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ANAEROBIC DIGESTION OF SOURCE-SEGREGATED DOMESTIC FOOD WASTE

by

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ABSTRACT

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Anaerobic digestion (AD) is an attractive waste treatment process in which both pollution control and energy recovery can be achieved. Source-segregated domestic food waste (FW) has a high organic content on a dry weight basis and is rich in lipids and proteins, indicating the potential for a good biogas yield with high methane content. Process instability, however, has often been reported in food waste digesters, which was mainly manifested by the accumulation of volatile fatty acids (VFA) and reduction of specific methane production. Trace element (TE) supplementation has been proved to be an effective way to rectify this problem and has been applied to industrial AD plants. This practice, however, was usually characterised by a trial-and-error approach due to the lack of a clear understanding of the impact of TEs on AD under different process conditions. The aim of this study was therefore to optimise TE dosing strategies for FW digestion at different loading rates, with particular attention to the role of cobalt (Co) and selenium (Se).

The limiting concentrations of Co and Se were studied in long-term continuously stirred tank reactor (CSTR)-type digester experiments at organic loading rates (OLR) from 1.8 to 5 kg volatile solids (VS) m^{-3} d^{-1}. In a digester operated at OLR 1.8 kg VS m^{-3} d^{-1} without TE addition, dosing of Co at a strength of 1 mg Co kg^{-1} fresh matter was effective to stimulate the complete degradation of accumulated VFA. Around 2500 mg L^{-1} VFA built up, however, after OLR increased to 2.5 kg VS m^{-3} d^{-1}; then dropped slightly by addition of Se at a strength of 0.05 mg Se kg^{-1} fresh matter. After stepwise increases in Se concentration to 0.2 mg kg^{-1}, VFA reduced to less than 1000 mg L^{-1}. In another 2 digesters, at OLR 3 and 4 kg VS m^{-3} d^{-1} respectively, TE washing-out was introduced for determination of the limiting Co concentration. All TE supplementation was ceased in these 2 digesters for around 300 days with the exception of continuous addition of 0.2 mg kg^{-1} of Se. VFA accumulation up to 30000 mg L^{-1} occurred in one digester immediately after the OLR increased from 4 to 5 kg VS m^{-3} d^{-1} and later up to 22500 mg L^{-1} in the other digester when OLR increased from 3 to 4 kg VS m^{-3} d^{-1}. By gradually increasing Co concentration in both digesters to 0.3~0.5 mg kg^{-1}, VFA started to be consumed. At the end of test, the recovered digester with OLR 5 kg VS m^{-3} d^{-1} was running stably with 0.2 mg kg^{-1} Se and 0.3~0.5 mg kg^{-1} Co addition, with a pH of 7.8, IA/PA ratio 0.4, specific methane production (SMP) 0.47 standard temperature and pressure (STP) m^3 CH_4 kg^{-1} VS d^{-1}, volumetric methane production (VMP) 2.37 STP m^3 CH_4 m^{-3} d^{-1}, and VFA concentration less than 500 mg L^{-1}. To further understand the effect of trace elements on VFA production, short-term trials were carried out to assess their function in VFA production. The results indicated that with accumulated VFA, supplementation of trace elements stimulated VFA production to a greater extent than VFA consumption.

Effect of organic loading rate on TE dosing strategy and digester performance was studied in 5 digesters, all of which had stable operation but different trace element addition histories. One pair digesters was run as control at OLR 5 kg VS m^{-3} d^{-1} over the course of the experiment, another pair operated with a gradual loading increase to 6, 7, 8 and 9 kg VS m^{-3} d^{-1}. A SMP of 0.46 $\pm$ 0.02 STP m^3 CH_4 kg^{-1} VS d^{-1} at OLR 8 kg VS m^{-3} d^{-1} was achieved. Volatile solids destruction (VSD) rates were similar between OLR 5 and 8 kg VS m^{-3} d^{-1}, at approximately 0.74~0.75, but reduced to 0.71~0.72 at OLR 9 kg VS m^{-3} d^{-1}. Residual methane production (RBP) test results showed that biogas production of digestate from OLR 5 and 7 kg VS m^{-3} d^{-1} were similar, whereas digestate from OLR 9 kg VS m^{-3} d^{-1} generated more biogas than OLR 5 kg VS m^{-3} d^{-1}, indicating lower conversion efficiency was achieved at OLR 9 kg VS m^{-3} d^{-1}. Nitrogen mass balance equations were developed to distinguish nitrogen distribution in digesters. These showed that microbial biomass density increased along with OLR increase, which in turn requires an increase in TE addition. The specific rate of biomass increase at OLR 9 kg VS m^{-3} d^{-1}, however, was lower than at 8 kg VS m^{-3} d^{-1}, reflecting the decrease in specific methane production and VSD rate. The results indicate that FW digester was able to operate at OLR 8 kg VS m^{-3} d^{-1}, without loss of performance when compared with OLR 5 kg VS m^{-3} d^{-1}. Loading 9 kg VS m^{-3} d^{-1} was regarded as overloaded due to the lower hydrolysis and acidification efficiency. The fifth digester, in which the same TE dosing was applied, was operated with random loading: a daily load between 2.5~7.5 kg VS m^{-3} d^{-1} was randomly introduced while weekly average OLR was maintained at 5 kg VS m^{-3} d^{-1}. Stable performance was observed in this digester with 2.27 STP m^3 CH_4 m^{-3} d^{-1} of 30-day rolling average VMP and 76% of VSD rate, and VFA concentrations less than 500 mg L^{-1}

Further research on essential TE supplementation for stable FW digestion at high loading was carried out. All TE additions were ceased except 0.3 mg kg^{-1} of Co and 0.2 mg kg^{-1} of Se, in two pairs of digesters at loading 5 and 8 kg VS m^{-3} d^{-1}, respectively. VFA accumulation occurred in digesters at the higher loading, which finally failed. VFA fluctuated around 4000 mg L^{-1} in digesters at OLR 5 kg VS m^{-3} d^{-1}, until the rest of trace elements in a full 11 trace elements recipe were reintroduced, when VFA degraded quickly to below 1000 mg L^{-1}.

The research provided new insight on optimising essential TE supplementation to FW digestion, especially at moderate and high loading rates, to ensure stable and high productive biogas production.

Keywords: anaerobic digestion, food waste, trace element, selenium, cobalt, organic loading rate, VFA accumulation

CONTENTS

LIST OF FIGURES

LIST OF TABLES

DECLARATION OF AUTHORSHIP

I, He Song, declare that this thesis entitled

ANAEROBIC DIGESTION OF SOURCE-SEGREGATED DOMESTIC FOOD WASTE

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
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- where I have consulted the published work of others, this is always clearly attributed;
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- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

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Date:

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ABBREVIATIONS

ACS	Acetyl-CoA Synthase
AD	Anaerobic Digestion
ASBR	Anaerobic Sequencing Batch Reactor
ATP	Adenosine Triphosphate
BFN	Biological Fixed Nitrogen
C/N	Carbon to Nitrogen ratio
CoA	Coenzyme A
COD	Chemical Oxygen Demand
CODH	Carbon Monoxide Dehydrogenase
CoM	Coenzyme M
COSHH	Control of Substances Hazardous to Health
CSTR	Continuously Stirred Tank Reactor
DBP	Daily Biogas Production
DI	Deionised
DW	Dry weight
EU	European Union
EPS	Extracellular Polymeric Substances
FAN	Free (unionised) Ammonia Nitrogen (NH_3-N)
FISH	Fluorescent In Situ Hybridisation
FM	Fresh Matter
FW	Food Waste
GC	Gas Chromatography
HRT	Hydraulic Retention Time
IA	Intermediate Alkalinity
LCFA	Long Chain Fatty Acid
MSW	Municipal Solid Waste
MS-OFMSW	Mechanically Sorted Organic Fraction of Municipal Solid
OFMSW	Organic Fraction of Municipal Solid Waste

OHPA	Obligate Hydrogen-Producing Acetogens
OLR	Organic Loading Rate
PA	Partial Alkalinity
POB	Propionate Oxidising Bacteria
RBP	Residual Biogas Potential
RT	Retention Time
SBP	Specific Biogas Production
SCOD	Soluble Chemical Oxygen Demand
SMA	Specific Methanogenic Activity
SMP	Specific Methane Production
SRT	Solids Retention Time
SS-OFMSW	Source Segregated Organic Fraction of Municipal Solid Waste
SS-DFW	Source Segregated Domestic Food Waste
STP	Standard Temperature and Pressure
TA	Total Alkalinity
TAN	Total Ammoniacal Nitrogen
TE	Trace Element
TKN	Total Kjeldahl Nitrogen
TWh	Terawatt Hour
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSD	Volatile Solids Destruction
VSS	Volatile Suspended Solids

CHAPTER 1 Introduction

1.1 Background

In 1999，the European Union (EU) landfill Directive (Council Directive 1999/31/EC) came into force and required all EU member states to reduce the amount of waste going to landfill to reduce negative impacts on the environment and human health. It is commonly agreed that one of the best ways for organic fraction of municipal solid waste (OFMSW) management is anaerobic digestion (AD), which can realise both energy and nutrient recovery by production of methane as an energy carrier and production of stabilised digestate as a bio-fertiliser (Banks and Zhang 2010). AD has clear advantages over other conventional treatment and disposal methods such as combustion, gasification and composing. The following list summarises the significant advantages over other waste treatment processes (Ward, Hobbs et al. 2008):

- A source of renewable and sustainable energy is produced in the form of biogas;
- Digestate produced is an improved fertiliser in terms of both nutrient availability to plants and its rheology;
- High degree of compliance with many national waste strategies implemented to reduce the amount of biodegradable waste entering landfill;
- Successful in treating wet wastes of less than 40% dry matter;
- Effective pathogen removal capacity. This is especially true for multi-stage digesters or if a pasteurisation step is included in the process.

Food waste (FW) has several values as anaerobic digestion substrate, such as readily degradable organic materials and high energy potential on a dry matter basis (Zhang, El-Mashad et al. 2007). A report from UK department for Environment, Food and Rural Affairs (Defra) indicated that in 2009 UK produced approximate 8.3 million tonnes of food and drink waste per year, 7.0 million of which was food. In England, this could generate at least 3-5 TWh electricity per year by 2020 (a heat equivalent of 6-10 TWh) (DEFRA. 2012). Since trace element (TE) supplementation was confirmed to play a significant role in FW anaerobic digestion, this strategy has been widely used for digesters with food waste as sole substrate. The effectiveness of TE addition is because trace elements are essential for AD but present in low concentration in source segregated

domestic food waste: for example, FW produced in the UK was shown to be deficient in Co and Se (Scherer, Lippert et al. 1983, Osuna, Zandvoort et al. 2003, Banks, Zhang et al. 2012, Zhang and Jahng 2012). In lab-scale or industrial-scale digestion, TE tends to be supplemented at excessive dosage, e.g. unnecessary supplementation strength and wide range of trace element species, to ensure stable performance. This, however, may increase the operating cost and waste natural resources. In addition, potential toxicity effect caused by introduction of certain TE into the environment by the land-application of digestate have also raised public concerns over soil and food contamination (Nagajyoti, Lee et al. 2010, Lessard, Renella et al. 2012). In order to achieve a more economic and effective control of the life cycle of the TE supplementation, optimising their dosing strategy by quantitative determination of essential trace elements required is needed to minimise the introduction of metals into the environments (Osuna, Zandvoort et al. 2003, Takashima, Shimada et al. 2011, Lindorfer, Ramhold et al. 2012, Zhang and Jahng 2012). On the other hand, the minimisation of TE supplementation should not compromise the efficient and stable food waste digestion, especially when digestion loading rate is designed high. There have been few studies, however, on optimising TE addition strategies for single-stage anaerobic digestion of food waste along, especially at high OLR (Sager 2007, El-Mashad, McGarvey et al. 2008, Banks, Zhang et al. 2012).

In this study, long-term semi-continuous digestion trials were conducted to determine the specific TE requirements, with particular attention on Co and Se, in mesophilic FW digesters over a wide range of loadings. VFA was the primary parameter to assess the effectiveness of TE supplementation; SMP, as well as RBP when OLR was in question, were mainly chosen to demonstrate the conversion efficiency of food waste; and the VMP was used to indicate the digester productivity.

1.2 Research Aims and Objectives

The aim of this study was to understand the link between trace elements (especially Co and Se) and OLR in food waste digestion in terms of digestion efficiency and stability, and in particular to examine the effects of Co and Se on AD performance at different OLR and their roles in process stabilisation at high OLR. The following objectives were identified in order to achieve the above aim.

1) To identify the individual effect of Co and Se on FW digestion at low and moderate loadings, especially when VFA had already accumulated in digesters

2) To test the effect of TE supplementation on VFA production and to examine the overall response of VFA-laden digesters to TE supplementation

3) To determine the maximum OLR for stable and efficient food waste digestion, and to assess if this could be achieved by supplemented with Se and Co only

4) To investigate process performance and operational stability of FW digestion under variable loading with TE supplementation

5) To clarify the link between organic loading rate, microbial biomass concentration and trace element requirements at different loading rates by nitrogen mass balance

CHAPTER 2 Literature review

2.1 Food waste as digestion substrate

Food waste constitutes one of largest components of the waste stream all over the world, in its broadest definition including food processing waste from agro-industrial sectors, as well as food preparation waste, uneaten food and leftovers from residences, commercial establishments such as restaurant, institutional sources like school cafeterias, and industrial sources like factory lunchrooms (Zhang, El-Mashad et al. 2007). According to a report by the Food and Agriculture Organisation (FAO, 2012), 1.3 billion tonnes of FW, or nearly one third of food produced, is wasted along the food supply chain across the world, and the amount of FW has been projected to increase annually due to economic and population growth. In the EU, approximately 200 million tonnes of waste are generated each year from agro- and food industries (Monier, Mudgal et al. 2011). It has been estimated that the annual food waste arising within UK households was around 7.0 million tonnes, or about half of the total UK food waste from all sources (Quested et al., 2013). The large quantity of domestic food waste arising is a universal issue: for example, the annual amount of domestic food waste in Asian countries is expected to be 416 million tonnes in 2025 (Melikoglu, Lin et al. 2013). Despite actions to minimise it, domestic food waste will still be a major part of the household waste stream and require proper treatment or recovery processes.

Domestic food waste can be incinerated with other combustible municipal wastes for generation of electricity and/or heat, but this leads to the loss of its chemical and energy values (Katami, Yasuhara et al. 2004). Due to the high water content of this material, the only effective way to recover energy from it is through biochemical conversion. Source-segregated domestic food waste is potentially an excellent substrate for biogas production via anaerobic digestion due to its high contents of biodegradable organic components (e.g. carbohydrates, lipids and proteins) and low level of contaminants (e.g. plastics and heavy metals). The main characteristics, with respect to digestibility, of different food waste streams from previous literatures are listed in Table 2.1.

Table 2.1 Characteristics of food waste streams

Parameter	Han and Shin (2004)	Zhang, El-Mashad et al. (2007)	Zhang, Lee et al. (2011)	Banks, Zhang et al. (2012)	Zhang, Xiao et al. (2013)
Source	Dining hall	Waste management company	University restaurant	Digestion facility	Farm
pH	-[a]	-	6.5±0.2	4.71±0.01	5.2±0.3
TS (%WW)	20.5	30.9±0.1	18.1±0.6	23.74±0.08	18.5±0.1
VS (%WW)	19.5%	26.4±0.1	17.1±0.6	21.71±0.09	17.0±0.1
C/N ratio	14.7	14.6	13.2±0.2	13.9±0.2	14.8
Carbon, C (% of TS)	51.4	46.78±1.15	46.67	47.6±0.5	46.78±1.15
Hydrogen, H (% of TS)	6.1	-	6.39	7.04±0.63	-
Oxygen, O (% of TS)	38.9	-	36.39	33.3±2.6	-
Nitrogen, N (% of TS)	3.5	3.16±0.22	3.54	3.44±0.04	3.16±0.22
Sulphur, S (% of TS)	0.1	0.81±0.03	0.33	0.15±0.01	-
BMP (mL CH_4 g^{-1} VS_{added})	-	435	479.5±21.3	-	-

[a] not available;

It was reported that, however, AD of source-segregated domestic food waste tended to suffer from severe VFA accumulation after extended periods of operation when it was

used as a sole substrate in anaerobic digestion. For example, a VFA concentration of above 15000 mg L^{-1} was observed at steady state when laboratory-scale food waste digesters were operated at a low OLR of 1.45 kg VS m^{-3} d^{-1} and a rather long HRT of 180 days (Climenhaga and Banks 2008). Similar problems also occurred in a pilot-scale trial using food waste over a period of 58 weeks: VFA accumulation appeared in both mesophilic and thermophilic digesters, at concentrations of more than 13000 and 45000 mg L^{-1} respectively, at an OLR of around 5 kg VS m^{-3} d^{-1} and HRT of 20-30 days (Banks and Stringfellow 2008). A full-scale digester (Valorsul SA, Lisbon, Portugal) with a capacity of 30000 tonnes of food waste per year, also suffered from severe instability with high VFA accumulation (Neiva Correia, Vaz et al. 2008).

Co-digestion can rectify this problem to certain extent when food waste is digested with other organic waste with lower nitrogen content and higher TE content, for example dairy manure (Van Horn, Wilkie et al. 1994, Zhang, El-Mashad et al. 2007, Zhang, Lee et al. 2011). El-Mashad, McGarvey et al. (2008) found digester fed with food waste was not stable at OLR of 4.0 kg VS m^{-3} d^{-1} or even at the reduced OLR of 2 kg VS m^{-3} d^{-1}, until dairy manure was added into digester. Biogas production increased significantly from 0.26 to 0.50 L g^{-1} VS when food waste was replaced with a mixed feedstock with 48% FW and 52% manure at OLR 4 kg VS m^{-3} d^{-1}; the VFA accumulation problem was also solved after the introduction of co-substrate. Besides co-digestion with other organic waste, problem of VFA accumulation was overcome by means of supplementation of trace element in the study of Climenhaga and Banks (2008), in which case a TE cocktail containing Fe, Cu, Zn, Mn, Mo, Co, Al and Se was added to digesters. That work showed the importance of trace elements in food waste digestion: reactors supplemented with trace elements demonstrated a stable digestion performance, while non-supplemented reactors failed. Comparisons in Table 2.2 indicated food waste as feedstock contained less amount of trace elements than manure and slurry, which was regarded to be one reason of food waste digestion failure.

It is clear from the above discussion that the characteristics of feedstock can affect the digestion process to a great extent. This knowledge is essential for the optimisation of anaerobic digestion process with regard to satisfying the nutrient requirements for the anaerobic microbial consortia. In order to successfully operate anaerobic digestion for

food waste treatment, the mechanism of anaerobic digestion and other factors affecting its performance also need to be considered.

Table 2.2 Concentrations Comparison of trace and heavy metals in different feedstock

Trace elements (mg kg^{-1} TS)	Food waste[b] (Banks, Zhang et al. 2012)	Pig manure (Sager 2007)	Cattle slurry (Sager 2007)
Aluminium (Al)	-[a]	0.70	1.67
Boron (B)	-	-	-
Cobalt (Co)	<0.25	4.0	2.1
Copper (Cu)	7.17±0.84	282	51
Iron (Fe)	227	2.08	1.97
Manganese (Mn)	84.25±12.64	358	180
Molybdenum (Mo)	0.46±0.04	5.3	3.5
Nickel (Ni)	7.16±2.95	12.5	6.3
Selenium (Se)	<0.29	3.37	0.59
Tungsten (W)	<1.05	-	-
Zinc (Zn)	32.86±10.95	1156	164
Potentially toxic element			
Cadmium (Cd)	-	0.46	0.27
Chromium (Cr)	6.93	6.9	6.6
Lead (Pb)	<10.53	1.9	4.1
Mercury (Hg)	0.01	-	-

[a] Not available;
[b] converted into TS basis from fresh matter basis

2.2 Overview of anaerobic digestion

2.2.1 Anaerobic digestion process

Anaerobic digestion is a spontaneous process mediated by microorganisms, to convert organic material into biogas (a mixture with methane and carbon dioxide as main

components) and digestate in the absence of oxygen. Generally, anaerobic digestion is described as a multi-step process of series and parallel reactions in which several key groups of microbes take part. The digestion is usually organised into four stages including hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 2.1), operated by different microorganisms (Zhang, El-Mashad et al. 2007, Demirel and Scherer 2008, Nagao, Tajima et al. 2012).

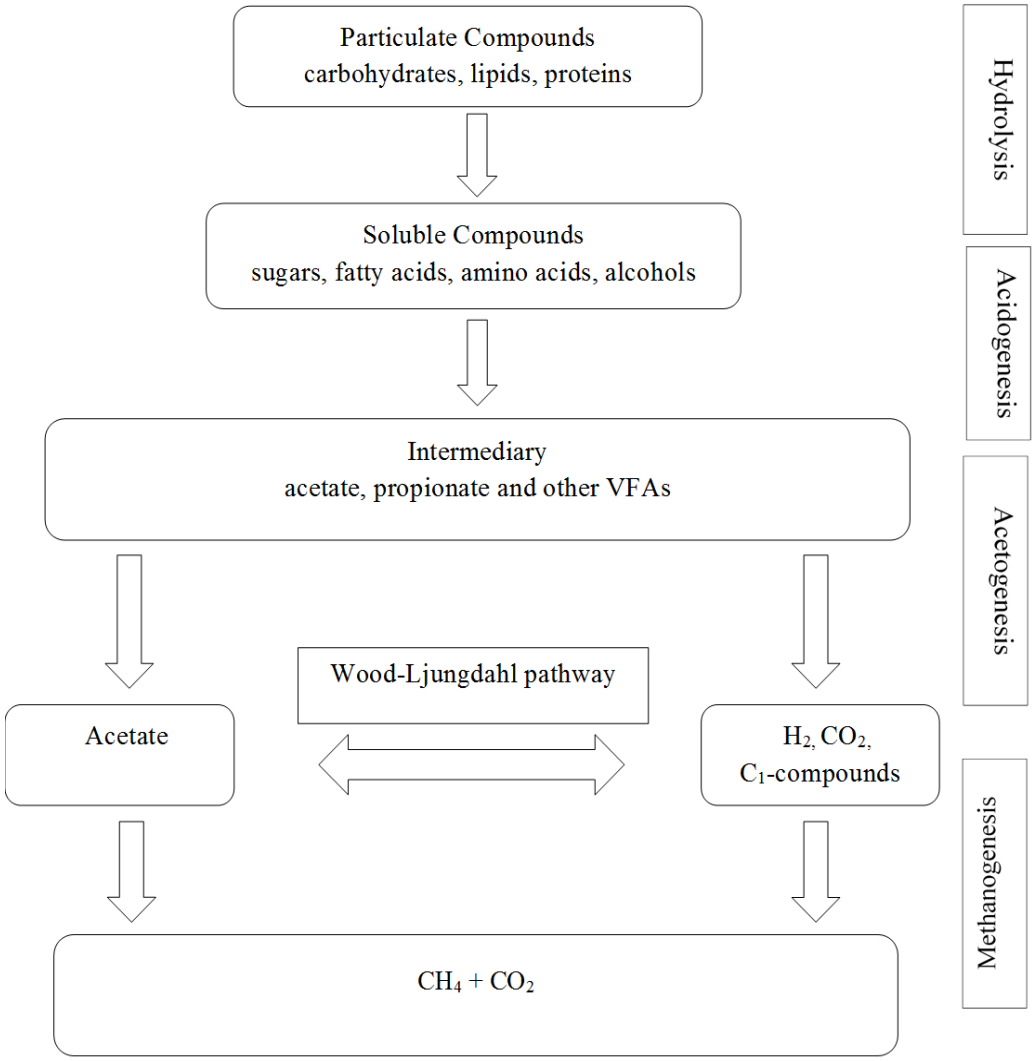


Figure 2.1 Anaerobic degradation of organic matter (modified from Demirel and Scherer (2008))

Hydrolysis

Particulate organic materials such as proteins, carbohydrates and lipids need to be hydrolysed to simple molecules, e.g. amino acids, sugars and long chain fatty acids

(LCFA), by extracellular hydrolytic enzymes before they can be used by the hydrolytic fermentative bacteria in the next stage (Schink 1997).

The rate of hydrolysis is affected by a range of environmental factors and operational parameters such as pH, temperature, substrate composition, hydraulic retention time and particle size. In many studies, hydrolysis of particulate organic material has been considered to be the rate-limiting step in anaerobic digestion, although the microbial community structure of hydrolytic bacteria still remains largely unknown (Pavlostathis and Giraldo-Gomez 1991, Vavilin, Rytov et al. 1996, Christ, Wilderer et al. 2000, Forster-Carneiro, Pérez et al. 2008, Vavilin, Fernandez et al. 2008, Zverlov, Hiegl et al. 2010).

In a well-balanced anaerobic digestion system, all products of previous metabolic stage are consumed by the next one without significant build-up of intermediate products. A faster hydrolysis rate compared to methanogenesis activity can induce accumulation of VFA and hydrogen which may lead to acidification of digester. Hence, maintaining the balance between hydrolysis and methanogenesis is important for process stability (Pavlostathis and Giraldo-Gomez 1991).

Acidogenesis

Acidogenesis is the quickest step of the anaerobic digestion process, referring to the production of organic acids, alcohol, hydrogen and carbon dioxide from soluble monomers without requirement for additional electron acceptor or donor. In this step, soluble compounds produced through hydrolysis are degraded by a diversity of facultative and obligate anaerobes through various fermentative pathways (Gerardi 2003, Vavilin, Fernandez et al. 2008).

Acetogenesis

There are two acetogenesis routes, and the main stream of acetogenesis in AD system is to degrade VFA with a carbon-chain length of 3 or more, alcohols and LCFA to produce acetic acid, carbon dioxide and hydrogen (Chernicharo 2007). This process is essential for beta-oxidation of LCFA produced in the hydrolysis of lipid, and is also involved for the anaerobic degradation of aromatic compounds. The microorganisms responsible for this are called obligate hydrogen-producing acetogens (OHPA) or proton-reducing acetogens.

They only function when growing in environments that maintain a low concentration of metabolic products such as hydrogen. Considering another fact that methanogens can be inhibited by fatty acids, the syntrophic relationships between methanogens and OHPA in an anaerobic digester are held in a fairly fragile state of equilibrium, and small perturbation in the concentration of the substrates or products of acetogenesis may lead to inhibitory effects of both groups (Mara and Horan 2003; Hattori 2008).

The other acidogenesis is named as homoacetogenesis, which utilises hydrogen and carbon dioxide to generate acetate. The homoacetogens responsible for this are obligate anaerobes that use the unique reductive Wood-Ljungdahl pathway. Acetate-oxidising bacteria can also accomplish the reverse reaction of homoacetogenesis and this step is vital in digesters with the acetoclastic methanogenic population inhibited or suppressed, e.g. at high total ammoniacal nitrogen (TAN) concentration (Demirel and Scherer 2008; Schnürer and Nordberg 2008). Several key metalloproteins/metalloenzymes have been identified in the reverse Wood-Ljungdahl pathway, such as formate dehydrogenase (FDH), corrinoid- and Fe/S cluster-containing protein (CFeSP), Carbon monoxide dehydrogenase (CODH), and Acetyl-CoA synthase (ACS) (Ragsdale 2008, Zhu and Tan 2009).

Methanogenesis

Methanogenesis is the final stage of anaerobic digestion, which is strictly performed by methanogenic archaea, namely methanogens. Three methanogenesis pathways have so far been discovered (Galagan, Nusbaum et al. 2002): 1) acetoclastic pathway with acetate as sole reactant: acetate is cleaved to a methyl group and an enzyme-bound CO, with the CO subsequently being oxidised to provide electrons for the reduction of the methyl group to methane; 2) hydrogenotrophic pathway with CO_2 and H_2 as reactants: CO_2 is reduced to methane using electrons provided by H_2; 3) methylotrophic pathway with C-1 compounds such as methanol or methylamines as reactants: one molecule of C-1 compound is oxidised to CO_2 to provide electrons for reducing three additional molecules to methane in the case of methanol. Methanogenesis is the catabolic activity of methanogens and an electrochemical gradient is generated which is in turn used in ATP synthesis. These pathways also share a common final step, i.e. the reduction of methyl-CoM to methane (Galagan, Nusbaum et al. 2002). Acetoclastic and hydrogenotrophic

pathways are the two dominant pathways involved in the anaerobic digestion of natural organic materials and therefore are described in more detail below. The information of the function of trace elements in these pathways is given in section 2.3.

Acetoclastic pathway

Acetoclastic pathway employs a set of enzymes and coenzymes and achieved via several biochemical reactions in a stepwise manner as shown in Table 2.3 (Ferry 1992, Ferry 1999, Ferry 2010).

As described in Eq.2.1, acetate firstly combines with coenzyme A to produce acetyl-CoA ($CH_3COSCoA$), identified as the activated form of acetate. This reaction is catalysed by adenosine monophosphate-forming acetyl-CoA synthetase or by the combined actions of acetate kinase and phosphotransacetylase.

The methyl group of acetyl-CoA is then transferred to H_4SPT to generate CH_3-H_4SPT, accompanying with enzyme-bound carbonyl group oxidised to CO_2, as shown in Eq.2.2. Carbon monoxide dehydrogenase/acetyl-CoA synthase complex (CODH/ACS) is the enzyme responsible for this reaction.

Subsequently, the methyl group of CH_3-H_4SPT is transferred to coenzyme M (H-S-CoM), by substitution of the hydrogen atom in the thiol group of coenzyme M (Eq.2.3). This reaction is catalysed by coenzyme M methyltransferase (Mtr).

The final step (Eq.2.4), common for all methanogenic pathways, is the reduction of methyl group by combining coenzyme B with coenzyme M via the catalysis of Methyl-CoM reductase (Mcr), yielding a heterodisulphide (CoM-S-S-CoB) and CH_4.

Table 2.3 Reactions in acetoclastic methanogenesis pathway

$CH_3COOH + CoA \rightarrow CH_3COSCoA + H_2O$	Eq.2.1
$CH_3COSCoA + H_4SPT \rightarrow CH_3\text{-}H_4SPT + CO_2 + CoA + 2[H]$	Eq.2.2
$CH_3\text{-}H_4SPT + H\text{-}S\text{-}CoM \rightarrow CH_3\text{-}S\text{-}CoM + H_4SPT$	Eq.2.3
$CH_3\text{-}S\text{-}CoM + H\text{-}S\text{-}CoB \rightarrow CoM\text{-}S\text{-}S\text{-}CoB + CH_4$	Eq.2.4

Hydrogenotrophic pathway

Biochemical reactions in this pathway, listed in Table 2.4, also involve several enzymes and cofactors (DiMarco, Bobik et al. 1990; Ferry 1999, Shima, Warkentin et al. 2002, Deppenmeier and Müller 2008, Thauer, Kaster et al. 2008, Ferry 2010). First, CO_2 attaches to methanofuran (MF) which is then reduced to formyl-MF (Eq.2.5) by the catalysis of formyl-MF dehydrogenase (Fmd). Ferredoxin (Fd) acts as electron donor for this reaction. Formyl-MF is then transferred to H_4MPT (Eq.2.6) with formyl-MF: H_4MPT formyltransferase (Ftr) as the catalyst for this transformation. This is followed by conversion of formyl-H_4MPT to 5, 10-methenyl-H^4MPT^+ (Eq.2.7), then 5, 10-methylene-H_4MPT (Eq.2.8) and 5-methyl-H_4MPT (Eq.2.9). In the conversion from 5, 10-methenyl-H^4MPT^+ to 5-methylene-H_4MPT, F_{420} acts as a coenzyme for hydride transformation in these two reactions. Subsequently, the methyl group is transferred to CoM (Eq.2.10) which is catalysed by methyl-H_4MPT: coenzyme M methyltransferase (Mtr). Finally, methyl-CoM is reduced to methane by methyl-CoM reducetase (Mcr).

Table 2.4 Reactions in hydrogenotrophic methanogenesis pathway

$CO_2 + MF + H_2 \rightarrow$ formyl-MF $+H_2O$	Eq.2.5
Formyl-MF + H_4MPT $\rightarrow$ 5-formyl-H_4MPT + MF	Eq.2.6
5-formyl-H_4MPT + $H^+ \rightarrow$ 5, 10-methenyl-$H_4MPT^+ + H_2O$	Eq.2.7
5,10-methenyl-$H_4MPT^+ + F_{420}H_2 \rightarrow$ 5,10-methylene-H_4MPT + $F_{420} + H^+$	Eq.2.8
5,10-methylene-H_4MPT + $F_{420}H_2 \rightarrow$ 5-methyl-H_4MPT + F_{420}	Eq.2.9
5-methyl-H_4MPT+HS-CoM $\rightarrow$ CH_3-S-CoM+H_4MPT	Eq.2.10
CH_3-S-CoM + HS-CoB $\rightarrow$ CH_4 + CoM-S-S-CoB	Eq.2.11

2.2.2 Effect of environmental and operational factors on anaerobic digestion

The performance of microbial consortium mediated anaerobic digestion can be influenced by a range of environmental and operational factors, with the most important ones listed below.

Temperature

Anaerobic digestion is very sensitive to temperature and normally carried out in one of two optimal temperature ranges identified: mesophilic (30-45 °C) and thermophilic (50-60 °C). It is reported that temperature has significant effect on the whole AD process, but methanogenesis is particularly vulnerable to sharp and/or frequent fluctuations in temperature. Maintaining a stable temperature in an AD system is therefore critical (Angelidaki, Ellegaard et al. 2003, Speece 2008, Weiland 2010). Food waste digester operated at mesophilic temperature is favourable because it gives more stable digestion with less extent of VFA accumulation (Banks and Stringfellow 2008, Yirong 2013).

pH

The pH of a digester is affected by substrate and operating conditions: for example an accumulation of intermediate product VFA by the imbalance of acidification and methanogenesis results in a decrease in pH, whereas degradation of protein may increase pH and buffering capacity through ammonia production (Veeken, Kalyuzhnyi et al. 2000). pH directly affects all the steps involved in AD, for examples acidogenic bacteria prefer pH 5-7 whereas pH 6.7-8 is beneficial to methanogens (Monnet 2003; Chernicharo 2007). The optimum pH for one-stage AD process was identified as between 6.7 and 7.6, which is favourable for methanogenic archaea (Speece 2008). This is because methanogens tend to grow very slowly at pH lower than 6.6. Therefore, pH of AD system is usually maintained according to methanogenic limits to prevent the predominance of acid-forming bacteria and avoid VFA accumulation (Angelidaki, Ellegaard et al. 2003, Speece 2008).

Ammonia

Ammonia provides readily available nitrogen sources for synthesis of amino acids, proteins and nucleic acids of microorganisms, and therefore essential for microbial metabolism in AD. As a base, ammonia also neutralises the volatile acids produced by fermentative bacteria, and thus helps maintain AD pH (Chen, Cheng et al. 2008, Sheng, Chen et al. 2013).

Ammonia, however, is inhibitory at high level. The effect of ammonia inhibition showed clearly in digesters fed with substrates rich in nitrogen, such as food waste, chicken

manure and slaughterhouse waste. Between free ammonia (FA) and its salt form ammonium ion, FA is usually considered to be the main cause of inhibition (Koster and Lettinga 1988, Angelidaki, Ellegaard et al. 2004, Chen, Cheng et al. 2008).

Although the inhibitory effect of ammonia has been reported in a number of studies, the concentrations at which inhibition commenced in these studies varies significantly, as shown in Table 2.5. It should be noted that some of the inhibitory levels in the Table were reported using total ammoniacal nitrogen (TAN), which is the sum of FA and ammonia ion.

A growing amount of evidence indicated that hydrogenotrophic methanogens have higher tolerance to ammonia than acetoclastic methanogens (Angelidaki and Ahring 1993, Hansen, Angelidaki et al. 1998, Schnürer and Nordberg 2008). Under high ammonia conditions the syntrophic acetate oxidation and hydrogenotrophic methanogenesis route has been observed for the conversion from acetate to biogas (Schnürer, Houwen et al. 1994, Schnürer, Zellner et al. 1999). Population structure analysis using fluorescent in situ hybridisation also confirmed that hydrogenotrophic methanogens was of dominance in stable mesophilic CSTR food waste digesters at elevated ammonia level (Banks, Zhang et al. 2012). These findings make the following two statements become invalid: 1) two third of methane formed in an anaerobic digestion is derived from the acetoclastic pathway (Gujer and Zehnder 1983); and 2) the syntrophic acetate conversion pathway was only observed under thermophilic condition (Zinder and Koch 1984).

Table 2.5 Inhibition levels of FA and TAN in continuously fed reactors

Temp.	Substrate	Reactor	Inoculum	Inhibition limit FA (mg N kg^{-1})	Inhibition limit TAN (mg N kg^{-1})	% reduction in CH_4 production	pH	Ref.
55	Soluble non-fat dry milk +NH_4Cl	CSTR	Acclim*		5770	64	6.40	(Sung and Liu 2003)
55	Cattle manure	CSTR	Acclim	600-800	NR		7.4-7.9	(Angelidaki and Ahring 1994)
55	Cattle manure	UASB	Acclim	500	7000	72		(Borja, Sánchez et al. 1996)
50	Cattle manure	CSTR	NR*	NR	1700	Initial inhibition	NR	(Zeeman, Wiegant et al. 1985)
55	Cattle manure	CSTR	NR	900	4000	25	NR	(Angelidaki and Ahring 1993)
35	Chicken manure diluted (44% to 10% TS)	CSTR	Acclim	1500	15000	0	7.5-8	(Niu, Qiao et al. 2013)
55				2000	4000	72	7.5-8	(Niu, Hojo et al. 2014)
55	Swine manure	CSTR	NR	1600	NR	70	7.97	(Hansen, Angelidaki et al. 1998)
60	Swine manure	CSTR	NR	2600	NR	96	8.15	(Hansen, Angelidaki et al. 1998)

55	OFMSW	Complete-mix reactor	NR	45	5700		7.20	(Kayhanian 1999)
37	Food waste	CSTR	NR	>1000	3400-3500	NR	>7.5	(Climenhaga and Banks 2008)
55	SS-DFW	CSTR	Acclim	843	3000	100	7.9	(Yirong 2013)
55	OFMSW	CSTR	Acclim	680-690	3000-3700	50	7.60	(Gallert and Winter 1997)
37	OFMSW	CSTR	Acclim	220-280	1830	50	7.60	(Gallert and Winter 1997)
55	OFMSW	CSTR with waste recirculation	Acclim	251		NR	NR	(Gallert, Bauer et al. 1998)
37	Co-dig$_1$	CSTR	non Acclim	548±33.9 645±41 1035±115	2795 2993 3700	100 91 85	8.1 8.2 8.4	(Pitk, Kaparaju et al. 2013)
55 38 38	Co-dig$_2$	CSTR	Acclim	860 100 750	2500 2000 5600	99 100 99	81 76 8.1	(Nordell, Vahlberg et al. 2013)

*Acclim = acclimatised; NR = Not Reported; Co-dig$_1$ = sewage sludge and sterilized solid slaughterhouse waste (2.5-10%); Co-dig$_2$ = OFMSW (59-82 % OLR) slaughterhouse (13- 18% OLR) and glycerol (0-28% OLR)

Retention time

Hydraulic retention time (HRT) indicates the time allowed for substrate to be transferred to biogas. A longer HRT ensures better conversion and hence energy recovery, but that also means a larger footprint and higher capital investment. Solids retention time (SRT) shows the residence time of microbial biomass in digester before it is washed out. The SRT at least should be higher than the doubling time of the microorganisms with the slowest growth rate to avoid the washing out of key microbes. Both HRT and SRT should be carefully controlled to ensure stable and effective digestion, and a SRT shorter than 10 days is not suitable for AD. At SRT longer than 10 days the breakdown of compounds started to improve as indicated by more stable biogas production (Zhang and Noike 1994; Sanders, Veeken et al. 2003; Appels, Baeyens et al. 2008). Furthermore, Sanchez, Borja et al. (2005) reported that an increase in SRT allowed better adaptation of microorganisms to the substrate and improvement of process performance as this ensured that slowly-growing organisms was not washed out. Increasing SRT or HRT can be achieved by increasing reactor volume, reducing the influent flow or recycling the sludge (microbial biomass). The HRT is usually coupled with SRT in one-stage CSTR-type digester treating organic solids.

Loading rate

Organic loading rate (OLR) is an important operational parameter for the AD process. A high OLR is usually desirable as this means high volumetric methane production, although overloading or shock loading may lead to instability and process failure for several interlinked reasons: 1) the breakdown of the delicate balance between VFA production by hydrolysis, acidogenesis and acetogenesis and VFA consumption by methanogenesis, the symptoms of which are VFA accumulation and pH decrease (Wheatley, Fisher et al. 1997, Lyberatos and Skiadas 1999); and 2) washing out of feedstock before it is converted to biogas (Rajeshwari, Balakrishnan et al. 2000), as indicated by a reduction in specific biogas and methane yields (Wheatley, Fisher et al. 1997, Gómez, Cuetos et al. 2006). Anaerobic digestion of readily degradable organic materials usually suffers from the first problem, and as a result irreversible

acidification of the digester may occur at impractically high OLR (Pavlostathis and Giraldo-Gomez 1991).

The effect of OLR on digestion productivity, stability and microbial communities has been studied using a wide variety of substrates as well as different digester types. In summary, higher organic loading was mainly achieved in UASB digesters using low VS substrates or in thermophilic CSTR digesters fed with municipal solid waste. The advantages of UASB or thermophilic digesters are obvious with respect to increases in OLR: microbial biomass retention is realised in UASB and higher metabolic activity is shown under thermophilic condition. The detailed results of studies are reviewed below.

A wide range of organic and hydraulic loading rates has been reported in the literature for UASB reactors, which has been widely adopted for treatment of high-rate substrates (Lettinga, Field et al. 1997). Improved process knowledge and operational experiences on formation and retaining of stable granules has made high loading possible, resulting in a more sustainable operation of these systems. The highest loading of 104 kg COD m^{-3} d^{-1} was applied to UASB type digesters of sugar solution under thermophilic process (Wiegant and Lettinga 1985). Syutsubo, Harada et al. (1997) reported that a COD loading of 30 kg COD m^{-3} d^{-1} was achieved with COD removal efficiency of 85% in thermophilic UASB reactors treating alcohol distillery wastewater. According to Soto, Ligero et al. (1997), excellent stability and high treatment efficiency was achieved with hydraulic residence time as low as 2h at an OLR of 6 kg COD m^{-3} d^{-1} in UASB digester treating with diluted wastewater (500 mg COD L^{-1} as sucrose), the percentage COD removals being 95% (30 °C) and 92% (20°C), while the percent COD converted to methane reached 67% (30 °C) and 48% (20°C), respectively. In a study using slaughterhouse effluent as substrate in mesophilic UASB reactor, high SCOD removals increased from 62% at OLR 10 kg COD m^{-3} d^{-1} to 92% at OLR 27 kg COD m^{-3} d^{-1}. However, after OLR increased higher than 30 kg COD m^{-3} d^{-1} SCOD removals decreased to below 70%. This decrease in performance could be attributed to insufficient time for substrate transfer from the liquid to biomass. (Torkian, Eqbali et al. 2003).

The characteristics of feedstock affect the achievable loading rate as well. According to report by Chelliapan, Wilby et al. (2011), in mesophilic UASB type digester treating pharmaceutical wastewater, efficient COD reduction (70-75%) was achieved under lower OLR (0.43-1.86 kg COD m^{-3} d^{-1}), however increasing OLR to 3.73 kg COD m^{-3} d^{-1} reduced the COD removal efficiency to 45%. Meanwhile, results indicated that a low VFA concentration of around 200 mg L^{-1} was present when OLR in the range 0.43 to 1.53 kg COD m^{-3} d^{-1}, but it was increased to more than 3000 mg L^{-1} when OLR reached 3.73 kg COD m^{-3} d^{-1}.

In semi-dry (10-20%TS) and dry (20-40%TS) thermophilic anaerobic digestion, high OLRs of 7-15 g VS m^{-3} d^{-1} had also been realised without irreversible acidification. But the methane yield was usually low (140-314 mL g^{-1} VS) and VS reduction was also low (31-48%) in these cases (Forster-Carneiro, Perez et al. 2007, Dong, Zhenhong et al. 2010, Fdez-Güelfo, Álvarez-Gallego et al. 2012). This is because that hydrolysis rate became limiting step at lower moisture contents during dry digestion. As transport of VFA from the acidogenic to the methanogenic stages can only take place through the liquid phase, lower moisture contents in the dry digester result in slower acidification and a stable treatment with low methane yield and low VS reduction.

A research work related to olive mill solid residue (Rincón, Borja et al. 2008) was operated at a fixed influent substrate concentration of 162 g total COD L^{-1} and 126 g VS L^{-1}, for the purpose of exploring the maximum methane production from anaerobic digestion by continuous increase OLR in CSTR type reactors under mesophilic condition. OLR was varied from 0.8 to 11 g COD L^{-1} d^{-1}, the maximum methane production rate was found to be 1.7 STP L CH_4 L^{-1} d^{-1} which was achieved under OLR of 9.2 g COD L^{-1}. OLR of 11 g COD L^{-1} d^{-1}, however, caused process failure by VFA accumulation and pH decrease.

Schmidt, Pröter et al. (2013) evaluated the performance of grain stillage digestion with TE supplementation in three different reactor systems under mesophilic condition: CSTR, anaerobic sequencing batch reactor (ASBR), and fixed bed reactor (FBR) respectively. During the study, OLR was increased from 1 to 10 g VS L^{-1} d^{-1} and HRT was decreased from 40 to 6 days when trace elements are supplemented

accordingly. No apparent difference could be identified between these three systems, even in CSTR system without biomass immobilization, a stable process at HRT's below 10-14 days was achieved.

Nutrients

Microorganisms in AD require nutrients for their growth and metabolism, and therefore optimal digestion performance. These can be categorised into macro-nutrients and micro-nutrients depending on the amount needed.

Macro-nutrients

In AD process, carbon, nitrogen, phosphorus and sulphur are included in macro-nutrients as these elements are required in substantial quantities. Deficiency in macro-nutrients may cause inadequate microbiological activity (Mara and Horan 2003, Mata-Alvarez 2003). Recommended C:N:P:S proportion for the growth and survival of microorganisms were proposed in the ratio of 600:15:5:3 (Fricke, Santen et al. 2007) or 600:15:5:1 (Weiland 2010).

Special attention needs to be given to the C/N ratio, which has been proven to be critical on the stability of digestion process. Low C/N ratio can result in inhibition due to an excess of nitrogen in the form of ammonia; while a very high C/N ratio can lead to nitrogen deficiency for biomass synthesis, loss of buffering capacity, or trigger the production of EPS which reduces the carbon recovery as biogas (Miqueleto, Dolosic et al. 2010, Yenigün and Demirel 2013). Optimal C/N ratio for AD of organic waste has been reported to be in the range of 20-35 (Khalid, Arshad et al. (2011; Wang, Yang et al. 2012), although the C/N of food waste was sometimes outside this optimum ratio, as shown in Table 2.1.

Micro-nutrients (Trace Elements)

Micro-nutrients or trace elements are required only in trace amounts, but are important to anaerobic microorganisms and may improve specific gas production or process stability at proper quantity, or contrarily become inhibitory or toxic to AD process when present at lower or higher concentrations. A range of TE, such as Co, Se, Fe, Ni, Mo and W, were reported to be essential to the AD process (Speece,

Parkin et al. 1983, Speece 1983, Fermoso, Bartacek et al. 2009, Worm, Fermoso et al. 2009, Banks, Zhang et al. 2012).

As reviewed in section 2.2.1, under anaerobic conditions, microorganisms utilise a set of unique enzyme systems, in which trace elements are often involved as part of a cofactor or electron carriers. On the non-enzymatic level, trace elements are also involved in membrane-bound electron transfer process of microbial anaerobic respiration (Zandvoort, Van Hullebusch et al. 2006).

Oleszkiewicz and Sharma (1990) indicated that TE supplementation requirement depends not only on their role in biochemical pathways, but also on concentration, type of metal and speciation. In a review by Zandvoort, Van Hullebusch et al. (2006), it is suggested the specific TE requirement is strongly dependent upon the speciation and bioavailability of the element to the microorganisms, as well as the specific methanogens in the process. Similar conclusions were given by Jiang (2006) and Schattauer, Abdoun et al. (2011).

Toxicity of trace elements is another important topic and has been mainly studied using feedstock from certain industrial processes, usually contaminated by heavy metals. In several other studies, the problem of TE overdosing was also raised when the substrate already contained relatively high background levels of trace elements. In this case surplus trace elements may have negative effect on anaerobic digestion process (Ishaq, Roussel et al. 2005, Hinken, Urban et al. 2008, Facchin, Cavinato et al. 2012).

Therefore, it is unlikely to propose a generic trace element supplementation strategy applicable universally to all anaerobic digesters treating different types of substrates. Optimisation of trace element supplementation still has to be specific to each type of substrates, although many studies have presented valuable results in terms of TE application in anaerobic digestion, as reviewed in section 2.3.

2.3 Trace element requirement for anaerobic digestion

Trace elements can be defined as 'any chemical element that occurs in very small amounts in organisms, but is essential for many physiological and biochemical processes' (Zandvoort, Van Hullebusch et al. 2006). Trace elements function as structural elements and catalytic centres in metalloproteins, metal-activated enzymes and electron carriers. Iron is almost ubiquitously required for life, but other metals and Se are associated more closely with specific microbial physiologies (Zerkle et al., 2005). The requirement for trace elements during anaerobic digestion can be explained according to the metabolic processes of anaerobic bacteria and methanogens. More information has been found for methanogens than for bacteria in this case probably for the following two major reasons (Ferry 1993, Deppenmeier 2002, Deppenmeier and Müller 2007, Thauer et al 2008). Firstly, biological methanogenesis is the terminal step in the mineralisation of organic materials under many anoxic environments, and therefore becomes an important part of the maintenance of the carbon cycle on earth. Secondly, from an evolutionary point of view, methanogens are close to the origin of life on earth, and live close to the thermodynamic limit. The trace element requirements of methanogens are explained below from two aspects – methanogenesis and biosynthesis, among which methanogenesis is discussed in more detail.

Studies of the trace element requirement of anaerobic bacteria have predominantly focused on the Wood-Ljungdahl pathway which is the reversed route of syntrophic acetate oxidation, especially its bifunctional carbon monoxide dehydrogenase / Acetyl-Coenzyme A synthase (Andreesen et al 1973, Lindahl and Chang 2001, Doukov et al 2002, Seravalli et al 2003, Ljungdahl 2009). It is worth noting that part of the Wood-Ljungdahl pathway is also used by methanogens in their methanogenesis and biosynthesis (Lindahl and Chang 2001, Smith and Ingram-Smith 2007), as well as by sulphate reducing bacteria to generate metabolic energy (Ragsdale and Pierce, 2008). The dependence of other anaerobic bacteria on trace elements has rarely been explored theoretically from the viewpoint of the anaerobic digestion process, except for a few examples on the effect of trace element supplementation on syntrophic propionate oxidation (de Bok et al 2003, Plugge 2009). This is mainly because the syntrophic oxidation of propionate is an

intermediate bottleneck step in anaerobic digestion, and these syntrophic bacteria have to carry out their metabolic activities at the thermodynamic limit (Kosaka et al 2006, McInerney et al 2008).

The supplementation of trace elements to anaerobic digesters is necessary when the trace element contents of the substrates are not sufficient for the metabolic processes of anaerobic digestion. The specific requirements for trace element supplementation, however, can be affected by their species, bioavailability, digester configuration and dosing strategy; and inhibition effects will arise if they are overdosed (Zandvoort 2006, Lenz et al 2008, Uemura 2010,). It appears that although the optimisation of trace element supplementation still has to be based on quantitative experimental trials, a fundamental knowledge of trace element functions in the process should provide a more theoretical basis to solve any trace element deficiency problem.

The literature reviewed in the section ranges from fundamental bioinorganic chemistry research to commercial anaerobic digestion practice. The quantitative trace element requirements in anaerobic digestion are introduced first, especially according to the well-studied methanogenesis, catabolic pathways of methanogens, and Wood-Ljungdahl pathway, and then the availability of trace elements are discussed, as well as the impacts of trace element supplementation in the AD process.

2.3.1 Quantitative trace element requirement of methanogens

Methanogenesis is the final and a most critical step of the anaerobic digestion process for biogas production, and was commonly regarded as the step most vulnerable to trace element deficiency (Takashima et al 1990, White and Stuckey 2000). As methanogens carry out the terminal steps in the anaerobic digestion process, whenever their consumption of acetate, hydrogen, or formate is slower than the production of these intermediate products due to trace element deficiency, further VFA accumulation will appear due to product-induced feedback inhibition and can adversely affect the overall process.

Theoretical estimation according to TE-containing enzymes and electron carriers involved in methanogenesis

All methanogenic archaea investigated to date rely strictly on methanogenesis for energy conservation and growth, and methanogenesis is regarded as one of the most metal-rich enzymatic pathways in biology (Zerkle et al., 2005; Glass and Orphan, 2012). As mentioned in section 2.2.1, the number of substrates utilised for methanogenesis is quite limited reflecting the narrow ecological niche methanogens occupy: most methanogens are only able to grow with H_2 and CO_2/formate, some can utilise methylated compounds, and some can grow with acetate. These different substrate classes are metabolised via distinct, but overlapping pathways (Ferry 1993, Thauer 1998, Deppenmeier and Müller 2008, Thauer et al 2008, Stock and Rother 2009), and methyl-S-CoM (2-methylthioethanesulfonate) is the central intermediate in the catabolic metabolism of methanogens. Depending on the substrate, methyl-S-CoM is formed by three different pathways (Deppenmeier 2002).

Although the specific metal requirements differ to a certain extent depending on the pathway involved, they follow the same trend as indicated in Figure 2.2: Fe is used most abundantly, followed by Ni and Co, and then smaller amounts of Se, Mo (and/or W) and Zn, (Zerkle et al., 2005; Glass and Orphan, 2012). No quantitative ratio of metal contents in each pathway or used in each cell, however, can be calculated because: 1) metalloenzymes may have different half-saturation constants (Km) value for their respective substrates which causes a change in enzyme ratios; and 2) metals are also required for anabolic pathway of methanogens. For example, Co-containing Acetyl-CoA synthase is used by hydrogenotrophic methanogens, although this enzyme system is not involved in their catabolic pathway (Lindahl and Chang 2001; Stock and Rother 2009). The methanogenic methionine synthase is also a Co-containing enzyme for at least one methanogen species (Banerjee and Ragsdale, 2003). Se has been found in certain tRNAs in hydrogenotrophic methanogens, apart from its function in hydrogenotrophic methanogenesis (Stock and Rother 2009). Mo is needed for nitrogen uptake in both diazotrophic and NH_4-based growth modes, although the requirement for Mo is much higher for diazotrophic growth (Scherer, 1989; Kessler et al., 1997). The information in the literature, however, is limited in this regard. Nevertheless, some studies have analysed the cellular metal contents of

some pure-cultured methanogens and the results roughly agree with the quantitative order of metal requirements for methanogenesis, which indicates metal requirements for methanogenesis have a strong influence on cell metal contents.

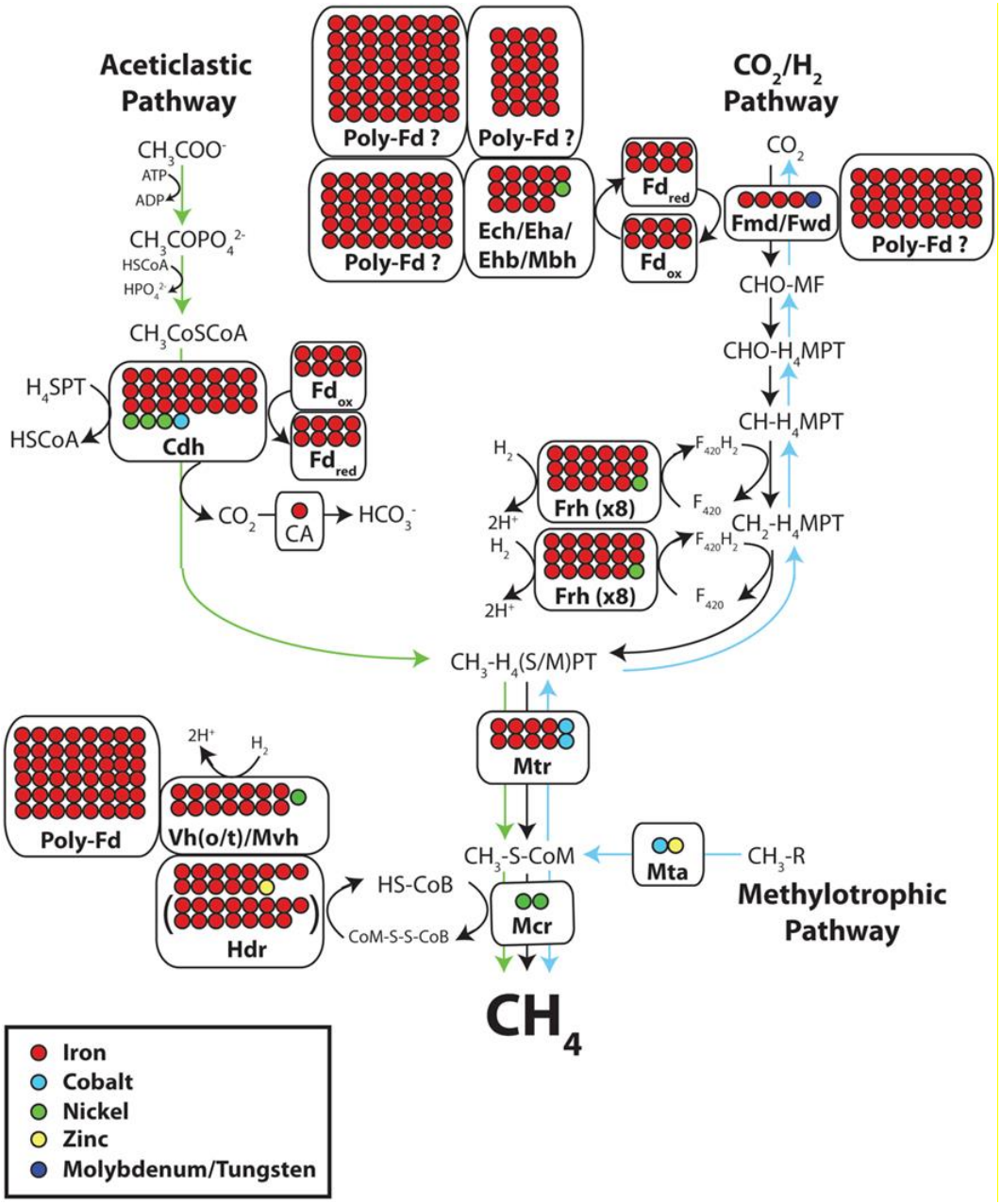


Figure 2.2 Metal content of metalloenzymes in the three methanogenesis pathways; each circle represents one atom (Glass and Orphan, 2012)

Note: when formate is used as an electron source instead of H_2 in the hydrogenotrophic pathway, F_{420}-reducing hydrogenase is replaced by formate dehydrogenase which contains Mo/W, Se, Fe and Zn.

Based on the metal content of enzymes in methanogenesis, the following general conclusion can be made: 1) roughly equal Fe, Ni, and Zn requirements for hydrogenotrophic, acetoclastic, and methylotrophic pathways; 2) higher Mo/W requirements for hydrogenotrophic and methylotrophic pathways than acetoclastic methanogenesis; 3) higher Co requirement for methylotrophic pathway than hydrogenotrophic and acetoclastic pathways; 4) only a few studies have investigated Zn requirements for methanogenesis, although Zn is present in at least two enzymes in the pathway; and 5) Cu and Mn are not needed for methanogenesis (Glass and Orphan, 2012).

It is worth noting that Ni and W have much more limited biological uses, but methanogenic archaea have significantly elevated Ni and W contents compared to non-methanogens. If the fractional contribution of each metal is calculated by dividing the total number of atoms of this specific metal in the cell by the total number of all the trace metals excluding iron, then the fractional contribution of Ni and W in methanogens is 9% and 5% respectively, compared to 2% and 2% for non-methanogens. Iron is not included in the fractional contribution calculation is due to its ubiquitous use in biological systems (Zerkle et al, 2005).

Se is also a trace element essential for many organisms. It is, however, not included in Figure 2.2 as it is not a metal and is therefore out of the scope of studies on trace metals. The most important and best characterised biological form of Se is the amino acid selenocysteine (Sec), the 21st genetically encoded amino acid. It is structurally identical to cysteine (Cys), only with the thiol group replaced by a selenol group. The use of Sec can be partly explained by its high nucleophilicity and the fact that the selenol group is mostly deprotonated at physiological pH due to its lower pKa value (5.2 for Sec, 8.3 for Cys) making it more reactive than Cys. Due to this trait, Cys is almost exclusively found in the catalytic site of numerous redox-active enzymes (Rother and Krzycki 2010). The only Archaea for which selenoproteins have been demonstrated, either by experimentation or prediction from genome sequence data are methanogens with obligatory dependence on the hydrogenotrophic pathway of methanogenesis, although far from all hydrogenotrophic methanogens employ Sec (Kryukov and Gladyshev 2004, Stock and Rother 2009). Stock and Rother (2009)

illustrated the application of Se in hydrogenotrophic methanogenesis as shown in Figure 2.3 below.

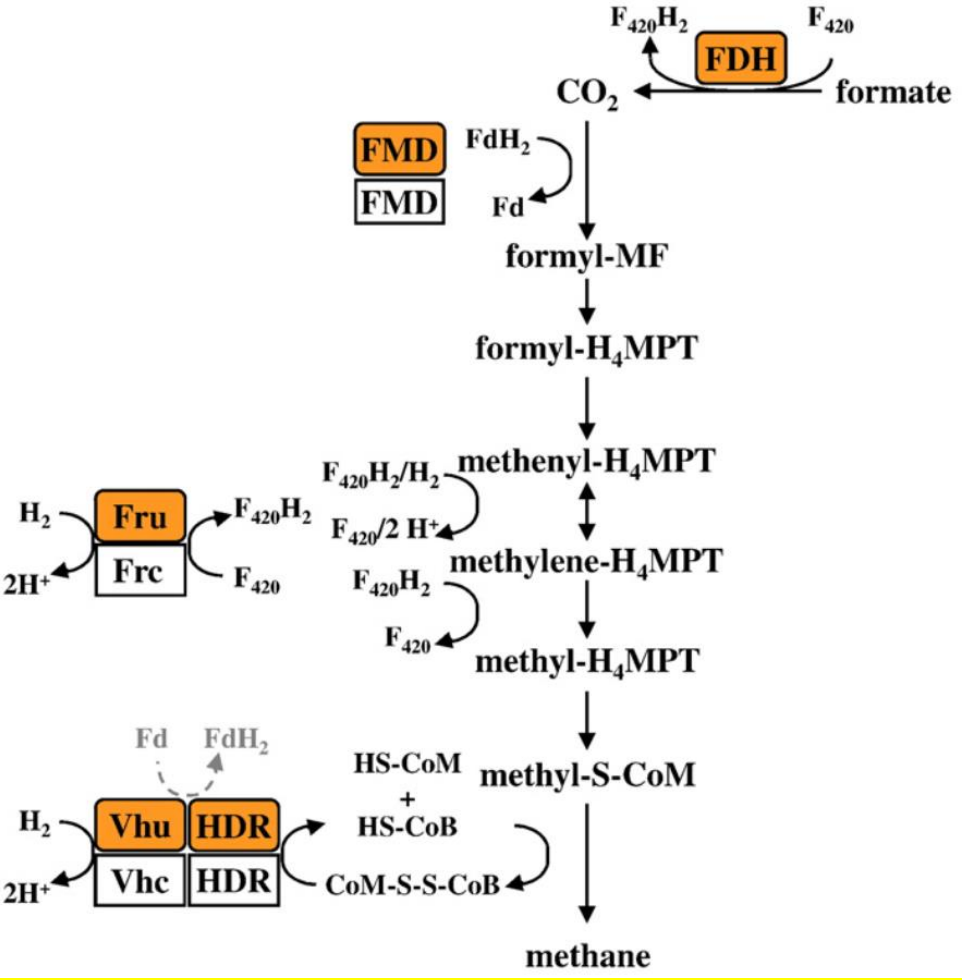


Figure 2.3 The pathway of CO_2 reduction to methane in Methanococcus species. Selenoproteins involved are round-boxed in orange (Stock and Rother 2009).

Direct determination of TE concentrations in microbial cells or in culture medium

In addition to the estimation of trace element requirements according to the types and concentrations of each metalloenzymes or other metal-containing functional molecules, two empirical experimental approaches are also applied. The first method is to quantify the concentration of each trace element in anaerobic microbial cells,

and the other is to identify the concentration of trace elements in the culture medium to provide optimal cell growth or substrate utilisation.

Both approaches help to identify the TE requirement for methanogens besides the central methanogenesis pathway and therefore provide some insight, but at the same time they have their respective limits. Regarding the first method, the metal concentrations in the cell may not directly link to their physiological functions, but may be actively transported or passively diffused into cells when they are available, i.e. luxury uptake. Although the second method helps to identify the concentration needed in the culture medium, this information cannot be directly used to estimate the functioning TE concentration in cells without some essential established parameters such as half-saturation constant or equilibrium constant for metal transport via the cell membrane. It is worth noting that the second approach is widely applied to identify the optimal TE dosing for the entire anaerobic digestion process (Fermoso et al., 2009; Mudhoo and Kumar, 2013; Thanh et al., 2006). The results obtained using these approaches are listed below.

Trace elements concentrations in microbial cells

Methanobacterium thermoautotrophicum was cultured at 65 °C using H_2/CO_2 and it was found that approximately 150 nmol Ni, 20 nmol Co and 20 nmol Mo were required to produce 1 g of dry cells (Schonheit et al. 1979). Another important finding of this study is that it demonstrated that the absence of Cu, Mn, Zn, Al and B in the culture medium did not affect the growth.

Scherer et al. (1983) determined the elemental composition of 10 methanogens cultured using defined media for hydrogenotrophic, methylotrophic and acetoclastic pathways and reached two conclusions: 1) in general, Fe (700-2800 ppm) >> Ni (65-180 ppm) > Co (10-120 ppm) ≥ Mo (10-70ppm) > Mn (≤ 25 ppm) on a TS basis; and 2) Zn and Cu concentrations were around the same level as Ni.

Unknown hydrogenotrophic methanogens selected from pond sediment were used to establish the TE requirement as well in defined culture medium (Zhang et al. 2003). The contents of Fe, Cu, Zn, Ni and Co in dry cells were 1472±187 ppm, 202±33 ppm, 201±10 ppm, 62±7 ppm and 12±2 ppm, respectively. Cu and Zn were proven to be

essential metals in this experiment which became limiting when the density of methanogens was still low. The cell concentrations of Cu and Zn obtained from Scherer et al. (1983) and Zhang et al. (2003) are remarkably higher than expected from their few known biochemical functions in methanogens, although these studies identified Zn could be regarded as a superacid catalyst in many enzymatic processes. Zn can also be used in carbonic anhydrase for methanogenesis (Glass and Orphan, 2012). Further investigation of this is desirable to establish the Cu and Zn requirement for methanogen but this is normally not an issue for anaerobic digestion processes using natural organic materials as substrate. This is because they are expected to contain sufficient Cu and Zn, in contrast to certain industrial effluents used as feedstock.

Trace elements concentrations in cultural media

Scherer and Sahm (1981) reported the growth of *Methanosarcina barkeri* on methanol as the energy source and found optimal growth of this strain depended on Co and Mo in the culture medium. In the presence of 10^{-6} M of Co and 5×10^{-7} M of Mo optimal growth occurred. Furthermore, it was demonstrated that Ni and Se each at a concentration of 10^{-7} M stimulated the growth of this methanogenic bacterium while the other elements B, Cr, Cu, Mn, Pb tested in the range of 10^{-7} to 10^{-3} M had no influence. The requirements for Co and Ni for optimal growth were in agreement with the result that the cells contained a Co containing corrinoid Factor III (0.1 - 0.2 mg 5-hydroxylbenzimidazolylcyanocobamide per g wet cells) and the Ni containing cofactor F_{430}.

Plugge et al (2009) studied the effect of W and Mo on the growth of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* in syntrophic cultures and the pure cultures of both the organisms. They concluded that the effect of W and Mo on the activity of formate dehydrogenase was considerable in both the organisms, whereas hydrogenase activity remained relatively constant. Depletion of tungsten and/or molybdenum, however, did not affect the growth of the pure culture of *S. fumaroxidans* on propionate plus fumarate significantly, although the specific activities of hydrogenase and especially formate dehydrogenase were influenced by the absence of Mo and W.

It should be noted that the metal ratio obtained is affected by the metal available in the substrate medium which in return will shift the metalloenzyme contents. It is therefore necessary to assess how and to what extent the available TE concentration can regulate the well-identified methanogenic and other pathways (Glass and Orphan, 2012; Choong et al., 2016).

2.3.2 Trace element requirement by anaerobic bacteria

Besides methanogenesis, the pathway for homoacetogenesis (Wood–Ljungdahl pathway) has been intensively studied both due to the important role it takes in energy conservation and autotrophic carbon assimilation which also contributes to global carbon cycle (Ragsdale and Pierce, 2008); and due to current trends in industrial biotechnology research related to CO_2 or syngas utilisation for chemical production (Thauer, 2015; Bengelsdorf et al., 2016). The other anaerobic acetogenesis routes, as well as acidogenesis and hydrolysis involved in anaerobic digestion, however, have not been widely studied with respect to their TE requirement apart from in the case of propionate degradation pathways.

Wood-Ljungdahl pathway

As mentioned in section 2.2.1, there are four key metalloproteins/metalloenzymes in this pathway: formate dehydrogenase (FDH), corrinoid- and Fe/S cluster-containing protein (CFeSP), Carbon monoxide dehydrogenase (CODH), and Acetyl-CoA synthase (ACS).

FDH of *Clostridium pasteurianum* was tested for this pathway and the results showed that this enzyme from *C. pasteurianum* is a molybdenum iron-sulphur protein containing 1 mol of molybdenum and 24 mol of non-heme iron and acid-labile sulphur in 1 mol of enzyme (Scherer and Thauer, 1978). A tungsten-containing FDH in *Desulfovibrio gigas* was reported as well, in its active side tungsten was bound to molybdenum atoms and selenoproteins. This tungsten enzyme follows the same catalytic mechanism as molybdenum-containing FDH (Raaijmakers, Macieira et al. 2002, Ragsdale and Pierce 2008, Stock and Rother 2009).

CFeSP was reported to connect the methyl and carbonyl branch in this pathway as a methyl-acceptor, then donate it to acetyl-CoA synthase (Ragsdale and Pierce 2008).

Svetlitchnaia, Svetlitchnyi et al. (2006) isolated the crystal structure of CFeSP from *Carboxydothermus hydrogenoformans*, illustrating its component as 1 mol Co-containing corrinoid cofactor and a single Fe_4S_4 cluster. In addition, CFeSP was assumed to be homologous to another Co-containing enzyme methyltransferase in methanogenesis as they have similar functions (Jablonski, Lu et al. 1993).

Two types of CODH were involved in anaerobic digestion: 1) monofunctional nickel CODH, containing 10 Fe and 1 Ni per monomer, physiologically functions in the direction of CO oxidation; 2) bifunctional CODH/ACS, containing 14 Fe and 3 Ni per monomeric unit (Ragsdale and Pierce 2008). In the Wood-Ljungdahl pathway, association of ACS with CODH forms a bifunctional CODH/ACS, to catalyse CO_2 reduction coupled with acetyl-CoA synthesis (Ragsdale 2007). In nature, acetyl-CoA synthase appears to associate tightly with CODH to form a heterotetrameric complex CODH/ACS. The structure of ACS itself could be described as a binuclear Ni-Ni centre bridged to a [4Fe-4S] (Nicolet, Lemon et al. 2000, Ragsdale 2007).

The trace elements identified as used in these protein/enzymes are Fe, Ni, Co, Se, Mo and W. There are also some studies showing the involvement of Cu and Zn in the Wood-Ljungdahl pathway, although other investigations demonstrated that the presence of Cu and Zn inactivated the enzymes (Doukov et al 2002, Seravalli et al 2003, Zhu 2009).

Propionate degradation pathways

The phylogenetic and functional diversity of syntrophic propionate oxidising bacteria (POB) present in the anaerobic digestion process have been investigated in a number of studies, with *Syntrophobacter* spp., *Pelotomaculum* spp. and *Smithella* spp. recognised as the main syntrophic propionate oxidising bacteria. These three distinct lineages of syntrophic propionate oxidisers could operate simultaneously, although an active POB community structure might be dependent upon propionate concentrations due to their different kinetic characteristics, and affected by the operational conditions (McMahon et al 2004, Worm et al 2009, Müller et al 2010). These bacteria belong to the *Syntrophobacteriales*, an order of the deltaproteobacteria subdivision, and to the family *Peptococcaceae* within the order *Clostridiales*. *Smithella propionica* converts propionate through a dismutated

pathway to acetate and butyrate after which butyrate is oxidised to acetate (de Bok *et al.*, 2001). All other known syntrophic propionate degraders oxidise propionate to acetate plus CO_2. They use the methylmalonyl-CoA pathway which generates per molecule propionate one ATP via substrate level phosphorylation and three electron pairs.

Among the characterised species which are capable of oxidising propionate under obligate anaerobic conditions, thermophilic *P. thermopropionicum* has been intensively studied in recent years. Kosaka et al (2006) analysed the genome of *P. thermopropionicum* and proposed a possible central catabolic propionate-oxidizing pathway (methylmalonyl coenzyme A pathway, MMA), and Acetyl-Coenzyme A synthesis was involved when pyruvate was degraded to acetyl-CoA, formate, and CO_2. Four hydrogenases and two formate dehydrogenases were detected in *P. thermopropionicum* (Kosaka et al 2008), which indicated the involvement of trace element Co, Ni, Fe, Se, Mo/W in the proposed pathway. It is also interesting to note that *P. thermopropionicum* could produce flagellum-like electrically-conductive filaments which participated in the congregation of *P. thermopropionicum* with *Methanothermobacterthermautotrophicus* cells when they were co-cultivated in the presence of propionate (Ishii et al 2005, Ishii et al 2006).

Other propionate degradation pathways were proposed in other studies (Tholozan et al 1988, Tholozan et al 1990, Lens et al 1996, de Bok et al 2001, Worm et al 2011). For example, de Bok et al (2001) investigated the pathway of propionate conversion in a syntrophic co-culture of *Smithella propionica* and *Methanospirillum hungatei* JF1 using 13C-NMR spectroscopy. During their experiment, the co-cultures produced acetate and butyrate from propionate, which did not give any evidence for the functioning of *Methanospirillum hungatei* JF1 in this co-culture. This might indicate that *Smithella propionica* could carry out this metabolic activity without the syntrophic relation with a hydrogenotrophic methanogen. They proposed that propionate was dismutated to acetate and butyrate via a six-carbon intermediate and the pathway required CoA derivatives which were likely coenzyme B_{12}-dependent reactions. This indicates Co, Ni, and Fe, as well as Mo or W, may be involved in this proposed pathway.

Two formate dehydrogenases were isolated from the syntrophic propionate oxidising bacterium *Syntrophobacter fumaroxidans* (de Bok et al 2003). Both enzymes were produced in axenic fumarate-grown cells as well as in cells which were grown syntrophically on propionate with *Methanospirillum hungatei* as the H_2 and formate scavenger. The purified enzymes exhibited extremely high formate-oxidation and CO_2-reduction rates, and low Km values for formate (0.01~0.04mM). Both enzymes contained W and Se, while Mo was not detected. This matches with the genome analysis of *Syntrophobacter fumaroxidans* which indicated that *S. fumaroxidans* could code for a cytoplasmic [NiFe]-hydrogenase, two cytoplasmic [NiFeSe]-hydrogenases, a [NiFe]-hydrogenase maturation protein, and two cytoplasmic formate dehydrogenases (Müller et al 2010).

Some trace element supplementation/deficiency experiments were carried out to distinguish the real trace element requirements for syntrophic propionate oxidisation. Plugge et al (2009) studied the effect of W and Mo on the growth of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* in syntrophic cultures and pure cultures of both organisms. They concluded that the effect of W and Mo on the activity of formate dehydrogenase was considerable in both organisms, whereas hydrogenase activity remained relatively constant. Depletion of tungsten and/or molybdenum, however, did not affect the growth of the pure culture of *S. fumaroxidans* on propionate plus fumarate significantly, although the specific activities of hydrogenase and especially formate dehydrogenase were influenced by the absence of Mo and W. Their results suggest a more prominent role for H_2 as electron carrier in the syntrophic conversion of propionate, when the essential trace metals W and Mo for the functioning of formate dehydrogenase are depleted.

Worm et al (2009) observed a propionate degrader shift from *Syntrophobacter spp.* to *Pelotomaculum spp.* and *Smithella spp.* in a UASB digester fed with synthetic propionate medium without Mo, W and Se. The authors argued that one of the reasons for the species shift was because *Pelotomaculum spp.* and *S. propionica* may need molybdenum for formate dehydrogenase activity whereas *Syntrophobacter spp.* need tungsten for formate dehydrogenase activity and molybdenum even has an antagonistic effect as was described for *S. fumaroxidans*. The medium, however, was deficient in both molybdenum and tungsten which could not support the authors'

explanation, especially as *Syntrophobacter spp.* was the dominant propionate oxidising species at the beginning of the experiment.

Boonyakitsombut et al. (2002) tested the effect of TE on propionate degradation in mesophilic conditions and showed the addition of Fe, Co and Ni (10, 1 and 1 mg L^{-1}, respectively) enhanced the propionate utilisation rate. The addition of Mo individually, however, reduced the propionate degradation rate.

According to Osuna, Zandvoort et al. (2003), the addition of trace elements to UASB reactors significantly stimulated the conversion of propionate in a mixture of VFA (acetate, propionate, butyrate, in a ratio 3:1:1). The effect of individual TE to propionate degradation could not be distinguished in this study, however, because the trace elements listed below were added simultaneously (concentrations are shown in brackets in the units of µg L^{-1}): $FeCl_2 \cdot 4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $MnCl_2 \cdot 4H_2O$ (500), $CuCl_2 \cdot 2H_2O$ (38), $(NH_4)_6Mo_7O_{24} \cdot H_2O$ (50), $CoCl_2 \cdot 6H_2O$ (2000), $NiCl_2 \cdot 6H_2O$ (142), and $Na_2SeO_3 \cdot 5H_2O$ (164).

Trace elements in other pathways for anaerobic bacteria

Studies on this aspect are patchy but Fe, Ni, Co, Se and Mo are still regarded as the main TE identified, with Cu and Mn identified occasionally. For example, Andreesen et al (1973) investigated the effect of trace elements on the fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum* and detected high corrinoid contents after addition of trace elements to the growth medium. Co and Zn are also required for transcarboxylase in *Propionibacterium shermanii* (Northrop and Wood, 1969). Co is necessary for the degradation of some amino acids, e.g. glutamate fermentation by *Clostridium tetanomorphum* (Schonheit et al., 2016). Two other amino-mutases containing Co were also identified from *Clostridium stricklandii* (Gruber et al., 2011). Se is used in glycine reductases found in *Clostridium* species (Stolz et al., 2006; Nancharaiah and Lens, 2015).

Information on the TE requirements of anaerobic bacteria can also be obtained from studies on dark fermentation for biohydrogen production. For example, Azbar et al. (2009) conducted batch experiments to optimise the basal medium for biohydrogen production from dark fermentation using cheese whey wastewater as substrate. The

study revealed that the optimal concentrations to stimulate hydrogen production with concurrent VFA production should be in the range of 1.25-2.5 mg L^{-1} for Co, Ni and Zn, 2.5-5 mg L^{-1} for Mn and 50-100 mg L^{-1} for Fe when chloride salts of TE were used. Although positive effects from trace metals were demonstrated in this study, it must be pointed out that the baseline metal concentrations from the inoculum were not taken into consideration in the study, and no control experiment was conducted. This hindered the quantitative analysis of TE effect on the basis of collective results.

The combined effect of Ca, Fe, Co and Ni was tested for hydrolysis and acidogenesis on particulate organic material using batch reactors, at concentrations of 500, 10, 1 and 1 mg L^{-1} as $CaCl_2$, $FeCl_2$, $CoCl_2$ and $NiCl_2$, respectively. The results showed that their supplementation simulated both COD solubilisation and organic acids production in both mesophilic and thermophilic temperatures (Kim et al., 2003). The supplementation of $FeCl_3$ individually has also been proved to accelerate the hydrolysis and acetogenesis at thermophilic digestion (Yu et al., 2015).

Mesophilic batch fermentation using excess sludge from a sewage treatment plant as substrate was conducted to test the effect of TE for selective butyric acid production. The result showed that all five TE tested had a positive effect and the extent of their individual influence was in the following order: Fe > Mn > Co ≈ Zn > Cu. The stimulated growth of *Clostridium* after TE supplementation was considered as the principal reason for the better butyric acid production performance (Liu et al., 2015).

2.3.3 Bioavailability of trace elements

TE essential for AD can be provided by substrates/co-substrates themselves or by direct supplementation. After entering the digester, the uptake of trace elements from surroundings to the cells by microorganisms is assumed to proceed mainly via the transport of free ions across the cell membrane (Zandvoort, Van Hullebusch et al. 2006). This occurs in two steps: a passive adsorption onto the biomass surface, followed by an energy-dependent transport into the cell. The first step is complicated and discussed in more detail in the following paragraph; a transporter in the cell membrane is usually required for the second step, and its quantity determines the maximum transfer rate.

Before essential trace elements actually reach the surface of microbial biomass present in a biofilm or suspended floc, they are subject to complex (bio)chemical processes such as precipitation as sulphides, carbonates and phosphates and the formation of inorganic and organic complexes with matters in bulk digester liquid or with extracellular polymeric substances (Van der Veen, Fermoso et al. 2007). Reduced bioavailability occurs in these cases, which limits the effective use of trace elements (Smith and Martell 1989). The bioavailability of trace elements needs to be analysed on a case-by-case basis due to a wide variety of substrate type and digester operational practices, and different opinions exist even for a single chemical process. For example, ageing of sulphide precipitants, which takes place during digester operation, was considered to lower the dissolution rates and therefore decrease the metal bioavailability (Gonzalez-Gil, Jansen et al. 2003). Jansen, Gonzalez-Gil et al. (2007), however, proposed that in most cases the dissolution rates of Co and nickel sulphides do not limit the methanogenic activity in anaerobic wastewater treatment.

The function of trace elements in the AD process therefore means only those available to microbial biomass represent useful TE, and the total TE concentration in digester is not a direct indication of this. With limited knowledge on bioavailability, however, the quantification of the optimum trace element concentrations range to maintain an optimised cell functions has to rely on total TE concentrations, especially for digestion systems treating complex solid organic waste streams. Although the total concentration may not present the readily available TE concentration in AD, the findings based on total TE remain reliable (Choong et al., 2016). In addition, the continuous supplementation of trace nutrients as soluble mineral solution makes sure that free metals are available for biomass to uptake at any time (Thanh et al., 2016). This approach was therefore followed for this study without focussing on the TE availability. As the physicochemical conditions of a specific digestion system do not vary much, the determination of total TE required for supplementation should provide sufficient information on other digesters of the same kind. The digester response to total TE addition applied also helps to clarify the mechanisms of TE to each microbial group involved in AD process, which is the first step to gain more insight and knowledge, i.e. the availability of TE, of this research topic.

2.3.4 Trace elements supplementation to FW digestion

TE supplementation to FW digestion has been studied widely in recent years. The main TEs confirmed as essential are Se, Fe, Co, Ni, Mo/W. In FW digestion, hydrogenotrophic methanogenesis was identified to be the principle route to methane formation, due to the inhibition of acetoclastic methanogenic activity at high ammonia concentration. In this case, acetate was oxidised to carbon dioxide and hydrogen via a reverse Wood-Ljungdahl pathway (Angelidaki and Ahring 1993, Schnürer and Nordberg 2008, Mayumi, Mochimaru et al. 2011).

Banks and Zhang (2010) carried out semi-continuous trial in CSTR reactors under mesophilic condition. The aim of this study was to explore the possibility of regulating the metabolic pathway leading to methane production by TE supplementation. The results showed Mo, W, and Ni were present in FW in sufficient quantities for moderate loadings, but may have to be supplemented in digestion at high OLR 5 kg VS m^{-3} d^{-1}. In their subsequent work in 2012, Banks, Zhang et al. (2012) found that supplementation of Se and Co could recover a FW digester suffering from a propionic acid accumulation caused by elevated ammonia concentration. Critical Se and Co concentrations were established as 0.16 and 0.22 mg kg^{-1} FM when digesters were fed at 5 kg VS m^{-3} d^{-1} giving specific methane production and volumetric biogas production 0.75 m^3 kg^{-1} VS and 3.75 m^3 m^{-3} d^{-1}, representing a significant increase in process performance and operational stability. FISH analysis showed the methanogenic groups in digester with TE supplementation were members of the order *methanomicrobiales*, indicating that the dominant metabolic pathway of FW digestion was via syntrophic acetate oxidation and hydrogenotrophic methanogenesis.

Co, Ni, Mo, B, Se and W were tested in (Feng, Karlsson et al. 2010), treating FW in mesophilic batch reactors, High strength of Se/W with low concentration of Co resulted in 7-15% increase in production of methane. The range of Se and W concentrations in the reactors after addition varied from 8 to 800 mg kg^{-1}, and 18 mg kg^{-1} to 1.80 g kg^{-1}, respectively, while Co concentration ranged between 60 mg kg^{-1} and 6 g m^{-3}. This study suggested that the archaea spp. in biogas reactors were more

sensitive to TE concentration when compared with the other members of the microbial community.

Zhang and Jahng (2012) reported the effect of TE supplementation on the long-term anaerobic digestion of FW in a CSTR reactor. Stable anaerobic digestion of FW was achieved for 368 days by supplementing 2.0 mg kg^{-1} Co, 5.0 mg kg^{-1} Mo, 10 mg kg^{-1} Ni, and 100 mg kg^{-1} Fe at OLR of 2.19-6.64 kg VS m^{-3} d^{-1}, a high methane production 352-450 mL CH_4 g^{-1} VS was obtained without significant accumulation of VFA, compared to process failure when digesting FW without TE supplementation.

Qiang, Lang et al. (2012) conducted mesophilic methane digestion of high-solid FW using continuous experiments. The continuous experiments carried out in a CSTR type reactor, which deteriorated due to lack of TE on day 50. In the second run, with a combination of 200 mg kg^{-1} Fe, 6.0 mg kg^{-1} Co, 5.7 mg kg^{-1} Ni, stable FW digestion was achieved, a giving specific biogas production of 0.86 m^{-3} kg^{-1} VS with a methane content of 55%.

Zhang, Wu et al. (2015) investigated the effect of TE (Fe, Co, Ni and Se) to FW digestion in semi-continuously fed digesters at OLR ranging from 1.0-5.5 kg VS m^{-3} d^{-1} at mesophilic condition, with high methane production of 465.4 mL g^{-1} VS was obtained with supplementation of 5.0 mg kg^{-1} Fe, 1.0 mg kg^{-1} Co, 1.0 mg kg^{-1} Ni, 0.2 mg kg^{-1} Se, VFA remained at a rather low level.

Zhang, Zhang et al. (2015) confirmed that the instable long-term anaerobic digestion of FW caused by propionate inhibition could be recovered by the supplementation of 100 mg kg^{-1} Fe, 2.0 mg kg^{-1} Co, 5.0 mg kg^{-1} Mo, 10 mg kg^{-1} Ni, as indicated by the increase CH_4 yield (from 384.1 to 456.5 mL g^{-1} VS), and the decreased propionate concentration (from 899.0 to 10.0 mg L^{-1}), and the increase of pH (from 6.9 to 7.4).

Ariunbaatar, Esposito et al. (2016) carried out BMP test to discuss the potential to enhance the anaerobic digestion of FW by supplementing TE (Fe, Co, Ni, Zn, Mn, Cu, Se and Mo) individually as well as in cocktails. Results showed that supplementation of TE increased the biomethane potential of a FW with low TE concentration background. The most effective elements were Fe with an increase of

39.2% of biomethane, followed by Se (34% increase), Ni (26.4% increase), and Co (23.8% increase).

Recent years a wide range of studies on food waste digestion were treating TE supplementation as a common practice. its supplementation strategy varied in terms of species and strength. However, since food waste digestion was influenced by many factors, it is not recommended to give a specific recipe of trace elements for stabling food waste digestion.

2.4 Conclusion

Although a large amount of research has been carried out on TE, metalloenzymes and the physiological functions of trace elements in anaerobic digestion have not yet been fully explored. Some general conclusions, however, can be drawn: 1) Fe, Co, Se and Mo work on all three groups of microorganisms in the AD system, i.e. acidogens (hydrolytic-fermentative bacteria), acetogens and methanogens. This is because the enzymes or electron carriers containing them work on a wide range of anaerobes, for example: Fe-containing Ferredoxin, Co-containing methyltransferase and Se and Mo-containing formate hydrogenase; 2) Ni-containing metalloenzymes are most commonly used in metabolic pathways involved in methane, carbon monoxide and hydrogen (the gases present in large quantities in early Earth) as reactants or products. There is limited evidence on its function in acidogens; 3) other trace elements seem to have less extended use in the AD system.

Despite the numerous studies on this subject, comparison is not always possible due to differences in the experimental conditions used and parameters given. The type of substrate (biochemical composition, trace element concentration), operating temperature etc affect the quantification of TE requirements (Fermoso et al., 2009; Demirel and Scherer, 2011). With regard to food waste, its composition is influenced by many factors such as location, season, culture and economic conditions. It was found in a comparative study that supplementation with Fe, Ni, Co and Se was beneficial to synthetic food waste prepared in Delft, the Netherlands; however only

Se supplementation was required in Tampa, Florida, USA due to the high background concentration of Fe, Ni and Co in that sample (Ariunbaatar et al., 2016).

Although it is difficult to compare and contrast different experiments, it is clear that there is a growing understanding of trace elements functions in anaerobic digestion, including: 1) a wide range of TE identified as essential for microorganisms in AD, but only a few require supplementation: for example Co and Se were identified for food waste digestion at moderate OLR, i.e. 3 kg VS m^{-3} day^{-1}. This is due to the natural abundance of other TE in substrates and/or their relatively low demand in the AD process; and 2) although in previous research TE dosing is mainly used to stimulate the rapid degradation of VFA and therefore to improve the digestion stability, a few studies have already shown the potential to increase OLR by resolving bottlenecks in the digestion process with TE addition. This is especially beneficial for readily biodegradable food waste and this finding therefore has practical significance for food waste digester productivity.

The advantages of TE supplementation make it a popular prevention and remedy measure for VFA accumulation in industrial AD plants; however the dosing should be minimised due to both environmental and economic issues associated with it. To identify the balance between minimal TE dosing and stable and efficient food waste digestion and to investigate the dynamic response of the anaerobic consortium in AD to Co and Se addition, the critical concentrations of essential trace elements (Se and Co) for food waste digesters operated at moderate/maximum loading were therefor studied in the current research in a programme of laboratory trials. The basic aspects of the trace element requirement, including limitation, stimulation and dosing strategy, were determined in the main experiments by the depletion approach, in which the digester was operated with one element depleted while other elements were still sufficient. This also helped to avoid the confusion between deficiency and toxicity of the element in question.

CHAPTER 3 Methodology

3.1 General

3.1.1 Reagents

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

3.1.2 Water

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

3.1.3 Laboratory practice

All laboratory operations were carried out using good laboratory practice, after first carrying out the appropriate risk assessments and, where necessary, COSHH assessment. All equipment, laboratory apparatus, and analytical instruments were operated in accordance with the manufacturer's instructions. All glassware was washed using detergent followed by rinsing with tap water and DI water. The glassware used for the acid digestion was soaked in a 12~15% nitric acid bath for a 24-hour period after which the glassware was rinsed with Milli-Q water.

3.2 Semi-continuous digestion experiments

3.2.1 Digesters

Digesters used in the study were of a continuously stirred tank reactor (CSTR) design. Different capacity digesters were used, 500-mL, 1-L, 5-L and 100-L, with 400-mL, 500-mL, 4-L and 75-L working capacity, respectively. Semi-continuous operation was achieved by removing digestate through an outlet port in the base plate before adding feed via the inlet in the top plate. All digesters were fed daily and digestate was removed at least once a week but more frequently at high organic loading rate (OLR) to maintain an approximately constant working volume. The quantities and times of feed added, digestate taken and trace element supplemented for each

digester were thoroughly recorded. Digestate TS, VS, VFA, pH, ammonia, and alkalinity were analysed at least once a week and more frequently in some cases, especially when digesters were at transient states.

500-mL and 1-L CSTR digesters

500-mL conic flasks and 1-L PVC bottles with stirrers were used as CSTR digesters for short-term trials. Flask trials were carried out in incubator whereas 1-L CSTR bottles were placed in water bath. Both types of trials were conducted under mesophilic condition with same stirring rate of 37~40 rpm.

5-L CSTR digesters

Digesters were constructed of PVC tube with gas-tight top and bottom plates (Figure 3.1). The top plate was fitted with a gas outlet, a feed port sealed with a rubber bung, and a draught tube liquid seal through which an asymmetric bar stirrer was inserted with a 40-rpm motor mounted directly on the top plate. Temperature was controlled at 37 ± 0.5 °C by circulating water from a thermostatically-controlled bath through a heating coil around the digesters.

Biogas production was recorded using tipping bucket gas counters with continuous data logging. Gas composition and gas counter calibration were carried out weekly by collecting the gas that had passed through the gas counter and measuring its volume. The biogas volume was measured using a weight-type water displacement gasometer (Walker, Zhang et al. 2009).

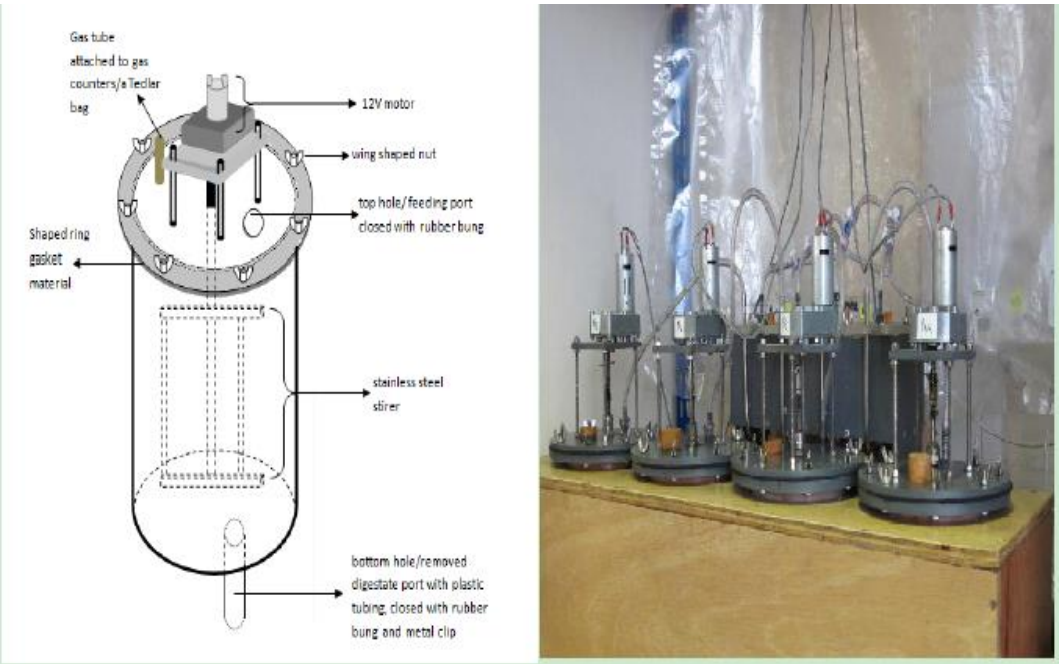
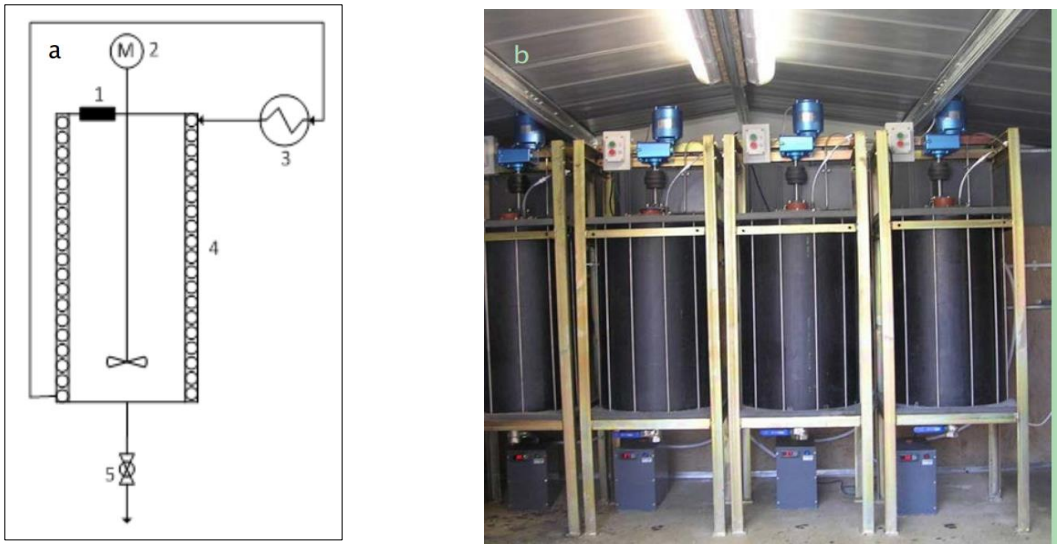


Figure 3.1 Mode and photo of CSTR anaerobic digester (5-L)

100-L CSTR digesters

The CSTR digester had a total volume of 100-L and a working volume 75-L, and was constructed from 40 cm inner diameter PVC pipe sealed at its top and bottom with plates incorporating feed and drainage ports. Digester temperature was controlled at 37 °C by recirculating water from a thermostatic bath through an internal heating coil. The digesters were sealed from the outside atmosphere by a draught tube through which an offset bar stirrer was inserted to allow low speed mixing at 26 rpm by means of geared motors (Parvalux, UK). Biogas production was measured using continuous gas flow meters (Walker, Zhang et al. 2009). The configuration of the system is shown in Figure 3.2.



a) b)

Figure 3.2 100-L CSTR digesters

a) schematic drawing (1. Feedstock inlet; 2. motor to propel the stirrer; 3. heater; 4. heating coil; 5. digestate outlet); and b) four digesters image

3.2.2 Feedstock and Inoculum

Food waste as feedstock

During the course of research, the source-segregated domestic food waste was obtained from 2 sources: the Biocycle digestion plant (Ludlow, South Shropshire, UK) operated by Greenfinch Ltd. and the waste transfer station (Otterbourne, Hampshire, UK) operated by Veolia Environmental Services. On each occasion that feedstock was required, a representative sample of around 200-300 kg of the waste, which was collected in biodegradable plastic bags, was obtained and taken to the laboratory. The food waste was taken out of the bags, and any obvious non-food contamination was removed along with large bones and seeds (Figure 3.3). The samples were then ground (S52/010 waste disposer, IMC Limited, UK) to a homogeneous pulp and frozen at -18 °C for its later use. When needed, the feedstock was thawed and stored at 4 °C and used over a short period.

a) food waste before grinding

b) food waste after grinding used for study

Figure 3.3 Pre-treatment of food waste before anaerobic digestion

Inoculum

There were 3 types of inoculum used during the course of study. In long-term CSTR-type 5-L digesters as discussed in chapter 5 and 6, inoculums were digestate of lab-scale FW digesters operated for over 700 days; the operational history of which were given in detail in Jiang (2013) and characteristics were listed in Table 3.1. Inoculum for 100-L breeder digesters was food waste digestate from a commercial AD plant. The digestate was collected a few months after that AD plant came into operation and no TE supplementation was applied at that time in that plant.

Inoculum for short-term CSTR digesters in Chapter 6 was digestate from 100-L breeder digesters. When used, these digesters were already fed with food waste for a period of more than 500 days. The detailed information on the operation and performance of those breeder digesters can be found in section 4.

Table 3.1 Inoculum characteristics of this study for long-term CSTR 5-L digesters

Digester name used in the current study	Digester name previously used in Jiang (2013)	OLR (kg VS m^{-3} d^{-1})	TE added	VFA (mg L^{-1})	pH	TAN (g kg^{-1})	IA:PA ratio
S1	F1	1.8	NO TE	15000	7.63	5.09	0.78
S2	F9	4.0	Se, Co, W, Ni, Mo	<500	7.64	3.61	0.26
S3	N3	3.0	Se, Co, W	<500	7.82	4.11	0.28
S4	F11	5.5	Se, Co, W, Ni, Mo, Fe, Cu, Zn, Al, Mn, B	<500	7.70	2.84	0.38
S5	F5	5.5	Se, Mo, Co	590	7.77	3.04	0.31
S6	F6	5.5	Se, Mo, Co	550	7.71	2.75	0.40
S7	F7	5.5	Se, Mo, Co, W	<500	7.67	3.00	0.33
S8	F8	5.5	Se, Mo, Co, W	<500	7.61	2.94	0.32

3.2.3 Trace element solution

11 trace elements were tested in this research work but not all of them were added to every digester. Instead, a range of TE mixes was investigated in this research work. As such, each individual trace element stock solution was made using the compound listed in Table 3.2, and kept in a refrigerator when not in use. The dosing strength of each trace element was chosen based on the results of previous study (Banks, Zhang et al. 2012), apart from Co and Se when they were supplemented with a changing strength. In that case, the dosing concentration for Co or Se was increased in a step

wise manner in order to investigate the optimal supplementation strengths for them at a range of OLR.

Table 3.2 Compounds and strengths used for trace elements supplementation

Trace elements	Compound used	Supplementation strength in the working condition (mg kg^{-1})
Cobalt (Co)	$CoCl_2 \cdot 6H_2O$	1 or variable
Selenium (Se)	Na_2SeO_3	0.2 or variable
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.2
Tungsten (W)	$Na_2WO_4 \cdot 2H_2O$	0.2
Nickel (Ni)	$NiCl_2 \cdot 6H_2O$	1
Iron (Fe)	$FeCl_2 \cdot 4H_2O$	5
Aluminium (Al)	$AlCl_3 \cdot 6H_2O$	0.1
Boron (B)	H_3BO_3	0.1
Copper (Cu)	$CuCl_2 \cdot 2H_2O$	0.1
Manganese (Mn)	$MnCl_2 \cdot 6H_2O$	1
Zinc (Zn)	$ZnCl_2$	0.2

3.3 Lab-based analytical methods

3.3.1 pH

pH was measured using a Jenway 3310 pH meter (Bibby Scientific Ltd, UK) with a combination of glass electrode and thermometer, calibrated in buffers at pH 7.0 and 9.2. The pH meter was temperature compensated and had a sensitivity of ±0.01 pH unit and accuracy of 0.01±0.005 pH units. Buffer solution used for calibration was prepared from buffer tablets (Fisher Scientific) which dissolved in 100 ml DI water.

The pH probe was rinsed with DI water before and after measurement, and placed into a mild acid solution to avoid cross contamination. Digestate samples were measured immediately after sampling to prevent changes in pH due to temperature change and the loss of dissolved CO_2.

3.3.2 Total solids (TS) and Volatile solids (VS)

TS and VS were measured according to Standard Method 2540G (APHA 2005). After thorough homogenisation, approximately 10 ~ 20 g of sample was transferred into a weighed dry crucible. Samples were weighed to an accuracy of 0.001 g (Sartorius BP210S balance, Sartorius AG, Gottingen Germany) and placed in an oven (LTE Scientific Ltd., Oldham UK) for drying overnight at 105 ± 1 °C. After drying the samples were transferred to a desiccator to cool for at least 40 minutes. Samples were then weighed again with the same balance, and then transferred to a muffle furnace (Carbolite 201, Carbolite, UK) and heated to 550 ± 10 °C for two hours. After this ashing step, samples were again cooled in a desiccator for at least one hour before weighing a fourth time.

After all analyses, crucibles were washed with detergent, rinsed with DI water, and dried in an oven at least an hour then transferred from the oven to a desiccator for cooling to room temperature and stored there for next analysis. The calculation formula for TS and VS are given as followed.

$$\%TS = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

$$\%VS \text{ (based on total wet weight)} = \frac{W_3 - W_4}{W_2 - W_1} \times 100\%$$

$$\%VS \text{ (based on total solids)} = \frac{W_3 - W_4}{W_3 - W_1} \times 100\%$$

Where W_1 is the weight of empty crucible, g;
W_2 is the weight of crucible and total wet sample, g;
W_3 is the weight of crucible and sample after drying at 105 °C more than 10 hours, g;
W_4 is the weight of crucible and sample after ashing at 550 °C for 2 hours, g.

3.3.3 Alkalinity

Alkalinity was analysed using Standard Method 2320 B (APHA 2005). Digestate was sieved to obtain a homogenous liquor sample and 2-5 g of this was added to 40 g DI water. Titration was done using a Schott Titroline Easy automatic digital titration burette system (Schott, Mainz, Germany), with the samples being magnetically stirred while the titration was carried out. The sample was titrated with 0.25 N H_2SO_4 to endpoints 5.7 then 4.3, allowing calculation of total alkalinity (TA), partial alkalinity (PA), and intermediate alkalinity (IA) (Ripley, Boyle et al. 1986). PA is a measurement of bicarbonate buffering while IA is attributed to the buffering capacity of volatile fatty acids.

The pH probe was calibrated before titration using buffers as described in 3.3.1, and washed with DI water between subsequent samples to avoid cross contamination. All measurements were on a wet weight basis, and the calculation formulas for alkalinity are as follows:

$$TA = \frac{(V_{4.3}+V_{5.7}) \times 0.25 \times 50000 \times a}{m}$$

$$PA = \frac{V_{5.7} \times 0.25 \times 50000 \times a}{m}$$

$$IA = \frac{V_{4.3} \times 0.25 \times 50000 \times a}{m}$$

Where *TA* is total alkalinity, mg $CaCO_3$ kg^{-1};

PA is partial alkalinity or bicarbonate alkalinity, mg $CaCO_3$ kg^{-1};

IA is intermediate alkalinity or volatile fatty acid alkalinity, mg $CaCO_3$ kg^{-1};

$V_{4.3}$ is the volume required to reach the pH value of 4.3, mL;

$V_{5.7}$ is the volume required to reach the pH value of 5.7, mL;

0.25 is normality of H_2SO_4;

a is correction factor for normality of H_2SO_4 solution;

50000 is conversion factor of 50000 mg $CaCO_3$ to 1 equivalent alkalinity;

m is weight of sample, g.

3.3.4 Volatile fatty acids (VFA) by gas chromatography

The method was based on SCA (1979): Determination of volatile fatty acids in sewage sludge. Pre-treatment of digestate samples was as follows: digestate samples were centrifuged at 14000 g (micro-centrifuge, various manufacturers) for 20 minutes and 0.4 mL of each supernatant was transferred by pipette (Finnpipette, Thermo Fisher Scientific, UK) to a vial, in which supernatant was diluted with milli-Q water, where final formic acid was also added to a concentration of 10% of total volume for analysis. If the samples at this point were turbid they were centrifuged again at 14000 g for 10 minutes to obtain a clearer liquid.

A standard solution set containing acetic acid, propionic acid, n-butyric acid, i-butyric acid, n-valeric acid, i-valeric acid, hexanoic acid, and heptanoic acid, at three dilutions to give individual acid concentrations of 50, 250 and 500 mg L^{-1} respectively, was used for calibration. Quantification of VFA was carried out by Shimazdu GC-2010 gas chromatograph (Shimadzu, Milton Keynes, UK) equipped with a capillary column type SGE BP 21 and a flame ionisation detector. The GC oven temperature was programmed to increase from 60 to 210 ℃ in 15 minutes, with a final hold time of 3 minutes, the temperature at the injector and detector were maintained at 200 and 250 ℃. Helium was used as the carrier gas at a flow rate of 190.8 mL min^{-1}. Total VFA concentration was reported as sum of the single compounds (acetic, propionic, n- butyric, i- butyric, n-valeric, i-valeric, hexanoic, and heptanoic acid).

3.3.5 Gas composition by gas chromatography

Biogas composition (CH_4 and CO_2) was measured periodically using a Varian star 3400 CX Gas Chromatograph (Varian, Oxford, UK). The device was fitted with a packed stainless steel SUPELCO 80/100 mesh porapack-Q column (Hayesep C) and used either argon or helium as the carrier gas at a flow of 25 mL min^{-1} with a thermal conductivity detector. Biogas composition was compared with a standard gas containing 35% CO_2 and 65% CH_4 (v/v) (BOC, UK) for calibration. During analysis, 5 mL sample was directly taken from the Tedlar bag (for semi-continuous experiments) or gas collection cylinder (for RBP tests) and was injected into the gas sampling loop of this instrument.

3.3.6 Gas volume

Volume of biogas in Tedlar bags was quantified by a water displacement gasometer (Walker, Zhang et al. 2009). In this device the biogas flowed from Tedlar bag to a water column under vacuum and the water displaced was introduced into a tank on a balance which allowed weight determination of discharged water. The procedure included the recording of initial height of the water column before the gas collected with Tedlar bag was introduced into the column from its headspace, and the weight of water displaced after the Tedlar bag was empty. The ambient temperature (T) and pressure (P) were recorded at the same time. The volume of biogas was calculated using equation below, and reported as the volume under standard temperature and pressure (STP) of 0, 101.325 kPa.

Weight Gasometer Governing Equation (Walker, Zhang et al. 2009)

$$V_{stp} = \frac{T_{stp}A}{T_{atm}P_{stp}} [((P_{atm} - P_{H_2O(T_{atm})} - \rho_{H_2O} g(H - h_1 - \frac{m_{H_2O}}{A\rho_{H_2O}}))(h_1 + \frac{m_{H_2O}}{A\rho_{H_2O}}) - (P_{atm} - P_{H_2O(T_{atm})} - \rho_{H_2O} g(H - h_1))h_1)]$$

Where

V_{stp} is biogas volume at standard temperature and pressure, m^3;

P_{stp} is standard pressure, 101325 Pa;

P_{atm} is ambient pressure, Pa;

T_{stp} is standard temperature, 273.15 K;

T_{atm} is ambient temperature, K;

$P_{H2O(Tatm)}$ is saturated water vapour pressure at temperature T_{atm}, Pa;

H is total height of gasometer, m;

h_1 is distance from the top of gasometer to liquid surface in gasometer, m;

A is cross-sectional area of water column in gasometer, m^2;

m_{H_2O} is mass of water displaced, kg;

p_{H_2O} is density of water, kg m^{-3};

g is gravitational acceleration, m s^{-2}.

For residual biogas potential (RBP) test, biogas produced from reactors was directly collected above a liquid barrier solution consisting of tap water acidified to pH 2 with HCl and containing 270 g L^{-1} of NaCl. The volume of gas in the gasometer was calculated each time a reading of the barrier solution level was taken.

$$V_{stp} = \frac{T_{stp}A}{T_{atm}P_{stp}}((P_{atm} - P_{H_2O(T_{atm})} - \rho_{H_2O}g(h_t - h_c))h_c$$

Where

V_{stp} is biogas volume at standard temperature and pressure, m^3;

P_{stp} is standard pressure, 101325 Pa;

P_{atm} is ambient pressure, Pa;

T_{stp} is standard temperature, 273.15 K;

T_{atm} is ambient temperature, K;

$P_{H2O(Tatm)}$ is saturated water vapour pressure at temperature T_{atm}, Pa;

h_t is total height of gasometer, m;

h_c is distance from the top of gasometer to liquid surface in gasometer, m;

A is cross-sectional area of water column in gasometer, m^2;

p_{H_2O} is density of acidified salt barrier solution, kg m^{-3};

g is gravitational acceleration, m s^{-2}.

The saturated water vapour pressure at temperature T was calculated using equation below:

$$p_{H_2O}(T) = 101324.6 \times 10^{z}$$

and

$$z = -7.90298\left(\frac{373.16}{T} - 1\right) + 5.02808\log_{10}\left(\frac{373.16}{T}\right)$$
$$- 0.00000013816\left(10^{11.34\left(1-\frac{373.16}{T}\right)} - 1\right) + 0.0081328\left(10^{\left(-3.49149\left(\frac{373.16}{T}-1\right)\right)} - 1\right)$$

The common errors associated with quantification of biogas volume from anaerobic digestion experiments were avoided in the above calculation. The calibration of tipping bucket gas counters using Tedlar bags, however, is still subject to some potential interference such as the effects of fluctuating gas flow rate, ambient temperature and pressure. The maximum error caused by those factors should be no more than 3% of the total gas produced (Walker et al., 2009).

3.3.7 Total ammoniacal nitrogen (TAN)

TAN was measured in accordance with the Standard Method 4500-NH_3 B and C (APHA 2005). 1.5-2 g sample, quantified by mass for accuracy using balance (capacity 200g ± 0.1 g), was added into a glass digestion tube with 40 mL DI water. Both blank (40mL DI water) and standard samples (10mL of 1000 mg L^{-1} NH_4Cl with 40 mL DI water) were used for calibration. Several drops of 10 M sodium hydroxide (NaOH) were added to the sample to raise the pH above 9.5 to make sure transformation of ammonium ion to free ammonia. The distillation was performed with a Foss Tecator Kjeltec system 1002 distillation (Foss Tecator A-B, Hoganas, Sweden). Erlenmeyer flask previously filled with 25 mL indicating boric acid solution, containing 20 g L^{-1} H_3BO_3, 10 ml L^{-1} mixed methyl red and methylene blue indicator solution, was used to collect the distillate. This distillate was titrated manually with H_2SO_4 (0.25 N) using a digital automatic titration system (Schott Titroline, Gerhardt UK Ltd) until a lavender colour was achieved, and the volume of H_2SO_4 consumed was recorded. Standards and blanks were distilled in the same way for quality control purpose. The calculation of TAN is as follows:

$$TAN = \frac{(V_{sample} - V_{blank}) * 14 * N * a}{m}$$

Where

TAN is concentration of total ammoniacal nitrogen, g N kg^{-1};

V_{sample} is volume of 0.25N H_2SO_4 used to titrate the sample, mL;

V_{blank} is volume of 0.25 N H_2SO_4 used to titrate the blank, mL;

N is normalities of H_2SO_4 solution;

a is standardisation factor of H_2SO_4 solution;

m is mass of the sample, g.

To obtain the free ammonia nitrogen (FAN) concentration, the equation from (Østergaard 1985) was used to calculate the FAN as shown in following equation

$$\frac{FAN}{TAN} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)$$

Where FAN is concentration of free ammonia nitrogen, g N kg^{-1};

TAN is concentration of total ammonia nitrogen, g N kg^{-1};

pH is pH of the samples;

T is absolute temperature of the samples, K.

3.3.8 Total Kjeldahl Nitrogen (TKN)

TKN analysis was carried out in duplicate in parallel with blanks and standard as follows: 1-3 g (weighed to ± 1 mg) of samples was places in a glass digestion tube. Two Kjeltab Cu 3.5 catalyst tablets (Foss Analytical) were added to improve acid digestion by lowering the activation energy of the reaction. 12 mL of low- nitrogen concentrated sulphuric acid (H_2SO_4) was added carefully to each digestion tube and then agitated gently to ensure entire mixing of sample and the acid. All digestion tubes were placed in heating block with exhaust system (Foss Tecator 1007 Digestion System 6, Foss Analytical, Hoganas Sweden) for approximately 2 hours at 420 ± 5 °C until the solution colour became clear blue-green. Once the reaction was completed, the tubes were cooled to around 50 °C, and then 40 mL DI water was gently poured to digestion tubes to prevent later crystallization on further cooling. Each sample was distilled using the same procedure of TAN distillation except larger amount of 10 M NaOH was added to raise the pH to above 9.5. The calculation formula for the concentration of nitrogen is the same as stated in 3.3.7.

3.3.9 Acid digestion for metals extraction

Fresh substrate and digestate samples for trace element determination were pre-treated in house in accordance with EPA method 3010 A (Analysts and Great Britain 1987) before sending out to commercial laboratory for analysis. The pre-treatment of samples by acid digestion was carried out using a small heating block (Gerhardt Kjeldatherm) in duplicate in parallel with blanks and standard. For each digestion tube, a known quantity (~10 g) of fresh sample/ DI water / standard trace elements solution was introduced into it first, and then15 mL of 35~36% w/v HCl and 5 mL 70% w/v HNO_3 were added into the tube sequentially and mixed gently. Tubes were placed into heating block, connected to the condenser system, and then digested at room temperature for 48 hours prior to heating. The key step of acid digestion involved gradually increasing the temperature first to 100 °C and then to the final temperature of ~ 200 °C for about 2 hours 10 mins. After cooling, each acid digestate was filtered using Whatman No.1 filter paper into a 50 mL volumetric flask. The

digestion tube and remaining residues on filter paper was raised with warm 12.5% HNO_3 which was also filtered into the same 50 mL flask. The volume was then made up to 50 mL with 12.5% HNO_3 when the content cooled down. The filtrate was then transferred into a PET bottle and sent out for analysis. All glassware and containers used in this experiment were acid washed in advance, and the reagents used were trace grade.

The concentrations of trace element in the resulting solutions were determined using ICP–MS at a UKAS accredited commercial laboratory (ALS Environmental Ltd, Coventry, UK). Spiked samples were prepared to identify possible matrix interferences for quality control purpose. The recovery calculated was within $\pm$20% for each metal indicating the metal determination method used was accurate.

3.3.10 Residual Biogas Production (RBP) test

The test was carried out in triplicate with 3 positive controls using cellulose as a standard material and 3 inoculum-only controls (Walker M. 2010). Anaerobic digester sludge from Millbrook Wastewater Treatment Works, Southampton was used as the inoculum for all reactors. The tests were conducted in static batch reactors with a total volume of 400 mL at a temperature of 35± 0.2 °C, maintained by a thermostatic water bath (Figure 3.4). The inoculum-to-substrate (I/S) ratio used was 4:1 on a VS basis and the test was run over a period of 28 days. No nutrient supplements were added, as the inoculum used was known to be sufficiently rich in the required nutrients. Biogas generated was collected in Perspex cylinders filled with a 75% saturated solution of sodium chloride acidified to pH 2 using hydrochloric acid to reduce carbon dioxide solubility. The height of the solution in the collection cylinder was recorded manually for a certain interval on a daily basis. Vapour pressure and salt solution density were taken into account in correction of gas volumes to a standard temperature and pressure (STP) of 0°C, 101.325 kPa (Walker, Zhang et al. 2009). Samples for gas composition analysis were taken from the cylinders each time when they were refilled, at intervals of no more than 7 days to avoid the risk of overfilling or losses of methane. The bottles were shaken each day before the gas level measurement was taken to provide mixing.

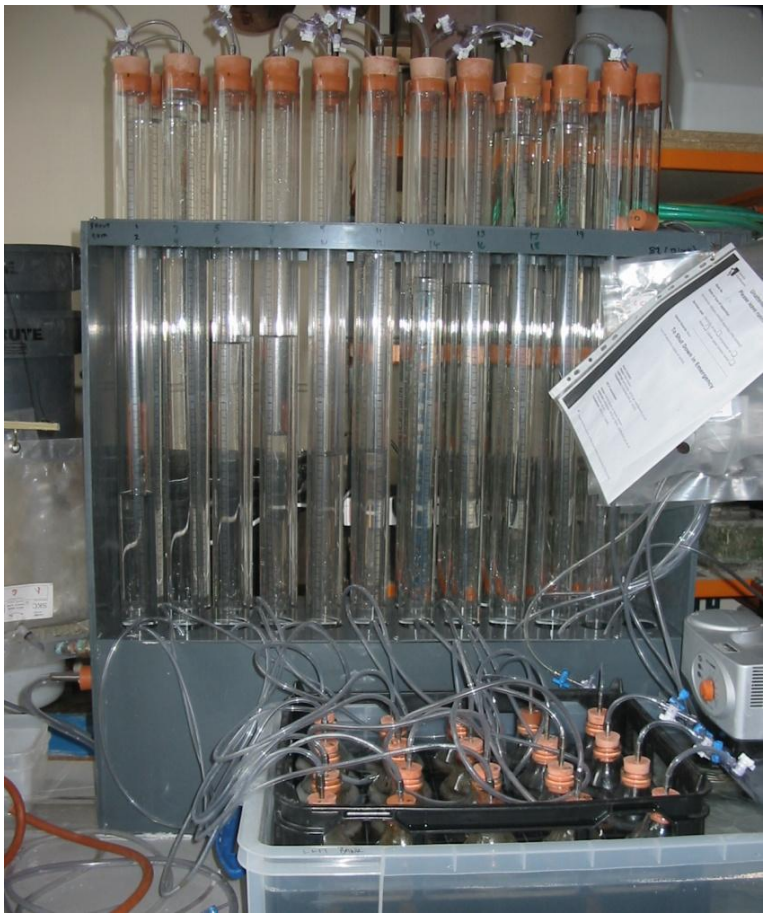


Figure 3.4 RBP reactors and test rig

3.4 Data analysis using mass balance approach

3.4.1 Volatile Solids Destruction (VSD) rate calculation

VSD, the ratio of VS removed to VS added, indicates the efficiency of volatile solids degradation in anaerobic digestion. It was calculated on a daily basis by mass balance in this study. The VS removed every day equalled the wet weight of digestate removed per day multiplying VS of digestate, and the VS added every day was calculated by multiplying wet weight of food waste added per day with VS of food waste. The wet weight of digestate removed was equal to that of food waste added per day minus the weight of biogas produced per day. The weight of daily biogas produced was estimated from the weekly average volume and gas composition in terms of total weight of methane and CO_2 produced. Water vapour

and other gases were ignored in this calculation, and therefore the results obtained might be slightly lower than the real values.

$$VSD = \frac{VS_{FW} \times W_{FW} - VS_{digestate} \times W_{digestate}}{VS_{FW} \times W_{FW}} \times 100\% \quad \text{Eq.3.1}$$

The digestate removal rate ($W_{digestate}$, g d^{-1}) from the digester can be calculated using the following equation:

$$W_{digestate} = W_{FW} - \frac{16 \times C_{methane} \times V_{biogas} + 44 \times (100\% - C_{methane}) \times V_{biogas}}{22.4} \quad \text{Eq.3.2}$$

Where VS_{FW} is the VS content of food waste, %;

$VS_{digestate}$ is the VS content of digestate, %;

W_{FW} is the weight of food waste added into digester every day; kg;

$W_{digestate}$ is the weight of digestate removed from digester every day, kg;

$C_{methane}$ is the methane composition of biogas, %;

V_{biogas} is the volume of daily biogas production, STP m^3.

3.4.2 Trace elements concentration during washing out

Concentration of trace element during washing out was modelled based on the following approximations: 1) the flow pattern of the digester followed that of CSTR model; 2) the density of macerated food waste was 1 kg L^{-1}; and 3) the hydraulic retention time equalled the working volume of digester divided by the volume of food waste added each day, which was calculated from the weight input. According to mass balance, the amount of trace elements in digester on day *n+1* was equal to the amount of trace element in digester in previous day *(day n)* minus that discharged with digestate on the day, then plus that introduced from daily food waste input on day *n+1*.

$$C_{n+1} = \frac{C_n \times (W_{digester} - W_{FW}) + C_{FW} \times W_{FW}}{W_{digester}} \quad \text{Eq.3.3}$$

Where C_n is TE concentration in digester on day n, mg kg^{-1};

n is the operational day n, start from day 0;

C_{FW} is TE concentration in food waste, mg kg^{-1};

W_{FW} is the weight of food waste added into digester every day; kg;

$W_{digester}$ is the total weight of digestate in digester, kg; assuming the density of digestate was 1 kg L^{-1}, its default value is 4 kg for 4-L working volume digesters, kg;

The initial TE concentration in digestate (C_0) was calculated according to the concentration of trace elements in food waste and that supplemented using stock trace element solution. The information of TKN of digestate and TKN of food waste previously used (before day 0 of the experiment in question) were also obtained in order to calculate to what extent the volume of feedstock was reduced due to digestion (Banks, Zhang et al. 2012). The equation for calculation is as follows.

$$C_0 = C'_{FW} \times \frac{TKN'_{digestate}}{TKN'_{FW}} + C'_{spiked} \quad \text{Eq.3.4}$$

Where C'_{FW} is the TE concentration of food waste used before day 0, mg kg^{-1};

$TKN'_{digestate}/TKN'_{FW}$ is the concentration factor, equal to the ratio of TKN in digester before day 0 to TKN in food waste used before day 0;

C'_{spiked} is the spiked TE concentration in digester before day 0, mg kg^{-1}, e.g. C_{spiked} of Co is 1 mg kg^{-1} (Banks, Zhang et al. 2012).

3.4.3 Microbial biomass concentration in digesters

The concentration of microbial biomass at different organic loading was estimated using equations given in Table 3.3. The calculation was based on the following two assumptions: 1) the degradation rate of nitrogen containing organic materials was equal to the overall food waste destruction rate; and 2) empirical microorganism formula was $C_5H_7O_2N$, and therefore nitrogen content of microbial biomass was 12.4% of VS of biomass. Any deviations from these assumptions, e.g. different degradation rates applying to different biochemical components, could cause errors in the calculation. This approach therefore was only regarded as a first attempt to investigate the link between microbial biomass concentration and other digester parameters.

Table 3.3 Equations of nitrogen mass balance

$N_{BFN} = N_{digester} - N_{TAN} - N_{residual\text{-}FW}$	Eq.3.5
$N_{digester} = TKN_{digestate} * W_{digester}$	Eq.3.6
$N_{TAN} = TAN_{digestate} * W_{digester}$	Eq.3.7
$N_{residual\text{-}FW} = N_{FW} * (1\text{-}VSD) *$ concentrated factor	Eq.3.8
$N_{FW} = TKN_{FW} * W_{FW}$	Eq.3.9
Concentrated factor $= TKN_{digestate}/TKN_{FW}$	Eq.3.10
$C_{biomass} = N_{BFN}/12.4\%$	Eq.3.11

Where N_{BFN} is the nitrogen content fixed by microbial biomass in digester, g;

$N_{digester}$ is the total nitrogen content in digester, g;

N_{TAN} is the total ammoniacal nitrogen content in digester, g;

$N_{residual\text{-}FW}$ is the organic nitrogen content in food waste residues in digestate, g;

N_{FW} is the total nitrogen content in daily feeding food waste, g;

$W_{digester}$ is the total weight of digestate in digester, kg; assuming the density of digestate was 1 kg L^{-1}, its default value is 4 kg for 4-L working volume digesters, kg;

W_{FW} is the weight of food waste added into digester every day, kg;

VSD is volatile solids destruction rate, %;

TKN_{FW} is the TKN concentration in food waste, g kg^{-1};

$TKN_{digestate}$ is the TKN concentration in digestate, g kg^{-1};

$C_{biomass}$ is Concentration of microorganisms in digester, g kg^{-1}.

CHAPTER 4 Food waste characteristics and its digestion at low OLR without TE supplementation

4.1 Introduction

The aims of this part of the experiment were to assess whether source-segregated domestic food waste collected over a period of several years showed consistent characteristics, and to confirm that VFA accumulation was a recurring problem in food waste digester without TE supplementation, even at low OLR. Key substrate characteristics for anaerobic digestion, TS, VS, TKN and several essential trace elements (especially Co and Se) were used for comparison of different batches collected and with previous reported food waste stream. The information obtained from this should help to verify whether the results and conclusions of this research have broad application for food waste produced in the same manner. In order to confirm that VFA accumulation occurred without TE addition even at rather low OLR, a long-term digestion trial was carried out in four 100-L CSTR digesters for nearly 600 days. In addition, the data obtained at this part of study, e.g. TKN and TE concentrations of food waste, were used to interpret the results of all digestion experiments, and the digestate produced from 100-L digesters also provided low-TE inoculum for short-term experiments, as described in Chapter 5-8.

4.2 Characteristics of food waste

Table 4.1 showed TS, VS, TKN and TE concentrations of 9 batches of food waste collected over a period of 3 years during this study. The values presented on solids and TKN were averages of replicate measurements and all data were reported on a fresh matter (FM) basis.

The solids contents were fairly consistent with less than 8% difference between different batches, and average values of 235 and 219 g kg^{-1} FM for TS and VS, respectively. No strong evidence was detected of the impact of seasonal variation on solids contents, and the ratio of VS/TS remained rather constant with an average of 92%. These solids contents were in the same range of typical food waste data reported in other studies (Zhang, El-Mashad et al. 2007, Banks and Zhang 2010, Banks, Zhang et al. 2012).

TKN was also analysed as it has a direct effect on ammonia concentration in digesters, and this is of great importance with regard to the microbial population and operation stability of digesters. The average value of TKN was 7.1±0.7 g kg^{-1} FM, which was approximately 10% lower than previous reports in Table 2.1. This indicated that materials rich in protein might have been diverted out of the food waste streams during the period of the study. The TKN results also showed some degree of variation, with Batch 1 and 4 having lower nitrogen content than others. This was because fruit and vegetable were present to a greater extent in those batches. This small variation was taken into consideration when the nitrogen mass balance was conducted for experiments on OLR, as discussed in Chapter 7.

Regarding TE concentration, the results indicated that food waste collected had relatively consistent low concentrations of Co and Se, moderate concentration of Mo, high concentration of Fe and varying concentrations of Ni; this reflected the results obtained from previous studies (Banks and Zhang 2010, Banks, Zhang et al. 2012). As this research mainly focused on Co and Se, their fairly low concentrations provided a steady baseline for testing addition strategies over a wide range of supplementation strengths.

Table 4.1 Characteristics of food waste used over the trial period (results are on a fresh matter basis apart from VS/TS)

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9
Days in use	0-160	160-293	293-349	349-497	497-580	580-700	700-776	776-877	878-1068
TS(g kg^{-1})	211.8	238.2	233.3	241.7	230.4	241.6	239.1	237.2	249.7
VS(g kg^{-1})	187.9	224.8	218.7	226.7	201	230.8	222.2	211.9	232.2
VS/TS (%)	88.8	94.4	93.7	93.8	90.3	95.5	92.9	89.3	93.0
TKN (g kg^{-1})	6.30	7.57	7.82	6.35	6.18	7.20	7.58	-[a]	-[a]
Trace elements									
Co (mg kg^{-1})	0.013	0.052	0.007	0.018	-[a]	0.013	0.022	0.024	
Se (mg kg^{-1})	0.050	0.059	0.063	0.019	-[a]	0.018	0.036	0.042	
Ni (mg kg^{-1})	0.125	0.330	0.105	0.017	-[a]	0.055	0.100	0.167	
Mo (mg kg^{-1})	0.129	0.197	0.178	0.120	-[a]	0.106	0.190	0.221	
Fe (mg kg^{-1})	36.3	100	16.6	-[a]	-[a]	-[a]	-[a]	-[a]	

-[a] not available

4.3 Performance of food waste digester at low OLR without TE addition

Food waste digestion was carried out in four 100-L CSTR digesters for nearly 600 days to observe whether VFA accumulation recurred at low OLR without TE addition. This experiment repeated previous studies to validate the importance of TE supplementation in FW digestion. The other objective of this experiment was to produce FW digestate with low TE content for experiments described in Chapter 6. For the purpose of TE addition experiments, an inoculum with low TE concentration was desired in order to exclude the effect of background TE from inoculum.

As shown in Figure 4.1, the OLR of all digesters was set at 2 kg VS m^{-3} d^{-1} at the beginning of the experiment, and to 3 kg VS m^{-3} d^{-1} after the initial acclimation period in order to wash out the remaining TE introduced with inoculum at a faster rate. The following OLR adjustments were based on VFA concentration in digester, for example feeding stopped in all digesters for 10 days to release stress from high VFA concentration between day 153 and day 163. BR1, BR2 ceased after 203 days' operation, whereas BR3 and BR4 continued operation until end of experiment with final OLR 2 kg VS m^{-3} d^{-1} and 1 kg VS m^{-3} d^{-1}, respectively.

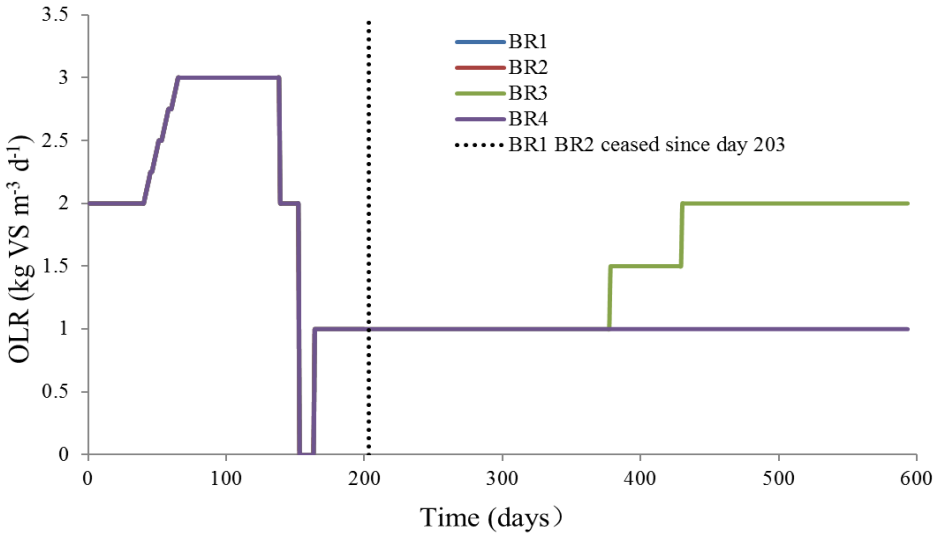


Figure 4.1 OLR of BR1-BR4

Stability indicator - VFA

As BR1 and BR2 ceased on day 203 and only BR3 and BR4 were used for the following experiments, only VFA profiles of BR3 and BR4 were presented in Figure 4.2. VFA profiles of BR3 and BR4 followed a similar pattern when they operated at the same OLR: VFA were accumulated after digesters were operated at 3 kg VS m^{-3} d^{-1} for about 1 HRT. Temporary feeding cease and following lower OLR with 1 kg VS m^{-3} d^{-1} were introduced when VFA in both digesters increased above 2000 mg L^{-1} to release VFA stress to digesters. However, these operations could only prevent VFA increase for a short period, and VFA increased up to 4000 and 6000 mg L^{-1} in BR3 and BR4 respectively. Acetic acid accounted for most of VFA accumulated, whereas propionic acid existed in small amount less than 2000 mg L^{-1}. VFA in BR3 further built up when its OLR increased to 2 kg VS m^{-3} d^{-1} after day 367. At the final stage of experiment, total VFA of BR3 and BR4 were around 3000 mg L^{-1} and 1000 mg L^{-1}, respectively.

The results of this experiment confirmed that in digester with food waste as single substrate, VFA accumulation is a common observation after long-term operation or operational condition change, e.g. OLR increase (Climenhaga and Banks 2008, Banks and Zhang 2010). This indicated single-substrate food waste digestion was prone to VFA accumulation even at low OLR.

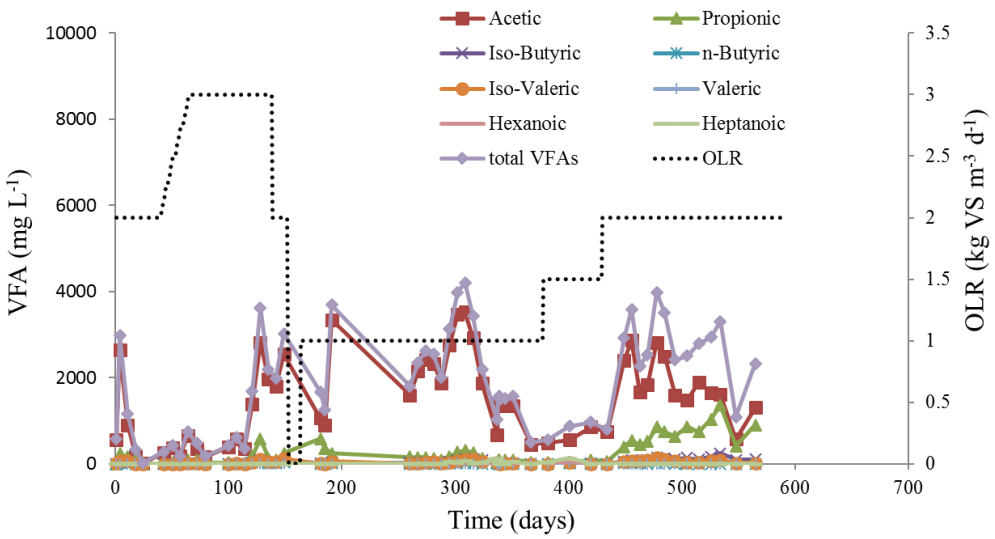


Figure 4.2 a) VFA profiles of BR3

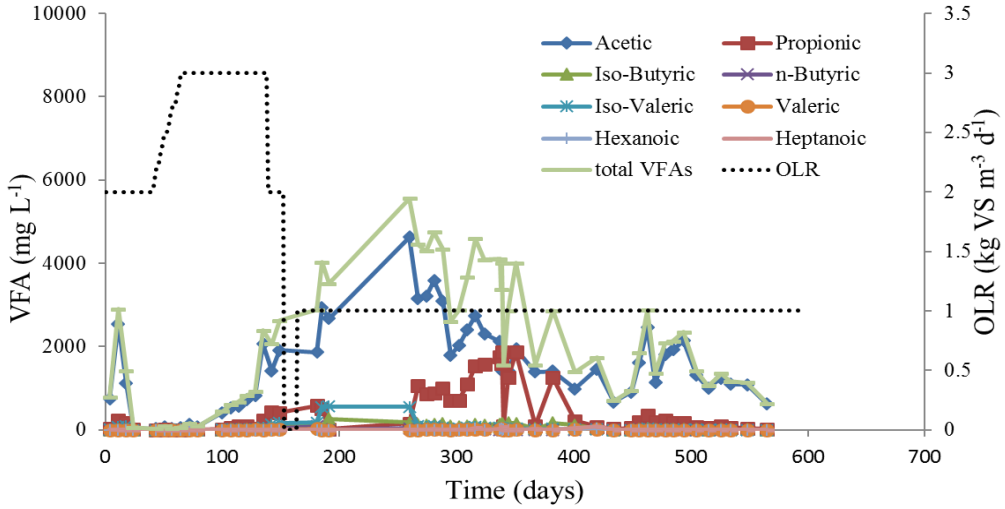


Figure 4.2 b) VFA profiles of BR4

Figure 4.2 VFA profiles of BR3 and BR4

General parameters

General parameters with reference to stability over the whole experimental period were shown in Figure 4.3. pH was stable with values of 7.80±0.02 in 4 digesters and insensitive to OLR increase or VFA production due to the buffering capacity of TAN.

IA:PA ratio increased up to 0.6~0.8 around day 160 to response VFA accumulation at that time. Since OLR decreased to 1 kg VS m^{-3} d^{-1}, IA:PA ratio also reduced and then fluctuated at 0.30~0.40. TAN concentration was 3.85 g kg^{-1} FM at the beginning of experiment, and increased gradually to reflect the TKN concentration in FW. The final TAN contents were 5.45 g kg^{-1} FM in BR3 at OLR 2 kg VS m^{-3} d^{-1}, and 6.16 g kg^{-1} FM in BR4 at 1 kg VS m^{-3} d^{-1}, respectively. The observation that TAN concentration had higher level in lower loading digester was investigated in detail in this research and more finding is discussed in the Chapter 7.

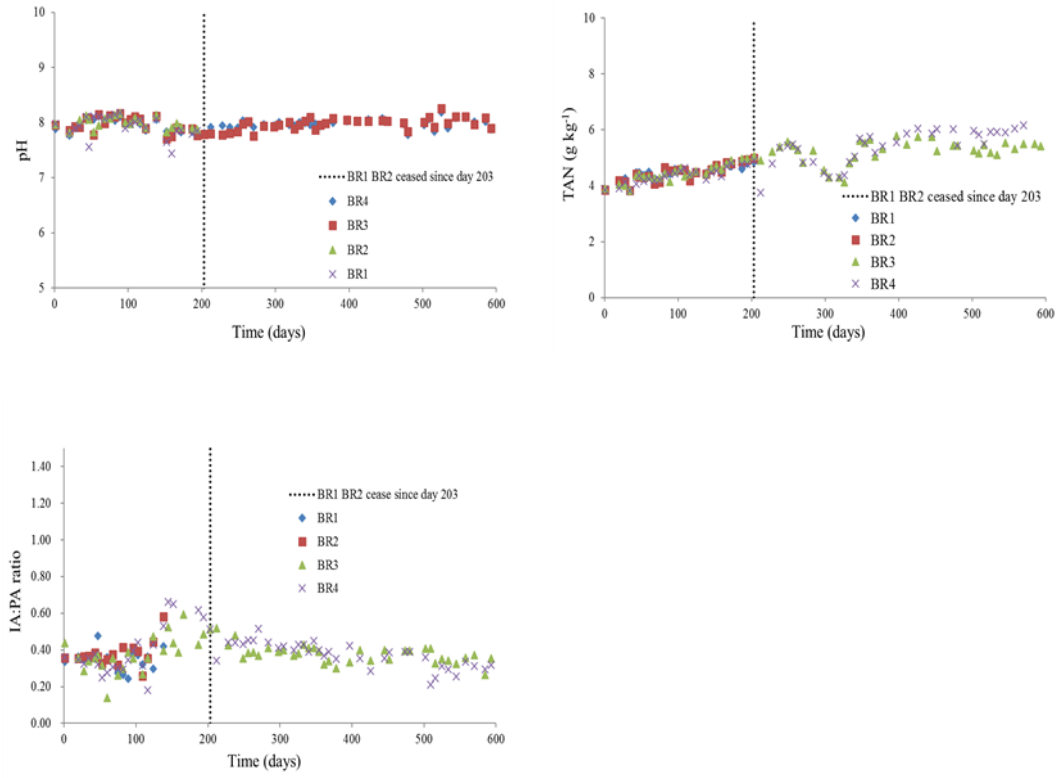


Figure 4.3 pH, TAN, IA:PA ratio of BR3-BR4

Trace elements concentrations of digestate

As the digestate from BR3 and BR4 was considered to be used as TE deficient inoculum for further study, as explained in Chapter 6, TE concentrations in digestate were analysed using method described in section 3.3.9. Table 4.2 presented TE concentrations in digester after BR3 and BR4 were operated for 180 days (3 HRTs), and the TE concentrations of BR3 and BR4 were comparable and in good agreement

with TE concentrations in FW (Table 4.1). This indicated that digestate from BR3 and BR4 could be used as TE-deficient inoculum from acclimated food waste digesters, and therefore the other objective of this experiment was achieved.

Table 4.2 Essential trace element concentrations in BR3 and BR4 after 180 days of operation

TE Concentration (mg kg^{-1} FM)	Co	Se	Mo	Ni
BR3	0.070	0.024	0.290	0.230
BR4	0.063	0.024	0.260	0.140

4.4 Conclusion

The food waste characteristics examined in this part of the study had fairly constant values, avoiding to a great extent the impact of their variability on long-term anaerobic digestion trial. The characterisation results also showed good agreement with previous studies regarding high VS/TS ratio (~ 90%) and TKN content (~7 g kg^{-1} FM) and low concentrations of certain TE (e.g. ~0.01 mg Co kg^{-1} FM and ~0.06 mg kg^{-1} FM Se).

Food waste digestion alone could not overcome the instability issue caused by VFA accumulation, even at low OLR of less than 3 kg VS m^{-3} d^{-1}. Once VFA accumulated, it was difficult to eliminate even by reducing the OLR or applying a temporary cessation of loading. According to the literature review in Chapter 2, it appears that TE deficiency is a direct cause of VFA accumulation in food waste digestion over extended operating periods. Based on these results, optimisation of TE supplementation to food waste digestion was studied in this research, as described in the following chapters.

CHAPTER 5 Effect of Co and Se on stabilisation of food waste digestion at low and moderate OLR

5.1 Introduction

This work continued previous research on the effect of TE supplementation on food waste digestion (Jiang 2013). Although the significance of beneficial effect of TE on FW digestion was identified, that study left some research questions for further clarification. An important one was on the role of Se and Co in FW digestion at moderate OLR. According to a diluting-out experiment Co and Se were highlighted as the essential TE for food waste digestion but they were lacking in food waste when operated at OLR of 3 kg VS m^{-3} day^{-1}. It was still not clear, however, if both or one of them was lower than the minimum requirement and induced VFA production. In addition, in that research no long-term digestion trial was carried out to confirm whether FW digesters could be operated with only Se and Co supplemented. For these reasons, this set of experiment was conducted. The digesters described in Jiang (2013) were directly used for this work without any disturbance (e.g. OLR or TE change), and some important operational and environmental parameters of those digesters were measured when the current work started, as shown in Table 3.1. The aim of this experiment was to investigate the role of Co and Se in stabilizing FW digestion at low and moderate OLR, and its design took into consideration the history of each digester. The general approach of this study was to supplement a single TE first in sufficient amount in order to identify the minimum requirement for the other TE: Co was used as the first TE for S1, and Se was used as the first element dosed to S2 and S3. A wide range of parameters were measured during this study, but VFA was monitored intensively as it is the most important and prompt one to indicate the stability of digesters and therefore the effect of TE addition.

5.2 Methodology

Three 5-L digesters (S1, S2, S3) with 4 L of working volume were used in this experiment and the detailed experimental design was shown in Table 3.1. Briefly, all digesters were still run according to previous plan (Jiang 2013) from the start point of this experiment (day 0) to establish a baseline.

At day 103, digester S1, with 12000 mg L^{-1} of VFA and no TE addition, was supplemented with Co at 1 mg kg^{-1} to test if accumulated VFA could disappear by single TE supplementation. Without success, a second element, Se was added later in a step wise dosing manner until its concentration reached 0.2 mg kg^{-1} to identify the function of Se in VFA consumption process.

Digester S2, which had been previously dosed with Co, Se, Ni, Mo and W, also changed operation plan from day 103: all TE supplements were removed with the exception of Se (dosing strength 0.2 mg kg^{-1}). The OLR of S2 increased from 4 to 5 kg VS m^{-3} d^{-1} from day 391 as no VFA accumulated after ceasing the supplementation of Co, Ni, Mo and W.

Digester S3 followed a same design as S2: it had been previously supplemented with Co, Se, W before day 103. The supplementation of Co and W was stopped after that, but Se was continuously applied at a concentration of 0.2 mg kg^{-1}. Again, with no apparent VFA accumulation for around 300 days, OLR of S3 was increased from 3 to 4 kg VS m^{-3} d^{-1}. S2 and S3 were then continuously monitored to investigate if VFA accumulation resulted due to OLR increase and Co diluting out. Where VFA accumulation occurred Co was again supplemented with step wise increase in concentration in an attempt to determine the optimal dosing concentration by testing the spontaneous effect of its dosing on VFA concentration.

Intermittent feeding (feeding at every other day) had to be adopted in digester recover period for S2 and S3. The dosing strengths of trace elements in this trial followed the recipe shown in Table 3.2, apart from Co and Se when their strengths were changing over time.

Table 5.1 The operational scheme of the digestion trial for S1, S2 and S3

Digester	Digester Initial OLR (kg VS m^{-3} d^{-1})	Objective	TE addition &OLR increase
S1	1.8	To test the requirement of FW digestion on Se at a loading of 2.5 kg VS m^{-3} d^{-1}, when Co was present in sufficient quantities	No TE addition at the start of the trial when OLR was 1.8 kg VS m^{-3} d^{-1}, but VFA concentration was around 12000 mg L^{-1} at this stage. Co was added and maintained from day 103 onwards, and OLR increased to 2.5 kg VS m^{-3} d^{-1} from day 179 after VFA decreased to a great extent. Se was added in a step wise manner from day 393 to test if this addition could further lower VFA concentration.
S2	4.0	To test the role of Co on FW digestion at loadings of 4-5 kg VS m^{-3} d^{-1}, when Se was present in sufficient quantities	Se, Co, W, Ni was added at the start of the trial when OLR was 4.0 kg VS m^{-3} d^{-1}, with less than 500 mg L^{-1} VFA at this stage. Se was added and maintained from day 103 onwards, and OLR increased to 5 kg VS m^{-3} d^{-1} from day 391 after VFA kept at a low level for more than 5 HRTs. Co was added in a step wise manner from day 427 to test if this addition could lower VFA concentration.
S3	3.0	To test the role of Co on FW digestion at loadings of 4-5 kg VS m^{-3} d^{-1}, when Se was present in sufficient quantities	Se, Co, W, was added at the start of the trial when OLR was 3.0 kg VS m^{-3} d^{-1}, with less than 500 mg L^{-1} VFA at this stage. Se was added and maintained from day 103 onwards, and OLR increased to 4 kg VS m^{-3} d^{-1} from day 391 after VFA kept at a low level for more than 5 HRTs Co was added in a step wise manner from day 546 to test if this addition could lower VFA concentration.

5.3 Results and discussion

5.3.1 Se requirement at low OLR when Co was not limiting

Stability indicator – VFA

As shown in Figure 5.1, before any TE added into S1, total VFA was above 12000 mg L^{-1}, in which propionic acid accounted for around 5000 mg L^{-1}, and acetic acid fluctuated between 3000~7000 mg L^{-1}. After 1.0 mg kg^{-1} Co supplemented from day 103 onwards, VFA concentration was decreased rapidly: from 12000 mg L^{-1} to 1000 mg L^{-1} in 40 days with acetic acid becoming the dominant VFA group. The concentration of acetic acid was continuously fluctuating around 1500 mg L^{-1} for a long period, and showed no obvious response to first Se addition with 0.05 mg kg^{-1} added on day 392, where acid peak reached 3500 mg L^{-1}. But it decreased to below 500 mg L^{-1} at the last stage of this experiment when 0.2 mg kg^{-1} of Se supplementation was adopted.

Propionic acid was mostly degraded after Co introduced until loading reached 2.5 kg VS m^{-3} d^{-1} for a period of 80 days. Afterwards it reappeared twice, both were respectively reduced by 0.05 and 0.1 mg kg^{-1} Se supplementation. Propionic acid concentration reduced to a very low level after a Se dosing of 0.2 mg kg^{-1} introduced since day 742, and total VFA concentration was less than 1000 mg L^{-1} onwards. This indicated that Se supplementation stimulated propionic acid degradation, but insufficient strength could not ensure its complete and timely degradation until a sufficient strength reached. The rest of other VFA species existed at very low levels throughout the experiment.

It is noteworthy that in the period that after the first Co addition from day 103 until OLR increased to 2.5 kg VS m^{-3} d^{-1}, acetic acid in digester reduced from 3000 to 1200 mg L^{-1} whereas propionic acid degraded from 5100 to 140 mg L^{-1}. This indicated that Co worked on both propionic and acetic acid degradation; but without sufficient Se the acetic acid accumulation still remained. Considering Se only works on the syntrophic acetic acid oxidisation and hydrogenotrophic methanogenesis pathway, this pathway should be the dominant pathway in this digester for methane production (Kryukov and Gladyshev 2004, Stock and Rother 2009).

The results from S1 showed that Co alone was unlikely to maintain stable food waste digestion even at low OLR. As another low concentration element present in food waste (Table 4.1), Se was still critical and a strength of 0.2 mg kg^{-1} was required for a stable process. This was in good agreement with the recommended concentration in Banks, Zhang et al. (2012).

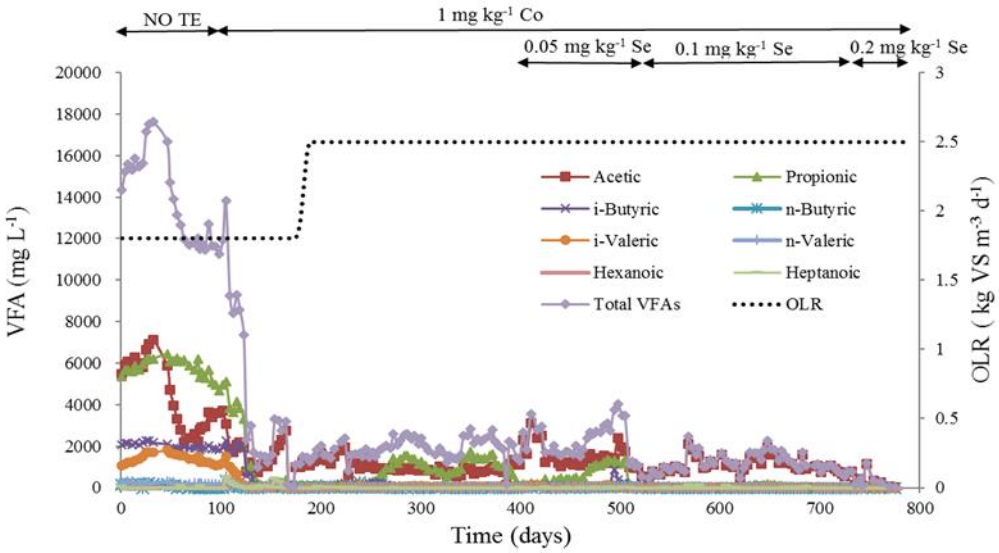


Figure 5.1 VFA and OLR changes in S1

General parameters

Figure 5.2 gave the results of operational parameters of S1 over 800 days. pH value of S1 remained within a safe range of 7.50-7.95 over trial period, the higher end reached after 1 mg kg^{-1} of Co supplementation induced VFA degradation. IA: PA ratio declined to below 0.4 from the initial value of 0.95, again, since 1 mg kg^{-1} Co introduced in day 103, then remained below 0.4 until the end. The methane percentage of S1 also responded to the Co addition, and increased from 51% to above 55% as no propionate accumulated afterwards.

The initial TAN concentration was above 4.50 g kg^{-1}, and fluctuated between 4 ~ 5 g kg^{-1} during the whole period. The switch of food waste batch (Table 4.1) accounted for this fluctuation.

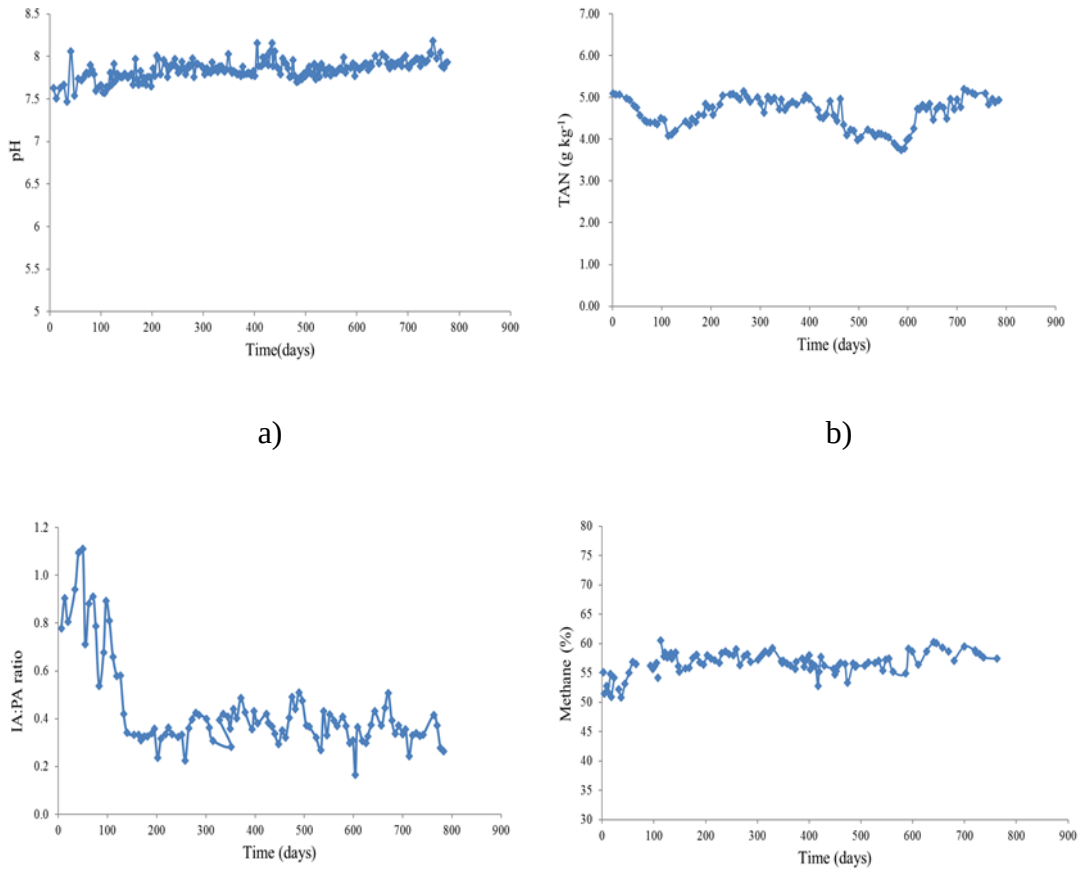


Figure 5.2 pH, TAN, IA:PA ratio and Methane (%) in digester S1

Biogas performance indicator – VMP, SMP

Both SMP and VMP increased 1.0 mg kg^{-1} Co introduced, from initial 0.38 m^3 CH_4 kg^{-1} VS d^{-1} and 0.68 m^3 CH_4 m^{-3} d^{-1}, respectively (Figure 5.3). This increase was caused by more complete food waste conversion after the product induced feedback inhibition was released after VFA decrease. After OLR increased to 2.5 kg VS m^{-3} d^{-1}, S1 showed relatively consistent SMP, whereas VMP increased reflecting the OLR increase; their values were around 0.43 m^3 CH_4 kg^{-1} VS d^{-1} and 1.00 m^3 CH_4 m^{-3} d^{-1} respectively at that stage. In the following period, Se supplementation showed no obvious affection to methane performance since only small amount of VFA existed in digester.

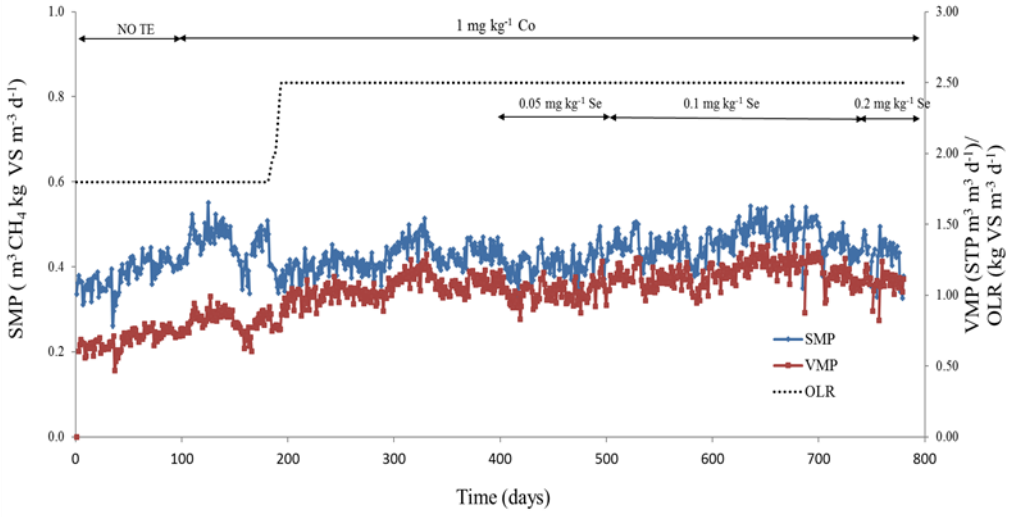


Figure 5.3 SMP and VMP in S1

5.3.2 Co requirement at moderate OLR when Se was not limiting

In this part of study, the effect of Co supplementation was tested in FW digester where sufficient Se existed. Two approaches were adopted for this in an attempt to identify its lowest concentration for maintaining stable FW digestion: Co washing out at the beginning of the experiment and then step-wise increase of Co supplementation strength when it was re-introduced.

Critical Co concentration determination by washing out test

The digesters which had OLRs initially of 4.0 kg VS m^{-3} d^{-1} in S2 and 3.0 kg VS m^{-3} d^{-1} in S3 both continued at previous loadings from day 1 until day 391. During this period all TE were stopped supplementing from day 103 except 0.2 mg kg^{-1} Se. Neither S2 nor S3 showed VFA accumulation before OLR increase introduced on day 391 (Figure 5.5&5.6). Calculated values of trace elements concentrations on particular days are listed in Table 5.3, used to compare with measured concentrations (Table 5.2) in the same sampling days. The measured concentrations were obtained by taking representative samples for acid digestion and ICP-MS determination. The measured values and calculated values comparison in S2 were plotted in Figure 5.4. The full data of calculated TE concentrations during washing out period are present in Appendix II. W was not an accredited test and therefore its determination was not carried out over this research and only calculated values were given in Appendix II.

Due to rapid VFA accumulation occurred in S2 after loading increase from 4 to 5 kg VS m^{-3} d^{-1} on day 392, Co was supplemented from day 427. That is the reason that the Co concentration of Table 5.3 started to increase in S2 since day 433. OLR increased in S3 from 3 to 4 kg VS m^{-3} d^{-1} on the same day as in S2, but no VFA accumulation to more than 1000 mg L^{-1} observed until day 467 (Figure 5.6).

It can be seen from Figure 5.4 that both calculated and measured values of TE concentration have good agreement. This confirmed the reliability of calculated concentration from equation model in section 3.4.2 and therefore the equation for TE concentration calculation during washing out can be used for same type of experiments to simulate the TE profiles.

VFA results from Figure 5.5, critical Co concentration in S2 at OLR 4~5 kg VS m^{-3} d^{-1} was determined to be 0.06 mg kg^{-1}; to be more accurate, 0.06 mg kg^{-1} was critical for OLR 4 kg VS m^{-3} d^{-1}, and after loading increased to 5 kg VS m^{-3} d^{-1}, VFA accumulated immediately. Similar critical concentration 0.08 mg kg^{-1} was obtained in S3 according to Figure 5.6, and after its OLR increased to 4 kg VS m^{-3} d^{-1}, VFA accumulated as well. VFA appeared in S3 on day 467, however, which was later than S2 on day 392. The reason for this delay could be that in lower-OLR digester S3, it took longer washing-out time to wash out Co to critical concentration, as well as the higher tolerance to Co deficiency for low OLR digester. In S2, Co reached its critical level faster than in S3, thus VFA accumulation appeared earlier. As another essential element supplemented, Se was fluctuated around 0.35 mg kg^{-1} in both S2 and S3.

Critical concentrations of Se and Co were previously tested (Jiang 2013). In that experiment 0.16 mg kg^{-1} Co and 0.22 mg kg^{-1} Se were demonstrated critically at moderate loading 3 kg VS m^{-3} d^{-1}, in which Co are almost twice higher than this study. The differences might be due to that Co and Se were washed out together in that study, whereas only Co was examined in this study. To be more specific, at OLR 3 and 4 kg VS m^{-3} d^{-1}, with 0.2 mg kg^{-1} Se supplementation, Co seemed to be critical when less than 0.06 mg kg^{-1}, whereas Se was critical when its concentration less than 0.2 mg kg^{-1} at lower OLR (2.5 kg VS m^{-3} d^{-1}). These two sets of Co and Se concentrations were found to be higher than their concentrations in food waste. This could be used to explain results of section 4.2.1 that without TE addition VFA

existed in 100-L digesters even at lower OLRs (1~2 kg VS m^{-3} d^{-1}). This explanation could also account for the high VFA concentrations that have been observed in a full-scale digester treating FW and in earlier pilot-scale and laboratory trials (Zhang and Jahng 2012).

It needs pointing out that critical dosage from this experimental stage were different from optimal concentration of Co required for stable process control. In this washing out stage, critical Co concentration was regarded as the minimal dosage needed to maintain stable process however less tolerance to circumstance changes. Optimal concentration of Co was determined in the following section, at which dosage stable process was achieved with proper buffering capacity in digester.

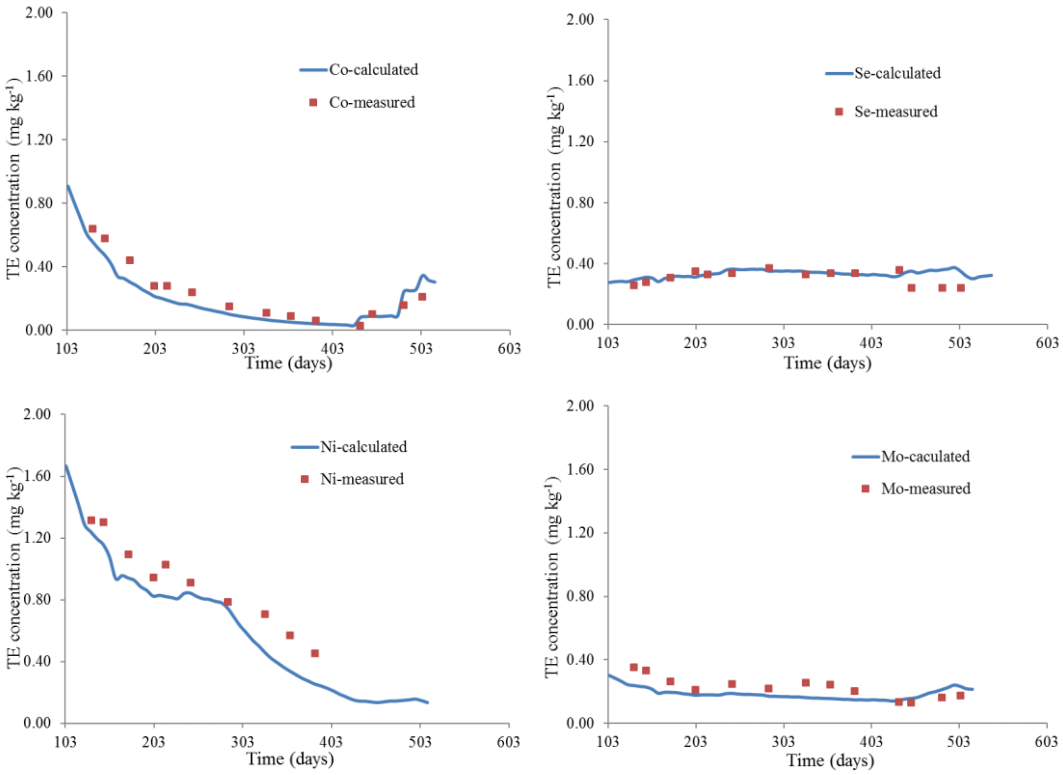


Figure 5.4 Calculated and measured TE concentrations profiles of S2

Table 5.2 Measured concentrations of TE in S2 and S3

Day	S2				S3	
	Co*	Se*	Ni*	Mo*	Co*	Se*
103	1.03	0.29	1.80	0.45	1.02	0.32
131	0.64	0.26	1.32	0.35	0.64	0.22
145	0.58	0.28	1.30	0.33	0.81	0.32
173	0.44	0.31	1.09	0.27	0.45	0.30
201	0.28	0.35	0.95	0.21	0.39	0.32
215	0.28	0.33	1.03	0.25	0.38	0.33
243	0.24	0.34	0.91	0.23	0.29	0.34
285	0.15	0.37	0.79	0.22	0.25	0.40
327	0.11	0.33	0.71	0.30	0.19	0.35
355	0.09	0.34	0.57	0.24	0.14	0.34
384[-b]	**0.06**	**0.34**	**0.45**	**0.20**	0.15	0.39
433	0.03	0.36	-[a]	0.14	0.13	0.36
447[-c]	0.10	0.24	-[a]	0.13	**0.08**	**0.35**
481	0.16	0.24	-[a]	0.16	0.07	0.24
503	0.21	0.24	-[a]	0.18	0.07	0.22

- All concentration units are mg kg^{-1} fresh matter
- [-a] not available
- [-b] the approximate day when VFA accumulated and corresponding TE concentrations in S2
- [-c] the approximate day when VFA accumulated and corresponding TE concentrations in S3

Table 5.3 Calculated concentrations of TE in S2 and S3

Day	S2				S3	
	Co*	Se*	Ni*	Mo*	Co*	Se*
103	1.02	0.27	1.80	0.32	1.02	0.27
131	0.56	0.29	1.24	0.24	0.94	0.36
145	0.47	0.31	1.16	0.23	0.65	0.38
173	0.30	0.31	0.94	0.20	0.56	0.36
201	0.21	0.31	0.82	0.18	0.41	0.36
215	0.19	0.33	0.82	0.18	0.32	0.36
243	0.16	0.37	0.84	0.19	0.28	0.35
285	0.10	0.35	0.75	0.17	0.22	0.35
327	0.07	0.35	0.46	0.16	0.16	0.37
355	0.05	0.34	0.34	0.16	0.12	0.38
384	0.04	0.33	0.26	0.15	0.10	0.37
433	0.08	0.32	0.15	0.14	0.08	0.34
447	0.09	0.35	0.14	0.16	0.05	0.33
481	0.24	0.36	0.15	0.21	0.04	0.33
503	0.34	0.35	0.15	0.23	0.04	0.32

* All concentration units are mg kg^{-1} fresh matter

Effect of Co on VFA degradation and methane production during it washed out and reintroduced

Stability indicator – VFA

Figure 5.5 showed VFA changes over the whole experiment, accompanied with Co and Se measured concentration in S2. After OLR increased from 4 to 5 kg VS m^{-3} d^{-1} which began on day 392 and was completed on day 412, an immediate increase in VFA which rose to 6000 mg L^{-1} by day 412 with most of the increase as results of propionic acid accumulation. pH dropped sharply to below 7.00 to reflect this VFA accumulation, corresponding increase were observed in IA:PA ratio (Figure 5.8). All parameters indicated that Co reached a critical level (0.06 mg kg^{-1} from both calculated and measured values) during that state. By the time stepwise increasing dosage of Co had been added the digester to not only compensate for the washout but also to compensate for the increase in loading.

Co reintroduced with strength of 0.05 mg kg^{-1} on day 427, and its dosing strength was gradually increased to 0.10 mg kg^{-1}, 0.15 mg kg^{-1}, 0.2 mg kg^{-1}, 0.25 mg kg^{-1} until 0.3 mg kg^{-1} on day 504. During the period of dosage increase, VFA did not show decline until reaching 30000 mg L^{-1} in total on day 500 except several slight drops following each dosage increase (0.1 mg kg^{-1} on day 441, 0.15 mg kg^{-1} on day 455, 0.2 mg kg^{-1} on day 476, and 0.25 mg kg^{-1} on day 483). Successive increases to the Co concentration in the digester were applied between day 509 and day 780 taking the concentration to around 0.3 mg kg^{-1}, this together with an interval feeding between day 509 to day 570 to bring the digester back under control. More than 30000 mg L^{-1} VFA were quickly consumed in the following 80 days. At this point the loading was immediately raised back to 5 kg VS m^{-3} d^{-1} and the digester operated at this loading with a reduced VFA concentration < 500 mg L^{-1} until day 780 almost 5 HRTs.

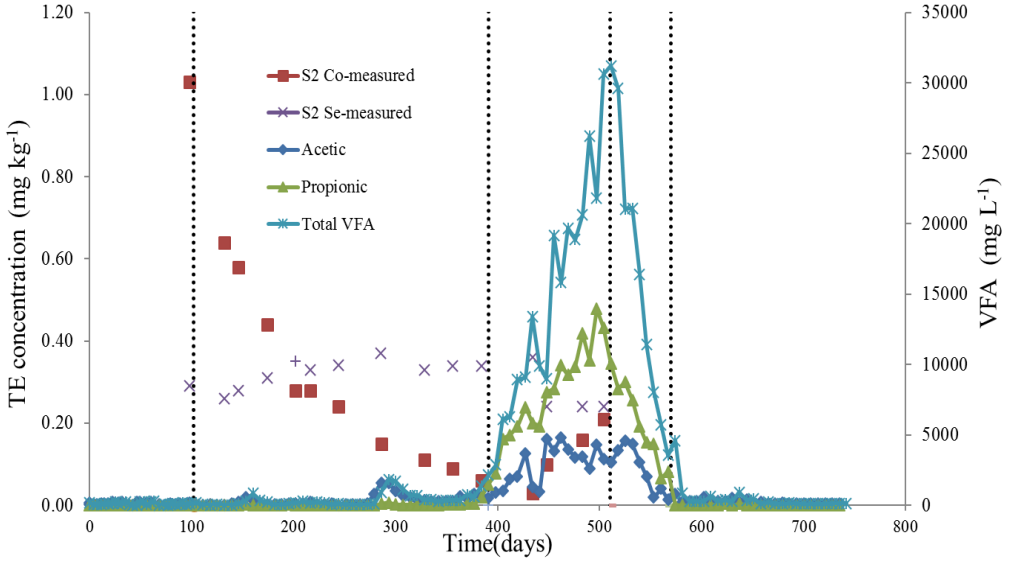


Figure 5.5 Measured Co and Se concentrations and acetic acid, propionic acid and total VFA in digester S2 at OLR 4~5 kg VS m^{-3} d^{-1}

note: TE washing out started from day 103; OLR increased from 4 to 5 kg VS m^{-3} d^{-1} from day 391; Co resupplied from day 427 until day 503 reaching 0,3 mg kg^{-1}, interval feeding performed between day 503 and 570

In S3, OLR gradually increased to 4 kg VS m^{-3} d^{-1} after TE washing-out started for almost 300 days. The digester continued to perform well for the following 60 days at which VFA showed severely increase to 4000 mg L^{-1} (Figure 5.6), and fluctuated approximate 60 days until another sharp increase in VFA occurred since day 540. At that point, 0.2 mg kg^{-1} Co was added to prevent the continuous VFA accumulation, and then Co was further increased to a concentration 0.5 mg kg^{-1} during the following 2 weeks. VFA, however, still sharply increased up to 22500 mg L^{-1} around day 576. Intermittent feeding were again adopted to lower in the loading (same operation as in S2, feeding every other day) until day 670. Since sufficient high strength Co added in S3 at that time, VFA decreased from 22500 mg L^{-1} to 800 mg L^{-1} in 40 days. Eventually digester was recovered with VFA concentrations reduced to < 1000 mg L^{-1} and therefore constant OLR was applied again at 4 kg VS m^{-3} d^{-1}. Digester S3 operated since day 670 at 4 kg VS m^{-3} d^{-1} until the end of experiments for more than 100 days with 0.5 mg kg^{-1} Co, 0.2 mg kg^{-1} Se, with < 500 mg L^{-1} VFA.

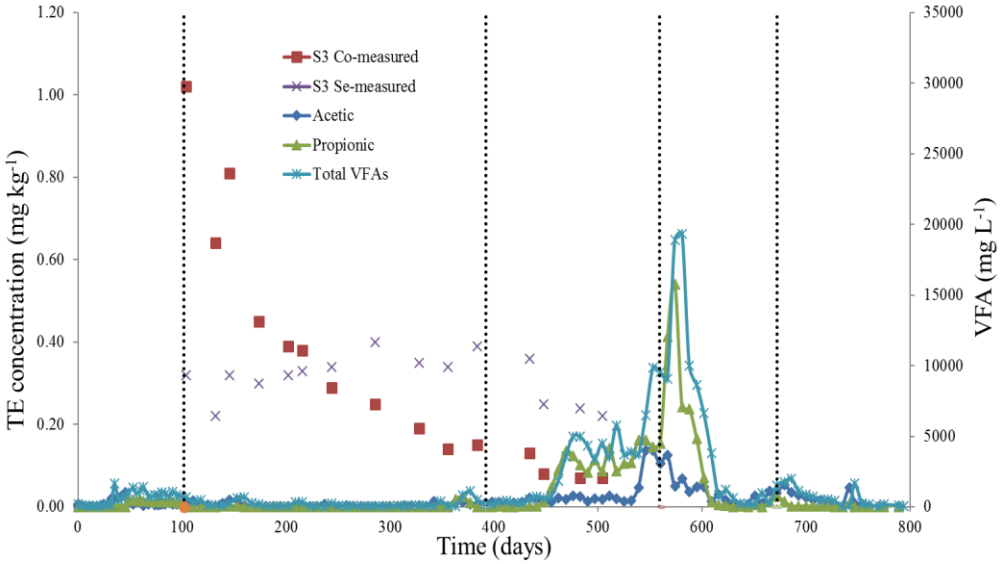


Figure 5.6 Measured Co and Se concentrations and acetic acid, propionic acid total VFA in digester S3 at OLR 3~4 kg VS m^{-3} d^{-1}

note: TE washing out started from day 103; OLR increased from 3 to 4 kg VS m^{-3} d^{-1} from day 391; Co resupplied from day 546 with interval feeding until day 670

During VFA increase period, propionic acid accounted for almost all of VFA in S3, and acetic acid showed no obvious accumulation after 0.2 mg kg^{-1} Co addition - it reached 5000 mg L^{-1}, and was degraded onwards. It is noteworthy there is no accumulation of acetate after Co was added at a strength of 0.2 mg kg^{-1}, whereas Propionic acid showed obvious degradation when Co strength increased higher up to 0.5 mg kg^{-1}. Similar observation was found in S2 where propionic acid has higher degradation rate then acetic acid when Co strength at 0.3 mg kg^{-1}.

In this study, non-reversible accumulation of propionic acid accounted for more than half of total VFA caused by Co washed-out, mainly because propionic acid oxidation and the following methanogenesis need Co-depending enzymes for their reactions. Propionic acid oxidation produces mixed products of acetate, CO_2, H_2 and formate, which take part in reactions of methane productions (Müller, Worm et al. 2010). Insufficient Co supplementation was unable to solve the issue of balancing propionic acid production and consumption. 0.3 mg kg^{-1} Co coupled with 0.2 mg kg^{-1} Se was suggested to be sufficient to stimulate propionic acid degradation.

Results of S2 and S3 suggested that at OLR 3~4 kg VS m^{-3} d^{-1}, FW digester could operate at a critical Co concentration of 0.06 ~ 0.08 mg kg^{-1} when there was 0.2 mg

kg^{-1} Se in digesters. This level of Co concentration, however, was not sustainable and not sufficient if instability initiated for instance by organic loading increase. The optimal Co dosing strength was 0.3~0.5 mg kg^{-1} according to this experiment. But it was further complicated by the changes in loading which could have exerted a higher Co demand, Also the test demonstrated that after long-term washed out, TE deficiency appeared earlier in higher OLR digester.

Based on above results, VFA accumulated times and maximal values it reached, accompanied with Co supplementation strategy were plotted in Figure 5.7 for comparison. In recovery period in S2 after each increase of Co dosage, VFA appeared shortly decline, and then climbed to higher level. VFA response to addition of Co was more straightforward in S3 with less dosing steps, 0.2 mg kg^{-1} Co addition stimulated VFA accumulation to 15000 mg L^{-1}. Further increase in Co to concentration 0.5 mg kg^{-1}, stimulated VFA degradation to 10000 mg L^{-1} in 4 days, followed by drastically increase to 22500 mg L^{-1}. Fluctuations of VFA were much more intensive in S3 than in S2 which might be caused by different strength and frequency of Co addition. With observation the direction of VFA changes were linked to TE supplementation strength, it is assumed that with adequate dosage Co addition, VFA accumulation could be resolved rapidly. This observation proved that Co limiting was much severe at higher OLR, as reported in (Banks, Zhang et al. 2012), that Co became limiting at higher

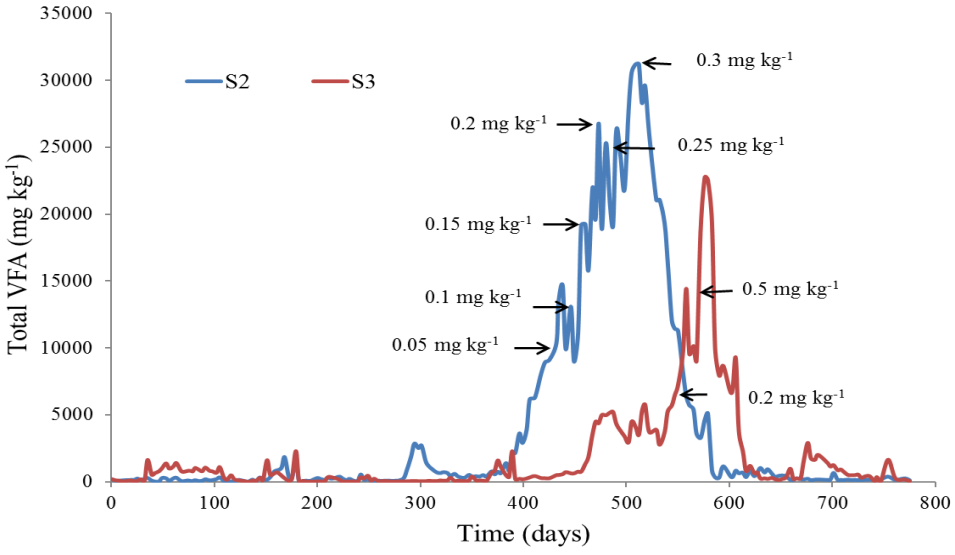


Figure 5.7 Total VFA changes and Co increase scheme in S2 and S3

OLR. It also raised the issue that the state of digester needs to be taken into consideration when applying TE supplementation strategy to seek a balance between TE effect on VFA-forming and VFA-consuming as well as the order their respond to TE addition.

In this study, insufficient Co addition was unlikely to stimulate methanogenesis for VFA degradation, but instead enhanced its accumulation. Sudden excessive Co supplementation strongly stimulated VFA accumulation to a great extent, in some case it could induce failure of digester. The exact response depended on digesters' adaptability to Co addition, e.g. sudden increase observed in S3 in Figure 5.6. Further investigation was required on the observation that when Co was supplemented into a Co-deficiency digestion system, its stimulation to methanogenesis was overwhelmed by its stimulation to VFA production. This topic is discussed further in Chapter 6.

General parameters

General parameters of S2 and S3 were shown in Figure 5.8. pH of S2 and S3 showed no differences after trace elements supplement (except Se) ceased until VFA accumulation occurred after OLRs increased, both showed gradual decline to below 7.0 (Figure 5.8-a). pH drop reflected changes in the ratio of IA:PA, which initially climbed even up to 9.0 in S2, then declined to below 0.5 (Figure 5.8-c). Methane percentages changes had similar pattern in both, remained above 55% in early stable stage, but declined in restored period in S2 and S3 (Figure 5.8-d). Especially in S2, methane percentage declined to even 35% from initial 57%, and then increased to above 55% after restored.

TAN concentration was always higher in S3 with 4.11 g kg^{-1} than S2 with 3.60 g kg^{-1} (Figure 5.8-b) and increased in S2 and S3 from day 150 caused by higher TKN content food waste used (Table 4.1), due to similar increase observed in S1. Afterwards ammonia showed decline since day 392 where OLR in S2 and S3 digesters increased, which showed inverse proportion to OLR in these 2 digesters. Similar pattern was observed in the following stages until stable process restored. In the end of experiment, TAN remained to be lower in OLR 5 kg VS m^{-3} d^{-1} digester of S2 than OLR 4 kg VS m^{-3} d^{-1} of S3, with 4.95 g kg^{-1} and 4.65 g kg^{-1} respectively,

whereas S1 at OLR 2.5 kg VS m^{-3} d^{-1} had higher TAN with 5.10 g kg^{-1}. Based on these 3 digesters results, ammonia concentration is hypothesized to have a reverse trend against OLR. Similar finding was observed in 100-L digester without TE addition, that ammonia level is lower in loading 2 kg VS m^{-3} d^{-1} than loading 1 kg VS m^{-3} d^{-1}. All of which were strongly against that ammonia inhibition to methanogenesis at higher OLR in food waste digester. However, this interesting hypothesis will be proved in section 7.3.4.

High ammonia concentration was reported to inhibit acetoclastic methanogens activities, acetate was then oxidised to carbon dioxide and hydrogen via a reverse Wood-Ljungdahl pathway, combined with hydrogenotrophic methanogenesis. In this study, due to high ammonia concentration in digester, the dominant methanogens for methane formation should mainly function on hydrogenotrophic methanogenesis. The observation that deficiency of Co was unable to reduce the accumulated acetic acid and propionic acid until certain concentration of Co added also implied that acetic acid degradation was via hydrogenotrophic methanogenesis. This raised the importance of Se, which was reported to exclusively function on Wood-Ljungdahl pathway, combined with hydrogenotrophic methanogenesis (Angelidaki and Ahring 1993, Schnürer and Nordberg 2008, Mayumi, Mochimaru et al. 2011).

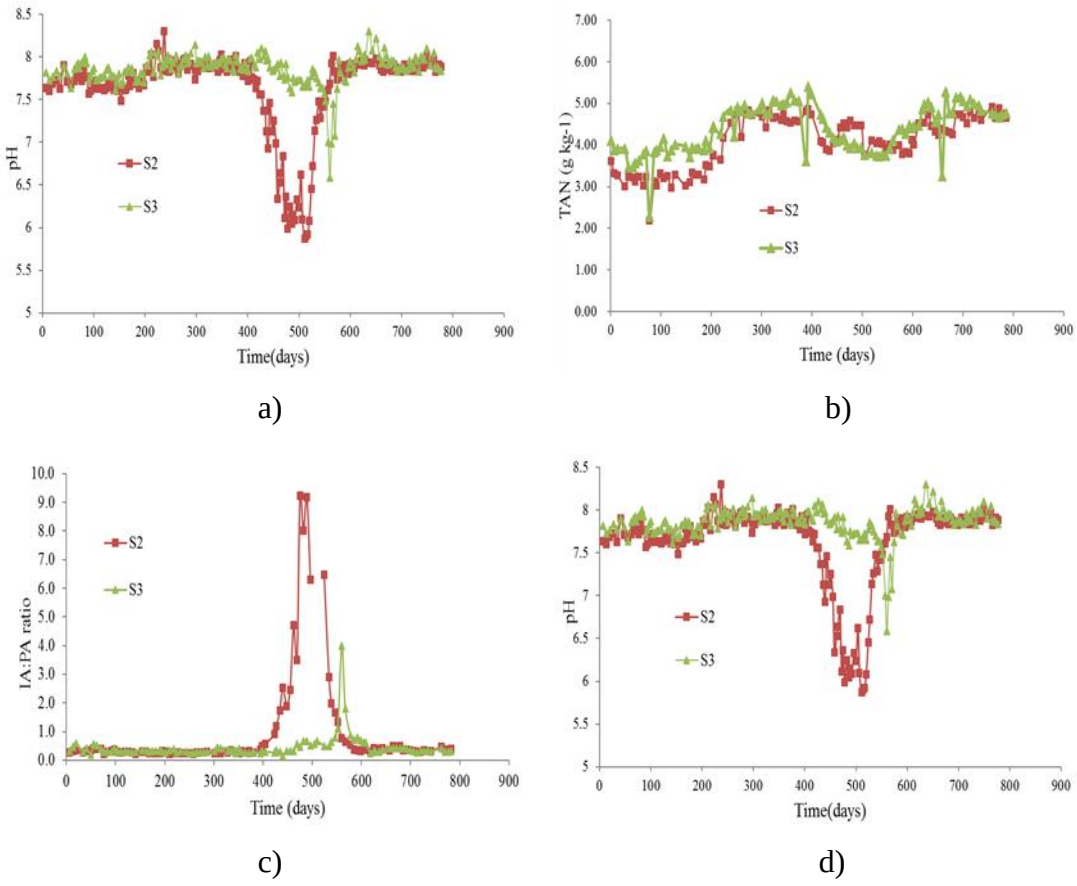


Figure 5.8 pH, TAN, IA:PA ratio and Methane (%) in digesters S2 and S3

Biogas performance indicator – SMP, VMP

Figure 5.9 showed the SMP and VMP in S2 during the trial period. Initially, S2 operated under OLR 4 kg VS m^{-3} d^{-1}, with Se, Co, Mo, Ni, W supplementation, generating VMP and SMP with 1.52 m^3 CH_4 m^{-3} d^{-1} and 0.42 m^3 CH_4 kg^{-1} VS d^{-1}. Both gas productions showed no apparent influence caused by TE washing out, indicating with enough TE addition, digester performance would not be enhanced. When VFA accumulation appeared after OLR increase, gas productions showed no increase but dropped sharply, VMP even once dropped to 0.11 m^3 CH_4 m^{-3} d^{-1}, with 0.02 m^3 CH_4 kg^{-1} VS d^{-1} SMP. During interval feeding period from day 509 to day 570, both VMP and SMP increased gradually. In the beginning period S2 switched back to OLR 5 kg VS m^{-3} d^{-1}, both VMP and SMP appeared gas peaks followed by decline, reaching stable production finally, with 2.37 m^3 CH_4 m^{-3} d^{-1} and 0.47 m^3 CH_4 kg^{-1} VS d^{-1}. In this trial higher SMP and VMP were observed after OLR increase and TE mixture replaced by combination of Se and lower concentration Co.

Figure 5.10 was the gas productions of S3, with 1.72 m^3 CH_4 m^{-3} d^{-1} VMP and 0.43 m^3 CH_4 kg^{-1} VS d^{-1} SMP in the beginning, as well no decline was observed during TE washed out period. After OLR increased to 4 kg VS m^{-3} d^{-1}, slight increase of VMP was seen, but its sharp drop appeared after quick VFA accumulation. During interval feeding period, both VMP and SMP increase were clear, and fluctuated regularly along with operation of feeding every other day. After OLR 4 kg VS m^{-3} d^{-1} reintroduced into S3, both VMP and SMP increased, were stable at 1.83 m^3 CH_4 m^{-3} d^{-1} and 0.46 m^3 CH_4 kg^{-1} VS d^{-1} until experiment finished, with combination of Se and lower concentration Co.

Based on gas production observation from S2 and S3, it was suggested that with adequate Co and Se addition into digesters, no gas production decreases were observed, as well as other elements seemed not to be limiting due to no loss of performance under moderate loading. Although increase of VMP and SMP is not confirmed due to TE supplementation in case of OLR increase, it could be confirmed that the simulation from Se and Co equalled the simulation from previous trace element mixture.

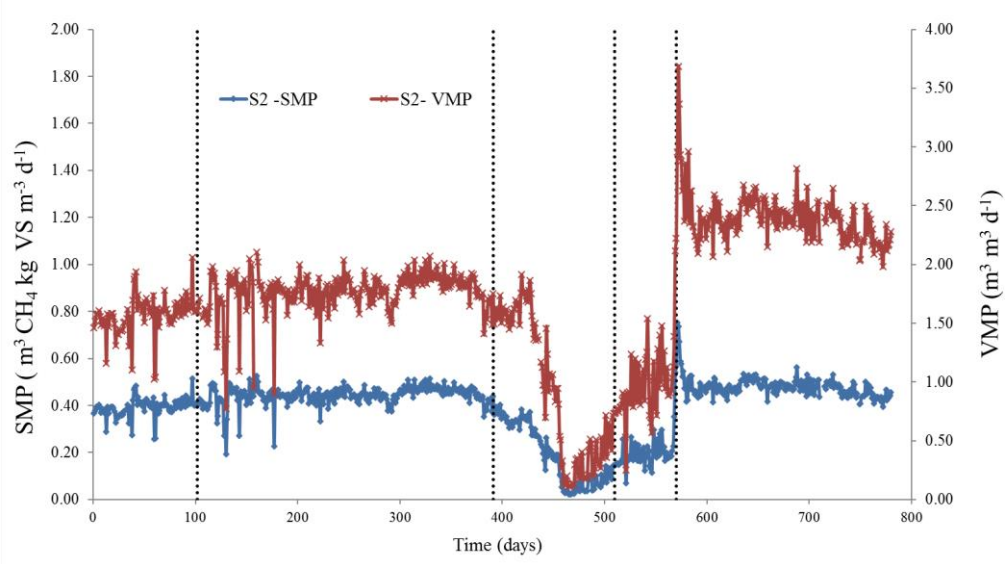


Figure 5.9 SMP and VMP of S2

note: TE washing out started from day 103; OLR increased from 4 to 5 kg VS m^{-3} d^{-1} from day 391; Co resupplied from day 427 until day 503 reaching 0,3 mg kg^{-1}, interval feeding performed between day 503 and 570

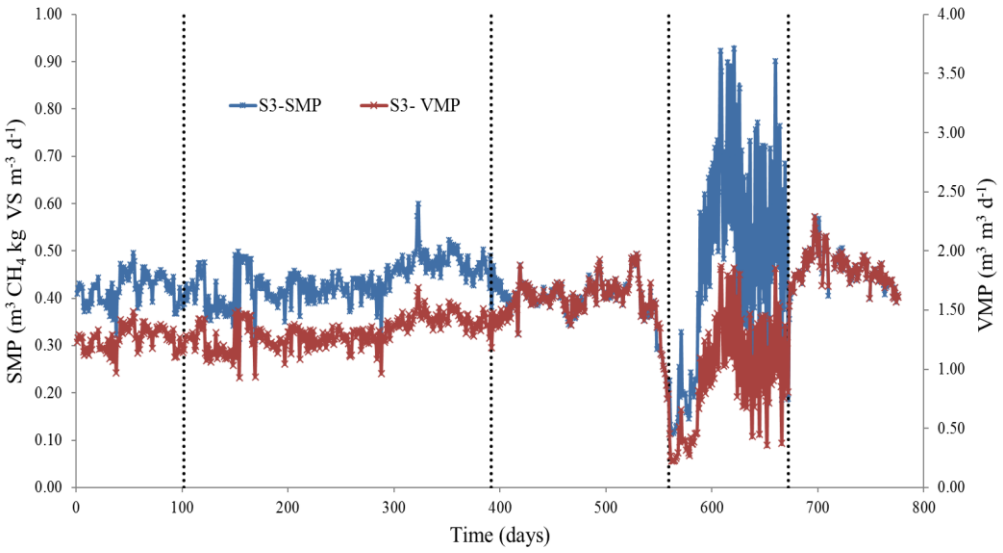


Figure 5.10 SMP and VMP of S3

note: TE washing out started from day 103; OLR increased from 3 to 4 kg VS m^{-3} d^{-1} from day 391; Co resupplied from day 546 with interval feeding until day 670

5.4 Conclusions and further discussion

According to the results of this set of experiments, Se was essential for FW digestion at loadings greater than 1.8 kg VS m^{-3} d^{-1} even when Co was supplied in sufficient quantity. 0.2 mg kg^{-1} Se was suggested as sufficient when the digester operated at loading 2.5 kg VS m^{-3} d^{-1}. At organic loadings between 3.0 and 5.0 kg VS m^{-3} d^{-1} both Se and Co were essential even though Co could be diluted out to around 0.06 ~ 0.08 mg kg^{-1} before VFA accumulation started. Recovery and continuing stable operation, however, required a much higher Co concentration. A concentration of Co at 0.3~0.5 mg kg^{-1} is recommended as the minimal strength to maintain stability and performance efficiency. With insufficient Co supplementation, propionic acid could not be degraded whereas acetic acid degradation showed no obvious inhibition. Since Co addition showed a stimulation effect on VFA production, careful investigation on the impact of trace elements supplementation on VFA production is needed, especially when OLR is not at a low level, to ensure a beneficial effect is achieved after TE addition.

Co is a trace element universally used by microbial biomass in the digestion process as there are three large classes of enzymes having cobalamin (also known as vitamin B_{12}, a Co-containing cofactor) in their cofactors: B_{12}-containing reductive dehalogenases, B_{12}-containing methyl-transferases and B_{12}-containing-dependent isomerases. Anaerobic microbes containing this type of reductive dehalogenases, such as *Desulfomonile*, *Desulfitobacterium* and *Dehalobacter*, are important in the detoxification of aromatic and aliphatic chlorinated organics. These reductive dehalogenases usually contain iron-sulphur clusters as well. The Co-dependent methyltransferases are essential for amino acid metabolism and CO_2 fixation by anaerobic microorganisms. The B_{12}-containing isomerases, e.g. ribonucleotide reductase, methylmalonyl-CoA mutase, iso-butyryl-CoA mutase and glutamate mutase, play important roles in anaerobic fermentation of small molecules in the absence of an external electron acceptor (Roth et al., 1996; Banerjee and Ragsdale, 2003; Pind et al., 2003; Gruber et al., 2011; Swanner et al., 2014; Takano et al., 2015).

TE supplementation is usually used to enhance VFA degradation and digestion stability, especially when VFA has accumulated in the digesters (Lindorfer et al., 2012; Ortner et al., 2014; Wall et al., 2014; Wei et al., 2014; Zhang et al., 2015). These results indicate, however, that the effect of TE may come into force in all 4 stages simultaneously. Although the TE addition stimulates the microbial activities for each stage if dosed at the right strength, the overall effect may not be preferable in some situations, e.g. when the production of VFA is faster than their degradation by methanogens. This is especially true when TE is applied to VFA-laden digesters. There are several possible reasons for this: 1) the acid-producing bacteria may have higher TE uptake rate and/or lower half-saturation constant compared to methanogens; 2) certain TE are particularly beneficial for bacteria, although not useful for methanogens; 3) the acid-producing bacteria may have a shorter doubling time overall than that of the methanogenic community; and 4) the acid-producing bacteria may be less stressed under VFA-laden conditions and therefore recover more promptly than methanogens.

This issue has been identified by a few other studies. For example, the optimal TE supplementation time was investigated by Yu et al. (2015) using Fe in thermophilic

batch digesters for wasted activated sludge treatment. The study found that the timing of Fe addition was essential with regard to balancing the process between hydrolysis-acidification and methanogenesis: an earlier supplementation was inhibitory to methanogens due to the inhibitory effect of VFA, whereas a delayed supplementation could not control the VFA accumulation by methanogenesis.

It would be much less complicated if trace elements were only required by methanogenesis. If this was the case, the overall effect would be very straightforward: methane production and substrate utilisation rates would increase. This is because TE supplementation helped stimulate the conversion of acetate and hydrogen to methane and therefore reduces the product-induced feedback inhibition to acetogenesis, which in turn helps acidogenesis and hydrolysis. In this case, however, it is very difficult to distinguish the direct effect of TE on acidification or the increased acidification caused by the disappearance of feedback-induced inhibition. However, due to the effect of TE on acetogenesis, acidogenesis and hydrolysis, the overall result could become uncertain, as it is affected by a range of factors including digester condition and operating arrangements. This is especially true when TE is added to VFA-laden digesters. Therefore the effect of TE directly on acidification was conducted as the follow-up action of this set of experiments, as described in Chapter 6.

CHAPTER 6 Effect of trace elements addition on VFA production

6.1 Introduction

Two sets of tests were conducted to investigate the mechanisms behind the observation described in Chapter 5: Co supplementation stimulated further VFA production when FW digesters at moderate OLR were laden with VFA. Test 1 was designed to directly confirm if TE addition promoted acidogenesis and acetogenesis and therefore stimulated VFA production. Heat pre-treated inoculum was used in this case to deactivate methanogenic activity. Test 2 was a comparative study used to provide preliminary understanding on how TE affected the balance between VFA production and consumption in VFA-laden digester. Food waste digestate without heat pre-treatment was used as inoculum for this test.

6.2 Methodology

Test 1

The objective of Test 1 was to assess the stimulation of trace elements to VFA production. Two pairs of 500 mL flasks were used as CSTR digesters with 400 mL working volume, which cultivated in an incubator (Weiss-Gallenkamp, UK) under mesophilic condition. Inoculum was from a 100-L food waste digester BR3 which worked for a period of over 500 days at an OLR of 2 kg VS m^{-3} d^{-1} without TE supplementation (section 4.2). Pre-treatment of inoculum was carried out by boiling this inoculum for 45 minutes after temperature reached 100°C in order to deactivate methanogenesis for VFA consumption. After inoculation, 1 pair of flasks were supplied with full (11 types) trace elements (Table 3.3) while the other pair were run as controlled with no TE received throughout the trial. Daily feeding with continuous stirring was performed at OLR 5 kg VS m^{-3} d^{-1} and representative digestate were sampled daily for VFA analysis. Carbon dioxide gas was used to flush flasks for anaerobic condition after daily feeding.

As a preliminary study to test the effect of TE on VFA production, the full range of essential trace elements were used in this trial rather than the combination of Co and

Se only. This was because the primary purpose of this test was to demonstrate, in general, that TE addition can stimulate VFA production, and therefore a full TE addition strategy was applied. More thorough investigation can then be carried out to identify which individual trace elements are effective in promoting VFA production in each specific digestion system, when supplemented. As Co and Se were recognised as two essential trace elements to be supplemented to food waste digesters for VFA degradation, their effect on VFA production was then specifically assessed in Test 2, as described in the next section.

Test 2

The objective of Test 2 was to investigate the digester responses to Co and Se supplementation under two different conditions: low and high VFA concentrations. The inoculum for Test 2 was the same as used in Test 1 but without heating pre-treatment. The inoculum was transferred from 100-L digester BR3 to 2 pairs of 5-L digesters which were the same design as used in long-term digestion trial (Figure 3.1). Daily feedings were performed at OLR of 2 kg VS m^{-3} d^{-1} for 14 days to ensure two pairs of digesters had comparable performance, and then OLR 5 kg VS m^{-3} d^{-1} were applied to both pairs. 1 pair of digesters were supplemented with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se from the beginning of the trial (day 0), whereas the other pair were supplemented with same trace elements on day 28 when total VFA accumulation to higher than 5000 mg L^{-1} occurred.

6.3 Results and discussion

Test 1

pH of all digesters was below 7.0 over the test period, which is unlikely the recommend range for methanogenesis, but favours fermentative bacteria. In addition, there was no methane production throughout the course of the experiment. Both these indicated that methanogens activity was inhibited.

Figure 6.1 showed VFA comparison between two pairs with and without TE supplementation. After 12 days of operation, VFA productions in flasks without TE

was around 38000 mg L^{-1} (Figure 6.1-a), almost 10000 mg L^{-1} lower than that from flasks with TE, as shown in Figure 6.1-b. The difference of VFA concentrations in these two pairs of flasks was confirmed to be caused by TE supplementation due to the inhibition of methanogenesis through heat pre-treatment, as methanogens were much more sensitive than acetogens. It is therefore demonstrated that supplementation of trace elements could stimulate VFA production.

The observation of this trial provided evidence that supplementation of TE stimulated acid-producing stages of anaerobic digestion system, and can cause VFA fluctuation during when digester is laden with certain level VFA. This explained the VFA fluctuation in S2 and S3 in Figure 5.7 that VFA showed slight drop afterward increased again during restored period.

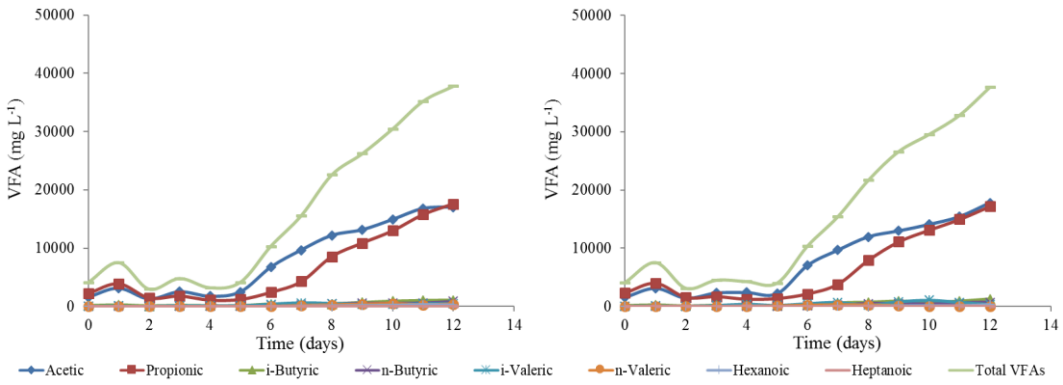


Figure 6.1-a VFA in 1st pair digesters (deactivated inoculum, without TE supplementation, OLR 5 kg VS m^{-3} d^{-1}

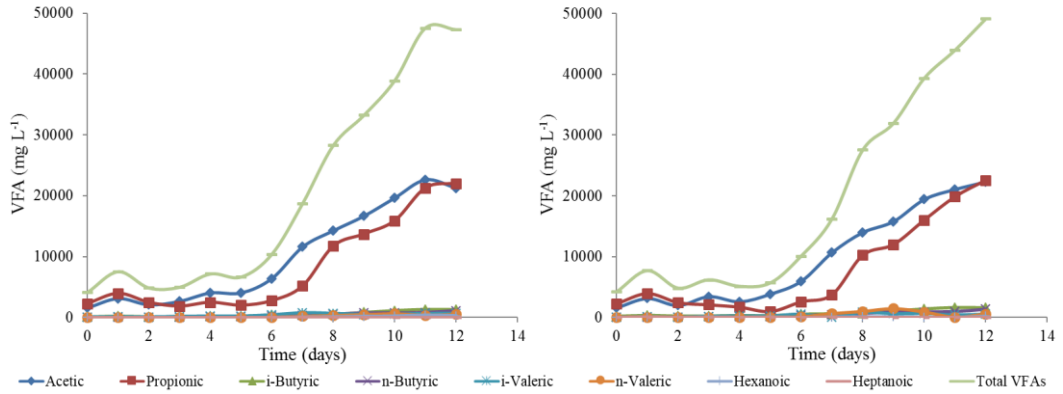


Figure 6.1-b VFA in 2nd pair digesters (deactivated inoculum, with 11 TE supplementation, OLR 5 kg VS m^{-3} d^{-1}

Figure 6.1 VFA comparison between FW digesters with/without TE supplementation when methanogenesis deactivated

Test 2

After inoculated using food waste digestate, 2 pairs of 5-L CSTR digesters were operated initially at OLR 2 kg VS m^{-3} d^{-1} for 14 days to attest the similarity of performance between digesters, and then OLR was increased to 5 kg VS m^{-3} d^{-1} for the rest of the experiment.

1st pair of digesters was supplemented with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se from the start point day 0 when digesters were inoculated until the end of experiment. The profile of VFA changes of each digester are shown in Figure 6.2-a, with first VFA peak appearing at around day 19, which was caused by sudden OLR increase in day 14. Acetic acid mainly accounted for this VFA perk, then was consumed soon. In the following 40 days, VFA increased incrementally up to 15000 mg L^{-1}, in which propionic acid accounted for more than 10000 mg L^{-1}. Propionic acid accumulation was properly caused by sudden OLR increase, due to digesters did not have sufficient acclimation time. After day 70, both total VFA and propionic acid showed decline. This pair of digesters, however, was still operational with pH of 7.50 $\pm$0.15, and SMP of 0.35 $\pm$ 0.02 m^3 CH_4 kg^{-1} VS d^{-1}.

2nd pair of digesters started without TE supplement. The first VFA peak appeared on day 19 in both digesters, almost reaching a level of 15000 mg L^{-1} (Figure 6.2-b), the magnitude of this VFA increase was greater higher than that of the 1st pair. The same dosage of 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were supplied to this pair of digesters on day 28 when VFA level reached 12000 mg L^{-1} without further decline. This supplementation action saw apparent VFA degradation during the following 2 days, followed by VFA sharply increase, of which acetic acid, propionic acid and other acids, all increased in digesters. In the end of trial, the total VFA reached 38000 mg L^{-1}, and this pair of digesters failed without apparent biogas production.

It is worth noting that different VFA profiles developed in these two pairs of digesters. With the supplementation of Se and Co from the beginning of the test for the 1st pair of digesters, all VFA produced could be consumed rapidly apart from propionic acid. As the most difficult VFA species for degradation (De Bok, Plugge et al. 2004, McMahon, Zheng et al. 2004), the accumulation of propionate was considered to be caused by the sharp loading increase. Its concentration then

stabilised towards the end of the experiment, indicating that the digesters started to acclimate to this OLR. More VFA species were present at high concentration in the 2nd pair of digesters, including acetic, propionic and butyric acids. Both loading increase and the imbalance between VFA production and consumption triggered by TE addition were considered to be the causes of this. VFA concentrations were still rising in the 2nd pair of digesters at the end of the experiment, 60 days after TE addition. It seemed that these digesters had reached a situation which was difficult to alter: as a bottleneck in the AD process, methanogens were not as functional as fermentative bacteria during OLR and TE deficiency, therefore more VFA accumulated and further inhibited methanogens. The elevated VFA concentrations in the digesters also caused product induced feedback inhibition to acetogens, resulting in more VFA species appearing in the digesters.

VFA profiles in the 2nd pair reflected VFA changes during the recovery period in S2 and S3 in section 5.3, after increasing the dosage of Co. In both cases VFA showed a slight fall then a severe climb to a higher level. It is evident from this experiment that under elevated VFA concentration, supplementation of TE stimulated VFA production to a greater extent than consumption. This was because the elevated VFA concentration inhibited methanogenesis whereas VFA production continued to be stimulated by Co and Se supplementation. In this case, TE supplementation induced further VFA accumulation and finally digester failure.

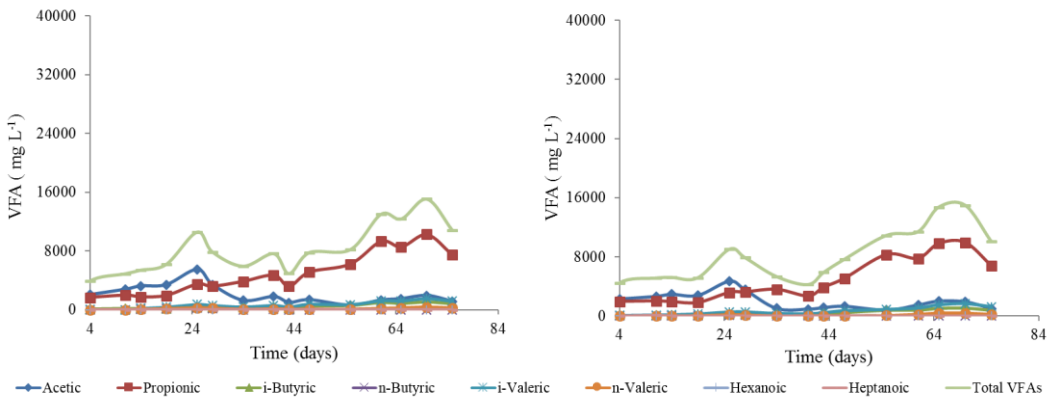


Figure 6.2-a VFA in 1st pair digesters with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se supplementation from initial day 0, OLR 2 (0-14), 5(14-end) kg VS m^{-3} d^{-1}

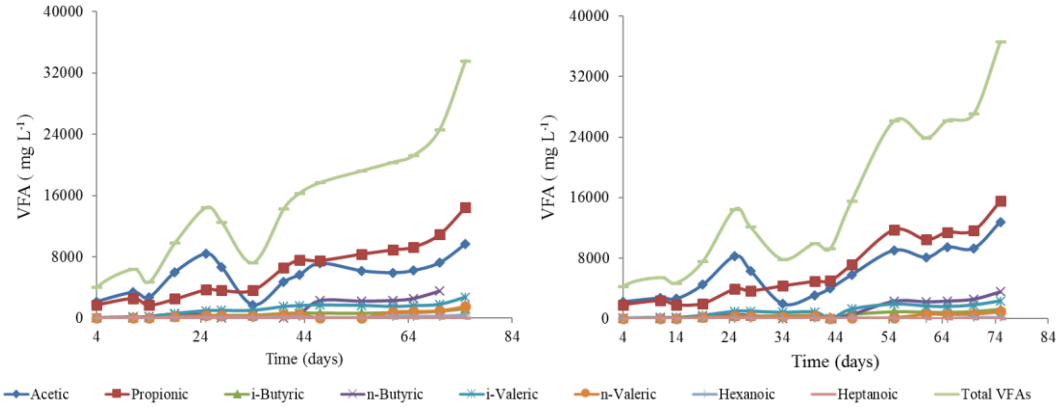


Figure 6.2-b VFA in 2nd digesters with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se supplementation from day 28, OLR 2 (0-14), 5(14-end) kg VS m^{-3} d^{-1}

Figure 6.2 VFA comparison between low /high VFA laden FW digesters when TE supplemented

6.4 Conclusion

In FW digesters, TE supplementation stimulated both VFA production and consumption. It was confirmed that when methanogenic activities were inhibited, supplementation of TE still functioned on VFA production, compared with control without TE addition. This knowledge has practical significance regarding TE supplementation strategy when digesters have been laden with VFA and methanogens are prone to that inhibitory effect. It showed that supplementation of Co and Se induced severe VFA accumulation and digestion failure when they were

added to VFA-laden digesters. TE supplementation strategy for VFA degradation therefore should be applied with caution.

Due to time constraint, OLR straight increased from 2 to 5 kg VS m^{-3} d^{-1} in Test 2, with very short acclimation period. In 1st pair digesters, total VFA was 5000 mg L^{-1} at the beginning and gradually increased to above 15000 mg L^{-1} at its peak, with propionic acid as the dominant species. This raised a question that whether this VFA accumulation was caused by sudden OLR increase and further acclimation could solve this problem, or 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were insufficient to maintain stable operation thus other elements should introduce. To clarify this, acclimated digesters were used for stability study with defined Co and Se supplementation strength in Chapter 8.

CHAPTER 7 Effect of organic loading rate on productivity and stability of mesophilic food waste digestion

7.1 Introduction

In parallel with the work carried out to explore the role of Co and Se in stabilising food waste digestion at low and moderate OLR as described in Chapter 5, another study was conducted on the investigation of FW digester performance with sufficient TE supplementation at high OLR. As discussed in the literature review, the maximum OLR achieved was variable and depended on the characteristics of feedstock and the operating conditions of the digestion system. There is no substrate analogous to FW, a readily biodegradable material with a ~20% solids content, which can be used to estimate the maximum loading of FW digestion, and therefore an experimental approach was required. This study aimed to assess the productivity, efficiency and stability of food waste digestion at high OLR with sufficient TE supplementation. In addition, the impact of variable loading on process stability and efficiency was tested at a moderate loading of 5 kg VS m^{-3} d^{-1}. This was to better simulate the real-world situation: commercial AD plants are likely to receive a fluctuating load, whereas in laboratory-scale trials operational conditions are usually carefully controlled and consistent. A pair of digesters operated at a constant loading of 5 kg VS m^{-3} d^{-1} was used as control for the above two sets of designs. VFA was again used as the most important parameter to indicate the stability of digesters; SMP, volatile solids destruction (VSD) rate and residual biogas potential (RBP) were used to evaluate the food waste conversion efficiency; VMP was employed to indicate the digestion productivity; and TAN was useful to estimate the change of microbial biomass density with OLR change.

7.2 Methodology

Five 5-L operating digesters of the type described in section 3.4.1 were used for this trial, with a known well-operated initial condition (Table.3.1) at OLR 5.5 kg VS m^{-3} d^{-1}. Trace elements recipe used for this study is described in Table 3.2. The operational scheme over this loading experiment started from day 1 until day 776. This experiment had been run for 775 days, with pH, TAN, biogas composition,

VFA, alkalinity measured once per week. TKN as well was measured in each operational scenario from steady state.

7.2.1 Constant OLR digestion test

2 pairs of digesters were used for this test: 1 pair (named as S5, S6) used as control; and another pair of digesters (named as S7, S8) were for investigation of maximum possible loading achievable with food waste and of digestion efficiency at a high loading. In control digesters (S5, S6), initially dosed with Se, Co, Mo, OLR was lower to 5 kg VS m^{-3} d^{-1} from day 105 whereas OLR of S7 and S8 increased stepwise until 9 kg VS m^{-3} d^{-1} for comparison. The control digesters were initially dosed with Se, Co and Mo and a mix of 11 trace elements (full) was supplemented to these controls on day 392 forwards. Digesters (named as S7, S8) for critical loading assessment were initial acclimated with Se, Co, Mo, W. Fe, Ni were added when their loadings increased to 6 kg VS m^{-3} d^{-1}, and afterwards 5 more TE (within a selection of 11 TEs in Table 3.2) were applied since day 524. The purpose of the full range of TE supplementation was to prevent TE deficiency to be the limiting factor of the digester operation in this test where the effect of OLR should be investigated. Figure 7.1 showed the entire operational scheme of the two pair digesters in respect of OLR and TE supplementation regime.

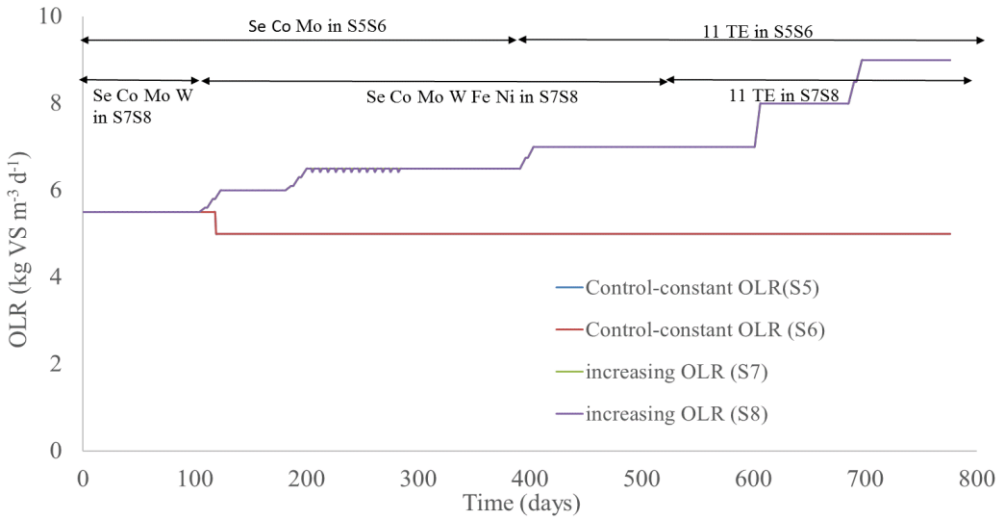


Figure 7.1 OLR and TE supplementation changes through the trial in S5-S8

7.2.2 Random OLR digestion test

Digester (named as S4) was supplemented with 11 (full) trace elements from the beginning and during the course of experiment. To simulate the fluctuating load of commercial plant, S4 was running with a varying loading to give a weekly average OLR at 5 kg VS m^{-3} d^{-1} from day 181. The loading applied each day was randomly chosen using a purpose-designed Excel spreadsheet routine. From day 181 to day 621, loading fluctuated from 2.5 to 7.5 kg VS m^{-3} d^{-1}, and afterwards the loading ranged from 0 to 10 kg VS m^{-3} d^{-1}. Digester performances were compared to the control digesters (S5, S6) which were fed at a constant loading of 5 kg VS m^{-3} d^{-1}. The overall operation during this period was shown in Figure 7.2.

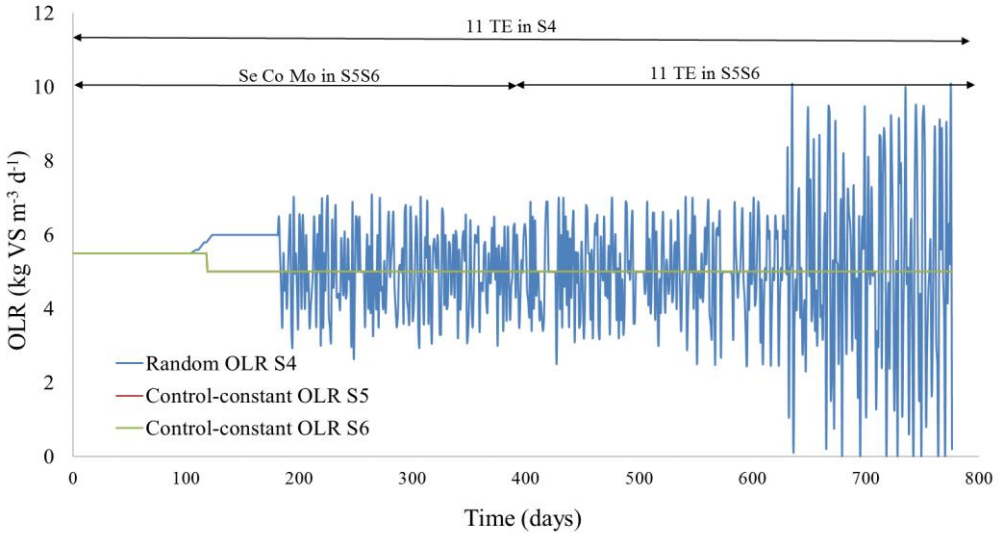


Figure 7.2 OLR and TE supplementation changes through the trial in S4, S5, S6

7.3 Results and Discussion

7.3.1 Stability parameters in all digesters

Stability indicator –VFA analysis

VFA profiles in these 5 digesters over 700 days were shown in Figure 7.3. No apparent accumulations were observed in all of these digesters, in particularly digesters S7 and S8 (Figure 7.3-b), which were operated at OLR 7 kg VS m^{-3} d^{-1} after

day 400 and then up to 9 kg VS m^{-3} d^{-1} at the end. Total VFA in both were stable less than 1000 mg L^{-1} most of the operating time.

VFA did not appear at concentration higher than 1000 mg L^{-1} most of the operating time in random loading digester S4 (Figure 7.3-c), both in periods of fluctuating loading from 2.5~7.5 kg VS m^{-3} d^{-1} and loading 0~10 kg VS m^{-3} d^{-1}. It is worth noting that during the long-term experiment, VFA peaks appeared in all constant loading (> 2500 mg L^{-1}, day 307) and increasing loading digesters (>1000 mg L^{-1} day 144), however no accumulation of VFA were observed in random loading digester, indicating its process stability.

For the short period of slight VFA increase to 2500 mg L^{-1} (mainly propionic acid) in digesters S5 and S6 (Figure 7.3-a) and the corresponding increase IA:PA ratio around day 307 (Figure 7.5), no obvious explanation can be given for this. It showed, however, that even under controlled conditions unexpected fluctuations in digester can occur.

This trial proved that FW digesters could be stable operated for long-term with sufficient TE addition when VFA was used as the most important stable indicator. No propionic acid or acetic acid accumulated at loading 9 kg VS m^{-3} d^{-1}, indicating effective volatile solids conversion and TE-depending enzymes involved in anaerobic digestion performed properly with given TE addition. In previous studies using FW as sole substrate in CSTR digesters, digesters were usually suffered from VFA accumulation or it had to be operated at very low loading to avoid VFA accumulation (Climenhaga and Banks 2008, El-Mashad, McGarvey et al. 2008). Successful operation in food waste digestion were achieved at moderate loading with TE supplementation of in recent studies (Banks, Zhang et al. 2012, Zhang and Jahng 2012, Wei, Zhang et al. 2014).

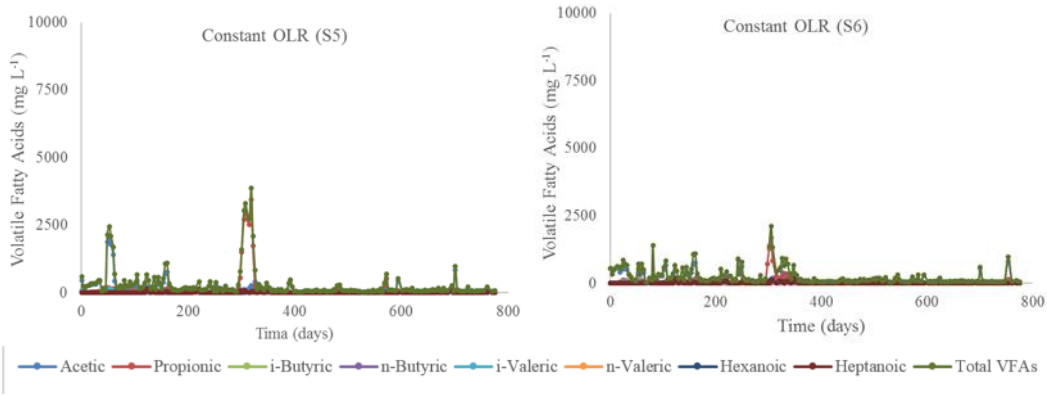


Figure 7.3-a VFA profiles in control digesters S5, S6 between day 0-775

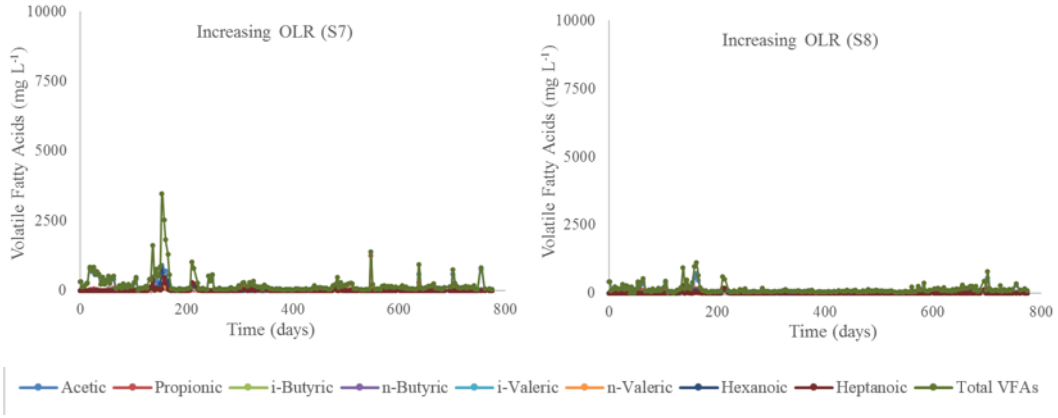


Figure 7.3-b VFA profiles in increasing OLR digesters S7, S8 between day 0-775

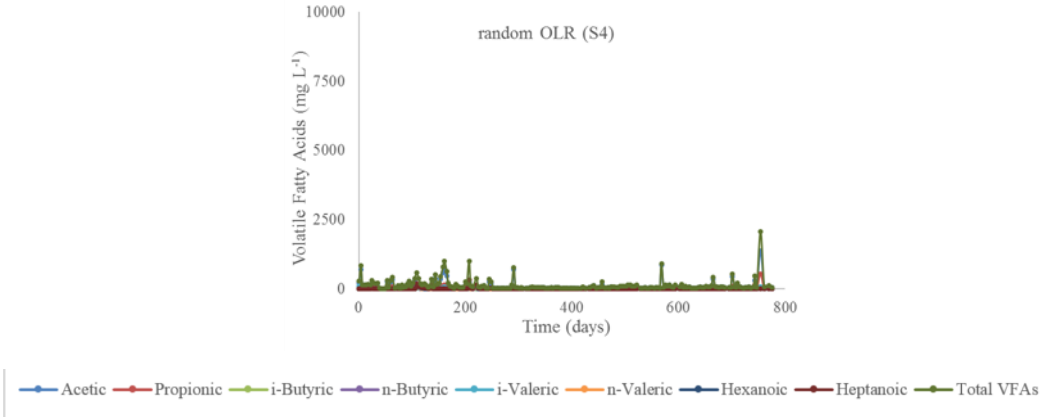


Figure 7.3-c VFA profile in random OLR digester S4 between day 0-775

Figure 7.3 VFA profiles in digesters of S4-S8 between day 0-775

High-loading food waste digestion was investigated in previous studies, when loading reached 9 kg VS m^{-3} d^{-1}, pH dropped to 6.54 due to high VFA accumulation,

experiments ceased (Jabeen, Zeshan et al. 2015), similar results were found in (Lim, Kim et al. 2008, Jiang, Zhang et al. 2013), all indicated that at higher OLR, VFA were not effectively consumed by methanogens. However, in this study, with proper TE supplementations, delicate balance between the rates of acetogenesis and methanogenesis was well maintained.

General parameters

In control digesters S5 and S6, pH was stable around 7.80 ± 0.20 over the whole experiment, IA:PA ratio was stable at 0.30 ± 0.10 (Figure 7.5).

In digesters S7 and S8, pH was slight lower than that of controls with time, especially after OLR increased to 6 kg VS m^{-3} d^{-1}, pH of S7, S8 were always 0.20 lower than their control. At higher loadings (8~9 kg VS m^{-3} d^{-1}), pH was maintained around 7.70±0.15. IA:PA ratio of S7 and S8 were stable around 0.30~0.40.

In random loading digester S4, similar performances were observed in both pH and IA:PA ratio, compared with its control S5, S6 at constant OLR 5 kg VS m^{-3} d^{-1}. Results from these two operational parameters implied that all 5 digesters had stable digestion, no obvious differences among these digesters however their loadings and TE supplementation were different in certain period. Especially in increasing OLR digesters S7, S8, no apparent response to each increase in loading, great buffering capacity in digesters were achieved. Random OLR digester S4 also showed well capacity to frequent loading changes. Methane percentages were stable around 58%±2% through testing period for all 5 digesters.

Figure 7.6 shows the TAN concentrations of these 5 digesters. In which the general fluctuations of TKN in all digesters over the experimental period were mainly caused by switch of different batches food waste since all TKN showed similar fluctuation curves, and no other operations were introduced to cause these TKN changes. However, it was observed that the TAN in digester S7 and S8 were always lower than their control digesters S5 and S6, when the OLR of S7 and S8 increased, TAN concentrations themselves showed decline as well. This observation was mentioned previously in section 5.3, in which TAN concentrations is 4.65 g kg^{-1}, lower in S2 at OLR 4 kg VS m^{-3} d^{-1} than S3 with 4.95 g kg^{-1} at OLR 3 kg VS m^{-3} d^{-1}, S1 had the

highest TAN concentration with 2.5 kg VS m^{-3} d^{-1} loading at 5.10 g kg^{-1}. Besides, in section 4.3 100-L digesters without TE supplementation trial, TAN was 5.45 g kg^{-1} at loading 2 kg VS m^{-3} d^{-1}, but reached 6.16 g kg^{-1} at 1 kg VS m^{-3} d^{-1} in the final period. Based on above observation, it can be proposed that TAN profile follows the pattern that the higher the OLR the lower the TAN in digesters. This hypothesis and the relationship between TAN and loading are discussed in section 7.3.4.

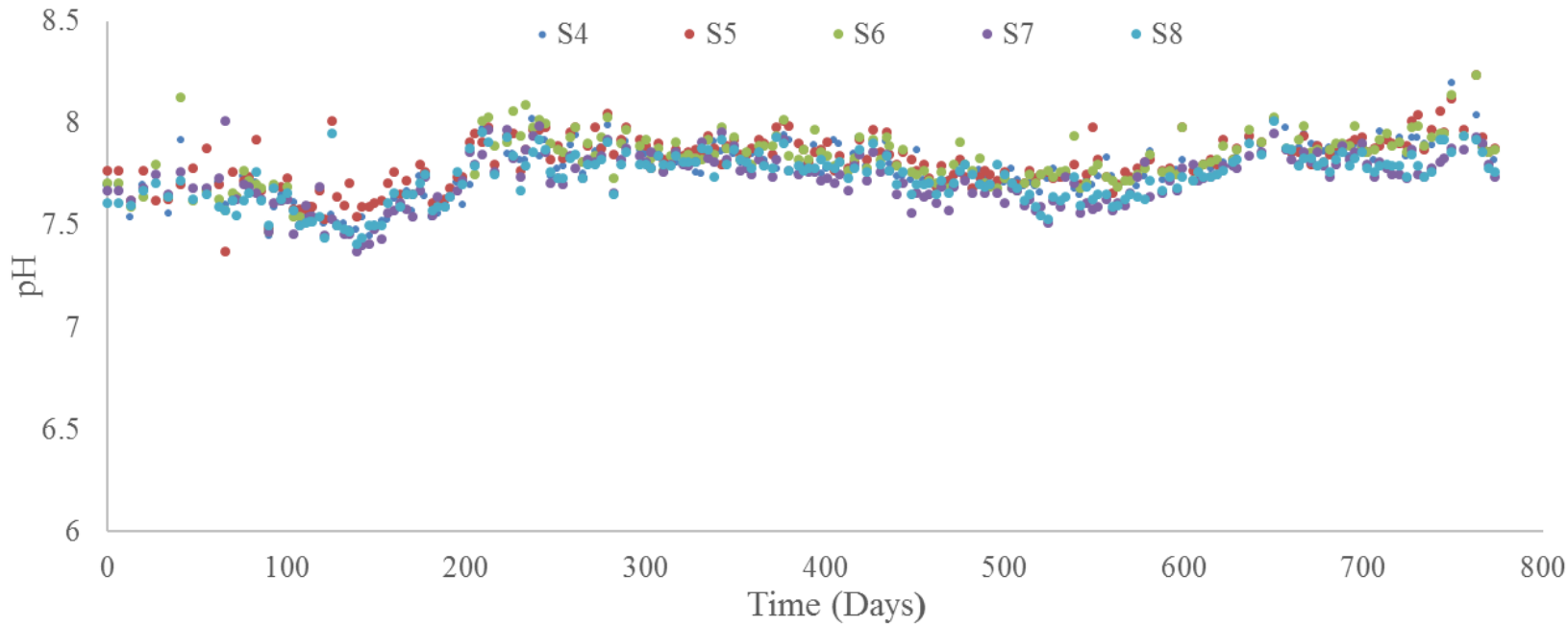


Figure 7.4 pH of digesters S4-S8 between day 0-775

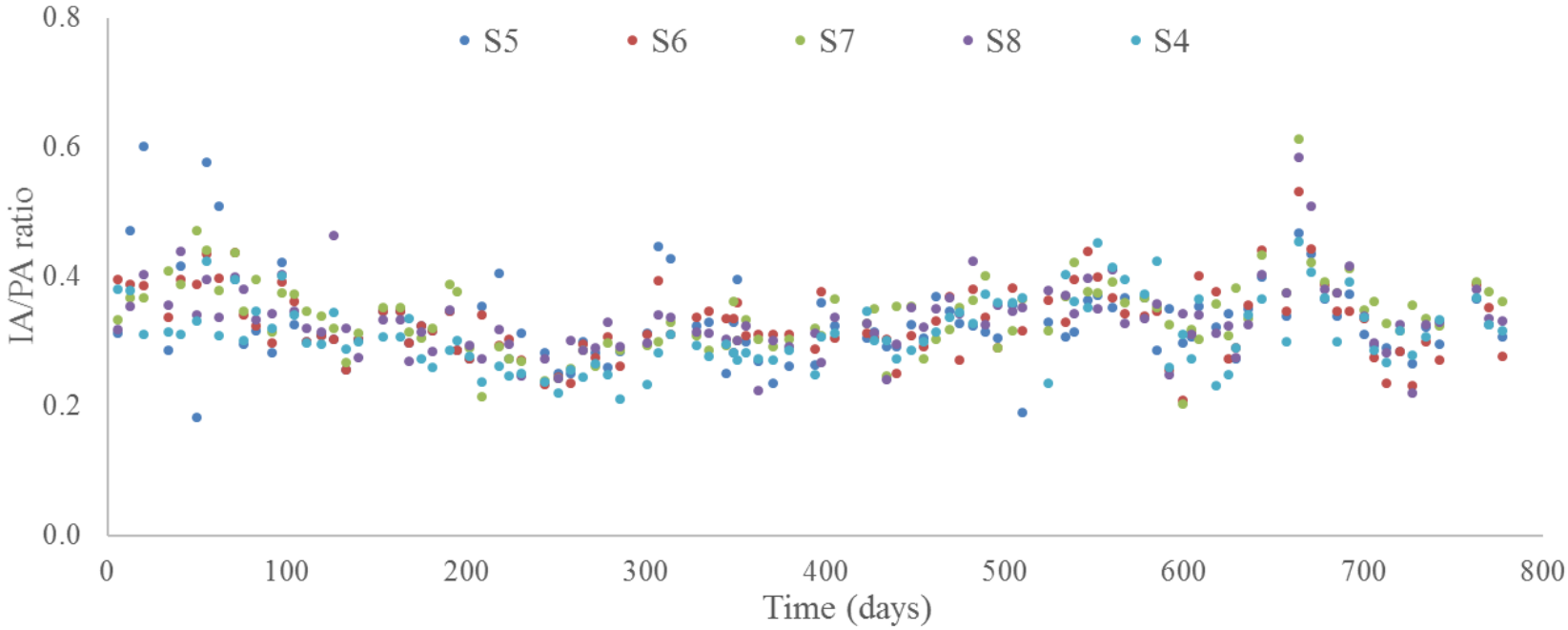


Figure 7.5 IA:PA ratio of digesters S4-S8 between day 0-775

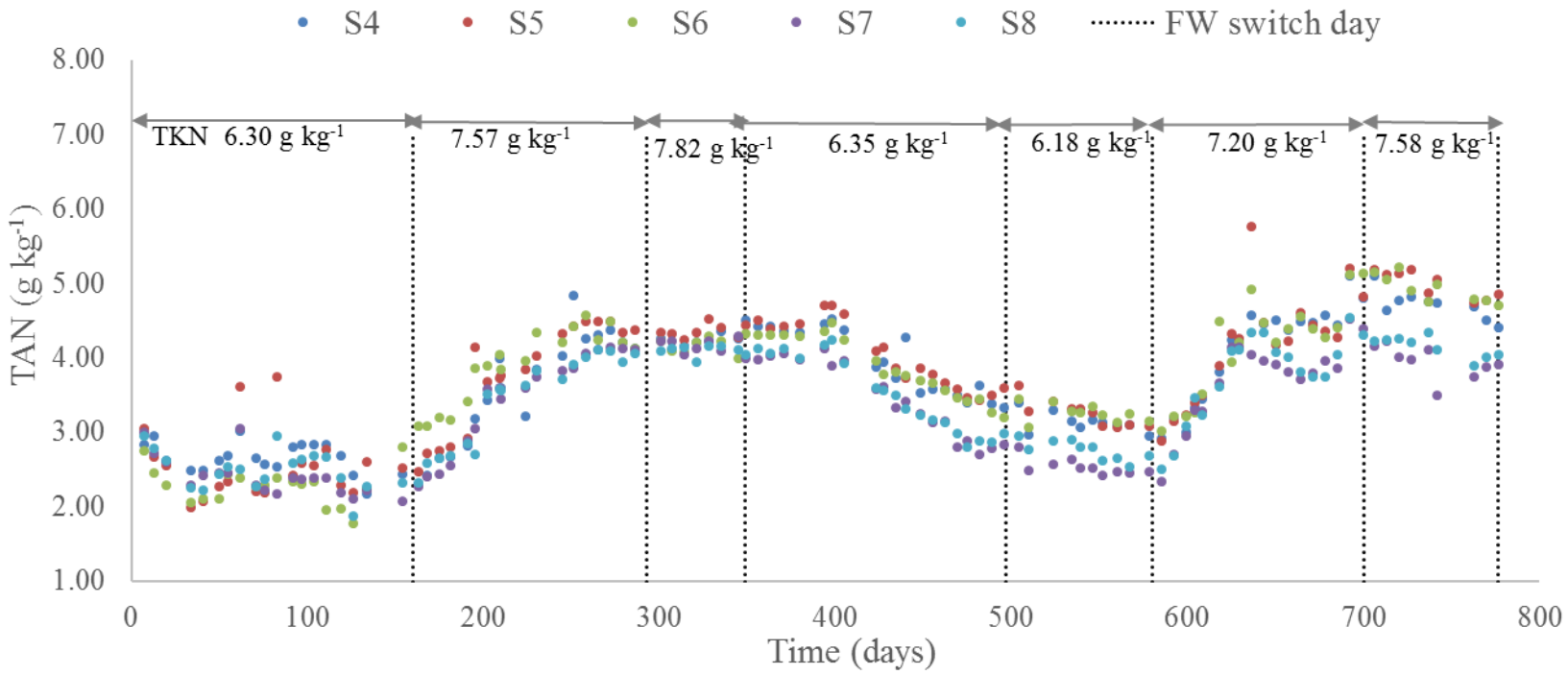


Figure 7.6 TAN of digesters S4-S8 between day 0-775

7.3.2 Performance comparisons between constant OLR and increasing OLR digesters

Specific methane production and volumetric methane production

For control digesters, S5 and S6, at loading 5 kg VS m^{-3} d^{-1}, had consistent VMP and SMP over the trial, fluctuating (Figure 7.7) around 2.29±0.20 m^3 CH_4 m^{-3} d^{-1} and 0.46±0.03 m^3 CH_4 kg^{-1} VS d^{-1} (Figure 7.8), respectively.

In increasing loading digesters S7 and S8, VMP gradually increased as shown in Figure 7.7, reflecting the increase in OLR applied to the digesters up OLR 8 kg VS m^{-3} d^{-1} reached, giving value of 3.85 m^3 CH_4 m^{-3} d^{-1}. After short gas peak after OLR further increased to 9 kg VS m^{-3} d^{-1}, VMP did not show any increase in both S7 and S8 from their steady state, with value of 3.73 m^3 CH_4 m^{-3} d^{-1}. Obvious decline of SMP were observed in digesters S7 and S8 with value of 0.42 m^3 CH_4 kg^{-1} VS d^{-1}, after compared with their control, approximately 10% FW conversion efficiency were lost.

Tables 7.1 gave the average performance values of S5-S8 from steady state at different loadings, in attempt to validate the accuracy of gas production values in case disturb from other factor, e.g. VS content of substrate. It is worth noting that when the loading reached 8 kg VS m^{-3} d^{-1} in S7 and S8, a new batch of food waste (Batch 6, feeding between day 580-700, Table 4.1) was used as feed and this batch of higher VS content (230.8 g VS kg^{-1} FM) food waste accounted for the following SMP increase in all digesters, with 0.48 and 0.46 m^3 CH_4 kg^{-1} VS d^{-1} at loading 5 and 8 kg VS m^{-3} d^{-1}. Methane percentage was detected to be around 62%~64% of biogas, indicating high proteins content existed in this batch Food waste

Table 7.1 Average steady state values of performance indicators for duplicate digesters (S5-S8) at different OLR

FW	Unit	Batch 2		Batch 3		Batch 4		Batch 5		Batch 6		Batch 7	
VS	$g\ kg^{-1}$	187.9		218.7		226.7		208		230.0		222.2	
TKN	$g\ kg^{-1}$	7.57		7.82		6.35		6.18		7.42		7.58	
Sampling days		178-182		314-321		504-509		575-580		677-682		765-777	
OLR	$kg\ VS\ m^{-3}\ d^{-1}$	5	6	5	7	5	7	5	7	5	8	5	9
SMP	$m^3\ CH_4\ m^{-3}\ d^{-1}$	0.44	0.42	0.48	0.46	0.45	0.43	0.46	0.46	0.48	0.46	0.46	0.42
VMP	$m^3\ CH_4\ kg^{-1}\ VS\ d^{-1}$	2.18	2.49	2.39	3.00	2.22	2.98	2.30	3.25	2.40	3.70	2.16	3.73
VSD	ratio	0.76	0.75	0.76	0.76	0.78	0.75	0.75	0.74	0.75	0.74	0.76	0.71
VFA	$mg\ L^{-1}$	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200

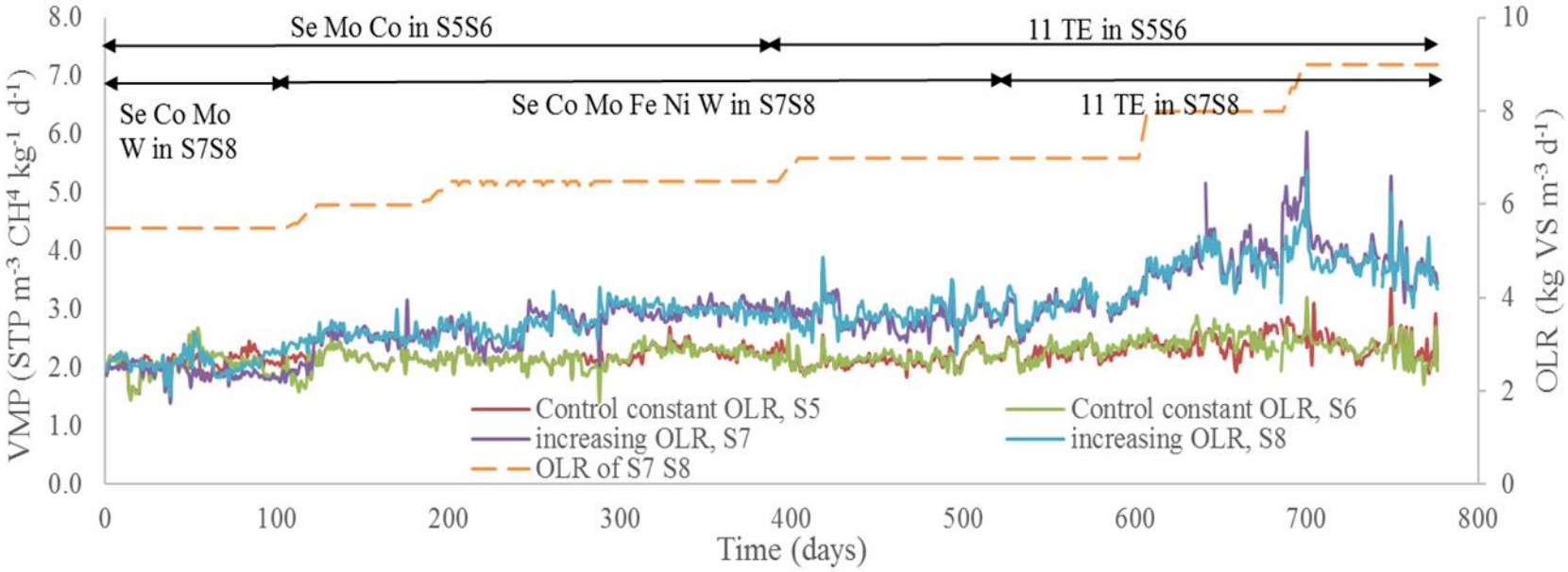


Figure 7.7 VMP comparison between constant OLR digesters and increasing OLR digesters

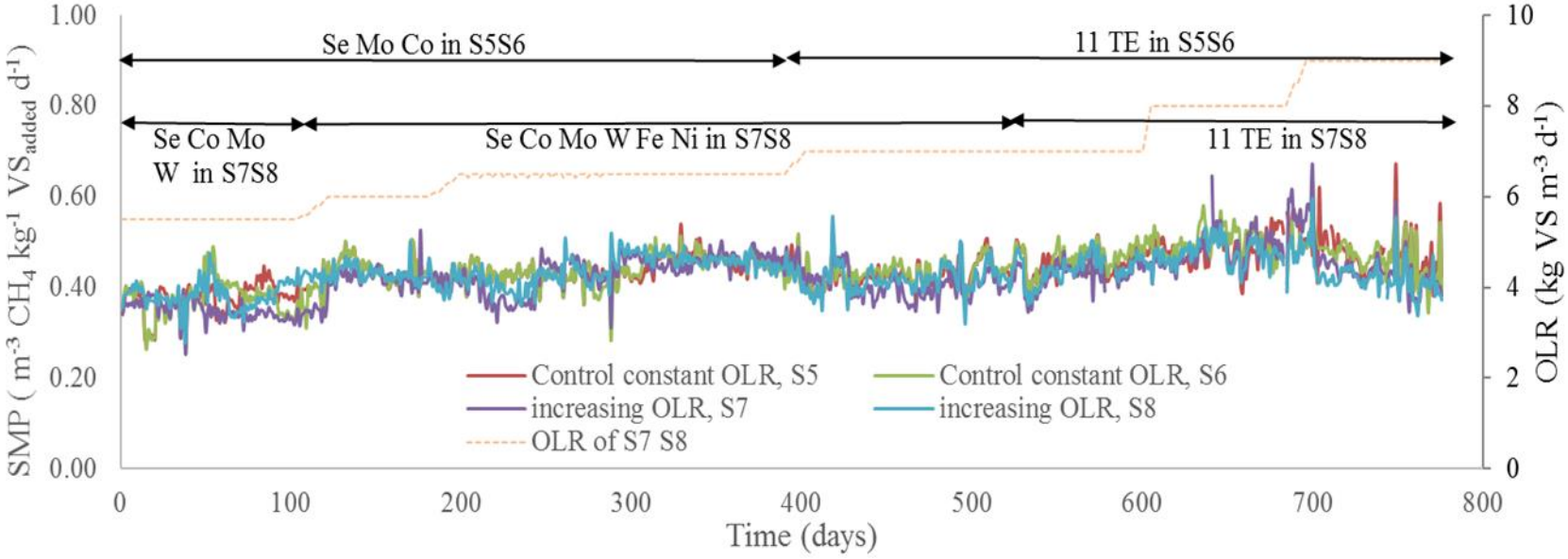


Figure 7.8 SMP comparison between constant OLR digesters and increasing OLR digesters

Volatile solids destruction rate

VSD rate, the ratio of VS removed to VS added, indicates the efficiency of volatile solids degradation in anaerobic digestion. Its calculation equation was described in section 3.4.1 based on mass balance.

Loading changes were introduced in all digesters from day 103, it decreased from 5.5 to 5 kg VS m^{-3} d^{-1} in S5 and S6, increased from 5.5 to 6 kg VS m^{-3} d^{-1} in S7 and S8. VSD drops were observed in all digesters during these days to response operational changes (Figure 7.9). After new batch FW introduced on day 160, VSD rates increased to above 0.75 in all digesters. Sudden increase in VSD rates were observed in all digesters around day 250, almost reaching 0.88. The corresponding SMP and VMP showed an increase in the same period. However, the reasons for this peak were still not clear.

In general, in digesters S5 and S6, VSD rates fluctuated around 0.76 ± 0.02 in most of experimental time (Figure 7.9), whereas the destruction rates in increasing loading digesters S7 and S8 were slightly lower than their controls especially when loading difference became larger. Obvious decline in rates was observed when OLR reached 9 kg VS m^{-3} d^{-1} in S7 and S8.

During the course of loading increase experiments, more TE supplied, from typical elements which were confirmed to be essential to anaerobic digestion, like iron, nickel, up to 11 trace elements when OLR reached critical levels. No sudden and stimulation of methane production was observed in all digesters when more elements introduced, mainly because digesters already reached optimal conversion rate.

As shown in Table 7.1, average VSD rate in digesters S7 and S8 at loading 5~8 kg VS m^{-3} d^{-1} had almost the same values as their control, fluctuated around 0.75. Their OLR, however, declined to 0.71 when loading reached 9 kg VS m^{-3} d^{-1}, compared the destruction rate of 0.76 from their control at 5 kg VS m^{-3} d^{-1}, equivalent to a 1.25% reduction in VSD per kg VS m^{-3} d^{-1} of OLR. If this reduction in VSD rate was taken into account then the SMP at loading 9 kg VS m^{-3} d^{-1} was 0.43 m^3 CH_4 kg^{-1} VS d^{-1}, very close to the value from measurement. This validated the accuracy of biogas measurement.

Based on above SMP and VSD rate results, in could be included that after loading reached 8 kg VS m^{-3} d^{-1}, the onwards increase in OLR will lead to a decrease in VSD rate, as well as SMP. To be precisely, food waste conversion efficiency will lose when its digestion was operated at loading 9 kg VS m^{-3} d^{-1}. However, without efficiency or productivity loss when digester was operated at 8 kg VS m^{-3} d^{-1}, which was assumed to be the maximum loading for food waste digestion.

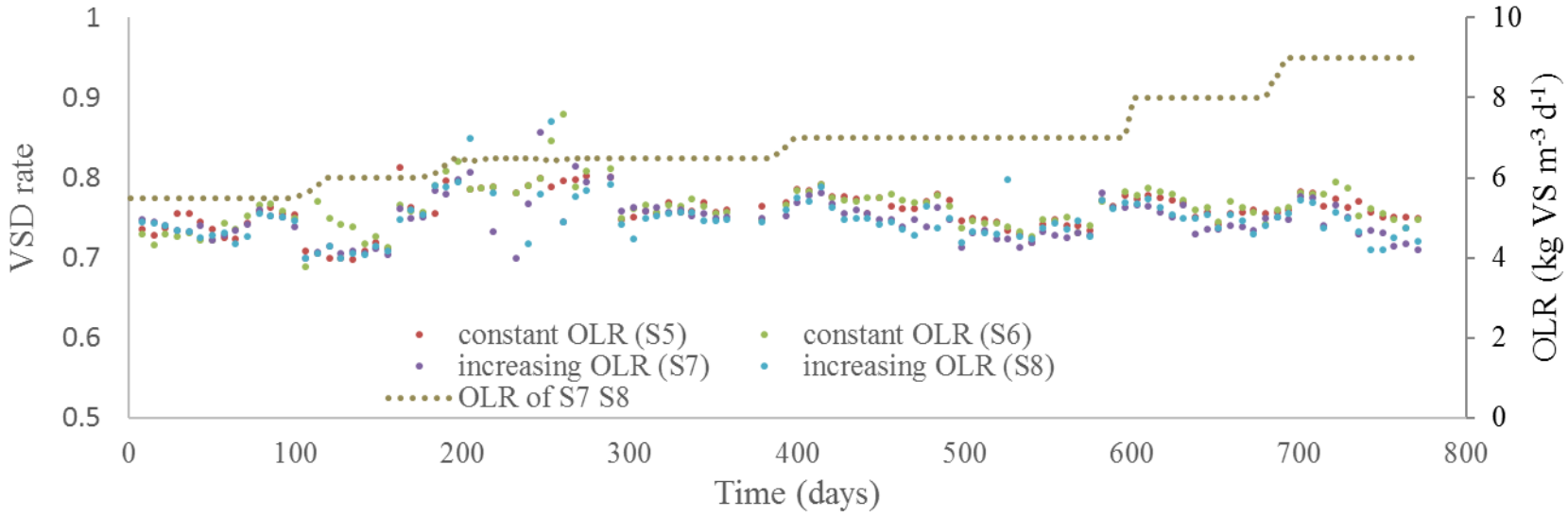


Figure 7.9 VSD rates comparison between constant OLR digesters and increasing OLR digesters

Residual biogas potential of digestate under different loadings

Residual biogas potential (RBP) test was carried out to determine the efficiency of digestion process, as well as to provide an indication of the environmental impacts arising from the use of digestate. According to the updated compliance requirements of PAS 110, specific biogas production (SBP) should not exceed the upper limiting 0.45 m^{-3} biogas kg^{-1} VS.

Digestate taken on day 580 and day 775 from increasing load digesters from stead state, when operating at OLR 7 kg VS m^{-3} d^{-1} and 9 kg VS m^{-3} d^{-1} respectively, and their control digesters of OLR 5 kg VS m^{-3} d^{-1} at the same time were tested over a 28-day period for the residual biogas potential.

Figure 7.10 had shown that at loading 7 kg VS m^{-3} d^{-1} and 5 kg VS m^{-3} d^{-1}, SMP results from RBP test had similar lag phase, and gave very similar final values, 0.124 and 0.126 m^{-3} CH_4 kg^{-1} VS respectively. This indicated that the same VS convert efficiency were obtained from these 2 loadings. SBP of OLR 7 kg VS m^{-3} d^{-1} was slight lower than OLR 5 kg VS m^{-3} d^{-1}, with 0.162 and 0.178 m^3 biogas kg^{-1} VS (Figure 7.11), which meant digestate from these digesters achieved the PAS 110 requirement to digestate on this aspect.

In Figure 7.12, SMP of OLR 9 and 5 kg VS m^{-3} d^{-1} gave values of 0.123 m^3 CH_4 kg^{-1} VS and 0.113 m^3 CH_4 kg^{-1} VS. Adding this value to the average SMP obtained from their digesters fed at 5 and 9 kg VS m^{-3} d^{-1} (Table 7.1) in the same period gave a total of 0.43 and 0.47 m^3 CH_4 kg^{-1} VS d^{-1}, close to the ultimate BMP value, 0.471 m^3 CH_4 kg^{-1} VS in a 28-day test (Yirong 2014), as the BMP result using the food waste from the same source. SBP from Figure 7.13 as well was compliance with the requirements of PAS 100, giving 0.178 and 0.154 m^3 biogas kg^{-1} VS respectively.

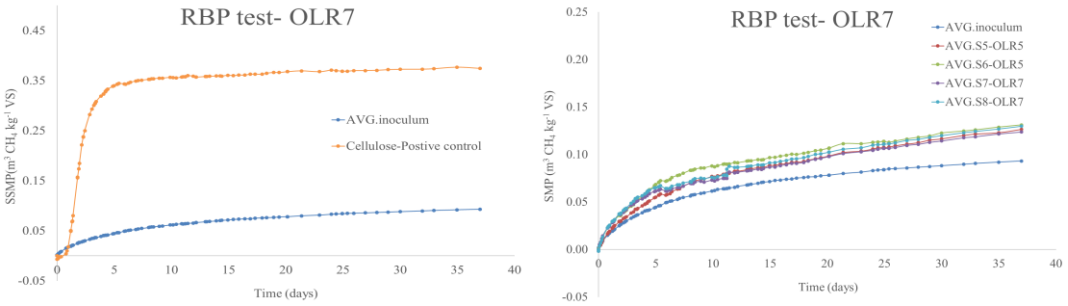


Figure 7.10 SMP in RBP test under OLR 5 and 7 kg VS m^{-3} d^{-1}

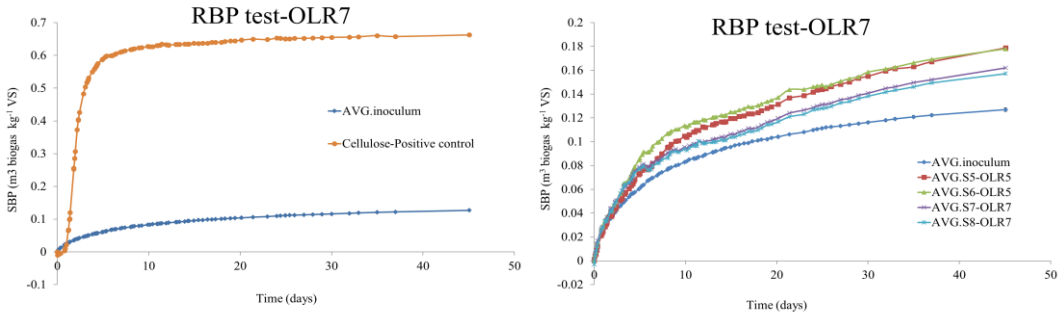


Figure 7.11 SBP in RBP test under OLR 5 and 7 kg VS m^{-3} d^{-1}

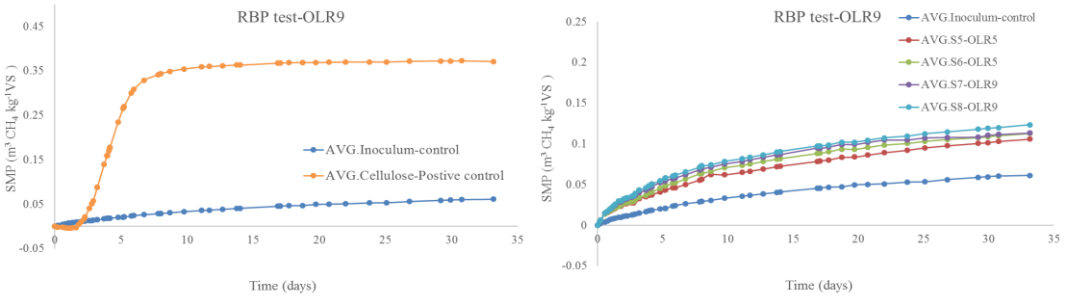


Figure 7.12 SMP in RBP test under OLR 5 and 9 kg VS m^{-3} d^{-1}

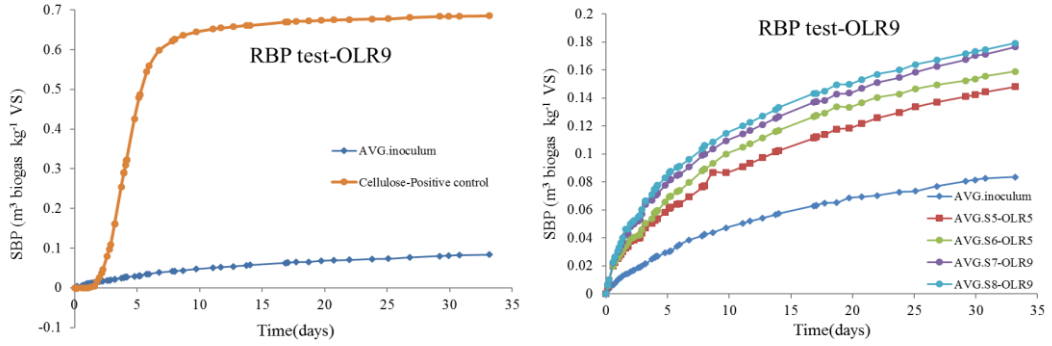


Figure 7.13 SBP in RBP test under OLR 5 and 9 kg VS m^{-3} d^{-1}

The bottleneck step of FW digestion at OLR of 9 kg VS m^{-3} d^{-1} was thought to be hydrolysis, as the HRT at this OLR was only 23 days. As mentioned above, the BMP result using the FW from the same source showed that methane production was 0.43 m^3 CH_4 kg^{-1} VS d^{-1} after day 23 (equal to HRT at OLR 9 kg VS m^{-3} d^{-1}), 9.5 % lower than the ultimate BMP value of 0.471 m^3 CH_4 kg^{-1} VS in a 28-day test (Yirong 2014). The extent of that decrease in methane production was comparable to the 8.6% decreased observed between OLR of 9 and 8 VS m^{-3} d^{-1}, reflecting the lower FW conversion efficiency.

In general, this result was in agreement with what could be achieved in CSTR digesters fed with food processing wastes (Schmidt, Pröter et al. 2013). It is difficult, however, to make a rigorous comparison between these studies due to different substrate characteristics, especially particle size, VS and moisture contents. This was because OLR determined HRT and SRT in once-through CSTR design (Fdez-Guelfo, Alvarez-Gallego et al. 2012). Depending on the substrate characteristics, each system presented an optimal HRT, below which the stability and/or conversion efficiency of the digestion was affected either by washing out of the slow-growing acetogens and methanogens or by shortening the duration for hydrolysis. The maximum loading achieved in this study therefore can only be regarded as a guide for FW digestion systems and the real limit for each substrate and digestion system has to be identified in practice.

7.3.3 Performance Comparisons between random OLR and constant OLR digesters

As comparisons between constant loading digesters S5 and S6 and increasing loading digesters S7 and S8, gas production and VS destruction are assessed between random loading digester and its controls in this section. Stability parameters have already been discussed in section 6.3.1.

30 day rolling volumetric methane production

In the period (day 0 – day 181) which S4 was running at OLR 5.5 and then 6 kg VS m^{-3} d^{-1}, the VMP in S4 is clearly higher than controls which worked at 5.5 kg VS m^{-3} d^{-1} until random OLR (range from 2.5~7.5 kg VS m^{-3} d^{-1}) introduced since day 181 (Figure 7.14).

Random loading operations introduced since day 181. Due to the variation of daily loading, it was difficult to directly compare its daily methane production with constant loading digester, in this case 30 day rolling volumetric methane production was used for comparison, using average volumetric methane production value of 15 days before and 15 days after's.

To evaluate the accuracy of methane production of random loading digester S4 calculated based on 30-day rolling average VMP. Actual and calculated values of S5 and S6 were shown in Table 7.2. Both values gave approximately similar values from the same period, supporting the accuracy of the methane production of S4. Measured values were in the range of ~ 10 % of calculated values, indicating a good agreement.

Table 7.2 Measured and Calculated values of VMP in constant loading digester S5 and S6

Time (days)	Measured value VMP*	Calculated value of VMP	
	Average VMP from Table 7.1	Average calculated VMP in S5	Average calculated VMP in S6
314-321	2.39	2.37	2.35
504-509	2.22	2.22	2.20
575-580	2.30	2.29	2.34
677-682	2.40	2.50	2.40
765-777	2.16	2.21	2.17

- * VMP unit: m^3 CH_4 kg^{-1} d^{-1}

No obvious production difference was found between S4 and constant loading digesters S5 and S6, all of their VMP fluctuating around 2.27 STP m^3 kg^{-1} d^{-1}. It is noteworthy that after larger loading range (0~10 kg VS m^{-3} d^{-1}) introduced in S4 since day 632, 30-day rolling VMP fluctuated more frequently in small magnitude, responding to loading variation frequency, implying good buffering capacity in

random loading digester. Additionally, after compared VMP of S4 its own gas production from different OLR range periods, it was observed that more smooth methane production was produced in loading range (2.5~7.5 kg VS m^{-3} d^{-1}), which could be recommended for real world operation.

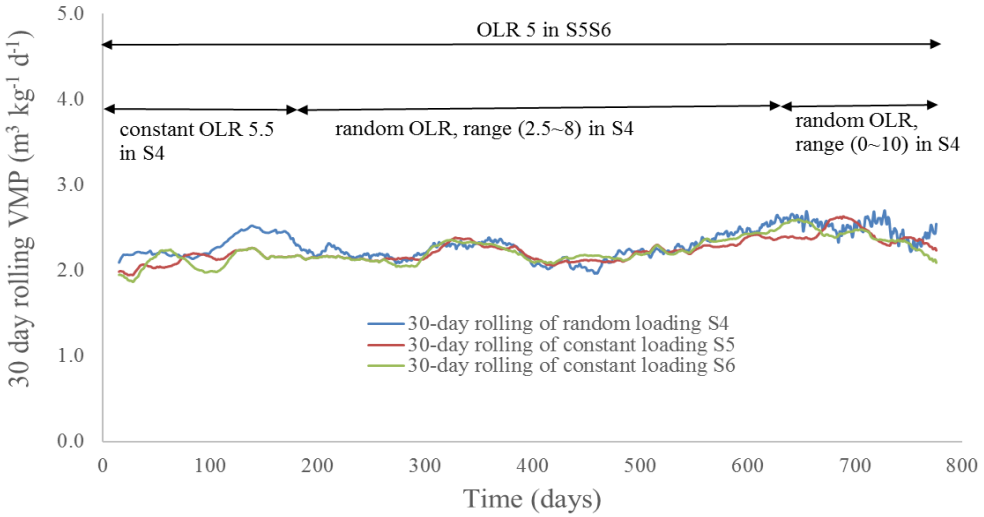


Figure 7.14 30 day rolling VMP in random loading S4 and constant loading digesters S5 and S6

Volatile solids destruction rate

Figure 7.15 showed the VSD rate comparison between random loading and constant loading digesters. The VSD curves were not smoothly due to the random loading applied. Random loading introduced on day 181, fluctuation of VSD rate was then observed in digester S4. Due to similar VSD rate fluctuation happened in its controls, it is unlikely to distinguish the reason for the fluctuation during these days. However, in the following experimental days, no apparent changes were observed in S4 until the end, in which VS destruction rate was stable around 0.76, almost the same as its control digesters S5 and S6.

Referring to VMP and VSD rates, for random loading digester, no loss of biogas performance and digestion efficiency were observed. Combined with well control of stability from section 7.3.1, it indicated that FW digester has well resilience to frequent loading variation with sufficient TE supplementation. Results from this

experiment is total different from previous studies, most of which indicated that transient loading could cause performance loss or process imbalance (Grimberg, Hilderbrandt et al. 2015, Kim and Lee 2015). According to their studies, loadings were always ranged in lower loadings with small magnitude. In the current case, however, given weekly average OLR at 5 kg VS m^{-3} d^{-1}, digester had well performance within loading range of 0 ~ 10 kg VS m^{-3} d^{-1}. This finding has significant meaning for commercial plant as stability control and performance efficiency are both achieved.

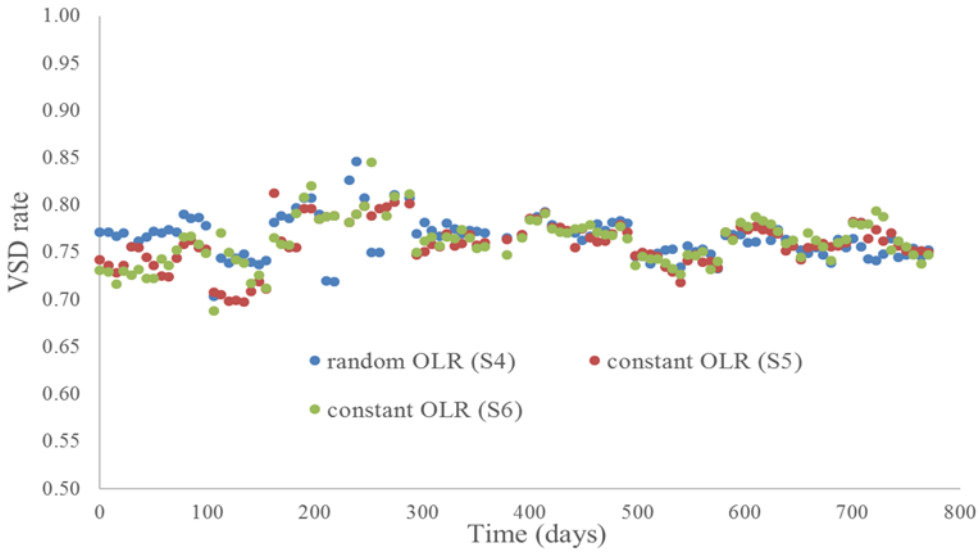


Figure 7.15 VSD rates comparisons between constant OLR digesters S5, S6 and random OLR digester S4 between day 181-775

7.3.4 Relationship among OLR, biomass concentration and TE requirements

TAN showed lower concentration at higher loading in FW digesters, as interesting finding that were mentioned particularly in section 5.3 and section 7.3. In anaerobic digestion, TAN mainly reflects the concentration of the TKN in the feedstock. Lower ammonia concentration was often observed at higher loading rate when a significant amount of nitrogen released from the hydrolysis in the form of ammonia was then used for cell synthesis by the microorganisms. According to Monod Equation, this decrease was due to more nitrogen was biological fixed by biomass at higher loading, namely biomass concentration increased in high loading digester based on the level

of biological fixed nitrogen (BFN). The detail of the Equation interpretation will be described in appendix III. Nitrogen distribution in digester raised attention in this study as it can be used to estimate microbial biomass density in digester and link to the required strength of TE supplementation.

Nitrogen exists in 3 forms in digester: ammonia nitrogen ($TAN_{digester}$), residual food waste ($N_{FW\text{-}residual}$), biomass in digester (N_{BFN}). Microbial biomass concentration in digester was indicated at BFN level, when the concentration of N_{BFN} changed, it reflected the biomass concentration in digester. Concentration of N_{BFN} in this study was calculated through mass balance equation described in section 3.4.3.

To evaluate the reliability of nitrogen mass balance equation developed in this study, estimated values based on VS Destruction rate was compared with the values from TKN of digestate and VS Destruction rate, data spreadsheet was listed in Appendix II. These 2 group of values were in the range of ~ 15 % of differences, indicating a good agreement.

To evaluate the biomass concentrations in different loading digesters, representative samples were taken from steady-state operational period for TKN determination, which were at loading 7, 8, 9 kg VS m^{-3} d^{-1} respectively, using the same method for food waste TKN determination in section 3.4.8. Digestate from loading 5 kg VS m^{-3} d^{-1} digester were also sampled at the same time for TKN determination. Since loading 6 kg VS m^{-3} d^{-1} was performed at earlier stage of this PhD study (before day 200), digestate samples were not kept for TKN measurement, some results from loading 6 kg VS m^{-3} d^{-1} were not presented, neither in Figure 7.16. In addition, when digester operated at 6 kg VS m^{-3} d^{-1}, its VSD rate was not as steady as when it operated at other loadings (Figure 7.15), therefore a representative sample was not ensured from digester at loading 6 kg VS m^{-3} d^{-1}. Therefore, the parameters used for calculation were based on measure TKN concentration in Table 7.4.

Table 7.3 showed the ratios of important parameters values from average of S7 and S8 to their control of average of S5 and S6 at the same period, all of figures from Table 7.3 were plotted in bar chart in Figure 7.16 for better comparison. Both BFN and DBP ratios showed positive increase, whereas negative increases were found in TAN, SMP and $SMP/VS_{removal}$ ratios. These comparisons revealed behaviour patterns

of these digesters performances. e.g. when OLR changes, $SMP/VS_{removal}$ which implies FW conversion efficiency, had similar values as loading 5, 7, and 8 kg VS m^{-3} d^{-1}, thus no methanogen activity inhibition was observed at 8 kg VS m^{-3} d^{-1}. In particular, when OLR increased up to 9 kg VS m^{-3} d^{-1}, specific increase rates in BFN and $SMP/VS_{removal}$ were found to below than that in OLR 8 kg VS m^{-3} d^{-1}, revealing that biomass growth rate decreased at OLR 9 kg VS m^{-3} d^{-1}. This is because methanogenic activity depends on the population density of methanogens. When population increase rate was inhibited, their methanogenic activity were inhibited. The reason for this decreases could be that biomass reached their full capacity after OLR reached 8 kg VS m^{-3} d^{-1}, or biomass washing out rate overwhelmed biomass grow rate, since HRT at loading 9 kg VS m^{-3} d^{-1} is short. This finding provided evidence that why OLR 9 is overloaded for FW digester.

Since the biomass growth rates appeared to increase with OLR, the relationship between biomass concentration and their requirements to trace elements strength could be revealed. As observed, when biomass concentrations increased, higher concentration of TE was required, for maintaining stable anaerobic digestion. Since increase of biomass concentration was caused by OLR increase, it could be concluded that after OLR increased, the strength requirements to trace elements increased. This was proved by results of section 5.2.3, in which Se became limiting when OLR increased from 1.8 to 2.5 kg VS m^{-3} d^{-1}, after Se strength increased in digester, VFA accumulation disappeared. Also in trace elements washed-out experiments, VFA appeared earlier in digester with OLR 5 kg VS m^{-3} d^{-1} than 4 kg VS m^{-3} d^{-1}, due to trace elements deficiency appeared earlier in higher loading digester.

It is observed that in anaerobic digester TE supplementation stimulated microbial capacity increase (Karlsson, Einarsson et al. 2012, Lindorfer, Ramhold et al. 2012), whereas biomass concentration increased when loading increase was rarely reported. In this study, it is clear indicated that when digester operated at possible high loading, microbial biomass concentration increased with organic loading. Moreover, the link between OLR and trace elements concentrations was developed through their relationship with biomass concentration, following the rule that along with OLR

increase, biomass concentration increased, the strength requirements to trace elements increase.

Table 7.3 Ratios of OLR, TAN, BFN, SMP and DBP of increasing loading/constant loading digesters, using performance values of OLR 5 kg VS m^{-3} d^{-1} as control 1

	OLR5	OLR7	OLR8	OLR9
OLR ratio	1	1.4	1.6	1.8
TAN ratio	1	0.78	0.83	0.75
BFN ratio	1	1.18	1.29	1.26
$SMP/VS_{removal}$ ratio	1	1.01	0.98	0.93
DBP ratio	1	1.38	1.62	1.67

7.4 Conclusion

Supplementations of 11 TE at their designated strengths were sufficient to maintain stability of FW digestion at high loading without any VFA accumulation. Maximal OLR was determined at OLR 8 kg VS m^{-3} d^{-1}, when FW digestion had no performance loss, whereas less productivity was shown at OLR 9 kg VS m^{-3} d^{-1}. As no VFA accumulation appeared at OLR 9 kg VS m^{-3} d^{-1}, stable operations were still achieved, but hydrolysis became the limiting step at short HRT.

Relationship between OLR and TE requirement was established in this study based on nitrogen mass balance. It became clear that high microbial biomass density was required at higher OLR, which meant the minimum dosing strength of TE supplementation was not a constant for a certain type of substrate, but relevant to the OLR applied.

Table 7.4 All parameters needed/calculated for/from nitrogen mass balance calculation

Sample Day[-a]	OLR	VS of FW (%)	TKN of FW (g kg^{-1})	TKN of digestate (g kg^{-1})	TAN of digestate (g kg^{-1})	SMP[-b]	DBP[-b]	VSD[-b]	SMP per $VS_{removal}$	BFN (g kg^{-1})
580	5.0	21.18	6.3	8.50	2.84	0.464	14.77	0.75	0.613	**3.54**
	5.0	21.18	6.3	8.50	2.79	0.463	14.77	0.75	0.613	**3.59**
	7.0	20.08	6.18	8.10	2.38	0.459	21.32	0.73	0.616	**3.53**
	7.0	20.08	6.18	8.16	2.47	0.464	21.84	0.74	0.622	**3.57**
679	5.0	20.55	7.20	9.78	4.60	0.473	15.65	0.75	0.627	**2.73**
	5.0	20.55	7.20	9.78	4.56	0.486	15.7	0.76	0.645	**2.87**
	8.0	20.55	7.20	9.90	3.72	0.462	25.01	0.74	0.622	**3.61**
	8.0	20.55	7.20	9.86	3.8	0.474	25.42	0.75	0.627	**3.59**
763	5.0	23.2	7.58	9.78	4.72	0.502	15.95	0.75	0.658	**2.71**
	5.0	23.2	7.58	9.78	4.74	0.480	15.34	0.766	0.623	**2.79**
	9.0	23.2	7.58	9.88	3.51	0.435	26.06	0.71	0.606	**3.51**
	9.0	23.2	7.58	9.75	3.60	0.424	25.88	0.72	0.583	**3.42**

[-a] Measured sample was chosen from period in which digesters stable operated for more than 2 HRTs
[-b] SMP, DBP, VSD was calculated based on weekly average, in which week digestate sampled

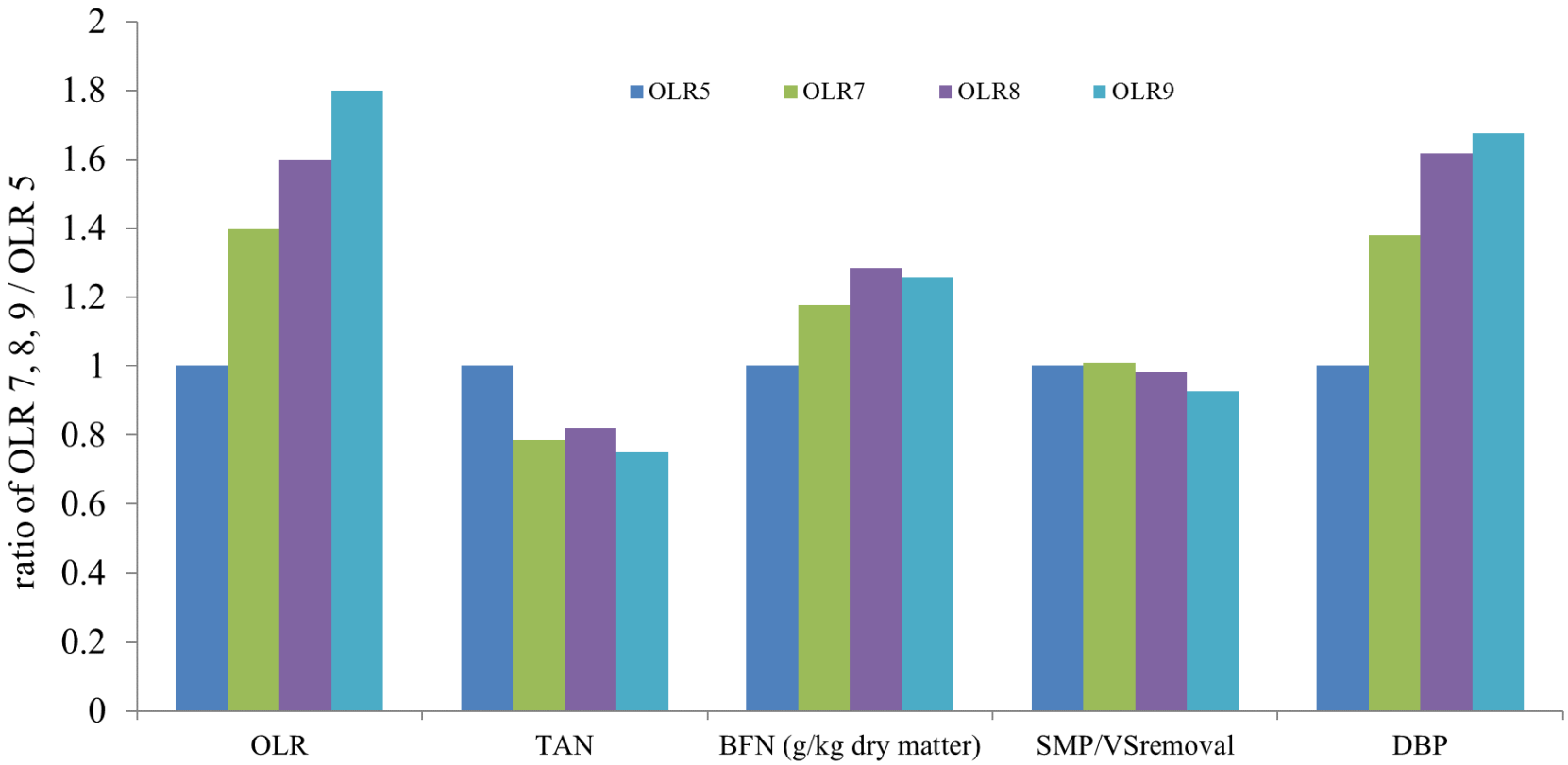


Figure 7.16 Ratios of OLR, TAN, BFN, SMP/$VS_{removal}$ and DBP of increasing loading/constant loading digesters, using performance values of OLR 5 kg VS m^{-3} d^{-1} as control 1. Comparisons between OLR5&7&8&9 kg VS $m^{-3}d^{-1}$, samples were chosen from stable operation days on 580, 679, 763.

CHAPTER 8 TE requirement at high organic loading rate

8.1 Introduction

In previous experiment, supplementation of 0.3 mg kg^{-1} of Co and 0.2 mg kg^{-1} of Se was confirmed to be sufficient for maintaining stable performance at moderate loadings range of 3~5 kg VS m^{-3} d^{-1}, as described in Chapter 5. It was also identified in chapter 7 that the density of microbial biomass was increased when digesters were operated at higher loading. Considering the relative stable TE concentrations in microbial cells, all of these raised the question on whether the TE combination confirmed at moderate loading (i.e. Co and Se), was sufficient for stable FW digester at higher loading which had been achieved with the supplementation of 11 trace elements. In this study, the performance of FW digesters operated at maximal loading with only certain strength of Co and Se addition was therefore tested.

8.2 Methodology

Five 5-L CSTR digesters S4-S8, were used for this experiment. This trial followed the digestion trial descried in Chapter 6 and from operated from day 776 until day 1068 when counted continuously. The initial experimental conditions of these 5 digesters were listed in Table 8.1. TE supplementation strategy was changed since the start point- day 776. All digesters were monitored to evaluate whether VFA accumulated after long-term TE washing-out. When VFA accumulation occurred, Co strength was increased to 0.5 mg kg^{-1} to test if VFA production was caused by deficiency of Co and could be rectified by this operation. If no degradation of VFA observed, other trace elements (from the full package of 11 TEs used) would be introduced.

Table 8.1 The operational scheme of the digesters trial

Digesters/ Initial condition	Objective	Operations during trial
S5, S6 OLR 5 kg VS m^{-3} d^{-1}, 11 TE addition, VFA< 500 mg L^{-1}	To determine whether 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were essential for OLR 5 kg VS m^{-3} d^{-1}	Keep OLR at 5 kg VS m^{-3} d^{-1} until end of experiment, stop TE supplementation except 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se from day 776 11 TE re-supplemented to digesters from day 1053 at time of VFA accumulation
S7, S8 OLR 9 kg VS m^{-3} d^{-1}, 11 TE addition, VFA< 500 mg L^{-1}	To determine whether 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were essential for OLR 8 kg VS m^{-3} d^{-1}	Decrease OLR to 8 kg VS m^{-3} d^{-1}, stop TE supplementation except 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se from day 776 increase Co concentration to 0.5 mg kg^{-1} from day 875 at time VFA increased to more than 3000 mg L^{-1}
S4 random OLR (weekly average 5 kg VS m^{-3} d^{-1}) 11 full TE addition, VFA< 500 mg L^{-1}	To determine whether 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were essential for random OLR (weekly average 5 kg VS m^{-3} d^{-1}) digester	OLR was kept in the range of (0~10 kg VS m^{-3} d^{-1}), given weekly average of 5 kg VS m^{-3} d^{-1}, stop TE supplementation except 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se from day 776, OLR was switched to daily constant OLR 5 kg VS m^{-3} d^{-1} from day 966

8.3 Results and discussion

8.3.1 Stability parameters in all digesters

Stability indicator –VFA

VFA concentrations in this trial were closely monitored as it was used as the indicator to determine if additional trace element addition was necessary. In this trial, when VFA accumulation appeared in digesters, increasing supplementation strength of Co was taken as the first strategy, in order to assess if VFA accumulation was

caused by Co deficiency. When this strategy failed, other trace elements dosing strategies were taken into consideration.

As shown in Figure 8.1-a and b, VFA accumulated in both pairs of digesters at OLR 5 kg VS m^{-3} d^{-1} and 8 kg VS m^{-3} d^{-1}, but appeared earlier in digesters at high OLR. About 2500 mg L^{-1} VFA production firstly occurred around day 810 in digesters S7 and S8 when they were only operated with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se addition for 1.5 HRTs. Co strength was then increased to 0.5 mg kg^{-1}, to ensure Co efficiency or availability in digesters. Propionic acid was consumed temporarily and remained below 5000 mg L^{-1} for another until day 1000, when excessive VFA accumulation observed. VFA accumulated to higher than 10000 mg L^{-1} in 20 days. pH dropped sharply and corresponding IA:PA ratios increased. Propionic acid accounted for most of the VFA accumulation, while acetic acid had a low concentration of less than 1000 mg L^{-1}. The failure of S7 and S8 implied that even in the presence of high Co and Se concentration, digesters was still unlikely to stable operate at maximal achievable loading when full TE applied, and trace elements for propionic acid degradation were also required.

After VFA accumulation appeared in S7 and S8, a small-scale test was carried out in sets of 1-L CSTR digesters, to identify which additional elements from the pool of 11 TE were required for stable FW digestion at 8 kg VS m^{-3} d^{-1}. However, no conclusive results were obtained from that test; the results and discussion were therefore presented in Appendix IV.

Compared with S7 and S8, the magnitude of VFA accumulation in S5 and S6 was less significant. VFA appeared in S5 and S6 at loading 5 kg VS m^{-3} d^{-1} after 3 HRT operations since day 776 when TE washed out except Co and Se (Figure 8.1-a). VFA accumulation in S6 reached 10000 mg L^{-1} on day 960, with propionic acid as the dominant species. Forming problem appeared in S6 since VFA accumulation, which caused the following observation and working capacity control difficult. The accumulated VFA, however, was consumed during the following 15 days, dropping to 3900 mg L^{-1} in S6 on day 982. As its duplicate S5 had less VFA production in which acetic acid accounted for most, around 2000 ~ 4000 mg L^{-1} in total but with very few propionic acid. In the following trial period VFA concentration changes

were observed, it remained around 4000 mg L^{-1}. To test if supplementation of more TE species could bring down VFA production before the end of experiment, 11 (full) trace elements as their initial TE strategy were introduced into S5 and S6 on day 1053, VFA concentration decreased to below 500 mg L^{-1} in the following 10 days in both S5 and S6. VFA behaviours in S5 and S6 were unable to give a confirmed conclusion that whether other element(s) except Se or Co was/were deficient at loading 5 kg VS m^{-3} d^{-1}, only that with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se, food waste digester could stable operate with elevated acetic acids concentration.

VFA production in S5 and S6 were different from digester operated with the same condition in S2 and S3 in section 5.3.2, in which no > 1000 mg L^{-1} VFA existed. It was also different from VFA behaviours in Test 2 in section 6.3, due to in Test 2, VFA accumulation were much severely, and propionic acid accounted for most, short acclimation period was the main reason. Besides, foaming existed in digester S6 when VFA accumulated in this digester, and occupied the head space of this digester, A high OLR can cause foaming because the excess feedstock is not fully degraded by microorganisms in the digester, resulting in the accumulation of hydrophobic or surface-active by-products (Ganidi, Tyrrel et al. 2009). In digestion of municipal wastewater biosolids, foaming usually occurs in digesters operating at OLR higher than 4.5 kg VS m^{-3} d^{-1} (Brown and Sale, 2002). In this study, forming should be caused by VFA production caused by TE washing out. Methanogens were unable to effective degrade excessive VFA due to their activity was reduced by lack of TE at loading 5 5 kg VS m^{-3} d^{-1}. However, why forming did not appear in S5 in the same time was not clear.

Figure 8.1-c shows the VFA profile of S4.VFA was always less than1000 mg L^{-1} in this digester throughout the trial. 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se were confirmed to be sufficient for digester at random loading which gave weekly average value at 5 kg VS m^{-3} d^{-1}. Stable operation achieved in S4 implied that with random loading, digester had higher adaptability with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se addition. In commercial plant, excessive TE supplementation and strict OLR control raised budget cost and technique difficult, successful operation in S4 with random loading and defined TE supplementation has significant meaning for waste management

industry. This finding also supported the hypothesis that VFA productions in S5 and S6 were due to their less buffering capacity in constant loading at 5 kg VS m^{-3} d^{-1}.

To conclude, at a constant loading of 5 kg VS m^{-3} d^{-1}, anaerobic digestion could be maintained by essential Co and Se supplementation in food waste digester, although a certain amount of VFA accumulation occurred in digesters with constant loading. Although the effect of shock or transient loading on anaerobic treatment has been widely investigated (Ketheesan and Stuckey, 2015), there has been little research on random loading variations as tested in this study. The only similar work found also confirmed that the anaerobic system was able to adapt to the periodic substrate perturbation and better results could be achieved compared to constant loading, and a long-term change in microbial community was used to explain the good performance in digester experiencing loading perturbation (Xing, Criddle et al. 1997).

It was obvious that Co and Se were unable to maintain stability for digesters at a maximum loading of 8 kg VS m^{-3} d^{-1}, and some other TE seemed to be limiting. This raised the issue of nutrient limitation and co-limitation. The initial Liebig's law of the minimum implies that there is a single limiting nutrient which controls the yield (de Baar, 1994), but this concept is frequently expanded to co-limitation due to the simultaneous scarcity of more than one nutrient. This alternative interpretation of multiple potentially limiting nutrients reflects the complexity of trace metal functions at the physiological and ecological levels, rather than on the biochemical level (Saito et al., 2008). In the context of anaerobic digestion systems, the extent of trace element deficiency was therefore related to OLR applied.

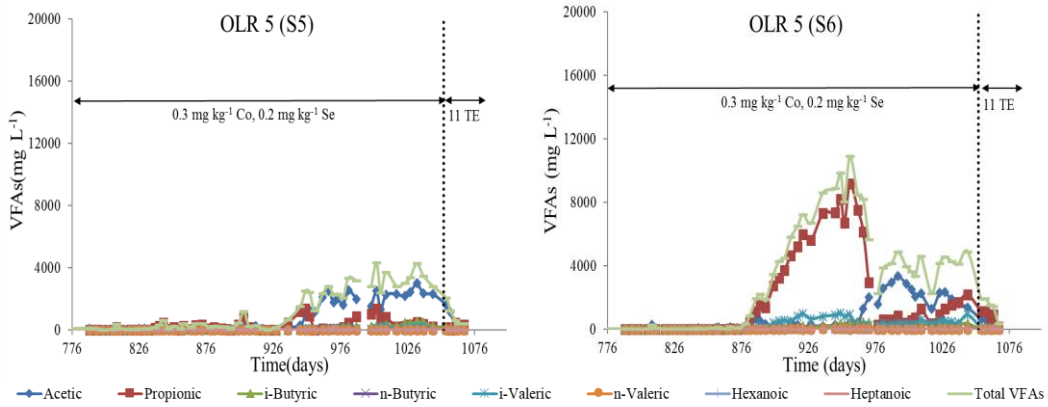


Figure 8.1-a VFA profiles of OLR 5 kg VS m^{-3} d^{-1} digesters S5 and S6 since day 776 until the end of trial

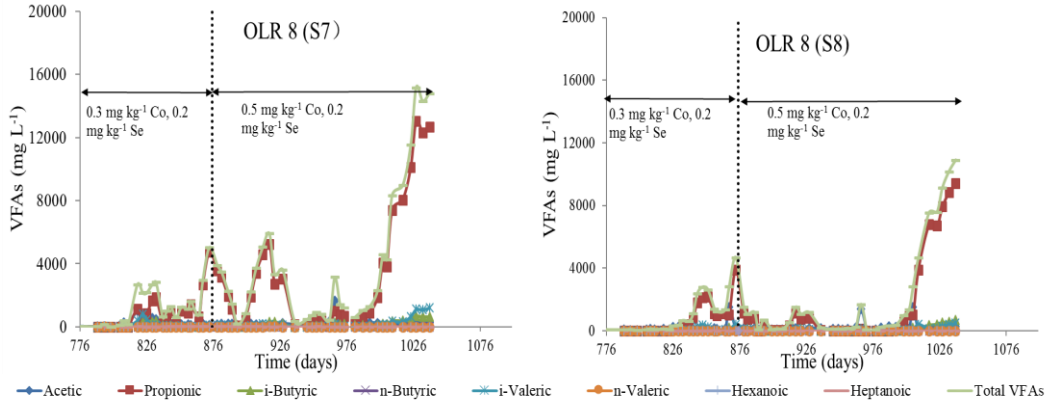


Figure 8.1-b VFA profiles of OLR 8 kg VS m^{-3} d^{-1} digesters S7 and S8 since day776 until the end of trial

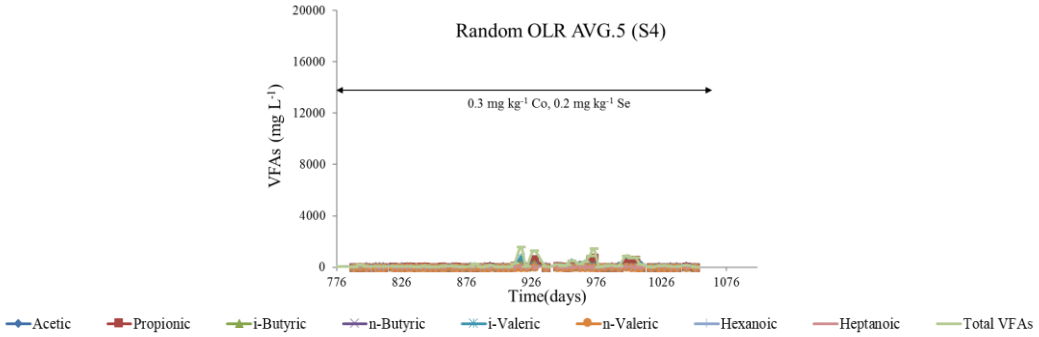


Figure 8.1-c VFA profiles of random OLR (AVG.5 kg VS m^{-3} d^{-1}) digesters S4 since day 776 until the end of trial

Figure 8.1 VFA profiles of S4-S8 since day 776 until the end of trial

General parameters

Figure 8.2 showed the pH values during this trial. When digester was operated with sufficient TE addition in the start period, pH was unlikely to response to TE washing out, fluctuating around 7.90 in S4-S6, and slight lower in S7 and S8. VFA production in the following period did not affect pH in S5 and S6 until the end of trial. pH in S7 and S8 at OLR 8 kg VS m^{-3} d^{-1} first dropped to below 7.50 then below 6.50 in 20 days. For digester S4 with random loading, which kept around 7.90 through the trial, loading changes which switched from random OLR to constant OLR on day 966 did not affected pH either.

Figure 8.3 showed the IA:PA ratio values of all digesters, all of which stabilised at around 0.30 initially. Certain VFA level existing in digester S5 and S6 showed no obvious affect to IA:PA ratio, except around 8000 mg L^{-1} propionic acid accumulation around day 950. IA:PA ratio decreased after this propionic acid consumed. In digesters S7 and S8 at OLR 8 kg VS m^{-3} d^{-1}, fluctuations of IA: PA ratio were frequent, in response to VFA accumulation. When massive VFA accumulated since day 1020, IA:PA ratio increased severely up to 4.00. No IA:PA ratio changes were observed in digester S4, random loading switching to constant one did not show effect.

TAN in all digesters showed decline trend after new batch FW used since day 776, since in last batch, TKN of FW is 7.58 g kg^{-1} (Table 4.1), and the following TAN increase around day 878 were also caused by the switch of FW batch. TAN behaviours in these 5 digesters followed the rules wo determined in Chapter 7, that at higher loading, microbial biomass fixed more nitrogen, which induce TAN concentration in digester lower. This was supposed to be beneficial for microorganism growth in stable digester, due to microbial activity mainly depends on their population, so higher methane production was generated at higher loading, e.g. at loading 8 kg VS m^{-3} d^{-1} with 11 TE addition. However, in this case, under condition of TE washing out, low buffering capacity caused by low TAN in digester, properly was one reason why VFA accumulated in S7 and S8 so fast that no time for other TE addition for rescuing.

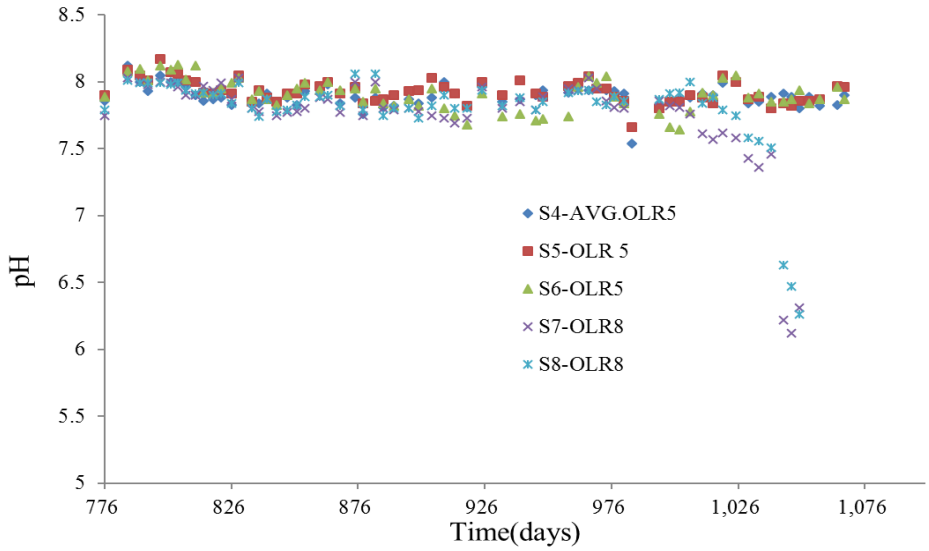


Figure 8.2 pH of digesters S4-S8, TE ceased from day 776 until the end of trial

Note: constant loading 5 kg VS m^{-3} d^{-1} was used in S4 after day 966

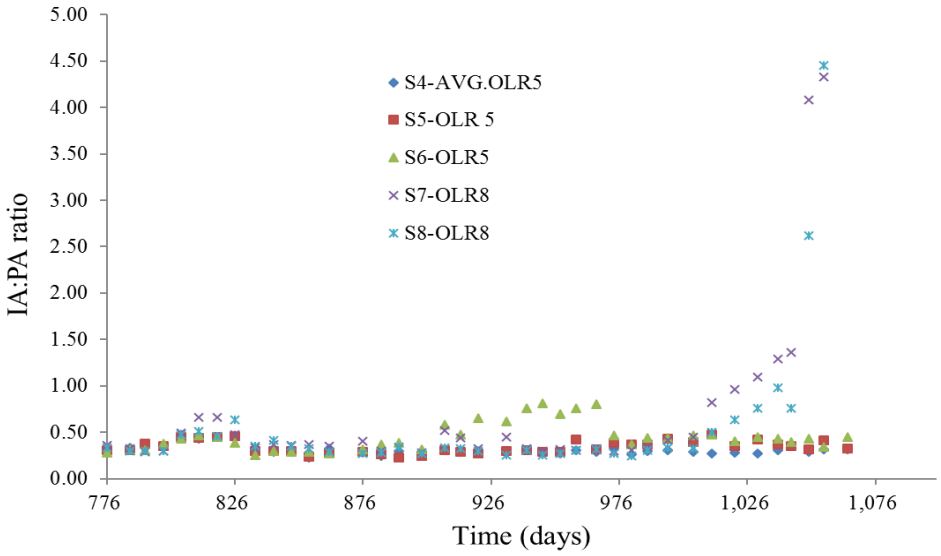


Figure 8.3 IA:PA ratio of digesters S4-S8, TE ceased from day 776 until the end of trial

Note: constant loading 5 kg VS m^{-3} d^{-1} was used in S4 after day 966

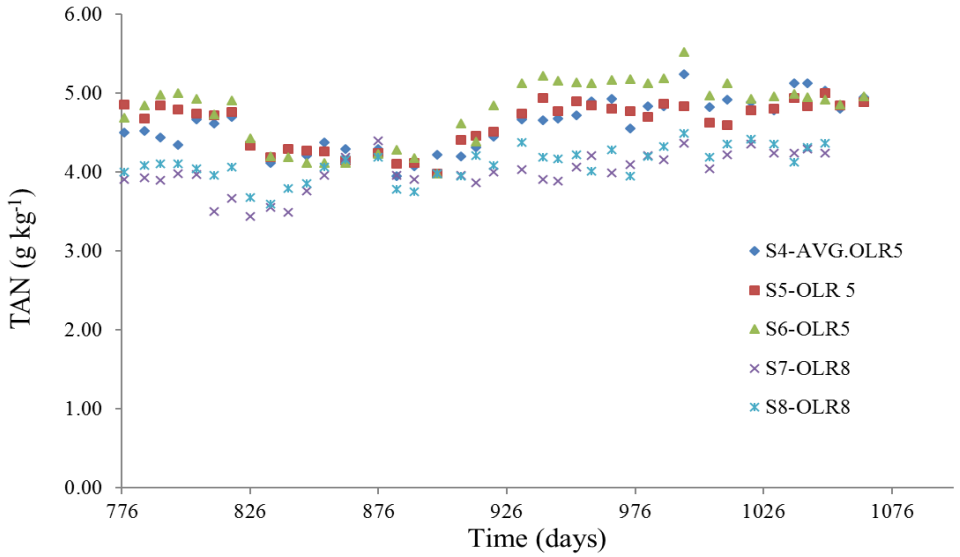


Figure 8.4 TAN of digesters S4-S8, TE ceased from day 776 until the end of trial

Note: constant loading 5 kg VS m^{-3} $d^{-1}$was used in S4 after day 966

8.3.2 Production performances in all digesters

Results of section 7.3.2 showed both VMP and SMP were unlikely to increase after full range of TE supplemented in digesters with excessive strength. In this study, these two gas parameters were monitored without excessive TE background, to explore whether production performance appear different with reduced essential trace elements.

In Figure 8.5, after fluctuation observed in initial period when trace elements washing-out except Co and Se, SMP in digester S5 and S6 showed slight decrease from initial 0.47 to later 0.44 m^3 CH_4 kg^{-1} VS d^{-1} with 4000 mg L^{-1} VFA production until initial 11 trace elements re-supplied into digesters since day 1053. Afterwards SMP appeared to increase slightly with 0.46 m^3 CH_4 kg^{-1} VS d^{-1}. SMP perk appeared in S6 around day 976 where propionic acid fast reduced. In S5 and S6 with defined TE supplementation, limiting biogas productivity appeared. SMP was lower than initial 0.47 m^3 CH_4 kg^{-1} VS d^{-1} themselves and also lower than S2 after it recovered by TE supplementation with value of 0.48 m^3 CH_4 kg^{-1} VS d^{-1}.

SMP of digesters S7 and S8 at loading 8 kg VS m^{-3} d^{-1} were gradually decreased from initial 0.45 to 0.41 m^3 CH_4 kg^{-1} VS d^{-1} around day 1000. In the period of excessive VFA accumulation in digesters, SMP declined to below 0.40 m^3 CH_4 kg^{-1} VS d^{-1}. After day 1053, methane productions were hardly observed in both S7 and S8, indicating digesters failure. Even no more than 10000 mg L^{-1} VFA observed before the last perk of VFA which induced failure, SMP in S7 and S8 still showed decrease, mainly because that deficiency of TE inhibited methanogens activity for methane production.

Similar observations were found in Figure 8.6, which showed VMP. In digesters at loading 5 kg VS m^{-3} d^{-1}, VMP appeared to have slight decrease tendency whereas obvious decline could be found in digesters at loading 8 kg VS m^{-3} d^{-1}. In digesters loading 8 kg VS m^{-3} d^{-1}, VMP gradually declined from initial 3.80 to 3.17m^3 CH_4 kg^{-1} d^{-1} around day 1000, afterwards decreased sharply to below 0.3 m^3 CH_4 kg^{-1} d^{-1} until digestion ceased.

Stable production performance was observed in digester S4 (Figure 8.7), in which 30 day rolling VMP was stable above 2.38 m^3 CH_4 kg^{-1} d^{-1} over the trial. TE removal showed no negative effect to VBP values, higher methane productivity was well maintained with 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se supplementation. Furthermore, switch of random to constant loading 5 kg VS m^{-3} d^{-1} on day 966, VMP showed apparent decline for acclimation then climbed back to around 2.44 m^3 CH_4 kg^{-1} d^{-1}, with slight higher gas performance than at random loading rate. Meanwhile, in control digesters S5 and S6, 30 days rolling VMP showed very gently decrease tendency compared with their initial performance, and obvious lower efficiency than S4 in the end. Based on the results, it is implied that with 0.3 mg kg^{-1} Co 0.2 mg kg^{-1} Se addition in digester S4, no loss of biogas performance was found, methanogens activity was not limited when other TE was washed out, whereas in constant loading digester S5 and S6 at loading 5 kg VS m^{-3} d^{-1}, same TE addition were unable to maintain same performance as they operated with 11 TE supplementation. However, random loading digester showed higher buffering capacity than constant loading, again, it is more convenient and should be recommended for commercial plant.

After compared stability and production between random loading and constant loading digesters from day 1 to day 1065, it is observed that random loading digester had higher resilience than constant loading one. Additionally, the recommended loading range for random loading digester should be limited in 2.5~7.5 kg VS m^{-3} d^{-1} with smooth biogas performance.

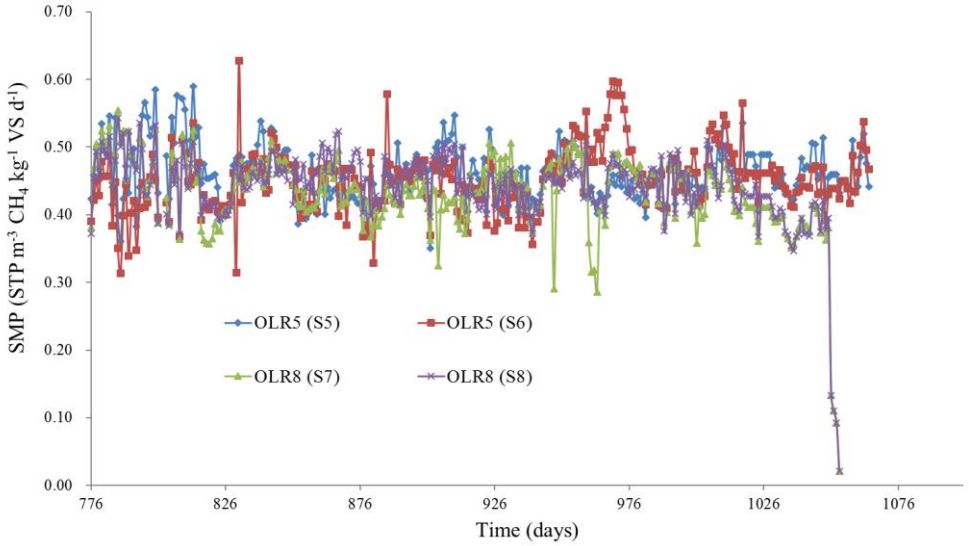


Figure 8.5 SMP of S5-S8 since day 776 until the end of trial

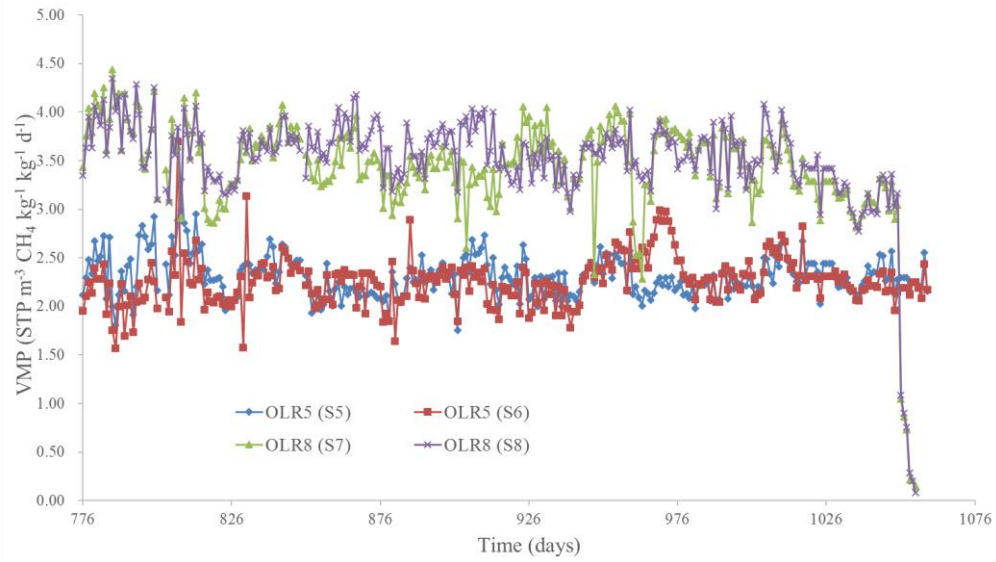


Figure 8.6 VMP of S5-S8 from day 776 until the end of trial

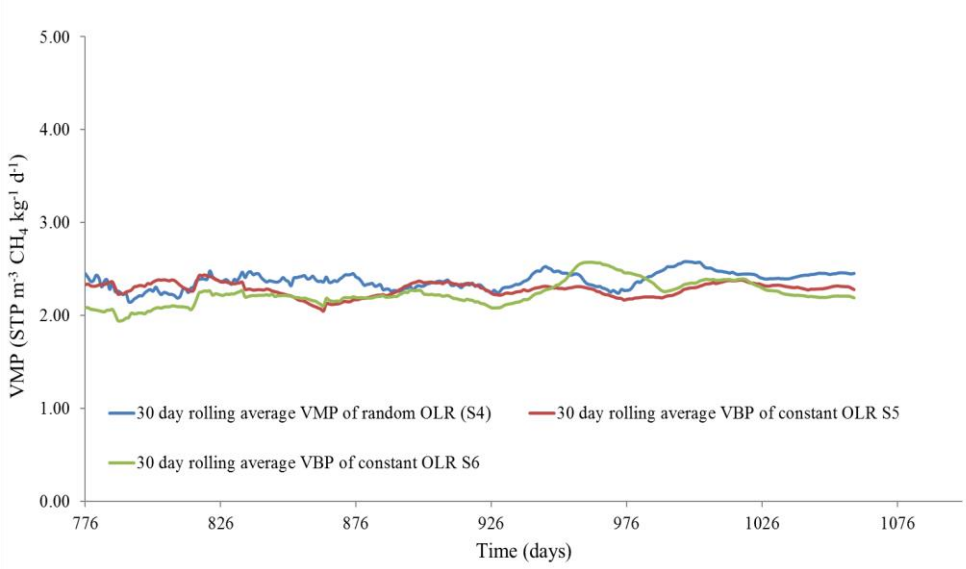


Figure 8.7 30-days rolling VMP of S4 and S5, S6 since day 776 until the end of trial
Note: constant loading 5 kg VS m^{-3} $d^{-1}$was used in S4 after day 966

8.4 Conclusions

Food waste digester was capable to operate at OLR 5 kg VS m^{-3} d^{-1} (including constant and random loading), with essential trace elements supplementation of 0.3 mg kg^{-1} Co and 0.2 mg kg^{-1} Se. Slight loss of performance and elevated VFA productions were observed in constant loading 5 kg VS m^{-3} d^{-1} digesters. Random loading digester has higher buffering capacity than constant loading digester, which should be recommended for commercial plant. For digesters at high loading of 8 kg VS m^{-3} d^{-1}, Co and Se alone were not sufficient to maintain stable digestion even Co strength was increased. Other trace elements became limiting at higher loading, however the species were not identified in this study due to time constraint.

In this study, TE deficiency caused propionic acid accumulation, but it was difficult to clarify which inhibition was the cause. In reported studies, other elements were reported to function for propionic acid degradation, e.g. Mo/Ni. However, further studies are needed for this.

The productivity comparison in digester S4 before and after random loading switched to constant loading in this chapter, is not conclusive one, since as its

controls, digester S5 and S6 had less efficiency performance, In this case, higher volumetric methane production after loading switch to constant one was not comparative.

CHAPTER 9 Conclusions and Recommendation

9.1 General Conclusions

- The research evaluated the effect of TE, in particularly Co and Se, on anaerobic digestion of FW. Experimental data indicated that 0.3 mg kg^{-1} of Co and 0.2 mg kg^{-1} Se were essential for maintaining operational stability and biogas efficiency when digester operates at loadings 3-5 kg VS m^{-3} d^{-1}.

- The maximum operational loading was determined as 8 kg VS m^{-3} d^{-1} for FW digestion with 11 recommended TE supplementation, without compromising process stability and FW conversion efficiency. Although a higher loading could be applied, the retention time was in this case reduced to an extent which could not allow complete hydrolysis. It is worth noting that the maximum loading of 8 kg VS m^{-3} d^{-1} achieved in this study should only be regarded as an approximate value because the exact maximum loading for any specific FW digester is affected by the particular characteristics of FW in question, e.g. moisture content.

- TE supplementation had a two-way effect on anaerobic digestion: although its contribution to VFA degradation is well known, it could stimulate VFA production to a great extent, especially when digesters were laden with VFA and methanogenic activity had already been inhibited. This needs particular attention when digesters are operated at moderate or high OLR.

- The TE requirement for digesters fed with the same type of substrate was not fixed: more TE species and/or higher TE strength were needed at higher OLR due to the increased microbial biomass density at higher OLR.

9.2 Specific Conclusions

- UK food waste had relatively constant characteristics with high VS/TS ratio (~90 %) and TKN content (~7 g kg^{-1} FM). Co and Se are present in food waste at concentrations below the recommended threshold for stable FW digestion.

- VFA accumulation was confirmed to be a recurring problem without trace elements addition, even at low OLR of 1 ~ 2 kg VS m^{-3} d^{-1}.

- At OLR between 3.0 and 5.0 kg VS m^{-3} d^{-1} both Se and Co were essential, even though Co could be diluted out to around 0.06 ~ 0.08 mg kg^{-1} before VFA accumulation started. This concentration was treated as the critical dosage to maintain stability of process with limited buffering capacity, as other factors could induce VFA accumulation, e.g. OLR increase.

- Acetic acid showed no obvious inhibition from insufficient Co supplementation, where propionic acid was unable to be degraded. However, with sufficient dosage of Co and Se addition, the propionic acid degradation rate was faster than that of acetic acid.

- A reliable element washing-out equation was developed and validated for quantification of TE concentration in digestate. Good agreement was found between calculated values of TE concentration and measured values.

- Reduced specific biogas productivity was shown in a food waste digester at OLR 9 kg VS m^{-3} d^{-1}; however, process stability was achieved. Hydrolysis became the limiting step due to short HRT at this condition.

- A digester with variable loading and TE supplementation showed good resilience to randomly applied loading changes at a constant average load with no loss of methane production or digestion imbalance observed in this study.

9.3 Recommendations

- A relationship between OLR, TE dosage demanding and microbial density is established in this study. At higher loading conditions, microbial density increases, thus their demand for trace elements increase. At higher loading, trace elements should be supplied with care.

- Food waste digestion at higher loading suffered VFA accumulation. Digestion with 11 trace elements is therefore currently recommended to ensure process stability and conversion efficiency. 8 kg VS m^{-3} d^{-1} is regarded as the maximum loading without productivity loss in CSTR digesters at mesophilic conditions.

- In commercial plant, excessive trace elements may be supplemented to ensure stable performance at moderate loading. For environmental and economic reasons, an optimal trace elements strategy is desired. 0.2 mg kg^{-1} Se and 0.3 mg kg^{-1} FM Co were recommended to maintain stable food waste digestion.

- Variable daily loading with recommended range (2.5~7.5 kg VS m^{-3} d^{-1}) can be conducted in the real world for commercial AD plants. Process stability and biogas productivity can be ensured by applying 0.2 mg kg^{-1} Se and 0.3 mg kg^{-1}Co.

9.4 Future Work

- The reason food waste digesters suffer trace elements deficiency at a maximum loading of 8 kg VS m^{-3} d^{-1} is still not clear. Further work on determining this/these trace element(s) species is needed.

- It is hypothesised that after food waste digester recovered from VFA accumulation, something unknown has happed to microbial communities inside the digester. In future, work referring to the microbial biomass should be carried out.

- Further work on trace elements supplementation at high VFA concentration needs be carried out in detailed studies.

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APPENDIX I Calculated values of trace elements concentrations in washing-out period by mass balance calculation

Table I-1 Full calculated values of trace elements in S2 during washed-out period

Days	Mo	Se	Co	Ni	W
initial	0.32*	0.27	1.02	1.80	0.20
104	0.30	0.28	0.91	1.67	0.18
111	0.28	0.28	0.80	1.54	0.16
118	0.26	0.28	0.70	1.42	0.14
125	0.24	0.28	0.61	1.29	0.12
132	0.24	0.29	0.56	1.24	0.11
139	0.23	0.30	0.51	1.19	0.10
146	0.23	0.31	0.47	1.16	0.09
153	0.22	0.31	0.41	1.08	0.08
160	0.19	0.28	0.34	0.94	0.07
167	0.20	0.30	0.33	0.96	0.06
174	0.20	0.31	0.30	0.94	0.06
181	0.19	0.32	0.28	0.93	0.06
188	0.19	0.32	0.26	0.89	0.05
195	0.18	0.32	0.23	0.86	0.05
202	0.18	0.31	0.21	0.82	0.04
209	0.18	0.32	0.20	0.83	0.04
216	0.18	0.33	0.19	0.82	0.04
223	0.18	0.33	0.18	0.82	0.03
230	0.18	0.34	0.17	0.81	0.03
237	0.19	0.36	0.16	0.84	0.03
244	0.19	0.37	0.16	0.84	0.03
251	0.19	0.36	0.14	0.82	0.03
258	0.18	0.36	0.13	0.81	0.03
265	0.18	0.36	0.13	0.80	0.02
272	0.18	0.36	0.12	0.79	0.02
279	0.18	0.36	0.11	0.78	0.02
286	0.17	0.35	0.10	0.75	0.02
293	0.17	0.35	0.09	0.69	0.02
300	0.17	0.35	0.09	0.63	0.02
307	0.17	0.35	0.08	0.59	0.02
314	0.17	0.35	0.08	0.54	0.01
321	0.17	0.35	0.07	0.50	0.01
328	0.16	0.35	0.07	0.46	0.01
335	0.16	0.34	0.06	0.42	0.01
342	0.16	0.34	0.06	0.39	0.01
349	0.16	0.34	0.05	0.36	0.01
356	0.16	0.34	0.05	0.34	0.01
363	0.16	0.34	0.05	0.32	0.01
370	0.15	0.33	0.05	0.29	0.01

377	0.15	0.33	0.04	0.27	0.01
384	0.15	0.33	0.04	0.26	0.01
391	0.15	0.33	0.04	0.24	0.01
398	0.15	0.33	0.04	0.23	0.01
405	0.15	0.33	0.04	0.21	0.01
412	0.15	0.33	0.03	0.19	0.01
419	0.15	0.32	0.03	0.17	0.01
427	0.14	0.31	0.03	0.15	0.01
434	0.14	0.32	0.08	0.15	0.01
441	0.15	0.34	0.09	0.14	0.01
448	0.16	0.35	0.09	0.14	0.01
455	0.16	0.34	0.09	0.14	0.01
462	0.18	0.35	0.09	0.14	0.01
469	0.19	0.36	0.09	0.15	0.01
476	0.20	0.36	0.09	0.15	0.01

*all concentrations are based on mg kg^{-1} fresh matter

Table I-2 Full calculated values of trace elements in S3 during washed-out period

Days	Co	W	Se
initial	1.02*	0.20	0.27
104	0.94	0.19	0.27
111	0.86	0.17	0.28
114	0.84	0.17	0.30
118	0.78	0.16	0.30
121	0.76	0.15	0.32
125	0.71	0.14	0.33
128	0.68	0.14	0.34
132	0.65	0.13	0.36
135	0.64	0.13	0.37
139	0.60	0.12	0.38
146	0.56	0.11	0.38
153	0.50	0.10	0.37
160	0.47	0.09	0.37
167	0.44	0.09	0.37
174	0.41	0.08	0.36
181	0.38	0.08	0.36
188	0.35	0.07	0.35
195	0.33	0.07	0.35
202	0.32	0.06	0.36
209	0.30	0.06	0.36
216	0.28	0.06	0.36
223	0.27	0.05	0.36
230	0.25	0.05	0.36
237	0.24	0.05	0.36
244	0.22	0.04	0.35
251	0.21	0.04	0.35
258	0.20	0.04	0.36
265	0.19	0.04	0.36
272	0.18	0.03	0.35
279	0.17	0.03	0.35
286	0.16	0.03	0.35
293	0.15	0.03	0.35
300	0.14	0.03	0.35
307	0.13	0.03	0.35
314	0.12	0.02	0.35
321	0.11	0.02	0.34
328	0.11	0.02	0.37
335	0.11	0.02	0.37
342	0.11	0.02	0.37
349	0.10	0.02	0.38
356	0.10	0.02	0.38
363	0.09	0.02	0.38
371	0.09	0.02	0.37
377	0.08	0.02	0.37

384	0.08	0.01	0.37
391	0.07	0.01	0.37
398	0.07	0.01	0.36
405	0.06	0.01	0.36
412	0.06	0.01	0.36
419	0.06	0.01	0.35
427	0.05	0.01	0.34
434	0.05	0.01	0.34
441	0.05	0.01	0.33
448	0.04	0.01	0.33
455	0.04	0.01	0.33
462	0.04	0.01	0.33
469	0.04	0.01	0.32
476	0.04	0.01	0.33
483	0.04	0.01	0.33
490	0.03	0.01	0.33
497	0.03	0.01	0.34
504	0.03	0.01	0.32

*all concentrations are based on mg kg^{-1} fresh matter

APPENDIX II BFN level calculation based on estimated TKN concentration in digestate

OLR	VS of FW (%)	TKN of FW ($g\ kg^{-1}$)	TKN of digestate * ($g\ kg^{-1}$) (estimated)	TAN of digestate ($g\ kg^{-1}$)	SMP	VSD	SMP per $VS_{removal}$	BFN ($g\ kg^{-1}$)	Difference (%)
5.0	22.48	7.58	10.0	2.52	0.437	0.75	0.573	**5.05**	
5.0	22.48	7.58	10.0	2.81	0.434	0.75	0.573	**4.76**	
6.0	22.48	7.58	10.0	2.07	0.432	0.75	0.573	**5.50**	
6.0	22.48	7.58	10.0	2.31	0.422	0.75	0.56	**5.26**	
5.0	21.18	6.3	8.4	2.84	0.464	0.75	0.619	**3.46**	**2.3**
5.0	21.18	6.3	8.4	2.79	0.463	0.75	0.618	**3.51**	**2.2**
7.0	20.08	6.18	8.5	2.38	0.459	0.73	0.628	**3.79**	**7**
7.0	20.08	6.18	8.4	2.47	0.464	0.74	0.627	**3.71**	**3.9**
5.0	20.55	7.20	9.6	4.60	0.473	0.75	0.630	**2.60**	**4.8**
5.0	20.55	7.20	9.5	4.56	0.486	0.76	0.639	**2.64**	**8**
8.0	20.55	7.20	9.7	3.72	0.462	0.74	0.624	**3.84**	**3.6**
8.0	20.55	7.20	9.6	3.8	0.474	0.75	0.632	**3.40**	**5.2**

5.0	23.2	7.58	10.1	4.72	0.502	0.75	0.669	**2.86**	**5.5**
5.0	23.2	7.58	9.9	4.74	0.480	0.766	0.626	**2.84**	**1.7**
9.0	23.2	7.58	10.7	3.51	0.435	0.71	0.612	**4.07**	**15.9**
9.0	23.2	7.58	10.6	3.60	0.424	0.72	0.585	**3.98**	**16.3**

*TKN in digestate is estimated by VS Destruction rate, all the other parameter values are measured values

APPENDIX III Monod Equation for biomass concentration calculation

From the mass balance inlet and outlet of the digester

$$\frac{dX}{dt}V = \left(Y\frac{dS}{dt} - k_d X\right)V - X \quad Q$$

Where X is biomass concentration; V is the bioreactor volume, m^3; Y is the yield coefficient, S is the substrate concentration, k_d is the endogenous coefficient,

Biomass accumulation = Total Biomass yield - Biomass in effluent

$$\tau = \frac{V}{Q}$$

τ is the hydraulic retention time, in our case, it equals to the solid retention time.

$$\frac{dX}{dt} = \left(Y\frac{dS}{dt} - k_d X\right) - X \quad /\tau$$

Then biomass accumulation from the reduction of substrate and endogenous respiration,

$$\frac{dS}{dt} = -\frac{\frac{dX}{dt}}{K} + k_d = -\frac{YX}{K} + k_d = -\frac{\mu_{max}X}{K} \quad \frac{S}{K_s + S} + k_d$$

μ_{max} is the max specific growth rate; K_s is the substrate saturation coefficient, K is the reaction rate, means substrate reduction rate;

Where assuming,

$$\gamma = \frac{\mu_{max}X}{K}$$

$$\frac{dS}{dt} = -\gamma\frac{S}{K_s + S} + k_d$$

Meanwhile the substrate reduction rate,

$$\frac{dS}{dt} = -\frac{S_0 - S_t}{\tau}$$

So

$$-\gamma \frac{S}{K_s + S} + k_d = -\frac{S_0 - S_t}{\tau}$$

When at time t, means the HRT = τ,

$$-\gamma \frac{S_t}{K_s + S_t} + k_d = -\frac{S_0 - S_t}{\tau}$$

Boundary conditions

When S_t is the concentration at time t, the estimated t is approximated τ, equals to the HRT,

$S_t << S_0$

Thus

$$-\gamma \frac{S_t}{K_s + S_t} + k_d \approx -\frac{S_0}{\tau}$$

Deduction

$$\frac{1}{\tau} = \mu_{max}\left(\frac{S_t}{K_S + S_t}\right) - k_d$$

Substitute this results into the initial equation

$$\frac{dX}{dt} = \left(Y\frac{dS}{dt} - k_d X\right) - X \quad /\tau$$

We obtained $$\frac{1}{X}\frac{dX}{dt} = \left(\frac{Y}{X}\frac{dS}{dt} - k_d\right) - \frac{1}{\tau}$$

Considering

$$\frac{dS}{dt} = -\frac{S_0 - S_t}{\tau}$$

Finally

$$X = Y\left[\frac{S_0}{\tau k_d + 1} - \frac{K_s}{\tau\mu_{max} - (\tau k_d + 1)}\right]$$

APPENDIX IV CSTR trial for TE effect on FW digestion in 1-L digesters

Introduction

Results from Chapter 8 indicated that FW digester suffered sharp VFA accumulation cause by TE deficiency at loading 8 kg VS m^{-3} d^{-1}, had stable performance with 0.3 mg kg^{-1} Co, 0.2 mg kg^{-1} Se supplementation at loading 5 kg VS m^{-3} d^{-1}, whereas this combination of trace elements was unable to sustain digestion at higher loading (eg. OLR 8 kg VS m^{-3} d^{-1} in section 8.3). Since VFA accumulation was not due to Co limiting, deficiency of other elements accounted for VFA accumulation at loading 8 kg VS m^{-3} d^{-1}. As FW digester stable operated with combination of Co, Se, Ni, Fe, Mo, W at loading 7 kg VS m^{-3} d^{-1}, it is proposed that the limiting elements is /are among these species. According to FW characteristics results from Table 4.1 and previous studies, Fe was assumed to be essential in FW to support Fe-depending enzymes activities. In this trial, Ni and Mo were treated as main elements, for the objective to determine whether VFA accumulations at loading 8~9 kg VS m^{-3} d^{-1} were caused by Ni/Mo deficiency. The results from this trial was meaningful for future work related to TE effect on high achievable loading FW digestion.

Methodology

4 pairs 1-L CSTR digesters, with 500 mL working volume, were used for this short-term trial, and their operation over this trial were shown in Table IV-1. Food waste digestate as inoculum for these 4 pairs digesters, were taken from S7 and S8 when they still stable operated around day 964 at loading 8 kg VS m^{-3} d^{-1}, both with <1500 mg L^{-1} VFA. All 4 pair digesters were running at previous loading but with different trace elements combination for a week, then increased loading up to 9 kg VS m^{-3} d^{-1} until experiments finished in the end or digester ceased. pH, TAN, and IA:PA ratio were monitored as general parameters, whereas VFA concentration was used as the main stability indicator.

Table IV-1 The operational scheme of this 1-L CSTR trials

Digesters/ Objective	Initial TE supplementation	Operation during trials
SS1, SS2, used as control	0.5 mg kg^{-1} Co, 0.2 mg kg^{-1} Se	After 7 days acclimation, digesters increase OLR from 8 to 9 kg VS m^{-3} d^{-1}, Co, Se from day 0 were supplemented to maintain until trial finished or digester failed
SS3, SS4, determined effect of Mo, with given strength of Se, Co	0.5 mg kg^{-1} Co, 0.2 mg kg-1 Se, 0.2 mg kg^{-1} Mo,	After 7 days acclimation, digesters increase OLR from 8 to 9 kg VS m^{-3} d^{-1}, Co, Se, Mo from day 0 were supplemented to maintain until trial finished or digester failed
SS5, SS6 determined effect of Ni, with given strength of Se, Co	0.5 mg kg^{-1} Co, 0.2 mg kg^{-1} Se, 1 mg kg^{-1} Ni,	After 7 days acclimation, digesters increase OLR from 8 to 9 kg VS m^{-3} d^{-1}, Co, Se, Ni from day 0 were supplemented to maintain until trial finished or digester failed
SS7,SS8 determined effect of Mo and Ni, with given strength of Se, Co	0.5 mg kg^{-1} Co, 0.2 mg kg^{-1} Se, 1 mg kg^{-1} Ni, 0.2 mg kg^{-1} Mo,	After 7 days acclimation, digesters increase OLR from 8 to 9 kg VS m^{-3} d^{-1}, Co, Se, Ni, Mo from day 0 were supplemented to maintain until trial finished or digester failed

Results and Discussions

Figure IV-1 to Figure IV-4 showed VFA profiles in digesters with different TE supplementation at loading 9 kg VS m^{-3} d^{-1}. In control digesters SS1 and SS2, it is obviously that 0.5 mg kg^{-1} Co, 0.2 mg kg^{-1} Se were not able to maintain digesters alive. After 100 days operations, VFA in both SS1 and SS2 reached level of more than 20000 mg L^{-1}, digesters failed. Propionic acids accounted for mostly of VFA accumulations, showed the same problem as S7 and S8 in Chapter 8.

Interesting finding were observed in the other 3 pairs testing digesters, the lack of parallelism existed in every pair. In pair of digesters with Co, Se, Mo supplementation (Figure IV-2), SS3 crashed at round day 89 whereas its duplicate SS4 kept stable performance more than 100 days, propionic acid started to accumulate. Similar propionic acid accumulation happened in SS5 with Co, Se, Ni supplementation (Figure IV-3), and SS8 with Co, Se, Ni supplementation (Figure IV-4). These results indicated that all these 4 combinations of TE supplementation were unable to maintain stability of digestion, which made following analysis difficult and complicated, and to be more specific, there were no conclusive results from this trial, that is why this trial only presented in Appendix.

However, some finding still could be observed from these non-parallel digesters. In digester SS5 and SS6, additional Ni supplementation was more effective to inhibit VFA accumulation than Mo in SS3 and SS4, due to VFA increase magnitude in SS3 was 10000 mg kg^{-1} higher than in SS5, as well as VFA appeared earlier in SS3 than SS5. Besides, in SS4 VFA accumulation appeared in the final stage, whereas SS6 still showed no VFA production. Another finding in SS7 with Se, Co, Mo, Ni supplementation, acetic acid appeared almost 5000 mg kg^{-1} in the middle of experiment, then reduced to very low level, SS7 stable operated until the end of trial.

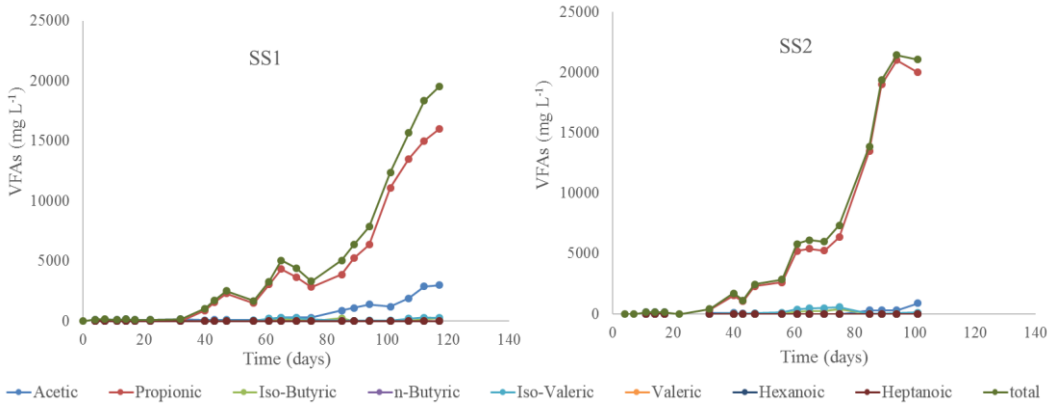

Figure IV-1 VFA in SS1 and SS2, with Co, Se supplementation, SS2 ceased on day 107

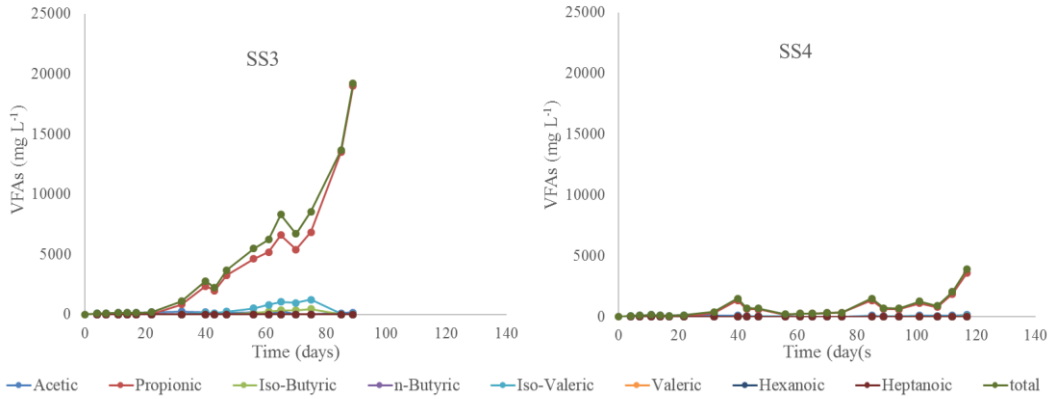

Figure IV-2 VFA in SS3 and SS4, with Co, Se, Mo supplementation, SS3 ceased on day 94

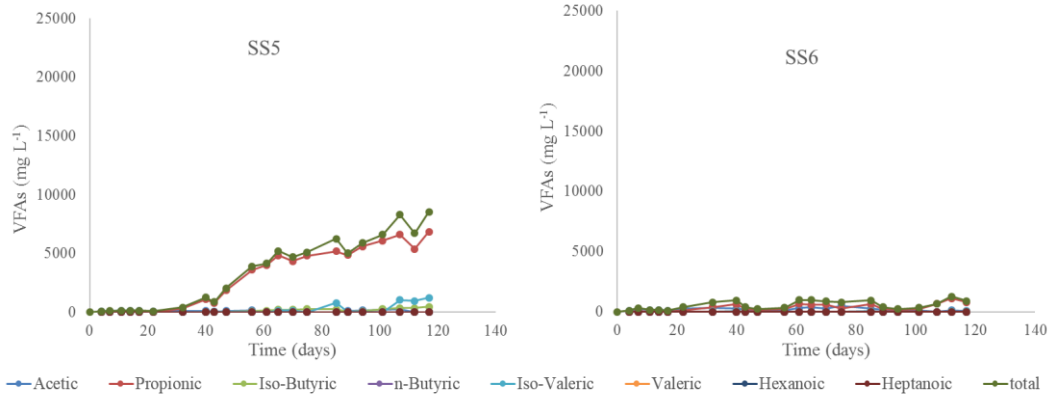

Figure IV-3 VFA SS5 and SS6, with Co, Se, Ni supplementation

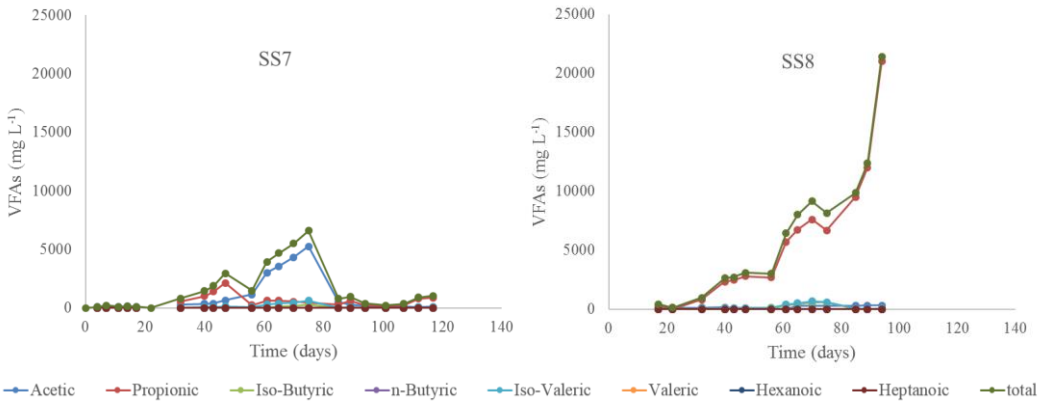

Figure IV-4 VFA in control SS7 and SS8, with Co, Se, Ni, Mo supplementation, SS8 ceased on day 101

Figure IV VFA profiles in digesters SS1-SS8

pH of these 4 pair digesters were the same around 7.90 initially, after loading increased to 9 kg VS m^{-3} d^{-1}, all of which showed different magnitude drops in pH, especially in SS1, SS2, SS3, SS8, pH below 6.50 indicated when digesters failed (Figure IV-5). IA:PA ratios were stable at 0.28~0.33 in stable periods (Figure IV-6), however in the following instable period, all ratios showed increase, in dying out digesters, IA:PA ratio climbed even great extent, which were not shown in Figure IV-7 due to Y-axis value limiting. TAN concentrations in these 4 pair digesters, showed no obvious difference, all fluctuated around 4.00 g kg^{-1} which were not plotted here. Both pH, and alkalinity fluctuations in these 4 digesters indicated that buffering capacity in digesters were decayed, stability of digestion was not achieved. All TE supplementation combinations in this trial showed no positive effect to process control.

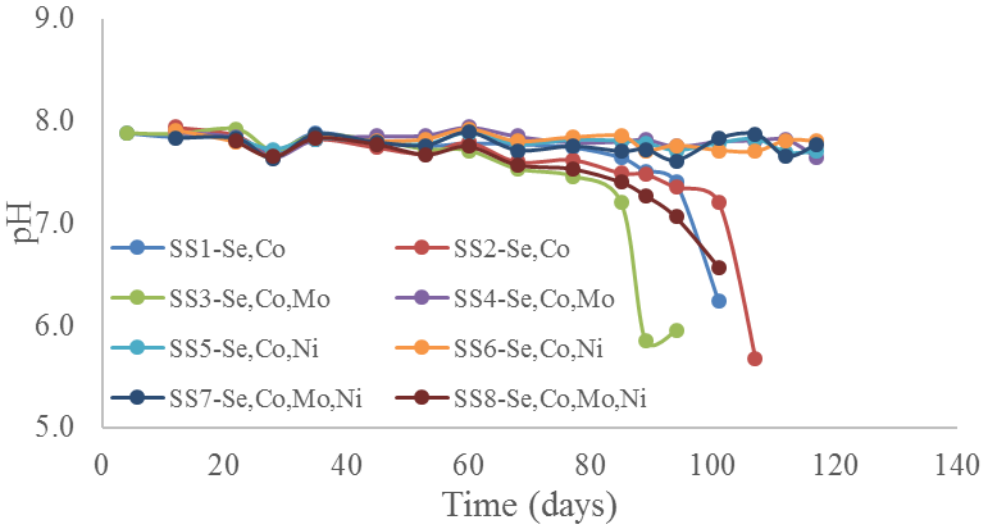

Figure IV-5 pH of SS1-SS8 during trials

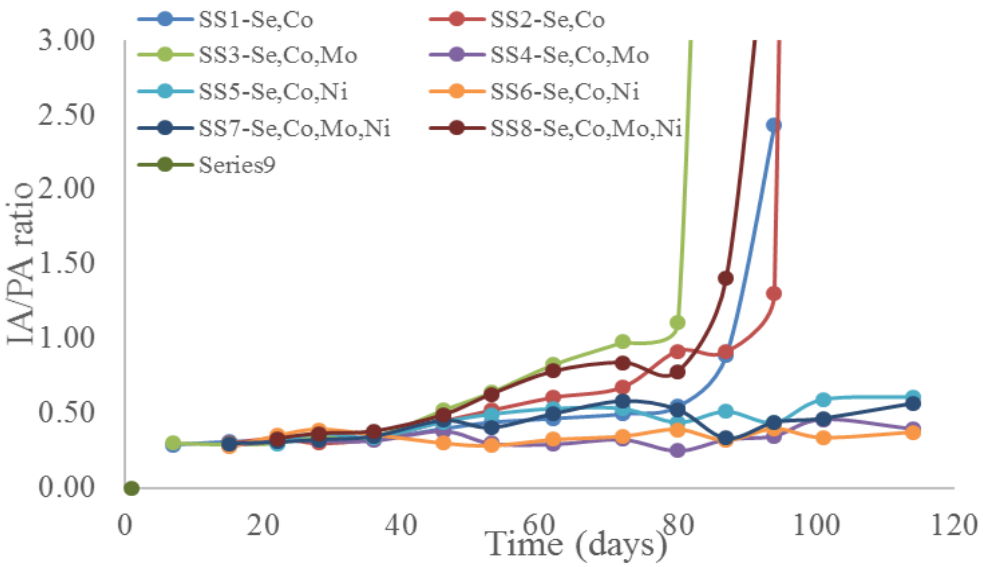

Figure IV-6 IA:PA ratio of SS1-SS8 during trials

Conclusions

Mo, Ni, or Mo/Ni combination with Se, and Co were not proper for stable operation at loading 9 kg VS m^{-3} d^{-1} in FW digestion. More elements from recommended species needed to be concerned in future work. However, Ni showed higher effect to VFA concentration control than Mo when digester under other elements deficiency

condition. Propionic acid accumulation accounted for almost total VFA production whereas no obvious acetic acid appeared, except in SS7, 5000 mg L^{-1} acetic acid appeared after 78 days (2.5 HRTs) operation.