and diversity of microbial populations[7]. During co-digestion of manure and straw at 37°C, 44°C and 52°C, for both bacterial and archaeal communities, species richness decreases in line with increases in digester temperature, and while this likely has impacts on community resilience, the reduction in diversity did not affect the overall performance of the digesters[10]. During co-digestion of food waste and straw the abundance of Proteobacteria and Bacteroidetes generally decreases under thermophilic conditions with the increase in Thermotogae, Synergistetes, and Firmicutes, and very little change in the archaeal community[7,46]. Temperature changes must be made slowly to reduce the impact of a changing microbial community on process outcomes, for example methanogens have been found to require temperature changes of less than 1 °C/d at

thermophilic temperatures, but can tolerate temperature changes of 3 °C/d at mesophilic temperatures[44,48].

1.1.3.2 Organic loading rate and hydraulic retention time

HRT and OLR are both measurements of digester feed rate. HRT is the length of time material would be in the digester and is typically expressed as the length of time it would take to replace the entire contents of the digester. OLR is the amount of organic material which is fed per volume of the system per time. High loading rates of organic material can lead to accumulation of VFAs and acidification of digesters due to higher activity of hydrolyzing and acidogenic bacteria compared to methanogens[4,44]. Firmicutes, represented largely by Bacillus and Clostridium, were the predominant phyla at low OLR[7], while at high OLR Gammaproteobacteria, Actinobacteria, Bacteroidetes, and Deferribacteres were the most abundant [49]. Similar to changes in temperature, the archaeal community does not change in response to small changes in OLR.[7] For biogas production, 4 kg of DS per cubic meter of reactor per day was reported as the highest rate that microorganisms can tolerate[44].

HRTs can be low while OLR are high and vice versa. The phyla Actinobacteria, Bacteroidetes, and Firmicutes show clear dynamics through different HRTs despite all being prominent phyla in AD. Actinobacteria, flourish at high HRT and are depleted at low HRT[50].

1.1.3.3 pH

The optimum pH for single stage AD is around 6.8–7.2[44]. Accumulation of VFA intermediates can reduce the pH below optimum, known as acidification, and reduce CH_4 production[4,44]. Acidogenesis and acetogenesis are greatly affected by excess H_2 and reduced pH[7]. As such monitoring of VFAs can be used in monitoring digester and microbial community stability[44].

1.1.3.4 Digester design

Anaerobic digesters come in a wide variety of shapes, sizes and configurations depending on space available and substrate composition among other constraints. Common digester designs include anaerobic baffled reactor (ABR), plug flow (PF), up-flow anaerobic sludge blanket (UASB) and continuously stirred tank reactor (CSTR) [3,51].

Digestion design can be split into single or multi-stage digestion consisting of two or more separate digesters in sequence. In single stage digestion, all 4 steps of digestion occur within the same vessel,

typically in multi stage digestion different steps occur in different vessels[52]. Multi-stage digestion is frequently found to be advantageous in allowing the separation and enrichment of the hydrolytic and fermentative bacteria and methanogenic archaea, allowing for the optimal growth conditions for different stages of the AD process to be conducted and ultimately increasing the efficiency of biogas production[3,5]. Acidogenesis occurs at a lower pH in one digester (5.5-6.5), and the effluent is fed into a second digester at a higher pH suitable for methanogenesis (7.0)[52,53]. Although typically resulting in higher biogas yields, and greater process stability multi-stage digestion requires much higher capital costs and is often considered not the most economical choice[52,53].

ABR's are multi-stage digesters consisting of a series of vertical baffles, through which wastewater moves upward and downward between the stages[3]. They require no moving parts or mechanical mixing, which reduces cost, but results in high retention times[3]. Due to their separate compartments they are considered more adaptable to accidental overloading, and more suitable for high strength, low solids effluents[3].

CSTRs, both continuously and semi-continuously stirred, are a simpler design and considered easier to operate making them popular at both lab and process scale[53]. The mechanical mixing allows for greater homogeneity within the digester and therefore greater control of process variables within the digester as a whole[53].

PF digesters can be in both a horizontal and vertical configuration, where the digester is fed from one end and pushed continuously towards the other end to the digestate outlet creating layers of reaction through the digester[54]. Although the reduced running costs associated with such a simple digester design are advantageous, homogenous feedstock composition is important in reducing the likelihood of localised inhibitor accumulation[54].

Other digester variables also include liquid vs solid AD[8,51]. Liquid or wet AD is typically operated at less than 15% DS content, whereas wet or solid-state AD (SS-AD) operates at greater than 15% DS[8,54]. As a result, SS-AD typically has a higher OLR, lower capital and running costs and produces less wastewater for further downstream processing[8,54,55]. However SS-AD comes with some technical challenges around mixing, which is why they often use unmixed digesters[54]. SS-AD can be particularly useful for high DS feedstocks such as lignocellulosic material, and biogas yields have been found to be comparable to liquid AD of switchgrass and corn stover[8].

1.1.4 Pretreatments

Pretreatments are a common step before the AD of high lignocellulosic feedstocks and can improve the digestibility and biogas potential[10]. However pretreatments increase the energy consumption associated with AD, and threaten the economic feasibility[10]. Pretreatment methods can be divided into three main categories: chemical, physical and biological pretreatments and they can be used alone or in combination[44].

1.1.4.1 Physical

The most common physical pretreatment methods used in AD are mechanical, thermal, and steam explosionl[44]. Physical pretreatments act by increasing the surface area available for the adsorption of hydrolytic enzymes, the first step in degradation of lignocellulosic material[8]. Ultrasonic and irradiation are also commercially viable pre-treatments, but are less commonly used[44]. Steam explosion, also known as high-pressure steam treatment, combines high temperature and rapid depressurisation, and is one of the most effective methods for physically breaking down lignocellulose[44,47]. It has been reported that steam explosion increases biodegradability of lignocellulosic materials by 20%[44]. High energy demand and high capital costs for the pressurised container could be considered the disadvantages of this method[44].

1.1.4.2 Chemical

Chemical pretreatments typically do not require high temperatures or pressures, reducing associated energy costs and have been found to increase methane production from wheat straw by 43%[47]. However, the costs are still high due to the cost of the chemical use and responsible disposal[44]. Frequently NaOH (sodium hydroxide) or KOH (potassium hydroxide) are used as alkali pretreatments in the breakdown of straw and other high lignocellulosic material[44].

1.1.4.3 Biological

Biological pretreatments utilize living organisms like fungi to aerobically break down the lignocellulose matrix before AD and have been shown to increase the biomethane potential of wheat straw by 30%[44,47]. Biological pretreatments are typically associated with lower energy consumption, lower waste disposal costs and high yield, however they can be slower and require higher capital costs[44].

1.1.5 Inhibition

AD is a complex web of metabolic interactions, disruption of any of which can unbalance the whole AD process and result in undesirable outcomes and products[44]. Many compounds that can act as inhibitors are still essential intermediate metabolites in AD, but disproportionate concentrations cause thermodynamic bottlenecks that are highly specific to the microbial community. For example: low levels of ammonia, produced in the degradation of proteins, are an essential nitrogen source as well as increasing the pH to buffer against high levels of VFAs in anaerobic digesters, but high levels of ammonia are a common inhibitor during thermophilic digestion[4,44,56,57]. However ammonia levels have been shown to be up to six times higher in thermophilic digestion compared to mesophilic with no negative effects[4], and in many cases bioaugmentation of ammonia tolerant methanogens has been found to enhance biogas yield under high ammonia[6].

VFA's are an essential intermediate metabolite that without, the stages of acetogenesis and methanogenesis within a mixed community anaerobic digester could not happen[44]. However high levels of VFAs can reduce digester pH, altering the thermodynamic feasibility of methanogenesis[44]. In particular propionate, and other odd chain carbon VFAs, have low degradation rates[44], and so can act as an indicator of a well-balanced, or unbalanced, microbial community.

Sulphates and sulphides are also inhibitors of AD as well as in the downstream use of biogas, by directly competing with methanogens during their reduction from sulphate to sulphide by sulphate reducing bacteria, as well as producing toxicity of sulphides to other microorganisms[57].

1.2 Why do we need AD?

2022 has been one of the warmest and driest years on record in the UK, and predictions and warnings that had been made years ago on the effects of climate change are coming into effect. The planet has now reached a level of warming 1.2 °C above 1850–1900 levels, resulting in heatwaves, flooding, and drought around the globe, and the average warming is expected to exceed 1.5°C shortly after 2030[58]. A decade ago the prediction was that continuation of the current trajectory was likely to result in an increase of 4-6°C above pre-industrial levels[59,60]. The Paris Agreement aims to keep warming to below 2°C and aim for no more of a rise than 1.5°C, and significant saving in carbon will need to be made in order to achieve this, far more than has already been pledged[58]. CO_2, CH_4 and N_2O (nitrogen dioxide) amount to roughly 80% of total radiative forcing from greenhouse gases (GHG)[61], although CO_2 and CH_4 are considered the main contributors to GHG emissions. CO_2 proportionally has the largest atmospheric concentration of 360 mmol mol, over 200 times the concentration of CH_4 (1.7 mmol mol), however CH_4 has a global warming potential 21 times that of CO_2 over 100 years, and over 50 times greater over 20 years, and contributes almost 20% of the GHG warming potential annually [62,63]. N_2O also has a substantial impact despite its low relative concentration (0.32mmol), with a global warming potential around 300 times greater than CO_2 and contributing another 6% to the impact [62]. The increase of CO_2, CH_4 and N_2O is not just caused by fossil fuel emissions but also from land use changes, in particular agriculture, and the disruption of the natural cycling that partially removals these gases from the atmosphere[61].

153 parties submitted new or updated nationally determined contributions (NDCs) in 2021, most of them including net-zero CO_2 or net-zero GHG emission targets for 2050, however analysis indicated that there was still over a 50% chance of exceeding 2°C[58]. The IPCC has stated that to limit warming to 1.5°C, a reduction in CO_2 emissions of 45% compared to 2010 by 2030 would be required. Still, the European Union has only proposed to reduce GHG emissions by 40%[64], and analysis of the updated NDCs predicts that global GHG emissions will likely increase by approximately 11% above 2010 levels, and close to 2019 levels, by 2030[58]. A recent estimate suggested that the 20 largest economies globally are predicted to fail their original NDCs by 1.1 billion tonnes of CO_2 per year in 2030[60].

The amount of further warming depends on the immediate actions taken, large changes will result in peak warming around 1.5 °C, whereas weak changes will see temperature continue to rise to 1.7, 1.8, 2.0 °C or higher[58]. Although various carbon capture technologies have reached the commercial market, they are expensive and often with high energy requirements making them counterproductive if the electricity is not from renewable sources[65]. Peaking of GHG emissions could

be achieved before 2030, but a strong action on current pledges and pledges that are yet to be made would be required to limit warming to 1.5°C [58].

It is important to note that although 50-65% of global CH_4 is anthropogenic, or man-made, through for example leaks or uncontrolled AD in landfills, 35-50% comes from methanogenic archaea in naturally anoxic environments such as wetlands and animals, ruminants especially and even termites [2,61,62]. However, there is increasing evidence that plants[66], fungi[67], algae[68] and cyanobacteria produce CH_4 in the presence of oxygen as a response of oxidative stress[69]. Particularly in plants it was found that for every 10°C increase in temperatures the concentration of CH_4 in the gaseous emissions doubled[66]. It is even hypothesized that all living cells have the capacity to generate CH_4[69], further highlighting the importance of restricting avoidable anthropogenic sources of atmospheric GHGs and the resulting temperature rises.

1.2.1 Population growth

Population growth and socioeconomic change are key considerations in predicting future GHG production. Changes in land use and management have effects on their ability to be either carbon sources or sinks. It has been found that changes in land management and use from virgin forest to arable land can decrease its action as a carbon sink by half[62].

With increases in population come increased in food production and food waste. It is estimated up to 40–50% of root crops, fruits, and vegetables; 35% of fish; 30% of cereals; and 20% of oil seeds, meat and dairy products are wasted annually[70]. In China it is estimated that volume of food waste increases by 10% every year due to their rapidly increasing population[55].

AD provides a large number of advantages to alternative technologies. Through AD, solid wastes are converted to gas, reducing the tonnage of wastes to be disposed of while generating a high calorie fuel in the form of CH_4. It is also associated with lower energy consumption than incineration, lower space requirements than landfill and lower overall costs as well as lower environmental impact.

1.2.2 Renewable energy targets

As a result of population growth and industrialisation of developing countries, it is predicted that global energy demand will rise by 50-65% by 2050[71], and that electricity will make up over 40% of the global energy consumption by 2040[70,71]. Currently approximately 84% of global energy is supplied by fossil fuels[71], with natural gas providing approximately one quarter of this[72]. However in the United States natural gas accounts for almost 40% of energy consumption[73], around half of

which is expected to come from shale gas reserves[74]. Fracturing for shale gas not only releases natural gas to be used as energy generating CO_2, process emissions and gas leakage releases CH_4 and the newly fractured shale provides methanogens with the chemistry and space to grow and contributes to biogenic CH_4 emissions[74]. The same can be said for oil and coal production, with methanogens isolated from oil production water and able to generate CH_4 from aromatic compounds found in abundance in both coal and oil[16,75]. It has even been found that up to 20% of residual CO_2 injected into oil reservoirs during enhanced recovery is converted to CH_4[76]. In addition to the process emissions, natural gas produced as a biproduct of extraction is often vented or inefficiently flared, responsible for the release of an estimated 142 billion m^3 of GHG[63].

While the percentage of energy supplied by renewable sources is expected to increase from 11% to 25% by 2050, global energy related CO_2 emissions are predicted to increase by almost 50% in the same period of time[71,72]. The global wind capacity increased by 10.8% in 2017[77], but electricity generated from solar voltaic and wind turbines is highly weather dependent and can be unreliable. For example, increased energy requirements for light and heat during winter months come at a time of fewer and less intense daylight hours. Hydro-electric is better tailored to energy storage and release on demand, but hydro-electric requires specific, and not that commonly found, geological formations to be commercially viable. It also requires the flooding of large swathes of land, which can be equally environmentally or economically damaging.

Biogas, be that used in the national gas grid or electrical grid via a CHP, from AD in comparison to other weather dependent renewable energy sources has high availability and high predictability and allows for the balancing of other less predictable, weather dependent, renewable energy sources[78].

1.2.3 Transportation

96% of global transport fuels are fossil fuel based[71]. While the growth of electric domestic vehicles has increased with improving battery technology, other areas of transport such as heavy goods vehicles, long distance haulage, aviation and marine transport are unsuitable for electrifying due to their higher energy requirements and therefore the uptake of renewable alternatives has been slow[64,71]. Also, while electric vehicles have the capability to be greener than petroleum-based vehicles, due to the method of electricity generation their carbon footprint is still significant.

For sections of the global transport system that are viewed as unsuitable for electrification, modification of existing infrastructure and the use of biofuels may be a better option. The UK has already launched a “Clean Maritime Plan” for 2050, aiming for zero GHG emission shipping[79].

Biofuels provide a greener, and liquid, alternative to either direct fossil fuels or electric vehicles and as such give greater potential uses. Biofuels in current commercial use are ethanol and biodiesel, both of which can be produced through AD.

Biodiesel use is currently limited in part due to the lack of engine compatibility with the majority of current vehicles, lack of infrastructure and the quality of the fuel[71]. These limitations can, and most likely will be solved with further investment, but competition for land use between food and fuel crops will still be a recurring issue as currently biodiesel is predominantly produced from hydrogenated plant oils[80]. In 2014, the EU set out targets to limit the share of biofuels from cereal, sugar and oil crops to 7%[14], and land use change and land management practices have implications for their ability to act as either carbon sources or sinks[62]. This poses a very difficult challenge to the transport fuel sector's carbon reduction targets due to the unavailability of commercially available alternative biofuels that can be produced in sufficient volumes[14]. The production of biofuel from AD of waste material provides a solution to this problem.

1.2.4 Waste and landfill

Although disposal of carbon containing waste without contributing to GHG emissions is impossible with current commercial technology, exploitation of the waste as a renewable energy source can reduce the impact. For example, it is estimated that China could meet over 30% of its annual natural gas demand by co-digestion of its food waste and wheat straw[46]. However the likely impact in biogas production of changing societal attitudes around waste management is highly regional specific[81]. For example, Austria has been collecting organic waste since 1995, and since 2009 inorganic waste is solely incinerated, generating electricity, and no longer sent to landfill[81]. Studies predict that more significant impacts on GHG emission reduction are made in reducing biogas output from landfills, than the impacts on reducing the countries reliance on natural gas as a result of putting all its organic matter through AD and using the biogas as an energy resource[81].

1.2.4.1 Municipal waste

In Europe it was estimated that 190kg of waste per capita was land-filled in 2009, at an estimated CH_4 production rate of up to 170 kg CH_4/tonne of organic material, and generating over 140 million tonnes of CO_2 equivalent in GHG emissions[81]. While there has been significant progress in ensuring most of the biogas from landfills is captured, presently the majority is flared with the view that releasing CO_2 is better than releasing the CH_4 rich biogas [81]

It is estimated that around 80% (by volume) of municipal waste is biodegradable and would therefore be suitable for AD[82]. Paper and card make up around 30% while organic waste constitutes up to 35%. Other reports estimate that up to 55% of municipal waste comes from kitchens, be that household or restaurants, alone[44,55]. Wood, textiles, rubber and biodegradable plastics also contribute to the organic fraction of municipal waste[82]. Although varying between regions, around 75% of municipal wastes undergo no further processing and enters landfill where CH_4 and CO_2 emissions from uncontrolled AD and chemical leaching into groundwater and air is of significant environmental concern[44,82]. The environmental damage of landfilling is further aggravated by the exclusion of large areas of land from agricultural use and the pollution of soil, converting the area from a carbon sink to a GHG source[82].

It is estimated that the area occupied by landfill continues to grow by around 6-8% each year[82]. While incineration is viewed as an alternative to placing municipal waste directly to landfill, by reducing the volume of waste to be disposed of and in some cases providing energy generation, it still produces large volumes of carbon and other harmful gases that need to be scrubbed out. Incineration of unsorted municipal waste is therefore unlikely to become either a viable substitute to fossil fuels for energy generation, or as a waste management strategy[82].

Although disposal of wastes without production of CO_2 and contribution to GHGs is impossible, production, capture and energy generation through biogas and AD of organic waste steams provides a more environmentally sustainable compromise[44].

1.2.4.2 Agricultural waste

Agricultural waste can be divided into two fractions, plant based (i.e. straw and food waste) and animal based (i.e manures). Globalisation of food production has led to large regional increases in specific agricultural wastes, in some cases up to 55% of total production[70]. Broadly speaking cereals, roots and tubers are the most wasted feedstocks in North America and Oceania, while oilseeds and pulses are the highest in North Africa, West and Central Asia[70]. In China it is estimated that roughly 220 million tonnes of just food waste and wheat straw alone is produced every year[46]. The AD of large quantities of food wastes could act as a promising source for the production of renewable energy in rural areas where a nationalised electrical grid may not exist and electricity is produced via diesel generators, which could in turn lead to indigenous development and a sustainable energy supply[70].

Animal manures and slurries are produced in large quantities and represent a significant pollution risk if not managed[83]. Animal manure is already frequently utilised as a fertilizer or soil amendment and in the EU animal manures must be stored within special tanks and applied at specific intervals to reduce run-off[83]. However, there is frequently significant nutrient loss during collection, storage and distribution[44], as well as high pathogenic load[84]. Nutrients, pathogenic bacteria and heavy metals leached from stored animal manures as a result of high rainfall enter the groundwater or local watercourses causing eutrophication[44,84]. Up to 70% of nitrogen can be leached within the first 24 hours, resulting in reduced fertilizer efficiency upon application[44], however immediate application bares little better outcomes. Secondary fermentation in the soil can emit heat and both thermally and chemically burn plant roots[84].

Lignocellulosic materials such as wheat straw, the second most abundant lignocellulosic waste globally[47], are commonly produced as byproducts of food production, and frequently burnt as a result of limited commercially viable options contributing approximately 18% of global CO_2 emissions per year[44,46,85]. For example, in the production of palm oil, extraction of the oil from the palm fruit leaves behind the byproduct palm pressed fibre[44]. This is commonly burnt to produce electricity on the farms and, as is common in the burning of solid fuels, is a large source of air pollution in the tropics[44]. Wastes from sugarcane, rice and coffee are also routinely burnt as fuel[85].

1.3 How is AD currently used in industry?

The first International Symposium on Anaerobic Digestion of Solid Wastes was held in Venice in 1992[51]. Since then, and in subsequent symposiums, there has been an increasing concern around waste disposal[51]. AD is currently considered to be the most reliable waste to energy model for converting organic waste to CH_4 with a wide variety of cheap and abundant waste streams from municipal, sewage sludge, food processing, animal husbandry and agriculture to name but a few[7]. Previously predominantly used in the treatment of sewage sludge, AD as grown in application to include a wide variety of potentially polluting, organic energy-rich waste streams[28]. Biogas has even been produced from a variety of waste paper sources[27].

However heterogeneity and homogeneity in feedstocks can both be bottlenecks in their implementation into AD[7]. For example, cheese production produces a considerable amount of waste, but the acidic pH and low alkalinity hampers stable treatment in AD, even with frequent intervention to stabilize the pH[86]. However co-digestion with cow-manure, a product high in alkalinity, nitrogen, and lignocellulosic material can be employed to improve digester stability[87].

Green energy subsidies have supported the rapid expansion of the biogas industry to provide a renewable energy source and reduce the volume of organic waste entering landfill. The capital cost of an AD system is now three times lower than that an incinerator, and according to statistics, by 2050, the production cost of AD will be reduced by approximately 38% compared to that during 2015[84]. In the 7 years between 2010 and 2017, the global biogas power generation capacity increased by 57.8%, generating 1,331,949 TJ of energy[84].

Regardless of feedstock and location, the majority of biogas produced is burned in combined heat and power engines.

1.3.1 Agricultural sites

The global livestock economy is steadily increasing, resulting in large scale land use change, increased atmospheric CH_4 release from methanogenesis within ruminant livestock, and ecological damage to water courses from animal manure. Livestock accounts for half of the global agricultural economy[84]. According to the statistics of the Food and Agriculture Organization of the United Nations, the global livestock and poultry production in 2019 was 0.85 billion for pigs, 1.51 billion for cattle, 27.54 billion for poultry, and 2.33 billion for sheep and goats[84]. It has been predicted that this will increase substantially by 2030 and continue to be mass produced[84]. As a consequence of rapid growth of the animal product industry, the volume of animal manure has also increased rapidly, with an annual global production of approximately 13 billion tons, almost 130 times that of human waste

produced in the United States[44,84]. Although progress has been made in identifying vaccine and chemogenomic targets for broad inhibition of rumen methanogens, which could have significant contribution to reducing the volume of emissions from the agricultural sector[59], rumen methanogenesis is not the only environmental impact of this industry.

AD is commonly used for animal manures on farms in co-digestion with lignocellulosic material such as bedding straw, helping to stabilize nutrient levels and reduce run off into watercourses and groundwater, threatening public health[44]. The low carbon to nitrogen (C/N) ratio in animal manures results in low biogas yields and poor digestion frequently as a result of inhibition from high ammonia levels[56], requiring co-digestion with a carbon rich substrate, such as lignocellulosic materials, another abundant waste, with high C/N ratios that cannot be the sole substrate for AD either[44]. Animal manure, in addition to being high in nitrogen, also contributes trace elements and buffering capacity to the AD process, producing a nutrient rich residue that can be used as a soil amendment[10]. Similarly to animal manure, food wastes typically have a low C/N ratio and benefit from co-digestion with lignocellulosic material[46].

The use of lignocellulosic material for biogas production is still somewhat limited because of their high content of recalcitrant lignocellulose, despite co-digestion with animal manures[10]. Large amounts of research has gone into increasing biogas yields from agricultural wastes, manure and straw, by optimising parameters such as temperature[10], pretreatments, and retention time.

1.3.2 Fertilizer and soil amendments

After AD and methanogenic reduction of carbon within the feedstock, a significant amount of solid material still remains called digestate. This digestate is typically high in nitrogen and phosphate and more suitable as a fertilizer or soil amendment than direct animal manure application, having high trace nutrient and carbon content, comparatively low pathogen content, and considered more biologically stable[51,84]. Digestate application to land can prevent soil hardening, decreases in soil carbon and soil productivity frequently caused by long-term application of chemical fertiliser[84].

Application of digestate can also help to reduce N_2O (another potent GHG) release from soil by encouraging growth of particular bacteria[88]. Studies into the large-scale production and distribution of N_2O-respiring bacteria find it to be prohibitively expensive and impractical. However, the use of N_2O-respiring bacteria could become feasible if adapted to an existing fertilization pipeline, such as digestate[88].

1.3.3 Wastewater treatment

AD has been used for over 100 years to treat sewage in one form or another and is critical in reducing the volume and tonnage of waste for disposal[89]. Modern wastewater AD in the UK collects and consists of multiple domestic and industrial waste streams from the cleaning of local waste waters, and is susceptible to large swings in temporal, seasonal and spatial, variation[89,90]. Primary sludge is produced from initial settlement of the wastewater[91]. After settlement, the effluent enters aeration tanks were bacterial growth is encouraged by bubbling of air resulting in removal of soluble organic matter, and producing secondary activated sludge or SAS[91]. This secondary stage of aeration is energetically costly, accounting for approximately 3% of total electricity use in the US each year, and has high running costs associated[89,92]. The SAS is also particularly difficult to digest due to the high content of filamentous bacteria[93]. The primary sludge and SAS is combined and fed into AD, and the total volume to be treated reduced to 0.5-2% of the original waste water entering the WWTP[94]. Although significantly reduced in volume from the original liquor entering the WWTP, on average up to 25kgDS of sewage sludge is produced per person each year[93]. After AD, the remaining digestate can be applied to land as a rich fertilizer if compliant to local regulations around pathogen count, or disposed of via landfill or incinceration[93,94].

1.3.4 Food waste

Food waste, as opposed to agricultural waste, can be defined as intended for human consumption but otherwise discarded with the exception of primary production losses, as opposed to agricultural waste which can be defined as inedible residues[95]. It is high in readily biodegradable organic material in the form of starch, fat and protein, in comparison to the recalcitrant nature of many agricultural and lignocellulosic residues[46,96]. The digestibility of food waste however can lead to digester instability due to acidification and ammonia inhibition, and as such the digesters are usually run at low OLRs[46,96].

1.3.5 Industry

Many industries now utilize AD as a method to reduce the nutrient load of their waste streams before discharge into local watercourses. Some are permitted to discharge liquid waste into local sewers that is then treated at local WWTP but others, and particularly solid wastes, undergo AD at source in industry specific digesters.

Pulp and paper

The pulp and paper industry uses large volumes of biomass and energy and produces considerable amounts of solid and particularly liquid waste, as part of their industrial process[97,98]. The water use in pulp and paper mills is in the region of 10^{e100} m^3 per ton of produced paper and the sludge generation ranges between 0.2 and 0.6 wet tons per ton pulp produced[99]. Accordingly, P&P mills are considered a major source of environmental pollution[97]. The generated effluents commonly have a high COD and low biodegradability, consisting of more than 700 organic and inorganic compounds[97]. AD of pulp and paper mill waste at full-scale is currently confined to the treatment of a few selected types of effluent, such as paper mill effluents and evaporator condensates from chemical pulping, however AD of the waste water shows a removal efficiency of over 70% of COD and reducing the environmental impact of the industry[3,99]. Approximately two thirds of all mill anaerobic reactors in the world treat effluents from recycled paper mills and one third from pulp mills[99]. Full-scale digesters for mill- derived sludges (biosolids) are almost non-existent[99], however investment into research is paving the way for upscaling for AD of new wastes at lab-scale[98].

Alcohol production

Alcohol production produces large volumes of liquid and solid wastes, consisting of a variety of complex organic materials and metals from the brewing, fermentation and distillery process[48]. The highly concentrated levels of complex organic materials, nitrogen, potassium and phosphorus makes discharge of the liquid wastes a significant pollution risk[48,100]. Approximately 3.4 million tonnes of solid waste from alcohol production is produced annually in the EU alone and AD has been found to be a more efficient use of waste recycling than previously used methods of incineration or diversion to animal feed[48]. Whiskey distillery and brewery wastes in particular are high in organic matter, however the solid waste, consisting of spent grain and yeast and commonly known as draff, is high in lignocellulosic material and benefits from pre-treatment[48]. While the solid waste fraction of both whiskey and beer production is similar and can be treated much the same in regards to AD, the process per litre of whiskey generates 8-21 litres of liquid waste called pot ale that can be high in metals such as copper and iron, and 3-10 litres of liquid waste per litre of beer[48,100]. In industry, the digester design and configuration are dependent on the specific site and waste stream being treated and there is not a universally utilized design, however the designs used are extremely successful with some whiskey distilleries reporting recovery of 95% of their site electricity demands and reduction of 95% of COD from liquid waste streams[48].

Sugar production

Sugar beet and sugar cane are the two main producers of sugar globally. Sugar cane requires tropical conditions for growth often in places where there is insufficient rural infrastructure to appropriately treat waste products, resulting in increased GHG emissions from soil in the form of N_2O, and compounding the environmental impact of sugar produced from sugar cane[101]. An alternative is sugar from sugar beets, which can and is grown in large quantities in temperate regions such as the USA and Europe[102]. While the transport and land environmental impacts of sugar from sugar beet are reduced compared to sugar cane, there is still significant waste produced during the extraction process. Approximately 250kg of sugar beet pulp remains per tonne of beet used, which was previously sold as livestock feed[103]. However, a more efficient use for the producer, is to utilise AD, producing electricity and heat that can be diverted back into the production of sugar from sugar beet and providing better financial returns[102]. This is done industrially at the UK's largest sugar producer British Sugar in Bury St Edmunds[104].

1.4 What is the future for AD?

One of the key aspects of de-carbonisation is energy storage. Wind farms and solar farms continue to grow, but these renewable energy generators are sporadic and unreliable, both temporally and spatially. Transporting electrical energy large distances through cables is also not particularly efficient and battery storage is unrealistic[77]. This excess of electricity generation can result in turbine curtailment, where turbines are turned off to protect the electrical grid from overloading.

Alternatives to battery storage have been proposed. One is using UK mines: when there is a surplus of electrical energy in the grid, large weights in retired coal mines are lifted and that electrical energy is stored as kinetic energy. Pumped hydroelectricity storage (PHES) is another, where water is pumped from a low reservoir to a high reservoir using grid surplus and released over a turbine to generate electricity during grid deficit, and is the largest energy storage option in current use[105]. While the efficiency is high, typically 75-80%, installation of new sites is dependent on specific parameters such as land topography and space available[105]. That being said, as of 2010, PHES had over 300 plants installed worldwide was the only commercially proven large scale energy storage technology[105].

The globe is moving towards the idea of a circular economy, and as part of this there is an urgent need for waste disposal minimization and biowaste valorisation[79]. Numerous industry reports have identified a long-term surplus of biogas, that could be better used in other high-value applications[92].

1.4.1 Biogas upgrading and power to gas (P2G)

A promising use of biogas is upgrading to biomethane, where it can be treated much the same as the natural gas so many energy economies rely upon[79]. Currently the majority of biogas produced is burnt at site of production to produce electricity with an approximately 38% efficiency[81]. This reduced efficiency, and the current technical challenges around ensuring power grid flexibility and distribution makes biomethane conversion and storage a compelling technology[92]. The number of biomethane plants in Europe has seen an increase of 51% in the two years between 2018 to 2020, most within Germany, France and the United Kingdom[79].

However, upgrading biogas to biomethane generates extra costs in comparison to direct biogas use in CHP units, and so policies such as feed in tariffs would likely be required to promote the growth of the biomethane industry[79]. Biogas typically constitutes approximately 55-65% CH_4 to 30-35% CO_2 as well as other trace gases such as H_sS, ammonia, and N_2[44]. The CO_2 and trace gases portion of biogas significantly reduces the calorific value of the biogas when burnt and needs to be scrubbed before

use for high efficiency. H_2S is toxic at low ppm and highly corrosive when combined with the water vapor in biogas, forming sulphuric acid, damaging engines/any metal parts and constituting a health hazard [44,106,107]. CO_2, although less toxic, can also combine with water vapor forming carbonic acid[107]. The calorific value of CH_4 is around 12,000 kcal/m^3 (36 MJ/m^3), while the calorific value of biogas is estimated around 5300 kcal/m^3 (20-25 MJ/m^3) [44,106]. Regulations around the required CH_4 content of natural gas are region specific, but many require a minimum CH_4 composition of 95%, and less than 3% CO_2 and 3% N_2, and the European Commission has recently issued a call to harmonise standards for gas quality across member states[106,107].

In general, the CH_4 recovery from physicochemical processes can reach > 96%[106]. In many biogas plants H_2S removal units are already in place, commonly based on biological H_2S oxidation by aerobic sulphate oxidizing bacteria[106], or by passing the biogas through beds of ferric oxide and iron[44]. There are several current commercially existing methods for scrubbing CO_2 from biogas, and many more under development, including alkaline and amine solutions, water and polyethylene glycol scrubbing, pressure swing adsorption, membrane separation, cryogenic separation[44,65,106,107]. The resulting concentrated stream of CO_2 also has potential for entry into the market as a commodity, or re-injection into AD to increase CH_4 yields[108].

Liquid scrubbing

Water scrubbing is the most commonly used technology for biogas cleaning and upgrading due to its low cost[92,106]. The process relies on the increased solubility of CO_2 and H_2S compared to CH_4 in water[106]. After a drying step, the CH_4 can reach up to 99% purity[106].

Solvents, often mixtures of methanol and dimethyl ethers of polyethylene glycol, can also be used instead of water[106]. They have a much higher solubility to CO_2 and H_2S than water and so less solvent is needed and the equipment can be smaller[106]. The final content of CH4 in the upgraded biogas using this technology can reach 98%[106].

Membrane technology

Membrane technology using cellulose acetate or polyamine membranes is a competitive alternative to liquid scrubbing[106]. The process relies on the selective permeability of a membrane allowing the separation of CO_2, H_2S and CH_4[106]. The CH_4 content in the upgraded biogas is commonly 95% but it can reach> 98% under certain conditions[106].

Photosynthetic scrubbing

Carbon capture technologies are currently expensive however research into CO_2 scrubbing using microalgae is promising[65,109]. Biogas upgrading using microalgae in photobioreactors, either alone or with bacterial or fungal co-culture raised the CH_4 content to over 80% and could be manipulated to over 90% when co-cultures and C/N ratios were optimised[110,111]. While the microalgae do not produce CH_4, the CO_2 was fixed for growth and photosynthesis and as such the relative percentage of CH_4 increased[111]. Proof of concept has also been established for CO_2 fixing by photosynthetic bacteria in co-culture with methanogenic archaea, increasing the CH_4 content as well as decreasing CO_2[112]. The electrosyntrophic co-culture was able to store solar energy as chemical energy in biomass and CH_4[112].

Electrolysis

Alternatively renewable electricity produced in surplus by solar or wind installations, can be used for water electrolysis and green H_2 production. While green H_2 is a fuel in itself, H_2 is highly volatile and difficult to store and transport[77]. Current UK heating infrastructure is based around natural gas and CH_4. Instead excess H_2 can be bubbled through anaerobic digesters for biogas upgrading[77,113,114].

Power to gas

Another option is power to gas (P2G), a hybrid technology combining multiple renewable technologies. By using surplus electricity to split water into H_2 and water, and combining H_2 and CO_2 into CH_4 either biologically or catalytically[14,77,115]. Scrubbing CO_2 from ambient air or chimney stacks at fossil fuel plants is expensive due to the low concentrations and high temperatures needed to liberate the bound CO_2, while CO_2 generated during AD is in effect a waste product[14]. Biomethanation in AD is limited thermodynamically to a maximum efficiency of 80%, and upgrading of biogas captured by current waste management strategies is too low to provide a significant substitution to the present natural gas consumption[81], but by using H_2 generated through electrolysis to convert waste CO_2 into biomethane this could be increased to more than 80%, making it more efficient than PHES or CAES (compressed air energy storage)[14].

Modelling of regions where energy generation by wind exceeds local requirements, and would otherwise be curtailed, such as the west coast of Ireland, finds that P2G in combination with AD a potentially more financially viable technology than biomethanation of biogas[14].

1.4.2 Gas fermentation

Biological fermentation of easily digestible carbohydrate feedstocks has provided the beginning of the chemical industries' movement away from petroleum-based chemicals. These biological processes function at relatively low temperatures and pressures in comparison to traditional metal catalysts, as well as having higher product selectivity and tolerance to gas contamination[116]. The feedstocks, also known as "farmed sugars", currently make up an estimated 10% of the worlds energy demand and commodity chemicals[117]. However the feedstock can be responsible for up to 60% of production cost, and the land cost associated has led to concerns over competition for food and the conversion of natural landscapes into arable land [118,119]. For example, in recent decades there has been extensive conversion of natural forest or semi-natural grassland to agricultural use in tropical Americas, reducing its capacity as a CH_4 sink by 50-65% [62].

An alternative to sugar fermentation is gas fermentation, where chemo-lithotrophic microorganisms build up useful products from gaseous C1 units[116,120]. Gas fermentation using autotrophic and acetogenic bacteria is able to utilise CH_4 and CO_2, both produced in abundance from AD, and carbon monoxide (CO) which can be produced from biogas, for the production of other valuable biological molecules[117,120]. CH_4 is the preferred feedstock for microbial gas fermentation from biogas, as it can act as both a carbon and energy source. CO_2 is less ideal as H_2 is required to provide activation energy to the process[116]. Methylotrophs were originally studied for their potential in bioremediation, and then to produce single cell protein for animal feed before spikes in the price of fossil fuels made it unviable[121].

Capturing and recycling CO_2 and CH_4 before they enter the atmosphere offers routes to carbon-negative manufacturing, a circular economy and reductions in GHG emisssion[118,120]. The first commercial scale gas fermentation plant producing ethanol was started up in May 2018[116].

1.4.2.1 Volatile fatty acids

VFAs are widely used building blocks for the manufacture of a wide range of chemicals including biosurfactants, bioflocculants, and bioplastics[9,77]. They are also currently mostly produced from fossil fuels[9]. Acetic acid in particular is the building block for a huge range and number of commercially relevant polymers, such as cellulose acetate, polyvinyl acetate and other synthetic fibres and fabrics[9,118], as well as biofuel precursors such as alkanes, acyl-esters and isopentanol[122], and is the main component of vinegar to name but a few[9]. Propionic acid is commonly used as a food preservative as well as a building block for many pharmaceutical compounds[9]. Butyric acid and its

esters are also commonly used in the food industry for flavourings ranging from buttery to fruity as well as in anti-cancer drugs[9].

Direct extraction of VFA's from the AD process is theoretically possible, but in practice challenging due to membrane fouling and particulate accumulation[123]. Short chain VFA's, consisting of 6 or fewer carbon atoms, such as acetate, propionate and butyrate, can easy be generated by gas fermentation from CH_4, CO and CO_2 in substantial amounts using both pure and mixed cultures, and has great potential in future biotechnology[80,122,124–126]. Reversal of the methanogenesis pathway to convert methane into readily available, clean, acetate has already been engineered[122]. VFA's have also been extracted directly from the AD of food waste without the need for gas fermentation, a difficult task due to membrane fouling, using a novel filtration and electrodialysis design[122]. Optimisation of the process allows manipulation towards higher CH_4 or VFA production[77].

1.4.2.2 Solvents and commodity chemicals

Acetone and isopropanol (IPA), like most commodity chemicals are derived from petrochemicals, and have a combined global market of more than US$10 billion[120]. Both molecules are industrial solvents as well as platform chemicals for the production of materials such as polymethyl methacrylate (acrylic glass) and polypropylene, and have been produced in industrially relevant amounts of up to ~3 g/L/h using gas fermentation of C1 gasses at small scale[120].

Methanol can already be produced from CH_4 thermo-chemically, however it is expensive to do so and requires higher purity than is present in biogas[127]. Biological methanol synthesis does not require the high temperatures and pressures of its catalytic counterpart and can be produced from CH_4 by many aerobic methanotrophs from phyla such as Proteobacteria and Verrucomicrobia, as well as anaerobic methylotrophs of Proteobacteria isolated from anaerobic digesters [127,128].

2,3-butanediol is another commodity chemical and precursor for nylon and rubber, with a commercial global market of around $43 billion USD[117,129]. Studies have shown that 2,3-butanediol is produced by the model acetogen *Clostridium autoethanogenum* from CO and H_2[129].

1.4.2.3 Bioplastics

Plastic waste, and in particular microplastics, is of growing international concern[130]. Over 140 million tonnes of plastic is produced each year globally and the recalcitrant nature of plastics that previously made them such a valuable commodity is proving their downfall as they continue to build up in municipal, agricultural and terrestrial environments[119,130]. Biobased plastics that are more amenable

to biological degradation and could contribute towards at least preventing the production of more petroleum-based plastics[119].

Polyhydroxyalkanoate (PHA) is a naturally occurring, biodegradable molecule that can act as a renewable substitute for many plastics, including single use plastic packaging[119,130]. The current high costs associated with producing PHA's in comparison to petroleum based plastics can, in part, be attributed to the high cost of sugar crop feedstocks[131], however a significant advantage is that PHA's degrade back into biogas in methanogenic bioreactors, reducing the release of microplastics into the environment[119], and it is estimated that the equivalent value of PHA is over 5 times that of biogas[132]. Proof of concept studies have now been able to produce high quality PHA, in high volumes from gas fermentation of CH_4, and pilot scale production has begun at US based Mango Materials[121,133].

Over 100 PHA molecules have so far been identified[119], one of which is poly-3-hydroxybutyrate (PHB). PHB's have been found to be produced by multiple genera and are non-toxic to both the environment and people, making them one of the most promising biopolymers [130,131]. Life cycle analysis (LCA) shows that the total energy requirement for PHB production is less from biogas than crops[119]. Based on estimates of current global biogas production, approximately 20−30% of the total plastics annual market could be replaced by microbial PHB production[119].

Both ethanol and lactic acid are already commonly produced via microbial synthesis and sugar fermentation. Ethanol is used as a substrate for polyethylene and butadiene production (nylon), and lactic acid is used as a substrate for the biodegradable plastic polylactic acid, commonly used in 3D printing[116,134]. However, both can also be produced through gas fermentation of CH_4[134].

1.4.3 Transport fuels

While small vehicle transport is amenable to electrification and decarbonisation, a large proportion of the global transport section, such as shipping, aviation, and heavy goods vehicles are more difficult to decarbonise sectors[64].

Direct use of compressed CH_4 in the transportation sector is restricted due to the low calorific value of CH_4 in comparison to longer chain alkanes and the lack of fuelling infrastructure required for its integration into vehicular transport [63,64,135]. For example, pentane (C_5H_{12}) has a higher heating value (3507 kJ/mol) than that of CH_4 (889 kJ/mol)[64]. The use of liquefied/compressed natural gas (LNG/CNG) as an alternative fuel in marine transport applications is possible due to larger carry capacity, and may in future be required due to stricter environmental regulations for other traditional fuels (i.e., diesel) [79]. However, land and air transport do not have the capacity to carry

large volumes of LNG. Also if large scale changes to existing transport fuel infrastructure can be avoided, the costs associated with transitioning from fossil to renewable transportation fuels will be much lower[135].

Biofuels generated for use in transport currently have a land cost attached due to the growth of crops purely for the conversion to biodiesel or ethanol, whereas biofuels generated from gaseous precursors generated during AD do not have this land cost associated. Microbial oil for biodiesel can be produced from acetic acid, which can be readily made from CO_2[136]. Biofuel candidates isoprenoids, branched hydrocarbons with fewer than 20 carbons, have been shown to be produced from CH_4 by methylotrophic bacteria[137], while ethanol can be generated from syngas (easily generated from CH_4) by acetogenic bacteria on a commercial scale, and has been used in commercial transatlantic flights[71,116,120]. Optimisation of operational parameters enhanced ethanol production from syngas from 0.15g/L to 2.3g/L in single organism fermentation, and yields of up to 8g/L have been reported from mixed cultures[138]. Ethanol however requires blending with gasoline and in general is not suitable as a fuel alone due to its hygroscopic and corrosive characteristics[135]. Longer carbon chain alcohols such as n-butanol and n-hexanol are candidates to replace ethanol due to their higher energy density and lower water solubility than ethanol[135]. Computational modelling of bioblendstocks for petrol engines have even generated a list of blendstocks that are likely to exceed the fuel efficiency of e10 petrol[139].

1.5 Aims

The overarching aims of this project are to build, modify and test a fleet of 5L, continuously stirred and automatically fed anaerobic digesters. Further to this, to investigate industrially relevant changes to operational parameters, and how these changes may impact process scale digestion.

Firstly, digesters modelling the process scale digesters used by Yorkshire Water, the industrial sponsor of this project, will need to be built and modified to ensure compatibility with real world feedstocks. The use of real-world feedstocks, a combination of primary, secondary and imported sludges, will no doubt provide challenges in comparison to commonly used synthetic wastewater, however it is essential to provide a fair reflection of the industrial process and microbial community.

Secondly, trialling the integration of unknown feedstocks into the YW AD process. The addition of new metabolites can have unforeseen consequences to the dynamics of the microbial community within an anaerobic digester and additional confidence can be gained by trialling at lab-scale, and assess the likelihood of the Humus material causing digester failure through disruption of the

microbial community by measuring the process parameters biogas production, solids reduction and COD reduction.

Thirdly, to trial an increase in organic load and feed rate to the anaerobic digester. The current YW assets are insufficient for the predicted growth of the population, and many assets are coming to the end of their lifespan over the next decade. The required processing of waste in the future could be met by increasing throughput through existing assets, however there are very realistic concerns around digester acidification that need to be addressed before this can be trialled at process scale, and to gain a better understanding of how and when digester performance destabilizes when operated at a lower HRT and higher OLR.

Chapter 2: Materials and Methods

2.1 Introducing System 60

System-60 is a fleet of 60 continuous, semi-automated, lab scale digester systems originally designed by Anaero Technology Ltd. Each digester has a 5 litre, single stage, capacity and a ~1.5L feeding syringe for automated feeding. Individual digesters are integrated into banks of 3, and into racks of 6. Triplicate banks of 3 were used for biological replicates. All parts were provided by Anaero Technology except when specified otherwise.

2.2 Digester design

The basic digesters were built by Anaero Technology for optimizing AD with synthetic feedstocks. Each digester has a 5-litre capacity, constructed from a cylinder of stainless steel with openings at the top, bottom and side (Figure 2.1). The tops of the digesters are sealed using a greased silicon gasket compressed between a Perspex headplate which is screwed into a welded plate at the top of the stainless tube (Figure 2.2). The lids include multiple ports for removal of samples without the lengthy and potentially contaminating process of removing the lid. These included a gas port, for sampling from the headspace, and a liquid sampling port that runs halfway into the digester and below the fill line. This allows for the removal of liquid digestate samples without contaminating the digester headspace with air. A third port allows for the insertion of a thermocouple into the contents of the digester. Below the digester a 1 ¾ inch stainless pipe connects the digester to the feeding syringe via a ball valve and non-return valve (Figure 2.3).

The opening to the side of the digester allows for the overflowing of the excess contents of the digester as the automated feeding system operates. The original design of the overspills proved impractical for long term experiments, the threading on the parts were unsuitable for making gas tight connections and this was originally mitigated using silicon sealant. Over longer-term experiments, the wear and tear would cause the silicon sealant to leak and come loose and so a different overspill design was designed and manufactured by the Biology Mechanical Workshops and retrofitted to all digesters.

Figure 2.1 – Front profile of the digester vessels detailing the general construction.

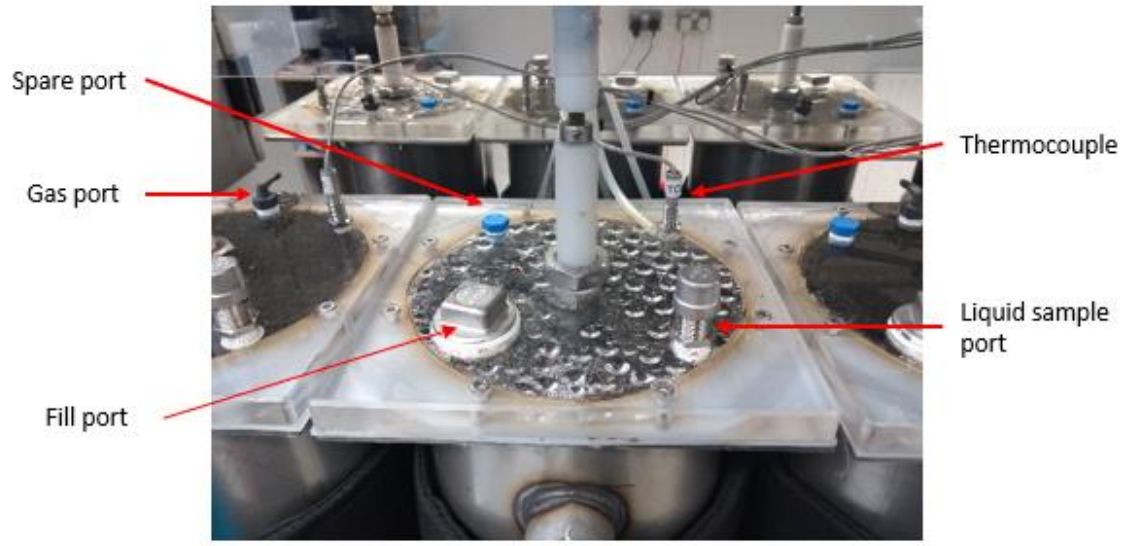


Figure 2.2 – Top profile of the digester vessels detailing the various ports for sample removal and temperature monitoring.

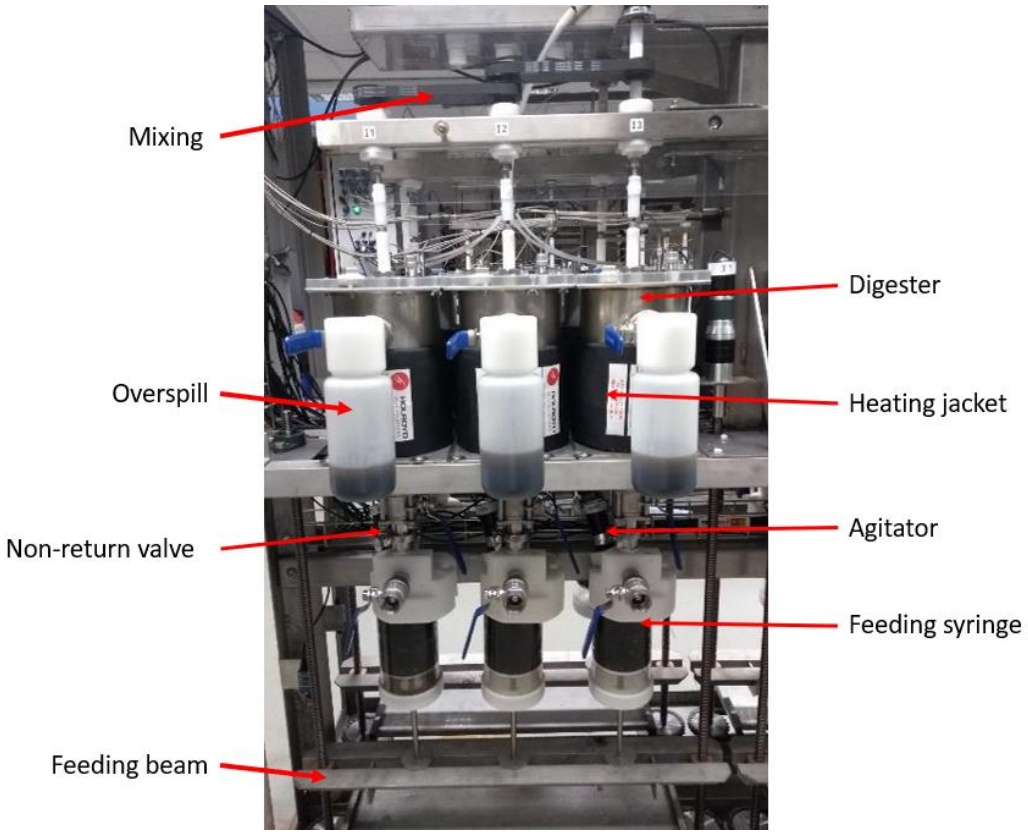


Figure 2.3 - A bank of 3 digesters.

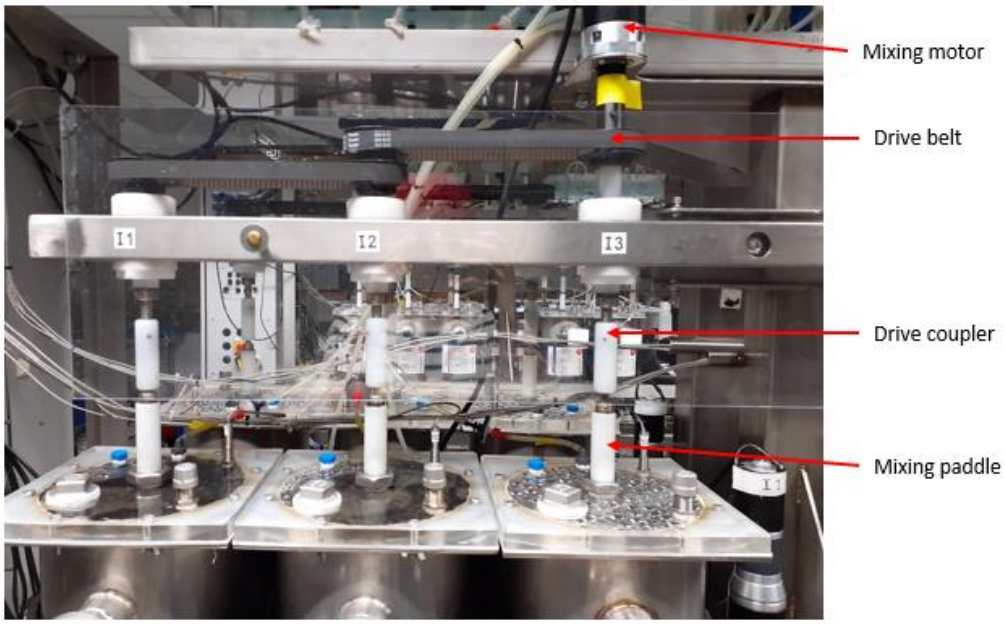


Figure 2.4 – Image detailing the series of mixing apparatus.

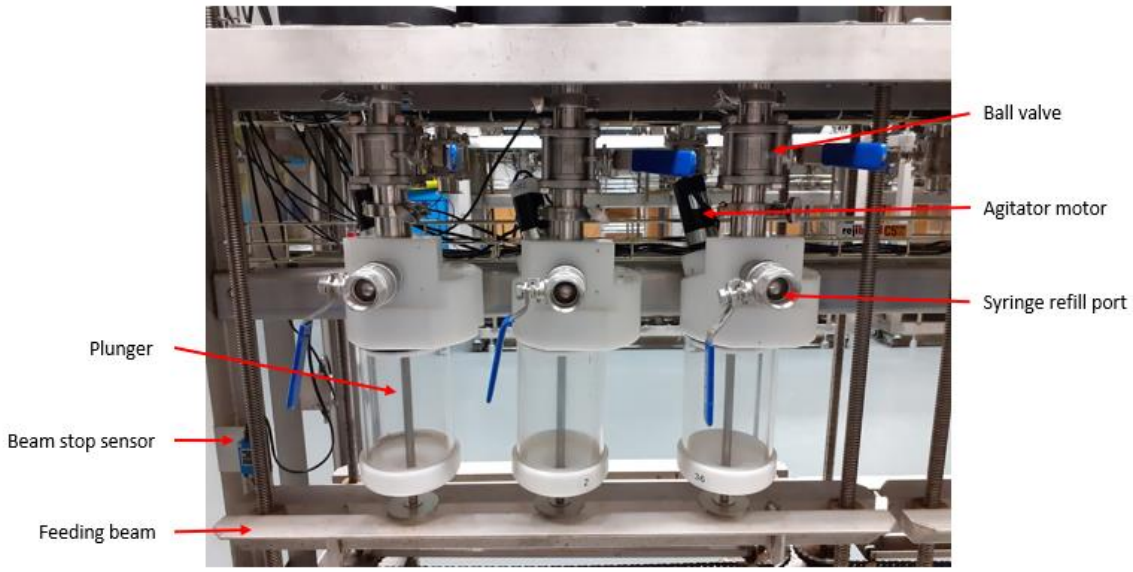


Figure 2.5 Image detailing the feeding syringes used to short term store the feedstock and automatically feed the digesters.

The temperature of the digesters is user determined and was maintained at approximately 35°C in these experiments in accordance with the average temperature of digesters at YW sites. Each digester is individually heated using a custom heating jacket (Holroyd Heat Solutions), and the desired temperature is maintained using internal thermocouples/temperature probes linked to a feedback loop in the programmable logic controller (PLC) (Figures 2.1 and 2.2).

2.3 Mixing

Digesters were continuously mixed at 50rpm in triplicate, using stainless paddles and motors provided as standard by Anaero Technology. A single motor controls one triplicate of digesters. The rotation from the motor is translated horizontally across all three digesters via toothed bands and then down into the digesters to stainless steel paddles to ensure triplicate digesters were mixed equally (Figure 2.4). By using paddles that extended the length and width of the digester it also ensured complete mixing and limited the likelihood of unmixed eddies within the digester.

2.4 Feeding mechanism

The feeding mechanism was designed such that the digesters are fed from the base and spill over from the top to ensure feed cannot bypass mixing and is more in keeping with the design of process scale digesters.

Each digester has its own feeding syringe connected via a ball valve to close the connection when necessary, for example for re-filling the feeding syringes, and a simple silicon non-return valve, a small silicon sheet with a cross scored from the centre, to discourage premature mixing of the digester contents and feeding syringe. Although simplistic, this allows material to only be fed in one direction.

The syringes consist of three main components, the syringe, plunger, and agitator motor. The agitator acts to mix the feed as it is pushed into the digester above via the plunger.

Although each digester has its own feeding syringe, the design means they are fed in triplicate. A single motor connects to a beam which is used to simultaneously raise the plungers of triplicate digesters. As the motor engages, it turns a screwing mechanism that is translated through a chain to both ends of the beam to ensure equal feeding between the three digesters. Positive pressure is created in the feeding syringe and feed is pushed up, out of the syringe into the digester. During feeding, the contents of the feeding syringe are mixed using an agitator motor located at the top of the feeding syringe to ensure that the feed is homogenised. The agitator motor is also programmable to start agitating the feed shortly before feeding which was preferable with thicker and harder to mix feedstocks.

The feeding beam was prevented from travelling too far and damaging the feeding syringes via a dedicated beam stop that uses dead man switches to prevent further feeding once the bar had reached a designated height.

The digesters were fed every 60 minutes, 24 hours a day between syringe refill points. The feed syringes had an approximate capacity of 1.5 litres which equates to 3-4 days at a 14 day HRT.

The feeding motors were programmed so that they would run for a specific length of time dictated by the estimated beam speed, the syringe diameter, and the desired hydraulic retention time. However, the beam speed varied from week to week depending on the friction of all the moving parts: the O-ring against the acrylic cylinder, the chain, the beam moving against the threaded rod and the rheology of the feed all act against the upward motion created by the feeding motor. This meant that while the PLC could be set to a particular HRT, there could be a large variance in the actual HRT. As a result, beam heights were measured at the beginning and end of each refill cycle to

measure the feeding rate more accurately. The actual HRT was back calculated based on the change in beam height to the nearest mm and the volume of feed that had entered the digester.

The PLC calculations for defining how long the feeding motor would need to run for are as follows where user inputs are highlighted in bold.

Digester volume (ml)/**HRT** (d)= Daily feed volume (ml)

Daily feed volume (ml)/ (π*$\mathbf{Syringe\ radius^2}$ (mm)/1000) = Daily beam travel (mm)

(Daily beam travel/**Estimated beam speed**)/(1440/**Feed cycle period**)*3600 = Seconds of feed time per hour

The feeding syringes had a capacity of approximately 1500ml which equated to a beam travel of approximately 150mm. Where one set of triplicate digesters was being fed at a different rate to another set of triplicate digesters that needed to be comparable, the volume of feed and thus beam travel needed over a set period of time was calculated and the beam's height set accordingly such that the feed within the syringe would be completely used up by the next refill point. By only adding the appropriate amount of feed into the feeding syringe we avoided possible variation in feedstock quality and composition that might occur from having it incubated at room temperature for differing amounts of time.

2.5 Gas flow and composition

Biogas flows out of the digesters through a port in the lid (Figure 2.2). The gas travels up to dual CH_4/ CO_2 composition sensors (Dynament) and then on to a gas flow meter (GFM) (Figure 2.6). Gas volume was measured using water displacement tip buckets that were manually calibrated monthly. The tip buckets fill with gas and tip back and forth, with an attached magnet disrupting the local circuit to allow the PLC to count tips. The system included a thermometer and barometer within the PLC, and converted the tips into STP gas measurements.

The GFMs were filled using tap water and a chlorine based biocide - Aqua Stabil water bath protective media (Jubalo) to prevent algal growth. Biogas enters the GFM through a port underneath a two-compartment tip bucket on a pivot. As gas fills one half of the tip bucket, the bucket pivots back and forth releasing the gas and activating a magnetic switch. In this way a tip, equating to a volume of gas, is recorded by the PLC. The temperature and room pressure are combined with the measured bucket volume to calculate the volume of gas and flow rate by the PLC. Gas was vented out of the building at normal atmospheric pressure.

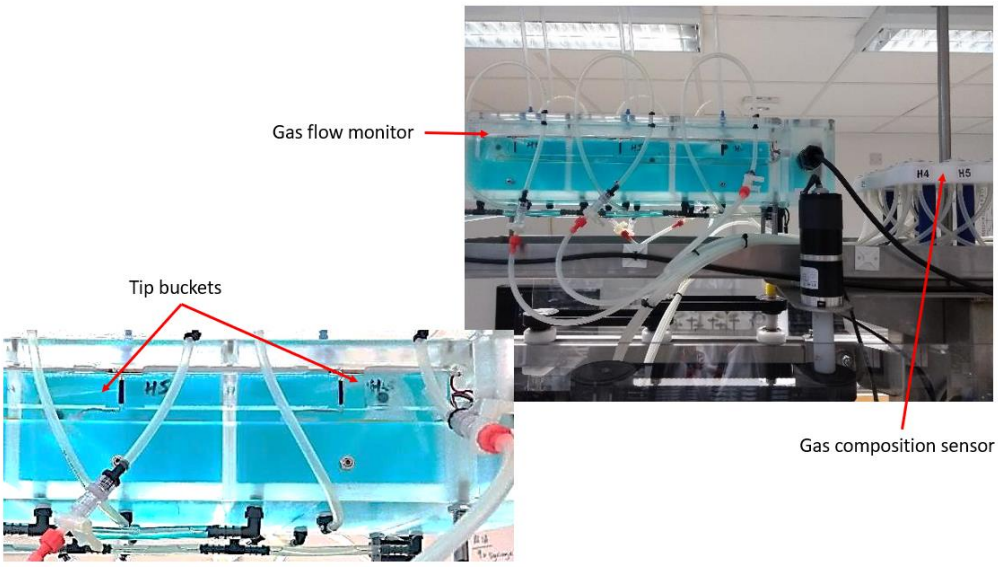


Figure 2.6 – Gas volume and composition monitoring system.

The gas composition sensors were not an original part of the design from Anaero Technology and were added later. The sensor housing and controlling circuit boards attached to Arduino control units including an ethernet connection were designed and built by Mark Bentley and Stephen Howarth from the Biology Mechanical and Electronic Workshops respectively.

The composition sensors were calibrated using a 2 point calibration of 100% N_2, 50:50 mix CO_2:N_2, 50.24:49.76% CH_4:N_2 (BOC) according to the manufacturer's instructions and recalibrated between experiments. The values for CH_4 and CO_2 were queried every 60 seconds by the PLC system via Modbus and recorded at every tip of the GFM.

GFM's were manually calibrated at the beginning of each experiment. This involved attaching a large syringe to push a known volume of air though the tip buckets, counting an even number of tips (minimum 4) and then measuring the volume of air to know the average amount of gas to cause a tip – each side of the bucket might require a different amount of gas to tip due to manufacturing tolerances.

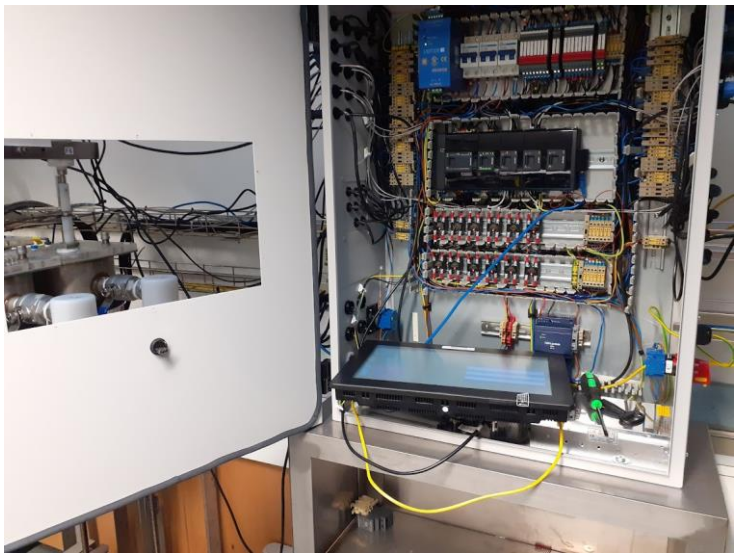


Figure 2.7 - The programmable logic controller (PLC) responsible for monitoring the inputs such as thermocouples, temperature, atmospheric pressure, beam speeds, and controlling the outputs such as heating jackets, mixing, feeding and agitator motors.

2.6 Digester control

Digesters were controlled via two Unitronics PLC systems and custom software written by Robin Proctor at LAB H4 Ltd and wired by Anaero Technology (Figure 2.7). The PLC was used to control the turning on/off of heating jackets, mixing motors, feeding motors and agitator motors while monitoring information from the thermocouples, beam stops, GFM's and gas sensors. The software also continuously recorded information on 40+ variables including gas flow, composition, room temperature, room atmospheric pressure, actual digester temperature, set digester temperature, and whether the agitator motors were engaged at the time.

2.7 Digester inoculation

For all experiments, digesters were seeded using inoculum from full-scale anaerobic digesters at Yorkshire Water's Esholt wastewater treatment works located at 53°51'09.6"N 1°43'15.1"W. The inoculum was added from the top into pre-warmed digesters until the digestate flowed out of the overspill so that each digester was "full" regardless of any variations in volume created during manufacturing.

Real world sludge was used as the feedstock and was also collected from Esholt wastewater treatment works from a sample point directly after thermal hydrolysis, either weekly or fortnightly. The sludge consists of a combination primary and secondary sludge as well as a larger than normal percentage of imports. This allowed for natural, seasonal variations to be reflected in the feedstock, while minimizing the extent of feedstock degradation. The thermal hydrolysis plant was built at Esholt by BioThelys and supplied by Veolia Water Solutions. Thickened sludge from conventional sewage treatment is filtered and thinned with 85°C water. The sludge feeds into one of 6 paired reactors and injected steam heats the sludge up to 165°C and pressurising up to 6-8 bar for 30 minutes. The sludge is then rapidly decompressed and heat exchangers cool the sludge before entering the digesters.

Both the digestate and feedstock was stored at 4°C in 30L vacuum-sealed food grade containers when not in use. The vacuum, thermal hydrolysis pretreatment and disposal of feedstock once the container had been opened helped to prevent any measurable degradation of the feedstock.

2.8 Humus flocculation & thermal hydrolysis treatment

Humic material at 1-2 DS% (w/w) was also collected from the Esholt wastewater treatment works. To thicken the material prior to steam explosion the flocculant Flopam FO 4490 VHM, a pre-THP thickener used at Esholt, was added at a rate of 1/1000 as advised by site operators, and the material was centrifuged at 6,000rpm and resuspended into part of the liquid fraction to increase the dry solids percentage.

Sewage sludge at the Esholt plant undergoes a thermal hydrolysis pre-treatment (THP) which involves heating the sludge up to a high temperature and pressure and then rapidly depressurising to break up larger particles and make the sludge easier to digest. In practice the humus would be added to the sewage sludge before this treatment, but the site was unable to process small batches. Instead, the humus was steam exploded separately by the Biorenewables Development Centre (BDC) in Dunnington, York and added to the sludge feedstock shortly before loading into the feeding syringes.

The material was processed in a single batch of 20 litres in a 100 litre stainless steel explosion vessel. The sealed vessel was heated to 160°C for 20 minutes before the material was cooled to 140°C and rapidly decompressed for explosion. This process is slightly different to that used by the Esholt site – there steam is injected to heat the sludge up to 165°C, held for 30 minutes and then decompressed. A small scale THP unit utilising the exact parameters of the Esholt site could not be sourced.

2.9 Sampling

40ml samples were taken from digesters and feedstock twice weekly for analysis. For the Humus (Chapter 4) and Esholt HRT reduction 1 (Chapter 5) experiment samples were taken from the overspill, which was mixed vigorously beforehand. For the Esholt HRT reduction 2 (Chapter 6) experiment samples were taken directly from the digester via the sample port, which was flushed twice using material from the digester before a sample was taken to prevent build-up within the port distorting downstream analysis. Samples were stored at -80°C.

2.10 Solids

A combination of techniques for calculating dry, volatile, and fixed solids percentage (DS%, VS%, FS%) were used depending on equipment availability, however all percentages are based on weight as a percentage of the original sample weight. The Humus (Chapter 4) and Esholt HRT reduction 1 (Chapter 5) experiments used a heated balance up to 120°C (Ohaus MB25) to calculate DS% and a Milestone Pyro Classic microwave furnace provided by Analytix Limited for calculating VS%/FS%. For the Esholt HRT reduction 2 experiment, crucibles were dried at 120°C overnight for DS% and ashed at 550°C for 1 hour using a Milestone Pyro Advance (ASTM D1506 Method B) for VS%/FS%. The following equations were used for calculating DS%, FS% and VS%:

$$Dry\ solid\ \%\ (DS\%)\ = \frac{(Dry\ sample\ -\ Crucible\ weight)}{(Wet\ sample\ -\ Crucible\ weight)} * 100$$

$$Fixed\ solid\ \%\ (FS\%) = \frac{(Ashed\ sample\ -\ Crucible\ weight)}{(Wet\ sample\ -\ Crucible\ weight)} * 100$$

$$Volatile\ solid\ \%\ (VS\%) =\ Dry\ solid\ \%\ -\ Fixed\ solid\ \%$$

When not in use, crucibles were dried in the drying oven and stored in a dehumidifier.

2.11 Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured using the commercially available LCK914 kits from Hach Lange according to the manufacturer's instructions. Samples were homogenous with a small

particle size and no pre-treatment was required. Where necessary samples were first diluted 50:50 using distilled water.

2.12 pH

Where applicable, pH of digester samples was analysed twice per week using a Seven Compact 220 pH/Ion meter calibrated according to the manufacturer's instructions.

2.13 Volatile fatty acid analysis

10ml sludge samples were spun down in 15ml Falcon tubes at 4000rpm (Eppendorf A-4-81 rotor). 2ml aliquots were taken from the supernatant and further pelleted using microcentrifuge at 10,000rpm for 10 minutes. A sterile 2ml syringe was used to filter the supernatant through a 0.22µm filter into a sterile 1.5ml micro-centrifuge tube. 1ml of filtered sample was transferred to a glass GC vial containing 7.5µl 80% w/w orthophosphoric acid to ensure full protonation of the organic acids.

1µl of prepared sample was injected into an Aligent 5890 GC-FID. The column was a Nukol 30 m x 0.25mm AD 0.25um (Sigma, 24107). Gases were pressurised as following: Air (BOC) – 30PSI, H_2 (supplied by hydrogen generator, 20H, Dominik Hunter) – 20PSI and He (Grade A, BOC) – 40PSI. Column head pressure was set to 20PSI. Detectors and injectors were both set to 200C. The temperature gradient for the oven was a two-step ramp followed by a hold; 75°C -> 150°C (10°C/min), 150°C->200°C (20°C/min) hold at 200°C for 10 minutes.

Standards were run every 10 samples to accommodate shifting peaks. Peak analysis was completed by Luna Pulford.

2.15 Statistical analysis

Where statistical analysis was appropriate, the relative standard deviation (RSD) also known as coefficient of variation was used. This measures the ratio of the standard deviation to the mean, and therefore the extent of variability in relation to the mean. The lower the value, the closer the data clusters around the mean. The use of this statistical analysis allowed the direct comparison of variation between assays with different ranges.

Relative standard deviation (RSD) = standard deviation / mean

Chapter 3: Digester design and test run

3.1 Introduction

Laboratory scale AD facilities are a vital tool towards better understanding the microbial community and AD process as a whole. Their flexibility allows them to test a wider range of conditions than AD at process scale, with few of the operational and economical risks, in the hope of improving process scale AD. Novel experiments can be designed and samples collected as frequently as required, often more frequently than would be practical at process scale and be processed immediately in house. There is also a greater level of control at lab scale, as operational parameters can be quickly altered without concern for economic repercussions.

Lab scale digesters are small enough to be able to run biological triplicates, controls, and multiple variations on a condition at the same time. At larger scale the infrastructure to do that does not currently exist and it would require a massive input of funding to build, let alone staff, and other logistical challenges such as disposal costs if material is not compliant would need to be considered. Also, particularly at process scale, the ability to run controls does not exist as generally single feed tanks lead into a single pump that feeds multiple digesters in sequence.

Sampling, assays, and analysis of the physical properties of material entering and exiting the digesters is important to understanding the stability and functionality of the microbial community and the level of variation in the feedstock. The analysis of multiple parameters together helps to build a better understanding of the microbial processes going on in the digester than any single analysis, and what drives them.

The volume of biogas produced by the digester is perhaps the easiest way to evaluate the efficiency of a digester, but volume alone can be misleading. The economic value to biogas comes from the CH_4 content, and so measuring both biogas flow rate and composition is preferable.

The gas produced is directly a result of the organic material that is put into, or fed to, the digester. This can be measured in several ways. Dry solids (DS) consist of fixed solids (FS), indigestible material such as sand or trace metals, and volatile solids (VS), organic digestible material such as microbial biomass or sewage sludge. By measuring the DS, FS, and VS we can gain an understanding of how these elements are changing through the digestion process. COD also measures the amount of digestible material that could potentially be turned into biogas.

In terms of understanding the flow of metabolites within the system, the concentrations of VFAs can provide insight, as does pH. Reduced gas flow rate or composition can be correlated to changes in VFA's or pH, which can be correlated back to changes in VS or COD in the feedstock or digestate.

Finally, DNA sequencing of the microbial community, either via metagenomics or 16S rRNA, can provide even more insight into the community and be correlated to biogas production and organic material destruction.

By performing multiple assays, a clearer picture of the processes and stability of the digester and microbial community is produced.

Scalability in lab-based AD is a frequent challenge. Many of the processes used at process scale waste-water treatment plants do not scale down to lab-scale, and many of the processes used at lab scale do not scale up to process scale. For example, process scale digesters used by YW have either recirculation pumps or gas mixing, if they are mixed at all. These systems scaled down to an appropriate size to work on a 5L digester would quickly become blocked or fouled when using real world sludges. Instead, a paddle attached to a motor was used for the lab scale digesters. Similarly, heating is provided to process scale digesters using heat exchangers and recirculation pumps. Again, these would not be practical on a small system, and so heating jackets were used instead. Other process scale features cannot be even mimicked at small scale. At process scale the digester contents are under massive pressure due to the volumes of the digesters, this likely has impacts on the microbial community and will have impacts on things like gas solubility. One feature that was as similar to process scale as possible was the method of feeding. At process scale feeding would be automated throughout the day and night, with a single pump feeding each digester for a set amount of time on rotation. The lab scale digesters were designed to feed automatically 24/7 for a short time each hour, the difference being at the same time rather than in rotation. The large pipe diameters also meant that real world feeds could be used without fear of blockages.

3.2 Aims

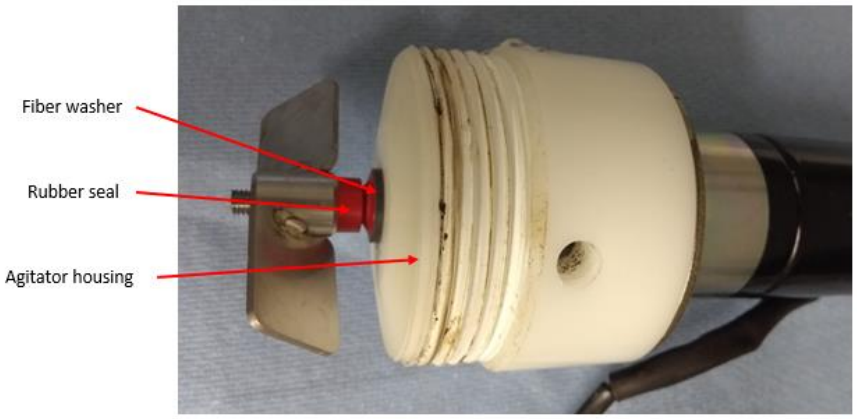
The overarching aims of this project are to build, modify and test a fleet of 5L, continuously stirred and automatically fed anaerobic digesters. Further to this, to investigate industrially relevant changes to operational parameters, and how these changes may impact process scale digestion.

Firstly, digesters modelling the process scale digesters used by Yorkshire Water, the industrial sponsor of this project, will need to be built and modified to ensure compatibility with real world feedstocks. The use of real-world feedstocks, a combination of primary, secondary and imported sludges, will no doubt provide challenges in comparison to commonly used synthetic wastewater, however it is essential to provide a fair reflection of the industrial process and microbial community.

3.3 Feed syringe modification

Real world feeds were collected from YW sites for use in System-60, however these feedstocks created problems with several components of the feeding syringe. Usually synthetic and homogenous feeds are used for lab scale AD and so modifications to the system were trialled and implemented. The heterogeneous nature of wastewater sludge meant that particles in the sludge were able to get between the seals and cause leaks and excessive frictional wear, and hair to wrap around moving parts. This was a particular issue around the agitator motors causing motor breakdown from the excessive torque as well as leaks. The original design featured a red rubber seal and fibre washer. Small particulates were able to infiltrate under the fibre washer and damage the acrylamide of the agitator housing and cause leaks (Figure 3.1A). First a stainless-steel plate was added to protect the soft acrylamide of the agitator housing from wear against moving parts and a "hair skirt" was 3D printed to protect the seals from attack by larger particles (Figure 3.1B and C). Both changes were designed and manufactured by Mark Bentley in the Biology Mechanical Workshop.

The O-rings supplied with the system proved unsuitable for use with high particulate feedstocks such as those from wastewater sites that typically have an element of sand or grit. The O-rings would roll rather than sliding smoothly up and down the Perspex of the feed syringe, leaving a coating of particulates on the inside of the feed syringe, damaging the Perspex and also creating excess friction which had consequences in increasing HRT (Figure 3.2). Several different solutions were trialled but ultimately the circular cross-section O-rings were swapped out for quad rings (also known as x-rings or four-lobed seals) with a square profile also thanks to the suggestion of the Biology Mechanical Workshops.



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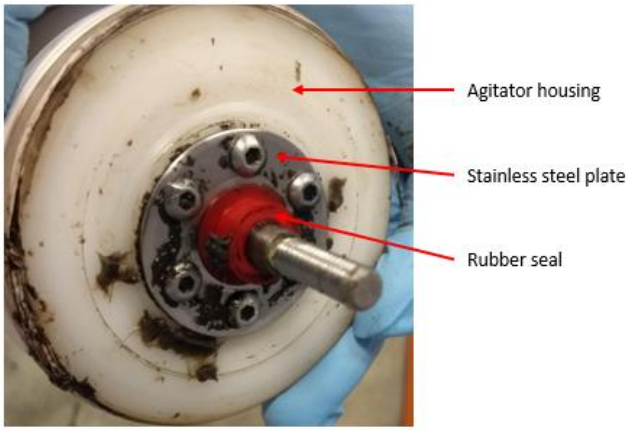

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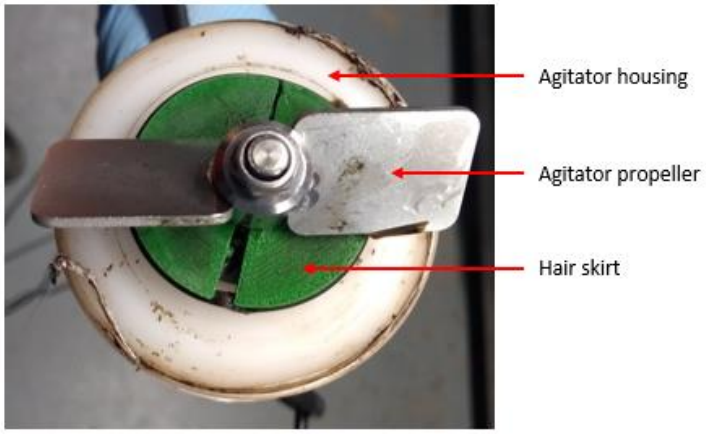


(C)

Figure 3.1 – Developing the agitator motor housing.

(A) The initial design of the agitator motor housing provided by Anaero Technologies.
(B) A stainless-steel plate was added to prevent wearing away of the white polyacrylamide housing from the friction.
(C) A 3D printed hair skirt to protect the red seal and shaft from accumulating hair and other material.

Figure 3.2 - The O-rings within the plungers of the feed syringes were not suitable for use with high particulate feedstocks.

3.4 Gas flow and composition modifications

Often displacement method gas flow meters are filled with sodium hydroxide (NaOH) rather than water. This allows the measurement of the volume of CH_4 produced as the CO_2 in the biogas dissolves into the NaOH. However, since we had gas sensors that would calculate the percentage of CO_2 to CH_4, this step was unnecessary. In general, it was determined that YW viewed the reduction of solids in the resulting digestate and conversion into gas was more of a priority than the quality of the gas, and so it was a priority to measure the total volume of biogas over the volume of CH_4. CO_2 would still have dissolved into the water within the GFM, albeit to a lesser extent than the NaOH and the measured gas flow rates may have been decreased initially. Based on the solubility of CO_2 at 18°C being around 20cc per gram of water[140], this was combatted by running digesters for several weeks before experiments officially started and then recalibrating the tip buckets.

Also, NaOH was deemed unsuitable as it would have eventually been saturated and no-longer absorbed the CO_2 resulting in an overestimation of gas volumes, which was foreseen as a risk due to the length of the experiments. NaOH has an absorption capacity of approximately 50g/L of CO_2 at a 5 w/w%[141], and the GFM a volume of 3L, resulting in an absorption capacity of approximately 150g of CO_2. Either the NaOH would become saturated, and CO_2 would have become an increasing fraction of the measured biogas over time, or the NaOH would have had to have been periodically replaced resulting in more down time.

Finally, the GFM's are housed above the digesters, and it was not considered safe to be lifting large volumes of NaOH overhead.

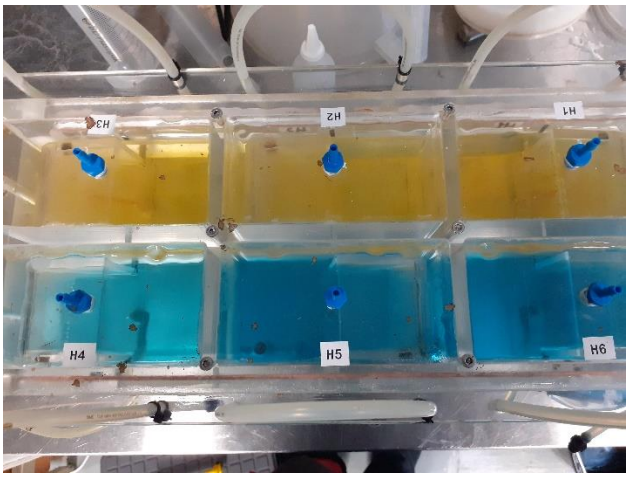


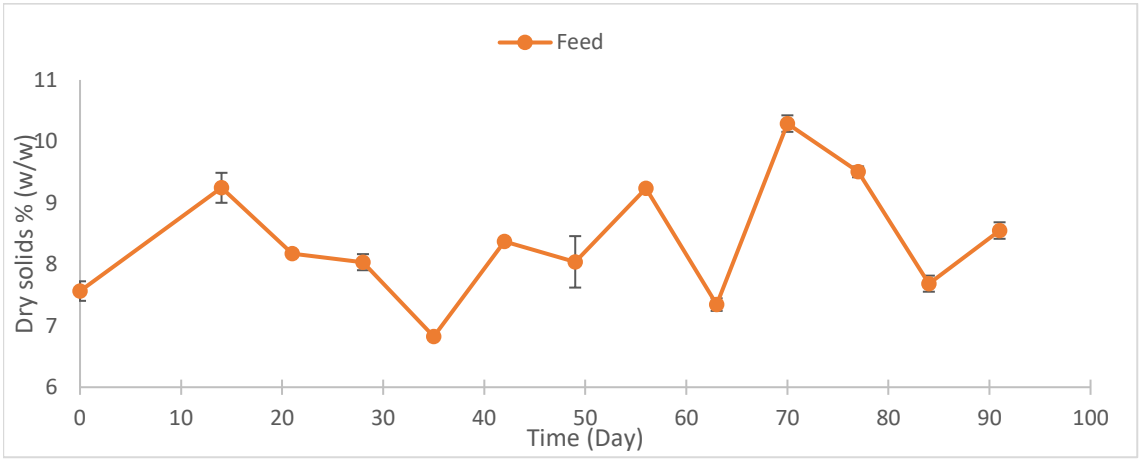
Figure 3.3 - The Aqua Stabil water bath protective media gave the water within the GFM's a blue colour. When the digesters were producing H_sS, the colour rapidly changed to a bright yellow colour.

Filling the GFM's with water did however create other issues, namely with bacterial and algal growth. This was solved by using Aqua Stabil water bath protective media (Jubalo), a chlorine-based biocide with a distinctive blue colour.

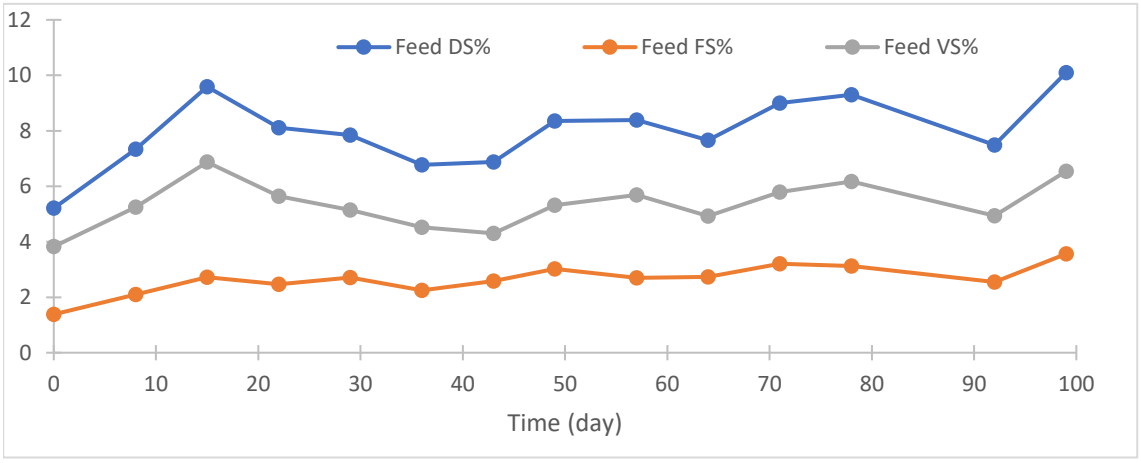
H_2S is a dangerous to human health gas in low doses and a large problem in AD. Sulphate-reducing bacteria produce H_2S and directly compete with methanogens and therefore H_2S is an indicator molecule that the delicate balance between the microbial community within the digester has shifted. Although H_2S sensors were not fitted, the chlorine-based biocide chosen turned from blue to yellow when digesters produced H_2S (Figure 3.3), and this became a warning system for H_2S production. The direct chemical or redox cause of the yellowing was not investigated past confirming causation.

3.5 Feed storage, collection, and characterization

Feed would need to be collected in bulk and stored between collections. Typically, collections would be weekly, but depending on site down time and staff availability could be fortnightly. To better understand how storage would affect the quality of the feed and how this might affect the results of any experiments, triplicate digesters were set up and run over 90 days. The feed was collected in multiple containers and was sampled twice per week for DS%, from the container being used to feed the digesters (Figure 3.4A). Containers of unused feed remained sealed under vacuum until used.



(A)



(B)

Figure 3.4 – Measurement of feedstock solids over 91 days

(A) DS% of barrels of feed collected at the same time. Feed was typically collected weekly. DS% from each collection date were averaged to better understand the variation between feed collected at the same point and the extent of any degradation that may occur. Error bars of the standard deviation applied.

(B) Breakdown of the solids % composition. The DS%, VS% and FS% of the feed were plotted together such that DS% = VS% +FS%

The DS% of the feed varied between collections, varying between 6.83% and 10.29% or a relative standard deviation (RSD) of 0.12, but less so between multiple barrels collected on the same day and stored up to two weeks, the largest variation being between 7.46% and 9.00% on day 49, or a RSD of 0.10 (Figure 3.4A). Comparison of the weekly DS%, VS% and FS% measurements also indicated that variation in the DS% was primarily due to changes in the VS% rather than FS% (Figure 3.4B).

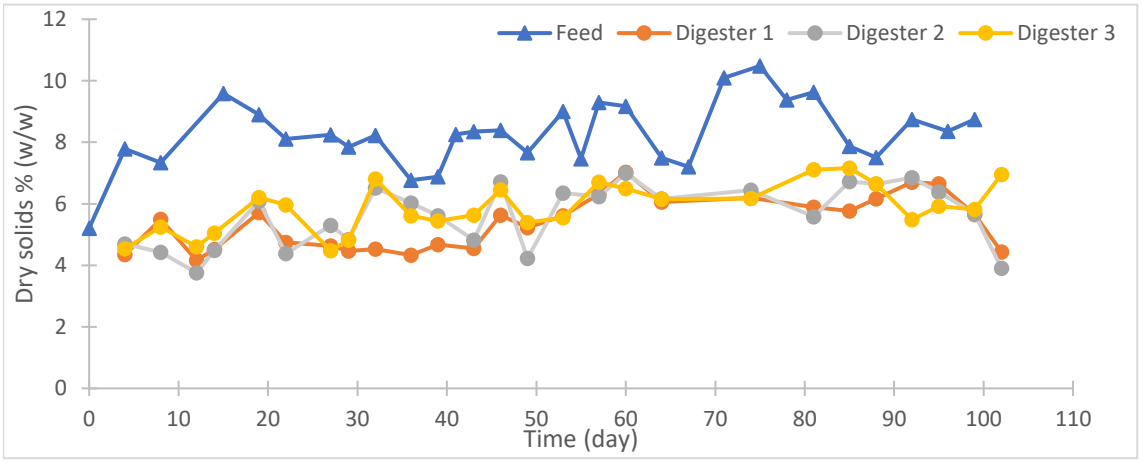
Therefore, it was concluded that storage at 4°C did not change the composition of the feed over the course of one or two weeks any more significantly than normal fluctuations in the feed composition

from site.

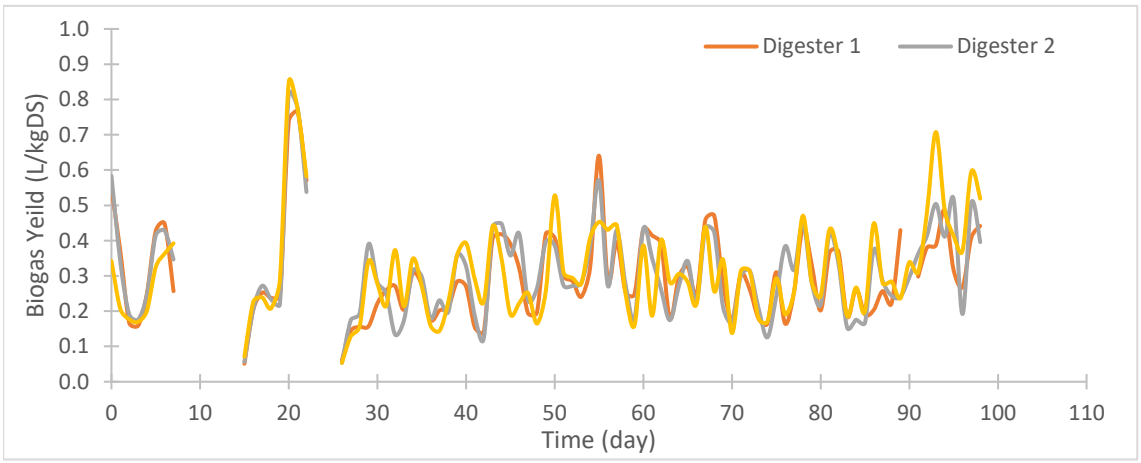
3.6 Initial operation and repeatability

The digesters were designed to run in biological triplicates. For an initial trial one bank of digesters was set up to check they functioned appropriately, breaking down solids into gas, and to assess the variation between the biological replicates. The HRT was set to 14 days, mixing to 50 rpm and temperature to 35°C.

The DS% were measured twice per week for both feed and digestate (Figure 3.5A),and used to calculate the biogas yield in conjunction with the biogas flow rate (ml/hr) and HRT (d) (Figure 3.5B). The FS%, VS% and COD were measured once per week (Figures 3.6A, 3.6B and 3.6C respectively). There was a reduction in DS%, VS% and COD between the feed and digestate of 32.4%, 40.8% and 37.2% respectively, and a minimal reduction in FS% of 3.8% on average over the course of the experiment. The biogas yield was calculated to be 0.3 L/kgDS over the course of the experiment. In this respect the digesters behaved as desired in converting solid material into biogas.



(A)

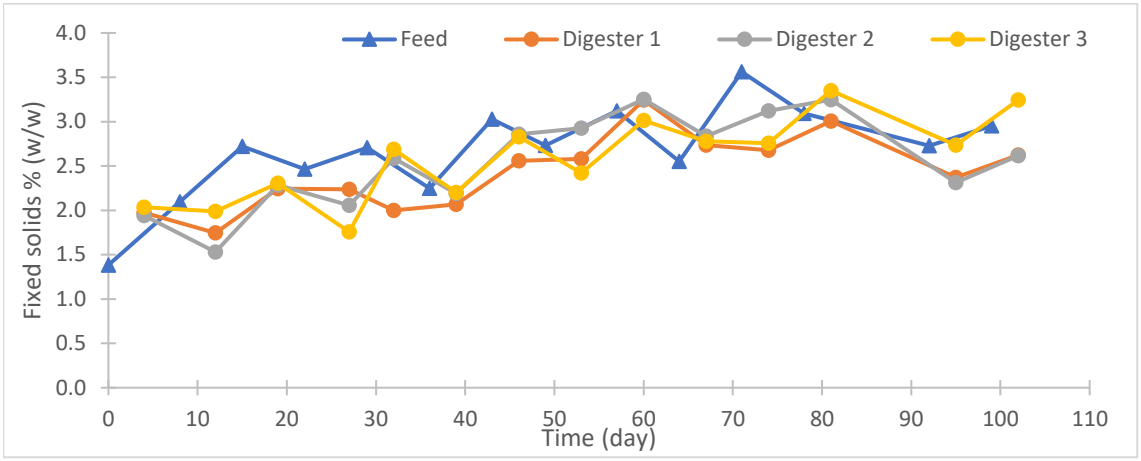


(B)

Figure 3.5 – Comparison of waste-water sludge AD using System 60

(A) Dry solids % was calculated twice per week for both feed and digestate on a per weight basis.

(B) Yield was calculated as litres of biogas per day per kg of dry solids fed. Gaps in biogas yield are due to missing HRT data due to software breakdown



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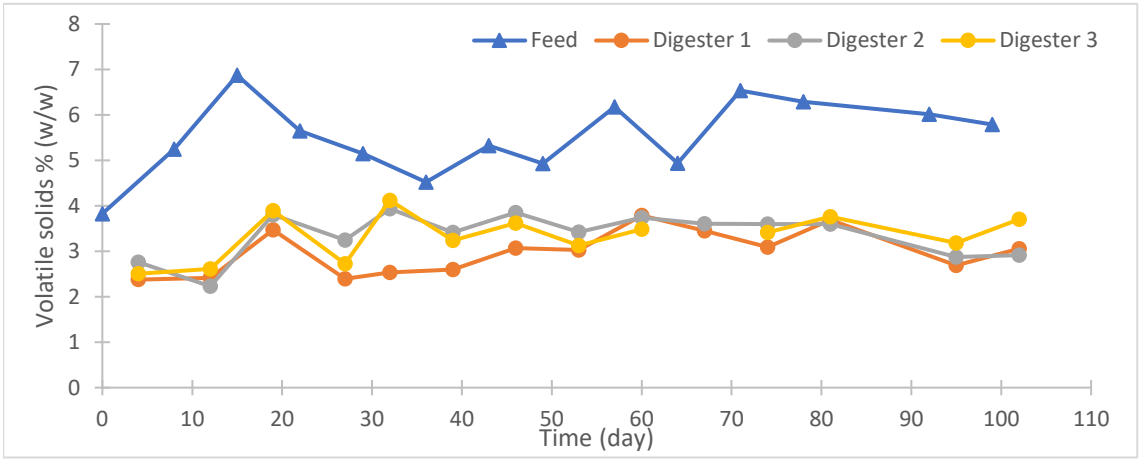

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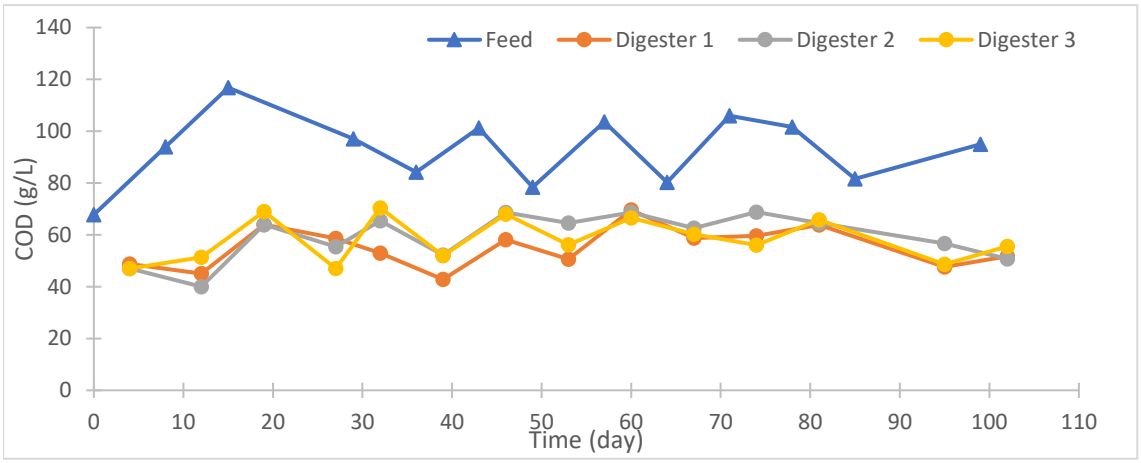


(C)

Figure 3.6 – FS%, VS% and COD of feed and digestate

The fixed solids % (A) and volatile solids % (B) were sampled weekly and calculated on a percentage by weight basis. The COD (g/L) (C) was calculated for both feedstock and digestate weekly.

3.7 Conclusions

Modifications to System 60 were identified and required for effective operation on real world wastewater sludge feedstocks.

Traditionally experimental anaerobic digestion is done under conditions that do not accurately reflect real world processes due to the difficulties in working at smaller scale, reduced resources and challenging feedstocks. These can include either singularly or in combination: batch rather than continuous feeding, the use of synthetic feedstock material, shorter experimental run times due to increased labour and <1L digesters[90].

Real world feeds were collected from YW sites for use in System-60, however some of the innate characteristics of the feedstocks created problems when used at smaller scale. High particulate content is a feature of sewage sludge feedstocks, in part, as a result of the settling and sand-filtration stages earlier in the treatment of wastewater. These particulates permeated seals and damaged moving components. However small changes to components and seals in the feeding syringe meant that real world feedstocks could still be used without any changes made to the feed such as sieving or filtering.

Typically, in small scale AD experiments only the CH_4 of biogas is measured, as this is currently the most valuable portion of biogas, using bubbling through NaOH to strip CO_2 out. This is not a technique that is used at full scale on YW sites. System 60 instead used distilled water and water bath protective media to prevent autotrophic microbial growth. This had the added benefit of being reactive to H_2S, a toxic and corrosive component of biogas, allowing rudimentary detection without the need for a dedicated H_2S gas sensor.

Although inoculum and feedstock is heterogeneous in nature, the robustness of the AD process and microbial community is resilient and reproducible despite heterogeneity.

Feedstock would need to be collected periodically and stored between uses due to the location of the WWTP in relation to System 60. When containers were filled at approximately 45°C, sealed and then stored at 4°C a natural vacuum formed, which if not disturbed unnecessarily, meant that storage of feed at 4°C for up to two weeks did not significantly affect composition with regards to dry/fixed or volatile solids. These data is in keeping with other reported observations around stored sludge[90,142]. Feedstock varied more between collections, than feedstock collected at the same time and then stored, highlighting the importance of regular collections to capture the daily changes in

feedstock composition. The digesters were resilient against spikes and variation in the feedstock. Increases in DS%, VS% and COD in the feedstock did not result in equal spikes in the digestate coming out, and the digestate was constantly and less variable product.

System 60 digesters show high reproducibility between biological replicates.

Laboratory scale AD facilities are a vital tool towards better understanding the microbial community and AD process as a whole. This short experiment demonstrated the reproducibility between biological replicates of digesters. The average biogas yields over 98 days for individual digesters Digester 1, 2 and 3 was 0.30, 0.31 and 0.31 respectively with an RSD of 0.02. Over shorter time periods there was greater variation between the digesters, for example on days 29 or 76, however over long time periods the biogas yield between triplicates is replicable.

Chapter 4: Humus trial

4.1 Introduction

The Esholt site, in addition to local sludge, treats waste from a number of different sources, including trade wastes. The AD process is a delicate balance between the 4 stages of hydrolysis, acidogenesis, acetogenesis and methanogenesis and can easily suffer from inhibition from ammonia, sulphides, VFAs, metals, and various other organic compounds[90]. The list of organic compounds reportedly toxic to AD is extensive, including benzenes, phenols, alkanes, aliphatics, alcohols, aldehydes, ethers, ketones and carboxylic acids to name a few broad groups[57]. There is also a large amount of variation in reported concentrations of inhibitory compounds, making predicting inhibition in a microbial community challenging[57]. These trade wastes could potentially contain toxic compounds with unforeseen consequences and their addition to the AD process should be treated with caution, making trialling at lab-scale an attractive option. Lab-scale trial of integrating new waste streams such as those with high fat content[143], into AD processes can be an excellent transitionary stage.

Yorkshire Water was required to treat a new trade waste, a humic residual material from the production of herbicides that shall be referred to as Humus. The cost of disposal of this waste was estimated at £600,000 per year, therefore addition to their AD process seemed preferable if possible. By adding the waste in higher concentrations than expected at operational levels to small lab-scale digesters, accumulation of inhibitory or toxic effects on the microbial community could be identified far in advance and an assessment made as to the financial benefits and drawbacks of treating the waste through AD. A limited list of compounds of concern to Yorkshire Water was provided to us (Table 4.1), however it is important to note that the list was not exhaustive, but also that the waste is 98% water, with the material being considered safe for direct discharge into a water course the majority of the time.

4.2 Aims

To trial the integration of unknown feedstocks into the YW AD process. The addition of new metabolites can have unforeseen consequences to the dynamics of the microbial community within an anaerobic digester and additional confidence can be gained by trialling at lab-scale, and assess the likelihood of the Humus material causing digester failure through disruption of the microbial community by measuring the process parameters biogas production, solids reduction and COD reduction

Table 4.1 – Humus chemical composition and COSHH R phrases

The Humus material for treatment contained a number of chemicals that caused concern among Yorkshire Water.

4-chlororthocresol, (Para-chloro-ortho-cresol), (4-chloro-2-methyl-phenol)	R23	R35	R41	R50		
2,4-dichlorophenol (2,4-DCP)	R21	R22	R34	R51	R53	
2-(4-chloro-2-methylphenoxy) acetic acid (CMPP)	R20	R22	R38	R41		
2,4-dichlorphenoxy acetic acid (2,4-D)	R22	R36	R37	R38		
4-(2,4-dichlorphenoxy) butyric acid (2,4-DB)	R21	R22				
2-(2,4-dichlorphenoxy) propionic acid (2,4-DP)	R21	R22	R38	R41	R43	
4-chloro-2-methyl phenoxyacetic acid (MCPA)	R22	R38	R41			
4-(4-chloro-2-methylphenoxy) butyric acid (MCPB)	R22	R23	R35	R41	R50	
Phenoxyacetic acid (PAA)	R37	R41				
alphachlorpropionic acid (ACPA)	R22	R35				
l-chlorpropionic acid (LCPA)	R22	R35				
Lactic acid (neutral pH)	R36	R37	R38			
Glycolic acid	R34	R36	R37	R41		
2-ethylhexanol (2EH)	R20	R21	R36	R37	R38	R41
n-Butanol	R22	R37	R38	R41	R67	
iso Butanol	R10	R37	R38	R41	R67	
Toluene	R11	R20				
Total Xylenes	R10	R20	R21	R38		
Isopropyl acetate (IPA)	R11					

Key
R10 Flammable.
R11 Highly flammable.
R20 Harmful by inhalation.
R21 Harmful in contact with skin.
R22 Harmful if swallowed.
R23 Toxic by inhalation.
R34 Causes burns.
R35 Causes severe burns.
R36 Irritating to eyes.
R37 Irritating to respiratory system.
R38 Irritating to skin.
R41 Risk of serious damage to the eyes.
R43 May cause sensitization by skin contact.
R50 Very toxic to aquatic organisms.
R51 Toxic to aquatic organisms.
R67 Vapours may cause drowsiness and dizziness.

4.3 Experimental design

Esholt receives approximately 25m^3 of Humus per day that would be required to enter AD at the site at approximately 1.5% dry solids. The Humus would be blended with approximately 1400m^3 of indigenous and other imported sludges with an average of 3.44% dry solids before dewatering and being treated by the THP reactors ahead of AD. This equates to approximately 48.16 tonnes of dry solids of feed and 0.38 tonnes of dry solids of Humus per day, or a 129:1 ratio.

With a view to run the System-60 digesters for 3 months (6 HRT's) at YW request, a minimum addition rate of Humus four times the estimated actual addition rate was chosen and a maximum addition of ten times the estimated actual addition rate. This equates to the digesters receiving a full years' worth of Humus material by the end of the experiment and YW was comfortable that a minimum concentration would be met.

Sewage sludge at the Esholt site undergoes thermal hydrolysis pre-treatment (THP) before addition to the digester (described in Chapter 2.8). It was likely that the Humus material would be blended into the sewage sludge before this THP step. Ideally this process would be emulated as much as possible. Hydrolysis of pre-THP feedstock combined with the Humus was deemed impractical due to the volumes that would need to be hydrolysed at small scale over the course of the experiment, therefore feedstock sludge was collected from a sample point post-THP before the sludge entered the digester and combined with the separately treated Humus in the lab (Figure 4.1).

120L of 1% DS Humus material was collected from site, thickened by centrifugation and subjected to steam explosion (described in Chapter 2.8), and then combined with the THP treated sewage sludge at time of refilling the digesters feeding syringes. On site the Humus could also be added post-THP of the feed should the THP process somehow chemically alter the material to become toxic to the microbial community in the digester, so a portion of the Humus was left untreated and added to the digesters.

12 digesters in 4 triplicate groups were set up as following:

(1) control: Esholt biology + THP-treated feed
(2) test: Esholt biology + THP-treated feed + 4* Untreated Humus
(3) test: Esholt biology + THP-treated feed + 4* THP-treated Humus
(4) test: Esholt biology +THP-treated feed + 10* THP-treated Humus
The digesters were initially run for 2 * 14d HRT on THP-treated feed from Esholt to stabilise conditions before any additions of Humus to the feedstock were made.

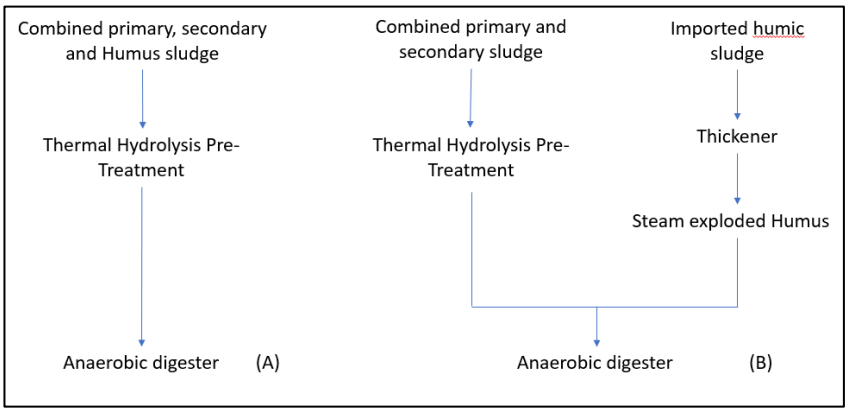


Figure 4.1

On site multiple sludges would be combined, undergo THP and then enter the anaerobic digester (A). As this was not possible for us to replicate on a small scale, hydrolysed sludge was collected from the Esholt site and combined with Humus that had been treated separately (B).

4.4 Results

The experiment was run until the Humus material was exhausted, which for the Untreated and THP*4 Humus addition digesters was 81 days. When the Untreated Humus was exhausted, the remaining THP treated Humus was diverted to the THP*10 digester and the Untreated and THP*4 Humus digesters were turned off. This meant that the Control and THP*10 digesters were run for 102 days and the THP*10 digesters received the equivalent dose of 1020 days of Humus material. Software failures on days 8-15 and 20-27 resulted in issues in HRT calculations and feeding.

Although the hydraulic retention time (HRT) was set to 14 days for all 4 feedstocks, this varied slightly between different feedstock triplicates for the reasons described previously in Chapter 2.4 (Figure 4.2A). The HRT was measured twice per week to ensure that the actual/ desired volume of feed being added to the digester was known. The average HRT across all 4 conditions for the length of the experiment was 14.1d, however excluding the several days of high HRT due to mechanical breakdown at the beginning of the experiment brings the average down to 13.7d.

In general, there was little variation in HRT and feed rate between the feedstock groups over the length of the experiment. There were periods of time of differing HRTs, for example between days 43 and 46. During this period the largest difference between HRTs for the control, untreated, THP*4 and THP*10 groups was 16.5, 17.2, 17.5 and 17.2 days respectively. Over time any differences evened out and the average HRT for each grouping for the length of the experiment was 14.1, 14.0, 13.9 and 14.1 days respectively. Further calculations of the exact volume of feed that on average

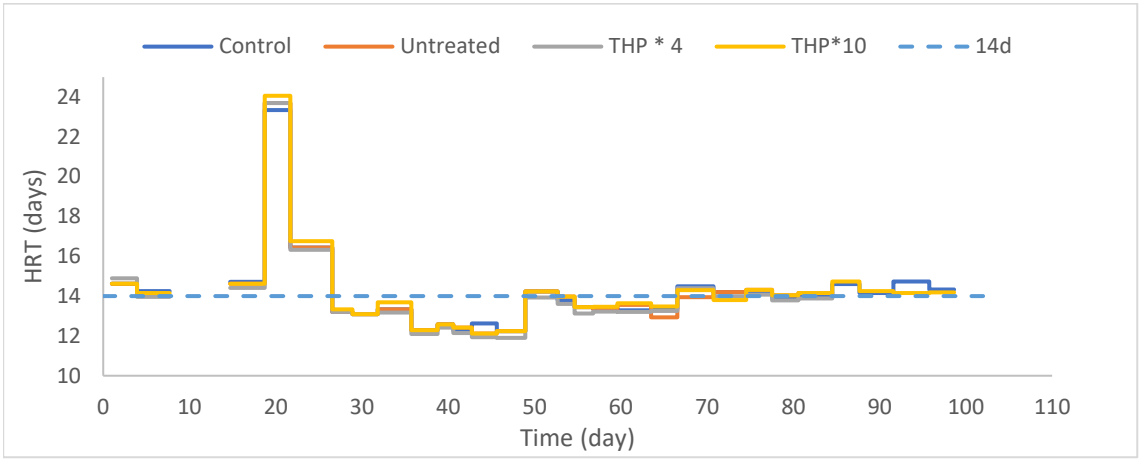
each feedstock group would have received finds a maximum differential of 6ml per day – (14.1d HRT = 354ml per day, 13.9d HRT = 360ml per day), 0.1% of the digester volume.

Gas Flow

All 4 sets of triplicate digesters showed consistent and replicable gas flow rates over the course of the experiment. The raw data for each digester were averaged to produce a single gas flow rate value for each day and then averaged across the biological replicate digesters in the group. There is a cyclical pattern to the gas production, with the gas rate increasing on days where the digesters were fed and decreasing over the next 3-4 days as the feed in the feeding syringe is used up indicating there may be some digestion of the feed within the feeding syringe within the first 24 hours (Figure 4.2B).

The average daily gas production for the length of the experiment for the Control, Untreated, THP*4 and THP*10 feeds was 396ml/hr, 350ml/hr, 400ml/hr and 431ml/hr respectively, giving an initial indication that the addition of the Humus material was not detrimental to the microbial community. Biogas yield was also calculated to account for varying HRTs and feedstocks (Figure 4.2C). The average biogas yield for the Control, Untreated, THP*4 and THP*10 feeds was 0.32, 0.29, 0.33 and 0.35 L/kgDS fed respectively, with all Humus variants showing no significant difference with the Control (t-test, $p=0.12$, $p=0.62$ and $p=0.14$ respectively), further supporting the hypothesis that the inclusion of the Humus waste to AD would not be detrimental to biogas production on site. The HRT was measured every 3-4 days, and the biogas yield was graphed with a 4-day rolling average.

In addition to 24-hour monitoring of gas flow, samples were taken and analysed regularly on both the feedstock going into the digesters and digestate coming out of the digesters. Although the addition of the Humus was not expected to significantly alter any of the analysis for the feed due to it making up such a small fraction of the overall feed, each of the 3 feedstocks with Humus added were analysed to confirm the presumed uniformity.



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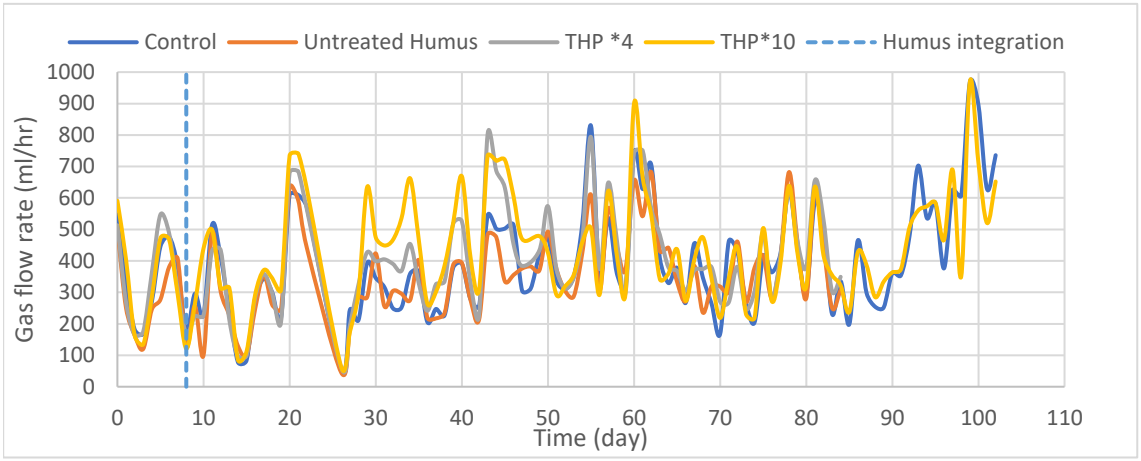

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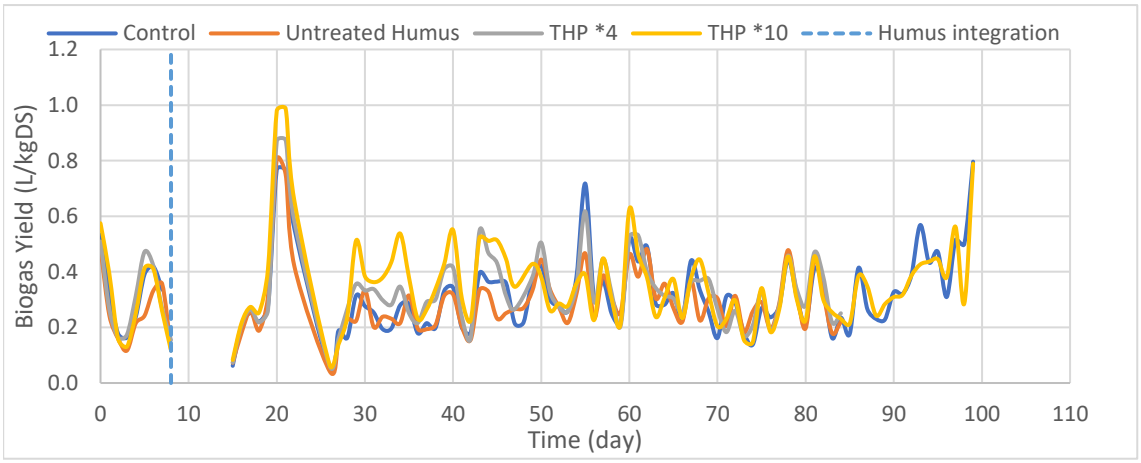


(C)

Figure 4.2

(A) The HRT of the experiment aimed to be 14d. This is impossible to enforce exactly, however the measured HRT was close for the majority of the experiment.

(B) Gas flow was averaged into a single value for each day for each digester and then averaged over biological replicates.

(C) Biogas yield calculated as litres of biogas per day per kg dry solids fed and graphed with a 4 day rolling average.

Dry solids

Samples from each digester and feedstock were taken weekly for dry solids measurements. The biological triplicate digester results were averaged, and effort bars of standard deviation applied (Figure 4.3A).

The 4 different feedstocks showed very little variation in DS% at each timepoint, with an RSD of 0.14 across the 15 sample points spread across 99 days. The largest variation between the 4 feedstocks was 0.32 on day 22. The average DS% for Control, Untreated, THP*4 and THP*10 feedstocks were 8.17%, 8.14%, 7.93% and 8.06% respectively across the duration of the experiment.

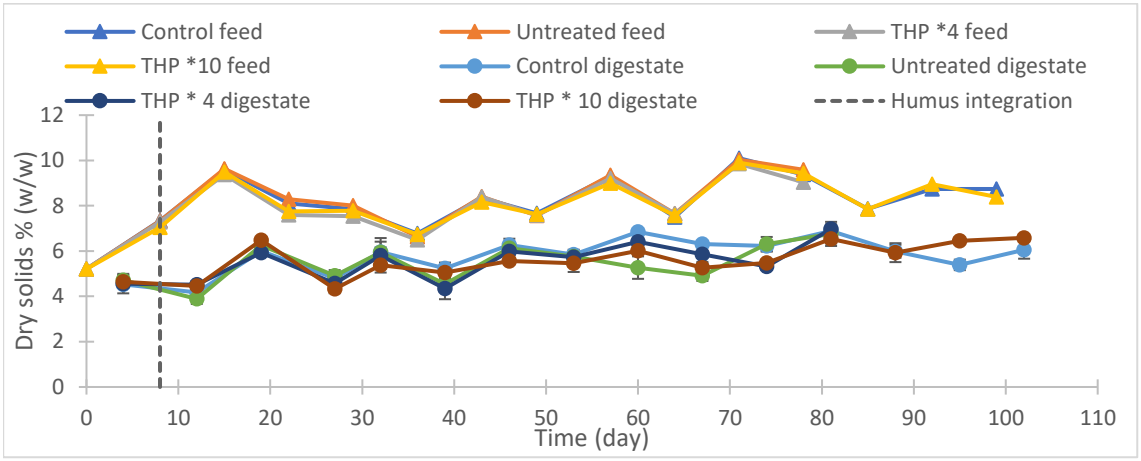
The DS% for the feedstocks averaged at 8.38%, 8.44%, 8.26% and 8.22% for the Control, Untreated, THP*4 and THP*10 feedstocks respectively for the length of the experiment, varying between a low of 5.21% on day 0, up to 10.10% on day 71. However, 80% of the timepoints fit within the smaller range of 3.1% between 6.49% - 9.59%, indicating that the feedstock is relatively stable week to week, with only the occasional large fluctuation. The RSD of the Control, Untreated, THP*4 and THP*10 feedstocks over the course of the experiment was 0.18, 0.21, 0.21, 0.17.

The DS% of the digestate was significantly lower and less variable than that of the feedstock entering the digester. The average DS% for the Control, Untreated, THP*4 and THP*10 digesters were 6.23%, 5.90%, 5.85% and 5.64% respectively, an average reduction of 25.61%, 30.07%, 29.18% and 31.43% respectively.

Fixed and Volatile solids

Samples were taken alternate weeks for fixed and volatile solids. The fixed solids (FS%) for both the feed and digestate appear stable over the course of the experiment but actually varied significantly, with RSD higher than either the DS% and VS% (Figure 4.3B). The fixed solids of the Control, Untreated, THP*4 and THP*10 feedstocks averaged at 2.79%, 2.75%, 2.72% and 2.74% respectively across the length of the experiment, with RSDs of 0.23, 0.27, 0.27 and 0.23 respectively. The fixed solids of the digestate averaged at 2.61%, 2.55%, 2.56% and 2.50% respectively, with RSDs of 0.15, 0.15, 0.11 and 0.13. This equates to an approximately 44% reduction in variability in the feedstock to the digestate across all digesters.

The average reduction in FS% from feedstock to digestate was 6.22%, 7.32%, 5.94% and 8.57% respectively. Ideally this would be 0% for all 4 as the fixed solids are indigestible and would indicate that the digesters are mixing perfectly and there was no settling of sand or grit etc, however this could be due to sampling error rather than different levels of mixing or settlement between the digesters.



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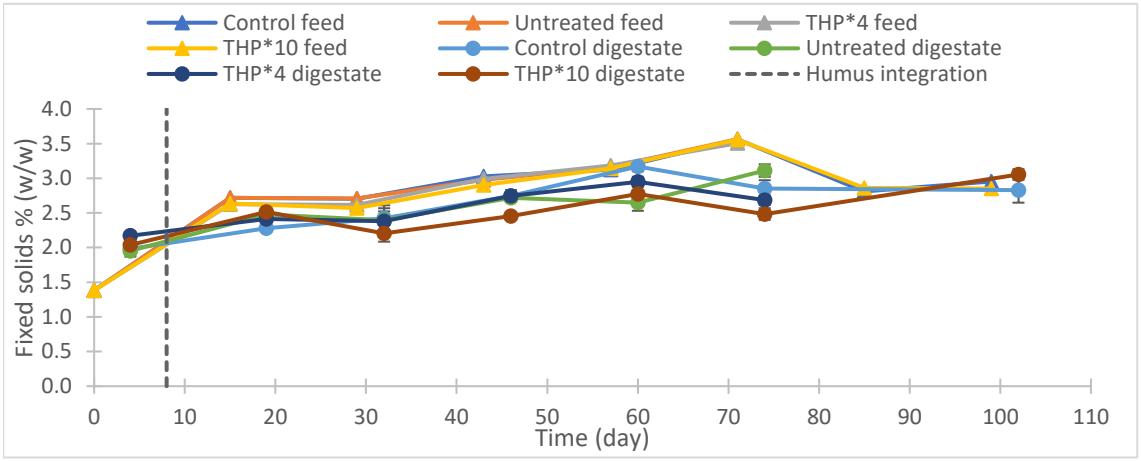

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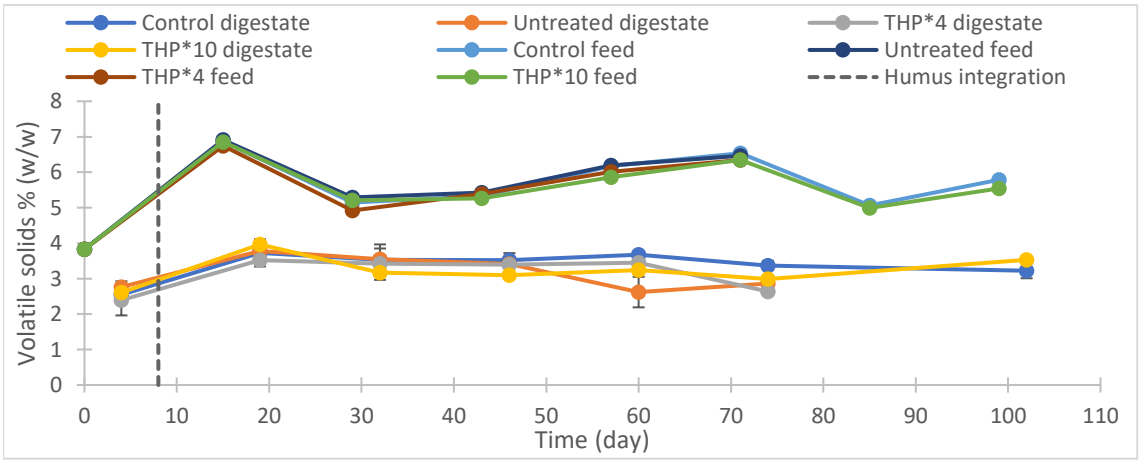


(C)

Figure 4.3

Dry solids % (A), fixed solids % (B) and volatile solids % (C) were calculated on a percentage by weight basis and error bars of standard deviation applied.

Graphically the feedstock volatile solids fraction appears to vary more than the fixed solids fraction across all feedstocks, however statistically it did not (Figure 4.3C). The average VS% for each feed, Control, Untreated, THP*4 and THP*10, was 5.59%, 5.69%, 5.54% and 5.49% respectively, with RSDs of 0.17, 0.19, 0.19 and 0.17 respectively, much lower than those of the fixed solids and dry solids.

The digestate volatile solids fraction averaged for each digester triplicate, Control, Untreated, THP*4 and THP*10 at 3.37%, 3.16%, 3.26% and 3.23% respectively with RSDs of 0.12, 0.15, 0.16, 0.13 respectively, representing an approximately 23% reduction in variability between the feedstock and digestate.

The average reduction in VS% was calculated using the average feedstock VS% and average digestate VS% for each digestate triplicate. For the Control, Untreated, THP*4 and THP*10 the average reduction in VS% was 39.75%, 44.36%, 41.09% and 41.15% respectively.

Based on these results the digesters were able to buffer against changes in the DS%, FS% and VS% of the feedstock across all conditions and generate a consistent digestate product.

Chemical oxygen demand

The chemical oxygen demand (COD) was also analysed every other week at the same timepoints as the fixed and volatile solids (Figure 4.4).

The average COD for the Control, Untreated, THP*4 and THP*10 feedstocks were 98.2 g/L, 99.6 g/L, 97.8 g/L and 95.9 g/L respectively, with RSDs of 0.15, 0.18, 0.16 and 0.15 respectively. The average COD for the Control, Untreated, THP*4 and THP*10 digestate was 60.5 g/L, 59.8 g/L, 59.5 g/L and 57.6 g/L respectively with RSDs of 0.12, 0.12, 0.10 and 0.12 respectively, representing a 29% reduction in variability between the feedstock and digestate. It also represents a reduction in the COD of 38.4%, 40.1%, 39.8% and 40.1% for the Control, Untreated, THP*4 and THP*10 respectively.

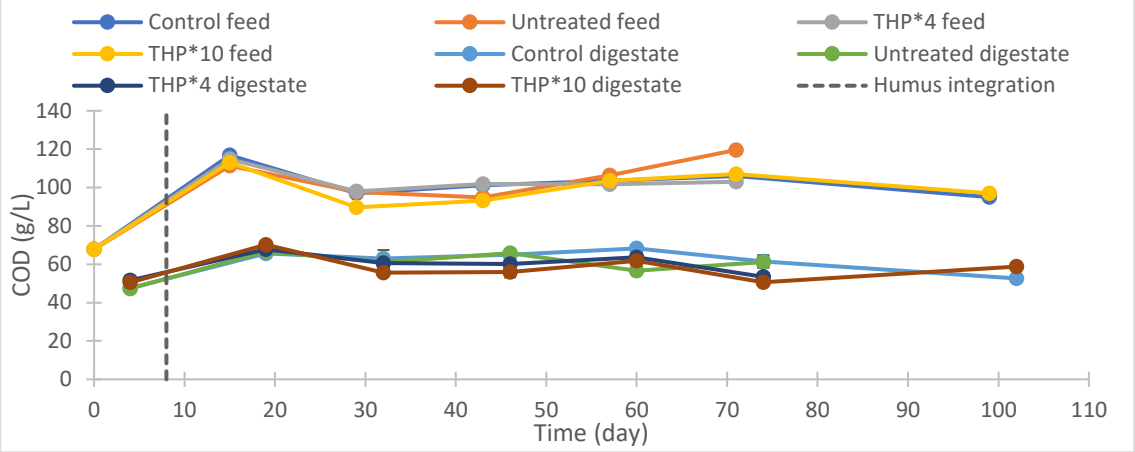
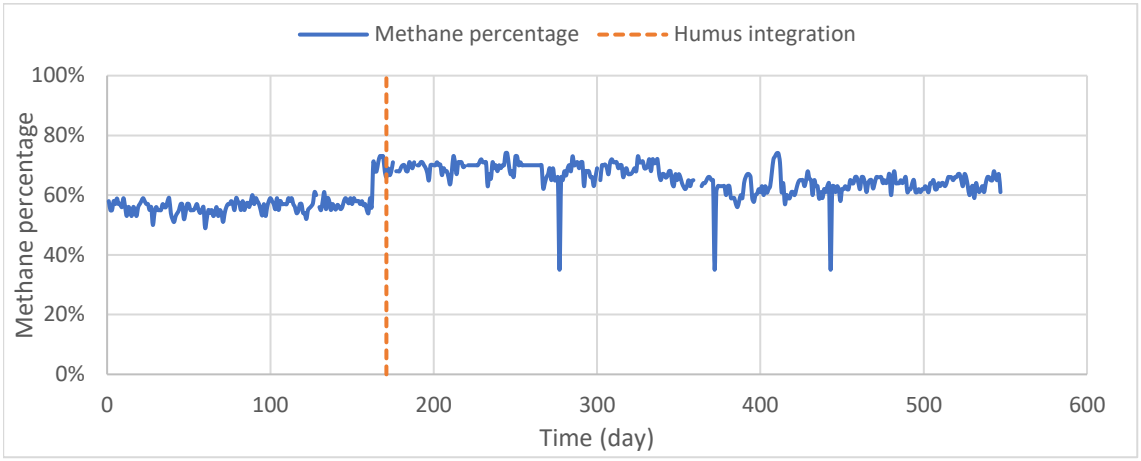


Figure 4.4 - The COD (g/L) was calculated for both feedstock and digestate on alternate weeks and error bars of standard deviation applied.

Scaling up to full-scale

Based on these results, no long-term negative effects were observed from the addition of THP treated Humus to THP fed digesters. These results were reported back to YW, and as a result YW modified their process with similarly no ill effects. Data provided by YW for the 6 months prior to addition, and the 12 months after the Humus material was integrated into their AD process included CH_4 percentage of biogas, biogas output (m^3/d) and feed volume (m^3/d). Although CH_4 percentage was not measured during the lab-scale experiment and was a cause for concern when upscaling to process scale, the CH_4 percentage of the biogas at the WWTP appeared to increase from an average of 56% in the 6 months prior to an average of 66% in the year after the addition of the Humus material (Figure 4.5A). This increase however began shortly before the Humus was added to the digesters and is likely the result of another separate process change that was not documented. Biogas yield was calculated using available data as cubic meters of biogas produced per cubic meter of feed and the raw data and the biogas yield with a rolling average of 14 days (the average HRT on site) was plotted (Figure 4.5B).

Although the average biogas yield in the year following the Humus addition dropped to 55.6 from 62.3 in the 6 months prior, there doesn't seem to be any indication the drop is directly linked to the Humus addition and the yield stabilises at this lower yield rather than continues to decrease. Based on these results, no long-term negative effects were observed from the addition of THP treated Humus to THP fed digesters at full scale.



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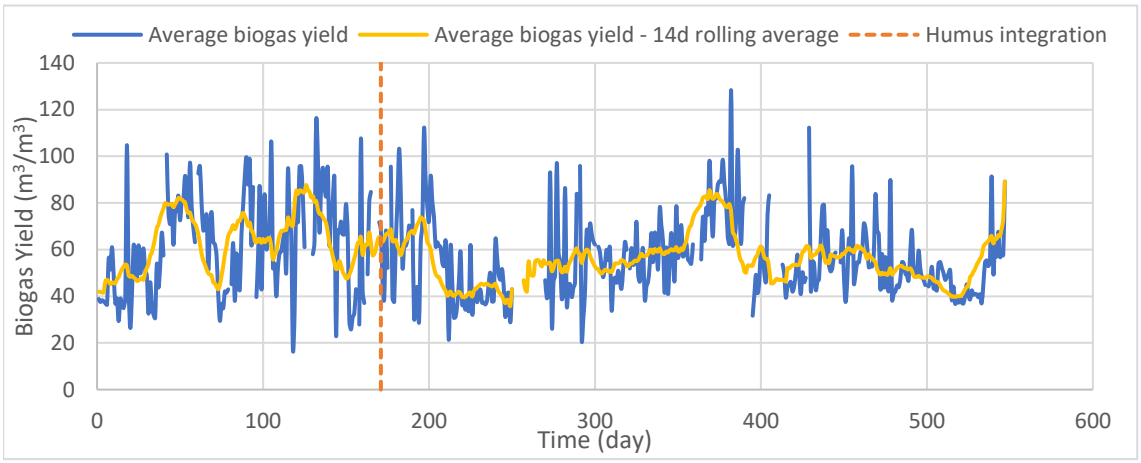


(B)

Figure 4.5 – Methane percentage (A) and biogas yield (B) from the process scale digesters 6 months prior and 12 most post integration of the Humus material into AD at the site.

4.5 Conclusions

The experiment was run for a total of 102 days, although the Untreated Humus and THP*4 Humus digesters were run for a shorter period of 81 days. The length of the experiment and concentrations of Humus material used meant that the digesters, and by extension the microbial community, was fed an equivalent of approximately 3 years' worth of Humus material in the space of 3 months without detriment to performance regarding solids destruction or biogas production.

The volume fed and physical characteristics of the feedstock varied between the 4 sets of digesters. The feed rate was set to a 14-day HRT, and the feeding mechanism rarely managed to achieve this precisely. However, when averaged over time, the feed rate was comparable, highlighting the importance of running experiments on this system over extended periods of time. The 4 different feedstocks also varied in terms of DS%, VS% and COD despite being predominantly the same

feedstock, further highlighting the importance of taking careful measurements around actual volume of feed fed in calculating and comparing biogas yields.

All digester conditions successfully produced biogas and reduced the organic load of the feedstock. All digesters also appeared to be able to buffer against variations in the feedstock across all the measurements taken based on RSD values. The Control group appears to be the poorest performing group out of the 4 in terms of dry solids destruction and COD reduction compared to the 3 groups with the Humus material added. In terms of biogas yield, the Control and Untreated Humus digesters resulted in marginally lower biogas yields than the two groups of digesters fed THP treated Humus. Investigating this further, particularly any microbial community changes as a result of Humus material, was beyond the scope of this study.

A lack of gas composition measurements at lab scale was not ideal, as a reduction in the CH_4 percentage of the biogas would not be ideal at full scale. However, AD is an essential part of reducing the tonnage of material that Yorkshire Water needs to dispose of via the land bank, landfill or incineration and costs of landfilling the untreated Humus material were deemed by YW to likely be greater than a marginal loss of CH_4 percentage and the Humus was added to their full scale digesters, with no long term detriment several years on (data not shown).

Chapter 5: Esholt HRT reduction part 1

5.1 Introduction

As the human population increases, the wastewater it produces requiring treatment increases. Building new WWTP to accommodate the increase in wastewater is expensive financially and ecologically – concrete releases large amounts of CO_2 at around 180kg/t, and construction of new sites requires large areas of land to be concreted over. Sites are built to be able to accommodate some population growth, however new ways are still needed for treating larger volumes of waste with fewer resources. One option is by increasing the throughput of the current facilities. The YW standard for AD is a 14d HRT, and most single stage, mesophilic, anaerobic digesters treating sewage sludge are run with a HRT of 15-30 days[144,145], but can this be lowered?

5.2 Aims

To trial an increase in organic load and feed rate to the anaerobic digester. The current YW assets are insufficient for the predicted growth of the population, and many assets are coming to the end of their lifespan over the next decade. The required processing of waste in the future could be met by increasing throughput through existing assets, however there are very realistic concerns around digester acidification that need to be addressed before this can be trialled at process scale, and to gain a better understanding of how and when digester performance destabilizes when operated at a lower HRT and higher OLR.

5.3 Experimental design

12 digesters were set up in 4 sets of biological triplicates at a 14d HRT with the aim of gradually decreasing the HRT to 12, 10 and then 8 days, leaving one set of triplicate digesters at each HRT as a long term control to identify any cumulative performance issues. HRTs at lower than 8 days were not deemed practical with the current system.

The experiment was designed so that should digesters fail after reducing the HRT, the next shortest HRT would still be stable, and the experiment could continue. If some of the digesters at lower HRT's became periodically unstable, one reason could perhaps be found in the composition of the feed. In theory this would mean Yorkshire Water could monitor for this particular destabilization agents and either increase the HRT in response, or work towards preventing it from entering the waste stream in the first place.

As the HRT of digesters was reduced, feeding syringes were refilled unequally so that the volume of feed within the feeding syringe reflected the volume required between feeding syringe refills. For example: feeding syringes were refilled approximately every 48 hours. Over 48 hours digesters on a 14d HRT would require ~715ml of feed, and digesters on an 8d HRT would require ~1250ml of feed. Although the feeding syringes can hold ~1,500ml of feed, the decision was made to ensure that those on a 14d HRT would only have ~715ml added so that, although some digestion in the feeding syringe was expected based on previous results, it would not impact unequally across all digesters despite these being operated at different HRTs.

5.4 Results

The experiment ran for a total of 51 days, equivalent to ~3.5 HRTs at a 14d HRT. However, the majority of the digesters were not run with a HRT of 14d for the length of the experiment (Figure 5.1A). 1 triplicate of digesters ran at an average HRT of 14.6d for the length of the experiment. At day 4, 3 sets of triplicate digesters were reduced to a 12d HRT. At day 15, 2 sets of the previous 3 sets of triplicate digesters were reduced to a 10d HRT. Finally on day 29, 1 set of the previous 2 sets of triplicate digesters were reduced to an 8d HRT (Figure 5.1A). As a result:

- The 14d digesters had an average HRT of 14.6d for the length of the experiment, 51 days.
- The 12d digesters averaged a 14.2d HRT for 4 days and then a 12.7d HRT for 47 days.
- The 10d digesters averaged a 14.3d HRT for 4 days, an average of 12.5d HRT for 11 days and then 10.6d HRT for 40 days.
- The 8d digesters averaged a 14d HRT for 4 days, an average of 12.4d HRT for 11 days, an average of 10.3d HRT for 18 days and an average of 8.5d HRT for 22 days.

Although the hydraulic retention time (HRT) was set to 14, 12, 10 and 8 days, this varied slightly between different feedstock triplicates for the reasons described previously in Chapter 3. The HRT was measured twice per week using the technique described in Chapter 2.4 to ensure that the actual/ desired volume of feed being added to the digester was known.

There was some variation within the set HRT's, which could have been exacerbated by moving from 2, to 3 to 4 feed syringe refills per week as the HRT became lower and digesters used the feed more quickly.

Software issues meant that the digesters were unable to feed for a short period of time between days 44 and 47. This extended the averaged HRT between these two dates.

The organic loading rate (OLR) was estimated using the COD (g/L) of the preceding feed sample, which was measured on average once per week (Figure 5.1B). Sampling was prioritized around days where the HRT was changed, however it would have perhaps been useful for calculating OLR to sample more consistently and frequently. Based on the measurements taken, the 8d HRT digesters theoretically achieved a maximum OLR of 12.76 gCOD/d/L between days 39-41 and averaged an OLR of 12.11 gCOD/d/L between days 29 and the end of the experiment.

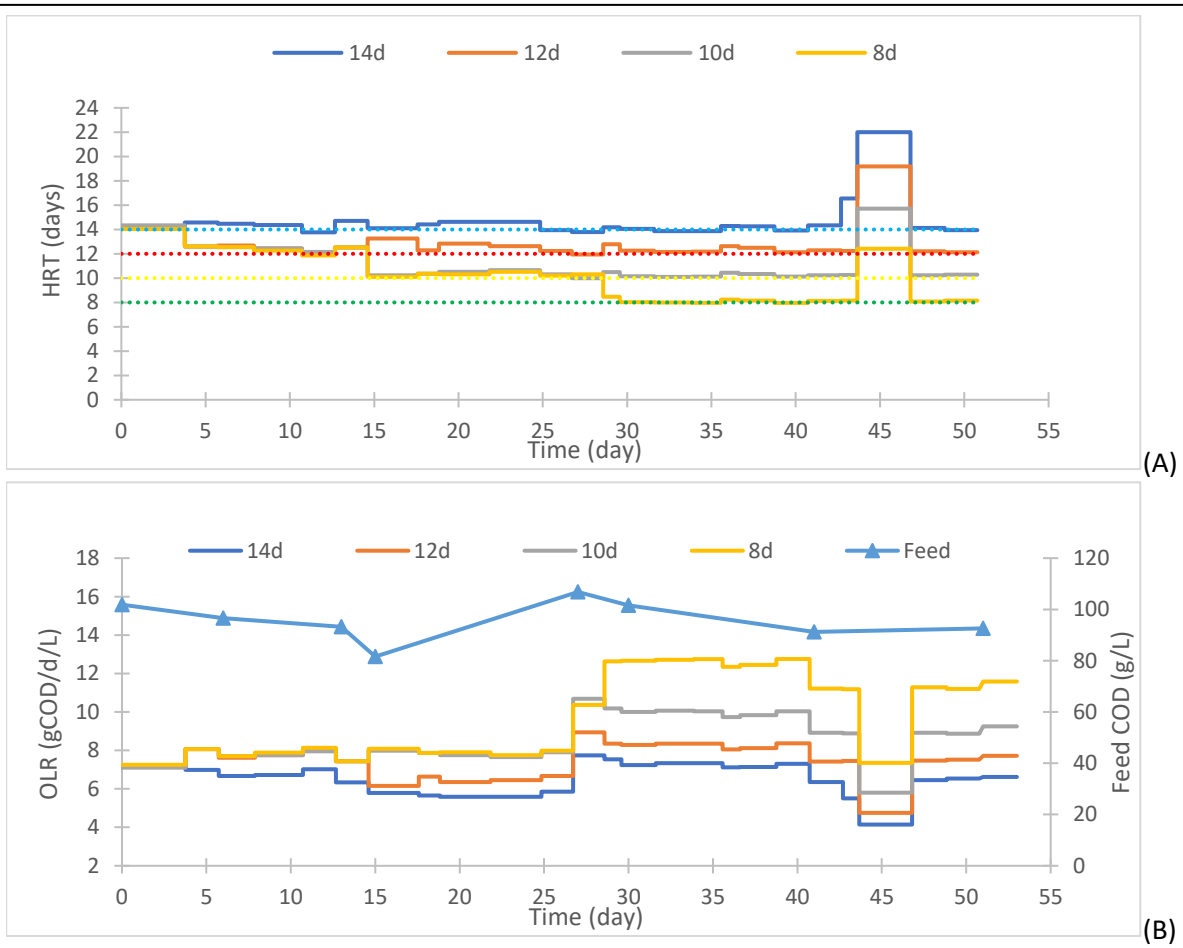
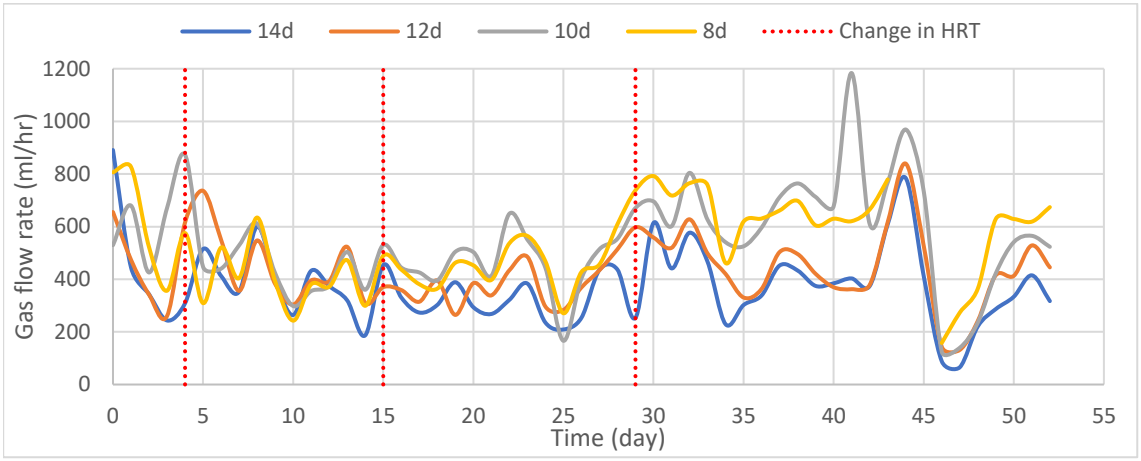


Figure 5.1 – Digester operating conditions - HRT and OLR

(A) The HRT of the digesters was set between 14d and 8d. The 4 sets of digesters started with the same HRT, 14d, until day 4. On day 4, one set of digesters was held at 14d, while the other 3 reduced to a 12d HRT. On day 15, one set of digesters was held at 12d, while the other 2 reduced to a 10d HRT, and on day 29 one set of digesters was reduced to an 8d HRT.

(B) HRT and COD was combined to estimate the organic loading rate (OLR) of each digester set.



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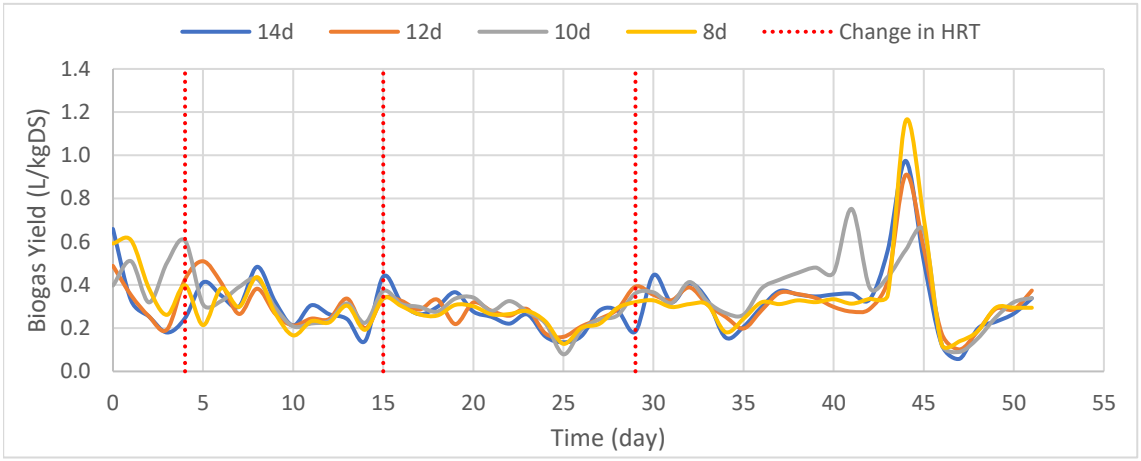


(B)

Figure 5.2 – Biogas flow rate and yields

(A) Gas flow rates were averaged into a single value for each day for each digester and then averaged over biological replicates.

(B) Average biogas yield calculated as litres of biogas per kg DS fed.

Gas Flow

All 4 sets of triplicate digesters showed consistent and replicable gas flow rates over the course of the experiment. The raw data for each digester was averaged to produce a single gas flow rate value for each day and then averaged across the replicated digesters in the group. As noted previously in Chapter 3, there is a cyclical pattern to the gas production, with the gas flow rate increasing on days where the digesters were fed and decreasing over the next 2-3-4 days as the feed in the feeding syringe is used up, indicating some digestion in the feeding syringe in the first 24 hours (Figure 5.2A). The raw average daily gas flow rate for the length of the experiment for the 14d, 12d, 10d and 8d digesters were 376ml/hr, 431ml/hr, 543ml/hr and 525ml/hr respectively, with digesters that ran faster producing more biogas, as would be expected if the microbial community remained stable.

However, the digesters are a closed system and biogas yield is a more appropriate measurement for comparing digesters at significantly different HRTs (Figure 5.2B). The average biogas yield post day 29, after the final change in HRT, for the 14d, 12d, 10d and 8d digesters was 0.34, 0.34, 0.37 and 0.34 L/kgDS fed. When visualised, the gas flow rate and biogas yield for the 10d HRT digesters is unusually high between days 37 and 41, which accounts for why the average biogas yield for 10d HRT digesters appears higher than the 14d HRT digesters (Figure 5.2B). When these 4 days are removed from the averages, the biogas yield for 10d digesters was reduced to 0.33 L/kgDS. The unusually large spike and slump in biogas yield around day 45 can be accounted for due to a rapid change in the HRT due to a temporary breakdown of the feeding mechanism.

In addition to 24-hour monitoring of the gas flow rate, samples were analysed regularly on both the feedstock going into the digesters and digestate coming out of the digesters.

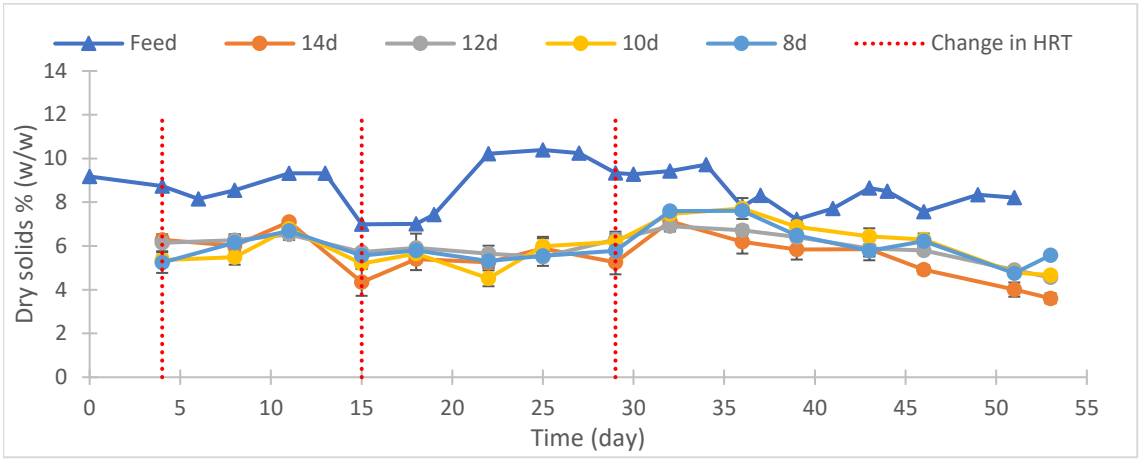
Dry solids

Samples from each digester were taken weekly for dry solids measurements, the feed was sampled twice per week. The triplicate digester results were averaged, and error bars of the standard deviation applied graphically (Figure 5.3A).

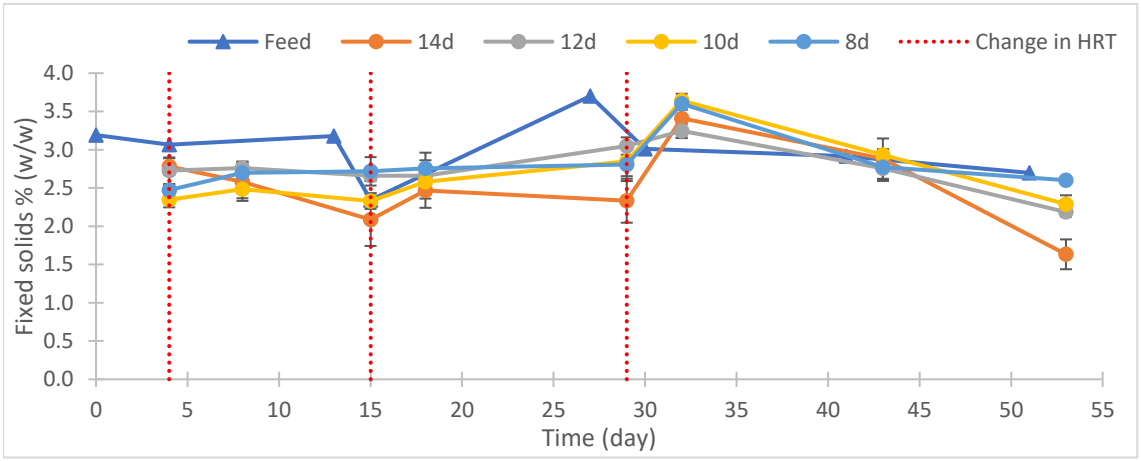
The feed averaged at 8.62 DS% across the 51 days, ranging between 10.39 DS% and 6.99 DS% and with an RSD of 0.12. Feed DS% was relatively stable except for a particularly low spot between days 15 and 19.

The DS% leaving the digesters proved to be less variable than the feedstock going in, as anticipated and observed in Chapter 3. The average DS% for the 14d, 12d, 10d and 8d digesters were 5.54%, 5.95%, 5.96% and 6.01% respectively with an RSD of 0.15, 0.08, 0.10, 0.08 respectively. This represents a 7.4%, 7.6% and 8.5% increase in DS% compared to the Control digesters.

Sampling more frequently than previous experiments provided a more detailed picture of how the DS% of the feed changes over time and how that is not reflected in the DS% of the digestate samples, or how delayed any reflection is. A sustained increase in feed DS% between days 22 and 36 results in a small increase in DS% in the digestate between days 32 and 43.



(A)



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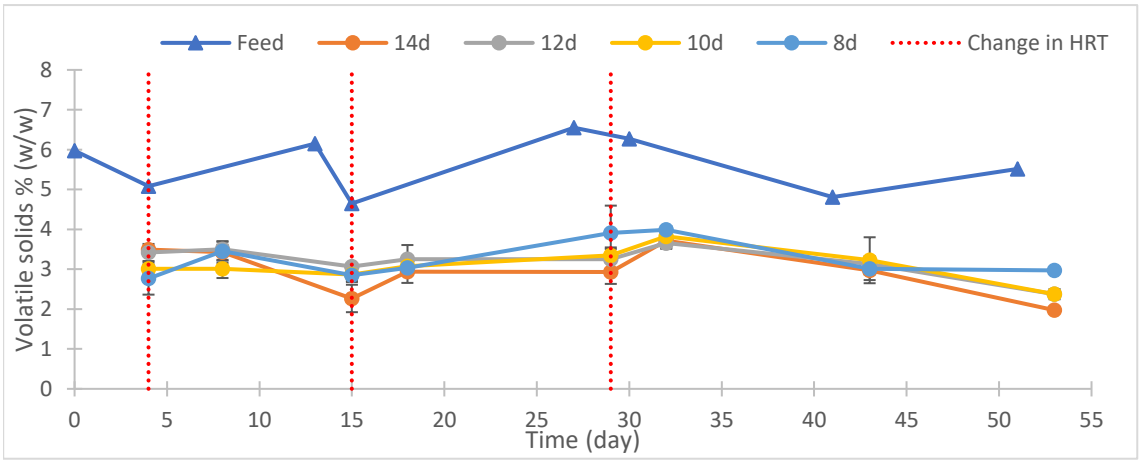


(C)

Figure 5.3 Feed and digester solids measurements

Dry solids % (A), fixed solids % (B) and volatile solids % (C) were calculated on a percentage by weight basis and error bars of standard deviation applied. The vertical lines on days 4, 15 and 29 indicate changes in HRT.

Fixed and Volatile solids

Samples were taken once per week for fixed and volatile solids, with priority placed on sampling before and after changes in HRT. The fixed solids (FS%) for both the feed and digestate were quite stable over the course of the experiment (Figure 5.3B). The average fixed solids for the feed was 3.01% with an RSD of 0.13. Unexpectedly there appeared to be a difference in FS% between the digesters at different HRTs. The average FS% of the digestate of the 14d, 12d, 10d and 8d digesters was 2.52%, 2.76%, 2.68% and 2.80% respectively with RSDs of 0.20, 0.10, 0.09 and 0.08. These values indicate that the FS% of the digestate of the 14d digesters was lower and more variable than that of the feedstock entering the digesters over the course of the experiment.

The FS% stayed relatively stable throughout except for days 15 and 27, when the FS% dropped to 2.34% and then jumped to 3.70%. There were also large variations in DS% and VS% at these timepoints. It is interesting to note that the jump in feed FS% is not reflected in the digestate sample on day 29, but does appear to be reflected in an increased FS% in the digestate samples on day 32 (Figure 5.3B).

The feedstock volatile solids fraction averaged at 5.62% and varied between 6.55% and 4.65% resulting in an RSD of 0.13 (Figure 5.3C). The average VS% for each digester triplicate, 14d, 12d, 10d, and 8d was 2.96%, 3.21%, 3.09% and 3.25% respectively with an RSD of 0.15, 0.12, 0.13, and 0.15. Again, this indicates that the digesters are producing digestate that is more variable than the feedstock entering the digesters.

Chemical oxygen demand

The chemical oxygen demand (COD) was analysed at the same timepoints as the fixed and volatile solids (Figure 5.4A). The feedstock COD averaged at 95.7 g/L and varied between 81.6 g/L and 106.8 g/L, with an RSD of 0.08 while the average COD for the 14d, 12d, 10d and 8d digestates was 49.3 g/L, 55.9 g/L, 54.8 g/L and 57.9 g/L respectively and RSDs of 0.17, 0.08, 0.11 and 0.09. This represents a 13%, 11% and 17% increase in COD compared to the Control digesters.

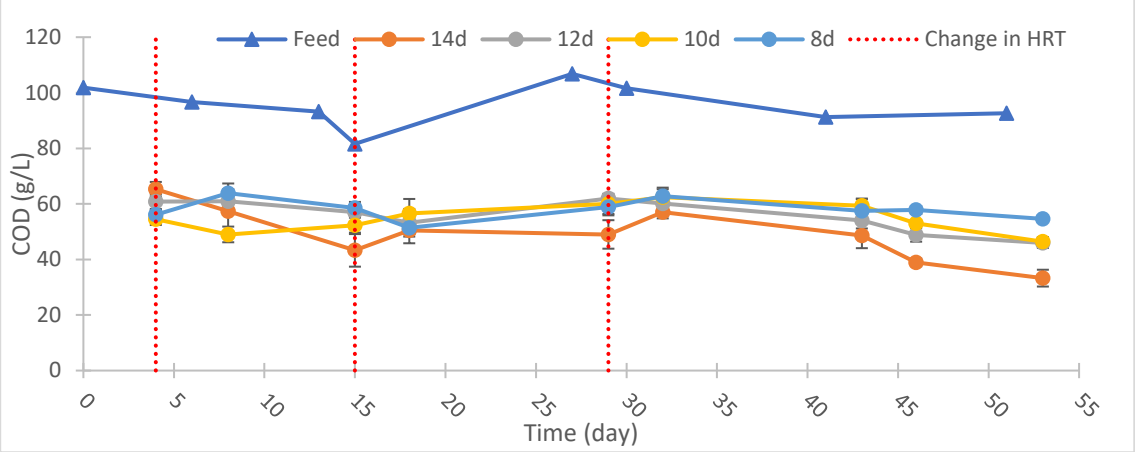


Figure 5.4 – Feed and digestate COD

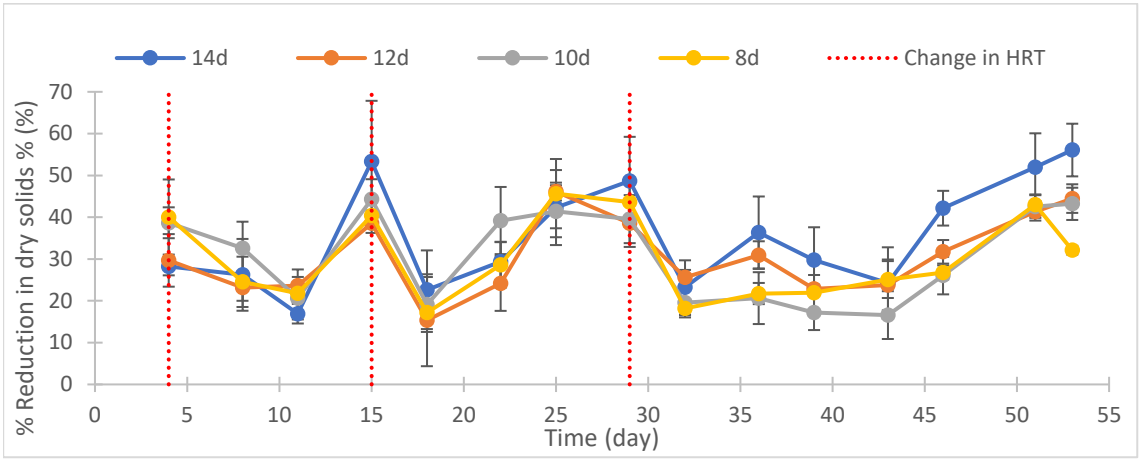
The COD (g/L) was calculated for both feedstock and digestate once per calendar week, with priority placed on sampling around changes in HRT. Error bars of standard deviation applied

Calculating percentage reduction

A key metric for YW revolves around the reduction of the organic load of the remaining digestate for disposal. The three key metrics for this are DS%, VS% and COD. % reduction was calculated using individual digester values and the preceding feedstock value and standard deviation error bars applied graphically (Figures 5.5A, B and C).

The average % reduction in DS% from feed to digestate was 35.77%, 31.05%, 30.97% and 30.35% respectively which does indicate some reduced digester efficiency as the HRT is reduced. The average reduction in VS% for the 14d, 12d, 10d and 8d digestates was 47.29%, 42.99%, 45.03% and 42.25% respectively, similar to the reduction in DS% in that reduction of HRT may result in reduced solids breakdown.

Reduction in DS% is consistent between all digester groups until day 32 (Figure 5.5A). Up until this point the average reduction in DS% for 14d, 12d, 10d and 8d HRT groups are 34%, 30%, 34%, and 33% respectively. After day 32 they are 38%, 32%, 26% and 27% respectively. It would make sense if the 8d HRT solids destruction deceased at this point, as it switched from a 10d HRT to an 8d HRT on day 29, but does not explain why the 12d and 10d HRT digesters also appear to decrease in their solids destruction. These variations in reduction of the organic load are not supported by any reduction in the biogas yield as discussed previously.



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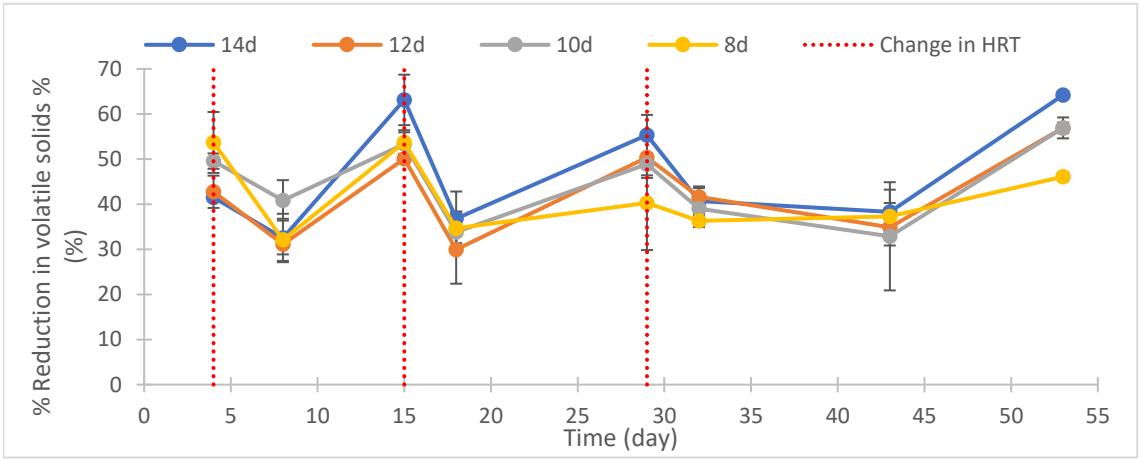

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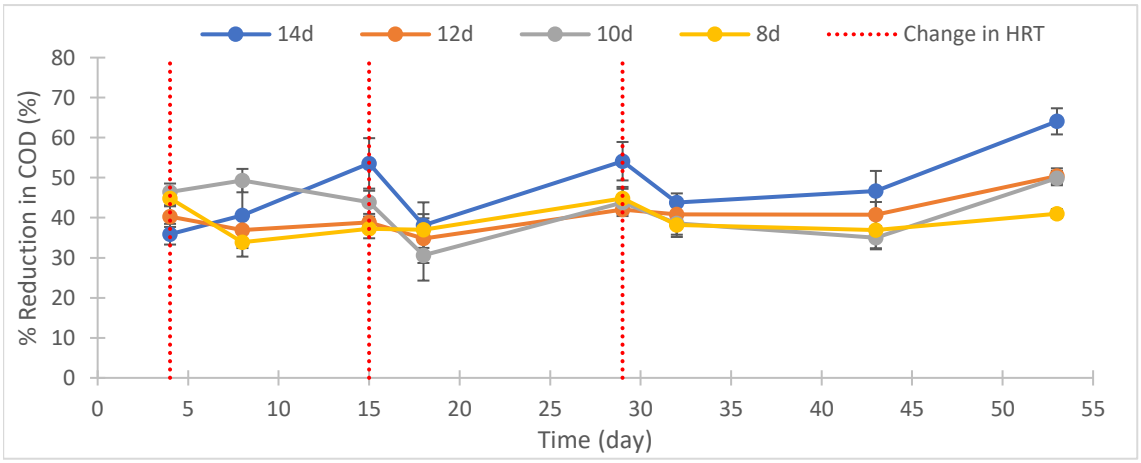


(C)

Figure 5.5 – Percentage reduction in DS%, VS% and COD

The reduction of the organic content, DS% (A), VS% (B), and COD (C), from feedstock to digestate was calculated for each digester condition and error bars of standard deviation applied.

5.5 Conclusions

The results of this trial are somewhat inconclusive due to the limited length of the experiment and a limited sampling regime. The biogas yield results indicated that there was no change in yield in response to a change in HRT, however the reduction in DS%, VS% and COD indicate lower HRTs result in lower organic matter reduction.

The experiment ran for a total of 51 days, 4 days of baselining, 25 days of reducing the HRT and only 18 days of feeding at an approximately 8d HRT, excluding the breakdown in feeding. Although the digesters were stabilised for several weeks prior to starting baselining, the data were not recorded, or sampling carried out on the digestate. The baselining of 4 days did result in similar biogas yields, however longer baselining with additional assays would give greater confidence that the digesters were behaving similarly before any changes were made to their operating conditions.

Increasing the frequency of DS% sampling of the feed and digestate, to twice per week minimum, gave a much clearer picture of the changes in feedstock over time and how this variability decreased in the digestate. This greater number of sample points, compared to the VS% and COD at closer to twice per month, may be the reason that the variability of the DS% decreased from feed to digestate, but the VS% and COD appears to not. It also highlighted that although biogas yields between the 4 digesters were the same over the final 18 days of the experiment, the apparent solids destruction and organic load reduction was not. The digesters at lower HRTs appeared to not generate the same reduction in DS%, VS% and COD that the 14d HRT digesters did, however it is difficult to draw a strong conclusion with only 4 timepoints. The jumps in chemical and physical characteristics in the feed, as well as irregular sampling also compounds this challenge in interpreting data. Results are too sporadic to be sure, but from FS% results it looks like reducing HRT/increasing feed rate reduced settlement even in well mixed digesters.

pH and VFA measurements would also be valuable tools in understanding changes in the balance in the microbial community as a result of increased feed rates. They would show quickly if the digesters were struggling to break down everything they were being fed, but VFAs would give a better picture of what was occurring in the digesters in terms of buffering capacity.

In conclusion an experiment in which the digesters run for longer than 22 days with additional analysis is required to be able to comfortably conclude that the microbial community was stable at an 8d HRT.

Chapter 6: Esholt HRT reduction part 2

6.1 Aims

To trial an increase in organic load and feed rate to the anaerobic digester. The current YW assets are insufficient for the predicted growth of the population, and many assets are coming to the end of their lifespan over the next decade. The required processing of waste in the future could be met by increasing throughput through existing assets, however there are very realistic concerns around digester acidification that need to be addressed before this can be trialled at process scale, and to gain a better understanding of how and when digester performance destabilizes when operated at a lower HRT and higher OLR.

To gain a better understanding of the stability of digesters running at an 8 day HRT in comparison to a 14 day HRT, through a longer experimental time course and greater depth sampling and analysis.

6.2 Experimental design

6 digesters were set up in 2 sets of biological triplicates at a 14d HRT with a view to gradually decrease the HRT on one set down to 12, 10 and then 8 days. The digesters would remain at each of the HRTs for a minimum of 14 days before the HRT was reduced again.

As previously described in Chapter 5, as the HRT of digesters was reduced, feeding syringes were refilled unequally so that the volume of feed within the feeding syringe reflected the volume required between feeding syringe refill cycles. This is to reduce any impact of feedstock digestion within the feeding syringes on biogas output. Liquid samples at full scale are removed from sample ports on the pipework leaving the digesters and for this reason, previously liquid samples from System 60 were taken from the overspill pots. For this study samples were removed directly from the digesters.

Covid self-isolation meant limited data points between days 120 and 140. The digesters were fed and maintained, but no measurements taken.

6.3 Results

Operational conditions

The experiment ran for a total of 164 days. The Control triplicate of digesters ran at an average HRT of 14.9d for the length of the experiment, equating to just over 11 HRTs. The Reduced HRT triplicate of digesters ran at an average of 14.1d HRT between days 0-14, 12.1d HRT between days 14-28, 10.6d HRT between days 28-70 and between days 70-164 an average HRT of 8.9d (Figure 6.1A). This equated to just over 10 HRTs at an 8.9d HRT.

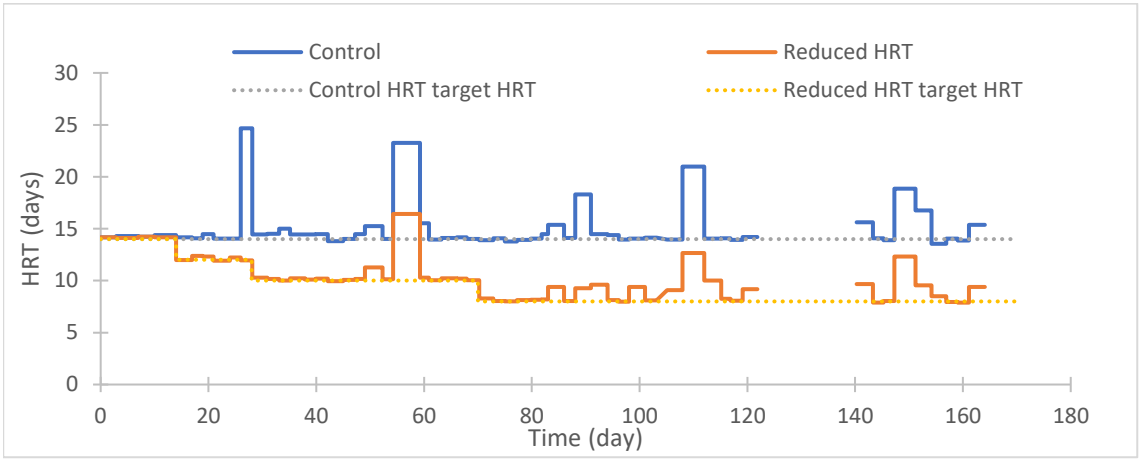
There was some variation within the set HRT's which was exacerbated as the HRT is reduced. This could have been the result of moving from 2, to 3 to 4 feed syringe refills per week as the HRT got lower and the digesters used the feed more quickly. It was more difficult to maintain a HRT of 8 days over a longer period of time than the previous experiment, with a higher frequency of software updates/breakdowns, causing jumps and greater variation in the HRT, over this period of time. The RSD for days 0-14 for the Control and Reduced HRT digesters was 0.01 and 0.05 respectively, increasing to 0.12 and 0.13 for days 70-164. Software or mechanical failures are responsible for the large jumps in HRT at days 28, 54, 88, 108 and 147.

The average OLR for the Control digesters was 6.86 gCOD/d/L over the course of the experiment. The Reduced HRT digesters averaged an OLR of 10.24 gCOD/d/L over the 164 days, with an average OLR of 7.17 gCOD/d/L between days 0-14, 9.15 gCOD/d/L between days 14-28, 9.74 gCOD/d/L between days 23-70 and between days 70-164 an average of 11.23 gCOD/d/L (Figure 6.1B). During the final 94 days where the HRT was set to 8 days for the Reduced HRT digesters the minimum measured OLR was 7.95 gCOD/d/L between days 108-112, while the highest measured OLR was 13.65 gCOD/d/L between days 77-80. Although graphically it appears that the OLR is significantly more variable for the final 94 days compared to the first 14, as it is with the HRT, this is not the case. The RSD for the Control and Reduced HRT digesters for days 0-14 was 0.10 and 0.15 respectively, and for the days 70-164, 0.11 and 0.13 respectively. Although the HRT, and therefore volume of feed, was more variable later in the experiment, the actual amount of COD fed was not.

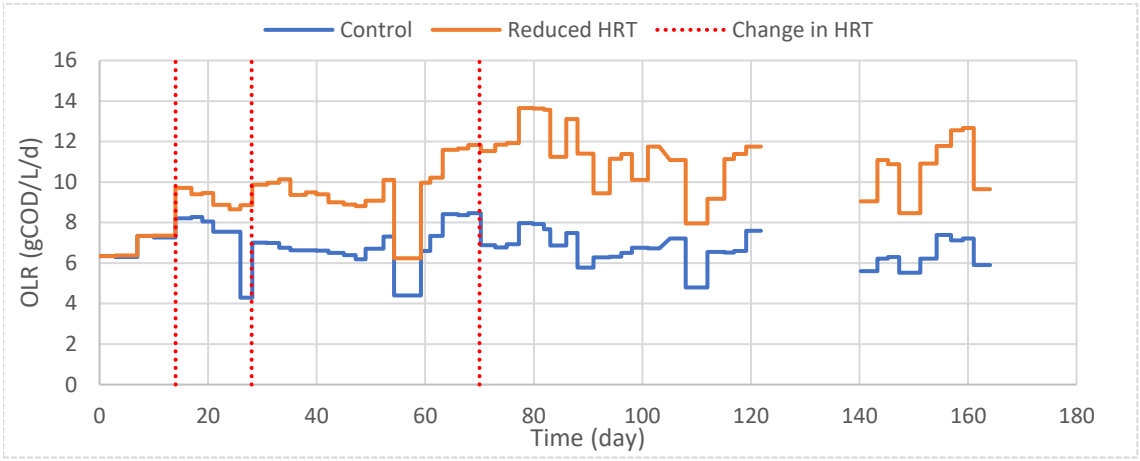
The internal temperature of digesters was also monitored to understand how the increased feeding and room temperature affected temperature stability within the digesters (Figure 6.1C). The digester temperature was set to 35°C, and at a 14d HRT between days 0-14 both Control and Reduced HRT digesters averaged a digester temperature of 34.99°C and 35.00°C respectively. Between days 70-164 the Control digesters still maintained their average of 34.99°C, while the Reduced HRT digesters averaged at 34.98°C. A drop of 0.02°C is not significant, however the Control digesters were

consistently warmer than the Reduced HRT digesters for 90% of the 94 days, compared to a 50:50 split at a 14d HRT. Variability in digester temperature did not change between conditions.

The average room temperature day 0-14 was 21.76°C, and between day 70-164 was 18.66°C, an average drop of 3.10°C. Variability in room temperature did not change between days 0-14 and days 70-164 and had an RSD of 0.01 for both. The feedstock was stored at 4°C before entering the digester refill syringes and then digesters, a significantly lower temperature than room temperature or digester temperature. Removal of data points on days that the feeding syringes were refilled, and in theory the feed was colder, did not alter any temperature averages. From this we can conclude that the drop in room temperature itself did not cause digester temperature to drop, since the Control digesters maintained their temperature and variability over time, however the increased feed rate of lower temperature feed from the feeding syringe may have a small impact on maintaining a stable temperature in these digesters.



(A)



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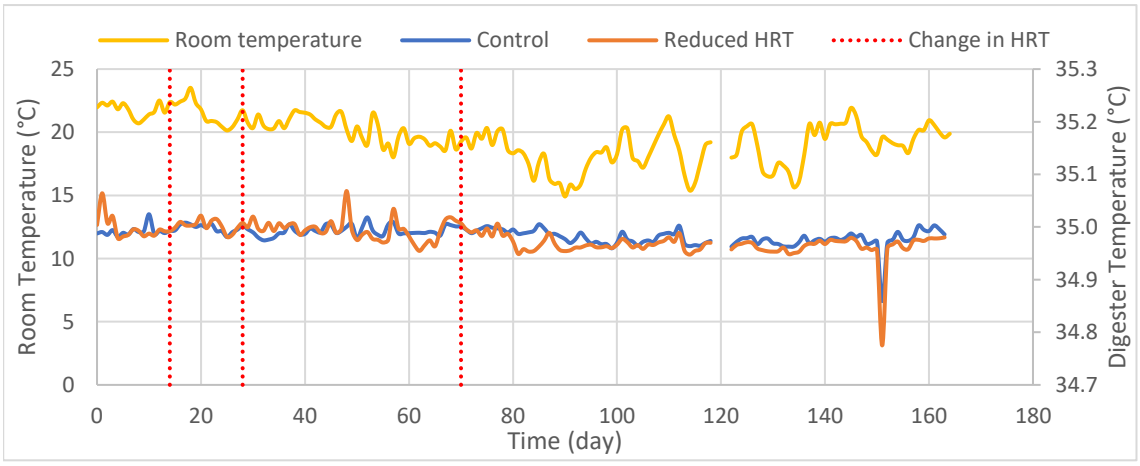


(C)

Figure 6.1 – Digester operating conditions

(A) The HRT of the digesters was set to be between 14d and 8d. The Control digesters were set at a 14d HRT for the length of the experiment. The Reduced HRT digesters started at a 14d HRT until days 0-14 when the HRT was reduced to 12d. At day 28 the HRT of the Reduced HRT digesters was reduced down to 10d and on day 70-164 they were set to a HRT of 8d.

(B) OLR of each set of digesters was calculated using the HRT and weekly feedstock COD measurements

(C) Average internal digester temperature and room temperature

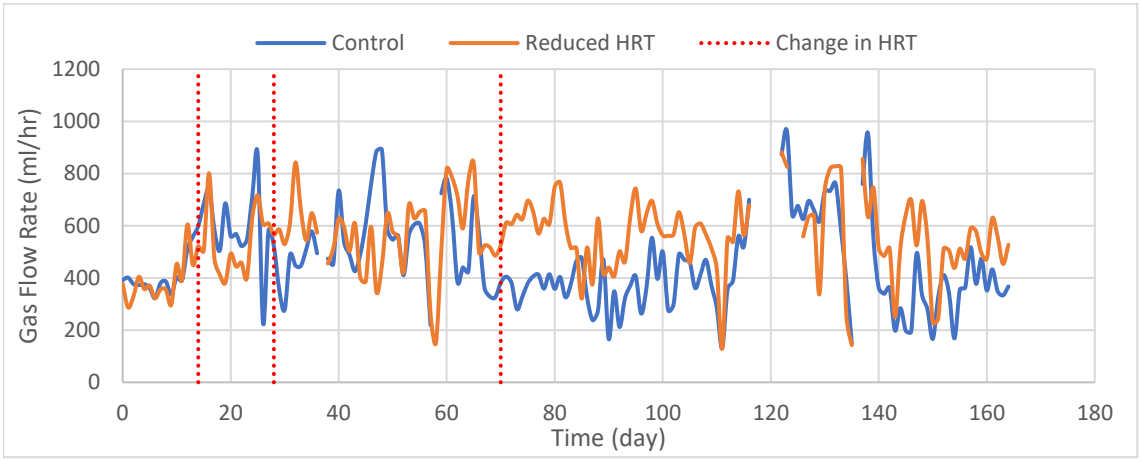
Gas flow, yield and composition

Both sets of triplicate digesters showed consistent and replicable gas flow rates over the course of the experiment. The raw data for each digester were averaged to produce a single gas flow rate value for each day and then averaged across the replicated digesters in each group. There is a cyclical pattern to the gas production as described previously (Figure 6.2A).

The average daily gas flow rate prior to changes in HRT for the Control and Reduced HRT digesters were 398ml/hr and 383ml/hr respectively, however when the HRT is reduced to 8d HRT the average daily gas flow rate was 423ml/hr and 562ml/hr respectively. When the digesters were fed more, and operated at higher OLRs, they produced more biogas.

The biogas yields for the Control and Reduced HRT digesters prior to day 14 and the first change in HRT were 0.34 L/kgDS and 0.33 L/kgDS (Figure 6.2B). The biogas yields for between day 70 and 164 were 0.30 L/kgDS and 0.28 L/kgDS respectively and were found to be significant (t-test, $p=8.02e^{-2}$). There was a 4.2% difference between the two sets of digesters at 14d HRT, and an 8.5% difference at 14d and 8d HRT. Interestingly the biogas yields of the 14d HRT digesters become more variable at the end of the experiment compared to the beginning (RSD of 0.27 and 0.31) while the Reduced HRT digesters become less variable over time (RSD of 0.32 and 0.28). This is in keeping with the OLR variability results, where the Control digesters exhibited an approximately 15% increase in variability between days 0-14 and days 70-164, and the Reduced HRT digesters exhibited an 12% decrease in variability. Variability in OLR is directly reflected in variability in biogas yield.

Biogas yield was also plotted as a 4 day rolling average, highlighting regions of apparent increased biogas yield for the Control digesters in comparison to the Reduced HRT digesters between days 14-60 (Figure 6.2C). There is no operational reason for this to be the case, and although may reflect changes in the microbial community as the HRT is reduced, it likely reflects poor calibration of gas flow meters. For this reason, without community measurements to support otherwise, the majority of the biogas flow rate measurements and biogas yield measurements focus around the periods of interest during days 0-14 and 70-164 rather than the experiment as a whole.



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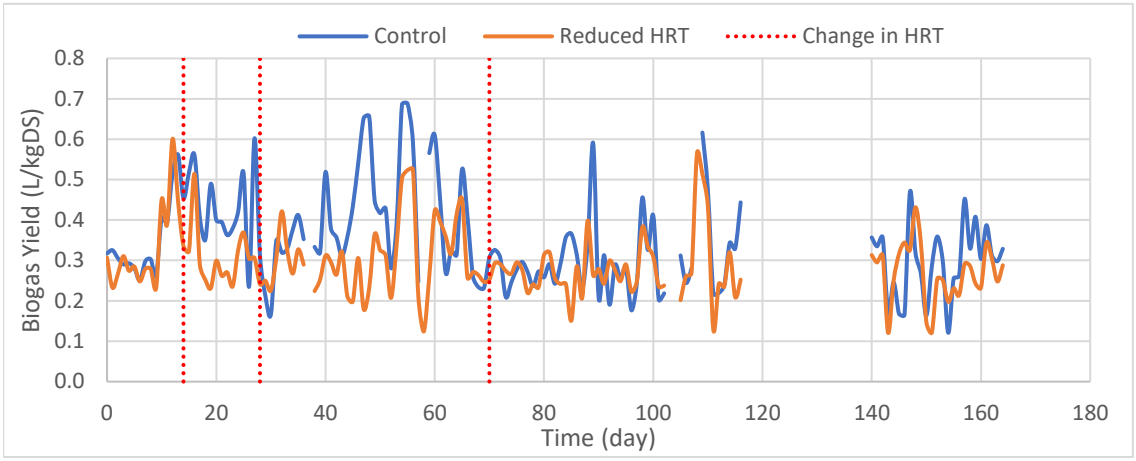

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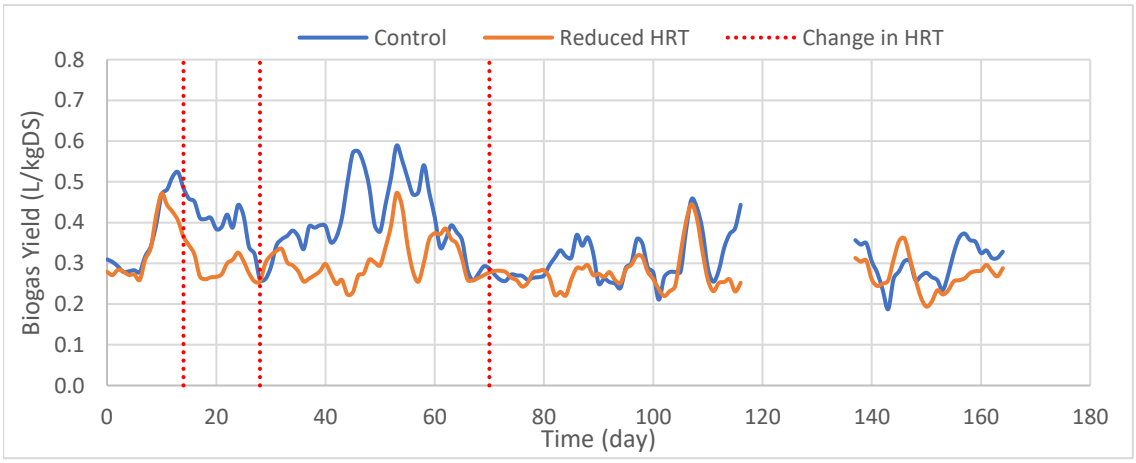


(C)

Figure 6.2 – Gas flow rates and biogas yields

(A) Gas flow rates were averaged into a single value for each day for each digester and then averaged over biological replicates.

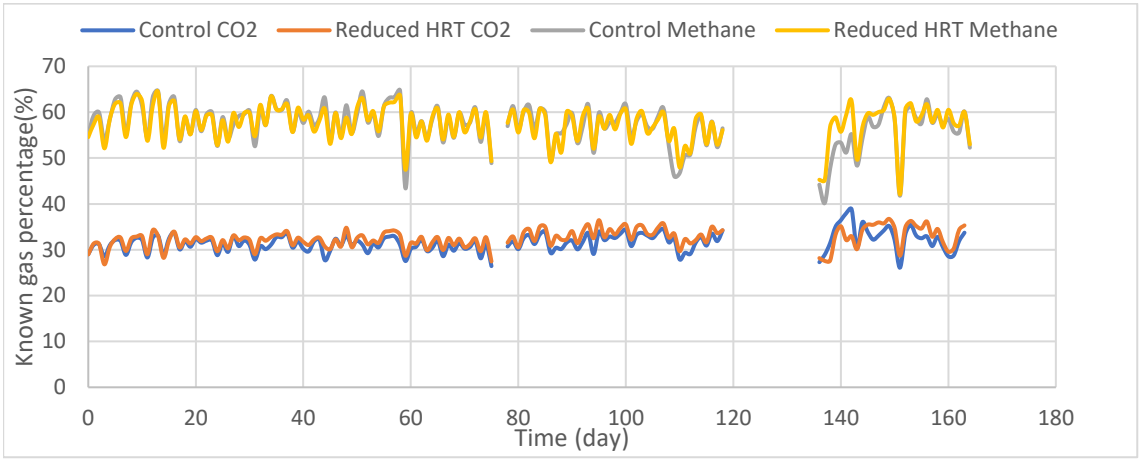
(B) Biogas yield calculated in litres of biogas per day per kg of dry solids fed.

(C) Biogas yield 4 day rolling average.

The absolute percentage gas composition of the biogas for CO_2 and CH_4 was also measured as described in Chapter 2. Prior to changes in HRT at day 14 the Control and Reduced HRT digesters had CO_2 concentrations of 31% and CH_4 concentrations of 60% and 59%. Between days 70-164 the Control and Reduced HRT digesters had CO_2 concentrations of 32% and 33%, and CH_4 concentrations of 56% and 57% (Figure 6.3A).

On average CO_2 and CH_4 made up 89% of the composition of the biogas, with the remaining 11% likely N_2 but not investigated (Figure 6.3B). This is higher than normal for biogas where the N_2 content is typically <5%. Gas composition of the biogas was directly impacted by the emptying of the overspills described in Chapter 2. Containers full of digested sludge were swapped out for empty containers full of air that entered the digester headspace every 1-2 days at the lower HRT's. Both the Control and Reduced HRT digesters had their overspills changed at the same time, even though the Control would be half empty and the lower biogas flow rate described previously would take longer to purge the overspill. This is reflected in the average CO_2+CH_4 composition of the Control digesters of 88% versus 90% for the Reduced HRT. If days where the overspills were changed are removed the CO_2 and CH_4 combined percentage increases to 93% (Figure 6.3C).

In addition to 24-hour monitoring of gas flow and gas composition, samples were taken and analysed regularly on both the feedstock going into the digesters and digestate coming out of the digesters.



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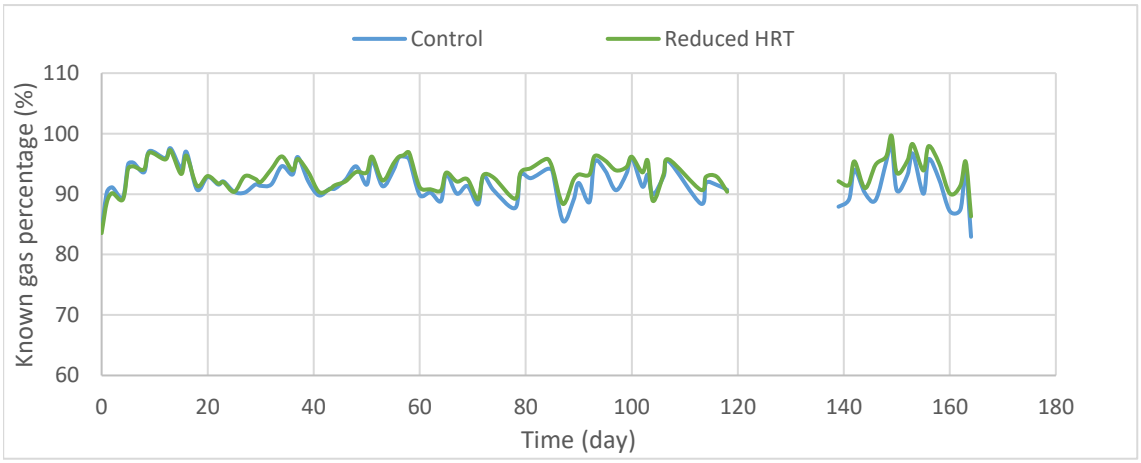


(C)

Figure 6.3 – Gas composition

(A) Absolute CH_4 and CO_2 percentages of the biogas

(B) Total known gas percentage (CH_4 + CO_2)

(C) Total known gas percentage with overspill emptying days removed

Dry solids

Samples from each digester and from the feed were taken twice per week for DS% measurements. The triplicate digester results were then averaged, and error bars of the standard deviation applied (Figure 6.4A).

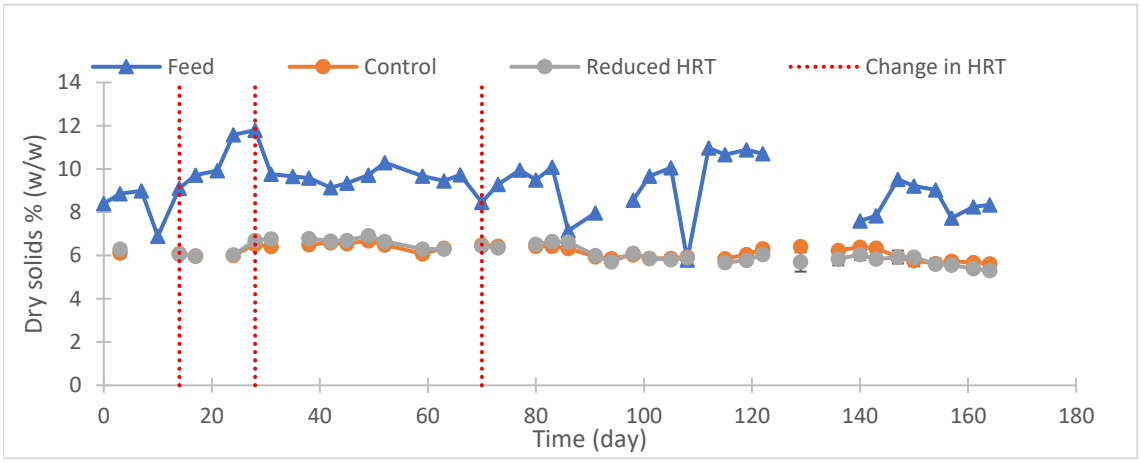
The feed averaged at 9.26 DS% across the 164 days, ranging between a maximum of 11.79 DS% on day 28 and minimum of 5.80 DS% on day 108. The RSD for the length of the experiment was 0.13 (Table 6.1).

The average DS% for the Control and Reduced HRT digesters were 6.16% and 6.13% respectively, and the percentage reduction in DS% from feed to digestate was 33.47% and 33.79% respectively for the entirety of the experiment, representing a seemingly small increase in dry solids destruction at lower HRTs. When split into days 0-14 before changes in HRT and days 70-164 after the final change in HRT, the percentage reduction from feed to digestate for the Control digesters was 34.05% and 34.49% and for the Reduced HRT digesters 33.23% and 35.81% (Table 6.1). An apparent 2.58% increase in dry solids destruction as a result of the reduced HRT. The DS% leaving the digesters proved to be much less variable than the feedstock going in with an average RSD of 0.05 and 0.07 for the Control and Reduced HRT digesters.

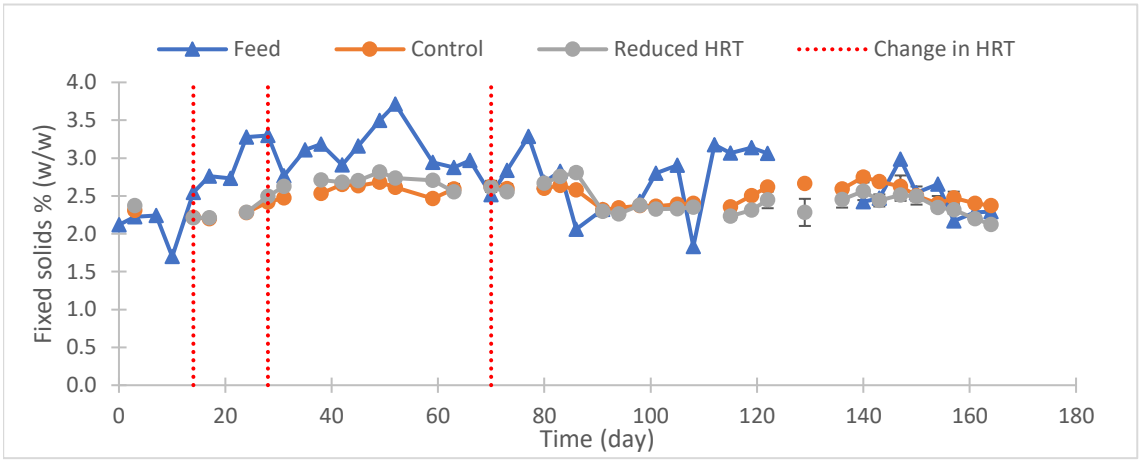
Fixed and Volatile solids

Samples were taken twice per week for FS% and VS% (Figure 6.4B and 6.4C). The FS% for both the feed and digestate varied more than the DS% for the whole course of the experiment (Table 6.1). The average FS% for the feed was 2.75% with an RSD of 0.16. The average FS% for the Control and Reduced HRT digesters was 2.49% and 2.46% respectively with an RSD of 0.06 and 0.08, representing a 9.26% and 10.30% reduction in FS%.

The feedstock VS% averaged at 6.53% and varied between 8.50% and 3.97%, with an RSD of 0.13 (Figure 6.3C and Table 6.1). The average VS% for the Control and Reduced HRT digesters were both 3.67% for the 164 days with an RSD of 0.06 and 0.08 respectively. Prior to the first reduction in HRT for days 0-14 the average VS% for the Control and Reduced HRT was 3.84% and 3.89% respectively, between days 70-164 it was 3.56% and 3.52% respectively. For the Control and Reduced HRT digesters the average reduction in VS% was 43.8%. This indicates that any increase in dry solids destruction seen in the previous section as a result of reducing the HRT was due to a loss of fixed solids, rather than greater destruction of organic matter.



(A)



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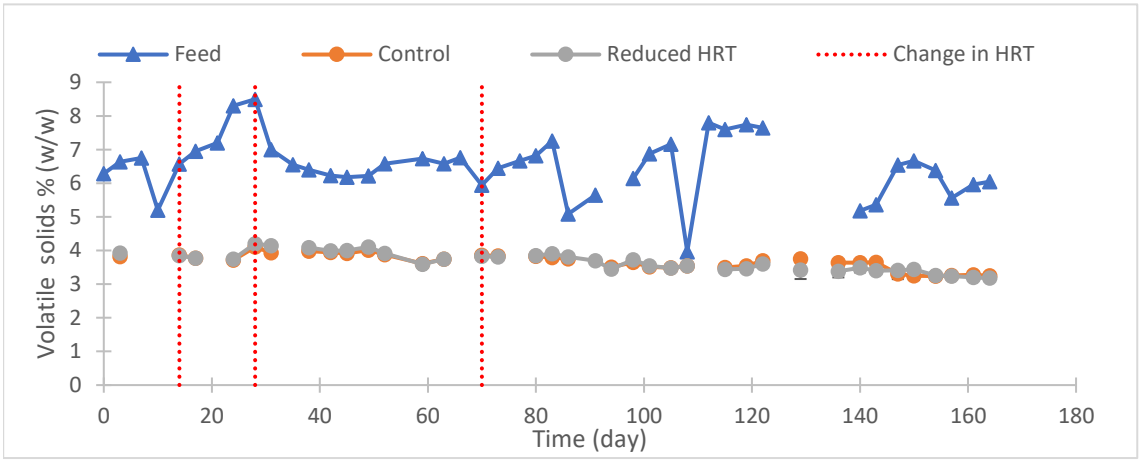


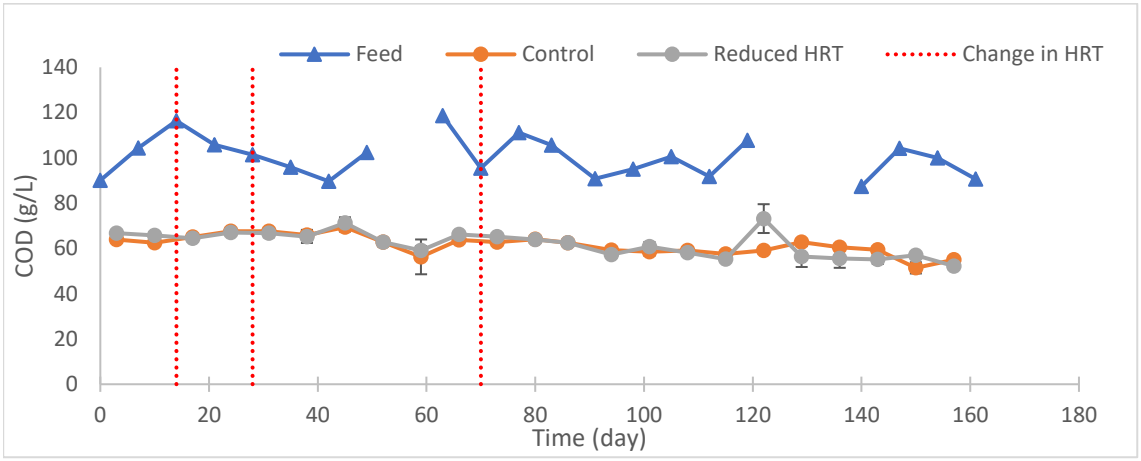
(C)

Figure 6.4 – Feed and digestate solids measurements

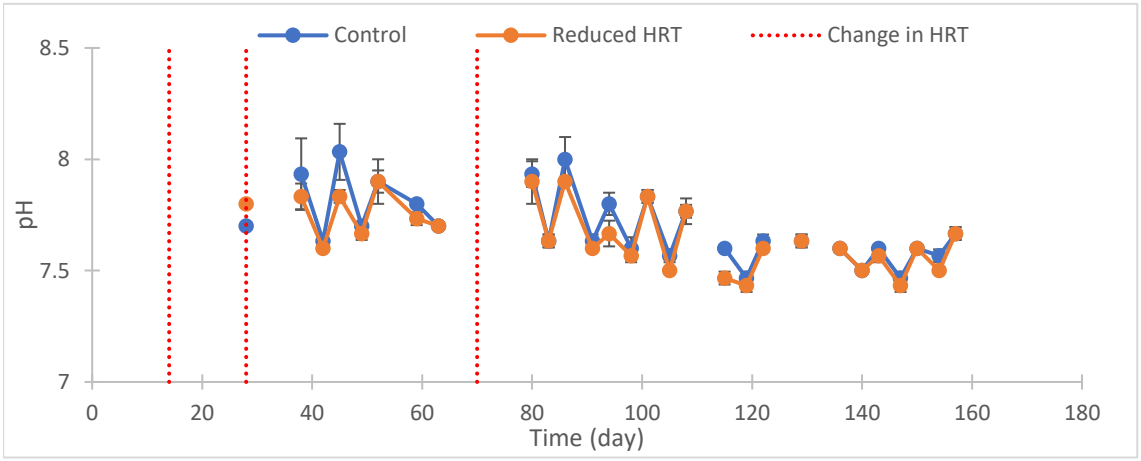
Dry solids % (A), fixed solids % (B) and volatile solids % (C) were calculated on a percentage by weight basis. The vertical lines on days 14, 28 and 70 indicate changes in HRT. Digester analysis was done in triplicate and error bars of the standard deviation applied.

Table 6.1 - Statistical analysis of DS%, FS% and VS%

	DS%	FS%	VS%
Feed - average	9.26	2.75	6.53
Feed - max	11.79	3.71	8.50
Feed - min	5.80	1.70	3.97
Feed - RSD	0.13	0.16	0.13
Day 0-164			
Control - average	6.16	2.49	3.67
Control - max	6.69	2.75	4.10
Control - min	5.62	2.20	3.24
average % reduction	33.47	9.26	43.81
Control - RSD	0.05	0.06	0.06
Reduced - average	6.13	2.46	3.67
Reduced - max	6.92	2.82	4.21
Reduced – min	5.30	2.12	3.18
average % reduction	33.79	10.30	43.83
Reduced - RSD	0.07	0.08	0.08
Day 0-14			
Control - average	6.11	2.26	3.84
Control - max	6.13	2.31	3.87
Control - min	6.09	2.21	3.82
average % reduction	34.05	17.64	41.11
Control - RSD	0.00	0.03	0.01
Reduced - average	6.18	2.29	3.89
Reduced - max	6.30	2.37	3.93
Reduced - min	6.06	2.21	3.85
average % reduction	33.23	16.52	40.41
Reduced - RSD	0.03	0.05	0.01
Day 70-164			
Control - average	6.07	2.51	3.56
Control - max	6.69	2.75	3.86
Control - min	5.62	2.32	3.24
average % reduction	34.49	8.75	45.48
Control - RSD	0.05	0.05	0.06
Reduced - average	5.94	2.42	3.52
Reduced - max	6.66	2.81	3.90
Reduced - min	5.30	2.12	3.18
average % reduction	35.81	11.93	46.02
Reduced - RSD	0.06	0.07	0.06



(A)



(B)

Figure 6.5 – Digester COD and pH

(A) The COD (g/L) was measured for both feedstock and digestate once per calendar week.

(B) The pH of digestate samples was measured twice per week where samples were available

Digester analysis was done in triplicate and error bars of the standard deviation applied.

Chemical oxygen demand

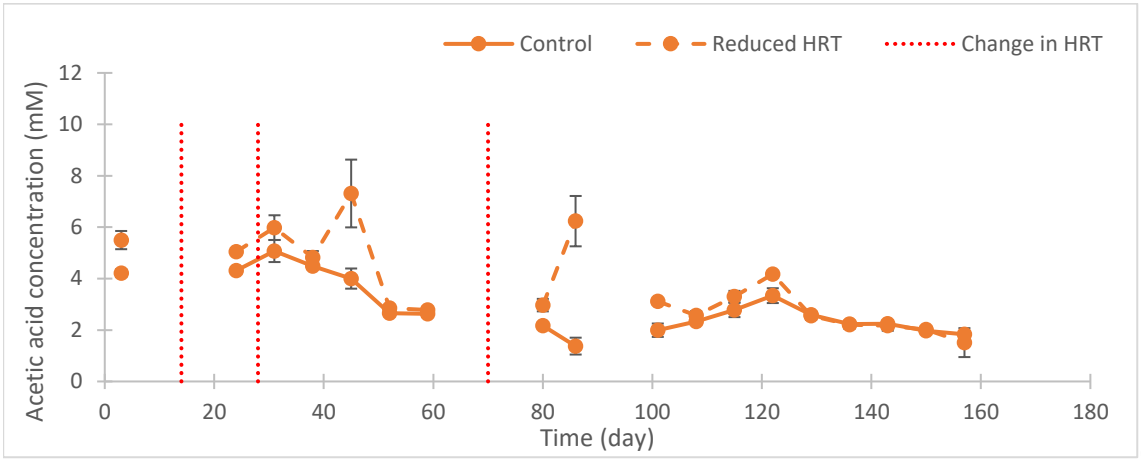
The chemical oxygen demand (COD) was analysed once per week for feedstock and digestate (Figure 6.5A). The feedstock COD averaged at 100.2 g/L and varied between 118.6 g/L and 87.4 g/L, with an RSD of 0.09 g/L while the average COD for the Control and Reduced HRT digesters were 61.6 g/L and 62.1 g/L respectively. The RSD was 0.07 and 0.09 respectively. The average COD for the Control and Reduced HRT digesters on days 0-14 only contained 2 timepoints and was 63.2 g/L and 66.2 g/L, while for days 70-164 it was 59.4 g/L for both sets of digesters over 14 timepoints, resulting in an average reduction of COD of 40.7% for both.

pH

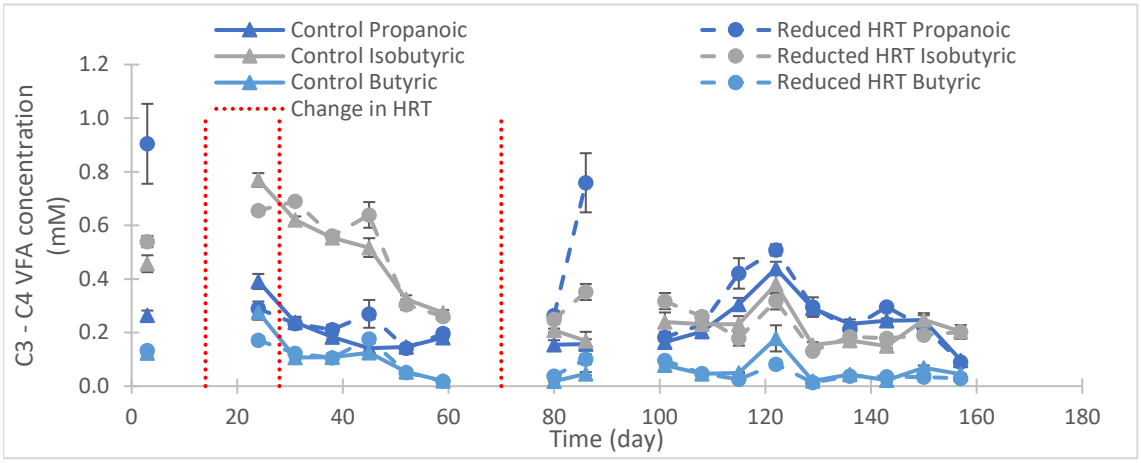
The pH of digestate samples was measured twice per week where samples were available (Figure 6.5B). Although there was a small amount of variation sample to sample, the average pH for the Control and Reduced HRT digesters was 7.7 and an RSD of 0.02 for both.

Volatile Fatty Acids

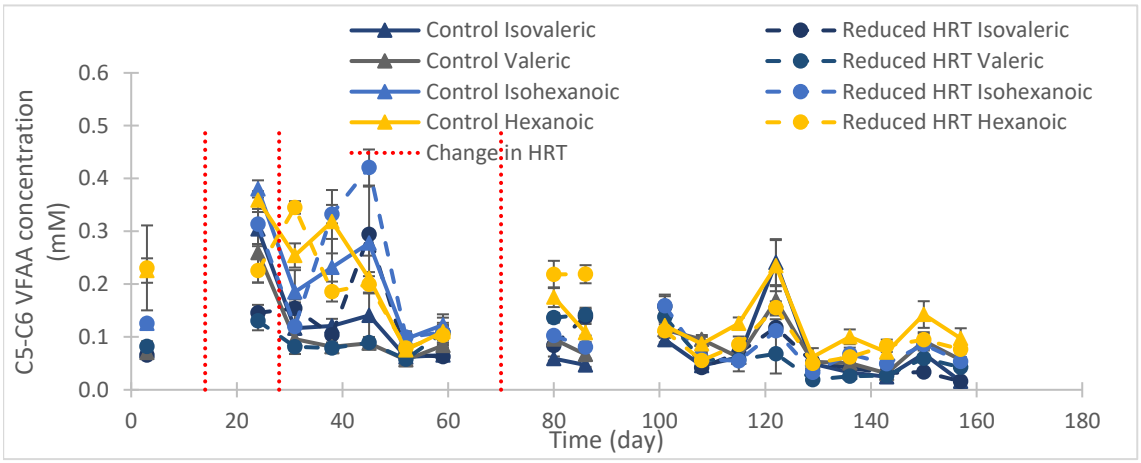
VFA's were sampled once per week at the same timepoints as COD. The acetic acid concentrations were plotted separately as their values were an order of magnitude greater than longer chain VFA's (Figure 6.6A-C). The average concentration of acetic acid was higher for the Reduced HRT digesters than the Control at 3.73mM vs 2.91mM over the course of the experiment, however this was partially due to increases at days 45 and 86 (Table 6.2). The error bars for these days were uncharacteristically larger and when the measurements for these timepoints were removed the average concentrations were much closer in value at 3.01mM and 3.35mM for the Control and Reduced HRT. This could indicate issues with sample preparation or digester instability, but also could just mean that the balance of nutrients have changed. However, there are no large changes in HRT around this timepoint. The average concentration of propionic acid for the Control and Reduced HRT were 0.23mM and 0.32mM respectively, with uncharacteristically larger error bars at the same timepoints as acetic acid (Figure 6.6B). C4 (isobutyric and butyric acid) and larger VFAs showed differences of ±0.01mM or less between the Control and Reduced HRT digesters (Figure 6.6C).



(A)



(B)



(C)

Figure 6.6 – Digester volatile fatty acid profiles

Concentrations of VFA's C2-C6 were measured weekly. Acetic acid was plotted independently (A) due to the disparity in concentrations of VFAs C3 and larger. VFA's C3-4(B) and C5-6(C) plots were split to allow for greater visual clarity.

Digester analysis was done in triplicate and error bars of the standard deviation applied.

Table 6.2 – Statistical analysis of VFA analysis results.

	Average (mM)	Day 0-14 (mM)	Day 70-164 (mM)	RSD
Acetic Acid				
Control	4.21	4.21	2.26	0.23
Reduced HRT	3.73	5.50	2.98	0.43
Propionic Acid				
Control	0.23	0.26	0.23	0.41
Reduced HRT	0.32	0.90	0.32	0.59
Isobutyric Acid				
Control	0.33	0.46	0.22	0.30
Reduced HRT	0.34	0.54	0.23	0.31
Butyric Acid				
Control	0.08	0.12	0.06	0.80
Reduced HRT	0.07	0.13	0.05	0.61
Isovaleric Acid				
Control	0.09	0.07	0.07	0.91
Reduced HRT	0.09	0.07	0.07	0.66
Valeric Acid				
Control	0.09	0.07	0.08	0.48
Reduced HRT	0.08	0.08	0.07	0.67
Isohexanoic Acid				
Control	0.14	0.13	0.09	0.60
Reduced HRT	0.13	0.13	0.08	0.46
Hexanoic Acid				
Control	0.16	0.23	0.12	0.41
Reduced HRT	0.14	0.23	0.11	0.55

6.4 Conclusions

Analysis of the biogas yield during the baselining period (days 0-14) and after the final changes in HRT have been made (days 70-164) indicates that the reduction of HRT from 14d to 8d is mildly detrimental to biogas production. During days 0-14 there was a 3% reduction in biogas between Control and Reduced HRT digesters, and during days 70-164 increasing to a 7% reduction in biogas production, however this 4% difference is significantly less than the 40% reduction in HRT gained. Therefore, if this is the case it may be economically acceptable to reduce the HRT regardless of the reduction in gas production. The gas flow meters are calibrated manually, and there is a lot of user discretion to this. Because of that, the gas values alone shouldn't be used as a measure of digester efficiency. Comparison of the multiple digestion metrics indicate that while baselining, during days 0-14, and both sends of digesters were on relatively the same HRT, the Control digesters outperform the Reduced HRT digesters in terms of biogas yield, CH_4%, DS% reduction, VS% reduction and COD reduction. After the final change in HRT at day 70, this changes. During days 70-164, although the Control digesters have a higher biogas yield and COD reduction, the Reduced HRT digesters have a higher CH_4%, DS% reduction and VS% reduction (Table 6.3). Since the biogas cannot come from nowhere, its perhaps more likely that the calibration on the GFM is not as sensitive as it needs to be. The average reduction of dry solids, volatile solids and COD were extremely similar for both the Control and Reduced HRT digesters, indicating that irrespective of differences in gas output, the physical material that is produced at the end of AD is the same regardless of the differing HRTs.

It was indicated by the previous experiment but clearly shown here that digesters that are fed continuously and regularly are stable to changes in feed rate and organic load. The digesters were able to cope with and even out spikes in the feedstock and rapid increases in VS% and COD in the feedstock do not result in equal spikes in the digestate coming out. The pH and VFA's also do not appear to show any acidification of the digesters which would indicate instability, either due to changes in feedstock or due to the reduction of the HRT.

In conclusion, AD is an essential part of reducing the tonnage of material that Yorkshire Water needs to dispose of via the land bank, landfill or incineration and a key requirement of this experiment would be that the digesters are still able to reduce the chemical (COD) and physical (volatile solids) material of the digestate. It would appear that regardless of the HRT, either 14d or 8d, this is the case.

Table 6.3 Comparison of Control and Reduced HRT digestion metrics

Comparison of the Control and Reduced HRT digester metrics during the baselining period (days 0-14) and after the final changes in HRT have been made (days 70-164) where the comparatively higher values have been highlighted in green to highlight trends.

	HRT(d)	OLR(gCOD/L/d)	Temperature (°C)	Biogas Yield (L/kgDS)	CH_4%	DS% reduction (%)	VS% reduction (%)	FS% reduction (%)	COD reduction (%)
Control day 0-14	14.3	6.81	34.99	0.34	61.32	34.05	41.11	17.64	36.94
Reduced HRT day 0-14	14.2	6.86	35.00	0.33	60.44	33.23	40.41	16.52	33.95
Similarity (%)	99.30	100.68	100.02	95.79	98.57	97.59	98.31	93.67	91.90
Control day 70-164	14.8	6.67	34.98	0.30	58.44	34.49	45.48	8.75	40.75
Reduced HRT day 70-164	8.9	11.22	34.97	0.28	59.28	35.81	46.02	11.93	40.72
Similarity (%)	60.14	168.07	99.96	91.53	101.45	103.83	101.18	136.36	99.94

Chapter 7: Discussion

The System 60 digesters were robust and repeatable results at lab-scale.

Laboratory scale (lab-scale) AD facilities are a vital tool towards better understanding the microbial community and AD process as a whole, allowing for the testing of a wide range of feedstocks and operational parameters without the risks associated with trialling at process scale. One of the benefits of lab scale digesters are that they are small enough to be able to run biological triplicates, controls, and multiple variations on a condition at the same time[10]. With this replication providing additional confidence to the results.

System 60, a fleet of 60, 5L continuously stirred digesters was designed, built, and trialled. The trialling of the digesters found several mechanical problems as a result of the feedstock and highlighted the importance of understanding the specific physiochemical characteristics of the feedstock and digestate utilised. For example, the feedstocks utilised throughout the studies presented here contained much higher fixed solids to volatile solids ratio (FS:VS) at 33:67 of the DS%, than would typically be found in other feedstocks. For example, in food waste the FS:VS can be as low as 5:95[55,146], while solid and liquid animal manures are typically higher at 16:84 and 24:76 respectively[146]. Sewage sludge is also considered a pseudoplastic or non-Newtonian fluid, showing high temperature-dependent viscoelasticity, that can be highly variable depending on primary/SAS ratios and shows no direct link to DS% or VS% which makes movement of the sludge through tubing challenging at small scale[93]. While using a feedstock directly from WWTPs rather than synthetic sludge for use in lab-scale digesters has been reported[90], it is uncommon.

The digesters proved to be highly replicable between biological replicates, be that a single triplicate of digesters as in Chapter 3, or between multiple triplicate digesters during baselining periods in Chapters 4,5 and 6.

Anaerobic digestion decreases variability, creating more stable and consistent end products.

Yorkshire Water provides a public service does not have a choice in the regional or temporal variability of the sewage it is required to treat. In comparison, commercial waste AD providers may be able to utilize a variety of feedstocks, carefully balancing their C:N ratios and organic loading rates to provide the digester with a stable feedstock and predictable biogas output. Careful analysis of the variability of the feedstocks from YW indicate this may not be required. Across all chapters in this

study the temporal variability in DS%, VS% and COD was reduced from the feedstock to the digestate in line with convention AD of sewage sludge to between 2-6 DS%[93].

AD typically generates a stable and consistent product solid digestate. Consistency is essential in adding market value, as a consistent product can be sold either as a fertilizer or as a feedstock into downstream processes. This provides support to the conclusion that System 60 digesters are able to function metabolically similarly to process scale digesters and can therefore be a valuable tool in de-risking commercially relevant decisions.

Loss of fixed solids % is consistent across all chapters of this study.

Across all chapters of this study there was a reduction in fixed solids between the feedstock and digestate. During Chapter 4 the reduction in FS% was approximately 7.01%, during Chapter 5 - 10.63%, and during Chapter 6 - 9.78%. This is not an inconsequential amount. Initially it was suspected that this was the result of sampling methods. For Chapters 4 and 5 the samples were removed from the overspill pots, well mixed before use. If they were not mixed thoroughly then there may have still been settlement of fixed solids at the bottom, however during Chapter 6 the liquid sample ports were used and samples were removed directly from the digester. It can be estimated that roughly 1.4kg of fixed solids were "lost" from the Control digesters running at a 14d HRT, and over 2kg for the Reduced HRT digesters, over the course of the experiment in Chapter 6. If this "loss" was due to settlement and grit accumulation in the digester, it would have become apparent when the digesters were emptied and cleaned, but it did not. Further work needs to be done to understand how the fluid dynamics within the digesters are affecting the fixed solids of the digestate, and whether there is any gravitational back-flow of fixed solids from the digesters back into the feeding syringes.

The results of laboratory-scale anaerobic digestion can still be directly applicable to process scale digestion.

Whether results based on lab-scale digesters scale to process scale is of large concern. There are a multitude of biological and physiochemical reasons why results from lab-scale and larger, do not scale to process scale, and often even more logistical or operational reasons. Scalability in lab-based AD is a frequent challenge, and literature directly linking scalability from lab-scale to process-scale is limited due to the timescales required to change operational procedure and sometimes governmental regulation. However, some applicable literature exists around introducing new waste

streams[143] and changing of operation parameters[147] at lab and then process-scale. At Lleida Municipal WWTP in Spain typically fat is removed from the wastewater to reduce the likelihood of build-up and pipe-blockages within the treatment plant, which is then incinerated or sent to landfill[143]. By trialling treatment and integration of the waste at lab scale, it was possible to trial the waste at full scale and prove the absence of negative effects and improve the overall economics of the WWTP[143]. At Treviso WWTP in Italy, operational process changes from mesophilic to thermophilic were modelled first using a pilot-scale (380L) stirred tank using digestate and feedstock from the WWTP, and then applied to the full scale digester[147].

On this occasion the results from lab-scale were able to effectively predict outcomes at process scale. We were able to trial the addition of Humus material at lab-scale and process scale, finding no inhibition of the AD microbial community in conversion of organic carbon into biogas in either, where inhibition is indicated by a decrease in gas production[57]. This resulted in predicted savings of over £600,000 per annum, and reduction of organic material in landfill, reducing the biogas produced and flared there.

The success of the project emphasises the utility of this model system for some investigations.

Increased OLR, and decreased HRT, did not result reduction of process performance indicating a stable microbial community.

Triplicate digesters were run at an average 8.9d HRT and OLR of 11.23 gCOD/d/L, producing a biogas yield of 0.3 L/kgDS for 94 days. In comparison, triplicate digesters were also run at a 14.8d HRT and OLR of 6.67 gCOD/d/L, also producing a biogas yield of 0.3 L/kgDS. Although experiments were designed so that the digesters would have at least 1 HRT, or 14 days, at the new HRT every time it was reduced, before reducing again, there were also periods of time where mechanical and software breakdown meant that the HRT's were increased and decreased at a much faster rate. Although accidental and undesirable at the time, this did provide us with rudimentary information on the robustness of the microbial community. Both digesters experienced rapid changes in OLR and HRT, with no apparent consequential changes to biogas output or solids destruction indicating that both microbial communities were stable under their respective conditions, containing enough buffering capacity and redundancy to deal with these changes.

One of the common reasons for digester failure is acidification from VFA accumulation, due to high OLR or low HRT[148]. Syntrophic fatty acid oxidising bacteria have a very specific metabolism centred around the β-oxidation of VFA's into acetate to be used in acetoclastic methanogenesis[148]. They are

typically found in low abundance and grow slowly, which make them susceptible to wash out at low HRTs[148]. Loss of these bacteria, or low uptake of acetate by methanogens, can result in high levels of VFAs and acidification of the digester[148,149]. However it would appear that given enough time, the microbial community is able to shift to accommodate the increased feed. Microbial communities have been widely observed to shift in response to physiochemical changes, including OLR and HRT[7].

OLR's of greater than 10 gCOD/d/L are not unreported, and indeed OLR's of nearly 18 gCOD/d/L have been reported during mesophilic co-digestion of glycerol and sewage sludge in CSTR's[94]. OLR's of 13.74 gCOD/d/L are also reported during thermophilic digestion of dairy wastewater[150]. However these experiments were completed in a two stage system allowing for the separation of acidogenic and methanogenic stages[94,150]. HRT's of 10d utilizing sewage sludge collected from a WWTP is also reported, however in these studies the OLR was between 0.6-1.8 gCOD/d/L, an order of magnitude lower than the OLR's reported here[144]. The most similar, and significant, study to the results reported here utilized a mesophilic, 0.9L CSTR fed daily with a mixture of primary and secondary sludge collected from a local WWTP, at an average 5-6 DS% and 61g/L COD[149]. Although the DS% and COD were around half the concentration of the feedstock utilised in this study (6-12 DS% and 87-119g/L COD), it is still much higher than reported in other studies and therefore more comparable. Much in a similar way to this study the HRT was stepwise reduced from a 20d HRT down to a 4d HRT, holding for a minimum of 3 HRT's at each step[149]. Interestingly they found that although COD and VS removal did reduce slowly as the HRT was reduced from 20d to 10d HRT, it was not until the HRT was reduced from 10d to 4d HRT that the COD and VS removal reduced more rapidly[149].

This reported stability in digester communities at high OLR has potential implications for green energy production and balancing of the electrical grid. One of the drawbacks of solar and wind energy generation is that it is unpredictable and uncontrollable, periods of high draw on the electrical grid do not always coincide with periods of high production from renewable sources[78,113]. However, electricity generation from CHP could in theory be increased and decreased as required by increasing and decreasing the OLR and HRT of anaerobic digesters accordingly. This approach has already been proposed and demonstrated using anaerobic trickle bed digesters supplemented with H_2 for grid balancing and energy storage[113]. A similar approach has also been validated at pilot scale for 210 days, utilizing a combination of co-digestion substrates glycerine, gelatine and pig manure, to vary the OLR between 0.71 – 6.33 gCOD/d/L and maximise methane yields[151].

Future work

A piece of future work of immediate importance is changing the method of emptying the overspill pots containing the digestate. The current method results in 1-2 litres of air entering the system every time they are emptied, which can be every 24-48 hours at low HRTs. This alters the gas composition readings and causes drops in the biogas flow rates by dropping the headspace pressure and potentially from introducing oxygen into an environment controlled by strict anaerobes. This could potentially be solved by changing the overspill to feature a weir such as found at process scale.

Alkalinity in combination with more in depth VFA analysis would give further insight into the changes in buffering capacity as the HRT decreases, and around any rapid changes in OLR and HRT. It would also allow further insight into how quickly the conditions can be changed, there appeared to be no harm using a minimum 14 day acclimatisation, but this could be reduced. It is assumed that the microbial community will have shifted in response to an increased OLR, however with 16S rRNA, or whole genome DNA sequencing would provide further insight into how the community responds. While metagenomic sequencing can be used to investigate relative abundance within the microbial community, transcriptomics would provide insight into the activity of the individual members of the community.

Further experimental work could also be done around sampling from specific sites. It is well known that different AD sites, and even different digesters within the same AD site, contain different microbial communities and may respond differently to reductions in HRT.

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