

Technical Report

Optimising inputs and outputs from anaerobic digestion processes

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Project investigators and organisation

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Abbreviations	
AAS	Atomic Absorption Spectrometer
ABPR	Animal By-Products Regulations
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ASTM	American Society for Testing and Materials
AT4	Four day Cumulative Oxygen Consumption
BMP	Biochemical Methane Potential
BMW	Biodegradable Municipal Waste
BSI	British Standards Institution
CHNSO	Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen
C:N	Carbon to Nitrogen ratio
COSHH	Control of Substances Hazardous to Health
CSTR	Completely Stirred Tank Reactor
CV	Calorific Value
DAF	Dissolved Air Flotation
DR4	Four day Dynamic Respiration rate
DRI	Dynamic Respiration Rate
DTPA	Diethylene Triamine Pentaacetic Acid
EA	Environment Agency
EC	Electrical Conductivity
FPD	Flame Photometric Detector
FW	Food Waste
GC	Gas Chromatograph
HAc	Acetic acid
HCL	Hollow Cathode Lamp
HEM	n-Hexane Extractable Material
HHV	Higher Heat Value
HPr	Propionic acid
IA	Intermediate Alkalinity
LCFA	Long Chain Fatty Acids
LHV	Lower Heat Value
MBT	Mechanical Biological Treatment
MSW	Municipal Solid Waste
NDF	Neutral Detergent Fibre
NDIR	Non-Dispersive Infrared Detector
NPK	Nitrogen, Phosphorus, and Potassium
PA	Partial Alkalinity
PPE	Personal Protective Equipment
PSD	Particle Size Distribution
PTE	Potentially toxic element
$r_{I/S}$	Inoculum-to-substrate ratio
RDF	Refuse Derived Fuel
SOUR	Specific Oxygen Uptake Rate
SBP	Specific Biogas Production
SMP	Specific Methane Production

Abbreviations	
SRB	Sulphate Reducing Bacteria
SRI	Static Respiration Index
SRT	Solids retention time
STP	Standard Temperature and Pressure
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TC	Total Carbon
TCD	Thermal Conductivity Detector
TIC	Total Inorganic Carbon
TK	Total Potassium
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TS	Total Solids
VBP	Volumetric Biogas Production
VFA	Volatile Fatty Acid
VMP	Volumetric Methane Production
VS	Volatile Solids
VSR	Volatile Solids Removal
WBP	Weekly Biogas Production
WMP	Weekly Methane Production
WRAP	Waste and Resources Action Programme
WW	Wet weight (equivalent to fresh matter)

Executive summary

Anaerobic digestion (AD) is an attractive waste treatment process in which both pollution control and energy recovery can be achieved. It involves the degradation and stabilisation of organic materials under anaerobic conditions by micro-organisms and leads to the formation of biogas (a mixture of carbon dioxide and methane) which is a renewable energy source, and a residual digestate that can be applied to land. A wide range of municipal, commercial, agricultural and industrial wastes have potential for anaerobic digestion because they contain high levels of biodegradable materials. Problems such as low methane yield and process instability are sometimes encountered in anaerobic digestion, however, restricting its use in certain circumstances.

The project aimed to provide a better understanding of some factors influencing the anaerobic stabilisation of biodegradable municipal waste (BMW). In particular the research addressed whether the process, as judged by volumetric biogas production, solids destruction and bio-stability of the residues, could be improved by co-digestion with other organic wastes from industry, commerce and agriculture. The research adopted a systematic approach based on an objective methodology to investigate the above factors. Two municipal waste streams (one source-segregated domestic food waste and one post-collection mechanically-recovered BMW) were selected as parallel substrates for a baseline study. These were blended with selected wastes arising from commercial, industrial and agricultural sectors. Both batch and semi-continuous anaerobic digesters were operated at laboratory and pilot scale. Their performance was monitored using a range of standard operating parameters and analytical measurements to assess the efficiency and long-term stability of the process. Suitable waste mixtures were suggested for stable and efficient anaerobic degradation, and optimal digestion conditions were identified based on the experimental results. Lastly, the digestate output was assessed for its biostability and potential value for land application.

Physico-chemical characterisation showed that source-segregated food waste had a high organic content and was rich in lipids and proteins, indicating the potential for a good biogas yield with a high methane content. These characteristics may also lead to inhibitory effects on the digestion process, however, as a result of the potential for volatile fatty acids formation and ammonia accumulation. The mechanically-recovered BMW had a lower organic matter content with a higher percentage of fibre, implying a lower biogas yield and methane content than food waste. The more favourable carbon to nitrogen (C:N) ratio of mechanically-recovered BMW should however lead to more stable digestion. The high nutrient content of food waste and its low concentration of potentially toxic elements (PTE) gave the digestate good properties as a fertiliser suitable for agricultural application. The mechanically-recovered BMW had higher concentrations of PTE and a lower nitrogen content, which may restrict its beneficial use.

Results from batch and semi-continuous experiments with mechanically-recovered BMW confirmed its operational stability: the organic loading rate (OLR) on the digesters based on volatile solids (VS) was successfully increased to $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ with a volumetric biogas production of $2.1 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ (corrected to standard temperature and pressure (STP)) and a methane content of 58%. The stability of operation was attributed to good buffering capacity, with a safe total ammonia nitrogen (TAN) concentration of 1.6 g l^{-1} and a pH of 7.4. It is possible that higher loadings could be achieved, as the TAN level will not rise

above 3.0 g l⁻¹ in a wet continuously stirred tank reactor (CSTR) process. It was not possible to determine an upper loading limit within the timescale of this project.

The digesters fed with food waste as a single substrate had a higher specific biogas yield and a higher percentage of VS removal compared with mechanically-recovered BMW in the early stages of continuous operation. As the duration of operation continued, however, there was a build-up of volatile fatty acids (VFA) and ammonia indicating long-term process instability. All laboratory-scale and larger-scale food waste digesters operated in the study showed similar patterns of accumulation.

Co-substrates were selected in the case of food waste to balance the C:N ratio, and for mechanically-recovered BMW to include readily-degradable high nitrogen content materials that would improve the overall biogas production and enhance the nutrient content of the digestate. The co-substrates for food waste were potato processing wastes, used office paper, card packaging, cattle slurry, and whey. Mechanically-recovered BMW was co-digested with blood and slaughterhouse waste (pig gut with flotation fat).

With respect to food waste digestion, the successful co-substrates capable of improving the performance were cattle slurry and card packaging. Addition of cattle slurry provides buffering capacity and essential elements, as well as a continuous inoculum of anaerobic microorganisms. Card packaging created a mixture with a similar nutrient composition to mechanically-recovered BMW, but without the gross physical contaminants (plastics, metal and glass) and PTE present in mechanically-recovered BMW. As well as lowering the overall nitrogen content of the feedstock mixture, card packaging also contributes some trace elements essential to the metabolic functioning of autotrophic methanogens. High nitrogen and high energy co-substrates produced less favourable conditions when used with mechanically-recovered BMW, and would be likely to restrict the overall loading, thereby negating any potential process advantages.

Pre-pasteurisation treatment did not show a significant effect on the rate of production or final biogas yield of the substrates tested. Whilst there is a requirement under the Animal By-products Regulations (ABPR) for pasteurisation of waste streams containing or contaminated by animal by-products to sanitise them and prevent pathogen transfer, the results of this work indicated that the option of pre-pasteurising waste streams before feeding them to the digester is unlikely significantly to affect the process efficiency of anaerobic digestion.

The research identified a requirement for addition of trace elements to digesters operating on food waste only. This is necessary to overcome deficiencies that will affect digestion performance by the inhibition of acetoclastic methanogenesis due to ammonia toxicity. It is proposed that further research is undertaken to develop methods of reducing the overall ammonia load in food waste digesters, or that co-substrates are used to balance the C:N ratio, provide essential elements and improve the overall efficiency of the digestion process.

1 Introduction

Anaerobic digestion (AD) is an attractive waste treatment process in which both pollution control and energy recovery can be achieved. It involves the degradation and stabilisation of organic materials under anaerobic conditions by micro-organisms and leads to the formation of biogas (a mixture of carbon dioxide and methane) which is a renewable energy source, and a residual digestate that can be applied to land. A wide range of municipal, commercial, agricultural and industrial wastes have potential for anaerobic digestion because they contain high levels of biodegradable materials. Problems such as low methane yield and process instability are sometimes encountered in anaerobic digestion, however, restricting its use in certain circumstances.

1.1 Aim and objectives

The overall aim of this project was to better understand the factors influencing the stabilisation of biodegradable municipal waste (BMW) in the anaerobic digestion process. In particular the research addressed whether the process, as judged by volumetric biogas production, solids destruction and bio-stability of the residues, could be improved by co-digestion with other organic wastes from commerce, industry and agriculture. Two municipal waste streams (one from source-segregated material and one after post-collection recovery) were selected as parallel baseline materials for anaerobic treatability studies, against which waste blending from commercial, industrial and agricultural sectors was compared. The study involved determination of the physico-chemical characteristics of the wastes and the operation of anaerobic digestion trials in both batch and semi-continuous processes using them as feedstock. The digestion performance resulting from different wastes / waste mixtures was then used to identify the basic factors controlling the efficiency and long-term stability of the anaerobic digestion process. Suitable wastes and waste blends for stable and efficient anaerobic degradation were suggested and the optimal digestion conditions were chosen based on the experimental results. Lastly, the digestate, the output of the digestion process, was assessed for its biostability and potential value for land application.

The specific technical and scientific objectives were as follows:

1. Characterisation of two municipal waste streams used in a baseline study: one from source-segregated material and one after post-collection segregation.
2. Identification and selection of potential co-substrate feedstocks from the commercial, industrial and agricultural sectors.
3. Characterisation of identified co-substrates for digestion.
4. Determination of biochemical methane potential (BMP) from the two municipal solid waste (MSW) substrates both in isolation and combination with co-substrates from commercial, industrial and agricultural sources, including a pre-pasteurisation step for animal by-products regulations (ABPR) substrates.
5. Laboratory-scale trials using both types of MSW and co-substrates to determine process loading rates and reactor conditions for optimisation of biogas production.

6. Digestion trials at a larger scale to validate laboratory data and establish standard operating conditions.
7. Assessment of the characteristics of digestates (including separation of the solid and liquid fractions) from different mixtures of substrates for their potential value as agricultural products.
8. Measurement of the biostability of the solids-separated fraction of digestates from different mixtures of substrates in relation to landfill acceptance criteria.

2 Analysis methodology for characterisation of wastes and digestate and for monitoring digester operation

A descriptive methodology was developed for the project in order to determine the characteristics of waste and digestate and to monitor digester operation. The criterion for the choice of tests was to maximise the understanding of the anaerobic digestion process and to assess the quality of the digestate for land application. Tests on the waste were to assess to what extent the physico-chemical properties, such as biochemical composition, macro-nutrient ratio, and essential trace elements influence its potential as anaerobic digestion feedstock. Tests on the digestate were to evaluate its potential value for land application and were therefore chosen to quantify nutrient availability and the concentration of contaminants likely to enter into the soil system. Tests on anaerobic digester operation were designed to examine the performance of the digestion process and identify the factors that control the rate and extent of anaerobic degradation. When combined with the other test results, the digester studies could be used to establish the overall mass and energy balance for the digestion system, and to help track the degree of degradation for each input component.

2.1 Suite of tests

The suite of tests adopted to carry out the research is shown in Figure 1.1; the superscripts following each test refer to the analysis methods given in Appendix 1. Composition analysis, total solids (TS) and volatile solids (VS), total organic carbon (TOC), total nitrogen (TN), biochemical methane potential (BMP) and dynamic respiration rate (DR4) are specified in the Environmental Agency (EA) guidance on monitoring mechanical biological treatment (MBT) and other pre-treatment processes for the Landfill Allowance Trading Schemes (England) 2005. Chemical Oxygen Demand (COD) is often used to express the loading on anaerobic digesters because of its stoichiometric relationship with methane production. This test is most appropriate for liquid wastewater samples, however, and is both more difficult and less accurate to perform with solid waste samples due to its limited range (0-400 mg l⁻¹, requiring small samples sizes and/or substantial dilution for most solid materials). It is generally accepted for solid substrate digestion that loading and degradation are more appropriately expressed in terms of volatile solids, sometimes referred to as organic dry matter (ODM) or Loss On Ignition (LOI)

Stability and potentially toxic elements (PTE) were assessed in accordance with criteria for landfill acceptance and from the proposed BSI PAS 110. The PAS 110 is being developed by the Waste and Resources Action Programme (WRAP) in association with the British Standards Institute, as the quality benchmark for digestates. The proposed BSI PAS 110 tests for pathogens and contamination by weed seeds and propagules were not included in the suite of tests for this project. This is because heating to 70 °C for 1 h in accordance with the Animal By-Products Regulations (ABPR) would without doubt reduce the pathogen indicator bacteria to zero in a laboratory-scale study, but in the industrial application the major risk is cross-contamination between clean and dirty ends of the process and this was something which could not be assessed in this project. In addition, at the scale of the project it is difficult to carry out meaningful plant response tests and therefore this test was excluded from the analysis list.

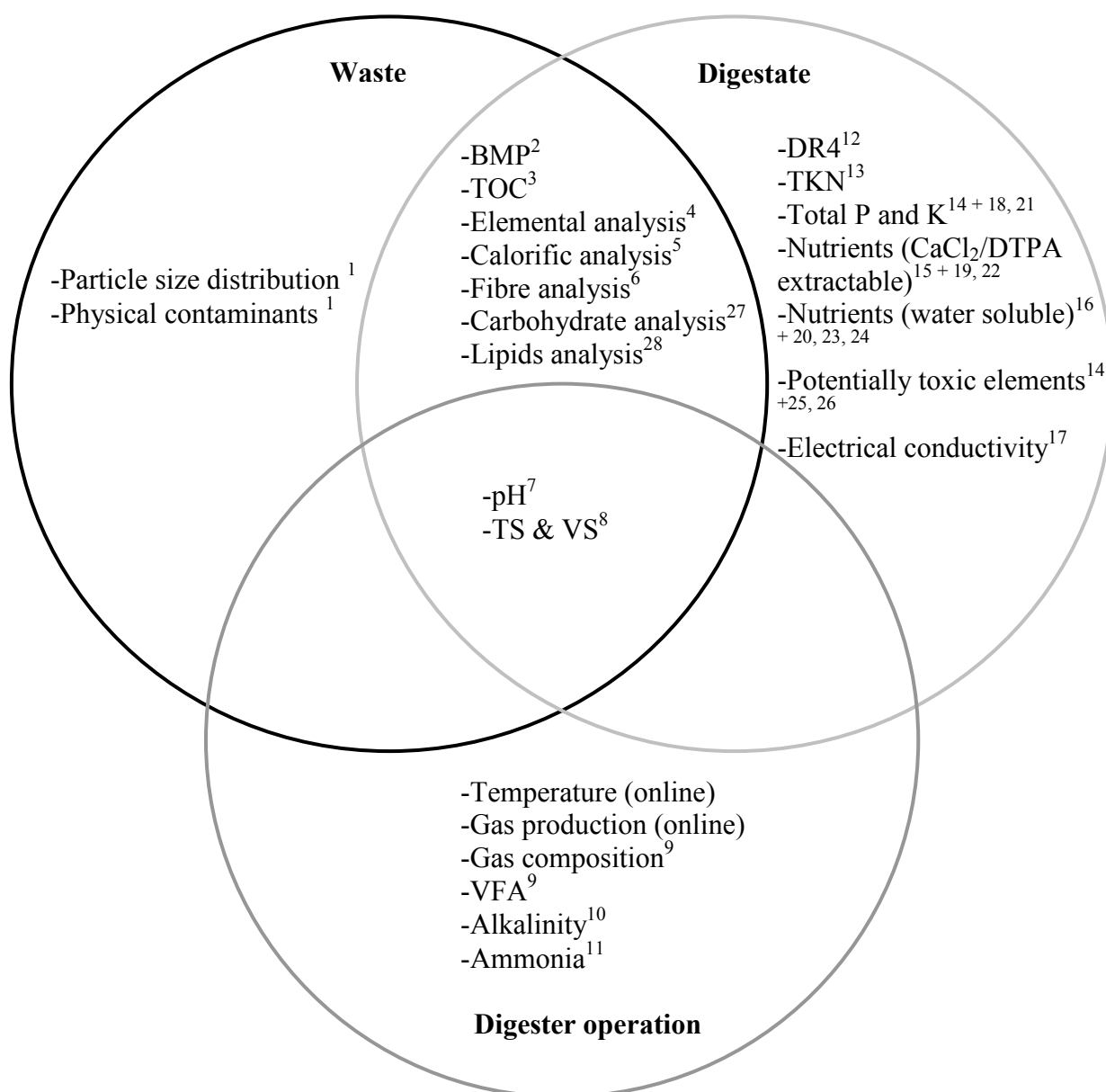


Figure 2.1 Suite of tests used in the research. Superscripts refer to method numbers in Appendix 1

2.2 Sample preparation

Considering the heterogeneity of waste and the fact that digestion is by an active microbial population, appropriate sample preparation is essential to achieve reliable analytical results. Therefore all sampling operations were carried out over a sufficiently short period of time and in such a way as to minimise any alternation in the characteristics of the samples.

2.2.1 Waste reception, homogenisation, sampling and storage

Waste streams were sampled and transported to the laboratory on the same day. The date and size of samples received were recorded on arrival, and they were kept in a cold room at 3 ± 1 °C overnight. Each sample was then thoroughly mixed to homogenise the waste,

with gentle breaking up of any lumps or agglomerates that had formed as a result of compression during transportation. About 10 kg of the waste was then sub-sampled using a simple random sampling approach before the analysis of particle size distribution and physical contaminants. Another 2-5 kg of sample was taken for the other tests. A larger sample size was necessary for the coarse and very heterogeneous wastes, and further sub-sampling took place after the bulk sample had been homogenised by grinding. After sampling, the remaining waste used in the digestion trials was packed with an identifying label and frozen at -18°C to prevent spoilage as a result of continuing microbial and chemical activity.

Once the sub-samples had been taken these were prepared for immediate analysis according to the methods given in Appendix 1, or preserved and stored at $3 \pm 1^{\circ}\text{C}$. Samples were stored in appropriate containers which were labelled with the sample description and the date of sampling. To maintain the sample integrity materials were analysed within 14 days of sampling.

2.2.2 Separation of the digestate into liquor and fibre fraction

The liquor and fibre fractions of the digestate were examined in parallel with the whole digestate in order to establish detailed information on the component parts. A typical commercial anaerobic digester separator has a mesh size of 0.5-1.5 mm, with material passing through the mesh being classified as liquor, whilst that retained on the surface of the mesh is classified as the fibrous material. In the laboratory, a plastic sieve with a 1 mm mesh was placed over a 1-litre glass beaker. The digestate material was poured (in 100 ml aliquots) onto the surface of the mesh, and was allowed to drain through the mesh for 10 minutes. Subsequently, a pressure of 1 kg was applied to the surface of the mesh for a further 5 minutes. The fibrous material was removed from the surface of the mesh, and the process repeated until sufficient quantities of the two materials had been collected.

The separation was carried out as soon as the digestate sample was collected. Once the separation process was complete, the liquor and fibre fraction were analysed immediately. If this was not possible the sample was stored at $3 \pm 1^{\circ}\text{C}$, for not longer than 14 days. At the start of the analysis the liquor was vigorously shaken by hand for 2 minutes.

2.2.3 Preparation of dried ground test samples

Some of the analytical procedures used to characterise the materials required the samples to be dried; these included TOC, elemental analysis, calorific analysis, and fibre analysis. In addition a reduction in particle size was required where analysis of the fresh samples had not been possible because of a lack of homogeneity. This included tests for total potassium, phosphorus, PTE, and essential trace elements.

The drying and grinding procedure was as follows: a portion of the test sample was dried at a temperature of $75 \pm 5^{\circ}\text{C}$ in a ventilated oven until a time when it crumbled to the touch. The dried sample was ground using a centrifuge mill with a 0.5 mm mesh sieve. In some cases it was necessary to reduce the sample dimensions prior to milling by coarse cutting using other mills or by hand. In all cases it was essential to ensure that the technique employed did not generate excess heat and that the final sample was representative of the original sample, i.e. the milling process did not exclude excessively hard particles or lose any dust.

Once dried and ground, the samples were stored in sealed polyethylene containers at room temperature until use.

When an analysis was carried out using a dried sample the results were expressed on a dry weight (dry matter) basis. This involved a separate determination of moisture content and this was carried out in parallel to the analytical test. If the results were required on a wet weight (fresh matter) basis this could be calculated simply by using the following expression:

$$C_w = \frac{C_d \times TS}{100}$$

where

C_w : Results on wet weight basis;

C_d : Results on dry weight basis;

TS: Total solids, %.

2.3 Quality control

Quality control of laboratory practices was undertaken specifically to achieve accurate and reliable analytical results. This covered waste sampling, accuracy and precision of results, and Health and Safety procedures.

To verify the bulk sampling procedure replicate samples were tested and results compared based on the solids and elemental analysis.

Accuracy of analysis was determined by known addition of a chemical standard to a sample and subsequent analysis to determine the percent recovery. Accuracy was expressed mathematically as percent recovery of the spiked standard material. For certain analytical tests known addition testing was done on a minimum of 10% of the samples. Quality control reference materials (hay powder for macro-nutrients and sewage sludge powder for PTE), were analysed monthly along with ordinary samples. When a result fell outside the control limits the cause of the failure was identified and rectified. In all cases replicate analysis of samples was carried out with variation expressed in terms of the standard deviation.

Good laboratory practice and appropriate Health and Safety procedures were followed in accordance with the University of Southampton's policies.

3 Physico-chemical characterisation of baseline waste streams

Two municipal waste streams, a source-segregated food waste and a post-collection mechanically-recovered biodegradable municipal waste (mechanically-recovered BMW), were selected for the baseline study. The physico-chemical characteristics were analysed in order to evaluate their anaerobic digestion potential (e.g. biochemical composition and elemental composition) and their land application potential after digestion (e.g. macro-nutrients and PTE content).

3.1 Collection and preparation of baseline waste streams

3.1.1 Source-segregated food waste

The food waste was collected from the Cwm Harry Estates, Newtown, Powys. This was a source-segregated collection scheme yielding 750 tonnes of material from households each year, which was sent to the Biocycle plant in Ludlow, Shropshire for anaerobic digestion. A 210 kg sample was taken from material delivered to the Biocycle plant on 30 March 2007. The waste was stripped from the biodegradable starch-based biobags and processed in a commercial shear shredder (RS404S, Untha Ltd, Germany) consisting of 4 counter-rotating shaft-mounted cutters with a 20 mm jaw spacing and an 80 mm rejection screen. Material rejected by the screen was subsequently recycled through the shredder until all material had passed the screen (i.e. minimum two-dimensional sizes were no larger than the aperture of the rejection screen). The sample was then transported, in sealed drums, on the same day to the laboratory. The shredded food waste was further processed by passing it through a macerating grinder (S52/010 Waste Disposer, Imperial Machine Company (IMC) Limited, Hertfordshire, UK). This produced a material of very homogeneous consistency which was further blended in a single container using a drill mixer, to give a mix any part of which could be considered representative of the entire batch.

The particle size distribution (PSD) of the mechanically macerated food waste was analysed using a wet sieving technique. The results (Figure 3.1) are expressed as the cumulative percentage oversize of the dry weight retained through a decreasing mesh size on the sieve. For example 10.1% of the material was larger than 1.4 mm and 4.4% was larger than 2.8 mm, therefore 5.7% was in the size range 1.4-2.8 mm. The results show that a substantial percentage (>80%) of the waste had a particle size less than 0.6 mm.

3.1.2 Post-collection mechanically-recovered BMW

100 kg of post-collection mechanically-recovered BMW was collected from Bursom recycling centre, Leicester, operated by Biffa Plc. Here the waste was continuously fed along a conveyor belt and into a 6.4 m diameter drum containing a large number of 5.5 kg steel balls. As the drum slowly rotated the balls broke down the waste into small pieces which passed through 80 mm slots in the drum and was fed into a trommel. This separated the material into two different sized fractions: 0-40 mm and 40-80 mm. The 40-80 mm fraction was passed through a magnetic separator for recovery of ferrous metals which were sent for recycling, and residuals went into a ballistic separator. This recovered plastic, paper and card which was baled as refuse derived fuel (RDF) and sent

as fuel to a cement kiln. The remainder went through an eddy current separator for recovery of non-ferrous metals for recycling, with the rest sent to landfill. The 0-40 mm fraction (mainly putrescibles) was put through a flip-flop slotted screen which removed excess water and then through a 5 mm grid. The material was then transferred to closed containers and, after a further wet densiometric separation stage to remove further plastics and glass, used as substrate for anaerobic digestion at Biffa's Wanlip plant. The waste sample for this project was taken from the closed containers before wet separation, on 2 March 2007.

The proportions of organic material and the different physical contaminants are shown in Table 3.1. The organic fraction, which represented 92% of the processed waste, had a mean particle size of 6.0 mm and more than 99% of the material was less than 13.2 mm, as shown in Figure 3.2. Overall the process was an effective mechanical sorting and particle size reduction process.

Table 3.1 Distribution of physical contaminants in different size fractions from the mechanically-recovered BMW taken from the Bursom recycling centre, Leicester

Size Fraction (mm)	Percentage on a wet weight basis (%)				
	Glass	Metal	Plastic	Non-combustibles	Biodegradable organic matter
20.0~13.2	3.60	0.00	8.06	0.00	88.3
13.2~6.7	2.55	0.21	8.70	0.56	88.0
6.7~5.0	2.34	0.27	3.74	0.87	92.8
<5.0	4.02	0.04	0.80	0.11	95.0
total	3.08	0.16	4.34	0.46	92.0

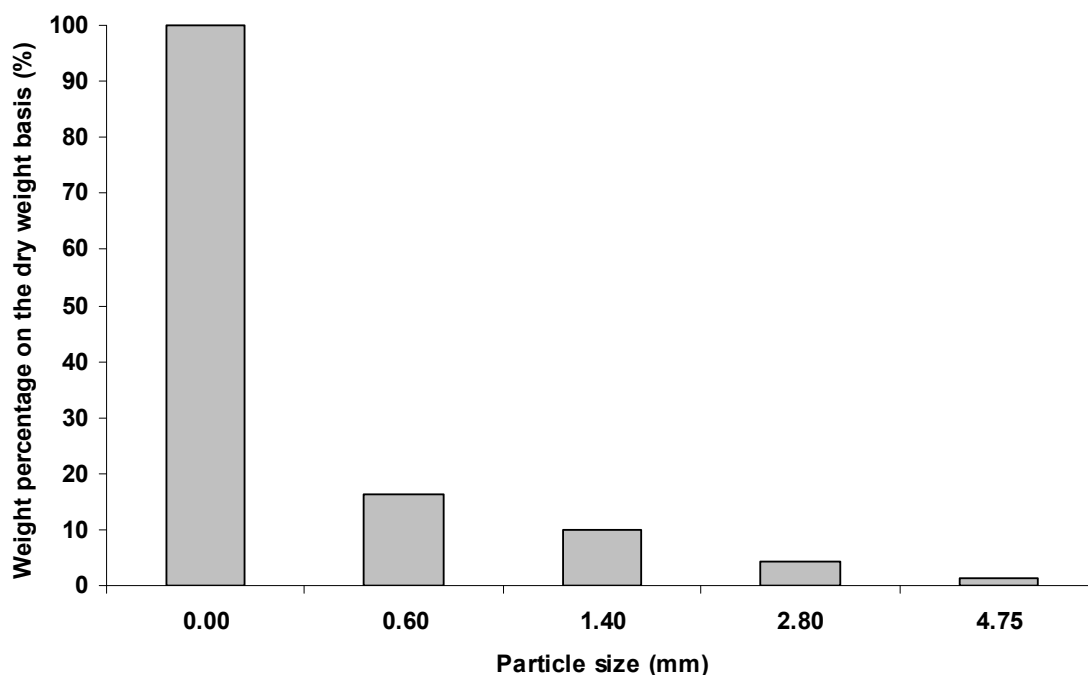


Figure 3.1 Cumulative oversize percentage by dry weight of particles of the food waste which sequentially passed through sieves of mesh sizes 4.75mm to 0.6mm.

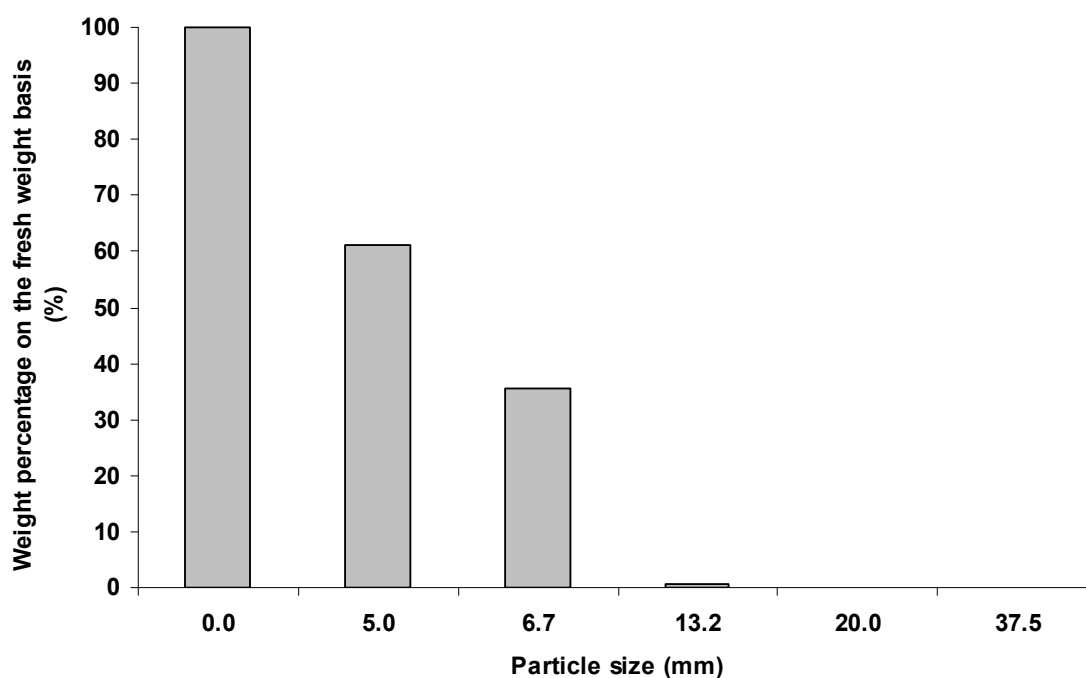


Figure 3.2 Cumulative oversize percentage by fresh weight of particles of mechanically-recovered BMW which sequentially passed through sieves of mesh sizes 37.5 mm to 5.0mm.

3.2 Determination of physico-chemical characteristics of the baseline waste streams

The baseline waste streams were characterised with respect to three categories of physico-chemical properties that: determine operational parameters for the anaerobic digestion process; predict the anaerobic digestion performance; and define the potential for land application of residue (digestate) after digestion. In addition, elemental analysis was also conducted to compare the experimental results with theoretical predictions using the Buswell equation for methane potential and the Du Long equation for calorific value. The analytical data are given in Tables 3.2, 3.3, 3.4 and 3.5; at least three replicates were taken for each sample to show the range of deviation from this average.

There were distinct differences in the physico-chemical characteristics of the two baseline waste streams. Source-segregated food waste had a much higher water content, also a higher volatile solids content and lower inert fraction compared to mechanically-recovered BMW when expressed on a dry matter basis. Food waste had a higher percentage of carbohydrates, lipids and proteins on a volatile solids basis. The absence of paper and card in food waste also gave it a lower fibre content.

The food waste had relatively high concentrations of chromium and nickel compared to other heavy metals in the sample; it is suspected that these came from pre-treatment equipment rather than the food waste itself. The mechanically-recovered BMW had concentrations of toxic heavy metals substantially higher than found in source-segregated food waste, and cadmium and mercury were present compared to being below the detection threshold in the food waste.

Table 3.2 Characteristics of the baseline waste streams

	Food waste	Mechanically-recovered BMW
pH (1:5)	4.71 ± 0.01	6.39 ± 0.01
TS (% WW)	23.74 ± 0.08	52.83 ± 0.63
VS (% WW)	21.71 ± 0.09	33.55 ± 0.63
VS (% TS)	91.44 ± 0.39	63.52 ± 1.89
TOC (% TS)	47.6 ± 0.5	34.8 ± 1.1
TKN (% TS)	3.42 ± 0.04	1.39 ± 0.08
TOC / TKN	13.9 ± 0.2	25.0 ± 1.6
Biodegradable C ¹ / TKN	13.6 ± 0.2	19.1 ± 1.6
CV (kJ g ⁻¹ TS)	20.7 ± 0.2	13.9 ± 0.2

¹ Biodegradable carbon was calculated by deducting lignin carbon from TOC. The formula for Lignin was chosen as C_{9.94}H_{12.82}O_{2.94}.

Table 3.3 Biochemical composition of baseline waste streams on a VS basis

	Food waste	Mechanically-recovered BMW
Carbohydrates ¹ (g kg ⁻¹)	453 ± 17	340 ± 7
Lipids ² (g kg ⁻¹)	151 ± 1	68.6 ± 5.4
Crude proteins (g kg ⁻¹)	235 ± 3	130 ± 7
Hemi-cellulose (g kg ⁻¹)	38.1 ± 3.7	52.2 ± 12.4
Cellulose (g kg ⁻¹)	50.4 ± 1.6	252 ± 36
Lignin (g kg ⁻¹)	16.5 ± 0.2	184 ± 26

¹ in equivalent glucose

² n-hexane extractable material (HEM).

Table 3.4 Nutrient and PTE content of the baseline waste streams on a TS basis

	Food waste	Mechanically-recovered BMW
TKN (g kg ⁻¹)	34.2 ± 0.4	13.9 ± 0.8
TP (g kg ⁻¹)	5.41 ± 0.32	2.17 ± 0.25
TK (g kg ⁻¹)	14.3 ± 0.8	4.26 ± 0.37
Cd (mg kg ⁻¹)	< 1.0	1.50 ± 0.37
Cr (mg kg ⁻¹)	29.0 ± 1.2	263 ± 11
Cu (mg kg ⁻¹)	7.20 ± 0.81	107 ± 10
Hg (mg kg ⁻¹)	< 0.010	0.179 ± 0.018
Ni (mg kg ⁻¹)	7.0 ± 2.9	97.0 ± 2.9
Pb (mg kg ⁻¹)	< 10	162 ± 10
Zn (mg kg ⁻¹)	33 ± 11	259 ± 4

Table 3.5 Elemental analysis of baseline waste streams

		Food waste	Mechanically-recovered BMW
% of TS	N	3.42 ± 0.04	1.39 ± 0.08
	C	47.9 ± 0.5	35.1 ± 1.1
	H	7.03 ± 0.63	5.06 ± 0.32
	S	0.15 ± 0.01	0.27 ± 0.04
	O	34.3 ± 2.5	25.1 ± 1.2
% of VS	N	3.76 ± 0.04	2.08 ± 0.12
	C	52.1 ± 0.5	51.9 ± 1.6
	H	7.70 ± 0.69	7.56 ± 0.48
	S	0.16 ± 0.01	0.40 ± 0.06
	O	36.4 ± 2.8	34.9 ± 1.9

To test the anaerobic treatability both batch (section 4) and semi-continuous (section 5) digestion trials were conducted using the baseline wastes as sole substrates.

4 Biochemical methane potential of baseline waste streams

A batch anaerobic digestion trial, also known as biochemical methane potential (BMP) test, was conducted for both of the baseline municipal waste streams in parallel with analysis of their physico-chemical characteristics (see section 3). The BMP value provides a baseline against which the specific methane yield of a digester can be compared for a particular substrate.

Although it is possible to estimate the methane potential of a substrate from theoretical considerations based on the biochemical composition of the material, this involves a substantial chemical testing procedure and a number of assumptions which may not be justified. As an alternative, the BMP test provides a practical way of measuring the generation of methane under optimal conditions. This takes place in a batch culture where the inoculum-to-substrate ratio is very favourable, providing buffering and an active methanogenic consortium. The test is carried out not only to determine the methane potential of the substrate but also to show the kinetics of the gas production; this cannot be established from theoretical considerations. Once the gas production curve has been determined practically it is then possible to fit this curve to mathematical expressions which describe its shape. Very often a first order rate equation is used allowing the rate of reaction to be expressed by means of simple constants. Other more complex mathematical models have also been used to simulate more accurately the shape of the gas production curve.

The method for the determination is detailed in Appendix 1 and was applied at two different inoculum-to-substrate ratios ($r_{I/S}$). For each of the baseline wastes, fifteen digesters each of 1.4 litres working capacity were used at a mesophilic ($36 \pm 1^\circ\text{C}$) temperature. These were set up as follows: six digesters were charged with waste at $r_{I/S}$ of 2; a further six digesters were charged with waste at $r_{I/S}$ of 4; and three digesters were operated as controls without substrate addition.

4.1 Further analysis of the baseline waste streams

The solids content and elemental composition of the two waste samples used in the BMP test were determined as a check on their homogeneity and to ensure that changes had not occurred on storage. The results from these two sets of analysis confirmed that the BMP samples were closely similar to the original material (Table 3.5 and 4.1).

4.2 Inoculum for BMP test

The digestate liquor from semi-continuous digesters fed on food waste and mechanically-recovered BMW (described in section 5) was used as the inoculum for the BMP test. The characteristics of the two inoculums are shown in Table 4.2.

Table 4.1 Solids contents and elemental analysis of BMP substrates

		Food waste	Mechanically-recovered BMW
TS (% WW)		23.48 ± 0.06	52.36 ± 0.63
VS (% WW)		21.50 ± 0.04	32.63 ± 0.66
VS (% TS)		91.55 ± 0.09	62.32 ± 1.04
% of TS	N	3.87 ± 0.27	1.66 ± 0.12
	C	48.3 ± 1.0	36.1 ± 0.6
	H	7.44 ± 0.36	5.36 ± 0.15
	S	0.15 ± 0.01	0.31 ± 0.04
	O	35.3 ± 1.3	27.0 ± 1.3
% of VS	N	4.23 ± 0.30	2.33 ± 0.17
	C	52.4 ± 1.1	50.3 ± 0.8
	H	8.14 ± 0.39	7.54 ± 0.21
	S	0.16 ± 0.01	0.44 ± 0.06
	O	37.5 ± 1.4	36.2 ± 1.9

Table 4.2 Physico-chemical parameters of BMP inoculums

	Food waste inoculum	Mechanically-recovered BMW inoculum
pH	7.51 ± 0.01	7.60 ± 0.01
TS (% WW)	3.78 ± 0.00	2.29 ± 0.01
VS (% WW)	2.41 ± 0.00	1.28 ± 0.01
VS (% TS)	63.8 ± 0.1	56.1 ± 0.5
PA (g CaCO ₃ l ⁻¹)	8.87 ± 0.06	4.66 ± 0.02
IA (g CaCO ₃ l ⁻¹)	3.35 ± 0.06	1.47 ± 0.05
TA (g CaCO ₃ l ⁻¹)	12.2 ± 0.0	6.14 ± 0.05
IA : PA (Ripley ratio)	0.38 ± 0.01	0.32 ± 0.01
TC (g C l ⁻¹)	14.3 ± 0.3	8.36 ± 0.08
TOC (g C l ⁻¹)	12.2 ± 0.1	6.66 ± 0.21
CV (kJ g ⁻¹ TS)	12.5 ± 0.00	11.8 ± 0.04
EC (S m ⁻¹)	1.59 ± 0.01	0.855 ± 0.006
Acetic acid (mg l ⁻¹)	160 ± 1	81 ± 1
Other VFAs (mg l ⁻¹)	< 5	< 5
Hemi-cellulose (% of VS)	6.68 ± 0.67	7.50 ± 1.76
Cellulose (% of VS)	8.37 ± 0.84	8.78 ± 0.13
Lignin (% of VS)	26.6 ± 0.3	26.0 ± 0.1
TAN (g NH ₃ -N l ⁻¹)	1.89 ± 0.01	0.708 ± 0.008
TKN (g N l ⁻¹)	3.30 ± 0.00	1.46 ± 0.01
Dissolved K (g l ⁻¹)	1.22 ± 0.01	0.574 ± 0.005
TK (g l ⁻¹)	1.31 ± 0.03	0.626 ± 0.014
Dissolved reactive P (mg l ⁻¹)	25.5 ± 0.1	11.3 ± 0.0
TP (mg l ⁻¹)	320 ± 25	112 ± 21

Table 4.2 continued Physico-chemical parameters of BMP inoculums

		Food waste inoculum	Mechanically-recovered BMW inoculum
Cd (mg l ⁻¹)		0.056 ± 0.006	0.063 ± 0.002
Cr (mg l ⁻¹)		3.20 ± 0.39	2.89 ± 0.22
Cu (mg l ⁻¹)		3.29 ± 0.10	4.16 ± 0.32
Hg (µg l ⁻¹)		3.48 ± 0.29	8.39 ± 0.11
Ni (mg l ⁻¹)		2.42 ± 0.13	2.39 ± 0.15
Pb (mg l ⁻¹)		1.15 ± 0.09	6.05 ± 0.14
Zn (mg l ⁻¹)		6.15 ± 0.37	16.2 ± 1.3
% of TS using air-dried sample	N	4.07 ± 0.07	3.71 ± 0.05
	C	34.0 ± 0.4	31.8 ± 0.4
	H	5.14 ± 0.09	4.74 ± 0.17
	S	0.43 ± 0.03	0.67 ± 0.04
	O	22.5 ± 0.8	23.4 ± 1.0
% of VS using air-dried sample	N	6.33 ± 0.11	6.18 ± 0.08
	C	52.9 ± 0.6	53.0 ± 0.7
	H	8.00 ± 0.14	7.90 ± 0.28
	S	0.67 ± 0.05	1.12 ± 0.07
	O	35.0 ± 1.2	39.0 ± 1.7

4.3 Methane production in BMP test

The BMP tests ran for 80 and 82 days with food waste and mechanically-recovered BMW respectively. The results are shown in Figures 4.1 and 4.2. The error bars represent standard deviation but are not plotted for every data point. It can be seen from Figures 4.1 and 4.2 that the methane production rates in both test sets can be clearly separated into two phases: an initial rapid production for the first 3 days followed by a slower rate over the rest of the test. The methane percentage in the biogas produced from the food waste was 60.4% and 61.0% for the inoculum-to-substrate ratios of 2 and 4 respectively. For the mechanically-recovered BMW a lower methane percentage was found with 55.1% and 59.0% at the ratios of 2 and 4 respectively. Within the two sets of results for mechanically-recovered BMW a clear difference was found, with a higher CO₂ production at the inoculum-to-substrate ratio of 2.

Table 4.3 gives the BMP values obtained, the theoretical BMP values estimated based on the biochemical composition of wastes, and the predicted maximum achievable BMP value calculated based on the elemental composition using the Buswell equation. The results from food waste at both ratios and BMW at the inoculum-to-substrate ratio of 4 were around 10% lower than the theoretical BMP values based on their biochemical composition. The reason for this was investigated by analysis of the digestates at the end of the test to establish the degree of degradation of the lipid and fibre components (see section 4.4). It is clear from Table 4.3 that the maximum theoretical BMP calculated using the Buswell equation is much higher than the experimental values, especially for BMW. This is because BMW contains a relatively large amount of lignin, which is a refractory organic component and difficult to break down biologically. If a carbon mass balance is carried out based on experimental values for VS destruction and the biogas composition, the predicted BMP for food waste is 0.467 STP m³ kg VS_{added}⁻¹ and for BMW the BMP is 0.349 STP m³ kg VS_{added}⁻¹. Both these values are within 5% of the

experimentally determined value which supports the accuracy of the analysis for volatile solids, biogas composition and elemental carbon.

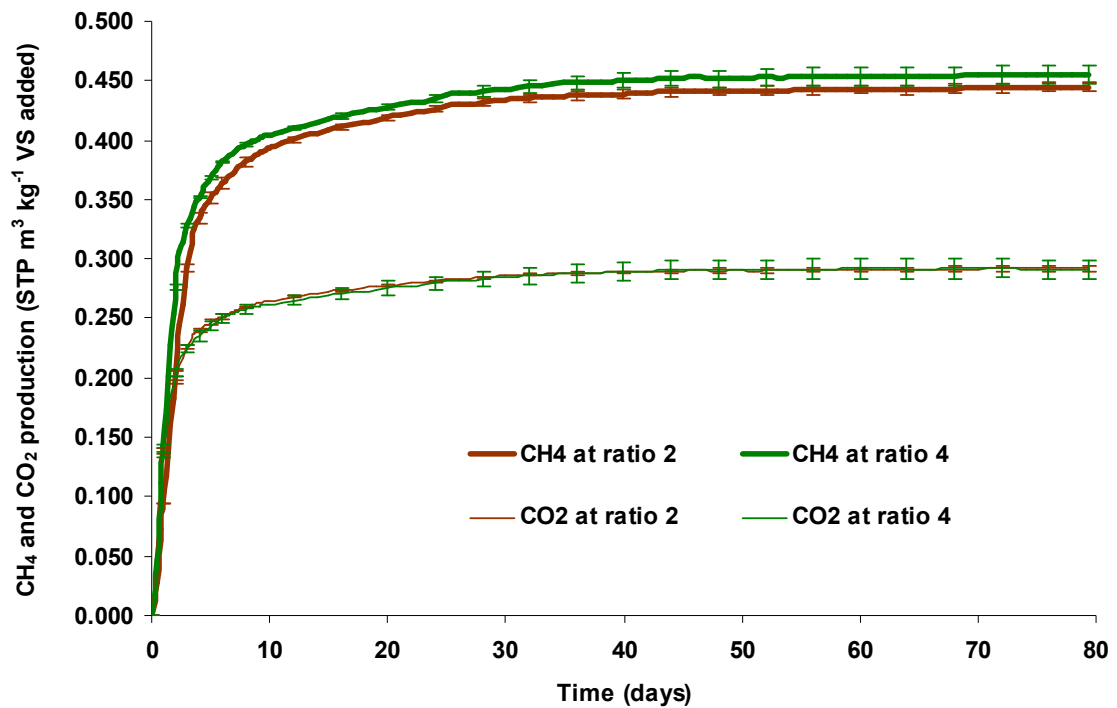


Figure 4.1 BMP assay of source-segregated food waste at inoculum-to-substrate volatile solids ratios of 2 and 4

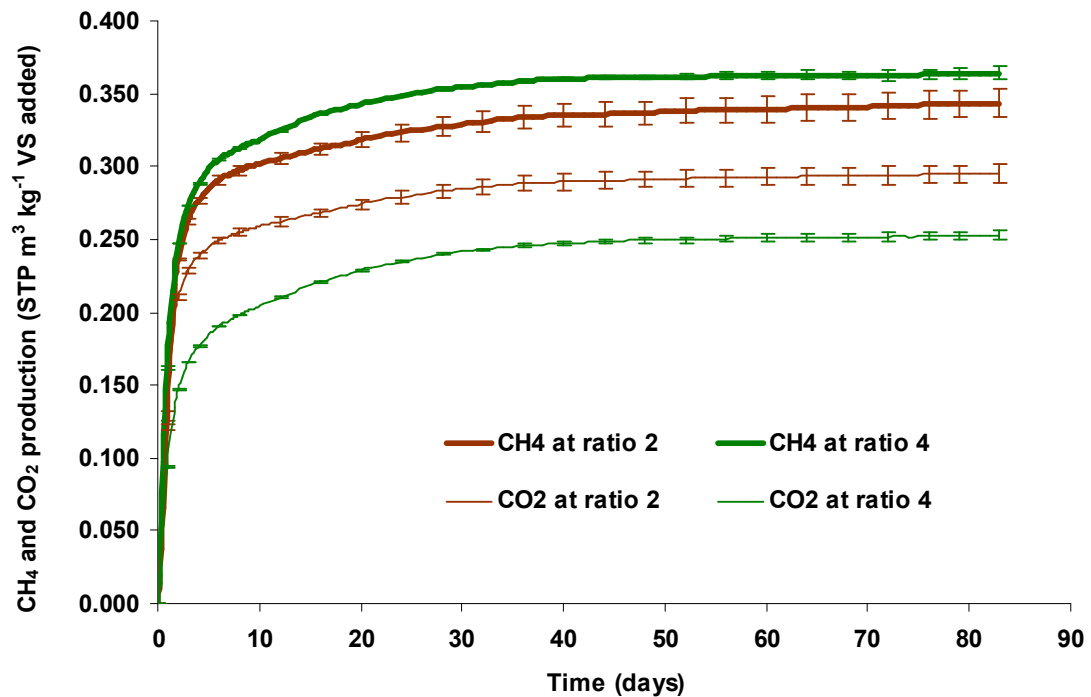


Figure 4.2 BMP assay of post-collection mechanically-recovered BMW at inoculum-to-substrate volatile solids ratios of 2 and 4

Table 4.3 Comparison of CH₄ production from BMP tests with the theoretical BMP value (STP m³ CH₄ kg⁻¹ VS_{added})

	Food waste		Mechanically-recovered BMW	
	Ratio 2	Ratio 4	Ratio 2	Ratio 4
Experimental value	0.445±0.004	0.456±0.007	0.344±0.010	0.364±0.004
Based on biochemical composition ¹	0.494		0.401	
Using Buswell equation ²	0.547		0.557	
Carbon balance	0.467		0.349	

¹ Angelidaki I. and Ellegaard L. (2003).² Symons, G.E. and Buswell, A.M. (1933).

To determine the kinetic constants, the specific methane production was modelled using two sets of assumptions: simple first-order degradation (Model 1), and a pseudo-parallel first-order model (Model 2). For model 1 the methane production is given by

$$Y = Y_m (1 - e^{-kt}) \quad [1]$$

where:

Y is the cumulative methane yield at time t

Y_m - is the ultimate methane yield

k - is the first order rate constant

Rao *et al.* (2000) suggested that when using organic solid waste materials as the substrate, it may be better to consider that the gas production curve corresponds to the rapid breakdown of readily degradable components followed by a much slower degradation of the fibrous proportion of the material. The methane production is therefore governed by two rate constants k₁ and k₂ rather than by a single constant:

$$Y = Y_m (1 - Pe^{-k_1 t} - (1-P) e^{-k_2 t}) \quad [2]$$

where:

Y is the cumulative methane yield at time t

Y_m is the ultimate methane yield

k₁ is the first order rate constant for the proportion of readily degradable material

k₂ is the first order rate constant for the proportion of less readily degradable material

P is the proportion of readily degradable material

The kinetic constants obtained from these two modelling approaches are given in Table 4.4. It can be seen that the simple first-order model (Model 1) gives only a moderately good fit to the experimental data (R² ≈ 0.962~0.989). A much better fit is obtained by model 2 (R² ≈ 0.996~0.998), especially for the mechanically-recovered BMW.

Table 4.4 Kinetic constants of CH₄ production from BMP tests from modelling

	Food waste				Mechanically-recovered BMW			
	Ratio 2		Ratio 4		Ratio 2		Ratio 4	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Y _m	0.445	0.445	0.456	0.456	0.344	0.344	0.364	0.364
P	1	0.87	1	0.83	1	0.83	1	0.80
k ₁	0.33	0.41	0.37	0.60	0.52	0.77	0.43	0.88
k ₂		0.04		0.05		0.05		0.06
R ²	0.989	0.996	0.979	0.998	0.967	0.996	0.962	0.998

4.4 Analysis of BMP digestates

The characteristics of the BMP digestates are given in Table 4.5. Plant nutrient concentrations (NPK) and PTE content was also analysed (Table 4.6) and compared with the calculated value according to the concentrations in each substrate and inoculum.

This set of analyses was also carried out to determine the partitioning of nutrients between the solid and the liquid phase (i.e. the percentages of nutrients readily available to plants), and as an overall check on the reliability of the analysis. The latter is possible because the BMP test is carried out in a closed system with respect to plant nutrients NPK and a mass balance can therefore be established for each component. The total ammonia nitrogen (TAN) was around 60~70% of total Kjeldahl nitrogen (TKN), the dissolved potassium accounted for 90% of total potassium (TK), and the dissolved reactive phosphorus was less than 10% of total phosphorus (TP). It also can be seen from Table 4.6 that the mass balance calculated values were very close to the measured values except for Cr, Ni, and perhaps Pb. The reason for the much higher Cr and Ni concentrations in BMP digestates was thought to be due to the release of these two metals from the stainless steel stirrers in the BMP digesters. The reason for higher Pb concentrations was not clear, but could possibly be due to the low accuracy of Pb measurement as a result of the high baseline drift of the Pb lamp used in the atomic absorption spectrometer (AAS).

Table 4.5 Characteristics of the BMP digestates

	Food waste			Mechanically-recovered BMW		
	Ratio 2	Ratio 4	Control	Ratio 2	Ratio 4	Control
pH	7.92±0.01	7.90±0.01	7.91±0.01	7.79±0.00	7.75±0.01	7.82±0.02
TS (% WW)	3.66±0.03	3.61±0.01	3.56±0.00	2.47±0.03	2.26±0.02	2.04±0.00
VS (% WW)	2.29±0.03	2.22±0.01	2.13±0.01	1.36±0.04	1.22±0.02	1.12±0.00
VS (% TS)	61.3±0.5	61.5±0.1	59.9±0.3	54.9±1.3	54.0±0.7	54.9±0.2
PA (g CaCO ₃ l ⁻¹)	10.5±0.1	10.1±0.1	9.73±0.14	4.69±0.02	4.86±0.14	4.59±0.33
IA (g CaCO ₃ l ⁻¹)	3.44±0.05	3.61±0.23	3.52±0.09	2.75±0.15	2.12±0.14	2.08±0.28
TA (g CaCO ₃ l ⁻¹)	14.0±0.1	13.7±0.1	13.3±0.1	7.44±0.15	6.98±0.02	6.67±0.09
IA:PA (Ripley ratio)	0.33±0.01	0.36±0.03	0.36±0.01	0.59±0.03	0.44±0.04	0.46±0.09
TC (g C l ⁻¹)	13.8±0.1	13.7±0.2	13.5±0.4	8.57±0.25	8.01±0.01	7.44±0.08
TOC (g C l ⁻¹)	10.7±0.2	10.6±0.1	10.5±0.4	6.89±0.22	6.49±0.02	5.88±0.18
VFAs (mg l ⁻¹)	< 5	< 5	< 5	< 5	< 5	< 5

Table 4.6 Comparison of nutrient and PTE concentrations in the BMP digestates with the calculated values based on mass balance

Food waste	Ratio 2		Ratio 4		Control	
	Calculated	Measured	Calculated	Measured	Calculated	Measured
TAN (g NH ₃ -N l ⁻¹)	-	2.49±0.03	-	2.37±0.05	-	2.25±0.02
TKN (g N l ⁻¹)	3.64	3.64±0.02	3.52	3.51±0.01	3.39	3.35±0.00
Dissolved K (g l ⁻¹)	-	1.31±0.01	-	1.24±0.01	-	1.16±0.01
TK (g l ⁻¹)	1.46	1.42±0.01	1.41	1.36±0.00	1.35	1.31±0.03
Dissolved reactive P (mg l ⁻¹)	-	20.8±0.2	-	19.4±0.1	-	17.5±0.5
TP (mg l ⁻¹)	379	334±9	355	328±4	329	306±6
Cd (mg l ⁻¹)	0.055	0.049±0.004	0.056	0.055±0.003	0.058	0.056±0.003
Cr (mg l ⁻¹)	3.48	9.93±0.21	3.39	8.88±1.00	3.30	16.8±0.7
Cu (mg l ⁻¹)	3.30	3.36±0.02	3.34	3.42±0.02	3.39	3.52±0.01
Hg (µg l ⁻¹)	3.39	3.43±0.07	3.48	3.36±0.10	3.58	3.39±0.10
Ni (mg l ⁻¹)	2.54	5.70±0.07	2.52	5.58±0.16	2.49	8.79±0.19
Pb (mg l ⁻¹)	1.12	1.41±0.05	1.15	1.69±0.07	1.18	1.52±0.08
Zn (mg l ⁻¹)	6.61	6.79±0.07	6.48	6.72±0.05	6.33	6.51±0.02

Table 4.6 continued Comparison of nutrient and PTE concentrations in the BMP digestates with the calculated values based on mass balance

Mechanically-recovered BMW	Ratio 2		Ratio 4		Control	
	Calculated	Measured	Calculated	Measured	Calculated	Measured
TAN (g NH ₃ -N l ⁻¹)	-	0.982±0.005	-	0.979±0.067	-	0.966±0.057
TKN (g N l ⁻¹)	1.59	1.61±0.01	1.54	1.53±0.01	1.49	1.47±0.00
Dissolved K (g l ⁻¹)	-	0.610±0.003	-	0.585±0.007	-	0.560±0.007
TK (g l ⁻¹)	0.675	0.671±0.016	0.661	0.646±0.010	0.639	0.620±0.004
Dissolved reactive P (mg l ⁻¹)	-	13.8±0.1	-	8.4±0.0	-	10.3±0.0
TP (mg l ⁻¹)	134	137±6	124	125±0	115	123±3
Cd (mg l ⁻¹)	0.079	0.077±0.004	0.068	0.072±0.001	0.064	0.065±0.002
Cr (mg l ⁻¹)	5.27	10.9±0.2	4.14	24.8±0.2	2.95	11.6±0.0
Cu (mg l ⁻¹)	5.17	5.03±0.06	4.78	4.73±0.01	4.25	4.38±0.02
Hg (µg l ⁻¹)	10.3	10.3±0.1	9.46	9.88±0.48	8.57	9.01±0.15
Ni (mg l ⁻¹)	3.40	6.20±0.10	2.92	11.5±0.2	2.44	6.06±0.05
Pb (mg l ⁻¹)	7.78	8.73±0.05	6.98	8.16±0.05	6.18	6.61±0.06
Zn (mg l ⁻¹)	19.1	19.3±0.0	17.8	17.8±0.1	16.6	16.7±0.1

The calorific value of the BMP digestates was also measured, and thus the total calorific load entering the digester, leaving the digester as digestate, and output as methane produced in the BMP system can be compared; these are shown in Table 4.7. For this calculation the gross calorific value of methane was taken as 36 MJ STP m⁻³. It can be seen from the results that the percentage of VS destroyed was approximately the same as the percentage of energy converted to methane.

At the end of the food waste BMP the quantity of holocellulose (the sum of hemi-cellulose and cellulose) was the same at the start. It is also apparent that the total amount of hemi-cellulose measured in the food waste digestate increased, although the amount of holocellulose remained constant. One possible reason for this is because the fibre analysis method adopted here is for forage crops, and whether it is entirely suitable for food waste digestate may need further investigation. The lipid component was almost fully degraded. For the mechanically-recovered BMW substrate, around 80% of holocellulose was degraded at both I:S ratios.(Table 4.8)

If the theoretical methane yield of the undigested holocellulose is taken into account when calculating the theoretical BMP values, these would be 0.458 and 0.376 STP m³ CH₄ kg⁻¹ VS for food waste and mechanically-recovered BMW respectively, which is very close to the experimental values.

Table 4.7 Calorific inputs and outputs from the BMP test for each digester and the percentage conversion of the total calorific load to methane

		Food waste			Mechanically-recovered BMW		
		Ratio 2	Ratio 4	Control	Ratio 2	Ratio 4	Control
Calorific input (kJ)	Substrate	340	170	-	172	86	-
	Inoculum	663	663	663	349	349	349
	Total	1002	833	663	521	435	349
Calorific output as CH ₄ (kJ)	Substrate	[253]	[130]	-	[107]	[56]	-
	Inoculum	74	74	74	53	53	53
	Total	327	204	74	160	109	53
Calorific output as digestate (kJ)	Substrate	[89]	[54]	-	[72]	[31]	-
	Inoculum	610	610	610	329	329	329
	Total	699	664	610	401	360	329
Percentage of energy converted to CH ₄ (%)	Substrate	[74.6]	[76.3]	-	[62.0]	[65.7]	-
	Inoculum	-	-	11.2	-	-	15.2
	Total	32.6	24.5	11.2	30.7	25.1	15.2
Percentage VS destroyed (%)	Substrate	[78.7]	[79.2]	-	[61.1]	[63.1]	-
	Inoculum	-	-	12.7	-	-	14.8
	Total	34.4	25.8	12.7	30.2	24.4	14.8

Note: Values calculated from measured results shown in []

Table 4.8 Comparison of the fibre and lipid contents in the BMP digestates with total load entering each BMP digester

Food waste	Start of BMP			End of BMP		
	Ratio 2	Ratio 4	Control	Ratio 2	Ratio 4	Control
Hemi-cellulose (g)	2.88	2.57	2.26	4.11	3.21	3.24
Cellulose (g)	3.66	3.24	2.83	2.35	2.87	1.61
Holocellulose (g)	6.54	5.81	5.09	6.46	6.09	4.85
Lignin (g)	9.26	9.13	8.99	9.76	10.5	10.1
Lipids (g)	3.58	2.34	1.10	1.02	0.96	0.95

Mechanically-recovered BMW	Start of BMP			End of BMP		
	Ratio 2	Ratio 4	Control	Ratio 2	Ratio 4	Control
Hemi-cellulose (g)	1.81	1.58	1.35	1.89	1.53	1.59
Cellulose (g)	3.83	3.65	1.58	0.98	1.16	0.67
Holocellulose (g)	5.64	5.23	2.92	2.87	2.69	2.26
Lignin (g)	6.31	5.48	4.66	6.70	5.55	5.44

5 Laboratory-scale digestion trial with baseline wastes as sole substrates

Four semi-continuous digesters each with a working volume of 35 litres were used in this part of the research. The digesters were constructed from a high density polyethylene (HDPE) cylinder with an inner diameter of 0.32 m and a height of 0.55 m, fitted with top and bottom flange plates. Each digester was heated to 36 ± 1 °C by means of an internal heating coil and the digesters were mixed using a picket fence stirrer connected by a gas-seal draught tube to a geared motor running at 35 rpm. Biogas production and digester temperature were measured continuously on each digester using a gas flow meter and a temperature sensor and the data logged via an interface to a computer. Fresh feed was added via a port in the top plate and digestate removed from the bottom of the reactor via a drain tube.

All four of these digesters had previously been used for a period of 16 months to digest hand-sorted biodegradable municipal waste from the Winchester area and the digestate was not changed before starting to feed the two baseline waste streams. At the start of the current trial two of the digesters were fed with the source-segregated food waste (FW), and the other two digesters with post-collection mechanically-recovered BMW. The digesters ran at an organic loading rate (OLR) of 2 kg volatile solids (VS) m⁻³ d⁻¹ and a mean solids retention time (SRT) of 30 days. In practice this meant that each day 1.16 litres of digestate was drained from each digesters and a calculated amount of waste was fed in so as to maintain the prescribed OLR. The digester was then made up to working volume using digestate liquor, which was separated from the drained digestate using a 1 mm sieve.

The uncoupled liquid and solids retention time was adopted in this study as it is the operational procedure used in the Biffa Wanlip anaerobic digester, where the mechanically-recovered BMW used in this project came from. This is a common practice for low solids anaerobic digestion of municipal solid waste (MSW) with high inert and low moisture contents. Food waste, on the other hand, can be digested with either coupled or uncoupled liquid and solids retention times. An uncoupled liquid and solids retention time for food waste digestion was used in this study to match both the mechanically-recovered BMW digestion, and the operating practice at the Defra Demonstrator food waste digestion plant in Ludlow at the time the current study began.

The digesters were monitored daily for biogas production and pH. Other digestate parameters such as total solids (TS), volatile solids (VS), volatile fatty acids (VFAs), Total Ammonia Nitrogen (TAN) and alkalinity, as well as biogas composition, were analysed twice to three times per week. The process efficiency was estimated by calculating the specific biogas production and volatile solids removals using data from the above analyses. The stability of digester operation was evaluated by reference to other parameters such as pH, VFA, TAN and alkalinity.

The initial purpose in running these digesters was to provide an inoculum for Biochemical Methane Potential (BMP) tests. To set up the baseline waste stream BMP tests (see section 4) 12 litres of digestate were drawn from each digester, then no further digestate was removed until the digester had returned to its 35-litre working volume as a

result of the daily feed additions. From this point (designated day 0) one of the food waste digesters was operated uninterrupted for a period of 284 days at an OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and a mean SRT of 30 days with recirculation of digestate liquor. The other food waste digesters had a further 24 litres of digestate removed on day 119 as an inoculum for the co-digestion food waste BMP test (see section 7). One of the mechanically-recovered BMW digesters had 24 litres of digestate removed on day 140 as an inoculum for the mechanically-recovered BMW co-digestion BMP tests (see section 7). The second mechanically-recovered BMW digester had 20 litres of digestate removed on day 144 to provide an inoculum for the semi-continuous mechanically-recovered BMW co-digestion trial (see section 8). After each of these digestate removals, no further digestate was removed from the digester until its volume had returned to the 35-litre mark as a result of daily additions of their respective feedstocks. Experimental results for the digestion trial are shown graphically in Figures 5.1 to 5.10, and the gaps in the experimental data are for those periods following digestate removal as described above. By the end of the trial, one of the food waste digesters had been fed daily for 9.5 retention times since day 0 at a fixed loading of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ without any disturbance.

5.1 Digestion performance of the two baseline waste streams

Both of the mechanically-recovered BMW digesters ran for more than four retention times before digestate was withdrawn on days 140 and 144, and during this time operational parameters appeared stable. The performance indicators were: specific methane production (SMP) $0.304 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$; specific biogas production (SBP) $0.529 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$; volumetric biogas production (VBP) $1.05 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$; methane percentage 57.5%; volatile fatty acids (VFA) concentration less than 100 mg l^{-1} ; total ammonia nitrogen 1400 mg-N l^{-1} and pH 7.5. The SMP accounted for 86% of the BMP value. The data indicated that most of the biodegradable part of mechanically-recovered BMW had been converted to biogas.

Food waste digester 1 (FW1) ran continuously with daily feeding for 9.5 retention times (284 days). Figure 5.3 shows that the initial pH of this digester was above 7.7. Between the second and third retention time (day 30 to 90) there was a build-up in total ammonia nitrogen (TAN) and VFA reaching 2500 mg-N l^{-1} (Figure 5.4) and 16000 mg l^{-1} (Figure 5.8) respectively, and the methane content of the biogas showed a corresponding drop to 58% (Figure 5.2). The build-up of VFA reduced the pH to 7.3 which shifted the total ammonia nitrogen (TAN) equilibrium from free ammonia (Figure 5.5) to the less inhibitory dissociated form (ammonium ion) leading to an increase in methanogenic activity. This increase subsequently consumed part of the accumulated VFA and gave an increased methane percentage in the biogas. The concurrent rise in pH and free ammonia concentration with reducing VFA concentrations probably initiated a second cycle of partial inhibition of methanogenesis, as seen by a fall in methane content of the biogas between days 120 to 140 (Figure 5.2). Recovery from this second occurrence was more rapid indicating acclimatisation of the methanogens to the unfavourable conditions. The system then appeared stable with good biogas production of $0.695 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ and volatile solids destruction of 83.9%. After 6 retention times (day 180), however, the concentration of propionic acid rose rapidly as shown in Figure 5.9, which led to a lowering of the specific biogas yield and of the biogas methane content (Figure 5.1 and 5.2). The digester finally became severely stressed with VFA rising to 20000 mg l^{-1} and pH falling to 7.1. At the same time the digester suffered a severe foaming problem caused by entrainment of fine gas bubbles, leading to expansion of the digestate volume by 25-

33% with a frothy surface layer filling the digester headspace. A number of measures were used to try and recover the digester. These included reducing the loading rate, stopping liquor recirculation, and finally stopping feeding the digester. None of these were successful.

The second food waste digester (FW 2) ran for 4 retention times before digestate was removed for BMP tests. The VFA profile for that period and for the period after recommencing feeding after digestate removal is shown in Figure 5.10. Although there is some lag (due to the lack of operational continuity) a trend of rising concentration of propionic acid can be seen towards the end of the trial.

Note: Gaps in the experimental data in Figures 5.1 - 5.10 are for periods following the removal of digestate as inoculum for other experiments, as described above.

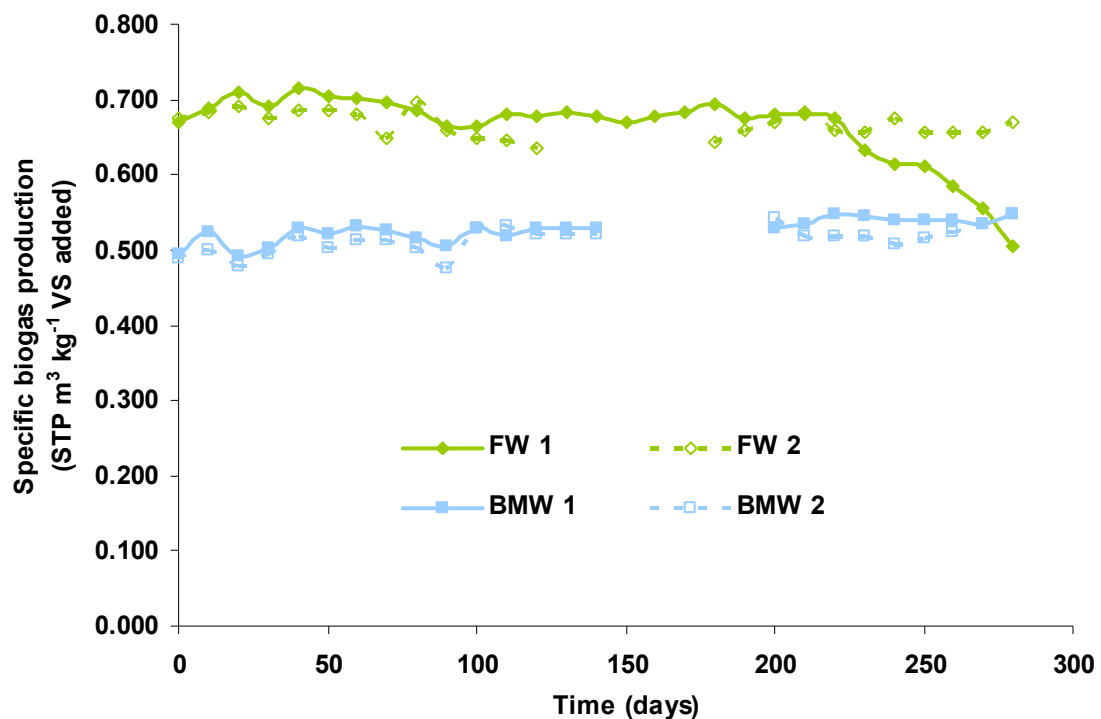


Figure 5.1 Weekly average specific biogas production in the baseline waste digestion trial

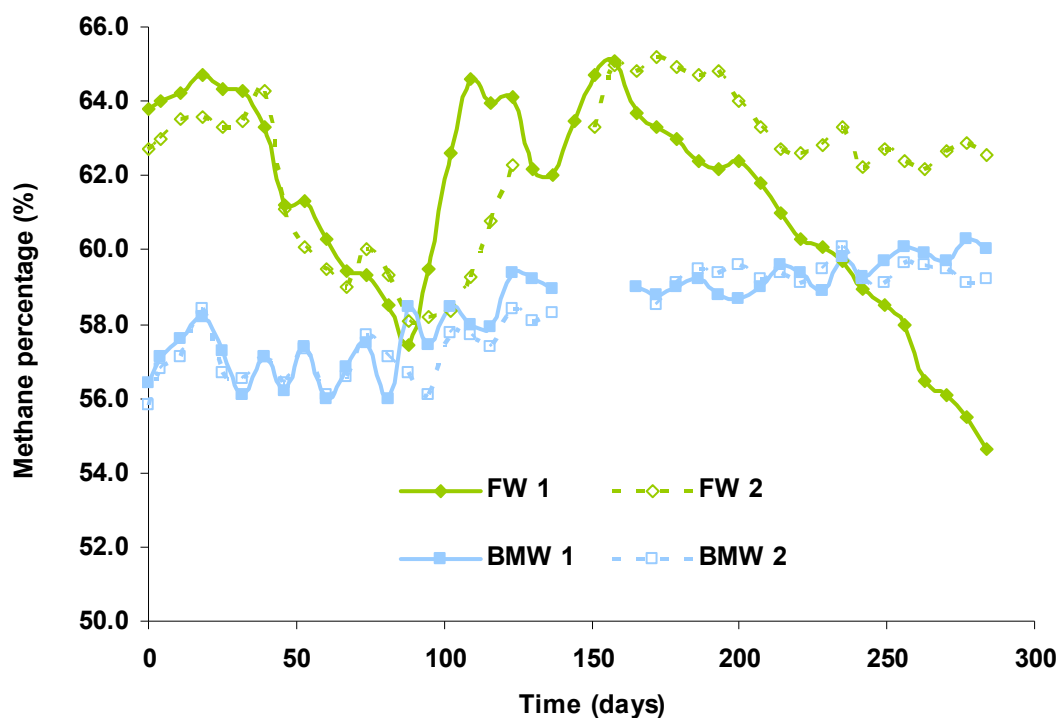


Figure 5.2 Weekly average methane content of biogas produced in the baseline waste digestion trial; Measurement was taken from the headspace before daily feeding

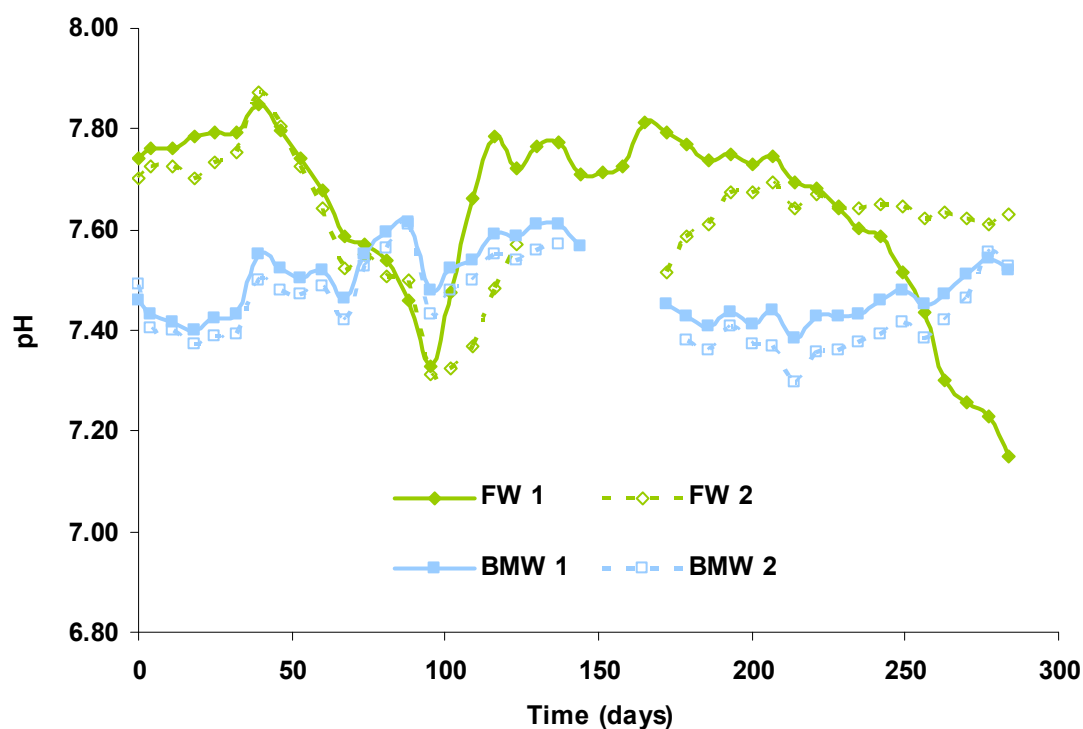


Figure 5.3 Weekly average pH values in the baseline waste digestion trial

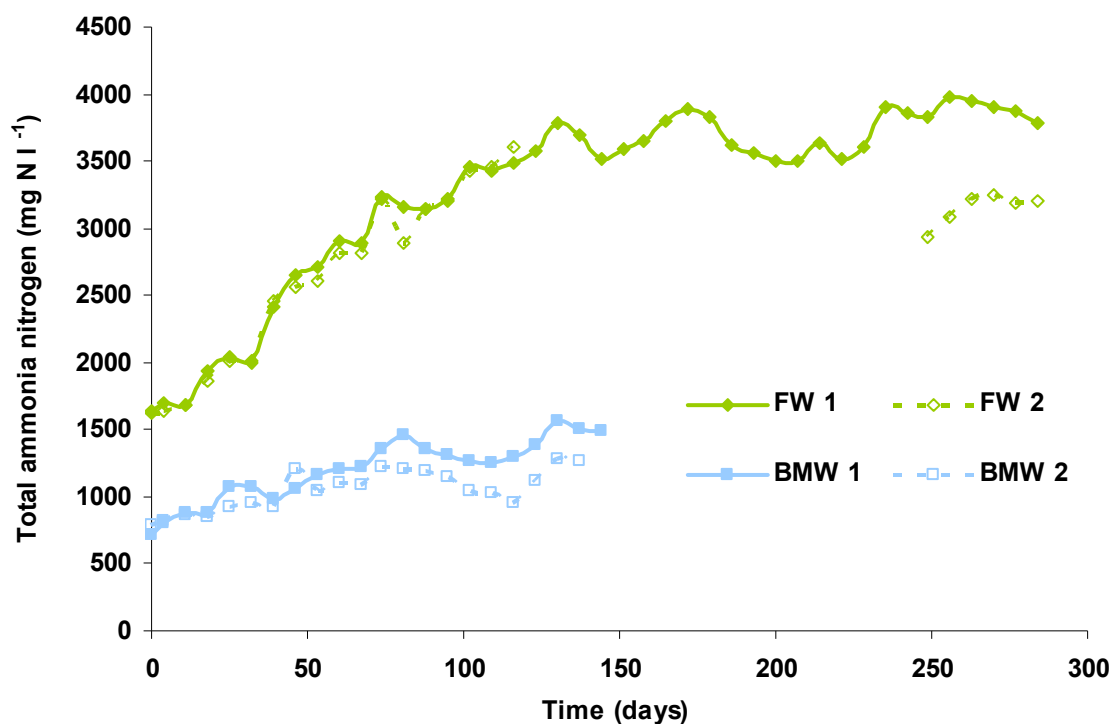


Figure 5.4 Total ammonia nitrogen in the baseline waste digestion trial

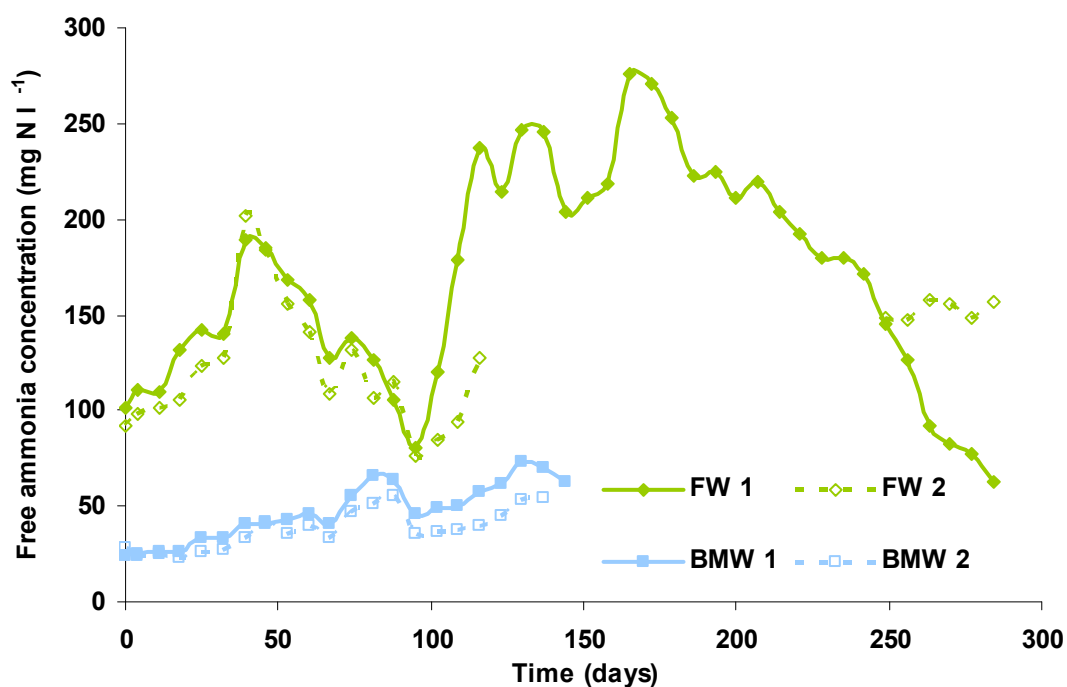


Figure 5.5 Free ammonia concentration in the baseline waste digestion trial

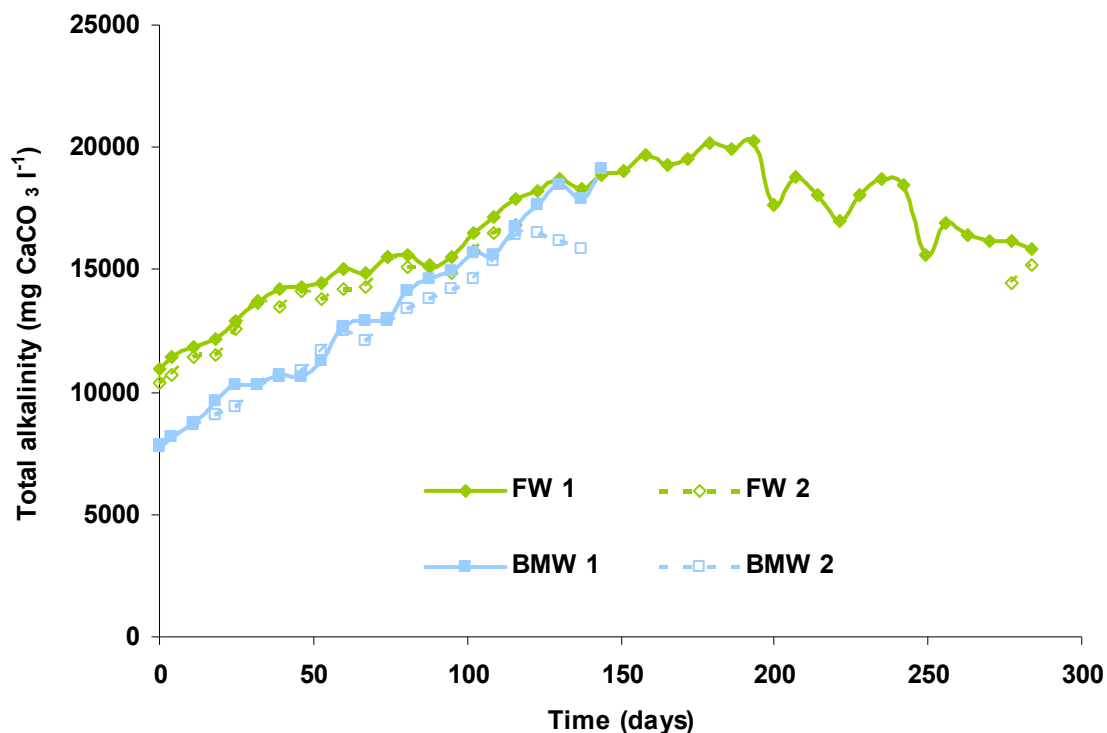


Figure 5.6 Total alkalinity in the baseline waste digestion trial

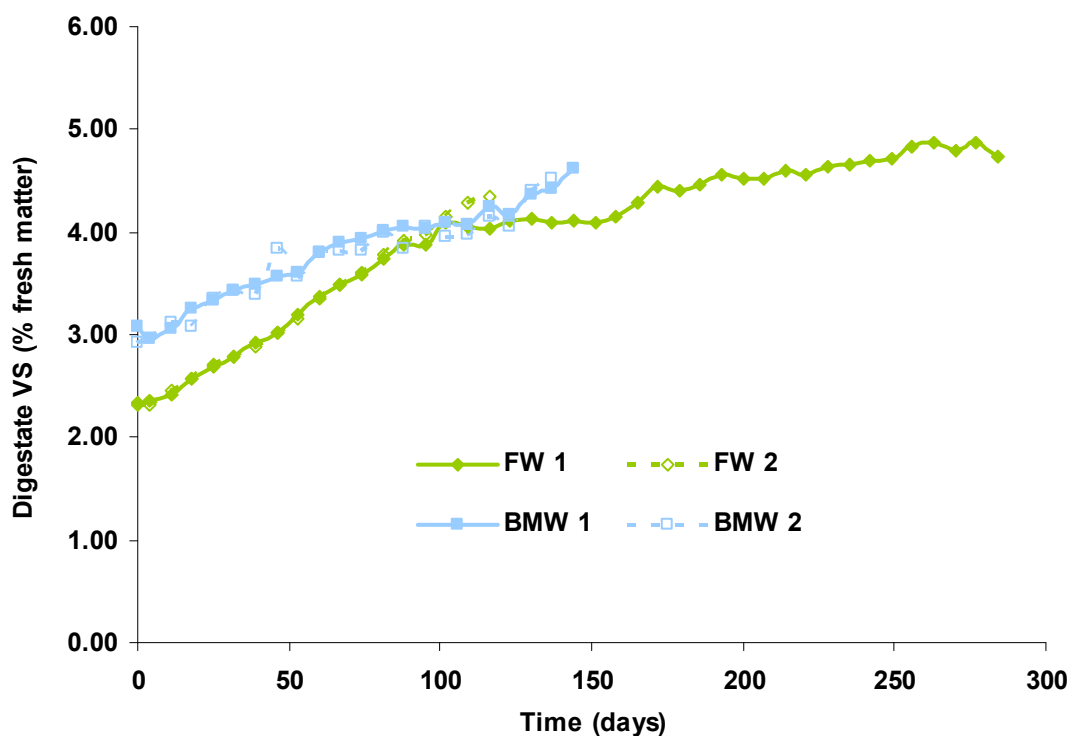


Figure 5.7 Digestion volatile solids content in the baseline waste digestion trial

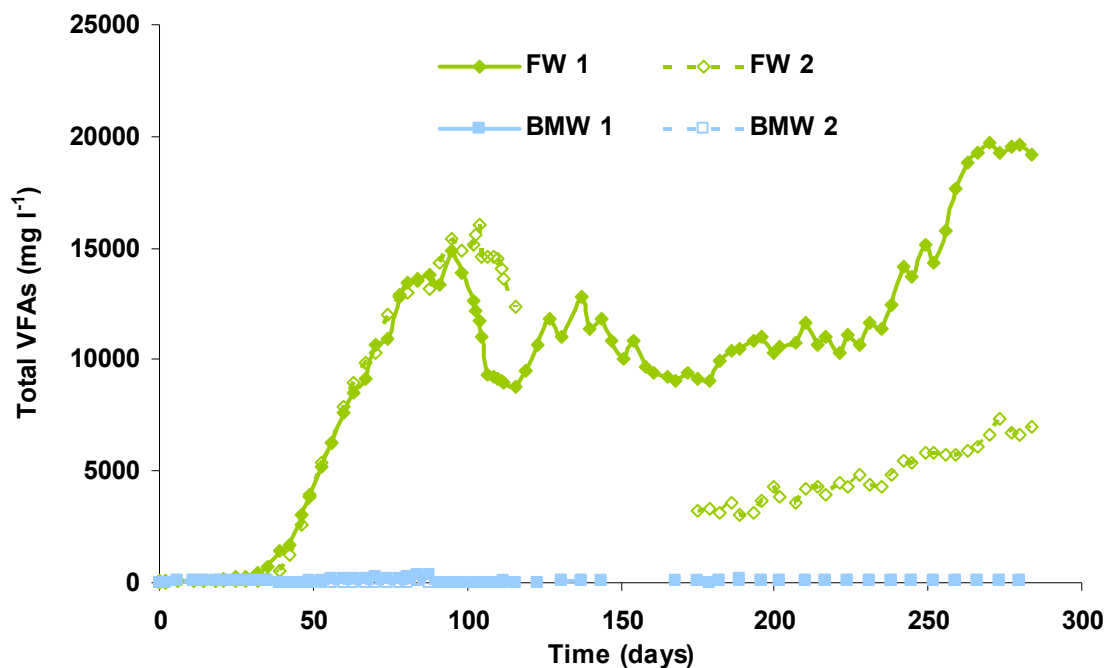


Figure 5.8 Total VFA concentration in the baseline waste digestion trial

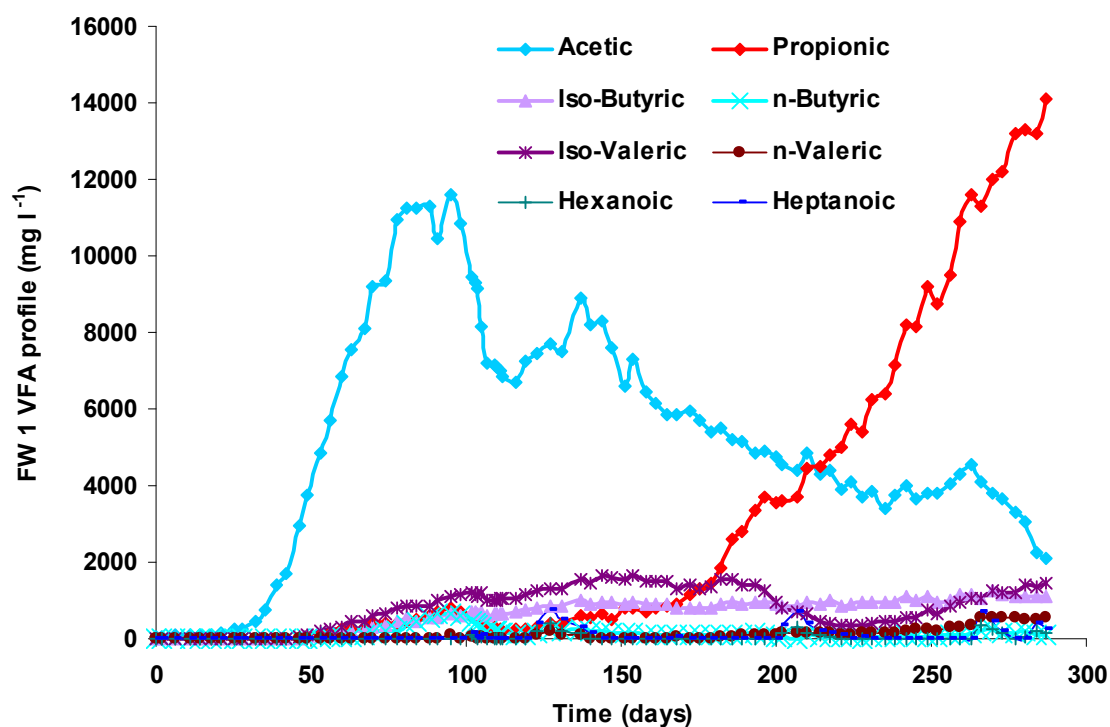


Figure 5.9 VFA concentration profile in food waste digester 1 (FW 1)

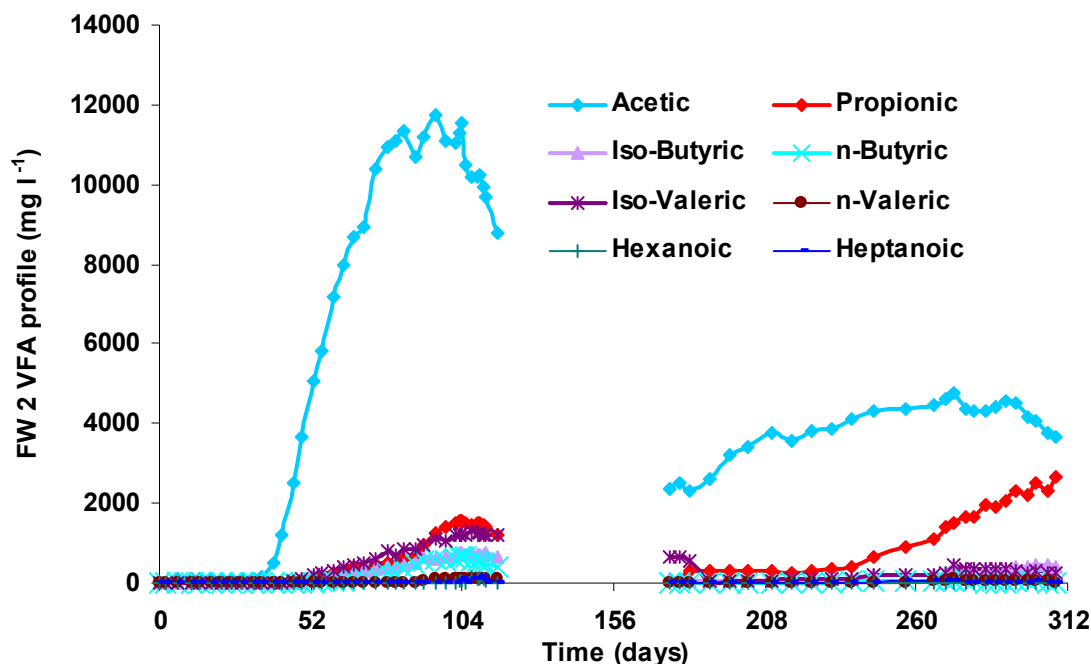


Figure 5.10 VFA concentration profile in food waste digester 2 (FW 2)

5.2 Kinetics of daily gas production in the digesters

The kinetics of biogas production curves were analysed by plotting these on a daily basis to show the pattern of biogas production over the feeding cycles. Examples of these are shown in Figure 5.11. There was very little difference in the rate of biogas production in the early stages of a feed cycle, possibly because at this point the amount of substrate was not the limiting factor and the rates of reaction were independent of the overall biochemical composition as readily available material was used first. The biogas production rate of the digesters fed with mechanically-recovered BMW began to slow down after approximately 10 hours indicating that readily available substrate was becoming limiting. In the digesters fed with the food waste the initial rate of reaction was maintained for almost for 18 hours reflecting the higher content of readily available material as VS, compared with the mechanically-recovered BMW.

The biogas composition and VFA concentration from each digester were also tested at intervals over each 24-hour period on day 42. The results, shown in Figure 5.12, indicated that the addition of food waste might affect the digester operation more than the addition of mechanically-recovered BMW. When adding food waste there was a fall in biogas methane percentage to around 48%; it then rose to around 62% but did not reach a plateau within the 24-hour period. Mechanically-recovered BMW, on the other hand, did not show such a severe drop in methane concentration and reached a maximum concentration within 5 hours from feeding (Figure 5.12). Changes in VFA over a 24-hour monitoring period were inconclusive for food waste digestate, possibly as any changes were small compared to the high background VFA concentration of 3000 mg l⁻¹. In the mechanically-recovered BMW digesters there was a small acetic acid peak of around 100 mg l⁻¹ 2 hours after feeding, which disappeared within 6 hours.

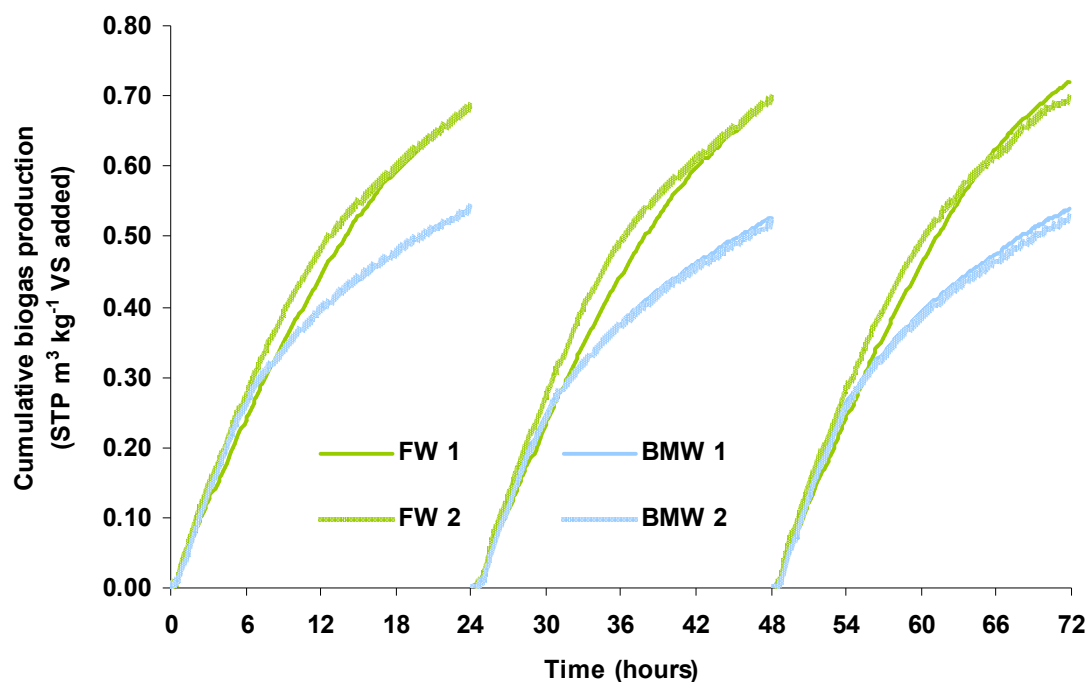


Figure 5.11 Typical cumulative biogas productions during a 3-day period between days 2 and 5

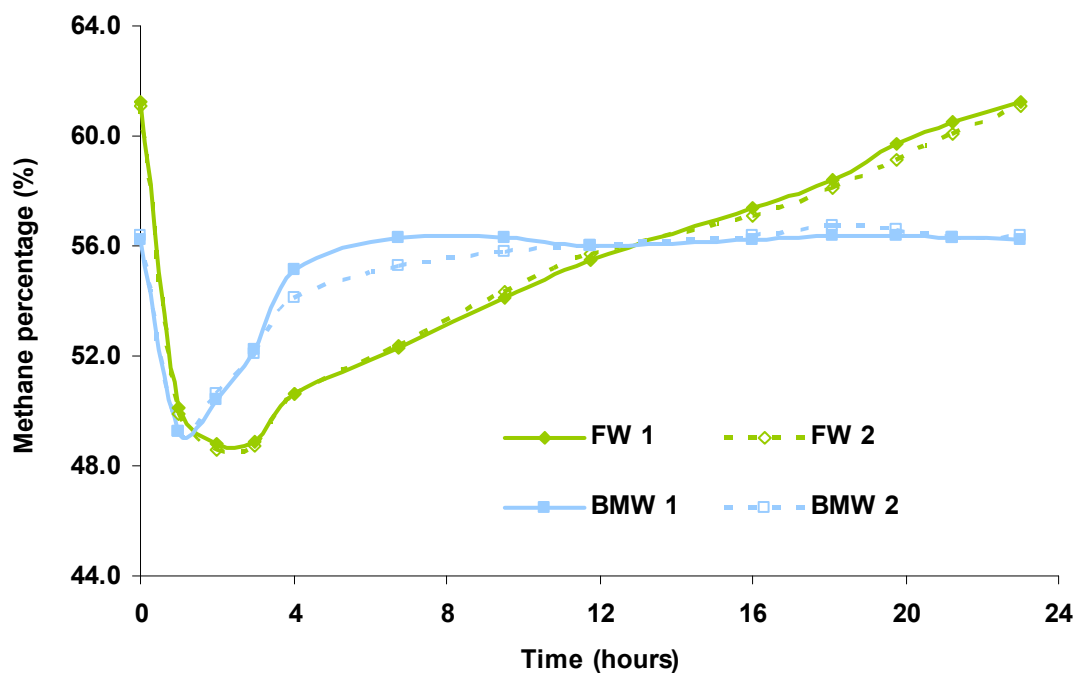


Figure 5.12 Methane percentage in biogas over a 24-hour period tested on day 42

5.3 Plant nutrient and PTE concentrations in the digestate

The plant nutrient and potentially toxic element (PTE) concentrations in the separated digestate liquor and fibre were analysed when food waste digester 2 and mechanically-recovered BMW digester 2 were taken down to use the digestate as an inoculum for co-substrate BMP tests, on days 119 and 140 respectively (Table 5.1). The percentage of fibre fraction in whole digestate was 1.2 % and 22% for food waste and mechanically-recovered BMW respectively. The digestate liquor from food waste digester 1 and mechanically-recovered BMW digester 1 were also tested for nutrient and PTE concentrations. The results can be compared with the concentrations in the digestate on the day 49 as an indication of the accumulation of these substances between the sampling dates.

As predicted from their physicochemical characteristics, food waste digestate had a higher plant nutrient and lower PTE content than mechanically-recovered BMW digestate in both fibre and liquor fractions. Since the operational regime included liquor recirculation, the nutrients and PTE tended to accumulate in the digestate liquor before reaching a steady state. In all cases in the digestate liquor the total ammonia nitrogen (TAN) was around 30-50% of total Kjeldahl nitrogen (TKN) and potassium was predominantly in the dissolved form (90%). The dissolved reactive phosphorus, however, was less than 10% of the total amount, despite the relatively low total phosphorus (TP) concentration compared with TKN and total potassium (TK).

Table 5.1 Plant nutrient and PTE concentrations in individual feedstocks and in digestate fibre from food waste digester 2 and mechanically-recovered BMW digester 2.

	FW feedstock	Mechanically-recovered BMW feedstock
TS (% WW)	23.7 ± 0.1	52.8 ± 0.6
VS (% WW)	21.7 ± 0.1	33.6 ± 0.6
VS (% TS)	91.4 ± 0.4	63.5 ± 1.9
TKN (g kg ⁻¹ TS)	34.2 ± 0.4	13.9 ± 0.8
TK (g kg ⁻¹ TS)	14.3 ± 0.8	4.26 ± 0.37
TP (g kg ⁻¹ TS)	5.41 ± 0.32	2.17 ± 0.25
Cd (mg kg ⁻¹ TS)	< 1.0	1.50 ± 0.37
Cr (mg kg ⁻¹ TS)	29.0 ± 1.2	263 ± 11
Cu (mg kg ⁻¹ TS)	7.20 ± 0.81	107 ± 10
Ni (mg kg ⁻¹ TS)	7.0 ± 2.9	97.0 ± 2.9
Pb (mg kg ⁻¹ TS)	< 10	162 ± 10
Zn (mg kg ⁻¹ TS)	33 ± 11	259 ± 4

Table 5.1 continued Plant nutrient and PTE concentrations in individual feedstocks and in digestate fibre from food waste digester 2 and mechanically-recovered BMW digester 2.

	FW 2 digestate fibre	Mechanically-recovered BMW 2 digestate fibre
Date	Day 119	Day 140
% of whole digestate	1.3	6.8
TS (% WW)	14.7 ± 0.1	35.0 ± 0.3
VS (% WW)	12.1 ± 0.0	21.2 ± 0.4
VS (% TS)	82.6 ± 0.1	60.5 ± 0.6
TAN (g NH ₃ -N kg ⁻¹ TS)	23.6 ± 0.1	4.76 ± 0.11
TKN (g kg ⁻¹ TS)	54.7 ± 0.4	16.2 ± 0.2
TK (g kg ⁻¹ TS)	18.0 ± 0.1	3.89 ± 0.27
TP (g kg ⁻¹ TS)	10.5 ± 0.2	3.40 ± 0.18
Cd (mg kg ⁻¹ TS)	< 1.0	1.36 ± 0.17
Cr (mg kg ⁻¹ TS)	10.9 ± 0.4	64.0 ± 2.6
Cu (mg kg ⁻¹ TS)	19.7 ± 0.0	146 ± 2
Ni (mg kg ⁻¹ TS)	11.4 ± 1.1	57.6 ± 2.2
Pb (mg kg ⁻¹ TS)	< 10	170 ± 7
Zn (mg kg ⁻¹ TS)	128 ± 7	438 ± 66

Table 5.2 Plant nutrient and PTE concentrations in the digestate liquor

	FW 1	FW 2	Mechanically-recovered BMW 1	Mechanically-recovered BMW 2
Date	Day 49	Day 49	Day 49	Day 49
TAN (g NH ₃ -N l ⁻¹)	2.67 ± 0.06	2.61 ± 0.02	1.19 ± 0.03	1.05 ± 0.02
TKN (g N l ⁻¹)	4.97 ± 0.05	4.89 ± 0.07	2.60 ± 0.03	2.42 ± 0.05
Dissolved K (g l ⁻¹)	1.95 ± 0.01	1.92 ± 0.01	0.838 ± 0.005	0.782 ± 0.004
TK (g l ⁻¹)	2.06 ± 0.03	2.04 ± 0.02	0.989 ± 0.007	0.921 ± 0.002
Dissolved reactive P (mg l ⁻¹)	20.5 ± 0.1	19.6 ± 0.1	12.4 ± 0.1	9.9 ± 0.2
TP (mg l ⁻¹)	466 ± 26	460 ± 7	210 ± 0	221 ± 4
Cd (mg l ⁻¹)	0.057 ± 0.003	0.055 ± 0.000	0.141 ± 0.000	0.146 ± 0.001
Cr (mg l ⁻¹)	4.21 ± 0.32	1.93 ± 0.06	5.09 ± 0.18	7.90 ± 0.26
Cu (mg l ⁻¹)	2.37 ± 0.02	2.18 ± 0.01	10.6 ± 0.0	11.7 ± 0.0
Hg (µg l ⁻¹)	1.81 ± 0.05	2.05 ± 0.04	21.4 ± 0.2	21.2 ± 0.8
Ni (mg l ⁻¹)	3.00 ± 0.19	1.47 ± 0.03	4.44 ± 0.24	6.22 ± 0.17
Pb (mg l ⁻¹)	1.01 ± 0.02	0.75 ± 0.01	16.3 ± 0.2	17.6 ± 0.1
Zn (mg l ⁻¹)	7.32 ± 0.09	6.93 ± 0.06	36.1 ± 0.1	37.2 ± 0.7

	FW 1	FW 2	Mechanically-recovered BMW 1	Mechanically-recovered BMW 2
Date	Day 119	Day 119	Day 140	Day 140
TAN (g NH ₃ -N l ⁻¹)	3.56 ± 0.02	3.80 ± 0.04	1.50 ± 0.01	1.47 ± 0.01
TKN (g N l ⁻¹)	6.34 ± 0.01	6.52 ± 0.00	3.32 ± 0.03	3.16 ± 0.02
Dissolved K (g l ⁻¹)	2.46 ± 0.01	2.53 ± 0.01	1.04 ± 0.01	1.02 ± 0.00
TK (g l ⁻¹)	2.63 ± 0.00	2.69 ± 0.01	1.20 ± 0.02	1.15 ± 0.00
Dissolved reactive P (mg l ⁻¹)	47.5 ± 0.3	44.0 ± 0.6	18.1 ± 0.0	19.4 ± 0.2
TP (mg l ⁻¹)	668 ± 40	695 ± 73	328 ± 6	297 ± 16
Cd (mg l ⁻¹)	< 0.06	< 0.06	0.168 ± 0.007	0.156 ± 0.001
Cr (mg l ⁻¹)	3.94 ± 0.10	1.70 ± 0.01	7.19 ± 0.08	10.9 ± 0.1
Cu (mg l ⁻¹)	2.39 ± 0.00	2.21 ± 0.02	19.2 ± 0.2	19.1 ± 0.1
Ni (mg l ⁻¹)	2.77 ± 0.28	1.47 ± 0.01	6.40 ± 0.16	9.05 ± 0.04
Pb (mg l ⁻¹)	< 0.6	< 0.6	18.5 ± 0.5	17.4 ± 0.1
Zn (mg l ⁻¹)	8.92 ± 0.04	8.79 ± 0.05	56.5 ± 1.0	55.2 ± 0.1

6. Physico-chemical characterisation of selected co-substrates

Commercial, industrial and agricultural waste materials that are available in large tonnages and evenly distributed throughout the UK were considered as potential co-substrates for digestion with municipal waste streams. A scoping matrix (Appendix 2) for selection of co-substrates was developed, including hierarchical classification of the waste streams within the major categories, tonnages, coverage nationwide, and characteristics for each waste type as regards processing in anaerobic digestion facilities.

6.1 Co-substrates

6.1.1 Co-substrates considered for use with source-segregated food waste

The ten co-substrates selected for co-digestion with source-segregated food waste were:

Cattle slurry: 20 kg of cattle slurry was collected from Parkers Farm, Hampshire. A particle size distribution (PSD) test on the wet slurry was not carried out as the solids fraction is composed of very fine fibres which do not move along the sieve surface effectively.

Rumen content: 20 kg of sheep rumen content was collected from an abattoir operated by R W Newman & Partners, Hampshire. A PSD test was not carried out due to the fibrous nature of the material which gives very different results depending on the orientation of particles as they pass over the openings of the sieve. Generally, the solids fraction in the rumen was forage feedstuff residues with a diameter of less than 1 mm and length from 1 to 10 cm.

Office paper: 5 kg of used office paper was taken from the recycling bins of the University of Southampton. This comprised white office paper which had mainly been printed. The manufacturing sources of the paper or the types of ink with which it had been printed were not known. A PSD was not carried out as paper is a thin two-dimensional material.

Paper pulp: 50 kg of paper pulp was collected from the manufacture of tea bags, Ahlstrom Paper Group, Chirnside mill, Berwickshire. A PSD was not completed for the same reason as cattle slurry.

Brewery waste, including spent grain and yeast: 5 kg of spent grain and 20 kg of yeast were collected from Palmers Brewery, Dorset. A brew of approximately 10,000 litres produces 1500 kg of spent grains and 55 kg of waste yeast. The brewery also produces a waste known as "the bottoms" which is any beer that is returned in the barrels, and this was estimated to be between 7-10% of the beer production. This fraction of waste was not included in the current study because of its very high water content. A PSD showed that the spent grain had a uniform grain shape with a diameter of approximately 1 mm and a length of 5 mm.

Whey: 20 kg of whey was provided by Greenfinch Ltd, Ludlow as material received from a cheese making factory

Potato waste: 2 kg of potato waste was collected from Forest Products Ltd, Dorset. This was potato chip rejected from crisp manufacturing. The material was two-dimensional with a thickness of approximately 0.5 mm.

Flourmill waste: 10 kg of waste was collected from the Rank Hovis flourmill in Southampton, Hampshire. The material was mainly wheat straw with a proportion of damaged or lost grain; it had about 3% contamination with material such as stones and feathers. PSD analysis was not carried out as the fibrous nature of the material gives very different results depending on the orientation of straw fibres as they pass over the openings of the sieve.

Card packaging: 100 kg of card packaging mixture was collected from Alton Materials Recovery Facility (MRF) run by Veolia Environmental Services, which was the reject material from the MRF. This mixed card packaging collection was sorted into corrugated cardboard, card packaging and other card. These materials were blended again at a defined proportion which consisted of 29.6% of corrugated cardboard, 62.5% of card packaging and 7.9% of other card on a fresh weight basis. This was calculated on behalf of Defra for the project, and was based on previous waste compositional studies.

6.1.2 Co-substrates considered for use with mechanically-recovered BMW

The five co-substrates selected for co-digestion with mechanically-recovered BMW were:

Biodiesel by-product: 10 kg of biodiesel by-product, believed to consist mainly of glycerol, was supplied by D1 Oils plc, Teeside, UK.

Animal blood: 20 kg of sheep blood was collected from an abattoir operated by R W Newman & Partners, Hampshire. Red offal was also collected at the same time and comprised liver, heart, lung and trachea. This was not selected as the mechanically-recovered BMW co-substrate as the material was all edible and was usually sold by the abattoir for either the human or pet food supply chains.

Slaughterhouse waste, including pig gut and flotation fat: 2 sets of pig gut, approximately 8 kg each, and 5 kg of recovered fat were collected from Grampian Country Pork–CASE, Somerset. The slaughterhouse produces approximately 800 tonnes of pig gut waste annually along with 2 tonnes of fat captured in the fat traps. The slaughterhouse did not operate a dissolved air flotation (DAF) unit for further fat removal from the wastewater and it was therefore assumed that the fat trap material was representative of the type of separable fat recovered from the wastewater stream. The amount recovered per year is considerably lower than might be expected from a meat processing factory, however, and the proportion of gut and fat in the mixture used in the current study was 9:1 on a VS basis. This is referred to as pig gut and flotation fat.

Poultry litter: 20 kg of poultry litter was collected from Vero Poultry, Hampshire. This contained a small amount of feather and egg contamination which were not separated out

before testing due to their organic nature and the probability that these would constitute a real part of this type of waste.

6.2 Determination of physico-chemical characteristics of co-substrates

The co-substrates were characterised for their physico-chemical properties according to the methods described in section 2. Analysis was carried out on representative samples, using at least three replicates within each sample. The analytical results are given in Table 6.1 and 6.2 for the food waste and mechanically-recovered BMW co-substrates respectively.

It can be seen from Table 6.1 that office paper, paper pulp, potato waste, flourmill waste and card packaging had a higher biodegradable carbon to TKN ratio than source-segregated food waste (which has a ratio of 13.6). Among these materials the low lignin content of potato waste, flourmill waste and office paper made them more promising co-substrates than the paper pulp and card packaging which had a high lignin content. Although cattle slurry, sheep rumen content, spent grain and whey had a similar, or even lower, C:N ratio than food waste they were still considered potential co-substrates because of their lower lipid content. Yeast, as recovered from the brewing process, had a very low C:N ratio and a high water content making it less favourable as a co-substrate. It was therefore rejected as a potential co-substrate for mixing with source-segregated food waste, but could be considered as a co-substrate for digestion with the mechanically-recovered BMW. On the basis of the experimental findings for source-segregated food waste, also taking into account the potential co-substrate characteristics, availability and tonnage quantities, it was decided that cattle slurry, card packaging and potato waste would be used as co-substrates in the laboratory-scale semi-continuous co-digestion trials with source-segregated food waste. The reasoning was as follows. It was apparent from the baseline digestion trial (described in section 4) that the high lipid and protein content of the food waste may lead to inhibitory effects on the anaerobic digestion process if fatty acids and/or ammonia accumulate in the digester under certain operational regimes. The card packaging and potato waste with their high fibre/carbohydrate content and low nitrogen content could be combined with the food waste to produce a more stable mix with a more favourable C:N ratio and a reduced lipid load. Although cattle slurry had a lower C:N ratio than the food waste it was still considered as a potential co-substrate because its known low methane potential, high fibre content and low lipid content make it unattractive as a substrate for energy production alone. The addition of high energy potential food waste could optimise this situation, however, leading to greater digester volumetric productivity and a more stable mix than food waste alone. Because of the large quantities of cattle slurry available the relative proportion of the food waste could be less than that of the slurry.

The high total Kjeldahl nitrogen (TKN) content and/or the high lipid content of sheep blood, pig gut with flotation fat, and poultry litter could lead to inhibitory effects on the anaerobic digestion process if treated alone. Co-digestion with mechanically-recovered BMW would lower the risk of the fatty acids and ammonia inhibition due to the higher C:N ratio and fibre content in mechanically-recovered BMW. Mechanically-recovered BMW would also benefit from the co-digestion with those materials which could raise the specific and volumetric methane production of the digestion system because of their low fibre content and their protein content which represents readily biodegradable material.

The by-product from bio-diesel production (glycerol) had a very high pH, no detectable nitrogen or fibre content and a low ash content. On the basis of the experimental findings of mechanically-recovered BMW, also taking into account the potential co-substrate characteristics, availability and tonnage quantities, it was decided that sheep blood and pig gut mixed with flotation fat would be used as co-substrates in the laboratory-scale semi-continuous co-digestion trials with mechanically-recovered BMW. The higher fibre content of mechanically-recovered BMW resulted in a lower methane potential than was obtained for food waste although the higher C:N ratio ensured stable digestion (described in section 4). Mixing this waste with animal blood or the pig gut/flotation fat mix would add a high energy component by increasing the lipid content and therefore the volumetric biogas productivity, whilst at the same time increasing the nitrogen content leading to a more nutrient rich digestate.

6.3 Physico-chemical characteristics of the mixture of baseline waste streams and co-substrates

The key characteristics of each baseline waste alone and with its corresponding co-substrate are listed in Table 6.3 and 6.4. These were calculated from analysis of the individual substrates, and were used to check that each co-digestion mix contained an appropriate amount of the co-substrate.

Table 6.1 Characteristics of the co-substrates considered for source-segregated food waste co digestion

Co-substrates	Cattle slurry	Rumen content	Office paper ⁴	Paper pulp	Brewery waste		Whey	Potato waste	Flourmill waste	Card packaging
					Spent grain	Yeast				
<i>Fundamental characteristics for anaerobic digestion</i>										
pH	7.83 ± 0.07 (1:5)	7.81 ± 0.04	9.10 ± 0.11 (1:20)	6.65 ± 0.03 (1:10)	4.84 ± 0.08 (1:5)	6.18 ± 0.06	4.49 ± 0.06	8.12 ± 0.01 (1:5)	6.26 ± 0.03 (1:10)	7.21± 0.03 (1:30)
TS (% WW)	9.31 ± 0.14	5.38 ± 0.18	96.6 ± 0.1	22.2 ± 0.0	21.9 ± 0.1	14.4 ± 0.2	6.07 ± 0.10	24.7 ± 0.0	88.2 ± 0.1	93.9 ± 0.1
VS (% WW)	6.52 ± 0.04	4.37 ± 0.18	76.2 ± 0.6	20.5 ± 0.0	20.9 ± 0.1	12.6 ± 0.2	5.48 ± 0.10	23.1 ± 0.0	84.6 ± 0.1	78.5 ± 0.4
VS (% TS)	70.0 ± 0.6	81.2 ± 0.60	78.9 ± 0.5	92.4 ± 0.0	95.3 ± 0.0	87.6 ± 0.5	90.2 ± 0.2	93.2 ± 0.0	95.8 ± 0.0	83.6 ± 0.5
TOC (% TS)	38.9 ± 1.0	41.6 ± 0.9	36.4 ± 0.7	39.3 ± 0.7	50.5 ± 0.5	42.5 ± 0.9	45.9 ± 2.0	43.4 ± 1.1	46.3 ± 0.9	41.6 ± 0.7
TAN (% TS)	1.15 ± 0.01	0.45 ± 0.02	-	-	-	-	-	-	-	-
TKN (% TS)	3.50 ± 0.05	2.04 ± 0.07	0.12 ± 0.01	0.62 ± 0.00	3.41 ± 0.04	8.78 ± 0.01	2.49 ± 0.07	1.53 ± 0.01	1.50 ± 0.04	0.144 ± 0.001
TOC / TKN	11.1 ± 0.3	20.7 ± 0.9	271 ± 18	63.6 ± 1.2	14.7 ± 0.2	4.52 ± 0.09	18.4 ± 1.0	28.0 ± 0.7	30.8 ± 1.0	288 ± 5
Biodegradable C ¹ / TKN	8.15 ± 0.32	14.2 ± 0.7	215 ± 20	35.2 ± 1.9	11.6 ± 0.3	4.51 ± 0.09	18.4 ± 1.0	27.5 ± 0.7	25.7 ± 1.0	42.9 ± 4.7
CV (kJ g ⁻¹ TS)	16.75±0.10	18.70±1.19	13.16±0.16	16.10±0.06	21.76±0.70	19.74±0.01	17.72±0.08	16.50±0.10	18.90±0.12	17.18 ± 0.36
<i>Biochemical composition on a VS basis</i>										
Carbohydrates ² (g kg ⁻¹)	21.9 ± 1.0	63.4 ± 6.4	86.5 ± 7.4	53.0 ± 6.9	104 ± 3	244 ± 30	812 ± 20	833 ± 35	497 ± 34	242 ± 19
Lipids ³ (g kg ⁻¹)	93.6 ± 0.8	25.7 ± 1.9	< 10	< 10	107 ± 2	< 10	36.3 ± 0.1	< 10	15.1 ± 0.4	< 10
Crude proteins (g kg ⁻¹)	276 ± 6	160 ± 8	9.8 ± 0.8	41.9 ± 0.1	224 ± 3	647 ± 1	175 ± 5	103 ± 0	98.0 ± 2.8	10.8 ± 0.0
Hemi-cellulose (g kg ⁻¹)	226 ± 6	310 ± 7	125 ± 10	123 ± 16	335 ± 1	20.7 ± 3.3	-	22.0 ± 0.5	180 ± 7	113 ± 5
Cellulose (g kg ⁻¹)	96.7 ± 8.5	146 ± 7	636 ± 22	398 ± 15	59.8 ± 9.7	13.2 ± 2.8	-	22.1 ± 2.3	109 ± 10	304 ± 6
Lignin (g kg ⁻¹)	226 ± 7	245 ± 6	145 ± 22	286 ± 15	166 ± 10	< 5	-	11.2 ± 2.3	122 ± 9	532 ± 2
<i>Nutrients on a TS basis</i>										
TKN (g kg ⁻¹)	35.0 ± 0.5	20.4 ± 0.7	1.2 ± 0.1	6.2 ± 0.0	34.1 ± 0.4	87.8 ± 0.1	24.9 ± 0.7	15.3 ± 0.1	15.0 ± 0.4	1.44 ± 0.01
TP (g kg ⁻¹)	8.58 ± 0.63	23.4 ± 0.6	0.068±0.005	3.47 ± 0.16	6.19 ± 0.61	25.7 ± 0.5	7.57 ± 0.15	3.59 ± 0.48	2.32 ± 0.17	0.134 ± 0.003
TK (g kg ⁻¹)	16.7 ± 0.2	12.6 ± 0.5	0.08 ± 0.00	0.10 ± 0.00	0.41 ± 0.01	31.6 ± 0.6	27.2 ± 0.4	23.8 ± 0.8	10.2 ± 0.1	0.221 ± 0.010

Table 6.1 continued Characteristics of the co-substrates considered for source-segregated food waste co digestion

Co-substrates	Cattle slurry	Rumen content	Office paper ⁴	Paper pulp	Brewery waste		Whey	Potato waste	Flourmill waste	Card packaging	
					Spent grain	Yeast					
Potentially toxic elements on a TS basis											
Cd (mg kg ⁻¹)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 0.05	
Cr (mg kg ⁻¹)	113 ± 2	17.7 ± 0.7	4.3 ± 0.3	24.9 ± 2.4	17.7 ± 0.1	< 2.0	< 2.0	6.9 ± 0.6	11.7 ± 0.0	9.1 ± 0.9	
Cu (mg kg ⁻¹)	58.4 ± 1.1	12.4 ± 0.0	7.4 ± 0.8	17.0 ± 0.5	17.6 ± 1.2	25.9 ± 0.6	< 4.0	9.8 ± 0.7	6.1 ± 0.2	20.3 ± 2.3	
Hg (mg kg ⁻¹)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.10	
Ni (mg kg ⁻¹)	44.8 ± 0.6	8.6 ± 0.4	6.0 ± 0.2	12.1 ± 1.2	6.3 ± 0.5	< 5.0	< 5.0	< 5.0	< 5.0	4.5 ± 0.5	
Pb (mg kg ⁻¹)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	2.9 ± 0.4	
Zn (mg kg ⁻¹)	231 ± 6	87.7 ± 1.8	5.6 ± 0.3	16.4 ± 0.2	71.8 ± 0.6	29.8 ± 0.7	4.2 ± 0.0	20.3 ± 0.5	27.4 ± 0.4	16.2 ± 4.3	
Elemental analysis											
% of TS	N	3.50 ± 0.05	2.04 ± 0.07	0.12 ± 0.01	0.62 ± 0.00	3.41 ± 0.04	8.78 ± 0.01	2.49 ± 0.07	1.53 ± 0.01	1.50± 0.04	0.14± 0.00
	C	39.2 ± 1.0	42.2 ± 0.9	36.7 ± 0.7	39.5 ± 0.7	50.6 ± 0.5	42.9 ± 0.9	46.1 ± 2.0	43.7 ± 1.1	46.4 ± 0.9	41.6 ± 0.7
	H	5.18 ± 0.15	5.61 ± 0.16	4.97 ± 0.20	6.14 ± 0.19	7.18 ± 0.19	6.53 ± 0.16	6.87 ± 0.35	7.18 ± 0.20	6.94 ± 0.19	4.76 ± 0.23
	S	0.31 ± 0.02	0.19 ± 0.02	< 0.05	0.22 ± 0.02	0.27 ± 0.03	0.54 ± 0.02	0.23 ± 0.03	0.06 ± 0.02	0.13 ± 0.02	0.21 ± 0.00
	O	23.1 ± 0.9	28.5 ± 1.3	32.2 ±1.0	33.7 ± 0.7	32.0 ± 1.6	29.1 ± 1.1	33.3 ± 1.2	38.8 ± 1.3	37.1 ± 2.8	36.9 ± 0.9
% of VS	N	5.08 ± 0.08	2.54 ± 0.09	0.16 ±0.01	0.67 ± 0.00	3.58 ± 0.04	10.3 ± 0.0	2.80 ± 0.08	1.64 ± 0.01	1.57 ± 0.04	0.17 ±0.00
	C	56.4 ± 1.4	52.5 ± 1.1	43.4 ± 0.8	42.6 ± 0.8	52.7 ± 0.5	46.6 ± 0.9	51.6 ± 2.2	45.9 ± 1.1	48.4 ± 0.9	49.8 ± 0.8
	O	32.8 ± 1.4	35.3 ± 1.7	35.0 ± 1.3	35.8 ± 0.8	33.3 ±1.6	32.4 ± 1.3	37.1 ± 1.4	40.4 ± 1.4	38.6 ± 3.0	44.1 ± 1.1

¹ Biodegradable carbon was calculated by deducting lignin carbon from TOC. The formula of Lignin was chosen as C_{9.94}H_{12.82}O_{2.94}.

² in equivalent glucose

Table 6.2 Physico-chemical characteristics of the co-substrates considered for mechanically-recovered BMW co-digestion

Recovered BMW co-digestion							
Co-substrates	Biodiesel by-product	Sheep blood	Pig gut and floatation fat			Poultry litter	
			Pig gut	Flotation fat	Mixture ¹		
<i>Fundamental characteristics for anaerobic digestion</i>							
pH	12.19± 0.01 (1:5)	7.23 ± 0.06	5.85 ± 0.03 (1:5)	5.61 ± 0.02 (1:5)	5.96 ± 0.04 (1:5)	8.89 ± 0.02 (1:5)	
TS (% WW)	99.2 ± 0.0	19.7 ± 0.3	19.9 ± 0.0	73.8 ± 0.4	20.8 ± 0.3	52.8 ± 0.5	
VS (% WW)	96.3 ± 0.0	18.9 ± 0.3	18.5 ± 0.0	71.3 ± 0.4	19.4 ± 0.3	24.3 ± 0.1	
VS (% TS)	97.0 ± 0.0	95.6 ± 0.1	93.0 ± 0.0	96.6 ± 0.0	93.2 ± 0.1	45.9 ± 0.7	
TOC (% TS)	32.1 ± 1.7	42.0 ± 0.7	44.1 ± 1.6	-	45.3 ± 1.7	25.6 ± 1.2	
TAN (% TS)	-	-	-	-	-	0.64 ± 0.01	
TKN (% TS)	< 0.05	14.7 ± 0.0	8.17 ± 0.00	0.53 ± 0.03	7.95 ± 0.12	3.45 ± 0.05	
TOC / TKN	∞	2.85 ± 0.05	5.40 ± 0.19	-	5.85 ± 0.24	6.70 ± 0.36	
Bio-C ² / TKN	∞	2.85 ± 0.05	5.27 ± 0.19	-	5.58 ± 0.23	5.33 ± 0.36	
CV (kJ g ⁻¹ TS)	18.86±0.15	22.91±0.25	24.85±0.03	-	26.21±0.01	8.63 ± 0.31	
<i>Biochemical composition on a VS basis (g kg⁻¹)</i>							
Carbohydrates ³	-	7.2 ± 0.6	27.1 ± 2.0	-	15.8 ± 1.1	84.1 ± 3.5	
Lipids ⁴	-	< 10	279 ± 1	-	349 ± 8	40.4 ± 0.6	
Crude proteins	< 5	965 ± 2	551 ± 0	34.3 ± 2.0	538 ± 8	490 ± 9	
Hemi-cellulose	-	-	51.5 ± 2.6	-	46.7±2.4 ⁵	214 ± 7	
Cellulose	-	-	51.2 ± 3.2	-	46.4±2.9 ⁵	69.8 ± 7.9	
Lignin	-	-	20.5 ± 2.4	-	18.6±2.2 ⁵	154 ± 5	
<i>Nutrients on a TS basis (g kg⁻¹)</i>							
TKN	< 0.5	147 ± 0	81.7 ± 0.0	5.3 ± 0.3	79.5 ± 1.2	34.5 ± 0.5	
TP	< 0.02	0.835±0.03	8.49 ± 0.06	-	8.10 ± 0.13	21.9 ± 0.1	
		6					
TK	< 0.02	3.71 ± 0.11	11.7 ± 0.0	-	10.9 ± 0.1	31.4 ± 0.1	
<i>Potentially toxic elements on a TS basis (mg kg⁻¹)</i>							
Cd	< 1.0	< 1.0	< 1.0	-	< 1.0	1.64 ± 0.05	
Cr	< 2.0	< 2.0	5.5± 0.1	-	14.6 ± 0.3	66.4 ± 0.2	
Cu	< 4.0	6.7 ± 0.3	43.0 ± 0.8	-	37.9 ± 0.5	56.5 ± 1.1	
Hg	< 0.010	< 0.010	< 0.010	-	< 0.010	< 0.010	
Ni	< 5.0	< 5.0	< 5.0	-	6.9 ± 0.3	38.0 ± 1.2	
Pb	< 10	< 10	< 10	-	< 10	14.3 ± 0.1	
Zn	< 2.0	16.3 ± 0.2	259 ± 1	-	250 ± 0	447 ± 14	
<i>Elemental analysis</i>							
% of TS	N	< 0.05	14.7 ± 0.0	8.17 ± 0.00	0.53 ± 0.03	7.95 ± 0.12	3.45 ± 0.05
	C	32.3 ± 1.7	42.1 ± 0.7	44.3 ± 1.6	-	45.6 ± 1.7	26.3 ± 1.2
	H	7.69 ± 0.40	7.33 ± 0.37	7.90 ± 0.21	-	8.04 ± 0.38	3.78 ± 0.22
	S	< 0.05	1.00 ± 0.02	0.64 ± 0.03	-	0.62 ± 0.03	0.55 ± 0.04
	O	34.5 ± 0.9	27.1 ± 0.9	25.6 ± 1.5	-	23.3 ± 1.7	25.8 ± 1.3
% of VS	N	< 0.05	15.4 ± 0.0	8.81 ± 0.00	-	8.38 ± 0.15	7.46 ± 0.11
	C	33.0 ± 1.7	44.1 ± 0.7	47.6 ± 1.7	-	49.0 ± 1.8	50.0 ± 2.6
	O	34.9 ± 0.9	28.1 ± 1.0	27.5 ± 1.7	-	25.2 ± 1.8	41.0 ± 2.8

¹ The ratio of pig gut and flotation fat in the mixture was 9:1 on a VS basis.² Biodegradable carbon was calculated by deducting lignin carbon from TOC. The formula of Lignin was chosen as C_{9.94}H_{12.82}O_{2.94}. ³ in equivalent glucose ⁴ n-hexane extractable material (HEM)⁵ Fibre contents in mixture of pig gut with flotation fat were calculated from the measured fibre results of pig gut, assuming no fibre in flotation fat.

Table 6.3 Carbon to Nitrogen Ratio and Biochemical Composition of food waste and co-substrates as different percentages of the input

Substrate	Food waste	Co-substrate									
		Cattle slurry	Rumen content	Office paper	Paper pulp	Brewery waste		Whey	Potato waste	Flourmill waste	Card packaging
						Spent grain	Yeast				
Individual materials - analytical results											
TOC / TKN	13.9	11.1	20.7	271	63.6	14.7	4.52	18.4	28.0	30.8	288 ± 5
Biodegradable C / TKN	13.6	8.14	14.2	211	35.2	11.6	4.52	18.4	27.5	25.7	83.1 ± 4.7
Carbohydrates (g kg ⁻¹ VS)	453	21.9	63.4	86.5	53.0	104	244	812	833	497	242 ± 19
Lipids (g kg ⁻¹ VS)	151	93.6	25.7	< 10	< 10	107	< 10	36.3	< 10	15.1	< 10
Crude proteins (g kg ⁻¹ VS)	235	276	160	9.8	41.9	224	647	175	103	98.0	10.8 ± 0.0
Hemi-cellulose (g kg ⁻¹ VS)	38.1	226	310	125	123	335	20.7	-	22.0	180	113 ± 5
Cellulose (g kg ⁻¹ VS)	50.4	96.7	146	636	398	59.8	13.2	-	22.1	109	304 ± 6
Lignin (g kg ⁻¹ VS)	16.5	226	245	145	286	166	< 5	-	11.2	122	532 ± 2
Mixed food waste and co-substrate - values calculated on a mass balance basis											
<i>30% of co-substrate in the mixture on a VS basis (except for cattle slurry which has 30% of food waste added)</i>											
TOC / TKN		11.8	15.4	18.5	17.4	14.1	8.82	15.0	16.1	16.4	19.2
Biodegradable C / TKN		9.45	13.7	17.1	15.1	13.0	8.68	14.7	15.8	15.4	14.9
Carbohydrates (g kg ⁻¹ VS)		151	336	343	333	348	390	561	567	466	390
Lipids (g kg ⁻¹ VS)		111	113	106	106	138	106	117	106	110	106
Crude proteins (g kg ⁻¹ VS)		264	213	167	177	232	359	217	195	194	168
Hemi-cellulose (g kg ⁻¹ VS)		170	120	64.2	63.6	127	32.9	26.7	33.3	80.7	60.6
Cellulose (g kg ⁻¹ VS)		82.8	79.1	226	155	53.2	39.2	35.3	41.9	68.0	126
Lignin (g kg ⁻¹ VS)		163	85.1	55.1	97.4	61.4	11.6	11.6	14.9	48.2	171
<i>20% of co-substrate in the mixture on a VS basis (except for cattle slurry which has 20% of food waste added)</i>											
TOC / TKN		11.5	14.8	16.6	16.0	14.0	10.1	14.6	15.2	15.5	17.0
Biodegradable C / TKN		8.99	13.7	15.6	14.5	13.2	9.89	14.3	14.9	14.7	14.4
Carbohydrates (g kg ⁻¹ VS)		108	375	380	373	383	411	525	529	462	411
Lipids (g kg ⁻¹ VS)		105	126	121	121	142	121	128	121	124	121
Crude proteins (g kg ⁻¹ VS)		268	220	190	196	233	317	223	209	208	190
Hemi-cellulose (g kg ⁻¹ VS)		188	92.5	55.5	55.1	97.5	34.6	30.5	34.9	66.5	53.1
Cellulose (g kg ⁻¹ VS)		87.4	69.5	168	120	52.3	43.0	40.3	44.7	62.1	101
Lignin (g kg ⁻¹ VS)		184	62.2	42.2	70.4	46.4	13.2	13.2	15.4	37.6	120

Table 6.3 continued Carbon to Nitrogen Ratio and Biochemical Composition of food waste and co-substrates as different percentages of the input

Substrate	Food waste	Co-substrate									
		Cattle slurry	Rumen content	Office paper	Paper pulp	Brewery waste		Whey	Potato waste	Flourmill waste	Card packaging
					Spent grain	Yeast					
10% of co-substrate in the mixture on a VS basis (except for cattle slurry which has 10% of food waste added)											
TOC / TKN		11.3	14.3	15.1	14.8	13.9	11.7	14.2	14.5	14.6	15.3
Biodegradable C / TKN		8.55	13.6	14.5	14.0	13.4	11.5	13.9	14.2	14.1	13.9
Carbohydrates (g kg ⁻¹ VS)		65.0	414	416	413	418	432	489	491	457	432
Lipids (g kg ⁻¹ VS)		99.3	138	136	136	147	136	140	136	137	136
Crude proteins (g kg ⁻¹ VS)		272	228	212	216	234	276	229	222	221	213
Hemi-cellulose (g kg ⁻¹ VS)		207	65.3	46.8	46.6	67.8	36.4	34.3	36.5	52.3	45.6
Cellulose (g kg ⁻¹ VS)		92.1	60.0	109	85.2	51.3	46.7	45.4	47.6	56.3	75.8
Lignin (g kg ⁻¹ VS)		205	39.4	29.4	43.5	31.5	14.9	14.9	16.0	27.1	68.1
5% of co-substrate in the mixture on a VS basis (except for cattle slurry which has 5% of food waste added)											
TOC / TKN		11.2	14.1	14.4	14.3	13.9	12.7	14.0	14.2	14.2	14.5
Biodegradable C / TKN		8.34	13.6	14.0	13.8	13.5	12.4	13.7	13.9	13.8	13.7
Carbohydrates (g kg ⁻¹ VS)		43.5	434	435	433	436	443	471	472	455	442
Lipids (g kg ⁻¹ VS)		96.5	145	143	143	149	143	145	143	144	143
Crude proteins (g kg ⁻¹ VS)		274	231	224	225	234	256	232	228	228	224
Hemi-cellulose (g kg ⁻¹ VS)		217	51.7	42.4	42.3	52.9	37.2	36.2	37.3	45.2	41.8
Cellulose (g kg ⁻¹ VS)		94.4	55.2	79.7	67.8	50.9	48.5	47.9	49.0	53.3	63.1
Lignin (g kg ⁻¹ VS)		216	27.9	22.9	30.0	24.0	15.7	15.7	16.2	21.8	42.3

Note: Values for food waste and co-substrate mixes used in laboratory-scale trials in bold and italics.

Table 6.4 Carbon to Nitrogen Ratio and Biochemical Composition of post-collection mechanically-recovered BMW and co-substrates as different percentages of the input

Substrate	BMW	Co-substrate		Pig gut and flotation fat		Poultry litter
		Bio-diesel by-product	Sheep blood	Pig gut	Mixture ¹	
<i>Individual materials – analytical results</i>						
TOC / TKN	25.0	∞	2.85	5.40	5.85	6.70
Biodegradable C / TKN	19.1	∞	2.85	5.27	5.58	5.33
Carbohydrates (g kg ⁻¹ VS)	340	-	7.2	27.1	15.8	84.1
Lipids (g kg ⁻¹ VS)	68.6	-	< 10	279	349	40.4
Crude proteins (g kg ⁻¹ VS)	130	< 5	965	551	538	490
Hemi-cellulose (g kg ⁻¹ VS)	52.2	-	-	51.5	46.7	214
Cellulose (g kg ⁻¹ VS)	252	-	-	51.2	46.4	69.8
Lignin (g kg ⁻¹ VS)	184	-	-	20.5	18.6	154
<i>Mixed BMW and co-substrate – calculated values</i>						
<i>30% of co-substrate in the mixture on a VS basis</i>						
TOC / TKN		31.8	8.16	12.3	12.9	13.9
Biodegradable C / TKN		25.9	6.75	10.2	10.6	10.7
Carbohydrates (g kg ⁻¹ VS)		-	240	246	243	263
Lipids (g kg ⁻¹ VS)		-	48.0	132	153	60.1
Crude proteins (g kg ⁻¹ VS)		91.0	377	256	252	238
Hemi-cellulose (g kg ⁻¹ VS)		-	36.5	52.0	50.6	101
Cellulose (g kg ⁻¹ VS)		-	176	192	190	197
Lignin (g kg ⁻¹ VS)		-	129	135	134	175
<i>20% of co-substrate in the mixture on a VS basis</i>						
TOC / TKN		28.9	10.6	14.9	15.4	16.3
Biodegradable C / TKN		23.0	8.55	12.0	12.4	12.6
Carbohydrates (g kg ⁻¹ VS)		-	273	277	275	289
Lipids (g kg ⁻¹ VS)		-	54.9	111	125	63.0
Crude proteins (g kg ⁻¹ VS)		104	295	214	212	202
Hemi-cellulose (g kg ⁻¹ VS)		-	41.8	52.1	51.1	84.6
Cellulose (g kg ⁻¹ VS)		-	202	212	211	216
Lignin (g kg ⁻¹ VS)		-	147	151	151	178
<i>10% of co-substrate in the mixture on a VS basis</i>						
TOC / TKN		26.7	15.0	18.7	19.0	19.8
Biodegradable C / TKN		20.8	11.8	14.6	14.9	15.1
Carbohydrates (g kg ⁻¹ VS)		-	307	309	308	314
Lipids (g kg ⁻¹ VS)		-	61.7	89.6	96.6	65.8
Crude proteins (g kg ⁻¹ VS)		117	212	172	171	166
Hemi-cellulose (g kg ⁻¹ VS)		-	47.0	52.1	51.7	68.4
Cellulose (g kg ⁻¹ VS)		-	227	232	231	234
Lignin (g kg ⁻¹ VS)		-	166	168	167	181
<i>5% of co-substrate in the mixture on a VS basis</i>						
TOC / TKN		25.8	18.8	21.4	21.6	22.1
Biodegradable C / TKN		19.9	14.5	16.5	16.7	16.9
Carbohydrates (g kg ⁻¹ VS)		-	323	324	324	327
Lipids (g kg ⁻¹ VS)		-	65.2	79.1	82.6	67.2
Crude proteins (g kg ⁻¹ VS)		124	171	151	150	148
Hemi-cellulose (g kg ⁻¹ VS)		-	49.6	52.2	51.9	60.3
Cellulose (g kg ⁻¹ VS)		-	239	242	242	243
Lignin (g kg ⁻¹ VS)		-	175	176	176	183

¹ The ratio of pig gut and flotation fat in the mixture is 9:1 on a VS basis.

Note: Values for food waste and co-substrate mixes used in laboratory-scale trials in bold and italics.

7. Determination of BMP for co-substrates

Two sets of biochemical methane potential (BMP) experiments were carried out for the determination of BMP values of co-substrates in combination with or in isolation from the baseline waste streams.

7.1 Determination of BMP for baseline waste streams with co-substrates

BMP assays were carried out on four of the food waste co-substrates and four of the mechanically-recovered BMW co-substrates, each mixed with their corresponding baseline municipal waste. Two sets of fifteen 1.4-litre working capacity digesters were used operating at a mesophilic temperature (36 ± 1 °C). Each set was used to test four of the co-substrates in triplicate with triplicate inoculum controls. The inoculum used was digestate liquor taken from semi-continuous digesters fed on the appropriate baseline wastes (see section 5). The tests used an inoculum-to-substrate ratio ($r_{I/S}$) of 4 on a volatile solids (VS) basis.

7.1.1 Inoculum of BMP test

The characteristics of the inoculum digestates are shown in Table 7.1. The digestates were taken from digesters which had been running for 119 days on food waste and 140 days on mechanically recovered BMW, both at an organic loading rate (OLR) of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$.

7.1.2 BMP test on the source-segregated food waste / co-substrate mixes

The four co-substrates used were: cattle slurry, used office paper, whey, and flourmill waste. The proportion of office paper and flourmill waste in the mixture was 20%, cattle slurry was 80%, and whey 17% on a VS basis.

The BMP tests ran for 130 days. Three quarters of the methane production in the test was due to the high background VFA concentration of the inoculum (equal to $13.9 \text{ STP m}^3 \text{ CH}_4 \text{ m}^{-3}$ inoculum or $0.335 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ of inoculum). This value also represented the residual BMP of the digestate liquor at the point the inoculum was taken for the BMP test. It can be seen from Figure 7.1 that there were some short-term increases in biogas production rate after the initial 20 days of rapid biogas production. This increase in rate was coupled with a slight drop in the biogas methane percentage, as shown in Figure 7.2. As this phenomenon was noted in both the test and control digesters it was likely to be related to the phased degradation of certain VFA species or other organic materials in the inoculum. This may result from the removal of product-induced feedback inhibition which occurs at high VFA concentrations. This phased degradation was also observed in the stressed digesters when they recovered from high VFA concentrations after the cessation of feeding in laboratory-scale food waste co-digestion trials (see section 8.2). The increases in biogas production rate occurred slightly later with office paper, flourmill waste and whey and slightly earlier with cattle slurry compared with the occurrence in the control. It was also noted in the laboratory-scale food waste co-digestion trials (see section 8.2) that cattle slurry could speed up the recovery of a stressed

digester. A second increase in the rate of biogas production in the BMP tests was observed at around day 60 to 80 though the extent was less than the first time.

The specific methane production and the methane percentage in biogas are given in Table 7.2. Food waste in combination with cattle slurry had a lower methane yield as a result of the high lignin content of cattle slurry and the lower percentage of food waste in the mix.

Table 7.1 Physicochemical characteristics of the co-digestion BMP inoculums

	Food waste inoculum	Mechanically-recovered BMW inoculum
pH	7.51 ± 0.04	7.55 ± 0.05
TS (% WW)	5.84 ± 0.02	6.57 ± 0.01
VS (% WW)	4.16 ± 0.02	3.28 ± 0.01
VS (% TS)	71.2 ± 0.2	49.9 ± 0.0
PA (g CaCO ₃ l ⁻¹)	8.76 ± 0.09	11.2 ± 0.4
IA (g CaCO ₃ l ⁻¹)	10.4 ± 0.1	4.97 ± 0.65
TA (g CaCO ₃ l ⁻¹)	19.2 ± 0.0	16.1 ± 0.3
IA:PA (Ripley ratio)	1.19 ± 0.02	0.45 ± 0.07
TAN (g NH ₃ -N l ⁻¹)	3.87 ± 0.01	1.50 ± 0.01
TKN (g N l ⁻¹)	6.52 ± 0.01	3.16 ± 0.02
TOC (g C l ⁻¹)	20.7 ± 0.4	15.5 ± 0.8
CV (kJ g ⁻¹ TS)	18.70 ± 0.06	10.56 ± 0.04
Acetic acid (g l ⁻¹)	9.72 ± 0.90	0.096 ± 0.003
Propionic acid (g l ⁻¹)	0.363 ± 0.039	< 0.005
Iso-Butyric acid (g l ⁻¹)	0.902 ± 0.080	< 0.005
n-Butyric acid (g l ⁻¹)	0.194 ± 0.026	< 0.005
Iso-Valeric acid (g l ⁻¹)	1.56 ± 0.14	< 0.005
n-Valeric acid (g l ⁻¹)	< 0.005	< 0.005
Hexanoic acid (g l ⁻¹)	< 0.005	< 0.005
Heptanoic acid (g l ⁻¹)	< 0.005	< 0.005
Total VFAs (g l ⁻¹)	12.7 ± 1.2	0.096 ± 0.003
% of TS using air-dried sample	N	11.1 ± 0.1
	C	36.0 ± 0.7
	H	5.55 ± 0.40
	O	21.7 ± 1.1
	S	0.46 ± 0.03
% of VS using air-dried sample	N	15.8 ± 0.0
	C	49.7 ± 1.0
	O	28.1 ± 1.6
Dissolved and total NPK	Shown in Table 5.2	Shown in Table 5.2
Potentially toxic elements	Shown in Table 5.2	Shown in Table 5.2

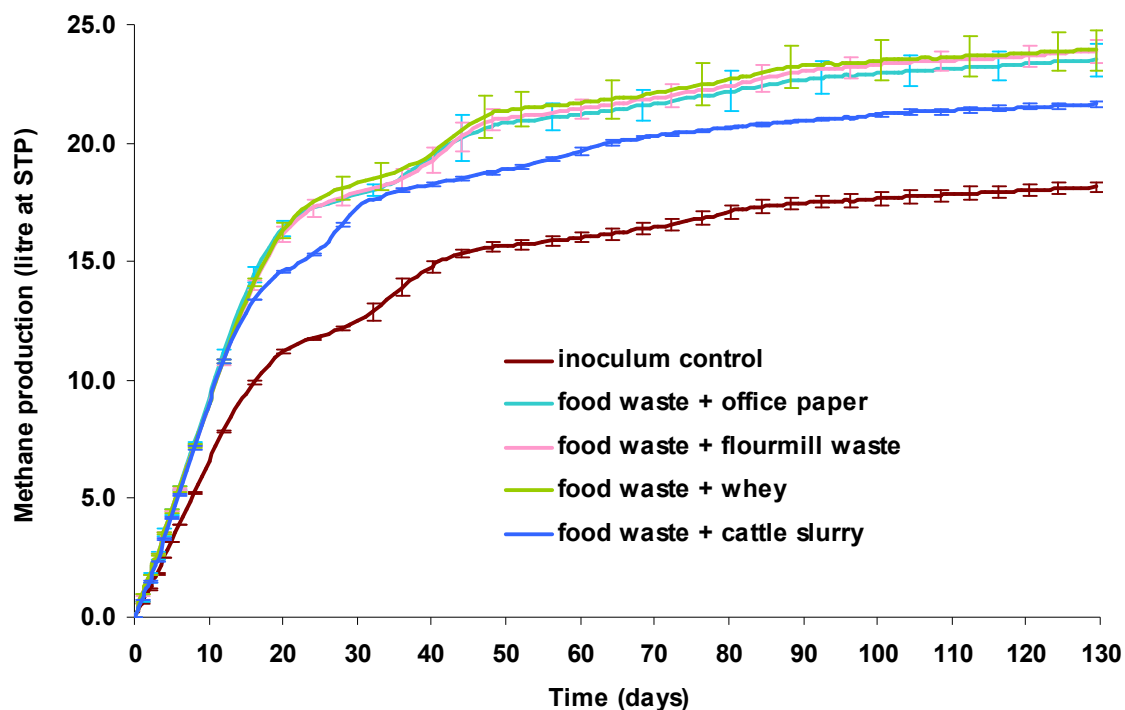


Figure 7.1 Methane production from source-segregated food waste in combination with selected co-substrates

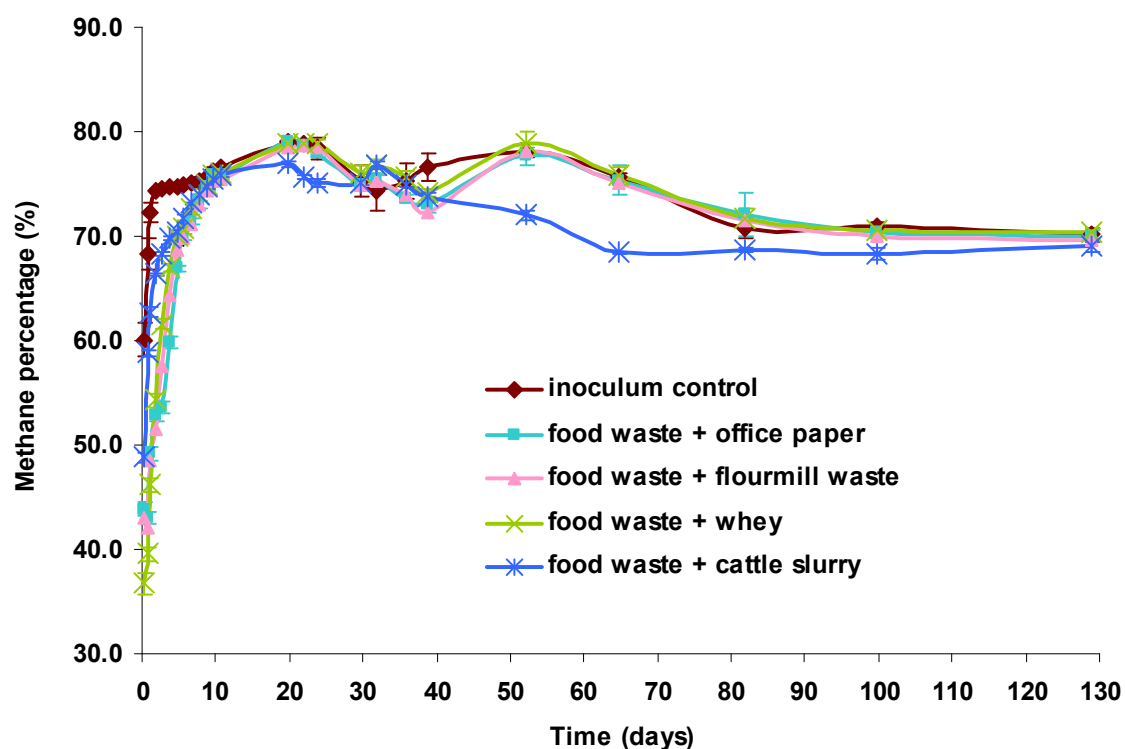


Figure 7.2 Percentage of methane in biogas produced in BMP tests using source-segregated food waste in combination with selected co-substrates as test materials

Table 7.2 Methane production and methane percentage in biogas from BMP tests using food waste in combination with co-substrates as test materials

Co-substrates with food waste	Office paper	Flourmill waste	Whey	Cattle slurry
% of co-substrates VS in mixture	20	20	17	80
BMP ¹	0.372 ± 0.013	0.398 ± 0.020	0.403 ± 0.016	0.264 ± 0.007
% of CH ₄ in biogas	56.0 ± 0.2	57.0 ± 0.7	57.5 ± 1.0	59.4 ± 0.2

¹ unit: STP m³ CH₄ kg⁻¹ VS

7.1.3 BMP test on mechanically-recovered BMW / co-substrate mixes

The four co-substrates used were: biodiesel by-product, sheep blood, pig gut with 10% of flotation fat (on a VS basis), and poultry litter. The moisture content of the biodiesel by-product, determined by Karl Fischer titration method, had not been measured when the BMP test was started and the ratio used was 20% of biodiesel by-product on a fresh weight basis to 80% of BMW on a VS basis; this was later established to be 19.4% of biodiesel by-product when calculated on a VS basis. The proportion of the other three co-substrates in each mixture was 20% on a VS basis.

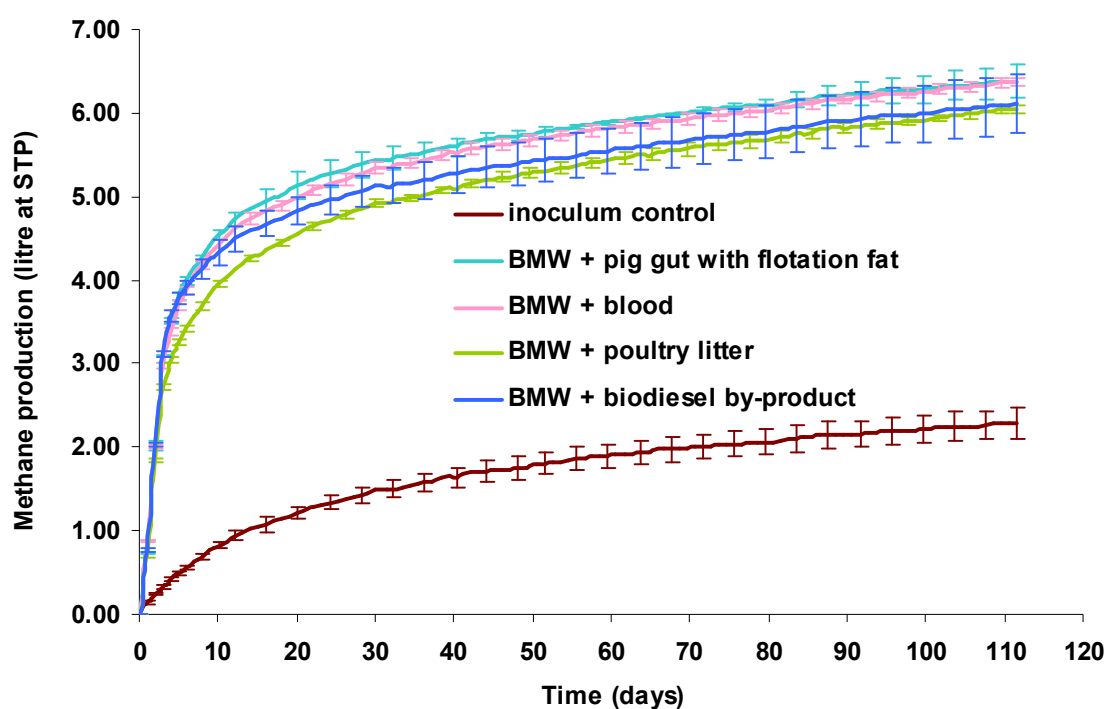


Figure 7.3 Methane production of mechanically recovered BMW in combination with co-substrates

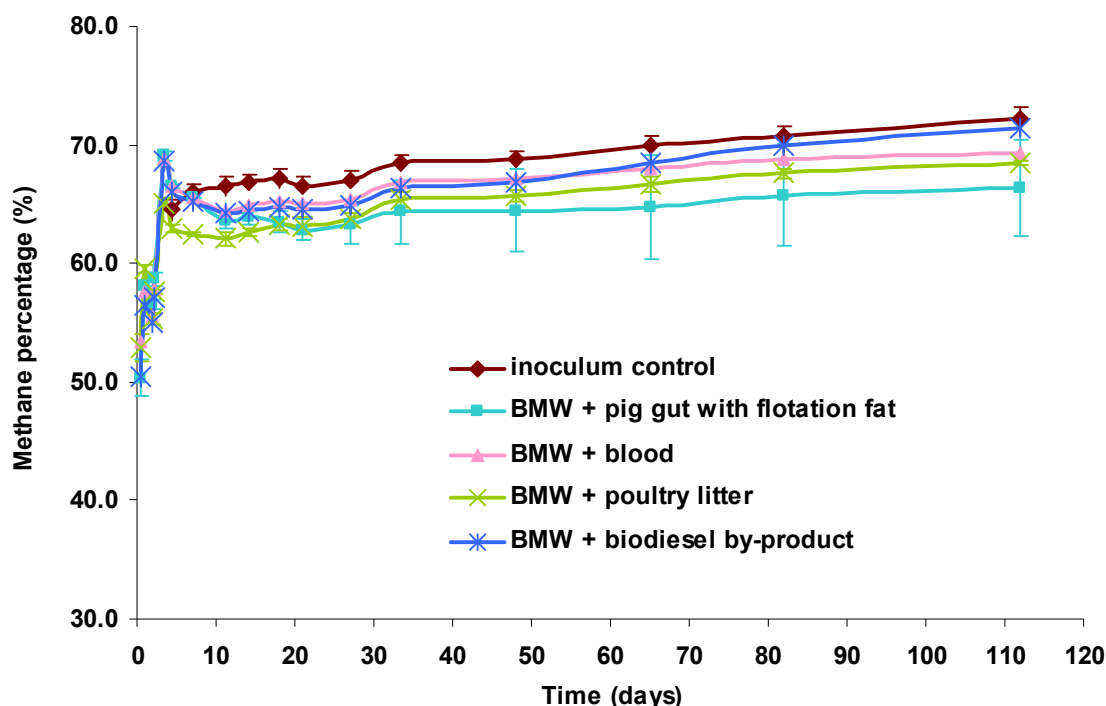


Figure 7.4 Percentage of methane in biogas produced from BMP test using mechanically recovered BMW in combination with co-substrates as materials

These BMP tests ran for 113 days. It can be seen from Figure 7.3 that the residual methane potential of mechanically-recovered BMW digestate liquor was $1.63 \text{ STP m}^3 \text{ CH}_4 \text{ m}^{-3}$ or $0.050 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$; this was one eighth of that of food waste digestate liquor. The percentage of methane in the biogas increased rapidly at the beginning of the test, as shown in Figure 7.4, with peaks indicating the shift in the digestion process from hydrolysis, acidogenesis and acetogenesis to methanogenesis and around 60~70% methane in the biogas mixes after 5-10 days incubation. There was a rapid initial biogas production rate in the first few days followed by a slower rate over the rest of the test.

The specific methane production and the methane percentage in biogas are shown in Table 7.3. Mechanically-recovered BMW in combination with poultry litter had lower methane production due to its higher fibre content. BMW with pig gut and flotation fat and with sheep blood showed higher methane production due to its higher protein and/or fat content.

Table 7.3 Methane production and methane percentage in biogas using mechanically recovered BMW in combination with co-substrates as test materials

Co-substrates with BMW	Pig gut with flotation fat	Blood	Poultry litter	Biodiesel by-product
% of co-substrates VS in mixture	20	20	20	20 ²
BMP ¹	0.358 ± 0.017	0.357 ± 0.005	0.329 ± 0.005	0.334 ± 0.030
% of CH ₄ in biogas	57.4 ± 2.0	58.7 ± 0.3	57.8 ± 0.4	57.4 ± 0.2

¹ unit: $\text{STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$

² The addition of biodiesel by-product was on a fresh weight basis; this is equal to 19.4 on a VS basis.

7.1.4 Discussion

In both sets of co-digestion BMP tests the methane production was lower than expected based on their theoretical BMP values. Table 7.4 lists the methane attributable to the co-substrates in the BMP tests, determined by subtracting the methane produced from corresponding baseline waste stream values, taken as 0.445 and 0.344 STP m³ CH₄ kg⁻¹ VS for food waste and mechanically-recovered BMW respectively.

A possible explanation is that the inoculum-to-substrate ratio ($r_{I/S}$) was calculated assuming that the microbial population in the inoculum was proportional to the VS content. When the inoculum comes from a sewage sludge digester in stable operation this is a reasonable assumption. In this case the inoculum came from a solid substrate digester with liquor recirculation: this allows volatile solids with a fine particle size to accumulate in the digestate, as some of the feedstock VS is from non-biodegradable organic materials such as lignin. VS content in the inoculum taken from the digester treating mechanically-recovered BMW increased from 1.28 % (Table 4.2) to 3.28 % (Table 7.1) between the two sets of BMP tests. A high proportion of non-degradable VS will lead to over-estimation of the actual ratio of 'active' VS to substrate. This explanation would also apply to the food waste BMP tests, although these were further complicated by the high levels of VFA within the digester.

The original aim of using acclimatised inoculum for the BMP tests was to provide microbial consortia specifically adapted to the baseline waste streams, in order to maximise the BMP value. No macro-nutrients or essential trace elements were added, as one of the purposes of the test was to assess whether these might be supplied by the co-substrate. The advantages of this approach failed to outweigh the difficulties, however, and made interpretation of the results harder. All subsequent BMP tests (i.e. for pre-pasteurisation) were therefore carried out using sewage sludge digestate from a municipal wastewater treatment plant as inoculum.

It is also noted from Table 7.4 that office paper appeared to have a surprisingly low BMP value; this may have been caused by inadequate sample preparation. The paper particle size was reduced to less than 0.5 mm using a centrifugal mill, leading to a loose but aggregated fibre structure which was difficult to disperse completely into the viscous food waste digestate liquor; this may have limited its bioavailability. Wet maceration was used when preparing card packaging for further BMP and semi-continuous laboratory-scale trials.

Table 7.4 Calculated and theoretical BMP values of co-substrates

Co-substrates	Experimental values calculated from batch co-digestion BMP experiments ¹	Theoretical BMP values based on biochemical composition ¹	Percentage of experimental value to theoretical BMP value (%)
Office paper	0.137	0.357	38.5
Flourmill waste	0.264	0.390	67.7
Whey	0.267	0.461	58.0
Cattle slurry	0.222	0.375	59.3
Pig gut with flotation fat	0.474	0.666	71.1
Sheep blood	0.450	0.482	93.5
Poultry litter	0.315	0.437	72.2
Biodiesel by product	0.344	-	-

¹ unit: STP m³ CH₄ kg⁻¹ VS

Note: BMP values of baseline waste streams are from $r_{I/S} = 2$

The soluble plant nutrient (N, P and K) content of the BMP digestates was analysed to compare the partitioning between the solid and liquid phase. Slightly lower concentrations were found with the co-substrates low in nutrients such as the biodiesel by-product. No clear trend could be concluded, however, due to the high background nutrient level introduced with the inoculum. The prolonged BMP test also changed the moisture content of BMP digestates making comparison difficult.

7.2 Determination of BMP using pasteurised waste streams

Where wastes contain or have been contaminated by meat or meat products, then processing of these wastes has to comply with the animal by-products regulations (ABPR). For digestion this is a relatively straightforward requirement as the process generates sufficient energy to allow a pasteurisation step in which the material, with a particle size of less than 12 mm, is held at 70 °C for 1 hour (EU ABP Regulation 1774/2002). The aim of this part of the research was to test the BMP of pasteurised wastes to assess whether pasteurisation / co-pasteurisation affected the kinetic and thermodynamic aspects of anaerobic degradation.

7.2.1 Set up

Tests were carried out on source-segregated food waste, mechanically-recovered BMW and the five co-substrates used in the laboratory-scale semi-continuous trials (cattle slurry, card packaging, and potato waste for food waste; sheep blood and pig gut with flotation fat for mechanically-recovered BMW) (see section 8). The pasteurised materials were tested in parallel with un-pasteurised materials. For completeness a mixture of food waste and cattle slurry pasteurised together was also compared with a mixture in which the two components had been pasteurised separately and then mixed. This was to see if there was any synergistic or antagonistic effect of heat treatment dependent upon the processing route. The characteristics of the materials used in the BMP tests have already been described (see section 3 and 6).

In total fifty-seven 1.4-litre working capacity CSTR digesters were operated at a mesophilic temperature (36 ± 1 °C) to carry out the BMP tests. The tests were all conducted with an inoculum-to-substrate ratio ($r_{I/S}$) of 4 on a VS basis, using an inoculum of digester sludge (strained through a 1 mm mesh) obtained from Millbrook wastewater treatment plant, Southampton. Each test material or material mixture was run in triplicate and four replicates of the sludge liquor without substrate were used as the inoculum control. The results are shown in Figures 7.5-7.15. The error bars represent the standard deviation but have not been plotted for every data point.

A test to monitor the time concentration profile of volatile fatty acids (VFA) and total ammonia nitrogen (TAN) was also set up in two digesters using pasteurised and un-pasteurised food waste material as substrates.

In addition, a positive control using cellulose as a standard reference material was also run as a validation check to ensure that the inoculum, inoculum-to-substrate ratio, biogas collection system, biogas sampling procedure, and biogas composition analysis gave accurate results. The cellulose used was from Sigma-Aldrich Company Ltd, UK, with a

product number of C6288, CAS number of 9004-34-6, and EC number of 232-674-9. It was a high purity cellulose powder used for partition chromatography, with a fibrous form and medium particle size.

7.2.2 Results from single substrate and waste mixes

It can be seen from Figure 7.5 that the methane production on the first day from un-pasteurised and pasteurised food waste was similar. Un-pasteurised food waste showed a higher methane production rate during the second day but subsequently the methane production rate from pasteurised food waste caught up resulting in the BMP values of 0.475 ± 0.031 and 0.473 ± 0.026 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised food waste respectively.

The methane production from the un-pasteurised and pasteurised cattle slurry during the test was similar, as shown in Figure 7.6. The BMP values were 0.267 ± 0.004 and 0.269 ± 0.004 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised cattle slurry respectively.

Methane production for un-pasteurised and pasteurised card packaging both exhibited a one-day lag at the start, as can be seen in Figure 7.7, and very similar rates thereafter. The BMP values were 0.266 ± 0.010 and 0.267 ± 0.005 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised card packaging respectively.

The rate of methane production from pasteurised potato (Figure 7.8) was slightly higher than from the un-pasteurised material at the beginning of the test but cumulative productions were the same by day 5 at which point the methane production levelled off in the un-pasteurised material and continued to increase, albeit at a low rate in the pasteurised material. The BMP values were 0.353 ± 0.004 and 0.395 ± 0.014 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised potato respectively.

The initial rate of methane production from un-pasteurised and pasteurised mechanically-recovered BMW was very similar, as shown in Figure 7.9. The un-pasteurised material showed a slightly higher methane production until the third day but after that time the cumulative methane production was roughly parallel. The BMP values were 0.349 ± 0.013 and 0.330 ± 0.019 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised material respectively.

The rate of methane production from un-pasteurised pig gut with flotation fat was higher than pasteurised pig gut with flotation fat at the start of the test (Figure 7.10) but methane production in the un-pasteurised test slowed down from day 7 onwards and continued to increase in the test with the pasteurised material. By day 16, the amount of methane produced was about the same in both tests. There was then a second short-term rate increase in methane production from un-pasteurised material which could possibly be attributed to the digestion of non-readily biodegradable intestinal material. A similar second short-term rate increase in methane production from pasteurised material was also observed from day 60 onward, perhaps for the same reason. The BMP values were 0.595 ± 0.014 and 0.575 ± 0.025 STP $\text{m}^3 \text{kg}^{-1}$ VS for un-pasteurised and pasteurised pig gut with flotation fat respectively.

The methane production rate from un-pasteurised sheep blood was initially higher than pasteurised sheep blood (Figure 7.11) and this may have been due to the lower specific surface area available for enzymic attack as a result of heat coagulation of the pasteurised

blood. From day 4 onward the methane volume produced from the digesters fed with un-pasteurised blood was less than that of the inoculum control, showing an apparent decrease in cumulative methane production when presented graphically. The apparent decline in cumulative methane production seen in both pasteurised and un-pasteurised blood tests was reversed as the test proceeded. In the un-pasteurised blood digesters methane production increased rapidly from day 33. The digestion of pasteurised blood started to recover from day 51 although the extent of inhibition was less than in its un-pasteurised control. The final BMP values were 0.418 ± 0.013 and 0.479 ± 0.026 STP $\text{m}^3 \text{kg}^{-1} \text{VS}$ for un-pasteurised and pasteurised blood respectively.

There is no clear explanation why the blood digestion behaved in this way, although the high free ammonia concentration might contribute towards the apparent inhibition; it was calculated that the total ammonia nitrogen (TAN) in the digestate could reach 2500 mg l^{-1} . The production of propionic acid during the degradation of blood protein might also be a reason, but as the digesters were sealed TAN, VFA, and pH were not measured to confirm this.

The results showed that for the materials tested pasteurisation showed a positive effect only on the methane yield of potato waste and sheep blood. The pasteurisation process had no impact upon the anaerobic biodegradation rate or the extent to which other substrates were degraded. Whilst there is a requirement under the Animal By-products Regulations (ABPR) to pasteurise waste streams containing or contaminated by animal by-products (ABP), the results of this work indicated that the option of pre-pasteurising waste streams before feeding to the digester is unlikely significantly to improve the process efficiency of anaerobic digestion.

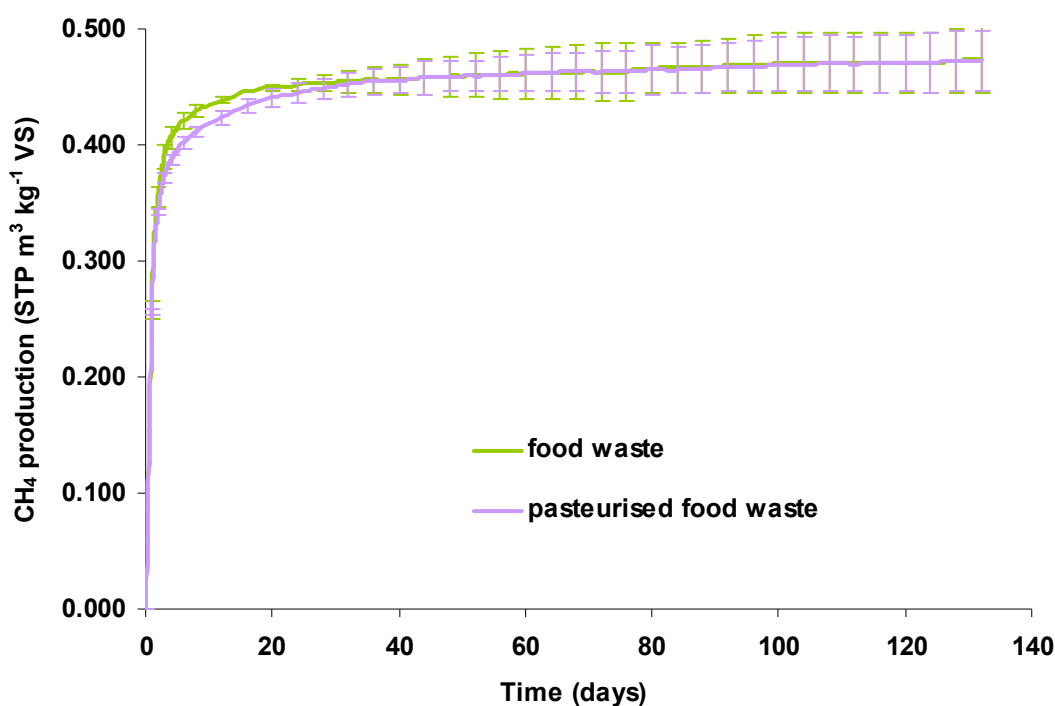


Figure 7.5 BMP assay of food waste and pasteurised food waste

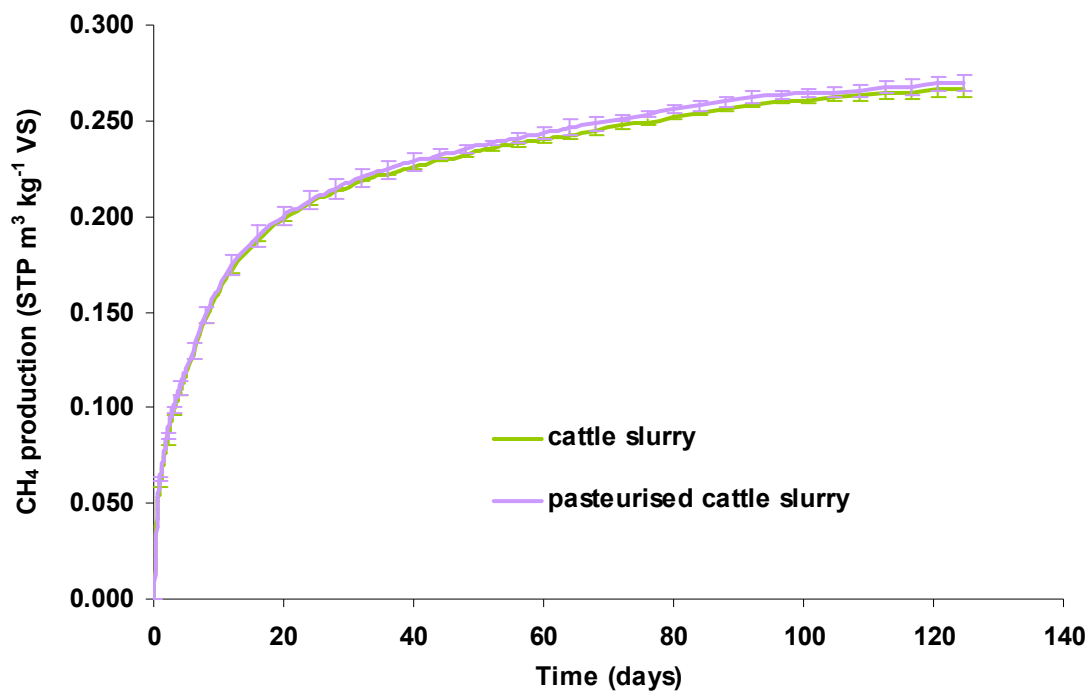


Figure 7.6 BMP assay of cattle slurry and pasteurised cattle slurry

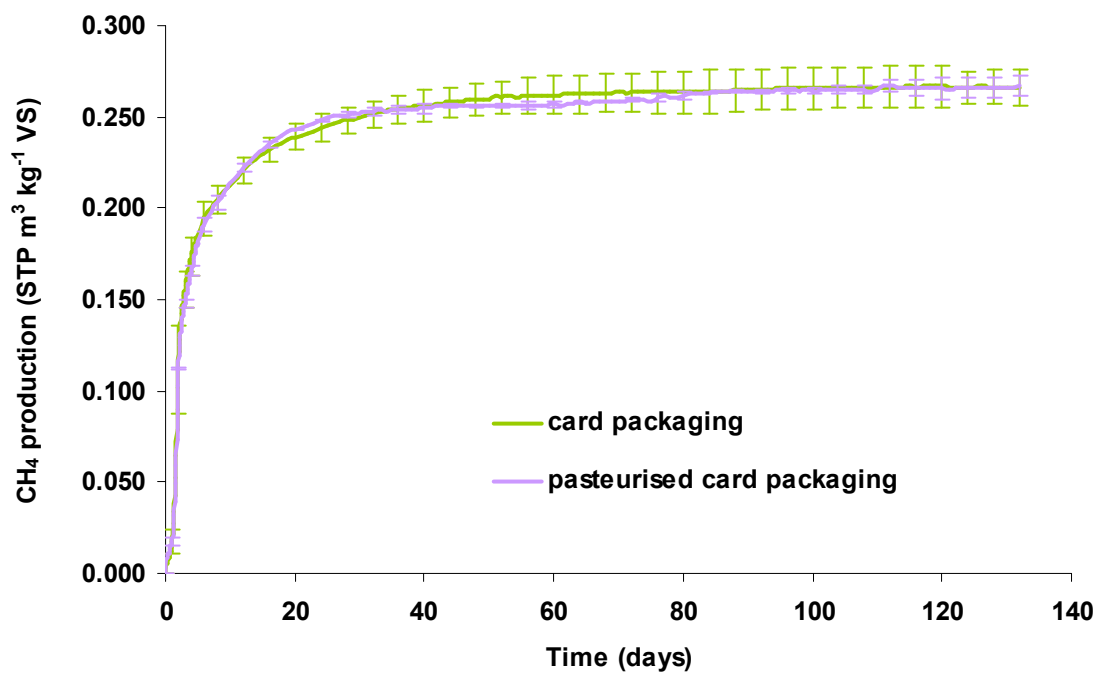


Figure 7.7 BMP assay of card packaging and pasteurised card packaging

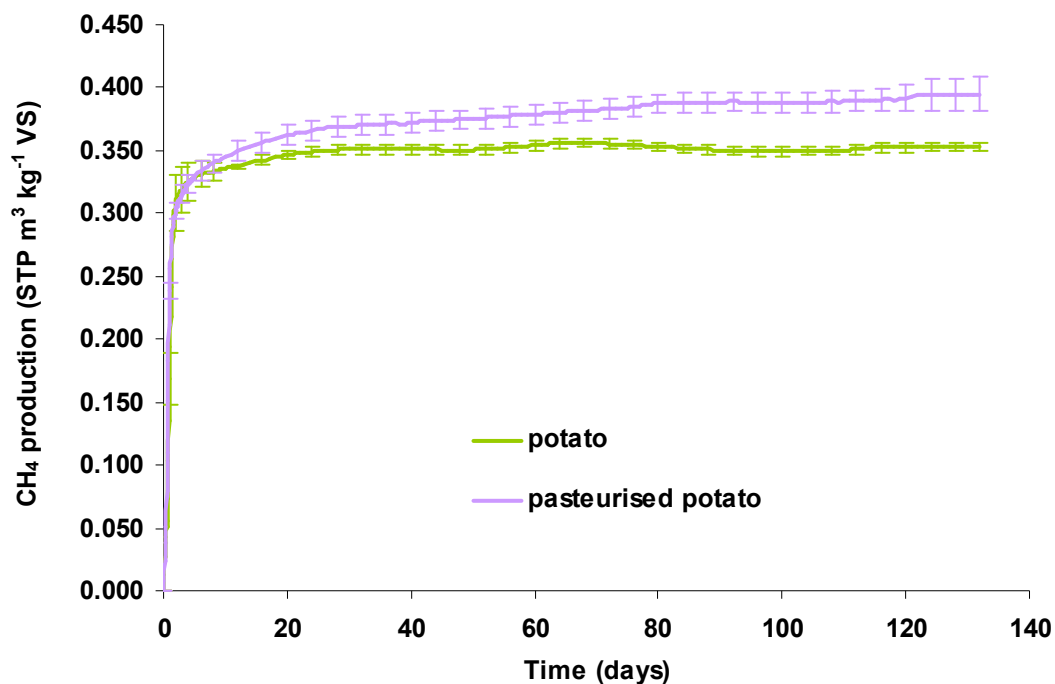


Figure 7.8 BMP assay of potato and pasteurised potato

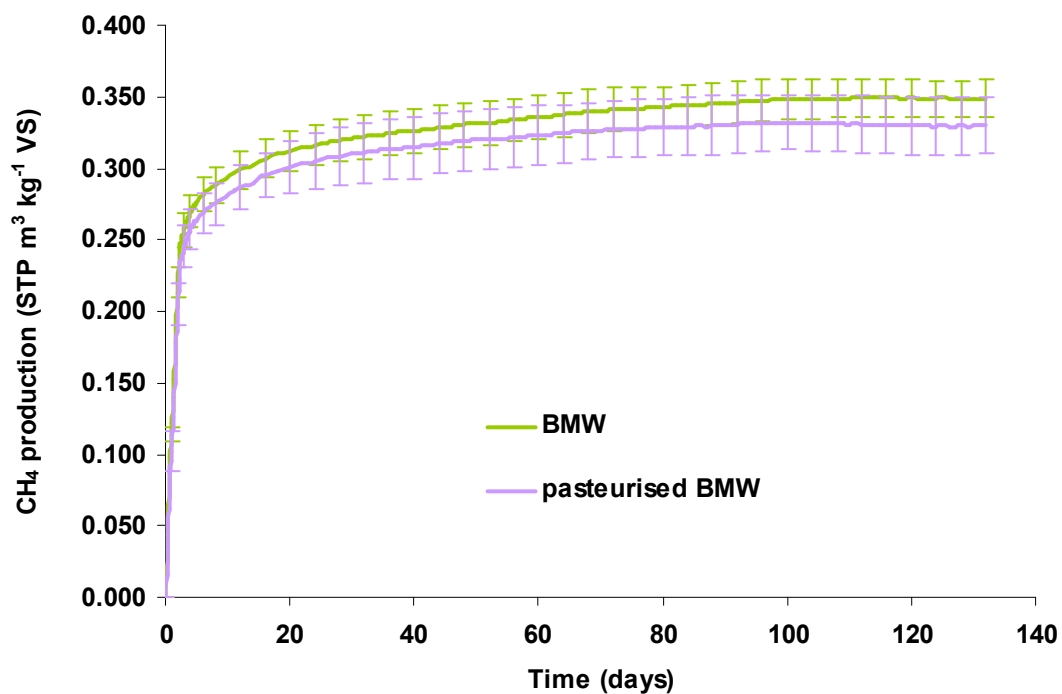


Figure 7.9 BMP assay of biodegradable municipal waste (BMW) and pasteurised BMW

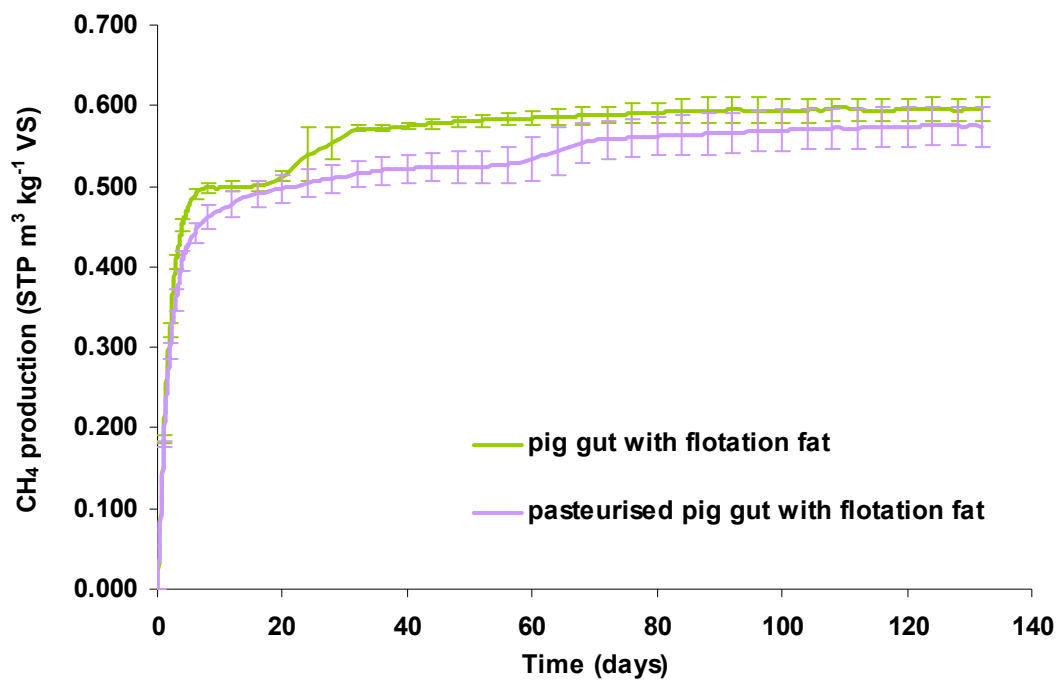


Figure 7.10 BMP assay of pig gut with flotation fat and pasteurised pig gut with flotation fat

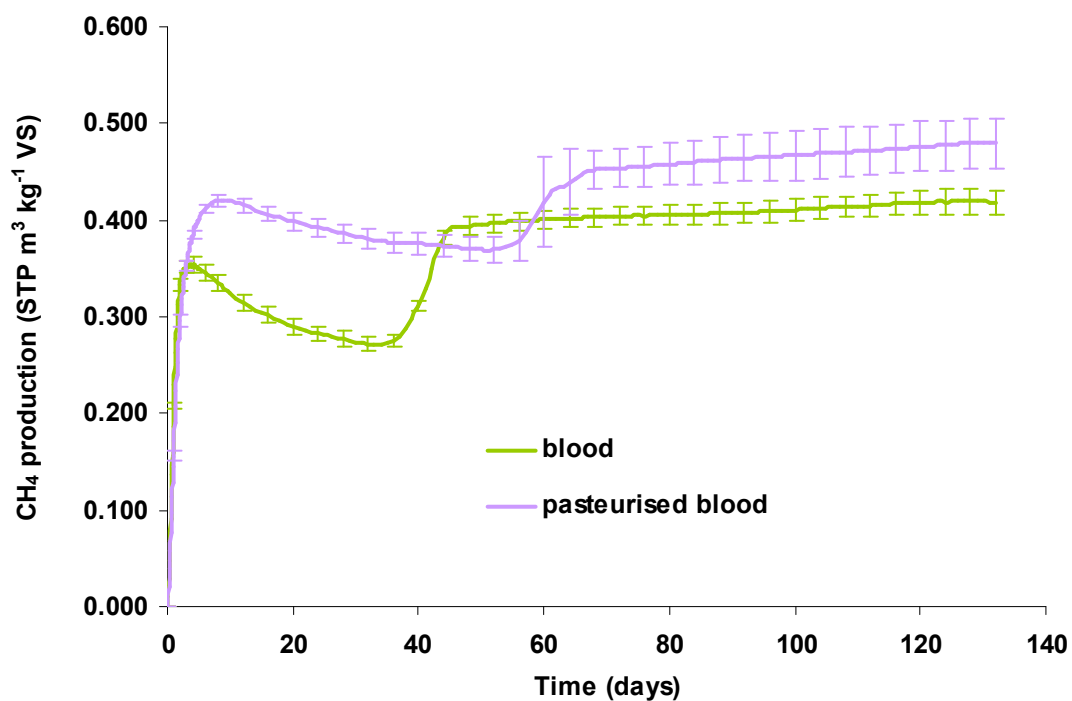


Figure 7.11 BMP assay of sheep blood and pasteurised sheep blood

7.2.3 Comparison of BMP data with theoretical methane yields

It can be seen from Table 7.5 that all of the test results except for the cattle slurry showed more than 80% of the theoretical methane yield calculated on the basis of the biochemical composition.

Calculated BMP values were also expressed on a fresh matter basis to take into account the inert and moisture fractions of the wastes (Table 7.6). In this case card packaging had highest methane yield because of its low moisture content of 6% and low inert content of 16% on a TS basis. Food waste and mechanically recovered BMW showed similar methane potential. The very low methane potential of cattle slurry, one tenth of that of card packaging, is the main reason why this is an unattractive substrate for energy production when digested alone.

Table 7.5 Comparison of experimental BMP tests with the theoretical BMP value calculated according to biochemical composition

Substrate	Theoretical BMP values ¹	Un-pasteurised		Pasteurised	
		Experimental BMP values ¹	Percentage of experimental value to theoretical value (%)	Experimental BMP values ¹	Percentage of experimental value to theoretical value (%)
Food waste	0.494	0.475±0.031	96.1	0.473±0.026	95.7
Cattle slurry	0.375	0.267±0.031	71.2	0.269±0.019	71.7
Card packaging	0.279	0.266±0.010	95.3	0.267±0.005	95.7
Waste potato	0.415	0.353±0.004	85.1	0.395±0.014	95.1
BMW ²	0.401	0.349±0.013	87.0	0.330±0.019	82.3
Pig gut with fat	0.666	0.595±0.014	89.4	0.575±0.025	86.3
Sheep blood	0.482	0.418±0.013	86.8	0.479±0.026	99.4

¹ Unit: STP m³ kg⁻¹ VS

² Mechanically-recovered

Table 7.6 BMP value of wastes on a fresh matter basis (Unit: STP m³ tonne⁻¹ fresh matter)

Substrate	Un-pasteurised	Pasteurised
Food waste	102	102
Cattle slurry	17.4	17.5
Card packaging	210	211
Waste potato	81.5	91.2
Mechanically recovered BMW	114	108
Pig gut with flotation fat	115	112
Sheep blood	79.0	90.5

7.2.4 Effect of co-pasteurisation of cattle slurry and food waste

The BMP of food waste and cattle slurry mixed in a ratio of 20:80% on a VS basis was tested as both separately pasteurised and co-pasteurised material. It can be seen from Figure 7.12 that the methane potential and rate of production of the pasteurised food waste and pasteurised cattle slurry when treated separately was similar to that when they were treated as a mixture. There was also no apparent synergistic or antagonistic effect as a result of heat treatment, as shown by comparison with the results of tests on the food waste and cattle slurry. The sum of the BMP values of the individually pasteurised

components was 0.310 ± 0.006 STP $\text{m}^3 \text{kg}^{-1}$ VS for the two materials tested individually. The BMP was 0.300 ± 0.008 STP $\text{m}^3 \text{kg}^{-1}$ VS if they were pasteurised individually but tested as a mixture, and 0.304 ± 0.002 STP $\text{m}^3 \text{kg}^{-1}$ VS if they were co-pasteurised and tested as a mixture.

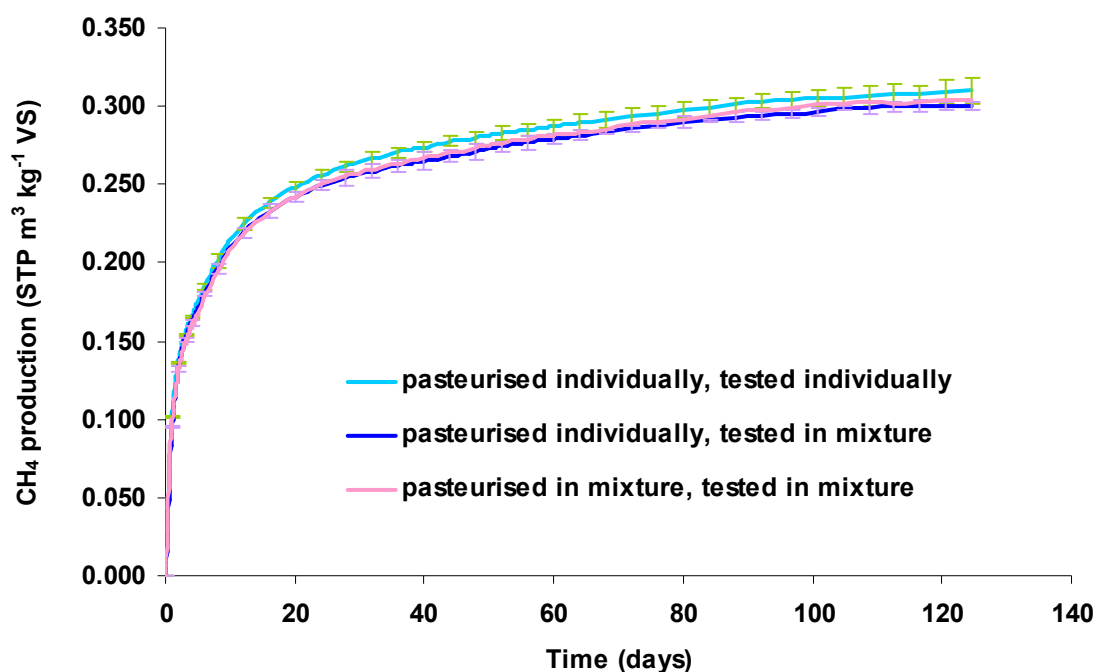


Figure 7.12 BMP assay for a mixture of pasteurised food waste and pasteurised cattle slurry, the pasteurised mixture of food waste and cattle slurry, and the calculated value of the total methane production from pasteurised food waste and pasteurised cattle slurry when daily values for each component are summed

7.2.5 BMP test on cellulose standard

Methane production from cellulose exhibited a three-day lag at the start of the test as shown in Figure 7.13. The lag phase probably indicated the time required for the inoculum to hydrolyse the complex macromolecular material. Methane production was then rapid with a 16-day BMP value of 0.361 ± 0.007 STP $\text{m}^3 \text{kg}^{-1}$ VS, reaching 87.0% of its theoretical BMP of 0.415 STP $\text{m}^3 \text{kg}^{-1}$ VS. Methane production continued to increase after 16 days, though at a much lower rate. The 64-day BMP value was 0.399 ± 0.007 STP $\text{m}^3 \text{kg}^{-1}$ VS, equal to 96.1% of the theoretical BMP. The final experimental BMP value of cellulose was 0.409 ± 0.006 STP $\text{m}^3 \text{kg}^{-1}$ VS, 98.6 % of the theoretical value. The closeness of the experimental value of the reference material to its theoretical value indicates that the BMP test method applied is reliable.

Carbon dioxide production was also monitored in this test to provide data on total biogas production. It can be seen from Figure 7.13 that this followed the same trend as that of methane, with an experimental value of 0.406 ± 0.017 STP $\text{m}^3 \text{CO}_2 \text{kg}^{-1}$ VS; this was equal to 98.0% of 0.415 STP $\text{m}^3 \text{CO}_2 \text{kg}^{-1}$ VS, the theoretical specific CO_2 production of cellulose under anaerobic condition.

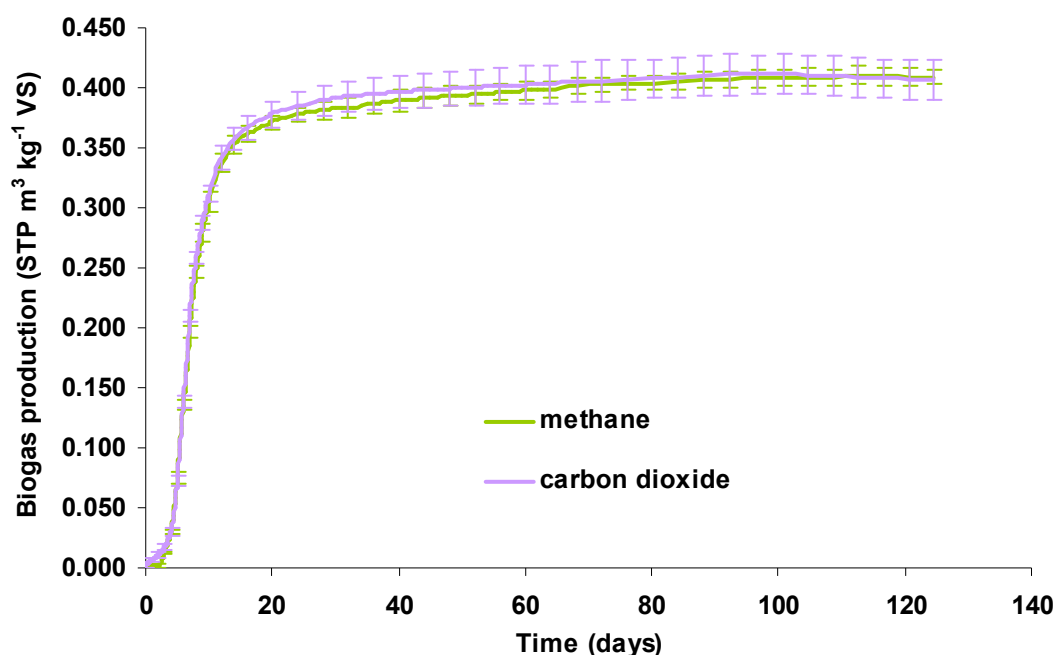


Figure 7.13 BMP assay of cellulose as positive control

7.2.6 VFA and ammonia profiles with time in pasteurised and un-pasteurised food waste BMP tests

It can be seen from Figure 7.14 that the VFA concentration in both the un-pasteurised and pasteurised food waste built up to around 550 mg l⁻¹ over a 12-hour period but then dropped very rapidly to less than 100 mg l⁻¹ after 1.5 days. This indicated that the inoculum had a balanced microbial population able to regulate the differential reactions likely to occur at the beginning of a BMP test, and inhibition due to the accumulation of intermediate products did not occur. As shown in Figure 7.15 acetic acid was around 55% of the total VFA on a mass basis, with propionic acid around 30%. Iso-valeric acid was the third largest followed by n-butyric and iso-butyric acid. There was no significant difference in the VFA profiles between pasteurised and un-pasteurised food waste.

The total ammonia nitrogen (TAN) concentration was also monitored. This gradually increased from 1500 (contributed by the inoculum) to 2000 mg l⁻¹ during the first 30 days of operation. The total Kjeldahl nitrogen introduced into the digestion system from the pasteurised and un-pasteurised food waste feedstock was only around 240 mg l⁻¹. TAN was not monitored in the inoculum control due to limitations on the number of digesters available and it was therefore not possible to have a complete mass balance. It can be assumed, however, that the inoculum may have contributed some additional TAN to the system.

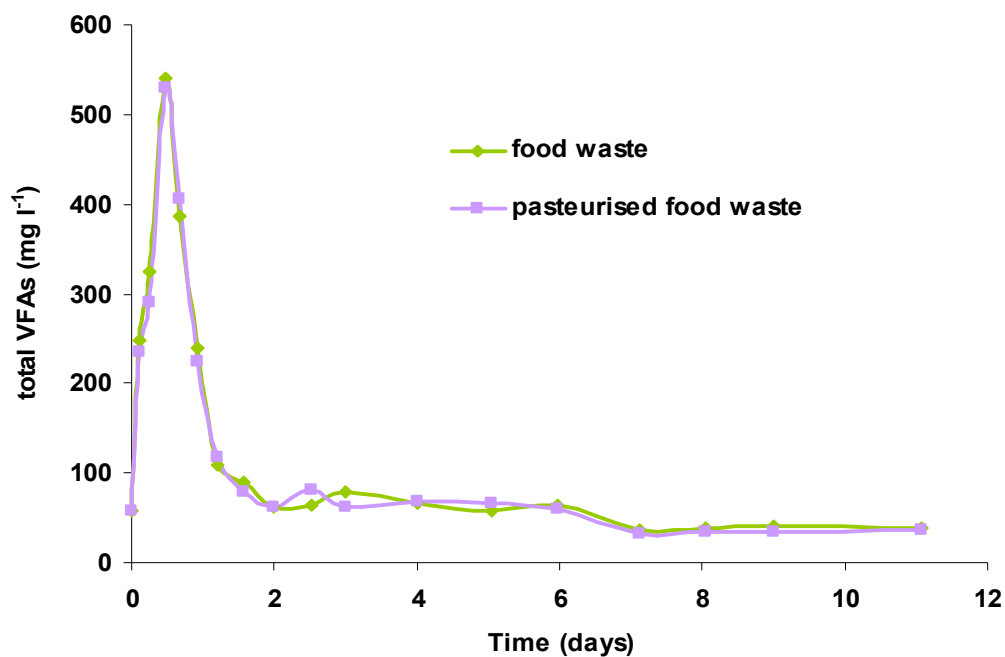


Figure 7.14 Total VFA concentrations in food waste and pasteurised food waste BMP digesters

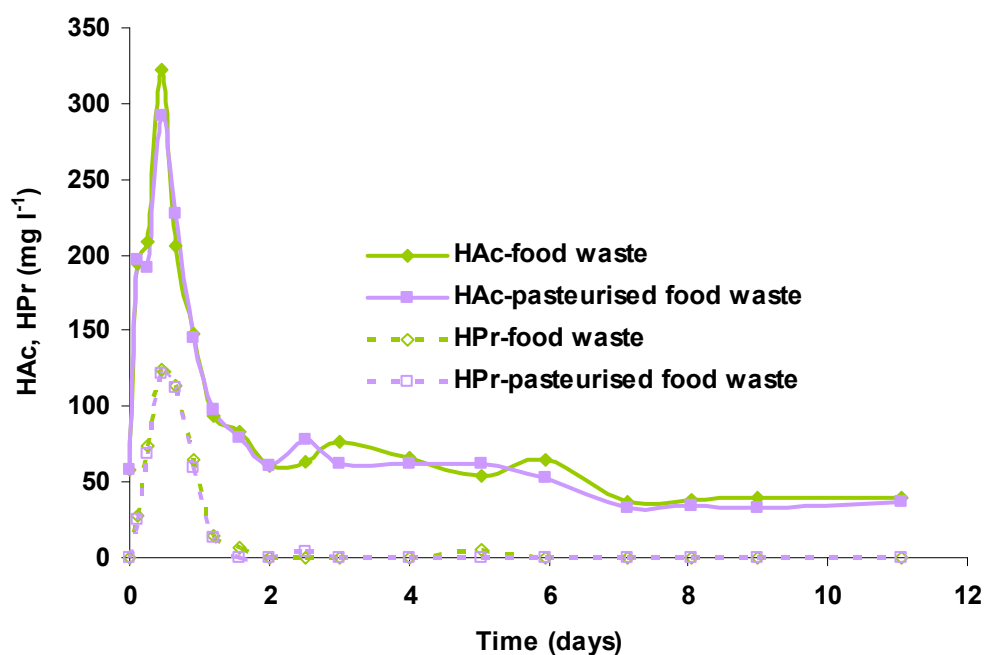


Figure 7.15 Acetic acid (HAc) and propionic acid (HPr) profiles in food waste and pasteurised food waste BMP digesters

7.2.7 Discussion of the pasteurisation experiments and BMP testing procedure

Of the materials tested, pasteurisation showed a positive effect on the methane yield only with potato waste and with sheep blood. The heat treatment made a big difference to the reaction kinetics of blood, but it is difficult to attribute this kinetic change to the effect of pasteurisation exclusively as the reason for the inhibition is not clear. The initially lower methane production rate from pasteurised blood may have been due to the lower specific surface area available for enzymic attack as a result of heat coagulation. It is likely that in this stage of the digestion of un-pasteurised sheep blood there was greater inhibition by the intermediate (e.g. VFA) and/or final digestion products (e.g. ammonia) than in the test with pasteurised blood. This effect might be overcome if a higher inoculum-to-substrate ratio was used or an inoculum acclimated to digesting blood had been chosen.

In the BMP test the inoculum-to-substrate ratio is kept high (4:1 on a VS basis in this case) to provide a robust consortium of bacteria to 'buffer' the differential rates in microbial reactions at the beginning of a batch fed test. In most cases immediate methane production is seen with little or no lag, and some time later there is a gradual tailing off in production rate. If the amount of inoculum is too low for the amount of substrate this response changes as the mass ratio of food to the acid-forming bacteria enables a very rapid onset of fermentation generating an unfavourable mass ratio of 'intermediate' food to methanogens. This leads to acidic conditions developing which are characterised by a lag in the methane production curve. It then takes time for the slower growing methanogens to 'catch up'. Where the initial substrate to inoculum ratio is very unfavourable, or the substrate contains a high proportion of readily fermentable components, the pH can drop to a point where methane production cannot start. With food waste that contains a very high proportion of readily fermentable materials some initial imbalance is inevitable even when the substrate to inoculum ratio is very favourable, but this does not necessarily have any adverse effect. In this case the accumulation of VFA (Figure 7.14) did not impact on the methane production curve (Figure 7.5) and the majority of the initial VFA peak was transformed into biogas within the first day. The measurement of the VFA profile is not an integral part of the BMP test but was carried out as an insurance test to explain any irregularities that might have occurred in the BMP gas production curve; in this case it was not necessary as the BMP curves themselves showed very typical responses.

8. Operation of laboratory-scale co-digestion trials with both baseline waste streams and co-substrates

The aim of this part of the research was to run laboratory-scale trials using both types of municipal waste streams and co-substrates to determine process loading rates and reactor conditions for optimisation of biogas production. Emphasis was placed on ensuring the stability of the long-term digestion operation, rather than increasing the loading rate to a point of maximum volumetric biogas production over a short period, as this would not necessarily reflect stable digestion conditions.

8.1 Operation of laboratory-scale mechanically-recovered BMW co-digestion digesters

Five digesters, each with a working volume of 4 litres, were set up using digestate liquor taken from the mechanically-recovered BMW digester 1 (see section 4) as inoculum. Mechanically-recovered BMW after further particle size reduction was the sole feedstock for the digesters over the first two months to confirm that the performance of the smaller-scale equipment was similar between units and to that of the 35-litre digesters. Two of the digesters were then fed with mechanically-recovered BMW and a mixture of pig gut and flotation fat in the ratio of 9:1 on a volatile solids (VS) basis. A further two digesters were fed a mixture of mechanically-recovered BMW and sheep blood. The proportion of the two co-substrates in each mixture was 20% on a VS basis. The fifth digester continued to be fed solely on mechanically-recovered BMW and acted as a control. A nominal solids retention time (SRT) of 30 days was maintained through liquor re-circulation and the total organic loading rate (OLR) on the digesters was initially $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$. The digesters were monitored daily in terms of biogas production and pH. Other digestate parameters such as solids, volatile fatty acids (VFA), total ammonia nitrogen (TAN), and alkalinity, as well as biogas composition, were analysed a minimum of once per week and often more frequently. The process efficiency was estimated by calculating the specific biogas production (SBP) and volatile solids removals (VSR) using data from the above analyses. Experimental results for the mechanically-recovered BMW co-digestion are shown graphically in Figures 8.1 to 8.15; day 0 was designated as the first day when co-substrates were added to the digesters, which were then run for 425 days. Between day 270-274 a total volume of 300 ml of digestate was taken from each digester for analysis and lukewarm tap water was used to make up the volume; this lowered the alkalinity and TAN concentrations for around one retention time.

Table 8.1 Organic loading rate in laboratory-scale mechanically-recovered BMW co-digestion trials

Time (days)	0~190	191~350	351~425
mechanically-recovered BMW + sheep blood	2	3	3
mechanically-recovered BMW + pig gut with flotation fat	2	3	4
mechanically-recovered BMW	2	3	4

Unit: $\text{kg VS m}^{-3} \text{ d}^{-1}$

8.1.1 Operation at 2 kg VS m⁻³ d⁻¹

The digesters ran at an OLR of 2 kg VS m⁻³ d⁻¹ for 190 days, more than 6 nominal SRT. During this time, the digester with mechanically-recovered BMW as the sole substrate showed very stable performance and the process efficiency was comparable to that of semi-continuous baseline waste digesters described in section 4. Some data on the biogas production of the mechanically-recovered BMW control are missing due to a gas leak.

Digesters running with the mixture of mechanically-recovered BMW and sheep blood showed some signs of inhibition at the end of the first solids retention time, as did the digesters with mechanically-recovered BMW and pig gut with flotation fat as feedstock shortly after. Although the quantity of co-substrate added was moderate it still led to a rapid build-up of TAN, as seen in Figure 8.8. This was not unexpected, as the physico-chemical characteristics of the mixture of mechanically-recovered BMW with sheep blood showed a very low C:N ratio of 8.6, and the mixture of mechanically-recovered BMW with pig gut and flotation fat also had a low ratio of 12.4 and a high lipid content of 125 g kg⁻¹ VS. Coupled with the high pH values, these TAN concentrations resulted in high free ammonia concentrations in both sets of digesters (Figure 8.9). This caused some inhibition of methanogenic activity, indicated by a reduction of the biogas methane content to 56% in the case of pig gut with flotation fat and 51% in the case of the blood co-substrate (Figure 8.6). This was accompanied by a build-up in VFA, reaching 3000 mg l⁻¹ in digesters fed with mechanically-recovered BMW and pig gut with flotation fat and 9000 mg l⁻¹ in digesters with mechanically-recovered BMW and sheep blood respectively (Figure 8.12). Acetic acid was the predominant VFA in both cases (Figure 8.13-8.16).

The pair of digesters fed with mechanically-recovered BMW and pig gut with flotation fat recovered from early signs of inhibition at the end of the second retention time, indicating acclimatisation of the microbial consortium to the feedstock. The system then appeared stable with methane production 10% higher than from the mechanically-recovered BMW control, and a VFA concentration of less than 200 mg l⁻¹ (Figure 8.12).

The digesters fed with mechanically-recovered BMW and sheep blood also showed some degree of recovery at the end of the second retention time with a fall in VFA concentration to less than 1000 mg l⁻¹ (Figure 8.12). The propionic acid concentration then built up suddenly and rapidly after the initial recovery and it became the predominant VFA species during the third retention time (Figure 8.15 and 8.16). This resulted in a second occurrence of process instability with a decrease in methane production to 0.214 STP m³ CH₄ kg⁻¹ VS_{added} (Figure 8.2) and in the percentage of methane in the biogas which fell to 55% (Figure 8.6). The presence of propionic acid is not unusual when using blood as feedstock; the digesters appeared able to regulate the unfavourable conditions imposed by the high propionic acid concentration and high free ammonia level during the fourth retention time when total VFA concentration reached around 4000 mg l⁻¹ (Figure 8.12) with propionic acid as 79% of the total (Figure 8.15 and 8.16). The VFA concentration later fell and levelled off at around 1500 mg l⁻¹ with acetic acid as the predominant species at the end of the sixth retention time.

The average specific methane production (SMP) during the sixth SRT was 0.319, 0.289, and 0.288 STP m³ CH₄ kg⁻¹ VS_{added} for digesters fed with mechanically-recovered BMW and pig gut with flotation fat, mechanically-recovered BMW and sheep blood and the mechanically-recovered BMW control respectively, equal to 80.1, 79.7 and 82.5% of the biochemical methane potential (BMP) values of the feedstock.

8.1.2 Operation at 3 kg VS m⁻³ d⁻¹

After 6 retention times the system appeared to be acclimatised to the conditions and at this point the OLR was increased to 3 kg VS m⁻³ d⁻¹. The mechanically-recovered BMW control adapted smoothly to the new OLR and showed a similar SMP to that obtained at an OLR of 2 kg VS m⁻³ d⁻¹ (Figure 8.2) with a 50% increase in volumetric methane production (VMP) (Figure 8.4). All stability parameters were maintained in the safe range during the loading change, with VFA less than 100 mg l⁻¹, total ammonia nitrogen (TAN) 1500 mg l⁻¹ and pH 7.4. The digester then ran at this loading for 160 days, or more than 5 retention times.

Both pairs of digesters fed with mechanically-recovered BMW and co-substrates experienced a shock as a result of the increase in loading: this can be seen from the rising VFA concentration (Figure 8.12). The digesters running with mechanically-recovered BMW and pig gut with flotation fat showed a rapid acclimatisation to the operational change as the VFA concentration reduced gradually from its peak value of 3500 mg l⁻¹. The digesters ran at this loading for 160 days. The VMP reached 0.99 STP m³ CH₄ m⁻³ d⁻¹ which was 50% higher than that achieved at an OLR of 2 kg VS m⁻³ d⁻¹ (Figure 8.4). The VFA concentration persisted at 1000~2000 mg l⁻¹ with acetic and propionic acids as the predominate species for the last 3 retention times at this loading, combined with an elevated TAN of 5000 mg l⁻¹ (Figure 8.8).

The higher loading had a more severe impact on the co-digestion of mechanically-recovered BMW and sheep blood. A transitional stable stage was observed during the fourth retention time at this loading (day 280~310) with VFA concentrations of 11000~15000 mg l⁻¹. The system maintained a high pH at around 8.0 (Figure 8.7) due to the high TAN concentration of 8000 mg l⁻¹, as shown in Figure 8.8. This provided buffering capacity and also maintained a moderate ratio of intermediate alkalinity (IA) to partial alkalinity (PA) at around 0.6, which was adequate to keep the digesters functioning. It is evident, however, that the SBP of this pair of digesters severely decreased at this OLR. Although the lowered TAN concentration around day 270 apparently mitigated the inhibiting effect of the ammonia, resulting in a short-term increase in biogas production, this fell again when the TAN rose to its previous level. The SBP of this pair of digesters was 0.320 STP m³ kg⁻¹ VS_{added} at the end of the fourth retention time at OLR 3 kg VS m⁻³ d⁻¹ (day 310) (Figure 8.1), only 60% of that at the OLR of 2 kg VS m⁻³ d⁻¹.

The performance of the pair of digesters fed with mechanically-recovered BMW and sheep blood diverged from the fourth retention time at this OLR (day 280). One digester (No. 1) appeared to acclimate further to the OLR of 3 kg VS m⁻³ d⁻¹, with a slight fall in VFA (Figure 8.12) and a lower percentage of propionic acid (Figure 8.15). This was combined with an increase in biogas production to 0.406 STP m³ kg⁻¹ VS (Figure 8.1) and a higher proportion of methane in the biogas from 56% to 59% (Figure 8.6). The other digester (No. 2), however, accumulated a large amount of acetic acid: rising from 5000 mg l⁻¹ at the beginning of the fifth retention time (day 310) to 25000 mg l⁻¹ at the end of the seventh retention time (Figure 8.16), resulting in a pH drop from 8.0 to 7.2 (Figure 8.7). This digester then remained stressed but stable until the end of the run, with a biogas production of 0.161 STP m³ kg⁻¹ VS (Figure 8.1) and a methane concentration of 43%

(Figure 8.6). The digesters continued to run at this loading for 235 days (nearly 8 retention times).

8.1.3 OLR increased to 4 kg VS m⁻³ d⁻¹

The OLR of the mechanically-recovered BMW control and the mechanically-recovered BMW and pig gut with flotation fat was increased to 4 kg VS m⁻³ d⁻¹ from day 351. The digesters ran at this loading for 75 days (2.5 retention times) until the end of the trial.

In the mechanically-recovered BMW control a drop in specific biogas production occurred at the end of the first retention time at this loading (Figure 8.1); this was accompanied by a rapid VFA accumulation to 5000 mg l⁻¹ (Figure 8.12) with acetic acid as predominate species (Figure 8.17), a pH drop (Figure 8.7), and a lower methane concentration in the biogas (Figure 8.6). As the VFA was consumed in the second retention time, specific biogas production also recovered to the level achieved at OLR 3, reaching a volumetric methane production of 1.25 STP m³ m⁻³ d⁻¹, double that achieved at an OLR of 2 kg VS m⁻³ d⁻¹. All stability parameters had returned to a safe range by 2 retention times after the loading increase from 3 to 4 kg VS m⁻³ d⁻¹, with VFA less than 150 mg l⁻¹ (Figure 8.17), total ammonia nitrogen (TAN) 1600 mg l⁻¹ (Figure 8.8) and a pH 7.4 (Figure 8.7).

The pair of digesters fed with mechanically-recovered BMW and pig gut with flotation fat showed a gradual rise in VFA after the loading increase, mainly contributed by propionic acid (Figures 8.13 and 8.14). Whilst the total ammonia nitrogen (TAN) remained at 4500 mg l⁻¹ (Figure 8.8), a drop in pH (Figure 8.7) was observed in response to VFA accumulation, with a fall in biogas production.

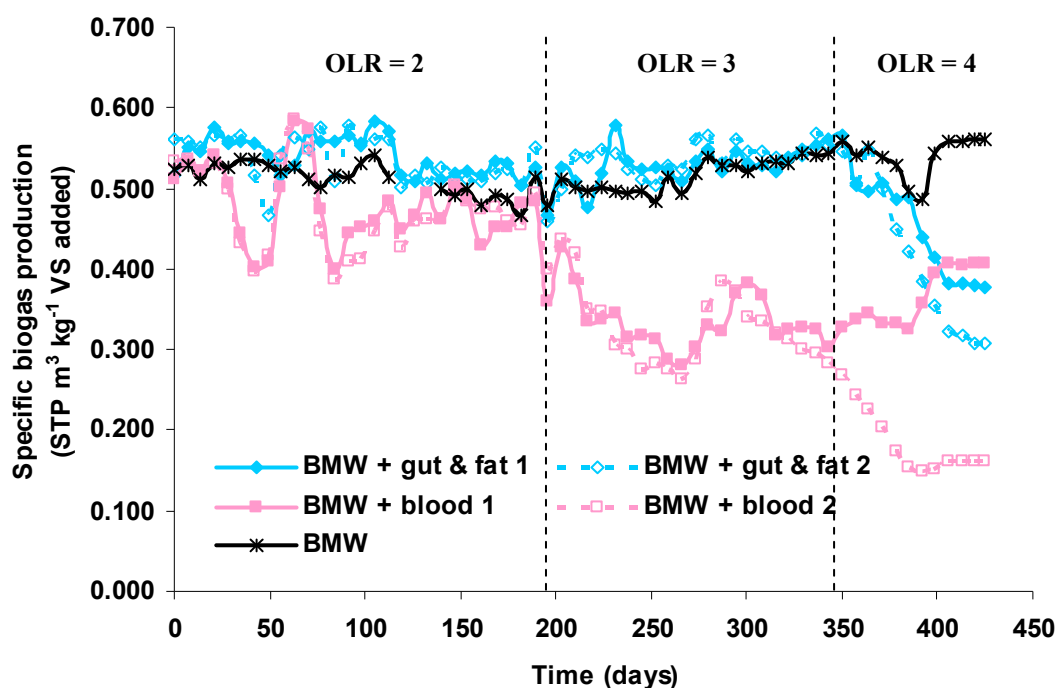


Figure 8.1 Weekly average specific biogas production in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

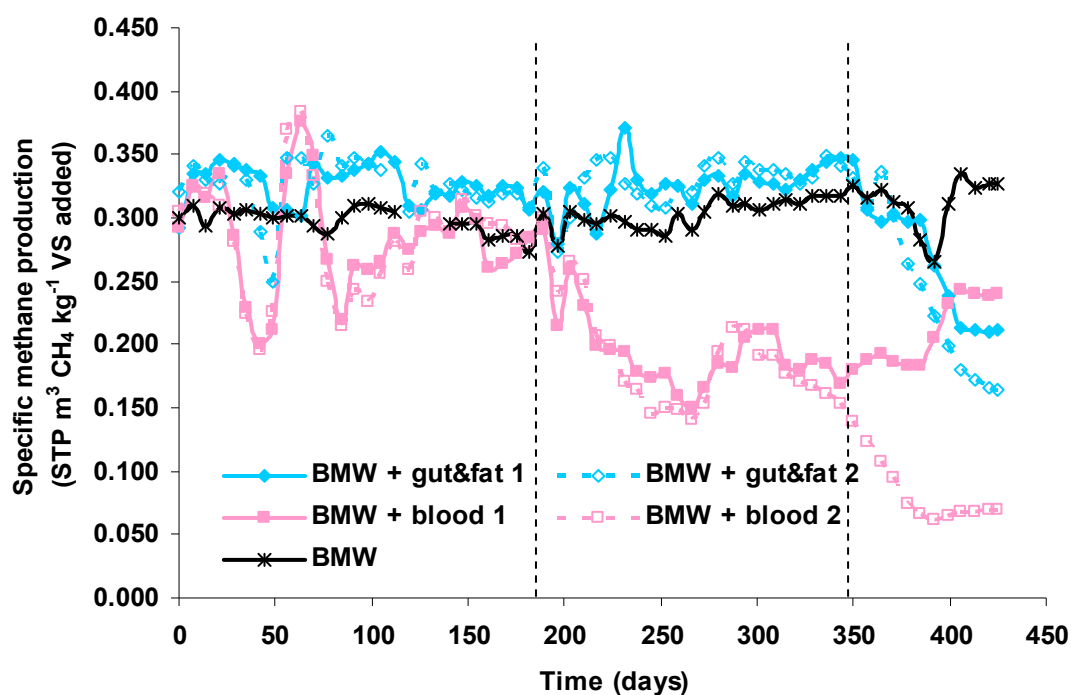


Figure 8.2 Weekly average specific methane production in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

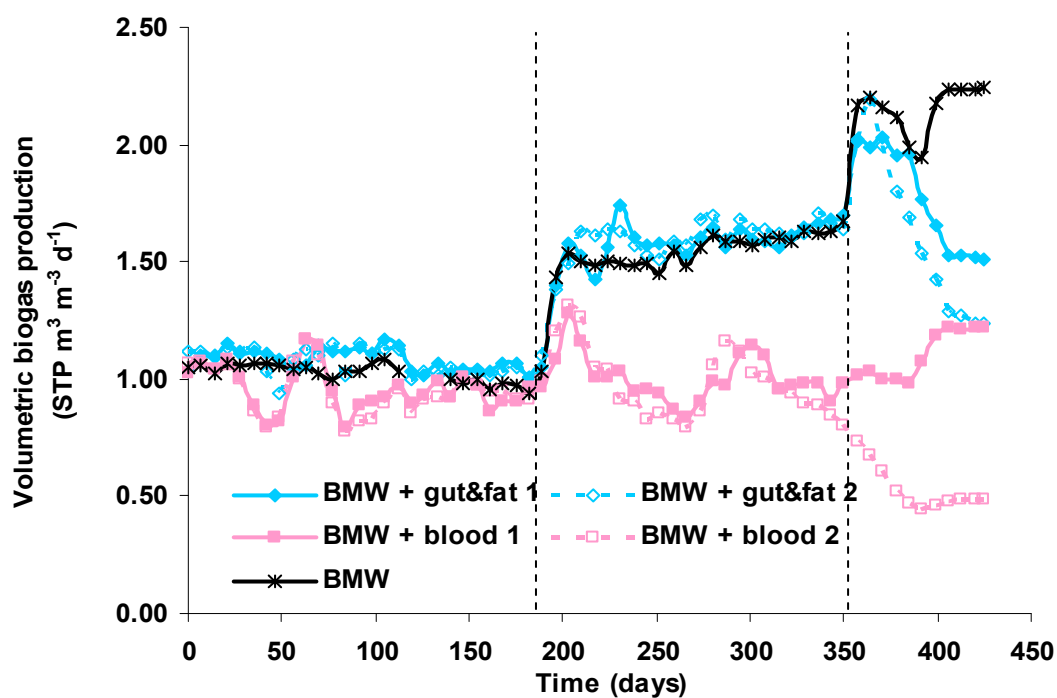


Figure 8.3 Weekly average volumetric biogas production in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

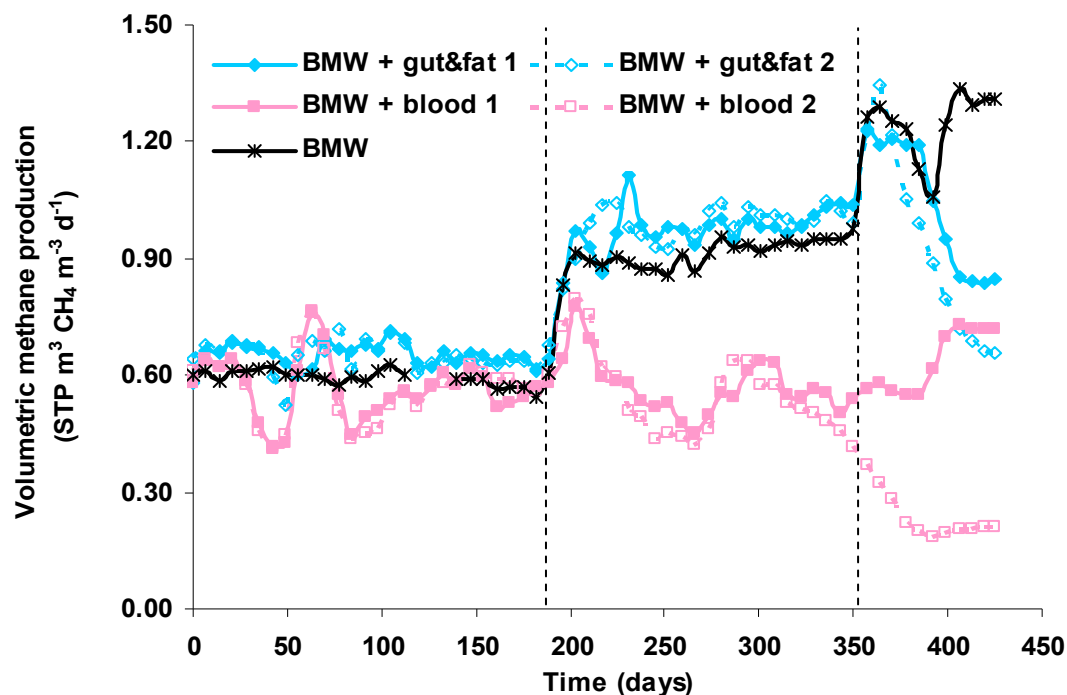


Figure 8.4 Weekly average volumetric methane production in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

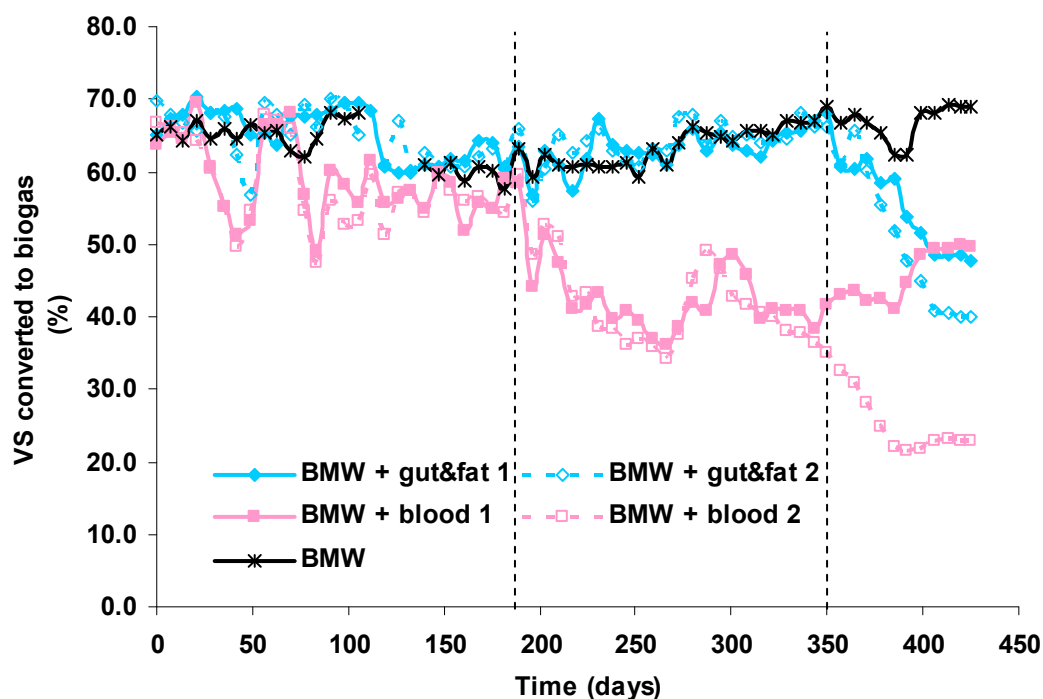


Figure 8.5 Weekly average mass conversion from feedstock VS to biogas in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

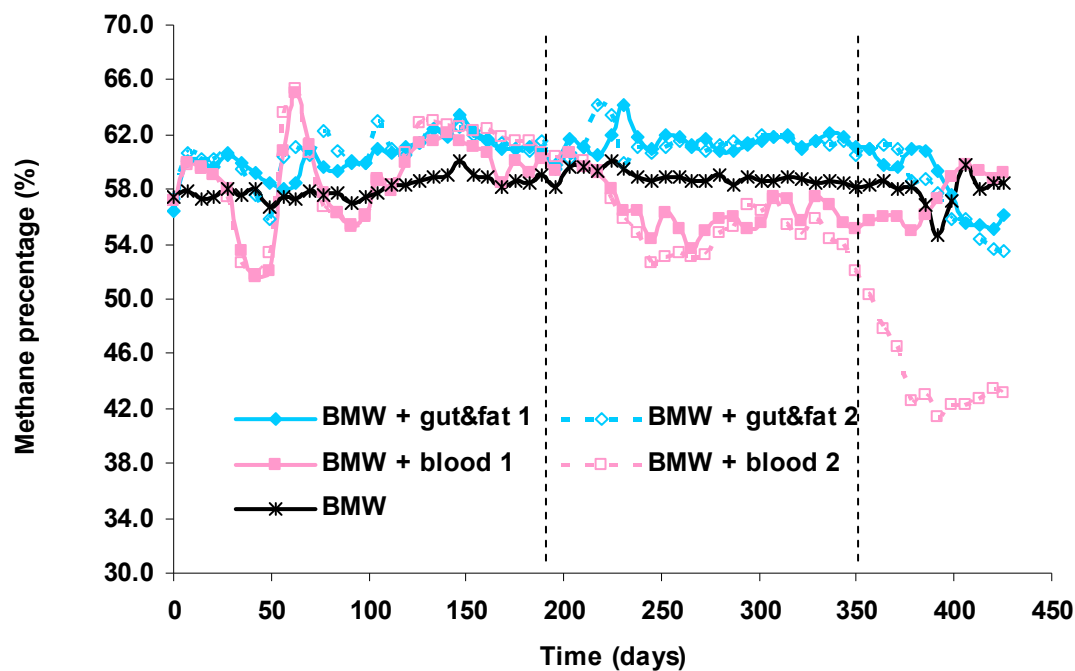


Figure 8.6 Weekly average methane content of biogas produced in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

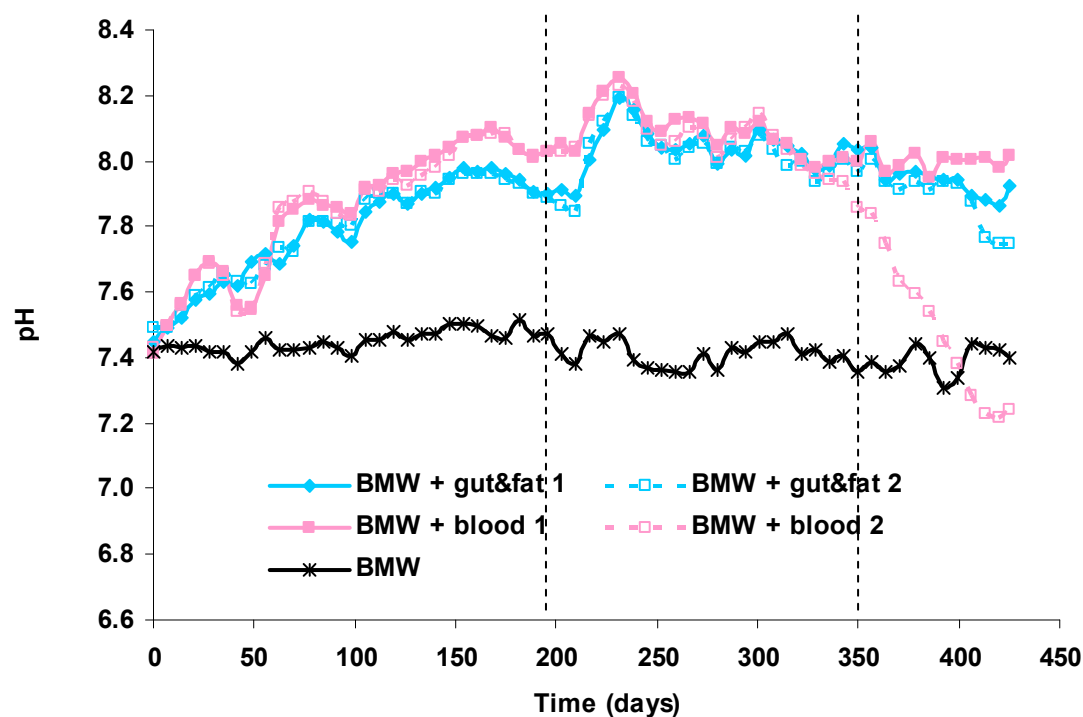


Figure 8.7 Weekly average pH values in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

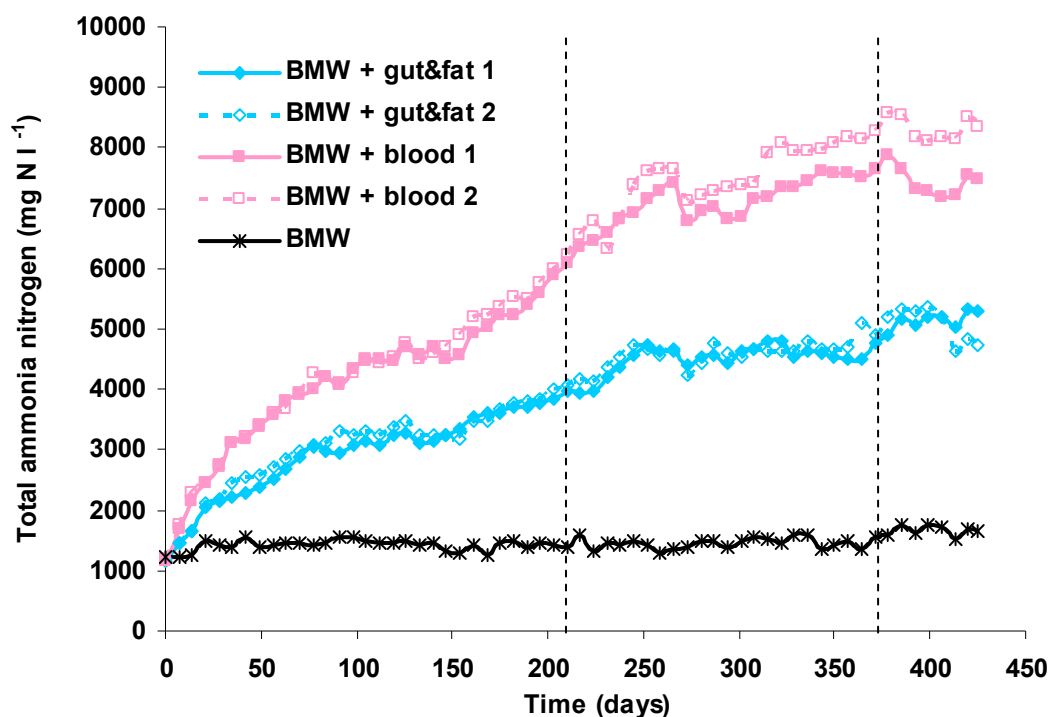


Figure 8.8 Total ammonia nitrogen (TAN) concentration in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

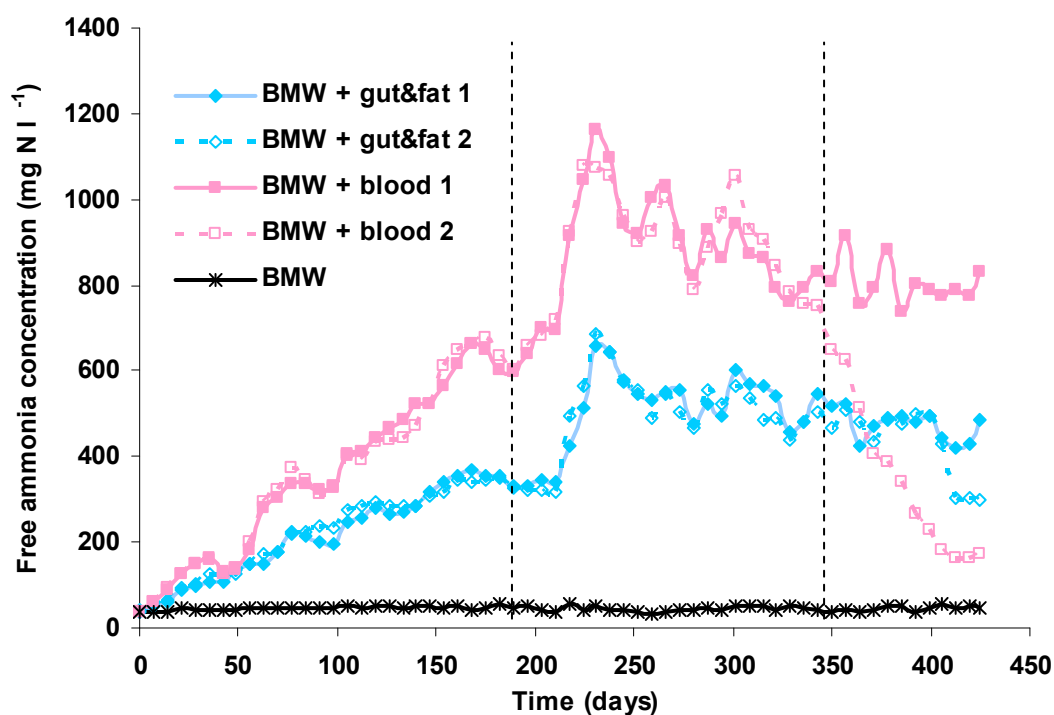


Figure 8.9 Free ammonia concentration in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

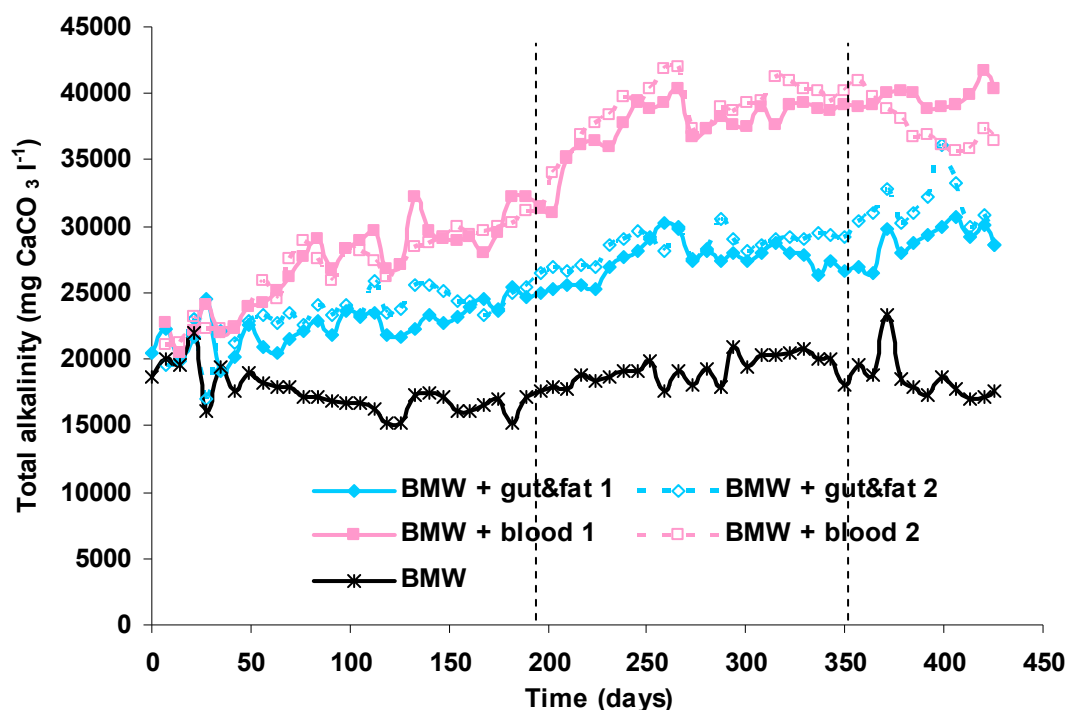


Figure 8.10 Total alkalinity in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

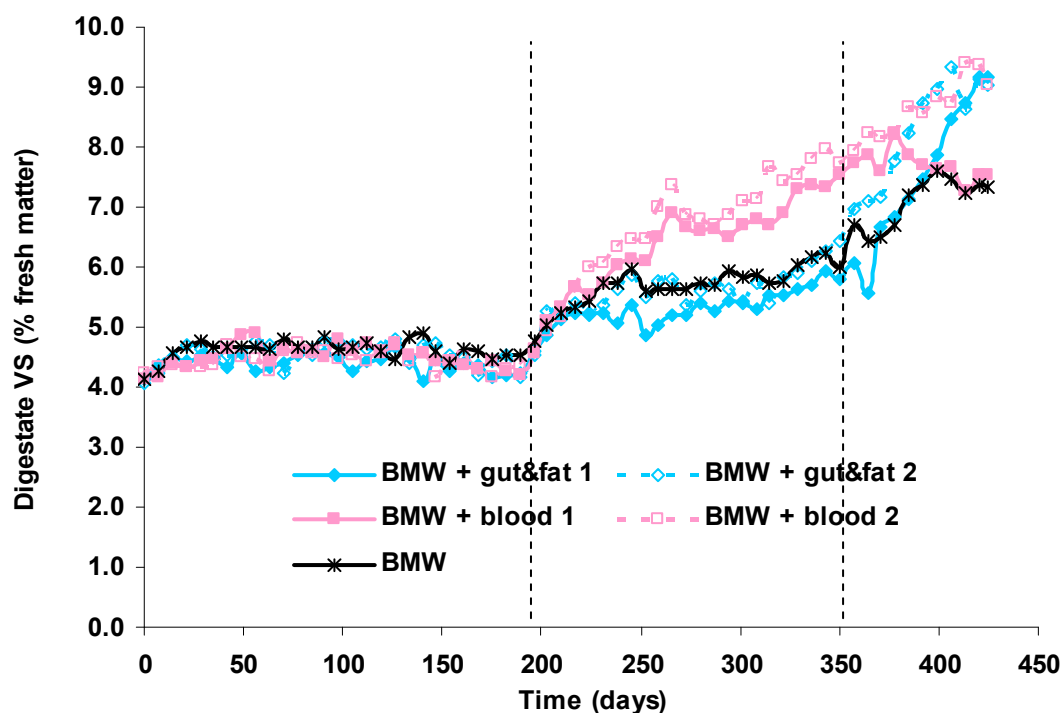


Figure 8.11 Digestate volatile solids content in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture

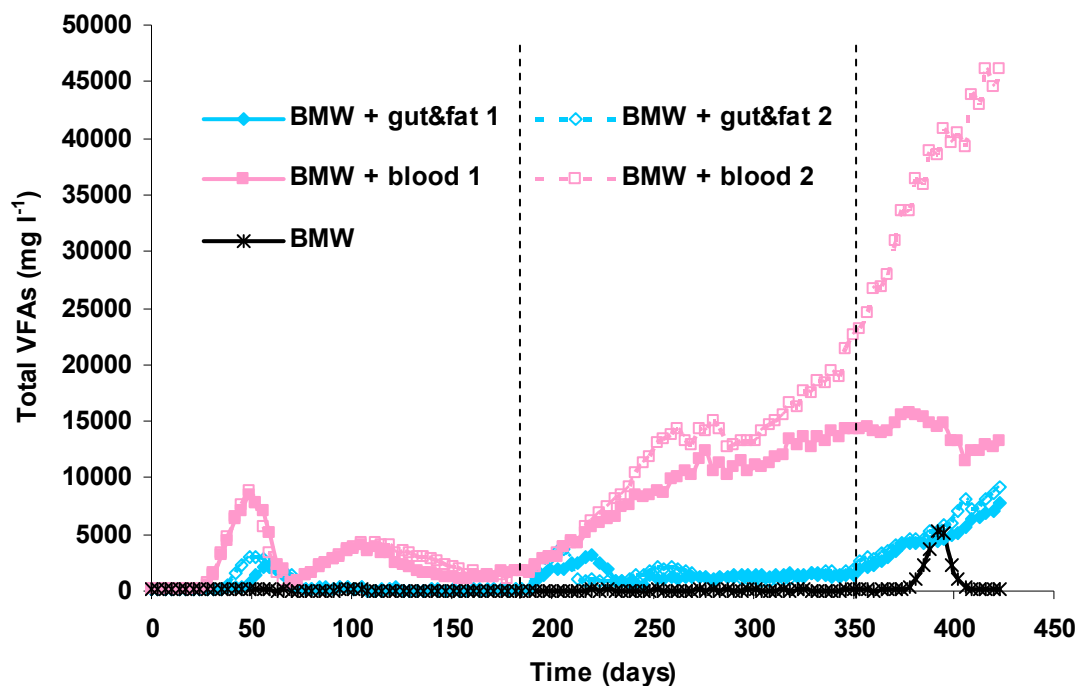


Figure 8.12 Total VFA concentration in co-digestion trials using mechanically-recovered BMW mixed with sheep blood and mechanically-recovered BMW with a pig gut and flotation fat mixture.

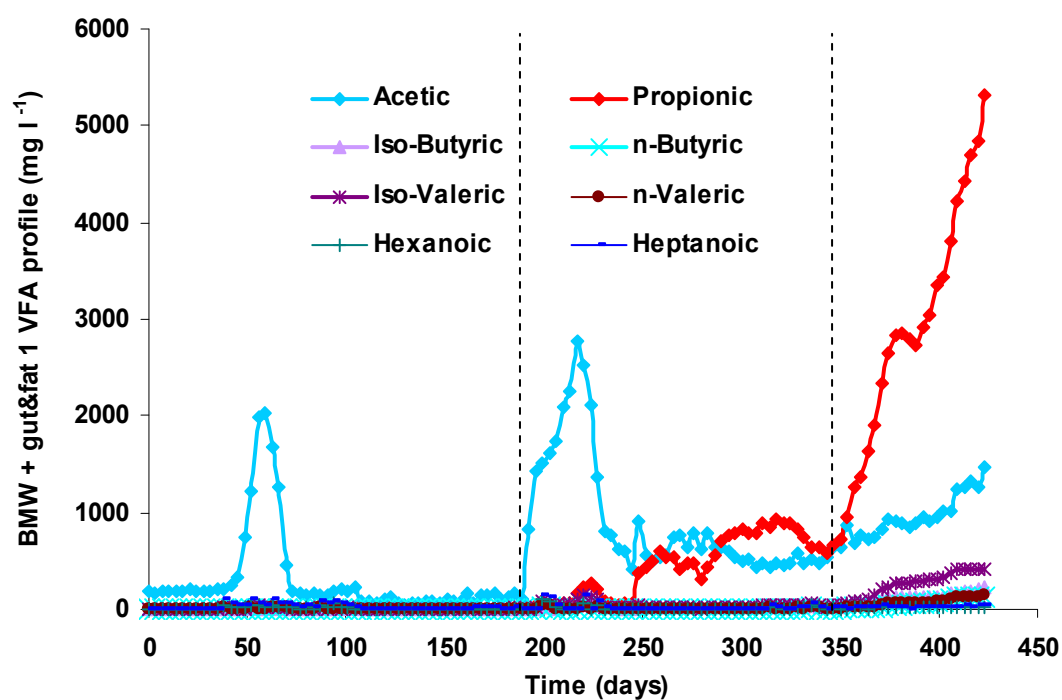


Figure 8.13 VFA concentration profile in the co-digestion trial 1 using mechanically-recovered BMW mixed with a pig gut and flotation fat mixture (digester No. 1)

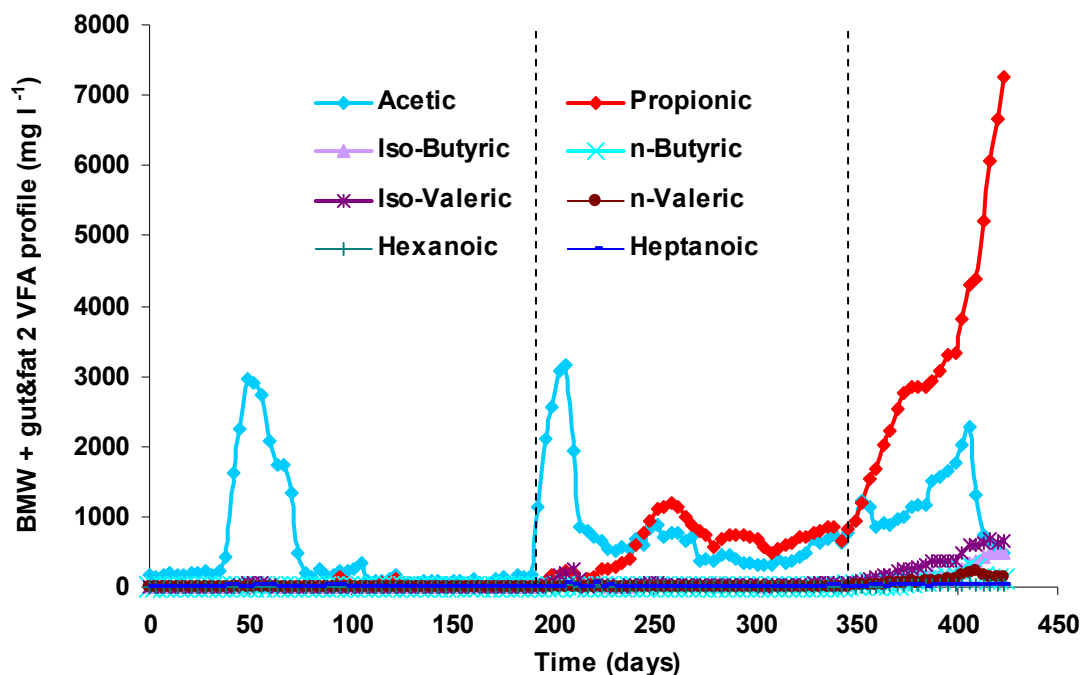


Figure 8.14 VFA concentration profile in the co-digestion trial 2 using mechanically-recovered BMW mixed with a pig gut and flotation fat mixture (digester No. 2)

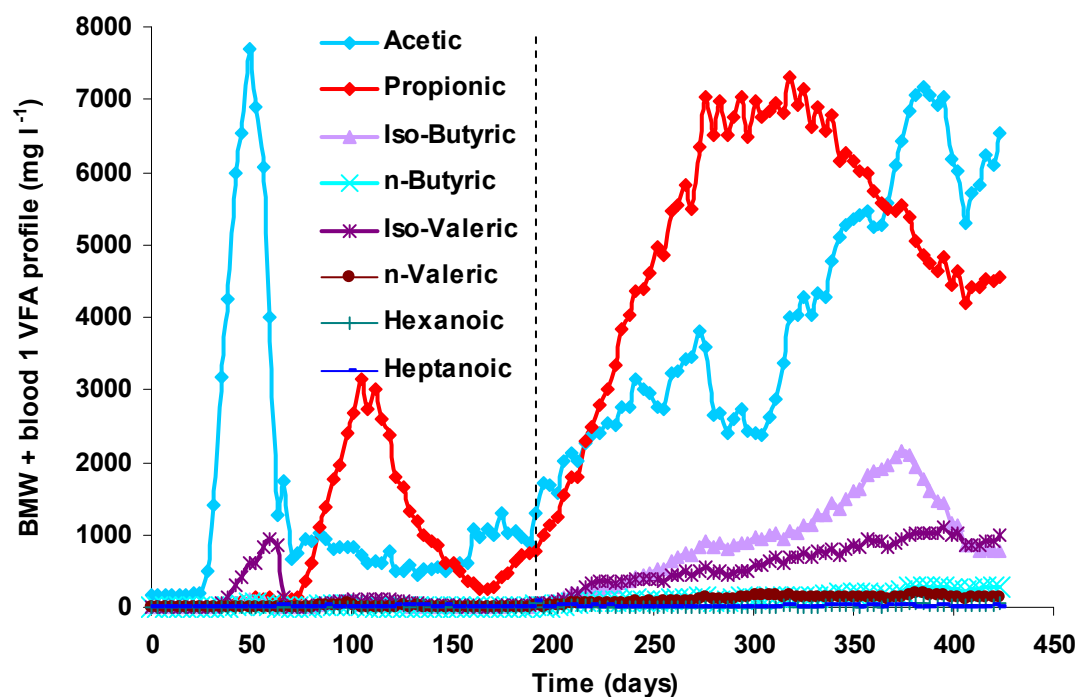


Figure 8.15 VFA concentration profile in the co-digestion trial using mechanically-recovered BMW mixed with sheep blood (digester No. 1)

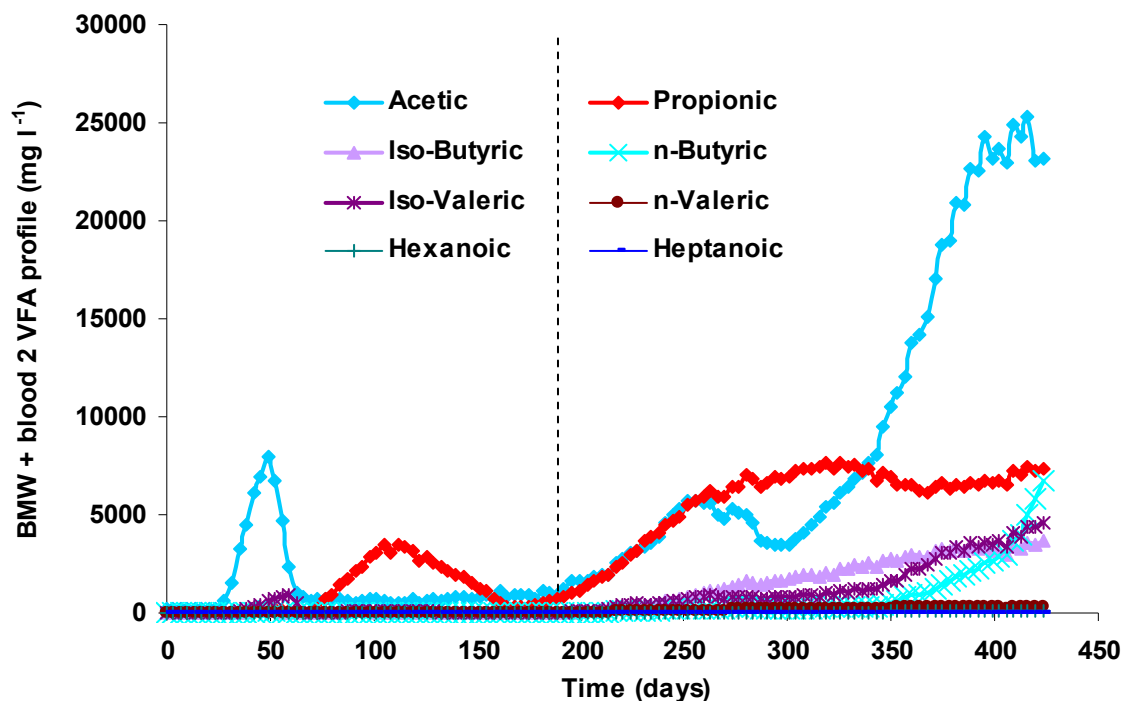


Figure 8.16 VFA concentration profile in the co-digestion trial 2 using mechanically-recovered BMW mixed with sheep blood (digester No. 2)

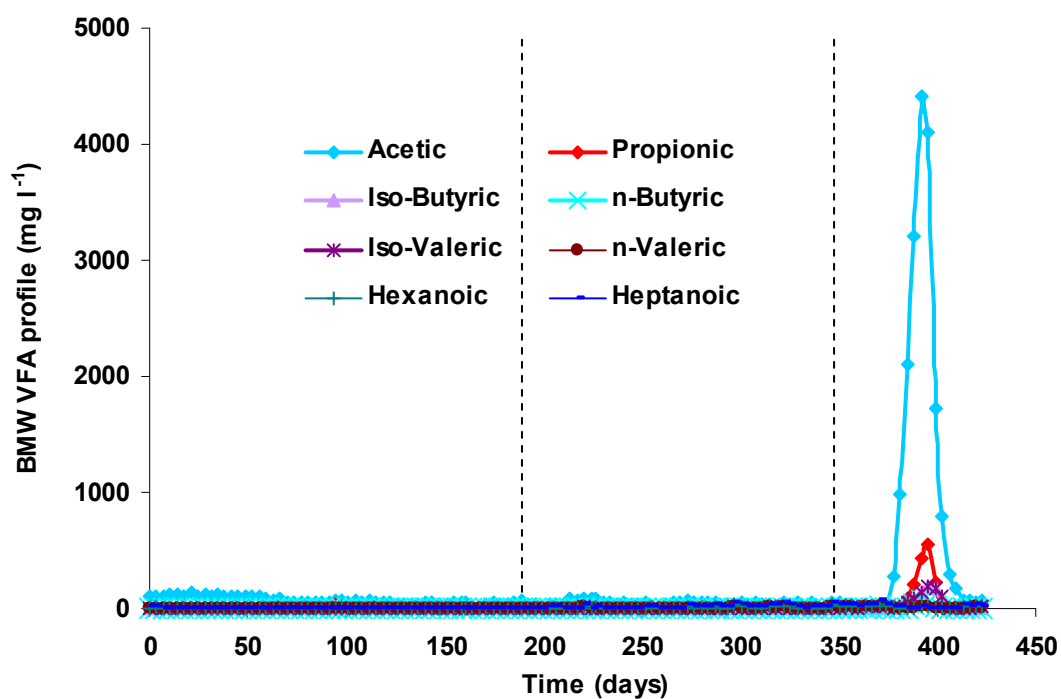


Figure 8.17 VFA concentration profile in mechanically-recovered BMW digestion control.

8.2 Laboratory-scale food waste co-digestion study

Seven digesters, each with a working volume of 4 litres, were set up using digestate taken from the baseline food waste digester 2 as inoculum (see section 5). Food waste was the sole feedstock for the digesters over the first week to confirm that the performance of the smaller-scale equipment was similar between units. From the start of the trial, one pair of digesters was fed with a mixture of food waste and cattle slurry, with a ratio of 20 parts of food waste to 80 parts of cattle slurry on a VS basis. The second pair of digesters was fed with a mixture of food waste and card packaging in the proportion of 78.4 parts of food waste, 6.4 parts of corrugated cardboard, 13.5 parts of card packaging and 1.7 parts of other card on a fresh weight basis. This gave a ratio of food waste to card packaging of 53:47% on a VS basis. To ensure homogeneity at the laboratory scale this material was prepared by wet maceration, reducing the total solids (TS) content from 94% to around 20%. A further pair of digesters was fed with a mixture of food waste and waste potatoes, in a ratio of 80 parts of food waste to 20 parts of waste potatoes on a VS basis. The seventh digester was fed solely on food waste and acted as a control. The total organic loading rate (OLR) on the digesters was $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ at the start of the trial and a nominal solid retention time (SRT) of 30 days was maintained by liquor re-circulation. The operational regimes changed as the experiment progressed, and detailed information on this is given in Table 8.2. The trial ran for 329 days.

Table 8.2 Operational regimes in laboratory-scale food waste co-digestion trials

Time (days)	FW+CS1	FW+CS2	FW+CP1	FW+CP2	FW+WP1	FW+WP2	FW
35	OLR = 2, FW:CS = 20:80 ^a	OLR = 2, FW:CS = 20:80	OLR = 2	OLR = 2	OLR = 2 c	OLR = 2	OLR = 2
63							
84							
98							
133	OLR = 2, FW:CS = 40:60	OLR = 2, FW:CS = 40:60	OLR = 3	OLR = 3			
175			Fed with cattle slurry	Ceased feeding			
231	OLR = 3, FW:CS = 40:60	OLR = 3, FW:CS = 40:60	OLR = 2	OLR = 2 ^b			
294	OLR = 3, FW:CS = 60:40	OLR = 3, FW:CS = 60:40	OLR=3	OLR=3			
329	OLR = 4, FW:CS = 60:40	OLR = 4, FW:CS = 60:40	OLR = 4	OLR = 4	Ceased feeding	Ceased feeding	Ceased feeding

Note: FW – Food Waste; CS – Cattle Slurry; CP – Card packaging; WP – Waste Potato;

a: The proportion of food waste and cattle slurry was on a VS basis;

b: No feeding at the first two weekends;

c: Fed with cattle slurry.

The digesters were monitored daily for biogas production and pH. Other digestate parameters such as solids, VFA, TAN, alkalinity, and biogas composition were analysed a minimum of once per week and often more frequently. The process efficiency was estimated by calculating the specific biogas production (SBP) and volatile solids removal

(VSR) using data from the above analyses. Experimental results for the co-digestion are shown graphically in Figures 8.18 to 8.44.

At the time the inoculum was taken, the 35-litre baseline food waste digester 2 was stressed; the performance in the co-digestion trials indicated possible recovery from this state when the food waste substrate was replaced by a mixed feedstock.

8.2.1 Co-digestion of food waste and cattle slurry

The digesters fed with food waste and cattle slurry achieved stable biogas production 0.5 SRT after introducing cattle slurry into the digester. The digesters had lower specific methane production of $0.218 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ (Figure 8.19) than food waste alone but a higher methane percentage of 62.7% (Figure 8.23) compared with their starting value. There was also a decrease in the total VFA concentration in the digesters which may in part be due to VFA being hydraulically flushed out of the system. The propionic acid (HPr) concentration in digesters decreased rapidly and this was coupled with a clear rise in the acetic acid (HAc) concentration. At the end of the first retention time HPr formed only 3.8% of total VFAs with HAc at 92% being the predominant VFA species (Figure 8.30 and 8.31). After 1.5 retention times HAc was the only VFA species in the digesters, and the total VFA concentration had dropped to less than 200 mg l^{-1} after 2 retention times.

The pair of digesters running on a food waste and cattle slurry mix were considered to be at steady state after 3 retention times, and therefore an attempt to improve the process efficiency was made by increasing the proportion of food waste in the mixture to 40% on a VS basis. The digesters had adapted to the changed feedstock recipe without VFA accumulation (Figure 8.30 and 8.31) over a period of 2.5 retention times. It can be seen from Figure 8.18 that the SBP increased by about 15~20% after the proportion of food waste was increased.

At the end of the sixth retention time (day 176) the OLR was increased to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ with the proportion of food waste to cattle slurry remaining at 40:60 in a further attempt to increase the volumetric biogas production. In one of the digesters this caused a rapid increase in VFA concentration followed by a fall (Figure 8.30), coupled with a fluctuating biogas production. Unstable biogas production in the other digester was also observed, although VFA concentrations remained below 200 mg l^{-1} (Figure 8.31); it may be that a VFA spike in this digester was not seen, if it vanished quickly between two sampling times. In Figure 8.20 it can be seen that after the OLR rose to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ the VBP increased by 40% to reach $1.13 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, but the SBP declined to 90% of that obtained at an OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$. The probable reason for this is because the cattle slurry had a relatively low solids content (only 7.5% of VS in fresh matter), and therefore the nominal liquid retention time was reduced to around 35 days, causing a higher proportion of undegraded material to be flushed out of the digesters.

After the digesters had run at an OLR of $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ for 2 retention times, the proportion of food waste in the mixture was increased from 40% to 60% on day 232. By increasing the food waste proportion the feedstock volume to achieve the required loading was reduced, mitigating the wash-out effect caused by the low solids content of cattle slurry. In this way it was possible to obtain the further increase in OLR without lowering the retention time to less than 30 days. The ratio of 60 parts of food waste to 40 parts of cattle slurry on a VS basis in the feedstock mixture was roughly equal to 40 parts of food

waste to 60 parts of cattle slurry on a volume basis. The digesters adapted smoothly to the changing proportion of feedstock mixture, and the VBP increased by 30% compared to the situation where the proportion of food waste was 40% (Figure 8.20). All the digester parameter remained in a safe range with VFA concentration less than 150 mg l^{-1} , TAN 1600 mg l^{-1} , and a pH of 7.5.

As an attempt to further improve the process efficiency, the OLR was increased to $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ at the end of the tenth retention time (day 295), with the proportion of food waste to cattle slurry remaining at 60:40. At the end of the trial the digesters had been running in this regime for more than one retention time without fluctuation in stability parameters. This is longer than necessary for any loading shock to appear, and it was therefore considered probable that the operation of the digesters corresponded to steady state conditions with a specific biogas production of $0.516 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS added}$ (Figure 8. 18) and a volumetric biogas production of $2.06 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ (Figure 8.20).

8.2.2 Co-digestion of food waste and card packaging

After one retention time the digesters fed with the mix of food waste and card packaging showed digestate parameters similar to when they started. During the second retention time the digesters appeared to have recovered from their initial stressed condition in which high VFA concentrations were present. The concentration of propionic acid (HPr) decreased rapidly coupled with increasing concentrations of acetic acid (HAc) and, to a lesser extent, iso-valeric acid.. The HAc was then consumed resulting in a decrease from 5200 mg l^{-1} at day 35 to 1100 mg l^{-1} at day 50 at which time HPr was less than 50 mg l^{-1} (Figure 8.32 and 8.33). The biogas production (Figure 8.18) and methane percentage (Figure 8.23) also showed a peak reflecting the change in VFA profile. A further rise of HAc concentration was observed at the end of second retention time when the concentration of iso-valeric acid was gradually reducing. Due to the low TKN content in card packaging, a sharp drop in the total ammonia nitrogen (TAN) level from the initial 3400 mg l^{-1} to 1800 mg l^{-1} was also observed by the end of the second retention time (day 60) (Figure 8.25); this may have contributed to some extent to the recovery process. The HAc concentration was stable at around 1500 mg l^{-1} during the third retention period although the TAN continued to fall.

The OLR to this pair of digesters was increased from 2 to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ at the beginning of the fourth retention time (day 99). The volumetric biogas production increased from 1.10 to $1.41 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ during the two weeks following this OLR increase (Figure 8.20), but the VFA concentration also increased soon afterwards (Figure 8.29) leading to a stressed situation. It is thought that this was a result of the continuing reduction in TAN concentration (Figure 8.25), which would have reduced the buffering capacity and contributed to the rapid decline in pH to a critical point (Figure 8.24). It was also noted that a high H_2 peak appeared in GC analysis for biogas composition when the methane percentage dropped to 25% in one digester (digester No.1).

After one retention time at an OLR of $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ (day 134), one of the digesters (No. 1) was fed with cattle slurry as the only substrate for 42 days, in an attempt to provide additional buffering capacity and nutrients essential for anaerobic digestion and as a source of anaerobic methanogenic bacteria. The second digester of the pair (No. 2) was not stressed to the same degree, and was therefore left without feeding in an attempt to recover it. In both digesters this recovery process lasted for 6 weeks, at which point both

digesters showed signs of returning to a satisfactory condition. It can be seen from Figure 8.32 that during this period the VFA concentration in digester No. 1 dropped from its peak value of 11000 to 1200 mg l⁻¹, the biogas methane percentage reached 60% (Figure 8.23), and the pH rose to 7.3 (Figure 8.24). In digester No. 2 during this period the pH climbed to around 7.5, VFA were almost completely consumed, and the TAN (Figure 8.25) rose due to degradation of the remaining organic materials in the digestate thus increased the buffering capacity. After the recovery process both digesters were again fed with the mixture of food waste and card packaging at an OLR of 2 kg VS m⁻³ d⁻¹. Digester No. 1 accepted this without any fluctuation in operational parameters, and within 2 weeks the VFA level had also dropped and remained less than 100 mg l⁻¹. On resuming feeding digester No. 2 showed a drop in pH to less than 7.0 (Figure 8.24) and the VFA concentration rose to 3000 mg l⁻¹ (Figure 8.33). At this point feeding of the digester was stopped over the following two weekends to allow a rest period between feeding periods. Continuous feeding then resumed and the digester operated at this loading for around 2 retention times and showed a similar digestion performance to the other one. It is interesting to note, however, that the persistent HAc level of around 1500 mg l⁻¹ observed during the first part of the run at an OLR of 2 kg VS m⁻³ d⁻¹ was undetectable after the re-acclimation at the start of the second part (Figure 8.29).

An explanation for the behaviour observed is as follows:

The digesters were started using an inoculum digestate from a long-term stressed food waste digester with a TAN concentration at 3400 mg l⁻¹ (Figure 8.25). This would have caused some growth limitation to the acetoclastic methanogens. Although the dose of card packaging lowered the TAN level it was evident from the existence of an acetic acid plateau in the system (Figure 8.29) that the population of acetoclastic methanogens was not entirely restored within the first three retention times. The increase in OLR from 2 to 3 kg VS m⁻³ d⁻¹ increased the load on autotrophic methanogens, and when this methane production route was unable to consume all the hydrogen produced, product-induced feedback inhibition would have caused the VFAs to build up. With the TAN concentration reducing to less than 1000 mg l⁻¹ there was insufficient buffering capacity to prevent the pH falling to a level at which the autotrophic methanogens were affected, bringing the situation to a critical point. The recovery stage allowed the accumulation of acetoclastic methanogens in the digesters and developed a healthier anaerobic microbial consortium operating via two different process routes.

After the digesters had run at an OLR of 2 kg VS m⁻³ d⁻¹ for 2 retention times (day 232) the OLR in both digesters was again increased to 3 kg VS m⁻³ d⁻¹. On this second attempt at increasing the OLR the VFA concentration (Figure 8.29) and all other operational parameters remained stable. The specific biogas production remained the same and the VBP increased by 50% (Figure 8.20).

The OLR was increased to 4 kg VS m⁻³ d⁻¹ at the end of the tenth retention time (day 295). At the end of the trial the digesters had been running at this loading for more than one retention time without any fluctuation in stability parameters and had apparently reached a steady state condition, with a specific biogas production of 0.545 STP m³ kg⁻¹ VS added (Figure 8.18) and a volumetric biogas production of 2.18 STP m³ m⁻³ d⁻¹ (Figure 8.20).

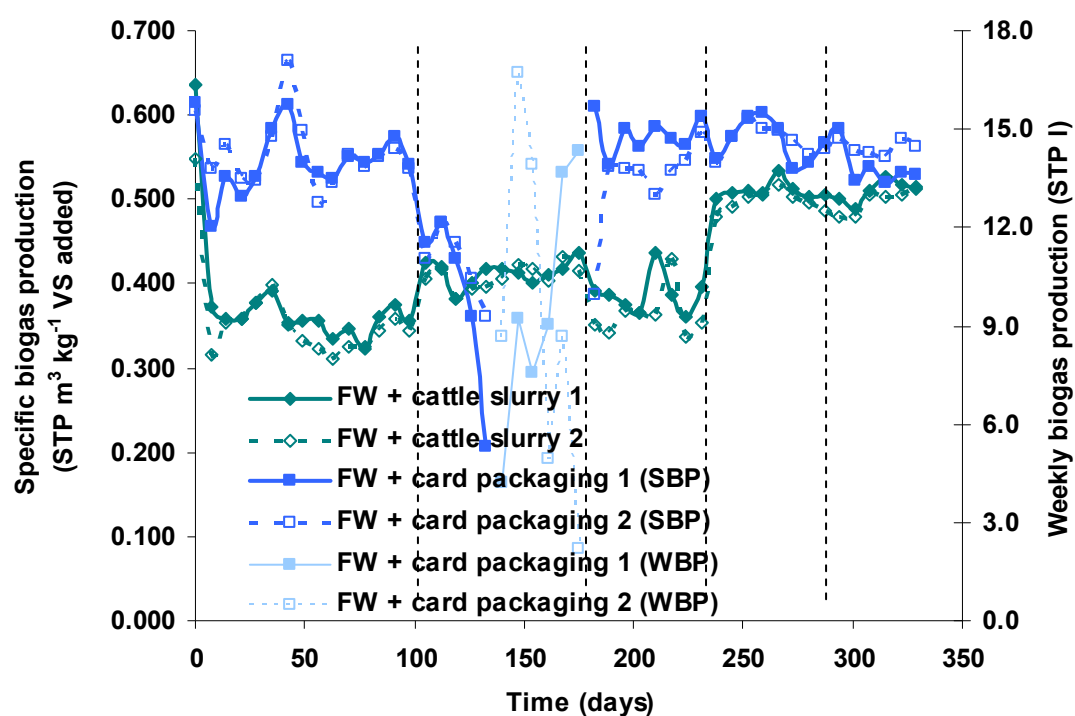


Figure 8.18 Weekly average specific biogas production (SBP) and weekly total biogas production (WBP) in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

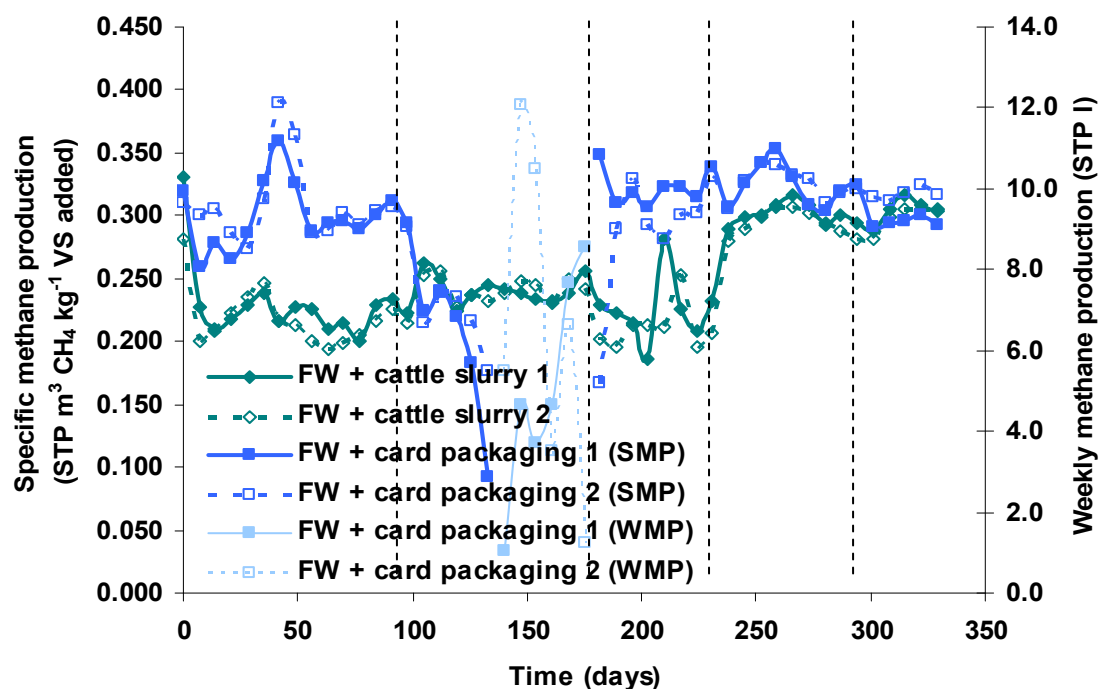


Figure 8.19 Weekly average specific methane production (SMP) and weekly total methane production (WMP) in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

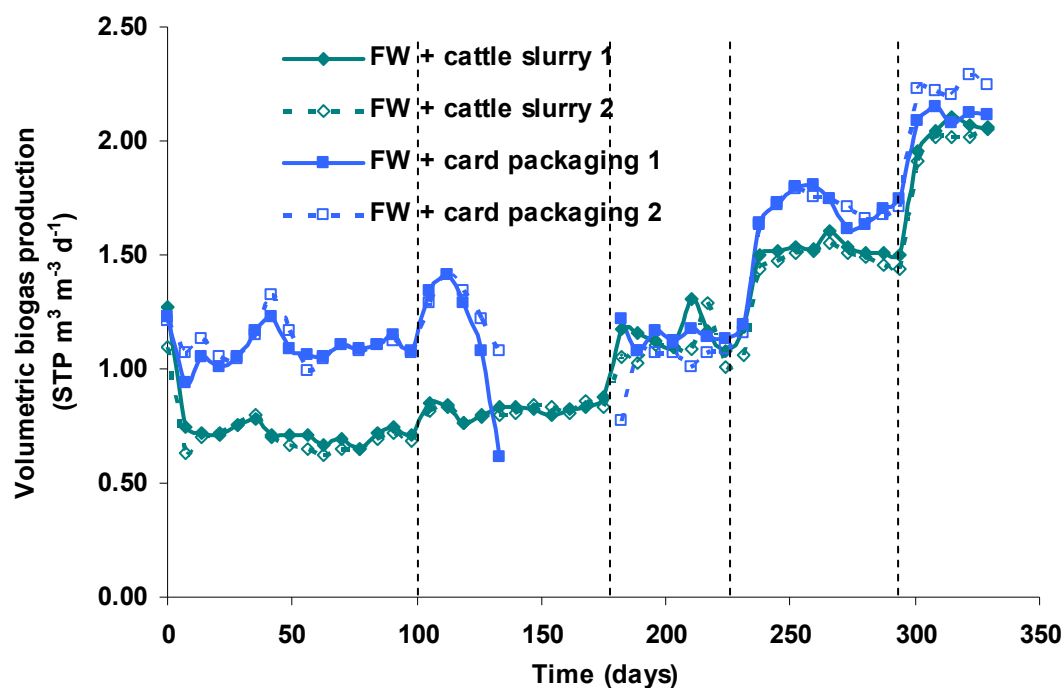


Figure 8.20 Weekly average volumetric biogas production in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

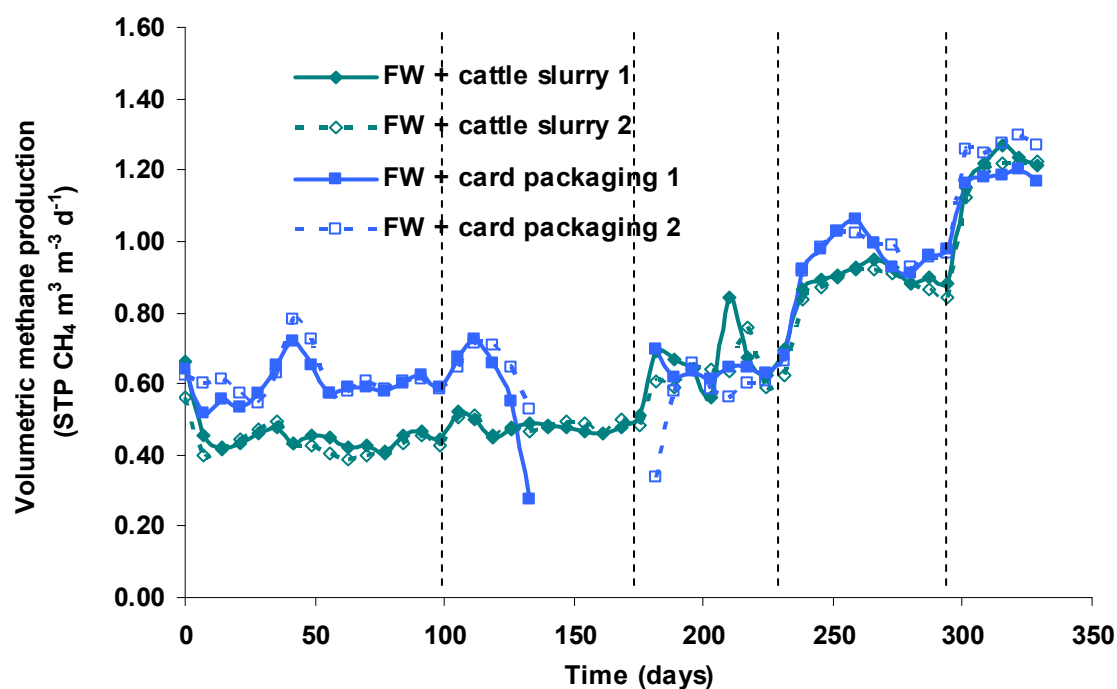


Figure 8.21 Weekly average volumetric methane production in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

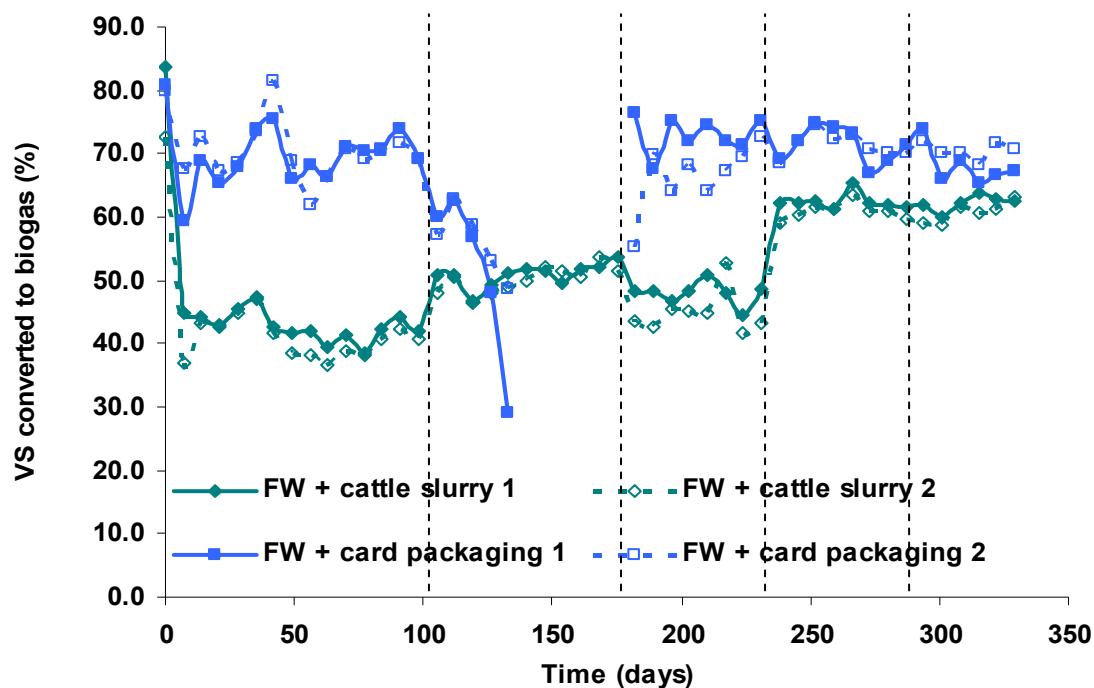


Figure 8.22 Weekly average mass conversion from feedstock VS to biogas in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

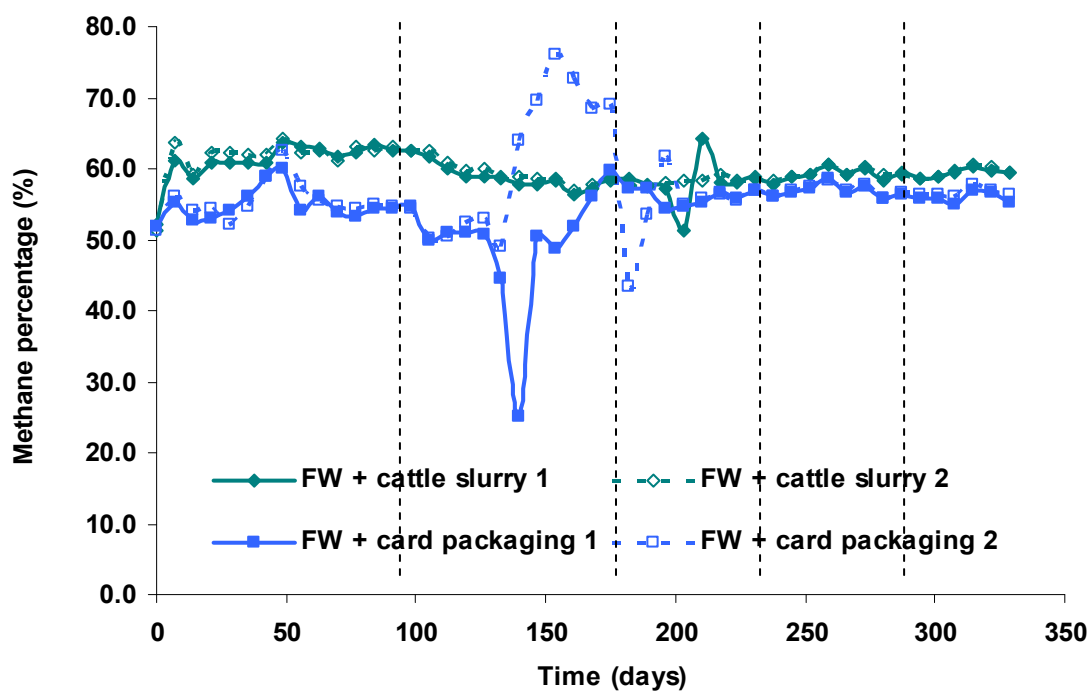


Figure 8.23 Weekly average methane content of biogas produced in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging

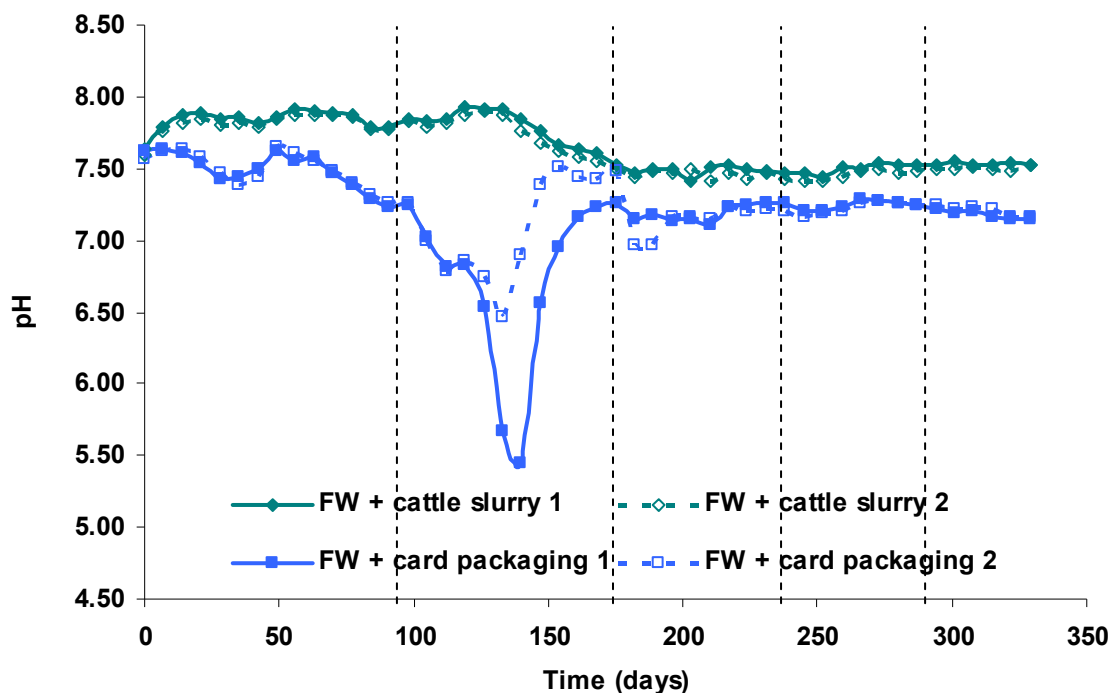


Figure 8.24 Weekly average pH values in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card-packaging waste

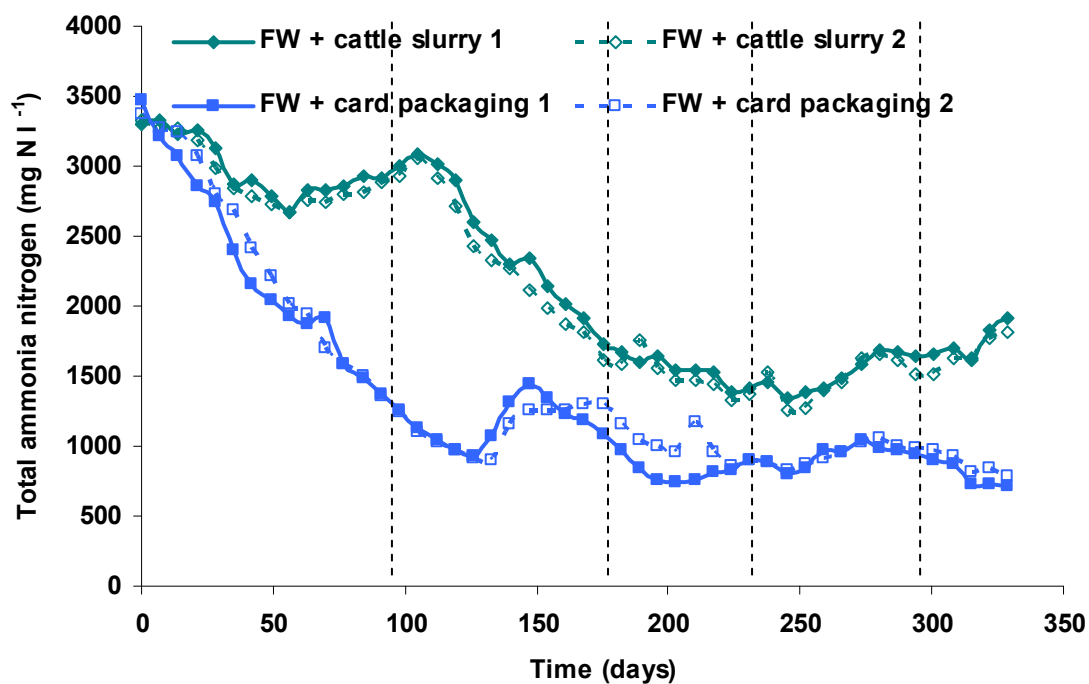


Figure 8.25 Total ammonia nitrogen (TAN) concentration in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging

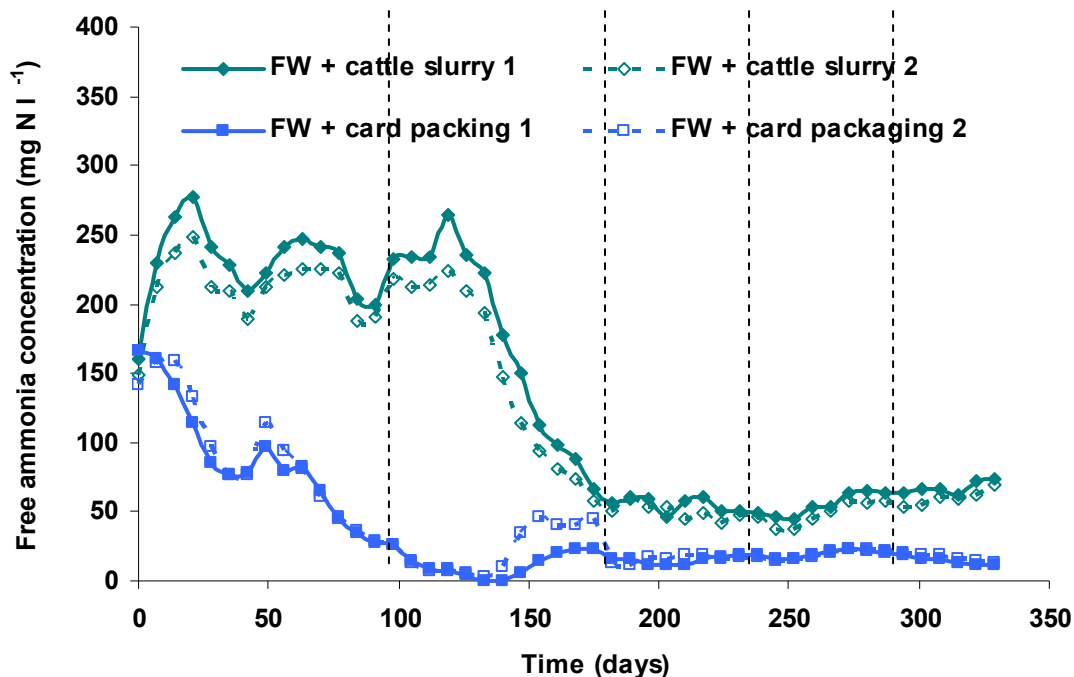


Figure 8.26 Free ammonia concentration in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

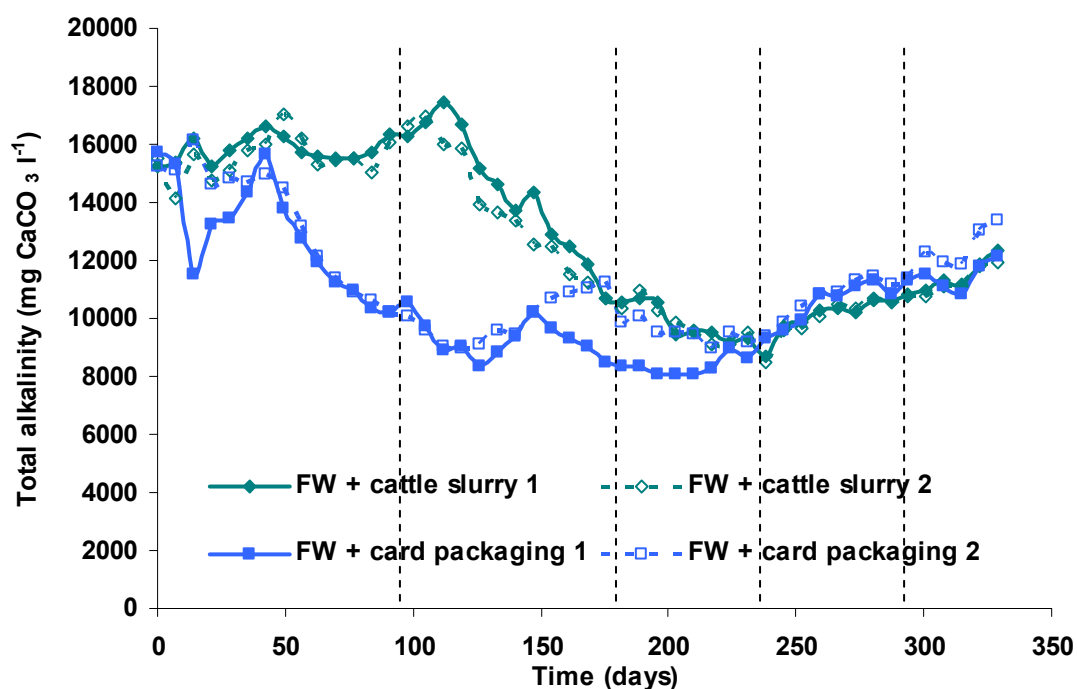


Figure 8.27 Total alkalinity in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging.

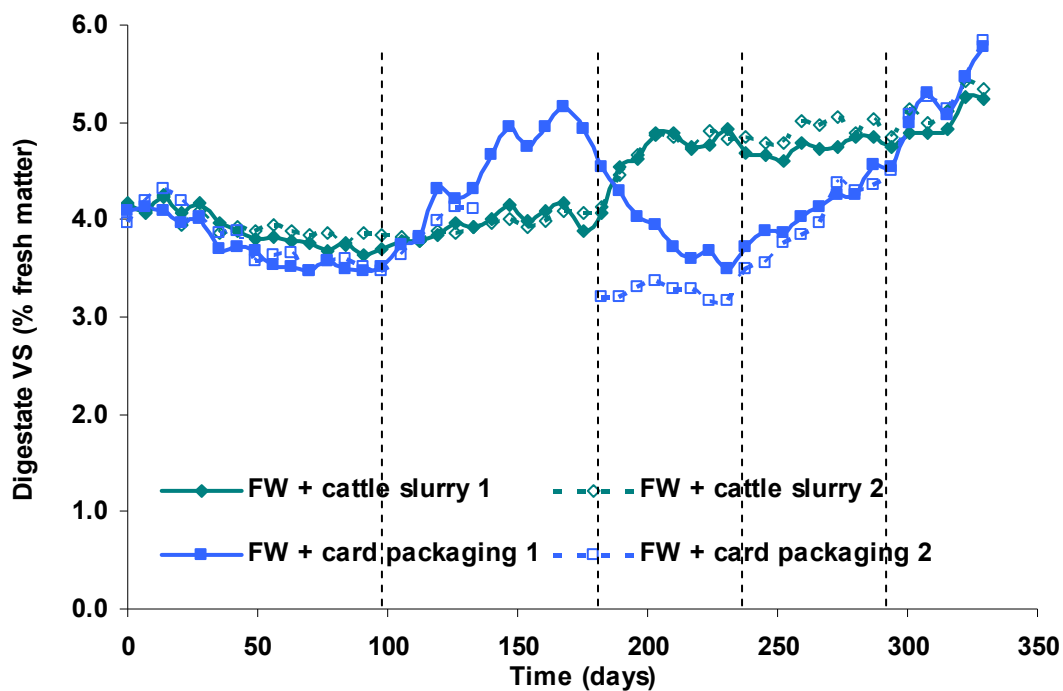


Figure 8.28 Digestate volatile solids content in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging

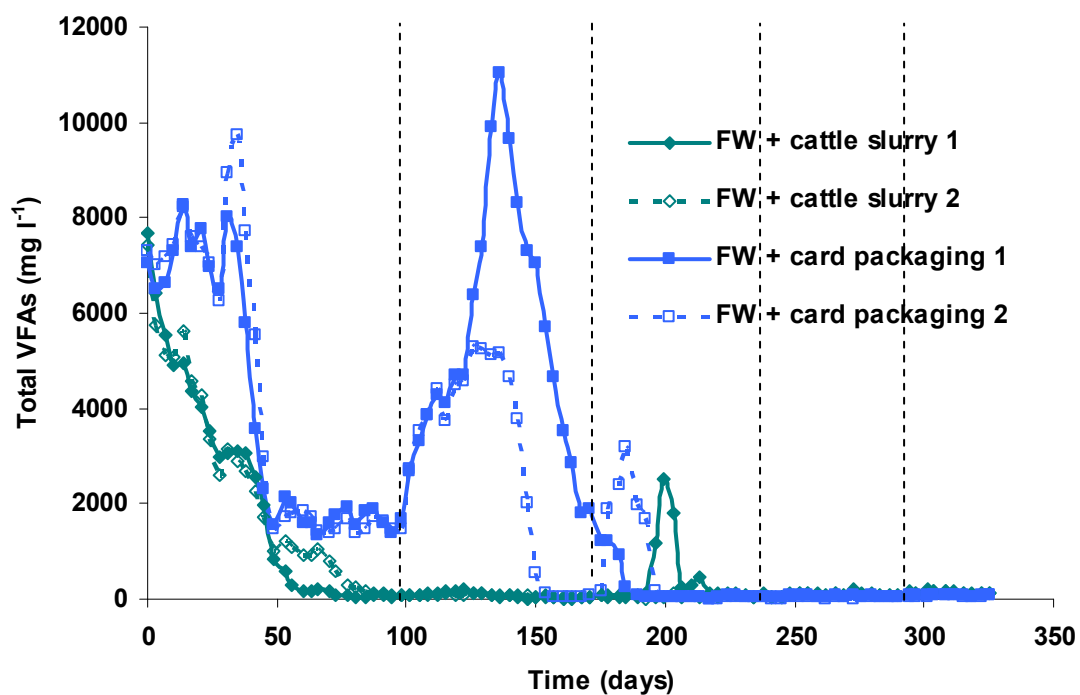


Figure 8.29 Total VFA concentration in laboratory-scale co-digestion trials using food waste mixed with cattle slurry and card packaging

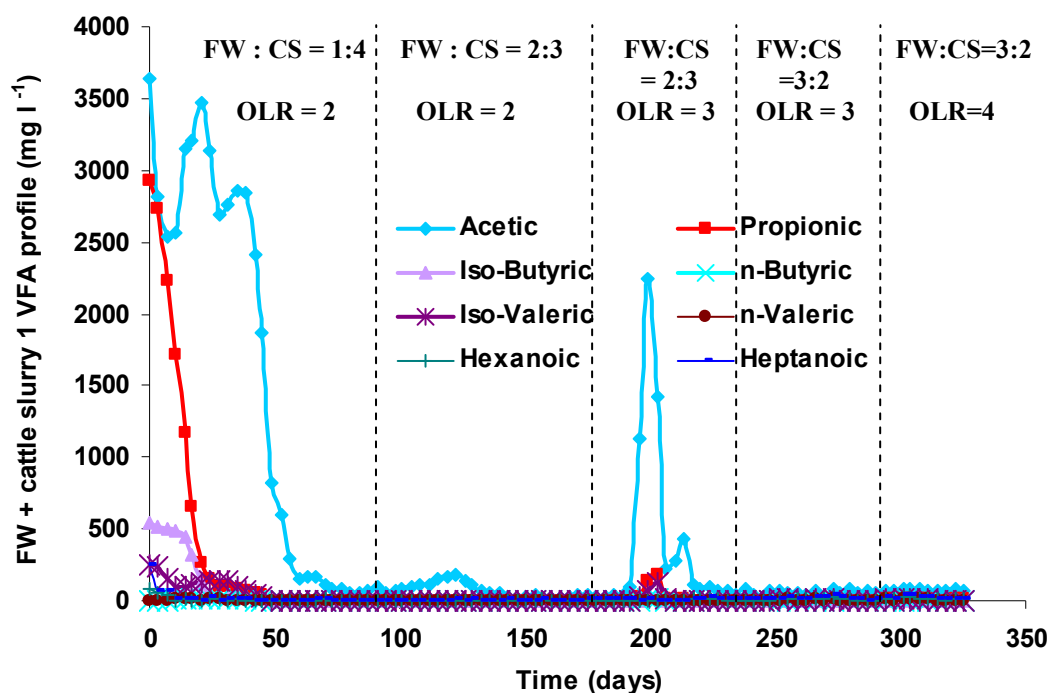


Figure 8.30 VFA concentration profile in laboratory-scale co-digestion trial using food waste mixed with cattle slurry (digester No. 1)

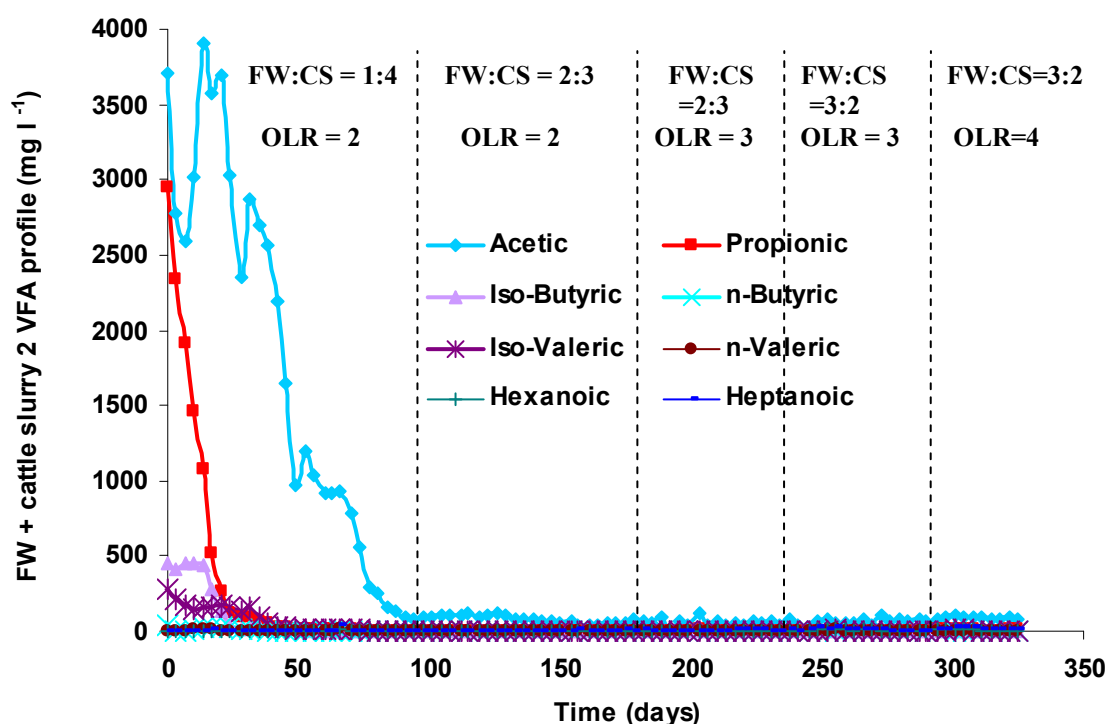


Figure 8.31 VFA concentration profile in laboratory-scale co-digestion trial using food waste mixed with cattle slurry (digester No. 2)

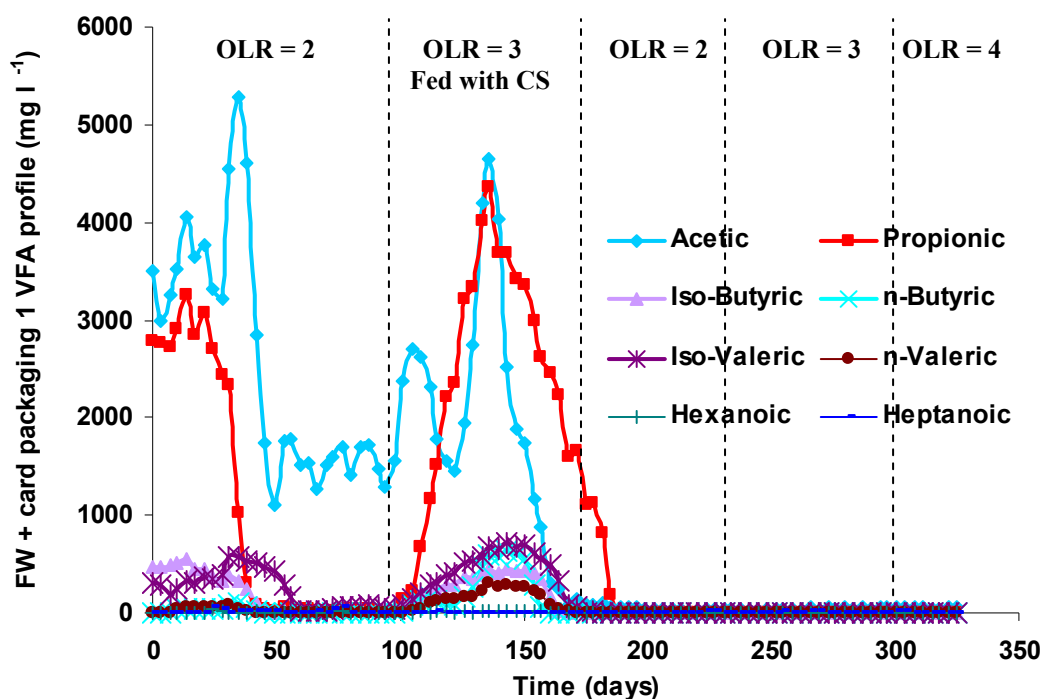


Figure 8.32 VFA concentration profile in laboratory-scale co-digestion trial using food waste mixed with card packaging (digester No.1)

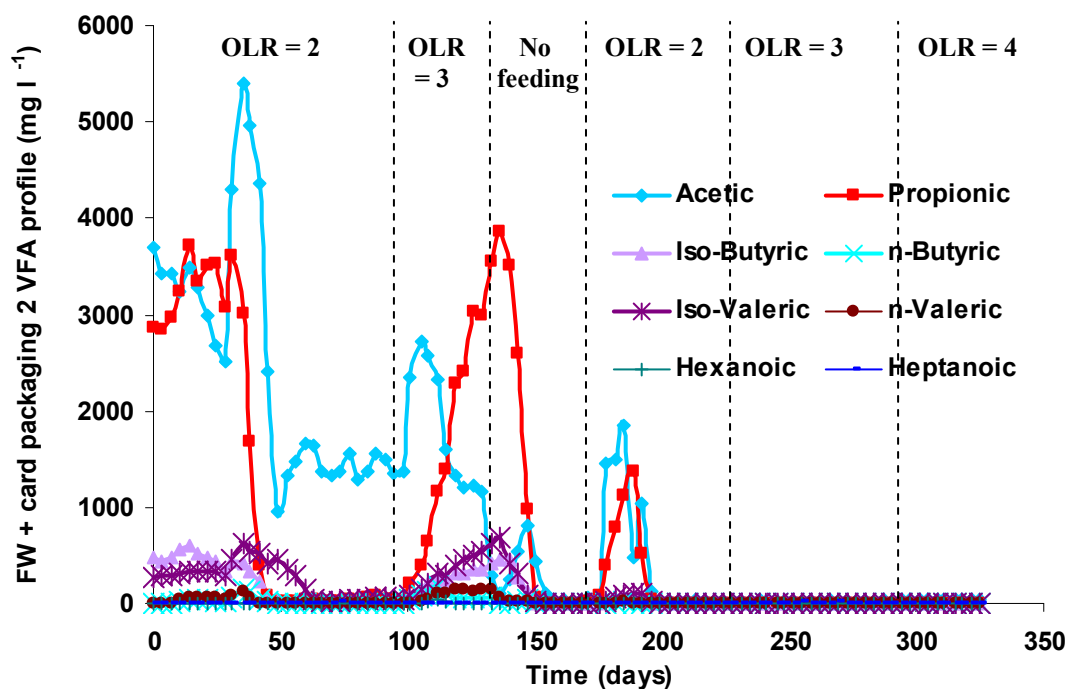


Figure 8.33 VFA concentration profile in laboratory-scale co-digestion trial using food waste mixed with card packaging (digester No. 2)

8.2.3 Co-digestion of food waste and waste potatoes

The digesters fed with a mix of food waste and waste potatoes showed a build-up in VFAs to a concentration of 12000 mg l⁻¹ over the first retention time (Figure 8.41). During the second retention time the digesters remained stressed with further increases in the total VFA concentration and the percentage of HPr in the VFA profile (Figures 8.42 and 8.43). One of the digesters (No. 2) failed at the end of the second retention time and feeding was stopped: the biogas production, biogas composition and digestate parameters continued to be monitored.

The other digester (No. 1) failed during the third retention time. This digester was then fed with cattle slurry for two weeks in an attempt to recover it. As no obvious recovery was observed, feeding to this digester was also stopped. After 6 weeks without feeding, digester No. 1 showed a rapid consumption of HAc (Figure 8.42) and a resulting increase in pH to 7.8 (Figure 8.37). There was also an increase in biogas production (Figure 8.34), biogas methane content rose to 70% (Figure 8.36), and total VFA dropped from 22000 mg l⁻¹ to 11000 mg l⁻¹ (Figure 8.42). A second biogas production peak appeared 3 weeks later and was linked to the rapid drop in HPr concentration, with a slight increase in HAc concentration that in turn gave rise to a third biogas production peak. After a period of 100 days in which no feed had been added the VFA concentration reduced to less than 200 mg l⁻¹. After 150 days without feeding there was a rapid consumption of HAc in digester No. 2 (Figure 8.43) resulting in a biogas production peak (Figure 8.34) and an increase in pH to 7.1 (Figure 8.37). Consumption of n-butyric acid followed, which raised the concentration of HAc to 5500 mg l⁻¹. This HAc concentration was subsequently reduced to less than 1000 mg l⁻¹ over the next retention period. There was a slight rise in HPr concentration but this dropped gradually towards the end of the trial in a pattern similar to digester No. 1.

8.2.4 Digestion performance of the food waste control

The digester fed with food waste only failed during the first retention time period, at the end of which the specific methane potential was less than 10% of its starting value (Figure 8.35). The pH had dropped to below 6.0 (Figure 8.37), the biogas contained less than 10% methane (Figure 8.36) and showed a high hydrogen peak on GC analysis. Because of the poor state of this digester, feeding was ceased after one retention period but the biogas production, biogas composition and digestate parameters were still monitored. At the end of the trial (day 329) all the VFAs accumulated in the control digester had been degraded, following more or less the same sequence as in the pair of digesters left to recover after feeding potato waste as co-substrate.

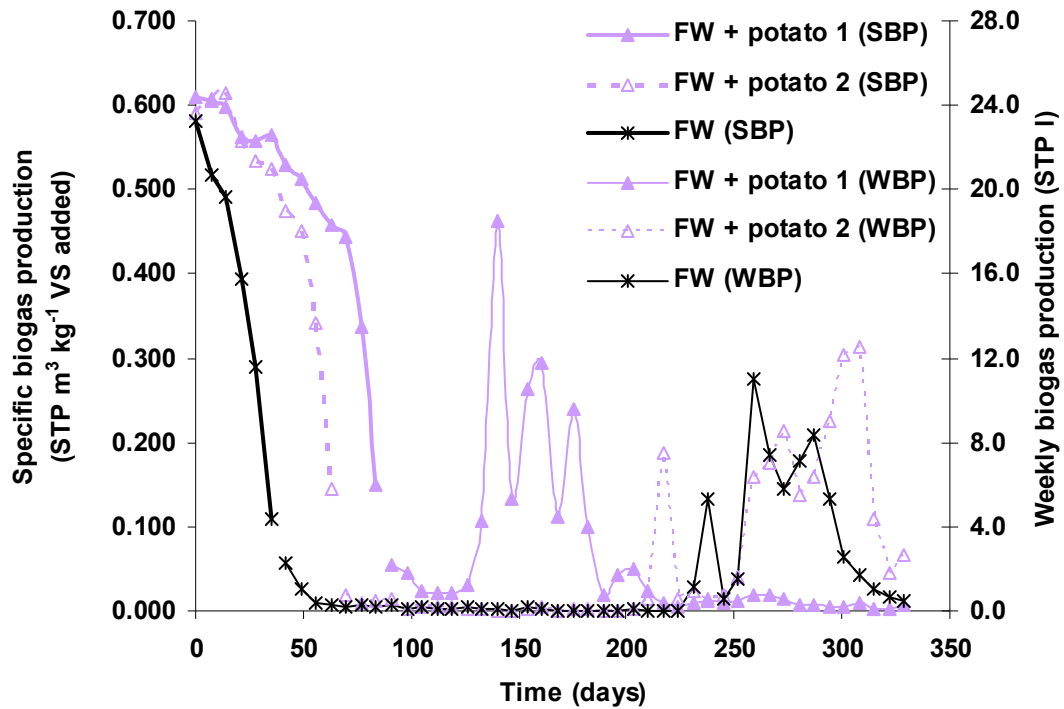


Figure 8.34 Weekly average specific biogas production (SBP) and weekly total biogas production (WBP) in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

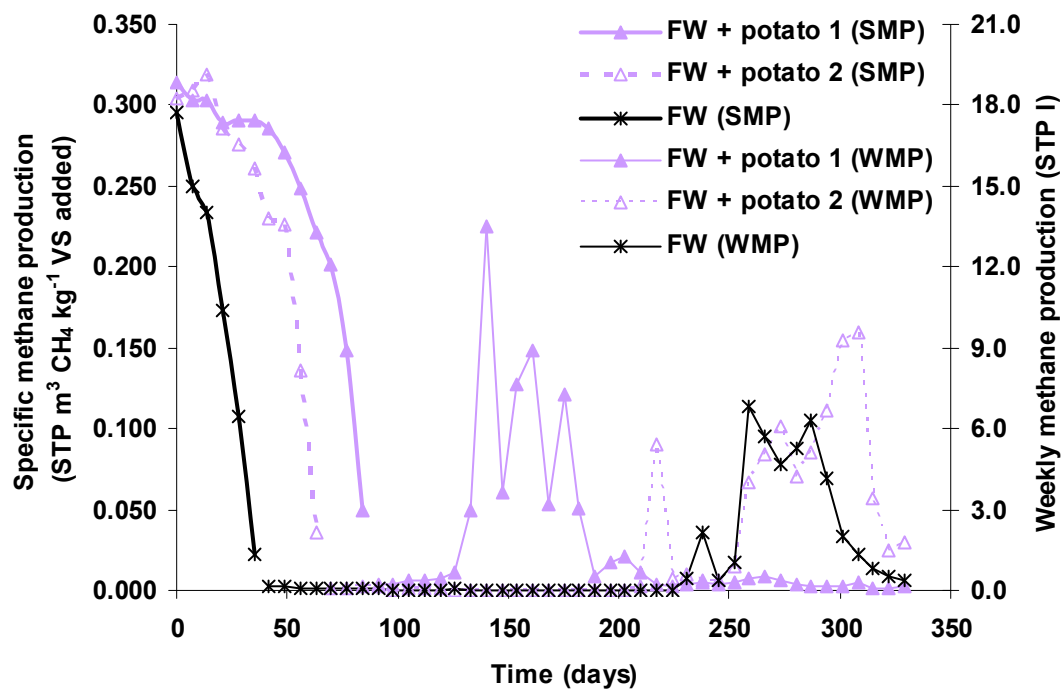


Figure 8.35 Weekly average specific methane production (SMP) and weekly total methane production (WMP) in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

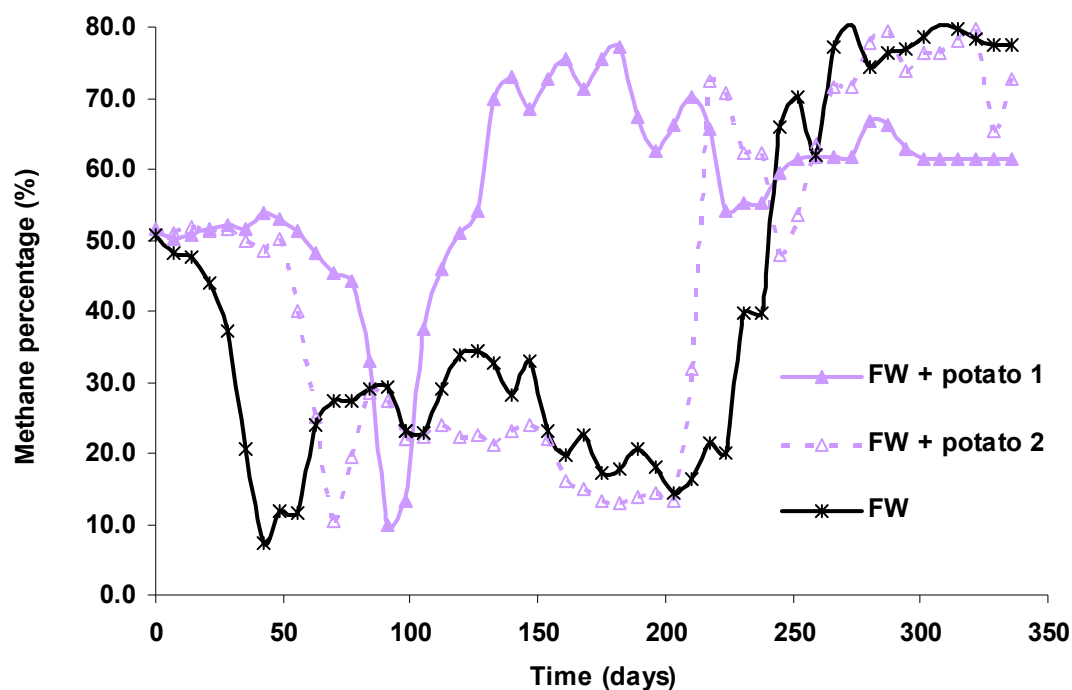


Figure 8.36 Weekly average methane content of biogas produced in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

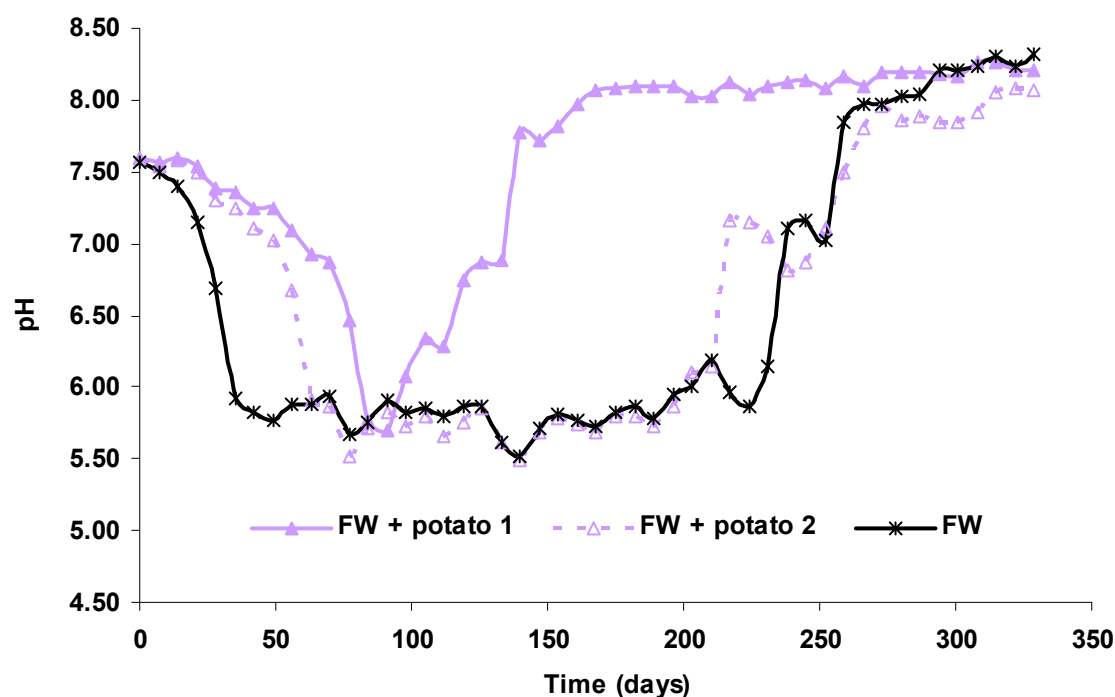


Figure 8.37 Weekly average pH values in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

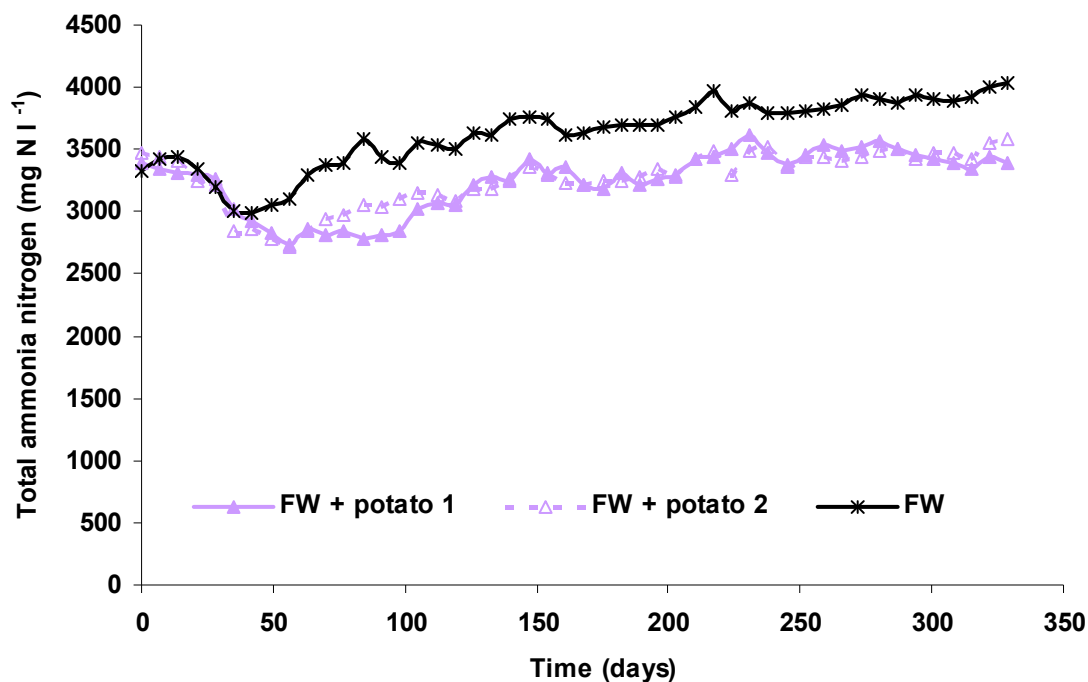


Figure 8.38 Total ammonia nitrogen (TAN) concentration in laboratory-scale co-digestion trial using food waste mixed with waste potato and in food waste digestion control

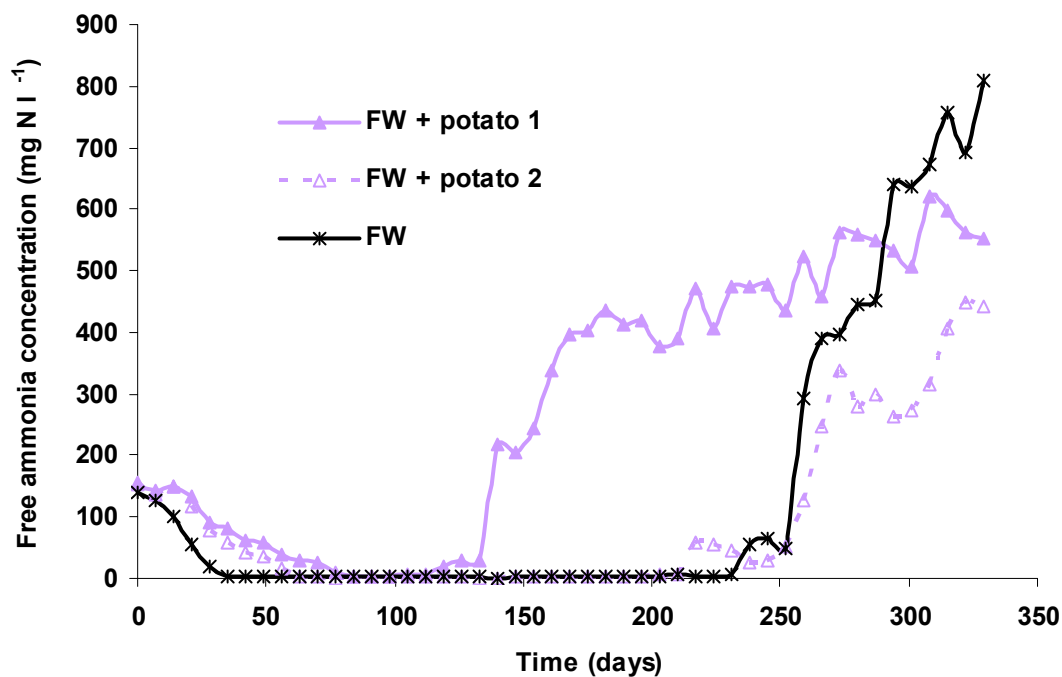


Figure 8.39 Free ammonia concentration in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

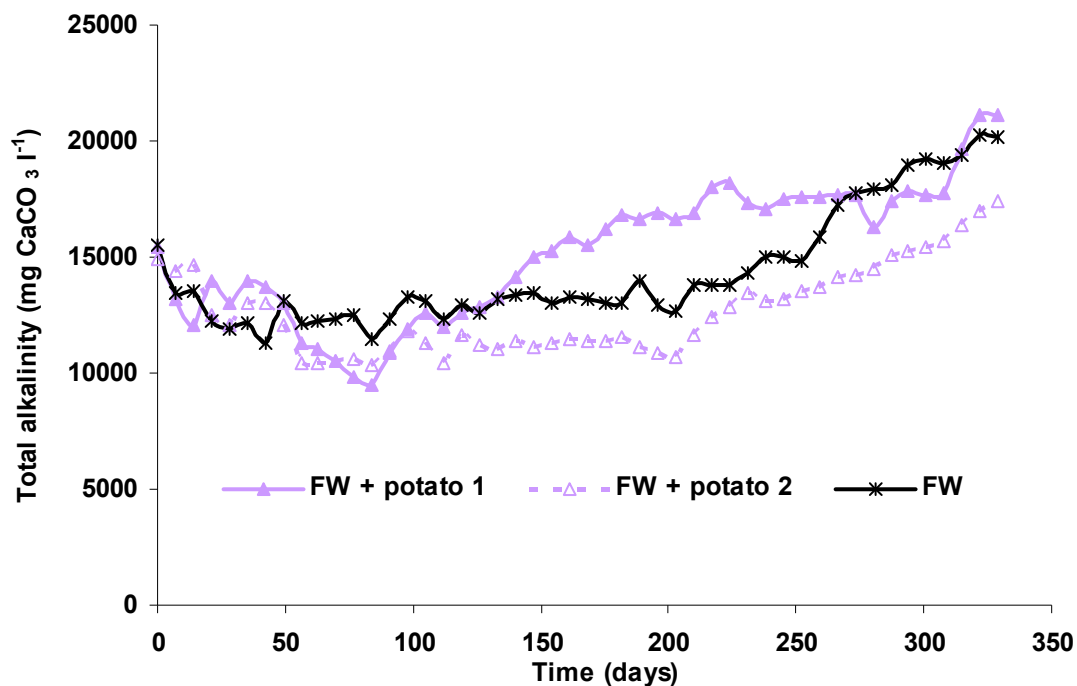


Figure 8.40 Total alkalinity in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

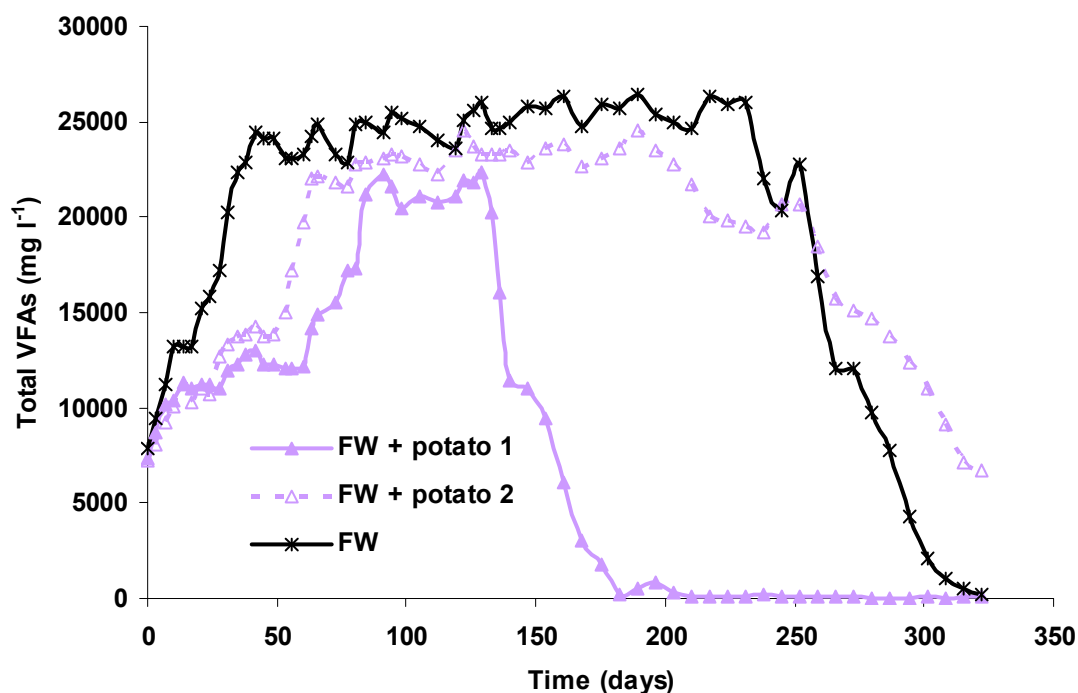


Figure 8.41 Total VFA concentration in the laboratory-scale co-digestion trial using food waste mixed with waste potato and in the food waste digestion control

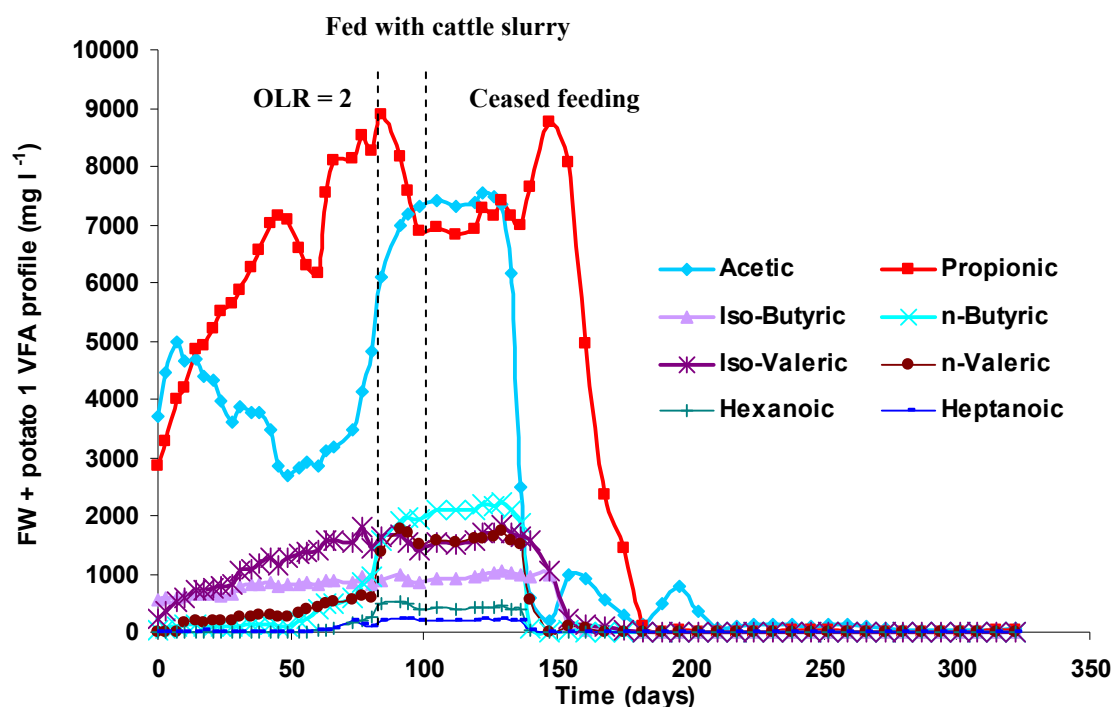


Figure 8.42 VFA concentration profile in the laboratory-scale co-digestion trial using food waste mixed with waste potato (digester No. 1)

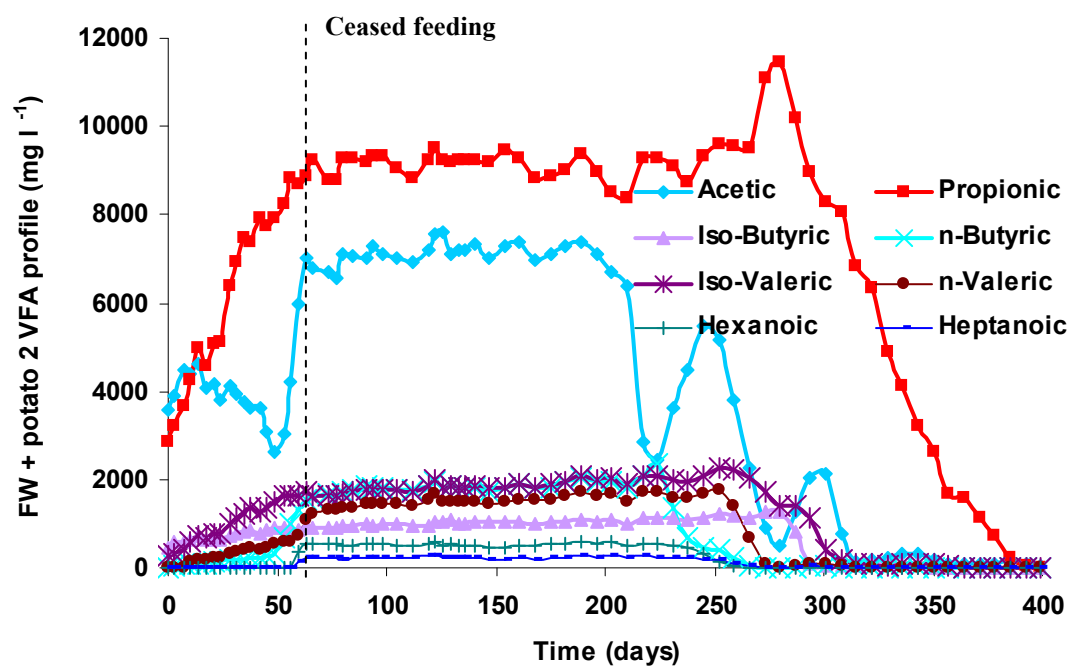


Figure 8.43 VFA concentration profile in the laboratory-scale co-digestion trial using food waste mixed with waste potato (digester No. 2)

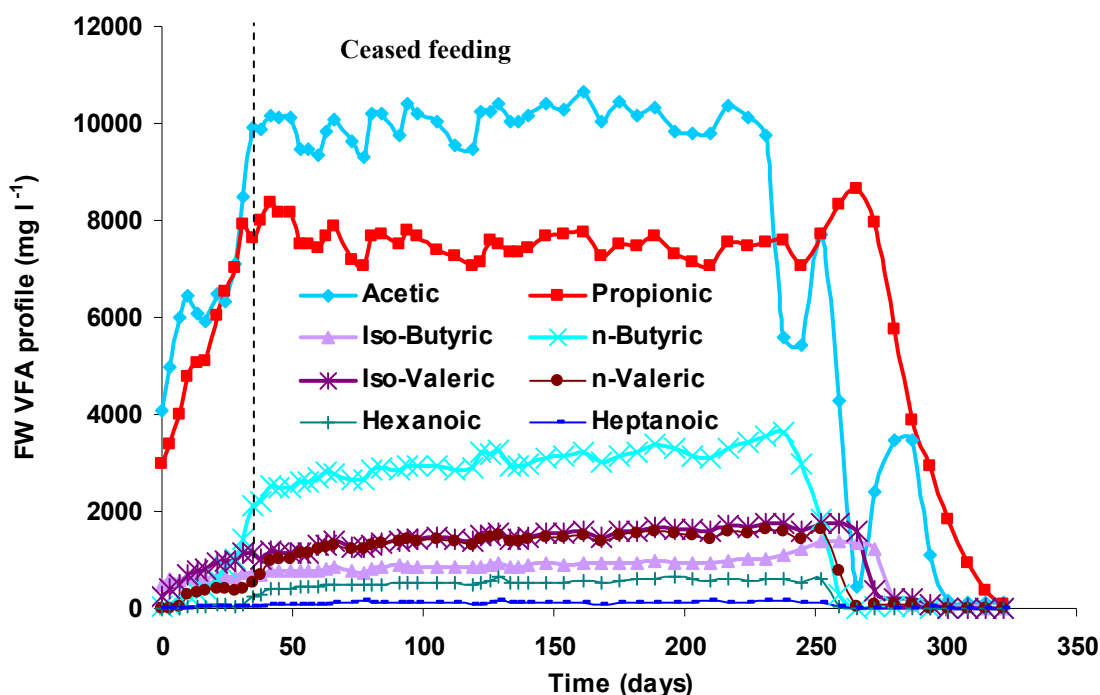


Figure 8.44 VFA concentration profile in the laboratory-scale food waste digestion control

8.3 Summary of results from co-digestion trials

The results from this long term co-digestion study indicated that mechanically-recovered BMW was a suitable feedstock for digestion alone: it had good buffering capacity with a safe total ammonia nitrogen (TAN) concentration of 1600 mg l^{-1} and a pH of 7.4. The volumetric biogas production reached $2.14 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ at the OLR of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$. The co-digestion of mechanically-recovered BMW and pig gut with flotation fat had higher specific methane production than the mechanically-recovered BMW control at an OLR of 2 and $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$, although the elevated VFA level of 1500 mg l^{-1} at an OLR of $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ indicated less favourable conditions as the OLR increased to 3 and then $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$. The co-digestion of mechanically-recovered BMW and sheep blood showed stress due to the inhibitory effect of the high TAN at even a moderate OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$, although this high TAN also provided enough buffering capacity to allow the digester to operate stably in this stressed condition.

The three food waste co-digestion trials with cattle slurry, card packaging and waste potato ran for 329 days. These were started using digestate from a stressed food waste digester as an inoculum. After running for two nominal solids retention times (SRT), it was evident that there were process benefits from adding cattle slurry and card packaging as co-substrates. The OLR of the pair of digesters fed with food waste and card packaging and the pair fed with food waste and cattle slurry was gradually and successfully increased from 2 to $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ with the proportion of food waste in the food waste with cattle slurry mix also being increased. The co-digestion of food waste and card packaging failed to stabilise on the first attempt to raise the OLR from 2 to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$, but the second attempt was successful. By the end of the study both pairs of digesters had been running smoothly at an OLR of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$, with SBP of 0.516 and 0.545 $\text{STP m}^3 \text{ kg}^{-1} \text{ VS added}$ and VBP of 2.06 and $2.18 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ for the co-digestion of food waste and cattle slurry and of food waste and card packaging respectively. The pair

of digesters fed with food waste and waste potato and also the food waste control both failed, as demonstrated by low biogas production and solids destruction. These were recovered after a period without feeding which in one of the food waste and potato waste digesters was preceded by the addition of cattle slurry. The digester with the cattle slurry addition showed a 3 times more rapid reduction in VFA concentrations than the other digester initially fed with food waste and potato waste mixture.

9 Operation of larger-scale food waste co-digestion

Co-digestion experiments with a food waste and cattle slurry mix and a food waste and card packaging mix were run alongside a digester fed on food waste as the mono-substrate and acting as a control.

The digesters used each had a working capacity of 75 litres, were completely mixed and were maintained at 36 ± 1 °C. The experimental run was started using sewage sludge digestate from Millbrook wastewater treatment plant, Southampton as the inoculum. The nutrient and potentially toxic elements (PTE) content of this inoculum is shown in Table 9.1. The feed ratio for the food waste and card packaging mix was 78.4 parts of food waste, 6.4 parts of corrugated cardboard, 13.5 parts of card packaging and 1.7 parts of other card on a fresh weight basis, equal to 53 parts of food waste and 47 parts of card packaging on a volatile solids (VS) basis. These ratios were the same as used in the laboratory-scale trials (see section 8.2), but in this trial the card packaging was not wet macerated. The feed ratio for the food waste and cattle slurry mix was 20 parts of food waste to 80 parts of cattle slurry on a VS basis, corresponding to the initial mixture in the laboratory-scale trials (section 8.2).

Table 9.1 Nutrient (NPK), PTE and solids content of the sewage sludge digestate used as the inoculum for the larger-scale food waste co-digestion trial.

	Whole digestate	Liquor fraction	Fibre fraction
TAN (g NH ₃ -N kg ⁻¹ TS)	39.1 ± 0.5	40.1 ± 0.3	-
TKN (g N kg ⁻¹ TS)	77.5 ± 1.5	79.3 ± 1.1	43.4 ± 1.0
TK (g K kg ⁻¹ TS)	2.90 ± 0.29	2.99 ± 0.13	1.26 ± 0.26
TP (g P kg ⁻¹ TS)	32.4 ± 3.5	33.2 ± 1.3	16.8 ± 2.6
Cd (mg kg ⁻¹ TS)	1.10 ± 0.21	1.10 ± 0.10	< 1.0
Cr (mg kg ⁻¹ TS)	67.3 ± 5.3	68.8 ± 5.3	40.1 ± 2.2
Cu (mg kg ⁻¹ TS)	462 ± 9	473 ± 1	247 ± 24
Ni (mg kg ⁻¹ TS)	52.9 ± 7.4	54.0 ± 7.5	32.5 ± 2.1
Pb (mg kg ⁻¹ TS)	83.8 ± 8.4	83.9 ± 0.7	61.9 ± 8.0
Zn (mg kg ⁻¹ TS)	718 ± 27	736 ± 24	380 ± 24
TS (% WW)	4.48 ± 0.07	4.10 ± 0.01	10.8 ± 0.6
VS (% WW)	2.81 ± 0.02	2.54 ± 0.01	8.56 ± 0.58
VS (% TS)	62.8 ± 1.4	62.0 ± 0.1	79.8 ± 0.5

The organic loading rate (OLR) used was 2 kg VS m⁻³ d⁻¹ and a nominal solids retention time (SRT) of 30 days was maintained through liquor re-circulation. The digesters were monitored daily for biogas production and pH. Other digestate parameters such as solids, volatile fatty acids (VFA), total ammonia nitrogen (TAN), alkalinity, and biogas composition were analysed a minimum of once per week and often more frequently. The process efficiency was estimated by calculating the specific biogas production and volatile solids removals using data from the above analyses. The stability of digester operation was evaluated also by reference to other parameters such as pH, VFA, TAN and alkalinity. The digesters ran for 308 days, or more than 10 retention times. Experimental results are shown graphically in Figures 9.1 to 9.15.

It can be seen that the specific biogas production (Figure 9.1) did not stabilise until around 3 retention times (day 90). Before this, two obvious peaks in specific biogas

production were observed for each of the digesters. The first appeared towards the end of the first retention time (day 20), and occurred simultaneously in all three digesters; this was followed by a fall in specific biogas production, a rapid accumulation of VFA (Figure 9.12), a drop in pH (Figure 9.7), and a lower methane content in the biogas (Figure 9.6). As this initial production of VFA was consumed a second biogas production peak appeared. This happened first in the digester running on food waste and cattle slurry (day 42), secondly in the digester with food waste as the sole substrate (day 49), and lastly in the one fed with food waste and card packaging (day 63). The second biogas production peaks also had an associated peak in biogas methane content, as shown in Figure 9.6. After the second biogas production peak this parameter stabilised, although there were slight fluctuations later in the digester fed with food waste and cattle slurry: these may have been due to differences between each batch of cattle slurry collected and used. The non steady state behaviour over the first three retention times may be explained as follows:

As the digesters were started from sewage sludge digestate it took time for the consortium of microorganisms to acclimate to the new feedstock and new environmental conditions in the digester. The robust acid-forming bacteria adapted to the substrate after the digesters had been running for around half a retention time and started to consume the accumulated feedstock at an accelerated rate, and in the process produced VFA faster than it could be consumed by the methanogens. At this point either the drop in pH provided optimal conditions for the methanogens to consume the VFA; or the moderate OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ simply allowed the more sensitive methanogens to catch up with the acidogens before the pH dropped further to a level which would have inhibited them.

The two digesters running on mixed substrates appeared to have reached a steady state after the first 3 retention times (day 90) and showed a similar specific biogas production to the parallel laboratory-scale co-digestion trials when operated at the same OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$. All operational parameters were also in the safe range, with TAN less than 2500 mg l^{-1} (Figure 9.8) and VFA below 100 mg l^{-1} (Figure 9.12) throughout the next 7 retention times (210 days) in the trial. The volumetric biogas production (VBP) was 0.70 and $1.2 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ with a methane percentage of 61% and 56% in the food waste co-digestion with cattle slurry and card packaging respectively.

The difference in TAN observed between the laboratory-scale and larger-scale co-digestion trial using a food waste and card packaging mix was because the card packaging used in the smaller-scale laboratory trial was pre-processed by wet maceration. The addition of water during this pre-treatment reduced the total solids content of the feedstock from 94% to 20%, thus increasing the rate at which TAN was hydraulically flushed out of the digesters.

Although the digester fed only on food waste appeared to reach a steady state for biogas production the TAN level exceeded 4000 mg l^{-1} by day 150 and continued to increase (Figure 9.8). By day 180 there were signs of stress with an elevated VFA concentration (Figure 9.15) from the previously stable level of around 200 mg l^{-1} between day 60 and day 180. The VFA concentration built up rapidly to above 1000 mg l^{-1} by day 210. Initially the VFA was predominantly in the form of HAc but later all the VFA species were present, with an initial rise in HPr concentration. The concentration of HPr then fell, accompanied by a further HAc accumulation. It can be seen from Figure 9.12 that by the

end of the trial VFA levels had been fluctuating around 4000 mg l^{-1} for about 3 retention times (98 days). Although VFA concentrations were not yet as high as previously seen in digesters fed only on food waste (see sections 5 and 8.2), the pattern of accumulation was similar. The pH in the digester remained high at 8.1 (Figure 9.7) due to sufficient buffering capacity provided by TAN (Figure 9.8).

By this point in the study it was also apparent that the food waste could be deficient in some essential trace elements needed for microbial metabolism. The concentrations of essential trace elements in both the laboratory-scale and larger-scale co-digestion trial were analysed and the results are given in Table 9.2. At the time of trace element analysis, the laboratory-scale mechanically-recovered BMW co-digestion trial had run for 259 days, the laboratory-scale food waste co-digestion trial for 161 days, and the larger-scale food waste co-digestion trial for 140 days. It can be seen from the modelled dilute-out profiles for essential trace elements (Figure 9.16) that concentrations of cobalt, selenium and tungsten had all dropped to below $1 \text{ mg kg}^{-1} \text{ TS}$ by day 210 when the VFA level built up to above 1000 mg l^{-1} . This corresponds to the concentration range at which laboratory-scale food waste co-digestion trials have also failed, as shown in Table 9.2.

The results indicate that the digestion of food waste by itself leads to an increase in VFA, probably as a result of wash-out of essential trace elements combined with an increasing level of TAN. This condition was prevented by co-digestion with cattle slurry and with card packaging, which could contribute essential elements and also reduce the ammonia concentration.

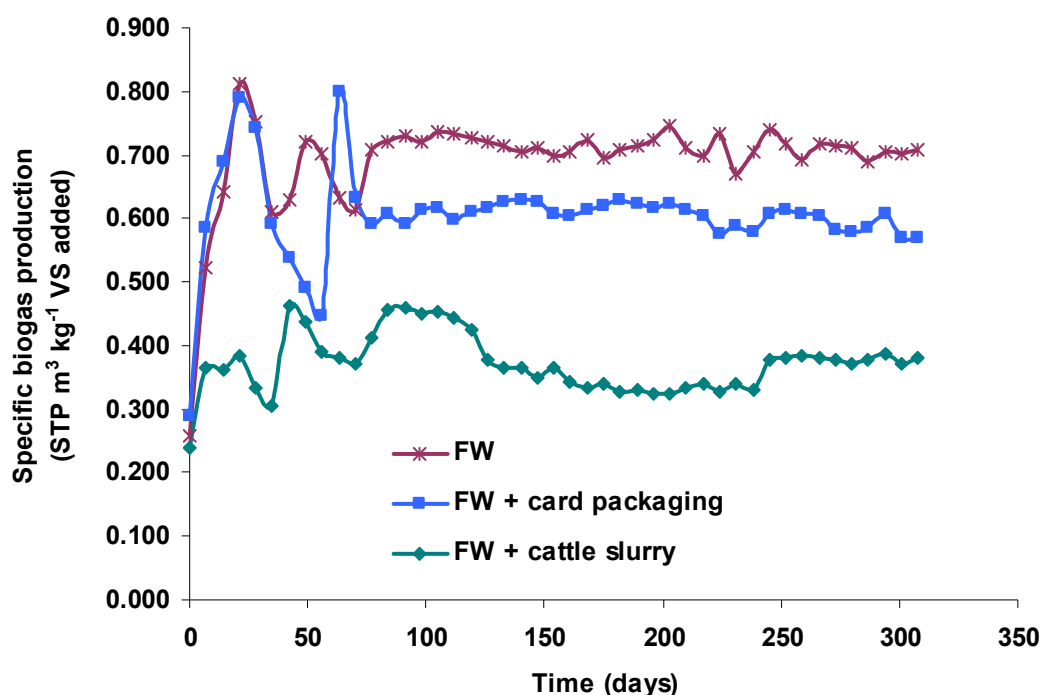


Figure 9.1 Weekly average specific biogas production in the larger-scale food waste co-digestion trials

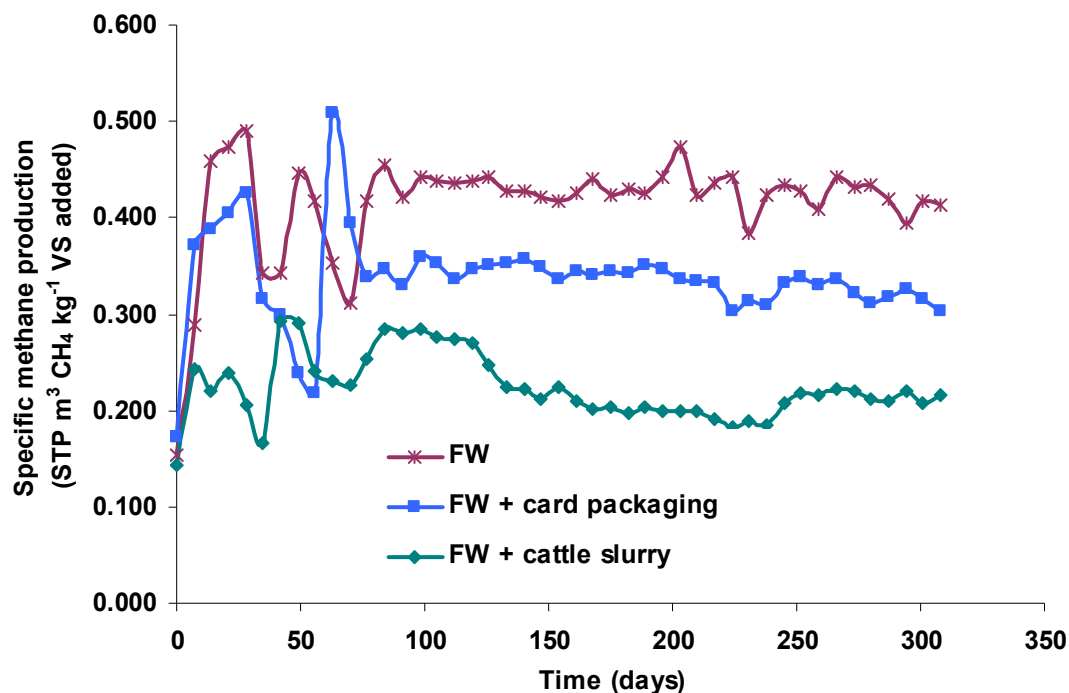


Figure 9.2 Weekly average specific methane production in the larger-scale food waste co-digestion trials

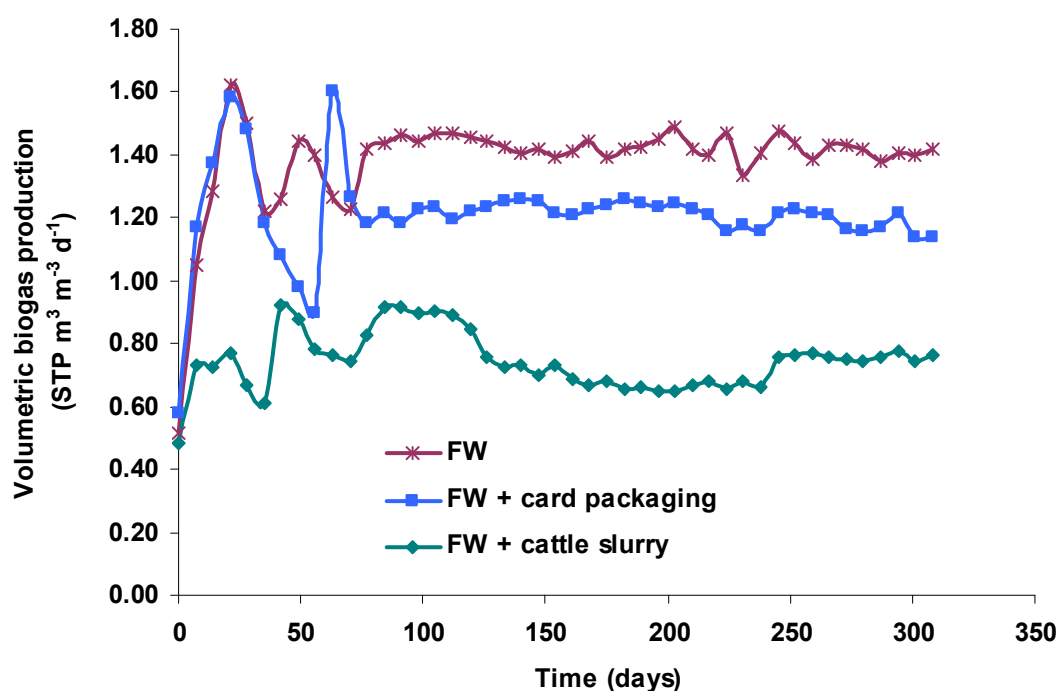


Figure 9.3 Weekly average volumetric biogas production in the larger-scale food waste co-digestion trials

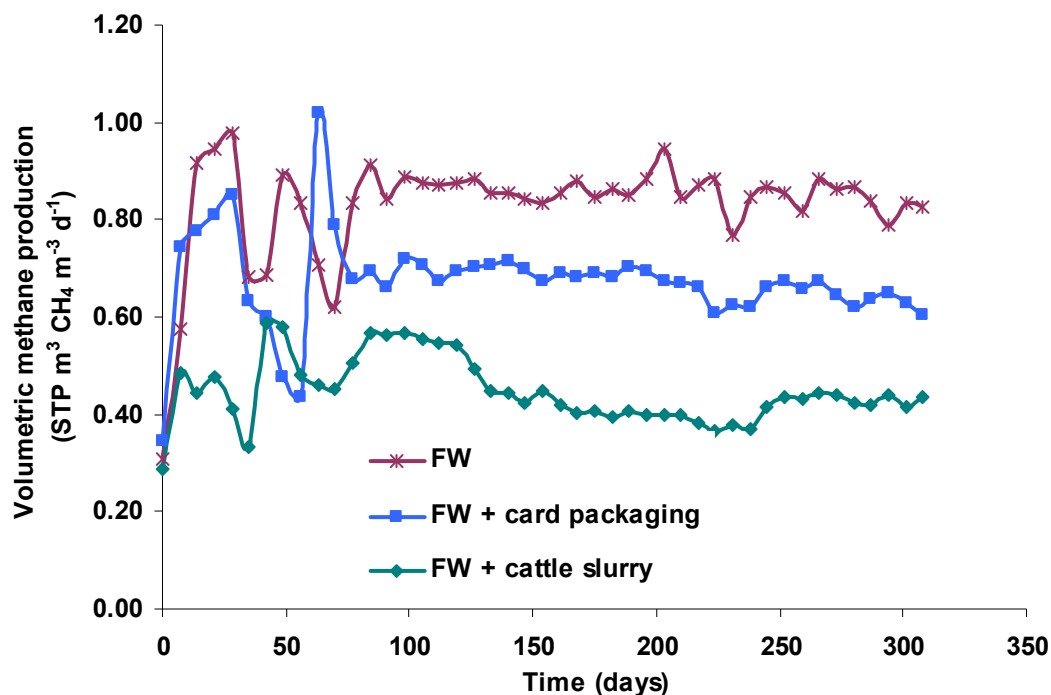


Figure 9.4 Weekly average volumetric methane production in the larger-scale food waste co-digestion trials

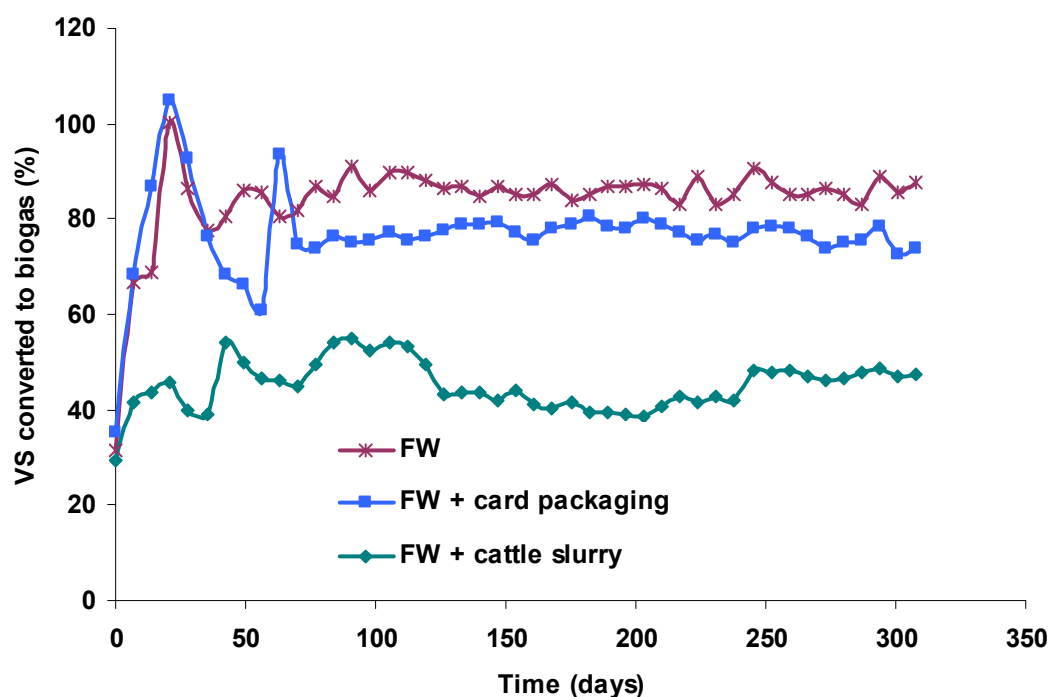


Figure 9.5 Weekly average mass conversion from feedstock VS to biogas in the larger-scale food waste co-digestion trials

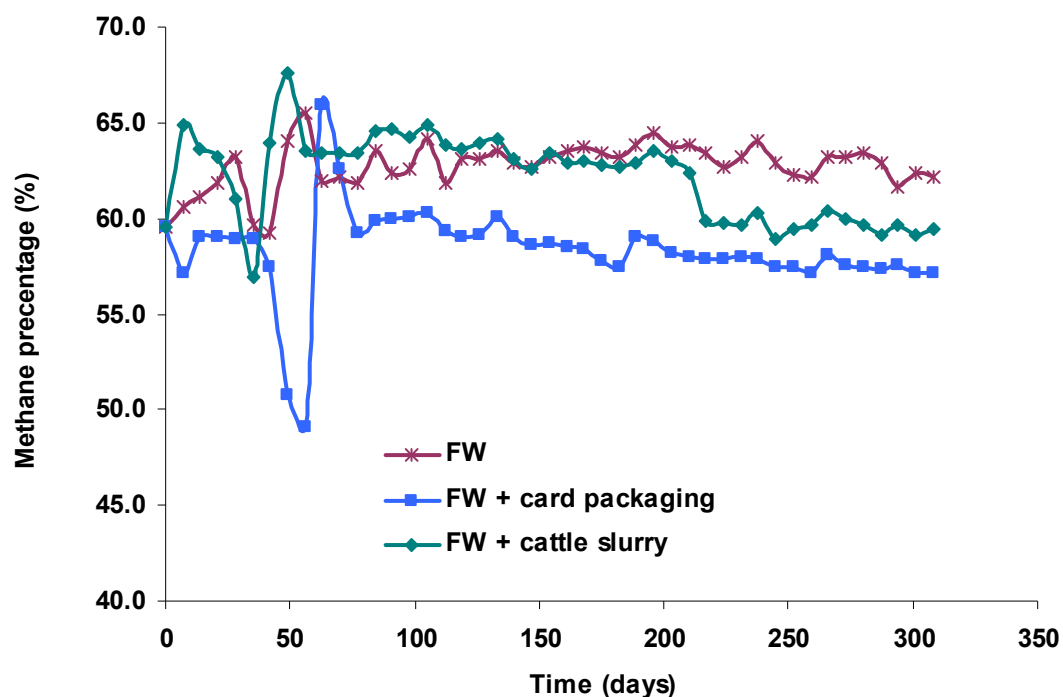


Figure 9.6 Weekly average methane content of biogas produced in the larger-scale food waste co-digestion trials; Measurement was taken from the headspace before daily feeding

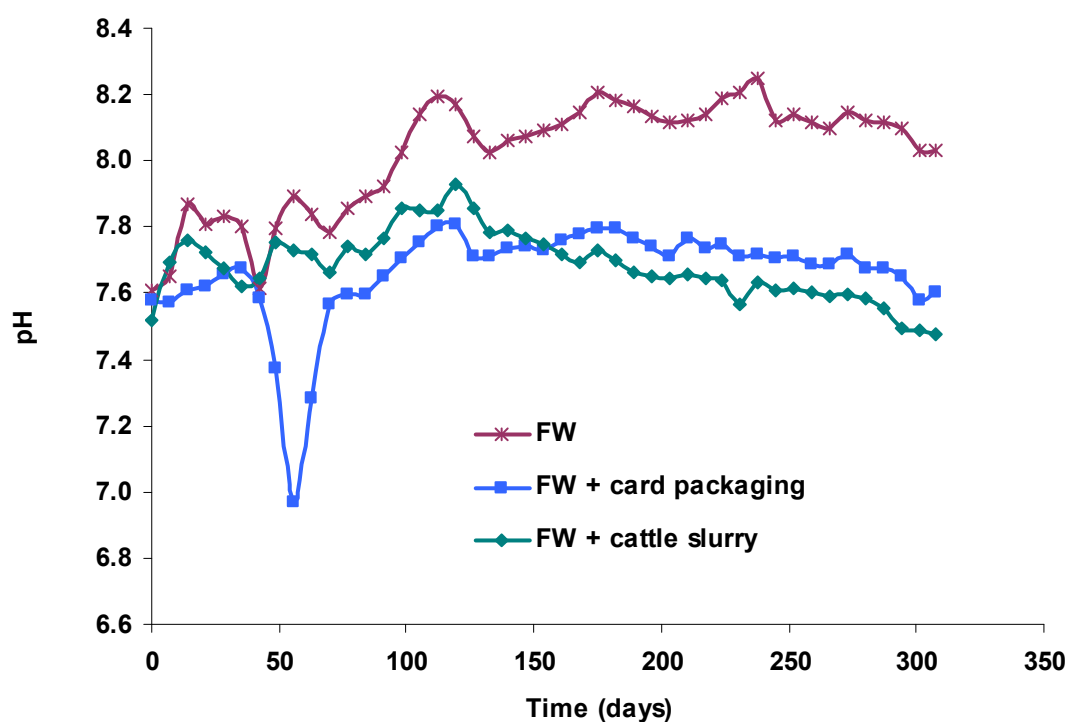


Figure 9.7 Weekly average pH values in the larger-scale food waste co-digestion trials

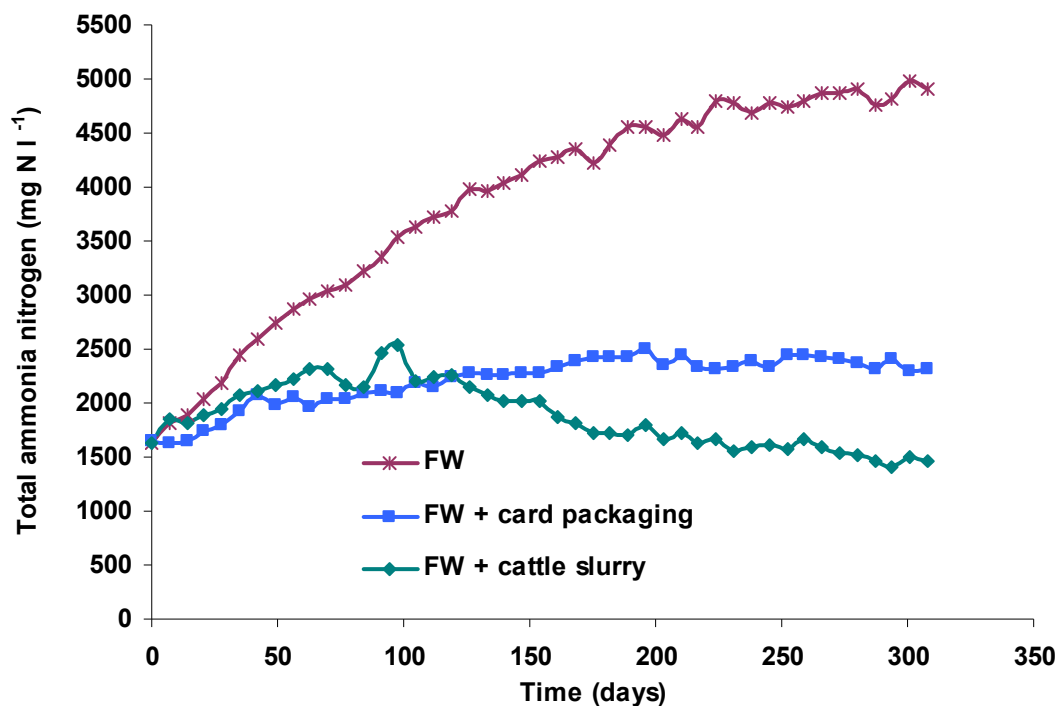


Figure 9.8 Total ammonia nitrogen in the larger-scale food waste co-digestion trials

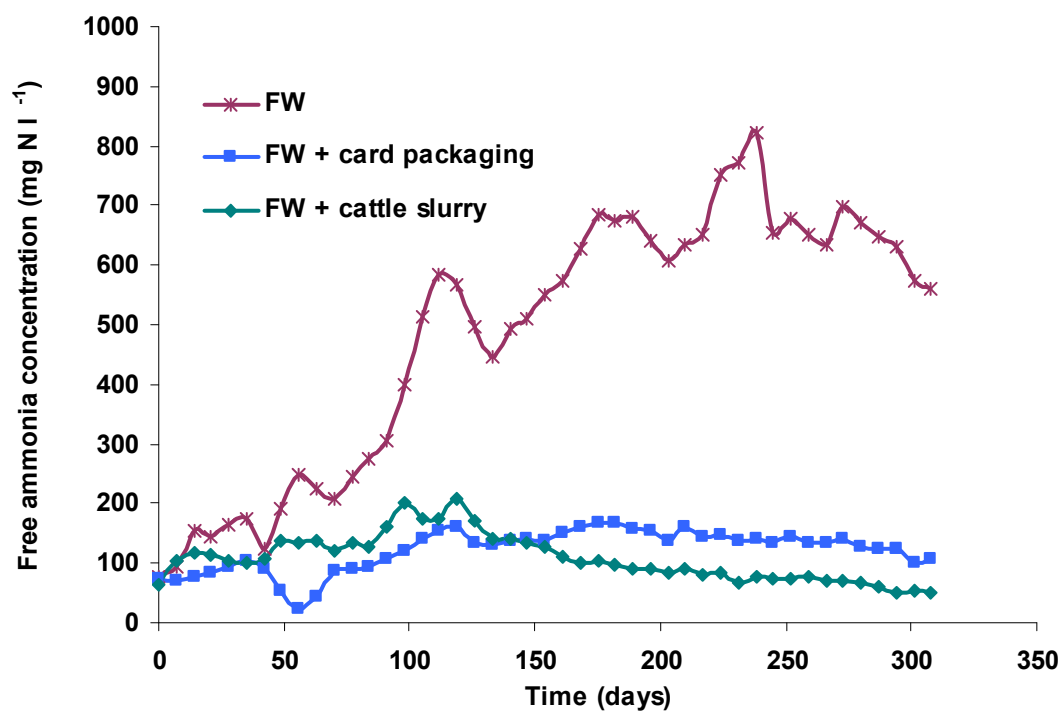


Figure 9.9 Free ammonia concentration in the larger-scale food waste co-digestion trials

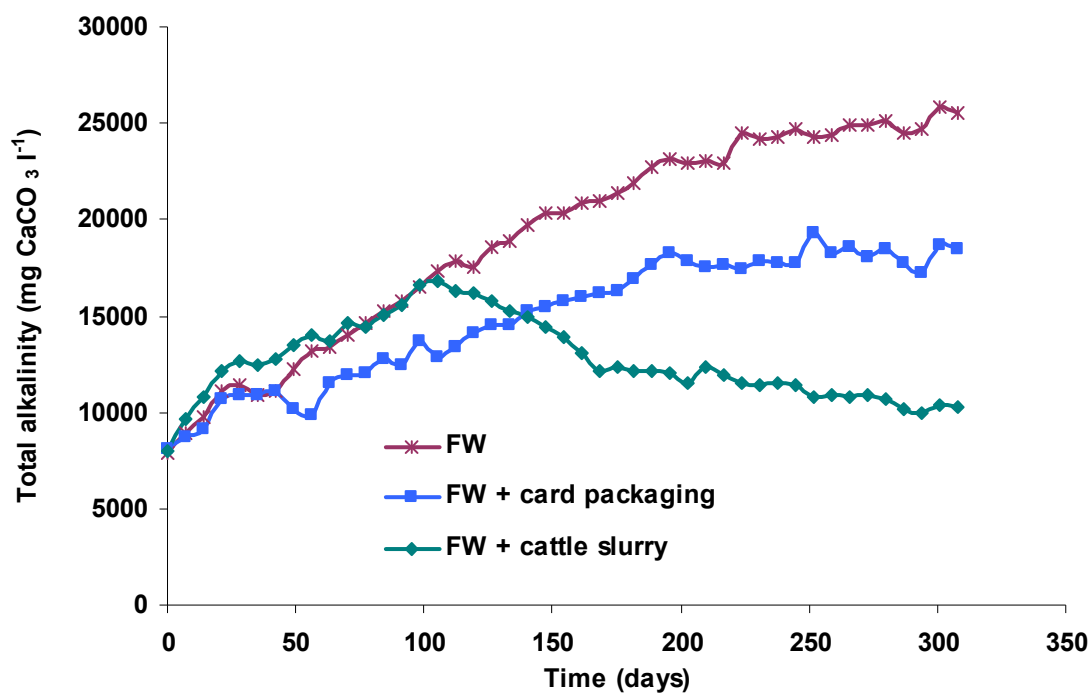


Figure 9.10 Total alkalinity in the larger-scale food waste co-digestion trials

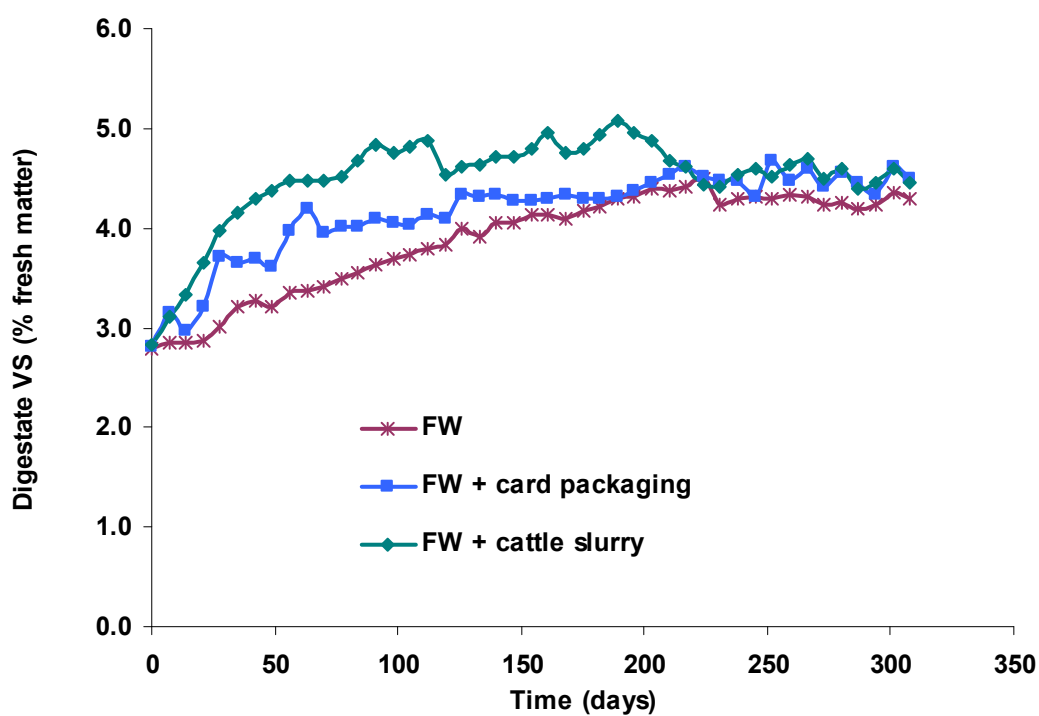


Figure 9.11 Digestate volatile solids content in the larger-scale food waste co-digestion trials

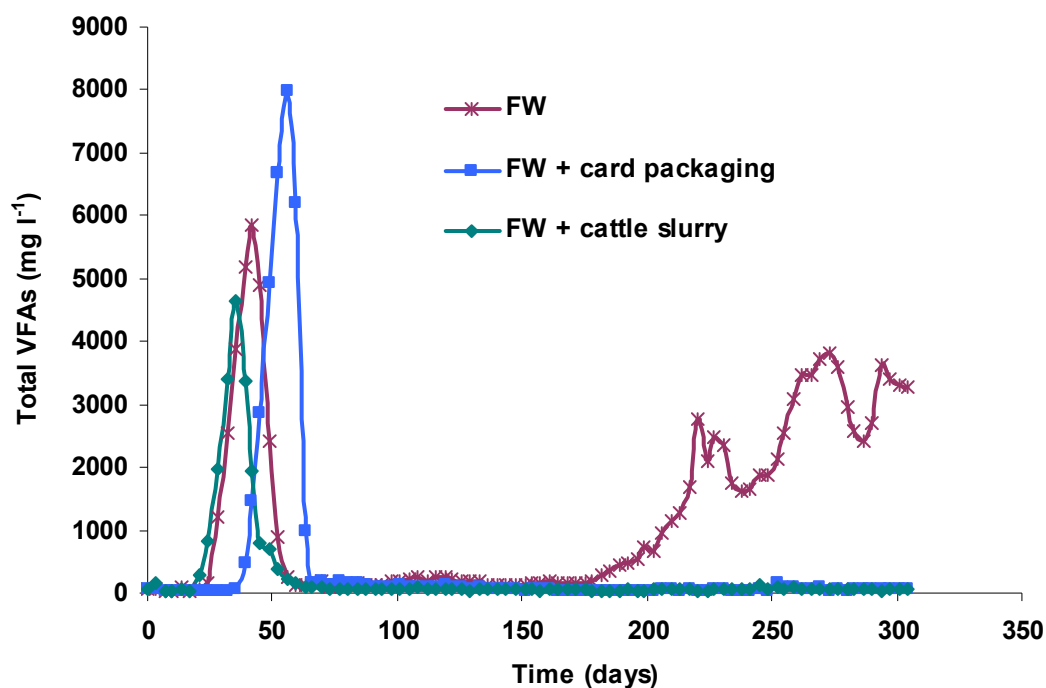


Figure 9.12 Total VFA concentration in the larger-scale food waste co-digestion trials

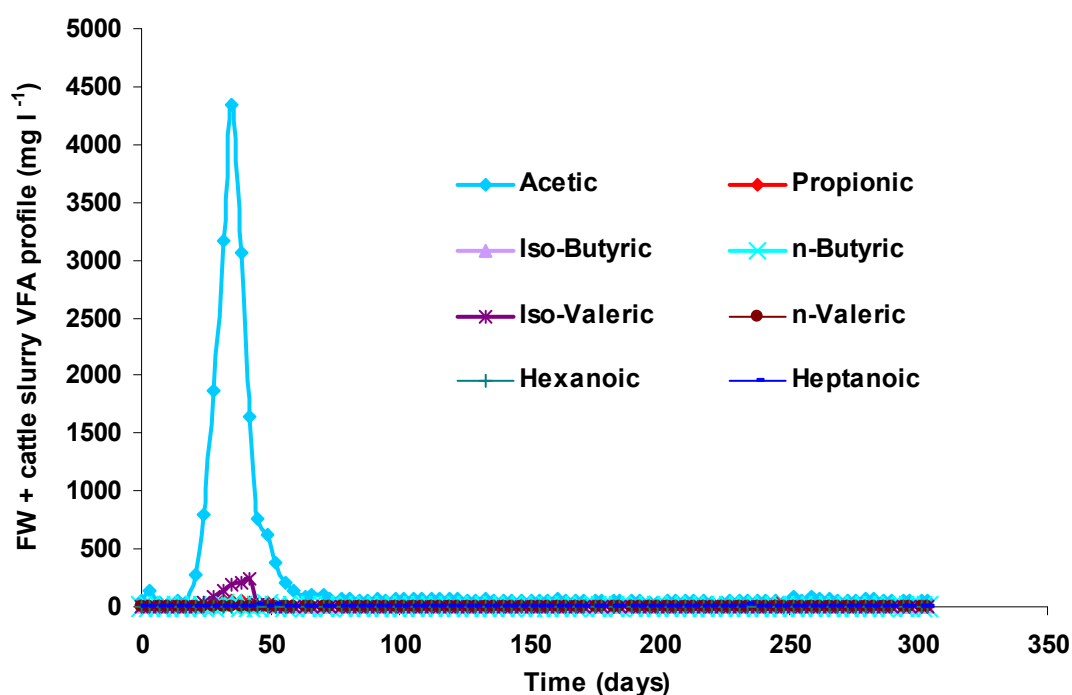


Figure 9.13 VFA concentration profile in the larger-scale co-digestion trial using food waste mixed with cattle slurry

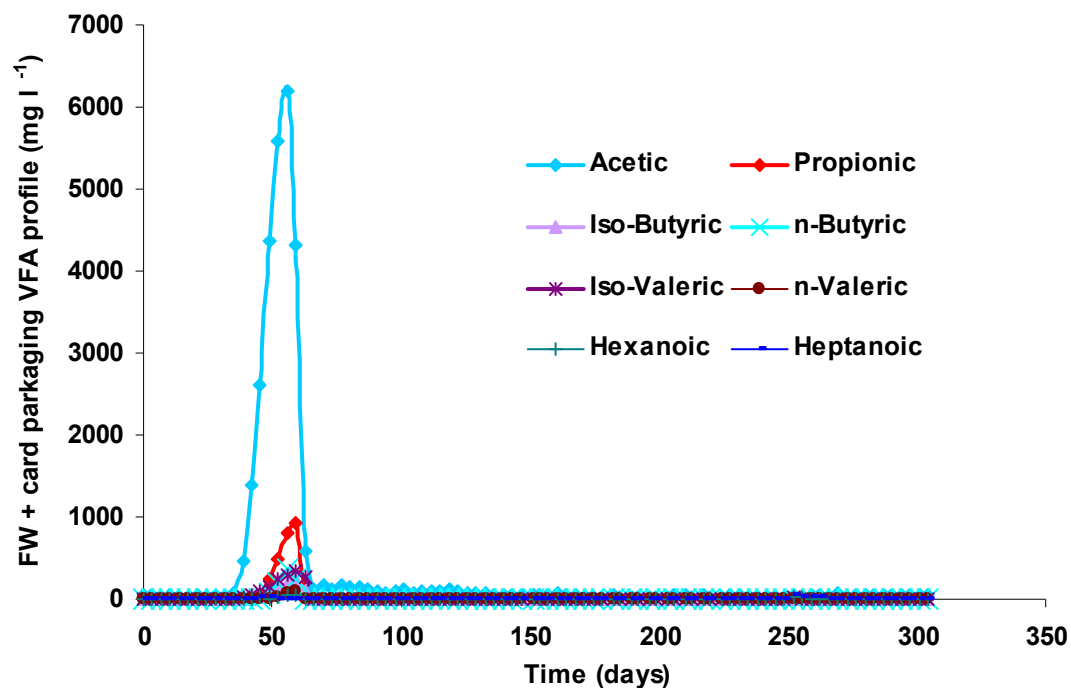


Figure 9.14 VFA concentration profile in the larger-scale co-digestion trial using food waste mixed with card packaging

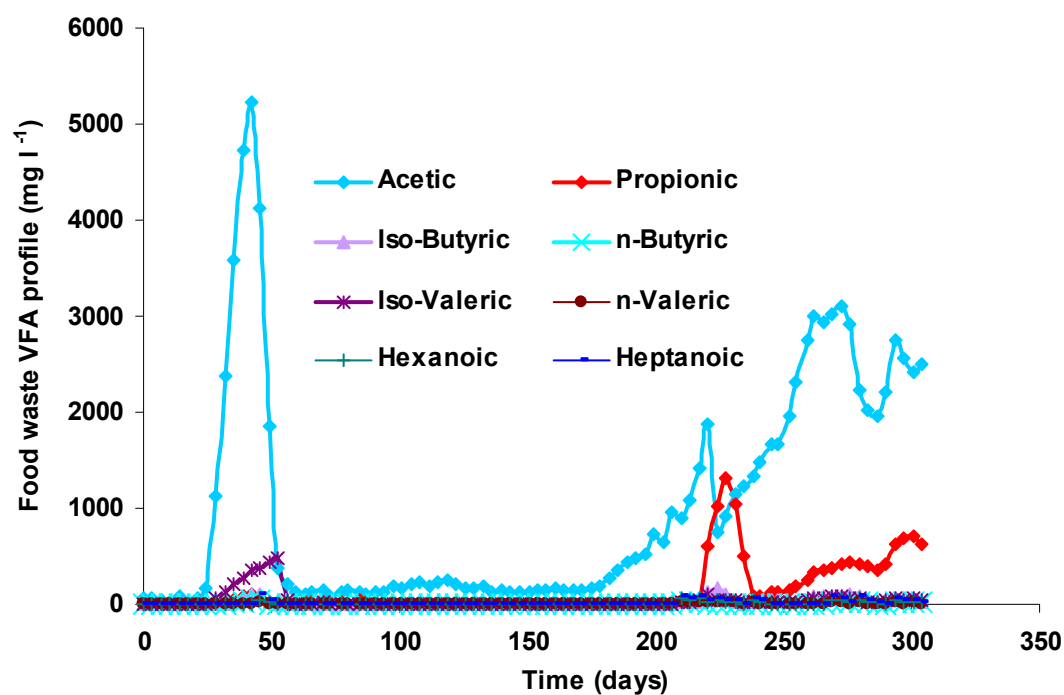


Figure 9.15 VFA concentration profile in the larger-scale food waste digestion control

Table 9.2 Concentrations of essential elements in whole digestate samples taken from laboratory-scale and larger-scale co-digestion trials.

Digester	Co	Cu	Mn	Mo	Ni	Se	W	Zn	pH	TAN	VFA	TA
<i>Laboratory-scale mechanically-recovered BMW co-digestion trials</i>												
BMW + gut & fat	8.9	512	1080	4.4	76.8	0.27	13	1080	8.0	4500	1600	30000
BMW + sheep blood	9.3	560	900	5.1	80.5	0.17	9.3	1020	8.1	7500	10000	40000
BMW control	10	565	687	4.2	69.9	<0.15	5.4	879	7.4	1400	100	18000
<i>Laboratory-scale food waste co-digestion trials</i>												
FW + CS	2.0	63.2	667	6.9	62.4	0.46	3.1	241	7.6	2000	50	12000
FW + CP 1	1.2	75.7	443	3.3	28.4	0.26	<0.25	173	7.2	1200	3500	9000
FW + CP 2	1.2	73.9	171	5.9	53.5	0.16	3.3	96.8	7.5	1200	50	11000
FW + WP 1	1.5	74.0	469	5.6	58.1	0.42	2.6	196	8.0	3400	6000	16000
FW + WP2	0.64	34.5	239	3.1	43.0	0.31	2.6	96.3	5.8	3200	24000	11000
FW control	1.3	140	255	7.9	132	0.33	6.1	121	5.8	3600	26000	13000
<i>Larger-scale food waste co-digestion trials</i>												
FW + CS	1.8	196	696	3.9	36.3	0.59	<0.25	314	7.8	2000	50	15000
FW + CP	1.3	178	163	3.3	36.5	<0.15	2.1	198	7.7	2300	50	15000
FW control	1.5	209	367	3.1	65.0	0.57	1.6	287	8.1	4000	150	20000

Units: essential elements: mg Kg⁻¹ TS; TAN: mg NH₃-N l⁻¹; VFA mg l⁻¹; TA: mg CaCO₃ l⁻¹.

Abbreviations: FW – Food Waste; CS – Cattle Slurry; CP – Card packaging; WP – Waste Potato

Note: At the time of sampling, laboratory-scale mechanically-recovered BMW co-digestion trial had run for 259 days, at OLR of 3 kg VS m⁻³ d⁻¹. Laboratory-scale food waste co-digestion trial had run for 161 days: the co-digestion of food waste and cattle slurry at OLR of 2 with 40% of food waste in the mixture on a VS basis; the co-digestion of food waste and card packaging No. 1 using cattle slurry as a recovery approach; the co-digestion of food waste and card packaging No. 2 in a static situation after recovery; the co-digestion of food waste and waste potato No. 1 in a static situation but at a recovery stage; the co-digestion of food waste and waste potato No. 2 in a static situation and stressed; the food waste control in a static situation and stressed. At the time of sampling, the larger-scale food waste co-digestion trial had run for 140 days.

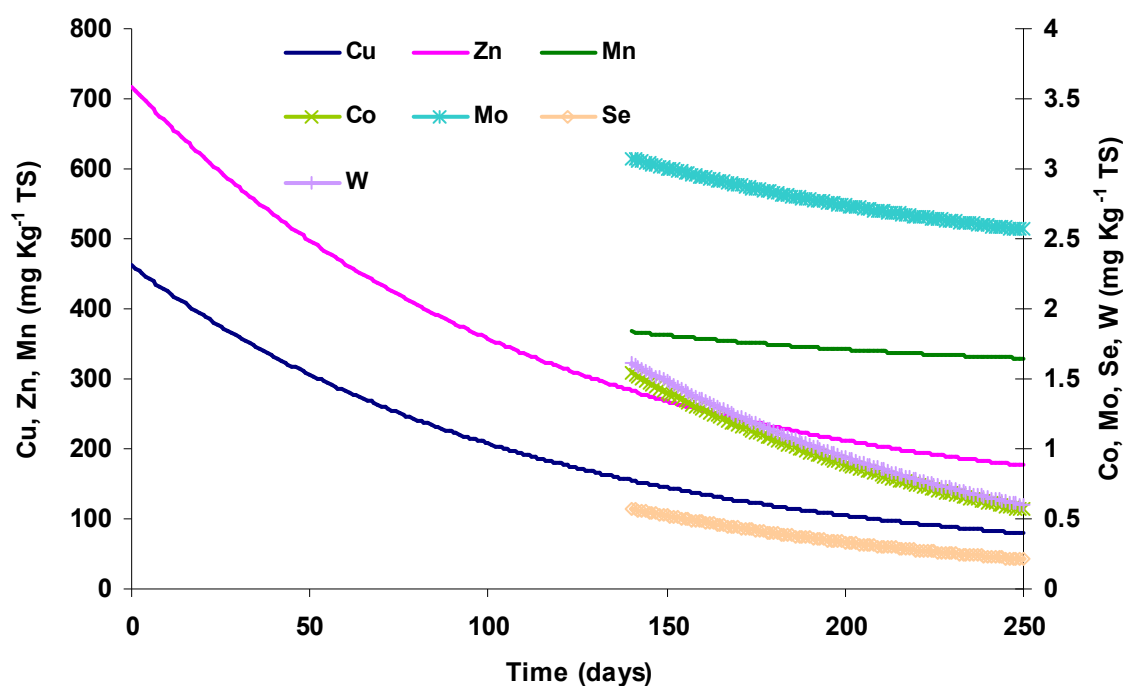


Figure 9.16 Simulated essential elements concentration-time profile in the larger-scale food waste only digester (day 140 was when a sample of digestate was taken for trace element analysis and day 210 was when VFA level rose to above 1000 mg l⁻¹.)

10 Digestate physico-chemical characteristics

The whole digestate, separated liquor and separated fibre from laboratory-scale and the larger-scale co-digestion trials were analysed for their plant nutrient and potentially toxic element (PTE) concentrations to assess their land application potential.

10.1 Digestate characteristics from laboratory-scale mechanically-recovered BMW co-digestion trials

The characteristics of the digestate liquor from the laboratory-scale mechanically-recovered BMW co-digestion trials are expressed on a volumetric and total solids (TS) basis in Table 10.1. The values for the fibre are given in Table 10.2 and for whole digestate in Table 10.3, in both cases expressed on a TS basis. Concentrations of N and K were 2~3 times higher in the digestate liquor than in the fibre due to liquor recirculation in the digesters. The concentration of P in fibre and liquor was about the same but the close association of P with fibre is well known. No clear trend was seen in the partitioning of PTE between digestate liquor and fibre: Cd, Pb and Zn had higher concentrations in the liquor and Cr, Cu, and Ni in the fibre.

The addition of pig gut with flotation fat mixture and sheep blood both increased the concentration of nutrients in the digestate. Because of the large proportion of mechanically-recovered BMW in the feedstock mixture the PTE content in the digestate was also high (Table 10.1 - 10.3), and the concentrations of Cu, Pb, Ni and Zn in the digestate liquor, and Cr, Cu, Pb, Ni and Zn in the digestate fibre and whole digestate were above the limit values in PAS 110.

Table 10.1 Characteristics of digestate liquor in laboratory-scale mechanically-recovered BMW co-digestion trials

	Mechanically-recovered BMW + pig gut and fat		Mechanically-recovered BMW + sheep blood		Mechanically- recovered BMW control
<i>On a volume basis</i>	Digester 1	Digester 2	Digester 1	Digester 2	
Nutrients					
TAN (g l ⁻¹)	5.21	5.21	7.18	8.16	1.72
TKN (g l ⁻¹)	7.42	7.30	9.28	10.0	4.65
TP (g l ⁻¹)	0.82	0.96	0.45	0.41	0.57
TK (g l ⁻¹)	1.8	1.8	1.3	1.4	1.5
Potentially toxic elements					
Cd (mg l ⁻¹)	0.15	0.18	0.13	0.12	0.18
Cr (mg l ⁻¹)	6.4	13	4.7	8.4	7.9
Cu (mg l ⁻¹)	43	48	34	35	49
Pb (mg l ⁻¹)	58	67	41	46	64
Hg (mg l ⁻¹)	0.073	0.085	0.057	0.062	0.094
Ni (mg l ⁻¹)	5.1	9.0	4.1	6.1	7.0
Zn (mg l ⁻¹)	99	110	73	80	110
Essential elements					
Co (mg l ⁻¹)	1.0	1.2	0.82	0.92	1.3
Fe (mg l ⁻¹)	900	1000	710	760	1000
Mo (mg l ⁻¹)	0.73	1.2	0.56	0.77	0.88
Se (mg l ⁻¹)	0.087	0.092	0.058	0.055	0.068
W (mg l ⁻¹)	<0.10	<0.10	<0.10	<0.10	<0.10

Table 10.1 continued Characteristics of digestate liquor in laboratory-scale mechanically-recovered BMW co-digestion trials

	Mechanically-recovered BMW + pig gut and fat		Mechanically-recovered BMW + sheep blood		Mechanically-recovered BMW control
<i>On a TS basis</i>	Digester 1	Digester 2	Digester 1	Digester 2	
Nutrients					
TKN (g Kg ⁻¹ TS)	65.6	75.4	72.3	108	39.9
TP (g Kg ⁻¹ TS)	6.7	6.9	4.6	4.0	4.7
TK (g Kg ⁻¹ TS)	15	13	13	14	12
Potentially toxic elements					
Cd (mg Kg ⁻¹ TS)	1.2	1.3	1.3	1.2	1.5
Cr (mg Kg ⁻¹ TS)	52	94	48	82	65
Cu (mg Kg ⁻¹ TS)	350	350	350	340	400
Pb (mg Kg ⁻¹ TS)	470	480	420	450	530
Hg (mg Kg ⁻¹ TS)	0.59	0.61	0.59	0.61	0.77
Ni (mg Kg ⁻¹ TS)	41	65	42	60	57
Zn (mg Kg ⁻¹ TS)	800	790	750	780	900
Essential elements					
Co (mg Kg ⁻¹ TS)	8.1	8.6	8.4	9.0	11
Fe (mg Kg ⁻¹ TS)	7300	7200	7300	7500	8200
Mo (mg Kg ⁻¹ TS)	5.9	8.6	5.8	7.6	7.2
Se (mg Kg ⁻¹ TS)	0.71	0.66	0.60	0.54	0.56
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0	<1.0
Solids content					
TS (% WW)	12.3	13.9	9.71	10.2	12.2
VS (% WW)	6.36	6.42	5.42	6.09	5.50
VS (% TS)	51.6	46.2	55.8	59.7	45.2

Table 10.2 Characteristics of digestate fibre in laboratory-scale mechanically-recovered BMW co-digestion trials

	Mechanically-recovered BMW + pig gut and fat		Mechanically-recovered BMW + sheep blood		Mechanically-recovered BMW control
	Digester 1	Digester 2	Digester 1	Digester 2	
Nutrients					
TKN (g Kg ⁻¹ TS)	24.2	21.8	32.4	40.2	16.5
TP (g Kg ⁻¹ TS)	4.8	4.8	2.9	2.8	4.3
TK (g Kg ⁻¹ TS)	6.6	6.9	4.4	4.7	5.5
Potentially toxic elements					
Cd (mg Kg ⁻¹ TS)	1.0	1.0	0.95	0.98	1.0
Cr (mg Kg ⁻¹ TS)	460	510	500	510	665
Cu (mg Kg ⁻¹ TS)	660	300	560	410	330
Pb (mg Kg ⁻¹ TS)	300	370	250	314	300
Hg (mg Kg ⁻¹ TS)	0.82	0.43	0.58	0.48	0.29
Ni (mg Kg ⁻¹ TS)	210	230	230	250	300
Zn (mg Kg ⁻¹ TS)	510	500	390	1400	530
Essential elements					
Co (mg Kg ⁻¹ TS)	13	13	13	13	16
Fe (mg Kg ⁻¹ TS)	8100	8200	7600	7500	10000
Mo (mg Kg ⁻¹ TS)	8.4	9.5	8.2	9.6	10
Se (mg Kg ⁻¹ TS)	0.44	0.37	0.33	<0.30	0.35
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0	<1.0
Solids content					
TS (% WW)	28.2	29.6	27.0	28.2	30.2
VS (% WW)	14.4	15.1	15.6	17.0	12.7
VS (% TS)	51.3	51.2	57.7	60.1	42.2

Table 10.3 Characteristics of whole digestate in laboratory-scale mechanically-recovered BMW co-digestion trials

	Mechanically-recovered BMW + pig gut and fat		Mechanically-recovered BMW + sheep blood		Mechanically- recovered BMW control
	Digester 1	Digester 2	Digester 1	Digester 2	
Nutrients					
TKN (g Kg ⁻¹ TS)	53.1	55.6	70.2	82.7	34.7
TP (g Kg ⁻¹ TS)	6.2	6.1	4.2	3.7	4.6
TK (g Kg ⁻¹ TS)	13	11	11	12	11
Potentially toxic elements					
Cd (mg Kg ⁻¹ TS)	1.2	1.2	1.2	1.1	1.4
Cr (mg Kg ⁻¹ TS)	160	250	150	190	220
Cu (mg Kg ⁻¹ TS)	430	330	400	360	380
Pb (mg Kg ⁻¹ TS)	430	440	380	420	470
Hg (mg Kg ⁻¹ TS)	0.65	0.55	0.59	0.58	0.65
Ni (mg Kg ⁻¹ TS)	85	120	85	110	120
Zn (mg Kg ⁻¹ TS)	730	690	670	930	810
Essential elements					
Co (mg Kg ⁻¹ TS)	9.3	10	9.4	9.9	12
Fe (mg Kg ⁻¹ TS)	7500	7500	7400	7500	8800
Mo (mg Kg ⁻¹ TS)	6.6	9.0	6.3	8.0	8.0
Se (mg Kg ⁻¹ TS)	0.64	0.55	0.54	0.41	0.51
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0	<1.0
Solids content					
TS (% WW)	16.4	20.0	13.8	14.6	16.3
VS (% WW)	8.48	9.33	7.68	8.72	7.47
VS (% TS)	51.8	46.5	55.5	59.7	45.8

10.2 Digestate characteristics from laboratory-scale food waste co-digestion trials

The characteristics of the digestate liquor from the laboratory-scale food waste co-digestion trials are expressed on a volumetric and TS basis in Table 10.4. The values for the fibre are given in Table 10.5 and for whole digestate in Table 10.6, in both cases expressed on a TS basis. The PTE levels in both the food waste co-digestates were below the upper limits specified in PAS 110 even when the mix contained a high proportion of cattle slurry which has a higher PTE concentration than food waste, particularly Zn.

In the laboratory-scale co-digestion trials the digestates from food waste mixes and mechanically-recovered BMW mixes had comparable total N concentrations; this reflected one criterion in the selection of co-substrates, which was to optimise the C:N ratio of the baseline waste streams. The digestate from food waste and cattle slurry digesters had higher P and K concentrations than digestates from food waste and card packaging digestate and from mechanically-recovered BMW co-digestion, mainly due to the high P and K concentrations in cattle slurry.

Table 10.4 Characteristics of digestate liquor in laboratory-scale FW co-digestion trials

<i>On a volume basis</i>	FW + cattle slurry		FW + card packaging	
	Digester 1	Digester 2	Digester 1	Digester 2
Nutrients				
TAN (g l ⁻¹)	1.70	1.62	0.87	0.93
TKN (g l ⁻¹)	3.89	3.90	2.92	2.95
TP (g l ⁻¹)	0.89	0.87	0.41	0.37
TK (g l ⁻¹)	1.6	1.7	1.2	1.2
Potentially toxic elements				
Cd (mg l ⁻¹)	<0.05	<0.05	<0.05	<0.05
Cr (mg l ⁻¹)	2.7	2.1	3.9	2.6
Cu (mg l ⁻¹)	3.6	3.7	5.6	5.5
Pb (mg l ⁻¹)	<1.0	<1.0	<1.0	<1.0
Hg (mg l ⁻¹)	<0.025	<0.025	<0.025	<0.025
Ni (mg l ⁻¹)	1.6	1.2	2.1	1.4
Zn (mg l ⁻¹)	13	13	6.3	5.8
Essential elements				
Co (mg l ⁻¹)	<0.10	<0.10	0.11	<0.10
Fe (mg l ⁻¹)	<200	<200	<200	<200
Mo (mg l ⁻¹)	0.34	0.32	0.44	0.35
Se (mg l ⁻¹)	<0.030	<0.030	<0.030	<0.030
W (mg l ⁻¹)	<0.10	<0.10	<0.10	<0.10
On a TS basis				
Nutrients				
TKN (g Kg ⁻¹ TS)	76.0	69.3	52.7	51.5
TP (g Kg ⁻¹ TS)	16.6	15.8	7.28	6.48
TK (g Kg ⁻¹ TS)	29.9	30.8	21.3	21.0
Potentially toxic elements				
Cd (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0
Cr (mg Kg ⁻¹ TS)	50.4	38.1	69.3	45.5
Cu (mg Kg ⁻¹ TS)	67.3	67.1	99.5	96.3
Pb (mg Kg ⁻¹ TS)	<18	<18	<18	<18
Hg (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50	<0.50
Ni (mg Kg ⁻¹ TS)	29.9	21.8	37.3	24.5
Zn (mg Kg ⁻¹ TS)	243	236	112	102
Essential elements				
Co (mg Kg ⁻¹ TS)	<2.0	<2.0	2.0	<2.0
Fe (mg Kg ⁻¹ TS)	<4000	<4000	<4000	<4000
Mo (mg Kg ⁻¹ TS)	6.4	5.8	7.8	6.1
Se (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50	<0.50
W (mg Kg ⁻¹ TS)	<2.0	<2.0	<2.0	<2.0
Solids content				
TS (% WW)	5.35	5.51	5.63	5.71
VS (% WW)	3.77	3.92	3.73	3.72
VS (% TS)	70.4	71.0	66.3	65.2

Table 10.5 Characteristics of digestate fibre in laboratory-scale FW co-digestion trials

	FW + cattle slurry		FW + card packaging	
	Digester 1	Digester 2	Digester 1	Digester 2
Nutrients				
TKN (g Kg ⁻¹ TS)	31.2	33.8	24.9	28.7
TP (g Kg ⁻¹ TS)	9.5	10	2.1	2.5
TK (g Kg ⁻¹ TS)	14	14	11	10
Potentially toxic elements				
Cd (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50	<0.50
Cr (mg Kg ⁻¹ TS)	130	90	36	34
Cu (mg Kg ⁻¹ TS)	40	41	22	33
Pb (mg Kg ⁻¹ TS)	<10	<10	<10	<10
Hg (mg Kg ⁻¹ TS)	<0.25	<0.25	<0.25	<0.25
Ni (mg Kg ⁻¹ TS)	58	41	19	17
Zn (mg Kg ⁻¹ TS)	180	240	36	41
Essential elements				
Co (mg Kg ⁻¹ TS)	2.4	2.1	<1.0	<1.0
Fe (mg Kg ⁻¹ TS)	<2000	<2000	<2000	<2000
Mo (mg Kg ⁻¹ TS)	4.4	4.1	2.3	2.5
Se (mg Kg ⁻¹ TS)	<0.30	<0.30	<0.30	<0.30
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0
Solids content				
TS (% WW)	15.1	15.8	11.9	11.2
VS (% WW)	11.4	11.4	9.10	8.74
VS (% TS)	75.5	72.1	76.6	78.2

Table 10.6 Characteristics of whole digestate in laboratory-scale FW co-digestion trials

	FW + cattle slurry		FW + card packaging	
	Digester 1	Digester 2	Digester 1	Digester 2
Nutrients				
TKN (g Kg ⁻¹ TS)	68.0	63.2	46.0	45.3
TP (g Kg ⁻¹ TS)	16	15	5.7	5.3
TK (g Kg ⁻¹ TS)	28	29	18	18
Potentially toxic elements				
Cd (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50	<0.50
Cr (mg Kg ⁻¹ TS)	62	45	59	42
Cu (mg Kg ⁻¹ TS)	63	64	77	77
Pb (mg Kg ⁻¹ TS)	<10	<10	<10	<10
Hg (mg Kg ⁻¹ TS)	<0.25	<0.25	<0.25	<0.25
Ni (mg Kg ⁻¹ TS)	34	24	32	22
Zn (mg Kg ⁻¹ TS)	230	240	89	83
Essential elements				
Co (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0
Fe (mg Kg ⁻¹ TS)	<2000	<2000	<2000	<2000
Mo (mg Kg ⁻¹ TS)	6.1	5.6	6.2	5.0
Se (mg Kg ⁻¹ TS)	0.48	<0.30	<0.30	<0.30
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0	<1.0
Solids content				
TS (% WW)	6.60	6.76	7.50	7.43
VS (% WW)	4.90	5.00	5.31	5.25
VS (% TS)	74.2	73.9	70.8	70.7

10.3 Digestate characteristics from the larger-scale food waste co-digestion trials

The characteristics of digestate liquor from the larger-scale food waste co-digestion trials are expressed on a volumetric and TS basis in Table 10.7. Values for the fibre are given in Table 10.8 and for whole digestate in Table 10.9, in both cases expressed on a TS basis.

Table 10.7 Characteristics of digestate liquor in larger-scale food waste co-digestion trials

	FW + cattle slurry	FW + card packaging	FW control
On a volume basis			
Nutrients			
TAN (g l ⁻¹)	1.51	2.30	5.00
TKN (g l ⁻¹)	2.95	3.96	8.21
TP (g l ⁻¹)	0.66	0.54	0.81
TK (g l ⁻¹)	1.8	1.5	3.0
Potentially toxic elements			
Cd (mg l ⁻¹)	<0.05	<0.05	<0.05
Cr (mg l ⁻¹)	1.1	3.1	4.7
Cu (mg l ⁻¹)	5.2	9.4	7.5
Pb (mg l ⁻¹)	<1.0	3.2	1.1
Hg (mg l ⁻¹)	<0.025	<0.025	<0.025
Ni (mg l ⁻¹)	0.62	1.7	2.9
Zn (mg l ⁻¹)	14	7.8	11
Essential elements			
Co (mg l ⁻¹)	<0.10	<0.10	<0.10
Fe (mg l ⁻¹)	<200	210	340
Mo (mg l ⁻¹)	0.20	0.32	0.31
Se (mg l ⁻¹)	<0.030	<0.030	0.042
W (mg l ⁻¹)	<0.10	<0.10	<0.10
On a TS basis			
Nutrients			
TKN (g Kg ⁻¹ TS)	65.2	70.6	138
TP (g Kg ⁻¹ TS)	14	10	14
TK (g Kg ⁻¹ TS)	38	29	50
Potentially toxic elements			
Cd (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0
Cr (mg Kg ⁻¹ TS)	23	60	79
Cu (mg Kg ⁻¹ TS)	110	180	130
Pb (mg Kg ⁻¹ TS)	<18	62	18
Hg (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50
Ni (mg Kg ⁻¹ TS)	13	33	49
Zn (mg Kg ⁻¹ TS)	300	150	180
Essential elements			
Co (mg Kg ⁻¹ TS)	<2.0	<2.0	<2.0
Fe (mg Kg ⁻¹ TS)	<4000	4100	5700
Mo (mg Kg ⁻¹ TS)	4.2	6.2	5.2
Se (mg Kg ⁻¹ TS)	<0.50	<0.50	0.71
W (mg Kg ⁻¹ TS)	<2.0	<2.0	<2.0
Solids content			
TS (% WW)	4.72	5.16	5.95
VS (% WW)	3.34	3.21	4.10
VS (% TS)	70.7	62.3	68.8

The physicochemical characteristics of digestate produced in the larger-scale food waste co-digestion trials were comparable with those in the laboratory-scale trials. The food waste digestate itself contained higher concentrations of plant nutrients than digestates from either of the co-digestion trials; this is not only due to the high NPK content in food

waste, but also because these elements are conserved in the digestion process while the food waste input loses a higher proportion of its solids than the mixed feedstocks.

Table 10.8 Characteristics of digestate fibre in larger-scale food waste co-digestion trials

	FW + cattle slurry	FW + card packaging	FW control
Nutrients			
TKN (g Kg ⁻¹ TS)	32.2	33.0	62.2
TP (g Kg ⁻¹ TS)	5.6	3.1	13
TK (g Kg ⁻¹ TS)	11	9.5	24
Potentially toxic elements			
Cd (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50
Cr (mg Kg ⁻¹ TS)	68	24	51
Cu (mg Kg ⁻¹ TS)	46	61	71
Pb (mg Kg ⁻¹ TS)	<10	14	<10
Hg (mg Kg ⁻¹ TS)	<0.25	<0.25	<0.25
Ni (mg Kg ⁻¹ TS)	32	13	28
Zn (mg Kg ⁻¹ TS)	140	50	130
Essential elements			
Co (mg Kg ⁻¹ TS)	1.3	<1.0	<1.0
Fe (mg Kg ⁻¹ TS)	<2000	<2000	2700
Mo (mg Kg ⁻¹ TS)	2.3	2.3	2.7
Se (mg Kg ⁻¹ TS)	<0.30	<0.30	<0.30
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0
Solids content			
TS (% WW)	11.4	12.7	14.1
VS (% WW)	9.70	9.33	11.2
VS (% TS)	85.1	73.6	79.3

Table 10.9 Characteristics of whole digestate in larger-scale food waste co-digestion trials

	FW + cattle slurry	FW + card packaging	FW control
Nutrients			
TKN (g Kg ⁻¹ TS)	58.8	65.3	136
TP (g Kg ⁻¹ TS)	13	8.9	14
TK (g Kg ⁻¹ TS)	34	25	50
Potentially toxic elements			
Cd (mg Kg ⁻¹ TS)	<0.50	<0.50	<0.50
Cr (mg Kg ⁻¹ TS)	31	53	79
Cu (mg Kg ⁻¹ TS)	100	160	130
Pb (mg Kg ⁻¹ TS)	<10	52	18
Hg (mg Kg ⁻¹ TS)	<0.25	<0.25	<0.25
Ni (mg Kg ⁻¹ TS)	16	29	48
Zn (mg Kg ⁻¹ TS)	270	130	180
Essential elements			
Co (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0
Fe (mg Kg ⁻¹ TS)	<2000	3200	5700
Mo (mg Kg ⁻¹ TS)	3.9	5.4	5.2
Se (mg Kg ⁻¹ TS)	<0.30	<0.30	0.70
W (mg Kg ⁻¹ TS)	<1.0	<1.0	<1.0
Solids content			
TS (% WW)	5.84	6.76	6.04
VS (% WW)	4.38	4.45	4.19
VS (% TS)	75.1	65.8	69.4

10.4 Conclusions from the digestate characterisation studies.

It can be seen from Tables 10.1 to 10.9 that the level of plant nutrients and PTE in the digestate largely reflected those in the corresponding substrate or mix. The digestate from mechanically-recovered BMW co-digestion trials was rich in essential elements and high in PTE; whereas the digestate from food waste co-digestion trial contained more nutrients. Each digester sampled in the laboratory-scale studies showed the same concentration of elements as the other in the pair. For the larger-scale trials digesters were not run in duplicate and the values given are from a single sample but are comparable with those found in the laboratory-scale food waste co-digestion trials.

11 Determination of the biostability of digestates

11.1 Test set-up

The biostability of digestate was determined based on its residual biogas production. Two methods were selected to measure this: the BMP method described in Appendix 1; and the BM100 method as specified in the Environment Agency's Guidance on monitoring MBT and other pre-treatment processes for the Landfill Allowances Trading Scheme (England and Wales) 2005. Materials for testing using the BM100 method were sent to an accredited laboratory and the BMP method was carried out in-house.

BMP assays were conducted on the following materials:

- digestate fibre from the laboratory-scale digester fed on mechanically-recovered BMW
- whole digestate from the larger-scale digester fed on food waste
- digestate fibre from the larger-scale digester fed on food waste and cattle slurry
- digestate fibre from the larger-scale digester fed on food waste and card packaging.
- digestate liquor from the larger-scale digesters fed on food waste and cattle slurry
- digestate liquor from the larger-scale digesters fed on food waste and card packaging.
- mixed food waste and card packaging co-substrate

The aim of the BMP test on the food waste and card packaging mixture was to determine whether the difference between the biogas production in this test and in the test on the corresponding digestate was similar to the specific biogas production in the corresponding larger-scale digester.

The tests were carried out in 1.4-litre working capacity continuously stirred tank reactor (CSTR) digesters at a mesophilic temperature (36 ± 1 °C). For whole digestate and digestate fibre these were operated at an inoculum-to-substrate ratio ($r_{I/S}$) of 2 with an inoculum of sewage sludge digestate (strained through a 1 mm mesh) from Millbrook wastewater treatment plant, Southampton. For food waste and card packaging mixture an inoculum-to-substrate ratio of 4 was used with the same inoculum. Each material or mixture was run in triplicate and four replicates of the inoculum without substrate were used as a control.

In the case of the digestate liquors, no inoculum sludge was added and the residual biogas was generated as a result of the microbial consortium already present. The test was set up immediately after the digestate had been drained from the digester and the liquor separated from the fibre. The test materials were run in duplicate and the biogas produced was collected using gas impermeable sampling bags, with biogas volumes measured according to the weight gasometer method (Walker *et al.*, 2009). Biogas composition was analysed by gas chromatography. All BMP tests ran for 100 days.

BM100 tests were carried out on the digestate fibre from the larger-scale digester fed on food waste and card packaging as well as on the corresponding feedstock mixture. The same material was used as in the BMP test. For the BM100 test the materials had to be air-dried and ground, and this test therefore started 8 days after the BMP test. The BM100 tests ran for 87 days.

A cellulose standard was also included in both the BMP and BM100 tests.

11.2 BMP test results

The results from the digestate BMP tests are shown in Figures 11.1-11.6. The residual methane production of digestate from mechanically-recovered BMW (Figure 11.1) and from food waste and card packaging (Figure 11.5 and 11.6) was only around 0.100 STP $\text{m}^3 \text{kg}^{-1}$ VS during the 100-day tests. This was as expected because the digestates were taken from well-functioning digesters running at a moderate loading rate. Digestate fibre from the digester fed on food waste and cattle slurry had a relatively high residual methane potential of 0.196 STP $\text{m}^3 \text{kg}^{-1}$ VS (Figure 11.3); this was because cattle slurry contains a relatively high proportion of lignocellulosic materials which are only slowly degraded, and since the material also has a high water content it has a relatively short retention time in the digester. Digestate liquor from the digester fed on food waste and cattle slurry had a lower residual methane potential (0.093 STP $\text{m}^3 \text{kg}^{-1}$ VS, Figure 11.4) than the digestate fibre, perhaps due to the lignocellulosic materials in the fibre component. Food waste digestate had the highest residual methane potential of 0.203 STP $\text{m}^3 \text{kg}^{-1}$ VS (Figure 11.2); this was probably because the larger-scale food waste digester had VFA concentration of 2600 mg l^{-1} (Figure 9.12) when the digestate sample was taken on day 286. There is currently no approved stability standard against which these results can be compared to ascertain whether a satisfactory degree of stabilisation has been achieved.

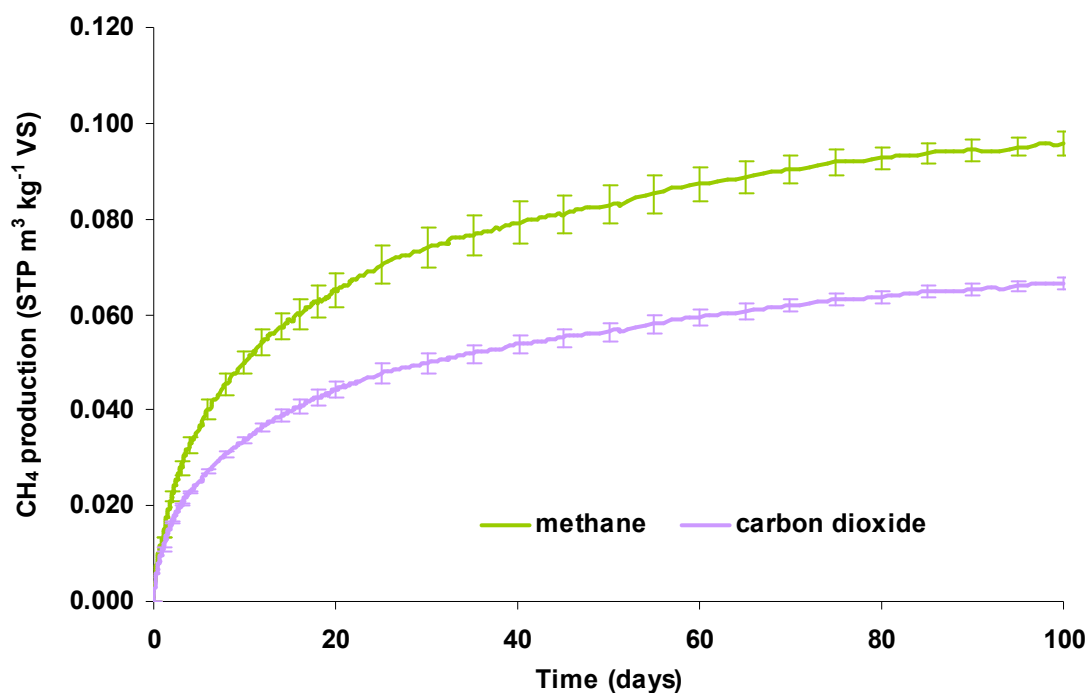


Figure 11.1 BMP test on the digestate fibre from the laboratory-scale digester fed with mechanically-recovered BMW only

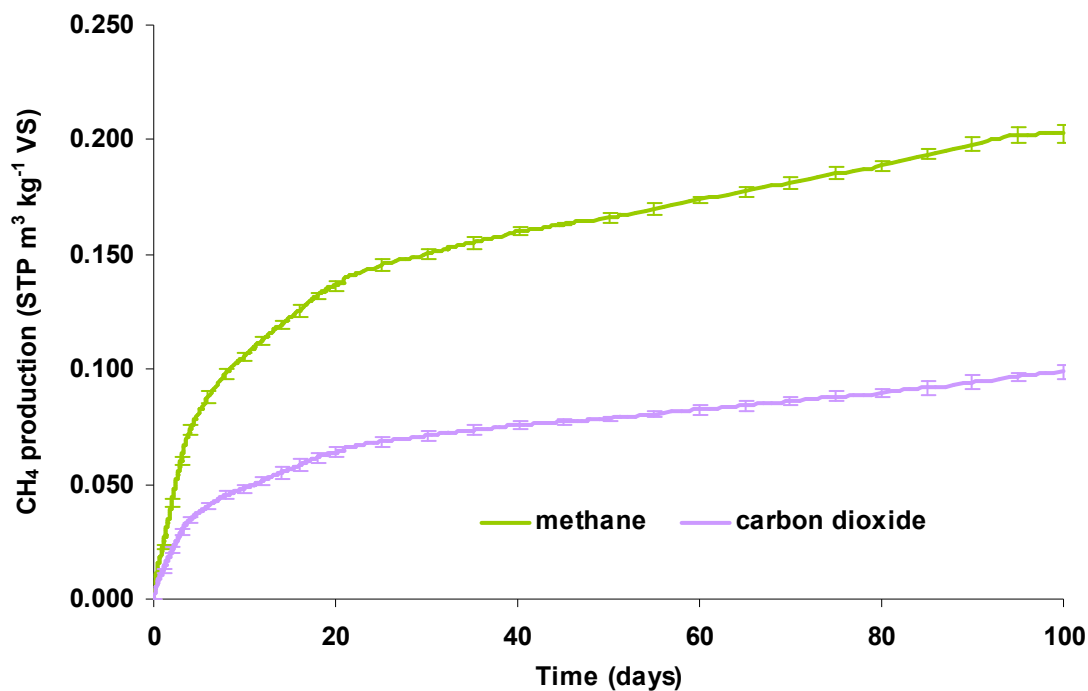


Figure 11.2 BMP test on the whole digestate from the larger-scale digester fed with food waste only

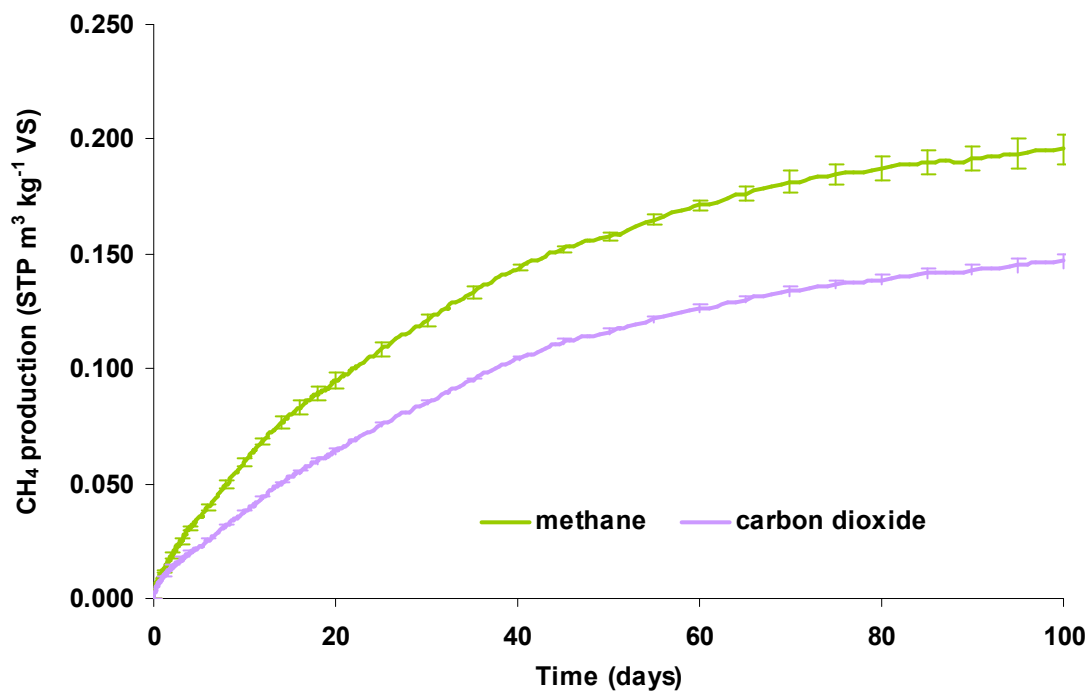


Figure 11.3 BMP test on the digestate fibre from the larger-scale digester fed with food waste and cattle slurry

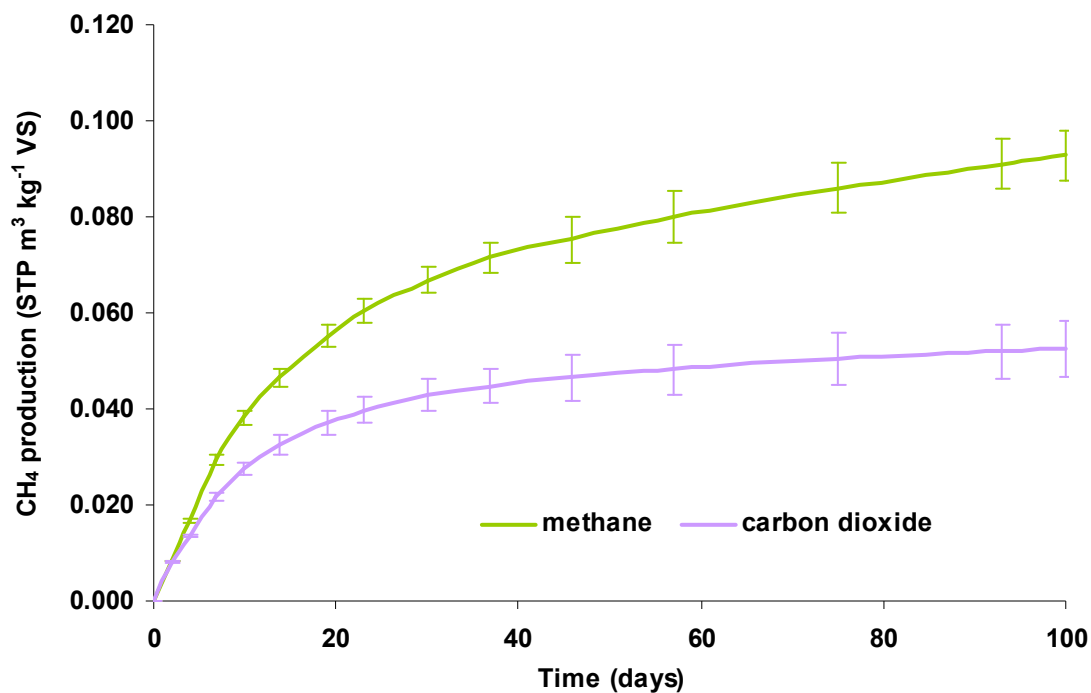


Figure 11.4 BMP test on the digestate liquor from the larger-scale digester fed with food waste and cattle slurry

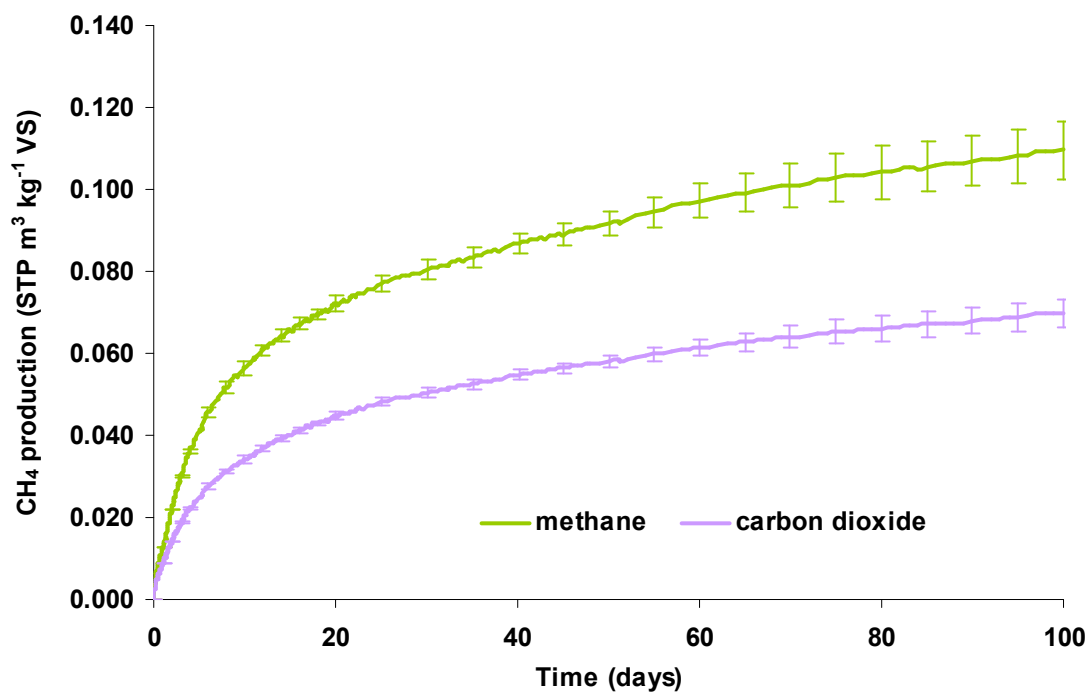


Figure 11.5 BMP test on the digestate fibre from the larger-scale digester fed with food waste and card packaging

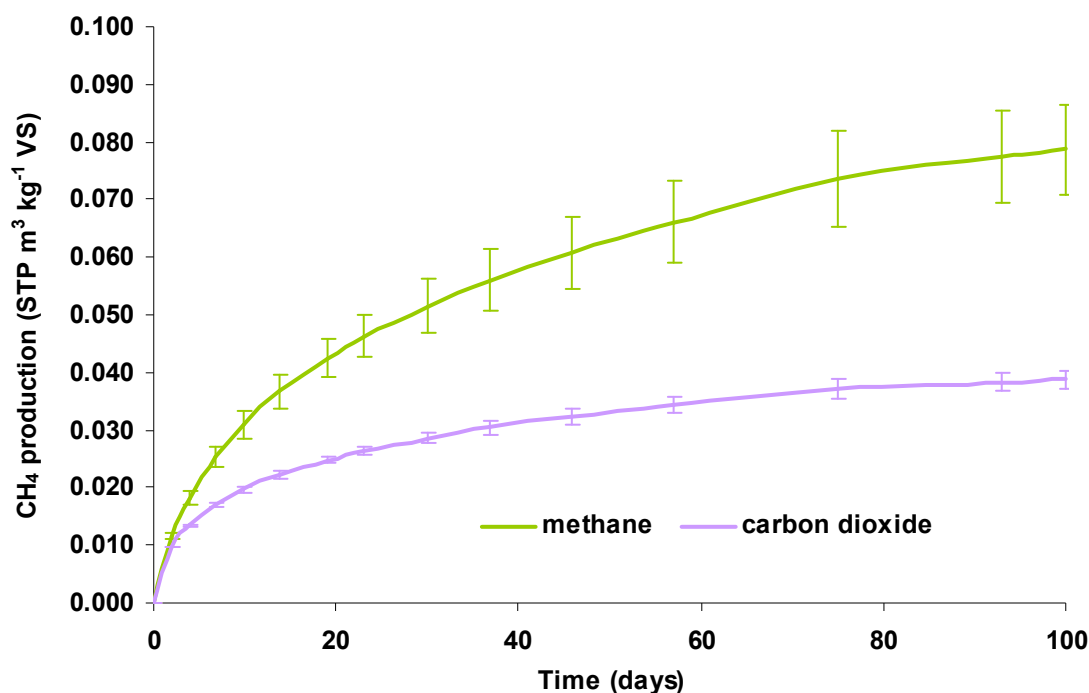


Figure 11.6 BMP test on the digestate liquor from the larger-scale digester fed with food waste and card packaging

11.3 Comparison of BM100 and BMP tests on digestate and feedstock

Figure 11.7 and Tables 11.1 and 11.2 show the results of the external testing for BM100. The report accompanying the test results is given in the textbox below. The BM100 test using the food waste and card packaging co-substrate mix and the cellulose standard showed a high variation between replicates. The percentage total of gases shown in Table 11.1 also indicates that volumes of gas were unaccounted for, perhaps because they were not identified in the gas analysis. It is possible that the missing volumes were comprised of hydrogen which is often produced as a result of shock loading followed by acidification, as seen in this test.

Table 11.1 Biogas composition in the first 14 days of the BM100 test (as supplied by test laboratory)

	Day 7				Day 14			
	N ₂ %	CH ₄ %	CO ₂ %	Volume ml STP	N ₂ %	CH ₄ %	CO ₂ %	Volume ml STP
input	34.4	0.0	27.0	559	39.3	8.4	47.8	510
input	34.4	0.0	23.4	854	48.8	0.0	45.6	603
input	33.1	0.0	27.4	804	74.2	0.5	28.2	294
output					43.4	50.0	9.6	565
output					46.9	45.4	10.8	521
output					43.1	47.7	12.7	561

Report on BMP100 test by accredited external laboratory

'The test was set up on Monday the 9th March 2009 which was ASAP after receipt of samples. 'Input' was the feedstock mixture, and 'output' the digestate fibre. 'Input' produced biogas over the first 5 days at a high rate which exceeded biogas outputs from many untreated waste types. Biogas production ceased during the first weekend due to acidification and pH of 'input' replicates were adjusted on Monday (day 7). It took about 3 days for biogas production to resume before acidification again limited biogas production during the second weekend. The pH of 'input' was adjusted again on Monday (day 14). Feedstock mixture ('input') is obviously highly putrescible and acidifies very easily during the initial stages of the test leading to cessation of biogas output. The BM100 test is typically carried out to completion (minimum 100 days for BMP mode) and initial problems with acidification for highly putrescible waste types are not considered to be significant in this context. pH was checked again on day 63.'

'The BM100 method followed the current standard BM100 protocol for waste samples. This method collects biogas produced using a barrier solution of acidified water. This gas collection method is likely to be updated to acidified water plus salt solution, and tests indicate that a higher proportion of biogas will be retained in the collection cylinder. Future biogas production and collection values may be increased as a result but results presented in this report are equivalent to other results obtained by using the current BM100 method.'

Methane measurements were taken as gas was emptied on each tube throughout the run. Mean seed result has been subtracted, seed values all low.

Results given combine:

- 8 measurements/replicate for 'input' sample
- 3 measurements/replicate for 'output' sample
- 2 measurements/replicate for standard cellulose

Table 11.2 BM100 results on feedstock ('input') and digestate fibre ('output') of the food waste and card packaging co-digestion (as supplied by test laboratory)

Sample	Biogas l kg ⁻¹ VS	Mean l kg ⁻¹ VS	c.v.	Methane l kg ⁻¹ VS	Mean l kg ⁻¹ VS	c.v.
cellulose	373.7	416.7	9.0	237.1	264.3	9.6
cellulose	434.7			287.4		
cellulose	441.7			268.3		
input sample	412.3	420.3	1.9	230.8	218.3	10.8
input sample	420.4			191.2		
input sample	428.2			232.9		
output sample	104.3	101.9	2.1	61.5	59.8	2.6
output sample	100.1			58.5		
output sample	101.4			59.5		

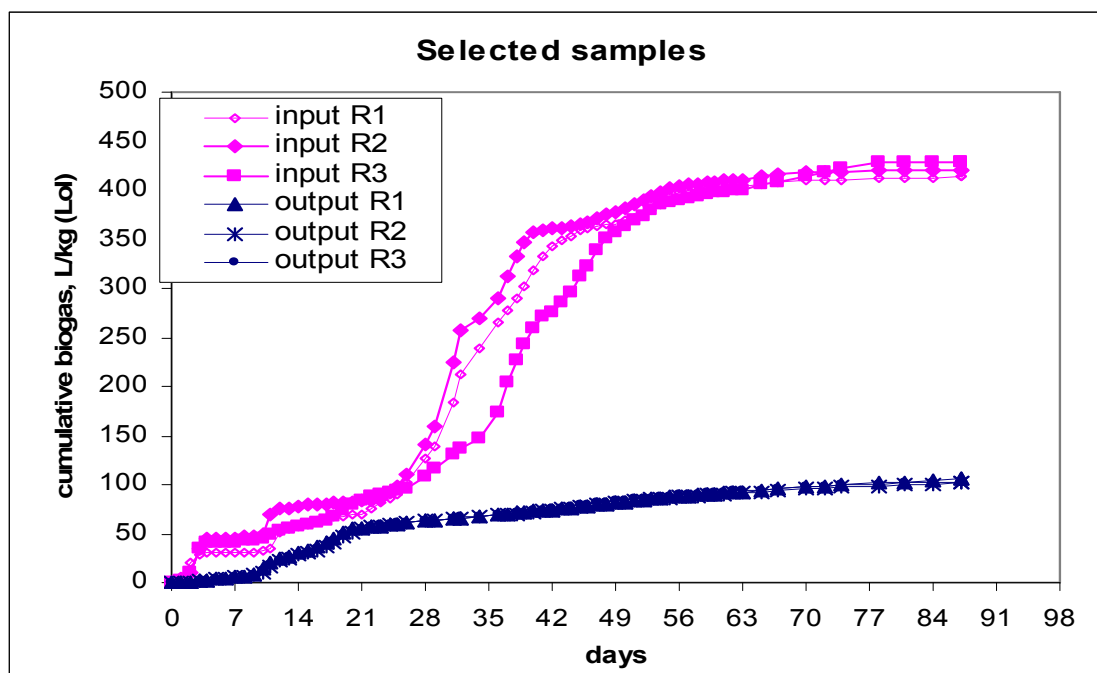


Figure 11.7 Cumulative biogas production in BM100 test (as supplied by test laboratory).

Input was the mixture of food waste and card packaging, and output was the digestate fibre from the larger-scale digester fed with the mixture of food waste and card packaging. Note: LOI = loss on ignition, equivalent to volatile solids.

Results from the BMP test using the same materials, but without drying, are shown in Figure 11.5 and 11.8. The 100-day biogas production values were 0.675 and 0.180 STP $\text{m}^3 \text{kg}^{-1}$ VS for feedstock and digestate fibre respectively, considerably higher than the values of 0.420 and 0.102 STP $\text{m}^3 \text{kg}^{-1}$ VS given by the BM100 test. The biogas production of cellulose in BM100 test was only 0.417 STP $\text{m}^3 \text{kg}^{-1}$ VS, which is half its theoretical value of 0.830 STP $\text{m}^3 \text{kg}^{-1}$ VS.

It is clear that the results for residual biogas or methane potential are very dependent upon the method used. The BMP procedure carried out at Southampton showed smooth biogas and methane production in the initial stage of the test, and consistency between replicates throughout the test; the biogas and methane potential of the reference material cellulose was 0.815 and 0.409 $\text{m}^3 \text{kg}^{-1}$ VS respectively, or 98% of the theoretical values.

As a further check on the BMP test a simple biogas mass balance was carried out. This was based on the volatile solids removal (75%, Figure 9.5) and the specific biogas production (0.591 STP $\text{m}^3 \text{kg}^{-1}$ VS, Figure 9.1) of the larger-scale digester fed on food waste and card packaging. The specific biogas production (SBP) should show good agreement with the difference between the input (food waste and card packaging mixture) and output (digestate fibre) values. The value of 0.622 STP $\text{m}^3 \text{kg}^{-1}$ VS obtained from the test results was only 5% different from the SBP, indicating that SBP could be used as a method of assessing stability in commercial digesters.

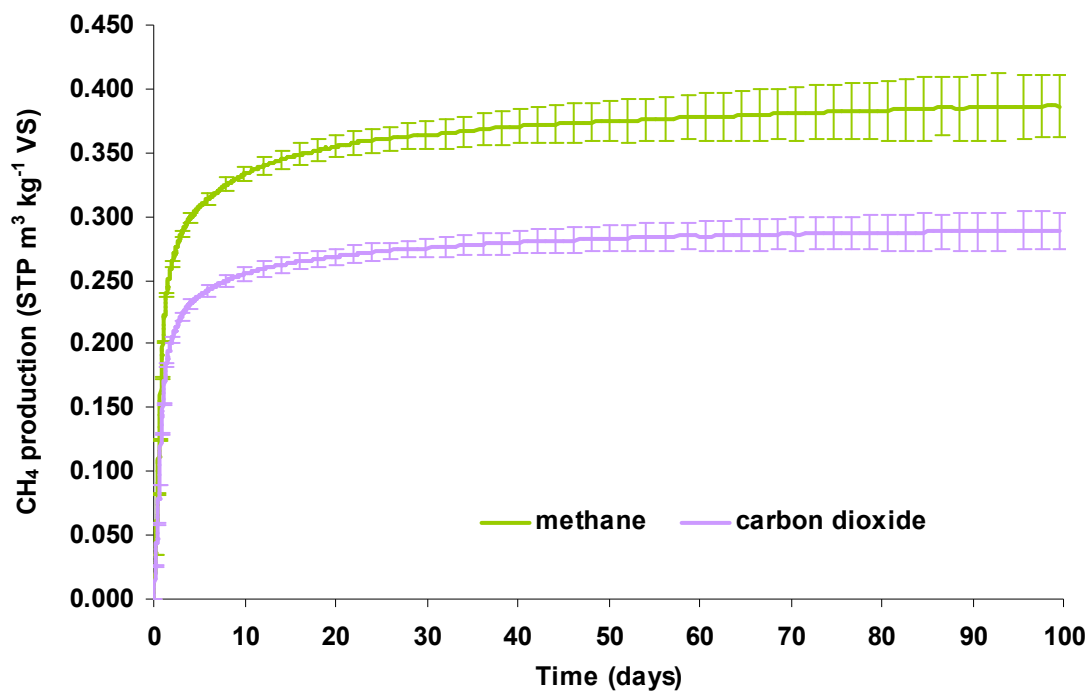


Figure 11.8 BMP test on the feedstock mixture of food waste and card packaging

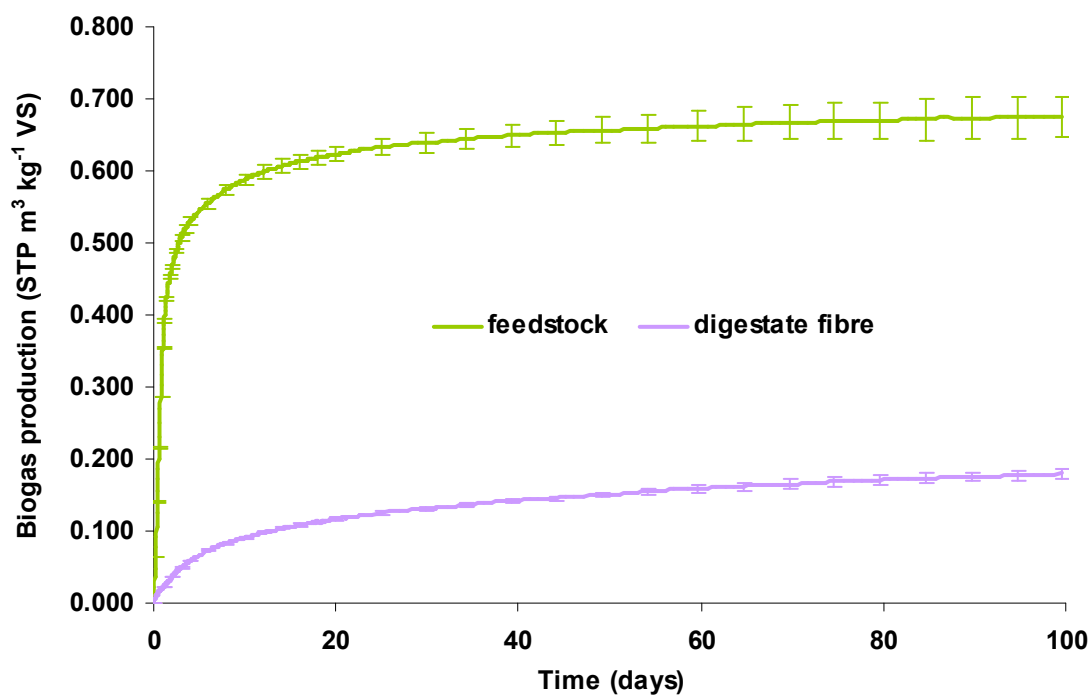


Figure 11.9 Biogas production in the BMP test on the feedstock mixture of food waste and card packaging and the residual biogas production from the corresponding digestate fibre from larger-scale co-digestion trial.

12 Summary results

Table 12.1 Comparison of the digester performance

	Process productivity			Process efficiency			Process stability					
	OLR	VBP	CH ₄ (%)	BMP ¹	SMP	Conversion rate (%)	pH	TAN	VFA	Dominate VFA species	Essential elements ²	Digester status
<i>Laboratory-scale baseline municipal waste digestion trial (section 3)</i>												
mechanically-recovered BMW	2	1.1	57	0.35	0.30	86	7.5	1.4	<0.1	HAc	-	Steady
FW	2			0.47							9.2	Failed
<i>Laboratory-scale mechanically-recovered BMW co-digestion trial (section 8.1)</i>												
mechanically-recovered BMW	4	2.2	58	0.35	0.32	91	7.4	1.6	<0.1	HAc	10.1,2,3	Steady
mechanically-recovered BMW + blood 1	3	1.2	59	0.36	0.24	67	8.0	7.5	13	HAc, HPr	10.1,2,3	Pseudo- steady
mechanically-recovered BMW + blood 2	3	0.5	43	0.36	0.07	19	7.2	8.5	46	HAc, HPr	10.1,2,3	Pseudo-steady
mechanically-recovered BMW + pig gut & fat 1&2	4	1.5	55	0.40	0.20	50	7.8	4.5	8.6	HPr	10.1,2,3	Sign of stressed
<i>Laboratory-scale food waste co-digestion trial (section 8.2)</i>												
FW	2			0.47						HAc, HPr	10.4,5,6	Failed
FW + cattle slurry 1&2	4	2.1	60	0.39	0.31	79	7.5	1.9	<0.1	HAc	10.4,5,6	Steady
FW + card packaging 1&2	4	2.2	56	0.38	0.30	79	7.2	0.9	<0.1	HAc	10.4,5,6	Steady
FW + potato waste 1 & 2	2			0.45						HAc, HPr	10.4,5,6	Failed
<i>Larger-scale food waste co-digestion trial (section 9)</i>												
FW	2	1.4	61	0.47	0.42	89	8.1	4.9	3.5	HAc, HPr	10.7,8,9	Pseudo-steady
FW + cattle slurry	2	0.70	61	0.31	0.21	68	7.5	1.5	<0.1	HAc	10.7,8,9	Steady
FW + card packaging	2	1.2	56	0.38	0.32	84	7.6	2.3	<0.1	HAc	10.7,8,9	Steady

Abbreviations and units:

OLR-organic loading rate ($\text{kg VS m}^{-3} \text{ d}^{-1}$); VBP-volumetric biogas production ($\text{STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$); BMP-biochemical methane potential ($\text{STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$); SMP-specific methane production ($\text{STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$); TAN-total ammonia nitrogen (g-N l^{-1}); VFA-volatile fatty acid (g l^{-1}).

1. BMP values listed can only be regarded as guidance because different batches of wastes were used as the study progress.

2. The numbers in the column shown which Table the concentrations of essential elements in each type of digestate can be found.

13 Discussion

13.1 Mechanically-recovered BMW

Mechanically-recovered BMW was a stable digestion substrate with a good biogas yield under the operational regimes utilised in this study. The BMP test on mechanically-recovered BMW ran for 82 days. The percentage of methane at the most favourable inoculum-to-substrate ratio of 4 was 59% and the final BMP value was 0.364 STP m³ CH₄ kg⁻¹ VS_{added}; this value was confirmed by a carbon mass balance which accounted for 96% of the carbon in the biogas and remaining in the digestate. The measured BMP was 91% and 65% of the theoretical BMP values calculated based on the biochemical composition and Buswell equations respectively, indicating that a proportion of the substrate is recalcitrant to anaerobic biodegradation in the timeframe of the test. A second BMP carried out on both pasteurised and un-pasteurised material gave BMP values of 0.330 and 0.349 STP m³ CH₄ kg⁻¹ VS_{added} respectively, confirming the earlier result for the un-pasteurised material.

In semi-continuous digestion trials at a 35-litre scale (see section 5) the mechanically-recovered BMW was shown to be a suitable substrate for digestion in terms of its stability. Both digesters in this part of the study were able to acclimate to the substrate while running at a loading of 2 kg VS m⁻³ d⁻¹ over 4 retention times before digestate was removed as an inoculum for other experiments. After this removal and once the digesters had returned to their original volume, biogas production and specific biogas and methane yields recovered their former values, with a slight increase in the biogas methane content. During this period the operational performance indicators were: specific methane production (SMP) 0.304 STP m³ CH₄ kg⁻¹ VS_{added}; specific biogas production (SBP) 0.529 STP m³ kg⁻¹ VS_{added}; volumetric biogas production (VBP) 1.05 STP m³ m⁻³ d⁻¹; methane percentage 57.5%; volatile fatty acids (VFA) concentration less than 100 mg l⁻¹; ammonia 1400 mg l⁻¹ and a pH 7.5. The SMP accounted for 86% of the BMP value. The data indicated that most of the biodegradable part of mechanically-recovered BMW had been converted to biogas.

As part of the 4-litre laboratory-scale co-digestion trials (see section 8) one digester was run on mechanically-recovered BMW for a period of 425 days during which the OLR was increased from 2 kg VS m⁻³ d⁻¹ (days 0-190) to 3 kg VS m⁻³ d⁻¹ (days 191-350) and then finally to 4 kg VS m⁻³ d⁻¹ (days 351-425). Throughout this period a solids retention time of 30 days was maintained by liquor recycling and solids wasting. At the two lower OLR of 2 and 3 kg VS m⁻³ d⁻¹ the SBP of ~0.52 STP m³ kg⁻¹ VS_{added} was similar to that observed in the 35-litre trial. The VBP of ~1.0 STP m³ m⁻³ d⁻¹ increased by 50% when the loading was increased to 3 kg VS m⁻³ d⁻¹. On increasing the loading to 4 kg VS m⁻³ d⁻¹ there was an initial drop in SBP, a rapid VFA accumulation, a fall in pH and a lower methane concentration in the biogas. During the second retention time at this loading the specific biogas production recovered to its former level and the VBP increased to 2.1 STP m³ m⁻³ d⁻¹, twice that achieved at OLR of 2 kg VS m⁻³ d⁻¹. At this loading the VFA concentration stabilised at less than 150 mg l⁻¹, total ammonia nitrogen (TAN) at 1600 mg l⁻¹ and pH at 7.4.

It is possible that higher loadings could be applied but this was not attempted. It should be noted, however, as the loading is increased the rheological properties of the digestate change as a result of the higher total solids (TS) in the digester liquor. The digester TS at the highest loading was 170 kg m^{-3} which was close to the limit of the mechanical mixing system of the small-scale digesters used in this study.

13.2 Food waste

Two sets of BMP tests were carried out on source-segregated food waste, the first of which ran for 80 days. The percentage of methane in the biogas at the most favourable inoculum-to-substrate ratio of 4 was 61% and the final BMP value was $0.456 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$. This value was confirmed by a carbon mass balance which accounted for 98% of the carbon in the biogas and that remaining in the digestate. The measured BMP was 92% and 83% of the theoretical BMP values calculated from the biochemical composition and Buswell equations respectively, indicating that only a small proportion of the substrate is recalcitrant to anaerobic biodegradation in the timeframe of the test. A second BMP carried out on both pasteurised and un-pasteurised material gave BMP values of 0.473 and $0.475 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ respectively, confirming the earlier result for the un-pasteurised material.

In the semi-continuous digestion trials at a 35-litre scale (see section 5) one of the food waste digesters ran continuously with daily feeding for 9.5 retention times (284 days). The digester showed some initial disturbance when food waste was first added but then stabilised allowing performance indicator parameters to be determined for pseudo-steady state conditions during days 150-180. The SBP was $0.695 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$, VBP $1.39 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$; VFA concentration between 9000-10000 mg l^{-1} ; ammonia 3800 mg l^{-1} , pH 7.7, and VS destruction 83.9%. The data indicated that most of the biodegradable part of the food waste had been converted to biogas, but with a large VFA pool remaining. From day 180 onwards, rises in propionic acid concentration, a reduction in SMP, and the development of severe foaming problems indicated the digester was failing. A second 35-litre digester showed a similar trend but with a delay in the onset of propionic acid accumulation (see section 5). The use of the digestate from the 35-litre trial as an inoculum for co-digestion work showed that recovery could be achieved but continued feeding with food waste alone (see section 8.2) resulted in the further accumulation of acetic, propionic and, to a lesser extent, n-butyric acids.

A 75-litre digester was also run on food waste for 308 days as part of the larger digester trial at an OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and a solids retention time of 30 days, starting from an inoculum of municipal sewage sludge digestate. As with the 35-litre digester there were initial disturbances during the first 60 days of operation as a result of the change of substrate from sewage sludge to food waste. After a further 4 retention times (120 days) of steady state operation, VFAs started to accumulate from the previously stable concentration of around 200 mg l^{-1} and had reached 4000 mg l^{-1} by day 270. Initially the VFA was predominantly in the form of HAc but later all the VFA species were present with an initial rise in HPr concentration. During periods of pseudo-steady state operation the performance parameters were: SBP $0.705 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$, SMP $0.425 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$, VBP $1.42 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, VMP $0.855 \text{ STP m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ d}^{-1}$, 86.5% VS

converted to biogas, 60.6% methane percentage in biogas, pH rising to 8.1, TAN rising to 4900 mg l⁻¹, free ammonia rising to 600 mg l⁻¹.

13.2.1 Operational characteristics of food waste digesters and possible reasons for long-term VFA accumulation

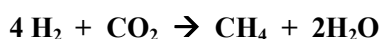
Food wastes have a very high recoverable energy (0.425 STP m³ kg⁻¹ VS_{added}), equivalent to 93 m³ of methane for each tonne of wet weight added to the digester, and can produce this consistently over extended periods of time. The experimental work revealed, however, that the digester is likely to accumulate high concentrations of volatile fatty acids, and in particular of propionic acid which appears after extended run times. There is also a build-up of ammonia in the digester as a result of the breakdown of organic nitrogenous materials. As food wastes are rich in proteins the ammonia concentrations found in digestate are higher than with many other waste types and typically can exceed 5000 mg l⁻¹. Similar conditions to those observed in this research have previously been reported in food waste digestion trials at both mesophilic and thermophilic temperatures (Banks *et al.*, 2008). Although digesters can operate with high levels of VFA and ammonia over extended periods without a great loss in biogas production, there is a risk that these conditions could result in sudden failure. This is most likely to happen when the concentration of propionic acid exerts sufficient acidity to overcome the pH buffering provided by the ammonia. If this happens the pH in the digester can fall to a critical point where the methanogenic population fails and the digester becomes 'sour' and biogas production ceases. To understand why these unusual conditions exist, it is necessary first to understand the biochemical routes by which methane is formed in the process and the conditions needed by the different groups of microorganisms that carry out each stage in the conversion. The AD process itself is based on a close microbial association between acid-producing bacteria, acid-degrading bacteria, and methanogens. These groups have to work together, as the energy yields from the biochemical reactions are very low and would not proceed without syntrophy between the methanogens and the acid-degrading bacteria: this second group is therefore sometimes referred to as syntrophic acid oxidisers (also known as acetoclastic bacteria). These bacteria are able to oxidise the longer chain volatile fatty acids to acetic and formic acid, and in the process release hydrogen and carbon dioxide into solution. The methanogens convert these products into biogas, and in doing so ensure that the syntrophic acid-oxidising bacteria are able to function. If either formic acid or hydrogen, accumulates in the system, the syntrophic acid oxidisers are unable to gain energy from their biochemical reactions, and therefore stop working. The removal of these intermediate products and their conversion into methane is therefore essential to the proper functioning of the system, and forms the contribution of the methanogens to the syntrophy. By these reactions carbon in the waste is transformed into a mixture of carbon dioxide and methane. This escapes from the liquid phase as a gaseous product, in which the methane still retains oxidisable carbon that can be reclaimed as energy by combustion. The syntrophic relationship has one further important aspect in that the biochemical energy released is very small. This limits the growth yield of the microbial population, and most of the original potential energy in the food waste ends up in the biogas, with very little excess microbial sludge yield.

As already mentioned, the methanogens convert acetic acid, formic acid, hydrogen and carbon dioxide into methane. One group of methanogens (the acetoclastic methanogens) exclusively uses acetic acid to form biogas effectively by cleaving the molecule into two gaseous elements.

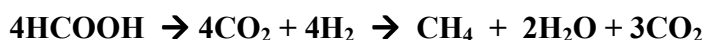


This is the group of methanogens that is most commonly encountered in anaerobic digesters and provides the principal route through which methane is formed (in sewage sludge digestion, 70% of methane is typically formed via this group).

The second group of methanogens (autotrophic methanogens) forms methane by combining hydrogen and carbon dioxide, and this group is therefore most important for ensuring the syntrophy with the acid degraders



There is one other important product of the degradation of the longer chain volatile fatty acids: this is formic acid, which can result from the degradation of VFA with an odd number of carbon atoms e.g propionic acid ($\text{C}_2\text{H}_5\text{COOH}$). Formic acid itself can also be converted to methane by the hydrogen and carbon dioxide route through a formate dehydrogenase enzyme system.



The accumulation of formic acid will result in propionic acid not being degraded in the system, in the same way as hydrogen accumulation will stop all longer chain VFA being degraded.

There are other minor routes for methane formation e.g from methanol, but they are not important in understanding the peculiarities of food waste digestion.

An explanation as to why propionic acid accumulates in food waste digesters is proposed, based on understanding these syntrophic reactions and on the experimental results from the research; examples are also given of where acetic acid is found at elevated levels in otherwise apparently well functioning digesters.

It is now well known that acetoclastic methanogens are inhibited at high ammonia concentrations (Karakashev *et al.*, 2006), and although much of the research in this area has been carried out on thermophilic digesters this phenomenon has also been shown and reported in mesophilic systems (Schnurer and Nordberg 2007). At the high ammonia concentrations reached in food waste digestion the acetoclastic methanogenic population is therefore likely to be lost or severely inhibited, and the direct formation of methane and carbon dioxide by cleaving of acetic acid is no longer possible. The autotrophic methanogens are not as sensitive to ammonia toxicity and can continue to operate at the concentrations of ammonia found in food waste digesters. For them to consume the acetic

acid, however, this has first to be converted to hydrogen and carbon dioxide. This conversion is carried out by bacteria which are syntrophic acetate oxidisers; the same organism can, however, also convert carbon dioxide and hydrogen to acetic acid.

Thus under high ammonia concentrations when the acetoclastic methanogens fail, the process of methane production does not stop, but the route through which methane forms changes. A stable system can exist based on this route (Banks, 1994; Karakashev *et al.*, 2006). The digester, however, is much more likely to run at an elevated level of acetic acids in the system (Wang and Banks, 2003; Angelidaki *et al.* 2005; Schnurer and Nordberg, 2007; Banks *et al.*, 2008)). The reason for this are uncertain, and currently under further investigation.

The major problem encountered in the current work which led to falling gas production and digester failure was the longer-term accumulation of propionic acid in the digesters. As already mentioned propionic acid is an uneven chain length VFA and the syntrophic propionate-oxidising bacteria that break this down produce a mixed product of acetic acid, H₂, CO₂ and formic acid.



Some of the autotrophic methanogens have been shown to convert formic acid to methane. If this population fails formic acid will accumulate, and if this happens then the syntrophic propionate-oxidising bacteria will be inhibited (product-induced feedback inhibition). Formic acid conversion to H₂ and CO₂ and subsequently to methane is thought to depend on a specific enzyme (formate dehydrogenase) and it is not uncommon for this to contain a selenium-containing amino acid which forms the complex molecule seleno-cysteine (Wood *et al.*, 2003). There may be other surrogate metals (e.g tungsten and molybdenum) that can form this metal enzyme complex, but even less is known about these. The hypothesis therefore is that as time progresses a digester running on food waste runs out of selenium (or other surrogate metals) as it dilutes out the original inoculum (usually sewage sludge) and as the quantity of selenium in the incoming food waste is negligible. If this hypothesis is correct then the enzyme system fails and formic acid cannot be converted and propionic acid accumulates as observed in our digesters.

The quantity of these trace metals required to maintain these enzyme systems fully functional is very small and wash-out of the original supply can take a long time. In the laboratory-scale systems operated at mesophilic temperatures for this study it was over a year before propionic acid started to accumulate. To gather some evidence to support this hypothesis the concentrations of essential elements in both the laboratory-scale and larger-scale co-digestion trials were analysed and it was found that cobalt, selenium and

tungsten had all dropped to below 1 mg kg^{-1} TS at times when VFA build-up was observed.

13.3 Co-digestion with mechanically-recovered BMW

A set of BMP tests was carried out on the mechanically-recovered BMW mixed with each of its four co-substrates (biodiesel by-product, sheep blood, pig gut and flotation fat, and poultry litter), with the co-substrates representing 20% of the total VS in the mix. The BMP values obtained were 0.334, 0.357, 0.358 and 0.329 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}$ respectively, compared to the previously determined BMP value of 0.344 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$ for mechanically-recovered BMW alone. Using these results a BMP value for the co-substrate could be estimated and compared to the theoretical value and to the result from an actual BMP test on the co-substrate with a sewage sludge inoculum. Blood had the highest estimated BMP of 0.450 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$, corresponding to 93.5% of the theoretical value, indicating its ready biodegradability. When the BMP for blood was determined as a single substrate with a sewage sludge inoculum, however, a value of 0.418 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$ was obtained, which is lower than the estimated value. Pig gut and flotation fat also had a high estimated BMP of 0.474 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$, but this corresponded to only 71% of its potential. When determined as a single substrate the BMP value for pig gut and flotation fat was 0.595 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$, which was considerably higher than the estimate made from the mixed substrate test. The values obtained from the single substrate tests are direct measurements and are therefore more reliable in terms of describing the properties of the material, but the mixed substrate test shows how the material may react in co-digestion. The above results show the difficulties in making predictions of gas production based on batch tests, and confirm the need to carry out continuous or semi-continuous fed simulation tests to obtain accurate gas production data and establish stability criteria.

When BMW was digested with blood as a 20% VS component in the mix in semi-continuous trials at an OLR of $2 \text{ kg VS m}^{-3} \text{d}^{-1}$ the ammonia rose to 6000 mg l^{-1} , but at this loading the VFA concentration stabilised and the SMP was 0.288 compared to 0.357 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$ in the BMP test, i.e. 81% of the measured potential. Increasing the loading rate to $3 \text{ kg VS m}^{-3} \text{d}^{-1}$ led to a rise in ammonia concentration to 8000 mg l^{-1} and VFA to over 15000 mg l^{-1} with an increasing proportion of propionic acid. VFA concentrations in one of the pair of digesters then rose higher still with a substantial fall in methane production. From the perspective of improving the biogas yield there was no advantage in adding blood as a co-substrate in the proportion used. It may however have some value in improving the nitrogen content of the final digestate product, as blood has an extremely high TKN level of $147 \text{ g kg}^{-1} \text{TS}$; the recommendation would be to add blood as a proportion of the mix so as to ensure that the total ammonia concentration in the digester does not exceed $3\text{-}4000 \text{ mg l}^{-1}$.

When BMW was digested with pig gut and flotation fat as a 20% VS component in the mix in semi-continuous trials at an OLR of $2 \text{ kg VS m}^{-3} \text{d}^{-1}$ the SMP was 0.319 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$ compared to the 0.358 STP $\text{m}^3 \text{CH}_4 \text{ kg}^{-1} \text{VS}_{\text{added}}$ expected from the BMP result, i.e. 89% of the BMP. The SMP of the mix was about 10% greater than for the mechanically-recovered BMW alone. The co-substrate added to the digester TAN and

there was some early evidence of VFA accumulation but this later stabilised. An increase in OLR to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ showed no reduction in SMP and at this load the VMP reached $\sim 1.0 \text{ STP m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ d}^{-1}$ with the TAN stabilising at $\sim 4500 \text{ mg l}^{-1}$ and VFA concentrations of $1\text{--}2000 \text{ mg l}^{-1}$. At the final loading of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ the TAN concentration was around 5000 mg l^{-1} and there was a fall in pH, increasing VFA, and a severe drop in SMP. The digesters had not recovered when the trial finished, but the reactor conditions indicated that this was not a safe loading for this mix.

Even at lower loadings there may be longer term implications for running on this type of co-substrate because of its high lipid content of $125 \text{ g kg}^{-1} \text{ VS}$. The lipids found in food waste consist mainly of triacylglycerides (Fernandez *et al.*, 2005). These consist of a glycerol backbone with three fatty acid chains attached, which is broken down by extracellular lipases excreted by acidogenic bacteria (Timberlake, 2003; Fernandez *et al.*, 2005; Cirne *et al.*, 2007). The glycerol fraction is then fermented to propionate, while the LCFA are sequentially oxidised to acetic acid, formic acid, hydrogen and carbon dioxide. Lipids may, however, potentially interfere with both of the two main rate-limiting steps in the AD process: hydrolysis and methanogenesis (Neves *et al.*, 2006). Firstly, the non-polar lipids and LCFA may adsorb to particulate substrate, making it 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.*, 2006). For a substrate consisting not solely of lipids, but also containing high quantities of readily degradable polysaccharides, the 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 of the LCFA requires a low partial pressure of hydrogen, this therefore creates an environment unfavourable for lipid degradation near the inlet. Fox and Pohland (1994) also looked 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). Although this study did not investigate LCFA inhibition, this is recognised as a long-term phenomenon and impact of lipid-rich substrates may not be realised for a substantial period of time.

13.4 Co-digestion with food waste

In the BMP test using office paper and flourmill waste these co-substrates represented 20% of the total VS, whereas in the mix with whey this was 17% and with cattle slurry 80%. The BMP values obtained were: 0.372, 0.398, 0.403 and $0.264 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ respectively compared to the previously determined BMP of the food waste of $0.455 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$. All of these co-substrates had relatively low BMP values when estimated from the combined BMP test. The estimated values were: 0.137 (office paper), 0.264 (flour mill waste), 0.267 (whey) and 0.222 (cattle slurry) $\text{STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ and the proportion of the theoretical BMP converted was 39, 68, 58 and 59% respectively. It is possible that conversion in this BMP test was hampered by high concentrations of

VFA and ammonia present in the food waste digestate used as inoculum, and these values should therefore be treated with caution. The BMP test carried out with pasteurised material used a sewage sludge inoculum, and the BMP for cattle slurry was $0.267 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ which is higher than the estimated value when the material was tested in a mixture with food waste using inoculum from the food waste digester. The BMP values for potato waste ($0.353 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$) and card packaging ($0.266 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$) were not determined as part of a mix with food waste, and the results therefore could not be compared. It was clear from the results for pasteurised and unpasteurised material, however, that pasteurisation had no effect on the BMP value for the food waste co-substrates.

In semi-continuous trials at a 4-litre scale, potato waste was used as a co-substrate for food waste on a 20% VS basis, card packaging at 47% VS and cattle slurry at between 40-80% VS. In all cases the 4-litre trial started with an inoculum taken from the 35-litre food waste digester which was already operating with elevated concentrations of VFA and ammonia. The trial with food waste and potato waste did not succeed in stabilising the digester and ultimately failed, as indicated by a loss of biogas production. Although the co-digestion delayed the onset of complete failure it is likely that the proportion of co-substrate in the mix was insufficient to lower the TAN to a non-inhibitory concentration, and the co-substrate itself may not have contributed missing essential elements. The high proportion of readily biodegradable starch may also have led to a further imbalance between VFA production and consumption.

In the trial where cattle slurry was 80% of the mix at a OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ the SMP was $0.22 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$, or 82% of the BMP value. On decreasing the proportion of cattle slurry to 60% the SMP increased by about 15-20% to $\sim 0.26 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ but on further increasing the load to $3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ the SMP for this mix was reduced to $\sim 0.23 \text{ STP m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{added}}$, reflecting the shorter hydraulic retention time in the digester. The VBP under these conditions was $1.1 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, which was considered acceptable for a commercial AD plant. It would not be possible to increase the loading above this at the mix ratio used without a further reduction in SMP. To increase the SMP without increasing the loading or changing the hydraulic retention time, the proportion of cattle slurry was decreased still further until it represented only 40% VS of the mix. This increased the VBP to $\sim 1.5 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$. The OLR with this mix was then increased to $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and stabilised at a SBP of $0.52 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ and a VBP of $2.1 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, out-performing that of a single substrate food waste digester. At all the loadings and with increasing proportions of food waste added to the cattle slurry the digester stability parameters of VFA, TAN, pH and alkalinity remained within very safe limits.

A larger-scale 75-litre digestion trial was undertaken with 20:80% VS mix of food waste and cattle slurry at an OLR of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ and showed a similar SMP of $0.21 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$. The VBP was between $0.65 - 0.75 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, with around 50% of the VS converted to biogas. Ammonia stabilised at around 1500 mg l^{-1} and VFA concentrations were less than 100 mg l^{-1} . Although the digester was very stable there were some fluctuations in performance due to the use of different batches of cattle slurry:

the properties of this material are variable depending on time of year and the diet of the cattle.

The 4-litre trial with food waste and card packaging on a 53:47% VS basis was able to improve the performance of the inoculum taken from the 35-litre food waste digester, taking about 1-2 retention times to reduce the VFA concentration to around 1500 mg l⁻¹ and TAN to around 1800 mg l⁻¹. During this period the methane content of the biogas was about 55%, the SBP fluctuated around 0.54 STP m³ kg⁻¹ VS_{added}, the SMP was 0.31 STP m³ kg⁻¹ VS_{added}, the VBP 1.1 STP m³ m⁻³ d⁻¹, and around 70% of VS was converted into biogas. Initially it proved difficult to increase the digester loading without inducing over-production of VFA that could not be buffered by the falling concentration of TAN. On a second attempt, the loading was successfully increased to 3 and then 4 kg VS m⁻³ d⁻¹ whilst maintaining a SBP of 0.55 STP m³ kg⁻¹ VS_{added}, and an SMP 0.32 STP m³ kg⁻¹ VS which is equivalent to 84% of the additive BMP of the two components of 0.38 STP m³ kg⁻¹ VS. The VBP at this point was 2.2 STP m³ m⁻³ d⁻¹ giving a 55% improvement in performance over that achieved in the digestion of food waste alone.

A larger-scale 75-litre digestion trial undertaken with food waste and card packaging on a 53:47% VS basis at an OLR of 2 kg VS m⁻³ d⁻¹ showed a SMP of 0.32 STP m³ kg⁻¹ VS_{added} similar to that in the 4-litre trial. The VBP was 1.2 STP m³ m⁻³ d⁻¹ with around 75% of the VS converted to biogas. Ammonia stabilised at around 2400 mg l⁻¹ and VFA concentrations were less than 100 mg l⁻¹.

A summary of the key factors in the waste substrates that are likely to influence the performance and stability of the digesters in light of the above discussion is given in Table 13.1

Table 13.1 Concentrations of total Kjeldahl nitrogen (TKN), lipids and essential elements in waste streams used in semi-continuous co-digestion trials.

Waste	Collection time	TKN ¹	lipids	Essential elements for microbial metabolism							
				Co	Cu	Mn	Mo	Ni	Se	W	Zn
BMW	Mar 2007	13.9	68.6	5.5	110	550	2.7	97	-	5.5	260
Blood	Oct 2007	147	<10	-	6.7	-	-	<5	-	-	16
Gut&fat	Nov 2007	79.5	349	-	40	-	-	6.9	-	-	250
FW	Mar 2007	34.2	151	<0.25	7.2	91	0.46	5.1	-	<0.25	49
	Dec 2007	26.0	129	<0.25	7.9	94	0.42	4.8	-	-	25
	Aug 2008	-	-	<0.25	7.0	69	0.51	7.2	-	<0.25	28
	Feb 2009	33.3	-	<1.0	7.6	-	<1.0	11	<0.30	<1.0	30
Slurry	Aug 2008	35.3	-	3.1	59	-	4.5	61	<0.30	<1.0	200
	Dec 2008	43.3	-	1.4	37	-	2.6	24	<0.30	<1.0	190
Card	May 2008	0.96	<10	0.59	32	-	1.5	5.9	<0.30	<1.0	26
Potato	Nov 2007	15.3	<10	-	9.8	-	-	<5	-	-	20
Sewage sludge		78.3	-	4.3	450	-	5.3	32	3.9	<2.0	720

Unit: TKN: g Kg⁻¹ TS; lipids: g Kg⁻¹ VS; essential elements: mg Kg⁻¹ TS

¹TKN is the nitrogen content of substance in organic and ammonia form.

The concentrations in sewage sludge are also given as this provided a source of nutrients and essential elements via the digester inoculum

14 Overall conclusions and suggestions

The research was not designed to consider the economics or engineering design features of anaerobic digestion plant. In making suggestions, however, some factors that might impinge on these are considered. For example the volumetric biogas potential (VBP) of a plant is of major economic importance as it determines the ratio of production of saleable energy to the capital investment made in the volumetric capacity of the plant. The situation is now more complicated due to energy subsidies and gate fees, but historically it was accepted that a VBP of at least $1 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ should be achieved and a value of $2 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ would be a very attractive investment. The methane production per tonne of wet waste is also important as this will have some influence on the distance over which it can be transported. A value of $100 \text{ m}^3 \text{ CH}_4 \text{ tonne}^{-1}$ is potentially a very good substrate, whereas one at $20 \text{ m}^3 \text{ tonne}^{-1}$ should only be used where transport requirements are minimal. The specific methane productivity (SMP) is related to the feedstock and is the proportion of the biochemical methane potential (BMP) that is achievable in continuously operating plant. The SMP should ideally be no less than 80% of the BMP. There is no ideal plant nutrient balance in a digestate as fertiliser requirements depend on soil type and crop being grown. A typical fertiliser for use on productive grass land would have an N:P:K ratio of 100:20:20, for spring cereals of 100:40:50, and for potatoes 100:100:100.

Mechanically-recovered BMW

- Mechanically-recovered BMW is a good substrate for anaerobic digestion and it should be possible to achieve a volumetric biogas productivity (VBP) of $2.2 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ at a loading rate of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$. This loading should be achievable in a 'wet' completely mixed single stage digester that will operate at a TS content in the digestate of around 17%. The methane yield of material on a wet tonne basis is $110 \text{ m}^3 \text{ tonne}^{-1}$.
- Mechanically-recovered BMW is probably also suited for digestion in a 'dry' type digester at a higher loading and volumetric gas productivity, but this was not investigated as part of the current research. Further work is needed to establish if 'dry' digestion systems have benefits compared to 'wet' systems when applied to waste streams of this type.
- The NPK ratio of the mechanically-recovered BMW digestate was 100:12:32 in digestate liquor, 100:26:34 in digestate fibre and 100:13:32 in whole digestate.
- The mechanically-recovered BMW digestate liquor exceeds the PAS 110 limit for the potentially toxic elements (PTE) Cd, Cu, Ni, Pb, and Zn. The digestate fibre exceeds the PAS 110 specification for the metals Cr, Cu, Ni, Pb and Zn. While this does not rule out land application for non-agricultural purposes, together with the presence of plastics and other physical contaminants it limits the potential of the material as a high-value product.

Source-segregated food waste

- Food waste has a very high specific methane yield of $0.425 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ which is equal to $93 \text{ m}^3 \text{ CH}_4 \text{ tonne}^{-1}$ on a wet weight basis.

- The high nitrogen content of food waste results in a high digester total ammonia nitrogen (TAN) and this can lead to raised concentrations of volatile fatty acids in the digestate. The overall loading may be limited by the unusual fermentation conditions that develop and the restricted microbial consortium that can adapt to these conditions. The maximum loading achieved in this research with food waste as a single substrate was $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$.
- Ideally the digestate TAN should be reduced to a level where it is non-toxic to the acetoclastic methanogenic population. This is probably less than 4000 mg l^{-1} but is dependent on pH and other factors. Further research needs to be carried out to assess methods of reducing digester ammonia levels and to provide a better understanding of the impact of high ammonia concentrations on the syntrophic communities in the digester.
- Food waste is probably lacking in essential trace elements and these need to be added to prevent the accumulation of VFA, and in particular of propionic acid. Trace elements likely to be deficient are Selenium and Cobalt. Other trace elements such as Molybdenum and Tungsten may also be important in mediating essential metabolic pathways when acetoclastic methanogens are inhibited by high digestate TAN. Further research is needed to establish the required trace elements and the correct dose rate.
- The maximum VBP that could be achieved with food waste at the limited loading of $2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ was $1.4 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$.
- The NPK ratio of food waste digestate was 100:10:36 in digestate liquor, 100:21:39 in digestate fibre, and 100:10:37 in whole digestate.
- Food waste digestate liquor and fibre were both within the PTE specification of the proposed PAS 110.

Co-digestion with mechanically-recovered BMW

- Co-digestion with mechanically-recovered BMW is possible with a number of waste materials, but for the two tested no improvement was obtained in the VBP.
- Co-digestion may restrict the achievable process loading if substrates are too rich in total Kjeldahl nitrogen (TKN) or lipids.
- The greatest potential advantage of co-digestion with mechanically-recovered BMW is to alter the nutrient balance in the digestate, improving its potential for use in land reclamation and other non-agricultural applications such as forestry and long-term cultivation of non-food crops.

Co-digestion with source-segregated food waste

- Co-digestion of food wastes offers the potential for reducing the TAN concentration in the digester allowing stable operation at higher loadings. The VBP achieved with a mix of food waste and card packaging at an OLR of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ was $2.2 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, which was a 54% improvement in performance over that achieved by digestion of food waste alone. Co-digestion of food waste and cattle slurry on a 60:40% VS basis at an OLR of $4 \text{ kg VS m}^{-3} \text{ d}^{-1}$ gave a VBP of $2.1 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, which outperforms a single substrate food waste digester by 45%.
- The 60:40% VS mix of food waste to cattle slurry (about 2:3 on a volume basis) gave a very good VBP of $2.1 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, but does not correspond well with the relative amounts of these materials produced in the UK at present. A 40:60% VS mix is about

5:1 on a volume basis, which is more representative of the proportions produced in the UK. This gave a VBP of $1.13 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$, which is acceptable for a commercial AD plant. It would therefore make good sense to blend food waste with cattle slurries in digesters, raising the volumetric productivity from less than 1 to potentially more than $2 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ making digestion economically feasible.

- Card packaging can be recommended as a co-substrate for food waste digestion. This mixture has a similar biochemical composition to mechanically-recovered BMW with a moderate TKN concentration, but without the physical and chemical contaminants present in mechanically-recovered BMW. Apart from lowering the overall TKN of the feedstock mixture, card packaging also brings some essential elements into the digester to ensure the function of autotrophic methanogens. It is possible however that the plastic content in some card packaging may make the digestate less attractive as a quality product.

General

- There is a need for a catalogue of waste types suitable for co-digestion with characterisation data so that the impacts of blends on the digestion process and the quality of the digestate can be estimated.
- The key parameters for stable digester operation and/or digestate quality are: TKN, C:N ratio, lipid content, and trace element profile and PTE concentration.
- The biochemical methane potential (BMP) obtained in practice cannot be reliably estimated either from the Buswell equation or from a knowledge of the biochemical characteristics of the waste; BMP values have to be determined experimentally.
- In semi-continuous studies with continuously stirred tank reactor (CSTR) digesters SMP was generally around 80-85% of BMP values at the loading rates achieved.
- There is currently no agreed method for testing the stability of digestate for land application, although it is likely that a method based on residual methane potential will be adopted for PAS 110 and a standard of acceptability defined. Tests carried out in CSTR digesters to assess the residual methane potential of the baseline waste streams and co-substrates gave values in the range of $0.09\text{-}0.20 \text{ STP CH}_4 \text{ m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$.

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Appendix 1: Analytical methods

Method 1 Determination of particle size distribution and physical contaminants

Principle:

The solid sample is graded using a specified nest of sieves by mechanical vibration shaking.

The physical contaminants include the recognisable fragments of glass, metal, plastic and non-combustibles (stones and ceramics). The biodegradable organic matter contains kitchen waste, garden waste, wood, leather, paper, cardboard, nappies and textiles. The fines that are not recognised in other categories are also considered as part of the organic fraction.

Apparatus:

1. Electromagnetic sieve-shaker, Endecotts Ltd, London, UK;
2. Test sieves, diameter 600 mm, aperture sizes as 37.5 mm, 20.0 mm, 10.0 mm, 6.7 mm, 5.0 mm and reception tray.
3. Balance, weighing range at least 10 kg with an accuracy of 0.1 g.

Procedure:

1. Assemble the sieves in ascending order of aperture size on top of the reception tray on the sieve shaker. Secure the sieves with the locking bars;
2. Connect the sampling bags onto the outlets of each sieve and the reception tray;
3. Switch on the equipment;
4. Distribute about 10 kg of sample slowly and evenly on the upper sieve over a 20-minute operation time;
5. Switch off the equipment and undo the locks;
6. Collect the sample resting on the sieves and the reception tray into the corresponding sampling bags;
7. Weigh the mass of sample in each size fraction on the balance tared with an empty sampling bag. Record the weights;
8. Clean and dry the sieves and the reception tray;
9. For each size fraction pick out the physical contaminants (glass, metal, plastics and non-combustible) from the sample. Sort each contaminant into a discrete pile for each type. Weigh and record the mass of each type in each size fraction.

Expression of results:

The mass fraction distribution of particle sizes and physical contaminants is determined by calculating as a percentage of wet weight.

References:

1. PAS 100:2005 Specification for composted materials;
2. Guidance on monitoring MBT and other pre-treatment processes for the landfill allowances schemes (England and Wales), Environment Agency, August 2005.

Method 2 Determination of Biochemical Methane Potential

Background:

The biochemical methane potential (BMP) assay is a procedure to determine the maximum methane yield of an organic material during its anaerobic decomposition by mixed microbial consortia under an optimal condition. This assay provides a simple means to monitor relative biodegradability of substrates. Various procedures have been developed for carrying out the test, dating back to methods using the anaerobic Warburg apparatus and serum-bottle techniques developed by Owen *et al.* (1979). There is also an outline procedure described in American Society for Testing and Materials (ASTM) (1992) and examples of results from this method can be found in Owens and Chynoweth (1992).

Some guidelines (Angelidaki *et al.*, 2009) are available from the international task group on Anaerobic Biodegradation, Activity and Inhibition (ABAI-group) for development of a standard assay procedure. These focus on ensuring that under the conditions of the test the degradation of the organic material is not limited by improper inoculum, substrate overloading, substrate toxicity, nutrient deficiency, pH, etc. A stock solution of micronutrients may be added to ensure that appropriate essential trace elements are available in the test matrix, and the composition of this is based on the known nutrient requirements of methanogens.

In all tests a sample of substrate is anaerobically incubated with an inoculum for a period of time, which can either be pre-determined or until a defined point e.g. where gas production has ceased relative to that of the inoculum control. In the latter case this can take at least 30 days for simple substrates (e.g. sugars and starches) and up to 120 days for recalcitrant lignocellulosic substrates (e.g. paper and wood). The ASTM E 1196 method suggests an incubation period of 56 days or longer if gas is still being produced. To account for biogas production from residual degradable matter in the inoculum, triplicate inoculum controls (inoculum blanks) containing only inoculum (or inoculum with the addition of micronutrients in the case when micronutrient supplementation is applied) are incubated and the gas is sampled and analysed simultaneously to allow subtraction of gas not attributed to the substrate. In some cases positive controls containing a standard material such as cellulose are also incubated and sampled simultaneously to ensure that inoculum and sampling procedures are not affecting the results.

Biochemical methane potential (BMP) analyses carried out in our laboratory follow a standard procedure as described below.

Materials and equipments:

1. Representative fresh sample for analysis;
2. Drying oven, capable of maintaining a temperature of $105 \pm 5^{\circ}\text{C}$;
3. Electric muffle furnace, capable of maintaining a temperature of $550 \pm 10^{\circ}\text{C}$;
4. Porcelain crucibles;

5. Analytical balance, with an accuracy of 0.01g;
6. Temperature controlled BMP digesters (2.0-litre total volume, 36 ± 1 °C);
7. Over-water gas collectors, leak-proof and filled with a 75% saturated solution of sodium chloride and acidified to pH 2 using hydrochloric acid to minimise the absorption of CH₄ and CO₂ into water from the biogas being collected in the gas collector;
8. Gas chromatograph for the analysis of biogas composition;
9. Standard biogas;
10. Suitable anaerobic inoculum (sewage sludge digestate from municipal wastewater treatment plant (WWTP) is suitable in most cases);
11. Various chemical compounds for preparation of trace element solution (optional, no need when using sewage sludge digestate from WWTP as inoculum in most cases).

Experimental procedures:

1. Where possible the test should be set up in triplicate for each substrate;
2. There should be at least 3 controls of inoculum without added substrate;
3. Collect a suitable anaerobic sludge as inoculum. This may be from sewage sludge digester in a municipal wastewater treatment plant or a laboratory anaerobic digester;
4. Sieve the inoculum through a 1mm mesh to remove particles and grit;
5. Determine the total solids (TS) and volatile solids (VS) of the inoculum;
6. Weigh the empty BMP digesters. Fill a fixed wet weight (1.4 kg for standard 2-litre BMP digesters) of inoculum into each empty digester. The inoculum should be kept homogeneous by constant mixing while sub-samples are taken for loading the digesters. Care should be taken throughout the filling procedure to avoid undue agitation of the inoculum so as to minimise oxygen transfer and to maintain anaerobicity. Secure the top plates to the inoculum-loaded digesters maintained them at 36 ± 1 °C;
7. Homogenise the test substrate if necessary by pre-processing using blender, grinder, or cutting mill;
8. Measure and calculate the quantity of volatile solids added to each digester using equation:

$$Total \cdot VS_{inoculum} (g) = \frac{Weight \cdot of \cdot inoculum \cdot added \cdot (g) \times VS_{inoculum} (\%)}{100}$$

9. Calculate the amount of substrate to be added to each test digester based on a suitable inoculum to substrate volatile solids ratio ($r_{I/S}$). It is noted from this project that a ratio of 4 is preferable of the BMP determination of waste material, the ratio maybe lower for the BMP determination of digestate.

$$Weight \cdot of \cdot substrate \cdot added \cdot (g) = \frac{Total \cdot VS_{inoculum} (g)}{r_{I/S}} \times \frac{100}{VS_{substrate} (\%)}$$

10. Place sufficient representative fresh substrate in a container and thoroughly mix it. Add the required amount of substrate from the container to the digester, which is placed on a balance to record the increase in weight. (Determine the TS and VS of the remaining substrate and inoculum in the container immediately after setting up the BMP test in order to

know exactly the quantity of TS and VS of the inoculum, the substrate and the ratio between them);

11. Pipette the required amount of trace element solution to each BMP digester (optional);
12. Put new gaskets on the BMP digesters. Secure the top plates to the digesters. Flush the headspace with nitrogen as a precaution against any aerobic decomposition of the substrate in the very early stages of the test. Connect each digester to its corresponding gas collector and switch on its stirrer;
13. Check and record the barrier solution level in the gas collectors at least twice per day. Record the ambient temperature and pressure at the same time to correct the gas volume to standard temperature and pressure (STP);
14. Take the biogas sample from the gas collector when the gas collector is full, or after 5 days (whichever is the shorter), and raise the barrier solution level in gas collector to its zero using a vacuum pump. Analyse the composition of gas sample by gas chromatography;
15. Calculate the methane volume produced in each time interval according to the gas collector readings and the biogas composition;
16. Check the BMP system regularly to ensure that the digester water bath is topped up with water and is maintaining a constant temperature of 36 ± 1 °C; that the stirrers in the digesters are all turning; and that there are no apparent gas or liquid leaks in the system;
17. Allow the experiment to run until there is no significant difference between the gas production of the control and test digesters.

Data analysis:

After each sampling, the value of the measured volume of methane produced by the digesters is converted to dry gas at 1 atm and 0°C (STP) and added to the previous measurements. The total cumulative methane volume produced by test substrate is corrected by subtracting the averaged inoculum control methane volumes from each test digester's total cumulative methane volume. Finally, the corrected cumulative methane yield is calculated by dividing the corrected volume by the weight of substrate VS added to each bottle.

To determine the kinetic constants, the specific methane production is often modelled using two sets of assumptions: simple first-order degradation (Model 1), and a pseudo-parallel first-order model (Model 2). For model 1 and 2 the methane production is given by

$$Y = Y_m (1 - e^{-kt}) \quad [1]$$

where:

Y: cumulative methane yield at time t, $\text{m}^3 \text{CH}_4 \text{ kg}^{-1}$ VS added;

Y_m : ultimate methane yield, $\text{m}^3 \text{CH}_4 \text{ kg}^{-1}$ VS added;

K: first order rate constant, d^{-1} .

$$Y = Y_m (1 - P e^{-k_1 t} - (1-P) e^{-k_2 t}) \quad [2]$$

where:

Y: cumulative methane yield at time t, $\text{m}^3 \text{CH}_4 \text{ kg}^{-1}$ VS added;

Y_m : ultimate methane yield, $\text{m}^3 \text{CH}_4 \text{ kg}^{-1}$ VS added;

k_1 is the first order rate constant for the proportion of readily degradable material, d^{-1} .

k_2 is the first order rate constant for the proportion of less readily degradable material, d^{-1} .
P is the proportion of readily degradable material

The parameters Y_m , k , and P may be estimated using a nonlinear regression fit to the yield data of a triplicate set. The regression can be performed on a computer using the Marquardt-Levenberg algorithm available in SigmaPlot or other appropriate software.

References:

1. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P. and van Lier, J.B. (2009) Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science and Technology* 59(5), 927-934.
2. ASTM, E1196-92 Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals, 1992 American Society for Testing and Materials, West Conshohocken, PA.
3. Owen, W. F., Stuckey, D. C., Healy, J. B., Jr., Young, L. Y. and McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research*, Vol 13, pp. 485-492.
4. Owens, J.M. and Chynoweth, D. P. (1993) Biochemical Methane Potential of Municipal Solid Waste (MSW) Components, *Water Science and Technology*, Vol. 27, No. 2, pp. 1-14.
5. Shelton, D. R. and Tiedje, J. M. (1984). General method for determining anaerobic biodegradation potential. *Applied and Environmental Microbiology*, Vol 47, No. 4, pp. 850-857.

Method 3 Determination of total organic carbon

Principle:

The total organic carbon (TOC) is obtained by the difference between the results of the measurements of total carbon (TC) and total inorganic carbon (TIC).

For liquid samples, the TC present is converted to carbon dioxide (CO₂) by high temperature catalytic combustion at 680°C in the pure oxygen environment with Pt on alumina as catalysis. The TIC is converted to CO₂ by acidification in a chamber containing phosphoric acid. The CO₂ generated from both these processes is measured by a non-dispersive infrared detector (NDIR).

For solid samples, the TC present is converted to CO₂ by high temperature catalytic combustion at 800°C in a stream of pure oxygen with Pt on alumina as the catalyst. The TIC is measured by determining the TC in the ashed sample remaining after combustion.

Apparatus:

1. Dohrmann DC-190 High-temperature TOC analyzer, Rosemount Analytical Inc., USA;
2. Analytical balance, with an accuracy of 1 mg.

Application:

1. Liquid sample matrices, especially those which are dirty, salty, or particulate-laden;
2. Solids, sludges, slurries, and waters with particulates greater than 0.5 mm are analysed using the 'boat' option.

Reagents:

1. *Potassium hydrogen phthalate stock standard solution, 50,000 mg C l⁻¹*: dissolve 106.4 g anhydrous potassium hydrogen phthalate (KHP) C₈H₅O₄K (dried at 105 °C) in deionised water, and dilute to 1000 ml using volumetric flask;
2. *Sodium carbonate stock standard solution, 5,000 mg C l⁻¹*: dissolve 44.17 g anhydrous sodium carbonate Na₂CO₃ (dried at 105 °C) in deionised water, and dilute to 1000 ml using volumetric flask;
3. *Phosphoric acid (H₃PO₄), concentrated.*

Calculation:

When determining TOC of the solid sample:

$$TOC = A - B \times (1 - \frac{VS}{100})$$

where:

TOC: total organic carbon, mg g⁻¹;

A: carbon concentration in dried sample, mg g⁻¹;

B: carbon concentration in ashed sample, mg g⁻¹;

VS: volatile solids, %.

Method 4 Elemental analysis for CHNSO

Principle:

The equipment used (elemental analyser) operates for analysis of CHN, and also S, using a flash combustion in which a sample contained within a tin capsule is dropped into a combustion/reduction reactor held at 900°C. This short flash combustion is accomplished when the tin capsule is exposed to a gas flow temporarily enriched with ultra high purity oxygen. The resulting oxidation raises temperatures to higher than 1700°C. The encapsulated sample, depending on its composition, combusts generating one or more of these gases: N_xO_x , CO_2 , H_2O , and SO_2 in the oxidation zone. Then N_xO_x is reduced to N_2 in the reduction zone. After passing the reactor, the gas mixture enters the gas chromatographic column where the different components are time-separated and then measured by detectors.

Oxygen in solid sample is converted to carbon monoxide by pyrolysis at 1060°C in the presence of metallised carbon but with the absence of oxygen gas. The carbon monoxide is then separated from the other pyrolozates under steady state conditions, and measured as a function of thermal conductivity.

Apparatus:

1. FlashEA 1112 Elemental Analyzer, Thermo Finnigan, Italy;
2. Analytical balance, with an accuracy of 0.1 mg.

Applications:

1. CHN analysis for solid sample;
2. S analysis for solid sample;
3. O analysis for solid sample;
4. CN analysis for liquid sample;
5. S analysis for liquid sample.

Analysis methods:

Shown in table M4.1. The detailed operational procedure is attached to the EA instrument.

Note:

EA analysis can be used to determine TOC of the solid sample:

$$TOC = A \times 10 - B \times \left(1 - \frac{VS}{100}\right) \times 10$$

where:

TOC: total organic carbon, mg/g;

A: carbon concentration in dried sample, %;

B: carbon concentration in ashed sample, %;

VS: volatile solids, %.

Table M4.1 Elemental analyser – summary of methods and principle of technique used

		Analytical determination		
		CHN	S	O
Reactors	Configuration	<i>Oxidation zone:</i> Chromium oxide <i>Reduction zone:</i> Reduced copper <i>SO₂ removal:</i> Silvered cobaltous/cobaltic oxide	<i>Oxidation zone:</i> Copper oxide <i>Reduction zone:</i> Electrolytic copper	<i>Pyrolysis zone:</i> Nickel plated carbon
	Temperature (°C)	900	900	1060
Adsorption filters			<i>H₂O removal:</i> Magnesium perchlorate	<i>H₂O removal:</i> Magnesium perchlorate <i>Acid gas removal:</i> Soda lime
Gas chromatographic columns		Multiseparation column	Sulphur separation column	Oxygen separation column
Detector	Type	Thermal conductivity detector (TCD)	Flame photometric detector (FPD)	Thermal conductivity detector (TCD)
	Temperature (°C)	75	90	65
Standards		L-Aspartic acid; Atropine; Nicotinamide	Cystine; Methionine; Sulphanilamide	L-Aspartic acid; Atropine; Nicotinamide
Catalyst			Vanadium pentoxide	

Method 5 Determination of calorific value by bomb calorimetry

Theory:

Calorific value can be defined as the amount of energy released on burning by each unit of combustible mass. There are two types of calorific value (i.e., the gross calorific value and the net calorific value), and bomb calorimeter measures the gross calorific value.

The gross calorific value, also known as higher heating value (HHV), is the amount of energy released on burning by complete combustion of a mass unit of sample, at constant volume in an oxygen atmosphere, assuming that the final products of combustion consist of O₂, CO₂, SO₂, and N₂ in the gas phase together with water, that contained in the sample and that generated from the combined hydrogen, in liquid form.

The net calorific value, also known as lower heating value (LHV), is defined as the amount of heat released by combusting a specified quantity and returning the temperature of the combustion products to 150°C. LHV assumes the latent heat of vaporization of water in the reaction products is not recovered. It is useful in comparing fuels where condensation of the combustion products is impractical, or heat at a temperature below 150°C cannot be put to use.

Both calorific values are related through the equation 1:

$$LHV = HHV_d - 2.442 \times (W + 9 \times H_d) \times 0.01 \quad [1]$$

where,

LHV: the lower heating value of the sample, kJ g⁻¹ fresh matter;

HHV_d: the higher heating value of the dry sample, kJ g⁻¹ TS;

W: the moisture percentage of the sample, % fresh matter;

H_d: the hydrogen percentage of the dry sample, % TS;

2.442: the heat of vaporization of water, kJ g⁻¹ water;

9: molecular weight ratio of water to hydrogen.

Principle:

A bomb calorimeter is a type of calorimeter used in measuring the heat of combustion in pure oxygen environment at high pressure. Electrical energy is used to light the sample. As the sample is burning, it will heat up the bomb vessel which is placed in the static polystyrene jacket. The temperature rise of the bomb vessel allows for calculating calorific content of the sample.

Apparatus:

1. CAL2k bomb calorimeter system: the filling station + bomb vessel + calorimeter + stainless steel crucible + cotton thread fuse, Digital data systems Ltd, South Africa;
2. Analytical balance with an accuracy of 1 mg.

Reagent:

1. Benzoic acid as standard, with a HHV of 26.454 kJ g⁻¹ TS.

Operation procedure:

1. Place the stainless steel crucible onto its stand and attach a cotton thread fuse to the ignition wire and crucible;
2. Put the stand into the bomb vessel. Secure the lid;
3. Fill the bomb vessel with pure oxygen in the filling station until the pressure inside reaching 3 MPa;
4. Put the vessel into the static polystyrene jacket of the calorimeter. Secure the lid. Ignition will happen automatically when the temperature inside the jacket is stable;
5. Record the calorific value shown on the screen of calorimeter. A blank is made to account for the electrical energy input and the energy released in burning the fuse;
6. Input this blank value in the operation program at the data entry space 'baseline';
7. Open the lid of calorimeter. Take the bomb vessel out.
8. Release the pressure of the vessel by pressing the gas value with the special tool;
9. Open the lid of the vessel. Clean the vessel and the crucible;
10. Leave the vessel until it cools to room temperature;
11. Select 'calibration' program using the calorimeter screen;
12. Weigh around 1.0 g of benzoic acid with an accuracy of 0.1 mg in the crucible. Record the weight and input it to the operation programme of the calorimeter;
13. Place the crucible onto its stand and connect the ignition wire and benzoic acid with a cotton thread fuse;
14. Repeat the steps 2-4;
15. Weigh around 1.0 g of sample with an accuracy of 0.1 mg. Record the weight and input it into the calorimeter;
16. Record the calorific value shown on the calorimeter screen;
17. Follow the steps of 7-10, and then start the next measurement.

Expression of the result:

When an analysis was carried out using a dried sample the results were expressed on a dry weight basis. This involves a separate determination of percent moisture content and this was carried out in parallel to the analytical test. The calorific value can be expressed either on a total solids (TS) or on a volatile solids (VS) basis; both of the expression can be calculated using the equation 1 in this method statement.

Notes:

1. When measuring the calorific value of liquid samples, benzoic acid may be added as a spike to assist the ignition;
2. The solid sample should be pressed inside the crucible if this is not done it will tend to 'splash' when igniting.

Method 6 Determination of fibre content

Principle:

Fibre is an inhomogeneous mixture of various macromolecules. Most of these are structural polysaccharides (e.g. cellulose, and hemicellulose, and pectin), but also non-carbohydrates like the aromatic lignin, non-digestible proteins and others are normally counted as fibre constituents.

The most commonly used terms, based on chemical analytical techniques, are Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL). All of these methods are based on parallel steps of chemical treatments to solubilise “non-fibre” components and final determination of the residue obtained. Depending on determination approach various kinds and amounts of fibre constituents are achieved in the residues.

Neutral Detergent Fibre (NDF) is defined to be the residue after treatment with a neutral detergent solution. In this procedure, sample is boiled for one hour with neutral detergent (ND). Enzymatic incubation before, during and after the ND treatment helps to break down protein and starch. The residue is then dried and ashed. The weight reduction by ashing is the sample content of hemicellulose, cellulose and lignin.

Acid Detergent Fibre (ADF) is defined to be the residue after treatment with an acid detergent solution. Sample is boiled with acid detergent (AD) for one hour, and dried and ashed. The weight reduction by ashing is the sample content of cellulose and lignin.

Acid Detergent Lignin (ADL) is defined to be the residue after initial treatment by the ADF method followed by removal of the cellulose fraction through extraction using 72% H_2SO_4 , and then dried and ashed. The weight reduction by ashing is the sample content of Lignin.

Apparatus:

1. FibreCap 2023 system;
2. Ashing crucibles (45 x 60 mm) x18;
3. Analytical balance with an accuracy of 1 mg.

Reagents:

1. Neutral Detergent Solution:

Disodium ethylene diaminetetraacetate dihydrate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) 18.61g x2

Sodium Borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) 6.81g x2

Sodium lauryl sulphate (sodium dodecyl sulphate, $\text{C}_{12}\text{H}_{25}\text{OSO}_3\text{Na}$) 30g x2

2-ethoxyethanol ($\text{C}_4\text{H}_{10}\text{O}_2$) 10ml x2

Disodium hydrogen phosphate, anhydrous (Na_2HPO_4) 4.56g x2

Alfa-Amylase solution – Termamyl 300L, type DX available from Foss Tecator x2

Place 18.61g of EDTA (Disodium ethylene diaminetetraacetate, $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) and 6.81g of Sodium Borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), in a beaker and add some distilled water and heat until dissolved. Add 30g Sodium Lauryl Sulphate, ($\text{C}_{12}\text{H}_{25}\text{OSO}_3\text{Na}$), 10ml of 2-ethoxyethanol ($\text{C}_4\text{H}_{10}\text{O}_2$) and 4.56g Disodium Hydrogen

phosphate, (Na₂HPO₄). Add water and heat until dissolved. Mix and dilute to 1000ml. Check pH which should be in the range 6.9-7.1. Adjust by NaOH if necessary.

Repeat this step twice to produce two 1000ml ND solutions.

2. Acid Detergent Fibre Solution: 1.00N H₂SO₄ with CTAB

Concentrated sulfuric acid 49.04 x2

Cetyl trimethylammonium bromide (CTAB, CH₃(CH₂)₁₅(CH₃)₃NBr) 20g x2

Weigh 49.04 g conc. H₂SO₄ into a 1000 ml volumetric flask containing 400 ml deionised water. Make up to volume with deionised water. Add 20 g of CTAB (Cetyl trimethylammonium bromide, CH₃(CH₂)₁₅(CH₃)₃NBr).

Repeat this step twice to produce two 1000ml AD solutions.

3. Acid Detergent Lignin Solution: Sulfuric acid, 72%

Concentrated sulfuric acid, 98%

Weigh 433 g of deionised water into 1000 ml volumetric flask and add 1201 g (or 653 ml) of conc. H₂SO₄ slowly with occasional swirling. The flask must be cooled in water in order to add the required weight of acid. Cool to 20°C and check if volume is right. If volume is too large, take out 5 ml solution and add 4.55 ml conc. H₂SO₄. If volume is too small, take out 1.5 ml solution and add 2.5 ml of deionised water. Repeat if necessary. Meniscus should be within 0.5 cm of the calibration mark at 20°C.

Sample preparation:

Solid samples are normally ground to less than 1.0 mm.

Semi-solid is difficult to handle particularly when there is a wide variation in particle size and/or hardness of constituents. Depending on the particular sample type, homogenizing, liquefying or ball milling may provide a suitable sample for analysis. If possible dry sample before milling.

Analytical Procedure for Neutral Detergent Fibre (NDF):

1. Label 18 capsules with an indelible pen and dry them with lids in the oven at 105°C for at least 30 minutes. Transfer to desiccator, cool for at least 5 minutes prior to weighing sample;

2. Weigh pre-dried capsule+lid (W₁), tare and weigh around 1 g of ground sample to an accuracy of ±0.1mg (W₂) into each capsule, secure lids. Place the capsules in the tray holder, and place the tray in place in the carousel. Triplicate analysis for each sample (totally 5 samples can be treated in a run), and the rest three capsules and lids are the control;

3. If the fat content is above 5%, samples should be de-fatted prior to analysis:

Add 1000 ml of ether to the extraction beaker. Place the tray holder with the capsules in the solution and agitate for 30 seconds. Lift the tray holder out of the solution and drain the capsules from solvent. Repeat three times in three different containers with solvent. Remove tray holder and allow capsules to drain and air-dry in fume hood.

For samples containing fatty substances that cannot be removed directly, the extraction shall be carried out after the detergent treatment using acetone (CH_3COCH_3);

4. Put 1000 ml of hot water (80°C) and 21-28 ml of 2% Amylase to the extraction beaker. Place the carousel with capsules into the beaker and gently agitate to mix well. Allow standing for 15 minutes at room temperature;

5. Drain the solution out of the capsules. Wash once with cold water and drain;

6. Place extraction beaker with 1000 ml of Neutral Detergent (ND) solution. Lower the carousel unit into the reagent sufficient to immerse the samples. Gently agitate to thoroughly disperse samples and then fully lower the carousel into the reagent.

7. Put the beaker on the hot plate and place condenser on top of the extraction beaker. Open cold water tap (0.4 l/min) for the reflux system. Let it boil gently for 30 minutes. Always measure boiling from the time when the solution has reached the boiling point (determined by the presence of small air bubbles breaking the surface of the liquid);

8. Remove the carousel from the beaker and dry the lid membrane with a piece of soft tissue. Discard half (500 ml) of the extraction solution. Add another 500 ml of fresh ND solution and 21-28 ml of 2% amylase solution;

9. Lower the carousel into the extraction beaker and agitate. Put the beaker back on the hot plate and fit the condenser on the top;

10. Bring the solution up to boiling and boil gently for 30 minutes again. Meanwhile, preheat ~3 liters of water to boiling;

11. Remove the condenser. Remove the extraction beaker from the hotplate. Remove the carousel from the beaker and empty the beaker and capsules of liquid. If solution is present on the lid membrane, it might be difficult to filter the capsule. Tap the whole extraction carousel against a hard surface and dry the lid membrane with soft tissue. Return the carousel to the empty extraction beaker and 'spin' rotate to remove all of the liquid from the capsules and discard;

12. Fill the beaker with 1000 ml of boiling water (to mark). Wash by partially lowering the extraction carousel into the water ensuring that the capsules refill, gently agitate the carousel and raise it to empty the capsules and lower to refill. Do not use so much water so that the lids are covered with water. Empty the capsules and extraction beaker. Dry the capsule lid with soft tissue if necessary. Repeat the washing procedure twice more;

13. Add 1000 ml of hot water (~80°C) and 21-28 ml of 2% amylase to the extraction beaker. Return carousel to the beaker and agitate. Allow standing for 15 minutes;

14. Wash the capsules twice with cold water following the above washing procedure;

15. De-fatting with acetone if necessary;

16. Put the capsule tray on the drying stand, and dry capsules in an oven at $105 \pm 2^\circ\text{C}$ for 5 h;

17. Cool the capsules to room temperature in a desiccator and weigh with a precision of ± 0.1 mg (W_3);

18. Place the capsules in pre-dried and pre-weighed (W_4) ashing crucibles. It is important that the crucible used is high enough so that all of the ash is retained inside the crucibles as a standing capsule can fall during ashing;

19. The pressure inside the capsule can increase during ashing. To avoid this, make a small slit in the capsule using a scalpel prior to ashing;

20. Ash the capsules in the ashing crucibles for 4 h at $600 \pm 10^\circ\text{C}$. Do not place capsules in hot furnace. Always try to heat them slowly by having them in a cold furnace from the beginning and then increase the temperature;

21. Cool the ashing crucibles slowly, at $\sim 200^\circ\text{C}$ place them in a desiccator. When room temperature is reached, weigh with a precision of ± 0.1 mg (W_5);

22. Calculation:

$$\%NDF = \text{Hemicellulose} + \text{Cellulose} + \text{Lignin} = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

where,

W_1 = Initial capsule weight, g;

W_2 = Sample weight, g;

W_3 = Weight of capsule + residue sample after extracting and drying, g;

W_4 = Weight of empty ashing crucible, g;

W_5 = Weight of total ash and ashing crucible, g;

C = Blank correction for capsule solubility;

D = Capsule ash, g.

The capsules can lose a small amount of weight during reaction with the reagents. A correction factor (C) to compensate for this loss is used in the formula for calculation of analytical results. Typically the correction factor (C) is > 0.9990 , corresponding to ~ 3 mg weight loss of a capsule during processing:

$$C = \frac{\text{blank} \cdot \text{capsule} \cdot \text{weight} \cdot \text{after} \cdot \text{extractions}}{\text{blank} \cdot \text{capsule} \cdot \text{weight} \cdot \text{at} \cdot \text{start}}$$

During the final ashing step some ash weight is obtained from the capsule itself (D). It is recommended to make an ash evaluation in each batch of samples being analysed. The ash weight contribution from the capsule is typically < 3 mg.

Analytical Procedure for Acid Detergent Fibre (ADF):

The first three steps follow the instructions for NDF 1-3;

4. Put 1000 ml of AD solution to the extraction beaker. Gently lower the carousel into the beaker ensuring all capsules have been wetted and then raise the capsules out again;

5. Place the carousel with capsules back into the AD solution avoiding getting fluid on the lid of the capsules. Dryness of the lids is essential;
6. Rotate the carousel gently and make sure that there is fluid in each cap;
7. Put the beaker on the hot plate and place condenser on top of the extraction beaker. Open cold water tap (0.4 l/min) for the reflux system. Let it boil gently for 60 minutes and rotate occasionally if desired. Always measure boiling from the time when the solution has reached the boiling point (determined by the presence of small air bubbles breaking the surface of the liquid);
8. Carefully take carousel out of the AD solution and carefully dispose of the solution down the sink with plenty of running water;
9. Place the carousel back into the empty beaker and spin it to displace the fluid. Dry the lids with soft tissue;
10. Fill the beaker with 1000 ml of boiling water. Lower the carousel into the boiling water and ensure all capsules have water in them. Twist the carousel backwards and forwards to rinse the capsules;
11. Remove the carousel from the water.
12. Dispose of the water down to the sink.
13. Replace the carousel back into the beaker and spin off any excess water. Dry the capsule lids with soft tissue;
14. Wash the capsules up to 4 times more with hot water following the above washing procedure. On the last rinse wash the lids of the capsules;
- 15-21. Follow the instructions for NDF 15-21;
22. Calculation:

$$\%ADF = Cellulose + Lignin = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

See the instruction for NDF 22 for the meaning of each symbol.

Analytical Procedure for Acid Detergent Lignin (ADL):

The first steps follow the instructions for ADF 1-14;

Note: Please do not fill the capsule with sample higher than half the capsule height. Otherwise, the acid is difficult to be washed out later and the capsules will burn in the oven when drying.

15. Place ~700ml of 72% sulfuric acid into the beaker;

16. Place the capsule tray onto the drying stand. Lower the tray with capsules into 72% sulfuric acid for 4 hours in fume cupboard;

17. Wash the samples in cold water for times until wash off all acid (wash in warm water later if necessary), and make sure the pH of the washing solution is neutral at last;

18. Follow the instructions for NDF 15-21;

19. Calculation:

$$\%ADL = Lignin = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

See the instruction for NDF 22 for the meaning of each symbol.

Method 7 Determination of pH

Principle of method:

pH is measured potentiometrically in the undiluted liquid sample or in a sample/water slurry for semi-solid or solid sample.

Apparatus:

1. pH meter with means for temperature compensation
2. Combination electrodes
3. Magnetic stirrer and Telfon-coated stirring bar
4. Plastic or glass containers, of sufficient capacity to accommodate the volume of the sample, deionized water and 10% air volume.

Reagents:

1. Buffer solutions: pH 4.0, 7.0 and 9.2.

Procedure:

1. Calibration of the pH-meter:
 - a. Calibrate the pH-meter as prescribed in the manufacturer's instruction;
 - b. Use at least two of the buffer solutions that bracket the expected pH of the samples and are approximately three pH units or more apart.
2. Liquid sample (when the aqueous phase constitutes at least 20% of the total volume of the sample):
 - a. Place the sample in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar;
 - b. Stir the sample at a constant rate to provide homogeneity and suspension of solids;
 - c. Thoroughly rinse and gently wipe the electrodes prior to measuring pH of the samples;
 - d. Immerse the electrodes into the sample beaker;
 - e. Record sample pH to one decimal place after stabilization is reached.
3. Semi-solid or solid sample:
 - a. Place a weight equivalent to 20ml of the sample volume into a container;
 - b. Add 40ml deionised water (or more in 20 ml aliquots if the tested material high in fibre), secure the cap and mix for 1 h on the magnetic stirrer;
 - c. Stop stirring just before the measurement;
 - d. Immerse the electrodes into the settling suspension;
 - e. Record the pH when the meter has stabilized and report the result as pH (1:*p*), where *p* equal to the proportion of water to sample used in the pH determination.

Notes:

1. Samples should be analyzed as soon as possible;
2. If the waste is hygroscopic and absorbs all the deionised water, begin the experiment again using 20ml of waste and 100 ml of deionised water;
3. Minimum stirring is required when measuring sample with high volatile component.

References:

1. BS EN 13037:2000 Soil improvers and growing media – Determination of pH;

2. SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. 9045D: Soil and waste pH (2004). Environmental Protection Agency, USA.
3. SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. 9040C: pH electrometric measurement (2004). Environmental Protection Agency, USA

Method 8 Determination of Total solids and volatile solids

Principle:

The test portion of sample is dried to constant mass in an oven at $105 \pm 5^\circ\text{C}$. The difference in mass before and after the drying process is used to calculate the total solids and the water content.

Then, the dried sample is heated in a muffle furnace at $550 \pm 10^\circ\text{C}$. The difference in mass before and after the ignition process is used to calculate the content of volatile solids and ash.

Apparatus:

1. Drying oven, capable of maintaining a temperature of $105 \pm 5^\circ\text{C}$;
2. Electric muffle furnace, capable of maintaining a temperature of $550 \pm 10^\circ\text{C}$;
3. Porcelain crucibles;
4. Desiccator with active silica gel desiccant with indicator;
5. Analytical balance, with an accuracy of 1 mg.

Procedures:

1. Place the crucibles in the drying oven for a minimum of 30 minutes. Put them in the desiccator to ambient temperature. If the crucibles are brand new, place them in the muffle furnace at 550°C for 30 minutes to burn off any organic residue and then put in the desiccator to cool;
2. Weigh the empty crucible using a balance of accuracy of at least 1 mg. Record the weight (W_1);
3. Add the sample to the crucible to make up around 2/3 of the capacity of crucible. Weigh the loaded crucible and record the weight (W_2). At least triplicate analysis should be done for one sample;
4. Place the crucibles containing the sample in the drying oven until constant mass has been reached, typically overnight;
5. Cool the crucibles with dried samples in the desiccator and weigh. Record the weight (W_3);
6. Place the crucibles with dried sample in the muffle furnace for 2 hours at 550°C .
7. Cool the crucibles with ash in the desiccator and weigh. Record the weight (W_4);
8. Clean the crucibles by washing thoroughly in water. Rinse with deionised water and dry.

Calculation:

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

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

and

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

Note:

Karl Fischer titration method should be used if sample contains high amount of volatile materials.

References:

1. BS EN 12880:2000 Characterisation of sludges – Determination of dry residue and water content;
2. BS EN 12879:2000 Characterisation of sludges – Determination of the loss on ignition of dry mass;
3. BS EN 13040:2000 Soil improvers and growing media – Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density;
4. BS EN 13039:2000 Soil improvers and growing media – Determination of organic matter content and ash;
5. BS ISO 5536:2002 Milk fat products – Determination of water content – Karl Fischer method.

Method 9 Determination of gas composition and volatile fatty acid by gas chromatography

Principle:

A gas chromatograph is an instrument for separating different components in a complex sample. It uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically.

Instrument analysis:

Table M9.1. Summary of parameters used for measurement of gas composition and VFA by gas chromatography

Test	Gas composition		Volatile fatty acid (VFA)	
GC model	GP-3800, Varian, USA		GC-2010, Shimadzu, Japan	
Standard	Standard biogas with 35% CO ₂ and 65% CH ₄		Mixture of VFAs in 10% of formic acid at concentrations of 50, 250, and 500 mg l ⁻¹ for each component	
Retention time (minutes)	CO ₂ CH ₄	2.34 3.27	Acetic Formic Propionic iso-Butyric n-Butyric iso-Valeric n-Valeric Hexanoic Heptanoic	6.33 6.92 7.32 7.67 8.39 8.86 9.64 10.81 11.94

Notes:

1. The detailed operational procedures are attached to the GC instruments;
2. A gas sample should be measured as soon as it is taken;
3. For VFA measurement, the sample should be centrifuged at 20800g for 10 min. Then the supernatant is mixed with pure formic acid at the ratio of 9:1;
4. The concentration of each targeted component is positively proportional with the peak area it creates.

Method 10 Determination of alkalinity

Principle:

Alkalinity is a measure of the buffering capacity of water, and digestate with high alkalinity is able to resist major shifts in pH.

Apparatus:

1. Automatic digital titration burette system with pH probe and magnetic stirrer;
2. 50 ml measuring cylinder;
3. 100 ml glass beaker.

Reagents:

1. *Standard buffer solutions*: pH 4.0 and 7.0;
2. *Standard sulphuric acid titrant, 0.1N or 0.25N*: Dilute 2.72 or 6.80 ml concentrated sulphuric acid to 1000 ml with deionised water.

Procedure:

1. Calibrate the pH reading with standard buffer solutions;
2. Measure a certain volume of homogeneous sample (50 ml is recommended) and place in the beaker with a Teflon-coated stirring bar;
3. Place the beaker on the magnetic stirrer and stir the sample with a moderate speed;
4. Fit the pH probe in the pH holder so that the sensing elements of the electrodes can be covered with a sufficient volume of sample but given adequate clearance for the magnetic stirring bar. Place the burette tip above the sample surface;
5. Allow pH reading to stabilize and record pH of the sample (not required for alkalinity calculation);
6. Set the titration ending point to pH 5.7, and press 'start' to start the titration;
7. Record the volume of sulphuric acid titrant used ($V_{5.7}$, ml) when the titration ends;
8. Set the titration ending point to pH 4.3 and start the titration. Record the volume of titrant used ($V_{4.3}$, ml) when the titration finishes;
9. Set the titration ending point to pH 4.0 and repeat the same procedure. The volume used is recorded as $V_{4.0}$ (ml);
10. Remove the pH probe, rinse thoroughly with deionised water;
11. Start the next measurement;
12. Place the pH probe into the storing solution after finishing all measurement.

Calculation:

$$TA = \frac{(V_{4.0} + V_{4.3} + V_{5.7}) \times N \times 50000}{V}$$

$$PA = \frac{V_{5.75} \times N \times 50000}{V}$$

$$IA = \frac{V_{pH4.3} \times N \times 50000}{V}$$

where

TA: total alkalinity, mg CaCO₃ l⁻¹;

PA: partial alkalinity (bicarbonate alkalinity), mg CaCO₃ l⁻¹;

IA: intermediate alkalinity (volatile acid alkalinity), mg CaCO₃ l⁻¹;

N: normality of acid;

V: the volume of sample, ml.

Note:

The ratio of IA:PA (Ripley ratio) is similar to the ratio of VFA to alkalinity, and can be acted as an index of the digester stability.

Method 11 Titrimetric determination of ammonia

General discussion:

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of organic nitrogen compounds and distilled into indicating boric acid solution. The ammonia in the distillate can be determined titrimetrically with standard H_2SO_4 .

Apparatus:

1. pH meter;
2. Kjeldahl distillation unit and tubes;
3. 250-mL Erlenmeyer flasks;
4. Analytical balance, with an accuracy of 1 mg.

Reagents:

1. *Standard Ammonium Chloride Solution*: dissolve 0.382 g anhydrous ammonium chloride (dried at 105°C for at least 2 h) in Milli-Q water, and dilute to 100 ml using volumetric flask: 1.00 ml = 1.00 mg N = 1.22 mg NH_3 . Stored in a stoppered glass bottle, this solution is stable for at least 1 month;
2. Borate buffer solution: Add 88 ml 0.1 N NaOH solution to 500 mL approximately 0.025 M sodium tetraborate solution (9.5 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O l}^{-1}$) and dilute to 1 l;
3. *Mixed indicator solution*: Dissolve 200 mg methyl red indicator in 100mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 ml 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly;
4. *Indicating boric acid solution*: Dissolve 20 g H_3BO_3 in water (heat if needed), add 10 ml mixed indicator solution, and dilute to 1 l. prepare monthly;
5. *Standard sulphuric acid titrant, 0.1N or 0.25N*: Dilute 2.72 or 6.80 ml concentrated sulphuric acid to 1000 mL with deionized water: 1.00 ml titrant = $14 \times \text{normality}$ mg N. (For 0.1N, 1.00ml = 1.4 mg N)

Procedure:

1. Starting up the distilling unit
 - a. Make sure that a digestion tube and a receiver flask are placed in their proper positions and that the safety window is pulled down. Check that the two valves at the rear of the unit are closed (handles parallel to the back);
 - b. Connect the tube labelled 'tap water' to the water tap, and put rest three tubes in the sink;
 - c. Switch on power, and open for steam by keeping the small black handle labelled 'steam' in the down position. (This valve should always be open when the unit is not in use);
 - d. Turn on the cold water tap to a flow of about 1.5litre/min for about half a minute and the water level should be visible through the top and then the bottom window at the left side of the distilling unit. Close the tap;
 - e. After a minute or so, open the water tap again for 10-15 seconds and watch the steam entering the digestion tube through the white Teflon tubing. After another minute open the water tap to a flow about 1.5litre/min and leave it open;
 - f. Let the distillation continue until about 150ml of distillate has been collected. Move the platform with the receiver flask to its lower position. Then close the steam valve on the front panel.

2. Sample preparation

a. Select sample volume and make up the volume to 100ml by deionised water if the sample volume less than 100ml. The following table is useful in selecting sample volume for the distillation and titration method;

Ammonia Nitrogen in Sample (mg l ⁻¹)	<200	200-500	500-1000	>1000
Sample Volume (ml)	100	50	25	10

b. Add 5 mL borate buffer solution and adjust to pH 9.5 with 10 N NaOH using a pH meter.

3. Distillation

- Remove the digestion tube using heat protective gloves and the receiver flask;
- Place the digestion tube with the sample to be distilled in its position and place the receiver flask with 25ml indicating boric acid solution on the platform;
- Close the safety window;
- Move the platform for the receiver flask to its upper position;
- Open the steam valve. The boric acid receiver solution in the distillate flask will soon be green indicating the presence of ammonia;
- When the indicating solution reaches the 150 ml mark of the flask, lower the platform for the receiver flask to its lower position. Close the steam valve and wait for a few more seconds to clean out the outlet tip;
- Replace the digestion tube and the receiver flask with the next ones and continue in the same manner with all the samples. When removing a digestion tube, the teflon tube through which the steam enters the sample, should be placed in the metal clip. This makes it possible to replace a new digestion tube without touching the teflon tube;
- It is better to titrate ammonia in distillate before distilling the next sample to allow the water to cool down in the distilling unit.

4. Titration

- Titrate ammonia in distillate with standard H₂SO₄ titrant until indicator turns to pale lavender;
- Carry a blank through all steps of the procedure and apply the necessary correction to the results;
- Carry a standard ammonia solution through all steps of the procedure to check the validation of the results.

5. Closing down the distilling unit

- Put an empty digestion tube and receiver flask into their position and close the water tap;
- Remove the drain trough under the tube holder and the platform for the receiver flask and clean it with water. Wipe the unit clean from any spillage. Close the safety window;
- Switch off power and open the valve in the middle position at the rear of the unit (handles vertical to the back) and drain out the water left in the distillation unit;
- Leave the steam valve open to prolong the life of the tubing in the valve.

Calculation:

a. Liquid samples: $mgNH_3 - N / L = \frac{(A - B) \times 14 \times N \times 1000}{mL(sample)}$

b. Sludge or sediment samples: $mgNH_3 - N / kg = \frac{(A - B) \times 14 \times N \times 1000}{g(dry \cdot wt \cdot sample)}$

where:

A = volume of H₂SO₄ titrated for sample, ml;

B = volume of H₂SO₄ titrated for blank, ml;

N = normality of standard sulphuric acid titrant.

Method 12 Determination of the four day dynamic respiration rate (DR4)

General discussion:

This test method provides a measure of the biodegradability over four days of any solid organic waste whether it is composed of readily biodegradable organic matter or treated stabilized or poorly biodegradable organic matter. DRI, or dynamic respiration index, is a reference to the method description where the test is aerated by passing air through the waste. This definition is used to differentiate the method from those where aeration is by diffusion of air into and out of the test material, which here are referred to as SRI or static respiration index. DRI is quantified by calculating the value of four day cumulative oxygen consumption (AT4), the cumulative oxygen consumption over four days. Reporting units are on both on a total solids (TS) and volatile solids (VS) basis, i.e. mg O₂/kg TS and mg O₂/kg VS respectively.

When WRc carried out a programme of research on behalf of the Environment Agency in which different methods for evaluating the stability were assessed for the purposes of setting landfill acceptance criteria or for estimating the diversion equivalence for MBT type processes. This research report (Godley *et al.* 2005) gave comparative results for the use of different test methods available and it is from this work that the EA guidance note was derived. DRI is also widely used in Europe as the means of expressing acceptability for landfill disposal, and it is stated in the second draft of the Europe Biowastes Directive which is currently being sub-summed into the European Soils Strategy.

The choice of a DRI test as opposed to the determination of specific oxygen uptake rate (SOUR) is based on the better correlation of the former with the Biochemical Methane Potential (BMP) test.

Principle:

The sample is mixed with a mature compost that provides a good source of microbes (seed) able to degrade the test material. The mixture is incubated under aerobic conditions by aerating the mixture in a vessel through which air is blown. The microbes degrade the test waste producing CO₂ as the decomposition product, which is evolved and found in the exhaust gas stream of the system. Either the oxygen consumption is measured directly or the CO₂ production is then measured as a measured and converted to oxygen consumption units.

Reaction vessels:

PVC cylindrical vessels of 160 mm diameter and 5 litres volume with a stainless steel perforated plate fitted in the bottom to support the test material and to allow an even gas purge in an upward direction. The vessel is sealed at the top with a gasket and top plate with a gas exhaust outlet and gas sampling loop.

Gas monitoring equipment:

Oxygen is measured directly using a Siemens OXYMAT 6 continuous gas analyzer. The measuring principle uses the paramagnetic effect of the oxygen molecule by comparison to a reference gas.

Seed compost source:

The seed compost is a mature compost derived from a commercial composting site (Veolia, Farham, Hampshire) which has been sieved through a 5mm aperture. The sieved material should be analyzed for:

1. Total solids and moisture content;
2. Volatile solids and ash content.

It should have an organic matter content (VS) of at least 25% of the total solids content and a biodegradability (AT4 value) of be between 2000 and 20000 mg O₂ kg⁻¹ VS.

Seed storage:

The compost seed can be stored in a cold room at <5 °C until required. The microbial activity is then restored before use by incubating the compost seed at room temperature for at least 48 hours before use.

Organic waste sample:

The digestate samples are taken from representative weights of the composite material and can be used fresh if they can be prepared so as to pass through a 5mm sieve, otherwise the samples are dried at 70°C and ground. In both cases the sample total solids (at 105°C) and volatile solids (at 550°C) are determined.

Control substrate cellulose:

A control substrate can be used to check the equipment; this should comprise a commercial cellulose material, which gives a moderate biodegradability response in the test. Typically cellulose from Sigma (product code C8002) has the approximate characteristics as follows:

Moisture content (% wet weight): 4.2

Volatile solids (% TS): 99.4

TOC content (% TS): 41.7

Total N content (% TS): <0.2

Biodegradability (DR4 test AT4: mg O₂ kg⁻¹ VS) 84900

The DR4 AT4 value was determined by the Environment Agency and is an approximate guide only.

Test mixture preparation:

1. Mixture of seed and test

The test mixture is prepared by mixing thoroughly 500 g by dry weight (TS) of test substrate with 500 g by dry weight of seed compost. The seed is also used as control and is composed of 1000 g by dry weight of the seed compost.

2. Nutrient addition (N and P) and moisture adjustment

The test mixture is supplemented with the following nutrients as a measure to ensure sufficient nutrient are present for microbial growth especially when the N and P content of the test substrate is not known. The amounts added should supplement any deficiency in the mixture:

40 ml of 2 M NH₄Cl – supplies about 0.28 g N per 100 g test substrate dry weight.

8 ml of 2 M KH_2PO_4 (adjusted to pH 7.0 with NaOH) – supplies 0.124 g P per 100 g test substrate dry weight.

The test mixture is supplemented with distilled or de-ionized water to give a final moisture content of 50% on a wet weight basis, i.e. 1000 g. The amount of water added takes into account the moisture already in the waste and seed, and that added in the nutrient additions:

$$\text{Water added (ml)} = 800 - (\text{g moisture in seed} + \text{g moisture in test} + 48)$$

The water and nutrient are mixed first and then added to the seed/compost mixtures and thoroughly mixed to ensure the mixture is evenly wetted. Then sub-samples are selected randomly from the prepared mixture.

Setting up the system:

1. Configuration

The configuration of the DR4 test system at Southampton University is shown schematically in Figure M12.1. Four respirometers are available in this system and these are set up as one for empty blank, one for seed control and two for test substrate mixtures.

2. Incubation temperature

The incubation temperature is controlled by an external thermostatic circulator which passes temperature controlled water through heating coils in contact with the reactor walls. The reactors themselves are contained in an insulated box from which they can be removed for filling. The temperature is variable but will be set at 35°C to be most comparable with the temperature of the anaerobic BMP method.

3. Air supply and flow

This is provided by a compressed air line feeding the base of each reactor, the flow is regulated by a needle valve rotometer. The air flow rate is set by the rotometer needle valves with typical value of 30-200 ml per minute. The exhaust gas flow is measured continuously using a microbridge mass airflow detector (AMW3100V, Honeywell, USA) and recorded via a data logger. The air is dewatered before passage to the airflow meter by means of a dessicant column.

4. Exhaust gas monitoring

A gas sampling loop is fitted to allow head space gas analysis from each of the reactors in turn. The sample time can be set as required, but typically this is set at 15 minutes per hour for each reactor. The head space gas is first passed through a desiccant column before being passed to the analyzer. The exhaust gas from the analyzer passes back to the head space of the reactor. When the sample stream switches from one reactor to the next there is a delay in returning the exhaust gas to the new reactor which corresponds to the dead space volume of the reactor gas sampling loop; this ensures there is no cross contamination between reactors.

5. Gas sampling and analysis

The exhaust gas from each respirometer is sampled for 1 minute every 15 minutes by diverting the flow using a solenoid valve into a gas impermeable sampling bag (Tedlar bag). The volume sampled is thus proportional to the flow at that time and the total volume of gas collected is 6.66% of the total gas passed. This gas sample is available

throughout the 4 day experiment or can be analyzed as a single composite at the end of the test. The gas sample is analyzed by gas chromatography for the content of CO₂, O₂, and also for trace gases such as methane.

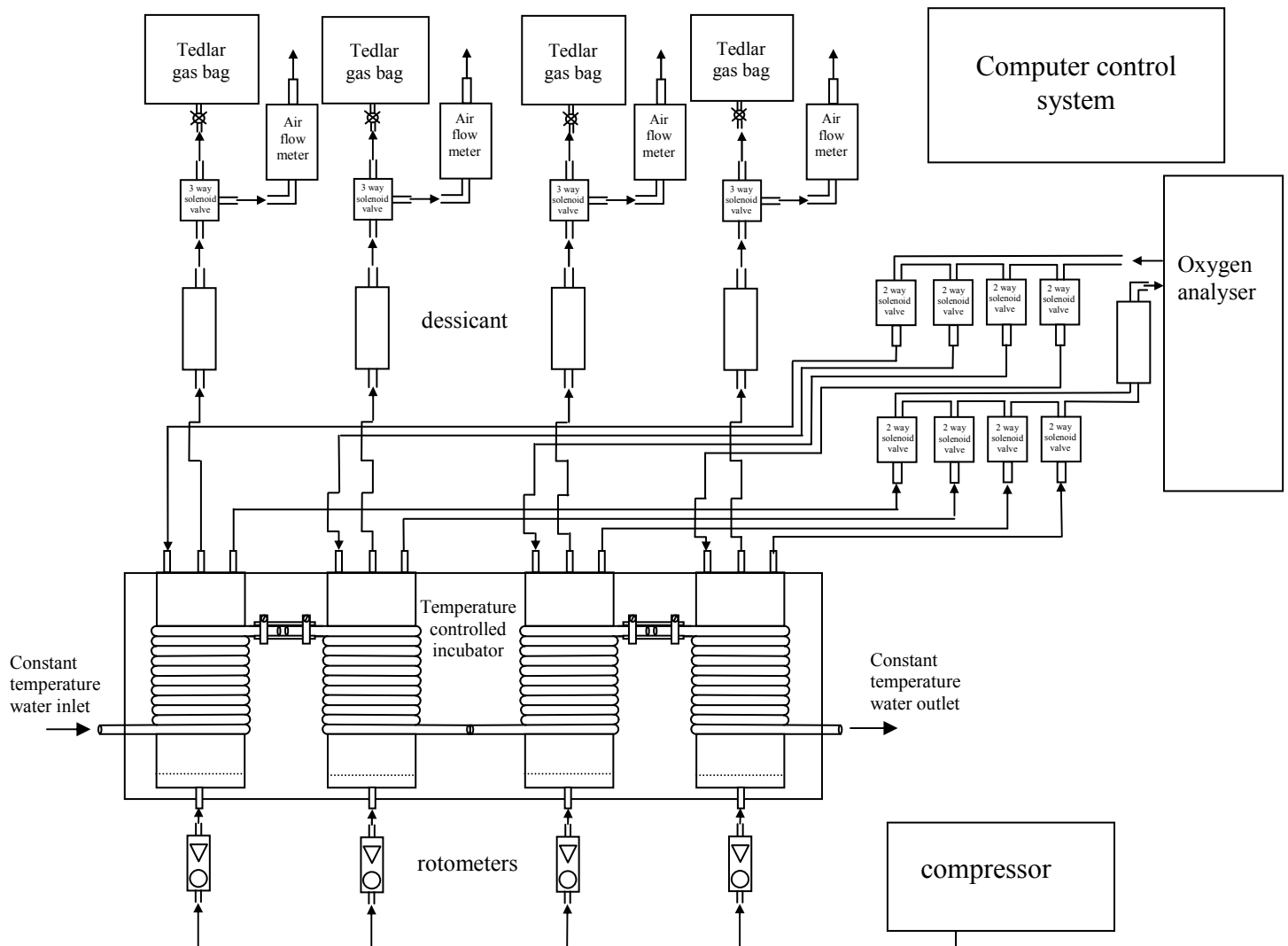


Figure M12.1 Set up for DR4 measurement by on-line O₂ analyser with gas sampling and mass airflow measurement

Estimating the AT4 from the DR4 measurement:

1. Drift compensation

The blank test containing no waste test material measures the O₂ present in the gas flowing through the system. Although the oxygen analyzer is very stable this gives a measure of instrument drift over the experimental period. The value in oxygen each of the reactor gas streams is compared to this control value on an hourly basis and any analyzer variability is corrected for drift.

2. Seed adjustment

The test results are then adjusted for the O₂ consumption from the seed. As only half the amount of seed was used in the tests compared with the seed control the test results are

adjusted by taking away half of the seed control O₂ production to give the O₂ production from the test material.

3. Expression of the results

The O₂ consumed can be assumed on a 1:1 molar ratio to CO₂ production as estimated from the concentration of CO₂ in the composite flow proportional gas samples collected from each reactor for 1 minute every 15 minutes over the 4 day operational period. The results are expressed directly in terms of an AT4 value (four day cumulative oxygen consumption) by summing the data of the four days of operation of the respirometer and expressing the results in terms of both VS and TS, i.e. mg O₂ kg⁻¹ VS and mg O₂ kg⁻¹ TS respectively.

References:

1. Assessment of options and requirements for stability and maturity testing of composts (2005). ADAS Consulting Limited Published by: The Waste and Resources Action Programme, The Old Academy, 21 Horsefair, Banbury, Oxon OX16 0AH ISBN 1-84405-057-2
2. Godley A. R., Graham A. and Lewin K., (2005) Estimating biodegradable municipal solid waste diversion from landfill: Monitoring biodegradable municipal waste removal in a Mechanical Biological Treatment process Environment Agency R&D Technical Report P1 - 513 (EP 0173)
3. Godley A. R., Graham A. and Lewin K., (2005) Estimating biodegradable municipal solid waste diversion from landfill: Screening exercise to evaluate the performance of biodegradable waste test methods, R&D Technical Report P1-513 (EP0173), WRC plc;
4. Guidance on monitoring MBT and other pre-treatment processes for the landfill allowances schemes (England and Wales). Environment Agency, August 2005

Method 13 Determination of Kjeldahl Nitrogen

General discussion:

The Kjeldahl method is a means of determining the nitrogen content (in organic and ammonia form) of substances. This method may be broken down into three main steps:

1. Digestion – the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulphuric acid. The end result is an ammonium sulphate solution;
2. Distillation – adding excess base to the acid digestion mixture to convert NH_4^+ to NH_3 , followed by boiling and condensation of the NH_3 gas in a receiving solution;
3. Titration – to quantify the amount of ammonia in the receiving solution.

Apparatus:

1. Tecator 1007 digestion system;
2. Kjeltac 1002 distilling unit;
3. Kjeldahl digestion tubes;
4. 250-mL Erlenmeyer flasks;
5. Analytical balance with an accuracy of 1 mg.

Reagents:

1. *Sulphuric acid concentrated*;
2. *Digestion catalyst: Kjeltabs Cu 3.5*;
3. *Standard Ammonium Chloride Solution*: dissolve 0.382 g anhydrous ammonium chloride (dried at 105°C for at least 2 h) in Milli-Q water, and dilute to 100 ml using volumetric flask: 1.00 ml = 1.00 mg N = 1.22 mg NH_3 . Stored in a stoppered glass bottle, this solution is stable for at least 1 month;
4. *Mixed indicator solution*: Dissolve 200mg methyl red indicator in 100ml 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 ml 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly;
5. *Indicating boric acid solution*: Dissolve 20 g H_3BO_3 in water (heat if needed), add 10 ml mixed indicator solution, and dilute to 1 l. prepare monthly;
6. *Standard sulphuric acid titrant, 0.10N or 0.25N*: Dilute 2.72 or 6.80 ml concentrated sulphuric acid to 1000 ml with deionized water: 1.00 ml titrant = $14 \times \text{normality}$ mg N. (For 0.1N, 1.00ml = 1.4 mg N)

Safety:

The Kjeldahl method requires the digestion of the sample using strong acid at high temperatures. Careful handling of the solutions is mandatory for laboratory safety. For added protection, acid digestions should be performed in a fume hood with adequate ventilation. Eye protection should be worn at all times and care should be taken when handling hot digestion tubes.

Sample weight:

The actual weight of sample required is dependent on nitrogen content and homogeneity. When homogeneity of sample is not a controlling factor the sample weight can be selected relative to the nitrogen content. Using a titrant concentration of 0.25 N the analytical sample should ideally contain 10-100 mg N. For selecting the size of sample a rough guide is given below:

Homogeneous solid samples	0.1-1.0 g
Non-homogeneous semi-solid samples	1.0-3.0 g or more
Liquid samples (depend on N content)	1.0-100 ml

Procedure:**1. Digestion**

- a. Switch on the digestion block and set the temperature to 420°C;
- b. Weigh an appropriate amount of sample to an accuracy of 0.1 mg, or measure a certain amount of sample if sample is liquid, into a digestion tube. A blank should be run through all steps of the procedure to compensate for any contribution from the reagents used;
- c. Add two Kjeltabs Cu 3.5;
- d. Carefully add 12 ml of concentrated H₂SO₄ and gently shake to 'wet' the sample with acid. If sample contains high-fat or carbohydrate, then use 15 ml H₂SO₄ and 1-3 drops of octanol as anti-foaming agent;
- e. Attached the exhaust system to the digestion tubes and secure it with PTFE tape;
- f. Set the water aspirator to full effect;
- g. Load the rack with exhaust into the preheated digestion block;
- h. After about 5 minutes turn down the water aspirator until the acid fumes are just contained within the exhaust head;
- i. Continue to digest until all samples are clear with blue / green solution. This will normally be after 60-120 minutes;
- j. Remove the tubes with exhaust still in place from digestion block and put them in the stand to cool for 10-20 minutes;
- k. Carefully add deionised water to the tubes to make up the volume to about 100 ml. For solid samples, this step should be done while the digestion mixture is still warm to avoid K₂SO₄ salting out.

2. Distillation

Starting up the distilling unit, which can be done when cooling the digestion mixture:

- a. Make sure that a empty digestion tube and a receiver flask are placed in their proper positions in the distilling unit and that the safety window is pulled down. Check that the two valves at the rear of the unit are closed (handles parallel to the back);
- b. Connect the tube labelled 'tap water' to the water tap, and put rest three tubes in the sink;
- c. Switch on power, and open for steam by keeping the small black handle labelled 'steam' in the down position. (This valve should always be open when the unit is not in use);
- d. Turn on the cold water tap to a flow of about 1.5litre/min for about half a minute and the water level should be visible through the top and then the bottom window at the left side of the distilling unit. Close the tap;
- e. After a minute or so, open the water tap again for 10-15 seconds and watch the steam entering the digestion tube through the white Teflon tubing. After another minute open the water tap to a flow about 1.5litre/min and leave it open;
- f. Let the distillation continue until about 150ml of distillate has been collected. Move the platform with the receiver flask to its lower position. Then close the steam valve on the front panel;
- g. Remove the digestion tube using heat protective gloves and the receiver flask.

Distilling the digestion mixture:

- a. Place the digestion tube with diluted digestion mixture in its position in distilling unit and place the receiver flask with 25ml indicating boric acid solution on the platform;
- b. Close the safety window;
- c. Move the platform for the receiver flask to its upper position so that the distillate outlet is submerged in the receiver solution;
- d. Gently press the alkali handle half way down to dispense about 50ml of 40% NaOH;
- e. Open the steam valve. The boric acid receiver solution in the distillate flask will soon be green indicating the presence of ammonia;
- f. When the indicating solution reaches the 150 ml mark of the flask, lower the platform for the receiver flask to its lower position. Close the steam valve and wait for a few more seconds to clean out the outlet tip;
- g. Replace the digestion tube and the receiver flask with the next ones and continue in the same manner with all the samples. When removing a digestion tube, the teflon tube through which the steam enters the sample, should be placed in the metal clip. This makes it possible to replace a new digestion tube without touching the teflon tube;
- h. It is better to titrate ammonia in distillate before distilling the next sample to allow the water to cool down in the distilling unit.

Closing down the distilling unit

- a. Put an empty digestion tube and receiver flask into their position and close the water tap;
- b. Remove the drain trough under the tube holder and the platform for the receiver flask and clean it with water. Wipe the unit clean from any spillage. Close the safety window;
- c. Switch off power and open the valve in the middle position at the rear of the unit (handles vertical to the back) and drain out the water left in the distillation unit;
- d. Leave the steam valve open to prolong the life of the tubing in the valve.

3. Titration

- a. Titrate ammonia in distillate with standard 0.10 or 0.25 N of H₂SO₄ titrant until indicator turns to pale lavender.

Calculation:

$$a. \text{ Liquid samples: } mgN / L = \frac{(A - B) \times 14.0 \times N \times 1000}{mL(sample)}$$

$$b. \text{ Solid samples: } \%N = \frac{(A - B) \times 14.0 \times N \times 100}{mg(dry \cdot wt \cdot sample)}$$

where:

A = volume of H₂SO₄ titrated for sample, ml;

B = volume of H₂SO₄ titrated for blank, ml;

N = normality of standard sulphuric acid titrant.

Notes:

1. When the sample content of fats and / or carbohydrates is high, 1-3 drops of octanol, an antifoam agent, should be used to control the tendency for foaming;

2. Pure substances of known nitrogen content can be used as the calibration substances, for example acetanilide (C_8H_9NO), L-aspartic acid ($C_4H_7NO_4$), or amino acids of known composition;
3. Kjeldahl digestion does not always recover all forms of nitrogen in a sample. Nitrate and nitrate ions (which are unlikely to be present in digestion) in a sample must first be reduced prior to acid digestion for quantitative recovery. Salicylic acid followed by sodium thiosulfate has been used to pretreat the mixture to ensure complete reduction. For detailed procedure, please refer to BS EN 13654-1:2001 Soil improvers and growing media – Determination of nitrogen – Part 1: Modified Kjeldahl method.

Method 14 Microwave assisted acid digestion for the determination of total nutrients and potentially toxic elements

Principle:

The objective of the method is to remove the organic matrix of the sample and leave the elements dissolved in the solution phase. The final elemental concentrations represent closely the total concentration of the element present in the sample.

A representative sample of up to 0.5 g dry matter is digested in 10 ml of concentrated nitric acid using microwave heating with a laboratory microwave unit. The sample and acid are placed in fluorocarbon microwave vessels. The vessels are sealed and heated in the microwave system. The temperature profile is specified to permit specific reactions and incorporates reaching 180 ± 5 °C in approximately less than 5.5 minutes and remaining at 180 ± 5 °C for 9.5 minutes for the completion of specific reactions.

After cooling, the vessel contents are diluted to volume. The diluted extract is allowed to settle overnight and then decanted for determination of extracted elements. The extracted phosphorus (P) is determined by spectrophotometer; potassium (K) and heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) by flame atomic absorption spectrometer (AAS); and Mercury (Hg) by cold-vapour atomic fluorescence spectrometry.

Reagent:

1. *Concentrated nitric acid, HNO₃*: purified and certified for trace element analysis.
2. *Concentrated hydrochloric acid, HCl*;

Apparatus:

1. Microwave digestion system (MARS X, CEM Corporation, NC, USA): Before use, all digestion vessels should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80 °C but less than boiling) for a minimum of two hours followed with hot (1:1) nitric acid (greater than 80 °C but less than boiling) for a minimum of two hours and rinsed with deionised water and dried in a clean environment;
2. Volumetric flasks, 50 ml capacity;
3. Funnels, suitable for 50 ml volumetric flask;
4. Storage containers, 50ml: Before use, glassware and plastic-ware should be cleansed by carefully immersing in warm (1:9) nitric acid for a minimum of 6 h and then rinsed in deionised water and dried in a clean environment;
5. Analytical balance, with an accuracy of 1 mg.

Microwave digestion procedure:

1. Weigh or pipette a well-mixed sample into the fluorocarbon digestion vessel. Adjust sample size to obtain a sample of between 0.3 to 0.5 g of solids. Triplicate for each sample.
 - a. For liquid samples, pipette 10 to 15 ml of the sample into the vessel. Record sample size. Rinse pipette with deionised water into the digestion vessel and bring all vessels up to approximately 20 ml to ensure constant sample volume of vessels during the microwave digestion;
 - b. For solid or semi-solid samples, weigh 0.1 to 5 g of sample into the vessel to achieve a final sample size that yields from 0.3 to 0.5 g of solids. Record sample weight. Bring all

samples up to approximately 5 ml to ensure equal sample volume of vessels during the microwave digestion;

c. It is important that all vessels contain the same volume of sample. Consequently, liquid sample should be digested with other liquid samples, and solid sample should be digested with other solid samples;

2. Prepare three blanks by adding 20 ml (for liquid sample analysis) or 5 ml (for solid or semi-solid sample analysis) of deionised water into three empty digestion vessels;

3. Pipette 10 ml of concentrated nitric acid into each vessel in a fume hood. If a vigorous reaction occurs, allow the reaction to stop before capping the vessel.

4. Cap the vessel and assembly the pressure relief membrane. Secure the vessel in its support module. Use the vessel with largest sample size as the control vessel for temperature and pressure control, and secure it in the control support module;

5. Place the support modules onto the microwave turntable. Connect the pressure sensor and the temperature sensor with the control support module;

6. Select the 'USEPA SW-846 3052' program and start the digestion;

7. At the end of the microwave program, allow the vessels to cool for a minimum of 1 h before removing them from the microwave unit.

8. Place the support module in the fume hood. Vent vessels by grasping the support module with one hand and loosening the Telfon vent fitting by slowly turning it in a counter clockwise direction. Remove the vessel from the support module;

9. Transfer the digested sample to 50 ml volumetric flask. Rinse the vessel and its cover with deionised water into the flask. Make up the volume;

10. Allow the diluted sample to stand overnight. Decant the supernatant into the storage container.

Determination of extracted elements:

1. Phosphorus (P): see the method of "Spectrophotometric determination of orthophosphate after microwave digestion";

2. Potassium (K): see the method of "Determination of potassium with flame atomic absorption spectrometry after microwave digestion";

3. Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn): see the method of "Determination of heavy metals with flame atomic absorption spectrometry after microwave digestion";

4. Mercury (Hg): see the method of "Determination of mercury with cold-vapour atomic fluorescence spectrometry after microwave digestion".

Expression of results:

1. When calculated on a real 'wet' weight basis:

$$C_w = \frac{(C_d - C_b) \times 50}{m}$$

where,

C_w : Concentration of nutrient (P or K) or potentially toxic elements in sample, mg kg^{-1} ;

C_d : Concentration of the corresponding element in the digestion supernatant, mg l^{-1} ;

C_b : Concentration of the corresponding element in blank, mg l^{-1} ;

50: The volume of final diluted solution, ml;

m: The mass of the sample, g.

2. To determine results on a dry weight basis, a separate determination of percent solids must be performed in parallel with the microwave digestion process:

$$C = \frac{C_w}{TS}$$

where,

C: Concentration of elements in sample on a dry weight basis, mg kg^{-1} ;

TS: Total solids, %.

Notes:

1. The method given here does not address the dissolution of silica that may be in the sample. To determine the total element concentration in silica complexes, 3 ml of hydrofluoric acid should be added with 10 ml of nitric acid;

2. When the digested sample cannot get clear supernatant after standing overnight, centrifuge the sample at 3000 rpm for 10 minutes.

Reference:

1. SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Method 3052 Microwave assisted acid digestion of siliceous and organically based matrices (1996), Environmental Protection Agency, USA;
2. BS EN 13650:2001 Soil improvers and growing media – Extraction of aqua regia soluble elements;
3. BS 7755-3.9:1995 ISO 11466:1995 Soil quality – Part 3: Chemical methods – Section 3.9 Extraction of trace elements soluble in aqua regia;
4. Recommended methods of mature analysis (A3769), John Peters, Cooperative Extension Publishing, US.

Method 15 Extraction of calcium chloride/DTPA soluble P and K

Principle:

Calcium chloride/DTPA soluble nutrients represent the nutrients available for plant, which including water soluble, exchangeable and adsorbed nutrients. In this test, the sample is extracted with calcium chloride/DTPA solution at $22 \pm 3^\circ\text{C}$ in an extraction volume ratio of 1:5 for 1 h. The extracted phosphorus (P) is determined by spectrophotometer and potassium (K) is determined by flame atomic absorption spectrometer (AAS).

Reagent:

1. *Concentrated extracting CaCl_2 /DTPA solution (CAT)*: dissolve 14.7 g of dihydrated calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and 7.88 g of Diethylenetriaminepentaacetic acid (DTPA, $\text{C}_{14}\text{H}_{23}\text{N}_3\text{O}_{10}$) in 800 ml of hot deionised water ($\sim 80^\circ\text{C}$) with stirring on a magnetic stirrer in a 1000ml volumetric flask. The reagents will dissolve within 2 h. Allow to cool to ambient temperature. Dilute to the mark with deionised water. The solution is stable at room temperature for a month. Any precipitation that occurs will disappear with warming and stirring;
2. *Extracting CaCl_2 /DTPA solution (CAT)*: dilute the above concentrated extracting solution with deionised water in the proportions of one part concentrated solution with nine parts deionised water. The final concentration of the extracting solution should be 0.01 M of CaCl_2 and 0.002 M of DTPA. The pH of the solution should be 2.6. Adjust the pH is necessary using nitric acid;
3. *Nitric acid, ~ 0.5 M*: put 35 ml of concentrated nitric acid into deionised water and make up to 1 l.

Apparatus:

1. 500 ml plastic bottles with screw cap. Cleansed by carefully immersing in warm nitric acid with concentration of 0.5 M for a minimum of 6 h and then rinsed in deionised water;
2. Analytical balance, with an accuracy of 0.01 g;
3. Rotary shaking machine with temperature control;
4. Vacuum filtration device and Whatman 40 filter papers.

Extraction procedure:

1. Place a weight equivalent to 60 ml of the sample volume to the nearest 0.01 g into the plastic bottle. Add 300 ml of deionised water into the plastic bottle. Secure the cap. Triplicate for each sample;
2. Prepare three blanks by adding 300 ml of extracting CaCl_2 /DTPA solution into each of the three empty bottles;
3. Shake the bottles on the rotary shaking machine at $22 \pm 3^\circ\text{C}$;
4. Filter the extraction solution through a filter paper. Discard at least the first 10 ml of filtrate. Rinse the filter device and change filter paper between bottles.

Determination of extracted nutrients:

1. Phosphorus (P): see the method of "Spectrophotometric determination of orthophosphate in CaCl_2 /DTPA extract";

2. Potassium (K): see the method of “Determination of potassium in CaCl₂/DTPA extract with flame atomic absorption spectrometry”.

Expression of results:

$$C = \frac{(C_e - C_b) \times 300}{60}$$

where,

C: Concentration of nutrient (P or K) in sample, mg l⁻¹;

C_e: Concentration of the corresponding nutrient in the filtered extract, mg l⁻¹;

C_b: Concentration of the corresponding nutrient in blank, mg l⁻¹;

300: The volume of deionised water added for extraction, ml;

60: The volume of the sample, ml.

Notes:

1. When paper filtration is too slow or even impossible, centrifugation at 3000rpm for 10 minutes needs to be used as a pre-treatment;
2. The filtered extract is stable for 3 days in a closed polyethylene bottle if stored in a fridge below 5°C.

Reference:

1. BS EN 13651:2001 Soil improvers and growing media – Extraction of calcium chloride/DTPA (CAT) soluble nutrients.

Method 16 Extraction of water soluble ammoniacal-N, P and K

Principle:

Water soluble nutrients represent the readily available nutrients for plant, like industrial fertilizer. In this test, the sample is extracted with deionised water at $22 \pm 3^\circ\text{C}$ in an extraction volume ratio of 1:5 for 1 h. The extracted ammoniacal-N and P are determined by spectrophotometer and K is determined by flame atomic absorption spectrometer.

Reagent:

1. Nitric acid, ~0.5 M: put 35 ml of concentrated nitric acid into deionised water and make up to 1 l.

Apparatus:

1. 500 ml plastic bottles with screw cap. Cleansed by carefully immersing in warm nitric acid with concentration of 0.5 M for a minimum of 6 h and then rinsed in deionised water;
2. Analytical balance, with an accuracy of 0.01 g;
3. Rotary shaking machine with temperature control;
4. Vacuum filtration device and Whatman 40 filter papers.

Extraction procedure:

1. Place a weight equivalent to 60 ml of the sample volume to the nearest 0.01 g into the plastic bottle. Add 300 ml of deionised water into the plastic bottle. Secure the cap. Triplicate for each sample;
2. Prepare three blanks by adding 300 ml of deionized water into each of the three empty bottles;
3. Shake the bottles on the rotary shaking machine at $22 \pm 3^\circ\text{C}$;
4. Filter the extraction solution through a filter paper. Discard at least the first 10 ml of filtrate. Rinse the filter device and change filter paper between bottles.

Determination of extracted nutrients:

1. Ammoniacal-N: see the method of “Spectrophotometric determination of ammonia in water”;
2. Phosphorus (P): see the method of “Spectrophotometric determination of orthophosphate in water”;
3. Potassium (K): see the method of “Determination of potassium in water with flame atomic absorption spectrometry”.

Expression of results:

$$C = \frac{(C_e - C_b) \times 300}{60}$$

where,

C: Concentration of nutrient (Ammoniacal-N, P, or K) in sample, mg l^{-1} ;

C_e : Concentration of the corresponding nutrient in the filtered extract, mg l^{-1} ;

C_b : Concentration of the corresponding nutrient in blank, mg l^{-1} ;

300: The volume of deionised water added for extraction, ml;

60: The volume of the sample, ml.

Notes:

1. When paper filtration is too slow or even impossible, centrifugation at 3000rpm for 10 minutes needs to be used as a pre-treatment;
2. The filtered extract is stable for 3 days in a closed polyethylene bottle if stored in a fridge below 5°C.

Reference:

1. BS EN 13652:2001 Soil improvers and growing media – Extraction of water soluble nutrients and elements.

Method 17 Determination of Electrical Conductivity

Principle of method:

Electrical conductivity (EC), a function of the ion concentration, is measured at room temperature using a self-contained conductivity meter in the undiluted liquid sample or in the supernatant of 1:2 (V/V) sample/water slurry for semi-solid or solid sample.

Apparatus:

1. Conductivity meter with temperature compensation and electrode;
2. Magnetic stirrer and Teflon-coated stirring bar;
3. Plastic centrifuge bottles, of sufficient capacity to accommodate the volume of the sample, deionised water and 10% air volume;
4. Analytical balance with an accuracy of 1 mg.

Reagents:

1. *Potassium chloride solution (0.100 M)*: dry KCl at 105°C for 2 h. Dissolve 7.456 g in deionised water and dilute to 1 l. The specific electrical conductivity of this solution at 25°C is 1290 mS/m.
2. *Potassium chloride solution (0.0100M)*: Add 100.0 ml of the above solution into a 1000 ml volumetric flask and dilute to the mark. The specific electrical conductivity of this solution at 25°C is 141 mS/m.

Procedure:

1. Calibration of the conductivity meter as prescribed in the manufacturer's instruction.
2. For liquid sample (when the aqueous phase constitutes at least 20% of the total volume of the sample):
 - a. Thoroughly mix liquid sample by inverting sample bottle several times;
 - b. Pour about 25 ml into a beaker;
 - c. Immerse EC electrode and temperature probe;
 - d. Record the EC value when the meter has stabilized.
3. For semi-solid or solid sample:
 - a. Place a weight equivalent to 20ml of the sample volume into a centrifuge bottle;
 - b. Add 40ml deionised water, secure the cap and mix for 1 h on the magnetic stirrer;
 - c. Centrifuge the mixture;
 - d. Immerse the electrodes into the supernatant;
 - e. Record the EC when the meter has stabilized and report the result as EC (water 1:2).

Note:

1. If the waste is hygroscopic and absorbs all the deionised water, begin the experiment again using 20ml of waste and 100 ml of deionised water. Modifications in the sample/water ratio must be denoted with the results.

Reference:

1. BS EN 13038:2000 Soil improvers and growing media – Determination of electrical conductivity.

Method 18 Spectrophotometric Determination of Orthophosphate after Microwave Digestion

Principle:

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely coloured molybdenum blue by ascorbic acid.

Calibration Curve:

Linear up to 1 mg l^{-1} using disposable cells with 1 cm path length.

Apparatus:

1. UV-Visible Scanning spectrophotometer (Ceceil 3000 series, Cecil Instruments Ltd., UK);
2. Analytical balance, with an accuracy of 1 mg;
3. 30 ml test tubes;
4. 100 ml volumetric flasks.

Reagents:

1-5. same as the reagents 1-5 in the method of “Spectrophotometric determination of orthophosphate in water”;

6. *Sodium nitrate solution, 0.63 M*: Dissolve 53.55 g of sodium nitrate (NaNO_3) into deionised water and make up to 1 l;

7. *Sodium hydroxide solution, 1 M*: Dissolve 40 g of sodium hydroxide (NaOH) into deionised water and make up to 1 l;

8. *Nitric acid, ~0.1 M*: put 0.63 ml of concentrated nitric acid into deionised water and make up to 100 ml.

9. *Phenolphthalein indicator* (0.5% solution in 50% isopropanol, w/v).

Procedure:

1. Take all the reagents out of the fridge;
2. Use standards of 0.25, 0.50, 0.75 and 1.00 mg l^{-1} by diluting 0.25, 0.5, 0.75, 1.0 ml of the stock standard with 0.63 M sodium nitrate solution in 50 ml volumetric flasks;
3. Dilute the sample with 4 times of deionised water first. If need dilute more, add 0.63 M sodium nitrate solution until the diluted concentration of phosphate is less than 1 mg l^{-1} (the optimal range is between $0.3\text{--}0.7 \text{ mg l}^{-1}$):
 - a. Decide the dilution factor (DF) by trial and error;
 - b. Put a calculated (by DF) amount of sample in a 50 ml volumetric flask and make up the volume to ~30 ml;
 - c. Add 1 drop of phenolphthalein indicator into the flask. Add 1 M NaOH solution drop by drop with shaking the flask until pink colour just appears;
 - d. Add one drop of 0.1 M HNO_3 solution to discharge the colour;
 - e. Make up the volume to the mark;

4. Pipette 10.0 ml of 0.63 M sodium nitrate solution (which is for reagent blank), standards and diluted samples into clean, dry test tubes in sequence.
5. Mix the reagents 2-5, when they reach room temperature, in the following proportions and order for 100 ml of the combined reagent: 50 ml 2.5 M H₂SO₄, 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. *Mix after addition of each reagent.* If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. This mixture is stable for 4 h.
6. Add 1.6 ml of the above combined reagent into each test tube and mix thoroughly with the liquid already in the tubes;
7. Switch on the spectrophotometer and let it warm up, then set its wavelength to 880nm;
8. Wait at least 10 min but no more than 30 min after adding combined reagent to the tubes, use the reagent blank (0.63 M sodium nitrate solution plus the combined reagent) to zero the spectrophotometer. Then measure absorbance of each standard and sample in sequence.

Plotting the calibration graph:

The relationship between absorbance and concentration is linear. Plot a graph of absorbance (as the *y*-axis) against the phosphate content (as the *x*-axis) in milligrams of phosphate per litre of the calibration solutions to give a straight line passing through the origin. Determine the slope (*s*) of the graph by Excel.

Expression of results:

$$C = \frac{A \times DF}{s}$$

where

C – concentration of orthophosphate, mg l⁻¹;

A – absorbance of the standard solution or the sample;

DF – dilution factor.

s – slope of the calibration graph, l mg⁻¹;

Notes:

1. p-nitrophenol indicator (0.25% solution in deionised water, w/v) also can be used to adjust pH when diluting the sample: add 5 drops of this indicator and add NaOH solution drop by drop with shaking until the yellow colour just appear.
2. Use acid-washed glassware for determining low concentrations of phosphate. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.
3. For highly coloured or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

Reference:

1. Clesceri Lenore S., Greenberg Arnold E., and Eaton Andrew D. (1998), 4500-P E. Ascorbic acid method, Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., 4-146-147.
2. ISO 6878: 2004 Water quality – Determination of phosphorus – Ammonium molybdate spectrometric method.

Method 19 Spectrophotometric Determination of Orthophosphate in CaCl_2 /DTPA Extract

Principle:

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely coloured molybdenum blue by ascorbic acid.

Calibration Curve:

Linear up to 1 mg l^{-1} using disposable cells with 1 cm path length.

Apparatus:

1. UV-Visible Scanning spectrophotometer (Ceceil 3000 series, Cecil Instruments Ltd., UK);
2. Analytical balance, with an accuracy of 1 mg;
3. 30 ml test tubes;
4. 100 ml volumetric flasks.

Reagents:

1-5. same as the reagents 1-5 in the method of “Spectrophotometric determination of orthophosphate in water”;

6. *Sodium hydroxide solution, 0.1 M*: Dissolve 4 g of Sodium hydroxide (NaOH) into deionised water and make up to 1 l;

7. *Standard hydrochloric acid titrant, 0.05M*: Dilute 4.9 ml of concentrated hydrochloric acid to 1000 mL with deionised water;

8. *Extracting CaCl_2 /DTPA solution (CAT)*: same as the reagent 2 in the method of “Extraction of calcium chloride/DTPA soluble P and K”, but adjust the pH to ~5 using 0.1 M sodium hydroxide solution;

9. *Phenolphthalein indicator* (0.5% solution in 50% isopropanol, w/v).

Procedure:

1. Take all the reagents out of the fridge;
2. Use standards of 0.25, 0.50, 0.75 and 1.00 mg l^{-1} by diluting 0.5, 1.0, 1.5, 2.0 ml of the stock standard with extracting CaCl_2 /DTPA solution in 100 ml volumetric flasks;
3. Dilute the sample with extracting CaCl_2 /DTPA solution until the diluted concentration of phosphate is less than 1 mg l^{-1} (the optimal range is between 0.3-0.7 mg l^{-1}):
 - a. Decide the dilution factor (DF) by trial and error;
 - b. Put a calculated (by DF) amount of sample in a 100 ml volumetric flask and make up the volume to ~50 ml;
 - c. Add 2 drops of phenolphthalein indicator into the flask. Add 0.1 M NaOH solution drop by drop with shaking the flask until pink colour just appears;
 - d. Add one drop of 0.05 M HCl solution to discharge the colour;
 - e. Make up the volume to the mark;

4. Pipette 10.0 ml of extracting $\text{CaCl}_2/\text{DTPA}$ solution (which is for reagent blank), standards and diluted samples into clean, dry test tubes in sequence.

5. Mix the reagents 2-5, when they reach room temperature, in the following proportions and order for 100 ml of the combined reagent: 50 ml 2.5 M H_2SO_4 , 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. *Mix after addition of each reagent.* If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. This mixture is stable for 4 h.

6. Add 1.6 ml of the above combined reagent into each test tube and mix thoroughly with the liquid already in the tubes;

7. Switch on the spectrophotometer and let it warm up, then set its wavelength to 880nm;

8. Wait at least 10 min but no more than 30 min after adding combined reagent to the tubes, use the reagent blank (extracting $\text{CaCl}_2/\text{DTPA}$ solution plus the combined reagent) to zero the spectrophotometer. Then measure absorbance of each standard and sample in sequence.

Plotting the calibration graph:

The relationship between absorbance and concentration is linear. Plot a graph of absorbance (as the y-axis) against the phosphate content (as the x-axis) in milligrams of phosphate per litre of the calibration solutions to give a straight line passing through the origin. Determine the slope (s) of the graph by Excel.

Expression of results:

$$C = \frac{A \times DF}{s}$$

where

C – concentration of orthophosphate, mg l^{-1} ;

A – absorbance of the standard solution or the sample;

DF – dilution factor;

s – slope of the calibration graph, l mg^{-1} ;

Notes:

1. p-nitrophenol indicator (0.25% solution in deionised water, w/v) also can be used to adjust pH when diluting the sample: add 5 drops of this indicator and add NaOH solution drop by drop with shaking until the yellow colour just appear.

2. Use acid-washed glassware for determining low concentrations of phosphate. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

3. For highly coloured or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

Reference:

1. Clesceri Lenore S., Greenberg Arnold E., and Eaton Andrew D. (1998), 4500-P E. Ascorbic acid method, Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., 4-146-147.
2. ISO 6878: 2004 Water quality – Determination of phosphorus – Ammonium molybdate spectrometric method.

Method 20 Spectrophotometric Determination of Orthophosphate in Water

Principle:

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely coloured molybdenum blue by ascorbic acid.

Calibration Curve:

Linear up to 1 mg l^{-1} using disposable cells with 1 cm path length.

Apparatus:

1. UV-Visible Scanning spectrophotometer (Ceceil 3000 series, Cecil Instruments Ltd., UK);
2. Analytical balance, with an accuracy of 1 mg;
3. 30 ml test tubes;
4. 100ml volumetric flasks.

Reagents:

1. *Orthophosphate stock standard solution, 50 mg l^{-1}* : dissolve 0.2195 g anhydrous potassium dihydrogen phosphate KH_2PO_4 (dried at 105°C) in deionised water, and dilute to 1000 ml using volumetric flask: $1.00 \text{ ml} = 50.0 \text{ } \mu\text{g PO}_4^{3-} - \text{P}$.
2. *Sulphuric acid, H_2SO_4 , 2.5M*: dilute 70 ml of concentrated H_2SO_4 (1.84 g ml^{-1}) to 500 ml with deionised water.
3. *Potassium antimonyl tartrate solution*: dissolve 0.2743 g of $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ in deionised water, and dilute to 100 ml. Store in a glass-stoppered bottle.
4. *Ammonium molybdate solution*: dissolve 20 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 500 ml of deionised water. Store in a glass-stoppered bottle.
5. *Ascorbic acid, 0.1 M*: dissolve 1.76 g of ascorbic acid in 100 ml of deionised water. The solution is stable for about 1 week at 4°C .

Procedure:

1. Take all the reagents out of the fridge;
2. Use standards of 0.25, 0.50, 0.75 and 1.00 mg l^{-1} by diluting 0.5, 1.0, 1.5, 2.0 ml of the stock standard with deionised water in 100 ml volumetric flasks;
3. Dilute the sample until the diluted concentration of phosphate is less than 1 mg l^{-1} (the optimal range is between 0.3-0.7 mg l^{-1});
4. Pipette 10.0 ml of deionised water (which is for reagent blank), standards and (diluted) samples into clean, dry test tubes in sequence.
5. Mix the reagents 2-5, when they reach room temperature, in the following proportions and order for 100 ml of the combined reagent: 50 ml 2.5 M H_2SO_4 , 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. *Mix after addition of each reagent*. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. This mixture is stable for 4 h.
6. Add 1.6 ml of the above combined reagent into each test tube and mix thoroughly with the liquid already in the tubes;
7. Switch on the spectrophotometer and let it warm up, then set its wavelength to 880nm;

8. Wait at least 10 min but no more than 30 min after adding combined reagent to the tubes, use the reagent blank (deionised water plus the combined reagent) to zero the spectrophotometer. Then measure absorbance of each standard and sample in sequence.

Plotting the calibration graph:

The relationship between absorbance and concentration is linear. Plot a graph of absorbance (as the y -axis) against the phosphate content (as the x -axis) in milligrams of phosphate per litre of the calibration solutions to give a straight line passing through the origin. Determine the slope (s) of the graph by Excel.

Expression of results:

$$C = \frac{A \times DF}{s}$$

where

C – concentration of orthophosphate, mg l^{-1} ;

A – absorbance of the standard solution or the sample;

DF – dilution factor.

s – slope of the calibration graph, l mg^{-1} ;

Notes:

1. If the sample is not within the range of pH 3 to pH 10, adjust it with sodium hydroxide or sulphuric acid solution, which can be done by using a pH meter, or: when adjust pH from higher than 10 to around neutral, add 2 drops of phenolphthalein indicator (0.5% solution in 50% isopropanol, w/v) as indicator until the pink colour just discharge; when adjust pH from lower than 3 to around neutral, add 5 drops of p-nitrophenol indicator (0.25% solution in deionised water, w/v) as indicator until the yellow colour just appear.

2. Use acid-washed glassware for determining low concentrations of phosphate. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

3. For highly coloured or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

Reference:

1. Clesceri Lenore S., Greenberg Arnold E., and Eaton Andrew D. (1998), 4500-P E. Ascorbic acid method, Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., 4-146-147.
2. ISO 6878: 2004 Water quality – Determination of phosphorus – Ammonium molybdate spectrometric method.

Method 21 Determination of Potassium with Flame Atomic Absorption Spectrometry after Microwave digestion

Principle:

Atomic absorption spectrometry (AAS) is an elemental analysis technique for determining the concentration of metal elements in liquid samples, which based on the absorption of UV or visible light by gaseous atoms. The metal elements in the liquid sample are atomised in a flame after injected into the AAS. Each element's atoms in the flame absorb precisely the wavelength sent by the source of light, a hollow-cathode lamp (HCL), special for this element. The absorption is proportional to the concentration of this element. Monochromator and photomultiplier tube are used as its detector. The concentration measurement with AAS is usually determined from a working curve after calibrating the instrument with standards of known concentration.

Potassium is partially ionised in the air-acetylene flame. To suppress ionisation, cesium nitrate is added to give a final concentration of 1000 mg l⁻¹ cesium in all solutions including the blank.

Apparatus:

1. Flame atomic absorption spectrometry (Spectr AA-200, Varian, Australia);
2. Analytical balance, with an accuracy of 1 mg;
3. Volumetric flasks;
4. 30 ml test tubes.

Reagents:

1. *Stock potassium nitrate solution, 1000 mg l⁻¹*: commercial;
2. *Cesium nitrate solution, 10g l⁻¹*: dissolve 1.466 g of cesium nitrate (CsNO₃) in the deionised water and make up to 100 ml;
3. *Diluted nitric acid solution (1+4)*: put 200 ml of concentrated nitric acid into deionised water and make up to 1 l.

Procedure:

1. Make the calibration solutions with the concentrations of 0.0, 0.2, 0.6, 1.0, 1.2, 1.6, 2.0 mg l⁻¹:
 - a. Pipette 0.00, 0.02, 0.06, 0.10, 0.12, 0.16, and 0.20 ml of the stock potassium nitrate standard into a series of 100 ml-volumetric flasks respectively;
 - b. Pipette 10 ml of cesium nitrate solution to each of the flasks;
 - c. Make up the volume with diluted nitric acid (1+4) for each flask;
2. Set up the atomic absorption spectrometer according to the operation instruction attached on the instrument. The working conditions for potassium determination are:
 - a. Lamp current: 5A;
 - b. Fuel: acetylene;
 - c. Support: air;
 - d. Wavelength: 766.5 nm;
 - e. Slit width: 1.0 nm;
3. Prepare the test portion by mixing 9 ml of sample with 1 ml of cesium nitrate solution;

4. After the lamp has warmed up (~30 minutes after setting up the AAS), calibrate the AAS by injecting the set of calibration solutions into it in ascending order;
5. Determination of test portion by aspirating it into the flame, and the concentration of test portion will be shown on the computer connected to the AAS. Measure each portion at least three times, and take the average (C_a);
6. If the potassium concentration of test portion is higher than 2 mg/l, dilute it proportionally with diluted nitric acid (1+4) to make up the volume of 9ml, and then mix it with 1 ml of cesium nitrate solution. For example, if the dilution factor (DF) is 5, then mix 1.8 ml of sample, 7.2 ml of diluted nitric acid (1+4) and 1 ml of cesium nitrate solution together as the test portion.

Expression of results:

$$C = \frac{C_a \times (9 + 1) \times DF}{9}$$

where

C – concentration of potassium, mg l⁻¹;

C_a – measured concentration shown on the screen, mg l⁻¹;

DF – dilution factor;

9 – portion of sample (or diluted sample) mixed with cesium nitrate solution, ml;

1 – portion of cesium nitrate solution mixed with sample (or diluted sample), ml.

Notes:

1. AAS should be re-calibrated after each 30 minutes of working time to avoid the baseline drift;
2. Spike analysis should be carried out with each sample type to assess the matrix effect. The percent recovery should be more than 95%. Otherwise, adjust the instrument parameters to overcome the interference.

References:

BS 7755-3.13: 1998 ISO 11047: 1998 Soil quality – Part 3: Chemical methods – Section 3.13: Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil – Flame and electrothermal atomic absorption spectrometric methods.

Method 22 Determination of Potassium in CaCl_2 /DTPA Extract with Flame Atomic Absorption Spectrometry

Principle:

Atomic absorption spectrometry (AAS) is an elemental analysis technique for determining the concentration of metal elements in liquid samples, which based on the absorption of UV or visible light by gaseous atoms. The metal elements in the liquid sample are atomized in a flame after injected into the AAS. Each element's atoms in the flame absorb precisely the wavelength sent by the source of light, a hollow-cathode lamp (HCL), special for this element. The absorption is proportional to the concentration of this element. Monochromator and photomultiplier tube are used as its detector. The concentration measurement with AAS is usually determined from a working curve after calibrating the instrument with standards of known concentration.

Potassium is partially ionised in the air-acetylene flame. To suppress ionisation, cesium nitrate is added to give a final concentration of 1000 mg/l cesium in all solutions including the blank.

Apparatus:

1. Flame atomic absorption spectrometry (Spectr AA-200, Varian, Australia);
2. Analytical balance, with an accuracy of 1 mg;
3. Volumetric flasks;
4. 30 ml test tubes.

Reagents:

1. *Stock potassium nitrate solution, 1000 mg l⁻¹*: commercial;
2. *Cesium nitrate solution, 10g l⁻¹*: dissolve 1.466 g of cesium nitrate (CsNO_3) in the deionised water and make up to 100 ml;
3. *Extracting CaCl_2 /DTPA solution (CAT)*: same as the reagent 2 in the method of "Extraction of calcium chloride/DTPA soluble P and K";
4. *Nitric acid solution, 3 M*: dilute 21 ml of concentrated nitric acid with deionised water in 100ml volumetric flask and make up the volume.

Procedure:

1. Make the calibration solutions with the concentrations of 0.0, 0.2, 0.6, 1.0, 1.2, 1.6, 2.0 mg l⁻¹:
 - a. Pipette 0.00, 0.02, 0.06, 0.10, 0.12, 0.16, and 0.20 ml of the stock potassium nitrate standard into a series of 100 ml-volumetric flasks respectively;
 - b. Pipette 10 ml of cesium nitrate solution to each of the flasks;
 - c. Pipette 0.5 ml of 3 M nitric acid solution to each of the flasks;
 - c. Make up the volume with extracting CaCl_2 /DTPA solution for each flask;
2. Set up the atomic absorption spectrometer according to the operation instruction attached on the instrument. The working conditions for potassium determination are:
 - a. Lamp current: 5A;
 - b. Fuel: acetylene;
 - c. Support: air;
 - d. Wavelength: 766.5 nm;
 - e. Slit width: 1.0 nm;

3. Prepare the test portion by mixing 9 ml of sample, 1 ml of cesium nitrate solution and 0.05 ml of 3 M nitric acid solution together;
4. When the lamp warm up (~30 minutes after setting up the AAS), calibrate the AAS by injecting the set of calibration solutions into it in ascending order;
5. Determination of test portion by aspirating it into the flame, and the concentration of test portion will be shown on the computer connected with the AAS. Measure each portion at least three times, and take the average (C_a);
6. If the potassium concentration of test portion is higher than 2 mg/l, dilute it proportionally with extracting $\text{CaCl}_2/\text{DTPA}$ solution to make up the volume of 9ml, and then mix it with 1 ml of cesium nitrate solution and 0.05 ml of nitric acid solution. For example, if the dilution factor (DF) is 5, then mix 1.8 ml of sample, 7.2 ml of extracting $\text{CaCl}_2/\text{DTPA}$ solution, 1 ml of cesium nitrate solution and 0.05 ml of nitric acid solution together as the test portion.

Expression of results:

$$C = \frac{C_a \times (9 + 1 + 0.05) \times DF}{9}$$

where

C – concentration of potassium, mg l^{-1} ;

C_a – measured concentration shown on the screen, mg l^{-1} ;

DF – dilution factor;

9 – portion of sample (or diluted sample) mixed with cesium nitrate solution, ml;

1 – portion of cesium nitrate solution mixed with sample (or diluted sample), ml;

0.05 – the portion of 3 M nitric acid mixed with sample (or diluted sample), ml.

Notes:

1. AAS should be re-calibrated after each 30 minutes of working time to avoid the baseline drift;
2. Spike analysis should be carried out with each sample type to assess the matrix effect. The percent recovery should be more than 95%. Otherwise, adjust the instrument parameters to overcome the interference.

References:

BS 7755-3.13: 1998 ISO 11047: 1998 Soil quality – Part 3: Chemical methods – Section 3.13: Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil – Flame and electrothermal atomic absorption spectrometric methods.

Method 23 Determination of Potassium in Water with Flame Atomic Absorption Spectrometry

Principle:

Atomic absorption spectrometry (AAS) is an elemental analysis technique for determining the concentration of metal elements in liquid samples, which based on the absorption of UV or visible light by gaseous atoms. The metal elements in the liquid sample are atomized in a flame after injected into the AAS. Each element's atoms in the flame absorb precisely the wavelength sent by the source of light, a hollow-cathode lamp (HCL), special for this element. The absorption is proportional to the concentration of this element. Monochromator and photomultiplier tube are used as its detector. The concentration measurement with AAS is usually determined from a working curve after calibrating the instrument with standards of known concentration.

Potassium is partially ionised in the air-acetylene flame. To suppress ionisation, cesium nitrate is added to give a final concentration of 1000 mg/l cesium in all solutions including the blank.

Apparatus:

1. Flame atomic absorption spectrometry (Spectr AA-200, Varian, Australia);
2. Analytical balance, with an accuracy of 1 mg;
3. Volumetric flasks;
4. 30 ml test tubes.

Reagents:

1. *Stock potassium nitrate solution, 1000 mg l⁻¹*: commercial;
2. *Cesium nitrate solution, 10g l⁻¹*: dissolve 1.466 g of cesium nitrate (CsNO₃) in the deionised water and make up to 100 ml;
3. *Nitric acid solution, 3 M*: dilute 21 ml of concentrated nitric acid with deionised water in 100ml volumetric flask and make up the volume.

Procedure:

1. Make the calibration solutions with the concentrations of 0.0, 0.2, 0.6, 1.0, 1.2, 1.6, 2.0 mg l⁻¹:
 - a. Pipette 0.00, 0.02, 0.06, 0.10, 0.12, 0.16, and 0.20 ml of the stock potassium nitrate standard into a series of 100 ml-volumetric flasks respectively;
 - b. Pipette 10 ml of cesium nitrate solution to each of the flasks;
 - c. Pipette 0.5 ml of 3 M nitric acid solution to each of the flasks;
 - c. Make up the volume with deionised water for each flask;
2. Set up the atomic absorption spectrometer according to the operation instruction attached on the instrument. The working conditions for potassium determination are:
 - a. Lamp current: 5A;
 - b. Fuel: acetylene;
 - c. Support: air;
 - d. Wavelength: 766.5 nm;
 - e. Slit width: 1.0 nm;

3. Prepare the test portion by mixing 9 ml of sample, 1 ml of cesium nitrate solution and 0.05 ml of 3 M nitric acid solution together;
4. When the lamp warm up (~30 minutes after setting up the AAS), calibrate the AAS by injecting the set of calibration solutions into it in ascending order;
5. Determination of test portion by aspirating it into the flame, and the concentration of test portion will be shown on the computer connected with the AAS. Measure each portion at least three times, and take the average (C_a).
6. If the potassium concentration of test portion is higher than 2 mg/l, dilute it proportionally with deionised water to make up the volume of 9ml, and then mix it with 1 ml of cesium nitrate solution and 0.05 ml of nitric acid solution. For example, if the dilution factor (DF) is 5, then mix 1.8 ml of sample, 7.2 ml of deionised water, 1 ml of cesium nitrate solution and 0.05 ml of nitric acid solution together as the test portion.

Expression of results:

$$C = \frac{C_a \times (9 + 1 + 0.05) \times DF}{9}$$

where

C – concentration of potassium, mg l⁻¹;

C_a – measured concentration shown on the screen, mg l⁻¹;

DF – dilution factor;

9 – portion of sample (or diluted sample) mixed with cesium nitrate solution, ml;

1 – portion of cesium nitrate solution mixed with sample (or diluted sample), ml;

0.05 – the portion of 3 M nitric acid mixed with sample (or diluted sample), ml.

Notes:

1. AAS should be re-calibrated after each 30 minutes of working time to avoid the baseline drift;
2. Spike analysis should be carried out with each sample type to assess the matrix effect. The percent recovery should be more than 95%. Otherwise, adjust the instrument parameters to overcome the interference.

References:

BS 7755-3.13: 1998 ISO 11047: 1998 Soil quality – Part 3: Chemical methods – Section 3.13: Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil – Flame and electrothermal atomic absorption spectrometric methods.

Method 24 Spectrophotometric Determination of Ammonia in Water

Principle:

Ammonia reacts with hypochlorite ions, generated in situ by the alkaline hydrolysis of sodium dichloroisocyanurate, and with sodium salicylate at about pH 12.6 in the presence of sodium nitroprusside to form a coloured compound. The compound is blue, but appears green against the yellow colour of the reagent blank. The absorbance of the compound is measured spectrophotometrically and related to the ammonia concentration in the sample by means of a calibration curve. Sodium citrate is used to mask possible interfering cations.

Calibration Curve:

Linear up to 4 mg l⁻¹ using disposable cells with 4 mm path length.

Apparatus:

1. UV-Visible Scanning spectrophotometer (Ceceil 3000 series, Cecil Instruments Ltd., UK);
2. Analytical balance, with an accuracy of 1 mg;
3. 10 ml volumetric flasks.

Reagents:

1. *Stock Ammonium Chloride Solution*: dissolve 0.382 g anhydrous ammonium chloride (dried at 105°C for at least 2 h) in Milli-Q water, and dilute to 100 ml using volumetric flask: 1.00 ml = 1.00 mg N = 1.22 mg NH₃. Stored in a stoppered glass bottle, this solution is stable for at least 1 month.

2. *Salicylate Reagent*: dissolve 13 g of sodium salicylate and 13 g of tri-sodium citrate dihydrate in about 90 ml of Milli-Q water in a 100 ml volumetric flask. Then add 0.097 g of sodium nitroprusside. Swirl to dissolve the solid and then make the volume up to the mark with Milli-Q water. Stored in an amber bottle, this reagent is stable for at least two weeks.

3. *Sodium dichloroisocyanurate reagent (DIC)*: dissolve 3.2 g of sodium hydroxide in about 50 ml of Milli-Q water in a 100 ml volumetric flask. Cool the solution to room temperature and add 0.2 g of sodium dichloroisocyanurate dihydrate to the solution. Make up to volume with Milli-Q water. Stored in amber bottle at 4°C this reagent is stable for at least two weeks.

Procedure:

1. Use standards in the range 1-4 mg l⁻¹ by diluting 0.1, 0.2, 0.3, 0.4 ml of the stock standard with Milli-Q water in a 100ml calibrated flask;
2. Pipette 8ml of sample, Milli-Q water (blank), or standard into a small flask (10 ml volumetric flask would be the best);
3. Add 0.8 ml of Salicylate reagent and mix well;
4. Add 0.8 ml of DIC reagent and mix well;
5. Add 0.4 ml of Milli-Q water (or dilute with Milli-Q to the mark if using 10 ml calibrated flask) and mix well;
6. Allow the colour to develop for at least 1 hour;

7. Measure the absorbance of the solution at 655 nm in a disposable cell with 4mm path length;
8. Ammonia contribution to the absorbance of standard or sample is calculated by subtracting the absorbance of blank from its total absorbance.

Plotting the calibration graph:

The relationship between absorbance and concentration is linear. Plot a graph of absorbance (as the *y*-axis) against the phosphate content (as the *x*-axis) in milligrams of ammoniacal-N per litre of the calibration solutions to give a straight line passing through the origin. Determine the slope (*s*) of the graph by Excel.

Expression of results:

$$C = \frac{A \times DF}{s}$$

Where

C – concentration of ammoniacal-N, mg l⁻¹;

A – absorbance of the standard solution or the sample;

DF – dilution factor.

s – slope of the calibration graph, l mg⁻¹;

Notes:

1. Samples should be adjusted to pH above 3 by dilution or neutralization before adding Salicylate reagent;
2. All calibrations and determinations should be carried out at the same temperature and the same colour development waiting time;
3. If abnormal things happen, such as precipitate or odd colour appears, then use titrimetric method to check the acceptability of this method. (The normal colour should be from yellow to green to blue as the ammonia concentration increases.)

Reference:

1. ISO 7150-1: 1984 Water quality – Determination of ammonium – Manual spectrometric method, by Subcommittee 2, Physical, chemical and biochemical methods.
2. Ammonia in Waters 1981, in Methods for the Examination of Waters and Associated Materials, by the Standing Committee of Analysts, a joint technical committee of the Department of the Environment and the National Water Council. London Her Majesty's Stationery Office, 1981: 21-24.

Method 25 Determination of Heavy Metals with Flame Atomic Absorption Spectrometry after Microwave digestion

Principle:

Atomic absorption spectrometry (AAS) is an elemental analysis technique for determining the concentration of metal elements in liquid samples, which based on the absorption of UV or visible light by gaseous atoms. The metal elements in the liquid sample are atomized in a flame after injected into the AAS. Each element's atoms in the flame absorb precisely the wavelength sent by the source of light, a hollow-cathode lamp (HCL), special for this element. The absorption is proportional to the concentration of this element. Monochromator and photomultiplier tube are used as its detector. The concentration measurement with AAS is usually determined from a working curve after calibrating the instrument with standards of known concentration.

Cobalt, iron and nickel have been found to cause depression of chromium absorbance. This can be overcome by the use of a nitrous oxide-acetylene flame. Phosphate suppresses lead absorbance significantly at concentrations ten times greater than lead. Its interference can be largely overcome by addition of EDTA solution so that the sample solutions are 0.1 M with respect to EDTA.

Apparatus:

1. Flame atomic absorption spectrometry (Spectr AA-200, Varian, Australia);
2. Analytical balance, with an accuracy of 1 mg;
3. Volumetric flasks;
4. 30 ml test tubes.

Reagents:

1. Stock cadmium nitrate solution, 1000 mg l⁻¹, commercial;
2. Stock chromium nitrate solution, 1000 mg l⁻¹, commercial;
3. Stock copper nitrate solution, 1000 mg l⁻¹, commercial;
4. Stock nickel nitrate solution, 1000 mg l⁻¹, commercial;
5. Stock lead nitrate solution, 1000 mg l⁻¹, commercial;
6. Stock zinc nitrate solution, 1000 mg l⁻¹, commercial;
7. Diluted nitric acid solution (1+4): put 200 ml of concentrated nitric acid into deionised water and make up to 1 l.

Procedure:

1. Make the calibration solutions in a series of 100 ml-volumetric flasks by following the calculated data in table M25.1. For examples, when making calibration solution level 2, pipette 0.10 ml of stock Cd solution, 0.50 ml of stock Cr solution, 0.20 ml of stock Cu solution, 0.40 ml of stock Ni solution, 1.00 ml of stock Pb solution and 0.06 ml of stock Zn solution into one 100 ml-volumetric flask labelled with level 2, and then make up the volume with diluted nitric acid solution (1+4).

The final concentrations of each metal in each level are listed in Table M25.2.

Table M25.1 Volume required for each reagents to make the calibration solutions

Calibration series	standard	Volume of stock solutions should be added in each standard level (ml)					
		Cd	Cr	Cu	Ni	Pb	Zn
Level 0		0.00	0.00	0.00	0.00	0.00	0.00
Level 1		0.05	0.25	0.10	0.20	0.10	0.02
Level 2		0.10	0.50	0.20	0.40	0.20	0.06
Level 3		0.15	0.75	0.40	0.80	0.40	0.10
Level 4		0.20	1.00	0.60	1.20	0.60	0.12
Level 5		0.25	1.25	0.80	1.60	0.80	0.16
Level 6		0.30	1.50	1.00	2.00	1.00	0.20

Table M25.2 The concentration of each metal in each calibration standard level

Calibration series	standard	Concentration in each standard level (mg/l)					
		Cd	Cr	Cu	Ni	Pb	Zn
Level 0		0.0	0.0	0.0	0.0	0.0	0.0
Level 1		0.5	2.5	1.0	2.0	1.0	0.2
Level 2		1.0	5.0	2.0	4.0	2.0	0.6
Level 3		1.5	7.5	4.0	8.0	4.0	1.0
Level 4		2.0	10.0	6.0	12.0	6.0	1.2
Level 5		2.5	12.5	8.0	16.0	8.0	1.6
Level 6		3.0	15.0	10.0	20.0	10.0	2.0

2. Set up the atomic absorption spectrometer for the determination of cadmium according to the operation instrument attached on the instrument. The working conditions for cadmium and the other five heavy metals are listed in Table M25.3:

Table M25.3 The working conditions for the determination of heavy metals

Working conditions	Targeted metals					
	Cd	Cr	Cu	Ni	Pb	Zn
Lamp current (A)	4	7	4	4	5	5
Fuel	Acetylene	Acetylene	Acetylene	Acetylene	Acetylene	Acetylene
Support	Air	Nitrous oxide	Air	Air	Air	Air
Wavelength (nm)	228.8	357.9	324.7	232.0	217.0	213.9
Slit width (nm)	0.5	0.2	0.5	0.2	1.0	1.0

3. When the lamp warm up (~30 minutes after setting up the AAS), calibrate the AAS by injecting the set of calibration solutions into it in ascending order;

4. Determination of cadmium concentration of test portion by aspirating it into the flame, and the concentration of test portion will be shown on the computer connected with the AAS. Measure each portion at least three times, and take the average (C_a);

5. If the cadmium concentration of test portion is over range, dilute it proportionally with diluted nitric acid (1+4). For example, if the dilution factor (DF) is 5, then mix 2 ml of sample and 8 ml of diluted nitric acid (1+4) together as the test portion;

6. After the cadmium determination, repeat the steps 2-5 to measure the concentrations of the other five heavy metals one by one.

Expression of results:

$$C = C_a \times DF$$

where

C – concentration of one species of heavy metal, mg l^{-1} ;

C_a – measured concentration shown on the screen, mg l^{-1} ;

DF – dilution factor for the metal ion.

Notes:

1. AAS should be re-calibrated after each 30 minutes of working time to avoid the baseline drift;
2. Spike analysis should be carried out for each metal ion with each sample type to assess the matrix effect. The percent recovery should be more than 95%. Otherwise, adjust the instrument parameters to overcome the interference;
3. When the suppression of lead by phosphate occurs, prepare an individual set of calibration solutions and test portions for Pb analysis by addition of stock EDTA solution to get a final EDTA concentration of 0.1 M.

References:

BS 7755-3.13: 1998 ISO 11047: 1998 Soil quality – Part 3: Chemical methods – Section 3.13: Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil – Flame and electrothermal atomic absorption spectrometric methods.

Method 26 Determination of Mercury with Cold-Vapour Atomic Fluorescence Spectrometry after Microwave digestion

Principle:

Elemental mercury vapour can be generated from the acid-digested sample by reduction with tin(II) chloride, and purged from solution by an argon carrier steam in a closed system. The mercury vapour is then injected into the cell of an atomic fluorescence spectrometer, where the mercury atoms are excited by radiation of a specific wavelength. The intensity of the fluorescence radiation is a function of mercury concentration.

Apparatus:

1. Cold-vapour atomic fluorescence spectrometry (PSA 10.025 Millennium Merlin, P S Analytical Ltd, Kent, UK);
2. Analytical balance, with an accuracy of 1 mg;
3. Volumetric flasks;
4. 30 ml test tubes.

Reagents:

1. *Stock mercury nitrate solution, 1000 mg l⁻¹, commercial;*
2. *Ultra pure water, with electrical resistance > 18Ω;*
3. *Diluted nitric acid solution (1+4):* put 200 ml of concentrated nitric acid, purified and certified for trace element analysis, into ultra pure water and make up to 1 l.
4. *Tin(II) chloride solution, 100 g l⁻¹:* Dissolve 10 g of dihydrated tin(II) chloride (SnCl₂·2H₂O, certified for trace element analysis) in 30 ml of concentrated hydrochloric acid in a 100 ml volumetric flask and fill to the mark with ultra pure water. Prepare this solution on the day of use.

Procedure:

1. Make the 2.00 mg l⁻¹ standard mercury solution by adding 0.200 ml of stock mercury solution (1000 mg l⁻¹) into a 100 ml volumetric flask and make up the volume with diluted nitric acid solution (1+4);
2. Pipette 0.00, 0.125, 0.625, 1.25, 2.50 ml of 2.00 mg l⁻¹ standard mercury solution into a series of 250 ml-volumetric flasks. Fill to the mark with diluted nitric acid (1+4) and mix well. These solutions correspond to mercury concentrations of 0, 1, 5, 10, 20 µg l⁻¹, respectively;
3. Set up the cold-vapour atomic fluorescence spectrometer for the determination of mercury according to the operation instruction attached on the instrument. The working conditions for mercury determination are:
 - a. Delay time: 15 seconds;
 - b. Rise time: 30 seconds;
 - c. Analysis time: 30 seconds;
 - d. Memory time: 60 seconds;
 - e. Slit width: 1.0 nm;
3. Calibrate the instrument by injecting the set of calibration solutions into it in ascending order;

4. Determination of mercury concentration of test portion. The concentration of test portion will be shown on the computer connected with the AAS. Measure each portion at least three times, and take the average (C_a);

5. If the mercury concentration of test portion is over range, dilute it proportionally with diluted nitric acid (1+4). For example, if the dilution factor (DF) is 5, then mix 2 ml of sample and 8 ml of diluted nitric acid (1+4) together as the test portion.

Expression of results:

$$C = C_a \times DF$$

where

C – concentration of mercury in sample, mg l^{-1} ;

C_a – measured concentration shown on the computer screen, mg l^{-1} ;

DF – dilution factor.

Note:

1. With the reagent blank and tin(II) chloride flowing to the gas/liquid separator, ensure that the system is equilibrated by monitoring for a stable fluorescence detector background. If sufficient time is not allowed, the detector baseline may change during an analysis cycle.

2. Spike analysis should be carried out with each sample type to assess the matrix effect. The percent recovery should be more than 95%. Otherwise, adjust the instrument parameters to overcome the interference.

References:

BS ISO 16772: 2004 Soil quality – Determination of mercury in aqua regia soil extracts with cold-vapour atomic spectrometry or cold-vapour atomic fluorescence spectrometry.

Method 27 Carbohydrate analysis

Carbohydrate determination was carried out by the phenol-sulphuric acid method (Dubois *et al.*, 1956)

Reference: Dubois, M., Gilles, K. A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28(3): 350-356.

Method 28 Lipid analysis

Lipid analysis used a Soxhlet extraction method (US EPA SW-846, 1998).

Reference: US EPA SW-846, Ed. (1998). Method 9071B: n-hexane extractable material (HEM) for sludge, sediment, and solid samples. Test methods for evaluating solid waste, physical/chemical Methods.