UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

School of Civil Engineering and the Environment

Anaerobic Digestion of Catering Wastes

by

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Thesis submitted for the degree of Doctor of Philosophy

October 2008

UNIVERSITY OF SOUTHAMPTON

ABSTRACT
FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

Doctor of Philosophy
ANAEROBIC DIGESTION OF CATERING WASTES

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This research addresses gaps in current knowledge regarding process issues associated with long term semi-continuous digestion of food waste as a sole substrate, and the role of trace elements and biomass retention in digestion of food wastes.

Source segregated food wastes were collected from a university catering facility and found, in characterisation studies, to have a total solids (TS) content of 28.1±0.25 %, a volatile solids (VS) content of 95.5±0.06% of TS and a chemical oxygen demand (COD) of 422±16 g $kg_{wet\ weight}^{-1}$. The total Kjeldahl nitrogen (TKN) and total lipid content were 22±1% and 3.8±0.24% of TS, respectively.

The substrate was then processed during a number of digestion trials using mesophilic continuously-stirred tank reactors (CSTRs), to establish the suitability of this substrate for CSTR digestion. It was found that although good specific methane production of 0.36 l gVS_{added}^{-1} was obtained from the substrate, the process was unstable at a hydraulic retention time (HRT) of 25 days, with methanogenic failure occurring after 80 days or when the organic loading rate (OLR) was increased.

Further digestion trials were initiated, therefore, to investigate the effects of trace element supplementation and extending HRT on process stability, areas for which there is little information in existing literature.

Reactors with hydraulic retention times of 25, 30, 50, 100, and 180 days supplemented with a trace element solution showed stable digestion for longer periods than duplicate control digesters without supplementation. The time points of failure in the control digesters were shown to be related to washout time, as calculated using the HRT. Trace element supplementation allowed stable operation at an OLR up to 3.5 gVS $l^{-1}d^{-1}$, with specific methane production ranging from 0.41-0.47 l gVS_{added}^{-1} and VS destruction of 63-77%. Supplementation with trace elements did not, however, guarantee indefinite stable operation, as digesters at the shortest (25 days) and longest (180 days) retention time eventually showed methanogenic failure. A slow methanogenic biomass growth rate and accumulation of inhibitory substances, respectively, were hypothesised as possible reasons for these failures. Analysis of metal concentrations in the digestate showed that cobalt was the metal most likely to be responsible for the observed benefits of the mixed trace metal supplementation as the concentration of this increased in the supplemented digester whilst decreasing in its non-supplemented control.

The relative importance of the liquid and solid fractions in maintaining stability were investigated in novel digestion trials in which solid and liquid retention times were uncoupled. Digesters with SRT of 25 days and HRT of over 150 days exhibited methanogenic failure after approximately 45 days. In contrast, reactors with SRT of over 150 days and HRT of 25 days maintained stable digestion, with specific methane production of 0.53 l gVS_{added}^{-1}, and also showed recovery from a thermal shock applied during the experiment. Inhibitory compounds such as VFA were kept low by flushing through the system while alkalinity was regenerated by the action of biomass kept in the system. The retention of solids may also have facilitated the retention of trace metals.

KEYWORDS: anaerobic digestion, food wastes, micronutrients, retention time, ammonia, inhibition, LCFA

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Climenhaga, M. A., and Banks, C. J. (2008). "Anaerobic digestion of catering wastes: effect of micronutrients and retention time." *Water Science and Technology*, 57(5), 687-692.

Climenhaga, M. A., and Banks, C. J. (2008)."Uncoupling of liquid and solid retention times in anaerobic digestion of catering wastes." *Water Science and Technology*, 58(8), 1581-1587.

List of acronyms and abbreviations

BMW	Biodegradable Municipal Waste
COD	Chemical Oxygen Demand
CSTR	Completely Stirred Tank Reactor
HRT	Hydraulic Retention Time
LCFA	Long Chain Fatty Acids
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
SRT	Solids Retention Time
TAN	Total Ammonia Nitrogen
TKN	Total Kjeldahl Nitrogen
TLC	Total Lipid Content
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids

Acknowledgements

This work was supported by funding from the UK Engineering and Physical Sciences Research Council (EPSRC) Sustainable Urban Environment (SUE) Programme grant GR/S79626/01, and the UK Overseas Research Students Awards Scheme (ORSAS).

I wish to thank my supervisor, Professor Charles J. Banks, for providing guidance and always challenging me to do better, and my co-supervisor, Dr. Sonia Heaven, for constant support and valuable insights throughout the project. I'd also like to thank all of the very helpful staff at Southampton's Staff Club catering facility for their cooperation and assistance on the food waste collection phase of this project.

I owe a huge debt of gratitude to colleagues and friends in the laboratory and office for your generosity in sharing equipment, advice, or a sympathetic ear whenever I needed it: Mark Walker, Yue Zhang, Elina Lapshina, David Neylan, Barbara Rincon, Marie Cornell, Caroline Lisk, Anne Stringfellow, Merissa Marius, Julie Vaclavik, Mike Rose, David Lynock, Rhys Jenkins, and Cliff Goodman. I'm especially grateful to Denise Cysneiros Karatzas, who has shared this journey with me from the beginning, and whose patience, readiness to help and cheerful attitude made a world of difference through the 'long, dark days' in the laboratory.

I'd also like to thank housemates, past and present, who have given me a home away from home – my UK family: Katia Maciel, Ramesh Satkurunath, Alexandra Makhnina, Ruchi Chaddha, Patrick Wiemann, Nicolas Choplain, Oliver Blacklock and Eleftheria Paliou.

Of course, I wouldn't have started, or finished, this PhD without the love, understanding and support of my fantastic family: thanks Mom & Sarah for your Buddhist words of wisdom during the tough times, Dad for your intellectual advice and invaluable sympathy as someone who's been through it before, and Rachel for providing me with inspiration and a role model.

Lastly, I'd like to thank previous supervisors and mentors who have supported and inspired me along the way: Professor Alfred Spormann of Stanford University; Dr. John Benemann; Matt Lyum and Quinn McCarty of Unisyn Biowaste Technology; David Merriman of MacViro Consultants (now Genivar); and Richard Cave of R. Cave and Associates (now Trow).

Thanks to all of you, who have lent a helping hand and kept me going through the ups and downs. I couldn't have done it without you.

1. Introduction

Anaerobic digestion (AD) is a biological waste treatment method with a number of benefits. Firstly, it has a positive energy balance: methane gas is produced in the process which can then be used as a renewable fuel, so that the process is a net generator of energy rather than consuming energy. Secondly, it can stabilise putrescible wastes and reduce pathogens (Kunte, 1997, 2003). Thirdly, the stabilised end products of the process can be applied to land (Lyum, 1999), thus recycling nutrients back to the soil.

It is a technology that is in widespread use in mainland Europe, where there are currently over 120 full scale plants treating approximately 4 million tonnes of biodegradable municipal waste (BMW) per year (De Baere, 2006).

In a report prepared in 2007 for the UK government's Waste and Resources Action Programme (WRAP), an increase in anaerobic digestion capacity was recommended for the sustainable management of part of the 6.7 million tonnes of food waste produced annually in the UK. Separate collection of food waste with treatment by anaerobic digestion, coupled to home composting and open windrow composting of garden wastes was found to be the best environmental and economic option for the management of organic wastes, as compared to either composting or anaerobic digestion of garden waste and food waste collected as a single stream (Hogg et al., 2007). Arising as a result of these recommendations, source-segregated food waste collection pilot programs are underway in 17 local authorities with support from WRAP and Defra. The food waste is to be sent to in-vessel composting or anaerobic digestion facilities. There is limited operating experience with AD of source segregated food wastes, however (Hogg et al., 2007). Apart from one facility in Portugal (Correia et al., 2008) and one in the UK (Banks et al., 2008), no other facilities were found that are currently processing a stream of 100% food waste. If the UK is to proceed with anaerobic digestion of source segregated food wastes, it is crucial to understand the possible process issues that could arise with this feedstock.

This work is part of an investigation into the best practicable options for local processing of wastes, at a small to mid-sized scale. The research described in this thesis focuses directly on the amenability of source segregated food wastes for treatment by anaerobic digestion.

1.1 Project Overview

This study focused on the biological process aspects of the anaerobic digestion of source-separated catering wastes. It is a necessary first step in the consideration of the potential for anaerobic treatment of source segregated organic wastes at an institutional scale. At the commencement of the project, a broad-ranging feasibility study for a pilot anaerobic digestion plant for the university was planned. During the course of the research, however, the focus was narrowed to address the important process issues that arose during the bench-scale trials.

The first element of research carried out included initial studies on catering waste produced at the University of Southampton's Staff Club catering facility. This included an assessment of the types of wastes produced by the catering facility and introduction of a food waste source segregation pilot programme. This was followed by an intensive source-segregated food waste collection period of one week during which all source segregated food wastes produced by the facility were collected, ground, and mixed to form a composite.

The food waste composite was characterised for various digestibility parameters. This characterised composite was then used as substrate for bench-scale digestion trials throughout the rest of the project.

Three successive bench-scale digestion trials were carried out in which food waste substrate was processed by anaerobic digestion in a completely-stirred tank reactor (CSTR) system. In all three trials, apparently stable digestion (as measured by production of methane and stable pH) was observed for a number of weeks or months, until a failure of methanogenesis occurred, in the form of a cessation of biogas production accompanied by a buildup of process intermediates.

In response to these methanogenic failures, investigations were initiated into the possible factors responsible for process failure and strategies for maintaining stable digestion. A number of possible causal or contributory factors were investigated by means of laboratory testing, review of literature and critical analysis of the existing data. The investigations into process failure led to elimination of some possible causes of failure, and pinpointing of others for further study. Trace elements and the importance of retention time were both pinpointed for further investigation.

The balance of the digestion trials carried out in this research involved investigations into the effects of trace element supplementation, varying hydraulic retention time, and uncoupling of solid retention time from liquid retention time.

1.2 Aims and Objectives

The aims of this research were to i) assess the suitability of source segregated food waste for processing by anaerobic digestion, and ii) optimise the process for the digestion of this feedstock.

The objectives of this project were to:

1. Create a methodology to allow the quantification of food waste generation.
2. Plan and implement trials to assess the amenability of source segregated catering waste for anaerobic digestion.
3. Identify and critically evaluate potential problems resulting from the digestion of this material.
4. Develop strategies for overcoming problems in digestion of this material.

The specific objectives for each of the individual investigations are outlined in their descriptions in Chapter 4.

2. Literature Review

This chapter explores previous work dealing with anaerobic digestion of the organic fraction of municipal solid waste, including source segregated food wastes similar to the catering waste feedstock used in the current study. It also includes a review of previous work in anaerobic digestion, not specifically focused on food wastes, but necessary for an understanding of the factors involved in the process that may be relevant for this particular feedstock.

2.1 Biological Conditions for the Anaerobic Digestion Process

2.1.1 Microbial Steps in the AD process

There are four main stages in anaerobic digestion (Veeken et al., 2000), consisting of the following:

i) Hydrolysis;

ii) Acidogenesis (or Fermentation);

iii) Acetogenesis; and

iv) Methanogenesis.

The different steps of the process are carried out by different microbial populations, in a mixed consortium of species carrying out different roles. This section provides a brief overview of the different steps of the process.

Hydrolysis

In hydrolysis, complex insoluble organic material is solubilized by extracellular enzymes excreted by hydrolytic microorganisms. This is an essential first step to allow the transport of macromolecules into microbial cells for further steps to take place. In the biodegradation of complex particulate substrates, hydrolysis is usually the rate-limiting step in the process (Sanders, 2001), and therefore determines the solids retention time in the reactor (Veeken et al., 2000).

Fermentation / Acidogenesis

In acidogenesis, soluble organic components are converted into organic acids (long-chain fatty acids and volatile fatty acids), alcohols, hydrogen and carbon dioxide. The

production of volatile fatty acids (VFA) is the fastest step in the process, and VFA are rapidly produced from soluble substrate (Mata-Alvarez, 2003).

Acetogenesis

In acetogenesis, the products of the acidogenesis stage are converted into acetic acid, hydrogen and carbon dioxide. Acetate is the most common precursor in methanogenesis. Homoautotrophic acetogenesis is the production of acetate from hydrogen and carbon dioxide (Madigan et al., 2003).

Methanogenesis

Methanogenesis is the production of methane from acetate as well as directly from other substrates including formic acid, methanol and carbon dioxide/hydrogen. It is carried out by methanogenic archaea. The various species have different ranges of substrate utilisation ability; for example *Methanobrevibacter arboriphilus* uses only H_2/CO_2 (autotrophic methanogenesis); species in the *Methanosaeta* genus use only acetate; *Methanospirillum hungatei* and *Methanobacterium formicicum* grow on H_2/CO_2 and formate; while *Methanosarcina* species can use a full range of H_2/CO_2, acetate, methanol and other one-carbon compounds (Stams et al., 2005).

Methanogens have a lower growth rate than acidogens and acetogens (Angelidaki et al., 1999) and are therefore slower to react to changes in operational conditions and are more sensitive to unfavourable conditions in the system than the bacterial populations.

2.1.2 Microbial Ecology in the AD process

The relationship between different microbial populations within a mixed environment is an important factor in biological processes such as anaerobic digestion of complex substrates. The process involves a number of steps in which intermediate products are produced and consumed before the formation of the final products. For example, complete mineralization of propionate and butyrate requires the involvement of acetogenic bacteria and both hydrogenotrophic and acetoclastic methanogenic archaea (Stams et al., 2005). If the products from one process reaction accumulate, the reaction will become slower and slower, leading to an accumulation of the reactants of that process. The accumulation of these reactants, which are products of a previous reaction step, in turn will affect the reaction rate of that process step.

During the degradation of complex substrates, different microbial species carry out the different reactions, and are dependent upon each other to provide the conditions necessary for the biochemical reactions required for growth.

Some of the main reactions involved in the steps of anaerobic digestion, starting from glucose as an example substrate, are summarized below (Conrad, 1999, Stams et al., 2005):

Acidogenic and fermentative reactions (glucose as example substrate)	*$\Delta G^{0/}$, kJ mol^{-1} substrate*
$C_6H_{12}O_6 \rightarrow 2/3\ CH_3CH_2CH_2COO^- + 2/3\ CH_3COO^- + 2\ CO_2 + 8/3\ H_2O$	- 248.0
$C_6H_{12}O_6 \rightarrow 4/3\ CH_3CH_2COO^- + 2/3\ CH_3COO^- + 2/3\ CO_2 + 2/3\ H_2O$	- 311.4
$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$ (Homoacetogenesis)	- 311.2
$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$	- 235.0
$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$	- 198.1

Acetogenic reactions	*$\Delta G^{0/}$, kJ mol^{-1} substrate*
$CH_3CH_2COOH + 2\ H_2O \rightarrow CH_3COOH + CO_2 + 3\ H_2$	+ 31.8
$CH_3CH_2CH_2COOH + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ H_2$	+ 48.3
$CH_3CH_2OH \rightarrow CH_3COOH + 2\ H_2$	+ 9.6
$2\ CH_3CH_2COO^- \rightarrow CH_3COOH + CH_3CH_2CH_2COOH$	0
$4\ H_2 + 2\ HCO_3^- + H^+ \rightarrow CH_3COO^- + 4\ H_2O$	- 104.6

Methanogenic reactions	*$\Delta G^{0/}$, kJ mol^{-1} substrate*
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	- 32.7
$CH_3COOH \rightarrow CO_2 + CH_4$	- 35.6
$4\ HCOOH \rightarrow 3\ CO_2 + CH_4 + H_2O$	- 29.8
$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$	- 79.5

The values on the right are Gibbs free energy values, which are a measure of how thermodynamically favourable a reaction is. The notation $\Delta G^{0/}$ denotes standard temperature (273 K) and pressure (1 atm) (or 1M concentration for solutes). Under conditions other than STP the nought and prime superscripts are not used.

Reactions with a more negative $\Delta G^{0/}$ value will proceed more readily at STP than those with a less negative or higher $\Delta G^{0/}$ value (Stams et al., 2005). Under conditions other than standard temperature and pressure or concentration, the ΔG value changes to favour a faster or slower rate of reaction. For example, a reaction may proceed more quickly at a temperature above 273 K, and the ΔG value will be lower under this condition, indicating that the reaction is more thermodynamically favourable. An important determinant of Gibbs free energy is the concentration of reactants and products. A reaction is more likely to proceed when the concentration of reactants is high and the concentration of products is low, but as the reaction proceeds and more of the reactants are converted to products, the equilibrium of the reaction shifts and the Gibbs free energy value increases, indicating a slower rate of reaction.

Syntrophy

The relationship between microbes that produce hydrogen and those that degrade it is important. For reactions that have positive Gibbs free energy values under standard conditions, decrease in the concentration of the reaction products will lower the Gibbs free energy value, and allow the reaction to proceed if product concentration is sufficiently low. The phenomenon by which two or more different microorganisms cooperate to degrade a substance that neither can degrade alone is termed syntrophy (Stevenson, 2006), and occurs, for example, between acetogens and methanogens: the consumption of hydrogen by hydrogenotrophic methanogens keeps the partial pressure of hydrogen in the medium low, which is a thermodynamic requirement for the degradation of the volatile fatty acids (VFA) butyrate and propionate by hydrogen-producing bacteria (Fox and Pohland, 1994, Conrad, 1999).

In a well-balanced digestion system, the overall process of conversion of complex organic compounds to carbon dioxide, methane and other reduced compounds including ammonia and hydrogen sulphide proceeds as the products from each metabolic stage are consumed in the next stage, without significant accumulation of intermediate products such as hydrogen and volatile fatty acids. These can, however, build up in the medium if they are being produced by acidogens more quickly than they can be utilized by methanogens. This can happen because methanogenic bacteria grow more slowly than acidogenic bacteria; acidogens have generation times on the order of 15-30 minutes, while methanogens have generation times ranging from 3-30 days (Gerardi, 2003). The problem is compounded by

the fact that excess VFA are inhibitory to methanogens, due to the lowering of pH in the medium (Veeken et al., 2000), thus resulting in a negative feedback loop of rising VFA concentrations as a result of, and a contributor to, falling methanogenic activity.

2.1.3 Nutrient Requirements for the Biological Process

The microbial consortia have nutritional requirements that must be met to maintain a balanced digestion process. The elements that are required fall into the categories of macronutrients – elements that are required in substantial amounts, and micronutrients – elements that are required only in trace amounts, but that are essential for the continuation of the process (Speece, 1996).

All living organisms require carbon, hydrogen, oxygen and nitrogen as the basic building blocks for synthesis of monomers and macromolecules. The most abundant element in the cell is carbon, comprising approximately 50% of dry weight of a typical bacterial cell, followed by nitrogen at about 12% of dry weight (Madigan et al., 2003). Hydrogen and oxygen are abundant in cells as water (H_2O) and as common elements in most cellular macromolecules.

Metabolism and growth for all species requires three things: a carbon source, an electron donor, and an electron acceptor. Carbon is required for anabolism, the synthesis of biomass, while electron donors and acceptors are required for catabolism, the generation of energy for the cell via transport of electrons (Madigan et al., 2003).

The most effective electron acceptor in biological systems is oxygen, the reduction of which gives the highest energy yield. In environments in which O_2 is not available, alternate electron acceptors such as nitrate, sulphate and carbon dioxide (NO_3^-, SO_4^{-2}, CO_2) are used. The metabolism of these compounds can be either *assimilative* or *dissimilative*; in assimilative metabolism, the nitrogen, sulphur or carbon atom (respectively) of the compound is used to build cellular macromolecules after energy requirements have been met by reduction of the compound. In dissimilative metabolism, a comparatively larger amount of the electron acceptor is reduced, and the reduced product is then excreted from the cell (Madigan et al., 2003).

Organisms that can meet their carbon requirements by assimilative reduction of carbon dioxide (CO_2) are termed autotrophs (Madigan et al., 2003). These organisms can use CO_2 as both carbon source and electron acceptor. Two major groups of strict anaerobes that can

grow autotrophically on CO_2 are homoacetogens and autotrophic methanogens (Madigan et al., 2003). Hydrogen is commonly the electron donor (Madigan et al., 2003).

Macronutrients

Along with the basic elements of carbon, hydrogen, oxygen and nitrogen, different organisms require varying amounts of the macronutrients phosphorus, sulphur, potassium, magnesium, sodium, calcium and iron. Table 2.1 summarises the main roles played by the different macronutrients.

Table 2.1
Macronutrients and Common Cell Functions (adapted from Madigan et al., 2003)

Macronutrient	Common form	Role in Microbial Cells
Phosphorus (P)	PO_4^{3-}	Nucleic acids and phospholipids
Sulphur (S)	H_2S, SO_4^{2-}, organic S compounds, metal sulphides (FeS, CuS, ZnS, NiS etc.)	Amino acids cysteine and methionine, some vitamins including thiamine, biotin, and lipoic acid; also found in coenzyme A, important electron carrier for cellular energy-yielding reactions including homoacetogenic acetate production
Potassium (K)	K^+ in solution or salt	Several enzymes, including those involved in protein synthesis
Magnesium (Mg)	Mg^{2+} in solution or salt	Stabilises ribosomes, cell membranes, nucleic acids; required for activity of some enzymes
Sodium (Na)	Na^+ in solution or salt	Not required by all organisms; stabilises cell wall and plays role in regulating diffusion of solutes in/out of cells
Calcium (Ca)	Ca^{2+} in solution or salt	Not required by all organisms; stabilises cell wall and plays role in regulating diffusion of solutes in/out of cells. Also important in heat stability of spores.
Iron (Fe)	Fe^{2+}(generally anoxic conditions) or Fe^{3+} (oxic conditions) in solution, FeS, $Fe(OH)_3$, other Fe salts	Cytochromes and iron-sulphur proteins active in electron transport for cellular respiration.

Trace Elements

Metals are important micronutrients. High doses of heavy metals can have a toxic effect, due to disruption of enzyme structure and activity (Oleskiewicz and Sharma, 1990). At low concentrations, however, metals are essential. A number of investigators have pointed to the importance of certain metals for maintaining a stable anaerobic digestion process (Florencio et al., 1994, Speece, 1996, Zandvoort et al., 2002, Zandvoort et al., 2005). Iron

has been listed as a macronutrient in Table 2.1 as it is needed in high concentrations in cells, but as it is a metal, it is also commonly grouped with the trace elements.

Although required in very small amounts, trace metals are essential for growth of bacterial biomass, as they are the building blocks for certain specialised cell molecules such as enzymes, cofactors and electron carrier molecules (Osuna et al., 2003).

For example, corrinoids are derivatives of vitamin B12, which contain a ring system with a central cobalt atom. A corrinoid is involved in the activity of methyl transferase, required for the transfer of methyl groups from compounds including methanol, in methanogenesis (Madigan et al., 2003). Cobalt is also found in carbon monoxide dehydrogenase (CODH), which plays an essential role in methanogenesis from acetate (Madigan et al., 2003). It is responsible for the cleavage of acetate and methylcob(III)alamin:coenzyme M methyl transferase, an intermediate in methanogenesis of all methanogenic substrates (Zandvoort et al., 2006).

Iron is part of cytochromes and ferredoxin in methylotrophic methanogens, and as iron-sulfur proteins in some enzymes, as noted in Table 2.1 (Takashima and Speece, 1990).

Another element found in CODH is nickel, which is also found in the cofactor coenzyme F_{430} (Takashima and Speece, 1990). Coenzyme F_{430} is a porphinoid (Shima et al., 2002) that plays a role in autotrophic methanogenesis, making nickel especially important when H_2 and CO_2 are the sole sources of energy. Nickel and/or iron is at the catalytic centre of most hydrogenases (Shima et al., 2002) and may be involved in membrane stability (Jarrell and Sprott, 1982).

Zinc is present in hydrogenase, CO dehydrogenase, formate dehydrogenase, and superoxide dismutase (Takashima and Speece, 1990).

Cobalt is also involved in the production of acetate from methanol by acetogens as this also requires methyl transferase. The cobalt requirement is lower for hydrogenotrophic methanogenesis (Zandvoort et al., 2006). Table 2.2 shows functions in cells for the different micronutrients.

Table 2.2
Micronutrients and Common Cell Functions (adapted from Madigan et al., 2003)

Micronutrient	Cellular Function
Chromium (Cr)	Required by mammals; no known microbial requirement
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Respiration, cytochrome c oxidase; photosynthesis, superoxide dismutases
Manganese (Mn)	Activator of many enzymes; some superoxide dismutases
Molybdenum (Mo)	Flavin-containing enzymes, nitrogenase, nitrate reductase, sulfite oxidase, some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} in methanogens, carbon monoxide dehydrogenase (CODH), urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases, senocysteine (amino acid)
Tungsten (W)	Some formate dehydrogenases
Vanadium (V)	Vanadium nitrogenases; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases, many DNA-binding proteins
Iron (Fe)	Cytochromes; catalases; peroxidases; iron-sulphur proteins; oxygenases; nitrogenases

Trace metals have been found to play a role in supporting the maintenance of granular sludge (Oleskiewicz, 1989) and in sludge settlement, through effects on surface charge (Agridiotis et al., 2007).

Trace element supplementation has been recommended during start-up or transitory conditions (Speece, 1996, Fish, 1999). Other investigators, however, recommended against supplementation during start-up, as low cobalt concentrations may prevent reactor instability by preventing the build-up of acetate from acetogenesis, which is also subject to cobalt limitation (Zandvoort et al., 2002).

In a review of case histories of trace metal stimulation in anaerobic digestion of various wastes and wastewaters, Speece (1996) noted that the symptoms of trace metal deficiency were similar to those of toxicity (reduced rates of gas production and elevated concentrations of VFA), and a trace metal deficiency could often be mistaken for toxicity. In many of these cases, trace metal supplementation was found to correct the problem and toxicity was shown to be unimportant. Waste substrates for which digestion was found to be improved by the addition of trace metals included whey, cattle waste, municipal sludge, wastewater from food processing, napier grass, ice cream wastewater, brewery wastewater, poultry waste, methanol and propionate. Table 2.3 is a partial reproduction of a summary

of earlier work compiled by this author on trace element supplementation with various waste feedstocks.

Table 2.3
Trace Metal Additions to Anaerobic Digesters on Waste Feedstocks, as compiled by Speece (1996)

Metal	System	Concentration	Unit	Substrate
Fe	CSTR	6.9-34.4	mg l^{-1} feed	Cattle manure
	Batch	300	mg l^{-1} reactor	Municipal and bean waste
	Batch	16-24	mM	Cellulose
	Expanded bed	0.15	mg l^{-1} feed	Whey
Ni	Expanded bed	1.3	mg l^{-1} feed	Whey
	Batch	0.6-6	mg l^{-1} reactor	Poultry waste
	CSTR	0.25	mg l^{-1} feed	Napier grass
Co	CSTR	1.7-8.3	mg l^{-1} feed	Cattle manure
	Expanded bed	0.0074	mg l^{-1} feed	Whey
	CSTR	0.19	mg l^{-1} feed	Napier grass

Among the benefits of trace element supplementation in the various cases were: decreased sensitivity to shock loading, decrease in VFA concentrations, increased gas production, better granule formation in UASB reactors, and increased resiliency during transient conditions such as start-up or change in loading rate. In wastewater feeds, this author recommended that Fe, Co and Ni be supplemented at rates of, respectively, 0.02, 0.004 and 0.03 g kg^{-1} acetate as preventative maintenance.

A requirement for the metals molybdenum, selenium, tungsten, calcium, magnesium, and sodium have been found for at least some species of methanogens, but the main metals that have been found to be important in the study of trace element limitation are cobalt, nickel and iron (Florencio et al., 1994, Zandvoort et al., 2003, Zandvoort et al., 2006). All three have been shown to be required by methanogens, and a stimulatory effect on acetate fermentation has been shown for each (Speece, 1996).

Metals partition into different fractions of a medium, and work has been done to investigate the fractionation of metals by a sequential extraction process to determine the distribution of metals among the different fractions of a medium (Zandvoort et al., 2006). The samples are subjected to increasingly stringent extraction agents and the resulting extracts are analysed for metals content to determine the amount in each of the following: the exchangeable fraction (extractant NH_4CH_3COO); the carbonate fraction (extractant

CH_3COOH); the organic / sulphide fraction (extractant H_2O_2) and the residual fraction (extractant 3:1 HCl/HNO_3).

In granular sludges studied by Zandvoort et al. (2006), the majority of cobalt was found in the organic/sulphides fraction, i.e. adsorbed to sludge or complexed to sulphide. This can be explained by the poor solubility at neutral pH of many metals, which will therefore precipitate or adsorb to non-polar substances such as cell membranes. The solubility equilibrium will allow metal sulphides to dissolve as metals are taken up by cells (Zandvoort et al., 2006).

Investigators researching the degradation of methanol in reactors deprived of cobalt observed a strong impact of cobalt deprivation on methanogenic activity of the sludge, in which after 55 days of operation methanol conversion efficiency was 55%, whereas the addition of cobalt for a further 33 days led to a conversion efficiency of 100% (Zandvoort et al., 2002). In a further experiment, they found a sudden deterioration in reactor efficiency as evidenced by increases in effluent VFA and methanol concentrations after 92 days of operation under trace element deprivation (Zandvoort et al., 2003).

Many of the recent trace element studies have been focused on a simple and defined feedstock such as methanol. The addition of trace elements, nutrients or alkalinity is commonly practiced in many laboratory anaerobic digestion studies on wastewaters that are known to be deficient in certain nutrients (Takashima and Speece, 1990), or in batch studies.

The effects of trace element supplementation have been less well studied for solid waste feedstocks. One exception to this is digestion of energy crops, which involve a single substrate and therefore there is a possibility of nutrient imbalance. Examples include mono-digestion of energy crops such as grass-clover silage (Jarvis et al., 1997), fodder beet silage (Neumann et al., 2008), sorghum (Richards et al., 1991) and maize (Lebuhn et al., 2008). For a feedstock of mixed solid wastes, however, less has been done and no recent studies were found dealing specifically with the effects of trace element supplementation with a mixed solid waste feedstock.

Lebuhn et al. (2008) found effects on reactor stability with trace element additions in mono-digestion of maize. They were able to increase OLR to over 3.5 gVS $l^{-1}d^{-1}$ and maintain stable digestion in reactors supplemented with a cocktail of trace elements, while parallel reactors operated under the same conditions but without trace element

supplementation showed acidification, required a lower OLR and had a high ratio of alkalinity from VFA to bicarbonate alkalinity.

Jarvis et al. (1997) operated two parallel 4.5 litre reactors fed grass-clover silage, under identical operating conditions except that one was supplemented with cobalt, while the other was not. Initially there was no cobalt supplementation to either reactor, and both performed well initially at a constant organic loading rate (OLR) of 3 gVS $l^{-1}d^{-1}$ and constant hydraulic retention time (HRT) of 20 days. After approximately 4 weeks (1.5 x HRT), gas production and pH dropped for both reactors, and therefore OLR was decreased to both reactors. Cobalt supplementation was introduced to one of the reactors beginning in week 7 of operation, while the other reactor did not receive supplemental cobalt. The reactor receiving cobalt quickly increased in gas production and pH, allowing a return to the previous OLR of 3 gVS $l^{-1}d^{-1}$ within two weeks, and gradual increases up to a maximum OLR of 7 gVS $l^{-1}d^{-1}$ at the end of the 80-week operating period, whereas the non-supplemented reactor required the reduced OLR of <1 gVS $l^{-1}d^{-1}$ to be maintained for a further three weeks, after which only a gradual increase was possible, with 3 gVS $l^{-1}d^{-1}$ being achieved only after 40 weeks of operation. The maximum OLR achieved for the non-supplemented reactor was 5 gVS $l^{-1}d^{-1}$ after 70 weeks, but following a rapid rise in acetate concentrations, OLR was again reduced to below 2 gVS $l^{-1}d^{-1}$ for the remainder of the 80-week operating period. While cobalt supplementation was introduced following a rapid increase in acetate concentrations in both reactors, it was observed that acetate concentrations in the cobalt-supplemented reactor decreased to below 50 mg/L within two weeks following the introduction of cobalt supplementation, whereas the reactor that did not receive cobalt supplementation required a further 15 weeks for acetate to drop to the same concentration (Jarvis et al., 1997).

They had previously found a stimulation of degradation following the introduction of effluent recirculation, and postulated that an increase in availability of trace elements due to retention in the recirculated process liquid may have been a factor.

These authors carried out batch assays using the digestate from the two reactors and found improvement of acetoclastic methanogenesis by the addition of cobalt. They did not find, however, any improvement in hydrogenotrophic methanogenic activity.

They tested the effects of adding iron, nickel, and molybdenum in parallel batch assays, but none of these single elements were as effective in increasing acetoclastic methanogenic

activity as cobalt – only a mixture of all elements including cobalt showed the same result as cobalt alone. The same result was found by Fish (1999), in which cobalt was the only element that showed the same effect on batch digestion as that of a mix of all elements. She found an optimum cobalt concentration of 0.15 mg l^{-1} in batch digestion (Fish, 1999).

Gonzalez-Gil et al. (1999) recommended continuous rather than pulsed additions of nickel and cobalt as they hypothesised that the precipitation and dissolution kinetics of metal sulphides could affect the bioavailability of these metals. In later work, however, the same research group found rapid dissolution of metal sulphides after uptake of cobalt and nickel, showing that sulphides could act as a pool for these metals and would not limit their uptake by biomass (Jansen et al., 2007).

Fish (1999) found that the addition of cobalt could be either stimulatory or essential depending on the inoculum to substrate ratio. With a sufficiently high inoculum to substrate ratio, stable digestion could be achieved either with or without trace element supplementation, but digesters receiving cobalt supplementation showed enhanced gas production and solids removal relative to the controls, indicating a stimulatory effect. At a lower inoculum to substrate ratio, however, digesters without cobalt supplementation failed, while those supplemented with cobalt showed stable digestion, showing that cobalt was essential in cases in which the inoculum did not provide microbial populations capable of utilizing the organic acids at a rate equal to their production. She concluded that the effects of cobalt would be most noticeable when the methanogenic population is stressed or has not been fully established.

Although micronutrient supplementation is not universally practised in commercial operations as the minimisation of costs is favoured, there are some facilities in which cobalt or other trace elements are dosed periodically (e.g., City of Toronto source separated organics processing facility)(van Opstal, 2006a, van Opstal, 2006b), and some energy crop digesters (Scherer, 2008). The addition of metals may not be necessary, however, if they are provided by a co-substrate. An energy crop digester for maize, sunflower, rye and grass which also digests cattle manure at 15% of the input has operated for one year without nutrient supplementation (De Baere, 2008).

2.2 Anaerobic Digestion of Food Waste

This section details some of the recent work dealing with food wastes as a substrate for digestion.

2.2.1 Previous Food Waste Digestion Studies

Although AD has been used for various organic feedstocks such as domestic and industrial wastewaters, animal manures, and sludges from biological wastewater treatment processes since early in the 20th century (Mata-Alvarez, 2003), anaerobic digestion of pure food waste has been less well studied until recently. This section outlines the recent work published on food wastes and similar feedstocks.

There have been a number of recent studies on the anaerobic digestion of market wastes and other food wastes containing primarily fruit and vegetables (Mtz.-Viturtia et al., 1995, Kim et al., 2000, Bouallagui et al., 2004, Parawira et al., 2004, Wang et al., 2005, Bouallagui et al., 2005). In most of these studies, a two-stage mode of anaerobic digestion was used; this is to allow for control of loading of volatile fatty acids to the methanogenic stage, due to the sensitivity of the methanogens to inhibition by high volatile fatty acids. Single-stage digestion, however, is still the preferred mode for digestion of biowastes at a commercial scale, as over 87% of plants in Europe are single-stage plants (De Baere, 2006). At a laboratory scale, single-stage digestion has recently been used for biodegradable municipal waste (BMW), previously referred to as the organic fraction of municipal solid waste (OFMSW) (Gallert et al., 2003, Bolzonella et al., 2003), wastewater and solid food wastes from food processing (Beccari et al., 1999, Carucci et al., 2005), and slaughterhouse wastes (Salminen and Rintala, 2002b, Siegrist et al., 2005). Beccari et al. (1999) studied the anaerobic digestion of effluent from olive mills involved in the production of olive oil. Research on this type of waste requires review, because although it is a liquid wastewater, it contains high amounts of lipids and some readily-degradable materials, similar to the food waste studied in the current research. In their studies they found that the high lipid content had an inhibitory effect on the degradation of olive mill effluent; these results are examined more fully in Section 2.3.4 concerning inhibition effects of lipids.

As noted above, two-stage digestion is one possible strategy to mitigate VFA inhibition effects. Mtz.Viturtia et al. (1995) compared two-phase with single-phase mesophilic digestion for fruit and vegetable wastes and found lower yields in the two-phase system.

They also found that as OLR was increased, the bulk of biogas production shifted from the second-stage methaniser to the first-stage hydrolyser. They concluded that a two-phase system did not have significant advantages over a one-phase system on wastes with a C:N ratio above 15, unless more sophisticated control mechanisms were also used. Later work by co-authors of this paper, however, has recommended two-phase systems for fruit and vegetable wastes, due to the susceptibility of one-phase systems to upset by VFA overproduction (Mata-Alvarez et al., 2000).

Bouallagui et al. (2004) carried out two-stage digestion of fruit and vegetable wastes from a market in Narbonne, France, reaching a maximum OLR of 10.1 g of chemical oxygen demand (COD) per litre per day to the acifidification stage, the effluent of which delivered an OLR of 0.72-1.65 g COD $l^{-1}d^{-1}$ to the methanogenic stage. Overall hydraulic retention time in the system was 13 days, but the methanogenic stage was operated in a fill-mix-settle-withdraw cycle that retained solids in the system. Solids retention time in the methanogenic stage was approximately 50 days.

Co-digestion of food wastes with other substrates has also been investigated by various researchers. Carucci et al. (2005) co-digested two types of food processing wastes from a frozen food factory in Italy: fresh vegetable waste (scraps from selection and washing of fresh vegetables, primarily spinach) and pre-cooked food waste (quality control rejects from the preparation of precooked products, containing high quantities of flour, grease and cheese). Co-digestion achieved better results than digestion of each of the types of waste as a single stream. For the single streams, they found inhibition of methanogenesis which they attributed to high content of potassium in the vegetable waste, and high content of lipids in the pre-cooked waste.

As part of an investigation into the potential for digesting fruit and vegetable wastes at wastewater treatment plans, Gomez et al. (2006) compared co-digestion of the fruit and vegetable fraction of MSW and primary sewage sludge with digestion of primary sludge alone, and found a better biogas yield and specific gas production with the co-digestion mixture. They found that low mixing conditions were more effective for digestion than static conditions or a high mixing regime.

Kaparaju and Rintala (2005) co-digested various potato wastes (peel, tuber and stillage from glue production) with pig manure to determine the technical feasibility of using on-farm digesters to treat industrial food wastes from potato processing. They found that the

addition of potato increased gas yield up to the maximum tested ratio of 20:80 VS_{potato}: $VS_{pig\ manure}$ at OLRs ranging from 2 - 3 g COD $l^{-1}d^{-1}$. From this work they were able to conclude that potato wastes could be co-digested successfully with pig manure at this ratio in farm-scale digesters.

A number of recent studies have been done in support of anaerobic digestion of biodegradable municipal waste at a commercial scale. Bolzonella et al. (2003) used high-solids digestion (20% total solids) at bench scale to simulate the process at a full-scale plant in Verona, Italy. They used a combination of mechanically sorted OFMSW from a refuse sorting plant and source-separated OFMSW from supermarkets and vegetable markets. The study was focused on plant start-up. The desired organic loading rate (OLR) of 9 kg total volatile solids (TVS) $m^{-3}d^{-1}$ was reached in approximately 30 days, with a specific biogas production of 0.23 $m^3\ kg_{TVS}^{-1}$.

Gallert et al. (2003) processed biowaste collected from households in the city of Karlsruhe, Germany in a bench-scale reactor as a test for increasing loading to a full-scale commercial plant operating on the same feedstock. Liquid pulp from the initial processing stages of the commercial plant was fed to a bench-scale reactor operating in CSTR mode on a 5-day feed schedule, the same mode as the commercial plant. The researchers were able to increase OLR stepwise from 4.3 to 19 g COD $l^{-1}d^{-1}$ while maintaining stable digestion, concluding therefore that it should be possible to increase load to the full-scale plant up to the desired OLR of 15 kg COD $m^{-3}d^{-1}$. This was borne out by results at the full-scale plant over the following year.

Lissens et al. (2004) tested a novel pre-treatment process (thermal wet oxidation) for biowastes, to increase methane yields from various components of BMW (food waste, yard waste, and post-digestion biowaste). Wastes were oxidised at temperatures of 185-220^{0}C and oxygen pressures of 0-12 bar. The thermal wet oxidation process resulted in higher methane yields in subsequent biochemical methane potential (BMP) tests, attributed to increased bioavailability of cellulose and lignin components.

Investigators have researched the effect of specific potential inhibitors, such as ammonia and cations. Kayhanian (1994) tested the performance of a thermophilic high-solids anaerobic digester processing a mix of paper, food waste and yard waste, under various concentrations of total ammonia nitrogen (TAN). TAN was varied by increasing or decreasing the C:N ratio of the feedstock by using greater or lesser fractions of nitrogen-

containing components (food waste and yard waste). Optimal digestion was found within the range of 600-800 mg l^{-1} TAN. At TAN concentrations beyond 1 g l^{-1} inhibition was observed; acclimation allowed operation at TAN concentrations up to 2.3 g l^{-1}, but failure occurred at 2.5 g l^{-1}. This is lower than the 4-6 g l^{-1} TAN that has been observed in stable digestion of livestock wastes (Angelidaki and Ahring, 1993), discussed further in Section 2.3.3.

Kim et al. (2000) studied the effect of cations and food waste particle size in a series of thermophilic batch activity tests. In the degradation of glucose at varying concentrations of sodium, they found that a sodium concentration of 5 g l^{-1} decreased the rate of methane production, and a sodium concentration of 20 g l^{-1} resulted in methane production of only 50% of the theoretical methane potential. In batch flask tests degrading food waste of various particle sizes, they found that substrate utilization rate was inversely proportional to particle size, i.e., the smallest particle size was the fastest to degrade.

Neves et al. (2004) carried out a series of activity tests with kitchen wastes from the canteen of the university of Minho, Portugal, comparing the performance of granular and suspended sludge at different waste:inoculum ratios from 0.5-2.3 $gVS_{substrate}\ gVS_{inoculum}^{-1}$. They found that the granular sludge exhibited a similar strong performance (in terms of methane production rate and methane yield) regardless of waste:inoculum ratio, while the suspended sludge only performed as well as the granular sludge at the lowest waste:inoculum ratio, with decreasing activity as the waste:inoculum ratio was increased. This was only true, however, in a buffered medium (37 mg $NaHCO_3\ gCOD^{-1}$); when the experiment was repeated with an initial alkalinity of 2 mg $NaHCO_3\ gCOD^{-1}$, both sludges acidified and showed low activity at all waste:inoculum ratios beyond 0.5.

These authors then went on to carry out a series of batch assays with a simulated food waste using different ratios of lipid, protein, starch and carbohydrate. They found that a high lipid content resulted in a lag in the onset of methane production (Neves et al., 2008a).

In a study of food wastes collected from restaurants in San Francisco, USA, biochemical methane potential (BMP) trials gave a total methane yield of 435 ml g_{VS}^{-1}, 80% of which was achieved after the first 10 days (Zhang et al., 2007). This showed restaurant food waste to be a good potential feedstock for anaerobic digestion, however since continuous digestion trials were not carried out, there was no investigation of long term stability for

this feedstock. It is therefore important to carry out continuous digestion trials to explore the potential of this feedstock for sustained stable anaerobic digestion.

In the UK, food waste digestion has been carried out at pilot scale. Kitchen waste was collected weekly from the village of Burford in Shropshire, and processed in 1500 litre CSTR digesters. Two digesters were operated in parallel at mesophilic and thermophilic temperature, to compare system stability and pathogen kill at the different temperatures. It was found that thermophilic digestion was less stable than mesophilic digestion, which had a greater buffering capacity and was more robust with respect to changes in operational conditions or accumulation of inhibitory compounds. Stable digestion was maintained at concentrations of VFA up to 27 g l^{-1} in the mesophilic digester; in thermophilic digestion VFA reached 45 g l^{-1}, at which point OLR was reduced from 4 kgVS m^{-3} to 1 kgVS m^{-3} to maintain process stability (Banks et al., 2008).

2.2.2 Substrate Parameters for Anaerobic Digestion of Food Wastes

Tables 2.4 and 2.5 show the range of values found in the characterisation of food waste mixtures and individual foods, respectively, by previous investigators. The parameters compared are Total Solids (TS), Volatile Solids (VS), Total Kjeldahl Nitrogen (TKN), Chemical Oxygen Demand (COD), and Total Lipid Content (TLC). These provide a basis for comparison with food wastes studied in the current research.

Table 2.4
Food Waste Parameters – Mixed Food Wastes and Biodegradable Municipal Wastes

Waste	Study	TS %	VS % of TS	TKN % of TS	COD g kg^{-1}	TLC % of TS
Precooked food waste	(Carucci et al., 2005)	42	95	2.2	504	12.9
Source-separated OFMSW	(Nordberg and Edstrom, 2005)	31	87	2.1		
Restaurant waste	(Zhang et al., 2007)	31	85	3.2		
OFMSW	(Kubler et al., 2000)	29	63	2.3-3.4		
Food Waste	(Kubler et al., 2000)	27	93	3.2-4.0		
Restaurant and supermarket waste	(Correia et al., 2008)	28	87	3.5		
Collected restaurant waste	(Neves et al., 2008a)	24	90	5	327	8
Household kitchen waste	(Banks et al., 2008)	23	92			
OFMSW	(Bolzonella et al., 2003)	20	62	1.4	114	
Canteen food wastes	(Schieder et al., 2000)	10-15	90	0.9	150	
Fresh vegetable waste	(Carucci et al., 2005)	11	78	4.3	110	1.4
Fruit & vegetable waste mix 1	(Bouallagui et al., 2005)	9	92	0.2	105	
Fruit & vegetable waste mix 2	(Bouallagui et al., 2005)	8	92	0.3	n.d.	
Source-separated OFMSW	(Pavan et al., 2000)	8	82	2.1	80	
Fruit and vegetable waste	(Mtz.-Viturtia et al., 1995)	6	88	0.1		
Averages		20 ± 11	85 ± 10	2.3 ± 1.5	199 ± 159	7.4 ± 5.8

Table 2.5
Food Waste Parameters – Single Foods

Waste	Study	TS %	VS % of TS	TKN % of TS	COD g kg^{-1}	TLC % of TS
Lard	(Neves et al., 2008a)	97	99	0.06	1632	100
Chicken breast	(Neves et al., 2008a)	33	97	15.8	306	2.6
Rice	(Wang et al., 2005)	10	56			
Noodles	(Wang et al., 2005)	45	93			
Wholewheat bread	(Veeken and Hamelers, 1999)	92	96		1230	
Potato peel	(Kaparaju and Rintala, 2005)	23	95	0.2	235	
Potato peelings	(Bouallagui et al., 2005)	12	89	n.d.	126	
Potato peel	(Raynal et al., 1998)	12	88		126	
Potato waste	(Parawira et al. 2004)	19	95	1.5		
Potato flakes	(Neves et al., 2008a)	93	96	1	1018	1.7
Potato stillage	(Kaparaju and Rintala, 2005)	48	96	0.03	600	
Vegetable roots	(Wang et al., 2005)	7	71			
Sugar beet	(Parawira et al. 2004)	11	84	3.3		
Salad waste	(Bouallagui et al., 2005)	8	91	n.d.	98	
Salad leaves	(Raynal et al., 1998)	8	91		58	
Cabbage	(Neves et al., 2008a)	6	82	n.d.	53	n.d.
Green peas and carrots	(Bouallagui et al., 2005)	18	95	n.d.	185	
Peas and carrots	(Raynal et al., 1998)	18	96		185	
Apple pomace	(Raynal et al., 1998)	38	95		370	
Orange peels	(Veeken and Hamelers, 1999)	90	85		1200	
Orange peels	(Wang et al., 2005)	24	97			

2.2.3 Considerations for AD of Specific Food Components

This section details some of the specific considerations relevant to the different feedstock components found in food wastes.

Metabolism of Carbohydrates

Carbohydrates include sugars, starches, and celluloses. They contain only carbon, hydrogen, and oxygen, usually in the ratio 1:2:1. Other than lignin, which requires specific

enzymes for degradation and can only be degraded by certain lignolytic species, most carbohydrates are readily biodegradable and serve as an excellent energy source (Timberlake, 2003).

An important factor to consider in anaerobic digestion of readily biodegradable materials, however, is that rapid biodegradability can lead to an accumulation of acidogenesis products including VFA and hydrogen. This can lead to an imbalance in the process due to lowering of pH, which then affects methanogenic activity. Some investigators have recommended that highly-degradable wastes such as fruit and vegetable wastes should be treated in 2-stage systems, to regulate the production of VFA and their subsequent feed to the methanogenic population (Mata-Alvarez, 2003, Bouallagui et al., 2005).

Metabolism of Proteins

The building blocks of proteins are amino acids (Timberlake, 2003). The most common pathway in anaerobic fermentation of amino acids is deamination (removal of an $-NH_2$ functional group) to form the corresponding volatile fatty acid (Fox and Pohland, 1994). The Gibbs free energy of reaction is positive under standard conditions for some amino acids, for example leucine:

$CH_3(CH_2)_3CHNH_2COOH + 3\ H_2O \rightarrow CH_3CH(CH_3)CH_2COOH + HCO_3^- + H^+ + NH_4^+ + 2\ H_2$

(Leucine) (Isovaleric acid)

$\Delta G^{0/} = +9.6$ kJ mol^{-1} (STP)

$\Delta G^{/} = -59.5$ kJ mol^{-1} (at H_2 partial pressure of 10^{-4}atm, pH 7)

For the fermentation of this amino acid, hydrogen partial pressure in the medium must be kept low, and therefore the presence of hydrogen-utilizing methanogens, as occurs in a one-stage digestion system, is favourable (Fox and Pohland, 1994). Hence, although two-stage systems are recommended for carbohydrates, one-stage systems are often favourable for the digestion of proteins.

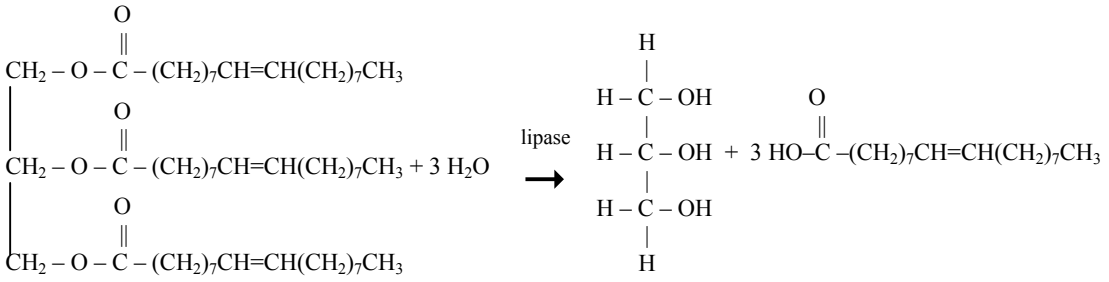
Since protein is approximately 1/10 nitrogen, for every 1 g l^{-1} of protein degraded, 0.1 g l^{-1} of NH_4^+-N will be formed. For each 0.1 g l^{-1} of NH_4^+-N formed, 0.56 g l^{-1} of NH_4HCO_3 alkalinity is formed (79g mol^{-1} NH_4HCO_3 / 14 g mol^{-1} N = 0.56), equivalent to 0.36 g l^{-1} of alkalinity as $CaCO_3$ (50 equivalents $CaCO_3$ ALK / 79 g mol^{-1} NH_4HCO_3 x 0.56) (Speece, 1996). This ammonium alkalinity can help to buffer the system.

Alkalinity is also generated in the reduction of sulphate and sulphite, with two moles of alkalinity produced for every mole of sulphate reduced, and one mole of alkalinity produced for every mole of sulphite reduced (Speece, 1996).

The reduced forms of both nitrogen and sulphur can, however, be inhibitory or toxic at high concentrations. This is discussed further in Section 2.3.

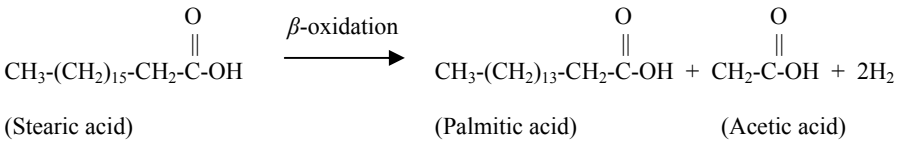
Metabolism of Lipids

The lipids are the group of non-polar substances that include triacylglycerides, waxes, glycerophospholipids and steroids (Timberlake, 2003). The lipids found in food waste consist mainly of triacylglycerides (Fernandez et al., 2005). Triacylglycerides consist of a glycerol backbone with three fatty acid chains attached by ester bonds (Timberlake, 2003). They are hydrolysed to long-chain fatty acids (LCFA) and glycerol by extracellular lipases excreted by acidogenic bacteria (Timberlake, 2003, Fernandez et al., 2005, Cirne et al., 2007). The nature of the fat (whether it is animal or vegetable in origin) influences which LCFA will be produced, but LCFA generally contain an even number of carbon atoms, usually between 14-24 (Fernandez et al., 2005).



After hydrolysis of the ester bonds, glycerol is then fermented to propionate, while the LCFA are primarily metabolised via the *β*-oxidation pathway (Fernandez et al., 2005, Li et al., 2005).

β-oxidation basically consists of the sequential cleavage of two-carbon fragments from a fatty acid chain, yielding acetic acid, hydrogen and a new fatty acid that has two less carbon atoms in its chain than the original fatty acid.



This is repeated until all of the fatty acid chain has been converted to acetate and hydrogen (and propionate in the case of LCFA with an odd number of carbons in the chain). In this way, *β*-oxidation provides substrates for both acetoclastic and hydrogenotrophic methanogenesis.

Lipids are attractive for biogas production as they are high in potential energy – for example, the theoretical methane yield from 1 g of oleate is 1.01 L CH_4, whereas the theoretical methane yield from glucose is only 0.37 L CH_4 g^{-1} (Kim et al., 2004). Lipids can be slow to break down; being non-polar, they do not dissolve in water, and can cause problems by adsorption onto cell membranes, resulting in sludge flotation and washout, and also interference with membrane function in methanogenic bacteria (Fernandez et al., 2005). In the treatment of wastewater containing high lipid concentrations, such as wastewater from slaughterhouses or food processing industries such as edible oil refining, a physicochemical pretreatment flotation process may be used to reduce lipids before anaerobic treatment (Pereira et al., 2001).

Lipids and their breakdown products can potentially be inhibitory to anaerobic digestion processes. This is discussed further in Section 2.3.4.

Processing of Mixed Food Wastes

Mixtures of readily biodegradable and slowly biodegradable substrates may be less degradable than pure slowly biodegradable substrates – Beccari et al (1999) found slightly higher methane production during the first 50 days of digestion of olive mill effluent (which has a high lipid content) than from a mixture of olive mill effluent with glucose, even though the COD of the mixture was nearly twice as high as that of the pure OME. They attributed the lower gas production to a temporary inhibition of methanogenesis by a build-up of VFA produced from glucose (Beccari et al., 1999). This has implications for the processing of feedstocks containing a mixture of readily biodegradable material (starches and sugars) and more slowly-degradable material (lipids). In a continuously-fed system, the constant input of readily-degradable material could possibly interfere with the degradation of more slowly-degradable materials by continuous production of VFA and

hydrogen, which creates an environment less favourable for the breakdown of LCFA (Fox and Pohland, 1994) due to product inhibition.

2.3 Inhibition

As described in previous sections, there are several elements and compounds that must be supplied in appropriate amounts for the anaerobic digestion process to proceed. Some substances, however, if present in excess, can have a negative effect on microorganisms, and result in slowing or cessation of growth by various mechanisms. This is referred to as inhibition, some types of which are described in the following sections.

2.3.1 Volatile Fatty Acid Inhibition

Volatile fatty acids are essential intermediates in the anaerobic digestion process and their production and degradation is necessary for the conversion of organic macromolecules to methane. At high concentrations, however, they can become inhibitory. Veeken et al. (2000) examined the effect of VFA on hydrolysis, in a system in which pH and VFA concentrations could be controlled separately. They found that pH affected the hydrolysis rate constant, while total and undissociated VFA concentration did not, showing that the inhibitory effect of VFA on hydrolysis was a result of the effect of high VFA on pH rather than a direct result of the VFA themselves. Propionic and butyric acids are generally agreed to be the most inhibitory VFA (Mata-Alvarez, 2003).

The salts of VFA have proton-accepting capacity at a low pH (below 5.75), and can lead to misleading results in the measurement of the amount of buffering capacity in a digestion system, when titration to a pH of 4.0 is used to measure alkalinity. This is because this titration will then include alkalinity from the association/dissociation of the salts of VFA, which does not actually buffer the system in the neutral range needed by methanogens (Lahav et al., 2002). The endpoint of 4.0 includes both carbonate and ammonium alkalinity, and alkalinity due to the salts of VFA. To separate the different buffering, Partial Alkalinity (PA) as measured to pH 5.75 is used as a measure of bicarbonate buffering, while Intermediate Alkalinity (IA) as measured between pH 5.75 and 4.3, is used as a measure of the buffering capacity attributable primarily to the salts of VFA. The ratio of IA to PA gives an indication of VFA in the system, and is sometimes referred to as Ripley's Ratio (Ripley et al., 1986).

2.3.2 Sulphide Inhibition

Sulphur species are also potentially inhibitory to digestion. Although sulphur is required by methanogens and is an obligate macronutrient (Gerardi, 2003), sulphur species can also interfere with the process in a number of ways. Firstly, sulphates formed from sulphur-containing amino acids during the acidogenesis phase act as a substrate for sulphate-reducing bacteria (SRB), such as the *Desulfovibrio* and *Desulfuromonas* genera, which compete with methanogens for carbon sources. The reactions involving sulphate as an electron acceptor to reduce hydrogen and volatile fatty acids to sulphides are more thermodynamically favourable than the formation of acetate or methane (Stams et al., 2005). This also means that sulphate reducers have faster doubling times than methanogens and are therefore capable of outcompeting the methanogens (Gerardi, 2003).

In addition, the presence of sulphate can increase the oxidation-reduction potential in the medium beyond that required by methanogenic bacteria. Oxidation-reduction (or redox) potential is a measure of the amount of transferable electrons available in a medium. Highly negative redox values indicate a large number of transferable electrons available (Madigan et al., 2003); redox potential above -300 mV can inhibit methanogenesis (Gerardi, 2003).

The reduction of sulphate to sulphide also causes problems for bacteria and archaea, as concentrations of dissolved sulphides above 200 mg l^{-1} are considered inhibitory (Tchobanoglous and Burton, 1991). Methanogens are more susceptible to sulphide toxicity than acidogens (Gerardi, 2003). Dissolved sulphides can form precipitates with trace metals, which decreases their bioavailability (Oleskiewicz and Sharma, 1990). However Jansen et al. (2007) found rapid replenishment of dissolved cobalt, nickel and iron from the dissociation of metal sulphides after uptake of metals by cells, indicating that the influence of sulphides on metal bioavailability may be less of a problem than previously believed.

2.3.3 Ammonia Inhibition

During digestion, organic nitrogen is reduced to ammonia (NH_3) and ammonium ion (NH_4^+). The distribution of the two species is pH dependent, with the ionised form dominating at lower pH (Mata-Alvarez, 2003).

Numerous investigators have studied ammonia inhibition in anaerobic digestion processes (Bhattacharya and Parkin, 1989, Angelidaki and Ahring, 1993, Kayhanian, 1994, Poggi-Varaldo et al., 1997, Grady et al., 1999, Fujishima et al., 2000, Sung and Liu, 2003). It is

generally accepted that high ammonia nitrogen can be inhibitory to methanogenesis, and that the unionised free ammonia species is the more toxic form. The actual value at which ammonia becomes inhibitory, however, is still a matter of some debate.

Although a TAN concentration of 1.2 g l^{-1} and above was earlier reported as inhibitory (Mata-Alvarez, 2003), later studies of full-scale biogas plants in Denmark found continued operation, although with decreased efficiency, at TAN of 4 g l^{-1} and up to 6 g l^{-1} with sufficient time for acclimation (Angelidaki and Ahring, 1993). Grady et al. (1999), however, stated that ammonium ion concentrations could reach up to 9 g l^{-1} for an acclimated population before toxicity would be observed. According to Hartmann and Ahring (2005), ammonia inhibition can begin at free ammonia concentrations beyond 0.65 g N l^{-1}.

The fraction of free ammonia relative to total ammonia concentration is dependent on pH and the dissociation constants of water and ammonia nitrogen, as shown in the following formula (Kayhanian, 1994):

$$NH_{3f} = \frac{\left(TAN \times \frac{K_a}{[H]}\right)}{\left(\frac{K_a}{[H]} + 1\right)}$$

Where:
NH_{3f} is the free ammonia nitrogen concentration, in mg l^{-1}
TAN is the total ammonia nitrogen concentration, in mg l^{-1}
K_a is a temperature dependent constant for the dissociation of ammonium
$[H]$ is the hydrogen ion concentration, 10^{-pH}

pK_a is the negative logarithm of the K_a constant, and is calculated as follows (Hansen et al., 1998):

$$pK_a = 0.9018 + \frac{2729.92}{(273.15 + T)}$$

Where:
T is the temperature of the medium, in degrees Celsius

The pK_a for the dissociation of ammonia is approximately 9.3 at mesophilic temperature (Grady et al., 1999) so at the pH values typical for anaerobic digestion most ammonia is in ionised form as ammonium ion.

The dissociation of ammonia is also highly temperature dependent; at mesophilic temperature (35^0C) and pH of 7, total ammonia concentrations may exceed 10,000 mg l^{-1} while free ammonia stays below 100 mg l^{-1}, whereas at the same pH at thermophilic temperature (55^0C), free ammonia will reach 100 mg l^{-1} if total ammonia exceeds 2,000 mg l^{-1} (Grady et al., 1999).

A total ammonia concentration of 2,000 mg l^{-1} will also result in free ammonia concentrations over 100 mg l^{-1} in a mesophilic system, however, if pH is between 7.5 and 8 (Grady et al., 1999).

There is some possibility for a system to self-correct, as the fraction of free ammonia decreases with decreasing pH. In systems with high concentrations of ammonia, ammonia inhibition can lead to high VFA production, which causes the pH to drop, which in turn shifts the ammonia/ammonium equilibrium in favour of lower concentrations of free ammonia, which can then stabilise the process (Angelidaki et al., 1993).

The temperature dependency of ammonia dissociation has implications for the stability of thermophilic digestion vs. mesophilic digestion of feedstocks high in nitrogen. In the pilot scale food waste digestion system noted earlier, Banks et al. (2008) found that although VS destruction and biogas yield were higher for a thermophilic digester than a mesophilic digester on the same feedstock, the thermophilic digester was unstable, with accumulation of propionate and high total VFA. The mesophilic digester, on the other hand, maintained operation with a TAN of approximately 5.2 g l^{-1} and VFA peaking at 27 g l^{-1}.

In a study of 18 full-scale biogas plants, Angelidaki et al. (2005) found a correlation between ammonia and VFA, with high concentrations of ammonia (4-6 g l^{-1}) associated with high concentrations of total VFA (also 4-6 g l^{-1}). They concluded that plants fed with substrate with a high ammonia concentration, such as pig manure, were more stressed and losing more potential biogas production in the digester effluent. They were still able to operate at these high concentrations, however, due to the self-regulating interaction of VFA and ammonia/ammonium described above.

Operation at high TAN has also been seen in commercial scale digestion in Sweden (Schnurer and Nordberg, 2007); the system has operated for several years with TAN of 5.2 g l^{-1}, with a VFA concentration of 2.3 g l^{-1}.

Ammonia may act as a non-competitive inhibitor, i.e., it does not compete with substrate for the same sites on the enzymes, but instead binds at other sites on the enzyme (Bhattacharya and Parkin, 1989, Mata-Alvarez, 2003). In a CSTR system subject to non-competitive inhibition, the system appears to operate normally with no significant changes until the toxicant is increased beyond a threshold concentration, at which point a sudden increase in effluent substrate concentration is observed and the system begins to fail (Bhattacharya and Parkin, 1989).

Bhattacharya and Parkin (1989) found that with ammonia additions to chemostats, a reactor on a shorter solids retention time (SRT = 15 days) was able to tolerate higher slug loadings of ammonia than reactors on the longer SRTs of 25 and 40 days, respectively. The 15-day reactor tolerated slugs of up to 8 g l^{-1} total ammonia nitrogen (TAN), while a TAN of 7 mg l^{-1} was the highest slug that could be tolerated by the long SRT reactors. With continuous ammonia addition, however, the situation was reversed: the reactor at an SRT of 40 days was able to acclimate to a TAN concentration of up to 5 g l^{-1}, while the 15-day SRT reactor showed failure beyond 2 g l^{-1} TAN. The difference between no-effect and complete failure was pronounced; for the 40-day SRT reactor, continuous addition of 5 g l^{-1} TAN showed little effect, but at 6 g l^{-1} TAN complete failure was observed. This agrees with the non-competitive inhibition hypothesis (Bhattacharya and Parkin, 1989).

Bhattacharya and Parkin (1989) measured the ratio of TAN:VSS as a measure of specific ammonia loading to biomass. They found that the studied systems failed when the TAN:VSS ratio exceeded approximately 16, and stated that a high biomass concentration could indicate a good potential for tolerating high TAN concentrations, but pointed out that this may not always be the case. Their results show that a high biomass concentration is advantageous for acclimation to high continuous ammonia concentrations, but systems with lower SRT and therefore lower biomass concentrations may be better equipped for sudden slugs of ammonia, due to the higher dilution rate. They found also that ammonia had a greater effect on acetoclastic methanogenesis than propionate utilisation (Bhattacharya and Parkin, 1989).

Lokshina et al. (2003) modelled inhibition by both ammonia and LCFA in the digestion of slaughterhouse waste and household kitchen waste, and noted that in actual systems both may contribute. They found that pH remained between 6.8 and 7.5 despite periodically

high VFA concentrations exceeding 2000 mg l^{-1}, due to the high buffering from the ammonia-bicarbonate buffer system.

Schnurer and Nordberg (2007) ran systems at high TAN and VFA to test for effects of high nitrogen on the pathway for methane production. At a TAN of 3.3 g l^{-1}, VFA was stable around 18 g l^{-1}, while an increase in TAN to 5.5 g l^{-1} led to a decrease in methane production from 0.4 l gVS_{added}^{-1} to 0.2 l gVS_{added}^{-1}, and a further TAN increase to 6.9 g l^{-1} resulted in complete process failure.

They determined that high ammonia concentrations were associated with a shift from acetoclastic methanogenesis to syntrophic acetate oxidation. In syntrophic acetate oxidation, acetate is first converted to carbon dioxide and hydrogen, from which methane is then formed by autotrophic methanogenesis. They found that these systems required a longer HRT because the doubling time of mesophilic syntrophic acetate oxidizing cultures (approximately 28 days) was longer than that of acetoclastic methanogenic cultures (2-12 days).

Possible control methods for high ammonia concentrations are dilution of digester content and increase in feedstock carbon:nitrogen ratio (Mata-Alvarez, 2003), such as by co-digestion with substrates low in nitrogen. It was recommended (Mtz.-Viturtia et al., 1995) that wastes with C:N ratios lower than 10 should not be treated in one-phase systems at loading rates beyond 3 g COD $l^{-1}d^{-1}$ due to instability caused by ammonia inhibition. A C:N ratio of 25:1 has been cited as optimal for microbial activity involved in conversion of vegetable biomasses to methane (Bouallagui et al., 2005).

2.3.4 Lipid and Long-Chain Fatty Acid Inhibition

Many investigators have found inhibition of various stages of the biomethanisation process by the first breakdown products of lipids, the long-chain fatty acids (Hanaki et al., 1981, Koster and Cramer, 1987, Angelidaki and Ahring, 1992, Broughton et al., 1998, Salminen and Rintala, 2002b, Lokshina et al., 2003, Mykhaylovin et al., 2005, Pereira et al., 2005, Li et al., 2005).

Lipids may potentially interfere with both of the two main rate-limiting steps in the AD process: hydrolysis and methanogenesis (Neves et al., 2006a). Firstly, the non-polar lipids and LCFA may adsorb to particulate substrate, making the substrate more resistant to enzyme attack and therefore slower to hydrolyse (Sanders, 2001). Secondly, adsorption of

lipids and LCFA onto bacterial cells can interfere with the mass transport of solutes such as acetate which then inhibits methanogenesis (Neves et al., 2006a).

Lipids have a propensity to float to the top of the digesters, and form aggregates with a low surface area to volume ratio, making them recalcitrant to attack by hydrolytic enzymes (Broughton et al., 1998). Also, lipid hydrolysis depends on extracellular lipases which act at the lipid-water interface, and when LCFA are produced by hydrolysis they remain at the lipid-water interface due to their amphiphilic structure (one end of the molecule is hydrophobic, while the other is hydrophilic), which interferes with the further action of the lipases by decreasing available surface area at the lipid-water interface (Cirne et al., 2007).

For a substrate consisting not solely of lipids, but also containing high quantities of readily degradable polysaccharides, these readily degradable materials provide a constant source of VFA while the more slowly degradable and less easily hydrolysed lipids may build up in the digester. This is similar to the example cited by Fox and Pohland (1994), of accumulation of fats and greases at the reactor inlet for anaerobic filters treating wastewaters containing lipids and polysaccharides. The easily-acidified substrates are degraded first, creating a high hydrogen partial pressure at the inlet. Since β -oxidation requires a low partial pressure of hydrogen, this therefore create an environment unfavourable for lipid degradation at the inlet.

A number of studies have been carried out with various LCFA; oleic acid or its salt, sodium oleate, are often used in investigations because oleic acid is one of the most abundant LCFA generally present in wastewaters and known to be inhibitory. While oleic acid is highly insoluble with an aqueous solubility of approximately 3 mg l^{-1} at 20 ^{0}C (Lalman and Bagley, 2001), sodium oleate has good solubility (Alves et al., 2001).

Lalman and Bagley (2001) reported an inhibition threshold of 30 mg l^{-1} for oleic acid and 300 mg l^{-1} for stearic acid on acetoclastic methanogenesis, at 21 ^{0}C. They were looking also for inhibition of hydrogenotrophic methanogenesis, but found that this was less sensitive to LCFA inhibition than acetoclastic methane production, which agrees with the results of other investigators (Hanaki et al., 1981, Koster and Cramer, 1987).

Pereira et al. hypothesized an adsorption of LCFA onto microbial cells. In their experiment, biomass degrading oleic acid as the sole substrate, that was first washed a number of times with basal medium and then incubated in the absence of any substrate, continued to produce methane, and indeed produced higher amounts of methane than non-

acclimated biomass subjected to the same treatment and then incubated with varying amounts of oleic acid (Pereira et al., 2001). After the completion of this batch test (all biomass-associated material mineralized), they incubated the same sludge with simple substrates (acetate, propionate, butyrate, ethanol and H_2/CO_2) and found enhanced activity of the sludge relative to its initial activity. This showed that the LCFA had not caused cell wall damage or irreversible toxicity, and lent evidence to their argument for mass transfer limitations, rather than toxicity, as the reason for the depressed activity in the presence of LCFA (Pereira et al., 2005).

In mineralization of biomass-associated LCFA, they observed a lag phase in the commencement of methane production from 100 to 500 hours for sludge from reactors processing oleic acid. They hypothesised that the lag could be due to transport limitations of solutes into and out of cells, rather than the growth of surviving bacteria after a bactericidal effect of LCFA (Pereira et al., 2004). They then quantified the kinetics of mineralization of LCFA associated to anaerobic sludge in batch trials, concluding that biomass-associated LCFA of up to 5 g COD-LCFA g_{VSS}^{-1} could be mineralized but that the mineralization was subject to inhibition beyond 1 g COD-LCFA g_{VSS}^{-1}. They noted that optimal LCFA mineralization would occur only when there was no other carbon source in the medium (which is rare in the anaerobic digestion of mixed substrates). They found further evidence for this assertion after conducting batch activity tests in which biomass with associated LCFA was incubated with acetate, propionate or butyrate. They found that the methane production rate indicated depletion of the added VFA before mineralization of the biomass-associated LCFA, in the case of acetate and butyrate. They concluded that their results support the use of anaerobic digestion modes involving sequential accumulation and degradation steps (e.g., alternating continuous feeding with allowance of a batch degradation period) for the treatment of lipid/LCFA-containing wastewaters.

Beccari et al. (1999) found a 25-day lag period before the commencement of degradation of oleic acid (OA) as a sole substrate in batch tests. The presence of OA resulted in a lag time of 40 days before the production of methane from glucose as a substrate, and increased the lag time for the production of methane from 25 to 50 days with olive mill effluent as the substrate. The addition of oleic acid had a strong inhibitory effect on the production of methane from glucose and olive mill effluent, but no effect on the production of VFA.

Lag times in the onset of methane production in batch tests have been found for lipid substrates including: slaughterhouse wastes - 10 days (Salminen et al., 2000); sheep tallow – 13 to 48 days (Broughton et al., 1998); and model waste containing triolein, starch, whey protein and cellulose – 10 to 40 days (Cirne et al., 2007) with the length of the lag time varying according to the concentration of lipid in the substrate.

In flask assays, digestion of a mix of glucose and olive mill effluent with a high lipid content was found to have slightly lower methane production than the olive mill effluent alone, even though the actual COD fed was almost twice as high for the mixture than the single substrate, and this was attributed to VFA inhibition due to rapid acidogenesis from the glucose (Beccari et al., 1999). The presence of readily degradable components interfered with the digestion of the lipids.

Triglyceride lipids are hydrolysed to glycerol and LCFA by microbial exolipases (extracellular lipolytic enzymes) (Broughton et al, 1998). While the glycerol is fermented to VFA, alcohols and formic acid, the LCFA are degraded by beta-oxidation carried out by syntrophic acetogenic bacteria (SAB). The activity of the SAB requires the concentration of acetate, formate and hydrogen in the medium to be kept low to prevent product inhibition and make the reaction thermodynamically favourable (Broughton et al., 1998).

Acetogenic bacteria, other than being active in LCFA degradation, are also responsible for the production of acetate from solubilised sugars, which are readily available from the hydrolysis of carbohydrates. Having a constant source of VFA from readily degradable carbohydrates may mean that the lipids build up as the more favourable substrates are consumed first.

A significant factor in LCFA degradation is the production and consumption of hydrogen. β-oxidation is thermodynamically unfavourable under standard conditions:

$$n\text{-carboxylic acid} \rightarrow (n\text{-}2)\ \text{carboxylic acid} + CH_3COOH + 2\ H_2 \qquad \Delta G^{0/} = +\ 48\ \text{kJ mol}^{-1}$$

(Fox and Pohland, 1994), requiring syntrophic removal of the reaction products. Methanogenesis provides a syntrophic complement to the process by the uptake of both acetate and hydrogen. This provides an argument for the use of single-stage, rather than dual-stage, reaction systems for the anaerobic digestion of wastes with a high lipid content (Fox and Pohland, 1994); although these authors also noted the possibility for lipid

inhibition of methanogenesis and stated that two stage reaction systems may sometimes be better.

Fatty acids that contain double bonds (unsaturated) must first be hydrogenated to saturated fatty acids (e.g., oleic acid is converted to stearic acid – both have 18 carbons but oleic has one double bond, while stearic acid contains only single bonds) before they are degraded by *β*-oxidation (Pereira et al., 2005). Some investigators have found this to be a rate-limiting step: Salminen et al. (2000) found that the saturation step converting oleic acid (18:1) to stearic acid (18:0) was the rate limiting step, after which the step from stearic to palmitic proceeded quickly, as evidenced by substantial concentrations of palmitic in the digestate (Salminen et al., 2000). Lalman and Bagley (2001) proposed the possibility of *β* –oxidation from oleic acid to palmitic acid occurring without an initial saturation step, due to their observation that no stearic acid was detected during degradation of oleic acid in their research, even though stearic acid produced from oleic would be expected to accumulate in the medium as its conversion to palmitic acid has a positive ΔG value under standard conditions.

To deal with the double bonds of unsaturated LCFA such as oleic acid in olive mill effluent, Andreozzi et al. attempted pre-treatment by ozonation, which selectively attacks the double bonds of unsaturated fatty acids and phenols. They found, however, that although the ozonation was effective in reducing oleic acid, there was greater inhibition of methanogenesis than from the untreated substrate, due to products arising from the ozonation process (Andreozzi et al., 1998).

Although Pereira et al. (2005) found that sludge from reactors fed with oleic acid (C18:1) as the sole carbon source had lower activity than sludge from reactors fed with palmitic acid (C16:0) as the sole carbon source, when analysing the LCFA associated with the sludge they found that, for both sludges, palmitic acid was the main LCFA, indicating that the saturation step and the first *β*-oxidation step had already occurred, but they used electron micrographs to show that the manner of LCFA accumulation differed for the two fatty acids – cells fed oleic were 'encapsulated' while in the reactors fed palmitic directly, there were individualized precipitates rather than encapsulation of the cells. This indicates that the unsaturated oleic acid was adsorbing onto cells where the saturation and initial *β* -oxidation steps were occurring. The investigators hypothesised that further degradation of the palmitic acid was inhibited by the continuous feed of oleic acid.

Following work on the degradation kinetics of biomass-associated LCFA and activity tests co-digesting VFA with biomass-associated LCFA, Pereira et al. recommended digestion in sequential accumulation and degradation steps for lipid-containing wastes. This recommendation arose from their observations indicating that optimal degradation of biomass-associated LCFA would occur only after shorter-chain VFA in the medium were depleted (Pereira et al., 2004).

The observations were used in later experimental design for other workers in the same group (Neves et al., 2008b): in work with co-digestion of cow manure and food waste, lipids from fish oil processing were fed as pulses rather than constantly with the regular feed. This was to address the issue of adsorption of lipids or LCFA to cells, leading to mass transfer limitations and inhibition of methanogenesis. They found that adding the fat as pulses rather than constantly allowed for enhanced methane production, but that at the highest pulse tested (giving a concentration of 18 g COD_{fat} l^{-1} in the reactor) the process was inhibited. This was reversible, however, after allowance of sufficient time for degradation of the fat.

In digestion of poultry slaughterhouse wastes, a substrate high in lipids and protein, and co-digestion of these wastes with the organic fraction of household municipal waste, Cuetos et al. (2008) observed methanogenic failure at a hydraulic retention time of 25 days. Analysis of the digestate revealed high concentrations of LCFA, beyond those previously reported as toxic. They also measured concentrations of total ammonia nitrogen, and calculated the concentration of free ammonia to be less than 15 mg l^{-1}, far below inhibitory levels, and thereby concluded that the cause of failure of the reactors was related to accumulation of LCFA and VFA, rather than ammonia inhibition. They subsequently restarted the system at an HRT of 50 days and lower OLR of 0.9 gVS l^{-1}, which they were then able to progressively increase to 1.7 gVS l^{-1} while decreasing HRT to 25 days again, after acclimation of the microbial populations.

In the field of ruminant microbiology, a number of papers have been published regarding inhibition of microbial methanogenesis by long-chain (14 carbons or more) (Templer et al., 2006) and medium-chain (8-14 carbon atoms) fatty acids (Machmuller, 2006). In this field, minimization of methane production is favourable, so there have been a number of studies in which feed to sheep and cattle was supplemented with sources of LCFA or MCFA such as oils from coconut, palm kernel or canola, with the aim of *reducing*

methane production (Machmuller, 2006). It has also been found that LCFA can reduce the degradation of fibre in the rumen (Machmuller, 2006).

A mix of iron, nickel and cobalt was shown to play a role in supporting maintenance of granular sludge in UASB reactors treating a high-lipid wastewater from food processing (Oleskiewicz, 1989). In other work, researchers investigated the effect of pre-incubation with iron on the production of methane from acetate, hydrogen and butyrate in the presence of oleic acid. They found that anaerobic consortia that had been incubated with substoichiometric amounts of Fe (III) in the form of ferric hydroxide were less susceptible to inhibition by oleic acid than by those to which no iron had been supplied. This was true for the utilisation of acetate and butyrate, but not for the utilisation of hydrogen, which was not inhibited by the presence of oleic acid (Li et al., 2005). These researchers suggested that the pre-incubation with ferric hydroxide may have selected for a microbial consortium that was less susceptible to inhibition by LCFA. They also suggested that the provision of iron may have resulted in a larger population of iron-reducing bacteria, which could produce hydrogen and/or oxidize acetate to provide alternate pathways for electrons to be transferred to methane under conditions where hydrogen-producing acetogens and/or acetoclastic methanogens were inhibited by high concentrations of LCFA. Their results and reasoning provide support for the hypothesis that trace elements may play a role in the response of acetoclastic methanogenesis to the presence of high concentrations of LCFA.

In LCFA degradation, methanogenic activity is required to reduce inhibitory concentrations of formate, acetate and hydrogen (Broughton et al., 1998). If LCFA are allowed to build up, inhibition of syntrophic acetogenic and methanogenic bacteria can occur (Hanaki et al., 1981, Koster and Cramer, 1987, Angelidaki and Ahring, 1992). LCFA toxicity has been shown to be reduced, however, by the addition of mineral compounds such as calcium chloride or bentonite clay (Hanaki et al., 1981, Broughton et al., 1998)

One mechanism suggested for this is that Ca^{2+} and Mg^{2+} ions complex with LCFA to form a precipitate, reducing the adsorption of LCFA onto bacterial cells (Pereira et al., 2001). The converse effect is that if lipids complex to metals, this will make the metals less bioavailable to methanogens.

2.3.5 Cation Inhibition

Cation toxicity is another factor to consider in anaerobic digestion. The ions calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}) have both stimulatory and inhibitory effects on digestion, depending on concentration. They are considered beneficial at concentrations in the range of 100-400 mg l^{-1}, but can exert toxicitiy at concentrations beyond 1500 mg l^{-1}, as stated by Gerardi (2003). Other investigators, however, found a high cation inhibition threshold of 5 g l^{-1} in batch thermophilic trials (Kim et al., 2000).

Inhibition of methanogenesis from fruit and vegetable wastes was attributed to high potassium concentration, as potassium is a regulator of osmotic channels and therefore affects the osmotic pressure of cells (Carucci et al., 2005). The potassium content of the waste was 55 g kg^{-1} TS, as compared to 1.9 g kg^{-1} TS for sludge or 3.0 g kg^{-1} TS for precooked food waste.

2.3.6 Other Inhibitory Compounds

Phenols and other compounds containing a benzene ring are also capable of inhibiting methane-forming bacteria (Gerardi, 2003). The phenolic compounds include chlorophenols, nitrophenols and tannins, which occur naturally in fruits and vegetables such as apples, bananas, beans, cereals and coffee.

Other compounds that can inhibit methanogenesis include recalcitrant compounds such as hydrocarbons and chlorinated compounds (Gerardi, 2003), but these are not expected to be present in any significant amount in the subject feedstock and therefore are not discussed further.

2.4 Co-Digestion

Many of the inhibitory substances discussed in Section 2.3 are not toxic or inhibitory in themselves, but only become inhibitory when they reach high concentrations. One way to achieve lower concentrations of these in the medium is to co-digest the substrate with another substrate that is low in the element of concern. In this way, the total proportion of the element of concern in the feedstock is lower and therefore lower concentrations of its conversion products or accumulant will be present in the digesting medium.

There are numerous examples of co-digestion. Two common substrates for co-digestion are animal slurries and wastewater sludges, due to their ready availability, presence of

existing infrastructure for digestion (e.g. manure digesters, municipal sludge digesters) and stability in digestion.

The potato waste / pig manure co-digestion work by Kaparaju and Rintala (2005) described in Section 2.2.1 is an example of using co-substrates for synergistic effect. They experienced difficulties with process stability in digesting potato waste alone or at high ratios of potato waste to pig manure (Rintala, 2006 *pers. comm*.), but were able to achieve improved gas yields and a stable process at ratios of up to 20:80 VS_{potato}:$VS_{pig\ manure}$.

Gomez (2006) found that biogas yield and specific gas production could be increased by co-digestion of fruit and vegetable wastes with primary sludge, as noted in Section 2.2.1.

Edelmann et al. (2000) co-digested food wastes from supermarkets with sewage sludge in digesters of a municipal wastewater treatment plant. The food waste addition resulted in not only an increase in volumetric gas production from the digester, but also an increase in specific gas production from the sludge, attributed to nutrient benefits from the food wastes.

Nordberg and Edstrom (2005) co-digested energy crops with biodegradable municipal waste (BMW) and achieved a daily loading of 6 gVS l^{-1} and methane yield of 0.33-0.38 L gVS^{-1} at an optimum ratio of 50:50 silage:BMW. They noted that the cobalt content of the BMW (0.8 mg $kgTS^{-1}$) benefited the digestion of the silage.

Zhang et al. (2008) are currently investigating the co-digestion of food wastes with used office paper and potato waste as a strategy to raise the C:N ratio of the input feedstock. Cattle slurry is also being investigated as a co-substrate for stability and the benefits of processing food wastes in slurry digesters for increased gas production.

2.5 Summary and Areas for Further Research

This literature review has identified a number of key areas necessary to understanding the process issues related to the anaerobic digestion of food wastes, and gaps in the literature where further information is needed.

As outlined in Section 2.1, the process fundamentals such as basic nutrient requirements and environmental conditions have been well studied for the anaerobic digestion of most feedstocks. There has been substantial research into the effects of trace element deprivation and supplementation in anaerobic digestion of defined feedstocks such as methanol

(Florencio et al., 1993, Zandvoort et al., 2002); or on mono-digestion of single solid substrates such as energy crops (Jarvis et al., 1997, Lebuhn et al., 2008). There is a gap in recent research, however, on the potential for trace element limitation for a mixed solid waste feedstock, and no studies dealing specifically with trace element limitation in anaerobic digestion of food wastes.

There is also little existing literature on the effects of trace element addition under inhibitory conditions, such as high concentrations of ammonia or LCFA. Fish (1999) suggested that cobalt addition would have the most effect during startup or conditions of stress; this is worthy of further study.

Section 2.2 summarises the current state of literature on anaerobic digestion of food wastes. A number of recent studies have been published on anaerobic digestion of fruit and vegetable waste feedstocks. Much less has been done, however, with source segregated food wastes with high contents of nitrogen or lipids, and still less on feedstocks containing both of these components. As described in Section 2.3, the breakdown products of both nitrogen and lipids can inhibit the anaerobic digestion process, and it is therefore necessary to gain a better understanding of their potential effects over long digestion periods. Although there have been some studies dealing with high-nitrogen or high-lipid food wastes, these primarily used batch or short-term digestion trials (Carucci et al., 2005, Zhang et al., 2007, Neves et al., 2008a). It is important to study the effects over longer term trials, to assess the potential for inhibition by ammonia and LCFA over long term continuous feeding. In fact, in studies dealing with longer-term semi-continuous feed trials, inhibition has been encountered (Salminen and Rintala, 2002b, Banks et al., 2008), pointing to a need for further study to better understand the potential process issues that can arise with these feedstocks.

This study, therefore, is intended to address the knowledge gaps concerning the role of trace elements in digestion of mixed waste feedstocks, and the potential process issues associated with long-term continuous digestion of food waste with high content of nitrogen and lipids.

3. Materials and Methods

This chapter describes the experimental methods used in this study.

3.1 Analytical methods

3.1.1 Chemicals Used and Preparation of Glassware

All reagents and standards were prepared using deionised water (Elix electrodeionisation system, 97% ionic rejection, Millipore Corporation, UK), except for standards and materials used for analysis of trace concentrations of metals. All glassware used was washed with detergent and rinsed with tap water followed by distilled water before reuse. All chemicals used were from Fisher Scientific (Loughborough UK) except where otherwise noted.

All glassware and apparatus used in the analysis of trace concentrations of metals was washed with detergent, rinsed twice with tap water and twice with deionised water, then soaked in an acid bath of 10% hydrochloric acid for at least twelve hours. Water used in the acid bath, subsequent rinsing and all trace metal analysis was purified by a reverse osmosis system (Milli-Q RiOs, resistivity 18.2 MΩ-cm at 25^0C, filter cartridge 0.22 μm, Millipore Corporation, UK), hereafter referred to as Milli-Q water. All acids used in trace metal analysis were trace analysis grade.

Glassware and apparatus used for lipid analysis was washed with detergent and heated to 550^0C to burn off any lipids or other organics that could affect analysis.

3.1.1 pH

pH of the digestate was measured using a Jenway 3310 pH meter (Jenway Ltd., Essex UK), immediately after sampling to avoid pH changes due to loss of CO_2 from the liquid. The meter has a sensitivity of 0.01 pH units and accuracy to 0.01 ± 0.005 units.

Calibration of the probe was carried out before each day's pH measurement, using buffer solutions of pH 4.0, 7.0 and 9.2, prepared from pH buffer tablets (Fisher Scientific) dissolved in 100 ml of deionised water. Fresh buffer solutions were prepared weekly and stored in closed containers between uses. Cross-contamination was avoided by thorough rinsing of the probe between each measurement, and storage of the probe in a mild acid solution between uses.

3.1.2 Alkalinity

A sample volume of 20 ml of digestate was made up to 50 ml with deionised water (to minimise foaming) and titrated with 0.25 N H_2SO_4 to pH 4.0 for total alkalinity, with constant stirring using a magnetic stirrer.

To separate the different buffering, Partial Alkalinity (PA) was measured to pH 5.75 as a measure of bicarbonate buffering, while Intermediate Alkalinity (IA) was measured between pH 5.75 and 4.3, as the buffering capacity attributable primarily to the salts of VFA. The ratio of IA to PA (Ripley's Ratio) gives an indication of VFA concentrations in the system (Ripley et al., 1986).

The probe was calibrated before each alkalinity titration using the buffer solutions noted above for pH measurement. Cross-contamination was avoided by thorough rinsing of the probe between each measurement, and storage of the probe in a mild acid solution between uses.

For alkalinity and all further analyses, at least five replicates were done during the first few weeks of analysis, until it was established that the variation between replicates was less than 10%.

The method is based on the Standard Method 2320B for Alkalinity (APHA, 2005). Alkalinity in mg $CaCO_3$/L was calculated based on the equation below:

$$ALK = \frac{A \times N \times 50{,}000}{V_s}$$

Where
A is the amount of acid used to titrate the sample, in mL;
N is the normality of titrant;
50,000 is the conversion factor of L to mL and 50 mg $CaCO_3$ to 1 milliequivalent alkalinity;
V_s is the volume of the sample, in mL.

3.1.3 Total and Volatile Solids

Substrate and digestate samples were analysed according to the following procedure:

After thorough agitation of the sample, approximately 10 g of sample was transferred into a weighed crucible by pouring (digestate samples) or spatula (substrate samples). Samples were weighed to an accuracy of 10±0.001 g (Sartorius LC6215 balance, Sartorius AG, Gottingen Germany) and placed in an oven (Vulcan laboratory oven, LTE Scientific Ltd.,

Oldham UK) for drying overnight at 105^0C ± 2^0 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, transferred to a muffle furnace (Carbolite Furnace 201, Carbolite UK, Hope Valley UK) and heated to 550^0C ± 10^0 C for a minimum of one hour (total cycle time for heating and cooling 3 hours). After this ashing step, samples were again cooled in a desiccator for at least one hour, before weighing a third time.

After all analyses, crucibles were washed with detergent, rinsed with deionised water, and stored in an oven until required for the next analysis. Crucibles were transferred from the oven to a desiccator for cooling to room temperature before each analysis.

Total and volatile solids were calculated according to the following formulae:

$$\%TS = \frac{W_3 - W_1}{W_2 - W_1} x100$$

$$\%VS = \frac{W_3 - W_4}{W_2 - W_1} x100 \text{ (as a percentage of fresh weight)}$$

$$\%VS = \frac{W_3 - W_4}{W_3 - W_1} x100 \text{ (as a percentage of TS)}$$

Where
W_1 is the weight of the empty crucible;
W_2 is the weight of the crucible containing fresh sample;
W_3 is the weight of the crucible and sample after drying at 105^0C;
W_4 is the weight of the crucible and sample after heating to 550^0C.

This is based on Standard Method 2540 G, "Total, Fixed, and Volatile Solids in Solid and Semisolid Samples"(APHA, 2005). The standard deviation of replicates analysed by this method was ± 0.1% or less.

3.1.4 Total Kjeldahl Nitrogen

Substrate and digestate samples were analysed for Total Kjeldahl nitrogen (TKN), which measures the nitrogen content in organic and ammonia form.

Reagents used:

1. H_2SO_4 concentrated sulphuric acid, laboratory reagent grade, low in N

2. Kjeltab Cu 3.5 catalyst tablets (Foss Analytical) containing 3.5 g K_2SO_4, 0.4 g $CuSO_4.5H_2O$
3. 40% NaOH concentrated sodium hydroxide, laboratory reagent grade
4. Indicating boric acid solution: 10 ml of mixed indicator (methyl red indicator dissolved in ethyl alcohol, methylene blue indicator dissolved in ethyl alcohol), 20 g boric acid (H_3BO_3) in deionised water to a total volume of 1 litre.

Method:

For each sample, a mass of 3-5 g was weighed to an accuracy of 1 mg into a glass digestion tube, and two catalyst tablets added to facilitate acid digestion by lowering the activation energy of the reaction. 12 ml of concentrated sulphuric acid (H_2SO_4) was added to each digestion tube and the tubes were gently agitated to ensure contact of the entire sample with the acid. One tube contained the Kjeltabs and acid with no sample, as a blank. Tubes were then subjected to acid digestion on a heating block with exhaust system (Foss Tecator 1007 Digestion System 6, Foss Analytical, Hoganas Sweden) at $420^0C \pm 5^0C$ until all tubes showed a clear blue-green solution, a time of at least two hours. PTFE tape was used to seal the tubes for the acid digestion step.

After cooling, the samples were subjected to steam distillation. For this step, a Foss Tecator Kjeltec System 1002 distillation unit (Foss Tecator AB, Hoganas Sweden) was used. Each cooled sample was distilled for a minimum of four minutes, after the addition of laboratory grade concentrated sodium hydroxide (NaOH, ca. 10M) to raise the pH beyond 9.5 and facilitate the volatilisation of ammonia. Distillate was collected in an Erlenmeyer flask containing indicating boric acid solution.

The distillate was then titrated with H_2SO_4 (0.25 N), until a colour change from green to lavender was obtained.

Nitrogen content of the sample was calculated according to the following formula:

$$\%N = \frac{(A - B) \times 14 \times 0.25 \times 100}{m}$$

Where
A is the volume of 0.25 N H_2SO_4 used to titrate the sample, in ml;
B is the volume of 0.25 N H_2SO_4 used to titrate the blank, in ml;
m is the mass of the original sample, in mg.

Using a figure for the molecular weight of nitrogen of 14 rather than14.0067 gave a difference of less than 0.05% in the final value.

This is based on Standard Method 4500-N_{org} B for organic nitrogen (APHA, 2005). The standard deviation of replicates analysed by this method was ± 6.3%.

3.1.5 Total Ammonia Nitrogen

Total Ammonia Nitrogen (TAN) was measured for digestate samples only. The procedure was similar to that described above for TKN analysis, with the exclusion of the acid digestion step. To minimise ammonia losses between the time of collection and analysis of samples, samples were analysed immediately or stored at 4^0C for no more than 2 hours between sampling and analysis.

For each digestate sample, 10 ml of sample was measured by mass to an accuracy of 10 mg, (My Weigh i500 balance, capacity 150 g ± 0.1 g, My Weigh Europe, Huckelhoven Germany) and added to a digestion tube with deionised water up to a volume of approximately 100 ml. The sample was then distilled and the distillate titrated according to the procedure described for TKN analysis.

Nitrogen content of the liquid sample was calculated according to the following formula:

$$NH_4N, mgl^{-1} = \frac{(A-B)\times 14.0\times 0.25\times 100}{V_{sample}}$$

Where
A is the volume of 0.25 N H_2SO_4 used to titrate the sample, in ml;
B is the volume of 0.25 N H_2SO_4 used to titrate the blank, in ml;
V_{sample} is the volume of the original sample, in ml.

Using a figure for the molecular weight of nitrogen of 14 rather than14.0067 gave a difference of less than 0.05% in the final value.

This is based on Standard Method 4500-NH_3 B and C (APHA, 2005).

3.1.6 Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured for substrate samples.

Reagents used:

1. H_2SO_4 concentrated sulphuric acid, with 10 g l^{-1} $AgSO_4$
2. H_2SO_4 concentrated sulphuric acid
3. Potassium dichromate solution ($K_2Cr_2O_7$, 0.2 M)
4. Ammonia iron(II) sulphate solution ($(NH_4)_2Fe(SO_4)_2$, 0.06 M with H_2SO_4 conc.,)
5. Ferroin indicator solution

Method:

Approximately 0.2 – 0.3 g of sample was weighed directly into Foss digestion tubes (as used in TKN analysis) and made up to approximately 10 g with deionised water. 20 ml of potassium dichromate solution was added, followed by 30 ml of concentrated H_2SO_4 containing 10 g l^{-1} $AgSO_4$. Two blanks were prepared in parallel using deionised water. After agitation, the tubes were connected to the digestion block as used in the TKN analysis (Foss Tecator 1007 Digestion System 6, Foss Analytical, Hoganas Sweden) and heated to 145^0C for a minimum of three hours. After cooling, contents of the tubes were quantitatively transferred to 250 ml volumetric flasks and deionised water added to make up the volume to 250 ml. From each flask, 10 ml of the sample was transferred by pipette (Finnpipette, Thermo Scientific, Finland) into an Erlenmeyer flask and made up to approximately 80 ml with deionised water. Ferroin indicator was added and the sample was then titrated with 0.06 M ammonia iron(II) sulphate solution until a colour change from blue-green to reddish brown was observed.

COD of the sample, in g/L was calculated according to the following formula:

$$COD = \frac{c \times 8 \times 25 \times (V_B - V_E)}{m}$$

Where:
c is the concentration of ammonia iron(II) sulphate solution, in mol l^{-1};
V_B is the volume of ammonia iron(II) sulphate solution consumed in titration of the blank;
V_E is the volume of ammonia iron(II) sulphate solution consumed in titration of the sample;
8 is the milliequivalent weight of oxygen (APHA, 2005), and 25 the dilution factor;
m is the mass of sample in g.

The method is based on Standard Method 5220C (APHA, 2005), with higher quantities of reagents to analyse a larger sample, and greater dilution. The standard deviation of replicates analysed by this method was ± 3.8%.

3.1.7 Total Lipid Analysis

Substrate samples were analysed for total lipids by Soxhlet extraction. All glassware and equipment used in the extraction was heated to 550^0C for one hour before analysis to ensure that there was no contamination by residual organic materials. Nitrile gloves were worn during the entire procedure.

Samples were dried and analysed for TS as described previously, then ground in a mill (ZM-1, Glen Creston, Middlesex UK). Approximately 6 g of ground sample was weighed into a paper extraction thimble, and approximately 0.5 g of glass beads were added to the thimble before folding over the top edges of the thimble to enclose the sample & beads. The thimble was then placed in a Soxhlet apparatus heated in a water bath on a hot plate. Glass beads were added to an extraction flask and the tare weight of the flask and beads was determined. 100 ml of hexane was added to the flask before connecting to the Soxhlet apparatus. Heat was then applied to the water bath and the system was allowed to reflux at a rate of approximately 20 cycles $hour^{-1}$ for at least four hours. The apparatus was monitored to ensure the solvent did not boil violently which could result in lipids being deposited back into the sample.

Following the extraction, the apparatus was disconnected and the extraction flask left under heating to evaporate the solvent. After evaporation of the solvent, the flask was dried for one hour at 105^0C and cooled in a desiccator for 30 minutes. The flask was then weighed again to measure the gain in mass due to lipids deposited in the flask during the extraction process. Lipid content was calculated according to the following formula:

$$\%TLC = \frac{W_2 - W_1}{m} \times 100$$

Where
TLC is total lipid content
W_1 is the mass of the extraction flask and beads before extraction procedure, in g
W_2 is the mass of the extraction flask and beads after extraction procedure, in g
m is the mass of the dry sample

Six replicates on food waste samples were performed, which gave a standard deviation of ± 5%. The method on the Standard Method 5520-D Soxhlet extraction (APHA, 2005) except that whole dried samples were used, rather than filtered samples. The standard deviation of replicates analysed by this method was ± 4.5%.

3.1.8 Trace Element Analysis

Substrate and digestate samples were analysed after acid digestion. All acids used in trace metal analysis were trace analysis grade.

Food waste substrate samples were prepared for trace element analysis by microwave digestion with trace metal grade nitric acid, in accordance with EPA method 3051A, *Microwave assisted acid digestion of sediments, sludges, soils and oils* (EPA, 1998). Samples were placed in acid-washed crucibles, weighed, dried overnight at 105^0C and weighed again to determine their total solids content as described previously for TS. Each dried sample was ground in the crucible using an acid-washed pestle. A quantity of 0.1-0.2 g of ground sample was then transferred into a teflon digestion vessel (XP-1500 Plus, CEM Corp.). To each digestion vessel was added 10 ml of concentrated nitric acid (HNO_3 70%) and the vessels were then left to stand in the fume hood for a pre-digestion period of at least one hour. Vessels were then placed in a microwave digestion unit (CEM Mars 5) and digested for ten minutes (112^0C for two minutes followed by 175^0C for eight minutes).

Anaerobic digestate samples were prepared for metals analysis using hydrochloric-nitric acid digestion, in accordance with DOE *Methods for the Determination of Metals in Soils, Sediments and Sewage Sludge and Plants by Hydrochloric-Nitric Acid Digestion* (DOE, 1986). Frozen samples were thawed and poured into acid-washed Pyrex beakers for drying and TS content determination. After determination of TS content, 7.5 ml trace metal grade hydrochloric acid (HCl 34%) and 2.5 ml trace metal grade nitric acid (HNO_3 70%) were added to each beaker. After addition of acids, the sample beakers were covered with watch glasses and left in a fume cabinet for a pre-digestion period of at least 36 hours. The samples were then heated on a hot plate for an acid reflux period of approximately 2 hours. The watch glasses were used to minimise evaporative losses of acid during the acid digestion period. After the hot digestion period a further 10 ml of the acid mix was added to each beaker and the beakers were again left in the fume cabinet for a final cold acid digestion period of 36 hours.

After digestion, the samples were filtered through Whatman glass fibre filter paper in an acid-washed vacuum filtration unit. The filtrate was quantitatively transferred to volumetric flasks and made up to 50 ml with Milli-Q water. All subsequent dilutions were prepared with the same Milli-Q water. Standards were prepared using Milli-Q water with hydrochloric and nitric acids to give the same acid concentrations in the standards as in the diluted samples.

The 50 ml digested metal stock contained 7% HNO_3 (5 ml x 70% = 3.5 ml, divided by 50 ml total stock volume) and 9.6% HCl (15 ml x 32% = 4.8 ml, divided by 50 ml total stock volume). This stock was diluted by a factor of 10 with Milli-Q water to give an acid concentration of 0.7% HNO_3 and 0.96% HCl.

For determination of metal content in the acid digest, inductively coupled plasma mass spectrometry was used for all metals except iron. For the determination of the content of iron in the acid digest, atomic absorption spectrometry was used.

An X-SERIES 2 ICP-MS (Thermo Fisher Scientific, Bremen, Germany) was set up in the standard configuration, using an ASX-520 autosampler (Cetac, Omaha, Nebraska, USA). The instrument was tuned for optimum sensitivity and stability using a 10ppb multi-element tuning solution (Thermo-Fisher CTUNEA250) containing a suite of elements including Li, Co and U. Calibration standards spanning the potential range of the samples were prepared using a trace metal standard (WP15) from the Assurance ICP/AAS multi-element standards range (SpexCertiPrep Ltd, Middlesex, England). Samples were run using the built in software PlasmaLab with the calibration standards and blank bracketing the unknown samples and interspersed at regular intervals acting as drift monitors. Data quality was monitored throughout the run by examination of the statistics produced after each analysis. Within-run reproducibility was typically better than 1% RSD for the 3 repeats.

Iron was measured on a Spectr AA-200 atomic absorption spectrophotometer (Varian, Australia) using a hollow cathode lamp, lamp current 10 mA, acetylene fuel, wavelength 248.3 nm, slit width 0.2 nm. Standards were 1, 5, 10, 15, 20 mg l^{-1} iron, prepared in the same acid matrix as the samples.

The samples were also sent to an independent laboratory (Severn Trent Laboratories Limited, Bridgend UK) for independent verification of the results. A relative percent

difference of 20% or less between the independent laboratory results and Southampton's results was found.

3.1.9 Volatile Fatty Acids

Digestate samples were prepared for Volatile Fatty Acid (VFA) analysis by centrifugation (Eppendorf 5417 C/R, Eppendorf, Hamburg Germany) at 17, 900 g (13,000 rpm) for 10 minutes and 0.9 ml of the supernatant transferred by pipette (Finnpipette, Thermo Fisher Scientific, UK) to vials with 0.1 ml formic acid for a concentration of 10% formic acid. Where dilution was necessary, deionised water was used and formic acid was added to a concentration of 10% of the total volume for analysis.

During the initial trials (CSTR digestion trials 1 through 3), centrifuged samples awaiting analysis were stored at 4^0C after the addition of formic acid. During later trials (DT4 and all subsequent digestion trials), samples collected for VFA analysis were immediately frozen in centrifuge tubes until the day of analysis, when the samples were thawed, centrifuged and added to formic acid immediately prior to chromatographic analysis. This gave better preservation of samples in the time interval between sampling and analysis. VFA were measured using a Shimadzu GC-2010 gas chromatograph, using a flame ionisation detector and an FFAP capillary column from SGE, model BP-21, 30 m length x 0.25 mm i.d. x 0.25 μm thickness. Detector temperature was 250^0C. Helium was carrier gas with split ratio of 100; total flow was 239 ml min^{-1}, column flow was 2.34 ml min^{-1}.

On each analysis run, standards were also analysed as blind samples to confirm accuracy, which was within 10%.

3.1.10 Biogas Composition

Biogas samples were collected and analysed weekly for gas composition by gas chromatography and compared to a standard mix of 35% CO_2/ 65% CH_4, on a Varian CP-3800 gas chromatograph with thermal conductivity detector, argon as carrier gas, with temperatures for oven, column and detector at 110^0C, 50^0C and 200^0C respectively. Weekly readings were checked for drift to ensure calibration. When necessary, calibration was performed by a trained laboratory technician.

During early digestion trials (DT1-DT3), biogas samples were withdrawn from the headspace of the reactors. During later digestion trials (DT4 and all subsequent digestion trials), biogas samples for GC analysis were withdrawn from the Tedlar bags to provide a

composite of the previous day's gas production. Three replicates were taken of each sample to check accuracy. Replicate readings were always within 1% of each other.

3.1.11 Soluble Sulphides

Soluble sulphides were measured in digestate samples using a Macherey – Nagel Visocolor colorimetric test kit with Macherey – Nagel PF-11 Filterphotometer. The kit is based on the methylene blue method for sulphide determination (APHA, 2005) which uses the reaction of sulphide with ferric chloride and dimethyl-p-phenylenediamine to produce methylene blue, which is then quantified photometrically. Samples were diluted by a factor of 10 immediately before analysis. Soluble sulphides were measured once per week during early digestion trials (DT2 and DT3), and were not measured during later digestion trials. Accuracy between replicates was within 30%, which was sufficient for the results as used Section 4.2.7.

3.2 Substrate Collection and Preparation

All food waste used as part of the research was collected from the central catering facility of the University of Southampton. This facility provided hot cooked food outlets, sandwich bars and space where staff and students of the university could consume food brought onto the premises. A methodology was developed which allowed a quantitative estimate of the food waste generated and a representative sampling to be achieved. The first part of this was through a familiarisation of the staff employed within the facility as to the aims and objectives of the research and to explain the proposed methodology through a series of face to face meetings with key personnel.

3.2.1 Food Waste Segregation and Collection

During the months of February and March 2005, food wastes were collected from the kitchen and dining areas of the facility in dedicated bins and buckets.

The first part of this collection period was used as a training period for staff to ensure that they were compliant with the source segregation requirements, i.e., to ensure that all employees using the waste bins understood what materials were to be disposed in them and were willing and able to follow the system. Measurement of the amount of contamination each day was used as a check on the effectiveness of compliance and source segregation.

The collected wastes were brought to the laboratory for weighing, quantification and removal of contaminants, and homogenisation.

Wastes were weighed on a digital platform scale (Adam CPW plus 150, capacity 150 kg ± 50 g, Adam Equipment, Indiana USA), subtracting the tare weight of the containers. During weighing, containers were visually inspected for contaminants such as plastic, metal, glass and textiles. These were then removed manually and weighed on a portable scale (CJ-600, capacity 150 g ± 0.1 g, JScale Co., USA) to calculate percentage contamination by weight.

After contaminant removal, wastes were homogenised by grinding in a commercial garbage grinder (S52/010 Waste Disposer, Imperial Machine Company Ltd., Hertfordshire UK).

During the first part of the collection period, wastes collected from different areas were characterised separately for total solids (TS) and volatile solids (VS) to give an indication of the range of possible values from these.

3.2.2 Collection, Preparation and Characterisation of Food Waste Substrate

Following the initial sampling, an intensive collection was carried out over 5 days, during which time source segregated food waste was collected from all of the areas of the facility. To achieve this, a separate food waste disposal bin was placed next to every regular waste disposal bin. All material collected in these bins was then homogenised and then frozen on the day of collection.

At the end of the collection period (March 2005), one half of all of the frozen samples were thawed overnight at room temperature. The samples were then mixed together in a single vessel using a hand-held electric drill mixer until a fully homogenised mix was obtained.

The mixed composite was characterised (Section 3.1.3) for total and volatile solids (TS and VS), total Kjeldahl nitrogen (TKN). The composite was divided into weekly feedstock portions of sufficient size to satisfy the feeding requirements of the set of bench scale anaerobic digesters and then frozen in 'snap top lid' plastic containers. COD, lipid analysis and trace element analysis were carried out at later points in the research using a number of subsamples of the composite. The second half of the homogenised and frozen food waste was thawed and mixed by the same procedure, and characterised by the same methods as the first composite in June 2007.

The freezing and thawing of the feedstock could affect it, due to the fact that water in the substrate will expand and break cell walls during the freezing process, leading to cell disruption and causing the release of cell contents. Investigators have assessed the effect of freezing and thawing on food waste as a method to enhance digestion, and found a 9% increase in the average rate of VFA production and a 6.7% improvement in methane yield versus digestion of fresh food waste (Stabnikova et al., 2008). The freezing of the substrate, therefore, may have had a slight positive effect on the digestion results versus those that would be obtained if the substrate had not been frozen. The length of the digestion runs and the amount of substrate required, however, necessitated some form of storage for the collected food waste, and since all runs used frozen and thawed substrate, there was no variability introduced by this storage method. Alternative storage options of storing the food waste at temperatures above freezing or adding some kind of chemical preservative would both have greater effects than freezing, as fermentation would have begun in substrate that was not frozen, and chemical preservatives would affect the organisms when the substrate was fed to the bioreactors.

The only alternative to storage would be to collect fresh food waste samples daily for feeding during the digestion runs. This would lead to wide variations in the substrate being fed to the reactors, however, introducing more variables into the study, and would necessitate daily analyses to characterise the substrate, rather than having one composite that could be characterised by multiple replicates. In addition, the logistical issues of daily waste collection, preparation and analysis before feeding to digesters each day would be prohibitive.

3.2.3 Modification of Food Waste Substrate

All digestion trials were carried out with the same food waste composite substrate, except for the digestion trial described in Section 4.5, for which the food waste substrate was pre-treated to reduce its lipid content. Approximately 8 kg of the food waste composite was thawed and subjected to a physical lipid reduction process as described below. The process was intended to remove or reduce floatable lipids. Chemical methods such as the use of non-polar solvents were not used, as this could affect the biomass in the digestion process and introduce new variables in the investigation. The objective of the physical lipid reduction process was to reduce lipid content while minimising any other form of alteration of the substrate.

Food waste was diluted with distilled water in a ratio of 1:1 water:food waste, and heated to a temperature of 70 ^{0}C for two hours in an autoclave (Prior Clave Midas 60, Prior Clave Ltd., London). The mixture was then allowed to cool and settle at 4 ^{0}C overnight, then centrifuged at 2500 rpm for 10 minutes. The pellet was separated from the supernatant, which was then filtered through a tea strainer (1 mm mesh) into a separating funnel, and let stand for one hour or more to separate. The bottom aqueous layer from the separating funnel was then drained through kitchen towel as a filter, and remixed with the pellet. The top lipid layer from the separating funnel was discarded.

Half of the substrate sample (4 kg) was subjected to the complete lipid reduction process as described above, while the balance (4 kg) was heated, cooled and centrifuged, but after centrifuging the supernatant was remixed with the pellet without the separation and filtration steps. The purpose of this was to have a treated control substrate, to be able to discern whether effects observed in the subsequent digestion trial were due to the reduced lipid content of the substrate or were an artefact of heating or other steps in the substrate modification process.

This substrate modification process was quite energy intensive, and therefore if this were to be a regular procedure for substrate treatment for anaerobic digestion, it would impact the energy balance of the process. The purpose, however, was not to introduce a process for treatment of substrate in regular operation, but to modify a specific batch of substrate to test how the process would perform with a substrate that was lower in lipid content.

3.3 Bioreactor Design and Setup

3.3.1 Inoculum

Except where otherwise noted, inoculum for all digestion trials was sludge from the anaerobic digester of the Millbrook Sewage Works, Southampton, treating co-settled primary and secondary municipal wastewater sludges. The sludge was collected in 15 litre plastic containers and sieved through 1 mm mesh on the day of collection. The sieved sludge was used as seed for reactor startup within three days of collection.

3.3.2 5 Litre CSTR Bioreactor Design

Bench-scale anaerobic bioreactors of 5-litre liquid capacity were constructed of unplasticised polyvinyl chloride (uPVC) tube, with internal diameter of 15.2 cm. Bioreactors were mounted in sets of four within an insulated wooden enclosure (Figure

3.1), containing a hot water heating loop connected to a thermocirculator to maintain a temperature of 33^0C in the reactors.

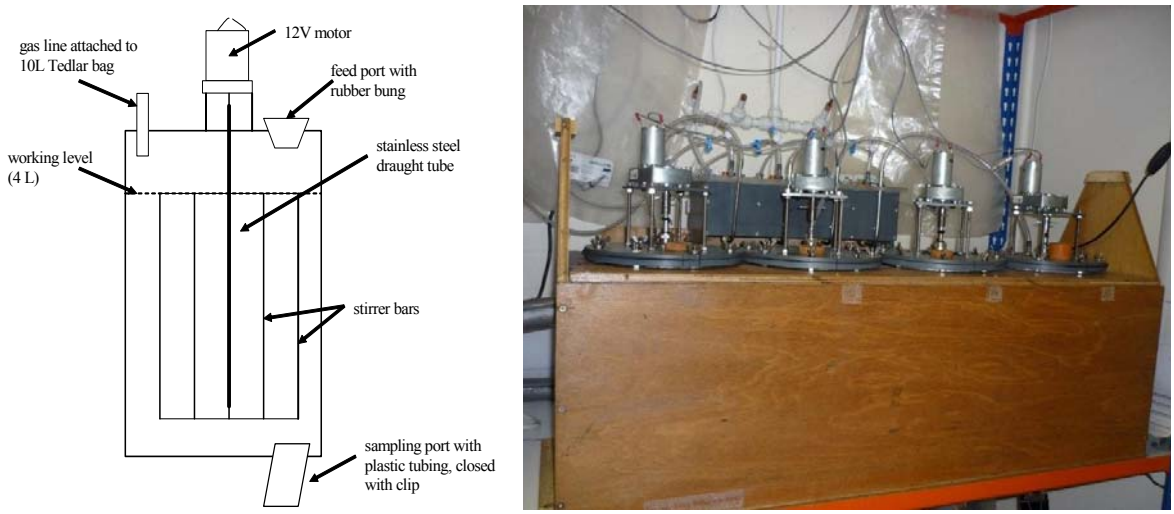


Figure 3.1
Schematic (left) and photo (right) 5-litre CSTR reactors.

Contents of the reactors were mixed continuously by an internal stirrer driven by a geared motor at 40 rpm. Feed addition was via a stoppered port in the reactor lid, while digestate removal was via a sampling port in the bottom of the reactor, consisting of a PVC pipe connected to a 19-mm diameter piece of flexible silicon tubing folded over and held closed with a metal clip.

Biogas production quantity was measured daily, via water displacement in a volume-calibrated cylindrical gas collector. During early digestion trials (DT1-DT3), gas collectors were connected directly to the headspace of the bioreactors by flexible tubing. During later digestion trials (DT4 and all subsequent trials), Tedlar bags (SKC 232, SKC Ltd., Pennsylvania USA) of 10 litre capacity were connected to the bioreactor headspace, then the collected gas was evacuated into a separate gas measurement cylinder for volume measurement by the same water displacement system. All gas volume measurements were corrected to standard temperature and pressure.

Digestate samples were withdrawn daily and an equivalent quantity of substrate plus tap water (during early digestion trials) or deionised water (during later digestion trials) was added, to maintain a constant volume in the reactors. The volume added and removed determined the hydraulic retention time (HRT) set for each reactor according to the following equation:

$$\text{HRT} = \frac{V_{reactor}}{Q}$$

Where
$V_{reactor}$ is volume of reactor, in ml; and
Q is daily flow of material through reactor (substrate + water in = digestate out), in ml d^{-1}.

Although food waste was measured by mass it was assumed to have specific gravity of 1.0 and therefore 1 g food waste was assumed equal to 1 ml for flow calculation purposes. This was confirmed by a series of five replicates in which a volume of 50 ml of food waste was weighed and found to have a mass of 50.0 ± 0.5 g, giving a density of 1 g ml^{-1}.

The organic loading rate (OLR) was determined according to the following equation:

$$\text{OLR} = \frac{mVS_{substrate}}{V_{reactor}}$$

Where
m is the mass of substrate fed per day, in g d^{-1}
$VS_{substrate}$ is the VS content of the substrate, in % of fresh weight
$V_{reactor}$ is the volume of the reactor, in l

The performance parameters of volumetric biogas production, specific biogas production and specific methane production were calculated according to the following equations:

$$\text{Volumetric Biogas Production} = \frac{V_{biogas}}{V_{reactor}}$$

Where
V_{biogas} is the volume of biogas produced per day, in l d^{-1}
$V_{reactor}$ is the volume of the reactor, in l

$$\text{Specific Biogas Production} = \frac{V_{biogas}}{OLR \times V_{reactor}}$$

Where
V_{biogas} is the volume of biogas produced per day, in l d^{-1}
OLR is the organic loading rate in gVS $l^{-1}d^{-1}$
$V_{reactor}$ is the volume of the reactor, in l

$$\text{Specific Methane Production} = \frac{V_{CH4}}{OLR \times V_{reactor}}$$

Where
V_{CH4} is the volume of methane produced per day, in l d^{-1}
OLR is the organic loading rate in gVS $l^{-1}d^{-1}$
$V_{reactor}$ is the volume of the reactor, in l

Volatile solids destruction at steady state was calculated as:

VS Destruction = $VS_{influent}$ - $VS_{effluent}$

Where
$VS_{influent}$ is the VS concentration of the input feed stream, in %
$VS_{effluent}$ is the VS concentration of the digestate effluent, in %

For many of the runs, however, reactors were not at steady state, as solids were being washed out. In this situation, VS destruction was calculated as:

VS Destruction = $VS_{expected} - VS_{measured}$

Where
$VS_{measured}$ is the actual VS concentration of the digestate effluent, in %
$VS_{expected}$ is the VS concentration that would be expected for the digestate, assuming no VS destruction and based solely on a washout rate determined by HRT, in %

$$\mathrm{VS}_{\mathrm{expected}} = \frac{M_t}{V_{reactor}}$$

Where
M_t is the mass of VS in the reactor at time t, in g
M_t was calculated on a spreadsheet for each day according to the formula:

$M_t = (1 - wf)M_{t-1} + m_{added}$

Where
M_t is the mass of VS in the reactor at time t, in g
M_{t-1} is the mass of VS in the reactor at time t-1 (i.e., the previous day), in g
m_{added} is the mass of VS fed daily to the reactor, in g
wf is the washout factor, equal to the amount washed out per day, dependent on HRT, as follows:
25-day HRT: wf = 4%; 30-day HRT: wf = 3.3% 50-day HRT: wf = 2%; 100-day HRT: wf = 1%; 185-day HRT: wf = 0.54%

The HRT and OLR used for the reactors during each of the 5-L reactor trials are shown in Table 3.1. Where feeding was increased to a reactor, two or more figures are shown in the OLR column showing the different OLRs used.

Table 3.1
Reactor Parameters, 5 l CSTR Reactors

Digestion Trial	Reactors	Flow, ml d^{-1}	HRT, d	OLR, gVS $l^{-1}d^{-1}$
DT1	M1	200	25	2.0
	M2, M3, M4	200	25	2.0, 4.0
DT2	M1, M2, M3, M4	200	25	1.45, 1.82
DT3	M1	200	25	1.45
	M3	200	25	1.45, 1.82
	M2	100	50	1.45
	M4	100	50	1.45, 1.82, 1.98, 2.25
DT4	M1, M2	200	25	1.45
	M3, M4	28	180	1.45
DT4a	M1, M2	100	50	1.45
DT5	M5, M6	100	50	1.45
	M7, M8	50	100	1.45
DT6	M6, M8	167	30	1.45
DT7	LR1, LR2, LC3, LC4	100	50	1.45

3.3.3 1.5 Litre CSTR Bioreactor Design

1.5-litre cylindrical bioreactors were constructed of the same material (uPVC tube) as the 5-litre bioreactors, with internal diameter of 10.5 cm, contained in an insulated wooden enclosure with heating loop (Figure 3.2). Lids were removable, fastened on top of the reactors by metal bars held in place by wing nuts, with the gas-tight seal maintained by means of internal rubber gaskets.

Contents of the reactors were mixed continuously by an internal stirrer driven by a geared motor at 40 rpm. Feed and water addition as well as sample removal were via the stoppered port in the top of the reactor. For periodic samples taken for total and volatile solids determination, the reactor lid was removed and the contents stirred manually before withdrawing sample by means of a ladle.

All 1.5 litre bioreactors were inoculated with digestate from existing 5 litre bioreactors, as part of an investigation into the effects of increasing OLR, described in Section 4.3.5. The bioreactor parameters are shown in Table 3.2.

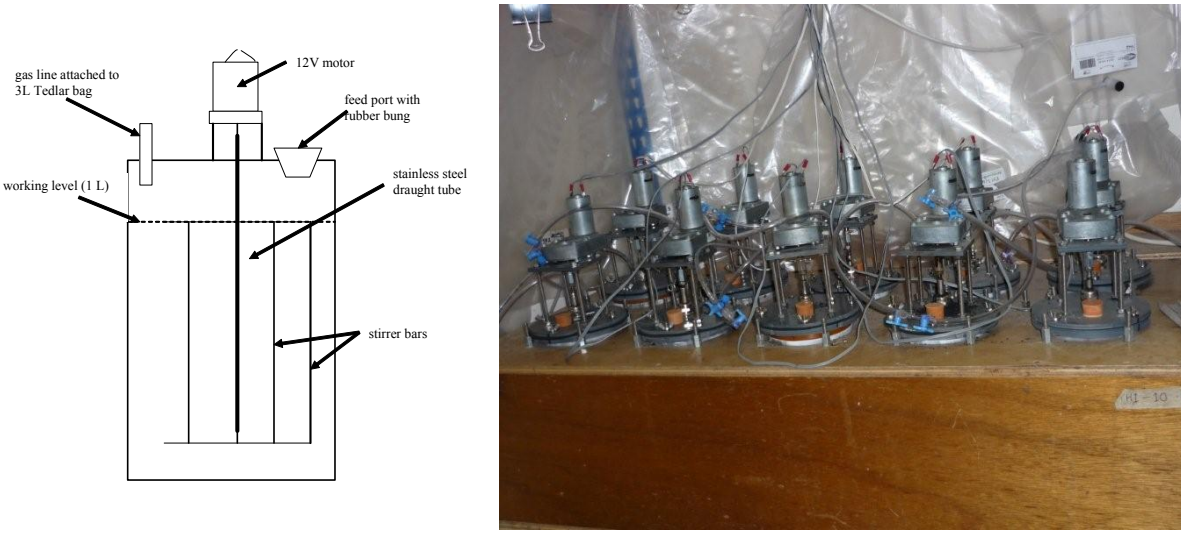


Figure 3.2
Schematic (left) and photo (right), 1.5-litre CSTR reactors.

Table 3.2
Reactor Parameters, 1.5 l CSTR Reactors

Digestion Trial	Reactor	Seeded from	Flow, ml d^{-1}	HRT, d	OLR, gVS $l^{-1}d^{-1}$
DT8	S1	M7, DT5	15	100	1.45
	S2	M7, DT5	15	100	1.45, 1.99, 2.49, 3.00, 3.51
	S3	M5, DT5	30	50	1.45
	S4	M5, DT5	30	50	1.45, 1.99, 2.49, 3.00, 3.51
	S5	M6, DT6	50	30	1.45
	S6	M6, DT6	50	30	1.45, 1.99, 2.49, 3.00
	S7	M3, DT4	8.3	180	1.45
	S8	M3, DT4	8.3	180	1.45, 1.99

3.3.4 0.8 Litre Centrifuge Bottle Bioreactor Design for Solid-Liquid Separation

Four bioreactors of 800 ml liquid capacity were constructed from 1000 ml polypropylene centrifuge bottles with a 40 rpm stirrer mounted on the sealable screw cap. The entire stirrer mechanism, including paddle stirrer, could be removed and replaced by a standard screw cap which allowed the reactors to be centrifuged at 6500 g (4000 rpm, Wifug 4000E centrifuge, Wifug Ltd., Bradford UK) for solid-liquid separation. Reactors were kept in a heated water bath in which temperature was maintained at 35^0C, except as otherwise noted.

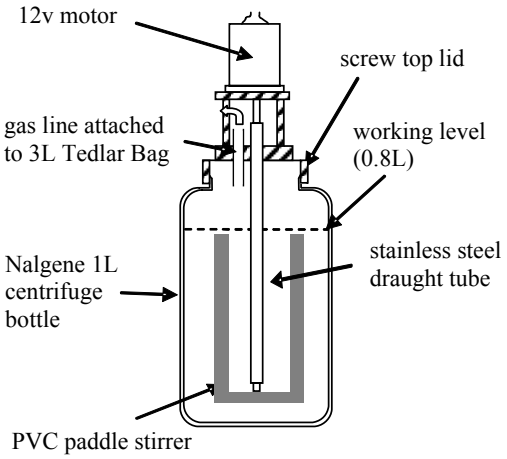


Figure 3.3
Centrifuge bottle reactor.

Two reactors were operated by retaining solids in the reactor and periodically flushing a portion of the liquid (Hydraulic Flush, HF), while the other two were operated by retaining liquid in the reactor but periodically wasting a portion of the solids (Solids Wastage, SW). Solid-liquid separation was carried out on a weekly basis, by centrifuging the reactors and decanting the supernatant through 1-mm mesh. A mass equal to 28% (based on a removal of 4% per day which corresponds to a 25-day retention time in CSTR reactors, multiplied by 7 days per week) of either the liquid or solid was removed each week, before recombining the liquid with the solids in the reactor and adding deionised water to bring the reactor back up to its working volume.

Table 3.3
Reactor Parameters, 0.8 l Centrifuge Bottle Reactors

Digestion Trial	Reactors	Solids Removed Weekly	Liquid Removed Weekly	Average SRT	Average HRT	OLR, gVS $l^{-1}d^{-1}$
CT1	1W	28%	1-2%	25 d	>150 d	1.45
	2W	28%	1-2%	25 d	>150 d	1.45
	3F	1-2%	28%	>150 d	25 d	1.45
	4F	1-2%	28%	>150 d	25 d	1.45

3.4 Trace Element Supplementation

During digestion trials investigating the effects of trace elements, reactors were supplemented with a trace element mixture. The composition of the trace element solution is shown in Table 3.4.

Table 3.4
Composition of Trace Element Stock Solution

Compound Added	Element Added	Concentration (mg/L)	
		Compound	**Element**
$FeCl_2$	Fe	200	88
$CoCl_2$	Co	200	91
$MnCl_2 . 4H_2O$	Mn	50	14
$AlCl_3 . 6H_2O$	Al	9	1.0
H_3BO_3	B	5	0.9
$ZnCl_2$	Zn	5	2.4
$(NH_4)_6Mo_7O_{24} . 4H_2O$	Mo	5	2.7
$CuCl_2 . 2H_2O$	Cu	3.8	1.4
$NiCl_2 . 6H_2O$	Ni	5	1.2
$Na_2SeO_3 . 5H_2O$	Se	19.4	5.8
EDTA		100	
HCl, conc.		1 ml/L	

This trace element solution is based on the recipe of Gonzalez-Gil et al. (2001), except that the metal amounts are lower than those of Gonzalez-Gil by a factor of ten. Lower concentrations of metals in the supplementation solution allowed the maintenance of lower metal concentrations in digestate with less dilution.

The stock solution was prepared by a laboratory technician following the glassware and chemical preparation procedures described in Section 3.1. The compounds listed in Table 3.4 were dissolved in Milli-Q water in a stirred and heated beaker. Ferrous chloride was added first and left with stirring and heating until dissolved, before adding all other components and diluting to 1 l in a volumetric flask.

Trace elements were delivered to 5 l reactors by using a calibrated pipette to add a known quantity of the stock solution above to approximately 50 ml of deionised water (equivalent to approximately 1% of reactor volume), briefly shaking the container, and pouring the solution into the reactor. For stock addition to the 1.5 l reactors, 15 ml of deionised water was used. For stock addition to the 0.8 l centrifuge bottle reactors, the trace element stock was pipetted directly into the reactor, after the addition of makeup water at the end of the weekly centrifuging routine described in Section 3.3.4.

The amounts added to reactors are shown in Table 3.5.

Table 3.5
Trace Element Supplementation to Reactors

	25d HRT	50d HRT	100d HRT	180d HRT	Centrifuge Reactors
Amount of stock added, in ml	1.5	1.5	1	1	1.5
Frequency of addition	Weekly	Biweekly	Biweekly	Monthly	Weekly

Washout curves were developed to show the predicted concentrations of each of the metals in the reactors, based on the rate of washout according to HRT and the frequency and dosage of trace metal addition to the reactors. The curves were plotted from a spreadsheet by which daily predicted concentrations of metals were calculated using the following formula:

$$C_t = \frac{M_t}{V_{reactor}}$$

Where
C_t is the concentration of the element in the reactor at time t, in mg L^{-1}
M_t is the mass of the element in the reactor at time t, in mg
$V_{reactor}$ is volume of the reactor, in L

M_t was calculated on a spreadsheet for each day according to the formula:

$$M_t = (1 - wf)M_{t-1} + m_{fw} + m_{supp}$$

Where
M_t is the mass of the element in the reactor at time t, in mg
M_{t-1} is the mass of the element in the reactor at time t-1 (i.e., the previous day), in mg
m_{fw} is the mass of element added to the reactor as part of the daily food waste feed, in mg
m_{supp} is the mass of element added to the reactor in the supplementation solution, in mg (equal to zero on days other than the weekly, biweekly or monthly days on which trace elements were added)
wf is the washout factor, equal to the amount washed out per day, dependent on HRT, as follows:
25-day HRT: wf = 4%; 30-day HRT: wf = 3.3% 50-day HRT: wf = 2%; 100-day HRT: wf = 1%; 185-day HRT: wf = 0.54%

4. Results

This chapter summarises the results and some brief discussion of the research undertaken.

4.1 Food waste Collection and Characterisation

4.1.1 Aims

The aim of this component of the study, as noted in Section 1.2, was to create a methodology to allow the quantification of food waste generation, using a university catering outlet as the study site. The objectives were the following:

i) to determine the total quantity of food wastes produced in one week by the facility, and estimate annual quantity of food wastes produced by the facility;

ii) to determine quantities of contamination in collected wastes, as an indicator of the potential success of a future food waste separation program and to ensure that contamination in the substrate composite was minimised;

iii) to characterise the substrate for use in this work and comparison with other studies.

4.1.2 Results

4.1.2.1 Total Quantity of Food Waste

The total amount of the homogenised composite resulting from the 5-day intensive collection period was approximately 300 kg. Based on this amount extrapolated over 50 weeks of operation per year, an annual quantity of approximately 15 tonnes of food waste could be expected from the study site. This is a very approximate estimate, as quantities would vary during busier or slower times during the academic year and during holiday closure periods. The intensive collection period was carried out over a week around the middle of the university term, and so is likely to represent an average rather than busy (end-of-term and graduation) or slow (summer or Christmas holidays) period.

4.1.2.2 Contaminant Levels

All food waste collected was examined and visible contaminants removed and weighed. Table 4.1 shows the average contamination, categorized according to the total mass of food waste collected on each day.

Table 4.1
Food Waste Collection Contaminant Levels

Mass Collected	Number of Collection Days	Contamination (Average and Standard Deviation)
< 5 kg	13	1.08 ± 1.04%
5-15 kg	11	0.31 ± 0.20%
30-80 kg (Intensive Collection Week)	5	0.12 ± 0.03%

Most of the collection days with less than 5 kg collected were during the first two weeks of the initial audit, when staff were being trained on the source segregation system, and therefore contamination was higher during this learning period. The quantities between 5-15 kg per day were collected during the later part of the audit period when staff were more accustomed to the system, and therefore better able to avoid depositing contaminants into the dedicated food waste bins.

The greatest quantities (30-80 kg) were collected during the intensive collection period. During this period, in addition to the food waste collected in dedicated rubbish bins in the food preparation and dining areas, excess unsold food from the hot food counter was emptied directly into the food waste collection bins at the end of the day. There was no non-food contamination in the hot food trays and therefore the proportion of overall contaminants was lower than for the other periods, in addition to the fact that staff members were most accustomed to the source segregation system during this period.

The overall findings of the contamination assessment were the following:

- In all categories, contamination was low, showing high effectiveness of the source segregation system trialled; and
- These initial results indicate that staff compliance would not be a barrier to implementation of source segregation of food wastes at this facility.

4.1.2.3 Characterisation of Food Wastes Collected During Initial Sampling Period

During the initial sampling period, TS and VS analysis were carried out for a number of subsamples from different areas of the kitchens and customer service areas of the Staff Club, to obtain initial estimates of the range and average values of food wastes from the subject facility. Table 4.2 summarises the results of these initial analyses.

Table 4.2
Food Waste TS and VS Results – Initial Sampling Period

Parameter	Unit	Number of Samples	Average ± Standard Deviation
Total Solids (TS)	%	29	24 ± 8.5
Volatile Solids (VS)	% of TS	29	94 ± 2.2

4.1.2.4 Characterisation of Food Waste Composite

The results of the characterisation analyses on the homogenised composite obtained in the intensive collection period are shown in Table 4.3.

Table 4.3
Replicate Analysis of Food Waste Characteristics – Composite

Parameter	Unit	Number of Replicates	Average ± Standard Deviation
Total Solids (TS)	%	7	28.1 ± 0.25
Volatile Solids (VS)	% of TS	7	95.5 ± 0.06
Total Kjeldahl Nitrogen (TKN)*	% of TS	6	3.8 ± 0.24
Total Lipid Content (TLC)*	% of TS	6	22 ± 1
Chemical Oxygen Demand (COD)*	g/kg	4	422 ± 16

** Standard deviation given as a guide only due to small number of replicates taken.*

The characterisation results show that this food waste is very high in volatile solids, with 95% of the TS being volatile. High volatile solids can indicate a high degree of biodegradability, which is expected for this material as it is made up almost purely of food.

The average value of 28% TS is within the range for mixed food waste and similar wastes found by other investigators, as shown in Table 2.4. It is higher than for most fruit and vegetable wastes which were below 15% TS, but lies in the range of 20-42% TS for restaurant wastes and organic fraction of municipal solid wastes (OFMSW). The VS content of 95% of TS is at the high end of the range, but is the same as found by Carucci et al. (2005) for precooked food waste.

At 3.8% of TS, the TKN content of this substrate is relatively high, but still within the range of biowastes and restaurant wastes surveyed.

It also, however, contains a significant percentage of lipids (22 ± 1 % of TS), which are slower to break down than carbohydrates. Many investigators have not reported the lipid content of their wastes, indicating that it is not considered to be an important parameter. A survey of the literature on LCFA inhibition, however, suggests that lipid content should be considered.

As noted previously, the annual quantity of food waste from the study site was roughly estimated at approximately 15 tonnes. The average capacity for commercial plants currently being built in Europe for anaerobic digestion of biodegradable municipal wastes is approximately 43,000 tonnes per year (De Baere, 2006). The current Defra demonstration project operated by Biocycle is for 5000 tonnes per annum and is probably the smallest scale of plant that could be operated commercially (*pers. comm.* M. Chesshire, Greenfinch Ltd.). The quantities expected from the study site are fairly insignificant relative to this pilot plant size. The costs for collection infrastructure, staffing, equipment and facility operation and maintenance for a facility for such low quantities would likely be prohibitive. A pilot plant solely for food wastes from the university, therefore, is not likely to be feasible. A source segregation program for food wastes from the university, however, could still be considered as part of a larger collection program that could include other institutions within the local area such as hospitals, schools, and local authority buildings. Source segregated food waste from the university is therefore worth study as a potential feedstock for anaerobic digestion, particularly in light of the low contaminant levels observed during the kitchen collection period.

4.2 Initial CSTR Semi-Continuous Digestion Trials

This section outlines the results of digestion trials DT1, DT2, and DT3 as described in Section 3.3. The aims of this component of the research, as noted in Section 1.2, were to plan and implement trials to assess the amenability of source segregated catering waste for anaerobic digestion, and furthermore to identify and critically evaluate any potential problems resulting from the digestion of this material.

4.2.1 Objectives

The objectives of the first three digestion trials were the following:

i) to acclimate the process through a series of step changes in loading rate;

ii) to determine the maximum loading that could be applied to a CSTR digester operating on this feedstock; and

iii) to establish whether HRT influences the stability of the process.

4.2.2 Digestion Trial 1

4.2.2.1 Method

Digestion Trial 1 (DT1) was carried out from April 2005 to July 2005. CSTR reactors of 5 litre working volume, as described in Chapter 3, were used. All reactors (M1 through M4) were operated on a hydraulic retention time (HRT) of 25 days.

4.2.2.2 Results

Figure 4.1 shows digestion results for reactor M1, which was operated at a constant OLR of 2.0 gVS $l^{-1}d^{-1}$ and for reactors M2, M3 and M4 to which the OLR was increased from 2.0 gVS $l^{-1}d^{-1}$ to 4.0 gVS $l^{-1}d^{-1}$ after a period of 25 days.

For graphs in Sections 4.2 and 4.3, error bars are omitted for clarity, although a scatter of up to 10% for alkalinity and VFA values can be assumed, according to the standard deviations for each of the methods (as described in Chapter 3). In most cases, however, potential variations of 10% or less are much less important than the large differences between stable operation and failure, for example large changes in alkalinity ratio and pH which are clearly process effects and not due to analytical variability. This was true for all digestion trials, and the findings are all based only on the major trends, and not on small variations which could be the result of analytical variability.

Reactors M2-M4 show an initial gas production spike upon doubling of the OLR, followed by a rapid decline accompanied by a drop off in pH from above 6.5 to approximately 5.5, indicating methanogenic failure. Feeding was stopped to these three reactors in response to the drop in gas production and pH, after which pH gradually rose again. Reactor M1 maintained a stable pH until approximately Day 80, when it also exhibited a drop in gas production and pH, at which point feeding was stopped.

The increase in OLR to M2-M4 resulted in a rapid increase in intermediate alkalinity simultaneous with a drop in partial alkalinity, indicative of a large increase in VFA concentration in the reactors. After the feeding was stopped, partial alkalinity rose and intermediate alkalinity declined. For three of the reactors, pH dropped below 5.75, the titration endpoint for partial alkalinity and therefore partial alkalinity was virtually zero. In

the control reactor (M1), to which feeding was continued, partial alkalinity was generally equal to or greater than intermediate alkalinity, except near the end of the run when this situation was reversed. The IA:PA ratio therefore exceeded 1 during this period, over which pH and gas production dropped off.

The total solids graph shows an overall decrease in TS from the beginning to the end of the trial.

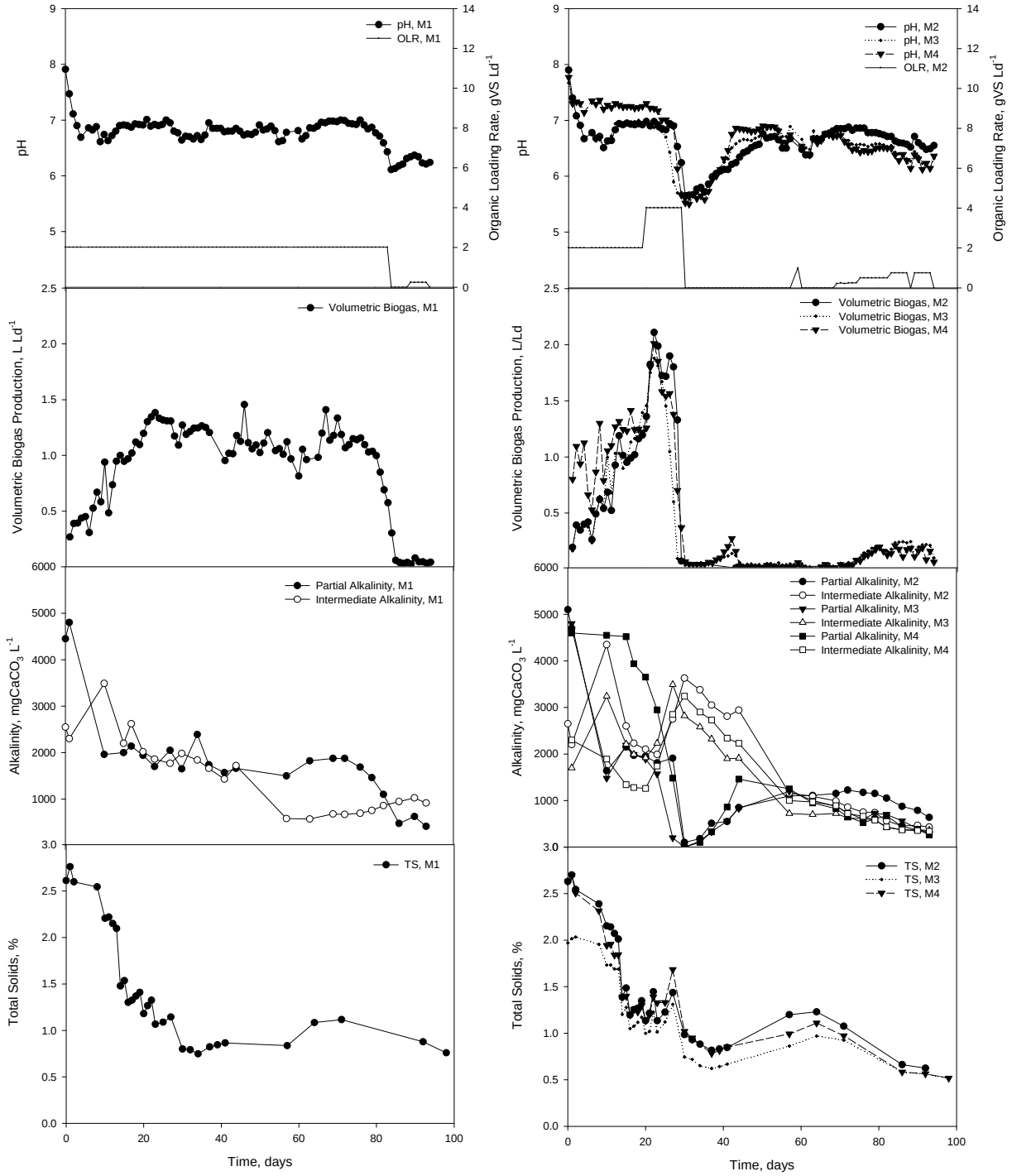


Figure 4. 1
Trends in pH, biogas production, alkalinity and digester solids in response to changes in OLR, DT1. Left: control bioreactor M1, constant OLR. Right: Bioreactors M2-M4, increase in OLR after 25 days.

Table 4.4 shows digestion parameters during the period of steady state operation for reactor M1, from days 25 to 75 of the digestion trial.

Table 4.4
DT1 Digester Performance Parameters, Control Reactor

Parameter	Average ± Standard Deviation
VS destruction, %	82 ± 4
Specific Biogas Production, L gVS_{added}^{-1}	0.57 ± 0.06
Specific Methane Production, L gVS_{added}^{-1}	0.36 ± 0.05
Specific Methane Production, L $gVS_{destroyed}^{-1}$	0.42 ± 0.09

The findings from this digestion trial were the following:

- While methane production and VS destruction for this substrate were good, the process was unstable.
- Doubling of OLR after one retention time resulted in process upset, indicating instability of the process.
- The control reactor, to which OLR was held constant, showed stable operation for a longer period but also showed failure after approximately 80 days.

Arising from these findings, it was decided that for the next digestion trial, the OLR increase would be smaller and occur later to allow more time for the bioreactors to stabilise before increasing the OLR.

4.2.3 Digestion Trial 2

The objective of this trial was to determine the maximum loading that could be applied to a CSTR digester operating on this feedstock. To attempt to avoid the same methanogenic failure observed during the first trial, more time for acclimation was allowed before increasing OLR. The incremental OLR increase was also smaller than in the previous trial.

4.2.3.1 Method

Digestion Trial 2 was carried out from August 2005 to November 2005. On this trial, feeding to all reactors was commenced at an OLR of 1.45 gVS $l^{-1}d^{-1}$ and maintained at this OLR for 2.5 retention times, before increasing feed to all reactors by 25% to an OLR of 1.82 gVS $l^{-1}d^{-1}$ beginning on Day 63.

Also during this trial, the effect of feeding the reactors on a 5 day per week basis rather than a 7 day per week basis was tested. This was tested because many commercial plants operate on a 5-day feeding basis (Kubler et al., 2000, van Opstal, 2006b), as well as some

laboratory studies (Salminen and Rintala, 2002b), while many laboratory studies as well as some commercial operations use a 7-day feed schedule (Banks et al., 2008). It has been argued that allowing two days without feeding allows VFA to be consumed by methanogenic bacteria (Lissens et al., 2004) while other authors have stated that more frequent feeding is better for not allowing VFA to accumulate (Carucci et al., 2005). On this trial, the two modes of feeding were tested to determine whether one would achieve better performance.

All reactors received the same OLR (1.45 gVS $l^{-1}d^{-1}$) on a weekly basis, but the daily feed differed according to the feeding schedule tested: M1 and M3 were fed 2.01 gVS l^{-1} for 5 days per week (Monday to Friday), and received no feed the other two days, while reactors M2 and M4 received 1.45 gVS l^{-1} seven days per week. There was no control reactor during this trial.

4.2.3.2 Results

Figure 4.2 shows digestion parameters for this period. Reactors are shown as pairs for clarity; reactors on a 5-day feed schedule are shown on the left, while reactors on a 7-day feed schedule are shown on the right. VFA profiles and volumetric biogas production for all reactors are shown in Figure 4.3. As for DT1, only the main trends are summarized.

In spite of the longer acclimation period allowed before increasing OLR on this trial, a similar pattern was observed to that seen during DT1, with all reactors showing a drop in pH and biogas, and drastic increase in intermediate alkalinity relative to partial alkalinity, after the OLR increase.

Figure 4.3 shows the VFA concentrations for reactors M1 through M4, respectively. Volumetric biogas production is also shown (secondary y-axis) to show the timing of methanogenic failure. All reactors showed an initial increase in acetic acid at the commencement of feeding, followed by low concentrations of VFA over the course of the following weeks up to the time of the feed increase, when acetic acid rose for all reactors. Acetic acid was the first VFA to rise, followed by the other VFA. Acetic acid concentrations decreased after a number of weeks of no feeding, while concentrations of propionic and butyric remained nearly static to the end of the trial.

The reactors fed on a 5-day basis performed similarly to the reactors fed on a 7-day basis, and failure was seen slightly later but not significantly later, indicating that a 5-day feed was no more detrimental to bioreactor health than the 7-day feeding regime. However, the

wide fluctuation in daily gas production made it very difficult to track patterns and mass balance calculations were more complicated under the 5-day feeding regime. Therefore, for further trials it was decided to continue with a 7-day feeding regime.

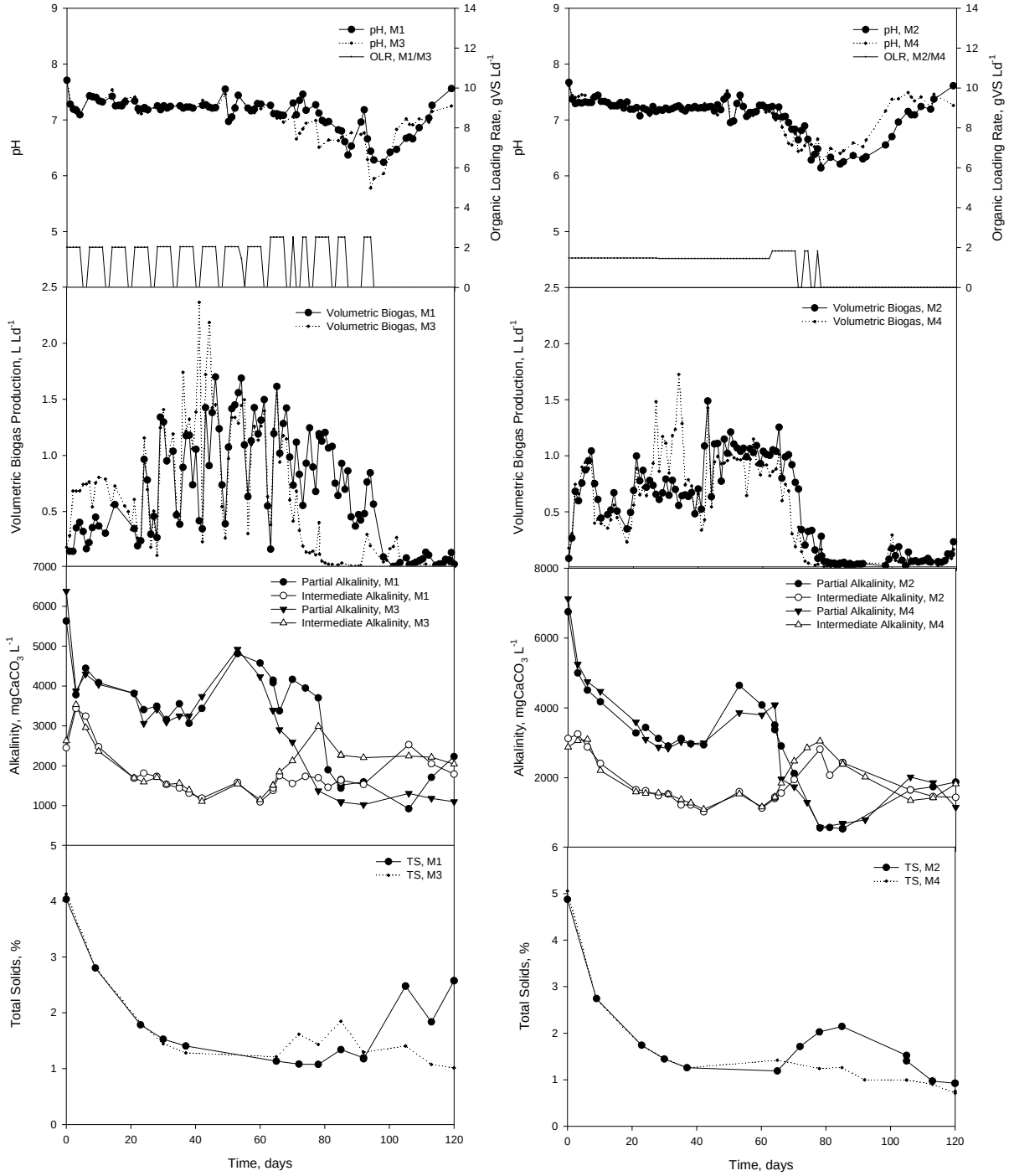


Figure 4.2
Trends in pH, volumetric biogas production, alkalinity and digester solids in response to changes in OLR, DT2.
Left: Reactors on 5-day feeding regime. Right: Reactors on 7-day feeding regime.
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A pattern of continual decrease of TS in the reactors was observed in both DT1 and DT2. This may be due to the high flow rate of dilution water, causing washout of biomass. As methanogens have a slower growth rate than acidogens, it was noted that a possible cause of methanogenic failure could have been the washout of methanogens at a rate faster than the growth rate. Extending hydraulic retention time, therefore, was considered for the following trial, consistent with work of other investigators that used an HRT of 50 days in digestion of poultry waste (Salminen and Rintala, 2002b), a feedstock high in protein and lipids.

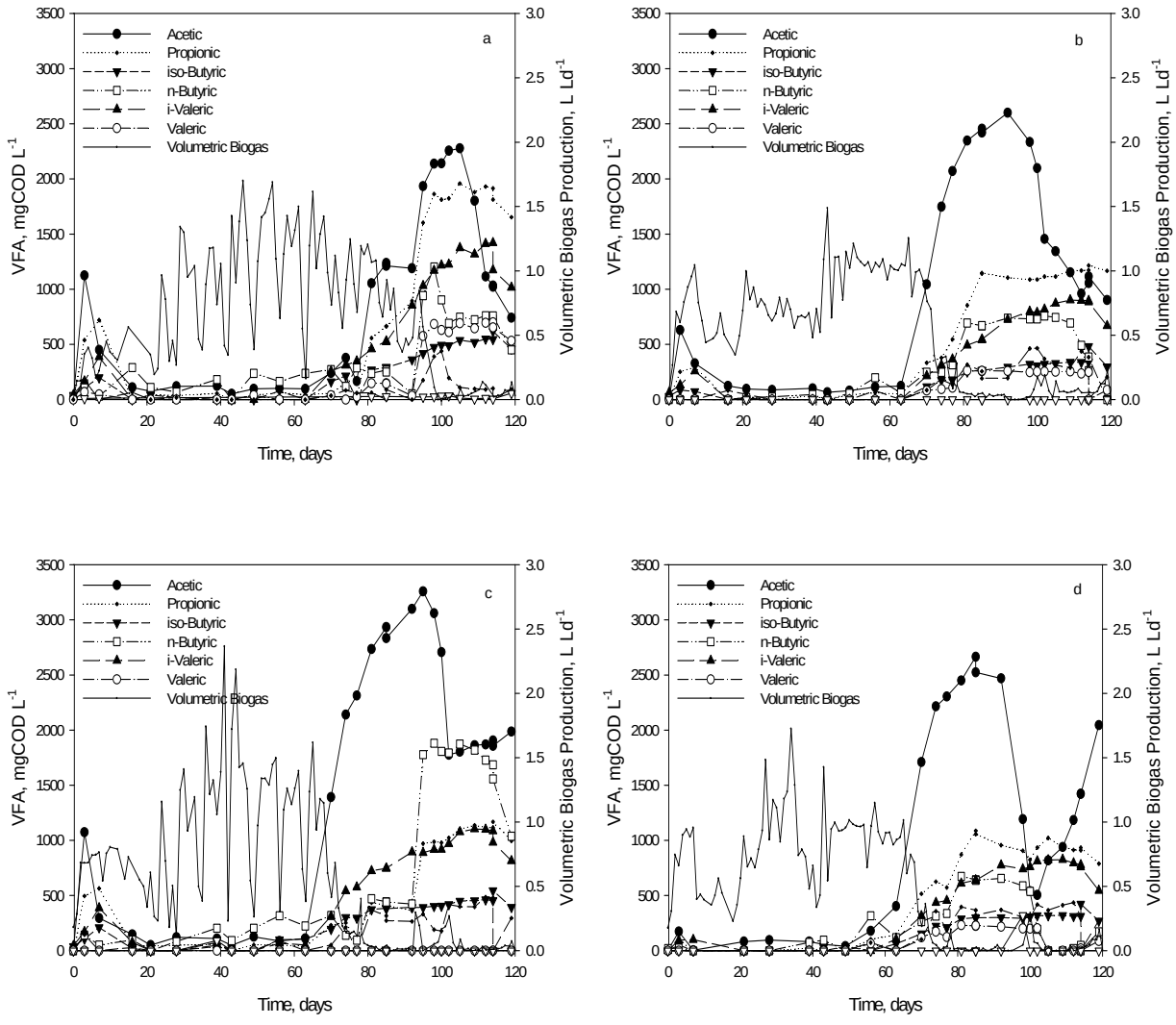

Figure 4.3
VFA concentrations and volumetric biogas production for bioreactors, DT2.
Left (a,c): reactors on 5-day feeding regime. Right (b,d): reactors on 7-day feeding regime.

The main findings from this digestion trial were the following:

- At an HRT of 25 days, the process was not sufficiently stable to allow an increase in OLR of 25%, even after 2.5 retention times.
- Reactor performance was similar between reactors fed 5 days per week and reactors fed 7 days per week; however the 5-day per week feed gave greater fluctuation in gas production.

Arising from these findings, the next digestion trial was designed to examine the influence of HRT on the stability of the process.

4.2.4 Digestion Trial 3

The objective of the third digestion trial was to examine the influence of hydraulic retention time by comparing reactor performance at two different retention times, 25 days and 50 days, to determine whether washout of methanogens was an important factor in reactor failure.

4.2.4.1 Method

Digestion Trial 3 was carried out from December 2005 to June 2006. Two of the reactors (M1 and M3) were operated at an HRT of 25 days, while the other two (M2 and M4) were operated at an HRT of 50 days. Initial OLR to all reactors was 1.45 g $VS.L^{-1}d^{-1}$. OLR was increased by 25% to 1.82 $gVS.L^{-1}d^{-1}$ to reactors M3 and M4 at Day 65. Two further feed increases of 25% each for reactor M4 occurred at Days 86 and 114.

4.2.5 Results

Figure 4.4 shows digestion parameters for all reactors for DT3. Reactors M1 and M3 (HRT = 25 d) began to decline in pH around Day 65, while M2 and M4 (HRT = 50 d) maintained a fairly constant pH at that point. After the pH had dropped, sodium hydrogen carbonate was added as a buffer to reactor M3 to raise pH and determine whether pH control could help in methanogenic recovery – this is the reason why pH rises from pH 6 back up toward 7.5 in the figure. Reactor M4 maintained a stable pH until approximately Day 114, the time of the third OLR increase, when it began to decline until feeding was stopped. pH for M2 stayed relatively stable up to Day 150.

Biogas production showed a similar trend to pH, with the 25-d HRT reactors declining in gas production commencing around Day 60, while the 50-d HRT reactors maintained

volumetric gas production that was stable (at constant OLR) or increasing (with increasing OLR).

Partial alkalinity for the two reactors on 50-day HRT stayed high for the earlier part of the trial while it dropped for the 25-day HRT reactors. The rise in partial alkalinity for M3 is attributable to the addition of sodium hydrogen carbonate noted previously. Intermediate alkalinity was similar for both sets of reactors but the difference in partial alkalinity results in a much higher IA:PA ratio for the reactors on HRT of 25 days.

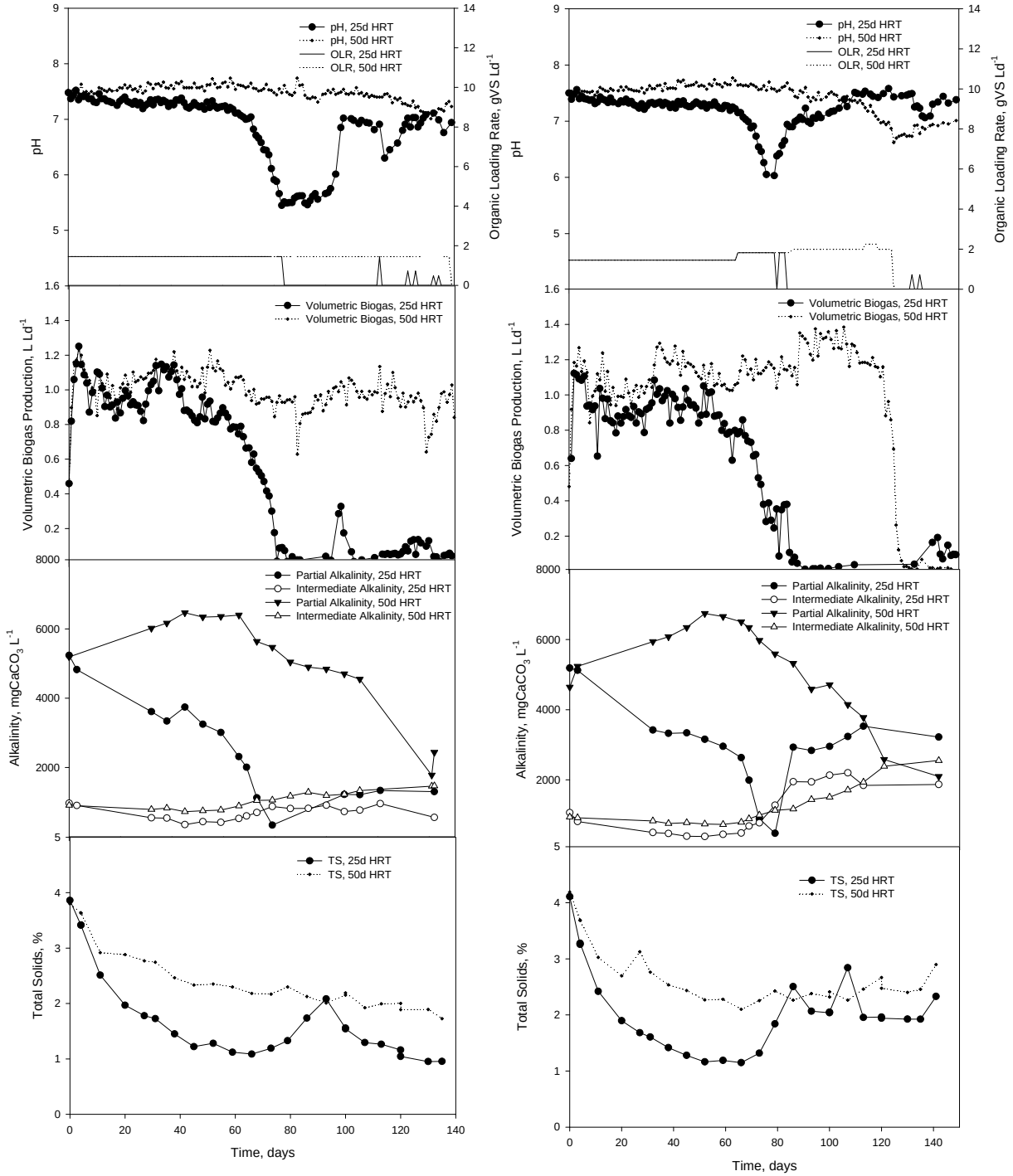

Figure 4.4
Trends in pH, volumetric biogas production, alkalinity and digester solids for reactors at different HRTs, DT3. Left: Reactors at constant OLR. Right: Reactors with increasing OLR.

Similar to DT1 and DT2, TS decreased from its initial concentration in the seed sludge. The 25-d HRT reactors showed a TS decrease at a faster rate than the 50-d HRT reactors, until the time of methanogenic failure when it rose again, likely attributable to a decrease in solids destruction.

Figure 4.5 shows the VFA curves for each of the four reactors. All four of the reactors show a similar rise in VFA, starting with acetic acid just before Day 60. Although both 25-d HRT reactors failed at this point, the 50-d HRT reactors continued to operate for a further 60 days.

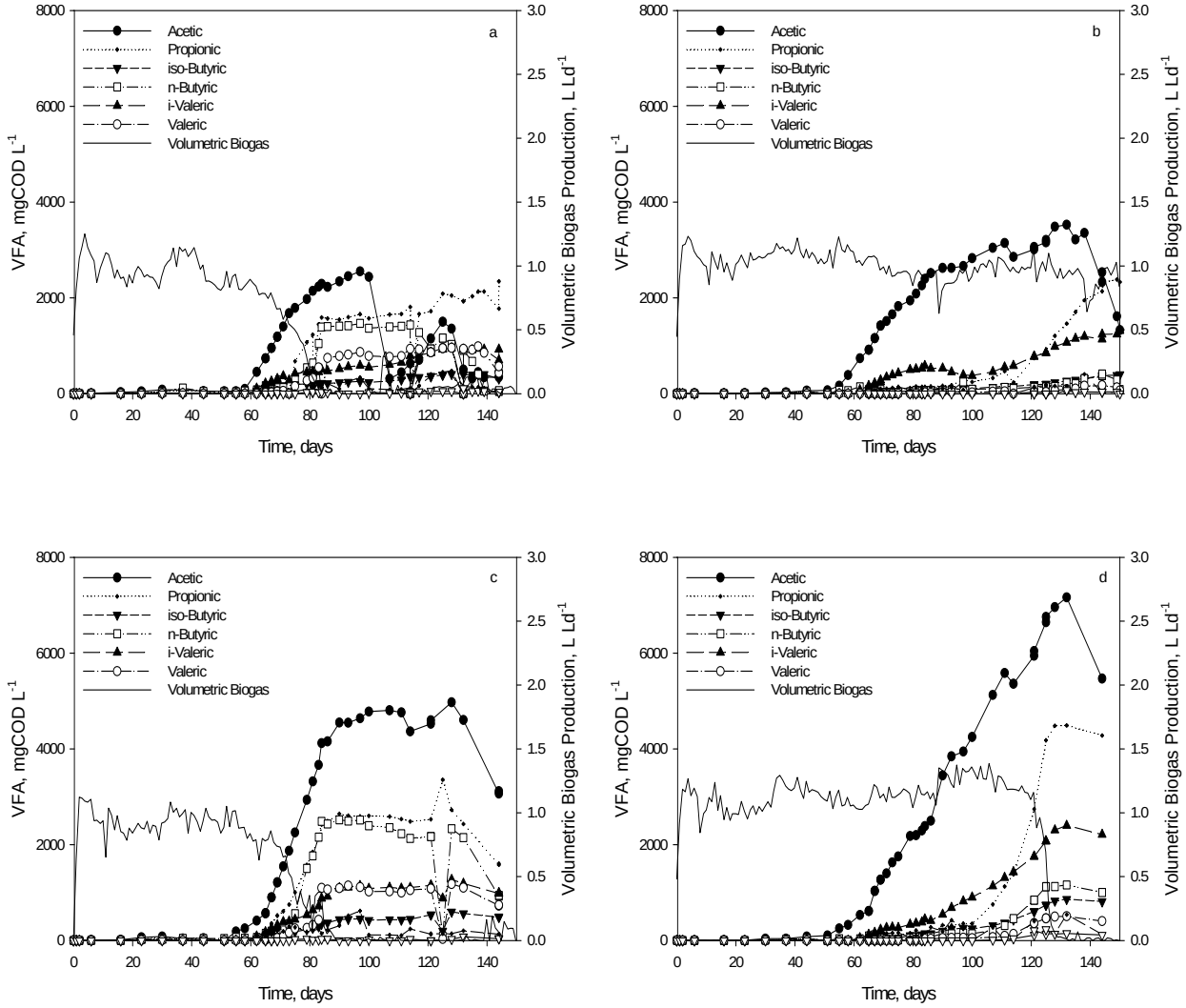

Figure 4.5
VFA concentrations and volumetric biogas production for bioreactors, DT3. Reactors on HRT of 25 days (a,c) or 50 days (b,d). Top: Reactors on constant OLR. Bottom: Reactors on increasing OLR.

The main findings from this digestion trial were:

- At an HRT of 25 days, the process was susceptible to failure irrespective of whether OLR was increased or held constant;

- An HRT of 50 days allowed operation for a longer period in spite of high VFA concentrations;
- An HRT of 50 days also permitted increase of the OLR from 1.45 to 1.98 before methanogenic failure occurred; and
- Although an HRT of 50 days permitted operation for longer at high VFA concentrations, it did not result in sustained stable operation for an indefinite period.

Arising from these findings, a number of possible factors that could contribute to digester failure were considered, as described in the following section.

4.2.6 Summary of Results of Routine Parameter Analysis for DT1, DT2 and DT3

The purpose of the digestion trials was to assess the digestibility of the catering waste feedstock. Achieving stable digestion of this feedstock in a single-stage mesophilic system, however, proved to be difficult.

All three trials showed fairly similar patterns of methanogenic failure, as evidenced by sudden declines in pH and gas production, accompanied by sharp increases in IA:PA ratio and VFA concentrations.

In all three trials, reactors with increased feed showed a marked increase in gas and VFA (particularly acetic acid) production, followed by a catastrophic failure approximately one week after the feed increase. This was true of all reactors on a 25-d HRT. During DT3, reactor M4 (50-d HRT) sustained two OLR increases before showing the same methanogenic failure upon the third increase in OLR.

There was also a pattern, however, of methanogenic failure even in the absence of an OLR increase, as evidenced by the failure of the control reactor M1 around Day 80 on DT1, and the failure of control reactor M1 around Day 65 on DT3.

During DT3, reactors M1 and M3 (HRT = 25 d) both showed signs of failure around the same time that reactors had failed on the previous trial. The failure occurred in both reactors even though one received an OLR increase while OLR was held constant for the other. In fact, during DT3 a rise in acetic acid concentration was exhibited by all reactors, and this increase appears to have commenced before the OLR increase. This may indicate a decrease in acetoclastic methanogenesis at that point. The 50-day HRT reactors,

however, were able to maintain digestion at that point while the 25-day HRT reactors failed.

On all trials, acetic acid was the VFA that showed the first large increase. All other VFA rose after feeding was ceased, and may possibly be due to product inhibition by concentrations of acetic acid, and/or hydrogen.

During DT3, Reactor M4 (HRT = 50 d) showed stability for much longer than the 25-day HRT reactors, and operated stably during two successive increases in feed. The failure followed a third increase in feed. It can be seen in the results that VFA were still increasing and had not yet stabilised at Day 114, the time of the third OLR increase.

Alkalinity was being maintained at a consistently high concentration (above 8000 mg l^{-1} $CaCO_3$) for the 50-d HRT reactors, as compared to the 25-d HRT reactors, for which alkalinity declined over the course of the trial to less than 4000 mg l^{-1} $CaCO_3$, and which failed much earlier than the 50-d HRT reactors.

During DT3, sodium bicarbonate buffer was added to M3 as a means of restoring alkalinity when the reactor had reached a pH of 6. Although the addition of buffer did raise the alkalinity and pH to the equivalent of the 50-d HRT reactors, this did not restore gas production or stimulate the degradation of VFA. On the contrary, VFA continued to increase after the addition of alkalinity.

4.2.7 Discussion of Possible Factors Involved in Digester Failure

To determine the possible cause(s) of the methanogenic failure observed in the three digestion trials, a number of parameters were systematically investigated concurrently with the digestion trials.

Organic Overload

The failure of methanogenesis as a result of feed overload is a likely hypothesis, as in most cases the failure occurred after an increase in OLR. It has been stated that readily-degradable materials such as fruit and vegetable wastes should be processed in two-stage digestion systems rather than one-stage digestion systems (Mata-Alvarez, 2003), since the production of volatile fatty acids by acidogenic and acetogenic bacteria may outpace the capacity of the slower-growing methanogens to degrade them. This can result in a drop in pH which makes the conditions further unsuitable for methanogenesis and may ultimately lead to failure (Bouallagui et al., 2005).

In the digestion trials, methanogenic failure usually followed an increase in OLR, lending support to the hypothesis that organic overload was the main factor involved in reactor failure. This is not a complete explanation, however, since there were two instances of failure in the absence of OLR increase: during both DT1 and DT3, the control reactor failed despite receiving no feed increase in either trial.

Also, in the case of M4 on DT3, an increase in OLR did not result in methanogenic failure, and gas production continued through two successive feed increases before failing on the third increase.

Therefore, OLR overload is likely a contributory factor, but is not the only factor involved. The system may have been previously unstable before any increase in OLR.

Temperature Variation, Chemical Shock or Other One-Off Events

There is the possibility that a single shock event was responsible for the methanogenic failures in each of the trials, such as temperature shock, chlorine shock from tap water, or toxicant shock from the feed.

During DT2, the heating system for the digesters was modified on Day 61, and temperature inside the reactors may have increased by 2 to 3 degrees Celsius as a result. During DT3, therefore, temperature in the digesters was measured weekly (data not shown) and it was found that temperature in the reactors stayed stable during the trial, and the gas production declines do not correspond with any variation in temperature.

During DT1 and DT2, tap water was used to dilute the feed and maintain constant flow. Tap water has advantages over deionised water that it contains more alkalinity and is more readily available. There is the possibility, however, of variation in the constituents of the tap water, since it is not defined like deionised water. There is a possibility that there was some kind of shock load of chlorine or other chemical in the tap water that was toxic or inhibitory to methanogens and caused the crash. To eliminate the possibility of chlorine shock or other interference from tap water, deionised water was used instead of tap water in the later stages of DT3.

The third one-off event possibility considered was some kind of toxicant load in the feed provided to the reactors. All feed was from a large single composite sample prepared from samples collected over a one-week period, ground and mixed together. The composite was then stored frozen in 2-L containers. The possibility that one of the containers contained

some kind of toxicant that could kill methanogens was considered. To check for the possibility of a toxicant in a single container of food waste, each time a new container of feed was begun, this was recorded and a 20 g sample of the feed was frozen for later analysis if necessary.

In all three trials, the pattern of methanogenic failure was fairly similar and happened during roughly the same time period. While the occurrence of three methanogenic failures as a result of single shock events is possible, it is less likely that they would all occur following a similar pattern and during the same time periods. Later digestion trials described in Sections 4.3 and 4.4 also confirmed that the failures were due to instability in the system rather than shock events.

Soluble Sulphides

Although sulphur is required by methanogens and is an obligate micronutrient, sulphur species are also potentially inhibitory to digestion (Speece, 1996, Gerardi, 2003), and can interfere with the process in a number of ways, as discussed in Chapter 2.

Since this feedstock includes sulphur-containing proteins, the possibility for inhibition from sulphide, the reduced form of sulphur, was considered. During the first two digestion trials of this investigation, odour from the failed bioreactors became quite offensive, which may have been due to the presence of mercaptans and other reduced sulphur compounds in the biogas (Iranpour et al., 2005).

A soluble sulphide concentration above 200 mg l^{-1} has been given as an inhibitory threshold (Gerardi, 2003). During DT3, sulphide concentrations in all four reactors were analysed weekly to determine whether sulphides were high enough to approach inhibitory concentrations and to see whether there was a pattern of increase.

Table 4.5 shows average weekly concentrations of soluble sulphides between days 35-80 of the trial. The sulphide concentrations are highest for reactors M2 and M4, the two reactors that maintained stable digestion for the longest.

It was found that the sulphide concentrations at later stages of the trial were actually lower than the initial concentrations from the seed sludge, and there was no apparent pattern of increase. Also, the sulphide concentrations were highest for reactors M2 and M4, the two reactors that maintained stable digestion for the longest.

Table 4.5
Soluble Sulphides – DT3

Reactor	Hydraulic Retention Time	Soluble Sulphide, mg l^{-1} Average ± Std Dev
M1	25 days	2.3 ± 1.0
M3	25 days	2.1 ± 0.9
M2	50 days	4.3 ± 1.7
M4	50 days	4.6 ± 2.1

The degradation of propionate and butyrate by acetogenic bacteria requires the syntrophic uptake of hydrogen by hydrogenotrophic methanogens, while sulphate-reducing bacteria can degrade these compounds directly, if sufficient sulphate is present (Stams et al., 2005). Therefore, degradation of propionate and butyrate in the absence of methanogenesis can be an indicator of high concentrations of sulphate reducers in the medium. During the digestion trials, however, concentrations of propionate and butyrate rose during or immediately following methanogenic failure, and remained static at their peak concentrations until the end of the trial in each case. This is evidence that sulphate reduction was not occurring to a significant extent at that time, although it does not eliminate the possibility that sulphate reduction was occurring up to the time of stopping feeding, and ceased after feeding was stopped due to the depletion of sulphate.

Therefore, inhibition by sulphide or competition from sulphate reducers does not appear to be the cause of methanogenic failure. Sulphides could, however, play a role in reducing bioavailability of micronutrients.

Cation Accumulation

An excess of cations such as sodium and potassium can cause stress on digestion systems and lead to digestion failure, as a result of interference with cell osmotic balance (Mata-Alvarez, 2003). Some investigators found inhibition of methanogenesis in digestion of fresh vegetable waste, which they attributed to a high content of potassium in the food waste (Carucci et al., 2005).

The food waste used as substrate in these digestion trials is from an institutional kitchen where significant amounts of salt are used in the preparation and serving of food, and therefore the food waste will contain salts, which may accumulate over time in the reactor. The possibility of cation inhibition or toxicity was therefore considered. Concentrations of

dissolved solids were monitored as an approximate measure of cations, on a weekly basis during days 35 – 80 of DT3, with results shown in Table 4.6.

Table 4.6
Total Dissolved Solids – DT3

Reactor	Hydraulic Retention Time	TDS, mg l^{-1} Average ± Std Dev
M1	25 days	5.2 ± 0.5
M3	25 days	5.4 ± 0.7
M2	50 days	8.9 ± 0.6
M4	50 days	9.2 ± 0.9

The dilution rate was higher in the reactors on an HRT of 25 days than the reactors with HRT of 50 days. Therefore, the total dissolved solid concentrations in the 25-day reactors were lower, as confirmed by the data above. If cation toxicity was the main cause of failure, the reactors with a higher concentration of dissolved solids, (ie., the 50-day HRT reactors) would be expected to exhibit a poorer digestion performance. The opposite, however, was true – in this trial the reactors on a 50-day retention time showed more stable digestion and higher gas production than the 25-day reactors, which had a lower cation concentration. Therefore, cation toxicity may be ruled out as the cause of digester failure.

Volatile Fatty Acids Accumulation

Volatile fatty acids, due to their effect on pH, can be toxic to methanogens at high concentrations (Mata-Alvarez, 2003). The VFA concentrations at which failure occurred, however, were not consistent during the different trials. Although VFA increased during every case of methanogenic failure, there is not a clear threshold value for failure. For example, during DT2 all reactors had total VFA concentrations of approximately 2500-3000 mg l^{-1} at the time of failure, whereas during DT3 reactors M2 and M4 continued to operate at much higher VFA concentrations – up to 6000 and almost 8000 mg l^{-1}, respectively, before failure or the cessation of feeding.

Propionic acid is a persistent VFA and known to cause inhibition (Fernandez et al., 2005). Some investigators recommend monitoring of the ratio of propionic:acetic, with a ratio of over 1.4 as an indicator of process failure (McCarty 1982, as cited in Fish 1999). At the time of methanogenic failure in the digestion trials for which VFA were monitored (DT2 and DT3), however, the concentration of propionic acid was lower than that of acetic acid,

giving a ratio of propionic to acetic below 1 in all cases except that of M2 during DT3, which was still producing methane at the time feeding was stopped. The data indicate that accumulation of propionic acid occurred as a symptom of the failure, rather than its cause.

Alkalinity Depletion

Alkalinity is necessary for buffering, to keep the system pH within a range suitable for methanogenic activity (Lahav et al., 2002). It was approximately 4000-5000 mg l^{-1} $CaCO_3$ around the time of the failures of the 25-d HRT reactors in the three trials, down from an initial concentration for seed sludge of approximately 8000 mg l^{-1} $CaCO_3$. During DT3, when two of the reactors were run at an HRT of 50 days, alkalinity had risen to a higher concentration than the initial concentration in the seed sludge (up to 9000 mg l^{-1} in M4, and 8800 mg l^{-1} in M2 before declining to 6500 mg l^{-1} around the time of methanogenic failure). The high IA:PA ratio observed was indicative of high VFA and may have signalled imminent failure. By stopping feeding at the time that the high ratio was noted, it may be possible to prevent failure, although VFA were continuing to rise and show a lag in most cases. In the case of M2 on DT3 gas production was persisting in spite of the high Ripley's Ratio.

During DT3, total alkalinity decreased over time in reactors with a 25-day HRT, while it increased gradually in the 50-day HRT reactors. This indicates that in the case of the 25-day reactors, alkalinity was being washed out before it could be replenished, whereas in the 50-day reactors, alkalinity was being regenerated at a pace exceeding the rate of washout.

All reactors showed a decrease in partial alkalinity to less than 3000 mg l^{-1} $CaCO_3$ prior to methanogenic failure. In the case of reactor M4 on DT3, partial alkalinity was high (4150 mg l^{-1} $CaCO_3$) at the initiation of declining gas production, but had decreased to 2600 mg l^{-1} $CaCO_3$ by the time of full methanogenic failure.

Some investigators periodically add alkalinity in the form of calcium carbonate or sodium hydrogen carbonate to digesters to maintain pH in the optimal range (Fernandez et al., 2005). The alkalinity concentration of 4000 mg l^{-1} as $CaCO_3$ observed prior to methanogenic failure, however, are actually high relative to the optimal alkalinity range for digestion of sewage sludge of 1500-3000 mg l^{-1} as $CaCO_3$ (Gerardi, 2003). Therefore regular additions of alkalinity would be unlikely to prevent failure; the drop in partial alkalinity observed during the trials was substantial and occurred within a few days. Also, acetic acid concentrations began to rise before the drop in partial alkalinity. During DT3,

alkalinity was added to digester M3 when it was at a pH of 6, which subsequently brought the pH in the medium up to neutral. This was not sufficient, however, to restore methanogenic activity.

Ammonia Toxicity or Depletion

The accumulation of high concentrations of ammonia from the breakdown of proteins in the feedstock is a possible factor that needs to be considered. Various investigators have researched the inhibitory effects of ammonia (Angelidaki and Ahring, 1993, Kayhanian, 1994), and give different inhibitory threshold concentrations for total ammonia nitrogen (TAN) ranging from 1200 mg l^{-1} (Mata-Alvarez, 2003) to 7 g l^{-1} (Grady et al., 1999).

TAN concentrations in the reactors were tested during the later period of DT3 and subsequent trials. During DT3, TAN concentrations for reactors M2 and M4 (on 50-day HRT) were higher than those in reactors M1 and M3 on the shorter retention time (data not shown). The reactors on longer HRT, however, maintained stable digestion for the longest time. The lower ammonia concentrations in the other two reactors (25-day HRT) can be attributed partially to washout of ammonia due to the higher flow rate. If ammonia was a critical inhibitor, therefore, it would be expected that the reactors on the shorter HRT would fare better as ammonia is kept at a lower concentration for these reactors.

Long-Chain Fatty Acid Inhibition or Toxicity

Many investigators have indicated inhibition of various stages of the biomethanization process by the presence of long-chain fatty acids (Salminen and Rintala, 2002a, Mykhaylovin et al., 2005, Pereira et al., 2005, Li et al., 2005). Some have suggested that LCFA, particularly oleic acid, can interfere with mass transport of solutes across cell membranes (Pereira et al., 2005).

The food waste substrate used in this trial contains lipids, which yield LCFA via β-oxidation (Angelidaki and Ahring, 1992). LCFA inhibition may be a factor that may merit further investigation and is discussed further in later sections.

Biomass Washout

Another possible cause of failure investigated is the washout of biomass. The retention time used for DT1 and DT2 was 25 days, which is at the upper end of the range of 15-25 days that is most commonly used for anaerobic digestion of wastewaters and solid wastes (Gerardi, 2003). Acidogens have generation times on the order of 15-30 minutes, while

methanogens have generation times ranging from 3-30 days (Gerardi, 2003). Especially under stressful conditions, growth can be slower and therefore there is a possibility that the methanogenic growth rate is not keeping up with the washout rate.

On DT3 two reactors were operated at an HRT of 50 days, twice the retention time of that used on previous trials. The reactors with the 50-day retention time clearly outperformed the reactors on a retention time of 25 days.

These results have shown, therefore, that hydraulic retention time affects process stability and should be investigated further.

Trace Element Depletion

A number of investigators have pointed to the importance of certain trace elements such as iron, cobalt, nickel, copper, and zinc for maintaining a stable anaerobic digestion process (Florencio et al., 1994, Speece, 1996, Zandvoort et al., 2002, Zandvoort et al., 2005). Although required in very small amounts, these metals are essential for growth of bacterial biomass, as they are the building blocks for certain enzymes, cofactors and corrinoids (Osuna et al., 2003).

In continuous anaerobic digestion investigations, supplementation of the feed with trace elements is common but not universal – some investigators add trace elements (Pereira et al., 2001) while others do not (Salminen and Rintala, 2002b). Speece (1996) recommended adding trace elements to remedy high effluent VFA concentrations in reactors treating industrial wastewaters.

The feedstock studied in this investigation is a complex waste containing a wide range of foods, all of which are intended for human consumption. It seems likely, therefore, that the food waste would contain sufficient amounts of all required trace elements. It is possible, however, that either the feedstock lacks all of the trace elements specifically required for methanogenic metabolism or that the elements are present but not bioavailable. Heavy metals are known to be precipitated by sulphides (Gerardi, 2003); long-chain fatty acids (LCFA) can also bind with metals (Pereira et al., 2001). Therefore although the substrate contains a broad range of nutrients and may contain all of the trace elements required, these may become non-bioavailable during the anaerobic digestion process. Periodic pulsing of trace elements could address this lack of available micronutrients.

4.2.8 Summary of Factors Considered

Table 4.7 summarises the stressors considered and investigated.

Table 4.7
Possible Factors Affecting Digestion of Catering Wastes

Possible Stressor and Rationale	Evidence / Test	Conclusion	Further Investigation
Rapid VFA increase from readily-degradable substrate (most failures after OLR increase)	VFA started to increase before OLR increase in DT3. Digesters receiving steady OLR eventually failed also.	Contributing factor. Food waste high in easily degradable carbohydrates for rapid VFA production	OLR to be kept constant or increased only gradually
Unknown toxicant load, thermal shock, other external one-off factor	Repetition of three digestion trials with similar results in similar timeframe	Likely that one-off events serve as indicator of unstable system, rather than sole cause	Minimise likelihood of future disturbance
Soluble sulphides	Weekly/biweekly sulphide monitoring	Sulphides not unusually high. On DT3, sulphides were higher in long-HRT digesters which failed later.	No
Cation toxicity	Reactors with higher dissolved solids in DT3 were more stable than those that were more dilute.	Not likely to be main factor	No
Ammonia Inhibition (Food waste high in N)	Weekly TAN monitoring: concentrations higher in long-HRT digesters that lasted longest	May be acting to provide buffering and allowing high VFA. Does not appear to be main cause of failure.	Monitoring of TAN concentrations during next trials
Long-Chain Fatty Acid inhibition	Literature review found substantial evidence of LCFA inhibition for feedstocks containing lipids.	Cannot be eliminated as a possible stressor in the system	Yes, more literature investigation
Washout of biomass and/or alkalinity	Weekly TS/VS and alkalinity monitoring - showing gradual decline in VS and alkalinity for short-HRT reactors	Dilution or washout effect apparent	Yes, more investigation into retention time (HRT and SRT)
Trace element limitation	VFA rose later in digestion trial, consistent with depletion of some substance	Possible contributing factor	Yes

The factors that emerge as meriting further investigation are trace elements, LCFA, ammonia and retention time. Therefore, subsequent digestion trials were designed to systematically investigate these factors.

4.3 Trace Element Supplementation Investigations

4.3.1 CSTR Digestion Trials

The digestion trial described in this section was summarised in a paper published in Water Science and Technology (Climenhaga and Banks, 2008a), attached in Appendix A.

The results of the previous digestion trials have pinpointed HRT and trace elements as factors for further study. It was found during DT3 that bioreactors on longer HRT of 50 days showed stable operation for longer than those on shorter HRT of 25 days. It was also determined that there was a pattern of stable operation up to a certain point at which rapid methanogenic failure occurred, which could be consistent with depletion of a certain nutrient essential to methanogenesis. These digestion trials were therefore designed to investigate the effects of varying HRT and trace element supplementation. The objectives of these trials were the following:

i) to investigate the role of trace element supplementation in the maintenance of stable digestion, by comparing the performance of trace element supplemented digesters with that of those with no trace element supplementation; and

ii) to compare the performance of reactors operated at different retention times.

4.3.1.1 Methods

CSTR bioreactors of 5 litre volume, as described in Section 3.3, were used. The reactors were operated on four retention times of 25, 50, 100 and 180 days, with two reactors operating at each retention time. The reactors were run as pairs that were duplicates of each other, except that one reactor from each pair was supplemented with a trace element mixture on a periodic basis (1.0 or 1.5 ml solution weekly, biweekly or monthly depending on HRT) while the other reactor was not. The trace element solution had the composition shown in Table 3.4.

4.3.1.2 Results

Total VFA (TVFA), methane production, ammonia, alkalinity and Ripley's ratio (Ripley et al., 1986) for each pair of reactors are illustrated in Figures 4.6 and 4.7.

The two reactors on a 25-day HRT (Figure 4.6) showed similar VFA and gas production trends at the commencement of the trial, until a steep increase in VFA occurred around Day 40 for the reactor without trace element supplementation. This increase in VFA was associated with a drop in methane production and eventual failure. Up to the time of the failure of the trace element-deprived reactor, the two reactors exhibited very similar behaviour in pH, ammonia, alkalinity and VFA profiles. The supplemented reactor showed stable digestion for three further retention times before also showing a rise in VFA and decrease in methane production between Days 110-120, at which point feed was stopped. Further investigations with this reactor are described in Section 4.3.2.

Both reactors in the 50-day HRT pair had a spike in total VFA production around Day 40-50, but the VFA were consumed over the following weeks. Both reactors exhibited very similar profiles for all parameters measured up to approximately Day 100 (two retention times) at which time the trace element-deprived reactor accumulated VFA and ceased to produce methane, while the trace element supplemented reactor maintained stable digestion.

The paired reactors on a 100-day HRT (Figure 4.7) also show a VFA spike around Day 40-50. The VFA concentration in the trace element deprived reactor, however, stayed high, while TVFA in the trace element supplemented reactor was consumed. Toward the end of one retention time (Day 90-100), TVFA in the trace element deprived reactor increased again at the same time as methanogenesis declined.

The paired reactors on a 180-day HRT were run for over 300 days. Both reactors in the pair continued to operate through two increases in TVFA. It is interesting to note that the trace element deprived reactor continued to operate in spite of higher VFA concentrations than those at which failure occurred for the reactors on shorter retention times. Reactors on this extended retention time showed very high alkalinity (over 18 g l^{-1}), and stable digestion at concentrations of TVFA (over 15 gl^{-1}) and TAN (over 5.7 gl^{-1}) that are considered by some investigators to be inhibitory or toxic (Grady et al., 1999, Gerardi, 2003). The reactor that did not receive trace elements showed methanogenic failure around day 300.

VFA profiles of all reactors are shown in Figure 4.8. With the exception of the trace element supplemented reactor on HRT of 25 days, marked increases in acetic acid around Day 40 and Day 100 were observed for all reactors. This cannot be attributed to external or equipment factors because the runs were commenced on different dates months apart, and therefore Day 40 for the 25-day HRT reactors falls on a different calendar date than Day 40 for the 50- and 100-day HRT reactors or the 180-day HRT reactors.

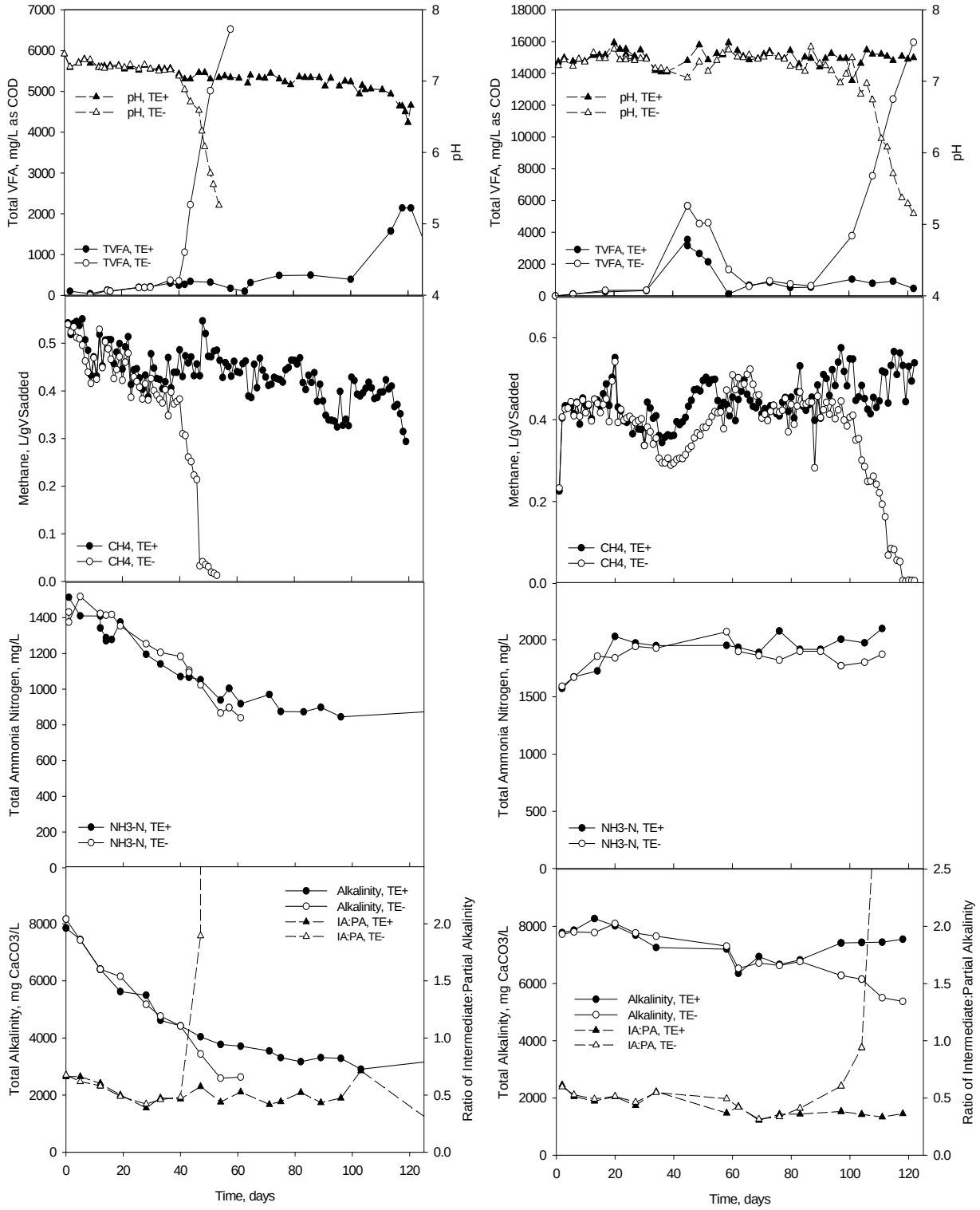

Figure 4.6
TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE+) or without (TE-) trace element supplementation. Left: HRT = 25 days. Right: HRT = 50 days.

Therefore, it is a phenomenon attributable to factors internal to the system. As the reactors are on the same OLR, at Days 40 and 100 all will have received the same cumulative VS, although the rate of washout and therefore accumulation of breakdown products such as ammonia differs for the different retention times.

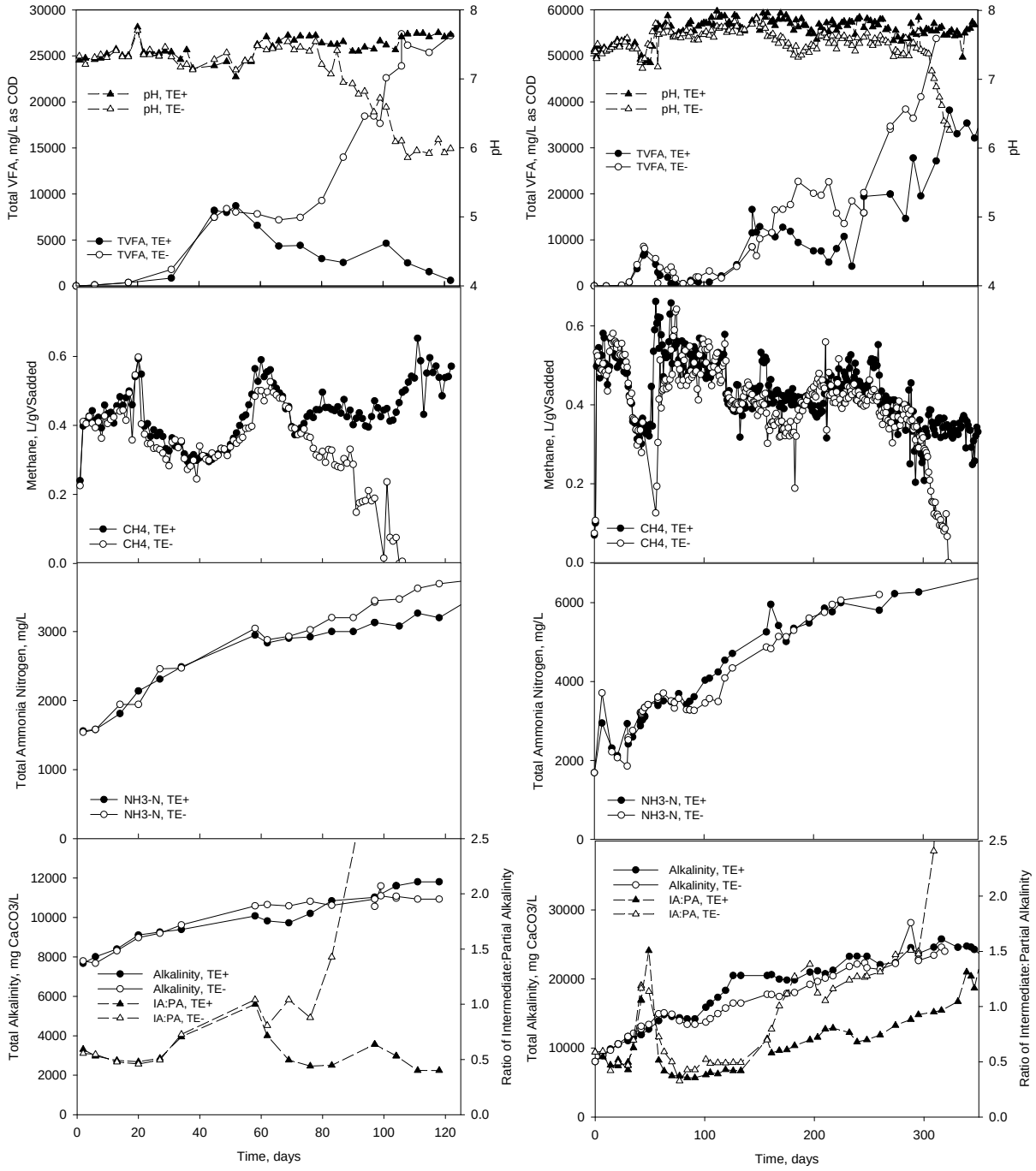

Figure 4.7
TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE+) or without (TE-) trace element supplementation. Left: HRT = 100 days. Right: HRT = 180 days.

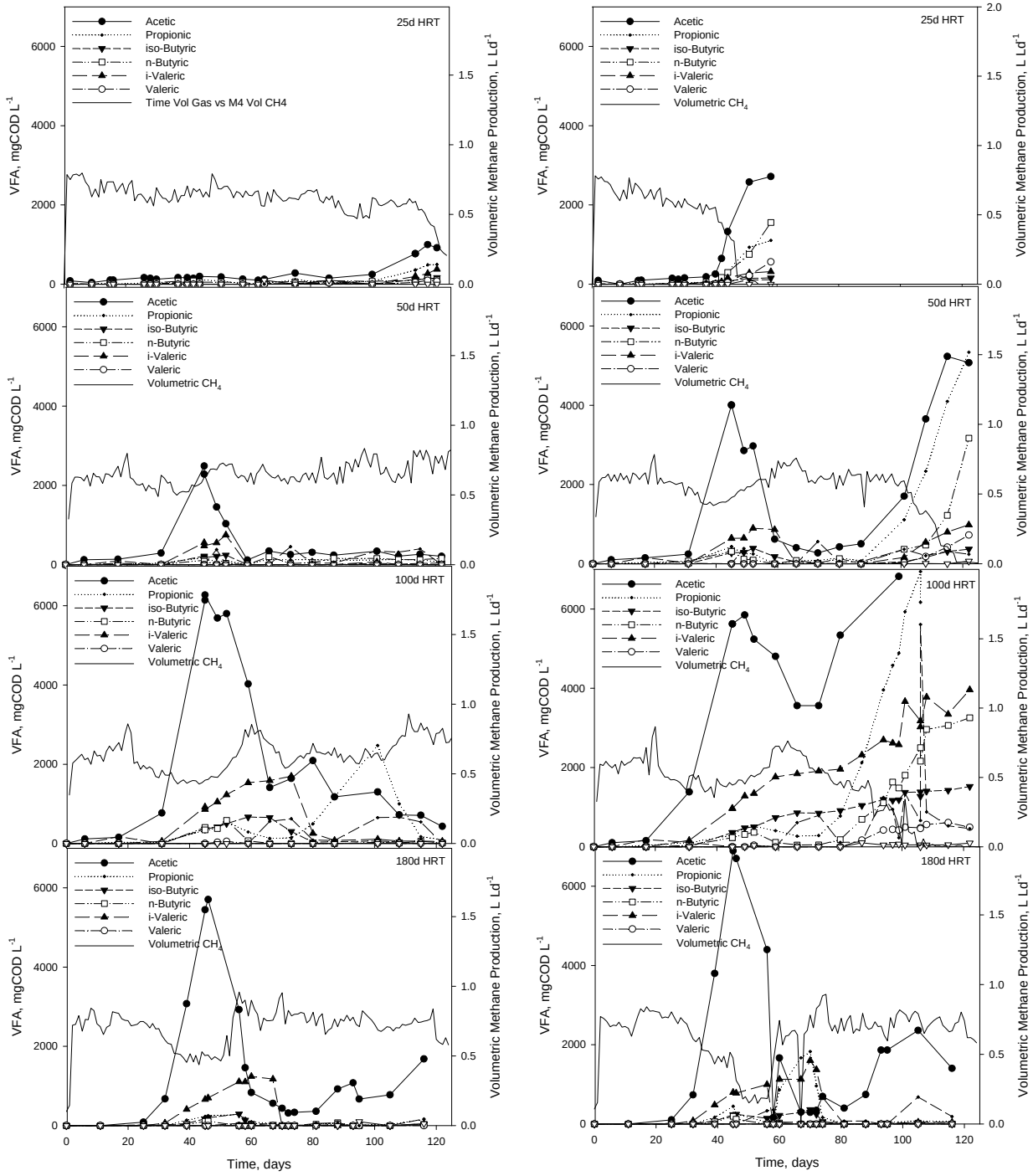

Figure 4.8
VFA concentrations and volumetric biogas production for reactors with (TE+) or without (TE-) trace element supplementation. Left: TE+ reactors. Right: TE- reactors.

These results showed that:

- Reactors supplemented with trace elements showed stable digestion and recovery or adaptation to high VFA concentrations, while reactors without trace element supplementation were subject to methanogenic failure; and

- Extending HRT allowed stable operation at higher concentrations of VFA and TAN than those in reactors on a shorter HRT that failed.

Concurrent with this trial, two other trials were commenced using the trace element-supplemented reactor on a 25-day HRT to further investigate the influence of HRT and trace elements. These trials are described in Sections 4.3.2 and 4.3.3.

4.3.2 Extension of HRT for Trace Element Supplemented Reactor

In this section, the effect of increasing HRT to a trace element supplemented reactor was tested. The objective of the trial was to determine whether extending HRT from 25 to 50 days would prevent methanogenic failure for a trace element-supplemented reactor.

4.3.2.1 Method

The trace element-supplemented reactor on an HRT of 25 days had shown stable operation for a period of 115 days (in excess of four retention times), which was nearly three times as long as its non-supplemented counterpart. At the end of this period, however, gas production declined and VFA increased in a pattern similar to that observed for trace element-deprived reactors, accompanied by a drop in pH to a minimum value of 6.5. At this point, feeding to the reactor was stopped for a period of 12 days. The reactor was then given a load of 1.45 gVS l^{-1} and again left without feeding for two more days to allow re-acclimation to the feed, before resuming operation at the previous OLR and a higher HRT of 50 days.

4.3.2.2 Results

The VFA profile for this reactor is shown in Figure 4.9. VFA concentrations in the reactor decreased and methane production increased back to its previous levels, indicating recovery of methanogenic activity. The reactor then showed stable operation for a further 130 days until the end of the trial.

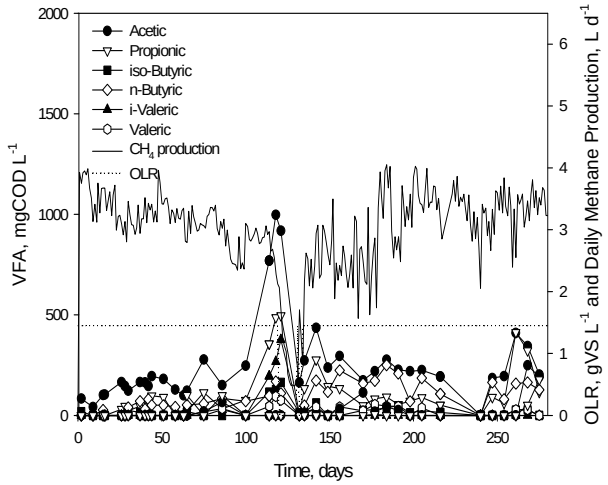

Figure 4.9
VFA and CH_4 production for a trace element-supplemented reactor, changing from HRT of 25 days to 50 days (transition between days 115-130).

These results showed that:

- While trace element supplementation was not sufficient to guarantee stable operation for a reactor on an HRT of 25 days, extending HRT to 50 days while maintaining trace element supplementation allowed recovery of the reactor and stable operation.

Concurrent with this trial, a trial investigating the effect of extending HRT without trace element supplementation was carried out, as described in the following section.

4.3.3 Depletion of Trace Elements from Previously Supplemented Biomass

Most of the bioreactors operated during these digestion trials were started up with fresh sewage sludge, with the exception of the experiment described in this section, involving an existing bioreactor as a control, and a second bioreactor seeded with effluent from the control bioreactor. The objectives of this experiment were the following:

i) to determine whether biomass that had previously been supplemented with trace elements and acclimated to the food waste substrate, when deprived of trace elements, would show the same pattern of methanogenic failure as that observed for digesters seeded with sewage sludge;

ii) to determine whether extending HRT without trace element supplementation would be sufficient for reactor recovery; and

iii) to observe whether methanogenic failure of the reactor would occur at the same predictable time point for trace element depletion as that for bioreactors seeded from sewage sludge.

4.3.3.1 Methods

Effluent biomass from a bioreactor previously operated with trace element supplementation was used to start up a second bioreactor. The seed bioreactor was the trace element supplemented reactor with a 25-day HRT from the digestion trials described in sections 4.3.1 and 4.3.2. Near the end of the run described in 4.3.1, effluent digestate removed as part of normal reactor flow was used to fill the second bioreactor. The reactor was filled over a period of approximately 25 days. No feed or other additions were made to the new reactor. At the end of the period of filling the bioreactor, feed to the seed bioreactor was stopped for a period of 12 days, after which both reactors were given one day's feed (1.45 gVS l^{-1}) for re-acclimation to the feed. Three days later, normal daily feeding at the rate of 1.45 gVS $l^{-1}d^{-1}$ was commenced to both reactors. Trace element supplementation was continued to the seed reactor, while no trace element supplementation was provided to the second bioreactor. Both reactors were operated on a 50-day HRT, extended from the 25-day HRT that had previously been used for the seed reactor.

This allowed for trace elements to be depleted by washout from a biomass that had previously been operating with trace element supplementation, for the purpose of comparing the performance after trace element depletion to the performance of biomass that continued to receive trace element supplementation.

4.3.3.2 Results

The results of the experiment are shown in Figure 4.10. The two bioreactors showed similar profiles in VFA and methane production for approximately 70 days after the commencement of the experiment. At around 75 days after the commencement of feeding, however, the reactor deprived of trace elements exhibited a VFA rise, followed by methanogenic failure by day 100. This was similar to those observed in trace element-deprived reactors started up with a sewage sludge seed, while the bioreactor receiving trace element supplementation continued to operate stably.

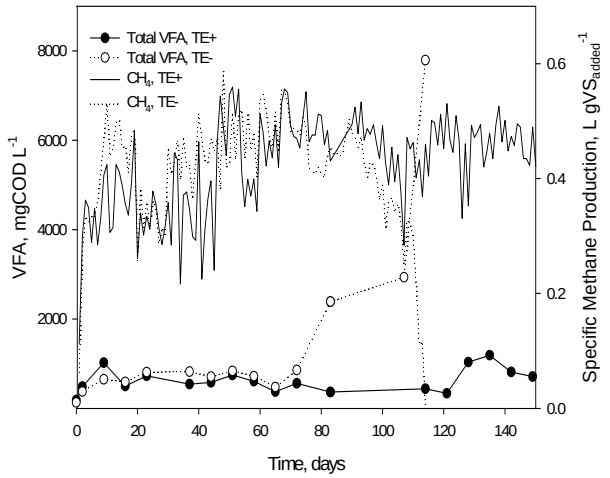

Figure 4.10
TVFA and CH_4 production, for reactors with (TE+) or without (TE-) trace element supplementation, after seeding from a reactor previously run with trace element supplementation.

These results showed that:

- Stopping trace element supplementation to biomass that had previously been supplemented with trace elements resulted in methanogenic failure after a washout period; and
- Extending HRT from 25 to 50 days was not sufficient to allow reactor recovery without the provision of trace elements.

Also, coupled with the results of the previous trials, it has been found that:

- An HRT of 25 days is insufficient for sustained stable operation, even when trace elements were provided.

Arising from these findings, the next digestion trial was designed to test a slightly longer HRT of 30 days, and to further test the effect of trace element supplementation.

4.3.4 Confirmation of Trace Element Effect and HRT

The digestion trials described in sections 4.3.1, 4.3.2 and 4.3.3 have shown that trace element supplementation can have an influence on the maintenance of stable operation. For all duplicate pairs of reactors, the reactor that was supplemented with trace elements showed stable operation over an extended period, while the other reactor of the pair

exhibited methanogenic failure. To confirm whether this was a repeatable phenomenon, a further new digestion trial was initiated with fresh sewage sludge inoculum. Trace element supplementation had proven effective in increasing reactor stability at retention times of 25, 50, 100 and 180 days. Methanogenic doubling times can sometimes exceed 25 days (Gerardi, 2003), and so it was hypothesised that the shortest HRT tested of 25 days may not be sufficient for stable operation, even with trace element supplementation; this was borne out by the results described previously. An HRT of 50 days was shown to be effective in previous trials; however a lower HRT would be desirable as a lower HRT allows smaller tank volumes and/or higher throughput. Therefore a retention time of 30 days was selected for this trial, to determine whether this HRT would be sufficient for stable operation with trace element supplementation, and to find the time point at which a reactor deprived of trace elements would fail.

Also, in the previous digestion trials nearly every reactor had a 'spike' (rapid increase followed by decrease) in acetic acid concentrations sometime between 40 and 60 days of operation, which occurred even in reactors started on different dates. Another purpose of this digestion trial was to observe whether this was a repeatable phenomenon.

The three objectives of the trial, therefore, were the following:

i) to seek confirmation of the previous results in which trace element supplemented reactors showed stable operation where duplicate reactors without trace element supplementation failed;

ii) to observe whether the peak in acetic acid production observed between approximately 40 and 60 days of digestion was a repeatable phenomenon; and

iii) to determine whether a 30-day HRT was sufficient to maintain sustained stable operation where a 25-day HRT had not been.

4.3.4.1 Methods

Two duplicate 5 l CSTR reactors were seeded with fresh sewage sludge and operated under the same conditions as those in Section 4.3.1, except that HRT was 30 days. One reactor was supplemented with trace elements on a weekly basis, while the other was not.

4.3.4.2 Results

The VFA and specific methane production for each of the two reactors are shown in Figure 4.11. Similar to previous results, the reactor deprived of trace elements exhibited

methanogenic failure after 90 days of operation, while the reactor supplemented with trace elements continued to operate stably throughout this trial and a subsequent trial described in the following section, for a total of 157 days of stable operation.

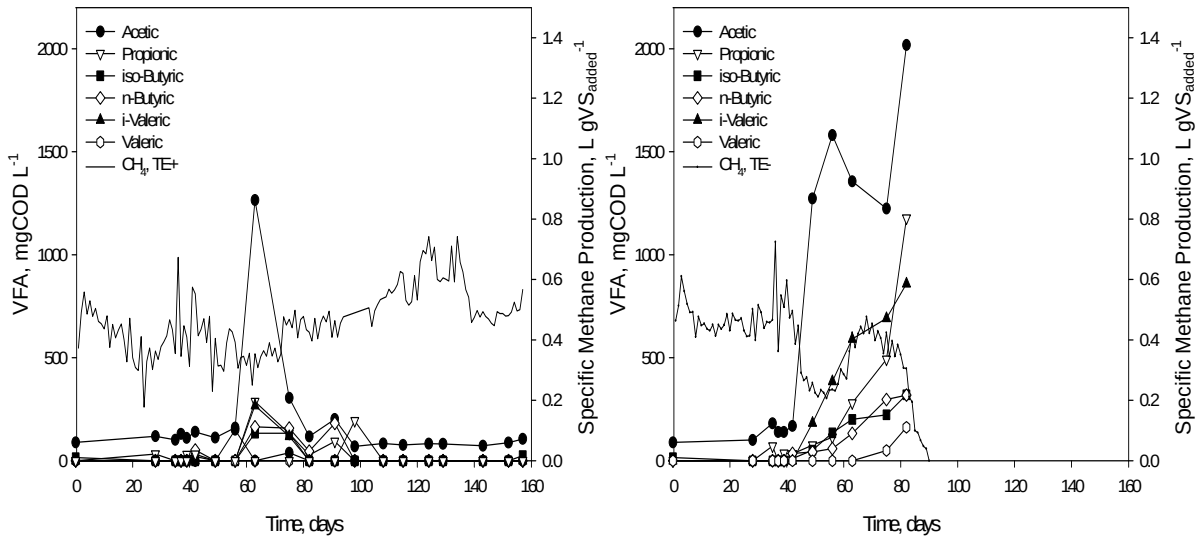

Figure 4.11
Volatile fatty acids and methane production for reactors supplemented with trace elements (TE+, left) and deprived of trace elements (TE-, right).

There was again a pronounced increase in acetic acid concentrations, as observed in earlier trials, beginning around day 45 for the trace element deprived reactor, and slightly later (around day 60) for the trace element supplemented reactor. The reactor supplemented with trace elements recovered from the spike, showing concentrations of acetic and other VFA declining again over the next few days. The reactor without trace element supplementation, however, did not show complete recovery from the acetic acid spike. Although the acetic acid concentration declined to an extent and methane production continued for several more days, the concentrations of other VFA (propionic, iso-valeric) continued to rise and methanogenic failure occurred.

During the previous trial with a trace element-supplemented reactor operated on an HRT of 25 days, the reactor operated stably for 115 days before showing signs of imminent failure in declining gas production and pH, and increasing IA:PA ratio. In this trial using an HRT of 30 days, stable digestion was sustained for a total of 157 days until the end of the trial. All digestion parameters at the end of the trial indicated that the reactor was healthy and could operate for longer. Based on these results it cannot be stated that stable operation

would have carried on indefinitely, but within the time frame tested, an HRT of 30 days was sufficient to maintain stable digestion with trace element supplementation.

The results of this trial showed that:

- The influence of trace element supplementation on reactor stability was a repeatable phenomenon;
- The acetic acid spike observed previously was also a repeatable phenomenon, although at slightly different times for the two reactors; and
- An HRT of 30 days was more effective for maintaining stable digestion with trace element supplementation than an HRT of 25 days.

The results of this and previous trials showed that trace element supplementation could allow stable operation with this feedstock at a constant OLR of 1.45 gVS $l^{-1}d^{-1}$, if an HRT of at least 30 days was used. The next trial was a test of whether these conditions would also allow stable operation at a higher OLR.

4.3.5 Increase of OLR in Final Phase of Digestion Period

Throughout the previous trace element supplementation trials, a steady OLR of 1.45 gVS $l^{-1}d^{-1}$ was maintained, to minimise disturbance and because it was unknown whether failure might easily occur if OLR was increased. This OLR, however, is quite low and a higher OLR would be desirable for commercial digestion of food wastes, as a higher OLR allows greater throughputs and smaller tank volumes.

The objective for this digestion trial was to determine whether stable digestion could be maintained, with trace element supplementation, at a higher OLR than that previously used.

4.3.5.1 Methods

Digestate from trace element-supplemented reactors from the previous trace element supplementation trials was used to seed 1.5 litre reactors (described in Section 3.4.3). Two new 1.5 litre reactors were started with biomass from each 5 litre seed reactor. The new reactors were operated on the same HRT as the seed bioreactors, and feeding was started immediately to the new reactors so that there was no break in feeding between the seed reactors and the new reactors. The reactors were operated on four retention times of 30, 50, 100 and 180 days, with two reactors operating at each retention time. The reactors at

HRTs of 50, 100 and 180 days were seeded from the reactors described in section 4.3.1, while the reactors on HRT of 30 days were started from a seed reactor started up as part of the confirmation trial described in section 4.3.4. The reactors were run as pairs that were duplicates, except that OLR was increased stepwise to one reactor of each pair, while for the other reactor of the pair, OLR was held constant or increased by one step only. Feed was increased at two-week intervals, to reach the top OLR tested of 3.5 gVS $l^{-1}d^{-1}$. In the case of the reactor on an HRT of 50 days, one OLR increase was commenced before splitting of the 5 litre reactor into the two 1.5 litre reactors.

Both reactors in each pair were supplemented with trace elements, to minimise the number of variables in the investigation.

4.3.5.2 Results

The top OLR achieved was 3.5 gVS $l^{-1}d^{-1}$ for reactors on a 50-day and 100-day HRT. An OLR of 3.0 gVS $l^{-1}d^{-1}$ was achieved for a reactor on a 30-day HRT. This is in contrast to DT3, before trace element supplementation was introduced, in which the top OLR achieved for reactors on a 50-day HRT before methanogenic failure was 2 gVS $l^{-1}d^{-1}$.

Figures 4.12 and 4.13 show volumetric methane production and TVFA, respectively, for reactors before and after dividing digestate from each 5 l reactor into two 1.5 l reactors, with OLR increased to one reactor of each pair. VFA profiles for constant and increasing OLR are shown in Figures 4.14 and 4.15, respectively. TAN and alkalinity are shown in Figure 4.16.

Although volumetric methane production increased with increasing OLR, the specific methane production on a VS_{added} basis remained the same. VFA profiles are similar between reactors at each HRT, irrespective of feed increase. None of the operational parameters were significantly different after the feed increase, as shown in Table 4.8.

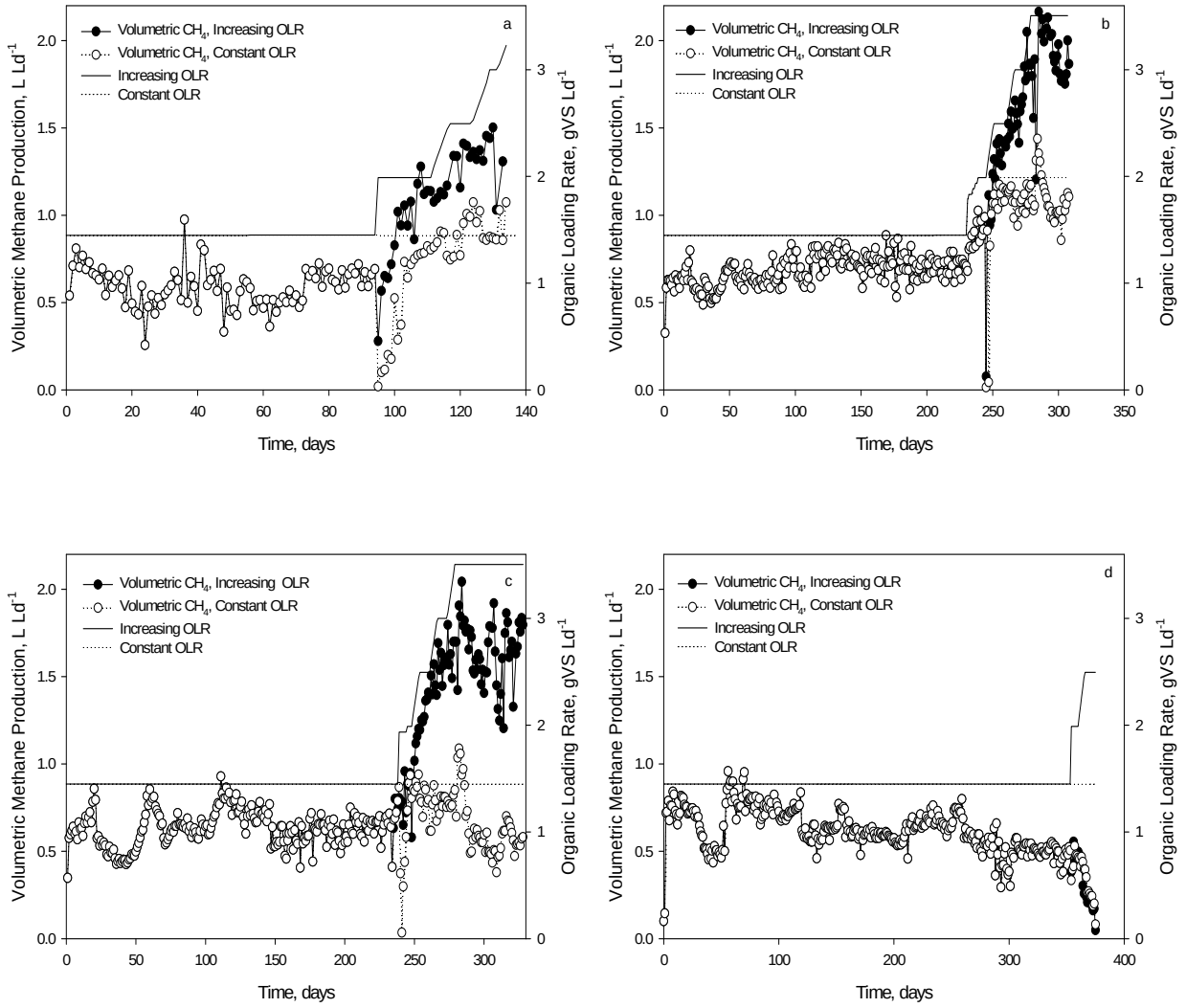

Figure 4.12
Volumetric methane production for reactors before & after an increase in OLR.
a) HRT = 30d (OLR increase from day 95); b) HRT = 50d (OLR increase from day 245); c) HRT = 100d (OLR increase from day 234); d) HRT = 180d (OLR increase from day 354)

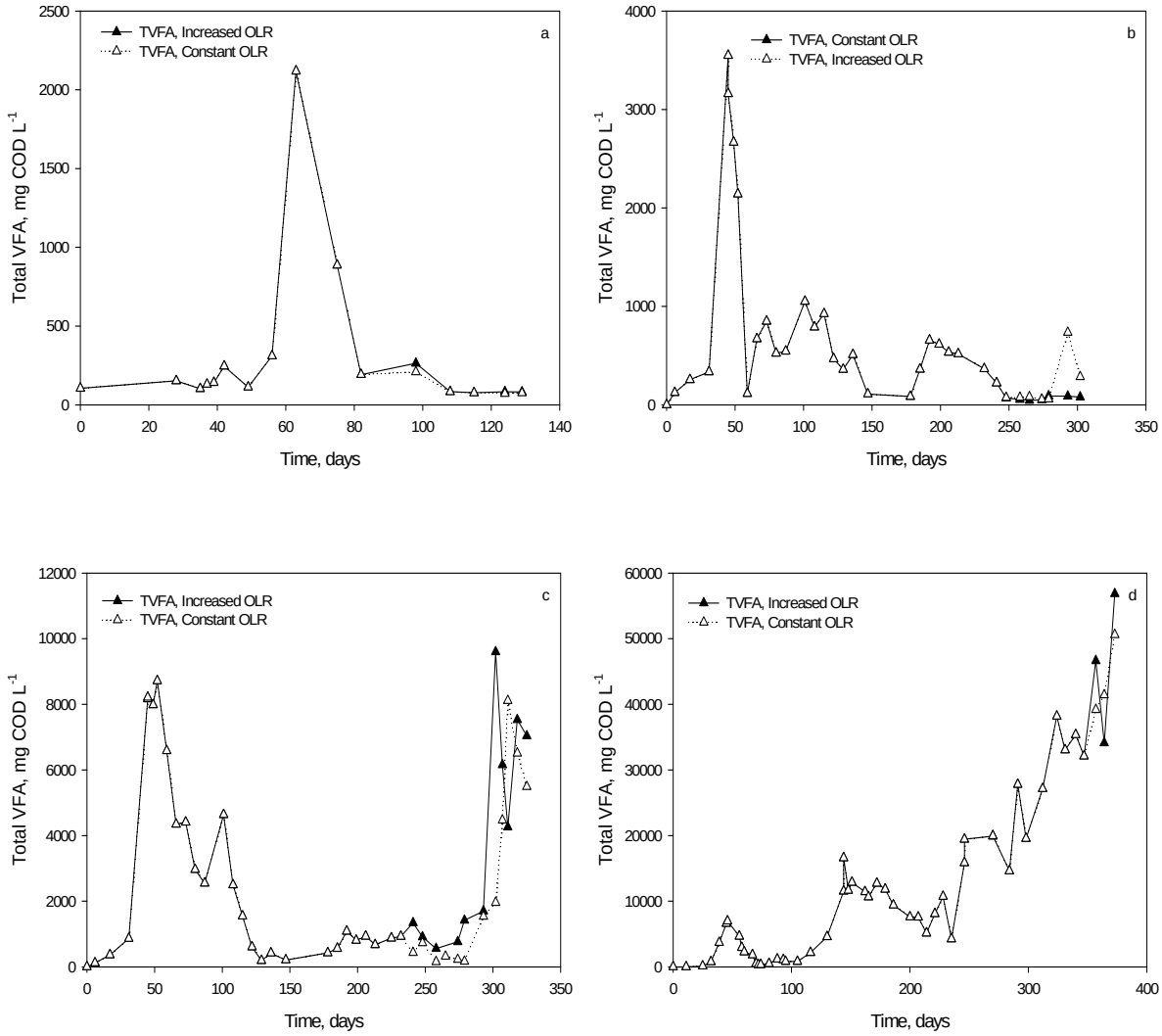

Figure 4.13
TVFA for reactors before & after increase of OLR: a) HRT = 30d (OLR increase from day 95); b) HRT = 50d (OLR increase from day 245); c) HRT = 100d (OLR increase from day 234); d) HRT = 180d (OLR increase from day 354)

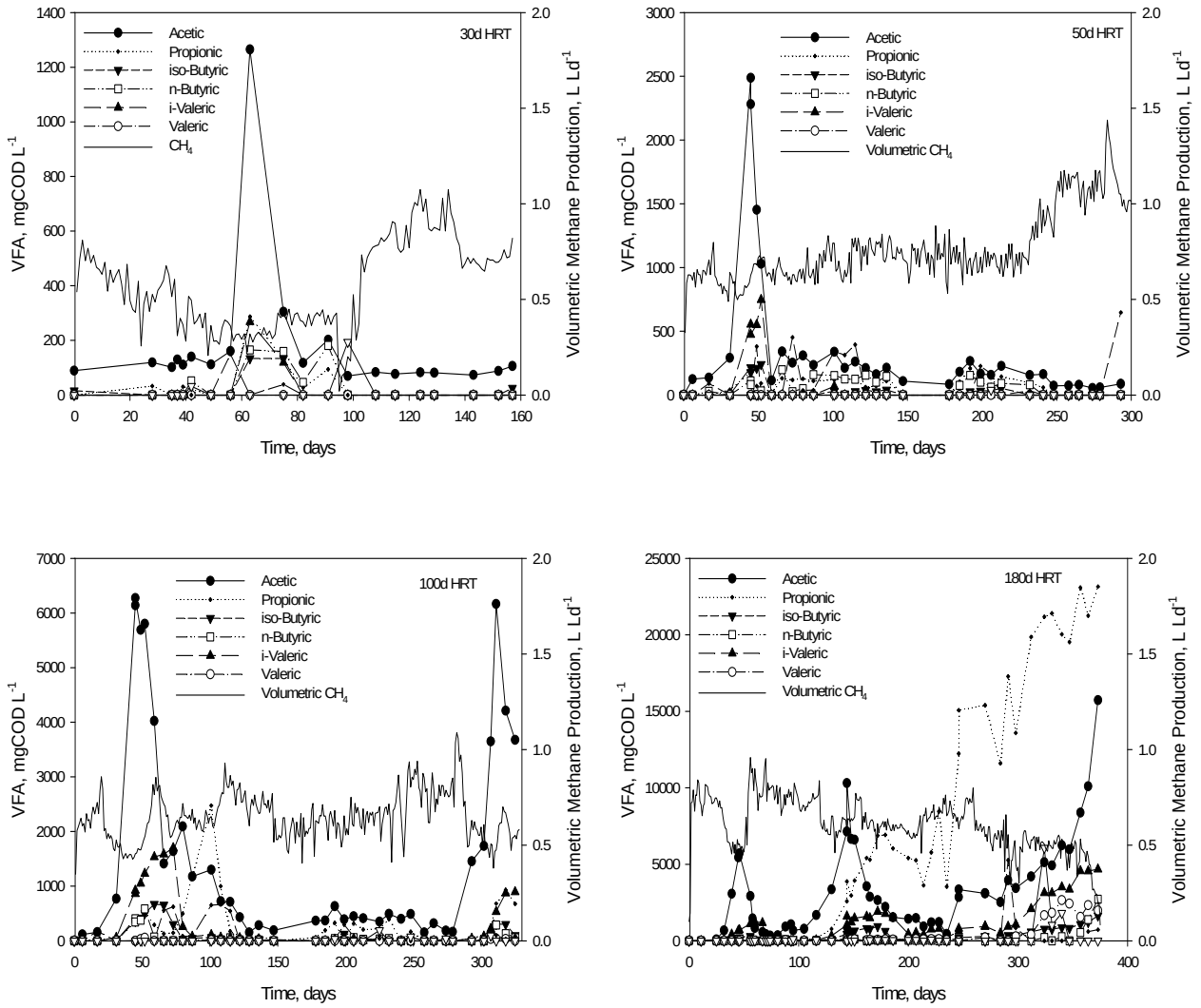

Figure 4.14
VFA concentrations and volumetric methane production for reactors on constant OLR.

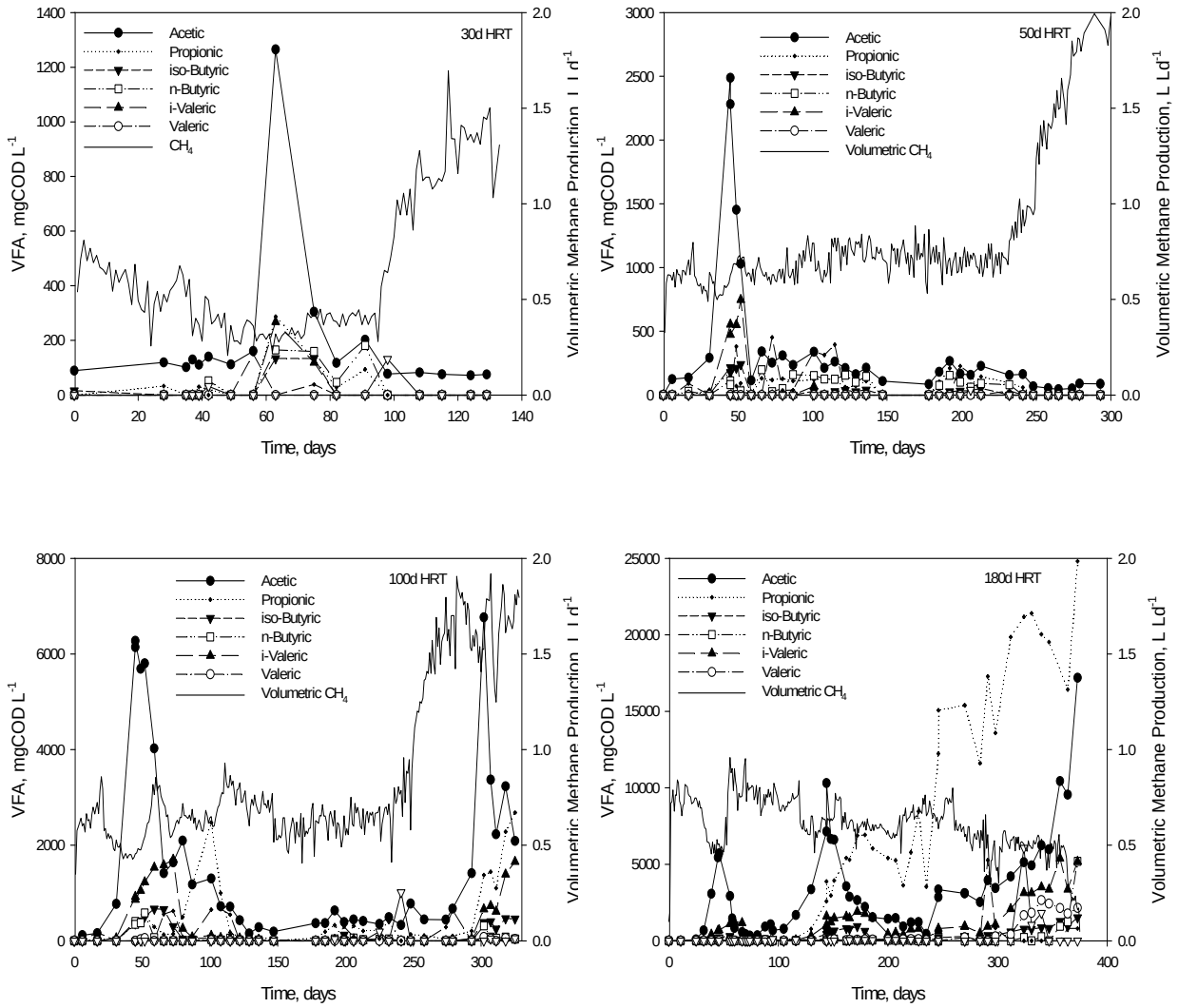

Figure 4.15
VFA concentrations and volumetric methane production for reactors with increasing OLR.

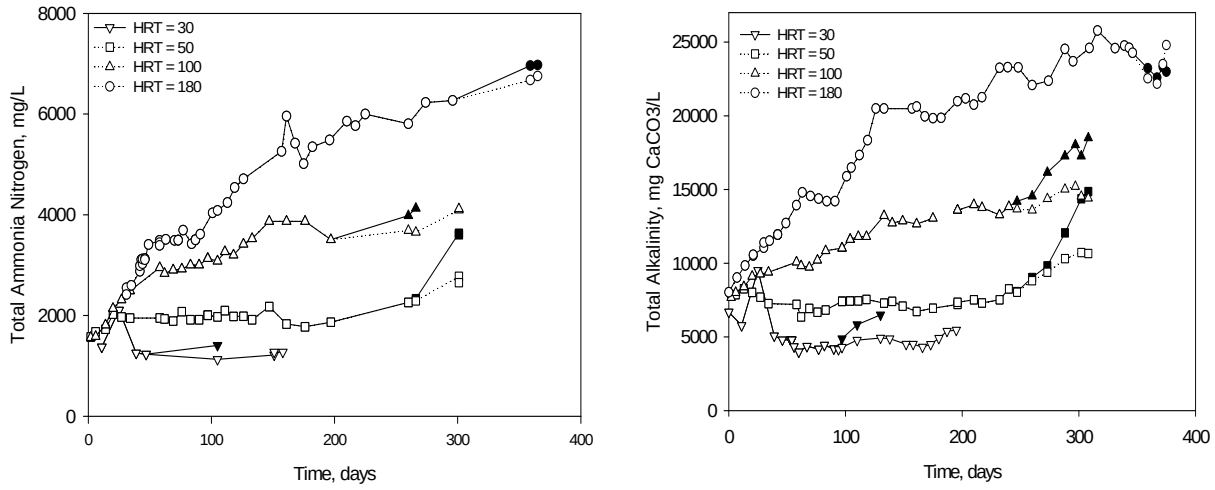

Figure 4.16
TAN (left) and total alkalinity (right) for reactors on HRT of 30, 50, 100 and 180 days.
Open symbols: OLR held constant; Closed symbols: OLR increased.

Table 4.8
Comparison of Digestion Parameters Before and After Feed Increase

Parameter	HRT	Average ± Standard Deviation		
		5-L reactor, before OLR increase	1.5-L reactor, no OLR increase	1.5-L reactor, after OLR increase
VS destruction, %	30	63 ± 20	74 ± 0	80 ± 3
	50	73 ± 18	82 ± 2	81 ± 3
	100	75 ± 14	87 ± 1	83 ± 3
	180	77 ± 13	84 ± 1	80 ± 2
Specific Biogas Production, l gVS_{added}^{-1}	30	0.71 ± 0.14	0.82 ± 0.15	0.84 ± 0.29
	50	0.77 ± 0.08	0.84 ± 0.17	0.85 ± 0.14
	100	0.72 ± 0.10	0.80 ± 0.17	0.77 ± 0.12
	180	0.71 ± 0.13	0.38 ± 0.15	0.29 ± 0.14
Specific Methane Production, l gVS_{added}^{-1}	30	0.41 ± 0.08	0.48 ± 0.10	0.52 ± 0.19
	50	0.47 ± 0.06	0.52 ± 0.10	0.52 ± 0.09
	100	0.44 ± 0.07	0.45 ± 0.12	0.46 ± 0.08
	180	0.43 ± 0.09	0.20 ± 0.09	0.15 ± 0.08
Specific Methane Production, l $gVS_{destroyed}^{-1}$	30	0.61 ± 0.19	0.59 ± 0.11	0.64 ± 0.22
	50	0.59 ± 0.09	0.59 ± 0.12	0.57 ± 0.10
	100	0.52 ± 0.08	0.52 ± 0.13	0.50 ± 0.08
	180	0.48 ± 0.09	0.23 ± 0.10	0.18 ± 0.09

The reactors on an HRT of 180 d showed eventual methanogenic failure at the end of the trial. Total VFA exceeded 20,000 mgCOD l^{-1} in the 5 l reactor from approximately day 250 onwards, yet the reactor continued to show stable gas production for a further 100 days. This was not indefinitely sustainable, however, as eventual methanogenic failure occurred in both 1.5 litre reactors after the transfer, irrespective of whether OLR was increased or not. Although acetic acid was the predominant VFA during the initial 150 days of the digestion trial, concentrations of acetic acid were exceeded by those of propionic acid from about day 160 to the end of the trial and eventual methanogenic failure.

Propionic acid is a persistent VFA and known to cause inhibition (Mata-Alvarez, 2003). The degradation pathway for the production of acetic acid and hydrogen from propionic acid is thermodynamically unfavourable as it has a positive Gibbs' free energy value under standard conditions, and requires the partial pressure of hydrogen in the medium to be kept low to proceed. Therefore the persistent presence of propionic acid in the medium may indicate insufficient uptake of hydrogen in the medium by hydrogenotrophic populations. A build-up of hydrogen in the medium can then lead to product inhibition of all reactions that require syntrophic hydrogen uptake, leading to further accumulation of intermediate products that in turn inhibit the degradation of their precursor compounds.

The extended period over which these reactors continued to operate, despite unfavourable conditions, is worthy of note. Although propionic acid concentrations exceeding 10,000 mgCOD l^{-1} (over three times as high as the toxic limit of 3,000 mgCOD l^{-1} quoted by Mata-Alvarez (2003)) were present in the medium from approximately day 250, the reactor continued to operate with an average specific methane production of 0.36 l CH_4 $g^{-1}VS_{added}$ $^{-1}$ for a further 100 days up to the time that it was split into two 1.5 l reactors at day 354. Fairly soon after the split into two reactors, however, partial alkalinity declined and methanogenic failure was seen for both new reactors, irrespective of whether OLR was increased or not. It is unlikely that the decline and failure was caused by the transfer of digestate into the 1.5 l reactors, as all other digestates showed good performance after transfer. The seed reactor was already showing an IA:PA ratio exceeding 1 from day 330, indicating an imbalance and a high likelihood of imminent process failure.

Visual Evidence of Lipid Accumulation

It should be noted that an accumulation of floating lipid material was observed in the reactors, which increased over time. Although performance of the reactors in terms of methane production and VS destruction remained high, the accumulation of lipids indicated some inhibition of lipid hydrolysis and/or accumulation of LCFA.

This trial showed that:

- With trace element supplementation it was possible to achieve stable digestion of this feedstock at an OLR up to 3.5 gVS $l^{-1}d^{-1}$;
- Extending HRT with trace element supplementation facilitates stable operation over a long period, but not indefinitely;
- A long HRT allowed stable operation at elevated VFA concentrations; and
- Lipid materials accumulated in the reactors over time, indicating possible inhibition of hydrolysis or accumulation of LCFA.

4.4 Trace Element Analysis

The previous sections have described research into the effects of supplementation of digesters with trace elements. The expected amounts of trace elements in digesters that were either supplemented with or deprived of trace elements were estimated based on rates of addition and washout. This section describes investigations to measure the actual amounts of trace metals in substrate and digestate. The digestate was from two parallel CSTR digesters operated on a 50-day HRT as described in Section 4.3.1; one of the digesters received biweekly trace element supplementation while the other digester received no trace element supplementation. The objectives of this investigation were:

i) to measure the actual concentrations of trace elements in the food waste, the digestate from the two reactors, and the supplementation solution;

ii) to confirm whether trace elements were washing out of the trace element deprived reactor over time, and whether they were being maintained in the trace element supplemented reactor.

4.4.1 Methods

Samples for trace metal analysis were prepared as described in Section 3.1.8. Nitric acid alone was sufficient for complete digestion of the food waste samples (EPA, 1998), whereas a more stringent extraction procedure of digestion with a mixture of hydrochloric and nitric acids (DOE, 1986) followed by filtration was required for the digestate.

4.4.2 Results and Discussion

To determine the amounts of micronutrients that were being delivered to the reactors on a daily basis, a sample of food waste was analysed by ICP-MS, after digestion with nitric acid as described in section 3.2. The results are shown in Table 4.9. Values are shown to two significant digits for the last two columns.

Table 4.9
Trace Element Amounts in Food Waste Composite

Metal	Measured Amount (ug/g TS)	Food Waste Daily Feed (g TS)	Daily Metal Addition from Food Waste (ug)	Total Over Two Weeks (ug)
Cobalt	0.044	7.63	0.34	4.7
Iron	480	7.63	3700	51 000
Manganese	4.5	7.63	34	480
Aluminium	8.1	7.63	62	870
Zinc	25	7.63	190	2700
Copper	2.5	7.63	19	270
Nickel	Not reported – contamination error			
Selenium	0.19	7.63	1.4	20

Of the elements shown, cobalt is present in the lowest amounts in the food waste.

A sample of the trace element solution was also tested by ICP-MS to determine the amount of the added micronutrients. One ml of trace element stock solution was added to 50 ml of deionised water and manually agitated following the same procedure as that used for trace element supplementation during the digestion experiments. After addition of nitric acid to give the same sample matrix as the digested food waste samples, this solution was analysed by ICP-MS.

The actual amounts of each of the metals measured are shown in Table 4.10, and the prediction curves shown in the figures in this section are based on these measurements.

Table 4.10
Trace Element Amounts Added by Biweekly Supplementation

Metal	Measured Amount (ug)
Cobalt	68.5
Manganese	19.8
Aluminium	0.7
Zinc	3.6
Copper	2.6
Nickel	1.8
Selenium	43.3

Trace metal analysis of the digestate from the two reactors showed that, for most metals measured, decreases in metal concentrations over time were similar between the two reactors.

Figure 4.17 shows the concentrations of individual metals in the reactors, along with washout prediction curves based on HRT in the reactor, as explained in Section 3.4. In the case of most metals, initial concentrations were sufficiently high that the amount of micronutrient supplied in the feedstock and the trace element solution made no difference to overall concentrations in the reactor. The 'Supplementation' and 'No supplementation' curves virtually lie on top of each other during most of the timescale shown for most metals. Metal concentrations in the two reactors were observed to decline following predicted washout curves, and there was no significant difference apparent in concentration between the reactor supplemented with the trace element mixture and the reactor to which no trace elements were added. This was because the actual amount of metal added was negligible in comparison to the concentration in the reactor, within the timescale of the experiment. This was the case for zinc, copper, aluminium, manganese and iron.

In the case of selenium, the supplementation curve is higher than the non-supplementation curve, but there was not a statistical difference in measured concentrations between the two reactors, as evidenced by the overlap in error bars.

For nickel, concentrations in the non-supplemented reactor were actually found to be higher than those in the supplemented reactor (data not shown). This was likely an artefact of the analysis, however, as readings from blank and highly diluted samples indicated that nickel contamination was likely an issue. Later analysis of shared equipment in the laboratory (data not shown) revealed traces of nickel contamination of pipettes.

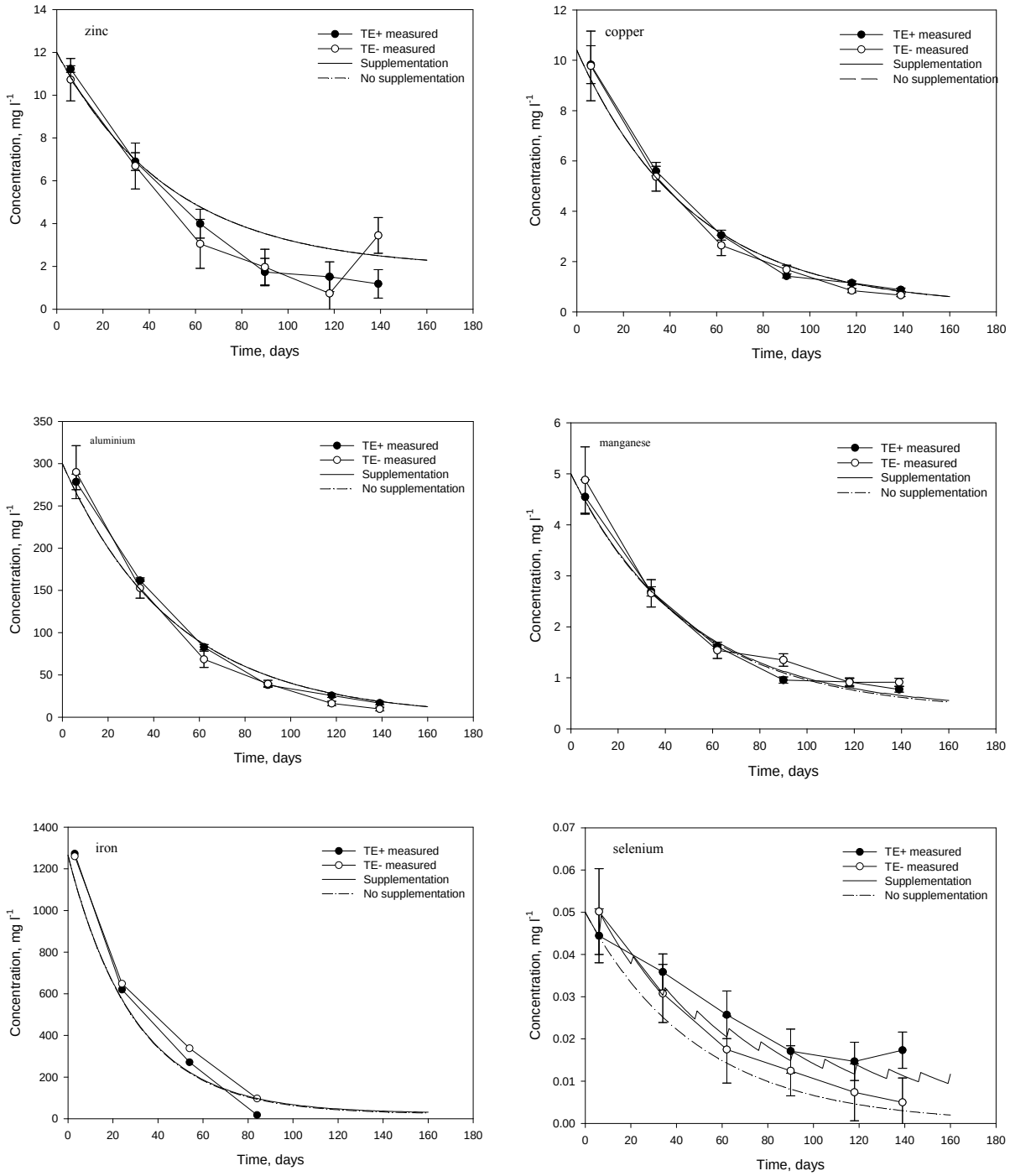

Figure 4.17
Trace element concentrations measured in reactors with (TE+) and without trace element supplementation (TE-). Average of two measurements, error bars show range. Smooth curves are predictions of element depletion based on washout for 50d HRT.

The results for cobalt are shown in Figure 4.18. Concentrations in the supplemented reactor were higher than in the non-supplemented reactor, and were similar to the prediction curve for concentration based on biweekly trace element cocktail additions.

The concentration of cobalt on a solids basis (in ug g^{-1}) increased in the trace element-supplemented reactor, while it decreased in the trace element-deprived reactor. These results appear to be consistent with a hypothesis of cells taking up the cobalt provided in the supplemented reactor, while cells in the other reactor did not have a source of cobalt, and therefore concentrations of cobalt in solids would be expected to drop, as was observed, indicating a cobalt limitation situation. These results are given only as an initial indicator, and further digestion runs and testing should be done to confirm the repeatability of these results.

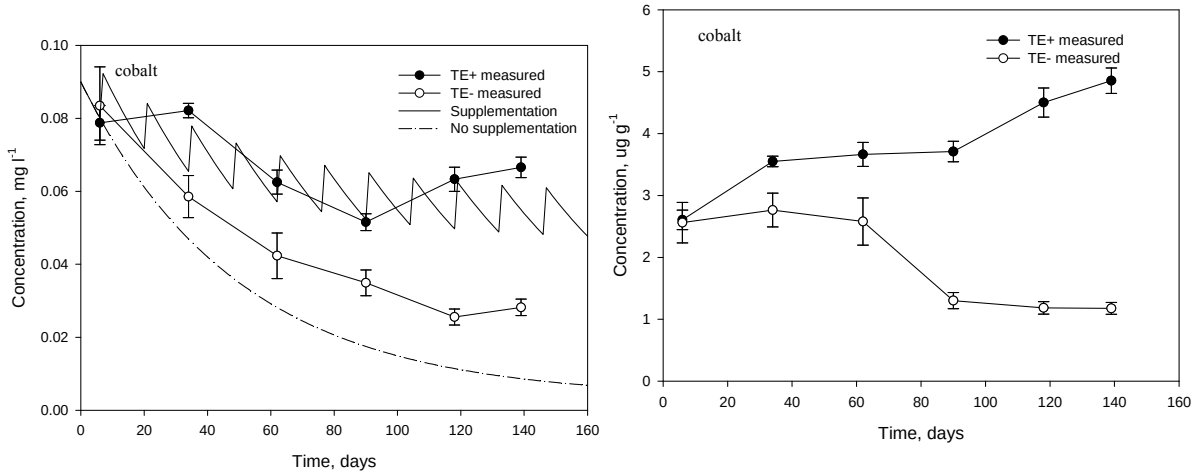

Figure 4.18
Cobalt concentrations measured in reactors with (TE+) and without trace element supplementation (TE-). Average of two measurements, error bars show range. Left: Concentrations in mg l^{-1}, compared to predictions based on supplementation. Right: Concentrations in TS, in ug g^{-1}.

The prediction curves were developed by using a simple spreadsheet model of accumulation and washout for each element, taking into account the amount of the element added daily in the food waste, as well as periodic trace element supplementation, as explained in Section 3.4.

The results in comparing the stability of reactors supplemented with the trace element solution versus reactors that were not supplemented with trace elements were consistent and repeatable. This allows it to be confidently stated that trace element supplementation did have an effect on reactor stability with this feedstock and using this mode of digestion. These metals testing results point to cobalt as the main micronutrient likely to be responsible for this effect. This is consistent with the results of a number of investigators who found cobalt to be one of the most important micronutrients for supplementation in

anaerobic digestion systems (Florencio et al., 1994, Jarvis et al., 1997, Zandvoort et al., 2002).

The initial preparation of the trace element stock solution had involved an error in measurement, so that all compounds were added in amounts ten times lower than the original recipe of Gonzalez-Gil et al. (2001). This meant that the actual amounts added during trace element supplementation were lower than had been planned, but this led to useful results. The trace element additions had initially been calculated to give relatively high concentrations of trace elements in the medium, following the recommendations of Speece (1996), which are guidelines intended to give generous concentrations of metals in a medium, whereas the actual lower amounts delivered were more effective in pinpointing a minimum threshold between operation and failure resulting from trace element supplementation.

This trial showed that:

- Trace element supplementation made little or no difference to metal content in the digestate except for cobalt;
- Cobalt increased in digestate solids over the course of the trial, indicating possible uptake by cells.

4.5 Investigations with Modified Substrate

During the CSTR digestion trials with trace element supplementation, it was noted that for almost all reactors, there was a marked increase in acetic acid concentrations between days 40-60 of the run. This was true of reactors with different retention times and started up on different dates, and therefore is thought likely to be due to factors internal to the system rather than external factors such as equipment problems.

The reactors were on different HRTs (ranging from 25 days to 180 days), and therefore the different washout rates would cause this peak in acetic acid concentrations to be observed at different timepoints for the different HRTs if it were attributable to factors directly related to HRT, such as population washout rates, depletion of nutrients or accumulation of inhibitory compounds. The single common factor in the trials was the OLR which was the same for all reactors, so that all reactors would have received the same cumulative feed in

the same time period. One possible hypothesis considered was that the acetic peak observed was an acclimation phenomenon. The process of β -oxidation of long-chain fatty acids to fatty acids of a chain length that is shorter by two carbons, with the accompanying production of acetic acid, is a process in which a lag time has been observed. For example, Beccari et al. (1999) found a lag time of 25 days for methane production from oleic acid as sole substrate in a batch trial with biomass acclimated to olive mill effluent, and in a second batch trial with glucose and oleic acid as co-substrates, they found that the presence of oleic acid delayed the onset of methane production by approximately 40 days, as compared to glucose alone which had no lag time. In batch incubations of aggregates of biomass and LCFA collected from a reactor fed on oleic acid, investigators observed a lag time of about 21 days before methane was produced from the LCFA (Pereira et al., 2005).

It was hypothesised that the 40-day peak observed in this study might be due to a delayed onset of lipid breakdown and β-oxidation, in which the first 30-40 days of the trial was an adaptation period in which little or no beta-oxidation was occurring, while the microorganisms acclimated to the food waste substrate. At the end of this time a sufficient population capable of beta-oxidation may have been established, with large amounts of acetic acid then being released into the medium through their activity in LCFA oxidation with the production of acetate.

The objective of this trial was to test the hypothesis that acclimation to lipids and onset of β-oxidation was responsible for the 40-day peak in acetic acid. This was done by comparing the performance of reactors fed with the normal lipid-rich food waste substrate against that of control reactors fed with a modified version of the food waste substrate containing lower concentrations of lipids.

4.5.1 Methods

A digestion trial was carried out with four 5 litre CSTR bioreactors, as described in Section 3.3.2. The reactors were again run as duplicate pairs with trace element supplementation to one reactor of each pair (1.0 ml solution weekly). All reactors were operated on an HRT of 50 days.

Substrate for the reactors was the food waste substrate, modified as described in Section 3.1.3. Two of the reactors received the lipid-reduced substrate, which had a lipid content of 12% of TS, while the other two reactors received the control substrate, subjected to the

same heating and separation procedures as the lipid-reduced substrate but without lipid removal. The control substrate had a lipid content of 22% of TS, the same as the non-modified substrate used in the previous digestion trials. The reactors were operated for a period of 60 days.

4.5.2 Results

Figure 4.19 shows the VFA and methane production results from the trial. All four reactors showed very similar results; the same acetic peak was observed for both the control and lipid-reduced substrate.

The results show that reducing the lipid content of the substrate did not significantly change the timing or magnitude of the acetic acid peak produced between days 40 and 60 of the digestion trial. Therefore, the portion of lipid content that was removed from the substrate was not responsible for the increase in acetic acid concentrations between days 40 and 60.

It should be noted that only physical means were used in reducing lipid content of the substrate. Chemical methods such as the use of non-polar solvents were avoided, as this could affect the biomass in the digestion process and introduce new variables in the investigation. The aim of the physical lipid reduction process was to reduce lipid content while minimising any other form of alteration of the substrate. The use of purely physical methods, however, means that only part of the lipids could be removed, so that the investigation was carried out with a lipid-reduced substrate rather than one that was entirely free of lipids, which would have been preferable. From this experiment, it can only be stated that the 40-day peak was not due to the fraction of lipids removed from the substrate, although it may still be possible that the presence of lipids in the substrate has some influence on the generation of an acetic acid peak during this time frame.

It also does not address the question of whether lipids play a role in the onset of methanogenic failure in reactors deprived of trace elements.

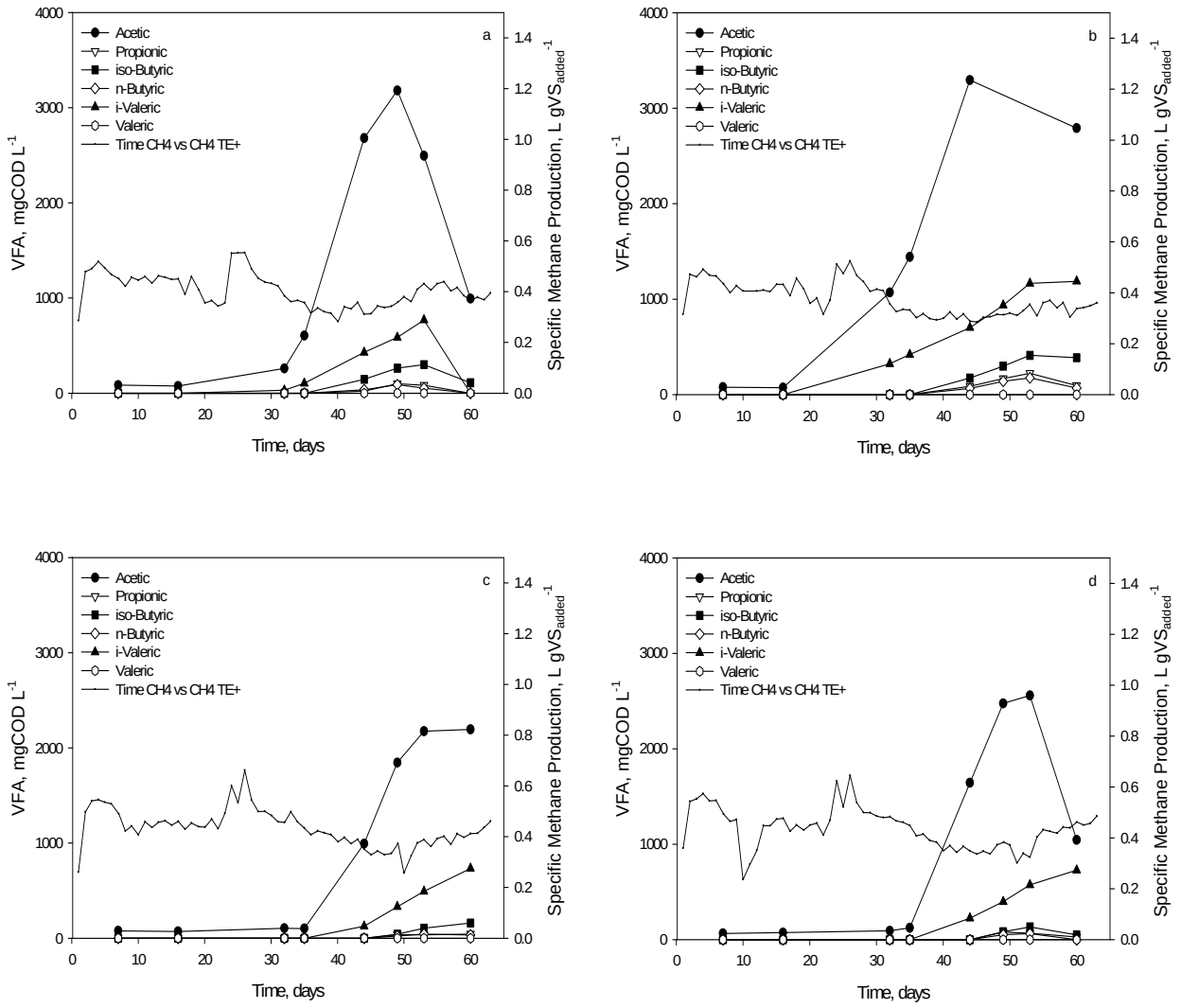

Figure 4.19
Volatile fatty acids and methane production for reactors on a lipid-reduced substrate (a,b) and control substrate (c,d).

The results of this trial showed that:

- Reducing the lipid content of the substrate did not affect the timing or magnitude of the peak in acetic acid characteristic of these digestion trials.

4.6 Uncoupling of Solid and Liquid Retention Times

From the results of previous 5 litre CSTR semi-continuous digestion experiments, it was evident that digesters operated on an HRT of 50 days or longer were more stable than those operated on an HRT of 25 days, indicating that keeping material in the reactor for a longer period was better for stable operation. The CSTR experiments could not distinguish, however, whether it was retention of the liquid fraction (i.e. alkalinity and soluble

compounds) or the solid fraction (i.e., biomass and unsolubilised substrate) that was most beneficial in maintaining stable operation.

Use of digestion modes designed to retain solids in the system is common in the treatment of liquid wastewaters (e.g. upflow anaerobic sludge blanket, anaerobic filter, expanded granular sludge bed reactor types). These work with a very short HRT which is less than the maximum growth rate for methanogens, and therefore mechanisms are required to maintain biomass in the reactor. Many solid waste digestion processes, on the other hand, do not uncouple solid and liquid retention times. The hydrolysis of particulate substrate is often rate-limiting (Sanders, 2001) and sets the retention time for both the solid and liquid fractions of these systems (Veeken et al., 2000). Less attention has been focused on the retention of biomass. Since, however, this CSTR research has shown that longer retention times led to more stable digestion, the experiment described below was designed to differentiate the relative importance of solid versus liquid retention time.

The objectives for this investigation were:

i) to investigate the relative contributions of the solid fraction (i.e. biomass and unsolubilised substrate) vs. the liquid fraction (i.e. alkalinity and soluble compounds) in maintaining reactor stability; and

ii) to determine whether this mode of digestion is subject to the same trace element requirement previously observed in CSTR digestion of this food waste.

The digestion trial described in this section was summarised in a paper, attached in Appendix B (Climenhaga and Banks, 2008b).

4.6.1 Methods

Reactors used were centrifuge bottle bioreactors with 800 ml liquid capacity, as described in Section 3.3.4. The reactors were operated with uncoupled solid and liquid retention times. Two reactors were operated by conserving all solids in the reactor and periodically flushing the liquid (hydraulic flush, HF), while the other two were operated in a liquid retention mode by retaining all liquid in the reactor but periodically wasting solids (solids wastage, SW). The HF reactors were operated with a liquid retention time (HRT) of 25 days, and a solids retention time (SRT) greater than 150 days. SW reactors were operated with HRT greater than 150 days, and SRT 25 days. The requirement for periodic sampling

of whole reactor contents for analysis set the retention time for solids in the HF reactors and liquids in the SW reactors, as opposed to an infinite retention time for those fractions.

The reactors were fed daily at an organic loading rate (OLR) of 1.45 gVS l^{-1} d^{-1}, the same OLR used in the semi-continuous digestion trials in 5 litre bioreactors. The reactors were run as duplicates, except that one reactor from each pair was supplemented with a trace element mixture on a weekly basis while the other reactor was not. Reactors were kept in a water bath with a constant temperature of 35 ^{o}C, except for a thermal shock episode between days 105-108, when temperature in the water bath rose to 50 ^{o}C. The organic loading rate (OLR) of 1.45 gVS $l^{-1}d^{-1}$ was constant throughout the trial except for adjustments during a three-week period following the thermal shock.

4.6.2 Results

Digestion results are shown in Figure 4.18. Although the two sets of reactors were similar in terms of alkalinity, TAN, VFA and specific methane production at the commencement of the study, they had diverged significantly after 40 days. Biomass growth in the SW reactors was not sufficient to keep up with removal rate on a 25-day SRT, as shown by the decrease in TS through the trial. These reactors showed an increase in TAN and VFA, as expected since these soluble compounds would be retained in the liquid fraction. Although total alkalinity (as titrated to pH 4.0) stayed fairly constant, the ratio of intermediate alkalinity to partial alkalinity rose rapidly between days 25-40, indicating the presence of high concentrations of VFA relative to bicarbonates in the system. This is confirmed by the rapid increase in VFA concentrations, ending in methanogenic failure as shown by the cessation of methane production. This was true for both reactors of this pair, regardless of trace element supplementation. Feeding to the SW reactors was stopped at day 67, following a number of days in which no gas was produced and total VFA continued to increase.

In contrast, the HF reactors maintained stable TS, TAN, alkalinity and VFA throughout the portion of the trial during which uniform conditions were maintained, a period of 100 days. Average specific methane production during this period was calculated to be 0.53 ± 0.06 l CH_4 $g_{VSadded}^{-1}$. This compares favourably to the specific methane production from CSTRs on the same OLR as shown in Table 4.8.

After 100 days, the temperature was raised from 35 ^{0}C to 50 ^{0}C from days 105-108. Feeding was stopped for a period of 7 days (days 109-115) following the thermal shock,

then resumed initially at full feed for one week (days 116-122) and then at 86% of full feed for two weeks (days 123-139) as a precautionary measure. The full OLR of 1.45 gVS $l^{-1}d^{-1}$ was then applied for the remainder of the run (days 140-150).

The thermal shock from days 105-108 resulted in an increase in VFA, indicating an imbalance during which the rate of acidogenesis and acetogenesis exceeded that of methanogenesis. VFA and methane production returned to the levels preceding the thermal shock by day 140, indicating recovery of the system.

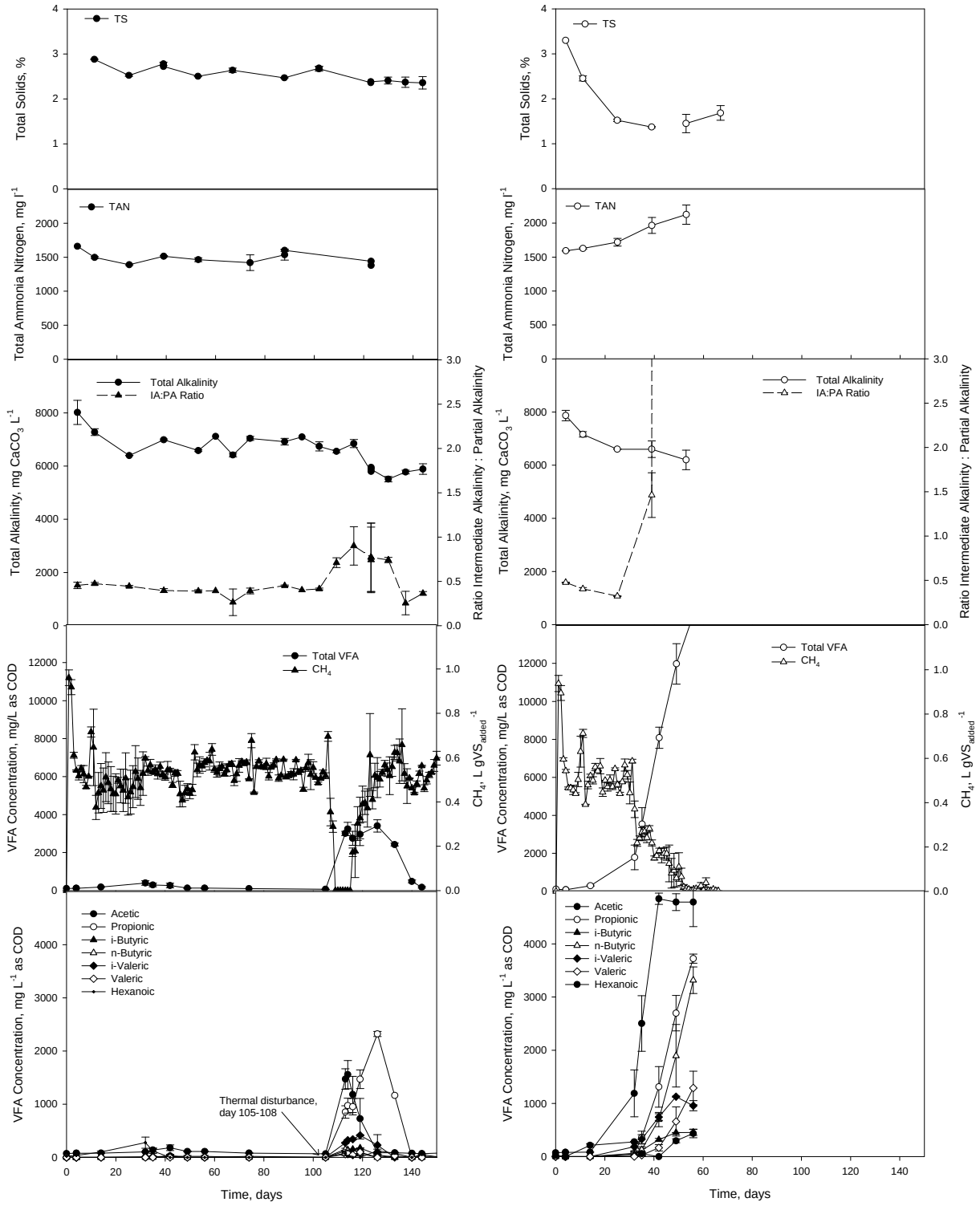

Figure 4.20
Total solids, total ammonia nitrogen (TAN) concentrations, total alkalinity and ratio of intermediate : partial alkalinity, total VFA, specific methane production, and concentrations of individual VFA for reactors operated in hydraulic flush mode (left) or solids wastage mode (right). Points are average of two reactors, error bars represent range of values between the two reactors.

Specific biogas production and specific methane production for the HF reactors (days 13-105 of the run) are shown in Table 4.11. No performance parameters are given for the SW reactors as they failed before a steady state was achieved.

Although an accumulation of floating lipid materials was noted during the CSTR digestion trials, no similar accumulation of lipids was seen in the HF reactors.

The specific biogas and methane production for the HF reactors was slightly higher than that for the CSTR reactors with trace element supplementation, although within the range of standard deviations they are not significantly different.

Table 4.11
Performance Parameters, Hydraulic Flush Reactors

Parameter	Average ± Standard Deviation
Specific Biogas Production, l gVS_{added}^{-1}	0.91 ± 0.06
Specific Methane Production, l gVS_{added}^{-1}	0.53 ± 0.05

Both HF reactors behaved similarly regardless of the presence or absence of trace element supplementation.

Previously it was found that trace element supplementation was required for stable digestion of this feedstock in CSTR digesters, as described in Section 4.3. In these experiments, however, regular supplementation with trace elements did not affect reactor performance – it was not required for hydraulic flush reactors, and was not sufficient to prevent failure for solids wastage reactors.

A possible explanation for this is the low solubility of micronutrients such as cobalt, iron and nickel within the pH range for anaerobic digestion. The major part of the mass of these metals are likely to adsorb to unhydrolysed particles and microbial cells rather than dissolving (Zandvoort et al., 2006), and therefore most of the initial mass of any micronutrient in the reactor would be conserved in the solid fraction. Therefore in the case of the hydraulic flush reactors, micronutrients would stay in the reactor rather than washing out over time with the liquid fraction, while in the case of the solids wasting reactors, micronutrients would be removed with the solids. Therefore, since all solids were retained in the hydraulic flush reactors, the concentrations of trace metals in the reactor would remain similar to that of the seed sludge, and therefore supplementation with trace metals would not be necessary for hydraulic flush reactors. Conversely, in the solid wasting

reactors, added micronutrients would be removed with the wasted solids and therefore would not have a beneficial effect. These results therefore confirm the trace nutrient requirements observed in earlier CSTR experiments.

In this work it was shown that keeping only the solid fraction of digestate in the reactor enabled robust and stable operation over an extended period, as well as resilience to disturbance. The flushing of liquid allowed the removal of VFA and other dissolved compounds, but sufficient bicarbonate alkalinity was maintained in the medium due to biomass activity. In contrast, removal of the solid fraction and extended retention of the liquid fraction resulted in accumulation of VFA and loss of methanogenic activity. Future work could be focused on decreasing the liquid retention time further, as process parameters such as VFA concentration and alkalinity indicated that the system was healthy and likely could operate at a higher hydraulic flush rate.

A solids retention mode of digestion has the benefit of allowing for syntrophic relationships, while reducing the likelihood of accumulation of potentially inhibitory substances such as VFA and ammonia. This mode of digestion, therefore, is promising for application to the processing of source-separated food wastes containing a mix of readily-degradable and slowly-degradable components.

This digestion trial showed that:

- Retaining solids in the reactor enabled robust and stable operation over a period of 150 days, as well as resilience to disturbance.
- Supplementation with trace elements was not necessary for reactors in which solids were retained, possibly due to retention of trace elements with the retained solids.
- The flushing of liquid allowed the removal of VFA and other dissolved compounds, but sufficient bicarbonate alkalinity for stable digestion was maintained due to biomass activity.
- In contrast, retaining liquids while removing solids resulted in accumulation of VFA and loss of methanogenic activity.

5. General Discussion

The work presented here has shown that while source-segregated food wastes are a promising substrate for anaerobic digestion, there are important process issues that still need to be considered. The anaerobic digestion of this waste stream by mesophilic CSTR digestion was subject to instability. Supplementation with trace elements including cobalt helped to improve the stability of the process; reactors for which trace element supplementation was provided operated for longer periods of time than their duplicate counterparts that were not supplemented.

For trace element supplemented reactors on HRT of 30, 50 and 100 days, no methanogenic failures occurred during the timeframe of the experiments. However, reactors on the shortest (25d) and longest (180d) HRT failed even with trace element supplementation. A potential strategy to address these failures was investigated in experiments in which solid and liquid retention times were uncoupled. Retaining solids while flushing liquids from the system was found to be effective for process stability.

5.1 Trace Element Supplementation and Stability

The role of metals in anaerobic digestion processes has been studied by a number of investigators, as discussed in Section 2.1.3. In this research, supplementation with a trace element solution containing cobalt was found to improve the stability of CSTR bioreactors at various retention times, allowing stable digestion periods two to three times longer than parallel reactors without supplementation.

When metal concentrations were measured in reactors with or without trace element supplementation, it was found that cobalt concentration in the reactor without trace element supplementation was between 0.02 mg l^{-1} and 0.03 mg l^{-1} at the time of failure, which is consistent with the result of 0.02 mg l^{-1} of cobalt found by Jarvis et al. (1997) at the time when a marked drop in pH and gas production was seen in grass-clover silage digestion, although complete failure of that system was not observed. Lebuhn et al. (2008) also had cobalt concentrations of 0.03 mg l^{-1} at the time of commencement of cobalt supplementation in response to decreased reactor performance in mono-digestion of maize.

5.1.1 Pattern of Failure Relative to Washout Prediction

The reasoning for the study of digestion at various extended retention times had initially been that a longer retention time (i.e., maintenance of methanogenic biomass and alkalinity in the system) was essential to prevent methanogens becoming overwhelmed by production of acidic intermediates by the acidogenic population, and that a higher biomass concentration would give better reactor performance. It was found, however, that with trace element supplementation, a bioreactor on a 50-day HRT showed equal performance in VS destruction, methane production and stability to that of bioreactors on 100 and 180-day HRT (Table 4.8). Conversely, without trace element supplementation, the bioreactors on extended HRTs of 100 and 180 days were equally susceptible to process failure to the shorter HRT reactors, after accounting for the different washout rates.

Figure 5.1 is a plot of the predicted depletion of any conservative element in CSTR reactors with no supplementation, with HRTs as used in these digestion trials. The approximate time of failure of the reactors on each of the trials is shown as arrows on the curves. From the curves it can be seen that the time of failure roughly corresponds to a washout of the element to approximately 20% of its initial concentration in most cases, except for the 100-day HRT case, where failure occurred at approximately Day 90, when the element would be expected to be at approximately 40% of its initial concentration, whereas failure would be predicted to be around Day 155 for a critical level of 20% of initial concentration.

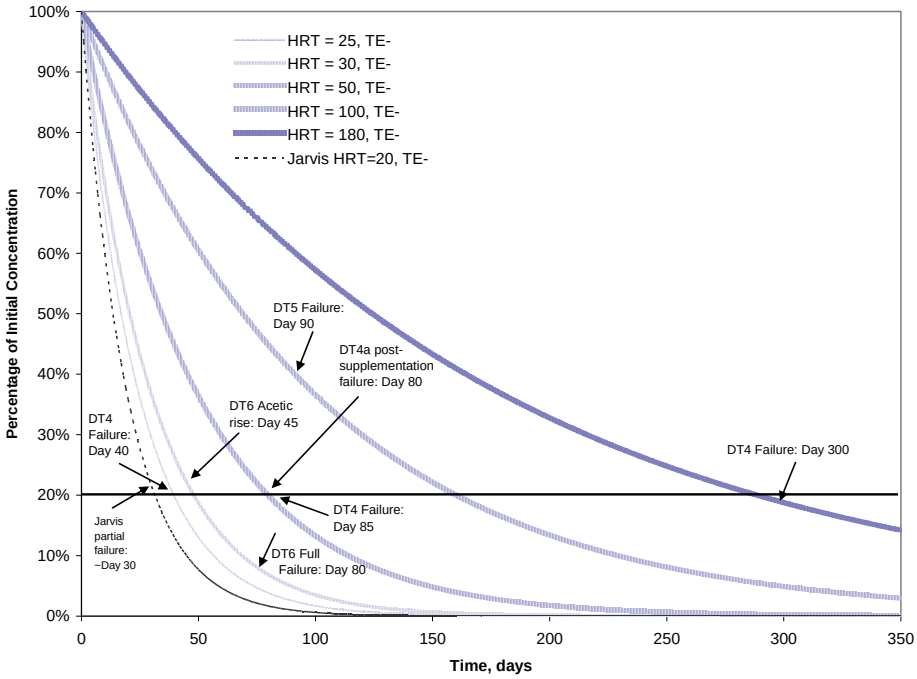

Figure 5.1
Predicted depletion, based on no weekly additions of trace elements, and approximate time of failure for reactors that did not receive trace element supplementation. HRT of 25, 30, 50, 100 and 180 days (these investigations). Also plotted is the time of drop in pH and gas production observed by Jarvis et al. (1997) for CSTRs fed grass-clover silage, with HRT of 20 days.

The results of Jarvis et al. (1997) are also included in the figure for comparison. It is interesting to note that the time of drop in pH and biogas production observed by those researchers is quite consistent with the prediction curves developed from the results of this research.

Although ICP-MS analyses of the digestate showed that for most metals the amount supplied was not significant in comparison to metal concentrations previously present, the consistency and repeatability of the results showed that supplementation had an effect. All reactors not provided with trace element supplementation exhibited methanogenic failure at fairly predictable time points according to their washout rates, while reactors with trace element supplementation continued to operate. The failures in reactors without trace element supplementation in these trials were accompanied by an accumulation of acetate, indicating a failure of acetoclastic methanogenesis, and providing evidence that the trace element supplementation was effective in supporting acetoclastic methanogenesis.

Cobalt is an essential element of vitamin B_{12} cobalamin, which is synthesized only by microorganisms (Martin et al., 1996). It is found in the enzymes methyl transferase and carbon monoxide dehydrogenase, which is involved in acetate degradation (Fish, 1999,

Madigan et al., 2003). The food waste in this study was low in cobalt, and therefore may not have provided sufficient amounts to meet methanogenic cell requirements under the conditions of this system. It was provided, however, in moderately high concentrations in the trace element cocktail, and was one metal for which the effect of supplementation could be seen in the digestate. The cobalt concentration on a TS basis increased over time in the reactor receiving trace element supplementation, while declining in the reactor that was not supplemented. This indicates that cobalt may have made the difference between operation and failure in the trace element supplementation trials. This is consistent with the findings of a number of other researchers on the importance in particular of cobalt for methanogenesis (Jarvis et al., 1997, Fish, 1999, Zandvoort et al., 2003, Osuna et al., 2003).

Interestingly, while some of these investigators found a strong effect of cobalt on degradation of methanol and other substrates, they did not find an effect with acetate (Zandvoort et al., 2003, Osuna et al., 2003) whereas other investigators found a pronounced benefit in acetate conversion upon addition of cobalt (Jarvis et al., 1997, Lebuhn et al., 2008). One difference between these findings is that the former set of studies were done in batch flask trials with single defined substrates, while the latter set used continuously fed systems degrading substrates from which acetate was one of the intermediates. Continously-fed systems require that methanogens are able to continuously manufacture sufficient amounts of acetate-degrading enzymes, and acetate degradation from complex substrates requires enzyme activity in the presence of other process intermediates. The beneficial effect of cobalt addition observed in the current study may be related to the rate of enzyme production and cell growth, in the presence of other compounds in the medium.

Micronutrients may play a role in support of biomass resistance to inhibition or toxicity by inhibitory substances in the medium – which may be in terms of growth rate or other mechanisms. Other investigators have found an effect of metal supplementation under unfavourable conditions, such as in reducing sensitivity of acetoclastic methanogenesis to inhibition by fatty acids (Li et al., 2005) and supporting maintenance of granular sludge in UASB reactors (Oleskiewicz, 1989). Supplementation with trace elements may have helped in resistance to some inhibitory factors.

Ammonia and LCFA have both been shown to inhibit acetoclastic methanogenesis, as described in Section 2.3. The following sections describe how these agents may have contributed to the results found in this study.

5.1.2 Role of Ammonia

Although ammonia is known to be inhibitory at high concentrations (Angelidaki and Ahring, 1993, Kayhanian, 1994, Gerardi, 2003), it can also have beneficial effects in terms of its contribution to the buffering capacity of the system. It was observed in these trials that high alkalinity concentrations allowed continued operation of the process at elevated concentrations of VFA. The high alkalinity was attributable in part to high concentrations of ammonia: as described in Chapter 2, ammonia contributes to carbonate alkalinity by the equilibrium between the protonated form (ammonium ion) and the dissociated form (free ammonia) and the formation of ammonium carbonate (Speece, 1996).

In the digestion trials in which reactors maintained stable operation while sustaining high concentrations of VFA, the concentrations of ammonia were also high. This is consistent with the results of other investigators who observed stable digestion at concentrations of ammonia and VFA beyond those previously considered toxic (Angelidaki and Ahring, 1993, Schnurer and Nordberg, 2007, Banks et al., 2008).

In comparing the graphs of alkalinity and TAN during the CSTR digestion trials in this research, it is apparent that they followed similar trends.

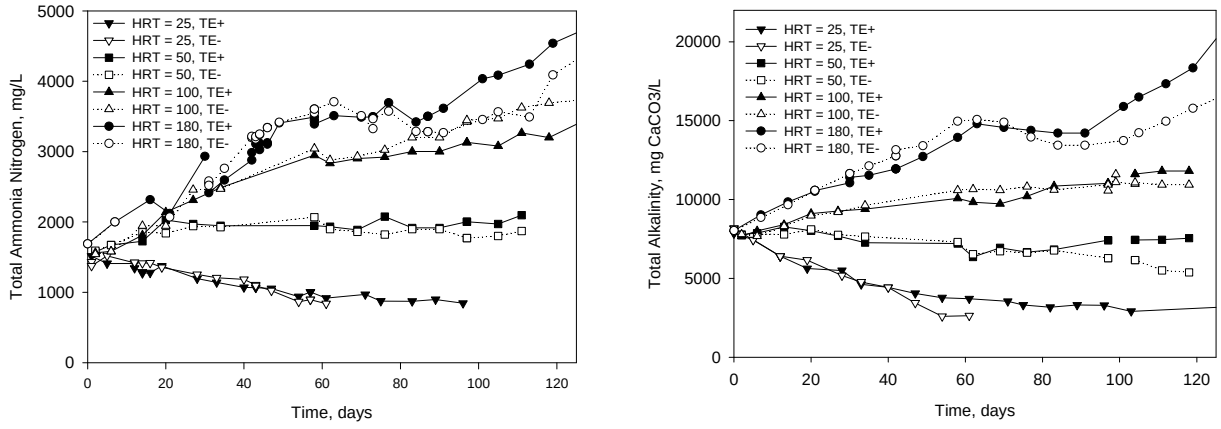

Figure 5.2
Trends in total ammonia nitrogen (TAN) and total alkalinity for reactors on varying retention times, DT4.

On the alkalinity graph, the TE+ and TE- reactors begin to diverge around Day 100 (or Day 40 for the 25d reactors); this was the time of methanogenic failure, with associated decrease in bicarbonate production and drop in pH.

The similarity in the appearance of the curves for TAN and alkalinity at the various HRTs strongly indicates an association between the two. Figure 5.3 shows regression plots between concentrations of TAN and alkalinity during DT4. The plots show a good regression line fit.

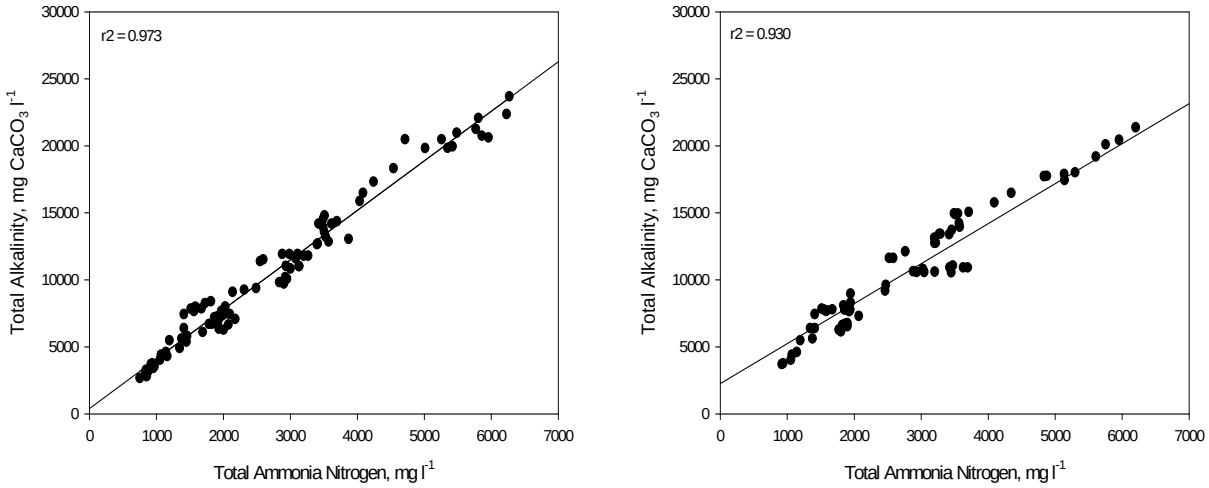

Figure 5.3
Regressions of total alkalinity vs. TAN during DT4.
Left: Reactors with trace element supplementation. Right: Reactors without trace element supplementation.

During the trace element CSTR trials, the 100-day HRT trace element reactor sustained total ammonia nitrogen (TAN) concentrations beyond 3 g l^{-1}, while in the 180-day HRT reactor TAN reached a maximum concentration of approximately 6.5 g l^{-1} at a pH of 7.7. Bhattacharya and Parkin (1989) reported digestion in chemostat cultures fed on acetate with addition of up to 5 g l^{-1} TAN, but failure at 6 g l^{-1} TAN. This was observed on a 40-day retention time, while at lower retention times failure happened at lower TAN concentrations; in the current study, extending retention time to 180 days allowed stable digestion at over 6 g l^{-1} TAN. In thermophilic digestion of cattle manure, 4 g l^{-1} TAN was found to be inhibitory, but acclimation over 6 months of operation allowed digestion at up to 6 g l^{-1} TAN, with reduced activity and a VFA concentration of 3 g l^{-1} as acetate (Angelidaki and Ahring, 1993); in another study, inhibition began at 3.3. g l^{-1} TAN, but the system continued to operate stably with a reduced methane yield, up to a TAN concentration of 6 g l^{-1}, with total VFA at 8 g l^{-1} (Hansen et al., 1998). In the current study, TVFA exceeded 15 g l^{-1} as COD for an extended period of operation in the 180-d

HRT reactors. This high VFA condition also agrees with previous findings of pilot-scale (1500 l digester) single-stage digestion of nitrogen-rich food wastes, in which system alkalinity was high and stable digestion at VFA up to 25 g l^{-1} was maintained in a mesophilic digester (Banks et al., 2008).

There is, however, an upper limit to the concentrations of TAN and free ammonia that can be tolerated before system performance declines, as observed by the previous investigators and in this study. The reactors on a 180-day HRT reached a maximum TAN concentration of 6.5 g l^{-1} at a pH of 7.7, but after this peak was reached, concentrations of VFA increased to beyond 40 g l^{-1}, particularly propionic acid, which exceeded 20 g l^{-1}, and methanogenesis eventually failed.

As the extent of dissociation and therefore of ammonia vs ammonium species is pH and temperature dependent, the concentrations of TAN that can be tolerated differ according to conditions in the system. Although the high amount of bicarbonate alkalinity resulting from high TAN allowed for systems to continue operating at very high concentrations of VFA, it is possible that high VFA concentrations were themselves attributable to inhibition by ammonia leading to incomplete conversion of these VFA to methane. Figure 5.4 shows the concentrations of total and free ammonia in reactors during the trials described in Section 4.3.5. It can be seen that the trends in free ammonia roughly follow those for total ammonia, i.e., increasing where TAN is increasing and staying constant where TAN is constant. Free ammonia fluctuates more than TAN, however, because free ammonia is highly dependent on pH (as demonstrated in the equations for free ammonia given in Section 2.3.3), and therefore small variations in pH have a significant effect on the free ammonia concentration.

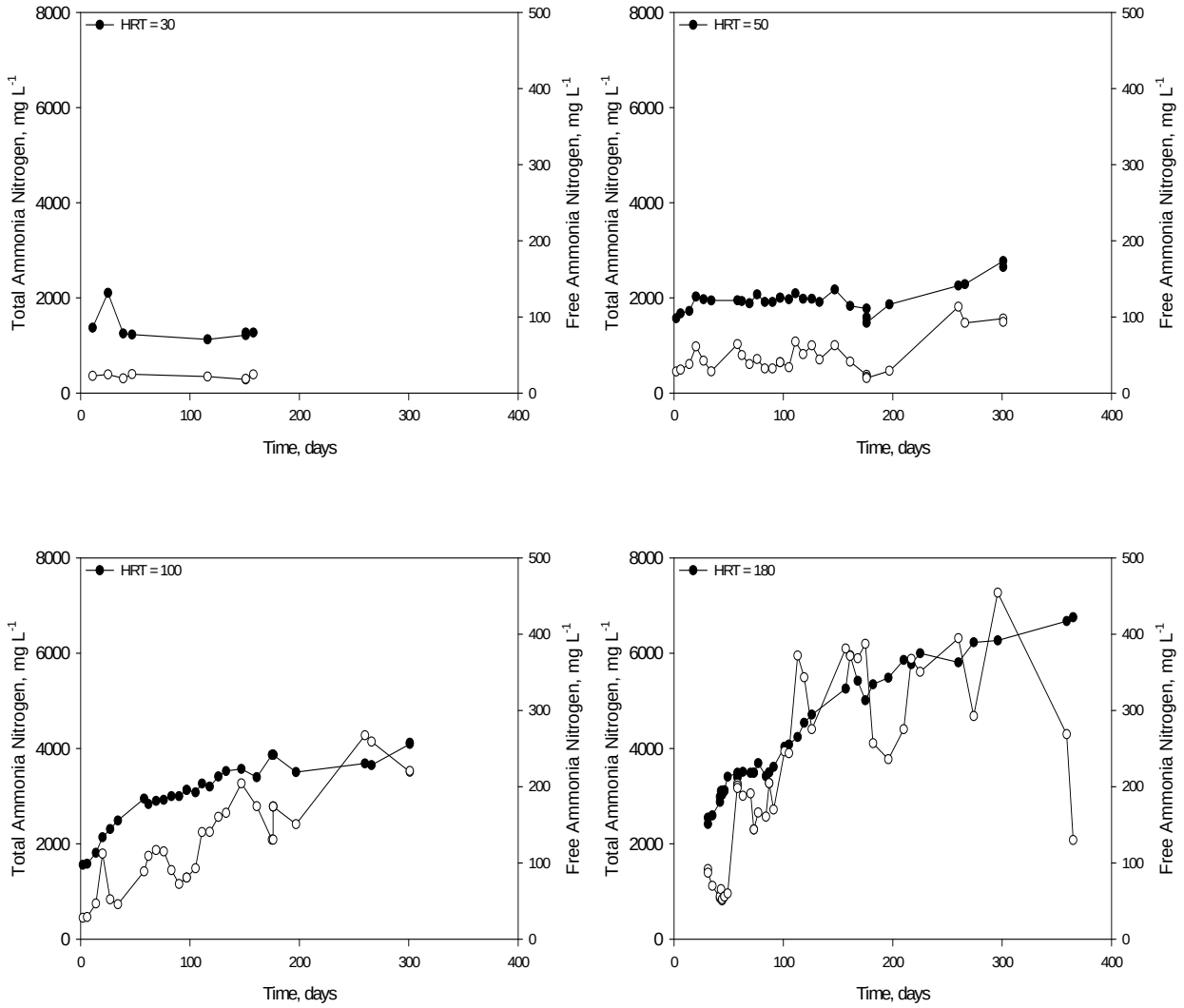

Figure 5.4
Concentrations of Total (closed circles) and Free (open circles) ammonia nitrogen for reactors on HRT of 30, 50, 100 and 180 days.

A lower pH means that a lower proportion of ammonia nitrogen will be present as free ammonia, and therefore a drop in pH results in a drop in free ammonia. This explains the last two points on the free ammonia curve for the reactor on HRT of 180 days in Figure 5.4 – although total ammonia nitrogen was high, VFA levels had increased sufficiently to lower the pH, resulting in a drop in free ammonia. This has been observed by other investigators as a self-regulating mechanism (Angelidaki and Ahring, 1993) in which inhibition by free ammonia results in high VFA concentrations, which then results in a lowering of pH with a resultant decrease in free ammonia concentration, allowing the system to continue to operate. Investigators have referred to an "inhibited steady state" at which the process is running stably but with a lower methane yield (Hansen et al., 1998).

In the current study, however, methane production was not affected until a threshold was reached at which a rapid decline in pH occurred and the system was unable to recover activity. This could be due to the rapid degradability of the subject feedstock, which resulted in the rapid production of VFA and transition to pH imbalance. The finding of little effect until the system passed a threshold at which rapid failure occurred, is similar to the results of Bhattacharya and Parkin (1989).

Two possible mechanisms for ammonia inhibition of methanogens have been suggested (Calli et al., 2005). The first is the direct inhibition of methane synthesizing enzymes by free ammonia. It has been stated to act as a non-competitive inhibitor (Mata-Alvarez, 2003); that is, it affects enzymes not by binding at the same site as substrate, but by binding at other sites on the enzyme. The second suggested mechanism is that free ammonia molecules diffuse into the cell and are rapidly converted under intracellular pH conditions to ammonium ion; accumulated ammonium can become toxic by altering intracellular pH. Under either mechanism, the system declines rapidly after a threshold concentration of the inhibitor is reached.

The results observed in this study may indicate inhibition by ammonia in the case of the 180-d HRT reactors, in which stable operation was observed under increasing TAN concentrations, at high VFA concentration, until a point was reached at which methanogenesis failed fairly rapidly. This would explain why trace element supplementation, while sufficient to support acetoclastic methanogenesis and allow continued operation in reactors at shorter retention times and with lower concentrations of ammonia, did not guarantee stable operation at the longest HRT. At this HRT, ammonia accumulated in the reactor until free ammonia reached a threshold at which it exceeded the system's tolerance.

5.1.3 Role of Long Chain Fatty Acids

Inhibition of methanogenesis by long chain fatty acids (LCFA) has been observed by a number of investigators (Koster and Cramer, 1987, Angelidaki and Ahring, 1992, Pereira et al., 2001). LCFA may act on methanogenesis by physical means, by association with cells and thus interference with the transport of solutes into and out of cells (Pereira et al., 2005), and also with hydrolysis by coating of substrate particles and making them more resistant to enzyme attack (Sanders, 2001). A visible accumulation of fatty particulate materials was observed in CSTR reactors in this study, which may have been a

combination of unhydrolysed substrate and also biomass with associated LCFA, as has been observed by other investigators dealing with anaerobic digestion of lipidic substrates (Hanaki et al., 1981, Hwu and Lettinga, 1997, Hwu et al., 1998, Pereira et al., 2005).

The mechanisms behind LCFA inhibition are still unclear. Although earlier investigators postulated LCFA toxicity to cells (Koster and Cramer, 1987), more recent investigators have provided evidence that LCFA inhibition is reversible, and that mass transfer limitations may be the primary cause of inhibition effects observed (Pereira et al., 2005). LCFA are degraded by syntrophic acetogenic bacteria, which require acetate, hydrogen and formate in the medium to be kept low to keep the reaction thermodynamically favourable (Broughton et al., 1998). If the concentration of any of these are too high, this would result in accumulation of LCFA, which in turn affects methanogenesis.

Investigators have found that cobalt can also have beneficial effects on acetogenesis (Florencio et al., 1994). Since syntrophic acetogenic bacteria are the populations responsible for LCFA degradation, it was considered that supplementation with cobalt in this study may have helped in conversion of LCFA to acetate, decreasing the concentration of, and thus inhibition by, LCFA.

A second possible scenario considered was competition for enzyme building blocks. If cobalt were involved in the enzymes required for the β-oxidation of LCFA, a high concentration of LCFA could mean competition between LCFA-oxidizing acetogens and methanogens for cobalt, which could result in a cobalt limitation for methanogens due to more successful cobalt scavenging by LCFA degraders. Supplementation of cobalt to the medium could then provide sufficient concentrations to both populations and allow acetoclastic methanogenesis to continue. Examination of the enzyme pathway for β-oxidation, however, reveals that although the pathway involves coenzyme A which is synthesised from a B-vitamin, it is pantothenate, which does not contain a central cobalt atom (Martin et al., 1996). This therefore eliminates the hypothesis of competition for cobalt for this enzymatic pathway. There may still be some mechanism related to enzyme competition among microbial populations, however, which could be influenced by higher concentrations of lipids and their breakdown products.

A third hypothesis for the influence of LCFA is inhibition via decrease of growth rate of methanogens. LCFA degrading bacteria also have a slow growth rate, requiring a longer hydraulic retention time; this is the reason that Salminen and Rintala (2002b) used

hydraulic retention times of 50-100 days for digestion of poultry slaughterhouse waste, which is high in lipids and protein. In the digestion trials in the current study, an HRT of 25 days was insufficient, which agrees with the results of Salminen and Rintala, who found that digesters running at 25 days HRT were unstable, while digesters at an HRT of 50 days operated stably for 280 days, although the loading rate was kept low (0.8 gVS $l^{-1}d^{-1}$). Cuetos et al. (2008) also observed methanogenic failure at HRT of 25 days, which they attributed to high LCFA and VFA. Extending HRT to 50 days allowed stable operation for these investigators also, although they were able to reduce HRT down to 25 days after acclimation.

In this study, a slow growth rate of either methanogens or LCFA degraders could have led to the results observed. Inhibition of the growth rate of methanogens would mean that the rate of reproduction of methanogens was unable to keep up with the rate of acetate production. A slow growth rate for LCFA-degrading bacteria would result in LCFA degraders being washed out at a rate exceeding their rate of reproduction, which would lead to a decrease in LCFA degradation, resulting in accumulation of LCFA to concentrations at which inhibition of methanogenesis occurred.

A slower growth rate could be counteracted by a combination of longer HRT and supplementation with cobalt, which supported both LCFA degradation and acetoclastic methanogenesis. Longer HRT would also allow more time for degradation of adsorbed LCFA before cells are washed out of the system. These help to explain why a longer HRT and trace element supplementation were effective in supporting continued methanogenesis and stable operation. This is only a hypothesis, however, as LCFA in the system were not measured and therefore no strong conclusions can be drawn regarding the role of LCFA. The results obtained in this study, however, agree with those of Salminen and Rintala (2002b) and Cuetos et al. (2008), and are consistent with the hypothesis that growth rate was affected by inhibition, which could be counteracted by enzyme production and increased growth due to cobalt supplementation.

5.1.4 Syntrophic Acetate Oxidation

In situations in which acetoclastic methanogenesis is inhibited, an alternate pathway of acetate conversion has been observed, termed syntrophic acetate oxidation (Schnurer and Nordberg, 2007). In syntrophic acetate oxidation, both carbon atoms in the acetate molecule are oxidized to carbon dioxide, which is subsequently a substrate for methane

production by hydrogenotrophic methanogens. Conversion of acetate to carbon dioxide followed by hydrogenotrophic methanogenesis would allow acetate degradation and methanogenesis to continue even if acetoclastic methanogenesis failed.

A changeover to syntrophic acetate oxidation has previously been observed in inhibition situations (Schnurer and Nordberg, 2007), where ammonia concentration was high. No work has yet been done on the effect of LCFA on syntrophic acetate oxidation.

A phenomenon observed on most of the digestion trials was a sharp increase in acetic acid concentration between days 40 to 60 of the trial, and the ability of the system to recover from this varied according to reactor HRT and/or trace element supplementation. One potential cause for this phenomenon that was considered is a change in the pathway of conversion for acetate, from acetoclastic methanogenesis to syntrophic acetate oxidation. The acetate may have accumulated as acetoclastic methanogenesis declined, but as syntrophic acetate oxidation proceeded the accumulated acetate was then taken up by the acetate oxidizers. Previous investigators monitoring a shift from acetoclastic methanogenesis to syntrophic acetate oxidation found that it occurred under conditions of TAN beyond 3.3 g l^{-1} and free ammonia beyond 130 mg l^{-1} (Schnurer and Nordberg, 2007). At the time that the acetate increase was observed in this study, TAN was below 2 g l^{-1} and free ammonia below 100 mg l^{-1} for reactors on HRT of 25 and 50 days, although the reactors on HRT of 100 and 180 days did have concentrations of total and free ammonia nitrogen in the range quoted by Schnurer and Nordberg. Also as previously noted, it has not been studied whether LCFA concentrations may have an influence on the onset of syntrophic acetate oxidation.

A similar 'spike' in acetic acid concentrations was observed by Jarvis et al. (1997) in bench-scale CSTRs processing grass-clover silage, when acetate rose from below 200 mg l^{-1} to above 2500 mg l^{-1} at approximately day 45 of their run. Cresson et al. (2006) also had a spike in acetic acid between days 40-60 in a biofilm reactor treating winery wastewater. Both of these groups of investigators subsequently introduced trace element supplementation and found reduced VFA concentrations. The cobalt supplementation may have helped in the production of more enzymes active in acetoclastic methanogenesis, and thus allowed continued direct production of methane from acetate, rather than via the syntrophic acetate oxidation pathway, which has a lower energy yield (Schnurer and Nordberg, 2007).

Another hypothesis that was considered during this study was that the acetate peak could perhaps be due to an onset of β-oxidation of LCFA, with associated production of acetate; following on from other researchers' observations of lag phases of up to 40 days for the onset of methane production from lipids (Beccari et al., 1999). The investigation described in Section 4.5 with a lipid-reduced substrate, however, revealed that the removal of half of the lipid content in the substrate did not affect the timing or magnitude of the acetate peak, thus providing evidence against the hypothesis of acetate production being due to the onset of β-oxidation of LCFA. It should be noted, however, that since lipids were merely reduced in the substrate, rather than removed altogether, the experiment does not eliminate the possibility of any effects of lipids. The possibility of some kind of acclimation mechanism to different components of the substrate should not be excluded; this is an area for further study.

The peak may be related to the difference in degradation rates of different components of the substrate and the growth rates of the populations that degrade the different components. The food waste substrate contains rapidly-degradable carbohydrates with more slowly-degradable proteins and lipids. As noted in Sections 2.1 and 2.3, acetate is formed from LCFA and VFA via syntrophic pathways which require a low hydrogen partial pressure in the medium to proceed at optimal rates. Acetate is also, however, one of the direct fermentation products of carbohydrates such as glucose, via reactions that are far more energetically favourable than the syntrophic pathways by which acetate is formed from fatty acids, as demonstrated by the different ΔG values for the reactions listed in Section 2.1.2. Acetate in the medium can be produced from all three of these sources: VFA, LCFA and carbohydrates; the energetics of the direct fermentation of carbohydrates would give a much higher energy yield and thus faster doubling time for carbohydrate-fermenting acidogens. The acetate peak may be related to the production of acetate in the medium from these rapidly growing populations before the methanogens had built up a population sufficient to degrade it.

It should be noted that there was one CSTR reactor for which no acetate peak was observed; this was the trace element supplemented reactor on an HRT of 25 days. As shown in Table 3.5, the frequency and amount of trace element supplementation for the reactors varied according to their HRT. The 25d HRT reactor had the most frequent trace element supplementation regime, with weekly additions of trace elements. Other reactors had biweekly or monthly trace element supplementation, and all others showed an acetate

peak. For all reactors there would be a decline in trace element levels between dosing events, and the more frequent dosing of trace elements to the 25d HRT reactor maintained a steadier concentration of cobalt. There was no interruption in acetoclastic methanogenesis in this reactor, which may have been related to the more frequent dosing of trace elements.

5.1.5 Trace Elements and Inhibition

Overall, the digestion trials have shown the effectiveness of trace element supplementation in supporting continued methanogenesis and system stability. The sections above outline some of the potential mechanisms by which the various factors may have been working in the system.

Trace element limitation

As explained in Section 5.1.1, the effect of trace element supplementation in these trials was quite clear: the failures followed a fairly predictable pattern associated with washout, failing at a time point at which concentrations of conservative elements in the reactor would have declined to 20% of their initial concentrations in the seed sludge. Furthermore, measurements of actual cobalt concentration in the digestate at the time of failure gave a value consistent with the results of previous investigators (Jarvis et al., 1997, Lebuhn et al., 2008). One possibility, therefore, is that failure in the reactors occurred purely due to limitation for trace elements (cobalt in particular) and that methanogenesis ceased due to lack of nutrients.

Testing of the hypothesis of pure trace element limitation, with no effect of inhibition from ammonia or LCFA, would require digestion runs with a substrate without significant content of protein or lipid, such as fruit and vegetable waste. Another way of testing this hypothesis is to look for reports of methanogenic failure of long-run digestion trials with fruit and vegetables as the only substrates. Few cases of failure have been reported; however as previously noted there is not a large body of existing work with long-run continuous systems digesting pure food waste. Trace element limitation may therefore not have been the primary mechanism involved in process failure; while trace element supplementation was clearly essential, its role may have been in conferring increased resilience to stress factors in the system.

Ammonia inhibition

As discussed in Section 5.1.2, ammonia inhibition may act on methanogenic enzymes via non-competitive inhibition, with free ammonia as the more toxic form due to its ability to diffuse into cells. The trace element supplementation may have conferred some degree of resistance to inhibition by providing cobalt that could be used in the production of more enzymes. This could account for the difference between failure and continued operation of reactors with and without trace element supplementation. However, in the case of reactors on HRT of 25, 30 and 50 days, concentrations of total and free ammonia were generally below those at which other investigators have documented toxicity. Therefore ammonia inhibition was not likely to be a primary mechanism in the case of reactors on retention times less than 100 days. In the case of the reactor on HRT of 180 days, which failed even with trace element supplementation, concentrations of free and total ammonia were indeed at a threshold at which other investigators have observed failure. At these high concentrations, the action of ammonia inhibition overcame the rate of enzyme production even with the trace element supplementation. Ammonia inhibition may therefore have been a primary reason for failure in this case.

To confirm whether ammonia inhibition was the cause of failure, a digestion trial could be carried out in which reactors could be fed with a substrate low in nitrogen for the control reactor, and the experimental reactor fed with the same substrate but also with defined amounts of nitrogen, for example as ammonium chloride. Numerous investigators have studied ammonia inhibition by this method (Angelidaki and Ahring, 1993, Kayhanian, 1994), but none have done it with and without the addition of trace elements; the results in this study indicate that it may make a difference and merits further study.

LCFA inhibition

Inhibition by LCFA has been observed by many investigators (Hanaki et al., 1981, Koster and Cramer, 1987, Angelidaki and Ahring, 1992, Broughton et al., 1998, Salminen and Rintala, 2002b, Lokshina et al., 2003, Mykhaylovin et al., 2005, Pereira et al., 2005, Li et al., 2005), and the food waste substrate used in this study has a high lipid content, therefore the possibility of LCFA affecting the process seems likely. It is also the inhibition mechanism, however, for which the least is known, especially for digestion of food waste. Most previous work on LCFA inhibition has been with UASB reactors treating wastewaters. A few have focused on wastes such as slaughterhouse wastes and sheep

tallow (Salminen and Rintala, 2002b, Broughton et al., 1998) which are high in lipid and protein but may have less risk of rapid overproduction of VFA from rapidly fermentable sugars, such as are found in this substrate. Cuetos et al. (2008) used a mixture of slaughterhouse wastes with OFMSW, and found failure at an HRT of 25 days which they attributed to LCFA.

Competitive inhibition by LCFA at enzyme sites where acetate should bind would cause an increase in acetate levels in the medium and decrease in methane production, which was indeed observed. Mass transfer limitation of cells by sorption of LCFA could affect all cells in the medium, but the most heavily affected cells could be the slowest growing, the syntrophs and the methanogens, as they would have the least ability to counteract the effects of LCFA by new cell material. Cobalt supplementation could assist in resistance for methanogens by promoting enzyme production and consequently the growth of new cells as more substrate is utilised. This would allow continued operation under conditions in which reactors without trace element supplementation failed.

One possible scenario, therefore, for the effects observed during the CSTR digestion trials in reactors without trace element supplementation is that long-chain fatty acids affected the growth rate of microbial cells, through mass transfer limitation or some other mechanism. Acetoclastic methanogens were most susceptible to the inhibition effect. After the concentration of cobalt had declined in the reactor to approximately 20% of its initial concentration in the seed sludge, methanogens were unable to continue sufficient production of cobalt-containing enzymes to keep up with the rate of acetate production by fermentation of carbohydrates and acetogenesis from other VFA and LCFA. Methanogenesis declined and concentrations of acetate increased, followed by increases of other VFA due to product inhibition by acetate and hydrogen.

In reactors which were supplemented with a trace element solution including cobalt, enzyme production could continue and keep up with the rate of acetate production. A hydraulic retention time of less than 25 days, however, was insufficient, either for methanogenic or LCFA-degrading cells to keep up with the rate of washout, or for sufficient alkalinity to be maintained in the medium. Experiments in which retention time for solids and liquids were uncoupled showed that it was the maintenance of biomass that was required on a longer retention time; this is discussed further in Section 5.2.

Trace element supplementation was sufficient to maintain stable digestion in reactors on long retention times; however for the reactor on the longest retention time of 180 days, ammonia in the medium accumulated to inhibitory concentrations. At these high concentrations, cobalt supplementation was not sufficient to counteract the toxic effects of free ammonia, and methanogenesis failed.

These are preliminary hypotheses, however, and further research is required to confirm or refute them. It is still possible that there were no inhibition mechanisms at work in the reactors on HRT of less than 180 days, and that the failures observed were attributable purely to a deficiency of trace elements. If this is the case, however, it would be expected that more examples of failure in long term continuous digestion of food waste should be reported, in cases where no trace element supplementation was provided.

Although there is a large body of research in the areas of nutrient limitation and inhibition of digestion by various compounds, there has been very little published assessing both together, i.e. the effect of trace metal additions under conditions of inhibition. There are several areas where future research could continue on from questions raised in this study. Some of these are:

i) Effect of trace element additions to microbial consortia with inhibitory compounds. Although many studies have been done with mixed consortia deprived of various trace elements, there have been few studies in which inhibitory substances were also added to assess the influence of trace element deprivation and supplementation under controlled inhibition conditions.

ii) Measurement of the rates of enzyme production in continuous systems with and without the addition of trace elements including cobalt, to further clarify the action of trace metal additions to continuous systems.

iii) Measurement of the growth rate of different populations involved in food waste degradation, particularly methanogens and LCFA degraders, and growth rate variation under different inhibition conditions.

iv) More investigation into the mechanisms of LCFA inhibition in the digestion of solid wastes.

v) Investigation into the role, if any, of syntrophic acetate oxidation under conditions of inhibition by LCFA.

vi) Investigation of synergistic effects of inhibitors such as ammonia and LCFA, and trace element limitation. As noted by Lokshina et al. (2003), although inhibition from LCFA and ammonia may be studied separately, in actual systems both can contribute.

vii) Investigations into the conditions under which acetate peaks occur in digestion systems, and the possible roles of inhibitory compounds and/or trace element supplementation in the generation of these peaks.

viii) Monitoring for and quantifying the prevalence of syntrophic acetate oxidation when acetate peaks occur in anaerobic digestion systems.

5.2 Retention Time Effects and Uncoupling of SRT from HRT

Trace element supplementation did not guarantee long-term stable operation of all reactors. Reactors on the shortest (25 days) and longest (180 days) HRT both eventually failed.

In the 25 day HRT case, although the trace element-supplemented reactor operated for nearly three times as long as the trace element-deprived control, it did not operate indefinitely, showing a decline in gas production and increase in VFA after 115 days. Methanogenic activity in the reactor recovered, however, after a 12-day break in feeding and resumption of operation at a higher HRT of 50 days. The reactor then showed stable operation for a further 150 days until the end of the trial.

This shows that, as discussed in Section 5.1, not only trace element supplementation, but also allowance of a sufficiently long HRT was required for stable operation of the reactor. An HRT of 25 days gave a washout rate that was too high to maintain stable operation. The instability could be due either to washout of alkalinity, which declined through the trial, or washout of methanogens. After the CSTR digestion trials, it was not definitively known which component was the critical factor, and therefore the solids retention trials were designed to address this question.

At the other end of the range, the trace element-supplemented reactor on the longest HRT of 180 days also showed eventual methanogenic failure, after a run of 345 days. Ammonia toxicity may have been a cause of the failure, as explained in Section 5.1. The

accumulation of ammonia and other dissolved compounds in the reactors was a function of hydraulic retention time, and therefore the long retention time for liquids may have been detrimental.

The centrifuge bottle reactor trials described in Section 4.6 were initiated to address the question of whether the requirement for an extended retention time was true for both the solid and liquid fractions. Solid and liquid retention times were uncoupled, with a retention time of 25 days for either solid or liquid, and extended retention time for the other component.

It was initially expected that both systems might fail, if both the solid and liquid fractions of the digestate were needed to maintain sufficient alkalinity (contributed by the liquid fraction) and sufficient biomass (contributed by the solid fraction). It was found, however, that for the solids wasting system, carbonate alkalinity quickly dropped. For the liquid flushing system, in contrast, sufficient alkalinity was maintained in the medium due to biomass activity, while VFA did not accumulate.

This indicates that it was the biomass fraction that was needed on a retention time above 25 days for the CSTR reactors. The longer SRT allows for slower growth of methanogens and/or LCFA degraders. Also, by the removal of liquids, the hydraulic flush mode provided a remedy to the problem of accumulation of inhibitory compounds as experienced in the 180 day HRT CSTR reactors. Lastly, keeping cells in the system allows time for degradation of biomass-associated LCFA.

While the effect of trace element supplementation was pronounced in the CSTR trials, the situation was different in the centrifuge bottle reactor trials. The solids wasting reactors failed regardless of trace element supplementation, while the hydraulic flush reactors showed stable operation regardless of trace element supplementation. One possible explanation for this is the low solubility of micronutrients such as cobalt within the pH range for anaerobic digestion. If these metals adsorb to organic solids – as demonstrated in the work of Zandvoort et al. (2006) in which the majority of cobalt was found adsorbed to sludge or complexed with sulphide - then much of the initial mass of any micronutrient in the reactor could be conserved in the solid fraction. Therefore in the case of the hydraulic flush reactors, micronutrients would stay in the reactor rather than washing out over time with the liquid fraction, while in the case of the solids wasting reactors, micronutrients would be removed with the solids. Also, if the added micronutrients were being taken up

by cells rather than staying in solution, the micronutrients would be conserved with the biomass in the hydraulic flush reactors, and removed with the biomass in the solids wasting reactors.

Another observed difference between CSTR and centrifuge bottle digestion trials was the extent of hydrolysis. While an accumulation of floating particles was observed in CSTR reactors, this was not seen in the centrifuge bottle reactors. The regular centrifuging of the contents of the reactor resulted in thorough mixing of the reactor, which may also have had an effect in increasing the contact between hydrolytic enzymes and substrate, and discouraging the formation of floatable aggregates such as were observed in the CSTR reactors. Longer SRT may also have allowed more time for the slower lipid degradation.

Although reactor designs that keep solids in the system are common for treatment of liquid waste streams, the situation is different in many commercial solid waste digestion systems, where wasting of solids and retention of liquids is common. Many commercial digestion facilities have a solid-liquid separation step following digestion, after which the wasted solids are composted or landfilled and the liquid is recirculated to the pre-treatment system or digester (Greenfinch Ltd., 2003, Blischke, 2004). Some high solids commercial systems, however, do recirculate solids as required (*pers. comm.* I. Wierinck, Organic Waste Systems NV). In this work, it was found that retaining only the liquid fraction while removing solids resulted in accumulation of VFA and loss of biomass.

In the case of plants treating wastes with a significant fraction of materials high in lignin such as green wastes or paper, the removal of solids would be a sound strategy to avoid the build-up of materials that are resistant to degradation. In the case of highly-degradable materials, however, such as the source-separated food waste substrate used in this study, it is apparent that keeping solids in the system is more beneficial for the regeneration of alkalinity and flushing of excess VFA and ammonia, and does not result in a build-up of recalcitrant materials.

The results found in this research support the use of reactor types that retain solids in the system, such as the anaerobic sequencing batch reactor (ASBR) used in the treatment of fruit and vegetable wastes by Bouallagui et al. (2005), or the hydraulic flush system of Wang and Banks (2003) for abattoir wastes. In those studies, two-stage systems were used, in contrast to the one-stage mode used in this research. The two-stage mode has the advantage of regulating the loading of volatile fatty acids to the methanogenic stage; it is a

strategy employed to minimise the likelihood of process upset due to rapid overproduction of VFA and has been used in many systems for food wastes such as fruit and vegetable or market wastes (Mtz.-Viturtia et al., 1995, Parawira et al., 2004, Kim et al., 2005). In the current work, however, it was found that in the case of the hydraulic flush (HF) reactors a one-stage system was sufficient to maintain stable VFA concentrations and a fair degree of robustness in the face of disturbance, when solids were maintained in the system while liquids were flushed through. This is a favourable result as one-stage systems are generally favoured over two-stage systems at a commercial scale, due to their lower complexity and costs; currently over 87% of AD capacity for biodegradable municipal waste in Europe is provided by single-stage plants (De Baere, 2006).

5.3 Co-Digestion

Although not trialled in this research, co-digestion with other less readily-degradable substrates is a second possible option for this feedstock. The use of co-substrates to mediate VFA concentrations, increase C:N ratio or increase gas production has been used successfully by a number of investigators (Kaparaju and Rintala, 2005, Gomez et al., 2006, Neves et al., 2006b).

Advantages of co-digestion for this feedstock would include:

i) Lower rate of VFA production in the system due to lower concentration of rapidly degradable substrate;

ii) Raising of the C:N ratio to decrease potential for ammonia inhibition and provide a better balance of nutrients for cell growth;

iii) Dilution of the lipid content in the feedstock, to decrease potential for inhibition by LCFA.

This is an area for further research for optimisation of digestion of food waste.

5.4 Significance of Errors

In any research, analytical and operational errors can arise and must be addressed. In this study, there were two main sources of error: analytical accuracy in measurement of digestion parameters, and prediction of trace element supplementation concentrations.

Due to the heterogeneity of digestate samples, it can be difficult to measure certain parameters with as much precision as would be possible with more homogeneous liquids.

The largest potential variation in measured values for digestate was 10% for alkalinity and VFA measurements, as shown by initial testing with multiple replicates. The potential lack of precision in these measurements, however, did not have an influence on the major trends which are the basis of all conclusions drawn. For example, the large changes in alkalinity and VFA concentrations that were noted at the points preceding methanogenic failure were clearly process effects and not the result of measurement inaccuracies of 10% or less.

There was an initial experimental error in the preparation of the trace element solution used to provide trace element supplementation to the reactors, with the result that the amounts delivered to the reactors were actually lower than expected by a factor of ten. This was addressed, however, by ICP-MS analysis of the trace element supplementation solution to determine the correct amounts of metals delivered, and analysis of metal concentrations in the digestate to confirm metal concentrations present in the reactors as a result of supplementation. As noted in Section 4.4.2, this actually led to more accurate pinpointing of the minimum threshold concentrations at which reactors could continue to operate.

6. Conclusions

In this study, source segregated food wastes were collected from a university catering facility and used as substrate in digestion trials.

Objective 1: Create a methodology to allow the quantification of food waste generation.

Food Waste Collection

The first objective was met by collecting and determining the total quantity of food wastes produced in one week by the subject facility. It was found that the total amount of source segregated food waste from a 5-day intensive collection from the campus facility was approximately 300 kg. Based on this amount extrapolated over 50 weeks of operation per year, an annual quantity of approximately 15 ± 5 tonnes of food waste could be expected from the study site. This is a very small amount relative to the tonnages required for full-scale digestion plants, but the site could be part of a larger collection program.

A subordinate objective of this component was to determine quantities of contamination in collected wastes, as an indicator of the potential success of a future food waste separation program and to ensure that contamination in the substrate composite was minimised. The amount of contamination in the waste samples was quantified and found to be below 2% of total mass in all collection categories, showing high effectiveness of the source segregation system trialled.

Objective 2: Plan and implement trials to assess the amenability of source segregated catering waste for anaerobic digestion.

Initial CSTR Digestion Trials

Processing of the catering waste in the initial digestion trials showed that:

- While methane production and VS destruction for this substrate were good, the process was unstable.
- At an HRT of 25 days, the process was prone to failure, and was not able to sustain any increase in OLR, or to maintain stable digestion beyond 80 days at a constant OLR.
- An HRT of 50 days allowed operation for a longer period, and permitted increase of the OLR from 1.45 gVS $l^{-1}d^{-1}$ to 1.98 gVS $l^{-1}d^{-1}$. However, it did not result in

stable operation for an indefinite period without additional nutrient supplementation.

Objective 3: Identify and critically evaluate potential problems resulting from the digestion of this material.

Trace Element Supplementation Investigations

After the initial digestion trials, subsequent digestion trials were carried out to investigate the roles of trace element supplementation and retention time. The objectives of these trials were to investigate the role of trace element supplementation in the maintenance of stable digestion, and to compare the performance of reactors operated at different retention times. The findings were as follows:

- Reactors supplemented with trace elements showed stable digestion and recovery or adaptation to elevated VFA concentrations, while reactors without trace element supplementation were subject to methanogenic failure.
- While trace element supplementation was not sufficient to guarantee stable operation for a reactor on an HRT of 25 days, extending HRT to 50 days while maintaining trace element supplementation allowed recovery of the reactor and stable operation. Extending HRT to 50 days without the provision of trace elements was not sufficient to allow reactor recovery.
- With trace element supplementation and HRT of at least 30 days it was possible to achieve stable digestion of this feedstock at an OLR up to 3.0 gVS $l^{-1}d^{-1}$, or 3.5 gVS $l^{-1}d^{-1}$ in the case of reactors on HRT of 50 and 100 days.
- Extended HRT allowed stable operation at elevated VFA concentrations.

Trace Element Analysis

Digestate samples from the CSTR digestion trials were analysed for the objective of measuring the actual concentrations of trace elements in digestate, to confim whether trace elements were washing out of the trace element deprived reactor over time, and whether they were being maintained in the trace element supplemented reactor.

Analysis of the digestate led to the following findings:

- Trace element supplementation made little or no difference to metal content in the digestate except for cobalt;

- Concentrations of cobalt increased in digestate solids for the trace element supplemented reactor over the course of the trial, while declining for the reactor without trace element supplementation.
- A decrease in cobalt concentration to below 0.03 mg l^{-1} in the reactor was associated with reactor failure.

CSTR Digestion Trial with Modified Substrate

A further CSTR digestion trial using a modified substrate was carried out, with the objective of testing the hypothesis that acclimation to lipids and onset of β –oxidation was responsible for the peak in acetic acid concentrations observed on previous trials. It was found that reducing the lipid content of the substrate did not affect the timing or magnitude of increases in acetic acid during these digestion trials.

Objective 4: Develop strategies for overcoming problems in digestion of this material.

Uncoupling of Solid and Liquid Retention Times

Trace element supplementation was one strategy used for overcoming problems in digestion of this material. The second strategy tested was the uncoupling of solid and liquid retention times. Using a mode of digestion in which solid and liquid retention times were uncoupled, it was found that retaining solids in the reactor enabled robust and stable operation over a period of 150 days, as well as resilience to disturbance. The flushing of liquid allowed the removal of VFA and other dissolved compounds, but sufficient bicarbonate alkalinity for stable digestion was maintained due to biomass activity. In contrast, retaining liquids while removing solids resulted in accumulation of VFA and loss of methanogenic activity. These results showed solids retention to be a successful strategy to enable stable digestion of this feedstock.

6.1 Future Work

Future work following on from these trace element supplementation investigations could focus on further quantification of metal concentrations in digestate and pinpointing critical concentrations for cobalt and other metals. There is a large potential for future work on the specific role of trace elements in supporting biomass under inhibitory conditions, such as under high TAN or LCFA concentrations, as discussed in Section 5.1.

The investigations with uncoupled SRT from HRT, discussed in Section 5.2, showed that extending solids retention time allowed stable digestion at a liquid retention time of 25

days. Future work could be focused on decreasing the liquid retention time further, as process parameters such as VFA concentrations and alkalinity indicated that the system was healthy and likely could operate at a higher hydraulic flush rate.

Co-digestion of this feedstock with other wastes with higher C:N ratio, lower lipid content and/or higher metal content was not investigated in this research. However co-digestion with other feedstocks has potential synergies as discussed in Section 5.3, and is a promising area for further research.

6.2 Recommendations

For this food waste, which contains high concentrations of readily-degradable carbohydrates combined with high nitrogen and lipids, anaerobic digestion in a single-stage mesophilic CSTR may not be the best option for bioprocessing, as it was shown to be susceptible to methanogenic failure. The substrate is, however, a promising feedstock for anaerobic digestion, as good performance in terms of methane yields and volatile solids destruction was seen in this study. The following are recommended to address the process stability issues noted in this research:

i) Regular supplementation with cobalt, if mesophilic CSTR digestion is to be used, or

ii) Use of a digestion mode that retains solids in the system, such as the hydraulic flush reactors tested in this study.

These recommendations can help in the design of systems for stable anaerobic digestion of source-separated food wastes. This knowledge will be very important as governments and industry in the UK and throughout the world proceed with programs to increase the use of anaerobic digestion for the sustainable management of organic wastes and generation of renewable energy.

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Appendix A

Paper: Anaerobic digestion of catering wastes: effect of micronutrients and retention time

Water Science and Technology 57(5) 2008

Anaerobic digestion of catering wastes: effect of micronutrients and retention time

M. A. Climenhaga and C. J. Banks

ABSTRACT

Source-separated foodwastes collected from a campus catering facility were processed in bench-scale single-stage anaerobic digesters. The feedstock contained a varied mix of fruits, vegetables, meats and fried foods. A constant organic loading rate (OLR) was maintained with differing hydraulic retention times (HRT). Regular addition of trace elements or prolonged retention time allowed stable digestion at high total volatile fatty acid (TVFA) levels. Reactors on HRT of 25, 50, and 100 days with no micronutrient supplementation exhibited methanogenic failure after approximately 40, 100 and 90 days respectively, while duplicate reactors with micronutrient supplementation maintained stable digestion. An extended HRT of 180 days has so far allowed continued digestion (for reactors with and without micronutrient supplementation) at levels of ammonia nitrogen exceeding 5.7 g l^{-1} and volatile fatty acid levels exceeding 15 g l^{-1}, usually considered inhibitory or toxic.

Key words | anaerobic digestion, catering wastes, foodwastes, micronutrients

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INTRODUCTION

Food wastes from domestic kitchens, commercial catering establishments and retail outlets represent a waste stream that holds significant potential as a resource for anaerobic digestion. This study used wastes from an institutional catering facility, containing high quantities not only of readily-degradable starches and sugars, but also more slowly-degradable lipids, as well as proteinaceous materials. The results indicate that a feedstock of pure foodwaste may not contain all nutrients required to meet microbial metabolic requirements.

METHODS

Substrate for the digestion trials was source-separated foodwaste from the main catering facility serving staff and postgraduates at the University of Southampton. Wastes were collected over a period of five days, from all areas of the facility including salad bar (primarily fruit and vegetables), hot food counter (cooked foods including meats and fried foods) and preparation kitchen (peelings, bones and fat trimmings). The wastes were ground in a commercial garbage grinder (S52/010 Waste Disposer, Imperial Machine Company Ltd.) and mixed to form a large composite. The composite was then frozen in 1500 g portions, stored at − 16°C and thawed as needed for use as substrate. Foodwaste was characterised for total solids (TS), volatile solids (VS) and total Kjeldahl nitrogen (TKN) by standard methods (APHA 2005). Chemical oxygen demand (COD) was measured following a modification of the standard method (APHA 2005) using larger quantities of the same reagents to analyse a larger sample.

Eight bench-scale anaerobic bioreactors of 5-litre liquid capacity were operated on a continuous basis for 125–200 days (runs of varying length). At the commencement of the trial, sludge from the secondary anaerobic digester of a wastewater treatment plant (Millbrook Sewage Works, Southampton) was collected and sieved through 1 mm mesh, then used as seed for the reactors. Digestate samples

doi: 10.2166/wst.2008.092

were withdrawn daily and an equivalent quantity of substrate plus distilled water was added to reactors, to maintain a constant volume and fix hydraulic retention time (HRT) in the reactors. A constant organic loading rate (OLR) of 1.45 g VSl^{-1} d^{-1} was maintained to all reactors. The reactors were operated on four retention times of 25, 50, 100 and 180 days, with two reactors operating at each retention time. The reactors were run as pairs that were duplicates of each other, except that one reactor from each pair was supplemented with a trace element mixture on a periodic basis (1.0 or 1.5 mL solution weekly, biweekly or monthly depending on HRT) while the other reactor was not. The trace element solution follows the recipe of Gonzalez-Gil *et al.* (2001).

Biogas production was measured daily, via water displacement in a volume-calibrated cylindrical gas collector after collection in Tedlar bags. Gas composition samples were withdrawn from the headspace of the reactors once per week, analysed by gas chromatography and compared to a standard mix of 35% CO_2/65% CH_4, on a Varian CP-3800 gas chromatograph with thermal conductivity detector, column temperature 50°C. Volatile fatty acids were measured weekly using a Shimadzu GC-2010 gas chromatograph, with flame ionisation detector and FFAP capillary column from SGE, model BP-21 12 m × 0.32 mm, with helium as carrier gas. Alkalinity measurement and proximate analysis were in accordance with standard methods (APHA 2005).

RESULTS AND DISCUSSION

The foodwaste contained (mean and standard deviation of five replicates) TS = 28.1 ± 0.25%; VS = 95.5 ± 0.06% of TS; TKN = 3.77 ± 0.24% of TS; COD = 450 $mg g^{-1}_{wetweight}$ (single measurement).

Total VFA, methane production, ammonia, alkalinity and Ripley's ratio (Ripley *et al.* 1986) for each pair of reactors are illustrated in Figures 1–4. The two reactors on a 25-day HRT (Figure 1) show similar VFA and gas production trends at the commencement of the trial, until a steep increase in VFA occurred around Day 40 for the reactor without trace element supplementation. This increase in VFA was associated with a drop in methane

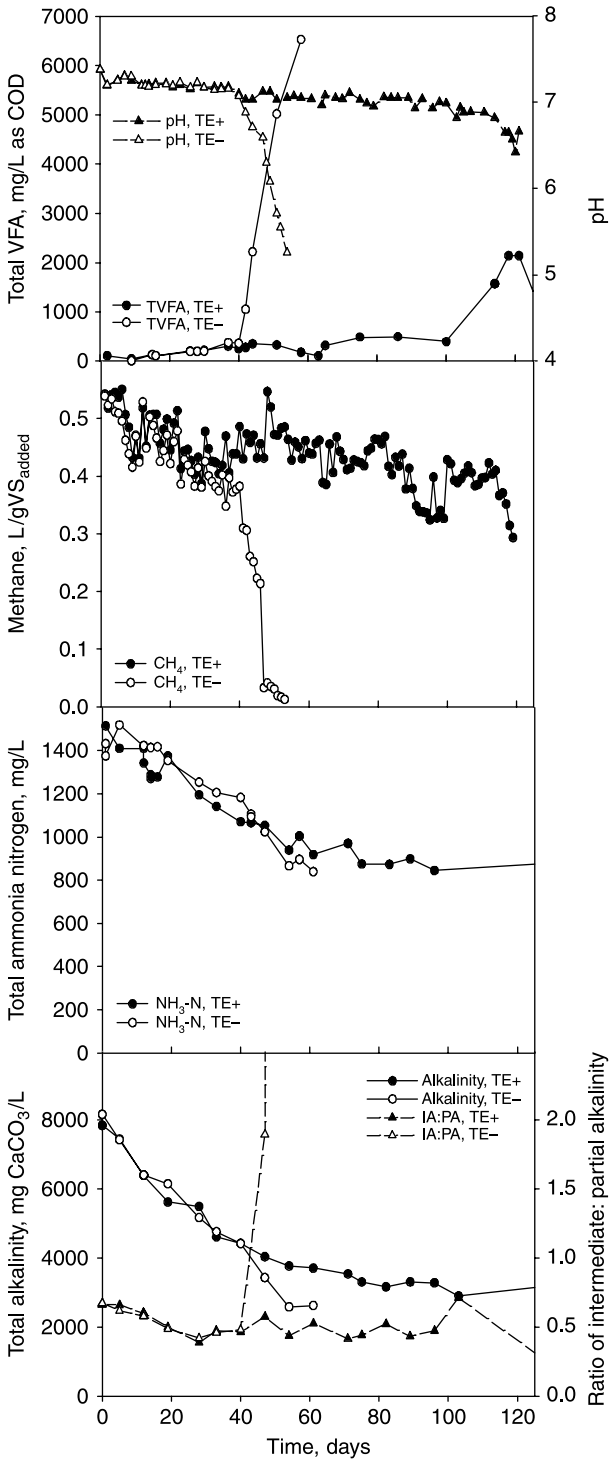

Figure 1 | TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE +) or without (TE −) trace element supplementation, HRT = 25 days.

production and eventual failure. Up to the time of the failure of the trace element-deprived reactor, the two reactors exhibited very similar behaviour in pH, ammonia, alkalinity and VFA profiles. The supplemented reactor showed stable digestion for three further retention times before also showing a rise in VFA and methane production failure between Days 110 and 120.

Both reactors in the 50-day HRT pair (Figure 2) had a spike in total VFA production around Day 40–50, but the VFA were consumed over the following weeks. Both reactors exhibited very similar profiles for all parameters measured up to approximately Day 100 (two retention times) at which time the trace element deprived reactor accumulated VFA and ceased to produce methane, while the trace element supplemented reactor maintained stable digestion.

The paired reactors on a 100-day HRT (Figure 3) also show a VFA spike around Day 40–50. The VFA level in the trace element deprived reactor, however, stayed at the higher level, while TVFA in the trace element supplemented reactor was consumed. Toward the end of one retention time (Day 90–100), TVFA in the trace element deprived reactor increased again at the same time as methanogenesis declined.

The paired reactors on a 180-day HRT (Figure 4) were run for over 200 days. Both reactors in the pair have continued to operate through two increases in TVFA. It is interesting to note that the trace element deprived reactor continues to operate in spite of higher VFA concentrations than those at which failure occurred for the reactors on shorter retention times. Reactors on this extended retention time show very high alkalinity (over $18\,g\,l^{-1}$), and are showing stable digestion at a TVFA concentration beyond $15\,g\,l^{-1}$ and total ammonia nitrogen (TAN) over $5.7\,g\,l^{-1}$, levels that are often considered to be inhibitory or toxic (Grady *et al.* 1999, Gerardi 2003). This trial is continuing, to investigate long-term stability.

The increases in VFA around Day 40 and 100 are observed for all reactors. This cannot be attributed to external or equipment factors because the runs were commenced on different dates months apart, and therefore Day 40 for the 25-day HRT reactors falls on a different calendar date than Day 40 for the 50- and 100-day HRT reactors or the 180-day HRT reactors. Additionally, the

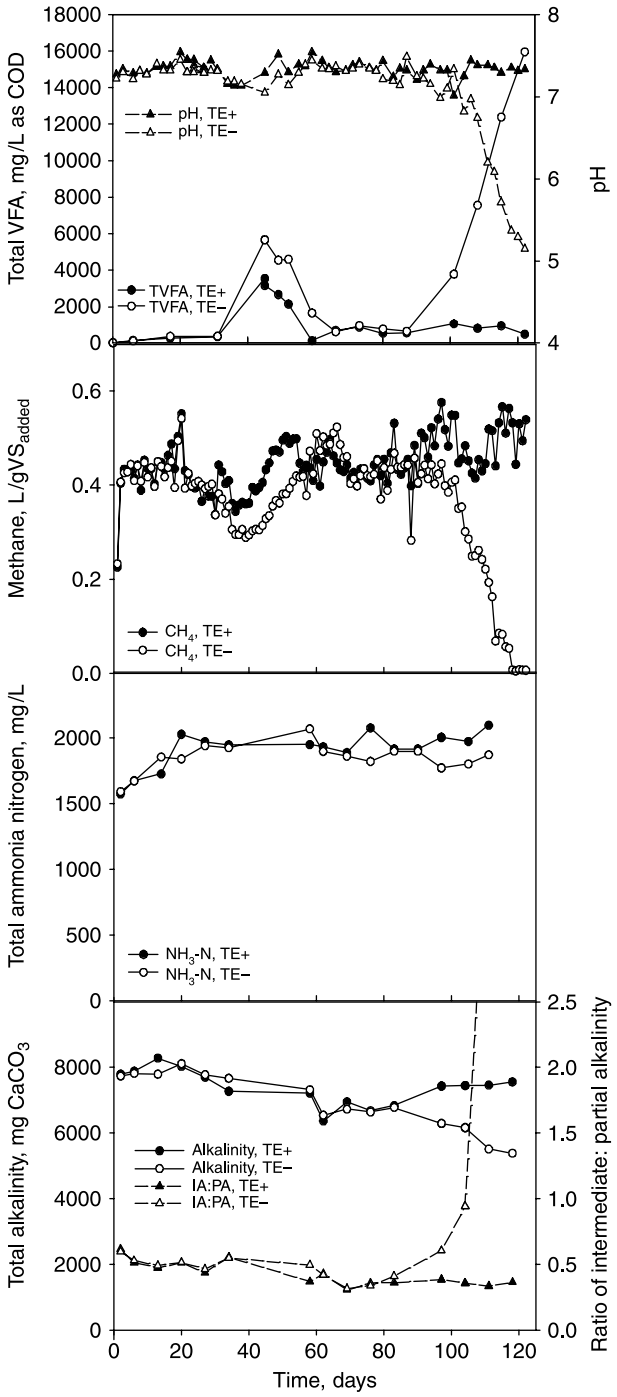

Figure 2 | TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE +) or without (TE −) trace element supplementation, HRT = 50 days.

phenomenon was observed on three previous digestion trials with HRT of 25 and 50 days (data not shown). Therefore, it is a phenomenon attributable to factors internal to the system. As the reactors are on the same

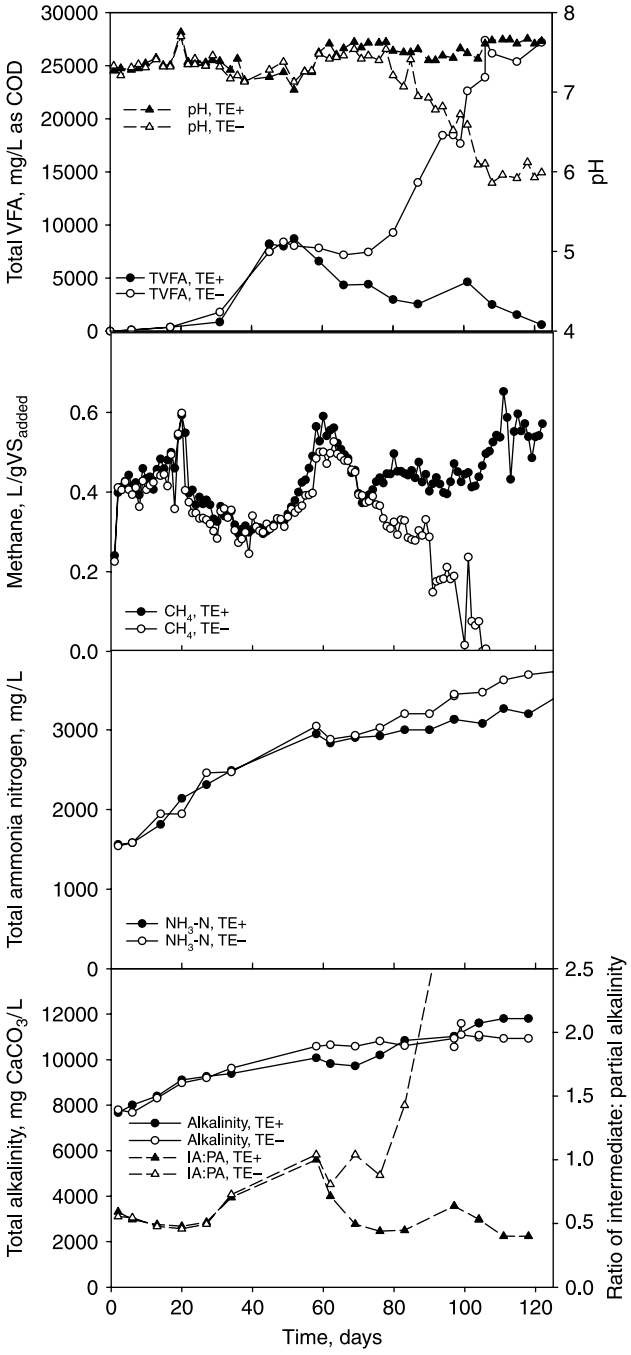

Figure 3 | TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE +) or without (TE −) trace element supplementation, HRT = 100 days.

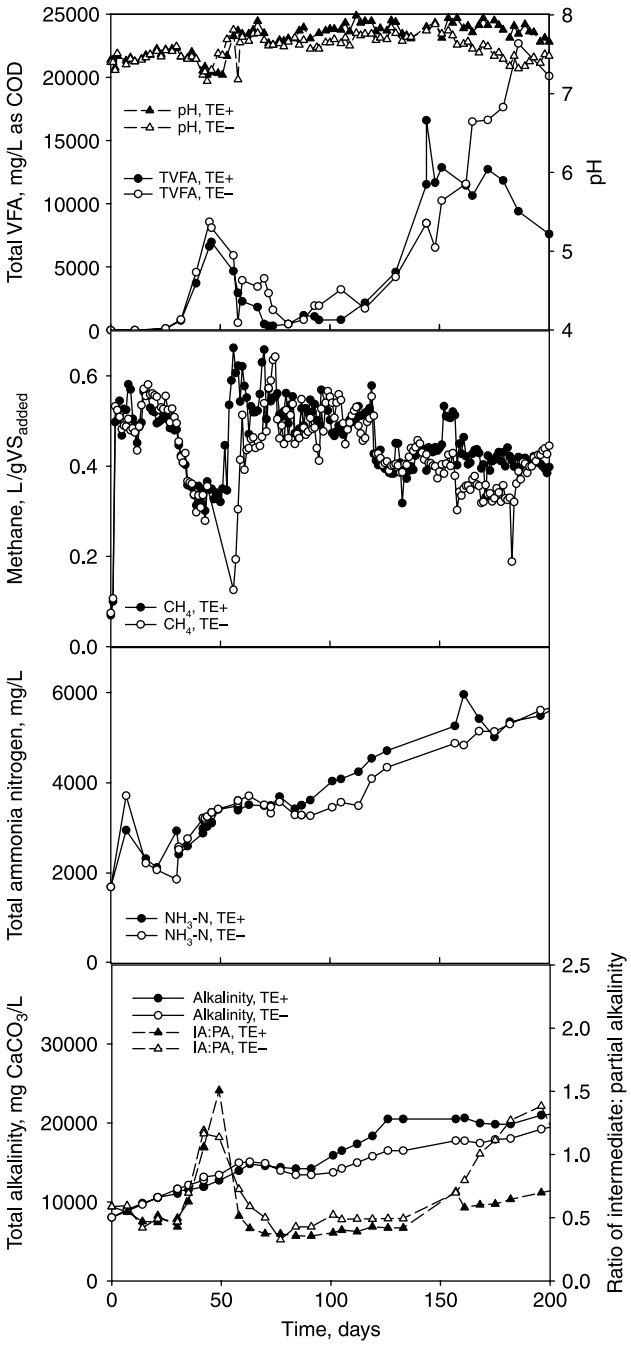

Figure 4 | TVFA and pH, CH_4 production, TAN, total alkalinity and alkalinity ratios for reactors with (TE +) or without (TE −) trace element supplementation, HRT = 180 days.

OLR, at Days 40 and 100 all will have received the same cumulative VS, although the rate of washout and therefore accumulation of breakdown products such as ammonia differs for the different retention times.

Role of trace elements

A number of investigators have pointed to the importance of certain trace elements such as iron, cobalt, and nickel for maintaining a stable anaerobic digestion process

(Florencio *et al.* 1994, Zandvoort *et al.* 2002), but these studies have generally been focused on a simple and defined feedstock such as methanol. The addition of trace elements, nutrients or alkalinity is commonly practiced in many laboratory anaerobic digestion studies on wastewaters that are known to be deficient in certain nutrients (Takashima & Speece 1990), or in batch studies, but in many cases no addition of other compounds is practiced, especially on studies with continuous feeding of diverse mixed feedstocks (Salminen & Rintala 2002, Carucci *et al.* 2005, Fernandez *et al.* 2005). Also, micronutrient supplementation in commercial operations is not commonly practised as the minimisation of costs is favoured. The feedstock studied in this investigation is a complex waste containing a wide range of foods, which might be expected to contain sufficient amounts of all trace elements required for cell metabolism. It is possible, however, that either the feedstock lacks all of the trace elements specifically required for methanogenic metabolism, or that the elements are present but not bioavailable. Heavy metals are known to be precipitated by sulphides (Gerardi 2003); long-chain fatty acids (LCFA) can also bind with minerals such as calcium (Pereira *et al.* 2001). Therefore although the substrate contains a broad range of nutrients and may contain all of the trace elements required, these may become non-bioavailable during the anaerobic digestion process.

A second possibility for the role of micronutrients is in support of biomass resistance to inhibition or toxicity by LCFA or other inhibitory substances in the medium. Other investigators have found an effect of micronutrient supplementation under unfavourable conditions; for example a mix of iron, nickel and cobalt was shown to play a role in supporting maintenance of granular sludge in UASB reactors treating food processing wastewater (Oleskiewicz 1989) and in other work the presence of substoichiometric amounts of ferric hydroxide reduced the sensitivity of acetoclastic methanogenesis to inhibition by fatty acids in anaerobic sediments incubated with vegetable oil (Li *et al.* 2005). Whether this is due to precipitation of LCFA by metals, thereby lessening their inhibitory effect, by direct support to microorganisms or by some other mechanism, is unknown and is being investigated further.

Role of ammonia/ammonium

The 100-day HRT reactors sustained total ammonia nitrogen (TAN) levels beyond $3\,g\,l^{-1}$, while in the 180-day HRT reactors TAN currently exceeds $5.7\,g\,l^{-1}$ at a pH above 7.5. Free ammonia, as calculated following Kaparaju & Rintala (2005), therefore exceeds $1000\,m\,gl^{-1}$, beyond the threshold of $300\,m\,gl^{-1}$ observed to have a severe inhibitory effect (Angelidaki & Ahring 1993). In this investigation, TAN appears to be more beneficial than detrimental, as it provides buffering capacity in the long HRT reactors, as opposed to the 25-day HRT reactors in which TAN is washed out and declines through the trial. Bhattacharya & Parkin (1989) reported digestion in chemostat cultures fed on acetate with addition of up to $5\,g\,l^{-1}$ TAN, but failure at $6\,g\,l^{-1}$ TAN. This was observed with cultures on a 40-day retention time, while at lower retention times failure was observed at lower TAN concentrations; in this study, extending retention time to 180 days has allowed stable digestion at over $5.7\,g\,l^{-1}$ TAN. In thermophilic digestion of cattle manure, $4\,g\,l^{-1}$ TAN was found to be inhibitory, but acclimation over 6 months of operation allowed digestion at up to $6\,g\,l^{-1}$ TAN, with reduced activity and a VFA concentration of $3\,g\,l^{-1}$ as acetate (Angelidaki & Ahring 1993); in this study, TVFA has exceeded $15\,g\,l^{-1}$ as COD in a reactor that continues to operate. This also agrees with previous findings of pilot-scale (1,500 L) single-stage digestion of nitrogen-rich foodwastes, in which system alkalinity was high and stable digestion at VFA levels up to $25\,gl^{-1}$ was maintained (Stringfellow *et al.* 2003).

CONCLUSIONS

This work has shown the importance of micronutrients in digestion of a mixed foodwaste feedstock. Reactors supplemented with trace elements showed stable digestion, while non-supplemented reactors showed methanogenic failure. Also, the extended retention time trial has shown an interesting result in the maintenance of stable digestion in spite of elevated concentrations of VFA (over $15\,gl^{-1}$) and total ammonia nitrogen (over $5.7\,gl^{-1}$). In addition to the role of micronutrients, system resilience at extended retention times is currently being investigated further. Current and future work will focus on the mechanisms by

which trace elements act to support stable digestion, and determination of the relative importance of the retention of soluble compounds versus the retention of biomass.

ACKNOWLEDGEMENTS

This work was supported by funding from the UK Engineering and Physical Sciences Research Council (EPSRC) Sustainable Urban Environment (SUE) Programme grant GR/S79626/01, and the UK Overseas Research Students Awards Scheme (ORSAS). The invaluable assistance of Denise Cysneiros, Mark Walker, Sonia Heaven and Yue Zhang are gratefully acknowledged.

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Appendix B

Paper :

Uncoupling of liquid and solid retention times in anaerobic digestion of catering wastes

Water Science and Technology 58(8) 2008

Uncoupling of liquid and solid retention times in anaerobic digestion of catering wastes

M. A. Climenhaga and C. J. Banks

ABSTRACT

Source-separated food wastes collected from a university campus catering facility were processed in bench-scale anaerobic digesters. The feedstock contained a varied mix of fruits, vegetables, meats and fried foods. Two modes of digestion were compared. The first was hydraulic flush (HF) mode, in which liquids were flushed through the reactor on a retention time of 25 days while solids were maintained on an extended retention time of over 150 days. The converse was a solids wastage (SW) mode, in which liquid retention time was over 150 days, and solids were wasted to maintain a retention time of 25 days. SW reactors exhibited methanogenic failure after approximately 45 days. HF reactors, in contrast, maintained stable digestion for a period of 100 days, and were robust enough to recover from a thermal shock applied over a three-day period in which the temperature was increased from 35°C to 50°C between days 105–108 of the experiment. Stable operation was regained by day 139 and continued until the end of the run on day 150.

Key words | anaerobic digestion, catering wastes, food wastes, hydraulic flush, solid–liquid separation, solids retention time

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INTRODUCTION

Anaerobic digestion of organic wastes from domestic and institutional sources is a growing field in Europe (De Baere 2006) and throughout the world. Socio-political and economic drivers favouring waste management technologies with a positive energy balance and potential to reduce emissions of greenhouse gases are creating favourable conditions for the expansion of anaerobic digestion (Hogg *et al.* 2007). New market opportunities for anaerobic digestion technologies include the use of the process for feedstocks which are new or for which few commercial examples exist, such as source-separated food wastes from food serving establishments. Successful continued development of the technology requires understanding of the particular challenges posed by the different feedstocks that could potentially be processed, and determination of optimal modes of digestion.

One important factor for consideration is the retention time for solids and liquids in the system. In treatment of liquid wastewaters, bioreactors are commonly designed to retain anaerobic biomass solids (e.g. upflow anaerobic sludge blanket, anaerobic filter, expanded granular sludge bed reactor types), as these work with a very short HRT which is less than the maximum growth rate for methanogens, and therefore mechanisms are required to maintain biomass in the reactor. In the anaerobic digestion of solid wastes, the hydrolysis of particulate substrate is often rate-limiting (Sanders 2001) and longer retention time is required.

In previous work (Climenhaga & Banks 2007) on digestion of source-separated food wastes using a CSTR design, the roles of hydraulic retention time and trace element supplementation were investigated. It was found that extending the hydraulic retention time resulted in higher levels of alkalinity in the system and facilitated more stable operation: bioreactors on a hydraulic retention time of 50 days showed stable operation for a longer period than

doi: 10.2166/wst.2008.497

those on a hydraulic retention time of 25 days. In both systems trace element supplementation was essential for sustained stable operation. The work described here focused on investigating the relative importance of the solid vs. liquid fraction in maintenance of alkalinity and stability of the system.

MATERIALS AND METHODS

Substrate for the digestion trials was source-separated foodwaste from the main catering facility serving staff and postgraduates at the University of Southampton (Highfield Campus, Southampton UK). Wastes were collected over a period of five days, from all areas of the facility including salad bar (primarily fruit and vegetables), hot food counter (cooked foods including meats and fried foods) and preparation kitchen (peelings, bones and fat trimmings). The wastes were ground in a commercial garbage grinder (S52/010 Waste Disposer, Imperial Machine Company Ltd., Hertfordshire UK) and mixed to form a large composite. The composite was then frozen in 1500 g portions, stored at −16°C and thawed as needed for use as substrate. Foodwaste was characterised for total solids (TS), volatile solids (VS) and total Kjeldahl nitrogen (TKN) by standard methods (APHA 2005). Total lipid content was analysed using a Soxhlet extraction (APHA 2005) using heptane as solvent. Chemical oxygen demand (COD) was measured following a modification of the standard method (APHA 2005) using larger quantities of the same reagents to analyse a larger sample.

Four bioreactors were constructed from 1000 ml polypropylene centrifuge bottles with a 40 rpm stirrer mounted on the sealable screw cap. The entire stirrer mechanism, including paddle stirrer, could be removed and replaced by a standard screw cap which allowed the reactors to be centrifuged at 4,000 rpm (Wifug 4000E centrifuge, Wifug Ltd., Bradford UK) for solid–liquid separation. A diagrammatic representation of the reactor design is shown in Figure 1.

The reactors were seeded with sludge from a wastewater treatment plant anaerobic digester (Millbrook Sewage Works, Southampton) and operated with uncoupled solid and liquid retention times. Two reactors were operated by conserving all solids in the reactor and periodically flushing a portion of the liquid (Hydraulic Flush, HF), while the other two were operated by retaining all liquid in the reactor but periodically wasting a portion of the solids (Solids Wastage, SW). Solid–liquid separation was carried out on a weekly basis, by centrifuging the reactors and decanting the supernatant through 1-mm mesh. A mass equal to 28% (based on a removal of 4% per day, multiplied by 7 days per week) of either the liquid or solid was removed each week, before recombining the liquid with the solids in the reactor and adding deionised water to bring the reactor back up to its working volume. HF reactors were operated with a hydraulic retention time (HRT) of 25 days, and a solids retention time (SRT) greater than 150 days. SW reactors were operated with HRT greater than 150 days, and SRT 25 days.

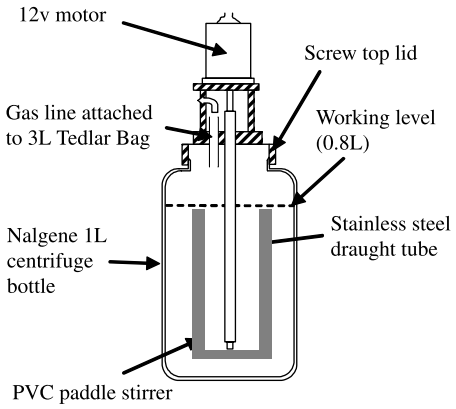

Figure 1 | Centrifuge bottle bioreactor.

Biogas production was measured daily, via water displacement in a volume-calibrated cylindrical gas collector after collection in Tedlar bags (SKC Ltd., Dorset UK). Gas composition samples were withdrawn from the headspace of the reactors once per week, analysed by gas chromatography and compared to a standard mix of 35% CO_2/65% CH_4, on a Varian CP-3800 gas chromatograph with thermal conductivity detector, column temperature 50°C. Volatile fatty acids (VFA) were measured weekly using a Shimadzu GC-2010 gas chromatograph (Shimadzu Europe Ltd., Manchester UK), with flame ionisation detector and FFAP capillary column SGE model BP-21 (SGE Europe Ltd., Milton Keynes UK), with helium as the carrier gas. Measurement of total ammonia nitrogen (TAN) and total solids were in accordance with standard methods

(APHA 2005). Alkalinity was measured using a three-point titration. While titration to 4.0 provides a measure of the total alkalinity in the system, this endpoint includes both carbonate alkalinity and alkalinity due to the salts of VFA, which have proton-accepting capacity at a low pH (below 5.75). Therefore, the total alkalinity can be misleading, as it includes alkalinity that does not actually buffer the system in the range needed by methanogens (Lahav *et al.* 2002). To separate the different buffering, Partial Alkalinity (PA) was measured to pH 5.75, while an endpoint of 4.3 was used to determine Intermediate Alkalinity (IA) due primarily to the salts of VFA (Ripley *et al.* 1986).

The reactors were run as duplicates, except that one reactor from each pair was supplemented with a trace element mixture on a weekly basis while the other reactor was not. The trace element solution followed the recipe of Gonzalez-Gil *et al.* (2001). Reactors were kept in a water bath with a constant temperature of 35°C, except for a thermal shock episode between days 105–108, when temperature in the water bath rose to 50°C. The organic loading rate (OLR) of $1.45\,gVS\,L^{-1}\,d^{-1}$ was constant throughout the trial except for adjustments during a three-week period following the thermal shock.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the food waste substrate used. Values are average and standard deviation of six replicates. In addition to being high in volatile solids, the substrate is high in lipid content due to the high level of fried foods in the collected feedstock.

Digestion results are shown in Figure 2. Though the two sets of reactors were similar in terms of alkalinity, TAN, VFA and specific methane production at the commencement of the study, they had diverged significantly after 40 days. Biomass growth in the SW reactors was not sufficient to keep up with removal rate on a 25-day SRT, as shown by the decrease in TS through the trial. These reactors showed an increase in TAN and VFA, as expected since these soluble compounds would be retained in the liquid fraction. Although total alkalinity (as titrated to pH 4.0) stayed fairly constant, the ratio of intermediate alkalinity to partial alkalinity rose rapidly between days 25–40, indicating the presence of high concentrations of VFA relative to bicarbonates in the system. This is confirmed by the rapid increase in VFA concentrations, ending in methanogenic failure as shown by the cessation of methane production. This was true for both reactors of this pair, regardless of trace element supplementation. Feeding to the SW reactors was stopped at day 67, following a number of days in which no gas was produced and total VFA levels continued to increase.

In contrast, the HF reactors maintained stable levels of TS, TAN, alkalinity and VFA throughout the portion of the trial during which uniform conditions were maintained, a period of 100 days. After this point, the temperature was raised from 35°C to 50°C from days 105–108, which resulted in an increase in VFA, indicating an imbalance during which the rate of acidogenesis and acetogenesis exceeded that of methanogenesis. Feeding was stopped for a period of 7 days (days 109–115) following the thermal shock, then resumed initially at full feed for one week (days 116–122) and then at 86% of full feed for two weeks (days 123–139), by which time VFA levels and methane production had returned to the levels preceding the thermal shock, indicating recovery of the system. The full OLR of $1.45\,gVS\,L^{-1}\,d^{-1}$ was then applied for the remainder of the run (days 140–150). Figure 2 shows the succession of VFA during the disturbance, in which acetic acid was the first VFA to rise, followed by propionic which continued to rise after acetic acid had returned to its previous levels, before also being consumed. Both HF reactors behaved similarly regardless of the presence or absence of trace element supplementation.

Previously it was found that trace element supplementation was required for stable digestion of this feedstock in CSTR digesters (Climenhaga & Banks 2007). In the current work, however, regular supplementation with trace

Table 1 | Characteristics of food waste substrate

Parameter	Average ± Std Deviation
Total Solids (%)	28.1 ± 0.25
Volatile Solids (% of TS)	95.5 ± 0.1
Total Kjeldahl Nitrogen (% of TS)	3.8 ± 0.2
Total Lipid Content (% of TS)	22.2 ± 0.2%
Chemical Oxygen Demand ($g/kg_{fresh\ weight}$)	422 ± 16

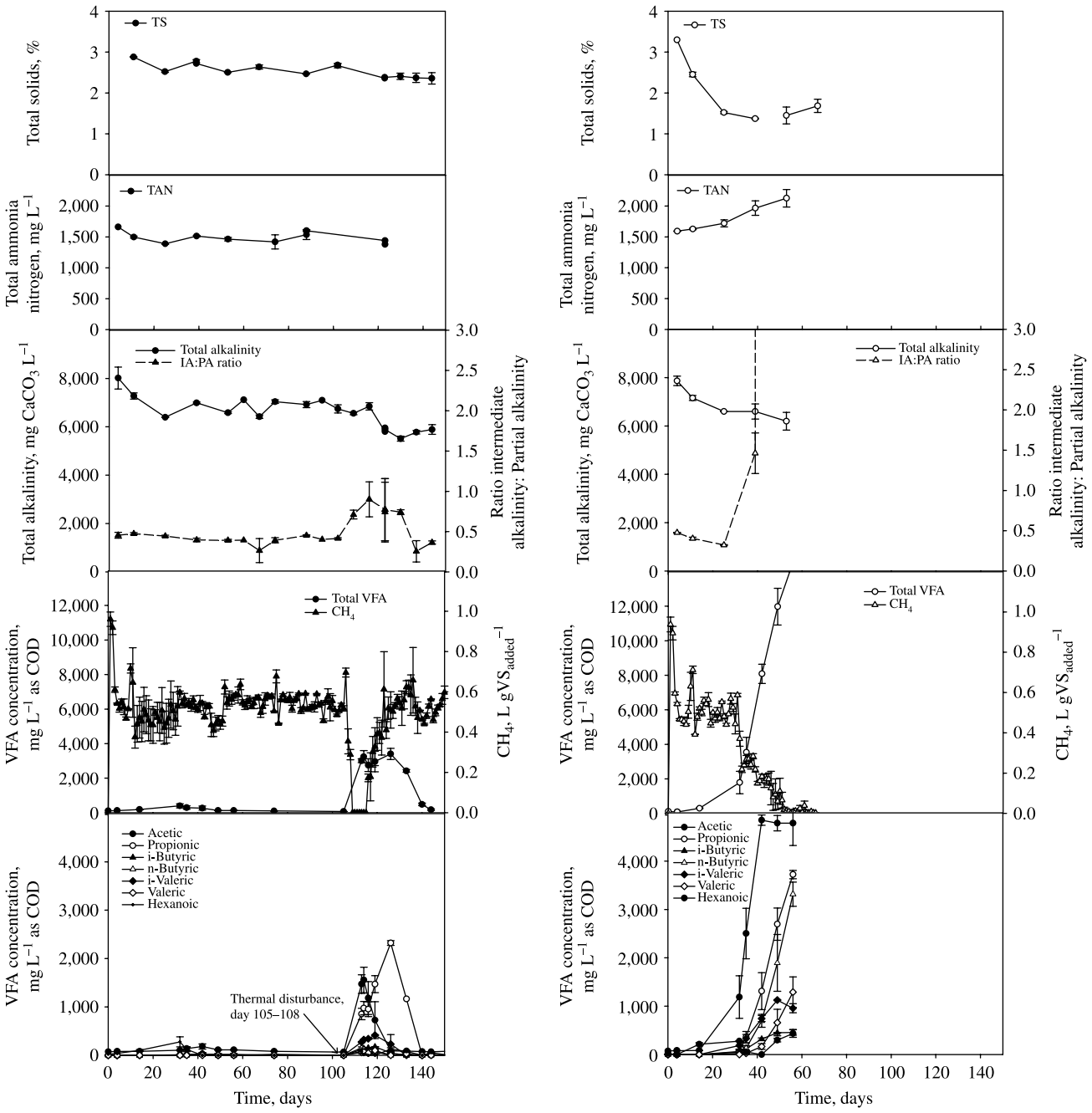

Figure 2 | Total solids, total ammonia nitrogen (TAN) concentrations, total alkalinity and ratio of intermediate: partial alkalinity, total VFA, specific methane production, and concentrations of individual VFA for reactors operated in hydraulic flush mode (left) or solids wastage mode (right). Points are average of two reactors, error bars represent range of values between the two reactors.

elements made no difference to reactor performance – it was not required for hydraulic flush reactors, and was not sufficient to prevent failure for solids wastage reactors. One possible explanation for this is the low solubility of micronutrients such as cobalt, iron and nickel within the pH range for anaerobic digestion. These metals are likely to adsorb to unhydrolysed particles and microbial cells rather than dissolving, and therefore most of the initial mass of any micronutrient in the reactor would be conserved in the solid fraction. Therefore in the case of the hydraulic flush

reactors, micronutrients would stay in the reactor rather than washing out over time with the liquid fraction, while in the case of the solids wasting reactors, micronutrients would be removed with the solids.

In comparing the relative performance of the two systems with extended SRT or extended HRT, some conclusions can be drawn as to the relative contributions that the liquid and solid components each make to the robustness of a CSTR system. If the SW system were to show better performance, this would provide evidence that the maintenance of soluble compounds was most important for stable operation. The fact that the HF system showed better performance provides support for the argument that the retention of biomass was most important for stable operation.

The fraction of alkalinity that is most useful within normal operating range for anaerobic digestion is bicarbonate alkalinity, as titrated to pH 5.75 (Ripley *et al.* 1986). Although bicarbonates are soluble compounds and were therefore expected to be flushed out with the liquid fraction, it was found that alkalinity could be regenerated by retaining the solid fraction, which contained active biomass and substrate. The shorter HRT also helped to prevent the build-up of VFA and ammonia, both of which are potentially inhibitory at high concentrations (Mata-Alvarez 2003).

Although reactor designs that keep solids in the system are common for treatment of liquid waste streams, the situation is different in many commercial solid waste digestion systems, where wasting of solids and retention of liquids is common. Many commercial digestion facilities have a solid–liquid separation step following digestion, after which the wasted solids are composted or landfilled and the liquid is recirculated to the pre-treatment system or digester (Blischke 2004). This work, however, has shown that retaining only the liquid fraction while removing solids results in accumulation of VFA and loss of biomass.

In the case of plants treating wastes with a significant fraction of materials high in lignin such as green wastes or paper, the removal of solids would be a sound strategy to avoid the build-up of materials that are resistant to degradation. In the case of highly degradable materials, however, such as the source-separated food waste substrate used in this study, it is apparent that keeping solids in the system is more beneficial for the regeneration of alkalinity and flushing of excess VFA, and does not result in a build-up of recalcitrant materials.

These results support the use of reactor types that retain solids in the system, such as the anaerobic sequencing batch reactor (ASBR) used in the treatment of fruit and vegetable wastes by Bouallagui *et al.* (2005). In that study, a two-stage system was used. The two-stage mode has the advantage of regulating the loading of volatile fatty acids to the methanogenic stage, and is a strategy employed to minimise the likelihood of process upset due to rapid overproduction of VFA. Two-stage digestion has been used in many recent studies on the processing of market wastes and other food wastes containing primarily fruit and vegetables (Mtz.-Viturtia *et al.* 1995, Kim *et al.* 2000, Bouallagui *et al.* 2004, Parawira *et al.* 2004, Wang *et al.* 2005). In the current work, however, it was found that in the case of the HF reactors a one-stage system was sufficient to maintain stable VFA levels and a fair degree of robustness in the face of disturbance, when solids were maintained in the system while liquids were flushed through. This is a favourable result as one-stage systems are generally favoured over two-stage systems at a commercial scale, due to their lower complexity and costs; currently over 87% of AD plants treating the organic fraction of municipal solid waste (OFMSW) in Europe are single-stage plants (De Baere 2006).

Single-stage digestion has been used at laboratory scale recently for feedstocks including OFMSW (Bolzonella *et al.* 2003; Gallert *et al.* 2003), wastewater and solid food wastes from food processing (Beccari *et al.* 1999; Carucci *et al.* 2005), and slaughterhouse wastes (Salminen & Rintala 2002; Siegrist *et al.* 2005). This last type of waste is high in proteins and lipids, and is relevant as the food waste feedstock used in this study also has a high lipid content. The primary breakdown products of lipids are long-chain fatty acids (LCFA); in single-stage digestion, the presence of methanogens within the same stage makes the degradation of LCFA by beta-oxidation more energetically favourable by the uptake of acetate and hydrogen produced in the process (Fox & Pohland 1994). Pereira *et al.* (2005) found an association of LCFA with microbial cells, and postulated that beta-oxidation may occur while LCFA is adsorbed to cells. They suggested solute transport limitations due to adsorbed LCFA as a possible mechanism for inhibition

effects observed by other investigators on hydrolysis (Neves *et al.* 2008) and methanogenesis (Koster & Cramer 1987; Hwu & Lettinga 1997). Keeping cells and unhydrolysed substrate in the system with the HF mode studied would allow sufficient time for degradation of the more slowly-degradable lipids and LCFA, as well as other compounds which may be affected by mass transfer limitations resulting from LCFA adsorption.

CONCLUSIONS

In this work it was shown that keeping only the solid fraction of digestate in the reactor enabled robust and stable operation over an extended period, as well as resilience to disturbance. The flushing of liquid allowed the removal of VFA and other dissolved compounds, but sufficient bicarbonate alkalinity was maintained in the medium due to biomass activity. In contrast, removal of the solid fraction and extended retention of the liquid fraction resulted in accumulation of VFA and loss of methanogenic activity. Future work could be focused on decreasing the liquid retention time further, as process parameters such as VFA levels and alkalinity indicated that the system was healthy and likely could operate at a higher hydraulic flush rate.

A solids retention mode of digestion has the benefit of allowing for syntrophic relationships, while reducing the likelihood of accumulation of potentially inhibitory substances such as VFA and ammonia. This mode of digestion, therefore, is promising for application to the processing of source-separated food wastes containing a mix of readily-degradable and slowly-degradable components.

ACKNOWLEDGEMENTS

This work was supported by funding from the UK Engineering and Physical Sciences Research Council (EPSRC) Sustainable Urban Environment (SUE) Programme grant GR/S79626/01, and the UK Overseas Research Students Awards Scheme (ORSAS). The invaluable assistance of Denise Cysneiros, Mark Walker, Sonia Heaven and Yue Zhang are gratefully acknowledged.

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