Improving the Biomethane Yield and Biogas Quality of Food Waste During Anaerobic Digestion by Sequential Process Optimisation and Biomethanation

by

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The candidate confirms that the work submitted is her own, except where the work has formed part of a jointly-authored publication and has been included. Also, appropriate credit has been given within the thesis where reference was made to the work of others.

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In the publications I and II, the candidate designed the experiments, collected data, analysed the results and drafted the manuscripts. Suruagy M. contributed in part to experimentation, data analysis and draft of Paper II. Dr. Ross, A. B. and Dr Camargo-Valero, M. A. reviewed and provided suggestions to all manuscripts. Papers I and II have been reproduced in some sections of Chapter 1, 2, 3, 4 and 5.

DEDICATION

This thesis is dedicated to my husband, Chebawaza Shekwaga and my children Godiya and Jaden. Your love and support made this journey worthwhile. I love you all so much.

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ABSTRACT

In spite of global efforts to reduce the generation of food waste, overwhelming quantities are still generated annually. In the United Kingdom for example, a third of the food crops produced annually for consumption end up in the bins. Anaerobic digestion (AD) is currently the most suitable technology for treating food waste, providing energy in the form of methane. However, the highly organic nature of food waste enriches the release of nutrients up to levels, which can be toxic or inhibitory to the acting microorganisms. As a result, the biomethane yields are much lower than the theoretical potential. This study investigates the possibility of improving the stability of AD and enhancing biomethane yield from mono-digestion of food waste, by a sequential optimisation of the biomethane production process.

The first level of optimisation was to identify suitable combinations of food waste particle size and microbial availability (inoculum-to-substrate ratio – ISR), to improve the process stability and biomethane yield. This investigation revealed that PS reduction (≤ 3 mm) resulted in a rapid digestion of food waste, and while this is expected to result in higher rates of acidification within the system, the variation in ISR helped to reduce such effects. Hence, an optimum condition of 1 mm PS and 3:1 ISR was determined; resulting in 38% increase in methane, and was used henceforth.

The second level of optimisation explored the potential for incorporating biomethanation into food waste AD. To optimise the conversion of the injected hydrogen to biomethane, three hydrogen injection points were investigated. As a result, 12.1%, 4% and 9.6% increases in biomethane yield were achieved, when hydrogen was added before hydrolysis, at the peak of acidification and during active methanogenesis respectively.

The third level of optimisation adopted the principle of acclimation to further improve the biomethane yield and explore the possibility of using formic acid (FA) as an alternative source of H_2. The H_2-acclimated systems performed better than the FA-acclimated systems, and yielded up to 81% biomethane against 65% without acclimation. Based on the results obtained in this study, it is possible to obtain up to 98% biomethane content, with continuous hydrogen acclimation. This reveals that the energy and revenue potential of food waste AD can be improved, by opening up multiple end uses beyond combined heat and power, such as gas-to-grid injection and vehicle fuel.

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LIST OF ACRONYMS/ABBREVIATIONS

AD	Anaerobic Digestion
ADBA	Anaerobic Digestion Biogas Association
ATP	Adenosine tri-phosphate
BMP	Bio-Methane Potential
C/N	Carbon-to-Nitrogen
CH4	Methane (also used as biomethane)
CHP	Combined Heat and Power
CIWEM	Chartered Institution of Water and Environmental Management
CO2eq	Carbon Dioxide equivalent
COD	Chemical Oxygen Demand
Defra	Department for Environment, Food and Rural Affairs
DfT	Department for Transport
DIC	Dissolve Inorganic Carbon
DOC	Dissolve Organic Carbon
EU	European Union
FA	Formic Acid
FAO	Food and Agriculture Organization of the United Nations
FIT	Feed-In Tariff
FW	Food waste
FWDs	Food Waste Disposers
GHGs	Greenhouse gases
GtG	Gas-to-Grid
HRT	Hydraulic Retention Time
IEA	International Energy Agency
IPCC	Intergovernmental Panel on Climate Change
ISR	Inoculum-to-Substrate Ratio
kWh	kilo Watt hour
LCFA	Long Chain Fatty Acid
LEC	Levy Exemption Certificates
mBMP	manometric Bio-Methane Potential
MSW	Municipal Solid Waste
Mt	Million tonne
MWh	Mega Watt hour
NNFCC	National Non-Food Crops Centre
OBHP	Obligate Hydrogen Producers
OHPMR	Over-head Pressure Measurement and Release
OLR	Organic Loading Rate
PS	Particle Size
REA	Renewable Energy Association
RED	Renewable Energy Directive
REGOs	Renewable Energy Guarantees of Origin
RHI	Renewable Heat Incentive
ROC	Renewable Obligate Certificate
RTFO	Renewable Transport Fuel Obligation
rWFD	Revised Waste Framework Directive

SAO	Syntrophic Acetate Oxidation
SRB	Sulphate Reducing Bacteria
TE	Trace Elements
TOC	Total Organic Carbon
VFA	Volatile Fatty Acids
WRAP	Waste Resources Action Programme
WwTW	Wastewater Treatment Works

CHAPTER 1

INTRODUCTION

This chapter presents a general overview of the problems of food waste generation and management technology in the United Kingdom (UK); outlining the research problems and research gaps addressed in this study. It starts out by establishing the global, European and UK generation of food waste, and then narrows down to the current food waste management practices adopted in the UK, as influenced by government policies. The use of anaerobic digestion (AD) as a suitable food waste management technology, its revenue potentials and limitations were briefly discussed, following which, the current research problems were identified and the possible intervention strategies detailed in the aim and objectives section.

1.1 Overview

Food waste comprises discarded foods; both uneaten and remnants from residences, waste foods from institutions such as canteens, cafeterias, lunchrooms and restaurants (Iacovidou et al., 2012; Kumaran et al., 2016; Zhang et al., 2007). Food waste is typically characterised with high moisture (>70%) and organic (>90% of its total solids) contents (International Energy Agency, 2015; Defra, 2011). The high moisture content provides an enabling environment for anaerobic microorganisms to feed on the organic content; in the process releasing harmful gases (CO_2, CH_4, H_2S, etc.). Therefore, food waste is a major environmental burden, as improper disposal poses a lot of threat to human health and the environment.

A third of the food crops cultivated annually (about 1.3 billion tonne - Bt) for human consumption is reportedly wasted or lost at some level within the food supply chain from production to consumption (FAO, 2011). This contributes significantly to municipal solid waste (MSW) production (about 25 – 70% by weight); that is waste collected by municipalities or other local authorities (IPCC, 2006; Pham et al., 2015).

Food waste generation and its characteristics vary from place to place; 40% of food waste in developing countries (such as in sub-Saharan Africa and South/Southeast Asia) was reported to be from the production and processing levels, while the same percentage in industrialised countries (such as in Europe and North America) comes from the retail and consumer levels (FAO, 2011). Notwithstanding, food waste collected at consumer level in

industrialised countries (about 222 million tonne – Mt) is almost equivalent to the average food production in sub- Saharan Africa (about 230 Mt – FAO, 2011). Generally, urbanisation and global population growth was projected to influence a continuous increase in food waste around the world (Uçkun Kiran et al., 2014).

Within the European Union (EU), 89 Mt of food was wasted in 2006, and estimated to increase to 126 Mt by 2020 (Pham et al., 2015; Lin et al., 2013). In agreement with FAO (2011), the European Commission (2010) reported that household (consumer level) contribute the highest to the total food waste collected; accounting for 42%, which is equivalent to 38 Mt or 76 kg per capita, while manufacturing was the next highest contributor to total food waste in the EU at 39% (35 Mt).

Recent studies by WRAP (2017) on the estimates of food surplus and waste arising in the United Kingdom (UK), quantify post-farm food wastage at 10 million tonnes, with about 60% believed to be avoidable. In addition, about a quarter of the food bought in the UK is wasted annually and household generated food waste is by far the largest contributor, sectoring out 71% of food waste arising (Figure 1.1).
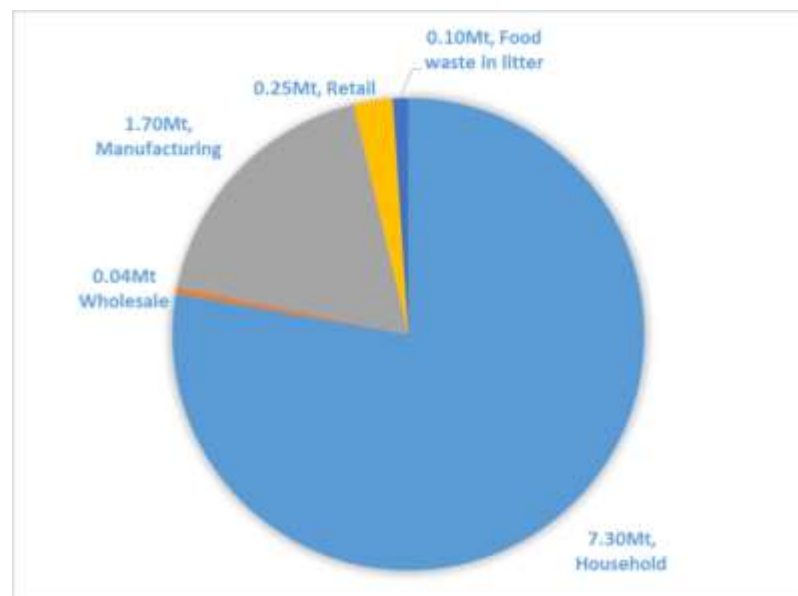
Source: Adapted from WRAP (2017).

Figure 1.1. Food waste arising in the UK in million tonnes/year.

An estimate of 18 Mt of municipal waste is sent to landfill annually, out of which 9 Mt is considered biodegradable, with food waste accounting for the highest composition by weight (Defra, 2015; Nguyen et al., 2014). Following the production, distribution and disposal of avoidable food waste, food waste

accounts for an average of 170 $MtCO_{2eq}$/annum greenhouse gas emissions; which is about 3% of the EU27's total annual GHG emissions in 2008 (European Commission, 2010). However, the amount of methane captured from anaerobic digestion of 1 tonne of food waste would potentially save 0.5 tCO_{2eq} from landfills (Defra, 2011; Evangelisti et al., 2014). In addition to the energy consumption, a significant amount of water (6% of the total UK's water) is used in food crop production, which is apparently wasted (Defra, 2011). Therefore, food waste diversion from landfills through the use of sustainable technology such as anaerobic digestion could significantly contribute to the reduction of greenhouse gases (GHGs) emitted from food waste, as well as economic costs.

1.2 Food waste management in the UK

The UK adopts the EU revised Waste Framework Directive (rWFD) (Council Directive 2008/98/EC) for their waste management policies, with an aim of moving towards a 'zero waste economy'. According to the rWFD waste hierarchy, priority is given to preventing the generation of waste through the adoption of technologies that enables lesser materials input, reusing materials and reducing hazardous materials used in design. However, where waste is already generated, then the priority is to prepare the material for reuse, which includes checking, cleaning, repairing and refurbishing the whole item or spare parts. Where this is not possible probably due to the nature of the waste such as food waste, then the waste should be turned into a new product such as compost. Otherwise, energy recovery technologies should be incorporated, which includes anaerobic digestion, pyrolysis and incineration with energy recovery. At the bottom of the hierarchy is disposal, which should only be an option where the waste material cannot be managed under all other four levels.

The rWFD also requires that 50% of household generated waste be recycled by 2020 (Defra, 2011). As a supplement, stringent requirements for landfill operations are described in the European Community Landfill Directive (Defra, 2010a); to reduce the disposal of organic wastes in landfills by 35% of the 1995 disposal levels by the year 2020 (Defra, 2011; House of Parliament, 2011). Hence, food waste disposal to landfill is expensive in the UK due to a continuous increase in landfill gate fees; for instance between 2009 and 2011, landfill gate fees increased from £40 – £74 to £68 – £111 (Lin et al., 2013).

Furthermore, the UK's Climate Change Act requires 80% reduction of greenhouse gases emissions by 2050 in comparison with 1990 levels (House of Parliament, 2008). Also the EU Renewable Energy Directive requires 15% of the energy delivered to consumers and 10% of energy used in transport to be generated from renewable sources by 2020 (Council Directive 2009/28/EC). All these regulations have in one way or another influenced food waste diversion from landfill and encouraged energy recovery. To track food waste arising and energy recovered from the adopted technologies, the food waste hierarchy was developed in the UK using similar drivers as the waste hierarchy described in the rWFD.

1.2.1 Food waste hierarchy

Figure 1.2 presents the food waste hierarchy for the UK, using food waste data from 2011 through 2015. While it is difficult to place current food waste management practice at a particular level on the waste hierarchy, overall efforts were directed at reducing the generation of food waste and keeping any generated food waste out of the landfill.

[Food waste data from 2011/15; food surplus data from 2015; table updated Jan 2017]	Household	HaFS*	Retail &** Wholesale	Manufac- turing	Farm	Total[7]
Total food waste	7.3 Mt	0.9 Mt	0.3 Mt[9]	1.7 Mt	nk	>10 Mt
Preventable food waste	4.4 – 5.7 Mt[1] (£13.0 bn)	0.7 Mt (£2.5 bn[2])	0.3 Mt (£0.8 bn)	0.9 Mt (£1.2 bn)	nk	> 6.3 Mt (>£17 bn)
Redistribution & animal feed	0.3 Mt[3] [n/a humans 0.3 Mt pets/ other animals	>0.001 Mt [>1kt to people] [n/a to animals]	0.03 Mt [5kt to people] [27kt to animals]	0.7 Mt [42kt to people] [635kt to animals]	nk	> 0.7 Mt
Recycling (AD/composting)	1.1 Mt[4]	0.1 Mt	0.1 Mt[5]	0.5 Mt	nk	> 1.8 Mt
Recovery (thermal, landspreading)	2.3 Mt[6]	0.2 Mt[6]	0.1 Mt	1.2 Mt	nk	> 3.8 Mt
Disposal (sewer, landfill)	3.5 Mt [1.6 Mt sewer 1.9 Mt landfill]	0.65 Mt [0.14 Mt sewer 0.51 landfill]	nk[5]	0.002 Mt [nk sewer 2,000t landfill]	nk	> 4.1 Mt
In addition: Rendering of animal by-products				0.6 Mt	nk	0.6 Mt
Other food by-products[8]				2.2 Mt		2.2 Mt

* HaFS = hospitality and food service; ** Retail includes wholesale; nk = not known; n/a = not applicable

Source: WRAP (2017).

Figure 1.2. Food waste hierarchy and corresponding food waste arising in the UK.

As presented in Figure 1.3, a significant effort has been directed towards reducing the amount of food waste generated; especially from households, through interventions such as the 'love food hate waste' website developed by the Waste and Resources Action Programme (WRAP). This provides information such as 'planning before food shopping', 'using leftovers for other meals' and 'understanding food date labels' (Love Food Hate Waste, 2017).
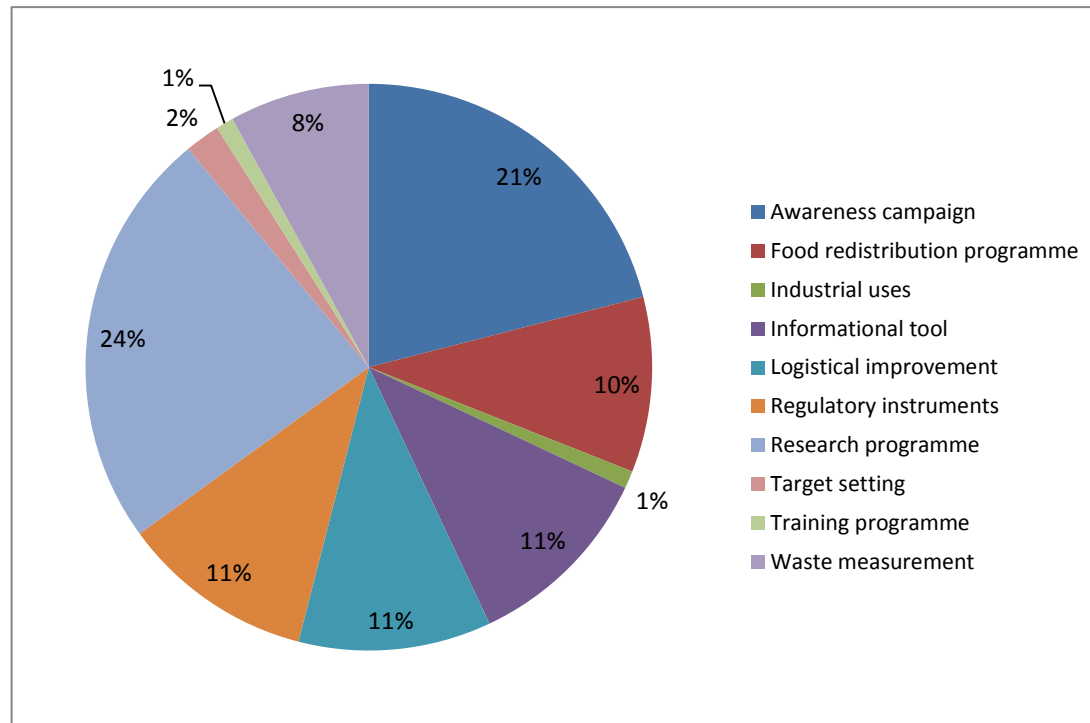
Source: European Commission (2010)

Figure 1.3. Instruments used in the UK to prevent food waste based on EU regulations

Food waste disposers (FWDs) connected under sinks, which help to separate food waste at source, macerate and transport it down to sewers to waste water treatment works (WwTW), were installed in about 6% of UK homes (House of Parliament, 2011). This technology have gained wide acceptance in other countries; such as the USA, with FWDs installed in about 50% of the houses and in New Zealand and Australia, with installation rates of over 30 and 20% respectively (CIWEM, 2003). However, it was advised against by Water UK (2009), stating that it increases the risk of sewer blockages, sewer flooding, odours, environmental pollution, excess water usage and rodent infestations; thereby, increasing the load of incoming waste water to the plant if it was encouraged. As such installation of these devices stalled in the UK and kerbside collection of segregated food waste was suggested as the most

sustainable and viable option for food waste collection in the UK (Water UK, 2009).

Household generated food waste in the UK was recorded at 8.3 million tonnes in 2009, whereby, 5.3 million tonnes were reported to be avoidable (Defra, 2011b; House of Parliament, 2011). In 2013 a lower value was recorded at 7 million tonnes (about 13 percent reduction) with 4.2 million tonnes avoidable (Defra, 2013; Defra, 2015). This means about 21% of avoidable food waste was actually avoided in 2013 compared to 2009. Notwithstanding, owing to diverse food habits and social statuses, food waste is still been generated, but, recycling and recovery technologies such as AD, composting, thermal treatments and land spreading have significantly reduced the amount of food waste going to the landfill.

As observed from Figure 1.2, AD and composting are the most preferred technologies for food waste management due to its nature. Moreover, AD is more widely adopted for food waste management than composting owing to the accrued benefits of the biogas and digestate. Consequently, about 1.6 million tonnes of food waste were recycled via the anaerobic digestion process in 2013; an improvement over 0.3 – 0.4 million tonnes recorded in 2010 (ADBA, 2015). Notwithstanding, recycling via anaerobic digestion only accounted for some 18 %, and the rest of it either ends up in incinerators or landfill. It is therefore, imperative that anaerobic digestion system be further enhanced for effective food waste treatment and optimised energy recovery.

Food waste itself can be transformed into other things using technologies beyond the AD, such as hydrothermal carbonisation (Pham et al., 2015), nevertheless, there is potential for utilising the remaining nutrients from such processes in AD. Compared with combustion for energy recovery, AD has the additional benefit of preserving the nutrients and producing a biofertiliser.

Furthermore, over 41% of the food waste generated in the UK is still disposed of, especially to landfills. This means there is a huge potential for energy recovery from food waste in the UK, by optimising the AD of food waste.

1.3 Anaerobic digestion in the UK

Anaerobic digestion is a naturally occurring process, during which microorganisms convert complex compounds in organic matter such as, proteins, carbohydrates and lipids to biogas in the absence of oxygen (Gould and Taglia, 2012; Defra, 2011); an extensive occurrence in landfills and stomach of ruminant animals (House of Parliament, 2011). The overall

anaerobic digestion process is summarised in Figure 1.4. Microorganisms that require little or no oxygen use up the available nutrients in organic materials in the form of carbon (for metabolic energy) and nitrogen (for the build-up of their cell structure) (Gould, 2012) and release gaseous by-products called biogas. The biogas produced is composed mainly of 60 – 70% methane (CH_4) and 30 – 40% carbon dioxide (CO_2) and little amounts of trace gases such as hydrogen sulphide (H_2S) and carbon monoxide (CO) (Kondusamy and Kalamdhad, 2014). The final methane is produced by two groups of microorganisms, namely; acetoclastic methanogens (contributing ~ 70%) and the hydrogenotrophic methanogens (contributing ~ 30%). When the feeding and metabolic activities by the microorganisms end, a liquid by-product is formed; referred to as the digestate, which is rich in macro nutrient, thereby, making it suitable as bio-fertiliser (WRAP, 2016).
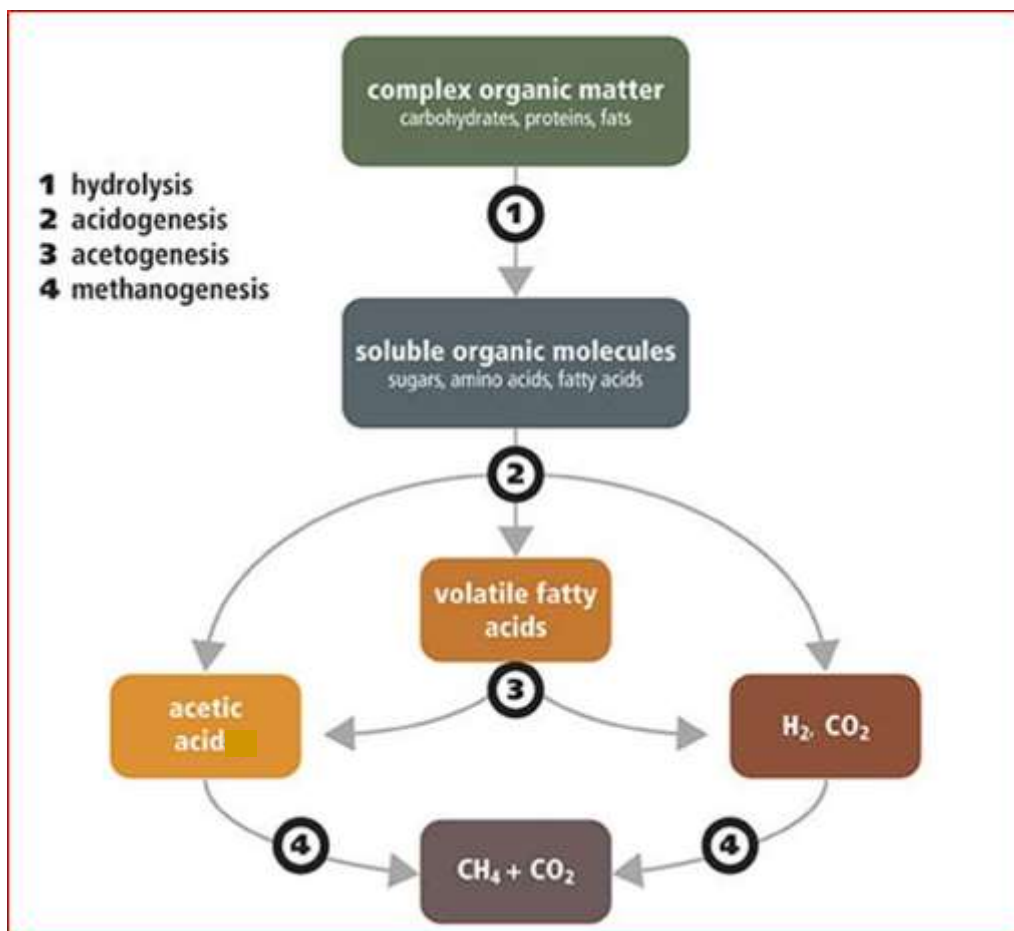
Source: Greene (2015).

Figure 1.4. A simplistic representation of the anaerobic digestion process

Anaerobic digestion is not a new technology as seen in Figure 1.5. It has been in existence since the 18th century; but was perhaps not clearly understood. It had a major breakthrough in the 1990s, primarily for organic waste

stabilisation (Gould and Taglia, 2012; Kumaran et al., 2016) and generation of renewable energy (Kondusamy and Kalamdhad, 2014; WRAP, 2012). Anaerobic digestion has therefore, received global attention, especially in past decades and is used to deal with organic wastes and reduce GHGs emission from landfills, by a more efficient capture and cleaning of the resulting biogas. It has been applied for sewage sludge treatment in the UK for over 100 years and is preferred over other biological unit processes, because it allows high organic loading rates and low sludge production (Batstone et al., 2002). However, only in recent years has it been explored for energy generation from other waste types and feedstock such as animal manure, maize silage and energy crops (Defra, 2011).
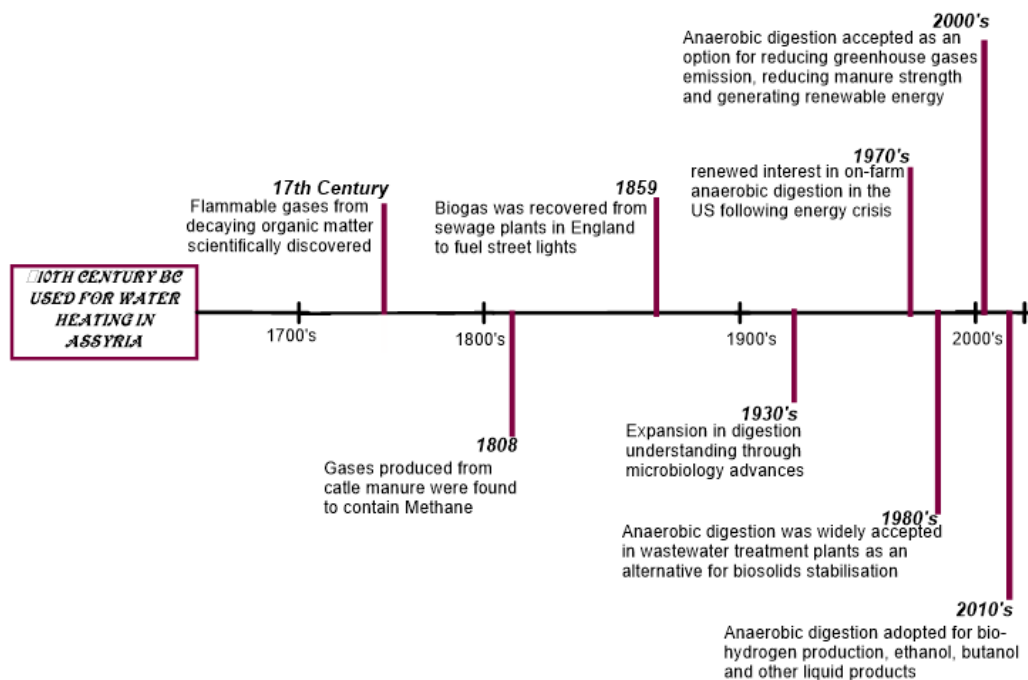
Source: Adapted from Gould and Taglia (2012) and updated with data from Defra (2015)

Figure 1.5. Anaerobic digestion history chart.

The main purpose for AD has been towards biogas production, but, following the identified potential for other valuable products from anaerobic digestion, it is constantly being optimised for diverse purposes. The products of anaerobic digestion can include volatile fatty acids (VFA – Karthikeyan et al., 2016; Komemoto et al., 2009), ethanol and butanol (Cazier et al., 2015), bio-hydrogen (Alemahdi et al., 2015; Chinellato et al., 2013), biogas (Kumaran et al., 2016) and digestate, which is inevitable whatever the route.

The number of anaerobic digesters treating solid organic waste in the UK has grown over the years. WRAP (2012) reported a total number of 233 AD plants in the UK treating 5.4 million tonnes of material annually; of which 36 digesters operated on farm waste, 51 on organic waste including food waste and 146 on sludge by wastewater treatment plants. However, the AD sector have seen continuous growth in the past years, as there are currently over 540 operational AD plants in the UK, out of which 82 inject biomethane into the gas grid (including 2 food waste AD plants), while the remainder have combined heat and power (CHP) plants, which convert the biogas to electricity with about 400 MWe total electrical capacity (REA, 2017).

The common substrates used for anaerobic digestion in UK are biogenic waste (sewage sludge, food waste, municipal organic waste); manures and slurries (agricultural/livestock by-products); by-products from food and agro-industries (animal by-products from abattoirs, solubles and breweries); and energy crops (maize whole crop silage, grass silage, sugar beet) (International Energy Agency, 2015). More recently, AD is being utilised in synergy with other renewable systems, to treat their respective by-products, such as by-products from bioethanol and biodiesel production processes (International Energy Agency, 2015) and hydrothermal processes (Salman et al., 2017).

1.3.1 Anaerobic digestion of food waste

As a result of high moisture and organic contents of food waste, AD and composting have been suggested as suitable options for handling food waste (Defra, 2011). However, anaerobic digestion is more widely employed for food waste treatment and energy generation (Banks et al., 2008; Kondusamy and Kalamdhad, 2014). It reduces environmental impacts and contributes to the production of renewable energy and the product alternatives obtainable from anaerobic digestion make it an interesting option for food waste management. In line with this, Evangelisti et al. (2014) conducted a life cycle assessment of the treatment/disposal of organic fraction of municipal solid waste from households in London at three scenarios; landfill, incineration and AD. They reported that AD was the most suitable in terms of acidification and total gas emission. When the generated biogas is used in CHP generation, it substitutes non-renewable heat and power and when the digestate is used as organic fertiliser, it substitutes inorganic fertiliser.

1.3.2 Anaerobic digestion incentives

To support the growth of anaerobic digestion a number of financial incentives were put in place depending on the end use of the biogas; which includes CHP generation, upgrade to biomethane for injection into the gas grid and upgrade to biomethane for use as transport fuel (Bright et al., 2011). The incentives available to support the use of biomethane includes (but not limited to) Renewables Obligation Certificate (ROC), Feed-In Tariff (FIT), Renewable Heats Incentive (RHI) and Renewable Transport Fuel Obligation (RTFO). Others include CHP Levy Exemption Certificates (LEC), Renewable Energy Guarantees of Origin (REGOs) and Green Gas Trading (NNFCC, 2018).

1.3.2.1 Renewable Obligation Certificate

The ROC is the main incentive provided to large scale AD operators, which was initiated to provide financial support to those who generate renewable energy. The banding levels within the ROC is regularly reviewed by the government and is different for different energy producing technology. As at 2011, AD was in the top banding at 2 ROCs/MWh (Renewable Obligation Certificates per megawatt hour) especially for biodegradable wastes, such as food waste. However, this incentive scheme is no longer open to new entrants, thereby, greatly impacting on the revenue generation from biogas when the end use is for CHP generation (REA, 2017).

1.3.2.2 Feed-In Tariff

The FIT was launched on 1 April 2010, with an aim of supporting small scale low carbon electricity generators, such as individuals, communities, businesses and organisations that are not involved in the traditional electricity market. At the time of launch, installed AD plants with a capacity of 500 kW or less received 12.1p per kilowatt hour (kWh), while installations with greater than 500 kW capacity received 9.4 p/kWh, and for electricity exported to the national grid, an additional 3.1 p/kWh was paid (Defra, 2011). As a result of complaints that the tariffs was not sufficient to support small-scale AD plants, the FIT was increased to 14 p/kWh for AD with 250 kW capacity or less, and 13 p/kWh for installations between 250 kW and 500 kW. Notwithstanding, the FIT is currently capped at 5 MW per quarter and with the complexities surrounding the queuing system for this payment, only very little is obtainable from the FIT (REA, 2017). Therefore, revenue generation from biogas to CHP,

through FIT is greatly limited, considering, most AD are usually below 5 MW capacity.

1.3.2.3 Renewable Heats Incentive (RHI)

Biogas upgrading for biomethane injection into the gas grid was initially supported by the Biomethane tariff, but is now embedded in the RHI. The RHI was the first approach to provide a secure financial support for installations generating renewable heat, over a long term. AD is eligible to receive a tariff of 6.5 p/kWh over a 20 year period; however, it only supports biogas combustion for AD installations below 200 kWth, while biomethane upgrade for gas-to-grid (GtG) injection receive this tariff regardless of the AD capacity (Bright et al., 2011; NNFCC, 2018).

1.3.2.4 Renewable Transport Fuel Obligation (RTFO)

The RTFO obliges fossil fuel suppliers to produce evidence that a specified percentage of their fuels for road transport in the UK comes from renewable sources, including biomethane. Biofuel suppliers are awarded Renewable Transport Fuel Certificates (RTFCs) for the volume of renewable fuels they supply. These can in turn be sold on to fossil fuel suppliers who have not supplied enough biofuel to meet their obligation for the year. In 2009/10; the second year of operation, the RTFO met its objective of driving a market for biofuels in the UK, as 3.33% of the UK's total road transport fuel supply was biofuel, which was slightly higher than the Government's target of 3.25% (NNFCC, 2018).

1.4 Benefits of biogas upgrade to biomethane and upgrading technologies

The current decline in incentives for the use of biogas in electricity generation and more robust incentive and opportunities for its upgrade to biomethane, has led to researches that optimise the biogas from anaerobic digestion in order to fully exploit its potential as a renewable energy source (Muñoz et al., 2015; Scarlat et al., 2018). For instance, biomethane injection into the gas grid facilitates low cost storage of biomethane and enables its use wherever it is needed (Scarlat et al., 2018), and as earlier discussed it holds more potential for higher revenue generation by AD operators.

Furthermore, the EU Renewable Energy Directive (RED) requires the UK to source 10% of energy used in transport from renewable energy by 2020 (REA, 2017). The department for transport (DfT) has recently consulted on proposals to amend the RTFO to implement the transport elements of the RED. The DfT implementation proposals involve introducing double certification for biofuels produced from wastes, residues and lignocellulosic materials including biomethane. The proposed change will give twice the financial support to these biofuels compared to conventional biofuels and no support to biofuels that do not meet the required sustainability standards. This and the advantage of obtaining a cleaner vehicle tailpipe emissions with biomethane than conventional transport fuels, have influenced the increase in the demand for biogas upgrade to biomethane for use as transport fuel (REA, 2017).

There are a number of physicochemical technologies adopted for cleaning biogas, however, the most common are adsorption (pressure swing adsorption) and absorption (water and chemical absorption), and membrane separation (Bright et al., 2011). Except for the chemical (particularly amine) absorption, which can both remove H_2S and CO_2, other processes have to be combined in series of two or more technologies to upgrade biogas (Ullah Khan et al., 2017). Physicochemical processes are relatively expensive and generate wastes than other forms of treatment, in addition, some amount of CH_4 is lost in the process (Ryckebosch et al., 2011).

Recent research reveals that CO_2 removal from biogas can also be achieved biologically in four ways. The first is by CO_2 dissolution to bicarbonate by activity of enzymes that facilitate this reaction (Muñoz et al., 2015). The second involves in-situ desorption of CO_2 in an aerated reactor using recycled sludge liquor and taking advantage of a higher CO_2 solubility than CH_4 (Muñoz et al., 2015). The third is CO_2 assimilation by microalgae for algal biomass production (Posadas et al., 2016; Posadas et al., 2015; Serejo et al., 2015; Toledo-Cervantes et al., 2016). And finally, anaerobic CO_2 removal (in-situ or ex-situ) by the activity of target microorganisms (hydrogenotrophic methanogens), which consume CO_2 and H_2 to produce CH_4; a process known as biomethanation (Voelklein et al., 2019). Furthermore, biomethanation potentially doubles the original methane mass, and upgrades the biogas to about 97 – 99% CH_4 content (Ryckebosch et al., 2011). Whereas, other processes only remove impurities, but does not improve the CH_4 mass, rather some amount of CH_4 in the biogas is lost and unwanted products accrues (with physicochemical treatments). This study seeks to upgrade biogas, while

also increasing overall methane yield, by in-situ biomethanation during the anaerobic digestion of food waste.

1.5 Research problem statement

Asides other high liquid feedstock (such as sewage sludge), food waste remains the largest feedstock in the UK AD market; with about 22.9% of food waste recycled through anaerobic digestion. However, there are only a few anaerobic digesters installed solely to treat food waste in the UK, with capacities ranging from 20,000 to 60,000 tonne/year (WRAP, 2015). This is because food waste composition poses some challenges during anaerobic digestion, even though it has high energy potential through anaerobic digestion (Banks et al., 2008). The prominent challenges are high ammonia formation in the digester, resulting from hydrolysis of the high protein content and low trace elements available to some key microorganisms both for metabolism and to withstand the high ammonia loads (Chen et al., 2015). Consequently, the organic acids are not effectively utilised and converted to biomethane, which leads to accumulation of ammonia and organic acids to level that become toxic to the microorganisms. Therefore, the biogas production rate reduces and in some cases, the digester breaks down completely after a period of time (Chen et al., 2015; Heaven and Banks, 2015; Zhang et al., 2014) and the produced biogas contains high CO_2 contents, requiring expensive cleaning.

1.6 Research gap statement

Different studies have been conducted to improve digester stability and biomethane yield from food waste, including co-digestion with low ammonia-prone substrates (Chen et al., 2014; Pagliaccia et al., 2016; Y. Zhang et al., 2012); ammonia stripping (De la Rubia et al., 2010; Serna-Maza et al., 2014; Walker et al., 2011), selective trace elements (TEs) dosing (Banks et al., 2012; Facchin et al., 2013; L. Zhang et al., 2012; Rajagopal et al., 2013; Wanli Zhang et al., 2015; Wanqin Zhang et al., 2015; Yenigün and Demirel, 2013; Zhang and Jahng, 2012), and more recently, addition of biochar (Cai et al., 2016; Meyer-Kohlstock et al., 2016).

What is clear is that these approaches mainly focus on improving acetoclastic methanogenesis, which results in the production of CH_4 and CO_2, hence, the quality of biogas produced remains relatively unchanged.

For instance, when TE was added to the mesophilic anaerobic digestion of food waste, Banks et al. (2012) reported a 3% difference in the biogas methane percentage between the control digesters at 55% compared to 58% for those supplemented with TE. Enhancing the hydrogenotrophic methanogenesis route, however, could improve both the CH_4 yield and quality of biogas to levels suitable enough for injection into the gas grid or transport fuel; but this route has been relatively under-explored. This approach has been tested with positive biogas upgrade on other substrates such as cattle manure (Bassani et al., 2015), maize leaf (Mulat et al., 2017), combination of cattle manure and whey (Luo and Angelidaki, 2013), sewage sludge (Pan et al., 2016) and anaerobic cultures (Rachbauer et al., 2017; Yun et al., 2017). But no previous work was found on biomethanation to upgrade the biogas from food waste as at the time of conducting this study.

1.7 Aim, objectives and scope

The aim of this research work is to explore the possibilities of improving biomethane yield and biogas quality from food waste AD. In this study, pre-treatment and in-situ treatment methods were explored to optimise the overall process. The overall aim was achieved by the following objectives:

1. To examine the effects of different waste streams and particle size (PS) reduction on the characteristics and biomethane potential (BMP) of food waste.
2. To optimise the BMP process and biomethane yield from food waste by the interaction of its PS and inoculum-to-substrate ratio (ISR).
3. To examine the feasibility of incorporating in-situ biomethanation to the anaerobic digestion of food waste as a means of improving the process stability, biomethane yield and the biogas quality.
4. To identify the most suitable injection point for hydrogen addition based on the volatile fatty acids (VFA) regime, which would yield the highest hydrogen conversion to biomethane and reduce the overall hydrogen demand for biomethanation.
5. To investigate the effect of hydrogen acclimation for improved in-situ biomethanation with food waste on the AD process and biogas quality.
6. To establish a statistical relationship between hydrogen utilisation and food waste's biogas upgrading to biomethane for alternative end uses such as gas-to-grid injection and transport fuel.
7. To examine the feasibility of using formic acid as an alternative source of hydrogen for in-situ biomethanation with food waste.

1.7.1 Scope

This research was limited to laboratory scale analyses using batch biomethane potential experiments. It also employs the use of statistical analysis and predictions of methane yield, based on the results obtained from the laboratory experiments.

1.8 Thesis structure

This thesis comprises 9 chapters, Chapter 1 provides the rationale behind the study reported and Chapter 2 provides a state of the art review of the literature relevant to the investigations conducted. In Chapter 2 the thermodynamics of the AD process is described in relation to how the interacting thermodynamic reactions could influence hydrogen utilisation. Based on the review of the thermodynamic pathways during anaerobic digestion, the potentials and possible competitions that could ensue, if hydrogen is added to the system are also reviewed. The technological options available to upgrade biogas to be fit for its desired end use are also discussed in this chapter. Furthermore, the role of hydrogen and FA as electron carriers during the AD process is discussed.

A comprehensive experimental design is described in Chapter 3 for all the experiments and statistical analysis conducted in the course of this study. A detailed description of the food waste source used and its sampling, processing and storage is described in Chapter 3. The potential for using food waste for biomethane production was estimated using a combination of theoretical models and experimental data. The experimental procedure for the addition of hydrogen and FA to the anaerobic digestion of food waste is also described in this chapter. This includes the source, form and concentration of hydrogen and FA added, as well as, the different process manipulations employed to obtain an optimised condition.

Chapter 4 focuses on objectives 1 and 2. It includes introduction, discussion of the results obtained and conclusions sections. It describes the influence of waste sampling, PS variation on the overall characteristics of food waste and its theoretical methane potential. Furthermore, it discusses the combined effect of PS and ISR on the experimental methane potential of food waste. At the end of Chapter 4, an optimal condition of food waste PS and ISR is established.

Objectives 3 and 4 are addressed in Chapter 5, which discusses the results obtained from the first stage optimisation of food waste biomethanation;

hydrogen injection based on the VFA regime. This chapter includes an introduction, experimental design, results and discussion and a conclusion section. Chapter 5 describes how the different thermodynamic stages of AD; as influenced by VFA regime, controls the competitive utilisation of hydrogen added to the system. Three hydrogen injection points were chosen to signify points before VFA production (before hydrolysis), at the peak of VFA accumulation (active acidogenesis) and depleted VFA intermediates (active methanogenesis). Based on the discussions in Chapter 4, Day0, Day3 and Day6 were chosen for hydrogen injection. At the end of this chapter, a suitable point of injection for hydrogen addition was identified.

Chapter 6 addresses objectives 5 and 6. It comprises of introduction, experimental design, results discussion and conclusion sections. This chapter discusses how hydrogen acclimation influence biomethanation and the process stability. The results obtained from three set of experiments with successive acclimation using hydrogen injection are discussed in this chapter. At the end of this chapter, a statistical relationship between hydrogen addition and biomethane yield is established.

Chapter 7 addresses objective 7. This chapter discusses how formic acid acclimation influence biomethanation and the process stability. The results obtained from three set of experiments with successive acclimation using formic acid are discussed in this chapter. It also gives a comparison between H_2-acclimated and formic acid-acclimated systems. At the end of this chapter, the most suitable electron carrier (hydrogen or formic acid) is identified.

Chapter 8 links together the findings described in the previous results' chapters, to obtain a feasible approach in terms of adopting biomethanation within a food waste anaerobic digestion framework. It presents a comparison of the energy balance for different biogas upgrading strategies. Furthermore, the global relevance of this research is discussed here within the context of its practical applicability to existing food waste AD systems in the UK. To achieve this, biomethane yields at a percentage acceptable by the UK for gas-to-grid injection was calculated, using the statistical relationship established in Chapter 6. The energy balance for hydrogen required to obtain the biomethane yield desired was calculated and compared with typical biogas upgrading technologies for different end use. At the end of this chapter, a feasible approach for adopting biomethanation in conventional AD systems is recommended. The limitations of this study are also described in this chapter.

Final conclusions from this study and recommendations for future research are presented in Chapter 9.

CHAPTER 2

LITERATURE REVIEW

This chapter gives a detailed review of the anaerobic digestion (AD) process. In this chapter, factors influencing the AD process are described in relation to how some of such factors can be optimised for a better anaerobic digestion process and ultimate methane yield from food waste. Furthermore, the interacting thermodynamic reactions occurring during AD process towards the production of biogas are discussed. The technological options available to upgrade the generated biogas to be fit for its desired end use are also comparatively discussed in this chapter. Based on the analysis of the thermodynamic pathways during anaerobic digestion, the potentials and possible competitions that could ensue, if an external source of hydrogen were added to the system (in-situ biomethanation) have been described. The role of hydrogen and formic acid (FA) as electron carriers during AD process is reviewed, so as to identify alternative source of hydrogen for the proposed biomethanation.

2.1 Anaerobic digestion and conditions for optimum operation

The AD process is naturally a self-stabilising process, but, inhibitions or even failure could occur if the system is subjected to stress conditions like temporal organic loading, pH changes, or presence of inhibitors (Giovannini et al., 2016; Gallert et al., 1998). Inhibition can either be substrate-induced by ammonia, volatile fatty acids (VFA), long chain fatty acids (LCFA), metals, sulphides, hydrogen and cations (Boe, 2006; González-Fernández and García-Encina, 2009) or external from temperature variation and pH; which could cause a shift in the microbial community. In most cases, inhibitions within the AD system are caused by compounds either already in the substrate or produced from the substrate during degradation, rather than external sources (Gallert et al., 1998). These compounds could cause inhibition by interfering with the metabolic enzymes of the microorganisms present, or with the cell membranes, thereby, initiating intercellular changes in salt concentrations or pH, or with the energy metabolism by the separation of growth and production of enzymes responsible for energy storage in cells (Adenosine triphosphate – ATP) (Gallert et al., 1998). While substrate-induced inhibitions can be managed by choosing the right proportion of feed to inoculum, it is also

important to choose external factors correctly as these can also influence the degree of toxicity of the substrate-induced inhibitions.

In general, the biogas production rate depends on (but not limited to) the following: reactor design, feedstock nature, carbon-to-nitrogen ratio, pH, temperature, organic loading rate, retention time and presence and level of inhibitory/toxic substances (Kondusamy and Kalamdhad, 2014).

2.1.1 Feedstock nature and pre-treatment

The nature of the feedstock to be digested can be said to influence all other factors of the AD system; including the reactor design, because it determines the biodegradability rate. Hence, AD are often classified according to the nature of the feedstock fed into the digesters. Dry anaerobic digesters treat feedstock with higher solid content typically 15 – 40% dry solids, while the wet anaerobic digesters use feedstock below 15 % dry solids (Defra, 2011a). The dry digester can use feedstock in their solid form and can be suitable for high dry solid materials such as farm residues and energy crops (Juniper, 2007), while wet digesters require further size reduction until a pulp is formed and is most suitable for feedstock with high moisture content such as food waste (Defra, 2011).

It was reported that for plants and animal-based feedstock, their ages before AD significantly influences the biogas yield. Non water-soluble substances in plant, such as: cellulose, polyamides, lignin and hemicellulose, which are generally more difficult to digest, increase with the age of the plant, while the easily digestible materials (water soluble), such as: proteins, amino acids, minerals and sugars decrease with the age of plants (Kondusamy and Kalamdhad, 2014). Therefore, older plants produce lower biogas compared to younger plants. Similarly, for animal-based waste products, the age and type of animal, the living and feeding conditions, and the storage and age of the waste products contributes to the biogas yield and quality (Kondusamy and Kalamdhad, 2014).

Notwithstanding, feedstock pre-treatment is often employed to improve biogas yield, by enhancing hydrolysis (Carlsson et al., 2012; Kondusamy and Kalamdhad, 2014).

2.1.1.1 Feedstock pre-treatment methods

Pre-treatment of feedstock is generally employed to improve its degradability and includes biological, chemical, thermal and physical pre-treatments (Pham et al., 2015; C. Wu et al., 2017). Various pre-treatment technologies are available for AD purposes, the choice of which is often dependent on the feedstock being fed into the AD (Pham et al., 2015).

Biological pre-treatment optimises the inoculation of microorganisms and enzymes that are responsible for substrate hydrolysis (Pham et al., 2015; C. Wu et al., 2017). It has recently gained a lot of research interest and employs the use of specific enzymes such as carbohydrase, peptidase and lipase and selective improvement of some anaerobic bacteria (Ariunbaatar et al., 2014; Carrere et al., 2016; C. Wu et al., 2017). Biological pre-treatment typically involves the use of multi-stage AD to separate the microbial activities and optimise the hydrolysis-acidogenesis. Therefore, it is interchangeably referred to as a process configuration and biological treatment (Ariunbaatar et al., 2014). Notwithstanding, biological pre-treatment are more commonly used for feedstock such as waste arising from pulp and paper industries as well as WwTW (Ariunbaatar et al., 2014).

Chemical pre-treatment employs the use of alkali, strong acids or oxidant for the lysis of organic compounds (Ariunbaatar et al., 2014). Because alkalinity is typically an important parameter for steady AD process, the use of alkali is the most preferred chemical used for such pre-treatments. Basically, by such treatments, hydrolysis is often enhanced. However, while it produces positive effects with recalcitrant feedstock, it is not suitable for highly biodegradable wastes that contain high amounts of carbohydrate, such as food waste (Ariunbaatar et al., 2014). This is because, it impacts on excessive VFAs production and accumulation, thereby, limiting methanogenesis (Ariunbaatar et al., 2014).

Thermal pre-treatment methods include microwave digestion, thermal hydrolysis, steam explosion and autoclaving (Carrere et al., 2016). It implies the application of heat to biomass for the disintegration of cell membranes and organic solubilisation (Ariunbaatar et al., 2014). Thermal pre-treatment is one of the most researched pre-treatment methods for AD feedstock (including food waste), with diverse technological designs. Thermal pre-treatment facilities are available in full scale; one of the most common being the thermal hydrolysis – Cambi, with over 30 facilities in operation (Carrere et al., 2016). The underpinning principle for thermal pre-treatment is withholding biomass in an air-tight container at high temperature and in some cases pressure from

a certain length of time. The temperature is more of an influencing parameter than the holding time, as it impacts on the release of inhibitory phenolic compounds among others, if run at high temperatures (Ariunbaatar et al., 2014; Carrere et al., 2016). Thus, optimum conditions of 120 – 160 °C and 20 – 30 minutes holding time was suggested (Carrere et al., 2016). Therefore, energy balance of this process is of key importance. However, where the end use of the biogas is for CHP applications, then this energy is off-set by the waste heat from the CHP plant. Hence, where other end uses such as injection to the gas grid and vehicle fuel is desired, a high energy input will be incurred.

Physical pre-treatment methods includes sonication, lysis-centrifuge, liquid shear, collision, high pressure homogeniser, maceration and liquefaction (Ariunbaatar et al., 2014; Carrere et al., 2016). It relates to the mechanical size reduction (such as grinding) of substrates for the release of compounds within the cell, thus, increasing the surface area (Pham et al., 2015; C. Wu et al., 2017). This increase in surface area enhances the direct contact between the anaerobic consortia and the intrinsic nutrients of interest (C. Wu et al., 2017). In essence, the time taken for the initial breakdown of the cell wall in order for the bacteria to digest these nutrients is greatly reduced. By this, the AD process and biomethane yield are enhanced. Notwithstanding, excessive reduction of particle size enriches acidification of the system, by the rapid production of organic acids consequently, reducing the methane yield (Izumi et al., 2010). Physical pre-treatment methods employed for food waste pre-treatment have been limited to mechanical size reduction at mesophilic temperature and high pressure homogeniser at thermophilic temperature (Pham et al., 2015).

Relative to other substrates, such as plants, the cellulose content of food waste is much less, as such, physical and thermal pre-treatments are the most adopted for food waste pre-treatment (C. Wu et al., 2017). The primary advantage of the thermal treatment over the mechanical size reduction is the potential removal of pathogens from thermal pre-treatment (Ariunbaatar et al., 2014). However, considering food waste does not contain faecal matter, mechanical pre-treatment is often adopted for food waste pre-treatment (Ariunbaatar et al., 2014). Therefore, mechanical particle size pre-treatment of food waste will be optimised in this study to improve the process kinetics and ultimate methane yield from food waste AD.

2.1.2 Reactor design

Anaerobic digesters can be classified according to the number of chambers (single or multi-stage), the feeding method (batch or continuous) and the mixing regime (plug flow or fully mixed).

2.1.2.1 Number of chambers

AD can be setup using a single reactor (single stage) or multiple reactors (multi stage) to optimise different thermodynamic digestion stages. In a single stage digester all the biological and physico-chemical reactions occur in one sealed holding tank, thereby saving space and cost. However, because the methanogenesis stage of AD is quite slow and there is different optimal pH for each stage of AD process, different reactors can be used to optimise the entire process, in which case it becomes a multi-stage digester (Defra, 2011). Multi-stage digestion is designed to improve the AD stability, organic loading rate (OLR) and ultimate yield, especially for complex feedstock such as food waste. The overall AD process can be grouped into acids production (acidogenesis) and methane production (methanogenesis). The problems arising from rapid acidification of highly organic feedstock and resultant inhibition on methanogenesis, are tackled by multi-stage AD systems, such that, these two processes occur in separate chambers (Grimberg et al., 2015). Therefore, in two stage AD systems, the first stage is often operated to allow acid fermentation at low pH around 5.5 to 6.5, and short hydraulic retention time (HRT, 2 – 3 days), while the second stage promotes steady methanogenesis, operated at relatively higher pH around 6 – 8 and longer HRT of 20 – 30 days (Xu et al., 2018). However, while multi-stage AD have been reported to produce more biogas, it increases i) the capital cost for building additional reactors, ii) the complexity of process controls to maintain the digesters in series at optimum operating conditions and iii) the footprint of the installation (Juniper, 2007).

2.1.2.2 Feeding method

According to the digester feeding, AD can either be a batch or continuous process. Batch processes involve a onetime loading of the feedstock over a fixed digestion period. They are designed for simplicity; however, the biogas production peaks at some point and decreases as the feedstock is consumed (Juniper, 2007). The continuous system on the other hand has a continuous biogas yield because the feedstock is fed continuously, which makes for

continual availability of food for the bacteria (Juniper, 2007). Anaerobic digesters are often designed using volatile solids loading rate calculated on the basis of monthly peaks (Appels et al., 2008). Hence, low solids loading in a continuous system could reduce the digester efficiency (Appels et al., 2008). Notwithstanding, the continuous biogas production enables downstream gas cleaning and energy recovery optimisation (Juniper, 2007).

2.1.2.3 Mixing regime

The mixing regime of the anaerobic digester design is key for optimum AD performance. Mixing provides a number of advantages including: i) enhanced contact between the acting microorganisms and the feedstock, ii) ensures uniform distribution of substrate, temperature, and other biological, chemical and physical parameters across the digester, and iii) avoiding scum formation and sludge settlement at the tank bottom (Appels et al., 2008). The mixing regime is most times related to the nature of feedstock. The mixing regime in dry AD is usually plug flow, since the reactors do not have internal mixers. Consequently, to achieve feedstock homogeneity, external mixing is used – i.e. mixing the feedstock before loading into the digester. Meanwhile, wet AD employs full mixing with internal mechanical stirrers or biogas injection (Appels et al., 2008; Juniper, 2007).

In this study, methane yield from food waste was optimised using wet single-stage mesophilic reactor design, by batch laboratory scale experiments with manual mixing.

2.1.3 Anaerobic digestion operating conditions

2.1.3.1 Temperature

Temperature is a significant factor that influences the kinds of microorganisms present within the AD system. Therefore, anaerobic digesters can be operated within three temperature ranges: the psychrophilic, which favours bacteria that can survive temperatures below 20 °C (Massé et al., 2003; Massé et al., 2010), the mesophilic, which favours bacteria that can live optimally between 35 – 40 °C and the thermophilic, which favour bacteria that can live optimally between 55 – 65 °C (Bright et al., 2011).

The conventional temperature adopted is the mesophilic, although at higher temperature, gas yield is enhanced with shorter retention time, consequently, yielding higher throughputs, lower digester foot print and a sterilised digestate

(Defra, 2011; Juniper, 2007). It has also been reported that thermophilic AD can help to meet the UK Animal By-Products (UK-ABPR) requirements for biogas producing processes; which requires waste to be held at 57 °C for 5 hours or at 70 °C for 1 hour (Juniper, 2007). Higher temperatures induce faster degradation rates and higher amount of soluble substances, hence, for materials with high initial nitrogen contents, there would tend to be higher build-up of ammonia within the system.

Increases in the level of free ammonia also influences pH increase, however, a simultaneous production of VFA buffers the pH. Whether or not mesophilic microorganisms are more susceptible to ammonia inhibitions remains rather controversial. Some researchers claim that at thermophilic temperature ammonia inhibition is lower (Gallert and Winter, 1997; Wang et al., 2016). It was also argued that thermophilic AD systems will be buffered by the VFA/ammonia pH control, however, the frequent change in pH affects methane yield (Appels et al., 2008). In contrast, Massé et al. (2003) and (2010) suggests that at lower temperatures (psychrophilic and mesophilic), the methanogens tend to acclimatise better to high ammonia concentrations, due to the tendencies for pH increases. Optimal temperature conditions of thermophilic hydrolysis/acidogenesis and mesophilic methanogenesis was suggested by Mao et al. (2015).

In addition, other factors such as higher net energy input, larger investments, decreased stability, vulnerability to environmental conditions, low-quality effluent, and poor methanogenesis have limited the wide scale adoption of thermophilic AD; except of course when feedstock with low nitrogen contents are being treated (Appels et al., 2008; Boe, 2006; Serna-Maza et al., 2015).

2.1.3.2 Carbon-to-Nitrogen (C/N) ratio

Carbon and nitrogen are the major elements within the classes of macromolecules required for cell growth. Various organic carbon compounds can be assimilated by many bacteria to produce new cell materials (Madigan et al., 1997). A typical bacteria cell comprises 50% carbon on dry basis, making it the major element of all classes of macro-elements. Nitrogen makes up about 12% of the cell, making it the next most abundant element after carbon and it is a major constituent of nucleic acids, proteins and several other constituents (Madigan et al., 1997). The C/N ratio is therefore, the relationship between the quantity of food available for microorganisms to feed on in terms of carbon content and the amount of nutrient required to build up the microorganisms in terms of nitrogen content. Some studies show that AD can

proceed effectively at a C/N ratio range of 15 – 20 (Zhang et al., 2013; Wu et al., 2010). However, an optimal C/N ratio in the range of 25 to 30 was suggested to have a balanced system (X. Wang et al., 2014); because anaerobic bacteria are able to consume 25 – 35 times as much carbon as they do nitrogen (Kondusamy and Kalamdhad, 2014). If the nitrogen in the feedstock is too high, high ammonia production during digestion could be experienced, leading to methanogenesis inhibition. Meanwhile, too low nitrogen content in the feedstock leads to low ammonia required for microorganism reproduction. Thus, leading to a reduced microbial community and thereby, limiting material digestion and biomethane yield (Doelle K, 2015; Kondusamy and Kalamdhad, 2014).

Feedstock with initial high protein contents; such as food waste, or high urea loads tend to have lower C/N ratio, while, materials with very high carbon contents such as straw and grass have high C/N ratio leading to the formation of more carbonaceous products, such as bicarbonates and thereby, increasing the alkalinity of the system. In order to obtain a balanced C/N ratio, materials with high carbon contents are usually co-digested with materials with high nitrogen contents (Mao et al., 2015).

2.1.3.3 Alkalinity and pH

Alkalinity relates to the capacity of a liquid media to resist changes in pH, which would make it more acidic. The initial production of VFA influences a reduction in the digester pH. However, the subsequent release of ammonia, CO_2 and bicarbonate, with further methanogenesis helps to counter the pH reduction (Appels et al., 2008). The fermentation of high protein- and lipid-containing substrates present in food waste result in the release of volatile fatty acids (VFAs), ammonia, CO_2 and H_2 (Gujer and Zehnder, 1983). The release of VFAs leads to an initial reduction in pH and alkalinity; however, ammonia and CO_2 helps to retain a high amount of bicarbonate in the liquid as ammonium bicarbonate (Banks et al., 2008), thereby, regaining the lost alkalinity (such as in Equation 2.1) and buffering the pH.

$$NH_3 + CO_2 + H_2O \leftrightarrow NH_4HCO_3 \hspace{3cm} 2.1$$
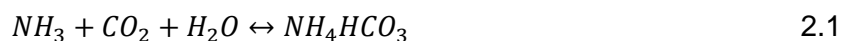
Therefore, during AD, pH is influenced by bicarbonate, ammonia and VFA transformations. Furthermore, the enzymatic metabolisms of the consortia of microorganism present during AD are governed by pH. The primary fermenters only require a pH not less than 5, while the secondary fermenters do well in a pH range of 6.8 to 7.6 and the methanogenic bacteria grow

efficiently in a pH range of 6.5 to 8.2. Hence, an optimal range of 6.8 to 7.4 is desired to allow coexistence of all acting groups (Kumaran et al., 2016; Mao et al., 2015). The pH at the start of AD also influences the composition of VFA produced, for instance, acetic and butyric acids were the main VFA products at low pH, while acetic and propionic acids were mainly formed at higher pH of 8.0 (Appels et al., 2008; Mao et al., 2015).

The pH is usually buffered by the gas phase CO_2 concentration and the liquid phase HCO_3-alkalinity (Fonoll et al., 2015). A 1.4 molar ratio of bicarbonate/VFA for a stable AD process was suggested, with particular emphasis on the ratio rather than the their relative levels (Appels et al., 2008). This ratio of bicarbonate/VFA is however, vague to apply because, the composition of VFA reported by Appels et al. (2008) was not specified. Different studies have presented different compositions of VFA as the total VFA, making it research specific. However, the effect of pH is particularly seen in the shift in the form of some substrates that induces inhibition, such as ammonia, VFA and sulphides (Rajagopal et al., 2013). For instance, ammonia is available in ionized form at lower pH (around pH 7), while at higher pH (>7.5), ammonia shifts to free ammonia. At high pH, free ammonia is dominant and VFA consumption is inhibited, leading to an accumulation within the system. This accumulation causes a reduction in the pH, leading to a reduction in the availability of free ammonia. With these interactions between pH, free ammonia and VFA, an inhibited steady state could occur; whereby, digestion is running stably, but at lower methane production (Chen et al., 2008; Rajagopal et al., 2013). When liquid piggery manure was digested at pH 8, Chen et al. (2008) recorded an accumulation of VFA to 316 mg/L, but, when the pH was lowered to 7.4, the VFA were reportedly reutilised and lowered to 20 mg/L, which was attributed to the ammonia-induced inhibition relief at a low pH.

2.1.3.4 Organic loading rate (OLR)

The OLR relates to the amount of organic matter (in terms of VS or COD) fed into the anaerobic digester over a period of time (hydraulic retention time – HRT), derived by dividing the daily VS loading by the reactor volume (Oliveira and Doelle, 2015). The OLR influences the rate of acidification and biogas production rate from a feedstock, hence, it has to be standardised for optimum biogas production, as well as preventing digester failure (Oliveira and Doelle, 2015). As such, for feedstock with high organic content, such as food waste, the OLR are usually lower than other conventional feedstock because of high

acidification. At HRT of 80 days, OLR for stable digestion of food waste of 2.25 kgVS/m^3/d was reported (Banks et al., 2011), and when trace elements were supplemented, 5 – 6.64 kgVS/m^3/d OLR were reported (Banks et al., 2012; Zhang and Jahng, 2012).

2.1.3.5 Volatile fatty acids toxicity

VFA intermediates in AD mainly comprise acetic acid, butyric acid, propionic acid and valeric acid. The VFA produced are ultimately transformed to methane and carbon dioxide by a number of syntrophic relationships. Among the four dominant acids, acetic and propionic concentrations have been used as indicators of process performance, because, they play dominant roles towards biogas production. A propionic – to – acetic ratio above 1.4 or an acetic acid concentration of 800 mg/L could lead to digester failure at any point during the digestion stages (Marchaim and Krause, 1993; Mawson et al., 1991; Zhang et al., 2014). Despite VFA being about the most important intermediates during AD, they can be toxic to anaerobic microorganisms. Siegert and Banks (2005) reported that VFA concentrations at 2 g/L inhibited hydrolysis during cellulose digestion and 4 g/L during glucose digestion yielded similar effect, but biogas production was affected at VFA concentrations above 6 g/L and 8 g/L for cellulose and glucose respectively.

2.1.3.6 Ammonium and ammonia toxicity

During AD, ammonia is formed by the breakdown of nitrogenous matter such as urea, protein and nucleic acids (González-Fernández and García-Encina, 2009). At low concentrations, ammonia is required for microbial growth, however, at higher concentrations, it causes a decrease in microbial activities (Rajagopal et al., 2013; Zhang et al., 2014). The methanogens are more sensitive to high ammonia concentrations; especially the acetoclastic methanogens, compared to fermentative bacteria (Chen et al., 2008; Zhang et al., 2014). AD tries to replicate the stomach of ruminant animals, however, in their case the rumen wall absorbs any excess ammonia, (Gallert et al., 1998; Rajagopal et al., 2013), this process have to be simulated in the AD system to eliminate ammonia inhibitions especially on the methanogens.

Inorganic ammonia in AD can exist either as ammonium (NH_4^+) or free ammonia (NH_3); of the two free ammonia is more toxic to microorganisms, because, while ammonium ion might only directly inhibit the methane producing enzymes, free ammonia can penetrate through the cell membrane

to cause imbalance in proton (Appels et al., 2008; Chen et al., 2008; Rajagopal et al., 2013). The accepted level of ammonia for AD is still a controversy, being interconnected with feedstock nature and other process conditions such as temperature and pH, as well as the acclimation period (Appels et al., 2008; Chen et al., 2008; Rajagopal et al., 2013; Yenigün and Demirel, 2013). Nonetheless, Gallert et al. (1998) claimed that ammonia inhibition is mainly due to temperature and pH-dependent ammonia concentration.

Furthermore, microbes will tolerate higher levels of ammonia when they become acclimated compared to an un-acclimated sludge (Chen et al., 2008; González-Fernández and García-Encina, 2009; Rajagopal et al., 2013). In this sense, the microbes develop a kind of adaptation mechanism to high levels of ammonia within the system. This adaptation mechanism is not yet clearly understood, but could possibly be due to a shift in the microbial population or perhaps some internal alterations in the metabolic structure of predominant methanogens; with the entrance of free ammonia. Certainly, the microbes need time to get adapted to high ammonia concentrations, for which Rajagopal et al. (2013) suggested about two months was required. In this light AD can proceed normally for small scale digesters, with gradual ammonia increase. Some researchers have actually proposed acclimation as a means of counteracting ammonia inhibition during AD by sludge recirculation (Gallert et al., 1998; González-Fernández and García-Encina, 2009).

Generally, in order to reduce the ammonia toxicity during AD, different approaches have been adopted, which are; physical stripping with nitrogen, air, biogas or steam (Jiang et al., 2013; Serna-Maza et al., 2014); chemical removal with the addition of ion absorbers or exchangers such as zeolite, activated carbon or clay (Gallert et al., 1998; Chen et al., 2008), and struvite precipitation (Rajagopal et al., 2013). Manure dilution have also been used as an approach to reduce ammonia inhibition, however, the resultant high volume of digestate produced, which results in increased cost of dewatering discourages its application (Chen et al., 2008).

2.1.3.7 Metals as micronutrients

Metals are important for microbial cell growth, and they play particular roles in enhancing biomethane production. Metals required for microbial cell growth are grouped into macro- and micro elements (see Table 2.1) depending on the level required by microorganisms. Both micro and macro elements are equally crucial to the microbial cell functions, they also help to stabilise AD

systems; by influencing the rates of different anaerobic reactions, in excess, however, metals can become toxic to the microorganisms (Xu et al., 2018).

Trace elements such as iron, cobalt, zinc and nickel have to be sufficiently available to initiate AD (Kondusamy and Kalamdhad, 2014) and other reactions involving different routes of VFA degradation requires different types and levels of trace elements.

Table 2.1. Metals requirement for microbial cellular activities (adapted from (Madigan et al., 1997)

Elements	Function in microbial growth
Macro elements	
Phosphorus (P)	Synthesis of nucleic acids and phospholipids
Sulphur (S)	Structuring of amino acids cysteine and methione. Also present in some important vitamins including: biotin, thiamine, lipoic acid and also coenzyme A; which is responsible for acetate degradation
Potassium (K)	Required by a variety of enzymes, including those that synthesize proteins
Magnesium (Mg)	Required for many enzymatic activities and helps to stabilize ribosomes, nucleic acids and cell membranes
Calcium (Ca)	Not essential for many microorganisms' growth, but helps to stabilize bacterial cell wall and heat of endospores
Sodium (Na)	Required by some organisms as a reflection of their habitat
Micronutrient	
Cobalt (Co)	For vitamin B_{12} and transcarboxylase (propionic acid bacteria)
Copper (Cu)	Proteins, particularly those associated with respiration
Manganese (Mn)	Required for activating enzymes
Molybdenum (Mo)	Available in many flavin-containing enzymes, molybdenum nitrogenase, nitrate reductase, sulfide oxidase and some formate dehydrogenases and oxotransferases.
Nickel (Ni)	Most hydrogenases, coenzyme F_{430}, urease and carbon monoxide dehydrogenase
Selenium (Se)	Some hydrogenases, formate dehydrogenase, and amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases and oxotransferases of hyperthermophile
Zinc (Zn)	Present in alcohol dehydrogenase, enzymes carbonic anhydrase, RNA and DNA polymerase, and many DNA binding proteins
Iron (Fe)[a]	Important for cellular respiration, catalases, iron-sulphur proteins (eg. Ferredoxin), oxygenase, peroxidases and all nitrogenases

[a]Needed in greater amounts than the others, sometime not regarded as trace element.

The amount of trace elements needed during digestion depends on the initial feedstock characteristics, the design of the reactor, nutrients availability and other controlling factors, therefore, it is often difficult to determine the actual elements and levels required (Kondusamy and Kalamdhad, 2014). Different studies on trace element supplementation for improved food waste digestion were conducted with different optimal levels (Banks et al., 2012; Facchin et al., 2013; Wanli Zhang et al., 2015; Wanqin Zhang et al., 2015; Zhang and Jahng, 2012; Zhang et al., 2012). Therefore, rather than adding trace elements into food waste digestion, this study aims to improve other controlling factors that would ultimately improve the digestion and methane yield, but do not greatly depend on the trace element levels.

2.1.3.8 Sulphide toxicity

In the absence of oxygen, organic and inorganic sulphur can either be fermented or reduced to dissolved sulphides, which can be translated to the biogas as hydrogen sulphide (Peu et al., 2012). This is principally progressed by sulphate reducing bacteria (SRB), impacting two levels of inhibition on the AD process; primary and secondary inhibition (Chen et al., 2008). Primary inhibition results from competition for organic and inorganic substrates, including hydrogen, acetate, butyrate and propionate, consequently, reducing methane yield, while secondary inhibition results from the backward inhibition of sulphides produced on various groups of bacteria; including SRB themselves.

The degree of primary inhibition posed by the SRB is different for the different groups of microorganisms present. The SRB cannot actively compete with hydrolytic and acidogenic bacteria during primary fermentation, because, they are unable to degrade natural biopolymers such as proteins, starch and lipids (Chen et al., 2008). Thus, they depend on the activity of other microorganisms to provide degraded products which they can utilise. Furthermore, the acidogens involved in the degradation of monomers are relatively more fast growing than the SRB, which also limits the SRB activity during primary fermentation (O'Flaherty et al., 1999). During secondary fermentation however, the kinetics and thermodynamics of the system are in favour of SRB outcompeting the acetogens for common substrates. Notwithstanding, this also depends on the COD/SO_4^{2-} ratio, relative population of SRB and other anaerobes and the sensitivity of SRB and other anaerobes to sulphide toxicity (Chen et al., 2008). Published data on the competition for acetate between the SRB and acetoclastic methanogens are quite contradictory. Some authors

suggest SRB outcompetes acetoclastic methanogens (Gupta et al., 1994; Rinzema and Lettinga, 1988; Stucki et al., 1993) and some authors suggest the opposite (Colleran and Pender, 2002; Isa et al., 1986; O'Flaherty et al., 1998). This competition is reported to be influenced by the COD/SO_4^{2-} ratio, such that, at a ratio of 2.7, acetoclastic methanogens dominated and at a ratio of 1.7, the SRB dominated. Between these values howvere, an active competition was reported (Chen et al., 2008). Furthermore, O'Flaherty et al. (1998) suggested that the different growth rates of the two microbial groups at different pH values influenced this outcome of the competition. Notwithstanding, dominance of acetoclastic methanogens over the SRB, was reportedly influenced by process conditions such as initial population, superior attachment abilities of the acetoclastic methanogens to films in membrane reactors, COD/SO_4^{2-} ratio and a lower affinity of SRB to acetate compared to other substrates (e.g. hydrogen). And the dominance of SRB over the acetoclastic methanogens was due to thermodynamic and kinetic advantages.

Secondary inhibition relates to the toxicity of sulphides produced from the SRB activity (primary inhibition). Within the AD system, sulphide is distributed between H_2S in the gas, H_2S, HS^- and S^{2-} in solution and insoluble metal sulphides (Isa et al., 1986). The form in which sulphide impacts toxicity is not very well established, although, dissolved H_2S (free H_2S) is mostly described as the inhibitory form, because it can permeate into cell membranes to disrupt proteins within the cytoplasm and also interfere with sulphur assimilation (Chen et al., 2008). Free H_2S concentration strongly inhibited specific acetoclastic methanogenic activity at a pH range of 6.4 to 7.2 (Chen et al., 2008). The toxicity of free H_2S controlled by the pH of the system. The toxicant concentration that causes 50% reduction in cumulative methane yield over a period of time (IC_{50}) was 250 mgH_2S/L at pH 6.4 – pH 7.2 and 90 mgH_2S/L at pH 7.8 – pH 8.0 (Chen et al., 2008). In agreement, free H_2S was said to induce inhibition at a pH range of 6.8 to 7.2, and above pH 7.2, inhibition was reportedly from the total sulphide concentration (O'Flaherty et al., 1998).

2.1.3.9 Hydrogen

Hydrogen has been postulated by many researchers to be used as a monitoring parameter for stable AD, because, it is present in several reactions during AD; acting as a vital intermediate during the entire process (Giovannini et al., 2016). Low concentrations of hydrogen is required as a thermodynamic prerequisite for breakdown of volatile fatty acids and alcohols to acetate

(Conrad, 1999; Siriwongrungson et al., 2007). For instance, hydrogen partial pressure of 5.82×10^{-5} atm was required under standard conditions of 1M acetate and butyrate, to effectively convert butyrate to acetate (Siriwongrungson et al., 2007), while hydrogen partial pressure of 10^{-5} atm was required for propionate degradation to acetate (FAO, 2015). However, the tendency for high hydrogen loads during AD is dependent on the organic material being digested. Clearly, hydrogen is about the most important regulating factor during AD, but perhaps the least monitored (see section 2.2.1).

2.1.3.10   Physicochemical interactions.

These reactions are not necessarily carried out by microorganisms, but are common occurrences in anaerobic digesters. They include liquid-liquid reactions (ion association/dissociation), gas-liquid exchanges (gas transfer) and the liquid-solid transformations (precipitations and solubilisation of ions). Liquid-liquid reactions are usually rapid, gas-liquid exchanges are rapid-medium, while the liquid-solid transformations are medium-slow, respectively (Batstone et al., 2002).

2.2 Thermodynamics of anaerobic digestion

The AD process involves a number of biochemical reactions taking place in sequential and parallel paths by a complex consortium of microorganisms (Batstone et al., 2002; International Energy Agency, 2015; Kondusamy and Kalamdhad, 2014). These thermodynamic interactions influence the release of hydrogen and its availability for use towards methane production.

2.2.1 Biochemical reactions

Biochemical reactions are catalysed by intra- and extra-cellular enzymes acting on the available organic material (Batstone et al., 2002). The AD process is generally controlled by four biochemical reactions; hydrolysis, acidogenesis, acetogenesis and methanogenesis (Kumaran et al., 2016). Preliminary disintegration (mostly in complex organic wastes like sewage sludge, cellulose, proteins, etc.) and hydrolysis are both extracellular activities, while further degradation to soluble materials, which subsequently leads to biomass growth and end product (e.g., biogas production) are carried out by intracellular activities (Batstone et al., 2002).

2.2.1.1 Hydrolysis

This is an extracellular enzymatic reaction carried out by exo-enzymes that are excreted by fermentative bacteria, whereby, insoluble organic polymers such as proteins, lipids and polysaccharides, are broken down to soluble monomers such as amino acids, monosaccharides and LCFA that are soluble in water (Batstone et al., 2002; Kondusamy and Kalamdhad, 2014; Kumaran et al., 2016). The hydrolysis step is generally accepted as the rate limiting step, as it affects the kinetics of the entire process; especially when a recalcitrant feedstock is being treated (Pan et al., 2016). Equation 2.2 is a typical representation of hydrolysis (Kondusamy and Kalamdhad, 2014);

Organic material breakdown to glucose and hydrogen

$$nC_6H_{12}O_5 + nH_2O \quad \rightarrow \quad nC_6H_{12}O_6 + nH_2 \qquad\qquad 2.2$$
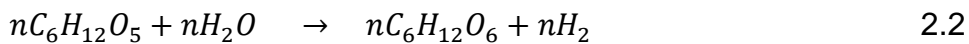
2.2.1.2 Acidogenesis

The soluble materials formed during hydrolysis are further converted to mixed organic acids; such as acetic-, butyric- and propionic-acid (Mosey, 1983), as well as by products such as alcohols, aldehydes, hydrogen, carbon dioxide, ammonia and hydrogen sulphide (Kondusamy and Kalamdhad, 2014; Zhang et al., 2014). The acidogenic bacteria are fast growing; with about 30 minutes minimum doubling time and produces intermediates according to Equations 2.3 – 2.7;

Glucose fermentation to ethanol

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \qquad\qquad 2.3$$

Glucose fermentation to acetic acid

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad\qquad 2.4$$

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad\qquad 2.5$$
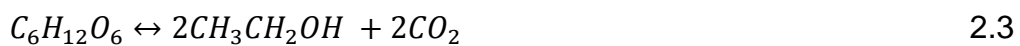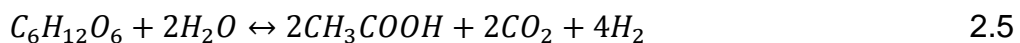
Glucose fermentation to propionic acid

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \qquad\qquad 2.6$$
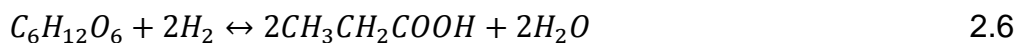
Glucose fermentation to butyric acid

$$C_6H_{12}O_6 \leftrightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \qquad\qquad 2.7$$
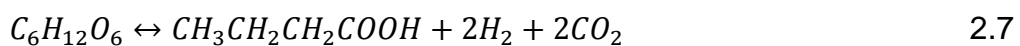
Acetic acid production is however, the most favoured route, which provides the highest energy yield for the bacteria growth, as well as providing prime substrate for acetoclastic methanogens towards methane production. The other reactions leading to the production of propionic and butyric acids are

practically bacterial responses to different hydrogen loads (Mosey, 1983). This would explain why VFA monitored during AD always shows higher levels of acetic acid than other VFA intermediates during the early stages of AD.

2.2.1.3 Acetogenesis

The organic acids from acidogenesis are further degraded into acetate, carbon dioxide and hydrogen by two groups of acetogens; the obligate hydrogen producing acetogens (OBHP), which breaks down propionic, butyric, valeric, and other LCFA to acetic acid releasing hydrogen in the process and the homoacetogens, which utilises H_2 and CO_2 to produce acetic acid (Fisgativa et al., 2016). Examples of acetogenesis include the following (FAO, 2015; Horan et al., 2005);

Conversion of butyrate to acetate

$$CH_3(CH_2)_2COO^- + 2H_2O \leftrightarrow 2CH_3COO^- + 2H_2 + H^+ \qquad \Delta G^0 = +48.3\ kj/mol \qquad 2.8$$
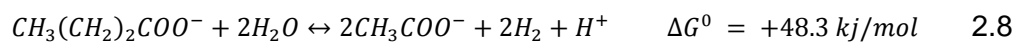
Conversion of propionate to acetate

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad \Delta G^0 = +76.1\ kj/mol \qquad 2.9$$
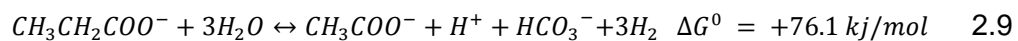
Studies show that the acetogenic bacteria grow quite slowly; having minimum doubling times of around 1.5 to 4 days. From the energy requirements; as seen in Equations 2.8 and 2.9, with Gibbs free energy > 0, the forward reactions would not be spontaneous and could very easily be stalled at high concentrations of dissolved hydrogen and acetic acid (Fukuzaki et al., 1990). Therefore, the rate of conversion of other VFA to acetic acid depends on the syntrophic relationship between the VFA conversion to acetic acid and the consumption of the resulting hydrogen produced. The butyrate-utilising bacteria have a much higher growth rate than the propionic utilising bacteria and are supported within a broader pH range of 6.8 to 7.6, while the propionate-utilising bacteria requires an optimal pH of 7.0 to 7.5 (O'Flaherty et al., 1998). Therefore, of the three predominant VFA produced during AD; acetic-, butyric- and propionic acids, propionic acid is the least degradable; it stays longer in the system during the digestion period, until other precursors have been almost completely depleted (Wang et al., 2006).

2.2.1.4 Methanogenesis

The final production of methane is carried out by a group of microorganisms called methanogens. During AD, primary fermenters (during acidogenesis) consumes the bulk of the total energy within the system; hydrogen and

formate serving as pools for the excess electrons released, thereby, severely limiting the energy available for secondary fermentation (Schink et al., 2017). In order for secondary fermenters to metabolise on the products from primary fermentation; such as alcohols and long-chain fatty acids, they establish a syntrophic relationship with the methanogens. Methanogens consume the hydrogen and/or formate, thereby, releasing energy and making it energetically possible for secondary fermentation to proceed (Schink et al., 2017). Methanogens are constrained to the utilisation of only a few substrates, mainly acetic acid and H_2/CO_2 (or formate) (Conrad, 1999) and this step has been regarded as the rate limiting step when the feedstock is easily biodegradable (Pan et al., 2016).

Two groups of methanogens are generally responsible for methane generation; they are the hydrogen-utilising (hydrogenotrophic) methanogens that utilise hydrogen and CO_2 to produce methane (Equations 2.10 – 2.12) and the second group is the acetate-utilising (acetoclastic) methanogens, which utilise acetate to produce methane (Equation 2.13) (FAO, 2015; Luo et al., 2012). Acetate can also be converted to methane through hydrogenotrophic methanagenesis, by syntrophic acetate oxidation bacteria to H_2 and CO_2, coupled with hydrogenotrophic methanogenesis (Bassani et al., 2015; Gao et al., 2015; Karakashev et al., 2006; Karlsson et al., 2012; Westerholm et al., 2011). Syntrophic acetate oxidation is said to be favoured when the AD is stressed, such as high ammonia and VFA levels; as in the case of food waste digestion (Banks et al., 2012). A low concentration of hydrogen (≤40 Pa) is required for syntrophic acetate oxidation (Demirel and Scherer, 2008), however, this is maintained by the hydrogenotrophic methanogens (Pap et al., 2015).

Hydrogenotrophic methanogenesis

a. Carbon dioxide reduction

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^0 = -130 \, kj/mol \qquad 2.10$$

b. Bicarbonate reduction

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad \Delta G^0 = -135.6 \, kj/mol \qquad 2.11$$

c. Syntrophic acetate oxidation – hydrogenotrophic methanogenesis

$$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2 \qquad 2.12$$
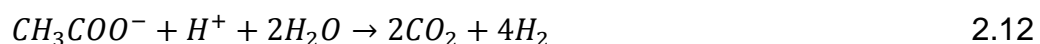
followed by Equation 2.10 and the combined reactions has $\Delta G^0 =$ $-22\ kj/mol$

Acetoclastic methanogenesis

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad \Delta G^0\ =\ -32.3\ kj/mol \qquad 2.13$$

Hydrogenotophic methanogens grow very fast, with about 6 hours doubling time, and were described by Mosey (1983) as the autopilot of the AD process, because they control the redox potential of the system and a lot more. As observed from the biochemical reactions, hydrogen is produced at virtually all stages of the AD process, especially acidogenesis/acetogenesis. This implies, the hydrogenotrophic methanogens can effectively metabolise at any stage of the AD process, provided all supporting conditions are right. After the hydrogenotrophic methanogens have used up most of the hydrogen, whatever hydrogen remains controls the mixture of acids formed by the acidogenic bacteria, as well as the overall acid production rate. Furthermore, the rate at which propionic and butyric acids are converted back to acetic acid, is controlled by the remaining dissolved hydrogen (Mosey, 1983).

The acetoclastic methanogens on the other hand, grow relatively slower, with about 2 – 3 days doubling time. They help to buffer the pH of the system by acetic acid removal and subsequent CO_2 production. Despite being a thermodynamically slower process, acetoclastic methanogenesis accounts for 70%, while hydrogenotrophic methanogenesis only contributes 30% to the overall methane yield (International Energy Agency, 2015; Kumaran et al., 2016). This follows the lower amount of hydrogen produced from the respective biochemical reactions, relative to the amount required for methane production.

For instance, for 1 mole of CH_4, 4 mole of H_2 is required via the hydrogenotrophic route, while for the same amount of CH_4, 1 mole of acetic acid is required via the acetoclastic route. Take glucose decomposition for an example, a complete fermentation would produce 2 mole of acetic acid and CO_2 each and 4 moles of H_2. Thus, 2 mole of CH_4 is obtainable from further decomposition of the produced acetic acid via acetoclastic methanogenesis, while only 1 mole of CH_4 can be obtained by hydrogenotrophic methanogenesis. Therefore, the hydrogenotrophic methanogens are primarily limited by the availability of hydrogen during the AD process. Consequently, from glucose fermentation, 3 mole of CO_2 is potentially left unconverted to CH_4. Although, part of this CO_2 is used for biomass growth and alkalinity within

the system, a good portion of it is translated into the biogas, thus, reducing the biogas calorific value.

2.2.2 Anaerobic digestion biogas and upgrading technologies

Biogas is typically composed of 60 – 70 % methane and 30 – 40 % carbon dioxide with little amounts of trace gases such as hydrogen sulphide and carbon monoxide; clean-up is therefore essential for removal of impurities (Kumaran et al., 2016). Biogas can be cleaned up to obtain bio-methane and used in engines for combined heat and power (CHP), injected into the gas grid and used just like natural gas or as vehicle fuel (Defra, 2011).

In 2012, the amount of biogas generated globally was 56 billion m^3 with Germany leading in biogas plants installations of up to 10,000 operational biogas plants (Kumaran et al., 2016). The UK generates about 7.4 TWh of biogas annually, from which, 2.1 – 2.3 TWh is used for electricity generation and 1.3 – 1.7 TWh of biomethane injected into the methane grid; projected to increase to a range of 1.9 to 2.6 TWh in 2016 (ADBA, 2015). Regardless of the end use, some amount of upgrade is required, particularly to remove moisture and other impurities such as H_2S and siloxanes for the following reasons: i) to increase the calorific value; ii) to align biomethane physical properties with natural gas; iii) to protect machines and iv) to reduce the carbon arising from gas utilisation (Bright et al., 2011).

The conventional methods for biogas clean-up and upgrade are physicochemical technologies including: absorption (water – 38% and chemical absorption – 23%), physical adsorption (pressure swing adsorption – 9%), organic separation (25%), and a growing number of membrane and cryogenic separation with 5% and 0.4% installations respectively (Bright et al., 2011; Corbellini et al., 2018). Except for the chemical (particularly amine) absorption, which can both remove H_2S and CO_2, other processes have to be combined in series of two or more technologies to upgrade biogas (Ullah Khan et al., 2017). Therefore, physicochemical processes are relatively expensive and generate wastes substances, in addition, some amount of CH_4 is lost in the process (Ryckebosch et al., 2011). Besides, physicochemical methods inducing up to 8% methane loss, they have high chemical and water demand, and releases CO_2 to the atmosphere during the regeneration of the absorbent media (Linville et al., 2016).

Biological biogas upgrade technologies are currently being explored, which includes: dissolution of CO_2 to bicarbonate by activity of enzymes that

facilitate this reaction (Muñoz et al., 2015); in-situ desorption of CO_2 in an aerated reactor using recycled sludge liquor and taking advantage of a higher CO_2 solubility than CH_4 (Muñoz et al., 2015); photosynthetic CO_2 assimilation by microalgae (Posadas et al., 2016; Posadas et al., 2015; Serejo et al., 2015; Toledo-Cervantes et al., 2016); anaerobic CO_2 removal (in-situ or ex-situ) by the activity of target microorganisms (hydrogenotrophic methanogens) that consume CO_2 and H_2 to produce CH_4 (also known as biomethanation – Voelklein et al., 2019). Biological biogas upgrading technologies are reportedly able to effectively remove both CO_2 and some H_2S from the biogas (Muñoz et al., 2015; Ryckebosch et al., 2011). However, except for biomethanation whereby, the original mass of CH_4 is potentially doubled, biological technologies also account for some CH_4 losses. This study therefore, seeks to increase the methane yield and upgrade the biogas from mono-AD of food waste by in-situ biomethanation.

2.3 Biomethanation

Hydrogen is rarely detected in the headspace during AD because it is produced in relatively small quantities and consumed rapidly. The high levels of CO_2 (30 – 40%) still contained in the biogas however, indicates the potential for additional hydrogen use towards methane production. Biomethanation is the biological conversion of CO_2 and H_2 to CH_4 by the selective activity of hydrogenotrophic methanogens (Voelklein et al., 2019). The addition of hydrogen to serve as the electron donor in this reaction is known as chemoautotrophic biological CO_2 conversion (Muñoz et al., 2015), otherwise known as and henceforth referred to as biomethanation. Biomethanation can either be ex-situ; carried out in a separate chamber using only the selected microorganism or in-situ; taking advantage of the existing group of hydrogenotrophic methanogens already present within the AD system (Voelklein et al., 2019). While the former holds the advantage of saving volumetric space required for an extra digestion chamber (1/10th of the anaerobic digester). It is however, limited by the impact of high hydrogen partial pressure on the other microorganisms present, as well as possible competition for the added hydrogen. In order to overcome the limitations of both in-situ and ex-situ systems, a hybrid system which combines in-situ and ex-situ biomethanation was proposed by Corbellini et al. (2018). In this system, hydrogen is injected into the anaerobic digester to partially upgrade the biogas and the output gas is then transferred into a second reactor enriched with an hydrogentrophic methanogen culture. This system was

proposed in order to enrich methane production in the first reactor (in-situ), without disturbing the kinetic processes due to factors such as increase in pH, while the complete biogas upgrade is achieved in the second reactor (ex-situ). Therefore, this system becomes particularly suitable for substrate with low acidification potentials such as cattle slurry and sewage sludge, which are not able to provide pH buffering with in-situ biomethanation.

Most studies on biomethanation have been lab scale ex-situ experiments with defined gas mixtures, usually a gas mix of 1:4 ratio of CO_2:H_2 according to the stoichiometric requirement for hydrogenotrophic methanogenesis. Furthermore, biomethanation has been conducted using synthetic media as inoculum (Liu et al., 2016; Rachbauer et al., 2017; Yun et al., 2017) and different reactor designs including: fixed bed, (Alitalo et al., 2015), trickle bed (Burkhardt and Busch, 2013; Burkhardt et al., 2015; Ullrich et al., 2018) and hollow fibre membrane (Ju et al., 2008). In general, there is only a limited information on in-situ biomethanation using conventional AD feedstock reported in literature (Bassani et al., 2016; Bassani et al., 2015; Luo and Angelidaki, 2012; Luo et al., 2012; Luo and Angelidaki, 2013; Pan et al., 2016) and no information on biomethanation with food waste as feedstock.

Addition of hydrogen is synonymous with increase in pH, especially significant for feedstock with low acidity such as cattle slurry (Luo and Angelidaki, 2012). However, the hydrogenotrophic methanogens are capable of withstanding pH as high as 8.2, at which level other hydrogen-utilisers could be outcompeted. The increase in pH could help reduce toxic H_2S production, and even enhance its removal by biomass assimilation (Muñoz et al., 2015), but it could limit some other processes such as acetoclastic methanogenesis, by the shift towards toxic ammonia production. Nonetheless, hydrogenotrophic methanogens have been reported to dominate methane production from acetate at high ammonia loads via the syntrophic acetate oxidation route, hence, ammonia inhibition becomes less of a concern when hydrogenotrophic methanogenesis are enhanced.

Although, yet to be proven economically viable, a number of studies have revolved around inhibiting the methanogenesis step during AD, to obtain biohydrogen from fermentation stages of AD (Guo et al., 2010; Wang and Wan, 2009; Zhang et al., 2016; Zhou et al., 2013) a process called dark fermentation. One of the reasons for its relatively low acceptability is that conversion of organics to hydrogen have lower energy recovery efficiencies compared with the traditional methane production (See Table 2.2). It is therefore, clear that if hydrogen is the desired product from AD of biomass,

feedstock with high protein content such as food waste would have lower energy recovery efficiency in comparison with other substrates. Hence, an upgrade of the biogas towards higher methane yield will be better for this kind of substrates. Biohydrogen from dark fermentation could however, be a low cost and sustainable source of hydrogen for the biomethanation process, by adopting a two-stage AD system as with the biological feedstock pre-treatment method described in Section 2.1.1.1.

Table 2.2 Energy recovery efficiencies from different organic materials from AD processes. Adapted from Ranbin Liu (2016).

Product	Parameter	Glucose	Lipid	Protein
Whole	Chemical formula	$C_6H_{12}O_6$	$C_3H_5(OH_3)$	$CH_3CH(OH)CH(NH_2)COOH$
	Calorific value(kJ)	2870	1609	2042
H_2	Production (mole)	4	3	2.67
	Calorific value(kJ)	1064	798	710
	Efficiency of energy recovery (%)	40	50	35
CH_4	Production (mole)	3	1.75	2
	Calorific value(kJ)	2625	1531	1750
	Efficiency of energy recovery (%)	91	95	86

2.4 Hydrogen as an energy carrier during anaerobic digestion

During primary fermentation (acidogenesis), some compounds are oxidised to CO_2, while some others are reduced to different compounds (e.g., SO_4 to H_2S) and a redox balance is achieved by production of hydrogen (Haghighatafshar, 2012). During secondary fermentation (acetogenesis), VFA are oxidised to acetic acid, giving off hydrogen in the process and a second group of acetogens (homoacetogens) that consumes hydrogen and CO_2 to form acetic acid, also utilise the available hydrogen. However, this is not as thermodynamically favourable as hydrogenotrophic methanogenesis, due to a relatively higher Gibbs free energy (Equation 2.14) compared to hydrogenotrophic methanogens (previously given in Equation 2.9) (Liu et al., 2016).

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad \Delta G^0 = -94.9 \, kJ/mol \qquad 2.14$$
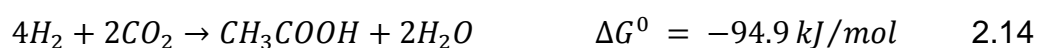
Notwithstanding, the products formed from the utilisation of hydrogen in the fermentation stages; whether by homoacetogenesis or hydrogenotrophic methanogenesis, ultimately results in the production of methane. The major competition for hydrogen that is more energetically favourable than hydrogenotrophic methanogenesis and does not effectively generate methane is the production of hydrogen sulphide by sulphate reducing bacteria (SRB). SRB can partially or fully degrade a wide range of organic compounds, including long chain and branched-chain fatty acids, organic acids, alcohols and hydrogen to produce H_2S, with an affinity reported in the following order; hydrogen > propionate > other organic electron donors (Chen et al., 2008). From Equation 2.15, we see that the Gibbs free energy for hydrogen sulphide formation is lower than methane formation from the hydrogenotrophic methanogenesis route; which is -130 kJ/mol.

$$4H_2 + SO_4{}^{2-} + H^+ \leftrightarrow HS^- + 4H_2O \qquad \Delta G^0 = -151.9 \, kJ/mol \qquad 2.15$$

The forward progression of Equation 2.14 is however, dependent on a number of factors, including: pH, substrate affinity, sulphate concentration, nature of feedstock and sulphide inhibition. The SRB are reported to operate optimally at a pH range of 6.8 to 7.2 (Luo et al., 2012), while a broader pH range of 6.5 to 8.4 is obtainable for hydrogenotrophic methanogens. However, according to O'Flaherty et al. (1998), the SRB and Methane producing bacteria (MPB) have similar growth rates between pH 7.0 and pH 7.5, within which other factors, such as substrate affinity, sulphate concentration, nature of the seed sludge and sulphide inhibition becomes the determinants of the competition outcome.

Individual bacterial communities cannot be rate limiting, instead, the availability of the nutrient within the system is what is usually limiting (Gujer and Zehnder, 1983). Hence, SRB growth within the AD would depend on the sulphur entering the system, as well as the electron donors; such as hydrogen and acetic acids. Furthermore, the microbial community will only autocatalyse to the solubilised substrates and not the complex substrate, hence, the SRB are often outcompeted during hydrolysis and acidogenesis (Chen et al., 2008). During initial feedstock degradation, whereby, CO_2 is the principal inorganic electron acceptor available, the only possible route for hydrogen consumption is by hydrogenotrophic methanogenesis or homoacetogenesis (Conrad, 1999).

The kinetics of competition for the available electron donors between SRB and MPB have received considerable attention. In comparison, the SRB apparently have a higher affinity than the MPB for hydrogen and acetate,

which are the primary methane precursors. This dominance enables SRB to maintain the pool of these substrates at concentrations too low for the MPB when sulphate is not limiting (Isa et al., 1986). Therefore, hydrogen availability indeed becomes a limiting factor for hydrogenotrophic methanogenesis after the primary fermentation stage, especially for high sulphur containing substrates. Notwithstanding, the SRB group (acetate or hydrogen utilising or otherwise) present within the system could also be limiting. In a study on the role of interspecies H_2 transfer to sulphate in anoxic paddy soil by Achtnich et al. (1995), they reported that when methanogenesis was inhibited, the addition of sulphate led to a decline in hydrogen concentration, while acetate was not affected, which implied the hydrogen-utilising SRB was dominant.

Elemental sulphur contained in food waste was reported within the range 0.15 to 0.44% of its dry mass, therefore, high amounts of H_2S are hardly recorded in AD of food waste (Defra, 2010b; Wang et al., 2014; Wanqin Zhang et al., 2015). Extreme cases such as macroalgal biomass, which contains much higher levels of sulphur accounting for up to 2.9 % of the dry mass (Peu et al., 2012), may produce higher levels of H_2S, but this is still dependent on the growth of the SRB and the AD conditions. Therefore, the SRB might not significantly compete with the hydrogenotrophic methanogens, when hydrogen is externally introduced into the food waste AD system. Moreover, although SRB has a high affinity for H_2, the addition of hydrogen will not necessarily influence more H_2S production, since the SRB can feed on other organic substances whether or not hydrogen is present. Also, to compliment the increase in hydrogen, additional sulphate would be required for the SRB to use as nutrient; this in turn is dependent on the elemental sulphur available.

2.4.1 Formate as an alternative energy carrier

During AD, methanoic acid widely known as formic acid (FA), is biologically formed from pyruvate cleavage; through pyruvate formate lyase, during primary fermentations by some strict anaerobes and Enterobacteriaceae (Pinske and Sawers, 2016; Schink et al., 2017). The same bacteria can further convert formate to H_2 and CO_2 by formate hydrogen lyase (Schink et al., 2017) and this equilibrium is controlled by environmental changes including pH and temperature (Reutemann et al., 2000; Schink et al., 2017). Formate breakdown to hydrogen and carbon dioxide is almost in equilibrium under standard conditions (1 M concentrations; gases at 1 atm) at pH 7.0;

$$HCOO^- + H^+ \rightarrow CO_2 + H_2 \qquad (\Delta G^{o\prime} = +4.6 \ kJ/mol) \qquad 2.16$$
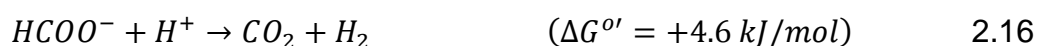
The above reaction is highly dependent on pH; being more favourable at lower pH. However, between pH 6.3 and pH 10.4; corresponding to the pH range between the two pK values of carbonic acid, bicarbonate rather than CO_2 is formed and is independent on the prevailing pH;

$$HCOO^- + H_2O \rightarrow HCO_3^- + H_2 \qquad (\Delta G^{o\prime} = +1.3 \ kJ/mol) \qquad 2.17$$

Above pH 10.4, the reaction tends towards the formation of carbonate ion as follows;

$$HCOO^- + OH^- \rightarrow CO_3^{2-} + H_2 \qquad (\Delta G^{o\prime} = +1.3 \ kJ/mol) \qquad 2.18$$

Therefore, only pH changes below pH 6.3 and above pH 10.4 becomes relevant to influence the route of formate reaction. Considering anaerobic digesters are typically operated within this pH range, it is expected that formate degradation is typically towards bicarbonate and H_2 production. Temperature on the other hand has only little impact on formate reaction. For instance, the free energy only changes by 5 kJ/mol, over a temperature range of 4°C to 80°C (Schink et al., 2017).

Formate can also be utilised by many hydrogenotrophic methanogens according the reaction in Equation 2.19, making it a probable route for formate degradation (Pan et al., 2016).

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad (\Delta G^{o\prime} = -130 \ kJ/mol) \qquad 2.19$$
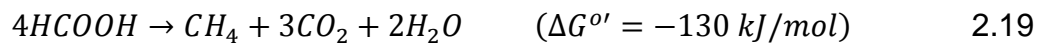
Chemically, FA is mainly produced by the hydrolysis of methyl formate and sometimes from the acidolysis of formate salts (Reutemann et al., 2000). While it is only partially miscible in hydrocarbons, it is completely miscible in water and many polar solvents (Reutemann et al., 2000). At room temperature, FA is relatively stable, but, its stability is dependent on its concentration; being more unstable as its concentration nears 100%, and temperature. When unstable it degrades by dehydration to carbon monoxide or dehydrogenation to carbon dioxide according to Equations 2.20 and 2.21 respectively.

$$HCOOH \rightarrow CO + H_2O \qquad (\Delta H_r = +10.5 \ kJ/mol) \qquad 2.20$$

$$HCOOH \rightarrow CO_2 + H_2 \qquad (\Delta H_r = -31.0 \ kJ/mol) \qquad 2.21$$
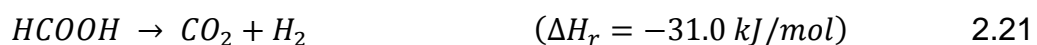
Dehydration of formate is predominant in the liquid phase and favoured by the presence of strong acids or oxide catalysts, while dehydrogenation is favoured by the presence of metal catalysts. Under hydrolysis however, formate can be degraded to bicarbonate and hydrogen (as in the biological process), with charcoal or palladium as active catalysts (Reutemann et al., 2000).

2.5 Potential for biomethanation during food waste AD

A number of researches have been conducted in order to stabilise and enhance the methane yield from AD of food waste, such as co-digestion with other waste types of low nitrogen content (Iacovidou et al., 2012), addition of trace elements (Banks et al., 2012; Facchin et al., 2013; Wanqin Zhang et al., 2015; Wanli Zhang et al., 2015) and also ammonia stripping (De la Rubia et al., 2010; Serna-Maza et al., 2014). However, these processes hardly influence significant changes in the biogas quality, meanwhile, adding hydrogen to the system could potentially increase the methane content of the biogas up to levels $\geq$ 95%, to make it suitable for other purposes such as injection into gas grids and transport fuels (Angelidaki et al., 2018).

The hydrogenotrophic methanogens have been classified as the auto-pilot of AD, because they help to regulate the formation of VFA; which are very important intermediates for the overall methane production (Mosey, 1983). However, hydrogen production and consumption during AD have so far been difficult to predict. This is due to the light molecular weight of hydrogen, and the rapid consumption of hydrogen in the liquid phase during material digestion. As such, there is hardly any model to the best of my knowledge that simulates the actual consumption pathway of hydrogen during digestion. Notwithstanding, the accumulation of propionic acid; such as is common with food waste digestion, could be used as a sign of possible hydrogen inhibition.

Considering microbial growth is a function of the substrate availability, increasing the supply of hydrogen could improve the metabolism and growth of the hydrogenotrophic methanogens, consequently, enhancing the syntrophic VFA (especially propionic acid) fermentation. Also, because it is the dissolved hydrogen in the liquid phase that causes inhibition, the low solubility of hydrogen could help to overcome VFA accumulation, such that the gas-liquid mass transfer of hydrogen gas as controlled by the hydrogenotrophs, determines the process stability. Therefore, enhancing the hydrogenotrophic methanogenesis route could improve the overall AD process and methane yield from AD of food waste.

The use of hydrogen to upgrade biogas (that is to increase methane yields and reduce CO_2 concentrations) is a novel approach in AD-related researches. Hydrogen gas can improve the methane yield and biogas calorific value from AD processes by enhancing the hydrogenotrophic route of methane production. The high level of CO_2 contained in the biogas from conventional AD processes, means additional cost for biogas upgrade using processes such as polyglycol absorption, chemical treatment, water scrubbing

and pressure swing adsorption; during which some amounts of CH_4 are also lost (Luo and Angelidaki, 2012; Luo et al., 2012; Wang et al., 2016). On the other hand, biogas produced from AD with hydrogen injection would have higher heating value, making it a suitable alternative to natural gas, while the unconverted hydrogen combined with the CH_4 can also significantly improve the combustion properties (Luo et al., 2012).

2.6 Energy balance for food waste anaerobic digestion with hydrogen addition

The typical energy balance for an AD system is determined based on the following components; biomass production or cultivation, biomass harvesting/collection, biomass transport, biomass pre-treatment or preparation, biogas production, biogas upgrade, by-product management, biogas transport and biogas use (Zhang, 2013). The overall system boundary for the energy balance of a typical AD system can be represented by Figure 2.1. Because the focus of this research is on food waste as the feed stock the crop production energy requirement reported in Figure 2.1 was not considered.
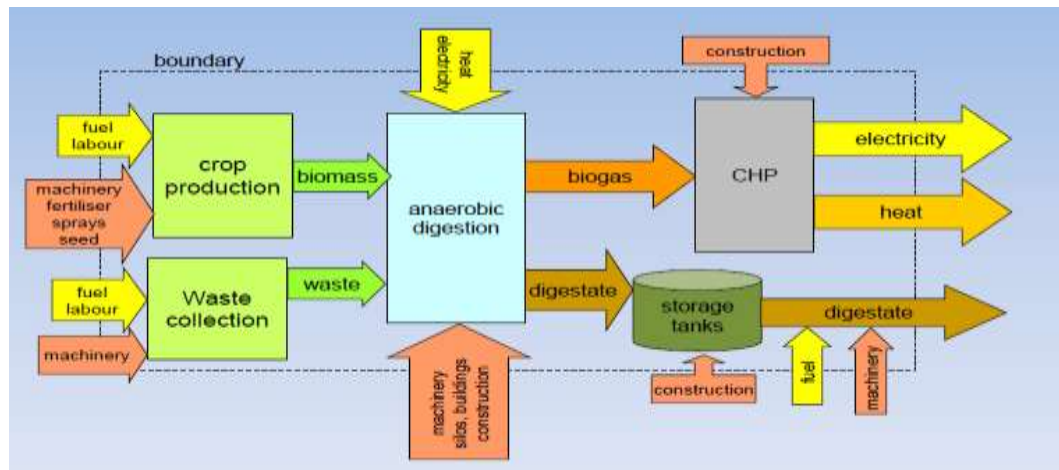
Source: Zhang (2013)

Figure 2.1. System boundary for a typical AD system

2.6.1 Waste collection

The energy inputs for waste collection includes vehicles and fuel used to collect food waste, and the energy required for pre-treatment such as sorting and size reduction. However, this is most times discounted as the waste is often collected by local authorities regardless of the end use.

2.6.2 Anaerobic Digestion

The energy inputs for the AD process itself includes the energy required to raise the digester temperature to the desired temperature and also the energy required to maintain the digester temperature, accounting for heat losses from the walls, floor and roof of the digester. It also includes energy required for process operations (where applicable), such as mixing, sludge pumping, biogas recirculation and so on.

2.6.3 Post Digestion Processes:

This includes the energy associated with the construction of CHP units for biogas conversion to electricity and heat, as well as storage tanks for digestate prior to digestate treatment and also accounting for the energy required for digestate treatment and farmland application, where possible.

Depending on the process optimisations, biogas from single stage AD of food waste contains methane in the range 55 – 73% (Banks et al., 2012; Oliveira and Doelle, 2015; Uçkun Kiran et al., 2014), and to be injected into the gas grid, it has to be upgraded to obtain over 95% biomethane (typically 97 – 98%) (Bright et al., 2011). With biomethanation, considering there is an existing infrastructure the only areas for additional energy input would be for hydrogen production, transport and storage (if necessary) and injection mechanism. Hydrogen production would impact about the most energy demand and has to be from a renewable source too, in order not to contradict the overall aim.

2.7 Hydrogen sources for biomethanation

Hydrogen is a clean fuel that does not emit GHGs when combusted, it is very light and has a relatively high calorific value of 120 MJ/kg compared to other gaseous fuels such as methane, ethanol and gasoline with calorific values of 50.0, 26.8 and 44.0 MJ/kg, respectively and can be derived from a vast range of feedstock (Kadier et al., 2016; Luo et al., 2012). Fossil fuels such as natural gas, coal and other light hydrocarbons, are yet the largest sources of hydrogen gas produced commercially (over 96%), through steam reforming and thermal conversions such as gasification (Kadier et al., 2016; Ramesh and Chowdhary, 2016).

Though hydrogen gas is a clean fuel and has great potential for direct applications, it is very light with a low volumetric energy value of 10.88 MJ/m^3, unlike methane which has 36 MJ/m^3 (Rachbauer et al., 2016; Luo et al., 2012).

To be used as transport fuel, firstly it has to be compressed at extremely high pressure and secondly it has a high tendency of leakage because of its tiny molecules; which means complex storage materials have to be provided. The compressed liquid hydrogen boils at –253 °C, making it very difficult to handle (Dodds and Mcdowall, 2012). Currently, other hydrogen storage forms such as metal hydrides are being researched, but this is yet undeveloped (Balat, 2008).

Due to the release of carbon dioxide accompanying fossil fuel sources, low-carbon and renewable technologies have to be employed for hydrogen production towards biomethanation applications, as listed on Table 2.3. So far, the low-carbon option for obtaining the hydrogen for biomethanation systems is the use of windmills (or excess energy from other renewable sources such as solar) to power water electrolysis cells, which generate hydrogen (Luo et al., 2012). Therefore, the possibility of obtaining the required hydrogen for food waste biomethanation using the most suitable method from the list presented in Table 2.3 need to be assessed, taking into consideration the amount of hydrogen gas required, scalability of the method, influence of the impurities from such processes and the economics of scale based on the energy balance.

Table 2.3. Emerging low carbon technologies for hydrogen production

Methods The process	Products formed	Limitations	References
Thermal conversions			
Wet/dry pyrolysis – Conversion of biomass to bio-char and gaseous products (H_2, CO_2, CH_4) 180 – 250 °C temperature and 2 – 10 MPa pressure.	Bio-char, tar and H_2, CO_2 and CH_4	Require high temperature and pressure.	(Wirth et al., 2015; Libra et al., 2011; Mumme et al., 2011)
Hydrothermal gasification – Conversion of biomass to synthetic gas (H_2, CO, CO_2, CH_4) in the presence of water; above its critical temperature (374.29 °C) and pressure (22.089 MPa).	Synthetic gas; having more of hydrogen gas and little amounts of tar.	Require high temperature (400 to 600 °C) and pressure (>22MPa).	(Acharya et al., 2014; He et al., 2014; Yanik et al., 2007)
Electrolysis			
Water electrolysis – Easiest method for pure hydrogen gas production from water; direct dissociation of H_2 from water.	Pure H_2 containing no sulphur and carbon in the product.	Require high energy input.	(Kadier et al., 2016; Lu and Ren, 2016)
Microbial electrolysis – The microbial breakdown of substrates to CO_2, protons and electrons at an anode, followed by the conversion of the protons and the electrons to H_2 at the cathode aided by applied voltage. It gives better conversion efficiency compared to other microbial conversions.	H_2, CH_4, H_2O_2 and FA.	Microbial selection and nature of substrate.	(Kadier et al., 2016; Lu and Ren, 2016; Shen et al., 2016)
Microbial conversions			
Biophotolysis – Naturally occurring process of hydrogen production by green algae or photosynthetic bacteria to split water into hydrogen and oxygen gas using solar energy.	H_2 and other gas mixtures	Oxygen produced inhibits the hydrogen producing enzymes, leading to low efficiency; explosive gas mixtures could be formed in the process and requires a large surface area	(Kadier et al., 2016; Ramesh and Chowdhary, 2016)
Photo fermentation – Nitrogen fixing bacteria uses solar energy to fix nitrogen in organic substances, releasing hydrogen gas in the process.	H_2, O_2 and other hydrogen containing fuels such as acetates.	Oxygen inhibition; energy intensive; low yield and requires large and complex designs of the anaerobic photo-reactors	(Kadier et al., 2016; Ramesh and Chowdhary, 2016)
Dark fermentation – The use of different organisms to hydrolyse organic substrates to hydrogen gas. It does not require light energy and can easily be adapted to various organic substrates.	H_2, CO_2 and other soluble hydrogen containing products such as acetic, butyric and lactic acids.	Thermodynamic limitations, which lead to lower substrate conversions, about 23 – 25% hydrogen recovery; requires critical reactor design.	(Kadier et al., 2016; Ramesh and Chowdhary, 2016; Shen et al., 2016)

2.8 Conclusions

In conclusion, mono-digestion of food waste produces methane below its theoretical maximum due to its intrinsic characteristics. Also, the AD system is a complex system with different interdependent factors which can individually be manipulated for improved biogas yield. Among such factors is the particle size pre-treatment. Considering, mechanical particle size reduction is the most widely adopted pre-treatment method for food waste, this factor will be optimised to influence improved digestion kinetics and ultimate biomethane yield.

Furthermore, food waste and the relative loading rates with respect to the inoculum-to-substrate ratio (ISR) influences the overall AD conditions monitored during this process. Therefore, it is important to pay close attention to this factor and how alternating it influences the overall process. Moreover, since particle size reduction is expected to increase solubilisation, it means a 'one-size-fit-all' approach cannot be adopted for the right choice of ISR. Therefore, the interaction between the particle size reduction and ISR will also be explored.

According to the literature reviewed, hydrogenotrophic methanogens are only primarily limited by the availability of hydrogen released from substrate decomposition. This implies that if hydrogen was added into the food waste AD hydrogenotrophic methanogenesis would be enhanced. Hence, the syntrophic relationship between the hydrogen producing acetogens and the hydrogen utilising methanogens would also be improved. By this, the problem of digester acidification common with food waste AD will be largely reduced.

However, to incorporate hydrogen production into AD processes for biomethanation, the hydrogen production unit has to be closely situated within the AD premise, to allow for direct supply as much as possible. In this regard, dark fermentation, which can be optimised using typical AD designs, proves to be about the most feasible option for this purpose.

CHAPTER 3

RESEARCH EXPERIMENTAL DESIGN

A comprehensive experimental design and statistical analysis conducted in the course of this study are described in this chapter. A detailed description of food waste source, sampling, processing and storage towards its use in biomethane production are given here. The experimental procedure for addition of hydrogen and FA to the AD of food waste are also described in this chapter, including the source, form and concentration of hydrogen and FA.

3.1 Characterisation of food waste for biomethane production.

3.1.1 Description of food waste source

Food waste samples used in this study were obtained from the University of Leeds Refectory, United Kingdom. The Refectory serves an average of 3,000 people daily (Monday to Friday). It is independent of the national university contracts scheme; hence, they have private contracts with food suppliers; mostly within close distances from Leeds. For instance, fruits and vegetables comes from Tadcaster, meat from Manchester, salad fillings from Holbeck, bread supply from Preston, Lancashire, milk from North Yorkshire, fish from Newcastle and so on (see Figure 3.1). Wherever possible, the food stuff are purchased with minimal waste potential. For example, to reduce meat-derived waste, meat is purchased boneless and potatoes purchased for boiling are purchased fresh and peeled (Tooley, 2017). This reduces most of the food-production-generated waste from the Refectory to mostly packaging. Customer eating habits, poor food selection choices, and even cooking style, still leads to large amounts of food waste collected daily from the Refectory; especially when an average of 3000 people are being served daily during week Days. On the average, 36,000 tonnes of food surplus is generated daily during term time; most of which is currently being sold off to composting companies (Tooley, 2017). Figure 3.2 gives a representation of the daily customer population distribution. This information was employed in the waste sampling design, to collect samples during the peak periods of the Day.

Source: GREAT FOOD at LEEDS (2018)

Figure 3.1. University of Leeds Refectory's food suppliers.

Source: **https://www.google.co.uk/maps/place/The+Refectory**
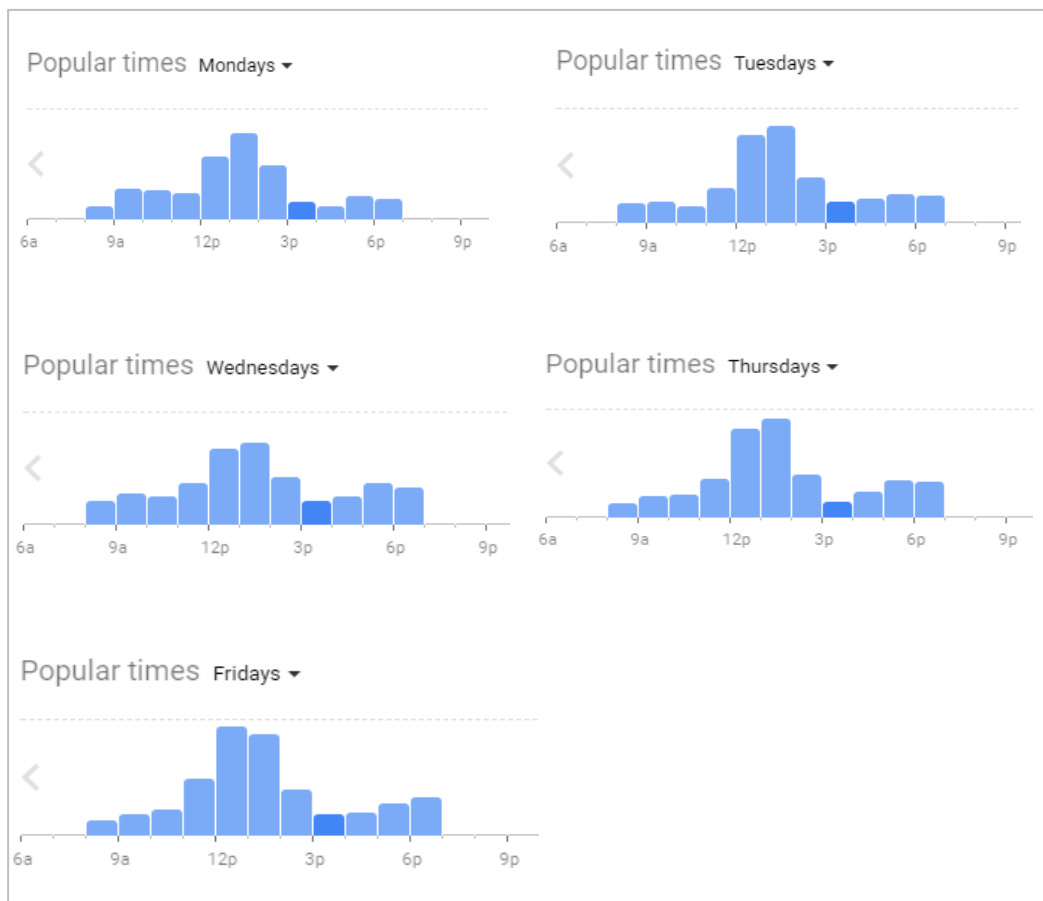
Figure 3.2. University of Leeds Refectory popular times; where 'a' means 'am' and 'p' means 'pm'.

3.1.2 Food waste characterisation

Waste characterisation generally involves three steps; sampling, sorting into desired material fractions, and material processing with data interpretation and application (Edjabou et al., 2015). Food waste characterisation is important prior to AD; because it influences the necessary pre-treatments and dilutions that might be required, as well as the amount of substrate required to achieve desired biomethane yield. The characterisation approach adopted in this study is presented in Figure 3.3.
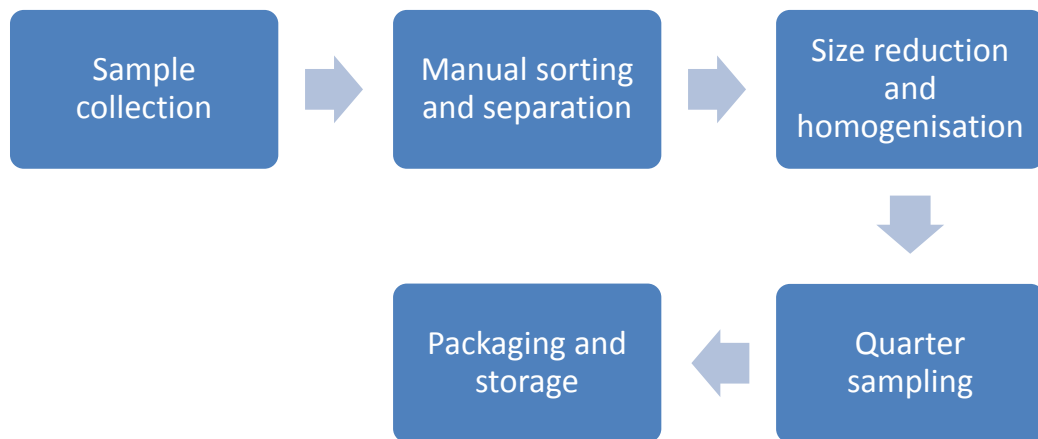
Figure 3.3. Food waste sampling and processing approach.

3.1.2.1 Waste sampling

The University of Leeds Refectory was chosen as the study area because it is the most visited commercial food hub by the University community and the samples from this study area can easily be compared with household and hospitality generated food wastes composition reported in literature. As it is quite difficult to analyse the whole waste quantity generated from this area, two sampling approaches were adopted; grab (one time collection) and composite (daily collection over a period of 5 days) sampling. The date and time of collection were recorded, in order to account for the seasonal variability. Waste samples were collected from both the kitchen and dining areas (leftovers in plates) of the Refectory in separately monitored bins and the overall weight of the sample collected was also recorded.

3.1.2.2 Waste sorting

The composition of food waste is a good indication of the calorific value of the waste. In order to understand the composition of the food waste collected and to identify what materials are dominant in the collected sample, the waste

samples collected were manually sorted into different material fractions and recorded as a percentage of the whole sample. Food waste sorting was done in the public health lab of the School of Civil Engineering, University of Leeds. The second sample; collected over a period of 5 days, was sorted daily after each collection and the biodegradable fraction was stored daily at -4 °C until the last day of sampling.

3.1.2.3 Food waste processing

It is important that the feed stock used in AD contains no impurities, that is, it should not contain materials that are not biodegradable. Therefore, after sorting, the non-biodegradable food materials were excluded and the remaining biodegradable food materials were processed. They were first minced using a manual mincing machine and then blended with a using a Nutribullet food processor to obtain a fairly smooth paste. Each portion was thoroughly mixed again to ensure that all aliquots were good representatives of the whole; 500 g aliquots were weighed into refrigerator bags, sealed and stored in the freezer at -20 °C in order to avoid any further microbial activity that could change the viability of the samples during downstream BMP trials. However, one of the bags was stored in the refrigerator at 4 °C for preliminary characterisation of the food waste sample. All experiment required for the characterisation were conducted within 14 days so as to reduce any possible error due to deterioration. For the composite sample, the sorted food waste sample was stored at 4 °C at the end of each day until the last day of collection, after which all the food waste samples were mixed together and blended.

3.1.3 Analytical procedures

Standard methods for examination of water and wastewater were employed for all analyses (unless otherwise stated), as described on Table 3.1 (APHA, 2005). Data obtained from all analyses were statistically tested for reliability using *t*-test analysis and where necessary, outliers were removed from the final data reported.

Table 3.1. Analytical methods adopted

1. **Physicochemical characteristics**	**Analytical method**
• Total solids and volatile solids	Gravimetric method as described in 2540B and 2540E of standard methods, respectively.
• Suspended solids and volatile suspended solids	Gravimetric method as described in 2540D and 2540E of standard methods, respectively.
• pH	Direct measurement, using HACH pH meter.
• Chemical Oxygen Demand (COD)	Titrimetric method as described in 5220C of standard methods[b].
• Alkalinity	Titrimetric method, using a Mettler Toledo (T50) equipment.
• Total Kjeldahl Nitrogen (TKN)	Kjeldahl method (4500-N_{org} B), using a Buchi distiller in the distillation step.
• Volatile fatty acids composition	GC analyzer as described in 5660B of standard methods.
2. **Elemental characteristics;**	
• Carbon, hydrogen, nitrogen, Sulphur and oxygen (CHNS-O)	FLASH 2000 Elemental Analyzer as described in the user manual (Thermo Scientific, n.d.)
3. **Biochemical characteristics**	
• Lipids	By acid solubilisation and extraction as described in AOAC Method 945.16.
• Carbohydrates	Subtractive method
• Proteins	Total Kjeldahl method
4. **Metals**	
• Metals composition	Microwave acid-assisted digestion for metals analysis by ICP-MS.

3.1.3.1 Physicochemical analysis

Food waste samples were diluted by pre-determined factors prior to some experiments, including: 1 in 5 mL dilution for pH and VFA, 1 in 500 mL for total kjeldahl nitrogen (TKN) and 1 in 1000 mL dilution for chemical oxygen demand (COD). The final results were corrected by the respective dilution factors (except for pH and VFA, which were reported without conversion).

The pH of all reactors was measured using a pH meter (HACH, 40d). VFA concentration was analysed by gas chromatography (GC – Agilent Technologies, 7890A) equipped with a flame ionization detector (GC-FID), auto-sampler and DB-FFAP column; length 30 m, diameter 0.32 mm and film thickness 0.5 µm, and helium as a carrier gas. The GC-FID operating conditions were; 150˚C inlet temperature and 200˚C FID temperature. Liquid samples were adjusted to pH 2.0 using phosphoric acid and allowed to rest for 30 minutes and then centrifuged at 14000 RPM (16,000 x g) for 5 min, using a Technico Maxi centrifuge. After centrifuging, the supernatant was filtered through 0.2 µm filter and the liquid analysed for VFA. The GC method was calibrated with SUPELCO Volatile Acid Standard Mix, which includes acetic-, propionic-, iso-butyric-, butyric-, iso-valeric-, valeric-, iso-caproic-, caproic- and heptanoic acids.

3.1.3.2 Elemental analysis

For elemental analysis, samples were dried at 40˚C and ground to powder, they were then wrapped in aluminium foil and stored in a dessicator until analysis was run using a FLASH2000 Elemental Analyzer.

3.1.3.3 Biochemical analysis

Protein content was calculated from the nitrogen content; analysed using the Kjeldahl method, which was then converted to protein by a standard conversion factor of 6.25 (ITW Reagents, 2007) in triplicates. Food waste (2 g) was weighed into respective digestion flasks, to which 1 catalyst tablet (K_2SO_4 + $CuSO_4$) and 25 cm^3 of concentrated sulphuric acid were added and digested for 2 hours. After digestion, samples were allowed to cool and transferred into distillation flasks; using distilled water to wash until an approximate volume of 400 cm^3 was obtained. Afterwards, 10 g of phenolphthalein indicator, approximately 1 g of anti-bumping granules and 1 cm^3 anti-foam agent were added. To 500 cm^3 conical flasks, 100 cm^3 of 4%

boric acid plus 3-4 drops of screened methyl red indicator were added; developing a purple colour. This was then attached to the distillation apparatus, and the outlet of the delivery tube completely submerged in the boric acid solution but not touching the bottom of the flask. Sodium hydroxide (NaOH, 50%) was added through a dropping funnel into each sample solution until it became alkaline (pink-purple in colour). The plug in the funnel was then replaced and sealed with distilled water and the mixture gently mixed by rotating the distillation framework. The tap of the condenser was then turned on and the distillation flask heated at a constant rate until a minimum of 250 cm^3 of distillate was collected in each conical flask. The distillates were then titrated with 0.25 mol dm^{-3} sulphuric acid; where 1 cm^3 of 0.25 mol dm^{-3} of sulphuric acid is equal to 0.007 g nitrogen.

The lipid content was determined by acid solubilisation (using hydrochloric acid) and Soxhlet extraction at 40 – 60 °C (using petroleum Spirit as solvent), according to the Soxhlet extraction AOAC Method 945.16 (McClements, 2003) in four replicates. Food waste (10 g) was added into four pre-weighed 250 cm^3 beakers each and 1 g anti-bumping granules and 50 cm^3 of 4 mol dm^{-3} HCl were added into each beaker. The solutions were heated up on a Bunsen burner and allowed to boil for about 3 minutes; until the samples turn dark brown and a layer of oil formed on the top. Whilst hot, they were carefully filtered through a No. 1 fluted filter paper and the beakers washed with approximately 25 cm^3 of boiling water twice. The filter papers were allowed to air dry overnight before extraction. Once dry, an approximate of 150 – 200 cm^3 of petroleum spirit was weighed into four round bottom flasks each, which had already been oven dried and weighed to obtain an absolute weight. The Soxhlet extraction apparatus was then set up and heated for a minimum of 6 hours, once the extraction was complete, the Soxhlet extractor body was removed and all solvent plus fat inside was poured into the round bottom flasks, and further heated up to separate the petroleum spirit from the total lipids, until an approximate 10 cm^3 of solvent remained. When all the solvent was removed, the outside of the flask was dried with tissue paper and placed in an oven set at 80 °C for 60 minutes and afterwards cooled in a desiccator and weighed accurately.

Carbohydrate values were obtained by differential method; deducting lipid, protein, ash and moisture content from the total weight of the samples.

3.1.3.4 Metals analysis

The concentration of the various trace elements and metals was determined by AOAC Method 2015.01, for heavy metals in food, by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS), using microwave-assisted acid digestion (nitric acid and hydrogen peroxide) (AOAC, 2013). Food waste (0.25 g) was carefully weighed into the bottom of a microwave (MARSXpress) PFA vessel, after which, 4 mL nitric acid and 1 mL 30% hydrogen peroxide were added accordingly. To the mixture, 1 mL of 50 mg/L Au + Lu (Gold and Lutetium) were added. The Au helps in stabilising the Hg in the preparation, and the Lu is used to assess the percentage recovery of the metals after digestion, such that the percentage of Lu recovered after the analysis, should give an indication of the potential loss in elements during microwave digestion. The vessel was then allowed to degas for 5 minutes, placed in the vessel liner and the cap screwed. A reference sample was prepared by spiking the mixture (containing food waste, nitric acid, hydrogen peroxide, Au and Lu) with 0.1 mL of mercury (Hg). The Hg percentage recovery was estimated by comparing the final Hg concentration of the spiked sample with the sum of the Hg concentration in the main sample and the concentration added to the spiked sample. This was done to ascertain the effectiveness of the method adopted to retain highly volatile metals such as mercury. A blank sample was also prepared with only nitric acid, hydrogen peroxide and Lu. All samples including the main food waste, spiked and blank samples, were prepared in 6 replicates. The microwave digester was set to a power level of 1200 W, temperature of 190 ˚C, 20 minutes ramp time and 10 minutes hold time. After digestion the entire content of the vessel was emptied into a volumetric flask and made to 100 mL with distilled water and sent for metals analysis using ICP-MS. The data output, showed average Lu and Hg recovery of 91% and 92% respectively.

The final metals concentration of each food waste sample was calculated by multiplying the value obtained from ICP-MS by the following conversion factor:

$$Conversion\ factor\ (cf, mL/g) = \frac{Volume\ of\ distilled\ water\ used}{weight\ of\ sample}$$

3.2 Theoretical methane potential (TMP)

TMP is the maximum methane potential of any biomass obtainable through AD, based on some theoretical models. Different models have been used to estimate the TMP of organic materials, either by the biochemical or elemental composition (Buswell Equation and Du Long formula), as well as estimations

based on the total oxygen demand (Nielfa et al., 2015). The most commonly used model is the Buswell equation by elemental composition. TMP assumes complete degradation of organic matter and does not account for internal enzymatic interactions during AD, therefore, the TMP values are very often higher than the experimental biomethane yield (Defra, 2010b; Nielfa et al., 2015). TMP values notwithstanding, are also useful for estimating materials' degradability potential and the extent of process optimisation required during experimental methane potential tests. As such, the anaerobic biodegradability of the material can be determined after the laboratory analysis by dividing the experimental methane potential by the theoretical experimental biomethane potential (Nielfa et al., 2015). The TMP values for the processed food wastes samples were thus estimated according to the Buswell equations (Kong et al., 2016);

$$TMP_{ele} \ (mLCH_4/g \ VS) = \frac{22.4 \ \times 1000 \times \left(\frac{c}{2} + \frac{h}{8} - \frac{o}{4} - \frac{3n}{8}\right)}{12c + h + 16o + 14n} \qquad 3.1$$

Where the letters c, h, o and n represent the subscripts of the corresponding elements; carbon, hydrogen, oxygen and nitrogen, in the empirical formula of the biomass, determined as follows;

$$Subscripts \ (c, h, n, or \ o) = \frac{Element \ (C, H, N \ or \ O)}{Element's \ molar \ weight} \qquad 3.2$$

3.3 Bio-methane potential (BMP) tests

The BMP test is often used to determine the ultimate methane production from diverse organic materials (Holliger et al., 2016). It is a laboratory scale AD test, conducted to determine the amount of biomethane obtainable from a biodegradable material under ideal conditions. It is also used as an indicator for the viability of biomass for AD (technical and economical) and a benchmark for predicting digester performance under field operations (Holliger et al., 2016). The BMP is somewhat like a verification of the TMP, to understand how much of the TMP is achievable under specific conditions (Nielfa et al., 2015).

In this research, different sets of BMP experiments were conducted to improve the biomethane yield of food waste, which includes; i) PS and inoculum-to-substrate ratio (ISR) variation; ii) addition of hydrogen gas and iii) addition of FA. Some guidelines for producing valid and reproducible BMP results have been established, including the VDI 4630 by the Association of German Engineers published first in 2006 and updated in 2016 (VDI, 2016) and the guidelines published by the Task Group for the Anaerobic Biodegradation, Activity and Inhibition group (ABAI-TG) of the International Water Association

(IWA) AD Specialist Group in 2009 and updated in 2016 (Angelidaki et al., 2009; Holliger et al., 2016). The guidelines by the ABAI-TG 2009 was used for all BMP experiment in this study (except otherwise stated), which focuses on all conditions surrounding the ultimate BMP production from any organic material including:

i.   Inoculum – choice, quality, preparation and storage;

ii.   Substrate – preparation and storage, analysis prior to BMP,

iii.   Test setup – reactor vessels, batch preparation, VS and ISR content, positive control, incubation conditions, gas measurements and data analysis.

As part of the substrate preparation, size reduction is employed to increase the surface area of substrates. Thereby, making it easily degradable by microorganisms, as such, it is very important during wet AD for improved biogas production rates (Angelidaki et al., 2009). It is often used as a feedstock pre-treatment method in commercial digesters, influencing the AD in the following ways: i) increased biogas yield, with a resultant decrease in the residues in the digestate, by enhanced degradability; ii) reduction in the technical digestion time, especially for substrates with low degradability; and iii) enhanced dewaterability of digester sludge (Palmowsky and Muller, 2000). However, too small PS could influence high levels of VFA within the AD system, which could inhibit methane production, therefore, the right choice of PS is of great importance.

For the BMP test setup, an adequate ISR is important. ISR is the ratio of the VS available in the inoculum (partly from actively degrading biomass) to VS available in substrate (Holliger et al., 2016). It is a key parameter because the inoculum provides the microorganisms required to consume the organic material, however, due to the potential of food waste to degrade into rapid accumulation of VFA and ammonia; that could inhibit the activities of the microorganisms, the right ISR must also be adequately selected.

To this effect, a 3^2 full factorial design was used for BMP assays; three PS ranges (< 1 mm, < 2 mm and < 5 mm) and three ISRs (2, 3 and 4) were set up in order to see the effect of these two factors on the methane yield. Due to the limitation arising from experimentation time, the optimal condition for PS and ISR was estimated using the grab food waste sample only. At the determined optimal conditions, BMP experiments were then conducted with the composite food waste sample and compared with the grab sample BMP data at the same conditions.

3.3.1 Food waste PS determination

Details on the food sampling, sorting and processing are given in section 3.1.2 of this chapter. The first food waste PS was the undersize of the processed sample from 1 mm sieve, the second PS was the undersize of the processed sample from a 2 mm sieve and the last was the homogenised sample after processing with PS ≤5 mm; having 95% solids recovery from a 5 mm sieve. The desired PS were obtained by sieving the homogenised food waste sample through the respective sieve sizes with manual application of pressure using a flat metal bar.

These sizes were chosen because smaller PS below 1 mm could encourage high VFA concentration, due to enhanced acidogenesis, while at higher PS, (above 5 mm) the biogas yields could be lowered due to poor feed stock degradation (Izumi et al., 2010).

3.3.2 Inoculum sampling

The use of sewage sludge digestate from a waste water treatment plant, instead of a food waste digester, offers better conditions for BMP analysis, due to a more diverse microbial consortia required to maintain a balance of the reactions occurring, especially at the start of the BMP experiment (Banks and Zhang, 2010). Furthermore, digestate from a food waste AD would typically be enriched by a defined process condition, as such, inhibitory conditions could be transferred to the new system if adopted. Hence, the inoculum used in this study was obtained from a mesophilic anaerobic digester treating sewage sludge at Yorkshire Water's Esholt Waste Water Treatment Work (Bradford, UK). The inoculum was filtered through a 1 mm sieve, to remove large materials and grits. Fresh digestate samples were first stored at 37 °C for 7 days to remove residual biogas from the digestate, followed by an acclimation with food waste for 30 days, achieved by adding 0.2 grams of food waste sample (as Volatile Solids – VS) per day in each litre of inoculum.

3.3.3 Determination of ISR

The ISRs used in this study were chosen because, the IWA ABAI-TG recommended an ISR between 2 and 4 to minimise acidification and inhibition problems (Holliger et al., 2016). They further recommended an ISR ≥4 for easily degradable substrates with rapid VFA accumulation potential, which is in agreement with an ISR of 4 for food waste recommended by Defra (2010b).

In contrast, however, Raposo et al. (2006) reported little variation in methane yield from AD of maize at ratios 3 and 2. Moreover, because different sources of inoculum could have different impact on the substrate degradation and also due to the possibility of having high VFA levels with food waste as a feedstock, the ISR was optimised in a range of 2 to 4.

3.3.4 Experimental set-up

There are two methods approved for BMP test by both the VDI 4630 and the IWA ABAI-TG; namely, the manometric (manual or automatic) and volumetric methods (Himanshu et al., 2017). These methods differ primarily by their gas collection and analysis, as determined by the reactor design. In the manometric method, the volume is held constant and the overhead pressure is measured and used to calculate the amount of gas produced, while in the volumetric method, the pressure is held constant and the volume of the gas produced is measured by a displacement volume device (Himanshu et al., 2017). Batch BMP assays are generally prepared in 100 mL to 2000 mL working volume depending on the homogeneity of the substrate; such that lower volumes around 100 mL are used for homogeneous substrate and a volume within the range of 500 mL to 2000 mL are used for heterogeneous wastes. However for increased reproducibility, reactor working volumes of 400 mL to 500 mL; which translates to a total reactor volume of 500 mL and 1000 mL respectively, was recommended by IWA ABAI-TG (Holliger et al., 2016). Hence, the experiments for optimising food waste PS and ISR were conducted by the volumetric BMP method, using an Automatic Methane Test System II (AMPTS II).

The AMPTS II equipment (Figure 3.4) uses 500 mL Duran bottles (as reactors) and a water bath to regulate the temperature. The system has downstream biogas cleaning, which removes CO_2 and measures and records real time methane production using volumetric flow rates. The CO_2 fixing solution was prepared by adding 5 mL of 0.4% thymolphthalein indicator to 1L of 3M NaOH; out of which 80 mL was transferred into 100 mL bottle for each reactor.

Frozen food waste samples were transferred to a refrigerator at 4°C to defrost a day before setup and then acclimatised to room temperature before they were used; there was no heat applied to the sample to defrost in order to retain sample characteristics.

Figure 3.4. AMPTS II equipment for BMP experimentation.

The amount of inoculum used was fixed at 300 mL per 1000 mL sample and the VS concentration in this amount of inoculum was calculated. For each ISR the amount of food waste required was calculated (Equations 3.3 – 3.5). Therefore, the calculated amount of food waste required was added to 300 mL of inoculum and made up to 1 litre with distilled water.

$$VS\ in\ 300\ ml\ inoculum\ (g) = \frac{VS\ (g)in\ 1000\ ml \times 300}{1000} \qquad 3.3$$

$$VS\ of\ food\ waste\ required\ (g) = \frac{VS\ in\ 300\ ml\ inoculum}{ISR} \qquad 3.4$$

$$Amount\ of\ food\ waste\ required\ (g) = \frac{VS\ of\ food\ waste\ required\ (g)}{VS\ in\ 1000\ g\ of\ food\ waste} \qquad 3.5$$

A blank sample was also prepared containing only the inoculum at 300 mL per 1000 mL sample. Bulk samples of 1500 mL were prepared and split into 3 equal portions (with constant manual mixing) of 500 mL; out of which 400 mL was used for the BMP analysis, while the 100 mL samples remaining were used to conduct the first day (Day0) analyses. Each test was conducted in triplicates on the first day of the setup, while the parameters monitored during the BMP process from each reactor were conducted in duplicates (except otherwise stated).

3.3.5 Process monitoring and analysis

The tests conducted and the days they were done throughout the BMP setup; as a way of monitoring the digester performance, are presented in Table 3.2; where 'X' indicate the performance of an activity. The experiment was ended when the cumulative methane yield was <1% and the last day of the setup is labelled as 'Day T' in Table 3.2. Standard methods for examination of water

and waste water as earlier described in section 3.1.3 were employed (except otherwise stated). The pH of the reactor content was measured immediately after collecting the sample using a HACH pH meter (HQ 40d) and alkalinity was analysed immediately afterwards using a METTLER TOLEDO (T50), equipped with an auto-titrator and 0.05 mol H_2SO_4/L as the titrant. The pH and alkalinity experiments were conducted as quickly as possible after sample collection to minimise changes due to atmospheric oxidation. Soluble chemical oxygen demand (sCOD) and soluble TKN (sTKN) were conducted on samples' filtrate, by centrifuging the samples at 2000 RPM (775 x g) for 5 minutes using an Eppendorf Centrifuge and filtering the supernatant through 0.45 µm and 90 mm diameter Whatson filter paper, respectively, followed by the standard methods for analysing COD (5220 C) and TKN (4500 – N_{org}, B) respectively. Total ammonia-nitrogen was determined by titrimetric method (4500 – NH_3, B – C of standard methods) using a Buchi distiller in the distillation step.

Table 3.2. Liquid analyses during grab samples BMP.

Tests \ Day	0	4	11	18	25	Day T
Solids (TS, VS)	X					X
pH	X	X	X	X	X	X
VFA	X	X	X	X	X	X
sCOD	X	X	X	X	X	X
TCOD	X					X
Alkalinity	X	X	X	X	X	X
TKN, sTKN	X					X
NH_3-N	X					X

3.3.6 Statistical and kinetic analysis.

All results from each group of BMP assay were individually analysed for statistical significance, using a one sample t-test. Where the results showed significant difference, further outlier test was conducted to remove outliers, before other analysis and graphical representations. To test the variation between the blank and the test assays, a two sample t-test was conducted. Furthermore, to understand the individual and combined effect of the two factors optimised (PS and ISR), a design of experiments (DOE) using a 2 factor 3 levels (3^2) full factorial design was created and analysed with the aid of Minitab 18 statistical software.

The Modified Gompertz (MGompertz) model (Equation 3.6) (Zwietering et al., 1990) was used to fit the cumulative methane curves, with Origin 2016 graphical and statistics software. The MGompertz model is widely adopted for fitting cumulative methane yields and preferred over the first order kinetic model, because, it provides additional information on the lag phase and daily maximum specific methane yield, which are important parameters for analysing efficiency of AD systems.

$$y = A exp \left\{ -exp \left[\frac{\mu_m \cdot e}{A} (\lambda - t) + 1 \right] \right\} \qquad 3.6$$

$Where; y = Cumulative\ methane\ yield\ (mLCH_4/gVS),$

$A = Maximum\ methane\ yield\ (mLCH_4/gVS)\ at\ time\ t,$

$\mu_m = Maximum\ specific\ methane\ yield\ per\ day\ (mLCH_4/g^{-1}VS\ Day^{-1})$

$\lambda = Lag\ phase\ (Day)\ and\ e = exp\ (1).$

3.4 Anaerobic digestion of food waste with in-situ biomethanation

After PS and ISR optimisation, AD experiments with hydrogen addition were conducted to optimise the hydrogenotrophic methanogenesis route of methane production, as a means of further improving the biomethane yield. Three hypotheses were developed for these sets of experiments; i) addition of hydrogen would improve biomethane yield, by enhancing the hydrogenotrophic methanogens via H_2/CO_2 consumption, ii) the VFA regime would influence the rate of the added H_2 utilisation and effective biomethane increase and iii) a stepwise acclimation of the system to increasing concentrations of hydrogen would further improve biomethane yield, via an adapted microbial community.

3.4.1 Hydrogen source

Due to safety and AD thermodynamic requirements, hydrogen used in these experiments was obtained from School of Chemical and Process Engineering (SCaPE), University of Leeds in a gas mix of nitrogen and hydrogen. Considering that typical BMP test reactors can be flushed with nitrogen gas to achieve an anaerobic environment; especially for small headspace reactors (Holliger et al., 2016), the $H_2:N_2$ gas mixture was used for these experiments, to simultaneously inject hydrogen and achieve an anaerobic environment. H-$_2:N_2$ gas mixtures of 5:95, 10:90 and 15:85 (%v/v) were used in this study. The

5:95 gas mixture was obtained from an installed gas line supplying the gas mixture at this ratio, and with the aid of a rotameter, the flowrate was set to 1000mL/min. The 10:90 and 15:85 gas mixtures were obtained using mass flow meters to calibrate the hydrogen and nitrogen gas volume (from different gas lines) according to the desired percentage distribution, at a gas flow rate of 1000 mL/min. The first and second hypotheses were tested using 5:95 gas mix, while the third hypothesis was tested using all three gas mixtures.

3.4.2 Dissolved and gaseous hydrogen calculation

Seo et al. (2012), put forward a method for determining dissolved hydrogen by titration. In their report, dissolved hydrogen was analysed using a reagent (MB-Pt) composed of methylene blue (MB) and colloidal platinum (Pt). This method was replicated in this study to estimate the amount of hydrogen dissolved from the respective gas mixtures. A solution of MB containing 0.3 g of MB in 98% ethanol was prepared; to give 99.2 g solution. An aqueous solution of 2% Pt (0.8 g) was then added to the solution; making a total of 100 g MB-Pt reagent and transferred to a storage vial with pipette cap. The concentration of dissolved hydrogen was calculated based on the weight of 1 drop of MB-Pt reagent (10 mg in this case), and the molar masses of MB and hydrogen. According to Seo et al. (2012), when the MB-Pt reagent was added to the hydrogen saturated water, it initially turned blue, however, with continuous drop wise addition of MB-Pt, the end of the titration was reached when the MB completely oxidises the dissolved hydrogen and the blue colour disappears (see Figure 3.5), thereby, making the water colourless again. Due to the limited amount of hydrogen used in this study, the time taken for one drop of MB-Pt reagent to be completely reduced was adopted using the following steps as detailed in Seo et al. (2012);

$$1 \, drop \, of \, reagent \, prepared \, weighed \, 10 \, mg = 0.01 \, g$$

$$molar \, mass \, of \, hydrogen = 1.008 \, g/mol = 1.008 \times 10^{-3} \, mg/\mu mol$$

$$molar \, mass \, of \, methylene \, blue = 319.85 \, g/mol$$

$$Amount \, of \, methylene \, blue \, in \, reagent \, prepare \, = \frac{0.3 \, g}{100 \, g} = 0.003$$

$$moles \, of \, hydrogen = \frac{0.01 \, g \times 0.003}{319.85 g/mol \times number \, of \, drops \, of \, reagent}$$
$$= 9.38 \times 10^{-8} \, mol/drop \, or \, 0.0938 \, \mu mol/drop$$

$$Therefore, concentration\ of\ hydrogen\ (mg/L)$$

$$= \frac{0.0938\ (\mu mol/drop) \times number of\ drops \times 1.008 \times 10^{-3}\ (mg/\mu mol)}{volume\ of\ sample\ (mL)} \times 1000$$

$$= \frac{0.0945 \times number\ of\ reagent\ drops}{volume\ of\ sample\ used\ (mL)}$$

A colour change was observed after 20, 13 and 5 minutes for 5:95, 10:90 and 15:85 gas mixtures respectively, However, all test reactors within each experimental group were only flushed with the gas mixtures for 5 minutes. This time was chosen considering that for the biomethanation experiments, 15 reactors would be required for each sample group (blank, control and test samples), making a total of 45 samples, therefore, the time required for the overall biomethanation assays preparation had to be shortened. Since all test reactors were flushed for 5 minutes with the respective gas mixture, the calculated concentration of dissolved hydrogen for each experiment was 3.16 x 10^{-4} mg/L, 4.85 x 10^{-4} mg/L, and 1.26 x 10^{-3} mg/L, at 5:95, 10:90 and 15:85 gas mixtures respectively.

Figure 3.5. Dissolved hydrogen experiment with MB-Pt reagent; the right bottle is the reagent in distilled water and the left is the reagent after bubbling with the H2/N2 gas mixture.

The hydrogen transferred to the headspace was calculated by direct GC measurement to obtain the percentage and multiplying by the headspace volume (88 mL). These were then converted to standard temperature and pressure (STP) values to yield concentrations of 3.96 mg/L, 6.98 mg/L and 11.86 mg/L at 5:95, 10:90 and 15:85 gas mixtures respectively.

3.4.3 Trial experiments for suitability of reactors.

The biomethanation experiments were set up with hydrogen injection using the composite food waste at the optimal conditions from the PS and ISR optimisation stage. An initial trial experiment was set up, with Day0 (day of setup) gas injection, using the 5:95 gas mixtures. This was done to measure the biogas yield and composition, study the possibility of gas leakages from the reactor, identify the possible sources of errors and suitability of proposed analytical methods and measure the influence of hydrogen gas addition on the cumulative gas yield. Therefore, for the trial run, liquid analyses were conducted only on the first and last days of the set up. Blank samples (inoculum only), control samples (inoculum and food waste) and test samples (inoculum and food waste with gas injection) were prepared in triplicates at 75 mL working volume each. The manometric and volumetric methods for BMP analysis were employed using 160 mL Wheaton bottles with rubber seals and crimps and 250 mL Duran bottles with rubber corks; having two bored holes and fittings for sample inlet and gas outlet, respectively. This was done in order to identify which setup would give minimal errors due to gas injection, process stability and gas collection.

The volume of inoculum was fixed at 75 mL per 250 mL bulk sample (that is 30%), and calculations for the amount of food waste required were the same as earlier discussed in section 3.3. Hence, one bulk sample was prepared for the control and test samples; at an ISR of 3, by adding 14.2 g of food waste to 210 mL of inoculum and topping up the mixture to 700 mL with distilled water. After the food waste-based bulk sample was prepared, it was split into the different reactors. The test reactors were then taken to gas source, where the gas mixture was bubbled through each reactor; with the help of a ceramic diffuser, for 5 minutes and sealed. The other reactors that did not require hydrogen gas (blank and control), were flushed with nitrogen gas to achieve an anaerobic environment. Balloons were then attached to all Wheaton bottles as a safety collection bag, should there be any gas or sample leaks or splash due to the pressure build-up within the reactor. The samples were digested for 21 days in a water bath set to 37 °C (Figure 3.6) and monitored for TS, VS, pH, alkalinity, sCOD, NH_3-N, VFA, CHNS-O, and biogas yield and composition. At the end of the trial experiments, the Wheaton bottles showed better gas withholding characteristics, process stability and minimum error from gas collection and analysis, as such, they were chosen for all biomethanation experiments. The results from the trial experiments are detailed in Appendix A.

Figure 3.6. BMP trial experiments with hydrogen injection for test of reactor suitability.

3.4.4 Hydrogen validation and leakage test

To ensure that the injected hydrogen was retained throughout the 21-Day digestion period, further hydrogen leakage test was carried out on the Wheaton bottles. Hydrogen could leak through the tiniest orifice, hence, to confirm that the hydrogen injected and generated during materials breakdown, was directly consumed by microorganisms and not lost to the atmosphere, samples were prepared at 75 mL working volume using distilled water; being the same solvent used in samples dilution, with 5:95 mixture bubbled through for 5 minutes. The same conditions used in setting up the biomethanation experiments were observed here and headspace gas measured on the GC by manual injection.

3.4.5 Experiments with hydrogen injection

Although the previous BMP experiments were conducted by the volumetric method, the results from the trial experiments and hydrogen leakage test showed that the Wheaton bottles were more efficient for hydrogen leak prevention. Hence, biomethanation experiments were run by manual manometric method (mBMP) using Wheaton bottles. The only limitation with this switch however, is that lower biogas yields are generally reported from mBMP (even within same laboratories), due to overhead pressure build-up; unlike the automatic manometric and volumetric methods, whereby, overhead

pressure measurement and release (OHPMR) is more frequent (Himanshu et al., 2017).

Replicate reactors of the same sample were prepared to make up for 6 points of analysis after the first Day (Day0) of the BMP set-up using Wheaton bottles. Samples were prepared in duplicates for some sampling points and triplicates for others as shown on Table 3.3, to give 45 BMP reactors in total.

Table 3.3. Total number of Wheaton bottles used for each assay

Sample	Day 0	Day1	Day2	Day3	Day10	Day15	Day21	Total
Blank	---	3	2	2	3	2	3	**15**
Control	---	3	2	2	3	2	3	**15**
Test	---	3	2	2	3	2	3	**15**
Total	**---**	**9**	**6**	**6**	**9**	**6**	**9**	**45**

3.4.5.1 Biogas composition and volume

The biogas generated was analysed for hydrogen, carbon dioxide and methane in order to justify whether or not the hydrogen was consumed at all and if it was consumed for other purposes such as hydrogen sulphide production. The headspace gas composition was measured by GC (Agilent Technology, 7890A) equipped with a thermal conductivity detector (GC-TCD) and Carboxen 1010 PLOT column; length 30 m, diameter 0.53 mm and film thickness 30 μm. The GC-TCD was operated at 200 °C inlet temperature and 230 °C detector temperature with Argon as a carrier gas (3 mL/min). Gas samples (G_v) were collected from the headspace of the reactors to analyse the composition using a 500 μL glass syringe. Two full syringes were drawn and expelled through a bottle of distilled water to flush the syringe and also ensure the needle was not blocked with septa cores. With the needle in the reactor, the syringe was pumped about seven times to mix the headspace gas sample and a full syringe was drawn, which was then set to 200 μL (bubbled through distilled water) and manually injected into the GC inlet column. The headspace gas composition within each reactor was measured in duplicate, such that each assay (blank, control and test) had either 4 or 6 GC results depending on the day of sampling as shown in Table 3.3. The GC method was calibrated with three standard gas mixtures; $50\%CH_4:3\%H_2:47\%N_2$, $20\%O_2:80\%N_2$ and $10\%CO_2:90\%N_2$ at predetermined intervals.

The volume of the biogas, was determined by water displacement method (Figure 3.7); such that, the volume of water displaced by the biogas was equivalent to the amount of the excess gas in the headspace. The headspace gas within the first week of setup was first analysed on the GC for its composition followed by volume measurement. This was done to avoid hydrogen losses within the initial digestion period; when hydrogen partial pressure was presumably highest, due to its rapid dispersion property. Subsequently, as the pressure within the reactors increased, manual extraction of gas samples for GC analysis could lead to losses due to pressurised gas leaks, and so, headspace gas volumes were measured to attain atmospheric pressure within the reactor, before analysis on the GC for gas composition.

1. Reactor	5. Rubber topper	8. Water collecting
2. Extraction syringe	6. Water displacement chamber	pipe
3. Gas inlet valve		9. Graduated glass cylinder collecting
4. Gas collecting pipe	7. Water outlet valve	displace water by the gas
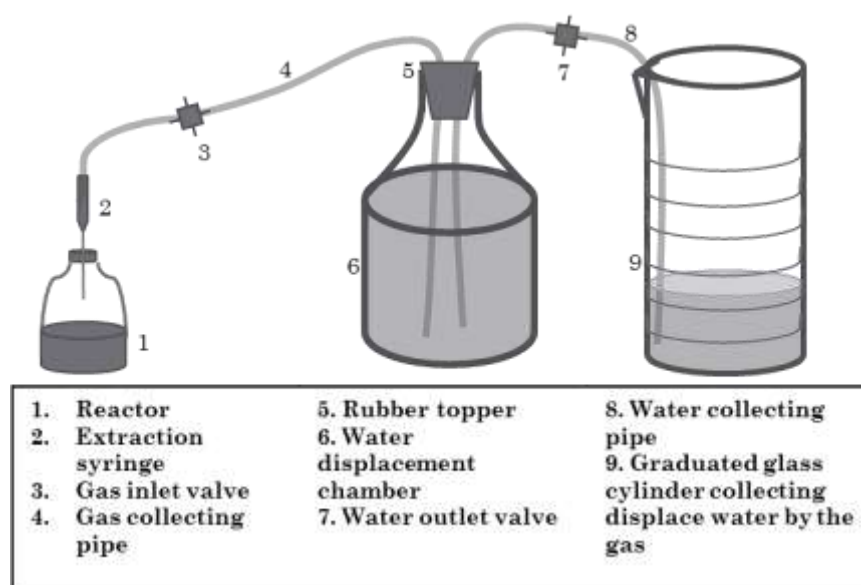
Figure 3.7. Headspace gas volume measurement by water displacement

The generated biogas was collected from the Wheaton bottles by injecting a needle into the bottle septa cap, after which the water displacement tank inlet valve was turned open. At this stage gas flow into the water tank was observed by the production of gas bubbles. The outlet of the displacement tank was then opened, and the pressure of the gas displaced an equal amount of water into a collecting measuring cylinder. This flow stabilised when the pressure across the water displacement system was the same as the atmospheric pressure; at which point no gas bubble was seen to be produced and no further increase in water level in the graduated cylinder was observed. The inlet and outlet valve were then closed to avoid any water drag, and the amount of biogas in excess of the head space volume was then recorded as the increase in water head displaced; measured directly from the calibrated

cylinder. The water displacement setup was calibrated with 10 mL of laboratory air before each analysis to ensure the system pressure was maintained.

The total volume of gas was then calculated as the sum of the volume used for analysis by GC, the volume measured by water displacement and the actual headspace volume. Recall that nitrogen gas was used to attain an anaerobic environment, hence, the generated biogas volume was calculated by adding up the percentages of the biogas components (H_2, CH_4 and CO_2) as obtained by GC and taking the resultant percentage of the total gas volume. Individual volumes of the biogas components were further estimated by taking the percentages of the respective gases from the total volume of biogas calculated.

3.4.5.2 Biogas conversions to standard temperature and pressure (STP)

The ideal gas law was employed to convert all gas volumes recorded to STP.

$$The\ ideal\ gas\ law\ is\ given\ as\ PV\ =\ nRT$$

$$Where\ P\ =\ pressure\ of\ the\ gas\ (atm);\ V\ =\ volume\ of\ the\ gas\ (L);$$

$$n\ =\ number\ of\ moles;$$

$$R\ =\ universal\ gas\ constant\ given\ as\ 0.08206\ L\ atm\ K^{-1}\ mol^{-1}$$

$$and\ T\ =\ temperature\ (Kelvin)$$

$$At\ STP\ (0°C,1\ atm)\ 1\ mole\ of\ a\ gas\ occupies\ 22.4\ Litres,$$

$$Hence,at\ the\ BMP\ operating\ temperature\ (37°C,310K),$$

$$1\ mole\ of\ gas\ will\ occupy\ \frac{310\times22.4}{273}=25.44\ Litres$$

$$If\ 1\ mole\ occupies\ 25.44\ L, then, n\ number\ of\ moles\ of\ measured\ volume$$
$$=\frac{measured\ volume\ (L)\times1\ mol}{25.44\ L}$$

$$Where\ number\ of\ mole, n\ (mol)=\frac{mass\ of\ gas, m\ (g)}{Molar\ mass, M\ (g/mol)}$$

The individual masses were then calculated using their respective densities at STP;

$$Mass\ of\ gas\ at\ STP, m\ (g)=n\ at\ STP\ (mol)\times M\ (g/mol)$$

The ideal gas law was therefore, rearranged to estimate the pressure of the respective gases (P_g) as;

$$P_g = \frac{n_g \times R \times T_g}{V_g}$$

The combined gas law was then used to estimate the volumes of the respective gases at STP;

$$Combined\ gas\ law: \frac{P_1 \times V_1}{T_1} = \frac{P_2 \times V_2}{T_2}$$

Replacing the left operand as the measured gas parameters and the right operand as the parameters of the gas at STP, the volume of the gas at STP (V_s) becomes;

$$V_s = \frac{P_g \times V_g \times T_s}{P_s \times T_g}$$

3.4.5.3 Liquid sampling and analysis

Liquid content of each reactor was analysed for total chemical oxygen demand (TCOD) and total organic carbon (TOC) on the day of setup and at the end of the digestion process, while pH, TS, VS, sCOD, VFA, dissolved organic carbon (DOC), elemental characteristics (C, H, N, S) and ammonia-nitrogen were analysed at all monitoring point (marked with an 'X' in Table 3.4). Taking a cue from the performance of previous BMP assays, elemental characteristics, pH, TS, VS and VFA were carried out directly on the sample without prior dilutions using standard methods as described earlier. The VS for all reactor contents was examined within 4 hours of opening the reactors.

Ammonium nitrogen concentration in each reactor content was determined by direct analysis on a HACH AP3900 Laboratory robot, however, to eliminate solids interference, samples for were diluted and centrifuged at 2000 RPM (775 x g) for 5 minutes, with an Eppendorf centrifuge and sieved through a 90 mm diameter Whatson filter paper.

The obtained results were then converted to free ammonia according to Equations 3.7 and 3.8 (Vanotti and Hunt, 2000);

$$Free\ ammonia\ (mgL^{-1}) = \left(\frac{17}{14}\right) \times \left(\frac{NH_4 - N \times 10^{pH}}{\frac{k_b}{k_w} + 10^{pH}}\right) \tag{3.7}$$

Where, k_b and k_w are ammonia ionisation constants defined by the operating temperature;

$$\frac{k_b}{k_w} = \left[\exp\left(\frac{6344}{273+T}\right)\right] \tag{3.8}$$

The total ammonia nitrogen was then estimated as the sum of free ammonia and ammonium nitrogen.

Prior validation for determination of total ammonia nitrogen (TAN) using the supernatant was conducted in comparison with previous ammonia results from the typical distillation/titration method using unfiltered samples (results given in Appendix B).

DOC and sCOD were analysed using the same sample dilutions, while the TCOD and TOC were analysed using the same dilutions. For TOC and TCOD, diluted samples were analysed straightway using the respective equipment, while for sCOD and DOC, diluted samples were further centrifuged at 2000 RPM (775 x g) for 5 minutes, using an Eppendorf centrifuge and the supernatants filtered through 0.45 µm filter. TCOD and sCOD were then measured using a HACH AP3900 Laboratory robot, while TOC and DOC were measured by the differential method with HACH IL550 TOC-TN equipment.

Table 3.4. Analyses run on liquid samples during biomethanation experiments

Sampling Day	Analysis								
	(C,H,N,S)	pH	TS, VS	VFA	sCOD	NH_3-N	DOC	TOC	TCOD
0	X	X	X	X	X	X	X	X	X
1	X	X	X	X	X	X	X		
2	X	X	X	X	X	X	X		
3	X	X	X	X	X	X	X		
10	X	X	X	X	X	X	X		
15	X	X	X	X	X	X	X		
21	X	X	X	X	X	X	X	X	X

3.5 Experiments with formic acid addition

Three sets of experiments, labelled as EF1, EF2 and EF3, were also set up with FA addition, to compare with the hydrogen acclimation experiments (hypothesis three). The same experimental setup, including monitoring periods and replicates as used in hydrogen experiments were adopted for these experiments. However, due to the volatility of FA, it was injected into the reactors; using a syringe and needle, after they were completely purged with nitrogen gas and sealed with rubber seals and aluminium caps.

3.5.1 Calculation of formic acid concentrations

The FA added was calculated based on the stoichiometric amount that would yield the equivalent amount of hydrogen used in the hydrogen addition experiments (dissolved plus gaseous at STP), if it were to completely degrade to CO_2 and H_2 (Equation 3.9).

$$HCOOH \leftrightarrow CO_2 + H_2 \qquad\qquad 3.9$$

1 mole of FA is required to yield 1 mole of hydrogen, therefore, the number of moles of hydrogen actually added was calculated as;

$$n_{H_2}(mol) = \frac{m_{H_2}}{2.016} = 0.496 m_{H_2}$$

And, the amount of FA required becomes;

$$m_{FA}(g) = n_{H_2} \times 46.025 = 0.496 \times m_{H_2} \times 46.025 = 22.8284\, m_{H_2}$$

Where, m_{H_2} and m_{FA} are the masses of hydrogen and FA added respectively and 2.016 and 46.025 are their corresponding molar masses in g/mol. The total hydrogen added was 3.48×10^{-4} g, 7.25×10^{-4} g and 1.09×10^{-3} g in experiments with H_2/N_2 gas mixtures 5:95, 10:90 and 15:85 respectively; therefore, the mass of FA was calculated accordingly as 7.94×10^{-3} g, 1.66×10^{-2} g and 2.48×10^{-2} g. The volume of FA required at 75 mL reactor working volume was then calculated using 1.220 g/mL density of FA at 20 °C (laboratory temperature), as 6.5 µL, 13.6 µL and 20.3 µL for EF1, EF2 and EF3 respectively.

CHAPTER 4

FOOD WASTE CHARACTERISATION AND OPTIMISATION FOR BIOMETHANE PRODUCTION: EFFECT OF PARTICLE SIZE AND INOCULUM-TO-SUBSTRATE RATIO.

4.1 Introduction

Food waste composition is highly inconsistent. It varies with geographical location, time of collection, peoples' culture, sampling method, as well as its definition by different authorities and researchers. These make the comparison of data from food waste characterisation between different studies rather ambiguous and bias. For instance, the UK food waste statistics by the Department for Environment, Food and Rural Affairs (Defra) and Waste and Resources Action Programme (WRAP) are often reported as a combination of food and drink waste (Defra, 2015; WRAP, 2017). Whereas, most of the research on food waste do not usually include drinks, however, these reports are often quoted in comparison.

Also, the commonly used term 'source segregated food waste', which is used to describe food waste separately collected at the point of generation, would typically be different for different locations such as household and canteen. Some researchers use the term 'kitchen waste' to define food waste collected from the kitchen section of any establishment; including households (De Vrieze et al., 2013; Feng et al., 2017) and others used this term for food waste collected from restaurants (L. Wang et al., 2014; Zhang et al., 2005). Meanwhile, kitchen waste could also pass for 'source segregated food waste' if it was collected without contaminants from kitchen areas.

The term 'fruits and vegetable wastes' have also been used to describe fruits and vegetable-based wastes (Alkanok et al., 2014; Bouallagui et al., 2003; Ganesh et al., 2014; L. Wang et al., 2014; Shi et al., 2017; Y. Wu et al., 2017), even though most of them were collected as source segregated wastes from canteens, supermarkets and households.

These discrepancies make it rather difficult to standardise the characteristics of food waste for biomethane recovery. In most cases, only the place of collection is mentioned, and the classification are based on the obvious food items collected; such as carrot, bread, and so on (Rajagopal et al., 2014; Zhang and Jahng, 2012), in which case the soft food items already mashed together are rarely mentioned.

A preferable approach towards food waste characterisation would be to describe the source in detail, followed by the characterisation of the food waste samples including i) the sampling time and season, ii) the sampling method and iii) the processing methods and PS range. This will help to reduce the bias in food waste characteristics' data comparison, towards better informed conclusions. The major drawback however, with an extensive food waste characterisation is usually the cost; especially for the different analyses to be run. This might be the reason most researches just analyse the physical parameters such as moisture content, total and volatile solids, and a few additional information relative to the research purpose. In this study, an extensive characterisation of food waste was conducted and the results from these experiments are discussed in this chapter.

4.1.1 Sample and Process Optimisation for Increased Methane Yield

Hydrolysis is generally thought to be the rate-limiting reaction during AD and can be improved by feedstock pre-treatment. Pre-treatment helps to increase solubilisation, thereby, improving the rate of methane production (Kondusamy and Kalamdhad, 2014). Mechanical pre-treatment, which mainly focuses on PS reduction, is widely employed in AD with documented resultant increase in methane yield, especially due to enhanced hydrolysis (Zhang et al., 2014). However, with the increase in feedstock solubilisation, it is also important to understand how the microorganisms respond to this change and whether or not an increase in microbial community will be necessary to efficiently utilise the hydrolysed feedstock.

Individual research has been conducted on the influence of PS (Izumi et al., 2010; Kim et al., 2000; Palmowsky and Muller, 2000) and inoculum-to-substrate ratio (Boulanger et al., 2012; Eskicioglu and Ghorbani, 2011; Pellera and Gidarakos, 2016) on the biomethane yield. However, it will be useful to also understand the interaction between these two factors on methane yield from food waste AD. By this, both the sample characteristics and the process can be optimised to obtain an increase in methane yield. One study that has considered these two factors was conducted using *Ulex europaeus* (plant species), whereby, biomethane yield was said to vary from 153 to 302 mL/g (Costa et al., 2016). Therefore, in addition to discussing food waste characteristics, the interactions between food waste particle size treatment (PS) and inoculum to substrate ratio (ISR) on the biomethane potential (BMP) test are also discussed in this chapter.

4.2 Objectives of chapter

- To study the effect of different waste streams and PS reduction on the characteristics of food waste.
- To understand the interaction of food waste PS and ISR on the biomethane yield from food waste.
- To compare the BMP processes of two food waste streams at optimal conditions of PS ad ISR.

4.3 Food waste sampling and sorting

Food waste samples were collected on two occasions from the University of Leeds Refectory. The first sample (grab sample), was obtained by a single visit to the Refectory on the 18th of April 2016 at 1:30pm, from the kitchen and eating sections. The second sample (composite sample) was collected over five days from Monday 23rd to Friday 27th January, 2017 between the hours of 12:00 and 14:00 (peak periods), from both the kitchen and the eating sections of the Refectory using separately monitored bins.

4.3.1 Grab sample

The composition of the waste collected by grab sampling from the Refectory is shown in Figure 4.1. This waste source composed of left-overs from kitchen and the eating sections, expired food samples from the refrigerator, and vegetable and fruit peels; which had the highest composition. A total of 20 kg of waste was collected, comprising mainly: bread, rice, vegetables, fruit peels, and egg shells. The components were then sorted into three groups in order to have a good representation of the whole waste; cooked foods, inorganic (non-degradable) substances, and fruits and vegetables. Each group was weighed as a percentage of the total mass as presented in Figure 4.1. The different characterisation stages; from sampling to packaging of final food waste sample are presented in Figure 4.2.

From Figure 4.2 (A and B) we observed that fruits and vegetables made up the bulk of the waste. The final blended sample was prepared without adding water (Figure 4.2C), and stored in refrigerator bags at 500 g each.
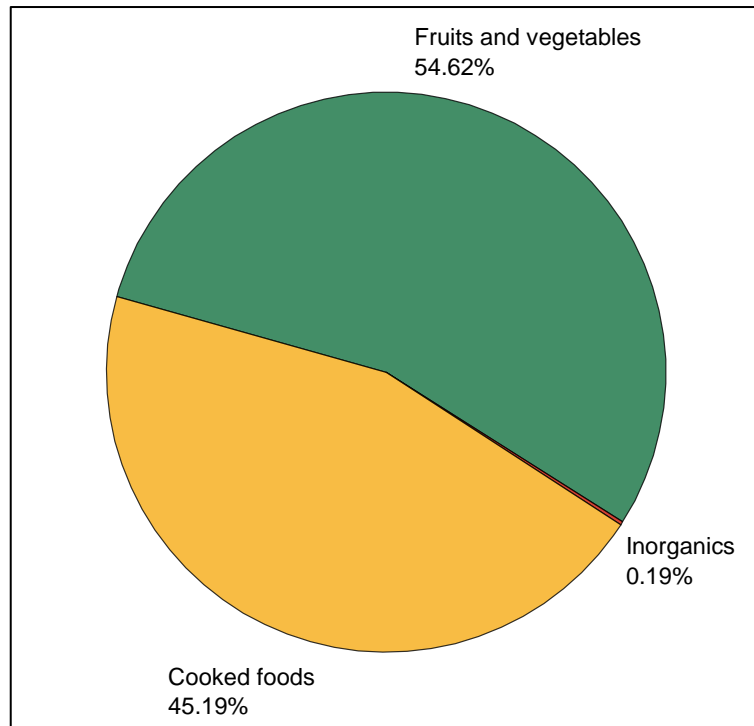
Figure 4.1. Waste composition of the Grab sample collected from the University of Leeds Refectory.

Figure 4.2. Grab sample collection and processing; A) waste sampling from the Refectory, B) sorted samples, C) processed sample, D) blended samples bagged for storage.

4.3.2 Composite sample

The composition of the waste samples collected daily from the Refectory is presented in Figure 4.3. This waste stream composed of a wide range of leftover items including fruits, vegetables, meat, bones, fish, sausages, bread, rice, paper packaging, tea bags, paper and plastic disposable cups, disposable food packs and ketchups. A total of 53.2 kg of waste was collected over the five-day collection period. Waste sorting was done daily after each collection into four categories; so as to have representative groups, since it was more heterogeneous than the grab sample.

The categories were: a) food waste, which comprised both cooked (spaghetti, rice, sausages, chicken, potatoes, mushrooms, okra and pizza) and uncooked foods (vegetables, fruit peels and bread); b) paper, which comprised paper wipes, tissue paper, paper packs, paper tea cups and a number of paper magazines; c) plastic, which comprised plastic packaging, plastic disposable spoons and cups, and plastic food wrappers; and d) others, comprising non-biodegradable materials, which were neither paper nor plastic, including wooden stirrers, metallic cutleries (probably emptied along with left-overs into the bin), tea bags and egg shells.
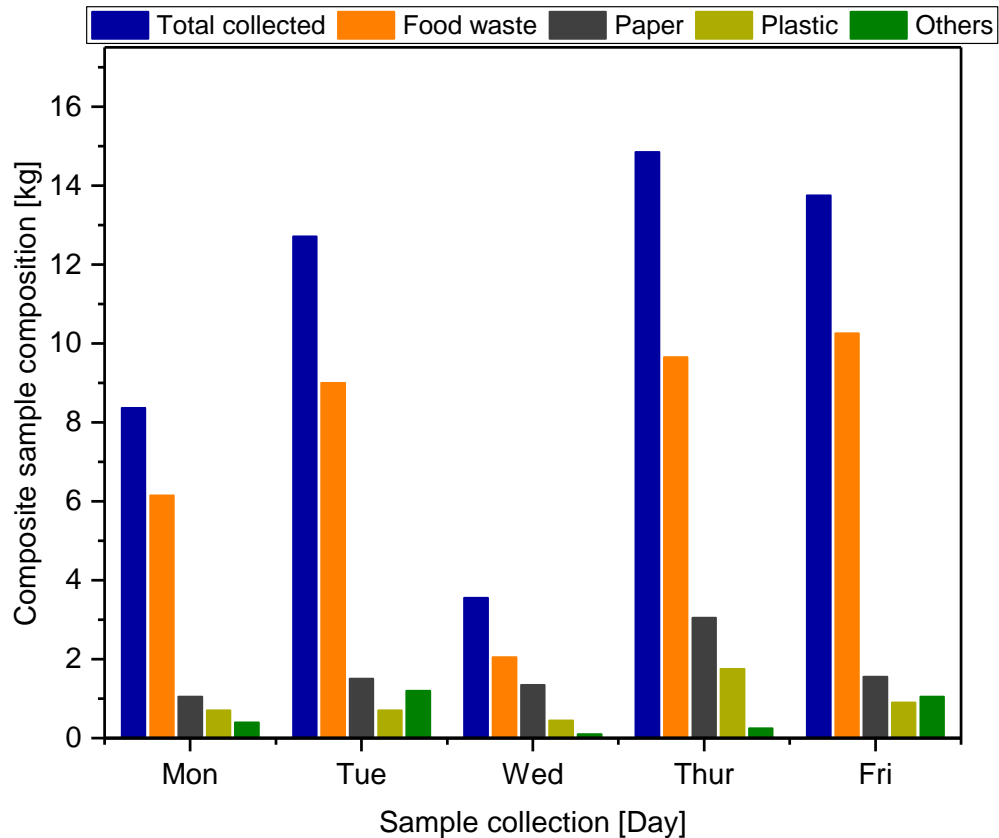
Figure 4.3. Waste composition of the composite sample collected from the University of Leeds' Refectory.

The different stages of the composite waste processing are presented in Figure 4.4. Stage A shows the separately monitored bin where all the leftovers on the plates and wastes from the cooking section were emptied; typical for each day of collection. Stage B shows the waste sorted into different categories; which was done daily after each sample collection. After sorting, all the waste categories were weighed and the food waste samples were kept aside for further processing, while the others were discarded. Stage C shows food waste outcomes having being first minced, and then blended with a nutribullet blender. The final blended sample was then stored in bags (Stage D); each weighing 500 g.

Figure 4.4. Composite sample collection and processing; A) waste sampling from the Refectory, B) samples sorted into categories, C) portions of the processed samples by first mincing followed by blending D) blended samples bagged for storage.

4.4 Analytical characterisation of processed food waste

All analytical procedures during the characterisation were carried out in triplicates; except for the metals analysis, which were run in 6 replicates. In addition, the effect of food waste PS reduction was only estimated on the physicochemical and elemental characteristics of food waste, which are the basic prerequisite analysis for AD.

4.4.1 Effect of size reduction on the physicochemical characteristics

Table 4.1 shows the physicochemical characteristics of the grab and composite food waste samples, at different PS ranges. From Table 4.1, we observe that size pre-treatment affected the characteristics of food waste. This gives a first-hand indication of the potential influence on the overall BMP process.

It was not surprising that total solids (TS) generally reduced, when the PS was reduced, since PS reduction consequently leads to a reduction in surface area. However, this difference was higher for the grab sample than the composite sample. This was perceived to be due to the different moisture contents of the two food waste streams. The high moisture content of the grab sample influenced high liquid separation to the lower PS when sieved. Meanwhile, the composite sample, which was much thicker, had relatively lower TS decrease with PS reduction. Notwithstanding, the volatile solids (VS) fraction of the TS remained approximately the same for all PS in the two food waste streams.

The chemical oxygen demand (COD) influences the degree to which a material can be digested anaerobically, therefore, its distribution among the PS is of key importance; higher COD denote higher biogas potential. Also, total kjeldahl nitrogen (TKN) and volatile fatty acids (VFA) could possibly influence the inhibition/promotion of some microorganisms inside the anaerobic digester. These three factors were also influenced by PS reduction.

Grab sample PS reduction influenced only little changes in COD, such that an overall reduction from 5 mm, through 1 mm, only yielded about 4% increase. Meanwhile, the successive reduction in the PS of the composite sample from 5 mm to 2 mm and 1 mm had COD increases corresponding to 38% and 43%. In agreement, Izumi et al. (2010), also reported increase in COD contents by 40% when food waste PS was reduced from 0.843 to 0.391 mm; even though they were much smaller PS. Typically, the applications of PS pre-treatment for AD feedstock are often reported to improve organic solubilisation and hence, the methane yield (Agyeman and Tao, 2014; Costa et al., 2016; Kim et al., 2000; Mshandete et al., 2006; Nges et al., 2016; Palmowsky and Muller, 2000; Sharma et al., 1988).

Table 4.1. Physicochemical characteristics of processed food waste on wet basis (values in bracket represent the standard deviation from the mean).

Parameter (n=3)		pH*	MC (%)	TS (g/kg)	VS (g/kg)	VS/TS (%)	COD (g-O_2/kg)	TKN (g/kg)	Total VFA (mg/kg)*
Grab sample	1mm	4.18	79.4 (0.04)	206.9 (0.4)	197 (0.4)	95.9	243.1 (24.2)	5.3 (0.1)	706 (6)
	2mm	4.18	79.1 (0.03)	209.0 (0.3)	200 (0.3)	95.9	235.5 (14.5)	4.3 (0.3)	709 (9)
	5mm	4.20	78.6 (0.25)	214.2 (2.5)	205 (1.4)	95.6	234.4 (37.8)	4.8 (0.2)	413 (26)
Composite sample	1mm	4.80	68.6 (0.02)	314.3 (0.2)	295 (0.3)	93.9	469.7 (0.0)	7.5 (0.6)	548 (23)
	2mm	4.84	68.1 (0.02)	318.7 (1.2)	300 (1.2)	94.1	452.7 (34.8)	8.6 (0.6)	501 (4)
	5mm	4.85	68.1 (0.30)	318.9 (3.0)	296 (4.1)	92.9	327.5 (17.8)	13.7 (1.0)	747 (3)
Other studies		4.10–4.71[a]	61.3–85.7[a]	217.5-294.0[a]	178.7-257.0[a]	80.6-98.2[a]	248.2–260.0[a]	11.9[a]	NR

All Measurements in wet basis. Total VFA comprise methanol, ethanol, acetic-, propionic, butyric-, iso-butyric, iso-valeric, iso-caproic, caproic and heptanoic acids.
*Dilution factor of 1 in 5 used in this study.
[a]Cited reference (Browne and Murphy, 2013; De Vrieze et al., 2013; Defra, 2010; Paritosh et al., 2017; Wang et al., 2014; Wanqin Zhang et al., 2015; WRAP, 2010)
NR – Not reported.

From Table 4.1 we can also observe that PS reduction accounted for reduced TKN contents of the composite sample; being less influenced by moisture content. It was expected that the higher changes in TS of the grab sample as a result of PS reduction, would lead to a lowering of the TKN levels, but this was not so. There was no obvious difference in TKN with PS reduction for the grab sample, perhaps, nitrogen was more available in its organic form. Hence, since the VS fraction of the grab sample was about the same for each PS, the TKN was not greatly affected.

Contrariwise, TKN reduced with reduction in the PS of the composite sample by 37.4% when reduced from 5 mm to 2 mm and 45.3% when reduced from 5 mm to 1 mm. It is not clear why this was so, perhaps, the relatively lower moisture content of the composite sample impacted on this. Water is seen as a universal solvent, which accounts for up to 90% of microbial cells by weight and the chemical reactions taking place within the cytoplasm of a cell occurs in aqueous environment (Madigan et al., 1997). As such, physical alterations to the solids and moisture content of food waste, could affect the transfer of elements within each PS range.

Similarly, while PS reduction influenced VFA increase in the grab food waste samples, reductions were observed in the composite sample. The reduction in the grab sample's PS from 5 mm to 2 mm resulted in 72% VFA increase, and a reduction from 5 mm to 1 mm resulted in 71% increase in VFA. In comparison, PS reduction of the composite sample from 5 mm to 2 mm and 5 mm to 1 mm resulted in 33% and 27% VFA decrease, respectively.

Further analysis of the composition of VFA also shows some slight variation between the two samples, as well as within the PS ranges of each sample as shown in Figure 4.5. VFA (greater than C1) generally increased with PS reduction, in both grab and composite samples. The detection of propionic acid only in the composite sample could be because propionic acid in foods mainly comes from food preservative. Hence, the grab sample which had foods mainly stored fresh, propionic acid was not detected in it. With most of the VFA distributed between acetic and butyric acids in the grab sample, it can be expected that VFA-induced inhibition would be less in the grab samples than the composite sample; wherein propionic acids was measured.
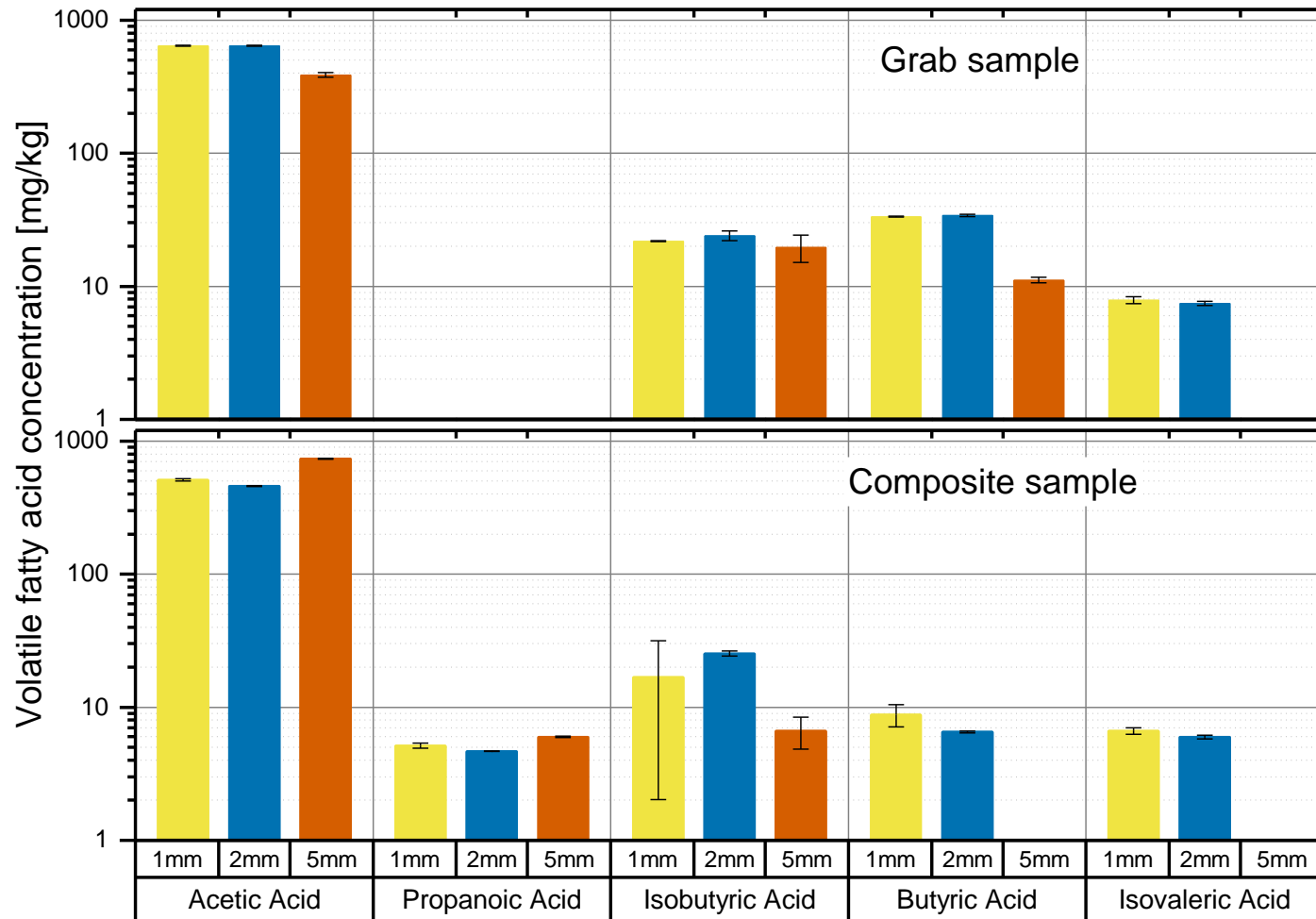
Figure 4.5. A comparison of volatile fatty acids composition between the grab and composite sample and within different particle size ranges of each sample; error bars indicate standard deviation from the mean.

Evidently, variations and sampling such as season and composition of waste collected affects the physicochemical composition of food waste, as well as the nutrient flow when PS reduction is employed. This effect was seen in Table 4.1, whereby, PS changes affected the physicochemical properties of the two food waste streams. In addition, the extent to which these changes occurred varied between the two streams, as a result of different moisture contents. Unlike the composite sample, the grab sample at all three PS ranges had relatively higher moisture content, which meant a similar degree of organic solubilisation was expected at all three levels. Thus, with the composite sample having lower moisture content, a slight change in the solids characteristic impacted greatly on the solubilisation of organic content, TKN and VFA. However, the reduction in TKN and VFA of the composite sample, implies potential reduction in ammonia and VFA-induced inhibition during AD process.

4.4.2 Effect of size reduction on elemental characteristics

Some differences were observed between the grab and composite samples; with the composite sample having higher values of each element, especially the carbon and nitrogen values (Table 4.2). For all elements, there were only very little changes in the values at different PS. However, these values on their own do not give enough information to conclude material suitability for AD. The C/N ratio and the stoichiometric representations, which were used to calculate the theoretical methane potential (TMP) are important figures use to determine whether or not the difference in the elemental values could significantly affect the AD process.

The C/N ratio increased by 29% with a PS reduction from 5 mm to 2 mm and an additional 3% increase was obtained with further reduction to 1 mm, while the composite sample PS reduction from 5mm to 2 mm only yielded 3% increase and a further reduction to 1 mm enriched an additional increase by 7%. Typically, a C/N ratio lower than 25 would potentially influence ammonia inhibition (Kondusamy and Kalamdhad, 2014), therefore, the grab sample may have a better process stability than the composite sample.

The changes in elemental composition observed in the grab sample following PS reduction can be attributed to the fact that these elements are largely chemically bound within the solids. Hence, reduction in total solids in the grab sample from 214.2 g/kg at 5mm to 209.0 g/kg and 205.9 g/kg at 2mm and 1mm respectively, resulted in a reduction in the elemental characteristics.

Table 4.2. Elemental characteristics of food waste samples (values in bracket represent standard deviation from the mean)

	Grab sample			Composite sample			Other studies
Element (n=3)	1mm	2mm	5 mm	1 mm	2mm	5 mm	
N (% of TS)	2.09(0.02)	2.10(0.03)	2.96(0.03)	4.44(0.10)	4.69(0.10)	4.85(0.07)	2.35 – 3.42[a]
C (% of TS)	47.47(0.57)	46.57(0.30)	50.87(0.07)	53.19(2.12)	53.02(0.73)	53.06(0.37)	32.85– 48.42[a]
H (% of TS)	7.63(0.11)	7.21(0.22)	7.21(0.14)	7.87(0.23)	7.79(0.09)	7.79(0.10)	6.9 – 7.03[a]
S (% of TS)	ND	ND	0.12(0.01)	0.33(0.18)	0.16(0.03)	0.13(0.03)	0.15 – 0.44[a]
O (% of TS)	42.80(0.69)	44.12(0.53)	38.83(0.24)	34.17(2.51)	34.35(0.92)	34.18(0.51)	34.13– 34.3[a]
N (g/kg-TS)	4.31	4.38	6.34	13.95	14.94	15.45	NR
C (g/kg-TS)	97.73	97.34	108.97	167.21	168.99	169.20	NR
H (g/kg-TS)	15.72	15.07	15.45	24.72	24.82	24.83	NR
S (g/kg-TS)	0.00	0.00	0.27	1.04	0.51	0.40	NR
C/N	22.7	22.2	17.2	12.0	11.3	10.9	14.7 – 24[b]
PSB$_{bio}$ (%)	0.00	0.00	0.09	0.23	0.11	0.09	NR
Empirical formula	$C_{26.5}H_{51.1}O_{17.9}N$	$C_{25.9}H_{47.2}O_{18.5}N$	$C_{20.1}H_{34.1}O_{11.5}N$	$C_{13.7}H_{24.4}O_7N$	$C_{13.2}H_{23.3}O_{6.4}N$	$C_{12.8}H_{22.5}O_{6.2}N$	NR
TMP (mL/gVS)	515.65	483.91	547.90	588.63	601.23	608.43	551 – 617.9[c]

[a]Source – Defra, 2010b; L. Wang et al., 2014; Wanqin Zhang et al., 2015; [b]Source – Browne and Murphy, 2013; Pagliaccia et al., 2016; L. Yang et al., 2015; Zhang et al., 2007; [c]Source – Wanqin Zhang et al., 2015; Wanli Zhang et al., 2015
NR – Not reported
TMP – Theoretical methane potential; PSB$_{bio}$ – predictable H_2S–S content in biogas

However, with the composite sample, whereby, PS pre-treatment influenced a TS reduction from 318.9 g/kg at 5mm to 318.7 g/kg and 314.3 g/kg at 2mm and 1mm respectively, the elemental characteristics were not greatly influenced. Therefore, the effect of PS on elemental distribution of food waste was influenced by the TS (or moisture) content of the original sample; so that samples with lower moisture content might require further PS reduction compared to samples with higher moisture content, in order to achieve significant changes in C/N ratios.

The TMP for both grab and composite samples at 5 mm PS were not so different, however, PS reduction impacted a reduction in TMP of both samples. Unlike the grab sample, the moisture content at all PS of the composite sample remained similar, on which basis no obvious change in TMP was observed with further reduction in PS. Notwithstanding, the increase in C/N ratio with PS reduction implies that a higher degradability was obtainable from both samples

The predictable H_2S–S content in biogas (PSB_{bio}) is the inherent ability of a feedstock to release H_2S during AD (Peu et al., 2012). Under normal conditions, methanogens and sulphate reducing bacteria have similar growth pattern, therefore, sulphur and carbon have similar degradability during AD (O'Flaherty et al., 1998). On this basis, the predicted biogas H_2S–S was determined by taking the molar ratio between total sulphur and carbon contents of the food waste according to Equation 4.1 (Peu et al., 2012).

$$Theoretical\ PSB_{bio}\ (\%) = \frac{\left(\frac{S}{32}\right)}{\left(\frac{C}{12}\right)} \times 100 \qquad\qquad 4.1$$

Where 32 and 12 are the molar masses of sulphur (S) and carbon (C) respectively.

For both grab and composite samples, the PSB_{bio} ranged between 0.00 to 0.23 % (Table 4.2). This means with complete sample digestion, only trace amounts of hydrogen sulphide was expected in the biogas. The PSB_{bio} was observed to increase with PS reduction of the composite sample, due to the initial increase in elemental sulphur content. Hence, the disadvantage of PS reduction could be with the potential increase in H_2S content of the biogas, but because this is only a small fraction, it's effect becomes relatively negligible.

In general, the elemental characteristics of the two waste streams were similar to values reported for food waste in literature (Defra, 2010b; L. Wang et al., 2014; Wanqin Zhang et al., 2015).

4.4.3 Biochemical characteristics of food waste streams

The biochemical characteristics of both the grab and composites samples at 5 mm PS range are given in Table 4.3. As with the physicochemical characteristics, the biochemical composition of the grab and composite samples clearly differ. The biochemical composition of the two food waste streams was a reflection of the kinds of food collected in each sample. The grab sample mainly composed of fruits, vegetables and cooked food (largely potatoes and rice), which influenced a higher percentage of carbohydrates. The composite sample was more heterogeneous, with good portions of a wide variety of food; including protein-rich foods such as meat and fish, thus, higher levels of protein, lipids and ash were recorded. While the higher lipid content of the composite sample could influence a higher methane yield, the high protein content implies a higher potential for ammonia-induced inhibition. Overall, the biochemical characteristics of both samples were within the upper limits reported in literature (Browne and Murphy, 2013; Esteves and Front, 2010; Paritosh et al., 2017).

Table 4.3. Biochemical characteristics of food waste samples on dry basis (values in bracket represent standard deviation from the mean).

Substance	n	Grab sample	Composite sample	Other studies[a]
Carbohydrates (wt %)	3	57.5 (1.7)	43.3 (0.6)	9.3 – 59.0
Proteins (wt %)	3	14.3 (0.8)	23.6 (1.1)	3.9 – 21.8
Lipids (wt %)	3	24.3 (0.5)	26.9 (1.5)	4.9 – 24.1
Ash (wt %)	3	3.9 (0.7)	5.2 (0.2)	1.2 – 5.9

[a](Browne and Murphy, 2013; Paritosh et al., 2017; Esteves and Front, 2010)

4.4.4 Metals characteristics of food waste streams

There are a number of ways metals get into foods and consequently, food waste. The sources of metals in food according to Reilly (2008) includes:

i) Soil – by direct plant uptake, agricultural practices such as, the use of fertilizers, sewage sludge and agrochemicals, and industrial contamination from surface runoff to water bodies (assimilated aquatic animals). Furthermore, leachates from mining operations and metal industries, emissions from coal burning and reuse of abandoned metal sites for agriculture are potential sources.

ii) During food processing – as a result of food contact with plant and equipment, during tin and aluminium canning. Also from catering

operations using metal cookware, coffee percolators, enamelled and ceramic wares. Printing and decorations on food and beverage containers, prints and colourings on plastic vessels and wraps also add to metals in foods.

iii)   Food fortification – by the addition of certain nutrients (including trace elements) for food quality preservation, such as ready to eat breakfast cereals, engineered foods and natural fortification with metals.

Typically, the soil (and water for aquatic life) is the primary source of trace metals in food waste, which, gives a representation of the environmental activities and the nature of the soil where it was grown. As such, metals in food can vary significantly within different locations. Notwithstanding, food waste are generally reported to lack sufficient trace elements required for optimum AD (Banks et al., 2012). Therefore, analysis of metals in food waste is important; in order to identify key metals' deficiency and potentials for process inhibition.

The metals concentration of the grab and composite food waste samples are summarised in Table 4.4, along with values typical for UK foods. Although, the metals' levels of both the grab and composite samples are within typical ranges reported for UK grown foods and similar to values reported in literature, but like other characteristics previously discussed, the trace metal contents were different for each food waste sample. Higher concentrations of TEs were measured in the grab sample, which can be attributed to the large proportion of bread and vegetable waste (as shown in Figure 4.2). Thus, the composite sample which had a good proportion of the different categories of food, had lower levels of trace elements.

Table 4.4. Metals concentration in food waste samples of this study in comparison with other studies.

Metal	This study (mg/kg fresh weight)[a]		Concentration of metals in foods within the UK[b] (mg/kg fresh weight)	Food categories[b]		
	Grab	Composite		Lower limit	Middle	Upper limit
Mn	4.9 (0.2)	2.0 (0.3)	0.02 – 8.0	Oil, fat	Fruit/vegetables	Bread/cereal
Fe	16.8 (2.1)	13.4 (1.9)	0.4 – 69	Beverage, oil, fat	Vegetables	Bread
Co	0.017 (0.005)	0.009 (0.001)	0.002 – 0.09	Fruit product	Green vegetables, meat products	Nut, offal, bread
Ni	1.0 (0.3)	0.5 (0.09)	<0.02 – 2.5	Dairy product, fresh fruit	Canned and green vegetables, preservatives	Nuts
Cu	8.0 (0.7)	4.0 (0.4)	0.05 – 40	Dairy product, oil, fat	Meat, bread, fruit	Nuts, offal
Mo	0.1 (0.02)	0.1 (0.01)	0.003 – 1.2	Fresh fruits	Green vegetables	Nut, offal
W	0.03 (0.007)	0.01 (0.003)	-	-	-	-
Zn	9.7 (2.3)	15.2 (2.6)	0.3 – 51	Beverages, fresh fruit, fruit products	Vegetables, milk	Meat, meat products, nuts
Se	ND	1.3 (0.3)	0.4 – 492	Beverages, fruits and vegetables	Bread, cereals, oil, fats	Fish, meat, offal, nuts
B	7.1 (2.3)	ND	<0.4 – 14	Carcass meat, offal, milk, eggs	Bread, cereals, fish, beverages	Fresh fruits, fruit products, nuts
Pb	0.2 (0.04)	0.2 (0.08)	<0.01 – 0.10	All foods	All foods	All foods

[a]Standard deviations in this study given in brackets. [b]Adapted from Reilly (2008).

Trace elements including iron (Fe), selenium (Se), cobalt (Co), tungsten (W), nickel (Ni), Molybdenum (Mo) and copper (Cu) have been reported to be crucial for AD (Wanqin Zhang et al., 2015). Furthermore, the key enzymes driving most of the anaerobic reactions are composed of heavy metals, with composition reported in the order: Fe>>Zn>>Ni>Co=Mo>Cu (Chen et al., 2008). Except for selenium, which was not detected in the grab sample, the key TEs concentrations were generally higher in the grab sample than in the control. Hence, although the composite sample had a higher TMP, it is also more likely to exhibit inhibitions due to TEs deficiency.

4.5 Impact of PS and ISR on the anaerobic digestion process

From the food waste characterisation experiments, it was clear that PS reduction impacts on some key AD factors such as C/N ratio, COD and TKN, therefore, the BMP experiments were set up for different food waste PS using the grab sample. In addition, the interaction between the food waste PS and ISR and the effect on the BMP process was analysed, so as to obtain an optimised condition for future BMP analysis.

4.5.1 VFA degradation

Considering that each experiment for the respective PS were set up differently; with different initial VFA concentration, the rate of VFA degradation was normalised against the initial concentration on the day of set up (Day0); as shown in Figure 4.6. By so doing, each experiment had a starting value of 1 and higher values could signify either of two things;

i)   The rate of VFA consumption was lower than the rate of VFA accumulation; such that, an increased rate of VFA consumption would bring this value closer to or lower than 1 and;

ii)   The amount of VFA produced during fermentation was relatively higher; such that, the higher values become a function of initial VFA produced rather than the rate of consumption.

The latter implies that such reactors would yield more methane if all the VFA were eventually consumed. But this was hardly the case with higher food waste PS (especially 5 mm), which although had the highest VFA peaks, produced the least amount of methane. Therefore, a reduction in PS

influenced faster VFA consumption, which led to reduced accumulation according to the former assumption.
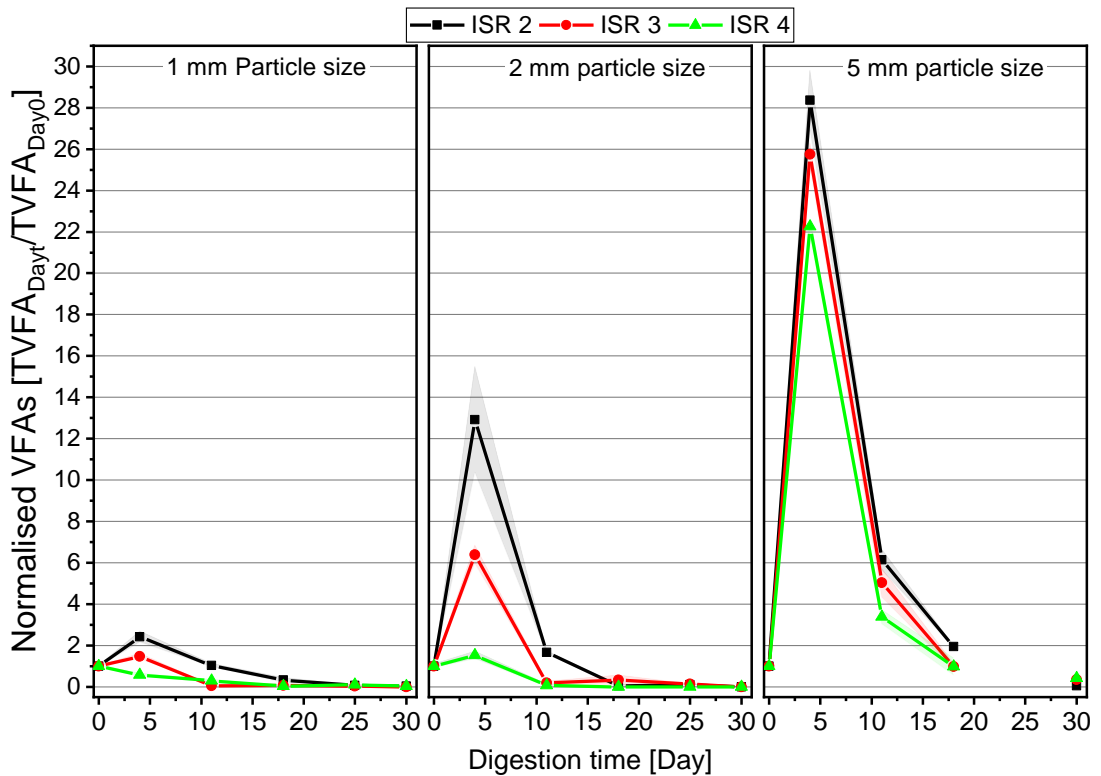
Figure 4.6. Total VFA degradation curves for PS and ISR optimisation experiments, normalised against the initial concentration at Day0. Disconnection between Day 30 and the rest of the data sets was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

In Figure 4.6, we observe that VFA accumulated up to as much as 30 times the initial concentration when 5 mm PS was employed. This reduced significantly with 2 mm PS treatment, which had VFA accumulation measuring up to 13 times its initial concentration. Further reduction to 1 mm PS resulted in VFA accumulating only less than 3 times its initial concentration. This is further explained by the lag in initial methane production within the early days of digestion at 5 mm PS for each corresponding ISR (discussed in section 4.5.3). This means with 5 mm PS, methane production progressed at an 'inhibited steady-state'; whereby, the process continued at a stable rate, but with low methane production (Angelidaki et al., 2016).

PS reduction influenced rapid consumption of VFA within the reactors at all ISR treatments. Within each PS, however, the VFA pattern at different ISR was a function of the amount of VFA produced during fermentation. It was not a surprise to observe that the VFA accumulation was higher at lower ISRs for all three PS in the ISR order 2 > 3 > 4. Considering lower ISRs meant relatively

more food waste loading within the same PS experiments, the VFA levels increased at lower ISR during fermentation.

The variation in ISR within each PS treatment was beneficial in identifying possible PS and ISR combinations that could help decrease the lag in methane production. Apparently, the reduction in food waste PS increased the amount of VFA produced, as well as its rate of consumption. This was due to increased solubility and microorganisms' access to feed by virtue of the increase in surface area.

Acetic and propionic acids are the main precursors to methane production (Zhang et al., 2014). To minimise the VFA-induced inhibition, a P/A ratio of 1.4 have been set as a benchmark (Buyukkamaci and Filibeli, 2004; Marchaim and Krause, 1993). The P/A trends for all BMP assays are shown in Figure 4.7.
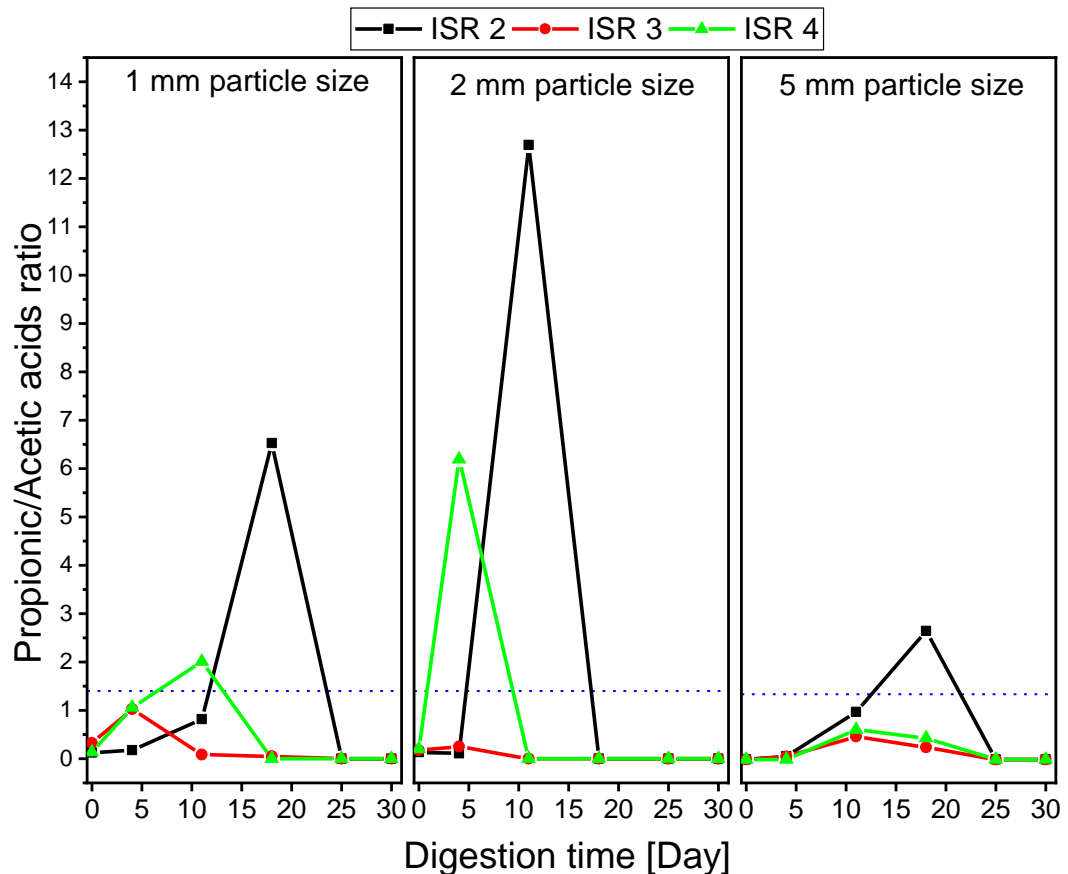
Figure 4.7. Propionic to acetic acid ratios for PS and ISR optimisation experiments using the grab sample; dotted lines indicate the acceptable limit of 1.4.

For 1 mm PS treatment (Figure 4.7), a combination with an ISR of 3 maintained the P/A below the 1.4 line. The increase observed with ISR 2 at the later stage of digestion was as a result of higher rate of propionic acid

accumulation compared to the rate of consumption by microorganisms responsible. However, the increase observed at ISR 4 could be due to higher rate of acetic acid degradation; since the system had high microbial presence. PS treatment of 2 mm (Figure 4.7) also showed similar trends as observed with 1 mm PS, only larger effects were observed.

However, for 5 mm PS, the P/A was very much lower at all ISR compared to values obtained at 1 mm and 2 mm PS treatments. Because the hydrolysis for 5 mm was not accelerated, the rate of acidogenesis was not excessively higher than the rate of acetogenesis. Hence, propionic and acetic acids accumulated at a similar rate, except with ISR of 2, which had a high organic load. Therefore, with PS reduction, the rate of acetic acid degradation was perceived to be higher than the rate of propionic acid degradation, which tended towards P/A levels higher than the 1.4 limit. However, at an ISR of 3, this was effectively managed below the threshold value at all PS.

4.5.2 Analysis of alkalinity and pH

The Alkalinity curves presented in Figure 4.8, illustrate the buffering capacity of respective digester contents to resist sudden changes in pH that would make it become more acidic (Fonoll et al., 2015).
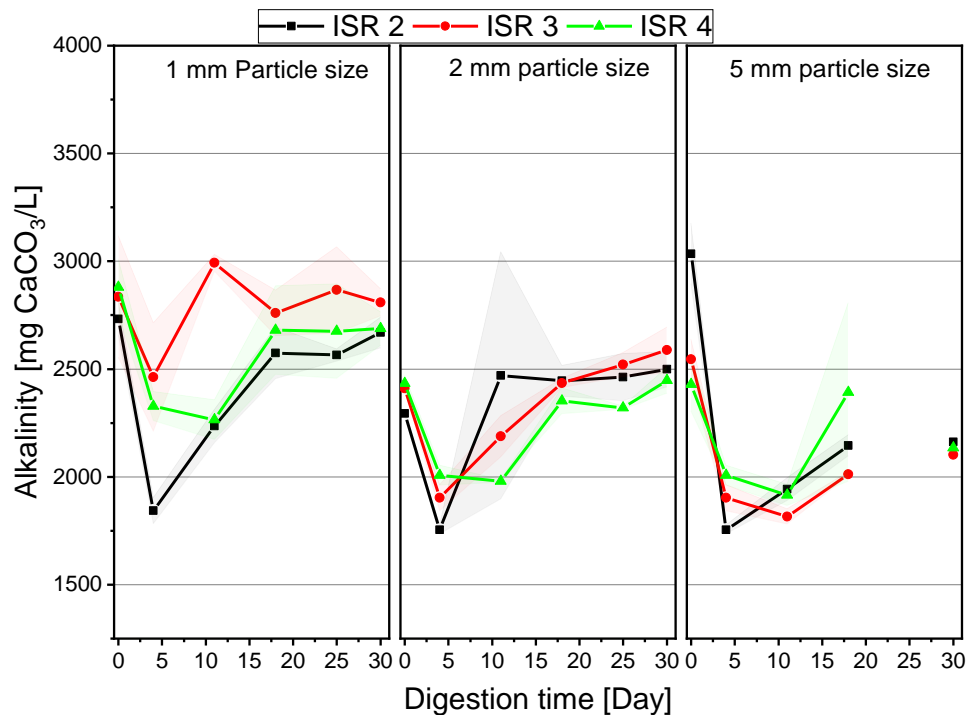
Figure 4.8. Alkalinity curves for PS and ISR optimisation experiments using the grab sample different PS and ISR treatments. Disconnection between Day 30 and the rest of the data sets was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

The initial reduction in alkalinity after all experimental setup, follows the production of VFA, for which some alkalinity was lost to buffer the low pH associated with acidification. Hence, as VFA were consumed, the alkalinity recovered. This implies, a continuous reduction in alkalinity can be an indication of inhibition of methanogens to convert the organic acids, thereby, leading to accumulation (Chen et al., 2015), or a limited production of alkalinity from the digested substrate (Appels et al., 2008).

For smaller PS of 1 mm and 2 mm, lower alkalinity recovery rates were observed at ISR of 2 and 4. The low recovery rates at ISR 2 supports the corresponding VFA accumulation and availability at this ISR for a long time during the digestion period. While the low recovery rates at ISR of 4 can be attributed to the limited amount of food waste to supply alkalinity from the release of carbon during degradation at this ISR. The reactors with low PS of 1 mm and 2 mm had better alkalinity recovery at ISR of 3 during the BMP process, which was perceived to have also contributed to a stable digestion.

With 5 PS mm, the initial high VFA accumulation impacted on relatively low alkalinity. Moreover, the higher organic loading and resulting acidification at ISR of 2 induced even lower alkalinity accordingly. However, low alkalinity recovery rates were observed at all ISRs. This would imply that lower carbon releases ensued with 5 mm PS decomposition. This supports the observations in Section 4.5.1 that hydrolysis rate was least with 5 mm PS, which also agrees with other studies relating to the impact of PS reduction on solubilisation (Agyeman and Tao, 2014; Costa et al., 2016; Kim et al., 2000; Mshandete et al., 2006; Nges et al., 2016; Palmowsky and Muller, 2000; Sharma et al., 1988).

The pH levels for the grab sample BMP experiments are shown in Figure 4.9. The pH levels were reflections of the VFA and alkalinity profiles of each respective reactor, such that, lower pH levels were associated with higher VFA production and/or accumulation during the digestion period. High VFA accumulation at 5 mm PS caused lower pH within the corresponding reactors; especially at ISR 2, which had the highest VFA peak. Reactors with PS 1 mm and 2 mm showed better pH stability at all ISRs.
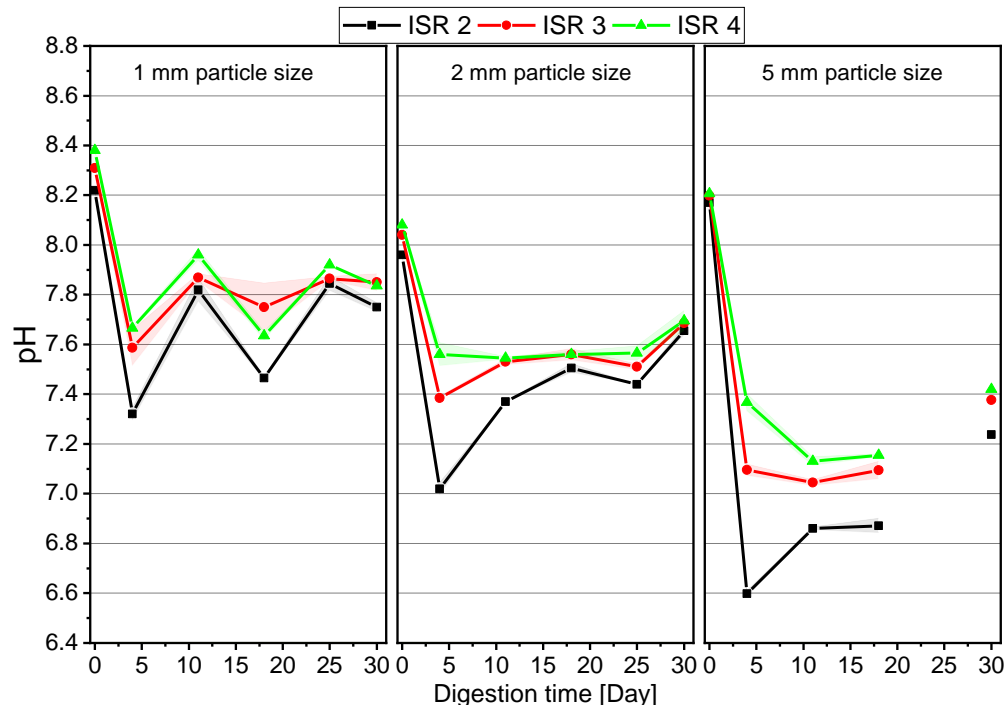
Figure 4.9. A comparison of pH patterns for PS and ISR optimisation experiments using the grab sample. Disconnection between Day 30 and the rest of the data sets was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

All enzymatic activity during AD is controlled by pH, and for stable AD, a pH between 6.8 to 7.4 is desired to allow coexistence of all acting groups (Kumaran et al., 2016; Mao et al., 2015). Although at ISR 2 significant drops in pH were observed, they, however, were recovered and maintained within optimal limits. In agreement with Defra (2010b) and Holliger et al. (2016), ISR below 3 has a high propensity to progress at acidic pH especially if it were employed in a continuous system.

4.5.3 Process kinetics and biomethane yields

Origin® 2016 graphical and statistics software was employed to fit the cumulative methane yields, and to derive the production rate (k-value, day^{-1}) for all observations, using its in-built standard Gompertz (SGompertz) model. The SGompertz model was then modified according to the modified Gompertz (MGompertz) bacterial growth model to derive other kinetic parameters such as the lag phase and maximum specific methane yield (Zwietering et al., 1990).
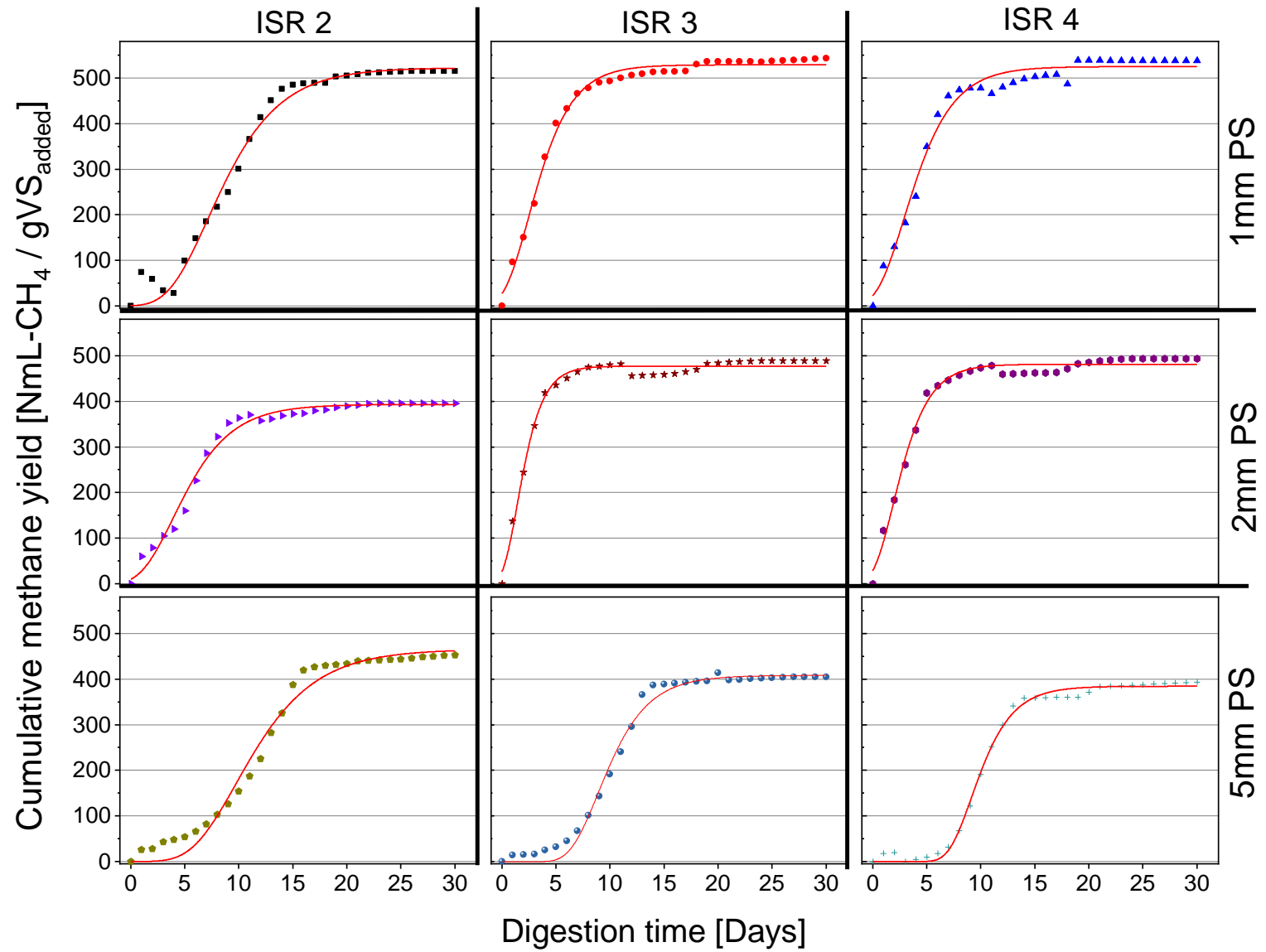
Figure 4.10. Biomethane yields from BMP experiments with grab sample fitted with MGompertz model (red lines).

Table 4.5. Process kinetics and biodegradability.

PS	ISR	K-value (Day^{-1})	R^2	Lag phase (Day)	Technical digestion time, T80 (Day)*	Theoretical methane potential (mLCH$_4$/gVS$_{added}$)	Experimental methane yield (mLCH$_4$/gVS$_{added}$)	Percentage biodegradability (%)
1 mm	2	0.27	0.99	3.5	12 (411.7)	515.7	514.6	99.8
	3	0.43	0.99	0.2	7 (434.2)	515.7	542.8	105.3
	4	0.40	0.98	0.4	6 (430.7)	515.7	538.3	104.4
2 mm	2	0.33	0.99	0.9	8 (316.6)	483.9	395.7	81.8
	3	0.53	0.99	0.1	5 (395.1)	483.9	493.8	102.1
	4	0.74	0.99	0.1	4 (390.8)	483.9	488.5	100.9
5 mm	2	0.25	0.98	5.8	15 (362.3)	547.9	452.9	82.7
	3	0.39	0.99	6.3	13 (323.8)	547.9	404.7	73.9
	4	0.46	0.99[a]	7.0	13 (314.7)	547.9	393.4	71.8

*Values in brackets represents the methane yield in mLCH$_4$/gVS$_{added}$, at the respective T80 point.
[a]R^2 values greater than 0.995, reported as 0.99

In recent years, the MGompertz model have been successfully employed for fitting cumulative methane yield, under the premise that methane production is directly proportional to bacterial growth (Boulanger et al., 2012; Meng et al., 2015; Pagliaccia et al., 2016; Pan et al., 2013, 2016; Ranjan et al., 2015; Wall et al., 2013; Wöhler-Geske et al., 2015; Xie et al., 2011; Zhao et al., 2014; Zhou et al., 2013).

The MGompertz fitting of the cumulative yields is presented in Figure 4.10, while k-values and lag time values are presented in Table 4.5. According to Table 4.5, the MGompertz growth model fit well into all experimental methane yields, with R-squared values in the range of 0.982 to 0.996. Regarding substrate PS, the first working document by ABAI-TG for the IWA, stated that it was fundamental for process kinetics rather than the actual determination of BMP (Angelidaki et al., 2009). This is in partial agreement with this study, whereby, both the kinetics and BMP were improved by food waste PS reduction.

The k-values at all PS and ISR combinations ranged from 0.25 to 0.74 day^{-1}, which is similar to k-values obtained from BMP experiments with food waste in the range of 0.28 to 0.45 day^{-1} (Pagliaccia et al., 2016). The interaction between the PS and ISR influenced the k-values, such that, higher values where obtained at ISRs 3 and 4.

It was observed that low ISR and low PS (and vice versa) was not a suitable combination, due to increases in lag phase and relatively lower cumulative methane yield. A lag time of 0.4 day was reported by Meng et al. (2015), and Pagliaccia et al. (2016) reported that when the initial pH was set to pH 8 and pH 7 (ISR of 1.67), lag times of 0.17 and 0.77 days were obtained respectively. However, Pagliaccia et al. (2016) added water to the food waste before homogenising at a ratio 1:1, which could have significantly affected the kinetic process.

Overall PS reduction from 5 mm to 1 mm resulted in up to 38% methane increase. And within each PS experimental group, varying the ISR also improved the methane yield. Thus, 5%, 25% and 15% increase in methane yields were obtained at 1, 2 and 5 mm when ISR was optimised respectively. Similarly, Mshandete et al. (2006) reported 23% increase in methane yield from sisal fibre waste when it was reduced from 100mm to 2mm. Izumi et al. (2010), also stated that smaller mean PS of food waste increased methane yield by 28%, as a result of enhanced solubilisation, when the mean PS was reduced from 0.843 to 0.391 mm. In a study on the effect of PS on thermophilic AD of food waste, Kim et al. (2000) concluded that PS was one of the most

important factors of food waste AD, after they observed an inverse relationship between food waste and maximum substrate utilisation rate, for PS reduction from 2 mm to 1.02 mm. Although, these studies were conducted at largely varied PS ranges, they all attributed PS reduction with increase in biomethane yield due to enhanced substrate solubilisation.

The lowering of the lag time following food waste PS reduction in this study, greatly improved the anaerobic biodegradability, resulting in up to 105.3% biodegradability compared to 82.7%, which was the maximum obtained at 5 mm PS. Furthermore, the technical digestion time (T80), which is the time required to achieve 80% of the total methane yield was greatly reduced with the reduction in PS. For instance, the T80 at ISR of 2 was 12, 8 and 16 days for PS 1, 2 and 5 mm respectively, at ISR of 3, they were 7, 5 and 13 days and at ISR of 4, they were 6, 4 and 13 days respectively. This means that shorter hydraulic retention times can be achieved with food waste PS reduction.

Lower T80 were achieved at 2 mm PS compared to 1 mm, as a result of a lower cumulative yield at 2 mm. Although, for 1 mm PS, the T80 were longer, the yields at these points were up to 30% higher than the corresponding yields from 2 mm PS. For 5 mm PS experiments, the T80 was high at all ISR, with corresponding low yields at these points. Hence, food waste PS reduction to as low as 1 mm improve methane yields and digester performance.

In agreement, Kim and his cohort in 2000 reported a decrease in maximum substrate utilisation rate from 0.0033 hr^{-1} to 0.0015 hr^{-1}, with an increase in food waste PS from 1.05 mm to 2.14 mm respectively. Izumi et al. (2010) also documented significant effect on methane yield with size pre-treatment, as a result of increase in total oxygen demand of up to 40%; with maximum methane yield obtained at PS of 0.6 mm. Palmowski and Muller (2000), studied the influence of PS reduction on biogas production using two waste types; high fibre substrate with low degradability (50% without size reduction) and substrates with high degradability (at 88% and 95%). Contrary to other studies, they reported that size reduction had no significant effect on the biogas yield when treating substrates with high degradability (such as food waste), but with high fibre substrates, a significant increase of 20% was observed in the biogas yield. They stated the reason could be because the highly biodegradable feedstock were already accessible by the microorganisms, hence, size reduction did not produce significant change. However, their study did not specify the final PS range of the reduced food-related waste.

4.5.4 Statistical analysis of PS and ISR interaction on methane yield

The Minitab 18 statistical software was employed to design and analyse the experiments, using a 2 factor, 3 levels (3^2) factorial design. The results obtained buttresses the arguments established in previous sections. Using the experimental yields, a response surface regression was conducted for the cumulative methane yield versus the ISR from the 3^2 factorial DOE (n=18 and $R^2 = 0.63$), which produced Equations 4.2 to 4.4 (Where P = PS). These equations were then used to predict the cumulative methane yields with PS 3 mm and 4 mm as shown in Figure 4.11.

$$Cumulative\ methane\ yield\ at\ ISR\ 2 = \ P^2 - 6.74P + 39.08 \qquad 4.2$$

$$Cumulative\ methane\ yield\ at\ ISR\ 3 = \ P^2 - 8.52P + 45.60 \qquad 4.3$$

$$Cumulative\ methane\ yield\ at\ ISR\ 4 = \ P^2 - 8.64P + 45.44 \qquad 4.4$$

The interaction plot of PS and ISR on the cumulative methane yield further demonstrates that a combination of large PS and high ISR (and vice versa) was not suitable for food waste BMP analysis. This was especially observed at PS 1 mm and 5 mm, whereby, the methane yield increased with an increase in ISR at 1 mm and an opposite effect observed at 5 mm. Generally, the biomethane yields reduced with an increase in food waste PS. Because, PS reduction increases surface area and consequently, the rate of VFA production, higher ISR helped to reduce excessive acidification from VFA production. This helped to reduce the backward inhibition caused by high VFA concentration on the methanogens, hence, the variation in ISR for each PS treatment significantly influenced the biomethane yield. For lower PS below 3 mm, a higher amount of microorganisms (inoculum) was required to consume the VFA produced. While for PS higher than 3 mm, a lower amount of microorganism was required to reduce excessive competition for limited substrates solubilised. Therefore, from Figure 4.11 we can identify the combinations at which a relatively balanced fraction of substrate to acting microbial load can be achieved during food waste digestion. And where the cost of PS reduction is a limiting factor, then the ISR at the working PS can still be optimised.
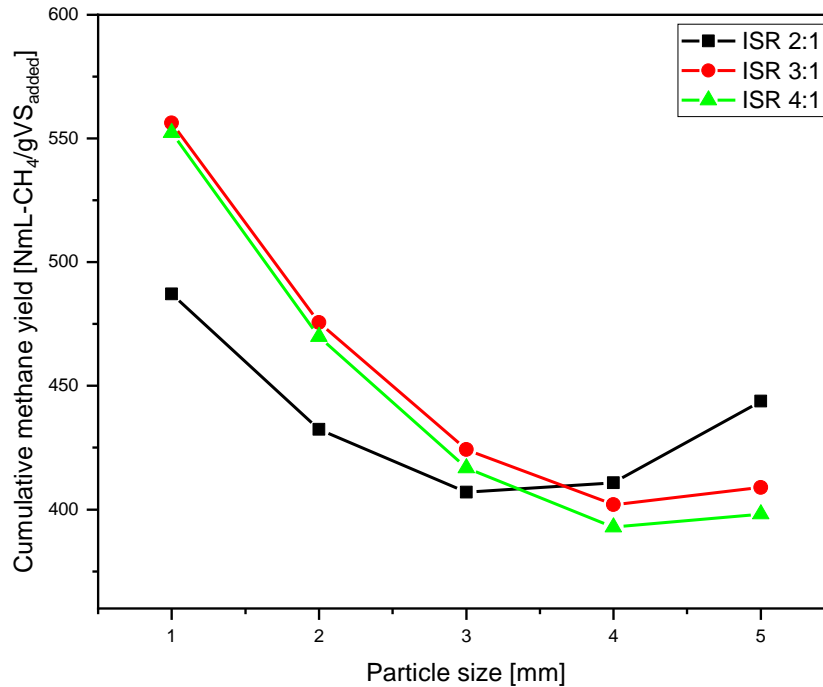
Figure 4.11. Predictive cumulative methane yield for food waste PS between 1 mm and 5 mm, using data from response surface regression equations.

Additional statistical analysis of means (ANOM) was used to understand the degree of influence the two factors had on the biomethane yield. Points outside of the decision limits (red lines), denotes that the mean at that point was significantly different from the grand mean. Hence, from Figure 4.12 it was established that both ISR and PS variations impacted on the methane yield, however, PS pre-treatment had the most significant effect on the methane yield.
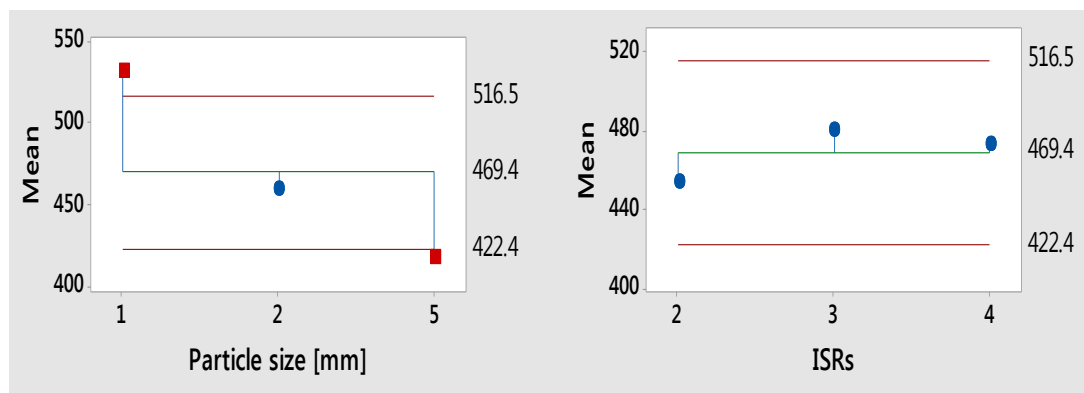
Figure 4.12. Two-way normal ANOM for mean of cumulative methane yield at α = 0.05 (as obtained from Minitab software)

According to the findings from this study, reducing the PS from 5 mm to 1 mm improved the methane yield, with the most yield obtained at 1 mm and the least obtained at 5 mm PS. In line with this, Kim et al. (2000) also established

that PS was one of the most important factors in AD of food waste. The findings from this study are also in agreement with the study on three food waste PS at 2.5 mm, 4 mm and 8 mm, whereby, methane production rate, specific yield and digestate dewaterability were highest at 2.5 mm PS (Agyeman and Tao, 2014). Therefore, food waste PS 1 mm at ISR of 3 was accepted as the most suitable combination for further BMP analysis of food waste using the composite sample.

4.6 Composite versus grab sample BMP process

Based on the initial characteristics of the grab and composite samples and the BMP experiments using grab sample, experiments were conducted with composite sample at the optimal conditions reached with the grab sample experiments. This was done with the assumption that the grab sample had better BMP characteristics in terms of the C/N ratio and metals content. As such, optimal conditions reached with grab sample could be replicated with the composite sample, rather than running the same series of experiments. As in the grab sample, BMP experiments were set up with composite sample at 1 mm PS at an ISR of 3.

4.6.1 VFA degradation and methane yield

As speculated, based on the initial characteristics of the grab and composite samples; such as a C/N ratio of 22.7 and 12.0 for the grab and composite samples at 1mm respectively, and relatively lower concentration of essential TEs in the composite sample, the BMP experiments with the composite sample progressed slower than with the grab sample as shown in Figure 4.13a. For the period when the VFA concentration remained very high (Figure 4.13b), the methane production progressed extremely slowly with the composite sample.

From the normalised VFA curve (Figure 4.13c), we observe that the VFA for the composite sample BMP experiments accumulated to as high as 34 times its initial concentration at setup. This value is similar to those obtained using the 5 mm grab sample. It therefore, supports the argument established in Section 4.4.2, that food waste with lower moisture content, might require extensive PS reduction to attain favourable AD process.

Acetic-, propionic- and butyric acids were the main acids that accumulated through time, with acetic acid alone accounting for about 75% of the total VFA within the first two weeks of digestion. This implies the acetoclatic

methanogenesis was probably the rate limiting step for the BMP process using the composite sample. The lower C/N ratio of the composite sample possibly led to high ammonia concentrations. And the essential TEs that could have helped the acetoclastic methanogens to utilise the VFA; despite the ammonia concentrations, were insufficient. Hence, acetoclastic methanogenesis was perceived to have been inhibited during the AD of the composite sample.

The excessive VFA accumulation with the composite sample, resulted in a longer T80 for the composite sample of 17 days, compared to 7 days for grab sample. Hence, although, the composite sample had a higher TMP, the methane yields from both samples were about the same at 542.8 and 544.6 $mLCH_4/gVS_{added}$ for the grab and composite sample respectively.
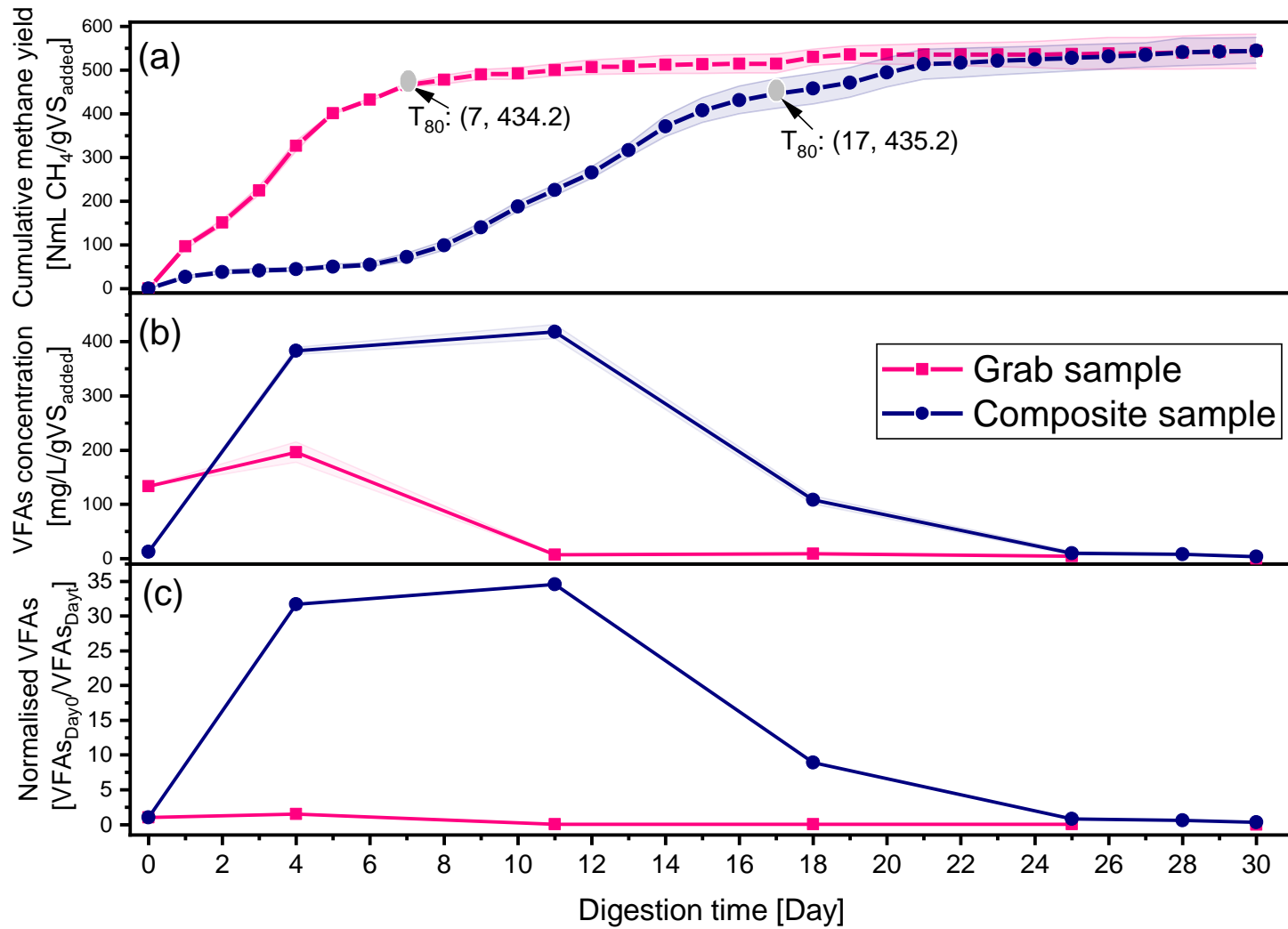
Figure 4.13. Cumulative methane yields from the grab and composite sample (a), in comparison with VFA trends for each corresponding sample (b and c). Shaded area around lines represent standard deviation from mean.

4.6.2 Analysis of pH and alkalinity

The pH and alkalinity curves for the BMP experiments using the grab and composite samples are shown in Figure 4.14. The excessive build-up in VFA influenced a relatively lower pH in the reactors digesting the composite sample (Figure 4.14a). This however, remained within the optimal pH range for AD, so that other factors; such as backward VFA inhibition on the acetoclastic methanogens, could have led to the lag in methane yield for the composite sample. The pH curves further depicts that the reactor treating the grab sample had quicker VFA depletion, while VFA accumulated for longer within composite sample reactor. The alkalinity trend was not so different between the two samples, thereby, implying a similar buffering effect within the grab and control reactors.
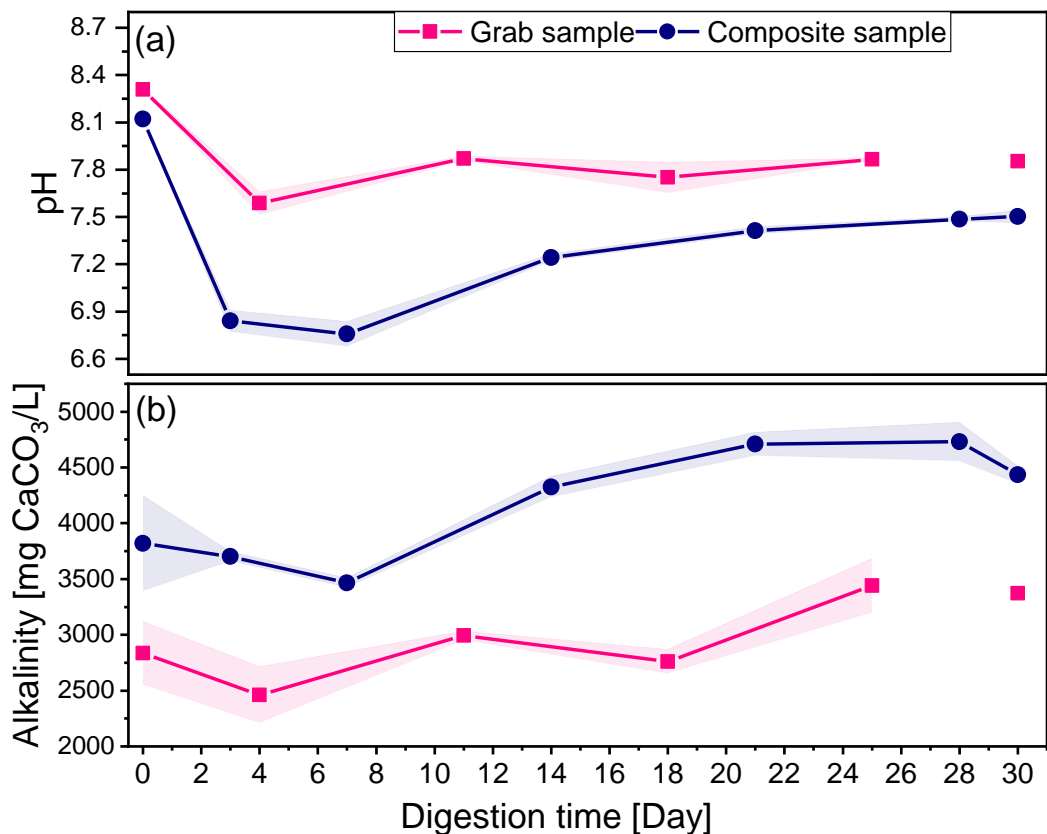
Figure 4.14. pH (a) and alkalinity (b) profiles for Grab and composite samples; shaded area around lines indicate standard deviation from mean. Disconnection between Day 30 and the rest of the data sets for the grab sample was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

4.6.3 Process kinetics

The MGompertz fiting for BMP experiments on grab and composite food waste samples are shown in Figure 4.15, with R^2 values equal to 0.992 and 0.994 respectively. It was not surprising that the grab sample showed better process kinetics than the composite sample.
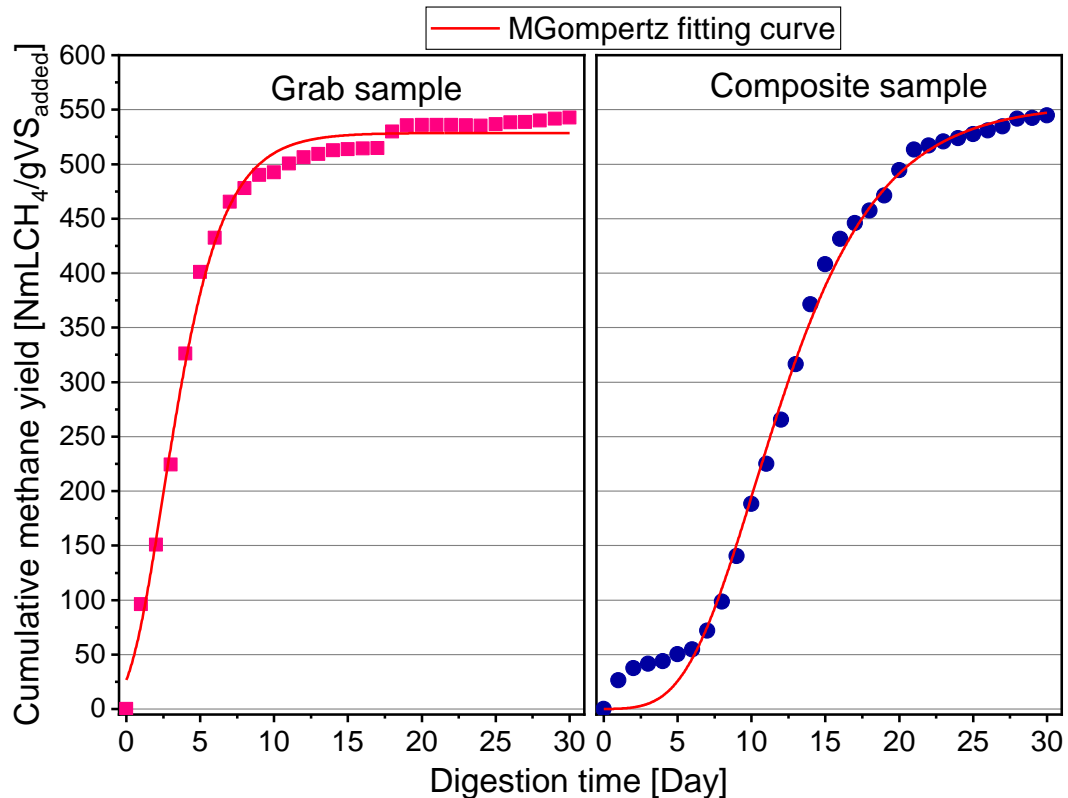
Figure 4.15. MGompertz fitting of the methane yield using the grab and composite samples at 1 mm PS and ISR of 3.

A lag time of 5.56 days (Table 4.6) obtained from the composite sample BMP experiment was a reflection of process inhibition, such that biomethane production progressed in restrained steady state. A higher k-value with the grab sample treatment was a result of the faster degradation rate and simultaneous methane production within the reactors. Therefore, although the methane yields were similar for both grab and composite samples, the grab sample had a better biodegradability, since a higher percentage was achieved compared to the composite sample.

The final biomethane yield between both samples were not significantly different; despite the relatively poorer process kinetics with the composite sample. This indicates that the composite sample has potential for even higher yields, should the conditions be more favourable.

Table 4.6. Comparing the process kinetics between grab and composite samples.

Parameter	Sample	
	Grab	Composite
K-value (Day^{-1})	0.44 (0.02)	0.22 (0.01)
Lag phase (Day)	0.21	5.56
Theoretical methane potential (mLCH$_4$/gVS$_{added}$)	515.7	588.6
Experimental methane yield (NmLCH$_4$/gVS$_{added}$)	542.8	544.6
Percentage biodegradability (%)	105.3	92.5

4.7 Conclusions

Food waste showed great potential for energy recovery in the form of biomethane, using AD technology. It was however, observed that variations in sampling seasons and composition of waste collected had an influence on the characteristics of food waste. This also impacted on the nutrients distribution when PS treatment was employed. The grab sample only contained the particular waste available at the time of collection, but the composite sample had a better representation of food waste from the source. The composite sample collected over a period of 5 days contained at least a portion of virtually every kind of food stuff processed and served within the Refectory. This basic difference in the sample collection influenced the overall characteristics of both samples. Moisture content variation was perceived to have impacted on physicochemical characteristics within each sample when the PS was reduced.

PS reduction experimentally improved the BMP of food waste, owing to the improvement of key AD parameters, such as: C/N ratio (up to 32% increase) and COD (up to 43% increase). This investigation revealed that PS reduction resulted in a rapid digestion of food waste, and while this was expected to result in higher rates of acidification within the system, the variation in ISR helped to reduce such effects. Hence, for lower PS ≤ 3 mm higher ISR of 3 and 4 were more suitable, while PS ≥ 3 mm, had highest yields at an ISR of 2. An overall optimal BMP process condition of 1 mm PS at an ISR of 3 was established for future food waste digestion. In general, PS reduction from 5 mm to 1 mm resulted in up to 38% methane increase. And within each PS experimental group, varying the ISR also improved the methane yield. As such, with ISR optimisation, 5%, 25% and 15% increases in methane yields

were obtained at 1, 2 and 5 mm respectively. Consequently, there were significant reductions in the lag phase following food waste PS reduction, which greatly improved the anaerobic biodegradability up to 105.3% compared to 82.7%; the maximum obtained at 5 mm PS.

Although, the composite sample had a higher TMP, it was also perceived to have had higher inhibition potential than the grab sample in terms of lower TEs and C/N ratio levels, as well as higher PSB_{bio}. These factors influenced a restrained methane production from the composite sample. Therefore, at optimal conditions of PS and ISR, the grab and composite samples had about the same methane yields of 542.8 and 544.6 $mLCH_4/gVS_{added}$ respectively. However, because of a higher TMP, with further process manipulations, a higher methane yield could yet be obtained from the composite sample and was henceforth utilised for further optimisation experiments.

Overall, following PS and ISR optimisation, the optimum conditions for an improved biomethane yield were 1 mm PS and ISR of 3 and these were used in the following experiments discussed hereafter in later chapters.

CHAPTER 5

FOOD WASTE BIOMETHANATION: EFFECT OF VOLATILE FATTY ACIDS REGIME

5.1 Introduction

Food waste AD experiments with hydrogen addition were conducted at different stages of VFA degradation and the results from these experiments are discussed in this chapter. AD for the production of biomethane follows four distinct but interacting steps; hydrolysis, acidogenesis (combination of hydrolysis and acidogenesis also referred to as primary fermentation), acetogenesis (secondary fermentation) and methanogenesis (methane production). Each step is controlled by different conditions, such as; pH and concentration of other products formed.

During primary fermentation, the feedstock is broken down to smaller individual molecules including: hydrogen, carbon dioxide, ammonia and VFA. The H_2 is quickly consumed by the hydrogenotrophs (all categories of hydrogen-utilising bacteria) actively present, therefore, part of the CO_2 produced is also removed. However, secondary fermentation and acetoclastic methanogenesis cannot progress conveniently, until the hydrogen partial pressure have been brought very low (FAO, 2015). Hence, for the time period when hydrogen is significantly available, VFA accumulation occurs; especially propionic acid.

For instance, under standard conditions, the partial pressure of hydrogen has to be maintained below 5.82×10^{-5} atm for butyric acids degradation (Siriwongrungson et al., 2007) and below 10^{-5} atm for propionic acid degradation to acetic acid (FAO, 2015). Notwithstanding, the rapid removal of hydrogen by the active hydrogenotrophs usually help to maintain the hydrogen partial pressure low enough to allow other biochemical processes to proceed accordingly (Pap et al., 2015).

Despite the hydrogenotrophic methanogens having a more rapid growth rate of 6 hours doubling time compared to 2 – 3 days by acetoclastic methanogens, the final stage of methane production is mostly attributed to the acetoclastic methanogenesis route (about 70%), as a result of the thermodynamic pathways yielding more acetic acid than hydrogen intermediates (Huang et al., 2015). In effect, CO_2 produced during digestion is much more than the

hydrogen available to the hydrogenotrophic methanogens to combine with for biomethane production.

The addition of hydrogen during AD (biomethanation), has been reported to influence an increase in methane yield through hydrogenotrophic methanogenesis (Bassani et al., 2016; Bassani et al., 2017; Kougias et al., 2017; Lecker et al., 2017; Luo and Angelidaki, 2013; Luo et al., 2012; Mulat et al., 2017; Rachbauer et al., 2016). Therefore, biomethanation can also be adopted for food waste systems to improve the digester performance and biomethane yield.

However, aside from hydrogenotrophic methanogenesis, there are two other possible sinks for hydrogen, namely: acetate formation by homoacetogens and sulphide production (sulfidogenesis) by sulphate reducing bacteria. Going by the process thermodynamics, hydrogen utilisation would follow the order; sulfidogenesis > hydrogenotrophic methanogenesis > homoacetogenesis. The metabolism of each hydrogen-utilising group however, would depend on the degraded state of the feedstock, availability of the combining elements; CO_2 for hydrogenotrophic methanogens and homoacetogens and sulphur/sulphate for sulphate reducing bacteria, presence of inhibitors (including hydrogen), as well as operating conditions such as pH (O'Flaherty et al., 1998). It was therefore, hypothesised that the stages of digestion; principally governed by the VFA regime, would have significant impact on how the added hydrogen would be utilised.

Biomethanation is rather a novel approach to biogas upgrading and a high energy input of 4.5 – 5 kWh/m^3 of hydrogen could be incurred from typical electrolysers (Rashid et al., 2015). It becomes important to optimise hydrogen utilisation (reduce overall hydrogen intake) for biomethane production. Therefore, to understand the influence of hydrogen injection point during biomethanation, three possible sinks of hydrogen, namely: biomethane, VFA and hydrogen sulphide (measured by elemental sulphur removal) were closely monitored.

It was perceived that high hydrogen loading could impact on the thermodynamic stability of the system leading to possible failure of other acting microorganisms (Mulat et al., 2017). As such, the experiments were conducted using low concentrations of hydrogen from a gas mixture of 5%-Hydrogen and 95%-Nitrogen. And as the bacteria acclimated to the initial concentration of hydrogen, the percentage of hydrogen was gradually increased (discussed further in Chapter 6).

5.2 Chapter objectives

- To examine the effect of introducing hydrogen gas into AD reactors treating food waste, towards improved biomethane yield.
- To identify the most suitable injection point for hydrogen addition, that would not necessarily inhibit the overall AD process, based on VFA regime.

5.3 Experimental set up with hydrogen injection

BMP experiments were designed using composite food waste sample at optimal conditions of PS and inoculum-to-substrate ratio (ISR) of 1 mm and 3 mm respectively (as discussed in Chapter four). Hydrogen was injected into the reactors in a mixture of hydrogen and nitrogen gas in a ratio of 5:95 (% v/v). This mixture was used under the assumption that pure hydrogen might cause adverse inhibition to the process. Also, nitrogen gas is conventionally used to attain anaerobic condition for BMP experiments. Hence, by flushing the reactors with the gas mixture, hydrogen was added into the reactors, while simultaneously achieving an anaerobic system.

Three hydrogen injection points were chosen to signify points before VFA production (before hydrolysis), at the peak of VFA accumulation (active acidogenesis) and depleted VFA intermediates (active methanogenesis). As established from previous experiments (Chapter four), VFA accumulation peaked around Day3, followed by a rapid decrease to a very low concentration around Day6. Based on this, the three hydrogen injection points were chosen as Day0, Day3 and Day6, labelled as Experiment1 (Exp1), Experiment 2 (Exp2) and Experiment 3 (Exp3) respectively.

Particulate organic matter cannot be consumed by microorganisms, unless they are solubilised to simpler forms (monomers). As such, hydrolysis is a very important step in material breakdown towards biomethane production. This step becomes rate-limiting when the materials are not quickly/efficiently solubilised, so that only a fraction of the biomass gets converted into biomethane (Pan et al., 2016). Injecting hydrogen by Day0 (Exp1) was used to assess the impact of increased hydrogen partial pressure on the hydrolysis of food waste, as well as the dominating pathway that ensues during the entire BMP process.

Most of the energy available during AD is utilised during primary fermentation, during which competition amongst acting microorganisms for substrates is

intense (Schink et al., 2017). Therefore, injecting hydrogen by Day3 (Exp2) was also used to assess the impact of hydrogen injection and the competition by the hydrogen consumers on the biomethane yield.

By Day6 however, over 80% of the VFA available is acetic acid, which means it is mainly methane production occurring after this time. Injecting hydrogen by Day6 helped to understand the bacteria response to sudden hydrogen surge, when other primary substrates had been consumed.

For each injection point, blank samples (reactors with inoculum only), control samples (reactors with food waste and inoculum flushed with pure nitrogen) and test samples (reactors with food waste and inoculum flushed with H_2-N_2 gas mix) were set up (Figure 5.1). In Exp2 and Exp3, bulk samples were prepared using 1L Duran bottles, until Day3 and Day6 respectively, after which the headspace gas was collected and the bulk samples split into Wheaton bottles, and flushed with the respective gases; following the succeeding steps.
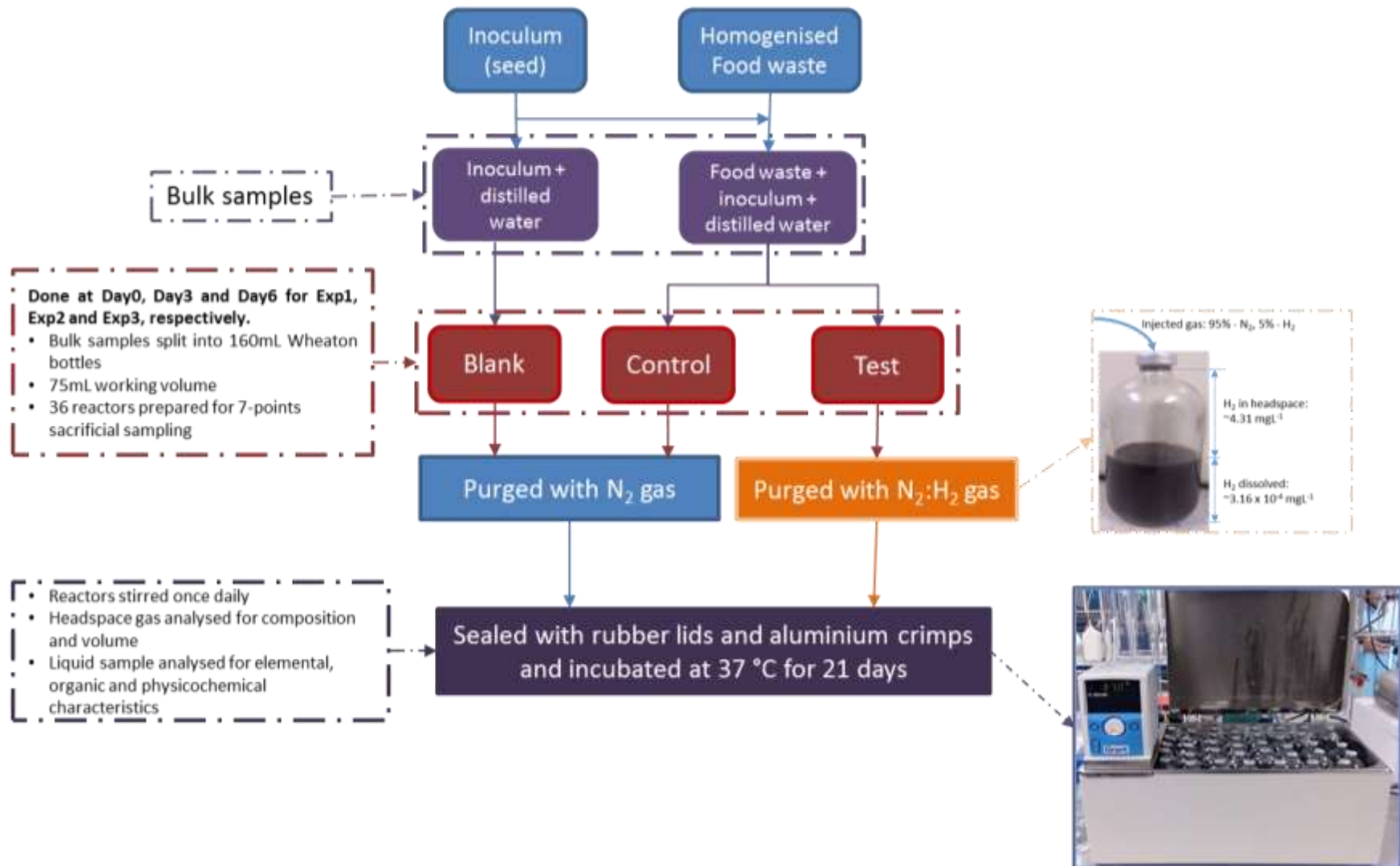
Figure 5.1. Experimental setup showing the preparation stages for BMP experiments with hydrogen addition.

5.3.1 Hydrogen validation and leak proof test of reactors

To ascertain that hydrogen was not going to leak from the reactors during the entire experiment, an identical experiment was designed using only distilled water. The reactors were set up in duplicates and flushed with H_2-N_2 gas mixture for 5 minutes and the headspace gas was analysed on the GC for each analytical point; as in the actual experiments with food waste. The data presented in Figure 5.2 indicate that there was an outlier arising from one of the Day1 samples.
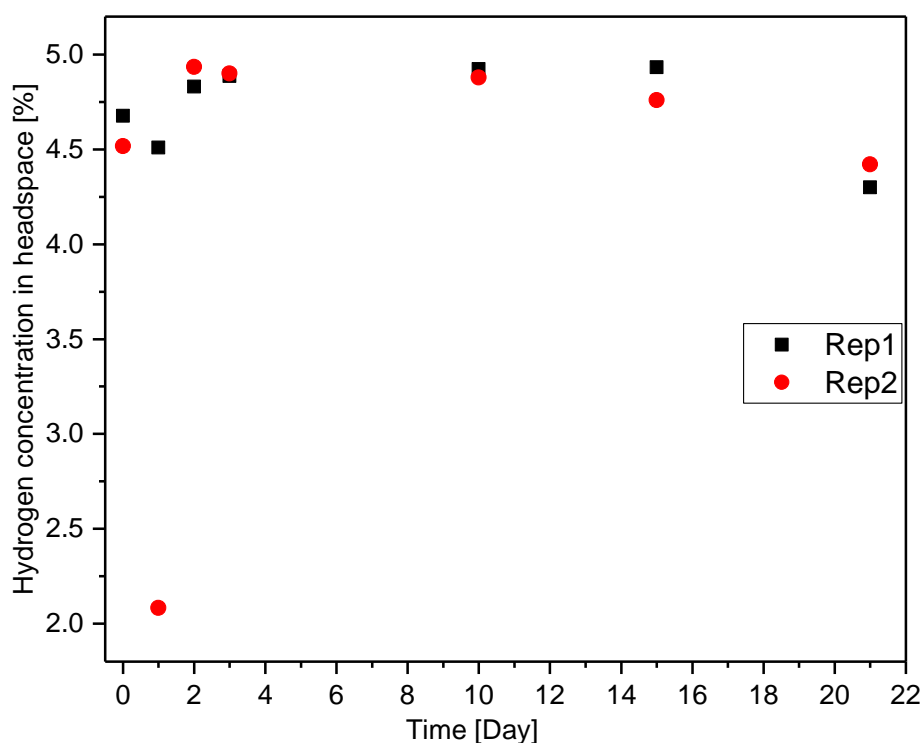
Figure 5.2. Percentage of hydrogen gas measured from the headspace of reactors containing only distilled water.

As this was the only point with such low percentage, it was assumed that this must have resulted from analytical error probably during gas purging, or manual injection into the GC inlet column. An outlier test was therefore, conducted to validate this, which gave a p-value of 0.00. Hence, the outlier 2.08% was removed from the data set and re-tested for an outlier as well as a one sample t-test, this time a p-value of 0.473 was obtained at 95% confidence level. The mean percentage hydrogen retained in the headspace was 4.73%, implying only 0.27 %-H_2 was lost from the original 5%-H_2 contained in the gas mixture. This was assumed to account for the amount of hydrogen dissolved in the liquid and any probable error from the GC measurement. Hence, the bottles were confirmed to be able to retain the hydrogen trapped throughout the BMP period.

Excluding the outlier value, most of the data obtained were between 4.7 and 4.9% of H_2. These figures emphasises that the method adopted for hydrogen injection had very minimal errors and did not significantly affect the amount of hydrogen contained. The mean value of 4.73% H_2 was hence, adopted as the percentage of hydrogen transferred to the headspace except otherwise stated.

5.4 Results discussion

5.4.1 Effect of hydrogen injection on hydrolysis

The impact of increased hydrogen partial pressure on the hydrolysis of food waste was measured using data from Exp1, as the percentage of the suspended volatile solids retained by Day1, from the initial total VS according to Equation 5.1 (Palaniyandi, 2009);

$$Percentage\ hydrolysis = \frac{Total\ VS_{Day0} - Soluble\ VS_{Day1}}{Total\ VS_{Day0}} \times 100 \qquad 5.1$$

The percentage hydrolysis for the control and test samples were 91% and 90% respectively, which does not reflect any significant inhibition on hydrolysis with hydrogen addition.

5.4.2 Stoichiometric hydrogen utilisation for biomethane

The headspace gas in the test reactor just after bubbling the N_2-H_2 gas mixture, was measured on the GC as 95.2% nitrogen and 4.8% hydrogen. Hence the volume of hydrogen gas in the headspace, at 88 mL headspace volume was 3.87 mL at standard temperature and pressure (STP), and the calculated volume of hydrogen dissolved was 3.516×10^{-3} mL; assumed to be negligible in the mass balance calculation.

According to the hydrogenotrophic methanogenesis pathway;

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
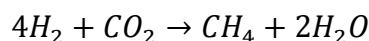
$$(4 \times 2.016)\ g/mol + 44.01\ g/mol \rightarrow 16.04\ g/mol + (2 \times 18.01528)\ g/mol$$

$$52.07\ g/mol \rightarrow 52.07\ g/mol$$

$$Taking\ individual\ masses\ as;\ mass\ (m)$$
$$= number\ of\ moles\ (n)\ \times Molar\ mass\ (M)$$

$$where;\ m_{H_2} = 8.064g\ ;\ m_{CO_2} = 44.01g;\ m_{CH_4} = 16.04g;\ m_{H_2O} = 36.03g$$

$$Hence, CH_4 : H_2 = 1.989, \; CH_4 : CO_2 = 0.364 \; and \; CO_2 : H_2 = 5.458$$

Hydrogen produced during AD is almost immediately consumed by the hydrogen consumers; relative to their abundance, such that excess dissolved hydrogen is transferred to the headspace; as a result of low H_2 solubility (15.5 mg/L at 25 °C). Additionally, until the dissolved and gaseous hydrogen are equilibrated to a very low partial pressure, the high hydrogen partial pressure could inhibit VFA degradation (Siriwongrungson et al., 2007; Conrad, 1999; Fukuzaki et al., 1990; Luo et al., 2012) and consequently, impact on acetoclastic methanogenesis, as a result of possible backward VFA-induced inhibition (Chen et al., 2008). Hence, hydrogen in the headspace gas could pass as an indication of dissolved hydrogen inhibition on AM. Based on these premises, the methane production from the day of setup (Day0) both in the control and test reactors until the point at which no hydrogen was detected in the headspace (Day3) can be attributed primarily to hydrogenotrophic methanogenesis. However, since it is difficult to state at what hour gaseous hydrogen was completely removed from the headspace, the mass balances for hydrogen consumption and methane production was limited to the days it was measured in the biogas.

In Exp1, according to the gas concentrations presented in Table 5.1, hydrogen was recorded in the headspace of the control reactor, implying that even without hydrogen gas injection, the system generated some hydrogen; for which the rate of consumption by the hydrogen consumers was lower than the rate of production, hence, the excess was transferred to the biogas. As such, assuming the amount of H_2 dissolved was negligible and there was no consumption of the added hydrogen gas in the test reactor, then by Day1, 4.5 mg-H_2/L would have been expected in the biogas of the test reactor; that is in addition to the supposed 0.6 mg/L produced in the control. However, 3.7 mg-H_2/L was recorded in the biogas, which implies that instead of a transfer of hydrogen to the headspace following food waste degradation, there was a reduction of the hydrogen added externally by 0.3 mg-H_2/L.

This resulted in a higher methane concentration in the test reactor at 12.4 mg/L, compared to 11.5 mg/L in the control. And with a CH_4:H_2 ratio of 1.99, the supposed hydrogen consumed to give these methane yields was 6.9% higher for the test reactor; at 6.2 mg-H_2/L and 5.8 mg-H_2/L in the test and control reactors respectively. Since the hydrogen in the headspace of test reactor only reduced by 0.3 mg-H_2/L, it means the remaining 5.9 mg-H_2/L utilised was hydrogen released during degradation, which is higher than the

calculated amount of hydrogen consumed in the control by 1.7 % (0.1 mg-H_2/L). This implies there was a higher activity by the hydrogenotrophic methanogens in the test reactor than the control.

Table 5.1. Concentration of biogas components (at STP) immediately following hydrogen addition until no hydrogen was measured in the headspace.

Experiment	Day	Control reactor (mg/L)			Test reactor (mg/L)		
		H_2	CH_4	CO_2	H_2	CH_4	CO_2
Exp1	0	0.0	0.0	0.0	4.0	0.0	0.0
	1	0.6	11.5	108.6	3.7	12.4	106.8
	2	0.4	21.9	200.7	1.0	27.8	179.8
	3	0.0	37.2	350.2	0.0	41.7	293.0
Exp2	3	0.0	0.0	0.0	4.0	0.0	0.0
	4	0.0	63.1	70.1	0.0	68.6	38.5
Exp3	6	0.0	0.0	0.0	4.0	0.0	0.0
	7	0.0	29.4	49.8	0.0	36.3	0.0
	8	0.0	53.4	69.4	0.0	57.9	35.9

Going forward, by Day2; taking the percentage gaseous hydrogen utilisation (U_H) between Day1 and Day2, U_H was 72% in the test reactor for 2.62 mg-H_2/L utilised and 25% in the control reactor for 0.14 mg-H_2/L utilised. This huge difference in the U_H and amount of hydrogen utilised by Day2 is again a good indication of an enhanced growth rate of the hydrogenotrophic methanogens, due to the availability of H_2 from the start of the experiment. This high U_H by Day2 influenced an increase in biomethane concentration by 26.9% and a corresponding reduction in CO_2 by 10.4%. Although, it can be argued that the hydrogen within the test reactor by Day2 was also utilised by other hydrogen consumers; such as the homoacetogens and sulphate reducing bacteria, further discussions in subsequent sections on the concentrations of acetic acid and sulphur depicts otherwise.

More so, the difference in the headspace hydrogen of the test reactor between Day0 and Day2 was 2.9 mg-H_2/L, which corresponds to 5.8 mg-CH_4/L. Adding this value to the methane concentration from the control by Day2, then the expected methane concentration in the test reactor would be 27.7 mg-CH_4/L, which is about the same as the actual concentration measured as 27.8 mg-

CH_4/L. Hence, it is reported here that virtually all the hydrogen added in Exp1 was consumed by the hydrogenotrophic methanogens to produce methane.

According to Gujer and Zehnder, (1983), the bacterial community within the AD system is autocatalytic, in that the amount produced will always be proportional to the flux of the substrates within the system. Hence, the availability of hydrogen at the start of the experiment influenced higher U_H between Day1 and Day2 at 67.6% in the test reactor compared to 50.8% in the control reactor. In addition, the subsequent increase in hydrogenotrophic methanogenic activity led to increase in CH_4 yield in the test reactor.

In Exp2, whereby, the process was presumably at a highly competitive phase, a different pattern from the Exp1 was observed. No hydrogen was measured in the headspace by the next day after injection; implying all of the injected hydrogen was consumed in one day. This was observed to be more of a competitive coexistence of acting hydrogen-consumers, rather than dominance by hydrogenotrophic methanogens. According to the concentration of individual gases presented in Table 5.1, if all of the injected hydrogen was utilised to produce methane, then the biomethane concentration in the test reactor would have been higher than the control by 7.9 mg-CH_4/L. However, the difference between the test and the control concentrations was 5.4 mg-CH_4/L; being less by 31%. Therefore only about 69% (2.7 mg-H_2/L) of the hydrogen added can be said to have been directly used for biomethane production.

However, the increase in methane yield could also have come from acetic acid degradation, since no hydrogen was immediately measured in the headspace by the next day. In which case, the removal of hydrogen becomes more of an outcompeting of the hydrogenotrophic methanogens, rather than a co-existence.

Furthermore, as would be discussed later, results from the VFA levels and compositions as well as the sulphur degradation, suggests the utilisation of hydrogen for other hydrogen-determining processes. Therefore, the competition for the injected hydrogen was perceived to be high In Exp2. Notwithstanding, the CO_2 level, which would have been 21.64 mg/L less in the test reactor; if all the hydrogen was consumed for biomethane production, was 31.62 mg/L lower. This was however, not surprising because, with the removal of CO_2 by the hydrogenotrophic methanogens, gas-liquid CO_2 transfer is used to regain lost alkalinity in the reactor content; as was the case for the other experiments.

Furthermore, around $0.28 - 0.42\%$ hydrogen and 3% CO_2 are said to be converted to biomass during biomethanation, such that the stoichiometric ratio of 4:1 (H_2:CO_2) for 1 mole of CH_4 production becomes 4:1.085 (Lecker et al., 2017). Therefore, some amount of CO_2 is often used by microorganisms as carbon source, for which around $6.4 - 8.5\%$ CO_2 losses due to biomass growth have been reported in various biomethanation studies (Burkhardt and Busch, 2013; Lecker et al., 2017; Luo et al., 2012; Rachbauer et al., 2016). Therefore, the reduction in CO_2 in Exp2 can also be attributed to biomass growth.

Exp3 demonstrated ultimate consumption of the injected hydrogen for biomethane production as with Exp1. At the time of hydrogen injection in Exp3, only trace amounts of acetic and propionic acids were remaining in the liquid reactor content. Hence, the process was actively in the methanogenesis phase, during which little or no competition was expected due to insufficient substrates. By Day 7 (next day after hydrogen addition), only 0.20 mg-H_2/L was measured in the headspace of the test reactor, indicating a much more rapid U_H than Exp1 of 94.9%. This high U_H was believed to be as a result of a well-established hydrogenotrophic community as with Exp2; but with lesser competition. Therefore, the less competitive environment did not allow for complete utilisation of the hydrogen by Day7.

For 3.8 mg-H_2/L utilised in Exp3, the biomethane yield in the test reactor was expected to be higher than the control reactor by 7.48 mg-CH_4/L, to give a biomethane concentration of 36.9 mg-CH_4/L. Instead, 36.3 mg-CH_4/L was measured in the test reactor; being less than the expected by only 1.6%. This indicates that the injected hydrogen was principally utilised by the hydrogenotrophic methanogens. Also, while the concentration of CO_2 in the control reactor was 49.8 mg/L by Day7, no CO_2 was measured in the headspace of the test reactor for the same day, which buttresses the assertion that the added hydrogen was ultimately utilised by the hydrogenotrophic methanogens. In agreement, during a biomethanation study on sewage sludge, with 5-days pulse hydrogen injection after Day 6 of digestion, high hydrogen uptake was reported (Agneessens et al., 2017). This, the authors reported was due to an adaptation of hydrogenotrophic methanogens to the injected hydrogen.

In general, the VFA regime influenced the utilisation of the added hydrogen for biomethane, such that before hydrolysis and after VFA intermediates removal proved to be the best options towards optimum utilisation for biomethane production.

5.4.2.1 Ultimate biomethane and carbon dioxide yields

The cumulative biomethane and CO_2 yields from Exp1, Exp2 and Exp3 are shown in Figure 5.3; where the dash lines are the yields from the control reactor and the solid lines are yields from test reactors. Lower yields were observed from Exp2 and Exp3 because, the biogas produced before the respective days of hydrogen injection was completely removed.

However, in all three experiments, the addition of hydrogen improved the quality of the biogas. The addition of hydrogen in the test reactor was responsible for the higher hydrogen consumption rate, biomethane yield and lower CO_2 concentrations in the test reactor. Evidently, the gas-liquid hydrogen mass transfer rate was influenced by the concentration of hydrogen available during the experiment. In Exp1, since the addition of hydrogen greatly increased the hydrogenotrophic methanogens' activity, it becomes logical to expect further increase in the hydrogenotrophic methanogens available throughout the digestion period. This explains the further reduction in CO_2 throughout the experiment, indicating a possibility that the hydrogenotrophic methanogens outcompeted other hydrogen-utilisers for the subsequent hydrogen released with further VFA degradation.

In Exp2, the BMP process progressed in the conventional manner until Day3; when the process was presumed to be in active fermentation stage, the intense competition at this stage for available substrates closed up the margin of increase in the biomethane yield. However, in Exp3, with the depletion of VFA within the system during active methanogenesis stage, competition was greatly reduced, such that, only the hydrogenotrophic methanogens were selectively enhanced when hydrogen was added.
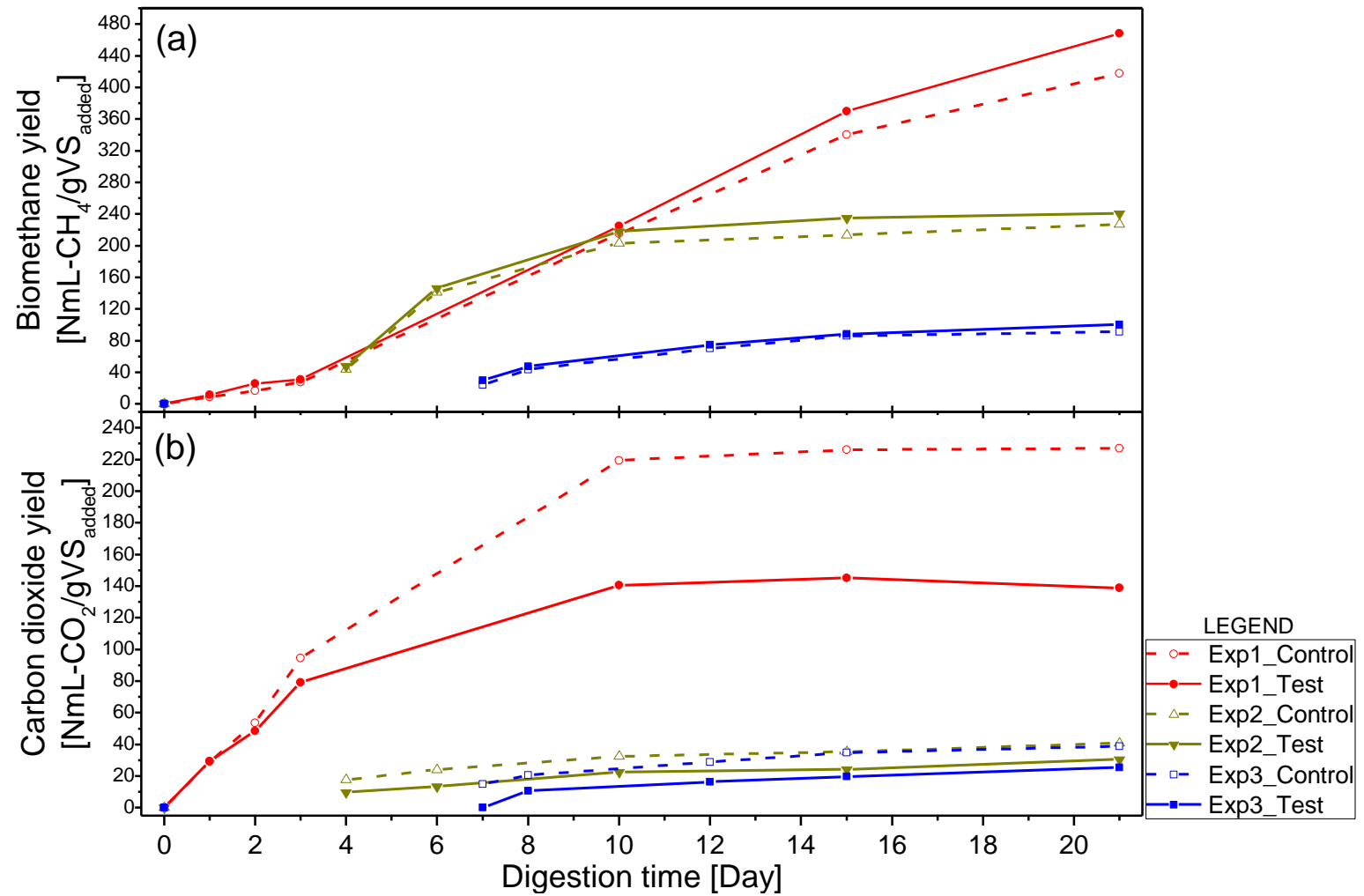
Figure 5.3. Biomethane (a) and carbon dioxide (b) yields from biomethanation experiments with hydrogen injection at Day0 (Exp1), Day3 (Exp2) and Day6 (Exp3).

The percentage increase in methane yield from the test reactor over the control for each experiment are presented in Figure 5.4. As seen, there were large CO_2 reductions in all test reactors by the next day after hydrogen addition, which depicts a higher rate of hydrogenotrophic methanogenesis in all test reactors; especially with Exp3, which recorded 100% CO_2 difference, after H_2 injection.

In Exp1, the change in CO_2 between the control and test reactors went from low to high because hydrogen was added before the initiation of digestion. So that as AD commenced, the excess hydrogen enhanced hydrogenotrophic methanogens, thereby, causing them to dominate hydrogen and CO_2 utilisation, throughout the entire process. In Exp2 the food waste was already solubilised into simpler organic forms available in large quantities. Hence, the CO_2 change was highest only for the days immediately following hydrogen addition. This margin reduced as the digestion progressed, which implies a competitive coexistence between the hydrogen consumers present for the hydrogen produced during further degradation.
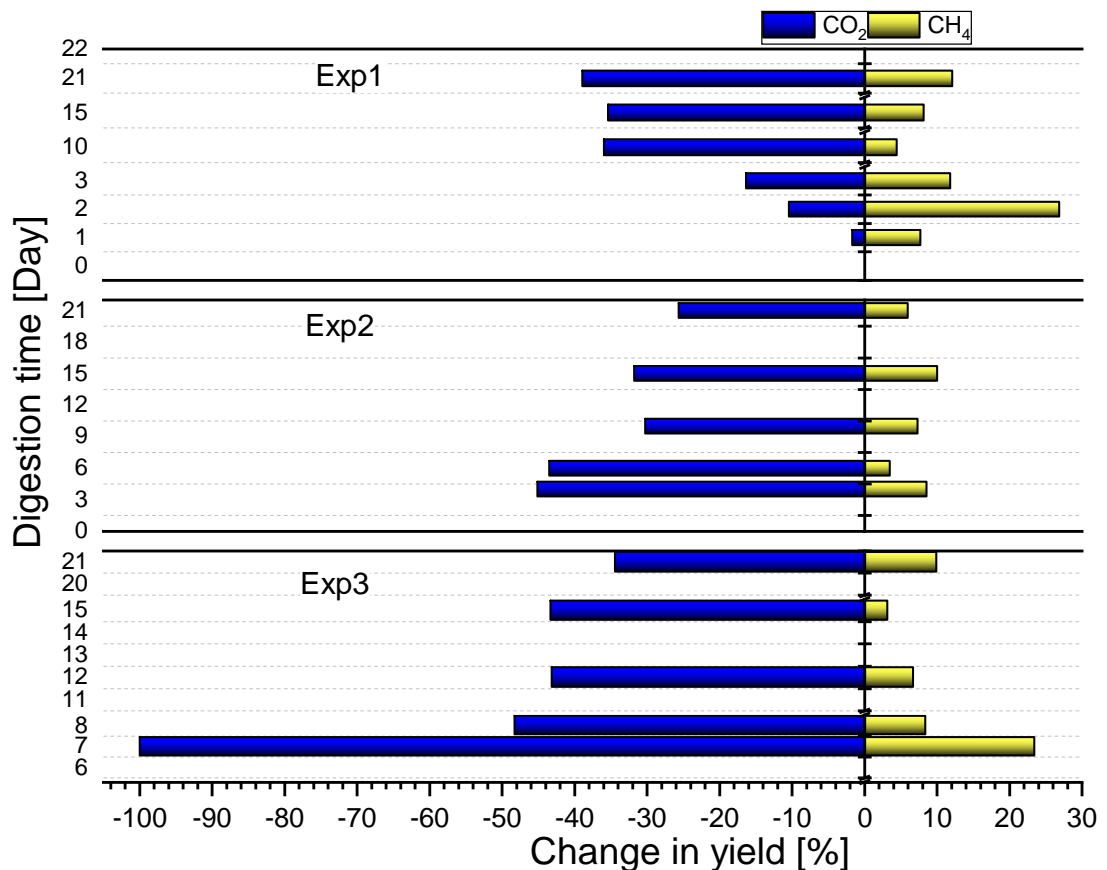
Figure 5.4. Percentage change in CH_4 and CO_2 volumes between the control and test reactors (i.e. ($Test_{CH4}$ − $Control_{CH4}$)/$Control_{CH4}$).

In Exp3, a similar trend was observed as with Exp2, whereby, initial high CO_2 difference was observed by the next day after hydrogen addition. However, the resulting reduction in the CO_2 margin between the control and test reactors was as a result of continuous acetic acid decomposition, and a lack of available hydrogen at this stage to facilitate further hydrogenotrophic methanogenesis.

With the addition of hydrogen, the total biogas yield changed from 644.7 to 607 NmL/gVS_{added} in Exp1, 268.2 to 271.4 NmL/gVS_{added} in Exp2 and 130 to 125.8 NmL/gVS_{added} in Exp3 respectively. The lower yields from Exp2 and Exp3 were due to the removed headspace gas prior to hydrogen addition. Clearly, more CO_2 removal was achieved in Exp1 and Exp3 than Exp2, judging by the reduction in the biogas volumes in Exp1 and Exp3. To further buttress this, it was observed that for the ultimate increase in methane yield in Exp1 and Exp3, there was a resultant reduction CO_2 by a factor of 1.7 and 1.5 respectively. But with Exp2, CO_2 only reduced by a factor of 0.8, which further elucidates that hydrogen added during Exp2 was highly competed for and was not optimally utilised for biomethane production. Therefore, an initial boost of the hydrogenotrophic methanogens by adding hydrogen at the start of the AD process and continuous addition of hydrogen after the depletion of intermediate VFA could help to improve the U_H for a faster biogas upgrade rate.

The biomethane content of the biogas increased from 65 to 77.1%, 84.8 to 88.8% and 70.2 to 79.8% in Exp1, Exp2 and Exp3, which corresponds to 12.1%, 4% and 9.6% biomethane increases respectively. It was not surprising to have high percentages of biomethane in Exp2 and Exp3, because of the removal of the biogas produced prior to the injection of hydrogen. This is also in agreement with other studies on food waste AD, whereby, up to 90% biomethane content was achieved using multi-stage digestion (Uçkun Kiran et al., 2014).

Although, Exp3 had a higher CO_2 conversion to biomethane than Exp2, when the initial CH_4 and CO_2 yields prior to the addition of hydrogen in these experiments were added to the final yields, the biogas quality from Exp3 was the poorest (Figure 5.5). This was due to the high amounts of CO_2 accumulated by Day6 compared to Day3, at 386.8 mL/gVS_{added} and 193.4 mL/gVS_{added} respectively.
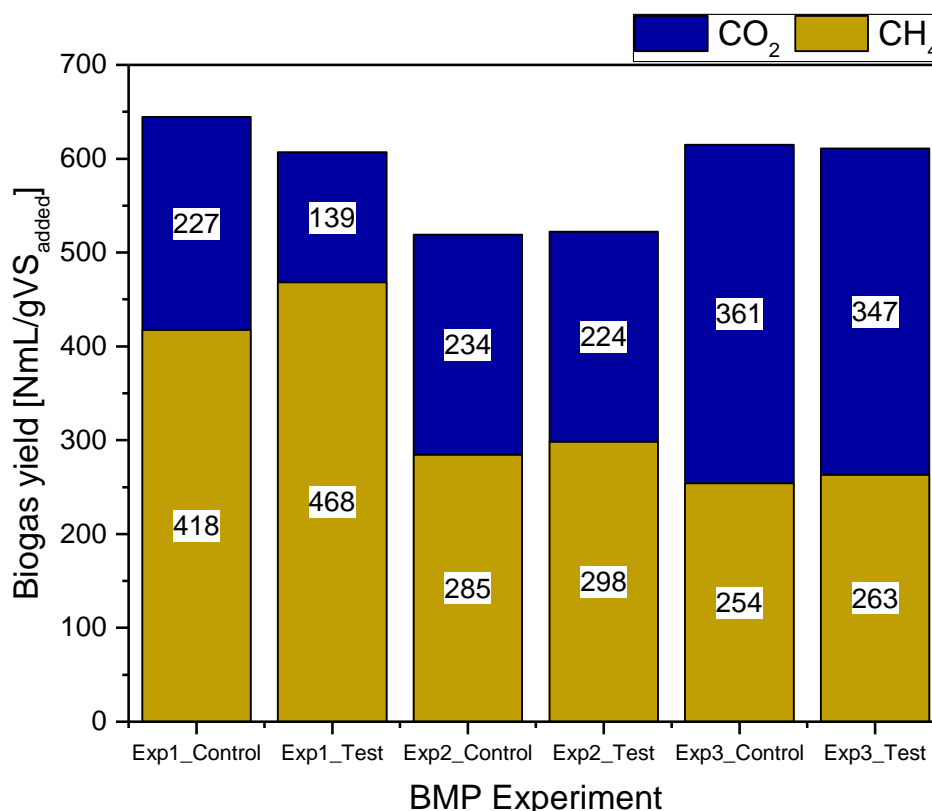
Figure 5.5. Final biomethane yields and percentages from Exp1, Exp2 and Exp3 including the initial biogas production from D3 and D6.

Perhaps, biogas recirculation and continuous hydrogen addition would help to improve the overall quality of the biogas produced from Exp3 (Bassani et al., 2016; Bassani et al., 2017; Burkhardt et al., 2015). In addition, the low biogas yields observed from Exp2 and Exp3 can be explained by the preparation method, whereby, the biogas had to be removed and the reactors completely bubbled with the respective set up gas; further expelling any dissolved gas. However, the higher biogas yield in Exp3 than Exp2 shows that further materials degradation occurred, which will be further described in section 5.4.5.2 on materials degradation.

5.4.3 Volatile fatty acids transformations with hydrogen addition

The total VFA reported here comprised acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids. Though, trace amounts of longer chain fatty acids including caproic, iso-caproic and heptanoic acids were detected, they were only negligible amounts; hence, were not added. The system's performance to excess hydrogen during acidogenesis was analysed only with Exp1 using the VFA concentration in the reactors up till day 2. This was so, because, it was only in Exp1 that hydrogen was added prior to acidogenesis. The

performance during acetogenesis and overall methanogenesis was studied and compared between the three experimental set-ups.

5.4.3.1 Primary fermentation (Hydrolysis and acidogenesis)

In Exp1, only the acetic-, propionic- and butyric- acids, were predominant, other acids remained relatively the same in both test and control reactors, with a difference in the range of 0.03 – 0.58 mg/L, throughout the digestion period. The total VFA concentration was 635.0 mg/L in the control and 644.6 mg/L in the test reactor respectively by Day1; with acetic, propionic and butyric acids higher in the test reactor by 0.5%, 6.0% and 4.2% respectively. According to Mosey, (1983), asides acetic acid, VFA produced during AD are mere bacteria responses to hydrogen surge loads. It was therefore, not surprising that VFA above two carbon atoms (C2) were higher in the test reactor by Day1, due to an initial system adjustment, as demonstrated by the relatively lower p-values presented in Table 5.2.

Table 5.2. P-values for 2 sample t-tests analysis of volatile fatty acids in the control and test reactors from Exp1 ($\alpha=0.05$, n=12).

Day	Acetic acid	Propionic acid	Butyric acid	Total VFA
Day 1	0.773	0.010	0.088	0.394
Day 2	0.848	0.774	0.118	0.721

By Day2, the concentrations of acetic, propionic and butyric acids within the control and test reactors increased to about the same levels in both reactors. The increased rate of hydrogen consumption in the test reactor, was believed to have slowed further propionic and butyric acids accumulation in the test reactor. While in the control reactor, hydrogen surge from primary fermentation influenced increased accumulation of propionic and butyric acids. For instance, by Day2, while propionic and butyric acids increased between Day1 and Day2 by 67% and 11% in the control reactor, they increased by 59% and 4% in the test reactor respectively, which explains the increase in p-values from Table 5.2. The p-values by Day2 suggests that there was no significant difference in the VFA intermediates produced during acidogenesis, also presented in Figure 5.6.
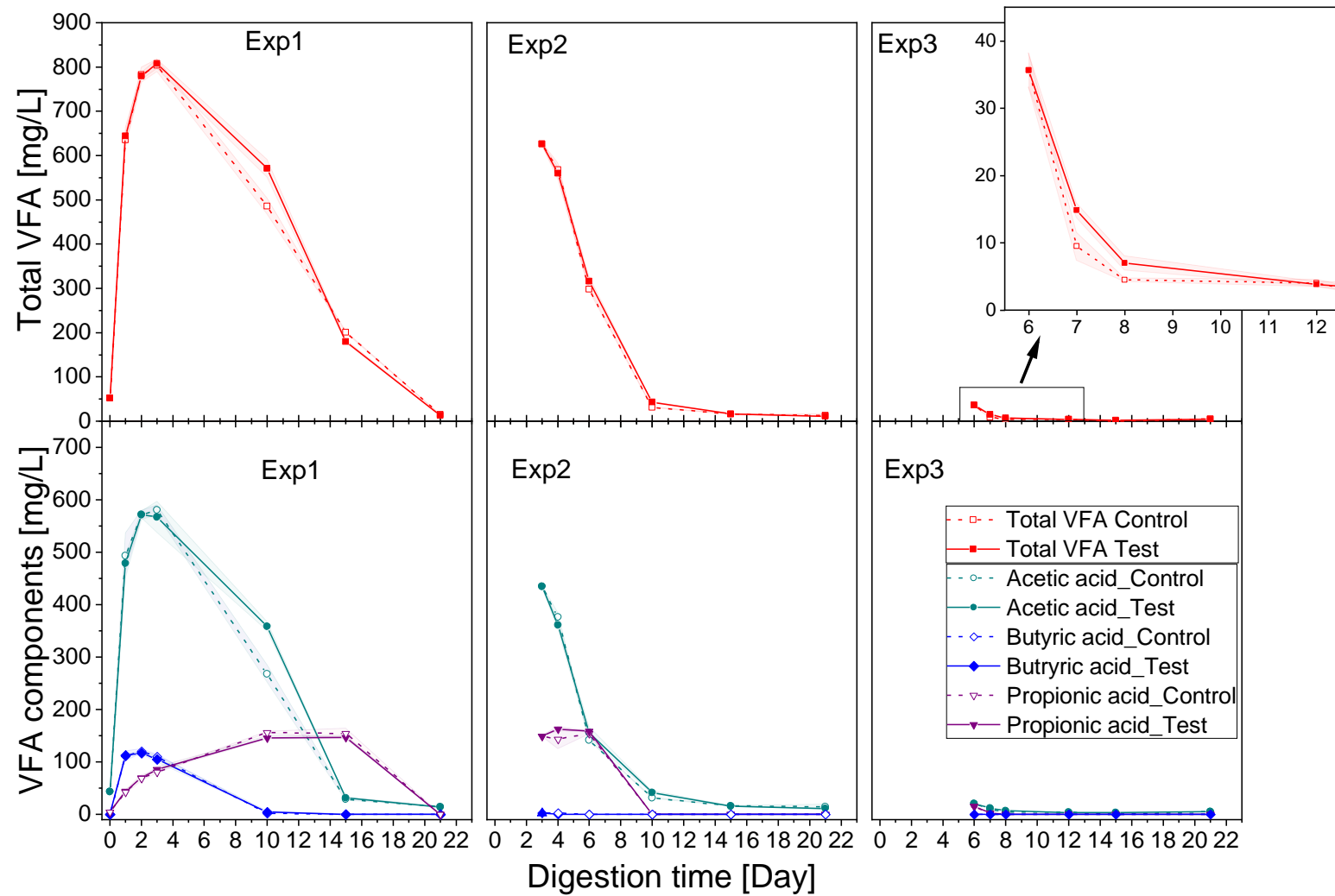
Figure 5.6. VFA concentrations in the test and control reactors' liquid contents; dash lines represent the control reactors, while solid lines represent the test reactors. Shaded area around lines represent standard deviation from mean.

5.4.3.2 Secondary fermentation (acetogenesis)

In Exp1, after the liquid phase H_2 concentration must have become low enough for the acetogens to metabolise, the increase in acetogenesis from Day3 may have led to the transformation of H_2 released during secondary fermentation to acetic acid by Day10 as seen in Figure 5.6. However, the acetic acid increase could also have come from degradation of the higher intermediates; following the relatively lower butyric and propionic acid concentration in the test sample. By Day10, it is highly unlikely that the injected hydrogen would still influence high hydrogen concentration in the liquid phase; taking a cue from the biogas composition earlier presented in Table 5.1. By this time, the excess hydrogen within the system had been removed, allowing the system to proceed normally with the continuous degradation of the solubilised materials. Hence, the acetic acid would be formed following its typical production route and not from the injected hydrogen gas.

Seeing that the hydrogenotrophic methanogenic activity had already increased from the early days of digestion, it was expected that the hydrogen produced during secondary fermentation would also be met by a rapid consumption and hence, increasing the rate of acetic acid production. Therefore, the higher acetic acid level in the test reactor of Exp1 by Day10, was due to the degradation of butyric and propionic acids onward from Day3. This was influenced by an enhanced syntrophic relationship between the hydrogenotrophic methanogens and acetogens.

In Exp2, no obvious change was observed on butyric acid however, propionic acid was observed to be 6.9% higher in the test reactor, suggesting that hydrogen addition at this stage had some level of inhibition on propionic acid degradation. This was only to a small extent because of the rapid removal of the injected hydrogen. As the hydrogen level reduced, allowing further propionic acid degradation, the acetogenesis rate increased, indicating that acetogenesis was only initially inhibited when the hydrogen was added, as a result of initial system adjustment to the injected hydrogen.

Acetic acid was also lower in the test reactor by Day4 (next day after injection); measuring 361.2 mg/L compared to 376.1 mg/L measured in the control reactor, which implies that the hydrogen added was not utilised for acetic acid production by the homoacetogens. The slight reduction in acetic acid could either be due to a backward formation of propionic acid, and/or an enhanced acetic acid breakdown to methane. Thermodynamically, acetic acid reduction observed here would be more as a result of the former than the latter.

In Exp3 primary fermentation had already occurred prior to adding hydrogen, hence, only a negligible amount of propionic acid was available at the time of hydrogen injection, and its degradation was not inhibited by hydrogen addition.

In general, rather than inhibit acetogenesis, the addition of hydrogen helped to accelerate acetogenesis especially in Exp1 and Exp3. This is in agreement with the observation by Luo and Angelidaki, (2013) during the co-digestion of manure and whey with the addition of H_2, whereby, there was no obvious acetogenesis inhibition with increase in hydrogen. Furthermore, they observed an increase in the key enzyme responsible for methane production from acetate and H_2/CO_2 consumption (Coenzyme F_{420}) by 20%, with hydrogen addition.

5.4.3.3 Acetate degradation (acetoclastic methanogenesis)

After the complete removal of the hydrogen added in Exp1 and Exp2, acetic acid was observed to increase in the test reactor going forward, as a result of an increased rate of propionic acid degradation, rather than inhibited acetic acid degradation.

In Exp3 acetic acid degradation was slower in the test reactor, for the days immediately following hydrogen addition. The acetic acid was 31% and 56% higher in the test reactor by Day7 and Day8 respectively. Because the primary source of carbon at this stage was acetic acid, its accumulation would imply that carbon was obtained from further materials degradation. As such the slow rates of acetic acid degradation in Exp3 might have been as a result of one or both of the following; i) inhibition on acetoclastic methanogenesis from the high level of hydrogen within the system, ii) increased release of acetic acid from additional materials degradation as carbon source.

During the hydrogen utilisation discussions, it was established that in Exp3, CH_4 increased in the test reactor, but, CO_2 was not released to the headspace of the test reactor until Day8. This suggest that, the CO_2 released from acetic acid decomposition was completely utilised for hydrogenotrophic methanogenesis. Hence, the slow acetic acid degradation rate in the test reactor of Exp3 would arise from further materials degradation. The VS degradation patterns with hydrogen injection are described in section 5.4.5.2, from where we also observe that materials degradation was enhanced in Exp3, which could also have influenced more acetic acid release.

5.4.4 Elemental Sulphur degradation

The elemental sulphur graphs from the three experiments are presented in Figure 5.7. The hydrogen-utilising community within the test reactors invariably increased following the initial high hydrogen loads, the question however, was, which of the communities where dominant? As earlier established, homoacetogenesis was not improved with hydrogen addition for all experiments, thus narrowing the competition down to the sulphate reducing bacteria and hydrogenotrophic methanogens. The sulphate reducing bacteria have a reputation of utilising a wide range of organic acids and hydrogen for their metabolism, with affinity in the order H_2 > propionic acid > other organic electron donors (Chen et al., 2008).

Hydrogen utilisation efficiencies discussed in earlier sections revealed that about all the hydrogen added in Exp1 was utilised for biomethane production. This was possibly because, the sulphate reducing bacteria were not able to directly degrade complex organic materials; such as lipids, carbohydrates and proteins, so they did not pose major competition during hydrolysis (Chen et al., 2008). Hence, in Exp1 sulphate reducing bacteria competition for hydrogen during hydrolysis was eliminated. The initial reduction in the elemental sulphur for both the control and test reactors in Exp1 therefore, follows initial hydrolysis. However, a relatively slower rate was observed in the test reactor for the first three days after hydrogen addition, as a result of the interim stall in materials degradation.

Since the hydrogenotrophic methanogens cannot directly utilise higher VFA, such as: propionic and butyric acids, the sulphate reducing bacteria was believed to compete for these as substrates. As such, although it has been established that the sulphate reducing bacteria did not competitively utilise the added hydrogen in Exp1, other organic acids were competed for. We recall from section 5.4.3 that acetogenesis was enhanced in Exp1 following an increased rate of propionic acid degradation. This was believed to be initiated by the sulphate reducing bacteria, because, as the VFA intermediates (propionic and butyric acids) reduced, elemental sulphur degradation consequently increased in the test reactor. In the control reactor, whereby, the VFA intermediates degradation were relatively slower, the elemental sulphur remained relatively stable between Day3 and Day10; until the concentration of VFA reduced significantly.
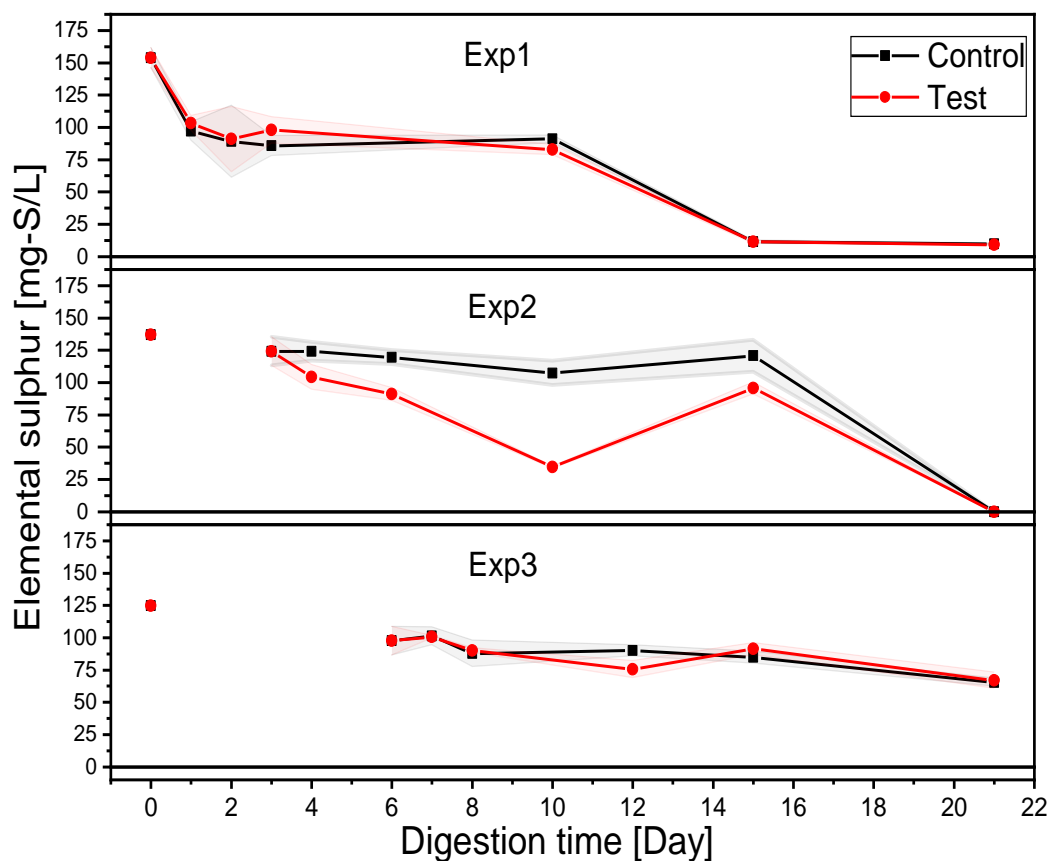
Figure 5.7. Elemental sulphur concentration in the reactor's liquid content (Dry basis). Shaded area around lines represent standard deviation from mean.

Hence, regardless of the fact that the sulphate reducing bacteria were not active during hydrolysis and initial fermentation, they were able to compete for other substrates afterwards. Otherwise, the elemental sulphur would have remained higher in the test reactor throughout the experiment.

Propionate degradation by sulphate reducing bacteria and typical obligate hydrogen producers (OBHP) goes according to Equations 5.2 and 5.3 respectively (FAO, 2015);

$$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + H^+ + 3HS^- \qquad \Delta G° = -151.3 \qquad 5.2$$

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad \Delta G° = +76.1 \qquad 5.3$$
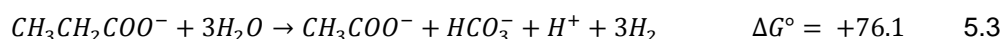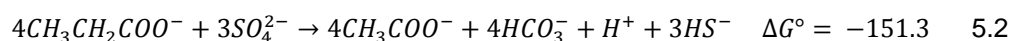
According to the above equations, SRB-induced propionate degradation is more thermodynamically favourable than OBHP-induced degradation. In addition, 1 mole of propionate reduced by SRB yields only 1 mole of acetate, whereas, propionate degradation by OBHP yields 1 mole of acetate and 3 moles of hydrogen. Also, butyrate degradation by sulphate reducing bacteria follows the reaction in Equation 5.4, and degradation by OBHP according to

Equation 5.5. Again, we see that butyric acid reduction by sulphate reducing bacteria reduces the amount of hydrogen released.

$$2CH_3(CH_2)_2COO^- + SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + H^+ + HS^- \quad \Delta G° = -55.7 \qquad 5.4$$

$$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+ \qquad\qquad \Delta G° = +48.1 \qquad 5.5$$
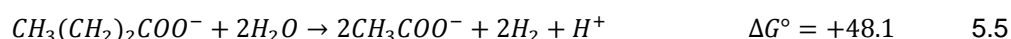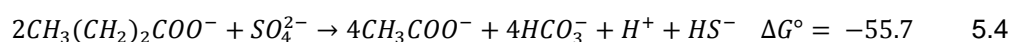
Therefore, VFA intermediates degradation by sulphate reducing bacteria had an impact on the methane yield by reducing the hydrogen released from such reactions, which could have been utilised by hydrogenotrophic methanogens. This must have influenced the reduction in biomethane margin between the control and test reactor by Day3 through Day10 in Exp1 (Figure 5.4).

The highest rate of sulphur degradation as a result of hydrogen addition was observed with Exp2. Having already gone through primary fermentation, the substrates availability was more in favour of the sulphate reducing bacteria than the hydrogenotrophic methanogens. Unlike the hydrogenotrophic methanogens, sulphate reducing bacteria had more readily available substrates in terms of hydrogen, VFA and initially solubilised sulphur. Therefore, high rate of elemental sulphur degradation was observed in the test reactor.

This high rate of elemental sulphur degradation in Exp2 corresponds with the high hydrogen utilisation also recorded for the same experiment, which implies high competition for the added hydrogen. As was seen previously in Section 5.4.2, hydrogen injected into the test reactor of Exp2 was completely removed by the next day. While this was not ultimately converted to methane, elemental sulphur reduced in the test reactor for the same time period. Therefore, the extensive removal of H_2 in Exp2 was predominantly through sulphate reducing bacteria utilisation.

Moreover, elemental sulphur in the test reactor continued to reduce after this time, indicating that other substrates such as propionic acid were also competed for by the SRB in Exp2. It was reported that sulphate reducing bacteria cannot compete effectively with the fermentative microorganism, which are relatively more fast growing (Postgate,1984 cited in Chen et al., 2008). In agreement, O'Flaherty et al. (1999), added sulphur to an AD treating glucose and lactose, and observed no changes in the sulphate degradation rates, implying the sulphate reducing bacteria did not grow on the substrates. In essence, the sulphate reducing bacteria's metabolism is more effective during acetogenesis and methanogenesis, which would explain the extensive

elemental sulphur degradation in the test reactor of Exp2, immediately after hydrogen injection.

Two levels of sulphate reducing bacteria inhibition on methane production can be identified; primary inhibition resulting from competition for common substrates such as hydrogen and acetate (Chen et al., 2008) and secondary inhibition as a result of the toxicity of produced sulphide on different microbial groups (Colleran et al., 1995). The outcome of the competition for substrates (primary inhibition) influences the sulphide concentration within the system (secondary inhibition). Hence, there is the possibility that the initial dominance of sulphate reducing bacteria led to high level of sulphides, consequently, a backward sulphide inhibition on the sulphate reducing bacteria was encountered, which accounts for the increase in elemental sulphur observed at Day15. At the end of the Exp2, elemental sulphur was neither measured in the test nor control reactors, which implies a higher sulphate reducing bacteria activity in both reactors of Exp2 and the addition of hydrogen in the test reactor, enhanced this process. However, elemental sulphur degradation in the control was only effective towards the end of the experiment, when the predominant VFA was acetic acid, implying that the major substrate utilised within the control reactor was acetic acid.

Furthermore, acetate degradation by SRB yields two moles of bicarbonate and sulphide, while the degradation of same by acetoclastic methanogens yields 1 mole of methane and bicarbonate each (Liu et al., 2018). Therefore, a higher amount of acetic acid used by the sulphate reducing bacteria in the control reactor, could also have contributed to the lower methane yield obtained accordingly. This was also supported by the relatively lower CO_2 removal factor for every increase in biomethane following hydrogen injection, of 0.8 obtained in Exp2, compared to 1.7 from Exp1. Therefore, the potential increase in methane yield attainable from the injected hydrogen in Exp2 was limited by a competitive utilisation, leading to a reduction in the HM growth potential. Thus, the growth of the sulphate reducing bacteria was presumably enhanced in Exp2, resulting in further competition for other VFA.

In Exp3, hydrogen mass balances earlier discussed proved that the added hydrogen was predominantly utilised for methane production. It was, however, unclear why this was the case, perhaps the limited amount of substrates for the sulphate reducing bacteria (in terms of VFA and oxidized sulphur) was responsible for this, since the elemental sulphur content of both the control and test reactors were not greatly degraded. Clearly, the sulphate reducing bacteria were outcompeted for the additional hydrogen in Exp3.

Overall, the percentage elemental sulphur degraded in both the control and test reactors within each experiment was about the same. Exp3 recorded the least elemental sulphur degradation, at 32.9% and 31.3% removal in the control and test reactors respectively. Hence, since the hydrolysis and the sulphate reducing bacteria activity was limited at this stage, the elemental sulphur was not greatly degraded. This justifies the initial assumption that elemental sulphur degradation was a function of hydrolysis and production of sulphides by the sulphate reducing bacteria. Meanwhile, 100% removal was recorded in both control and test reactors of Exp2 and in Exp1 93.5% and 94.1% removal was recorded from the control and test reactors respectively. These results; showing about the same level of elemental sulphur degradation in both the control and test reactors, suggest that the injection of hydrogen did not necessarily enhance the potential for sulphides production. However, the competition posed by sulphate reducing bacteria could significantly reduce the growth potential of the hydrogenotrophic methanogens (as observed in Exp2). As such, for a continuous system, this can effectively reduce the efficiency of hydrogen conversion to biomethane. Nevertheless, by optimising the periods before hydrolysis and during active methanogenesis to inject hydrogen, the competition for the injected hydrogen posed by the sulphate reducing bacteria can be effectively reduced during in-situ biomethanation.

5.4.5 Effect of VFA regime on the AD process stability

5.4.5.1 pH and alkalinity

The pH profile for all three experiments are presented in Figure 5.8; clearly showing that the VFA regime influenced the pH of the BMP process. Acidification from hydrolysis led to a sharp reduction in pH, which continued to reduce with the accumulation of VFA for all experiments. However, in the test reactor of Exp1 (Figure 5.8a), only a slight increase in pH was observed by Day2 and Day3 following the initial hydrogen. So that, regardless of the ongoing biomethanation, the continuous accumulation of VFA helped to buffer the excessive increase in pH. And the relatively lower pH observed by Day10 is in relation to the increased acetogenesis for the same period in the test reactor, as earlier discussed.

The increase in the pH of both the control and test reactors in Exp2 (Figure 5.8b) and Exp3 (Figure 5.8c), was a result of CO_2 removal within the system, which ensued when the bulk sample was split into the Wheaton reactors. As

such, Exp3 in which more CO_2 was displaced, higher pH increases were observed in both the control and test reactors. In comparison with the control, the additional increases in pH within the test reactors of Exp2 and Exp3 indicates the progression of biomethanation in the test reactors during both experiments. This effect was higher in Exp3 than Exp2, and implies a higher hydrogenotrophic methanogenic activity in Exp3 than in Exp2.

An optimal pH range of 7.0 to 7.5 is required for acetoclastic methanogenesis to proceed effectively (O'Flaherty et al., 1998), therefore, the excessive increase in pH in the test reactor of Exp3 above pH 7.5 could also have contributed to the slower rate of acetic acid degradation observed therein. As such, until Day12 when the pH in the test reactor became relatively favourable, acetic acid degradation was slower in the test reactor than the corresponding control of Exp3.

Increase in pH level is typical with biomethanation processes (Tian et al., 2018), and thus, a good indication of hydrogenotrophic methanogenesis occurring. However, this can be impacted by the nature of the feedstock, and the digestion stage during addition of hydrogen according to the results here obtained. In a study on hydrogen addition to AD of manure, Luo et al. (2012) reported pH increase between 8.2 and 8.3 due to bicarbonate consumption. With further investigations, Luo and Angelidaki (2013), demonstrated that the addition of acidic whey to manure, helped to buffer the system to maintain the pH below 8.0. Therefore, food waste is a much more suitable feedstock than the widely used feedstock for biomethanation, such as, sewage sludge and cattle manure, due to the buffering effect provided by VFA production.
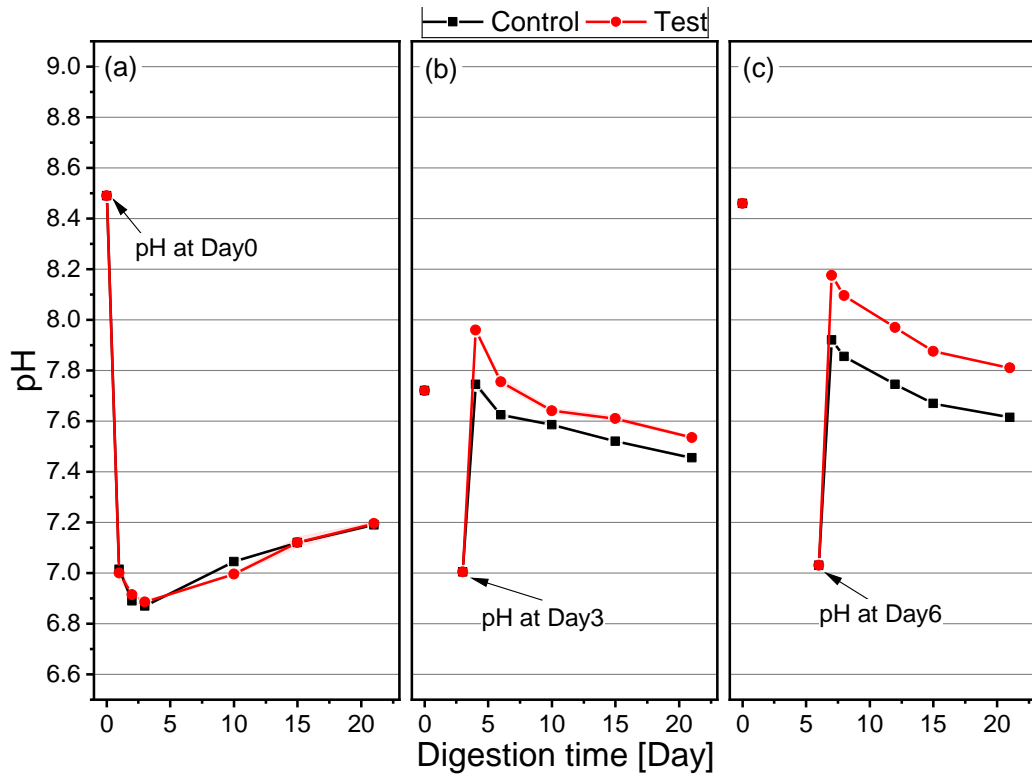
Figure 5.8. Influence of VFA regime on pH during biomethanation: *(a) Exp1, (b) Exp2 and (c) Exp3.* Shaded area around lines represent standard deviation from mean.

The alkalinity profiles for all three experiments are presented in Figure 5.9, to complement the alkalinity profiles, dissolved inorganic carbon (DIC) for each respective experiment have also been placed beside the alkalinity graphs in Figure 5.9. The removal of bicarbonate during biomethanation results in DIC reduction and thus, provides a good indication of hydrogenotrophic methanogenesis (Agneessens et al., 2017).

A build-up in VFA or enhanced removal of bicarbonates could lead to reduction in alkalinity (Appels et al., 2008). Since there was no VFA build-up in the test reactors for Exp1, the relatively lower alkalinity in the test reactor after the complete removal of the injected hydrogen, indicates an increased rate of bicarbonates removal.

This was also supported by the DIC profiles for the same experiment, whereby, the lower levels of DIC in the test reactor as digestion progressed, indicates enhanced removal of bicarbonates. This means the hydrogenotrophic methanogenic activity was enhanced throughout the digestion period, hence, supporting the arguments earlier presented that injecting hydrogen before hydrolysis resulted in the dominance of the hydrogenotrophic methanogens for hydrogen produced during secondary fermentation.
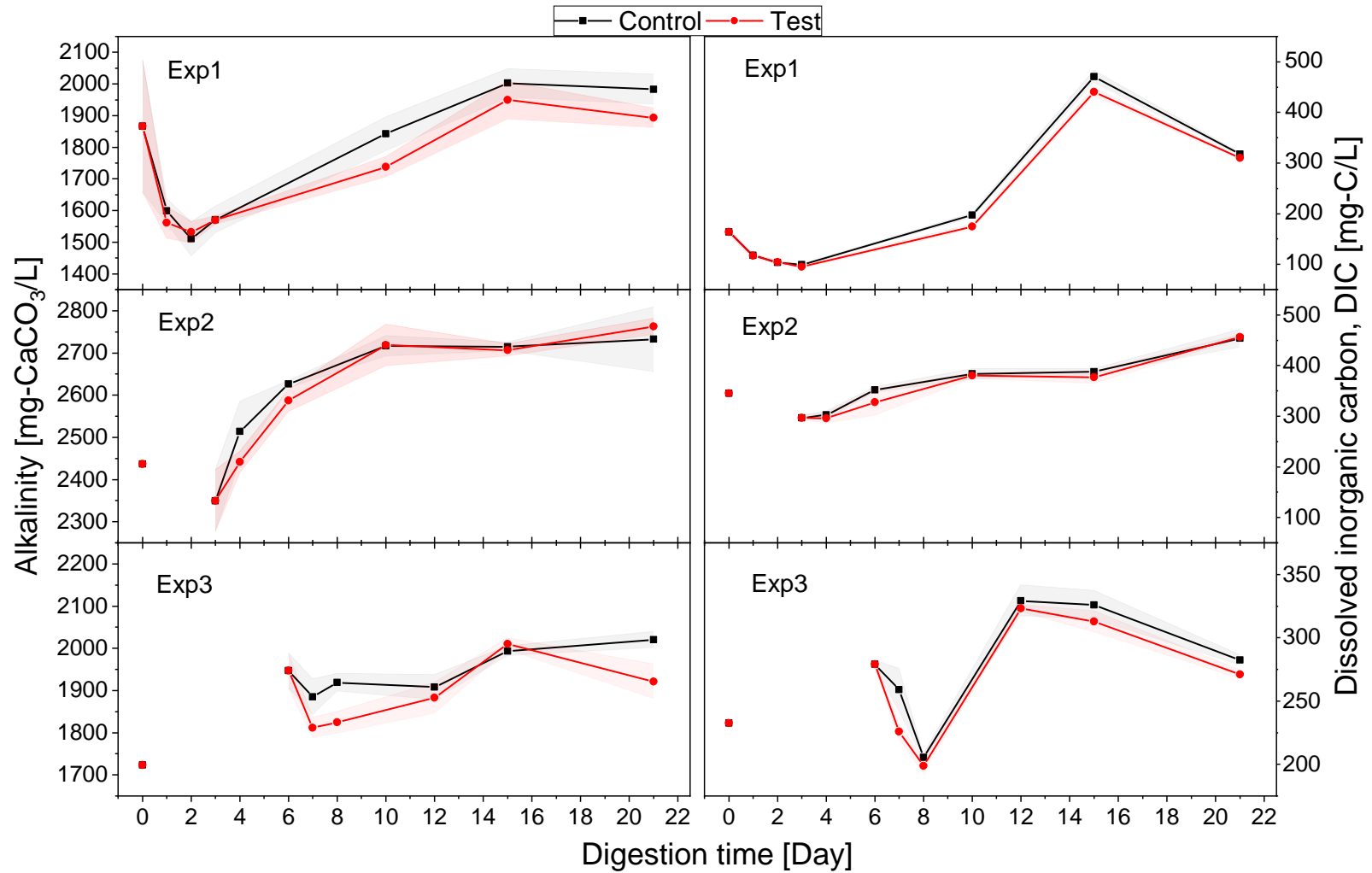
Figure 5.9. Plots of alkalinity (left) and dissolved inorganic carbon, DIC (right) in the control and test reactors' liquid contents of Exp1, Exp2 and Exp3. Shaded area around lines represent standard deviation from mean.

In Exp2, the alkalinity increased both in the control and test reactors after the setup. At this point of hydrogen injection, VFA concentration were presumably accumulated to high levels. Hence, the conversion of VFA to methane influenced a resultant increase in both control and test reactors. However, the alkalinity recovery was slower in the test reactor, indicating an enhanced hydrogenotrophic methanogenesis.

This was also supported by the increase in bicarbonate removal within the test reactor in the early days of setup; as presented in the DIC profile. Notwithstanding, the alkalinity and DIC quickly stabilises to about the same level as the control. Hence, although hydrogenotrophic methanogenic activity was believed to have been enhanced, the competitive coexistence of sulphate reducing bacteria, did not allow for optimum hydrogenotrophic methanogenesis during further VFA breakdown.

In Exp3, a reduction in both the control and test reactors was observed after setup, due to huge amount of CO_2 removed from both systems. And the slower alkalinity recovery in Exp3 compared to Exp1 and Exp2, was due to a lower amount of VFA available to enrich the alkalinity. However, a much lower alkalinity was measured in the test reactor for the days immediately following hydrogen injection. This indicates a more enhanced hydrogenotrophic methanogenic activity with hydrogen injection in Exp3 than Exp2, especially by the next day (Day7), whereby, no CO_2 was measured in the headspace. But, with continuous material degradation, the alkalinity gradually increased in the test reactor. The DIC profiles clearly shows the reduction in bicarbonate content of the test reactor in Exp3, therefore, indicating enhanced hydrogenotrophic methanogenesis as in Exp1.

In general, the alkalinity and DIC graphs show the extent to which hydrogen was utilised for biomethane production. These results further prove that the VFA regime prior to hydrogen injection, influences how hydrogen is utilised during biomethanation. In addition, with food waste as a feedstock, the alkalinity of the systems was not completely lost.

5.4.5.2 Materials degradation

5.4.5.2.1 Volatile solids destruction

The volatile solids (VS) profile during the BMP processes is presented in Figure 5.10. The percentage VS destruction measured between Day0 and Day1 was 17% and 14% for the control and test reactors in Exp1 respectively

(Figure 5.10a). This implies the possibility of an inhibited hydrolysis phase in the test reactor. However, analysing hydrolysis based on the VS content might be insufficient, as VS destruction during BMP can be a function of the conversion of feedstock to methane and the resultant increase in biomass produced. Hence, considering the biomethane yields were higher in the test reactor than the control from Day1, the higher VS content of the test sample could not have been a result of inhibited hydrolysis.
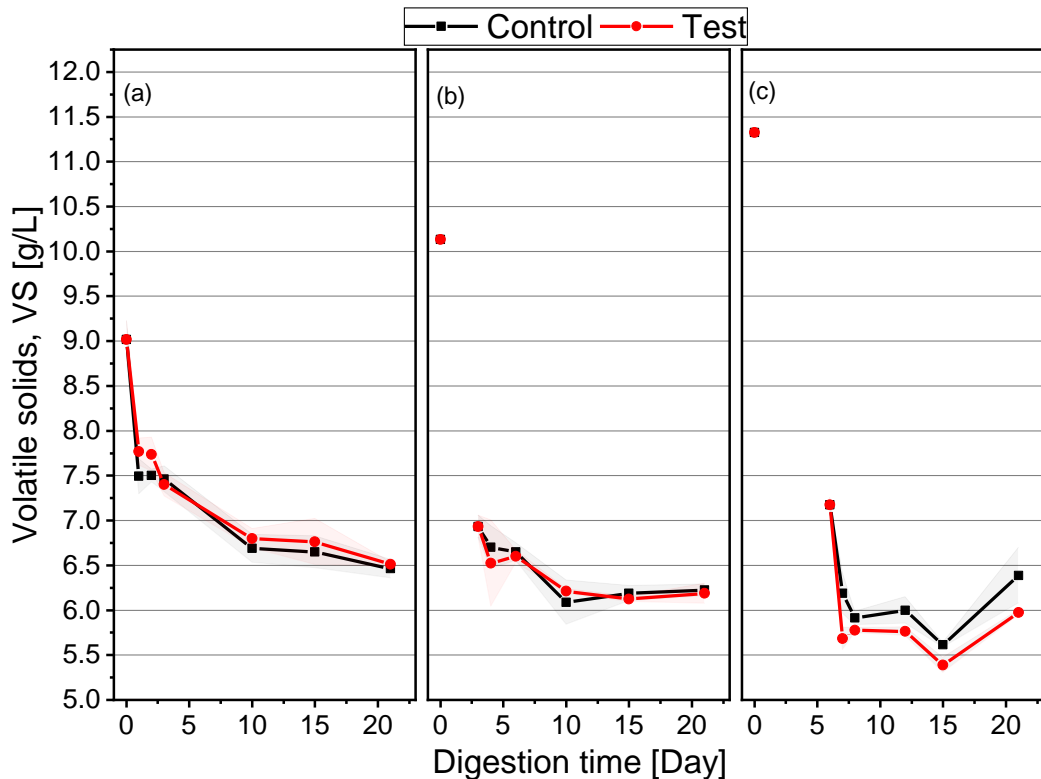
Figure 5.10. Effect of VFA regime on volatile solids destruction during food waste biomethanation: *(a) Exp1, (b) Exp2 and (c) Exp3.* Shaded area around lines represent standard deviation from mean.

Thus, when hydrogen was added before hydrolysis in Exp1, since the materials required by the hydrogenotrophic methanogens were readily available in the form of H_2 and CO_2, further materials breakdown seemingly ceased. So, the higher VS measured on Day1 and Day2 in the test reactor becomes directly related to natural microbial convenience selection, rather than hydrolysis inhibition. This was also supported by the earlier mentioned percentage hydrolysis of 90% and 91% measured in the test and control reactors respectively. Hence, there was an interim stall in direct materials breakdown to CH_4, further suggesting that the CH_4 produced was principally a direct consequence of hydrogenotrophic methanogenesis.

The VS degradation accelerated with the removal of the injected hydrogen in the test reactor of Exp1. The higher levels of VS measured afterwards could be due to a number of reasons such as an increased hydrogenotrophic methanogenic activity leading to higher biomass concentration. Luo and Angelidaki (2012), also reported that biomethanation enriched the hydrogenotrophic community however, this was not reported in terms of biomass yield.

When hydrogen was added in Exp2 and Exp3, there was limited CO_2 to utilise, hence, increased dependence was on materials degradation for CO_2 supply. This explains the lower levels of VS measured immediately following hydrogen addition in both experiments. In the case of Exp2, there was still sufficient amount of VFA to be utilised for this purpose, as such the VS destruction was not significantly different between the control and test reactor onwards after hydrogen removal in the test reactor. In Exp3 however, an increased VS destruction was observed in the test reactor due to highly limited substrate availability.

5.4.5.2.2 Dissolved organic concentrations

The dissolved organic concentrations presented in Figure 5.11, further supports the observations made with the VS destruction. The high DOC and sCOD concentration in the test reactor immediately following hydrogen addition in Exp1 demonstrates that food waste was hydrolysed. Notwithstanding, the rate of organic carbon consumption slowed as a result of readily available food forms for the hydrogenotrophic methanogens; in the form of H_2 and CO_2. With an increase in the hydrogenotrophic community, higher rate of sCOD removal was observed going forward, in the test reactor than the control.

In Exp2 whereby, food waste had already undergone some solubilisation and coupled with the competition for hydrogen, the dissolved organics degradation was not so different between the control and test reactors. In Exp3, however, the increase in DOC and sCOD for both the control and test reactors indicates an enhanced materials solubilisation to help stabilise the process, however, this was greater in the test reactor.

This increased rate of materials solubilisation in the test reactor influenced a higher dissolved organic content. This increase in the organic content could also have contributed to the slow acetic acid degradation rate reported in 5.4.3.3.
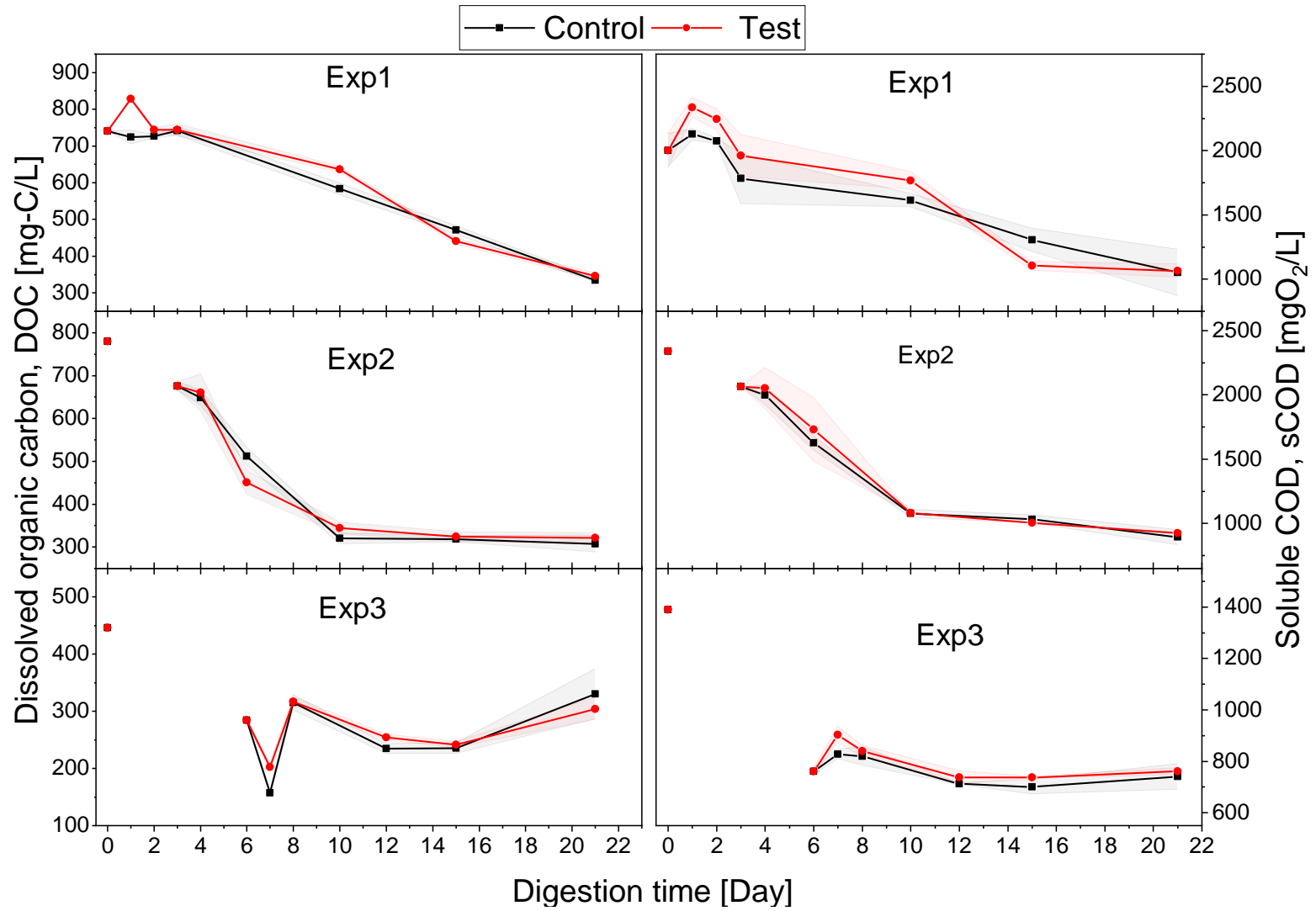
Figure 5.11. Dissolve organic concentrations during food waste biomethanation experiments, as influenced by VFA regime. Shaded area around lines represent standard deviation from mean.

5.4.5.3 Ammonia

Figure 5.12 represents the ammonia-nitrogen curves for the three sets of experiment during the digestion period. Addition of hydrogen was observed to have some effect on the ammonia levels; especially for Exp2. When hydrogen was added before hydrolysis in Exp1, the ammonia concentration only reduced for the period when the added hydrogen was actively consumed, and increased rapidly afterwards. The lower ammonia level observed in the test reactor of Exp1 could either be due to the temporary switch to H_2 and CO_2; which was established to have stalled further materials degradation for the same time period, or the use of ammonia to provide alkalinity, or a combination of both.

When hydrogen was added after hydrolysis in Exp2, the ammonia level was observed to reduce in the test reactor throughout the digestion period. In this case, the lower ammonia level was a result of its utilisation to regain lost alkalinity in the form of ammonium bicarbonate (Banks et al., 2008). Ammonium bicarbonate is thermally unstable and can easily be dissociated especially in the presence of organic acids, however, since, there was neither production of organic acids nor further ammonia release from typical hydrolysis, the ammonia level remained low in the test reactor, throughout the digestion period.

In Exp3, the initial reduction in total ammonia-nitrogen levels observed in both the control and test reactors after setup might have been a result of more ammonia utilisation to enhance alkalinity; since the substrate available was highly insufficient at this time, unlike Exp1 and Exp2. However, the ammonia levels in the test reactor remained relatively higher than the control throughout the digestion period. This was similar to the effect observed with dissolved organic concentrations for the same experiment; following an increased breakdown of materials.
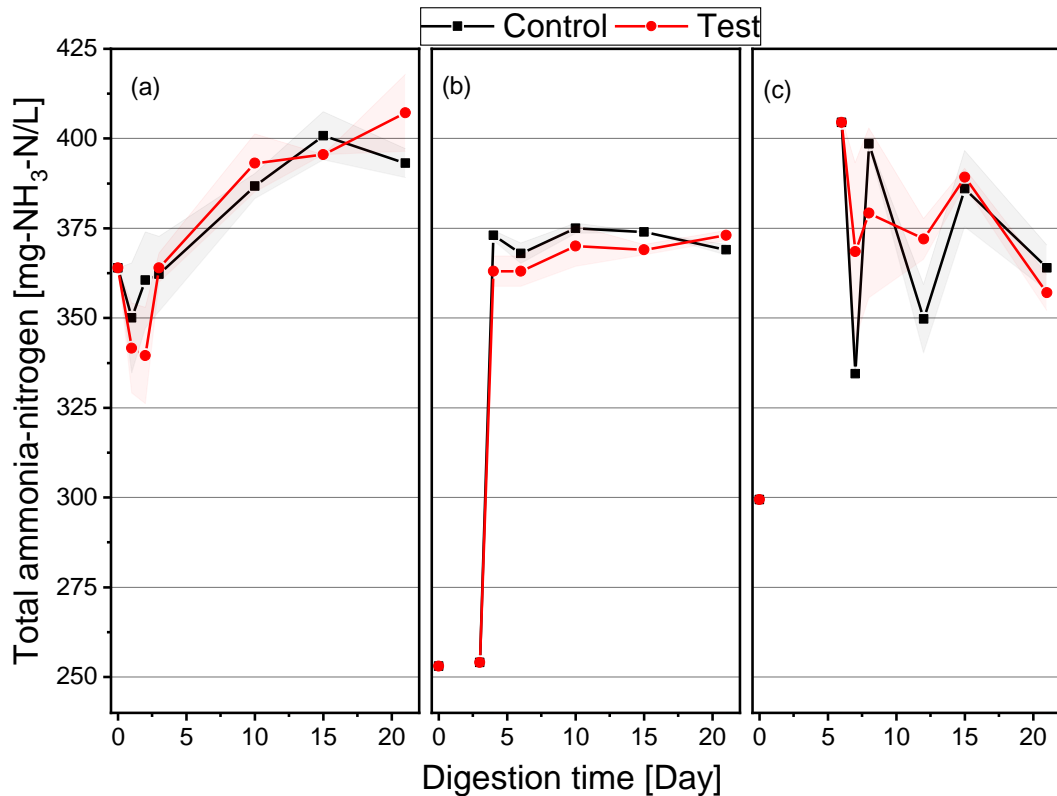
Figure 5.12. Effect of hydrogen addition and VFA regime on total ammonia concentrations of the control and test reactors; *a) Exp1, b) Exp2 and c) Exp3.* Shaded area around lines represent standard deviation from mean.

Clearly, the stage of digestion and the VFA regime prior to hydrogen addition plays an important role on the different forms in which ammonia is available within the system. Perhaps the impact of biomethanation on ammonia was not large enough due to the low levels of hydrogen used in this study, so that with higher hydrogen loads, the changes in ammonia levels can be more appreciated.

5.5 Conclusions

According to the findings in this chapter, food waste is a suitable feedstock for AD with in-situ biomethanation and the high organic content of food waste was of advantage to help regain lost alkalinity and stabilise pH. Hydrogen injection enhanced hydrogenotrophic methanogenesis, however, the VFA regime had huge influence on the outcome of the completion for the injected hydrogen.

Injecting hydrogen before hydrolysis at Day0 (Exp1) influenced an autocatalysation towards an enhanced hydrogenotrophic methanogenesis before other hydrogen consumers became relatively active and the useful nutrient was depleted. In Exp1, hydrogenotrophic methanogens dominated

the process. Since the sulphate reducing bacteria cannot metabolise during hydrolysis, there was a direct assimilation of the readily available H_2 and CO_2 by the hydrogenotrophic methanogens, rather than dependence on substrate-based nutrients. Typically, by Day3, the AD system was already autocatalysed to the substrate, which implies there was a well-defined community of all acting microorganisms. Therefore, there was an extensive competition for hydrogen when injected at Day3 in Exp2. As a result, the competition followed the most favourable thermodynamic pathway for the available substrate; which was by sulphate reducing bacteria utilisation. Hydrogen injected by Day6 in Exp3 was predominantly utilised for hydrogenotrophic methanogenesis. By this time, most of the VFA had completely depleted, leaving only acetic acid and trace amounts of propionic acid, hence, there was limited competition for the added hydrogen.

The biomethane content of the biogas increased from 65 to 77.1%, 84.8 to 88.8% and 70.2 to 79.8% in Exp1, Exp2 and Exp3, which corresponds to 12.1%, 4% and 9.6% biomethane increases respectively. This confirms an autocatalysation towards an enhanced hydrogenotrophic methanogenesis in Exp1. Also Exp3 whereby, most of the useful VFA intermediates had depleted before hydrogen injection, produced the second highest increase in biomethane yield. Moreover, for the increases in ultimate methane yield in Exp1 and Exp3, there was a resultant 1.7 and 1.5 times reduction CO_2 respectively, but with Exp2, there was only 0.8 times reduction in CO_2, which further elucidates that hydrogen added during Exp2 was highly competed for. Day0 injection was therefore, chosen as the optimal injection point for hydrogen and adopted in further optimisation experiments.

CHAPTER 6

FOOD WASTE BIOMETHANATION: EFFECT OF HYDROGEN GAS ACCLIMATION

6.1 Introduction

Biomethanation processes have recorded relative successes in biogas upgrade. The primary limitation of this process is the hydrogen gas-liquid mass transfer rate. This can however, be enhanced by the mixing regime (Luo and Angelidaki, 2012; Yun et al., 2017), an extended gas residence time (Savvas et al., 2017) and also the hydrogen injection design, such as trickling filters (Rachbauer et al., 2016). Moreover, it is believed that the hydrogen gas-liquid mass transfer rate can be enhanced by an improved hydrogenotrophic methanogens' population, which can be achieved by acclimation (Mulat et al., 2017).

Exposing AD consortia to increasing levels of inhibitory elements, allow them to adapt to and overcome the inhibitory effects; a process known as acclimation (Gao et al., 2015; Liu and Sung, 2002; Rajagopal et al., 2013; Yenigün and Demirel, 2013). Acclimation has been suggested as a method of improving the tolerance of AD microbial consortia to inhibiting/toxic substances including: ammonia, long chain fatty acids (LCFA), metals and phenolic compounds (Chen et al., 2008). This is generally brought about by a shift in the microbial population or internal changes that occur in the predominant microbial species (Chen et al., 2008).

This principle was also adopted in this study, to acclimate the AD consortia to increasing concentrations of hydrogen gas. In effect, the gradual increase in the concentration of this electron carrier to an acclimated population would help the microbial consortia to adapt to high hydrogen loads and also improve the hydrogenotrophic methanogens' population.

6.2 Chapter objectives

- To analyse the influence of acclimating food waste AD system to increasing concentrations of hydrogen.
- To establish a statistical relationship between hydrogen utilisation and biomethane production for food waste biomethanation experiments.

6.3 Experimental set up

Experiments were designed using the composite food waste sample at optimal conditions described in Chapters 4 and 5; 1 mm PS, ISR of 3 (Chapter 4) and Day0 hydrogen injection (Chapter 5). The schematic representation of the experimental design for hydrogen acclimation is shown in Figure 6.1. Three sets of experiments with hydrogen addition were conducted to analyse the impact of acclimation on hydrogen conversion to biomethane and labelled as EH1, EH2 and EH3 respectively. As in Chapter 5, each set of experiment had a blank, control and test reactor and both control and test reactors were treated to the same inoculum and food waste dosing condition. Hence, for the acclimation stages all the reactors had an acclimated inoculum. Hydrogen was added to the test reactors using a gas mix of hydrogen and nitrogen at 5:95, 10:90 and 15:85 (% v/v) (see Figure 6.1).

The effect of acclimation only on the AD process was measured as the changes in process characteristics of the control reactors in EH2 and EH3 in comparison with EH1. And the combined effect of acclimation and increasing concentration of hydrogen, was measured as the characteristic changes in the test reactors accordingly.

For easy comparison of effects of change within experiments, some of the graphs have been plotted as normalised values (by dividing the respective value by the value at the start of the experiment), rather than actual parametric values between experiments.
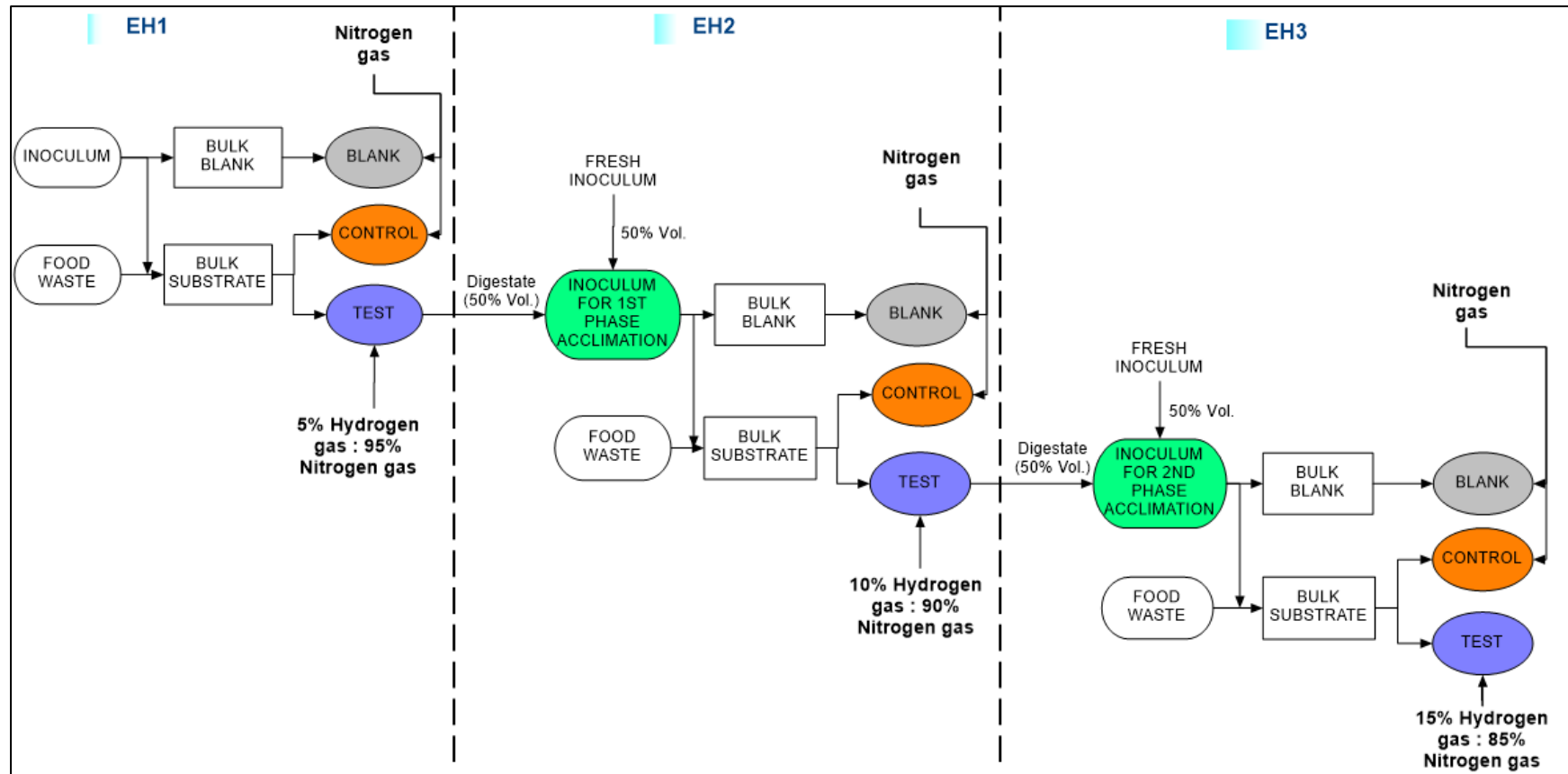
Figure 6.1. Schematic representation of hydrogen-based biomethanation acclimation experimental setup.

6.4 Effect of increasing levels of hydrogen gas on biogas characteristic

6.4.1 Hydrogen gas utilisation

The rates of headspace hydrogen removal and the corresponding changes in CH_4 and CO_2 yields are presented in Figure 6.2. A detailed discussion on hydrogen utilisation in EH1 was given in Chapter 5 (as Exp1). Hydrogen was measured in the headspace of the control reactor in EH1, but during the acclimation phases in EH2 and EH3, hydrogen was not detected in the control reactors (Figure 6.2). This implies the addition of hydrogen in EH1 improved the hydrogenotrophs, which led to an increased rate of hydrogen utilisation during the acclimation phases in EH2 and EH3.

By implication, VFA degradation (as will be discussed later) as well as biomethane production was also enhanced in both the control and test reactors of EH2 and EH3.
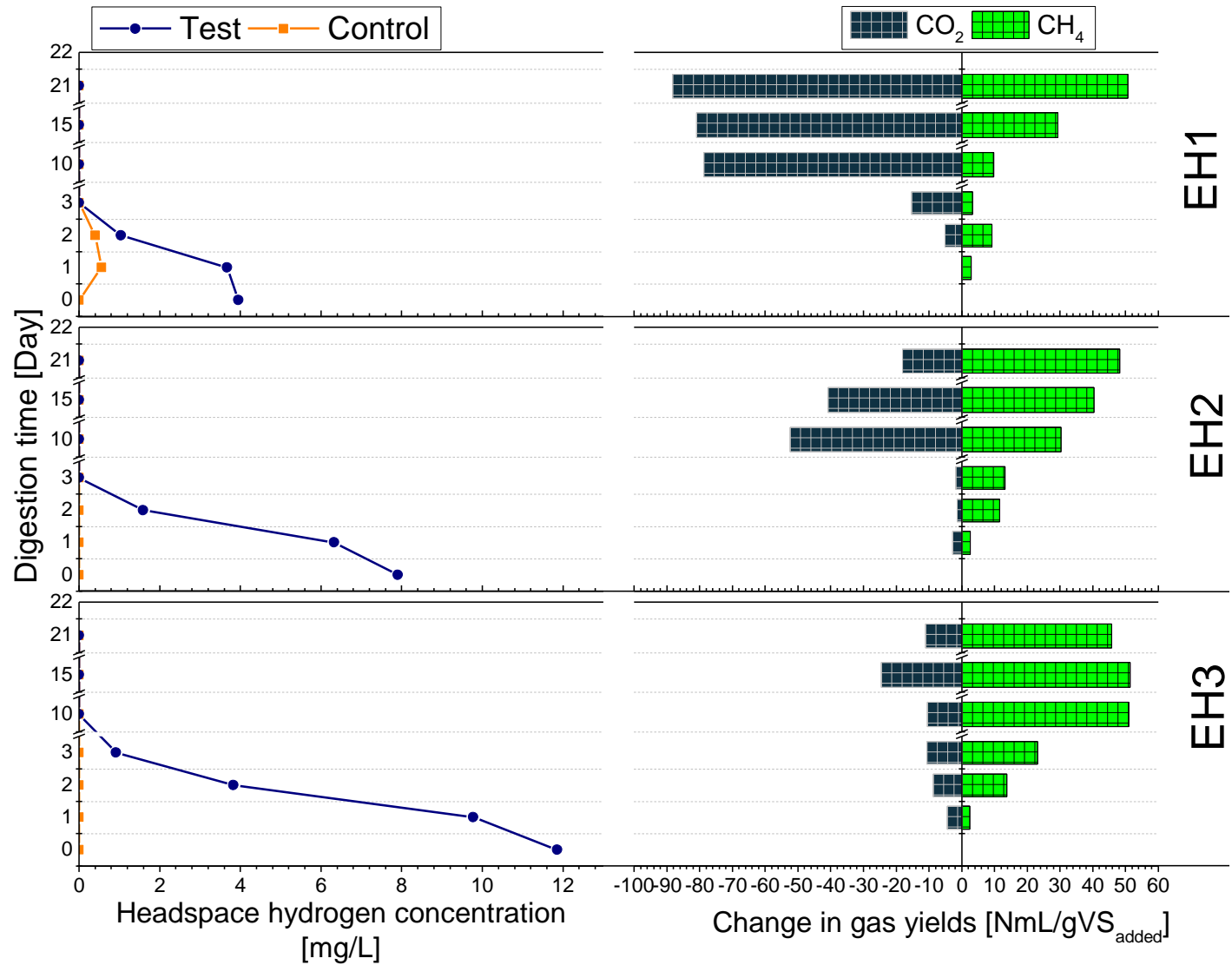
Figure 6.2. Headspace H$_2$ concentration (line graphs), as an indication of hydrogen gas-liquid transfer and Change in CH$_4$ and CO$_2$ yields (bar graphs), taken as a test yields minus control yields.

Therefore, because the control reactors in EH2 and EH3 were equally improved by the acclimated inoculum, the percentage change in biomethane between the test and control reactors reduced through the acclimation phases. Since hydrogen was not measured in the control reactors during the acclimation phases, the gaseous hydrogen utilisation rates were only analysed for the test reactors. In EH1, the percentage gaseous hydrogen removal in the test reactor by Day1 and Day2 was 7.2% and 71.6%, measuring 0.28 and 2.63 mg/L respectively. In the first phase of acclimation (EH2), the percentage gaseous hydrogen removal by Day1 and Day2 was 9.3% and 74.8%, measuring 0.65 and 4.74 mg/L respectively. Going forward to phase 2 acclimation in EH3, the percentage gaseous hydrogen removal by Day1 and Day2 was 20.9% and 60.8%, measuring 2.58 and 5.94 mg/L respectively. This successive increases in the percentage and concentration of gaseous hydrogen removed due to acclimation, confirms hydrogenotrophic population was enhanced at every stage of acclimation. This also explain why hydrogen was not measured in the headspace of the control reactors in EH2 and EH3.

In all experiments, the highest hydrogen gas removal was between Day1 and Day2; and is understandably so, considering the system had to adjust to the initial high hydrogen load at the start of the experiment (between Day0 and Day1). So that, when the system adjusted to the hydrogen load, rapid consumption ensued.

The graphs on the right presented in Figure 6.2, show the difference between the yields from the test and control reactors of each respective experiment that is, EH1, EH2 and EH3. Progressing from EH1 through EH3, the change in biomethane yield improved in the early days of digestion, when gaseous hydrogen was made available. This shows that the increase in the amount of hydrogen utilisation impacted on biomethane production. Interestingly, the change in CO_2 yields also decreased through EH1 and EH2 by acclimation. This means that with the use of the acclimated inoculum alone, CO_2 production reduced in the control reactors of EH2 and EH3. Therefore, the difference between the CO_2 yields, between the test and control reactors also declined.

However, it is unclear why this was so, perhaps, the improvement of the hydrogenotrophic methanogens influenced more methane production via the H_2/CO_2 route by syntrophic acetate oxidation and less acetoclastic methanogenesis. Additionally, more CO_2 could also have been utilised for

biomass growth, by virtue of the increase in hydrogenotrophic methanogenesis (Lecker et al., 2017).

To work out the hydrogen utilisation towards biomethane production in the acclimation phases, it was postulated that since the inoculum condition in the control and test reactors within each experimental setup were the same, the difference between the biomethane yields, should theoretically be equivalent to the biomethane that would be produced from the gaseous hydrogen added/utilised. Hence, the theoretical biomethane yield on this basis was estimated as the biomethane yield expected from the utilised gaseous hydrogen up till Day2 in EH2 and Day3 in EH3. This was then compared with the actual change in biomethane yield between the control and test reactors from EH2 and EH3, using three guiding conditions.

Firstly, if the theoretical yield from hydrogen utilised was lower than the actual difference between the test and control reactors, then VFA degradation was enhance and also contributed to biomethane production in the test reactor. Secondly, a higher theoretical yield meant hydrogen was either utilised for other products such as sulphides or higher VFA, or VFA degradation was inhibited. Lastly, where the theoretical yield equals actual biomethane difference, the increase was primarily from the utilised gaseous hydrogen.

In EH2, hydrogen was not measured in the headspace of the test reactor by Day3, and the actual difference in biomethane yield was higher than the theoretical by 60% by Day2, signifying that VFA degradation was also enhanced. It is unclear if the additional biomethane produced was through acetoclastic methanogenesis or syntrophic acetate oxidation. Although, lower CO_2 yield obtained in the test reactor suggests syntrophic acetate oxidation could have been the favoured route. The possibility of an enhanced VFA degradation was also supported by the non-detection of H_2 in the headspace of the control reactor, to signify rapid H_2 removal and consequently, a relatively less inhibited VFA degradation.

In EH3, hydrogen was measured in the headspace of the test reactor by Day3, and the actual difference in biomethane yield was also higher than the theoretical yield from H_2 utilisation by 15% by Day2 and reaching 60% by Day3; again, signifying improved VFA degradation. Effectively, by acclimation the hydrogen utilisation rate was perceived to be enhanced, thereby, limiting the availability of dissolved hydrogen to cause inhibitions at any time.

In general, the addition of hydrogen to acclimated inoculum in EH2 and EH3 was met with more rapid gaseous hydrogen removal, which increased as the

acclimation progressed from phase 1 to phase 2, in EH2 and EH3 compared with EH1 (Figure 6.2). In agreement, during a batch biomethanation study using mesophilic sludge, pulse injection of hydrogen over 5 consecutive days enriched hydrogenotrophic methanogens' adaptation, subsequently, increasing the hydrogen uptake rate (Agneessens et al., 2017).

6.4.2 Biogas yield from H_2 acclimation

The biomethane and carbon dioxide yields from H_2-based acclimation experiments in EH1, EH2 and EH3 are presented in Figure 6.3, which shows the yield from the test reactors in solid lines and the corresponding control yields in dash lines. By acclimation, the control reactors were observed to improve in biogas production rate and the quality. This was especially so for CO_2 reduction, whereby, the non-acclimated control reactor in EH1 had the highest amount of CO_2 in the biogas, but with acclimation the CO_2 reduced.

For instance, considering the control reactors only, biomethane yield increased from 417.6 $NmL\text{-}CH_4/gVS_{added}$ in EH1 to 435.4 $NmL\text{-}CH_4/gVS_{added}$ in EH2 following the first phase acclimation. This further increased to 453.3 $NmL\text{-}CH_4/gVS_{added}$ in EH3 after the second acclimation phase. Correspondingly, the CO_2 yield reduced from 227 $NmL\text{-}CO_2/gVS_{added}$ to 154 $NmL\text{-}CO_2/gVS_{added}$ and 129 $NmL\text{-}CO_2/gVS_{added}$, moving from EH1 to EH2 and EH3 respectively. Consequently, by acclimation only, biogas was improved from 64.8% biomethane in EH1, to 73.9% in EH2 and finally 77.8% in EH3. This improvement in biogas quality by virtue of acclimation clearly depicts that hydrogenotrophic methanogenesis was enhanced. One major proof to this assertion is the fact that unlike in EH1, whereby, hydrogen was measured in the headspace of the control reactor up till Day2, the control reactors in EH2 and EH3 did not record any gaseous hydrogen. And the rapid removal of hydrogen within the system, impacted on the rate of VFA fermentation and conversion to biomethane.
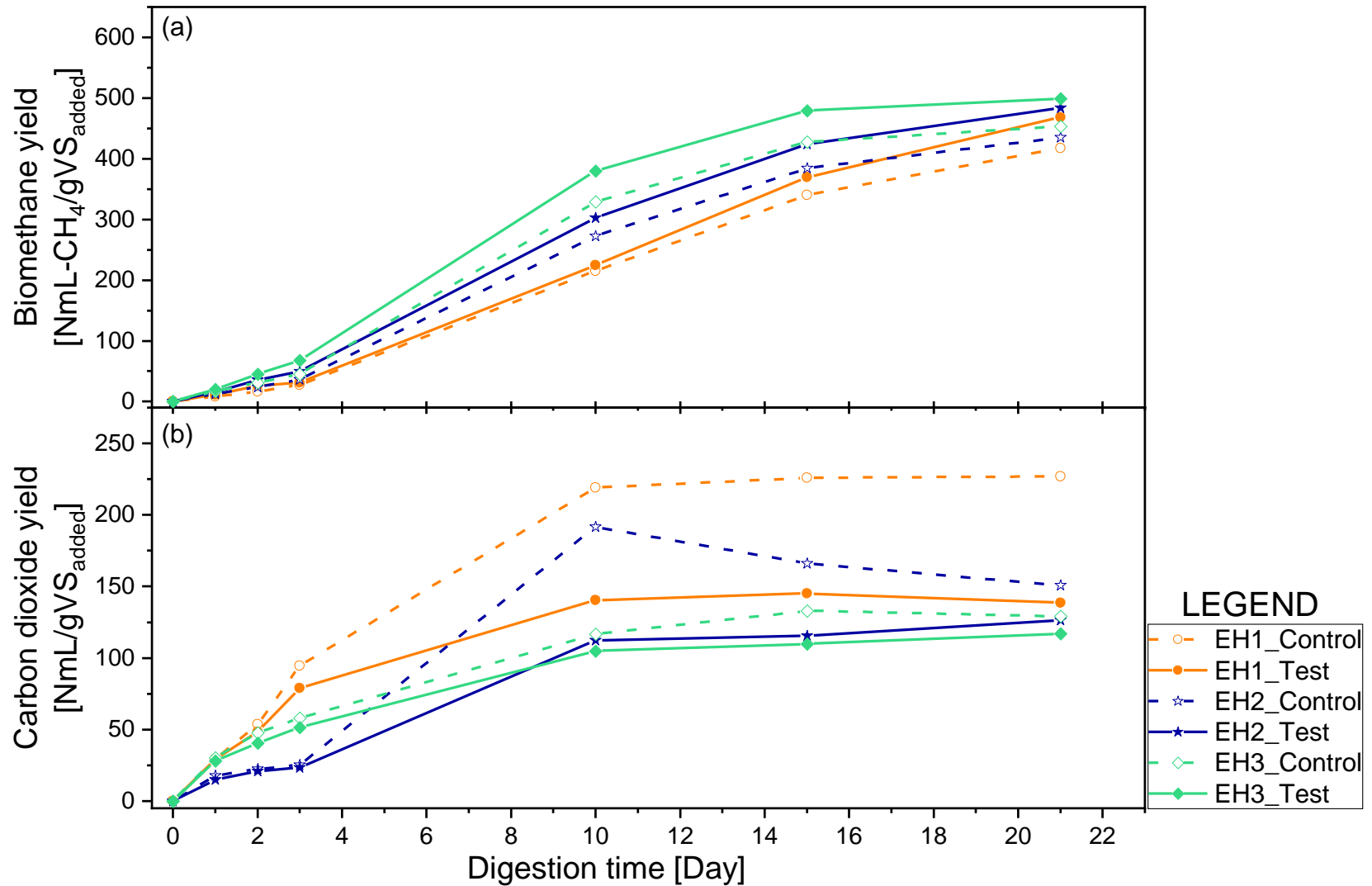
Figure 6.3. Biomethane (a) and Carbon dioxide (b) production curves from all hydrogen-based acclimation experiments: dash lines represent control yields and the solid lines represent test yields.

The biogas quality was further improved by the combined effect of acclimation and increase in hydrogen in the test reactors over the control. The biomethane contained in the biogas of the test reactors improved from 77.2% in EH1, to 78.1% in EH2 and 81.0% in EH3, corresponding to 468.3, 483.6, and 499.0 $NmL-CH_4/gVS_{added}$. In comparison with the control reactors, the increase in percentage biomethane was 12.4%, 4.2% and 3.2% in EH1, EH2 and EH3 respectively. The reduction in the biomethane margin, was a result of the corresponding improvement in the control reactors. This improvement indicates that hydrogenotrophic methanogenesis was further enhanced at every acclimation phase.

The findings from this study are similar to batch biomethanation studies whereby, more than one-time hydrogen injection was made. For the batch mesophilic biomethanation with maize leaf as substrate, final biomethane yield ranged from 76.8 – 100%; an improvement over 59.4% obtained without biomethanation (Agneessens et al., 2017). However, yields that tended towards 100% CH_4 as a result of excessive H_2 loading enriched homoacetogenesis, consequently, inducing VFA inhibition and accumulation (Agneessens et al., 2017). Furthermore, during a batch thermophilic biomethanation study using two types of maize leaf as substrate, biomethane yield increased from 64.4% and 65.2% to 87.8% to 89.4% respectively (Mulat et al., 2017). In agreement, with the use of cattle manure as substrate, Bassani et al. (2015) recorded biomethane increase from 69.7 to 88.9% at thermophilic temperature and 67.1 to 85.1% at mesophilic temperature respectively.

Contrariwise, short term adaptation of H_2/CO_2 to AD reactors containing sewage sludge, digested manure and granular sludge all influenced lower methane production rates and biomethane yields from such systems (Pan et al., 2016). Their study was however, conducted within a week, hence, the decrease was probably as a result of the microorganisms being in the decay phase when used in subsequent experiment. More so, the nature of feedstock in this study being food waste in digested sewage sludge inoculum, could also have influenced the positive outcome in biomethane yield.

Ultimately, H_2 acclimation helped to reduce CO_2 yields and improve biogas quality in this study. The addition of H_2 to acclimated system resulted in both CH_4 increase and CO_2 decrease in the test reactors compared to the control, which agrees in general with previous studies on biomethanation (Angelidaki et al., 2018).

6.5 Effect of increasing concentrations of H_2 gas on the biomethanation process

6.5.1 VFA profile

The VFA profile for experiments with H_2 gas is presented in Figure 6.4. Sequel to biomethanation with 5% H_2 in EH1, the rate of VFA degradation improved by virtue of both acclimation and increasing concentration of hydrogen in EH2 and EH3. It was perceived that acclimation improved material solubilisation, leading to faster VFA degradation. This was also supported by the higher biomethane production discussed earlier in Section 6.4.2 and increased DOC removal (discussed further in Section 6.5.2) in both the control and test reactors in EH2 and EH3.

The initial total VFA available at the start of each experiment was the same in the control and test reactors measuring 52.1, 15.8 and 21.2 mg/L in EH1, EH2 and EH3 respectively. Accounting for the concentration of VFA at the peaks, the control reactors measured 803.0, 694.9, and 705.2 mg/L, while the test reactors measured 807, 715.5 and 726.2 mg/L in EH1, EH2 and EH3 correspondingly, indicating lower VFA build-up for succeeding experiments. The relatively higher concentrations in the test reactor of each experiment at the peak point were not surprising, considering the possible interim shift in substrates utilisation until hydrogen was completely depleted. Although, VFA increases in the test reactors were negligible, Luo and Angelidaki, (2013) also reported relative VFA accumulation, during biomethanation using cattle manure and whey as co-feedstock.

However, as the digestion progressed, the rate of VFA degradation consequently increased in the acclimated reactors, especially the test reactors. This implies acclimation improved the rate of VFA degradation as a result of an increased hydrogenotrophic methanogenic activity.

Figure 6.4. Total volatile fatty acids profile for hydrogen-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

6.5.1.1 VFA composition in H₂ acclimated experiments

In this section, butyric acid is presented as a combination of iso-butyric and butyric acids and valeric acid presented as a combination of iso-valeric and valeric acids. The composition of the predominant VFA; acetic, propionic, butyric and valeric acids for H$_2$-utilised biomethanation experiments are presented in Figure 6.5.
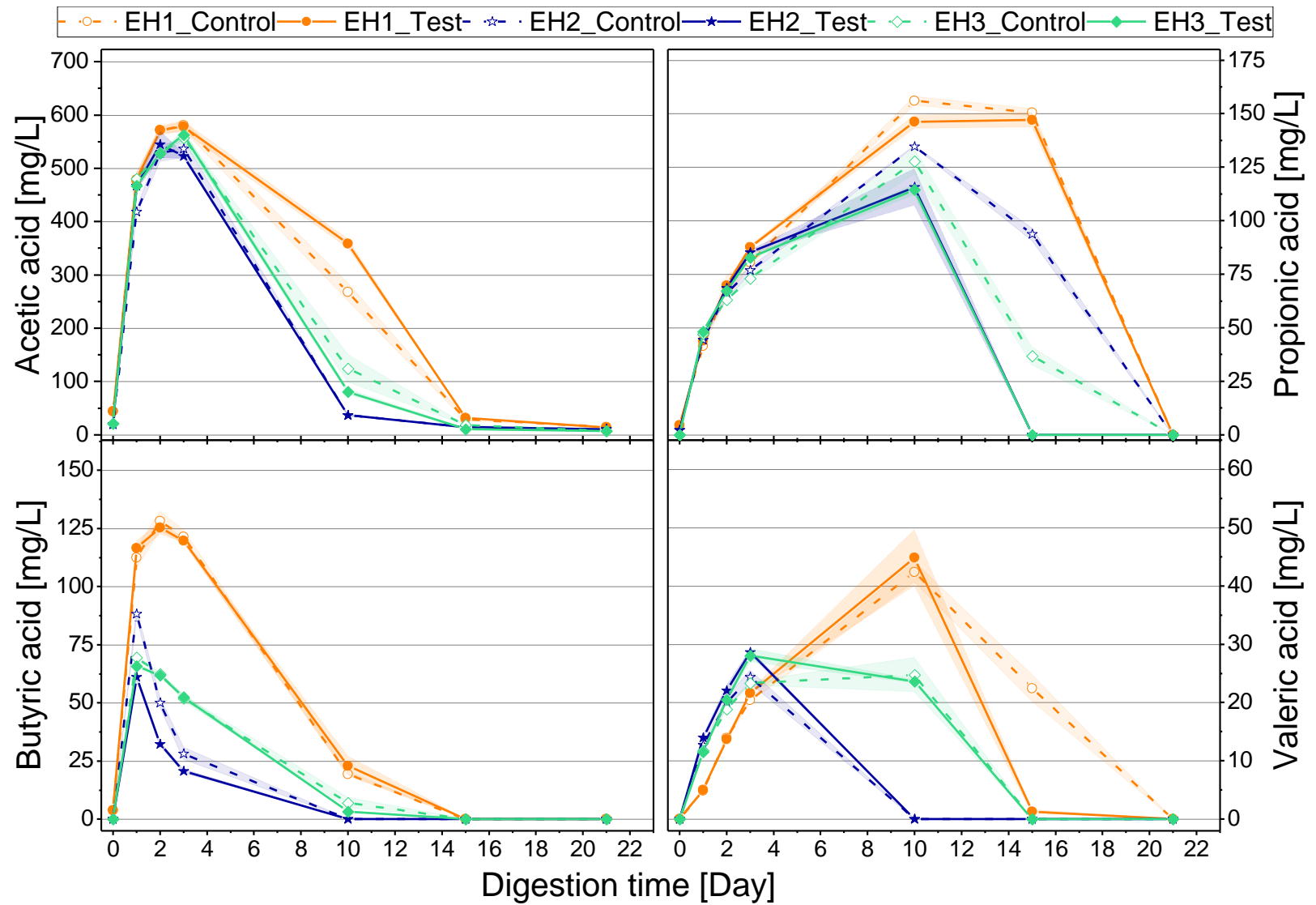
Figure 6.5. Effects of hydrogen acclimation on Volatile fatty acid composition: test values presented in solid lines and control in dash lines. Shaded area around lines represent standard deviation from mean.

Addition of hydrogen in EH1 prior to acclimation resulted in slightly higher concentrations of VFA with longer carbon chains than acetic acid, including; propionic, butyric and valeric acids, for the periods hydrogen was also measured in the headspace. Build-up of VFA (except acetic acid) have been established to be a result of high hydrogen loads within the system (Mosey, 1983), therefore, it was not surprising to have higher concentrations of these acids at the initial stages of digestion when the hydrogen concentration was still quite high. After this period, these acids were observed to degrade relatively faster in the test reactor than the control, as a result of the improved hydrogen consumption rate within the system.

By acclimation alone, VFA accumulation generally reduced through the acclimation phases, especially for the higher VFA. The reduction in the build-up of higher VFA in the acclimated control reactors, buttresses the presumption that hydrogenotrophic methanogens were enhanced by acclimation. Consequently, VFA-induced inhibitions were also reduced in the succeeding acclimation phases. This could be one of the factors responsible for the increase in biomethane yield recorded in the control reactors by virtue of acclimation only. Interestingly, early stage production of acetic and propionic acids were not obviously influenced by either acclimation, but there was a shift from butyric to valeric acid production.

Acclimation reduced the rate of higher VFA accumulation when hydrogen was measured in the headspace, and as the hydrogen depleted, the rate of VFA degradation also increased. Therefore, they were not accumulated to very high peaks. Valeric acid was the only exception, with increasing accumulation rate at the early stages of digestion, however, it was also followed by a rapid degradation as the hydrogen was consumed, and hence, lower peaks were observed throughout the digestion period. Within each acclimation experiment, further addition of hydrogen resulted in lower acetic, butyric and propionic acids but led to higher levels of valeric acid. This further proves that acclimation enhanced acetogenesis rather than inhibiting it.

Hydrogen production and consumption has been established to be a key influence on VFA degradation. But propionic acid has been reported to have the most significant inhibitory effect on the methanogenesis process, which informed the establishment of a propionic-to-acetic acid ratio of 1.4 as a threshold value above which could indicate possible inhibition (Appels et al., 2008). The potential for the higher VFA to tend towards valeric acid instead of propionic acid due to acclimation, reduced the inhibitory potentials at any time during the digestion period.

Valeric acid would typically degrade to acetic acid, propionic acid and hydrogen (Flotats et al., 2003), which means its decomposition should ideally influence an increase in propionic and acetic acids. This was the case in EH1, whereby, valeric acid decomposition influenced high loads of propionic acids in both the control and test reactors up till Day15. But as acclimation progressed in EH2 and EH3, propionic acid accumulation declined in both the control and test reactors. This means hydrogen acclimation also improved overall propionic acid degradation rate throughout the digestion period and was helpful to avoid excessive VFA accumulation with subsequent increases in hydrogen concentrations. In fact, for the acclimation experiments in EH2 and EH3, the test reactors, which had additional hydrogen loads were observed to have faster propionic acid decomposition than the corresponding control reactors.

For all three experiments, initial production of propionic and valeric acids were faster in the test reactors, however, the resulting decomposition of these acids were also faster in the test reactors than their corresponding control reactors. This further support the observation that the addition of hydrogen improved the syntrophic relationship between the hydrogenotrophic methanogens and hydrogen producers, hence, limiting VFA-induced inhibitions. In essence, hydrogen addition and acclimation to food waste AD reactors can be said to have improved the growth of both the hydrogenotrophic methanogens and the acetogens. In agreement with findings presented in this study, Bassani et al. (2015), reported that VFA remained stable throughout the biomethanation process using cattle manure as feedstock. However, excessive loading of hydrogen above the stoichiometric requirement of 4:1 $H_2:CO_2$, VFA inhibition and excessive accumulation ensued (Agneessens et al., 2017). This followed an enrichment of homoacetogenesis, which consequently, increased acetic acid resulting in sharp pH drops.

6.5.2 Dissolved organic carbon degradation

For an unacclimated inoculum, the addition of hydrogen was observed to initially influence an increase in the DOC, due to a temporal shift in the nutrients' utilisation to H_2 and CO_2 in EH1 (Figure 6.6). With gradual acclimation in EH2 using the digestate from EH1 and using the digestate from EH2 in EH3, the rate of DOC removal increased in the control reactors, implying that acclimation enhanced the destruction of DOC. By this effect, the rate of DOC removal by virtue of acclimation (in the control reactors) was not

so different from the removal rate observed when the concentration of hydrogen was simultaneously increased in the test reactors.
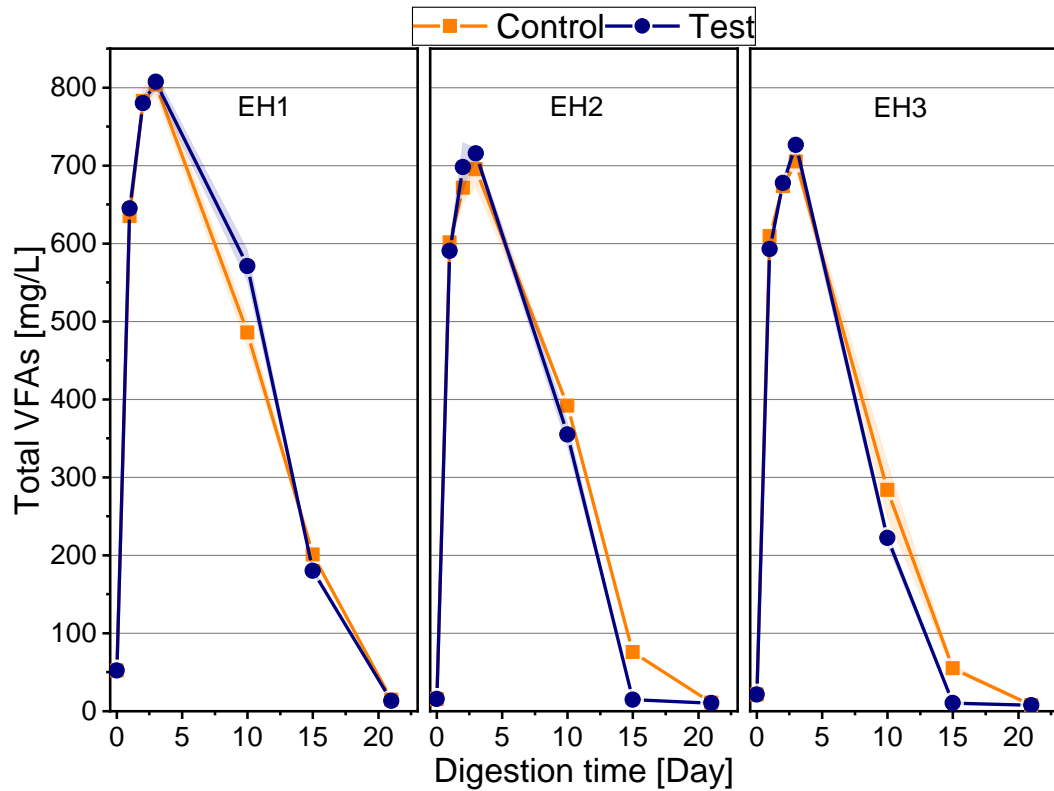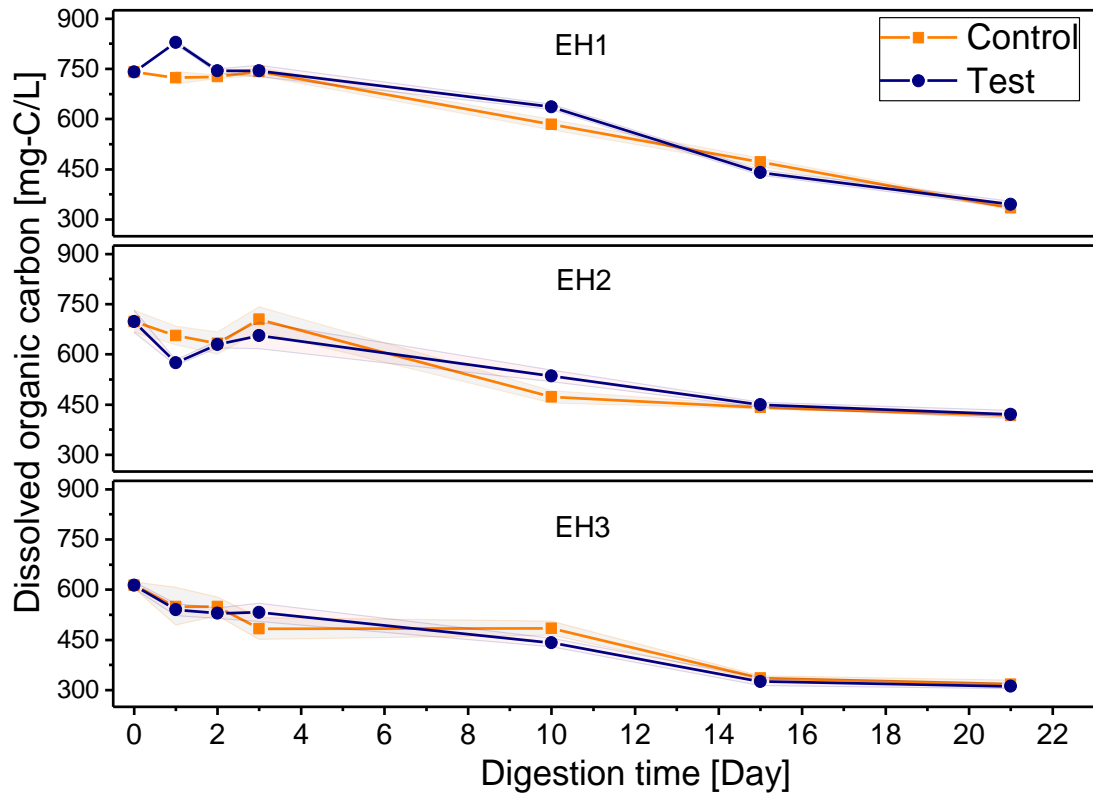

Figure 6.6. Dissolved organic carbon profiles from hydrogen-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

6.5.3 pH and Alkalinity

6.5.3.1 pH

With hydrogen addition, acclimation did not greatly impact on the pH profile as seen from Figure 6.7, whereby, the pH within the control reactors for EH1, EH2 and EH3, followed similar pattern especially in the early stages of digestion. Increasing the concentration of hydrogen within the acclimated reactors (test reactors) however, resulted in increase in pH for all test reactors, which indicates an increase in hydrogenotrophic methanogenesis, by virtue of increased CO_2 removal. This was not excessively increased as a result of the VFA-induced buffer, which helped to maintain the pH within optimal limits. As such, even with the successive increase in the hydrogen injected from 5% to 15%, the pH of the system was not increased beyond optimal limits.

This further proves that in-situ biomethanation could be a feasible method of managing the low pH common with food waste digestion, rather than dosing

with alkaline chemicals to increase the pH, as employed in some studies (Chen et al., 2015).
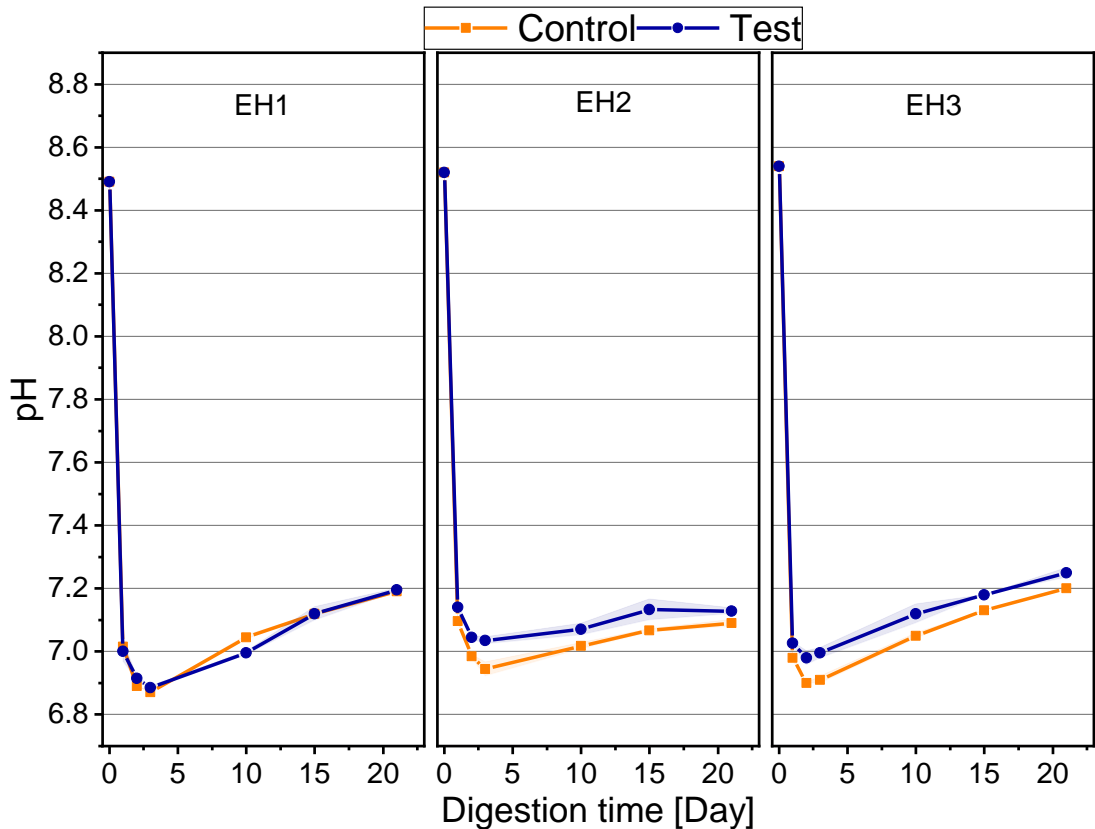
Figure 6.7. pH profiles from hydrogen-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

6.5.3.2 Alkalinity

For the experiments with H_2, the alkalinity at Day0 was 1867, 2140 and 1568 mgCaCO$_3$/L for EH1, EH2 and EH3 respectively. To compare the effect of increasing concentrations of hydrogen on the alkalinity, the effective change was used, by dividing the alkalinity at each monitored point by the alkalinity at the start of the experiment. Hence, at the start of the experiment a value of 1 was obtained and as the AD progressed, values above 1 indicated increase in alkalinity and below 1 indicated reduction in alkalinity (Figure 6.8).

From Figure 6.8 we observed that with acclimation, the system's alkalinity was improved and rather than a reduced alkalinity obtained at 5%-H_2 in EH1, the alkalinity increased with the increase in hydrogen concentration. As such, by virtue of acclimation, the system became more resistant to changes induced by initial VFA production due to an acclimated environment.
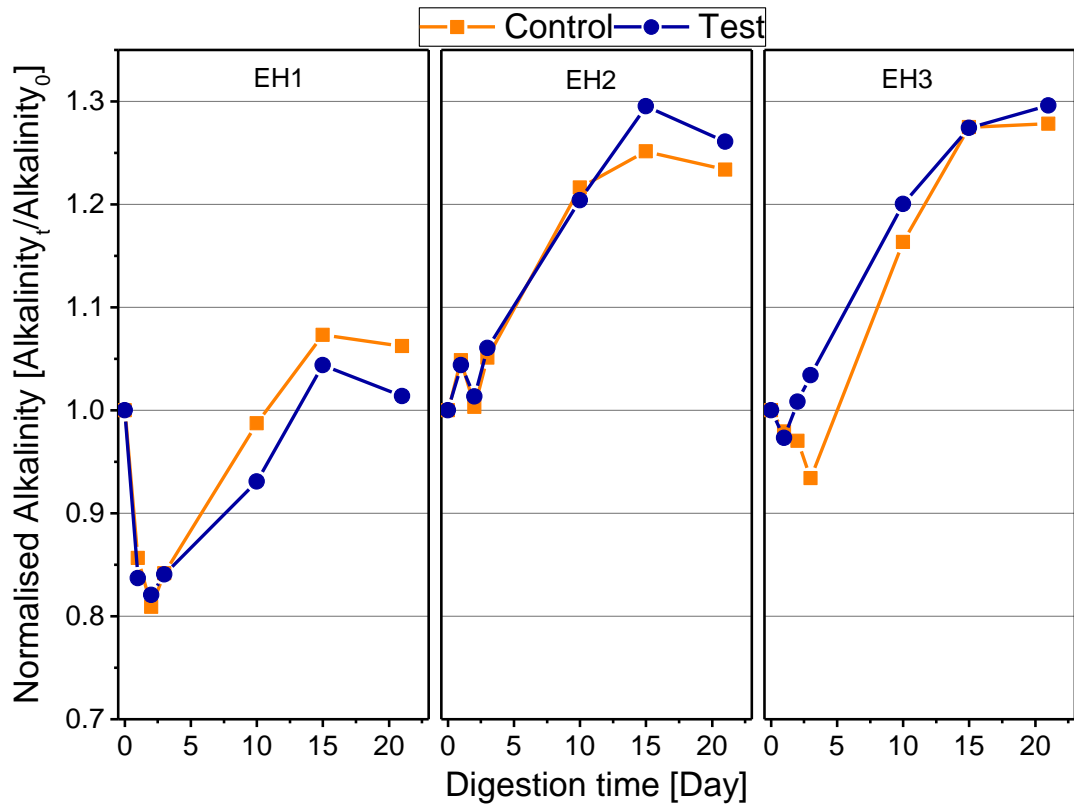
Figure 6.8. Normalised alkalinity patterns from hydrogen-based acclimation experiments, each point represents the ratio of the alkalinity measured at that point (Day t) to the alkalinity measure on the day of set up (Day 0).

The additional effect of increasing hydrogen concentration, also led to increase in alkalinity within the test reactors, whereby, from 5% to 10% H_2 (EH1 to EH2) the change in alkalinity in the test reactor was about the same as the control, while increasing the H_2 concentration from 10% to 15% (EH2 to EH3), the test reactor alkalinity further increased higher than the control. This was also supported by the pH trends observed for the acclimated reactors. This improvement in alkalinity could be due to an increased rate of biomethane production. However, biomethation is thought to influence the removal of liquid phase alkalinity because of the removal of CO_2. While this was the case in EH1, acclimation helped to overcome this effect in EH2 and EH3. Hence, despite the increased rate of CO_2 removal in EH2 and EH3, the alkalinity also increased. It is not clear what might have influenced this, perhaps in addition to acclimation, the nature of the feedstock used in this study (food waste) might also have contributed to this.

At the end, by virtue of acclimation, the Day_t/Day_0 alkalinity ratio of the control reactor increased from 1.06 in EH1 to 1.23 in EH2 and 1.28 in EH3, which implies the final alkalinity increased from 6% without acclimation in EH1 to 23% and 28% in EH2 and EH3 respectively. Furthermore, the increase in

hydrogen concentration led to a corresponding increase in the alkalinity within the test reactors. For instance, for EH1, the final alkalinity was 5% lower in the test reactor than the control, but with EH2 and EH3, the test reactors' alkalinity increased to be 3% and 2% higher than their corresponding control reactors. So that, without acclimation, the extensive removal of CO_2, influenced a reduction in the alkalinity of the test reactor, but with acclimation, the system was able to resist the sudden changes associated with VFA production.

Biomethanation studies are said to be prone to loss of alkalinity due to the removal of bicarbonate (Angelidaki et al., 2018). However, most biomethanation studies primarily explored substrates with low protein base, such as sewage sludge and cattle slurry, perhaps the heterogeneous characteristics of food waste as in this study, provides an advantage over other feedstock, in terms of alkalinity recovery.

6.5.4 Ammonia profile

For the H_2-acclimation experiments, total ammonia-nitrogen at the start of the experiment was 364 mg/L, 361.5 mg/L, and 255.8 mg/L for EH1, EH2 and EH3 respectively. As with alkalinity analysis, the change throughout the AD period in comparison with the initial value is represented in Figure 6.9. System stresses from high ammonia loads in protein-rich substrates, such as food waste have been reported to influence acetate degradation towards more hydrogenotrophic methanogensis (by syntrophic acetate oxidation) and less acetoclastic methanogens as an adaptation strategy (Gao et al., 2015). This could also have impacted on the increased hydrogenotrophic methanogenesis activity reported earlier throughout the digestion process of the test reactor for EH1. Apparently, acclimation was able to effectively reduce the ammonia-nitrogen concentration within the system, judging from the control curves in EH2 and EH3 of Figure 6.9. The initial increases in the ammonia levels immediately after setup buttresses the point that hydrolysis was enhanced when an acclimated inoculum was used leading to more release of ammonia than consumption.
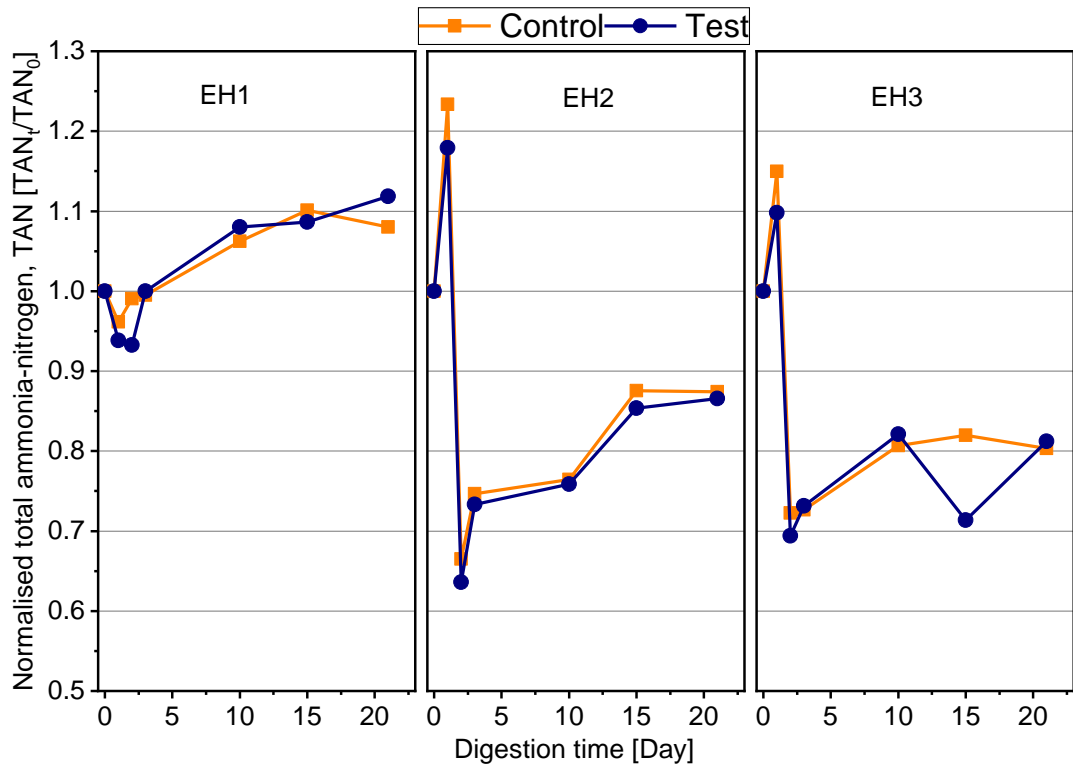
Figure 6.9. Normalised TAN patterns from hydrogen-based acclimation experiments, each point represents the ratio of the TAN measured at that point (Day t) to the TAN measure on the day of set up (Day 0).

In this study, we realise that acclimating the food waste digester to increasing loads of hydrogen, helped to reduce ammonia concentrations throughout the digestion period. This means that the potential for ammonia-induced inhibition becomes lowered. In effect, the final ammonia concentration in the non-acclimated system in EH1 increased by 8% and 11.9% in the control and test reactors, which corresponds to an actual increase by 29 and 43 mg/L respectively. Hence, although the ammonia in the test reactor was initially lowered with the injection of hydrogen; due to the microbial shift towards H_2 and CO_2, but as digestion progressed more ammonia was released in the system.

However, by acclimation in EH2, ammonia levels reduced both in the control and test reactors, such that with the use of the acclimated inoculums alone, the final ammonia level in the control reactor reduced by 12.6%, which corresponds to an actual reduction by 46 mg/L. The addition of hydrogen to the acclimated reactor produced a similar result at 13.4% reduction (49 mg/L). Further acclimation in EH3 also resulted in further decrease in ammonia level by 20% and 19% in the control and test reactors, also corresponding to actual decrease by 50 and 48 mg/L respectively. These results implies that by acclimation with hydrogen, ammonia levels reduced; regardless of the

simultaneously addition of hydrogen. The high removal rate of ammonia, is in agreement with the increase in alkalinity, to imply the enhanced utilisation of ammonia to produce alkalinity.

Other studies have tried to enable the AD system withstand high loads of ammonia by gradually increasing the ammonia concentration (ammonia acclimation) until a maximum tolerable level was obtained (Gao et al., 2015), and also the enrichment of ammonia-tolerant methanogenic culture (bioaugmentation) (Fotidis et al., 2017). However, with hydrogen acclimation, the need for ammonia acclimation does not arise, since it consequently reduces the ammonia concentrations.

Moreover, with side stripping of ammonia using biogas, to reduce the ammonia concentrations during food waste digestion, a high temperature of 70°C and a pH of 10 were required; under which 48% of the total ammonia nitrogen was removed after 138 days (Serna-Maza et al., 2014). But with in-situ biomethanation in this study, up to 20% ammonia removal was obtained at mesophilic condition, with pH maintained around 7.2.

Moreover, the pH within the H_2-based systems were all around pH 7.2, hence, the free ammonia content was also not greatly impacted with acclimation; they were only slightly lower in the acclimated reactors.

6.5.5 Elemental sulphur decomposition

The normalised sulphur measured over the initial concentration at the start of the experiment is presented in Figure 6.10. The elemental sulphur concentration at the start of the experiment was 154, 157 and 58.2 mg/L (dry basis) for EH1, EH2 and EH3 respectively. The rate of sulphur degradation in the control reactors following previous acclimation reduced, especially at the early stages of digestion. Although, a lower ratio was observed with EH3 at the early stages, the actual amount of sulphur degraded by Day1 was 56.8, 29.3 and 38.4 mg/L for EH1, EH2 and EH3 respectively. Hence, the low ratio (reaching 0 by Day2) was mostly due to the initial low concentration of sulphur available at the start of the experiment and was completely depleted by Day2.
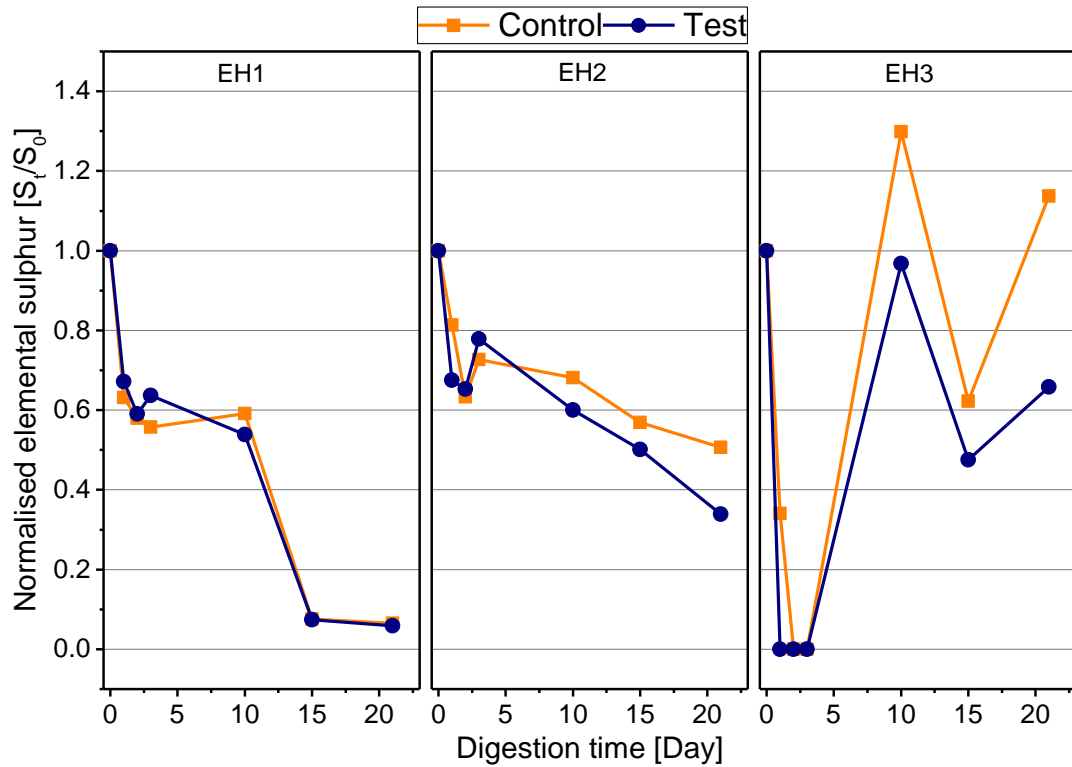
Figure 6.10. Normalised elemental sulphur patterns from hydrogen-based acclimation experiments; each point represents the ratio of the elemental sulphur measured at that point (Day t) to the elemental sulphur measure on the day of set up (Day 0).

What was interesting was the recovery of elemental sulphur at the later stages of digestion for the acclimated reactors in EH2 and EH3. The percentage of elemental sulphur degraded in the control reactors at the end of the experiments were 94%, 49% and -13% for EH1, EH2 and EH3 respectively. This means that a backward elemental sulphur recovery from sulphides was enhanced by acclimation, thereby, releasing the hydrogen in the sulphides, and consequently improving hydrogenotrophic methanogenesis. This might explain the increase in methane and decrease in CO_2 yields observed in the control reactors in EH2 and EH3.

Additionally, the increase in the concentration of added hydrogen was observed to influence a higher rate of elemental sulphur degradation in test reactors of EH2 and EH3, than the corresponding controls. Perhaps the sulphate reducing bacteria were also somewhat enabled from previous experiments and carried on to the later, hence, with the addition of hydrogen some early stage sulphides production was possible. The higher elemental sulphur recovery in the control reactors than the test reactors for EH2 and EH3, was believed to be responsible for the reduced CH_4 margin between the control and test reactors for EH2 and EH3 compared to EH1.

Like the observations with the control reactors, the percentage of elemental sulphur degraded in the test reactors were 94%, 66% and 34% for EH1, EH2 and EH3 respectively. Again, this implies a lower potential for sulphides production with acclimation and a potential increase in hydrogen concentration. Hence, although the sulphur degradation rate initially increased, hydrogen was recovered at the later stage of digestion, thereby, increasing biomethane production via hydrogenotrophic methanogenesis. Therefore, acclimation of food waste AD reactors to increasing concentrations of hydrogen could reduce the potential of sulphides production. In agreement Strevett et al. (1995), found biomethanation was able to remove both CO_2 and H_2S from the biogas, although, a hollow fibre membrane was incorporated in the digester design.

These results might imply that long term acclimation of food waste digesters to increasing concentrations of hydrogen could help reduce hydrogen sulphides potential in the biogas. Hence, it becomes important to acclimate the system during AD with biomethanation, so that the backward production of elemental sulphur could be optimised.

6.6 Kinetic and statistical analysis of H_2-based biomethanation

6.6.1 Kinetic analysis

The kinetic parameters obtained from the SGompertz and MGompertz fitting models for hydrogen-based biomethane yields are summarised in Table 6.1. The kinetics generally improved for the hydrogen-based acclimation experiments. By acclimation only, the k-value and maximum specific methane yield increased through the acclimation phases, consequently, the lag times reduced. This implies that early days methane production improved through the acclimation period, and emphasises that hydrolysis was not inhibited. The addition of hydrogen to the acclimated systems (test reactors) did not behave contrary to the control reactors, but were slightly improved in terms of lag time and maximum specific methane yield for each corresponding acclimation phase. Notwithstanding, these changes were only small because of the resultant improvement in the control reactors, which is also in line with other parameters such as DOC degradation. On the contrary, Pan et al. (2016) reported reduction in maximum specific methane yield and increase in lag time by hydrogen adaptation. However, they suggested it was due to a short

adaptation period of one week, during which the microorganisms were assumed to be in the decay stage.

Table 6.1. Kinetic analysis of biomethane production from hydrogen-based biomethanation experiments.

Condition	Experiment	k-value	Lag time (Day)	Max. specific CH$_4$ yield	R-squared
Acclimation only	EH1_Control	0.19	3.2	31.5	0.99
	EH2_Control	0.22	2.5	37.3	0.99
	EH3_Control	0.27	2.2	45.5	0.99
Acclimation + hydrogen	EH1_Test	0.17	3.1	32.9	0.99
	EH2_Test	0.21	2.2	39.6	0.99
	EH3_Test	0.27	1.8	51.2	0.99

6.6.2 Statistical relationship between hydrogen addition and biomethane yield

The statistical relationship between percentages of hydrogen in the gas mixture utilised was established by linear regression using the MiniTab18® statistical tool. Regression equations from nine data points obtained from biomethanation experiments (using the three gas mixtures – 5%, 10% and 15% H$_2$) were used for each linear regression fitting, with R^2 values in the range of 0.88 to 0.99. The resulting regression equations (Equations 6.1 to 6.4) were then used to predict the level of acclimation required to obtain 100% methane yield in the biogas; assuming all conditions remained favourable.

Figure 6.11 shows the trend in biogas upgrade for both the control (acclimated only) and test (acclimated + additional hydrogen) reactors and the predicted biomethane yields at 100% biomethane content for the H$_2$-based experiments respectively.

$$\%CH_4 \ in \ biogas \ (Test) = 74.65 + 40.1 \cdot (\%H_2 \ added) \qquad 6.1$$

$$\%CH_4 \ in \ biogas \ (Control) = 66.4 + 75.3 \cdot (\%H_2 \ utilised \ in \ preceding \ acclimation \ stage) \qquad 6.2$$

$$Biomethane \ yield \ (Test) = 452.9 + 307.2 \cdot (\%H_2 \ added) \qquad 6.3$$

$$Biomethane\ yield\ (Control) = 399.8 + 356.0 \cdot$$
$$(\%H_2\ utilised\ in\ preceding\ acclimation\ stage) \qquad\qquad 6.4$$
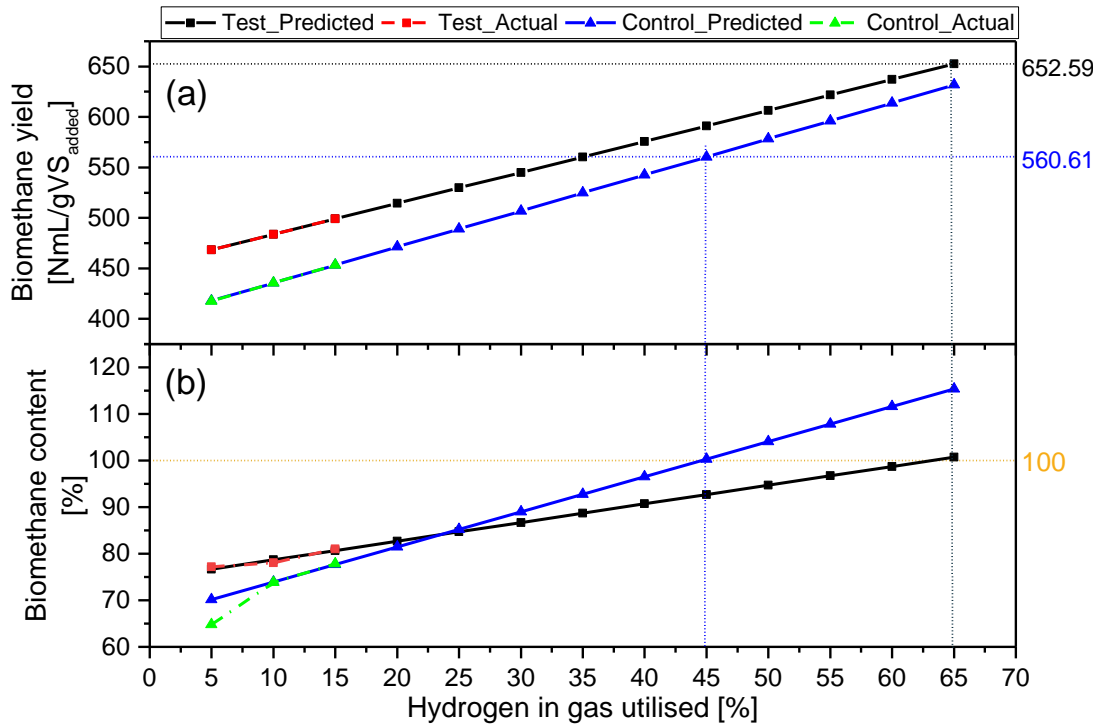
Figure 6.11. Predicted and actual biogas upgrade and biomethane yields from stepwise hydrogen acclimation.

The control reactors showed quicker upgrade trend than the test reactors. The large residual between the predicted and actual control values at 5% hydrogen in Figure 6.11b was because the experiment was started with 5%-H_2, hence, the actual value was from a non-acclimated sludge, while the predicted is the value obtained assuming an acclimated sludge was utilised. Going by Figure 6.11b, 100% biomethane was obtainable by using an inoculum that has undergone a stepwise acclimation with hydrogen up to 45%-H_2; without the need to add hydrogen after this point. However, with hydrogen addition at this point, only about 93% biomethane yield is obtainable. This trend is probably due to the additional competition and system adjustments that ensues from the addition of hydrogen in the test reactors, thereby, influencing slower upgrade rate in the test reactor than the control. However, this does not necessarily cumulate to higher biomethane yield in the control reactor. As seen from Figure 6.11a, the yield from a stepwise acclimation up till 45% H_2 gas usage could yield about 560.4 NmL-CH_4/gVS$_{added}$ (100% biomethane), whereas, continuous upgrade to 65%-H_2 usage will be required to obtained 100% biomethane (equivalent to 652.6 NmL-CH_4/gVS$_{added}$) in the test reactor. Notwithstanding, except where the supply of hydrogen is sufficient and the requirement for biomethane to gas grid or as transport fuel stringent, achieving

560.4 NmL-CH$_4$/gVS$_{added}$ using an inoculum that has undergone stepwise acclimation with hydrogen up to 45%, could be less energy and cost demanding than for stepwise increase in hydrogen up to about 65%, which will result in only 15% biomethane increase.

6.7 Conclusions

Acclimation of the AD system to increasing concentrations of hydrogen was effective towards improving both the AD process, kinetics and biogas upgrade. Acclimation led to successive increase in pH, however, this was not increased beyond the optimal limit required for AD. VFA degradation was also improved with acclimation, thus, limiting VFA-induced inhibitions that is common with food waste AD. As a result, the kinetics of the process improved with successive acclimation, as reflected by the reduction in lag time from 3.2 in EH1 to 1.8 in EH3.

Furthermore, hydrogen acclimation enhanced elemental sulphur recovery, which further improved the hydrogenotrophic methanogenesis both in the control and test reactors of all experiments. Consequently, H$_2$-based acclimation upgraded the biogas to yield 81% biomethane against 65% obtained without acclimation within the same experiments.

CHAPTER 7

FOOD WASTE BIOMETHANATION: EFFECT OF FORMIC ACID AS AN ALTERNATIVE ELECTRON CARRIER

7.1 Introduction

The source of hydrogen is still a major drawback to full-scale biomethanation adaptation. Water electrolysis using surplus energy from other renewable energy sources, is arguably the most efficient option for biomethanation currently employed (Ullah Khan et al., 2017). Notwithstanding, this energy surplus is not available at all times (International Energy Agency, 2006), as such, there are still some complexities surrounding hydrogen production, storage and utilisation for biomethanation. Hence, it is important to also identify alternative sources of hydrogen that can be adopted into the biomethanation process to achieve the same results; such as formate.

Formate and hydrogen have been identified as significant substrate (electron carriers) utilised by the hydrogenotrophic methanogens for methane production. Asides formate having a higher solubility, during AD, both hydrogen and formate are stoichiometrically and thermodynamically available in nearly equivalent amounts (Pan et al., 2016; Schink et al., 2017). In fact, formate was argued to be the preferred electron carrier in aqueous solutions, while hydrogen was postulated to dominate only with microbial aggregates, such as sediments and sewage sludge flocs; because of its lack of polarity and small size (Schink et al., 2017).

Similarly, when interspecies distance between hydrogenotrophic methanogens and syntrophic acetate oxidising bacteria was high, formate was said to be the principal electron carrier, and vice versa (Fotidis et al., 2013). Essentially, hydrogen and formate can be alternatively or simultaneously utilised as electron carriers for biomethane production. Hence, formate was utilised in comparison with hydrogen-based biomethanation systems, as an alternative source of hydrogen for biomethanation.

7.2 Chapter objectives

- To investigate alternative means of introducing hydrogen into AD reactors in the form of FA.
- To analyse the influence of acclimating food waste AD system to increasing concentrations of FA in comparison with hydrogen.

7.3 Experimental set up

The same procedure used in the H_2-based assays was also adopted in the FA-based assays; 1 mm PS, ISR of 3 and Day0 FA injection. Hence, three sets of experiments with FA addition were conducted to analyse the impact of acclimation on FA conversion to biomethane and labelled as EF1, EF2 and EF3 respectively (see Figure 7.1). As in Chapter 6, each set of FA experiment had the same inoculum condition. Hence, for the acclimation stages both the control and test reactors of each experiment had an acclimated inoculum. After bulk samples were split into the respective reactors (Blank, Control and Test), the reactors were all flushed with nitrogen gas to obtain an anaerobic environment as described in section 3.5 (Chapter 3). FA was added to the test reactors using 0.087, 0.183 and 0.271 mL-FA/L FA loading (see Figure 7.1). These values correspond to the thermodynamic amount of FA required to obtain the same amounts of hydrogen used in the H_2-based biomethanation experiments. Furthermore, some of the graphs have also been plotted as normalised values (by dividing the respective value by the value at the start of the experiment) rather than actual parametric values between experiments.
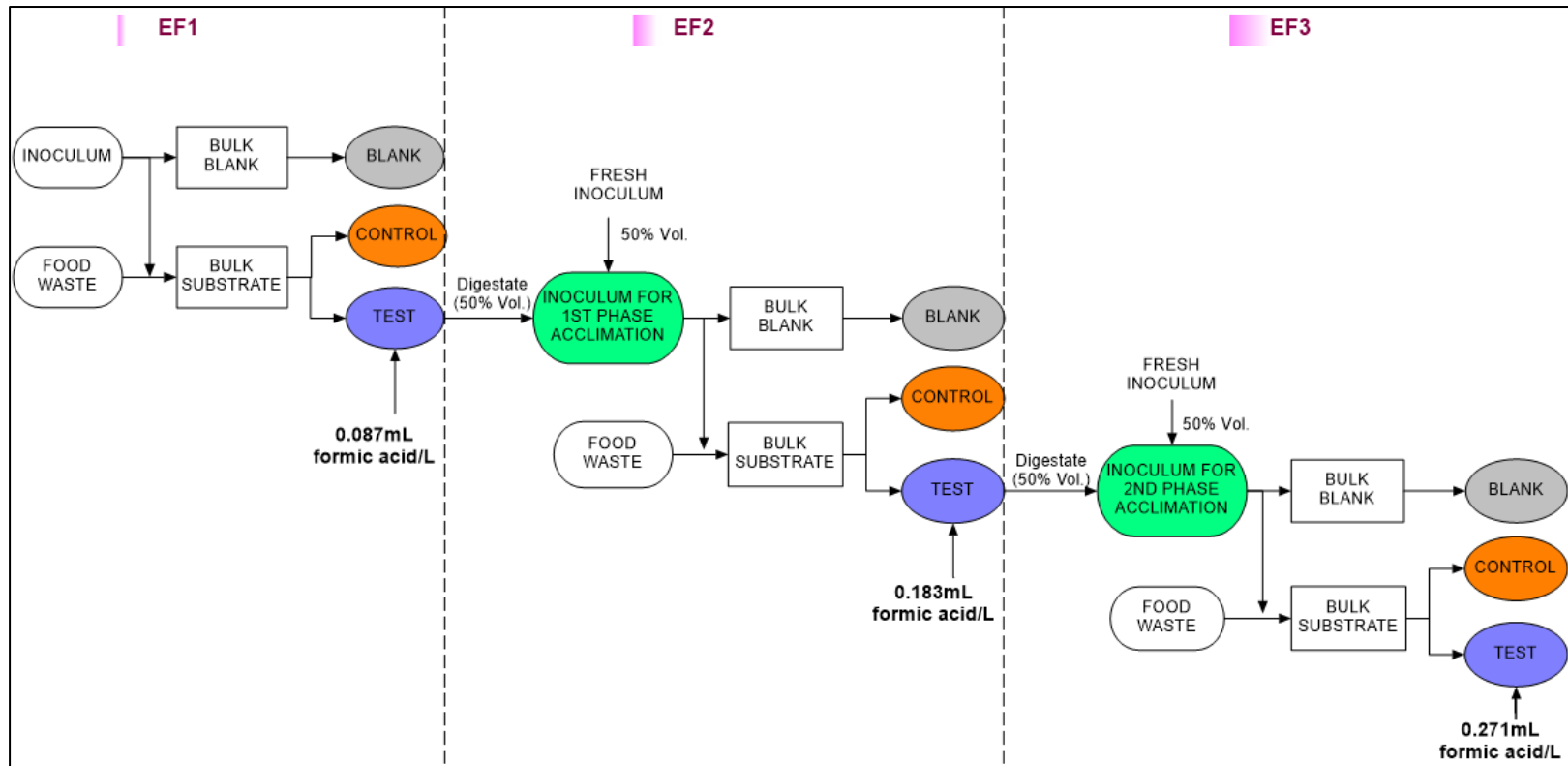
Figure 7.1. Schematic representation of formic acid-based biomethanation acclimation experimental setup.

7.4 Effect of increasing levels of formic acid on biogas characteristic

7.4.1 Formic acid utilisation

In the first phase of FA addition to the AD system in EF1, hydrogen was not detected in the headspace of the test reactor. However, progressing through the acclimation phases, both the control and test reactors recorded trace amounts of hydrogen in the headspace by Day1. The non-detection of hydrogen in the test reactor of EF1 and small amounts detected in the test reactors of the acclimated experiments indicate that the added FA did not completely split into H_2 and CO_2 as anticipated. Or perhaps it did split to H_2 and CO_2, but they were only available in the liquid content of the reactors. Furthermore, the detection of hydrogen in the corresponding control reactors of the acclimated experiments implies that hydrogen consumption rate was not as improved with FA-based assays compared with H_2-based assays discussed in Chapter 6.

By acclimation only, 0.47 and 0.40 mg-H_2/L was recorded in the headspace of the control reactors in EF2 and EF3 respectively. Compared with the H_2-based experiments; whereby, hydrogen was not detected in the control reactors at the acclimation phases, it means the FA-experiments were not as effective in improving the hydrogenotrophic methanogens as the H_2-experiments. In addition, the combined effect of acclimation and increasing FA concentration in the test reactors, yielded gaseous hydrogen by Day1, measured at 1.18 and 0.74 mg/L in EF2 and EF3 respectively. In support, Yang et al. (2015) reported that low concentrations of formate could result in only limited supply of hydrogen, which is quickly utilised for hydrogenotrophic methanogenesis. But higher concentrations results in more hydrogen production; being favoured by different reaction routes.

Assuming a complete utilisation of the gaseous hydrogen measured by Day1, the actual difference in biomethane yield was 15% and 10% lower than the theoretical yield by Day2 in EF2 and EF3 correspondingly. This could be as a result of one or both of the following; i) the measured hydrogen was utilised for other purposes asides biomethane production, ii) the measured hydrogen was converted to biomethane but VFA degradation to biomethane was strained.

Generally, acclimation with hydrogen was more effective in improving the early stage methane production rates than FA. This was believed to be as a result of the forms in which they were made available. For instance, H_2 was added in gaseous state and was limited in solution due to its low solubility, therefore, impacting a minimal degree of inhibition. Whereas, FA was added in liquid state and was perhaps limited in complete conversion to H_2 and CO_2 by thermodynamic conditions such as pH and temperature, therefore, impacting a higher degree of inhibition in the liquid content of the reactors.

7.4.2 Biogas yield from FA acclimation

The biomethane and corresponding CO_2 yields from the FA acclimation experiments are presented in Figure 7.2; showing test yields in solid lines and the control yields in dash lines. As seen from Figure 7.2, the addition of FA in EF1 had a negative impact on the biomethane yield, whereby, the biomethane production rate was lower in the test reactor than the control. As acclimation progressed through the two phases, biomethane production rate was observed to reduce even further, with longer lag times. This negative impact on biomethane production with FA acclimation follows a perceived inhibition on the digestion process, especially on VFA fermentation. Interestingly, CO_2 yields reduced with FA acclimation, in EF2 and EF3. The reduction in CO_2 implies that there was some degree of improvement in hydrogenotrophic methanogenesis.

The CO_2 in the control reactors reduced with acclimation from EF1 to EF2 and then to EF3, which led to biogas upgrade from 66.7% biomethane yield to 69.0% in EF2 and 69.6% in EF3. Furthermore, with two phase acclimations in EF2 and EF3, cumulative biomethane yield in the test reactors were only slightly higher than the control reactors in EF2 and EF3 accordingly. Notwithstanding, the test reactors for each corresponding experiment recorded higher CO_2 yields, so that, although there were slight increases in the biomethane yields of the test reactors over the control in EF2 and EF3, the biogas upgrade were lower, recording 66.6%, 68.9% and 67.9% biomethane in EF1, EF2 and EF3 respectively.

In general, FA acclimation helped to reduce CO_2 yields and improve biogas quality, but H_2 addition was more effective. The addition of H_2 to acclimated system resulted in both CH_4 increase and CO_2 decrease in the test reactors compared to the control, while FA addition resulted in relative increase in CO_2 of the test reactor within each experiment; regardless of acclimation. In agreement, maximum methanation rates of 314.6 and 640 NmL/gVS/d with

H_2/CO_2 and 19.6 and 6.5 NmL/gVS/d with FA were reported during a comparative study on methane production from H_2/CO_2, acetate and FA using digested manure and sewage sludge as inoculum respectively (Pan et al., 2016). Also in agreement with this study was a research on methane production from selected C1 to C5 organic acids, where FA was reported to exhibit self-substrate inhibition during digestion (Y. Yang et al., 2015).

Hence, although FA was suggested to be produced in almost equilibrium amounts as hydrogen during primary fermentation, FA might not digest very well when added externally. Furthermore, FA is the strongest of the alkyl carboxylic acids, and very easily form esters with primary, secondary and tertiary alcohols (Reutemann et al., 2000). This property of FA could be responsible for the self-induced inhibition on biomethane yield, (Yanti et al., 2014). However, Pan et al. (2016) and Yang et al. (2015) related the low methane yields from formate to the source of sludge, containing more of the hydrogen-utilising methanogens than the formate-utilising counterparts.

The biogas quality of the control reactors that were subject to an acclimated inoculum only, was relatively better than the quality of the corresponding test reactors. The cumulative methane yield from the unacclimated control reactor in EF1 was 472.5 NmL-CH_4/gVS$_{added}$ and 489.5 NmL-CH_4/gVS$_{added}$ from 1st phase acclimation in EF2 and 482.9 NmL-CH_4/gVS$_{added}$ from 2nd phase acclimation in EF3.
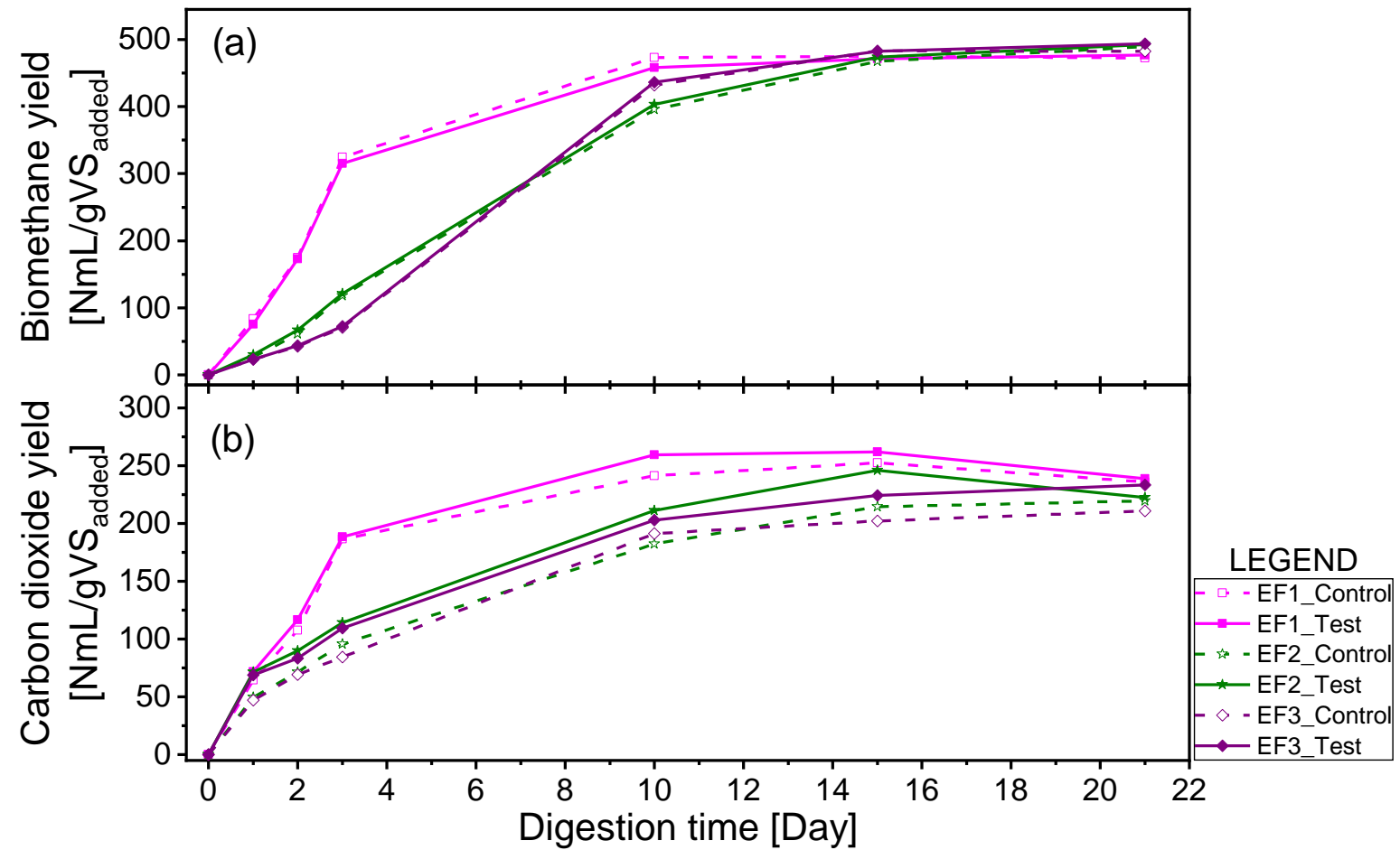
Figure 7.2. Biomethane (a) and carbon dioxide (b) production curves from all formic acid-based acclimation experiments: dash lines represent control yields and the solid lines represent test yields.

The cumulative methane yield from the test reactor was 476.7 NmL-CH$_4$/gVS$_{added}$ before acclimation in EF1, with 1st phase acclimation (EF2) it was 492.7 NmL-CH$_4$/gVS$_{added}$ and with further acclimation at the 2nd phase (EF3) it increased to 494.1 NmL-CH$_4$/gVS$_{added}$.

7.5 Effect of increasing concentrations of FA on the biomethanation process

7.5.1 VFA profile

In comparison with H$_2$, VFA accumulation within FA-systems was observed to increase in the acclimated reactors in the order EF3 > EF2 > EF1 (Figure 7.3). It was observed that FA addition had inhibitory effects on the VFA degradation and acclimation did not necessarily curb this effect. Consequently, other key process parameters, such as pH and alkalinity; as we shall see later in Section 6.5.3, were greatly impacted, which led to low biomethane recovery and biogas upgrade from FA treatment.

Figure 7.3. Total volatile fatty acids profile from formic acid-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

7.5.1.1 VFA composition in FA acclimated experiments

Acclimation with FA led to the accumulation of most of the VFA components (Figure 7.4). When FA was added before acclimation in EF1, the VFA components in the test reactor were generally lower than the control reactor throughout the digestion period (except propionic acid). This was believed to be a result of hydrolysis inhibition, because, the biomethane yield was also lower in the test reactor; though having higher CO_2 yield compared to the control. Going forward to the first phase of acclimation in EF2, VFA accumulation was observed in both the control and test reactors. While the acetic acid accumulation indicated possible inhibition on acetoclastic methanogenesis, the accumulation of higher VFA was perceived to be a result of inhibition on the fermenting microorganisms. Consequently, methane production rates reduced in the control reactor as acclimation progressed from phase 1 (EH2) to phase 2 (EH3).

The first phase of acclimation (EF2) did not impact on acetic acid accumulation, but on higher VFA, so that the inhibition in biomethane production at this stage was probably due to inhibition on VFA fermentation. The coupled effect of acclimation and addition of FA in EF2 at a concentration higher than the previous EF1 experiment, impacted more on butyric and valeric acids than propionic and acetic acids. Hence, addition of FA to the acclimated system led to further inhibition on VFA degradation, resulting in the accumulation of longer chain fatty acids especially butyric acid, followed by valeric acid, while acetic and propionic acids remained relatively unchanged.

The continuous build up in propionic acid in both control and test reactors of EF2; which peaked by Day10, follows the degradation of the longer chain fatty acids and was believed to have imposed further inhibition on the methanogens. Consequently, it took longer time for the VFA to degrade and produce the relevant substrate for methane production such as acetic acid and hydrogen. This explains why a lower methane production rate was observed for EF2 treatment compared to EF1. With further acclimation in EF3, a similar pattern as observed in EF2 was seen, whereby, butyric and valeric acids increased at the early stages of digestion, while the propionic acid remained relatively unchanged in the early stages; only increasing as butyric and valeric acids decreased. Acetic acid was observed to continuously increase at the early stages of digestion, which means the inhibition was also affecting the acetoclastic methanogens at this stage, consequently, reducing the biomethane production rate even more.
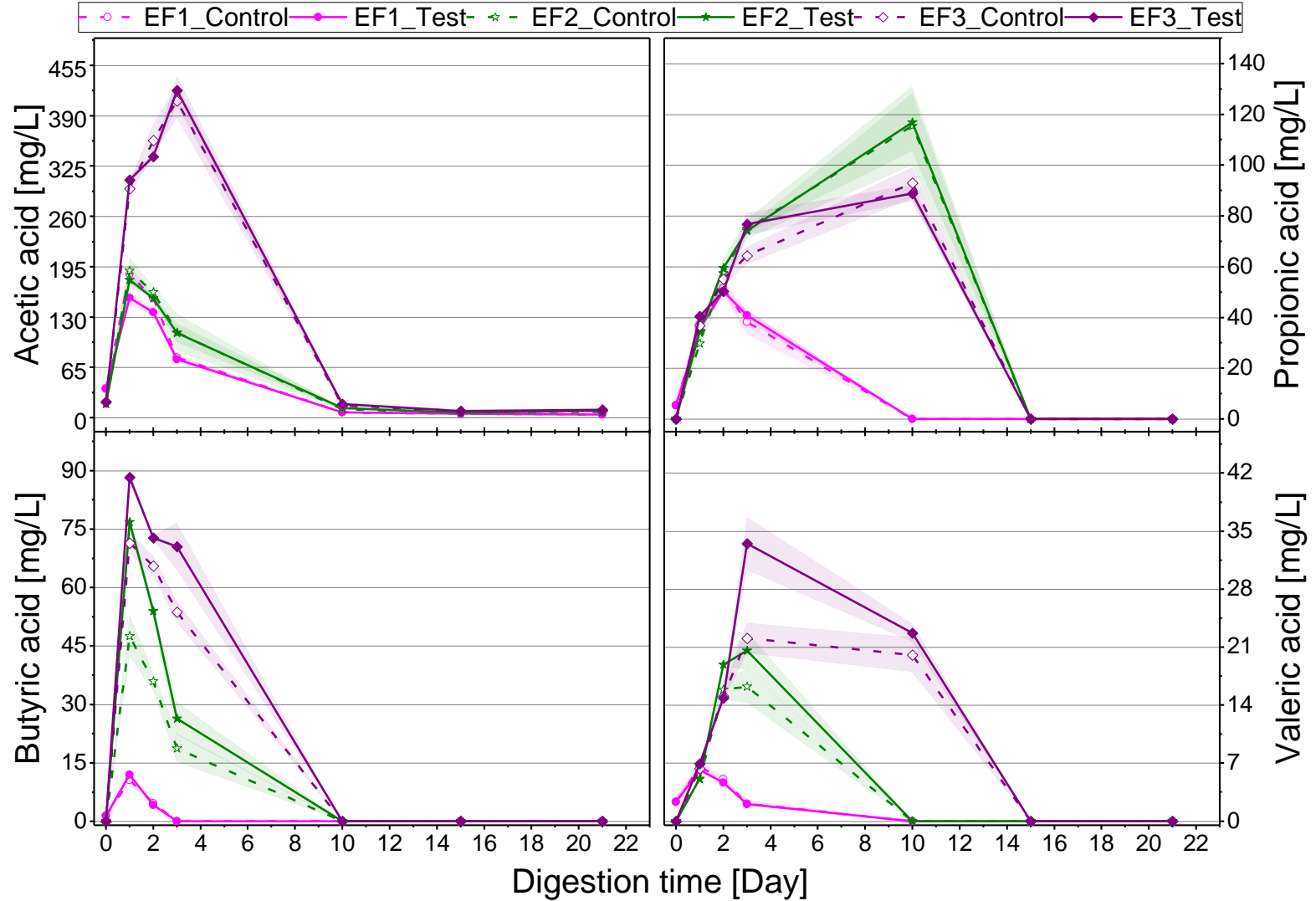
Figure 7.4. Effects of formic acid acclimation on Volatile fatty acid composition: test values presented in solid lines and control in dash lines. Shaded area around lines represent standard deviation from mean.

The resultant increase in acetic acid could also be related to the formation of acetic acid via homoacetogenesis, which was said to be a preferred route with high formate concentrations compared to hydrogenotrophic methanogenesis; owing to the production of more dissolved hydrogen (Y. Yang et al., 2015).

The addition of FA to the acclimated inoculum from EF2 further increased the inhibitory effects on VFA degradation. The longer chain VFA (especially valeric acid) took even longer to degrade in EF3 than EF2, which explains why the propionic acid peaks of both the control and test reactors from EF2 were higher than the corresponding peaks from EF3, as a high amount of valeric acid was yet available in EF3 up till Day 10. This shift from propionic acid accumulation in EF2 to more butyric and valeric acids in EF3, was perceived to have aided some sort of recovery in the methane production rate in EF3 than EF2 as shown in Figure 7.2.

In general, acclimation and increasing concentrations of H_2 improved the overall VFA fermentation, while FA acclimation produced some inhibitory effects on VFA fermentation, therefore, resulting in restrained methane production. This was possibly because, while dissolved hydrogen availability was limited by gas-liquid hydrogen mass transfer in the hydrogen experiments, FA was immediately dissolved and combined with the organic acids produced to form higher VFA. The use of FA for biomethanation was therefore, limited by its propensity to higher VFA formation rather than hydrogen and the rate at which this occurs.

The conditions required for FA to almost equilibrate H_2 and CO_2 production are standard conditions of 1 M concentrations, 1 atm and pH 7.0 (Schink et al., 2017), which was hardly the case in these experiments, perhaps if these conditions were met, a better outcome could have been obtained with the use of FA. Furthermore, the gradual recovery of the system in EF3 is an indication that FA might eventually yield positive outcomes, but a longer acclimation period might be required.

7.5.2 Dissolved organic carbon degradation

Contrary to biomethanation with hydrogen, the addition of FA influenced reduction in the rate of DOC degradation (Figure 7.5). When FA was initially added in EF1, the DOC degradation rate was high in the early days of digestion, especially in the test reactor. However, with acclimation in EF2, the degradation rate reduced in both the control and test reactors and with further acclimation in EF3, the DOC greatly increased both in the control and test

reactors. These were also supported by the high VFA build up with increasing FA concentration and acclimation described earlier in Section 7.5.1.1.
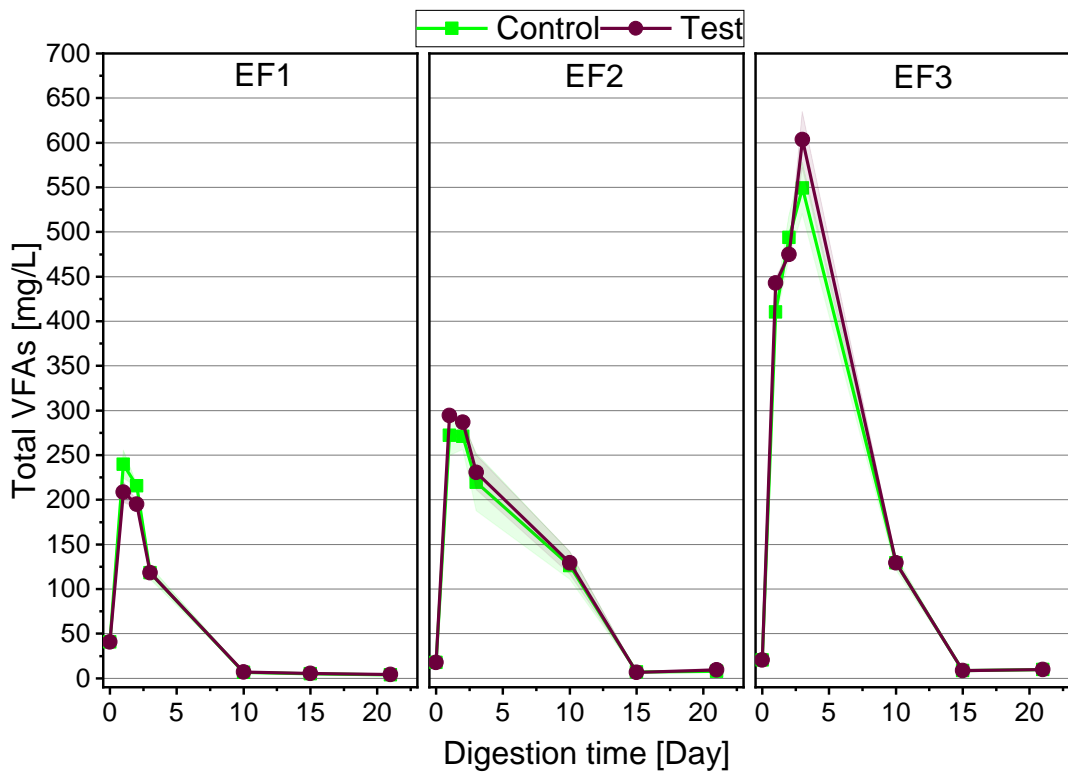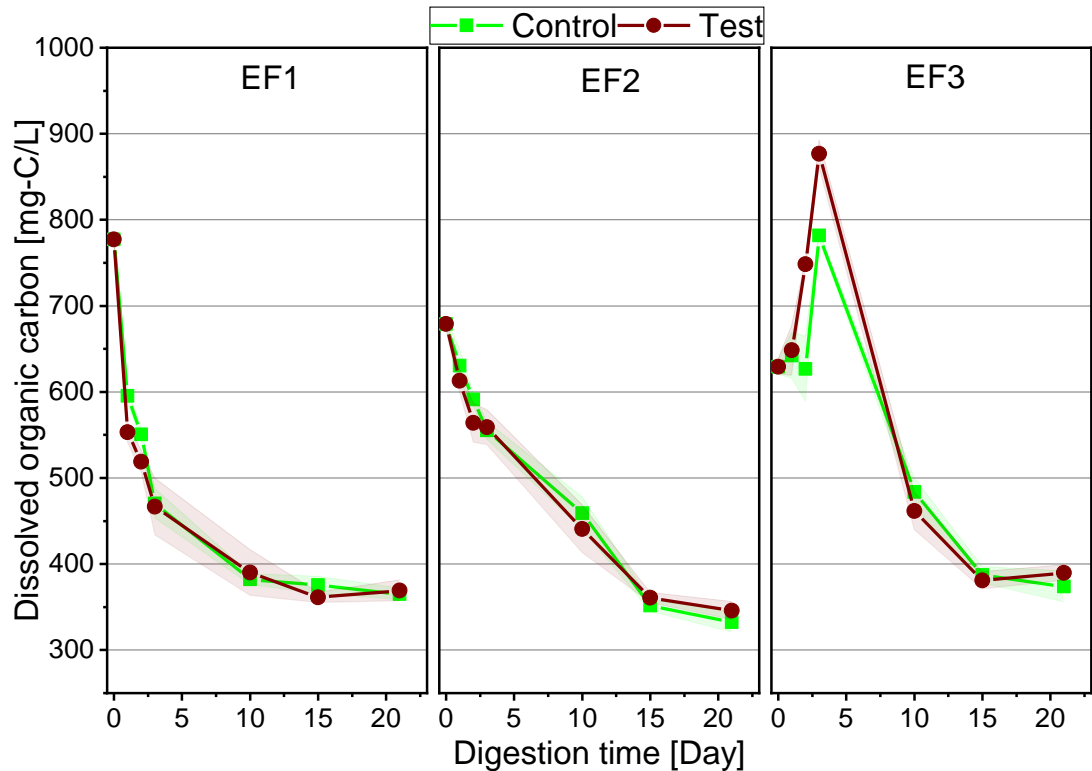

Figure 7.5. Dissolved organic carbon profiles from formic acid-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

Therefore, addition of FA and subsequent acclimation negatively impacted on materials degradation. However, the addition of hydrogen influenced a momentary shift in the substrates utilisation to H_2 and CO_2, and subsequent acclimation helped the system to adapt to the increasing hydrogen loads, thereby, improving materials degradation.

7.5.3 pH and alkalinity

7.5.3.1 pH

Acclimation and increase in FA concentration influenced further lowering of the pH. In Figure 7.6 we see that acclimated reactors in EH2 and EH3 had lower pH through the AD period. This was due to inhibition on VFA degradation, such that as the VFA accumulated within each successive experiment, the pH levels consequently lowered. And the test reactors, which had both acclimated inoculum and additional FA, had even lower pH levels. This was either because of the release of CO_2 with the addition of FA.

Although, biomethanation was slightly enhanced with the addition of FA, this was not large enough to cause an increase in the pH. Furthermore, despite that the pH in all the acclimated reactors were within the optimal limits, the tendency for continuous lowering of the pH with addition of FA could result in future digester breakdown; if FA was adopted.
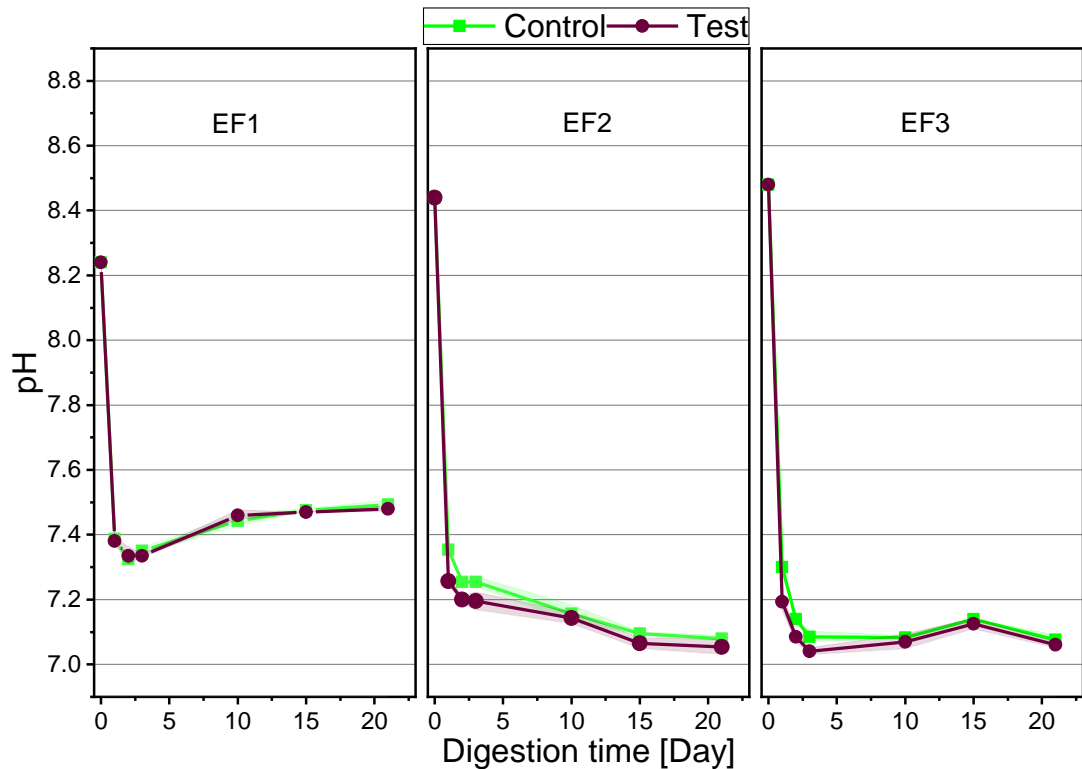
Figure 7.6. pH profiles from formic acid-based acclimation experiments. Shaded area around lines represent standard deviation from mean.

7.5.3.2 Alkalinity

Alkalinity profile during FA-based biomethanation experiments, like other parameters showed a different trend than H_2-based systems. The alkalinity of the test reactors was higher than the control reactors for all experiments (Figure 7.7), probably due to the simultaneous release of CO_2 from FA breakdown. The normalised alkalinity for EF1 reveals the alkalinity increased more than the initial alkalinity in both the control and test reactors; however, the test reactor had higher concentrations, which results from the release of CO_2 from FA. With acclimation, the overall alkalinity recovery rate declined, however, the test reactors having additional FA recorded higher alkalinity levels than the corresponding control reactors in EF2 and EF3. It is not surprising that low alkalinity ratios were measured in the acclimated experiments judging by the excessive VFA build-up and the low methane production rates.
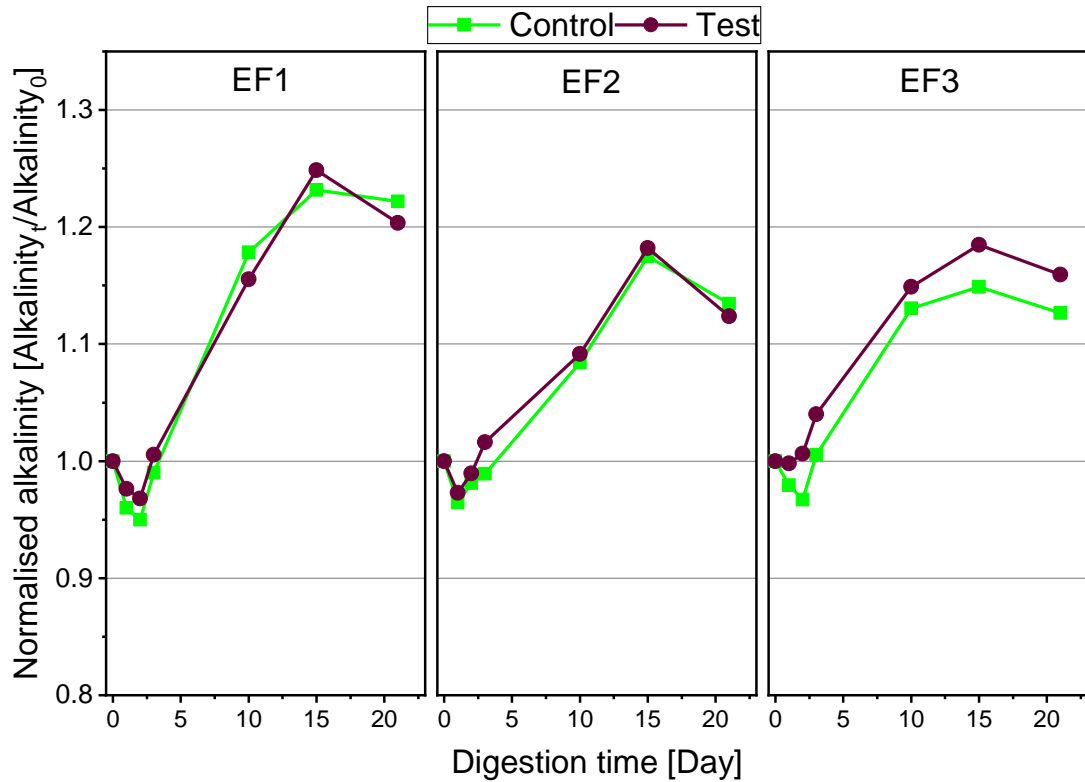
Figure 7.7. Normalised alkalinity patterns from formic acid-based acclimation experiments. Each point represents the ratio of the alkalinity measured at that point (Day t) to the alkalinity measure on the day of set up (Day 0).

7.5.4 Ammonia profile

FA-based acclimation presented in Figure 7.8 produced a relatively undefined pattern in ammonia-nitrogen; however, it generally tended towards increasing concentrations. Further studies on the FA-acclimation would be required to fully understand the effect of injecting FA to the system and how it interacts with other elements within the system.
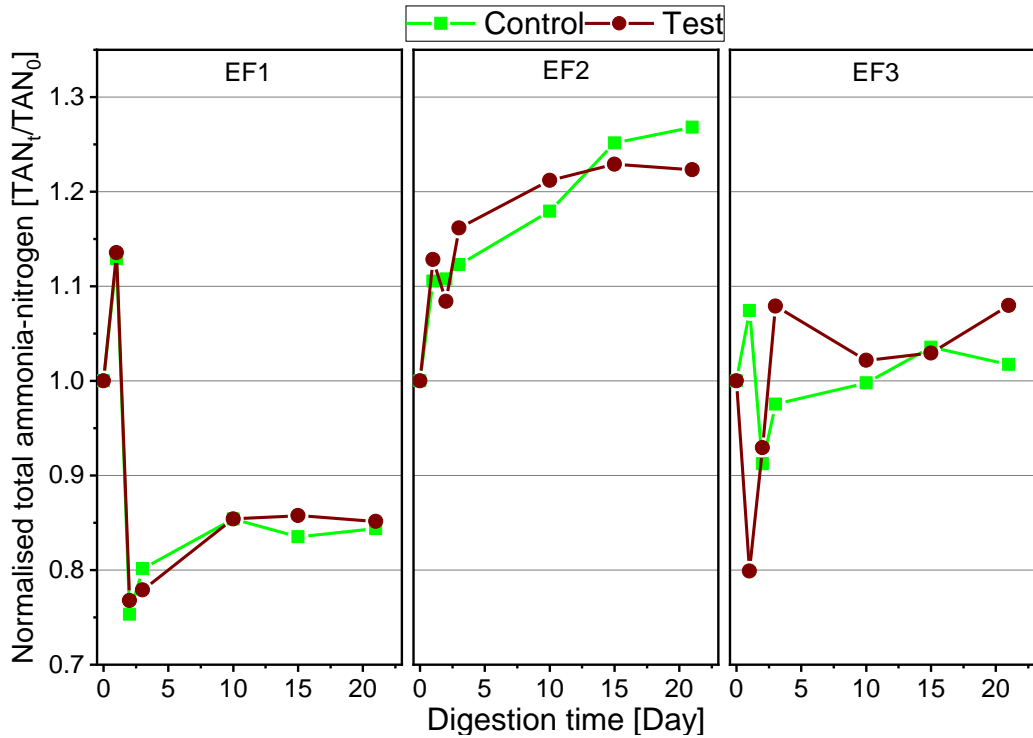
Figure 7.8. Normalised TAN patterns from formic acid-based acclimation experiments. Each point represents the ratio of the TAN measured at that point (Day t) to the TAN measure on the day of set up (Day 0).

7.5.5 Elemental sulphur decomposition

In the FA-based acclimation experiments, the concentration of elemental sulphur at the start of the experiments was 116.7, 99.7 and 112.7 mg/L (dry basis) in EF1, EF2 and EF3 respectively. The elemental sulphur levels measured during the digestion have been normalised against the starting value in Figure 7.9.

Like H_2-based experiments, an initial reduction in elemental sulphur was observed in the non-acclimated reactors in EF1. But as the digestion progressed, elemental sulphur was regained in the FA experiments, whereas a continuous decline was observed for the non-acclimated hydrogen experiments. Perhaps there was a backward VFA-inhibition on the sulphate reducing bacteria, to influence elemental sulphur recovery in the FA non-acclimated control and test reactors.
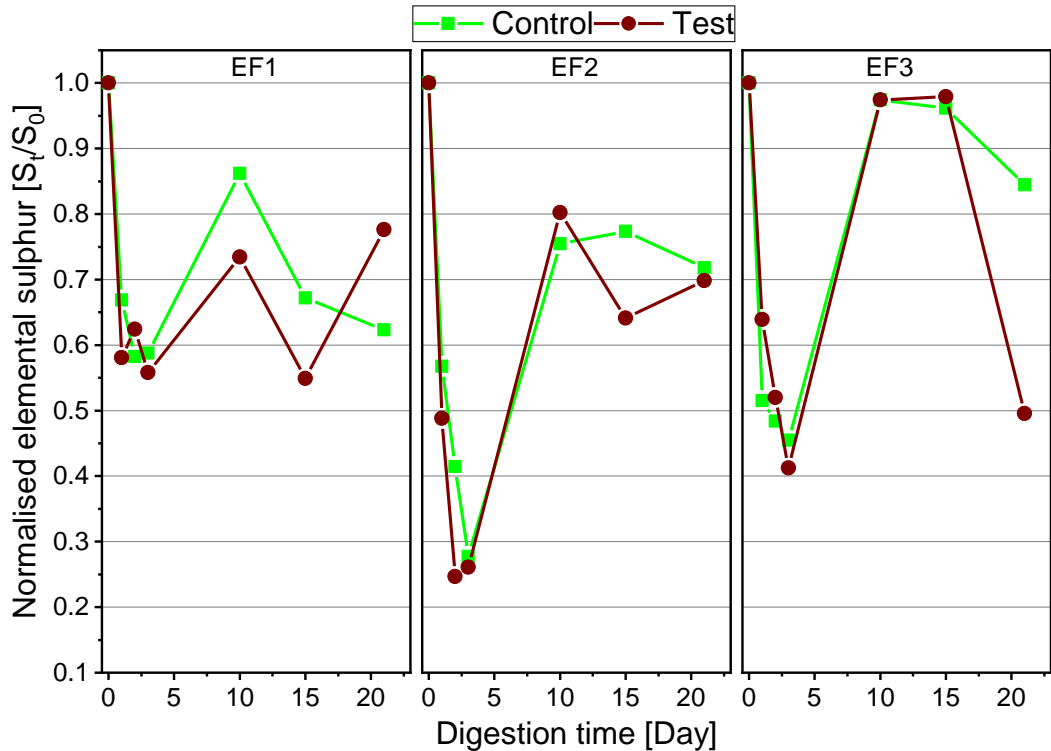
Figure 7.9. Normalised elemental sulphur patterns from formic acid-based acclimation experiments; each point represents the ratio of the elemental sulphur measured at that point (Day t) to the elemental sulphur measure on the day of set up (Day 0).

When the systems were setup with acclimated inoculums in EF2 and EF3, initial elemental sulphur rate increased; however, this was accompanied by a higher recovery in both the EF2 and EF3 experiments than EF1. So that with acclimation only, the systems were able to recover elemental sulphur, for instance, the overall elemental sulphur degraded was 37.7%, 28.2% and 15.5% in the control reactors of EF1, EF2, and EF3. However, the coupling effect of acclimation and increase in FA influenced further elemental sulphur degradation, such that the test reactors recorded 22.4%, 30.2% and 50.5% reduction in elemental sulphur in EF1, EF2 and EF3 respectively. In effect, the test reactors of all acclimated systems recorded a higher percentage of elemental sulphur degradation than each corresponding control reactor.

With acclimation in EF2, the early stage elemental sulphur degradation intensified, however, with further acclimation in EF3, the sulphur degradation reduced in the early stages of digestion, and the elemental sulphur recovery was highest. This was like the elemental sulphur recovery recorded with hydrogen experiments at the second phase of acclimation in EH3. One effect common with both hydrogen and FA acclimation, however, was the elemental sulphur recovery during the process, influencing periods of high and low peaks of elemental sulphur. Therefore, acclimation and increasing concentrations of

hydrogen and FA reduced the potential of sulphides production; however, this effect was higher with H_2-based acclimation. These results might imply that long term acclimation of food waste digesters to increasing concentrations of hydrogen could help reduce hydrogen sulphides potential in the biogas.

7.6 Kinetic analysis of FA-based biomethanation

The kinetic parameters obtained from the SGompertz and MGompertz fitting models for FA-based biomethane yields are summarised in Table 7.1. The relative differences in the kinetic values of the hydrogen-based systems and the FA-based systems was probably due to the inoculum, although from the same source, the time of these experiments were different. Hence, the initial conditions and degree of activity of the sludge were likely not the same. Notwithstanding, since the raw inoculum was similar for each group of experiment (hydrogen and FA), the kinetic changes within each experimental group were analysed accordingly.

The opposite of hydrogen-based biomethanation outcome was observed with FA injection. By acclimation only, the k-value and maximum specific methane production reduced in the control and test reactors, as the experiment progressed from EF1 to EF2. However, progressing from EF2 to EF3, they both improved. This imply that a longer acclimation time could be required if FA were to be the desired electron carrier. Nevertheless, the lag time continued to increase through the acclimation phases, so that although the system was able to recover methane production in EF3, there was yet a relative inhibition to hydrolysis with FA-acclimation in both the control and test reactors.

Comparing the digestion kinetics between some C1 to C5 acids including: formate (C1), acetate (C2), propionate (C3), pyruvate (C3), lactate (C3), butyrate (C4) and valerate (C5), FA was reported to demonstrate self-inhibition, consequently, yielding the longest lag time; which increased with an increase in FA (Y. Yang et al., 2015).

Table 7.1. Kinetic analysis of biomethane production from formic acid-based biomethanation experiments.

Condition	Experiment	k-value (Day^{-1})	Lag time (Day)	Max. specific CH$_4$ yield	R-squared
Acclimation only	EF1_Control	0.77	0.6	133.9	0.99
	EF2_Control	0.29	1.0	52.5	0.99
	EF3_Control	0.37	1.9	67.2	0.99
Acclimation + FA	EF1_Test	0.77	0.6	132.8	0.99
	EF2_Test	0.30	1.0	53.7	0.99
	EF3_Test	0.37	1.9	66.8	0.99

7.7 Conclusions

The acclimation of the AD system to increasing concentrations of hydrogen was effective towards improving both the AD process, kinetics and biogas upgrade. Hydrogen acclimation enhanced elemental sulphur recovery, as well as, materials solubilisation and utilisation, which led to an improvement in hydrogenotrophic methanogenesis both in the control and test reactors of all experiments. Notwithstanding, the acclimation of food waste AD to gradual increases in H$_2$ and FA produced different process outcomes. The addition of FA had some inhibitory effect on the system, which resulted in high VFA accumulation, as well as lower DOC removal. Furthermore, while the addition of hydrogen resulted in pH increases, the addition of FA reduced the pH of the reactors. Considering, food waste digesters are always prone to acidic pH ranges from VFA production, FA might not be a suitable source of hydrogen for the purpose of food waste biomethanation, because, external use of chemicals would be required to provide alkalinity and increase the pH. The availability of the two electron carriers (H$_2$ and FA) in the dissolved form was a major determinant to the outcome of the process. H$_2$ was made available in gaseous form and limited in solution by the gas-liquid mass transfer rate, while FA was added in its liquid state and perceived to have dissolved into the liquid upon addition, thus, producing inhibitory effects. It was therefore, difficult to establish a relationship between hydrogen gas and FA for biomethanation during food waste AD experiments due to these limitations. In general, H$_2$-based acclimation upgraded the biogas to yield 81% biomethane against 65% obtained without acclimation within the same experiments, while FA-based acclimation only improved the biomethane content from 66.7% (without acclimation) to 69.9 % (with acclimation).

CHAPTER 8

GENERAL DISCUSSION

This chapter links the findings described in preceding results' chapters together, to obtain a feasible approach for optimising the AD of food waste. Furthermore, the relevance of this research is discussed here within the context of its practical applicability in the food waste AD industry. This study was not just limited to autonomous optimisation processes; the best option at one stage was carried on to the next stage, until the final stages. Figure 8.1 gives a schematic representation of the strategies adopted in this study towards optimising the biomethane yield of food waste, showing the interconnectivity of each stage with the next.
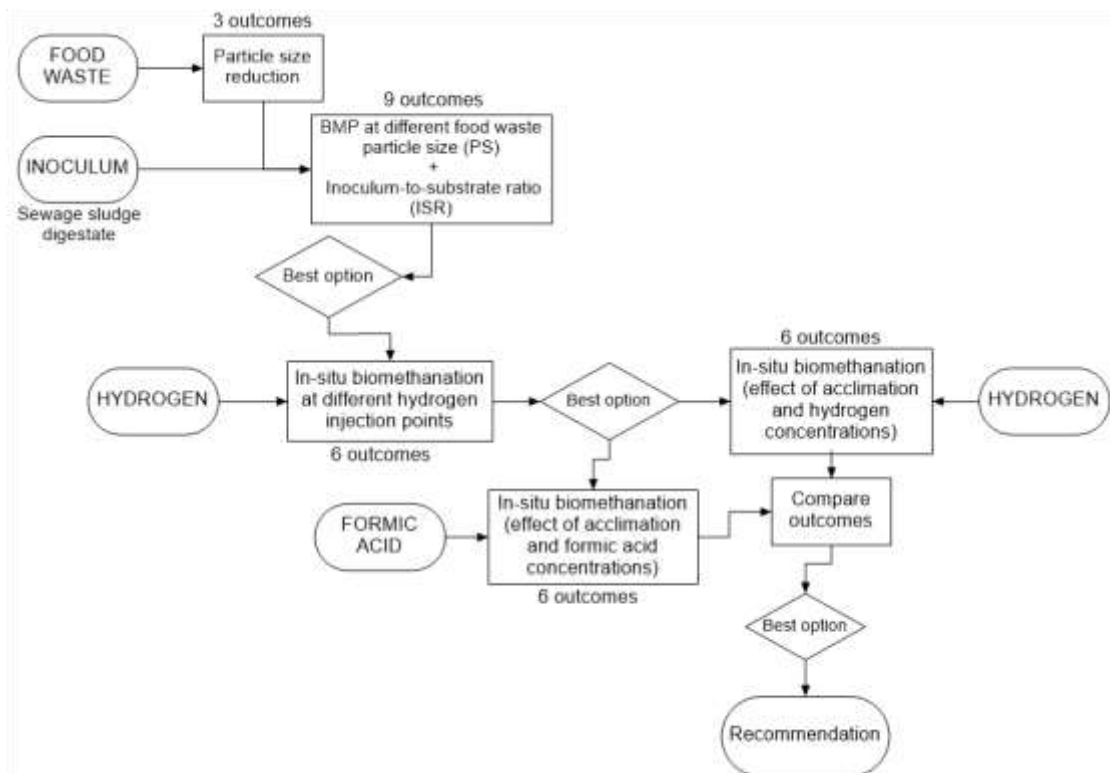
Figure 8.1. Overall optimisation process flow chart adopted in this study

8.1 Food waste sampling, pre-treatment and inoculum-to-substrate ratio optimisation towards improved methane yield.

Independent studies have previously been conducted on the influence of PS (Agyeman and Tao, 2014; Izumi et al., 2010; Kim et al., 2000; Palmowsky and Muller, 2000) and ISR (Boulanger et al., 2012; Eskicioglu and Ghorbani, 2011;

Pellera and Gidarakos, 2016) on the BMP kinetics and methane yield. However, it was important to understand how these two factors interact and impacts on the overall BMP process and methane yield. By this, both the sample characteristics and the reactive environment were optimised in this study, to improve the digestion performance of food waste.

Two food waste streams were analysed based on the sampling variation; the first was a one-time collection of the available food waste at the University of Leeds Refectory (grab sample) and the second was composed of food waste samples collected over a period of one week from the same source (composite sample). Therefore, the grab sample only contained the particular waste available at the source, as at the time of collection, while the composite sample had a better representation of the typical household and hospitality generated food waste composition. Both food waste samples were mechanically pre-treated to three PS groups: 1 mm, 2 mm and 5 mm, in order to improve their digestive properties.

Generally, both food waste streams showed great potential for energy recovery in the form of biomethane using AD technology; based on characteristics such as high COD content and theoretical methane potential. It was however, observed that the sample composition and sampling time among other factors had some influence on the characteristics of food waste collected and also impacted on the nutrients distribution when the PS were reduced. These basic differences in the sample variation influenced the overall characteristics of both samples in terms of their physicochemical, elemental, biochemical, as well as their metals characteristics. In particular, moisture content variation influenced the nutrients distribution when PS reduction was employed as pre-treatment. Unlike the composite sample, the grab sample at all three PS ranges had relatively higher moisture content, which influenced a similar degree of organic solubilisation at all three levels. Thus, with the composite sample having lower moisture content, a slight change in the solids characteristic impacted greatly on the solubilisation of organic content, TKN and VFA. However, the PS reduction of the composite sample resulted in lower TKN and VFA content, consequently, reducing the potential for ammonia and VFA-induced inhibition during AD process.

Despite the composite sample having a higher theoretical methane potential; 588.6 – 608.4 mL-CH$_4$/gVS, compared to 515.6 – 547.9 mL-CH$_4$/gVS from the grab sample, it exhibited more inhibitory potentials than the grab sample, which was not surprising, considering its initial characteristics. For instance, as opposed to an optimal C/N ratio of 25 – 30, C/N ratio was in the range of

10.9 to 12 in the composite sample, while in the grab sample, it ranged from 17.2 to 22.7, which was also supported by higher TKN levels in the composite sample (8.6 – 13.7 g/kg), compared to the grab sample (4.3 – 5.3 g/kg). This was perceived to have influenced higher ammonia release and related inhibition during AD of the composite sample than the grab sample.

PS reduction also experimentally improved the BMP of food waste, owing to the improvement of key parameters suitable for AD, such as C/N ratio and COD. For instance, by PS reduction from 5 mm to 1 mm, C/N ratio improved from 10.9 to 12 in the composite sample and 17.2 to 22.7 in the grab sample, similarly, the COD also improved by 43% and 4% in the composite and grab samples respectively. Consequently, PS reduction fastened the degradation process, resulting in quicker VFA production within the reactors, but adjusting the ISR helped to identify suitable conditions to suppress excessive VFA accumulation. Hence, for lower PS ≤ 3mm higher ISRs of 3 and 4 were more suitable, while PS ≥ 3mm, had optimal yields at an ISR of 2. By optimising the PS and ISR of the grab sample, the overall methane yield improved by 38% from 393.4 $NmL\text{-}CH_4/gVS_{added}$ (at 5 mm PS and ISR at 4) to 542.8 NmL/gVS_{added} (at 1 mm PS and ISR at 3), therefore, an overall optimal BMP condition of 1 mm PS at an ISR of 3 was established for food waste digestion and utilised for further optimisation processes.

At optimal conditions of PS and ISR, an identical methane yield of 543 and 545 $NmL\text{-}CH_4/gVS_{added}$ was obtained from the grab and composite samples respectively; despite the composite sample having a much higher theoretical methane potential than the grab sample. Because the composite sample was more representative of the conventional household and hospitality generated food waste and it showed more inhibitory potentials compared to the grab sample; such as a longer lag time, it was used for further optimisation tests, in order to obtain results that could be adapted into the conventional AD process.

8.1.1 Energy demand for PS reduction

Although, PS reduction would seemingly increase the AD energy demand, a potential increase in methane yield to 38% will increase the energy output to make up for the energy demand from size reduction. The VS content at 5 mm and 1 mm for the grab sample were 20.5% and 19.7% respectively. Therefore, for 393.4 and 542.8 $NmL\text{-}CH_4/gVS_{added}$ obtainable at 5 mm and 1 mm PS, the yield per tonne of food waste becomes 1846 and 2755 $m^3\text{-}CH_4$ respective.

The gross calorific value of methane is 39.8 MJ/m^3, as such, the energy value of the methane yield from 5 mm and 1 mm PS was 76,376 and 109,649 MJ/tonne, equivalent to 21,216 and 30,458 kWh/tonne respectively (where 1 kWh = 3.6MJ). The efficiency for methane conversion to electricity (CHP) was estimated to be 35% (Scarlat et al., 2018), hence, without further PS reduction (5 mm), an energy output of 7,426 kWh/tonne was obtainable. Meanwhile, with further PS reduction to 1 mm, the energy output increases to 10,660 kWh/tonne, which is 43.5% higher than the energy output at 5 mm. At the time of writing this thesis, there was no data on energy required for PS reduction to support whether or not the increased energy output achieved in this study can sufficiently cover the energy input.

8.2 Food waste AD with in-situ biomethanation: optimising the point of hydrogen injection for improved hydrogen utilisation efficiency

Having established a suitable PS and ISR combination for food waste AD, further process optimisation towards biomethane increase was done by injecting hydrogen to enhance CO_2 conversion to biomethane via hydrogenotrophic methanogenesis (biomethanation). In-situ biomethanation have been trialled with other feedstock, such as synthetic media, cattle slurry and sewage sludge (Rachbauer et al., 2016; Burkhardt et al., 2015; Luo and Angelidaki, 2012; Zabranska and Pokorna, 2018; Yun et al., 2017), but there was no report on its application with food waste as an AD feedstock. Biomethanation was thought to be an adoptable method to improve the AD of food waste since it enhances hydrogenotrophic methanogenesis, resulting in increases in pH. Therefore, food waste digestion which is prone to acidic pH levels would benefit from this process.

It was suggested that hydrogen required can be produced from water electrolysis, using surplus energy from other renewable processes such as wind and solar energy. However, this surplus is usually not available at all times, hence, the cost of and energy demand for hydrogen production for biomethanation is still relatively high. It was therefore, important to identify the point of hydrogen injection that would give the highest conversion to biomethane, as a result of limited competition for the added hydrogen.

Therefore, to apply biomethanation in this study, the different stages of AD were optimised to identify the most suitable point at which to add the external hydrogen. Aside from hydrogenotrophic methanogenesis, there are two other

possible sinks for hydrogen, which are acetate formation by homoacetogens and sulphide production (sulfidogenesis) by sulphate/sulphur reducing bacteria. Going by the process thermodynamics, hydrogen utilisation would follow the order sulfidogenesis > hydrogenotrophic methanogenesis > homoacetogenesis.

The metabolism of each hydrogen-utilising group however, primarily depends on the degraded state of the feedstock, availability of the combining elements; CO_2 for hydrogenotrophic methanogens and homoacetogens, and sulphur/sulphate for sulphate reducing bacteria, presence of inhibitors (including hydrogen), and operating conditions such as pH. It was therefore, hypothesised that the stages of digestion; principally governed by the VFA regime, would have significant impact on how the added hydrogen would be utilised.

Three hydrogen injection points were chosen to signify points before VFA production (before hydrolysis), at the peak of VFA accumulation (active acidogenesis) and depleted VFA intermediates (active methanogenesis). During the PS and ISR optimisation experiments, VFA accumulation peaked around Day3, followed by a rapid decrease to a very low concentration around Day6. Based on this, using gas mixture of 5%-H_2:95%-N_2, three hydrogen injection points at Day0, Day3 and Day6 were chosen and labelled as Experiment1 (Exp1), Experiment 2 (Exp2) and Experiment 3 (Exp3) respectively. Furthermore, to understand the influence of adding hydrogen into the system, the three possible sinks of hydrogen; biomethane, VFA and hydrogen sulphide (measured by elemental sulphur removal) were closely monitored.

According to the findings from this optimisation phase, food waste was proven as a suitable feedstock for AD with hydrogen addition and the initial high protein content of food waste was instrumental towards regaining lost alkalinity (due to VFA production) and stabilising the pH. Hydrogen injection enhanced hydrogenotrophic methanogenesis in all experiments, however, the VFA regime had huge influence on the outcome of the competition for the injected hydrogen.

With Exp1, the injected hydrogen was predominantly used via hydrogenotrophic methanogenesis. The sulphate reducing bacteria were outcompeted due to their limited metabolism during hydrolysis. Therefore, there was a direct assimilation of the readily available H_2 and CO_2 by the hydrogenotrophic methanogens, rather than dependence on substrate-based nutrients.

In Exp2, when hydrogen was injected at Day3, the AD system was already autocatalysed to the initial substrate, which implied there was a well-defined community of all acting microorganisms and therefore, an extensive competition for the injected hydrogen ensued. In essence, the competition for the additional hydrogen was controlled by the most favourable thermodynamic pathway for the available substrates. Despite the sulphate reducing bacteria metabolism being more thermodynamically favourable, because, the initially produced biogas was completely removed from the system, the hydrogenotrophic methanogens and homoacetogens were perceived to have been limited by CO_2 availability. Hence, the sulphate reducing bacteria outcompeted the hydrogenotrophic methanogens and homoacetogens; being favoured by both the thermodynamics and substrate availability. This was observed by an extensive degradation of elemental sulphur, with the addition of hydrogen and a relatively insignificant increase in methane yield and acetic acid level, when hydrogen was added.

In Exp3, when hydrogen was added at Day6 most of the VFA had completely depleted, leaving only acetic acid and trace amounts of propionic acid. Since there were only limited substrates in the form of propionic and butyric acids, no major differences were measured with elemental sulphur depletion when hydrogen was added. To further buttress this, CO_2 was not measured in the headspace gas of the test reactors until the second day of digestion, which only increased with further degradation of acetic acid. Also, because the gaseous CO_2 had been removed from the system before adding hydrogen, there was no CO_2 to utilise as carbon source by the hydrogenotrophic methanogens. This had to be provided by the elemental carbon and acetic acid; being the only carbon sources available. In general, due to the limited amount of substrates at this stage, competition for the injected hydrogen was greatly reduced so that the hydrogenotrophic methanogens were selectively enhanced when hydrogen was added in Exp3.

Overall, it was observed that at the end of the experiment, for the increase in ultimate methane yield in Exp1 and Exp3, there was a resultant decrease in CO_2 by a factor of 1.7 and 1.5 respectively, but with Exp2, CO_2 only reduced by a factor of 0.8, which further elucidates that hydrogen added during Exp2 was highly competed for. Furthermore, the biomethane content of the biogas increased from 65 to 77.1%, 84.8 to 88.8% and 70.2 to 79.8% in Exp1, Exp2 and Exp3, which corresponds to 12.1%, 4% and 9.6% biomethane increases respectively. It was not surprising to have high percentages of biomethane in Exp2 and Exp3, because of the removal of the biogas produced prior to the

injection of hydrogen. Hence, injecting hydrogen in Exp1 influenced an autocatalysation towards an enhanced hydrogenotrophic methanogensis before the other hydrogen consumers became relatively active. And in Exp3 whereby, most of the useful VFA intermediates had depleted before hydrogen injection, produced the second highest increase in biomethane yield.

It is clear that the VFA regime influences the competition for hydrogen during biomethanation and injecting at the start of the experiment will give a higher hydrogen utilisation efficiency towards biomethane production. Day0 injection was therefore, chosen as the optimal injection point for hydrogen and adopted in further optimisation experiments.

8.3 Food waste AD with in-situ biomethanation as impacted by acclimation and hydrogen source

Exposing AD consortia to increasing levels of inhibitory elements, allow them to adapt to and overcome the inhibitory effects; a process known as acclimation (Gao et al., 2015; Liu and Sung, 2002; Rajagopal et al., 2013; Yenigün and Demirel, 2013). Acclimation has been suggested as a method of improving the tolerance of AD microbial consortia to inhibiting/toxic substances including; ammonia, long chain fatty acids (LCFA), metals and phenolic compounds (Chen et al., 2008). This is generally brought about by a shift in the microbial population or internal changes that occur in the predominant microbial species (Chen et al., 2008). This principle was also adopted in this study, to acclimate the AD consortia to increasing concentrations of hydrogen gas and FA. In effect, the gradual increase in the concentration of these electron carriers to an acclimated population would help the microbial consortia to adapt to high hydrogen and FA loads and also improve the hydrogenotrophic methanogens' population.

Furthermore, formate and hydrogen have been identified as significant substrates (electron carriers) utilised by the hydrogenotrophic methanogens for methane production. Asides formate having a higher solubility, during AD, both hydrogen and formate are stoichiometrically and thermodynamically available in nearly equivalent amounts (Pan et al., 2016; Schink et al., 2017). Hence, FA was utilised in comparison with hydrogen-based biomethanation systems, as an alternative source of hydrogen for biomethanation.

The acclimation of food waste AD to gradual increases in hydrogen and FA produced different process outcomes. The acclimation of the AD system to increasing concentrations of hydrogen was effective towards improving both

the AD process kinetics and biogas upgrade. This was achieved as a result of an enhanced materials solubilisation and destruction, such as: VFA and DOC, together with elemental sulphur recovery. This led to an improvement in methane production both in the control and test reactors of all acclimated experiments. The addition of hydrogen to the acclimated inoculum resulted in pH increase for all test reactors, indicating an increase in hydrogenotrophic methanogenesis. However, this was not excessively increased as a result of the VFA buffer; which helped to maintain the pH below pH 7.5. The alkalinity by virtue of hydrogen acclimation also improved successively, as such, the system was more resistant to changes induced by initial VFA production due to an acclimated environment.

In comparison, the addition of FA had some inhibitory effect on the system, which resulted in poor VFA degradation, as well as lower DOC removal. VFA accumulation within FA-systems was observed to increase with successive acclimation. FA addition had some inhibitory effects on the VFA degradation, and acclimation did not necessarily curb this effect. Consequently, other key process parameters, such as pH and alkalinity were negatively impacted, which led to low biomethane recovery and biogas upgrade from FA treatment. FA-acclimation influenced reduction in pH to levels around pH 7 and the overall alkalinity recovery rate declined; however, the test reactors having additional FA recorded higher alkalinity levels than the corresponding control reactors during acclimation, indicating the release of CO_2 from FA degradation as well as a lower rate of hydrogenotrophic methanogenesis. It was not surprising that lower alkalinity recovery were measured in the acclimated experiments, because of the excessive VFA accumulation and low methane production rates recorded.

Therefore, acclimation and increasing concentrations of hydrogen improved the overall VFA fermentation, while FA acclimation produced some inhibitory effects on VFA fermentation, resulting in restrained methane production. One common effect with both hydrogen and FA acclimation, however, was the elemental sulphur recovery during the process, as a result of which, hydrogenotrophic methanogenesis improved at the later stages of all experiments. Considering this effect in the AD process, biomethanation (by virtue of acclimation) can be a possible means to reducing sulphides production during AD.

The kinetic process of the system was also impacted in the opposite of hydrogen-based biomethanation outcome with FA injection. By acclimation only, the k-value and maximum specific methane production reduced as the

experiment progressed from EF1 to EF2, however, progressing from EF2 to EF3, they both improved. To imply that a longer acclimation time could be required if FA were to be the desired electron carrier. Nevertheless, the lag time continued to increase through the acclimation phases, so that although, the system was able to recover methane production in EF3, there was yet a relative inhibition to hydrolysis rate with FA-acclimation. Similar effects were also observed within the test reactors of the FA experiments, but with relatively improved lag times. However, beyond just improving the k-values and methane production rates, hydrogen addition at each acclimation stage also improved the technical digestion time (T80), from about 15 days to 10 days following 15% hydrogen addition. This means that with in-situ biomethanation and acclimation, the hydraulic retention time for conventional food waste digestion can also be reduced, thereby, enabling a higher throughput and energy recovery.

The availability of the two energy carriers (H_2 and FA) in the dissolved form was a major determinant to the outcome of the process. Hydrogen was mostly available in gaseous form and limited in solution by the gas-liquid mass transfer rate, while FA was dissolved into the liquid upon addition, thus producing inhibitory effects. This influenced a biogas upgrade to about 81% biomethane against 65% obtained without acclimation in the H_2-acclimated systems, while FA-acclimation only improved the biomethane content from 66.7% (without acclimation) to 69.9 % (with acclimation).

Therefore, the use of gaseous hydrogen for in-situ biomethanation during the AD of food waste was chosen as the preferred source of hydrogen. And the entire optimisation process adopted in this study was useful to influence food waste biogas upgrade from about 65% CH_4 to 81% CH_4. A mechanistic relationship between hydrogen addition, acclimation and increase in biomethane yield was established (discussed later). It defined a mathematical potential for biogas upgrade to ~ 98% biomethane yield, if it were to be used GtG injection or transport fuel.

8.4 Mass and energy balance for hydrogen addition into food waste AD

The revenue from biogas in the UK is usually dependent on government incentives supporting its diverse end use; such as electricity, upgrade for GtG and transport fuel. Although these incentives are quite volatile, biogas upgrade for application in transport and GtG currently hold the best prospects

for biogas. For these purposes, biogas has to be purified to obtain over 95% biomethane; typically 97 – 98% (Bright et al., 2011), therefore, biogas upgrade to 98% biomethane was chosen in this study, giving a tolerance margin of 2% in account of process efficiency.

The mass and energy balances for hydrogen addition in this study were therefore, calculated according to: the amount of hydrogen gas required, scalability of the method and the economics of scale based on the net worth of the biomethane gas.

8.4.1 Hydrogen gas required

The amount of hydrogen required to obtain biogas with 98% biomethane was calculated and used to estimate the energy balance of the proposed system. According to Equation 6.2 (Chapter 6), by virtue of continuous acclimation, the composition by volume of gas mixture required to obtain a biogas with 98% biomethane was 40%-H_2 and 60%-N_2. Experiments in this study were set up using a gas flow rate of 1000 mL/min for 5 minutes, however, for the purpose of scale, 1 minute purge time (i.e. 1000 mL) was assumed; which could be less in practical terms. Therefore, the corresponding amount of hydrogen required was calculated, assuming the experiments in this study were replicated with stepwise acclimation (at 5% interval) from 5% to 40%-H_2.

The amount of hydrogen used in the biomethanation experiments was calculated as the sum total of the amount of hydrogen required for each acclimation stage (i.e. from 50 mL to 400 mL), which was 1800 mL using a 160 mL reactor volume and hence, equivalent to 11.3 L/$L_{reactor}$. Alternatively, in terms of solids content, the total hydrogen required was 10.9 L/gVS_{added} (36.9 L/g-FW). For a 21 day digestion period; as in this study, the hydrogen injection rate was 0.5 L/(L·day), but, considering hydrogen was measured in the headspace for up to 3 days after setup, then for a 3 day gas retention time, the hydrogen injection rate becomes 3.7 L/(L.day).

The injection rate calculated in this study was much lower than rates adopted to achieve maximum hydrogen H_2 and CO_2 conversion in other studies, such as: 5.05 – 5.29 L/(L·day) for batch in-situ biomethanation with grass (Voelklein et al., 2019); 4.52 L/(L.day) for a mesophilic trickle bed reactor using immobilised hydrogenotrophic methanogens from digested sludge (Burkhardt and Busch, 2013); 6 L/(L.day) for a mesophilic AD using an enriched methanogenic culture (Luo and Angelidaki, 2012); 25.2 L/(L·day) for a thermophilic AD using 2 solid state bioreactors composed of vermiculite

shales and granular perlite respectively (Alitalo et al., 2015); and 40.2 L/(L·day) for a thermophilic AD using anaerobic sludge (Díaz et al., 2015).

The lower hydrogen injection rate calculated for in this study was probably because, the hydrogen injection point in this study was optimised to identify the point at which the hydrogen injected was primarily used for biomethane production. Hence, competitions by other hydrogenotrophs were greatly minimised. Furthermore, the continuous acclimation of the system to gradually increasing H_2 concentrations, rather than a continuously high loading, further improved the dominance of the hydrogenotrophic methanogens and hydrogen utilisation rates, therefore, reducing the overall amount of hydrogen required.

8.4.2 Scalability of hydrogen injection with food waste digestion

As earlier stated, water electrolysis and dark fermentation, currently stands out as the feasible and sustainable renewable sources of hydrogen for the purpose of biomethanation and thus, were analysed here as the potential sources of hydrogen for this study.

Hydrogen production by water electrolysis currently contributes about 4% of overall annual hydrogen produced around the world and was estimated to increase to about 22% in 2050 (International Energy Agency, 2006). There is therefore, a growing interest and demand for water electrolysis, which would boost the future of biomethanation systems. Over 26% of the EU's electricity from wind is temporarily surplus, which can be used for electrolysis, and hence, have been suggested as the most viable option for hydrogen generation for biomethanation (Ullah Khan et al., 2017). The conventional industrial electrolyser requires about 4.5 – 5 kWh energy input per m^3 of hydrogen (Rashid et al., 2015). The use of this excess electricity in synergy with biomethanation during AD eliminates to a great extent the energy required for hydrogen production.

Alkaline electrolysers are currently the most commercially available water electrolysers and have up to 150 MW capacity, sufficient to meet the hydrogen demand from this study. However, because of the current distance in separation between the respective renewable energy installations, transportation of electricity from source of production to the AD plant still pose some challenges. As such, dark fermentation seem to be a cheaper and more easily adoptable option; since its operation is similar to the conventional AD. Hydrogen yields in a range of 57 to 283 mL/gVS was reported from food waste in a review by Uçkun Kiran et al. (2014) and the amount of hydrogen required

for progressive acclimation in this study was around 303 mL/gVS. Thus, dark fermentation can potentially be used to provide the hydrogen required.

8.4.3 Economics of scale based on the energy balance

The desired use of the biogas determines what level of upgrade is required and the technology adopted to achieve such upgrade. The possible end uses for biogas are electricity, GtG and transport fuel (Bright et al., 2011). In all cases, some amount of upgrade is required, particularly to remove moisture and other impurities such as H_2S and siloxanes. The four main reasons for upgrading biogas are; i) to increase the calorific value; ii) to align biomethane physical properties with natural gas; iii) to remove impurities in order to protect machines and iv) to reduce the carbon arising from gas utilisation (Bright et al., 2011). In this study only the impact of upgrading technologies for CO_2 removal and biomethane production (improving calorific value and reducing carbon arising), for the different possible end uses was considered.

An extensive review of biogas upgrading, utilisation and storage was reported by Ullah Khan et al. (2017) and the data from this review have been adopted in this study for the energy balance of physicochemical biogas upgrading systems in comparison to this study.

8.4.3.1 Comparative energy demand and revenue generation from biogas upgrade

The final energy worth of biomethane for the respective end use depends on the efficiency of its conversion for the different purposes (i.e. electricity, GtG or transport) and the level of carbon emissions avoided when it is used (Bright et al., 2011). A summary of the potential energy input and output from different physicochemical biogas upgrading technologies, in comparison with this study, is given in Table 8.1. Furthermore, the option of direct use of biogas for electricity from reactors without hydrogen addition, through typical CHP plants was also compared with the biomethanation yield, to estimate the final energy potential. According to the characteristics of food waste discussed in Chapter 4, the VS content of the composite food waste used in this biomethanation study was 29.5%, hence, to convert the methane yield to tonne of food waste for energy calculations, this value was adopted. The methane yield from food waste biomethanation experiments was 417.6 mL-CH$_4$/gVS$_{added}$ without the addition of hydrogen, which is equivalent to 1414 L/kg-FW (1414 m^3/tonne-FW), and the estimated yield with hydrogen addition to obtain 98%

biomethane content was 1952 m^3/tonne-FW. The methane yield without hydrogen addition (1414 m^3/tonne) was used for the physicochemical upgrading technologies; assuming they were employed to upgrade the biogas from its initial content without hydrogen addition. Therefore the biomethane yield was obtained by subtracting the potential losses in methane for each upgrading technology from Table 8.1.

The gross calorific value of pure methane is 39.8 MJ/m^3, however, because of the assumed CO_2 content of the final gas, the calorific values becomes lower. Therefore, the resulting calorific value from the respective upgrading processes was calculated by correcting the calorific value of the pure methane with the expected methane percentages in the final gas (summarised in Table 8.1).

Estimating the calorific value of biomethane, is dependent on the efficiency of the processes adopted for its desired end use (Bright et al., 2011). The final energy output was estimated by first converting the biomethane calorific value to kWh units; dividing by a factor of 3.6, and then the efficiencies of the end use processes were adopted. The reported efficiency of biomethane use for GtG was 99.75% (Bright et al., 2011), conversion to electricity (CHP) was 35% (Scarlat et al., 2018) and 98% efficiency was assumed for use as transport fuel.

Table 8.1. Comparative energy outputs and caloric values from conventional upgrading technologies and this study.

Upgrading technology	Energy input (kWh/m³)	Methane loss (%)	Final yield (m³CH₄/tonne)	Methane purity (%)	Calorific value (MJ/tonne)	Energy output from End use[a] (MWh/tonne)			Energy benefit compared to biomethanation (MWh/tonne-FW)[b]
						CHP	GtG	Transport	
Absorption (high pressure water scrubbing – HPWS)	0.2 – 0.43*	5.13*	1341.5	98	52304	5.1	14.5	14.2	2.3 – 6.6
Absorption (Amine scrubbing)	Not given	0.1*	1412.6	99	55659	5.4	15.4	15.2	2.0 – 5.7
Absorption (organic physical scrubbing – OPS)	0.4 – 0.51*	4*	1357.4	97	42404	4.1	11.7	11.5	3.3 – 9.4
Adsorption (pressure swing adsorption – PSA)	0.24 – 0.6*	4*	1357.4	97.5	52674	5.1	14.6	14.3	2.3 – 6.5
Membrane separation – MS	0.27–0.38*	6*	1329.2	91 – 99**	52373	5.1	14.5	14.3	2.3 – 6.6
Cryogenic separation – CS	0.42*	0.65*	1404.8	98	54793	5.3	15.2	14.9	2.1 – 5.9
No biogas upgrade	-	-	1414	65	36580	3.6	-	-	3.8 – 17.5
Biomethanation (this study)	4.5 – 5.0[c]	-	1952	98	76136	7.4	21.1	20.7	-

[a]1 MWh = 3600 MJ
[b]Values obtained by comparing the various energy outputs with the outputs from biomethanation (e.g CHP$_{Biomethanation}$ –CHP$_{HPWS}$)
[c]Energy input for water electrolysis at kWh per m³ of hydrogen produced
*Data obtained from Ullah Khan et al. (2017)
**91% reported by Ullah Khan et al. (2017), and 97 – 99% was reported by Muñoz et al. (2015), therefore, the maximum of 99% was adopted.

From Table 8.1, if we compared biomethanation to other physicochemical upgrading technologies, the energy input for biomethanation is higher by about 4.2 – 4.5 kWh/m^3, however, this culminates to a potential increase in energy output by 2 to 9.4 MJ/tonne-food waste. Furthermore, upgrading the biogas from AD of food waste opens up multiple income streams for the AD operator compared to the singular CHP (electricity) option for the biogas not upgraded, with a potential increase in energy output by 3.8 to 17.5 MJ/tonne-food waste. Therefore, profits can be optimised by adopting whatever end use becomes most profitable according to the available incentives and much more so with biomethanation than other physicochemical technologies. Hence, by incorporating in-situ biomethanation, the energy output from AD of food waste can be optimised, with up to 17.5 MJ/tonne energy increase.

Besides conventional physicochemical technologies incurring up to 8% methane losses, high chemical and water demand elevates the biomethane production cost to 20 – 72%, and during the regeneration of the adsorbent media, CO_2 is released to the atmosphere (Linville et al., 2016). This high parasitic CO_2 from upgrading technologies reduces the carbon savings especially from GtG applications (Bright et al., 2011). For instance, in 2011, the use of biomethane in electricity generation had the highest CO_2 savings at a conversion factor of 0.54 kgCO$_2$/kWh compared to transport fuel and GtG at 0.25 and 0.185 kgCO$_2$/kWh. However, as more renewable sources of electricity becomes available, biomethane for GtG was reported to be a suitable technology for carbon savings when it replaces natural gas (Bright et al., 2011). Furthermore, when biomethanation replaces the current biogas upgrade technologies, it can significantly increase the carbon savings from biomethane for GtG applications.

8.4.3.2 Comparative cost analysis of biogas upgrading technologies

It is quite difficult to compare the capital investment cost for hydrogen utilisation in biogas upgrade using hydrogen from renewable energy sources to conventional physicochemical processes, due to the limited information available on cost of hydrogen production from water electrolysis and dark fermentation. Notwithstanding the capital investment cost and maintenance cost for the upgrade technologies given here were adapted from values reported in different studies (Balat, 2008; International Energy Agency, 2006; Ullah Khan et al., 2017). According to Ullah Khan et al. (2017), capital investment and maintenance costs for physicochemical processes ranges from 0.12 – 0.4 €/Nm^3 biogas and 15,000 – 56,000 €/year respectively.

For electrolysis, the cost of hydrogen production is equivalent to the cost of the electricity input and the investment cost of the electrolyser. Although, surplus electricity from renewable sources have been suggested as a viable option to reduce this cost, it is, however, only available to for a limited time annually, which will greatly impact on the hydrogen supply. Therefore, the cost of electricity required still has to be taken into consideration. Alkaline electrolysers are currently the most commercially available electrolysers and have been used here to estimate the cost of hydrogen production for biomethanation according to data from IEA (2015). The investment cost for alkaline electrolysis (including gas turbine) was estimated at US$3500/kW at 60,000 hours lifespan (equivalent to US$0.06/kWh). An energy input of 4.5 – 5 kWh/m^3-H$_2$ was presented in Table 8.1, therefore, the cost of producing 5 kWh/m^3-H$_2$ energy becomes US$0.3/m^3 (equivalent to €0.27 at an exchange rate of €1 = US$1.13). Since the electrolysers are usually built with a life span based on the hours of usage, the maintenance cost was taken as the possible cost of compressed hydrogen storage for up to 30 days (where necessary) at US$36.93/GJ (Balat, 2008), which is equivalent to US$443.16/year (equivalent to €392.13/year).

For dark fermentation, the capital cost would be related to installing a pre-digestion reactor with volume equivalent to about a third of the actual digester's volume. Notwithstanding, this cost in practical terms does not measure up to the cost of incorporating a water electrolyser, hence, only the cost of hydrogen production from water electrolysis was considered, to account for the extreme scenario. By this, the highest possible cost of hydrogen production and utilisation (between dark fermentation and water electrolysis) for biomethanation was used in comparison with the cost of other physicochemical biogas upgrade processes. The capital and maintenance costs for different biogas upgrade systems are detailed in Table 8.2.

Table 8.2. Cost of biogas upgrade arising from different upgrading technologies.

Biogas upgrade technology	Capital cost (€/m³)	Maintenance cost (€/year)
Absorption (high pressure water scrubbing – HPWS)	0.13[a]	15,000[a]
Absorption (Amine scrubbing)	Not given	Not given
Absorption (organic physical scrubbing – OPS)	Not given	39,000[a]
Adsorption (pressure swing adsorption – PSA)	0.4[a]	56,000[a]
Membrane separation – MS	0.12[a]	25,000[a]
Cryogenic separation – CS	0.17[a]	Not given
Alkaline electrolysers	0.27[b]	392.13[c]

[a] Source: Ullah Khan et al., (2017); [b] Adapted from IEA, (2015) at an exchange rate of €1 to US$1.13; [c]Adapted from Balat, (2008)

As observed from Table 8.2, except for the investment cost for hydrogen production by alkaline electrolysis for biomethanation, physicochemical biogas upgrade technology have a higher parasitic cost than incorporating biomethanation. Furthermore, a relatively lower energy yield and carbon capture, as well as wastes produced from such physicochemical processes, makes biomethanation a much better technology for biogas upgrade to biomethane.

8.4.4 Position of current study within researches for improved AD and methane yield from food waste

A number of researches reported in literature have been conducted to improve the sole AD of food waste; most of which are yet at the research phase including: PS pre-treatment, trace element dosing, alkaline treatment, ammonia stripping, ammonia acclimation and addition of biochar. Table 8.3 presents the novelty and position of the current study within the context of improving process stability, biomethane yield and biogas upgrade during sole food waste AD.

Table 8.3. Novelty and position of current study amongst AD of food waste for biomethane researches: focussing on mesophilic mono-digestion.

Intervention	Previous studies		This study	
	Intervention	Effects	Intervention	Effects
Pre-treatment	Food waste PS reduction[1]	Increased microbial degradation; Reduced methane production with excessive PS reduction.	Food waste PS + ISR	Improved microbial degradation; Improved process stability; Up to 38% increase in methane yield.
In-situ/post treatment	Trace element dosing[2]	Positive and negative impact on methane yield depending on elements and dosage; No reported influence on biogas upgrade.	Biomethanation: Optimising H_2 injection point	Obtained 4 – 12% biomethane increase and biogas upgrade; Identified injection point with highest rate of hydrogen conversion to biomethane.
	Effect of alkalinity sources[3]	Significant increase in methane yields; No reported influence on biogas upgrade.	Biomethanation: Influence of H_2 acclimation	Biogas upgrade from 65 to 81% CH_4; Improved digester stability; Up to 20% digester total ammonia nitrogen reduction; Improved elemental sulphur assimilation, hence, reduction in gaseous H_2S; Predictive analysis of biomethanation from food waste for GtG and transport fuel.
	Ammonia stripping[4]	Not effective at mesophilic temperature and requires pH=10.	Biomethanation: Influence of FA acclimation	Not effective in improving process stability and biogas upgrade
	Addition of biochar[5];	Reduced lag phase; Increased maximum methane production rate; No significant biogas upgrade.		

[1]Source: (Izumi et al., 2010); [2]Source: (Banks et al., 2012; Facchin et al., 2013; Wanli Zhang et al., 2015; L. Zhang et al., 2012); [3]Source: (Chen et al., 2015) [4]Source: (Serna-Maza et al., 2014); [5]Source: (Cai et al., 2016; Meyer-Kohlstock et al., 2016).

8.4.5 Application of current study in large scale (continuous) operation

An extensive report on the production of biomethane gas and injection to the national grid was produced by Bright et al. (2011), which reveals biomethane injection to the gas grid is very well practised in other EU countries including Germany, Luxembourg, Netherlands, Sweden and Austria than the UK. This is probably, due to more stringent gas quality standards in the UK than others with a well-developed practice. According to WRAP's 2017 spreadsheet on operational AD in the UK (available online – WRAP, 2019), there are currently about 10 AD plants in the UK injecting biomethane to the gas grid; 2 of which are food waste AD plants. Other food waste AD plants primarily use the biogas to operate CHP engines. However, this study reveals that biomethanation can be adapted into full scale food waste AD plants in the UK, which can increase the UK's GtG facilities.

The hydrogen injection point optimisation and acclimation experiments showed that hydrogen injection and utilisation can be optimised by injecting at the start of the AD setup; in which case, start-up food waste ADs would benefit the most. Notwithstanding, injection can also be optimised during continuous digestion, with the incorporation of digestate recycling to influence acclimation and higher utilisation of the hydrogen by the hydrogenotrophic methanogens, thereby, outcompeting other hydrogen users.

A synergistic approach among renewable energy sources would be the best option for hydrogen production where possible. If that were the case, water electrolysis would give the purest and most consistent quantity of hydrogen for biomethanation. However, these systems are not yet fully developed, therefore, for current practice, dark fermentation would be cheaper and more easily incorporated, since it requires similar technical knowhow as in the AD system.

8.5 Research limitations

1. The early stages of feedstock and inoculum optimisation were conducted using standard AMPTS II system by Bioprocess control. However, because this system only delivered the changes in biomethane yield without accounting for the corresponding CO_2 produced, a manual manometric method for BMP (mBMP) (Himanshu et al., 2017) was adopted in the biomethanation optimisation stages,

using Wheaton reactors. The mBMP systems typically give lower gas yields compared to volumetric and automatic manometric methods due to the impact of the headspace pressure, since the gas content is not continuously collected (Himanshu et al., 2017; Mass et al., 2016). As such, relatively lower methane yields for the same BMP conditions were obtained from the mBMP systems.

2. During the optimisation of the hydrogen injection points, Exp2 and Exp3 where first digested in bulk samples using Duran bottles and had to be transferred to the Wheaton bottles for further biomethanation experiments. As such, the headspace gas from each bulk sample reactor was completely removed before adding hydrogen. This limitation led to some losses in biogas yield in Exp2 and Exp3.

3. It was difficult to establish a relationship between hydrogen gas and formic acid for biomethanation during food waste AD experiments due to the limitations in their relative forms. Hydrogen was used here in gaseous form, while FA was added to the system in its liquid form, which influenced a completely different impact on the system for the two sources.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

1. The results obtained from food waste characterisation for biomethane production, indicated that factors such as sampling and processing methods, affects the AD process and biomethane yield. For easy comparison of data and experimental repeatability, a more robust approach (than just grab sampling) should be adopted for food waste collection from the desired source. This will help to establish a representative feedstock composition, especially, for new anaerobic digester installations. In general, food waste from the University of Leeds Refectory has great potential for biomethane recovery through AD.

2. Food waste pre-treatment by size reduction impacted on both the characteristics of food waste and the AD process. The reduction in particle size influenced a faster degradation of food waste. However, while this was expected to result in higher rates of acidification within the system, the variation in inoculum-to-substrate ratio helped to reduce such effects. An optimum condition for a relatively stable BMP process and increase in biomethane yield was reached at 1 mm food waste particle size and an inoculum-to-substrate ratio of 3:1. At these conditions, the cumulative biomethane yield was increased by 38%, in comparison with the yield at 5 mm PS and inoculum-to-substrate ratio of 4:1. When used for electricity generation, about 43.5% increase in energy output is obtainable. In practice, particle size reduction would incur additional energy demand, however, it is assumed that the potential increase in energy output would sufficiently cover the energy input for particle size reduction.

3. This study showed that food waste was a very suitable feedstock for in-situ biomethanation. The tendency for food waste digestion to influence high volatile fatty acids levels, was an advantage, as it helped to stabilise the excessive rise in pH during biomethanation. This made the incorporation of in-situ biomethanation to food waste AD quite feasible. More so, there was no adverse effect on hydrolysis and the resulting volatile fatty acids degradation. At the end of the experiments, 77% biomethane content in the biogas was obtained with the addition

of hydrogen, compared to 65% biomethane content measured in the reactor without hydrogen treatment.

4. It was found that the digestion phases; as governed by volatile fatty acids composition greatly impacted on the digestion process and biomethane yield from in-situ biomethanation. The addition of hydrogen before hydrolysis enabled the dominance of the hydrogenotrophic methanogens throughout the AD process. In contrast, adding hydrogen at the supposed stage of volatile fatty acids accumulation resulted in its rapid utilisation, due to high competition by different hydrogen-utilising group of microorganisms. Adding hydrogen after this stage, however, behaved more like the system with hydrogen addition before hydrolysis, due to the limited substrates available for other hydrogen-utilising microorganisms. Therefore, up to 12.1% increase in biomethane yield was obtained when hydrogen was added before hydrolysis. While the least increase in biomethane yield of 4% was obtained when hydrogen was added during the volatile fatty acids accumulation stage. This clearly shows that for the same amount of hydrogen injected, different energy outputs can be achieved. Taking into consideration, the energy input associated with hydrogen production, it becomes important to optimise the energy output from biomethanation systems. In practice, to maximise the energy output from food waste biomethanation, hydrogen should preferably be added prior to hydrolysis. Where the feedstock (food waste in this case) is fed continuously into the digester, the feedstock and hydrogen loading should be done simultaneously.

5. Acclimation of the AD reactors to increasing concentrations of hydrogen was effective towards improving both the AD process kinetics and biogas upgrade. This was achieved following an enhanced materials solubilisation and utilisation, as indicated by the volatile fatty acids and dissolved organic carbon profiles. As a result, improvements in methane production were obtained both in the control (without hydrogen) and test (with hydrogen) reactors of all acclimated experiments. The addition of hydrogen to the acclimated inoculum resulted in pH increase for all test reactors, indicating an increase in hydrogenotrophic methanogenesis. However, this was not excessively increased as a result of the volatile fatty acids buffering capacity; which helped to maintain the pH below pH 7.5. The alkalinity by virtue of hydrogen acclimation also improved successively, making the system

was more resistant to changes induced by initial organic acids production. Furthermore, acclimation resulted in successive recovery in elemental sulphur, which is believed to have led to the release of hydrogen. Thus, biomethanation was further enhanced by the perceived utilisation of hydrogen released during elemental sulphur recovery. By acclimation, up to 81% biomethane content was achieved, against 65% obtained without acclimation. In practice, rather than establishing a fixed hydrogen loading rate that would be stoichiometrically required to combine with the carbon dioxide released during AD, acclimation to gradual increases in hydrogen is recommended. This will help to reduce the system shock (and possible break down) that could be encountered with very high hydrogen loading rates. Furthermore, it will influence a systemic growth of the hydrogentrophic methanogens over other hydrogen-utilising competitors, thus, maximising the energy output from the hydrogen injected.

6. A statistical relationship between percentages of hydrogen in the gas mixture utilised was established by linear regression using the MiniTab18® statistical tool. According to the regression equations, in order to obtain biogas with approximately 98% biomethane content, a gas mixture of 40%-H_2 and 60%-N_2 would be required, which translates into 0.5L-H_2/($L_{reactor}$·day). However, this should have gone some acclimation stages, until the system conveniently withstands this amount of hydrogen.

7. In comparison with hydrogen, the addition of formic acid had some inhibitory effects on the AD system, which resulted in poor volatile fatty acids degradation, as well as lower dissolved organic carbon removal rates. The acclimation of the system to gradual increases in formic acid did not necessarily curb these inhibitory effects. Consequently, other key process parameters, such as pH and alkalinity were negatively impacted, which led to low biomethane recovery and biogas upgrade from formic acid treatment. Formic acid-acclimation resulted in a reduction in pH to levels around pH 7 and the overall alkalinity recovery rate declined. However, the test reactors, which had additional formic acid, recorded higher alkalinity levels than the corresponding control reactors during acclimation. This was perhaps due to the release of carbon dioxide from formic acid degradation, as well as a lower rate of hydrogenotrophic methanogenesis. The availability of the two electron carriers (hydrogen and formic acid) in the dissolved form was a major

determinant to the outcome of the respective biomethanation processes. Hydrogen was mostly available in gaseous form and limited in solution by the gas-liquid mass transfer rate, while formic acid was added in its liquid form, thus readily assimilated into the solution. In general, formic acid-acclimation only improved the biomethane content from 66.7% (without acclimation) to 69.9 % (with acclimation). Hence, hydrogen gas was a better electron carrier than formic acid for the purpose of incorporating biomethanation into food waste AD.

9.2 Recommendations

Based on the limitations of the research conduction, the following recommendations have been drawn for future research;

1. Further research should be conducted in a continuous system, to fully understand the impact of adding hydrogen to food waste AD and also, allow easy replicability in large scale.

2. Hydrogen sulphide gas was analysed in this study as a function of the elemental sulphur oxidation in the solids, but this does not accurately measure the potential hydrogen sulphide yield in the biogas. Therefore, future studies should include the direct measurement of hydrogen sulphide gas, in order to have a direct reference for hydrogen utilisation by sulphate reducing bacteria. This is an important factor to measure in biomethanation processes especially with the typical and commercially available processes that uses high-sulphur containing feedstock, such as, sewage sludge and cattle slurry.

3. Microbial analysis for the population within the system would be important in future studies, so as to directly quantify the influence of adding hydrogen on the microbial groups present.

4. Hydrogen production from water electrolysis seem to be the most feasible option for pure hydrogen generation, however, where this is not possible due to cost and other operational factors, dark fermentation passes as the next most cost effective and environmentally sustainable option for hydrogen production towards biomethanation here proposed.

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APPENDICES

A.

Results from trial BMP experiments with hydrogen injection using Wheaton and Duran bottles

Sample	Bulk sample at Day0 (start, n=3)			Wheaton bottle samples at Day21 (finish, n=6)			Duran bottle samples at Day21 (finish, n=6)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
pH	7.67	6.76	6.76	6.4	6.51	6.48	4.91	6.41	
VS (g/L)	6.35	7.35	6.88	5.20	5.45	5.38	5.60	6.25	5.84
TS (g/L)	9.81	11.08	10.50	8.55	8.95	8.83	8.80	10.00	
Alkalinity (mgCaCO$_3$/L)	550	620	594.6	1130	1175	1155.5	143	153	148.25
sCOD (mg-O$_2$/L)	3345	3720	3532.5	678	693	684.2	1351	2307	1752.5
Total VFA (mg/L)	35.91	35.51	36.56	3.24	5.03	4.34	602.0	2694.3	708.1
CH$_4$ (mL/gVS$_{added}$)				388.2	400.6	394.4	16.3	28.5	22.2
CO$_2$ (mL/gVS$_{added}$)				138.5	139.5	139.0	53.	161.9	123.2
O$_2$ (mL/gVS$_{added}$)							52.5	218.1	119.5

B.

Results from total ammonia-nitrogen (TAN) validation experiments, comparing results from the filtered and unfiltered samples; centrifugation achieved with an Eppendorf Centrifuge.

Pre-treatment	Inoculum bulk sample (mg NH_3/L, n=3)			Substrate bulk sample (mg NH_3/L, n=3)		
	Min	Max	Mean	Min	Max	Mean
Unfiltered sample	290	340	320	280	308	295
Shaken and filtered	252	308	280	238	266	252
2,000 RPM, 3mins	280	308	294	280	294	289
2,000 RPM, 4mins	280	350	308	168	238	196
2,000 RPM, 5mins	294	336	317	252	336	299
2,000 RPM, 6mins	308	336	317	280	280	280
2,000 RPM, 7mins	322	322	322	266	280	275
2,000 RPM, 8mins	308	322	317	280	280	280
2,000 RPM, 9mins	322	322	322	266	280	271
2,000 RPM, 10mins	280	308	299	252	280	271