UNIVERSITY OF SOUTHAMPTON

Faculty of Engineering and the Environment

ANAEROBIC PROCESSING OF BABY MAIZE STOVER FOR BIOENERGY PRODUCTION UNDER THERMOPHILIC AND MESOPHILIC CONDITIONS

by

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ABSTRACT

FACULTY OF ENGINEERING AND THE ENVIRONMENT

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There is a growing consensus that purpose-grown crops should not be used for energy generation, especially if this involves competition for land and resources that could be used for food or animal feed (OECD, 2010). This highlights the need for the use of agricultural or industrial residues as sources of biomass. Tropical Power Ltd opened Africa's first gird-connected anaerobic digestion (AD) plant in Kenya which uses baby maize stover as a substrate. The aim of this work was to assess the digestion of the novel substrate in AD, baby maize stover from Kenya.

This research conducted laboratory experiments and modelling, and investigated different approaches (e.g. operating temperatures, pre-treatments and trace elements (TE) supplementation) to improve the overall energy balance and digestion performance of this substrate, in comparison with traditional maize silage as a model for ligno-cellulosic agro-wastes. For laboratory experiments, this work was carried out over 3 hydraulic retention time (HRT) in ten 4 L continuously-stirred tank reactors (CSTR) under thermophilic (55℃) and mesophilic conditions (35℃). The pre-treatment was a thermophilic pre-hydrolysis step before mesophilic digestion. Modelling was conducted by hand calculation and modelling software 'AD assessment tool'. Laboratory experimental data and data from the biogas plant in Kenya were used for the modelling.

In laboratory experiments, the greatest methane yield of baby maize stover was found in mesophilic digesters with 5 TE (Fe, Co, Ni, Se, Mo) which was 0.333 L CH_4 g^{-1} volatile solids (VS) at organic loading rate (OLR) 3 g VS L^{-1} day^{-1}. In contrast, the methane yield in mesophilic digesters with 3 TE (Fe, Co, Ni) was significantly lower than that of thermophilic digesters. 5 TE supplementation helped to provide stable operation and higher methane yield. Even with 5 TE supplementation, however, mesophilic digesters showed signs of failure after 150 days. The volatile fatty acids (VFA) accumulation in thermophilic digesters with 5 TE only fell after W dosing, and it appears W may also help the stable operation. The two-stage system was tested three times under different conditions and the gas production from pre-hydrolysis improved every time; however, specific methane yield in the two-stage system remained less than that of single stage digestion.

In modelling, firstly, overall energy balance of the biogas plant in Kenya from 08.2015 to 07.2016 was assessed. The percentage of calculated fuel consumption per actual fuel consumption was 95.3 % so the modelling assumption was close to actual operation. Secondly, rationalisation of the biogas plant design based on actual feedstock availability was conducted because the biogas plant received only 21 % of target feeding and was too large for actual feed availability. This rationalisation was conducted by hand calculation and modelling software AD assessment tool. The required digester volume was 1360 m^3 which was 24 % of actual digester volume and less than total volume of 1520 m^3 for the hydrolysers in the main plant. While hand calculation considered a more limited range of parameters (energy requirement for heating and energy output as methane) than the AD assessment tool (transportation, heat, CHP, electricity, methane, process loss), both modelling results clearly indicated the net energy output in single-stage mesophilic digestion was greater than that of single-stage thermophilic digestion.

When mesophilic digesters fed on baby maize stover at OLR of 3-4 g VS L^{-1} day^{-1} received appropriate TE, the specific methane yield was close to that of thermophilic digesters. In terms of overall energy balance, single mesophilic digestion was thus better than single-stage thermophilic digestion and two-stage system for this substrate.

Keywords; anaerobic digestion, maize, baby maize stover, biogas, trace elements, mesophilic, thermophilic, energy balance

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DECLARATION OF AUTHORSHIP

I, Chihiro Masusawa declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Anaerobic processing of baby maize stover for bioenergy production under thermophilic and mesophilic condition

I confirm that:

• this work was done wholly or mainly while in candidature for a research degree at this University;

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• I have acknowledged all main sources of help;

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Signed: ……………………………………………………………………

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Abbreviations

AD	Anaerobic digestion
ADF	Acid Detergent Fibre
ATP	Adenosine Triphosphate
BMP	Biochemical Methane Potential
C/N	Carbon to Nitrogen ratio
CEDAR	The Centre for Dairy Research
CoA	Coenzyme A
COSHH	Control of Substances Hazardous to Health
CST	Capillary Suction Time
CSTR	Continuously Stirred Tank Reactor
CV	Calorific Value
DG	Digester
DI	Deionised
DM	Dry Matter
EU	European Union
FDH	Formate Dehydrogenase
FIC	Frozen Image Centrifuge
GC	Gas Chromatography
H1	Hydrolyser 1
H2	Hydrolyser 2
HHV	Higher Heat Value
HRT	Hydraulic Retention Time

IA	Intermediate Alkalinity
KPLC	Kenya Power and Lighting Company
LCFA	Long Chain Fatty Acids
NDF	Neutral Detergent Fibre
NREL	National Renewable Energy Laboratory
OLR	Organic Loading Rate
PA	Partial Alkalinity
PVC	Poly Vinyl Chloride
RPM	Revolutions Per Minute
SBP	Specific Biogas Production
SMP	Specific Methane Production
STP	Standard Temperature and Pressure
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TE	Trace Element
ThMP	Theoretical Methane Potential
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VBP	Volumetric biogas production
VG	Vegpro Group Ltd
VMP	Volumetric methane production
VS	Volatile Solids
VFA	Volatile Fatty Acids

WMO	World Meteorological Organization
WW	Wet Weight
WWTP	Wastewater Treatment Plant

1. Introduction

In Chapter 1, the background, overall aim and objectives of the research are described.

The need for sustainable energy sources is increasing due to depletion of fossil fuel reserves, rising CO_2 emissions, and climate change (United Nations, 2015). Bioenergy is a form of renewable energy which is produced from biomass such as crops and agro-wastes. In 2016, renewable electricity accounted for 24.5 % of global electricity production and bioenergy made up 2.0 % of the renewable electricity (REN21, 2017).

Lignocellulosic crop materials such as maize (*Zea mays*) are in widespread use in Europe and elsewhere as feedstocks for renewable energy production via anaerobic digestion (Amon *et al.*, 2007). There is a growing view, however, that the cultivation of purpose-grown energy crops is not acceptable where this competes with production of human food or animal feeds (Herrmann, 2012), especially as the latter come under increasing pressure over the next few decades due to population growth and changing patterns of consumption. In addition, a report by the Organisation for Economic Cooperation and Development (OECD) has estimated that approximately 160 million hectares of land would be needed to meet the predicted demand for biofuels in 2050, if these are produced from energy crops alone (OECD, 2010). 160 million hectares is equivalent to 6.6 times the land area of the UK, while the UK's agricultural land area is around 17 million hectares which is only 10.6 % of 160 million hectares (FAO, 2017c). These figures highlight why the use of energy crops is undesirable, and illustrate the growing importance of agricultural or industrial residues as potential sources of biomass for bioenergy and biofuel production.

Stover from the production of baby sweet corn is a novel ligno-cellulosic agro-waste which could be digested to produce biogas as a source of renewable energy. Maize for baby sweetcorn production (\`baby maize\`) is planted commercially in many regions of the world, particularly in warmer and drier climates areas of India and Africa. The Food and Agriculture Organization of the United Nations (FAO) reported that baby maize stover could be used as a livestock feed (Wadhwa, 2013); however, this approach has not been adopted by farmers due to a failure to demonstrate cost-effectiveness (Devendra and Sevilla, 2002). Nouala studied the use of baby maize stover as a basic animal feed in Africa, with and without supplementation (Nouala et al., 2004; Nouala et al., 2008; Nouala et al., 2009). The weaknesses of baby maize stover for livestock feeding were its low digestibility, and the low crude protein content which caused poor productivity in animals: farmers would therefore need to buy supplements to increase the nutritional value of the feedstock, but these were not affordable. For this reason, research into alternative ways of gaining value from this substrate is justified.

In 2013, the Government of Kenya has set a target for new installed electricity of 5538 MW, of which 48 % is expected to come from renewable energy including biogas (MoEP, 2013). In 2015 Tropical Power Ltd, one of the sponsors of this study, opened Africa's first grid-connected biogas plant in Kenya with the aim of using baby maize stover as substrate (Energy, 2015), along with land application of the digestion residues as a soil conditioner and fertiliser substitute. It has previously been reported that the digestate from biogas plants increases the yield and nutritional quality of baby maize (Malav *et al.*, 2015), and this approach could therefore help to contributed to reduced CO_2 emissions and a circular economy (Kamadi, 2017). There is little information in the literature on the digestion performance of baby maize stover, however, and the purpose of this study was therefore to assess this material as a substrate for biogas production.

1.1. Background to the research

The research was based both on practical experimentation to determine the methane yields from different processing options using conventional and modified anaerobic digestion technologies; and on evaluation of the energy and nutrient balance of the whole system with the aid of a modelling tool that has been developed at the University of Southampton for this purpose. Baby maize, along with other fresh vegetables, is grown under irrigation in the Rift Valley region of Kenya, giving multiple crops per year. Baby maize is grown on a rotational basis and requires a break crop after every second harvest; this is usually sorghum (*Sorghum bicolor*).

Tropical Power (www.tropicalpower.com) is a UK-based engineering procurement and design company with subsidiaries in Kenya and Ghana, which has the goal of developing renewable energy production from biomass. In Kenya the company has commissioned Africa's first grid-connected AD plant at Gorge Farm, Naivasha in which the aim is to produce biogas from digestion of the baby maize stover. The AD plant is fed on baby maize stover, the break crop, and vegetable trimmings from the pack house and the biogas is used in a combined heat and power plant to generate electricity and heat. There is a use for some of the heat, in heating greenhouses used to breed parasites that attack the red scale mite which infests roses (also grown for export at Gorge Farm). Some heat is also required to maintain the digester temperature. After hand-picking of the baby maize, the baby maize stover (or the break crop) is harvested by a forage harvester and transported to the digester: this equipment is operated by the company and fuel usage figures are available. After digestion and solid-liquid separation the digestate is applied back to land using a tank trailer

1.2. Overall aim

The overall aim of this research is to determine the methane production potential and net energy yield from anaerobic digestion of wastes arising from the cultivation of baby maize in Kenya, and to identify successful strategies for stable operation.

1.3. Objectives

1.3.1. Laboratory digestion studies

- To establish the energy potential of the input material
- To determine: volumetric methane productivity, specific methane yield, volatile solids destruction, rheological characteristics of digestate (e.g. viscosity and dewaterbility) and stability of operation in continuous digestion at mesophilic and thermophilic temperatures
- To assess the potential benefits of thermophilic digestion of baby maize stover as a pre-hydrolysis before mesophilic digestion
- To assess the effects of trace element additions and source of inoculum/adaptation (NB this was not an original objective. From the laboratory experimental results, it seemed trace element additions and inoculum source had effects on the anaerobic processing of baby maize stover. Therefore, this objective was added.)

1.3.2. Modelling

- To evaluate the overall energy balance of the agricultural system at Gorge farm and make recommendations on how to improve its operation
- To obtain validated data and results for energy inputs and outputs that can be used in assessment of the use of baby maize stover as an anaerobic digestion substrate in Kenya

2. Literature review

Chapter 2 presents the literature review for this research. Each section is related to the theme of anaerobic processing of baby maize stover under thermophilic and mesophilic conditions. Section 2.1 provides basic information on anaerobic digestion, thermophilic digestion and two stage AD processes. Sections 2.2 to 2.4 describe some maize-derived substrates (i.e. maize silage, maize stover and baby maize stover) and summarise previous work on these materials. Section 2.5 discusses the roles of and requirements for trace elements in anaerobic digestion. In Section 2.6, approaches to modelling for overall energy balance are described. Section 2.7 summarises the identified research gaps and research questions.

2.1. Anaerobic digestion

Anaerobic digestion is degradation of organic material in the absence of oxygen to produce biogas which primarily consists of methane and carbon dioxide, plus water vapour and traces of other gases. This technology is becoming popular because the process allows production of energy from a variety of organic wastes. In addition, AD produces not only biogas but also a digestate with potential value as a fertilizer and soil conditioner, and in some circumstances can be manipulated to produce volatile fatty acids and hydrogen. Normal stable anaerobic digestion converts carbon all the way through to gas, however, H_2 or volatile fatty acids are able to obtain by altering the operating conditions.

Figure 1 shows the general degradation route of particulate organic material to biogas in the anaerobic digestion process.

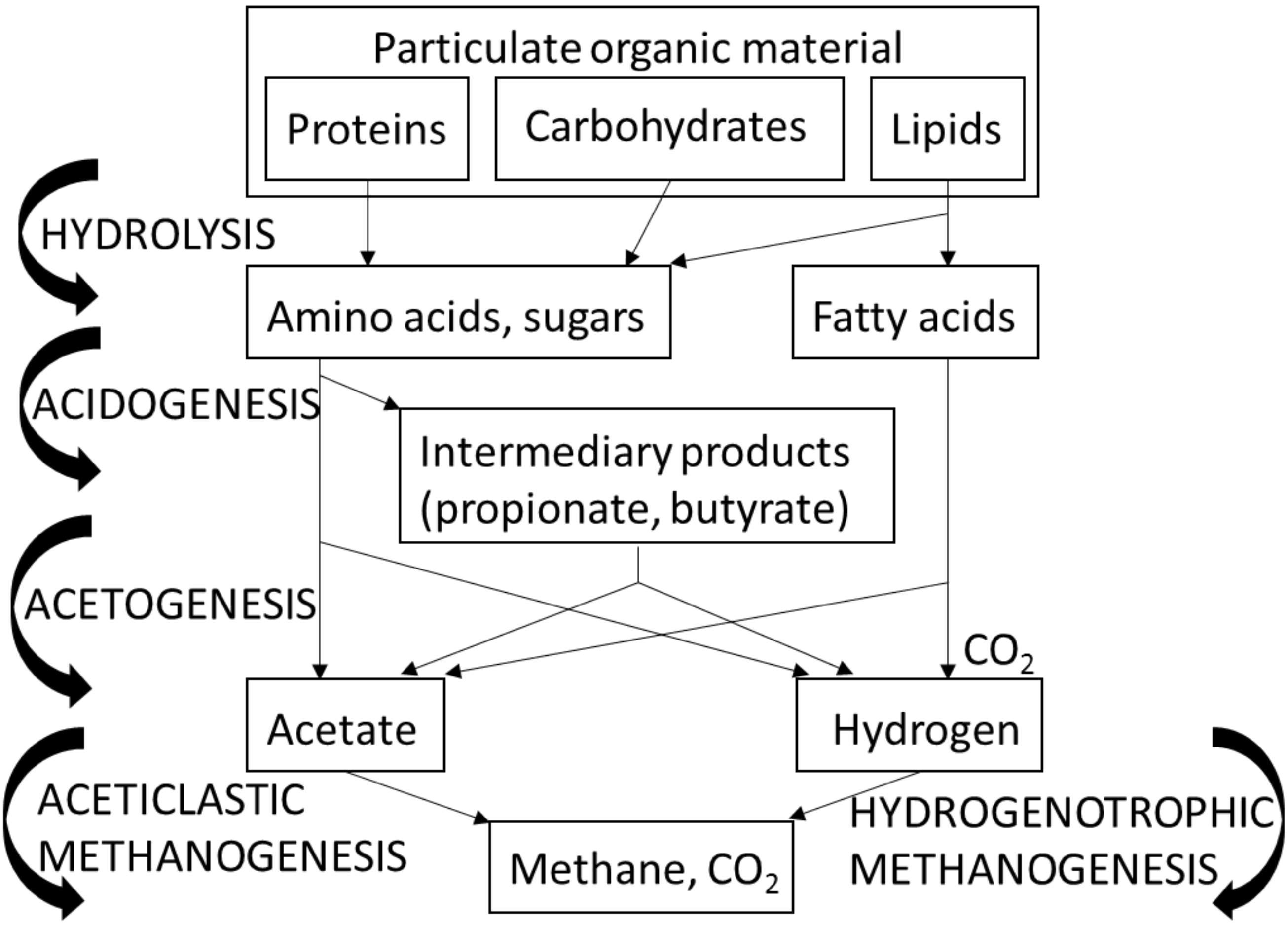


Figure 1. Anaerobic digestion of particulate organic materials to biogas (Mata-Alvarez, 2003)

Hydrolysis

Hydrolysis is the first step in anaerobic biodegradation. Hydrolysis converts complex biopolymers into soluble products (amino acids, long chain fatty acids and sugars) by enzymatic hydrolysis (Mata-Alvarez, 2003). Extracellular enzymes (protease, lipase, cellulase, etc) conduct this solubilisation and the products of hydrolysis can be used as a substrate by acidogenic and fermentative microorganisms (Mara and Horan, 2003).

Acidogenesis

In this stage, the solubilized monomers are fermented to produce volatile fatty acids (propionic, butyric and valeric acids), acetate, H_2 and CO_2. The degradation of amino acids produces ammonia. Acidogenesis is the fastest step (Mata-Alvarez, 2003).

Acetogenesis

The obligate hydrogen-producing acetogens (OHPA) degrade long chain fatty acids (LCFA: the organic acids have more than five atoms of carbon) and volatile fatty acids (VFA) to produce acetate, CO_2 and H_2 (Mata-Alvarez, 2003).

Aceticlastic methanogenesis / hydrogenotrophic methanogenesis

Methanogenesis is the last stage and two main processes produce methane, which are aceticlastic methanogenesis and hydrogenotrophic methanogenesis. There are other processes such as methanol and other organic compounds, however, these process are not very common as aceticlastic and hydrogenotrophic methanogenesis. Methanogenesis is very sensitive to temperature. AD is generally carried out at around 35^oC (mesophilic) and 55^oC (thermophilic) because maximum methanogenesis activity was found at these temperatures (Mata-Alvarez, 2003).

Aceticlastic methanogenesis produces most of the CH_4 in the whole process. Aceticlastic methane-producing archaea use acetate as a substrate and produce methane and carbon dioxide. The reaction pattern is simple but this reaction can be inhibited by low pH. The appropriate pH for AD is 6.4-7.6 which is better for methane-producing archaea (Speece, 2008). When acidogens produce more acids than are consumed by methanogens, it causes low pH. If pH is less than 6.6, methanogens grow very slowly. Therefore, AD process usually maintains at favourable pH for methanogens to prevent dominance of acidogens and VFA accumulation (Speece, 2008). Hydrogenotrophic methanogenesis uses H_2 to produce methane. This hydrogenotrophic methanogenesis grows slowly, and higher tolerance with ammonia than aceticlastic methanogenesis (Angelidaki and Ahring, 1993). When the ammonia concentration is high enough to aceticlastic methanogens, the microorganisms population may consist largely of hydrogenotropic methanogens.

2.1.1. Different types of reactor and system configuration used in AD

There are number of different types of reactor used in AD. Banks and Stentiford (2007) reported the classification of reactors shown in Figure 2. AD process can operate as wet or dry systems. The classification depends on the solids concentration of the feedstock. If the solids concentration is less than 15%, it is generally considered a wet system. If the solids concentration is more than 15%, it is a dry system (Banks and Stentiford, 2007). The process can be carried out in single stage or two stage systems. Single stage takes all reactions in a single reactor, and two stage uses two reactors in series. The feeding system maybe continuous or batch mode. The digestate is completely mixed, plug flow or intermediate. These variations are discussed in the following sections. The reactor temperature maybe ambient, mesophilic or thermophilic.

The wet, single-phase and mixed mesophilic digester is most commonly used for agricultural biogas plants, and approximately 90% of biogas plants in Germany use this system (Weiland, 2010). This study adopted this as the most common system and also tested a two stage process to assess anaerobic processing of baby maize stover under mesophilic and thermophilic conditions.

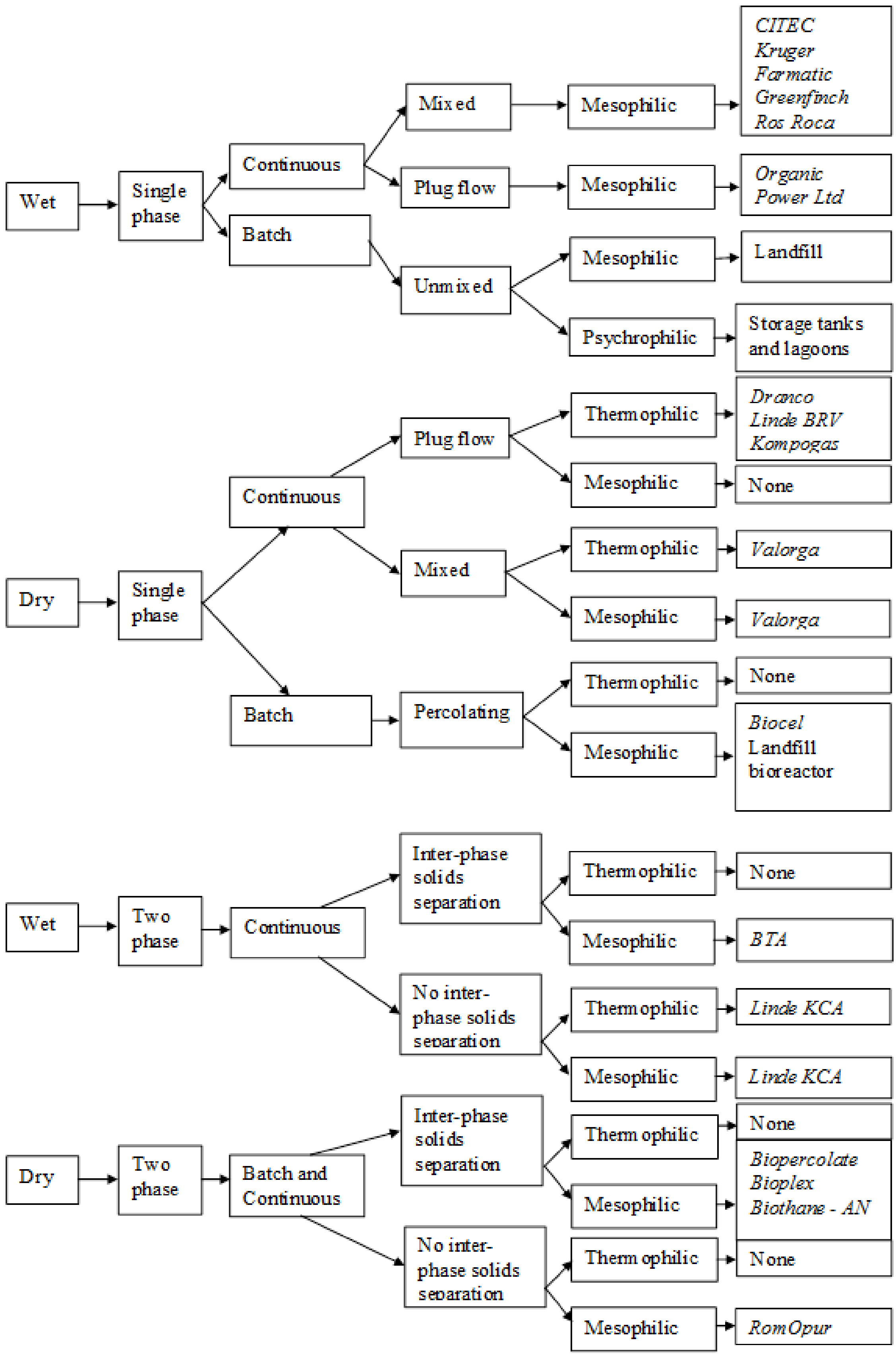


Figure 2. Classification of process alternatives for anaerobic digestion, adopted from (Banks and Stentiford, 2007)

2.1.2. Thermophilic digestion

Thermophilic digestion occurs under thermophilic conditions (e.g. between 50-65 °C). Anaerobic microorganisms are very sensitive to temperature, which strongly affects methanogenic activity. If the temperature is less than 50 °C, toxicity of ammonia decreases and thermophilic microorganisms grow very slowly. The low growth rate may cause wash out of microorganisms when the growth is slower than the reactor's hydraulic retention time (HRT) (Weiland, 2010). Cavinato compared digestion at 55 °C and 47 °C which is often applied to European biogas plants (Cavinato *et al.*, 2010). The study compared data from a full-scale biogas plant (1400 m^3) at 47 °C, and a pilot-scale plant (380 L) at 55°C and 47°C. The substrate was mixture of 27% cattle manure, 18% maize, 37% fruit processing waste and 18% bread. The research confirmed that biogas production under thermophilic conditions improved from 0.45 to 0.62 m^3 kg^{-1} VS and methane concentration increased from 52 to 61%.

De Baere reported the advantage of thermophilic digestion is faster chemical kinetics than mesophilic digestion, thus, hydraulic retention time can be shorter and required reactor volume is smaller (De Baere, 2000). Thermophilic digestion for municipal wastewater treatment has a positive impact on removal of pathogens and the digestate could be applied to soil directly as fertiliser due to the higher degree of pathogen removal (Riau *et al.*, 2012). This may be of more importance to digestion of wastewater and municipal waste-derived substrates than crop wastes: but crop wastes may also benefit from destruction of weed seeds and plant pathogens.

On the other hand, the energy balance has to be considered. Thermophilic digestion does not necessarily change the ultimate methane yield from organic matter (Weiland, 2010). Thermophilic temperature may break down substrate more, however, the main advantage is faster reaction. Thermophilic digestion needs more energy for heating. Thermophilic digestion is usually carried out at a temperature of 55°C which is 20 °C higher than mesophilic digestion (35°C). In many cases the energy demand is equal to the energy production from the substrate (Mata-Alvarez, 2003).

Some researchers assessed thermophilic digestion of agro-wastes. Giuliano *et al.* (2013) compared mesophilic and thermophilic digestion of a range of mixed substrates including livestock effluents (cattle slurry, cow manure), energy crops (maize silage, triticale), and agro-wastes (potato and onion). This study used 4 continuous stirred tank reactors (CSTRs) each with a working volume of 230 L. Reactor 1 was for mesophilic digestion at OLR 4 g VS L^{-1} day^{-1}, reactor 2 was for mesophilic digestion at OLR 2 g VS L^{-1} day^{-1}, reactor 3 was for thermophilic digestion at OLR 2 g VS L^{-1} day^{-1}, reactor 4 was for OLR at 4 g VS L^{-1} day^{-1}. The reactors operated for 390 days in all and completed 3 HRT, but the data was not duplicate. The substrates were changed three times; the first run was only livestock effluents, the second run was 50% livestock effluents and 50% energy crops, and the third run was 50% livestock effluents, 25% energy crops and 25% agro-wastes. The highest biogas yield of 0.54 ± 0.01 m^3 kg^{-1} VS was found in reactor 3 during the third run. The lowest one from the third run was 0.47 ± 0.01 m^3 kg^{-1} VS from the reactor 2. Giuliano *et al.* (2013) reported the mixture of livestock effluents, energy crops and agro-wastes, and thermophilic digestion increased biogas production.

Almeida Streitwieser (2017) also compared mesophilic and thermophilic digestion. This study used a CSTR which was the Cole Parmer Fermentation System KH-29207-00. The substrate was a mixture of 80% fruit waste and 20% cow manure. The fruit waste was shell, peel, seeds and fibres from blackberries, soursops, naranjillas and tree tomato. The waste came from local agribusiness. The organic loading rate was 1.5 kg COD m^{-3} day^{-1}. The residence time was 16 days. The methane concentration increased from 64 to 72 % and biogas production increased from 0.7 to 1.3 L day^{-1} under thermophilic conditions. The author suggested that the increase in methane production was caused by the 8 % increase in biogas methane content to 72% in thermophilic conditions is very high, and would require e.g. a significant change in substrate composition or some dissolution of the carbon dioxide component; but no mechanisms to account for this for this were put forward. This research only used 1 reactor and did not complete 3 HRT. During mesophilic digestion, the reactor received around 30 mL day^{-1} NaOH to keep the pH above 6.5. Although the research claimed that thermophilic digestion showed greater biogas production, the comparison between mesophilic and thermophilic digestion was not well reported and the author did not provide sufficient data to support the validity of the results.

Fountoulakis *et al.* (2008) carried out anaerobic processing of typical Mediterranean agro-waste under mesophilic and thermophilic conditions. The study carried out biochemical methane potential (BMP) tests in duplicate at 35°C and 55°C. The working volume of the batch experiment was 120 mL. The substrates were olive mill wastewater (OMW), wine grape residue (WGR) and slaughterhouse wastewater (Swinnen and Maertens). The BMP test compared the mixture of OMW:GR, OMW:SW and WGR:SW for 60 days. This study reported the methane yields of thermophilic digestion were 14-35% greater than for mesophilic digestion.

The above researchers reported thermophilic digestion had positive impacts for higher methane yield, however, the overall energy balance was not considered. Some researchers did not complete 3 HRT or not duplicate. Mesophilic and thermophilic conditions were not compared well. This study compared mesophilic and thermophilic completed 3 HRT in 10 CSTR to assess the impacts of thermophilic digestion.

2.1.3. Two stage AD process

The concept of two stage digestion was invented by Pohland and Gosh (1971). The two stage AD process includes a first stage for hydrolysis and acidogenesis, and a second stage for methanogenesis (Mata-Alvarez, 2003). Pretreatment can be categorised into four main groups: mechanical, chemical, thermal and biological. Mechanical methods include milling, ultrasound and microwave. Chemical methods are carried out by the use of acid or alkaline. Thermal pretreatment uses high temperature and/or high pressure. Biological pretreatment uses enzymes or microorganisms.

Mata-Alvarez discussed the advantages and disadvantages of two stage systems (Mata-Alvarez, 2003). Technically, the design is more flexible but complex. Because of the more complex system, it is more difficult to control operation. The failure of hydrolysis causes formation of methane and hydrogen in large extent and the hydrolysis gas is emitted to the atmosphere from the reactor (Weiland, 2010). That is an energy loss and has a negative climate effect. From the biological aspect, a two stage system is not expected to have a higher reaction rate and higher biogas yield. The main advantage of two stage systems is biological reliability for degrading substrates. The best environment for anaerobic microorganisms is different and their growth rates are affected by that. The optimum pH for each microorganism is 5.5-6.5 for acidogens and 7.8-8.2 for methanogens (Kim *et al.*, 2003; Song, 2016b). Therefore, in terms of pH, a two stage system may appear preferable (Pohland and Ghosh, 1971).

After the work by Pohland and Gosh (1971) which invented 2 stage systems, numerous papers have been published to demonstrate the advantages of two stage system.

Verrier compared single and two stage systems under mesophilic and thermophilic conditions (Verrier *et al.*, 1987). The substrates were carrot peelings and French bean wastes. In the primary digester, the highest acidogenesis was observed at pH 6.5 under mesophilic and thermophilic conditions. Thermophilic digestion tended to produce acetate and ethanol and mesophilic digestion mainly produced valerate and propionate. The two stage process under mesophilic conditions showed 90% degradation of wastes which was the highest value achieved. Single and two stage thermophilic digestion did not show significant differences.

Pavan and Mata-Alvarez compared one and two stage systems with pilot continuous mixed reactors (Mata-Alvarez, 2003). The substrate was waste from fruits and vegetable markets. Although the one stage failed at 3.3 kg VS m^{-3} day^{-1}, the two stage system was stable at 7 kg VS m^{-3} day^{-1}. Mata-Alvarez noted that the failure of the single stage system might be caused by the heterogeneity of wastes and non-continuous feeding.

Dichtl *et al.* (1997) studied anaerobic stabilisation of sewage sludge. The study considered degradation of organic materials, reduction of solids, pathogen removal and biogas production. They found the two stage system gave the best results. The two stage system consisted of the primary digester at 50-55°C with high loading, and second digester at 35-37°C. This is known as temperature phased anaerobic digestion (TPAD).

Lee *et al.* (2009) tried TPAD with the first stage at 70°C and the second stage at 35°C, 55°C and 65°C, with a substrate consisting of artificial composted kitchen garbage. The highest methane yield was 0.351 L CH_4 per g VS $_{added}$ for operation at 70°C and 55°C. Methane conversation efficiency and VS destruction were approximately 65% and 64% at 55°C, respectively. The TPAD of 70°C and 35°C showed the lowest methane yield of 0.143 L CH_4 per gram VS $_{added}$. Lee also mentioned that the stability and methane yield were affected by HRT.

Two stage systems have been applied for solid manure, municipal and industrial organic wastes, however, energy crops digestion is not studied well (Weiland, 2010). Figure 3 shows typical two stage agricultural biogas plant, and the system showed greater gas yields and less residual methane potential of digestate (Weiland, 2010).

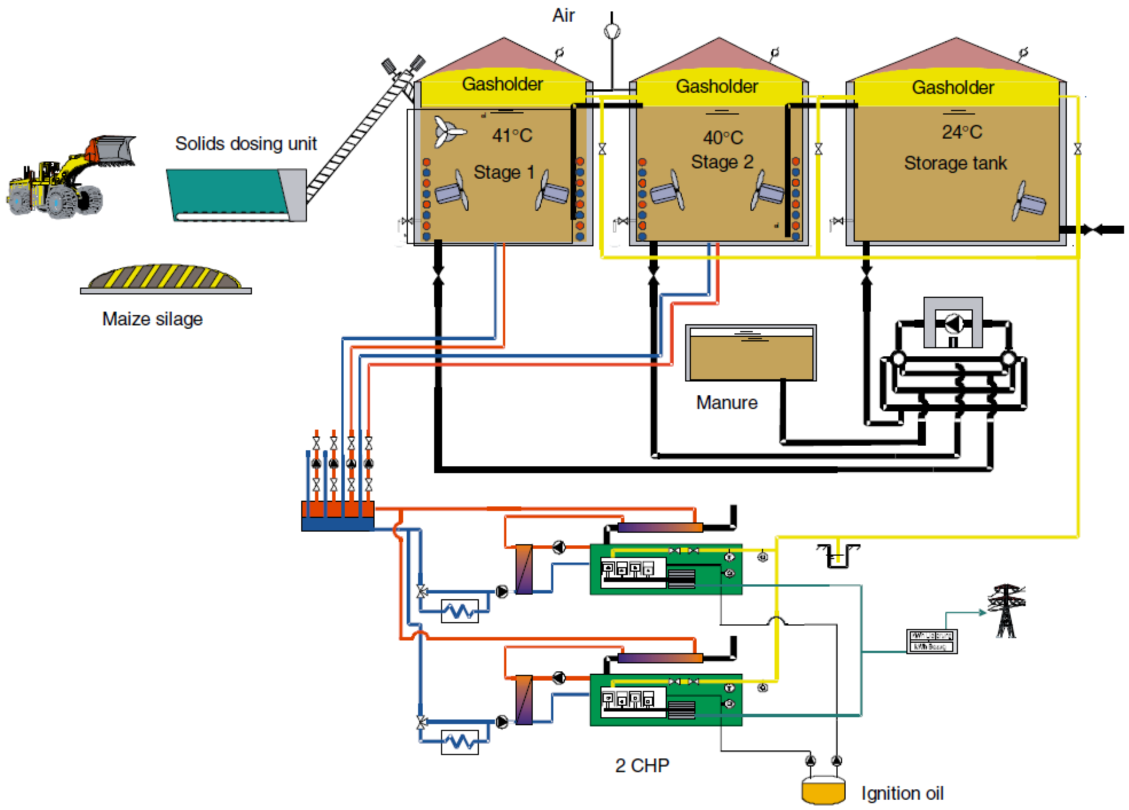


Figure 3. Typical two-stage agricultural biogas plant, adopted from (Weiland, 2010)

The simpler one stage AD is often preferred in industrial applications because biological reliability could also be supported by enough buffering, mixing, controlling OLR or co-digestion (Mata-Alvarez, 2003). Because the AD process also relies on syntrophy and inter-species electron transfers, separation into two stages may also have disadvantages.

2.2. Maize as a digestion substrate

Maize (*Zea mays*) is a member of the Poaceae, a family that contains all of the grasses including the cereals. It is an annual plant that prefers a warm environment for growing, and is one of the most widely produced grains all around the world. According to the Food and Agriculture Organization of the United Nations (FAO), 1037.8 million tonnes maize was produced globally in 2014. Kenya produced 3.5 million tonnes of maize in 2014 (FAO, 2017a).

Maize is very widely used as a substrate for anaerobic digestion in Germany, and the average mass percentage of total substrate was 50% followed by whole crop cereal silage 10.7%, grass silage 10.5%, early rye silage 9.8% and cereal grains 3.1% (Murphy, 2011).

Maize silage is an ensiled substrate and ensiling is the most usual storage process for AD (Amon *et al.*, 2007). This storage is one of the potential advantages for AD. It is possible to produce energy when demand and price for energy is the highest because energy crops are easy to store (Pakarinen *et al.*, 2008). In addition, there was an increase of 1-18% in the methane yield of maize, sorghum, forage rye and triticale after ensiling (Herrmann *et al.*, 2011). Amon also reported silaging of maize increased the methane yield by about 25% compared to non-silaged maize. He explained that this might be caused by degradation of sugars and crude fibre. Maize was chopped, compressed and stored under anaerobic condition for silaging. Sugars of maize silage were degraded and formed lactic acid, acetic acid, methanol, alcohol, and CO_2. They were precursors to produce methane. Decomposition of crude fibre during silaging process increased the nutrients availability for the methanogenesis. On the other hand, deliverable D12 of the CROPGEN project by the University of Jyvaskyla reported silaging had negative effect because the loss of total solids (TS) in the silage process is related to energy losses (JyU, 2006). They reported the average losses in dry matter amount were 11-17% during ensiling. Sugars were expected to be degraded into lactic acid, however, soluble fraction did not show any losses. The authors noted that this might be related with wash out due to rain fall.

In addition, there is considerable past experience with maize digestion in the Environmental laboratories at University of Southampton e.g. (Cysneiros *et al.*, 2008; Cornell, 2011; Cornell *et al.*, 2012; Cysneiros *et al.*, 2012a; Cysneiros *et al.*, 2012b) where this study was carried out. Therefore, this research used maize silage as a conventional substrate to compare its performance with that of a novel feedstock, baby maize stover.

2.3. Maize-derived waste substrates

There is a growing view that purpose-grown energy crops are not acceptable as they compete with the production of human foods and animal feeds. The use of residues from agriculture instead of conventional energy crops such as sugar- and starch-based crops has therefore been gathering attention (Sawatdeenarunat *et al.*, 2015).

2.3.1. Maize stover

Maize stover is an agro-waste that consists of the leaves, stalks, tassel, husk and cobs of maize which are normally left in the field after harvesting. Maize stover has attracted attention for many years as a potential biomass fuel source, particularly for bioethanol production (Barten, 2013).

The major problems associated with the use of maize stover are difficulty of degradation (Michael *et al.*, 2007) and effects on soil health (Sheehan *et al.*, 2003). Regarding the difficulty of degradation, maize stover is a lignocellulosic material which includes cellulose, hemicellulose and lignin. Cellulose is long chain of glucose which is linked by β 1-4. Hemi-cellulose is branched polymer of pentose and hexose sugars. Lignin is a complex hetero polymer of phenolic compounds and is a recalcitrant substance. These components form a matrix which is resistant to hydrolysis (Zaldivar *et al.*, 2001). Especially, the cellulose and lignin structure makes hydrolysis difficult and is a barrier to the use of maize stover as an energy source (Wyrick, 2006). Extensive research has been carried out on improving hydrolysis to facilitate the use of maize stover and other lignocellulosic materials as biomass feedstocks. Hydrolysis as a pretreatment is described in section 3.8.

Turning to soil health, maize stover is normally left in the field after the grain is harvested, where it can contribute to soil carbon and fibre. Maize stover covers and protects the soil from washing away or blowing away when it is rainy or windy. In addition, the stover's nutrient and mineral content cycles and buffer the soil's organic matter (Wilhelm *et al.*, 2011). The mineral nutrients are incorporated into maize as the plant grows (Abendroth, 2011). Baby maize stover's N, P, K were 7.50, 0.69 and 9.98 mg g^{-1}, respectively (Hoskinson *et al.*, 2007). Therefore, the potential effect of removal of maize stover on agricultural sustainability should be carefully considered (Johnson *et al.*, 2010). The amount removed varies because it depends on regional yield, climatic conditions and agricultural practices (Wilhelm *et al.*, 2004). For instance, United States' National Renewable Energy Laboratory (NREL) reported that 4.91 tonnes per hectare of residue must be left for typical tilling operations in Iowa (Sheehan *et al.*, 2003).

2.3.2. Baby maize stover

Baby maize stover (Figure 4) is an agricultural waste or by-product from production of baby maize, also known as baby sweetcorn. Baby maize is the immature ear of fully grown standard maize and is a popular Asian vegetable (Kaiser, 2011). Thailand is one of the main countries producing and exporting baby maize to USA, Europe and some other Asian countries (Bakshi *et al.*, 2016). Recently, baby maize production has substantially increased in India due to the low cost of production (Kumar and Bohra, 2014). Baby maize is harvested two or three days after silk emergence, while the ears are still immature. The size is 5-10 cm long and 0.8-1.7 cm in diameter (Kaiser, 2011). The life cycle is around 60-70 days after sowing, and the reproductive phase starts after 45 days (Kumar and Bohra, 2014). Bakshi *et al.* (2016) reported the average baby maize production was approximately 7.5-8.7 tonnes ha^{-1}. Only 15% of this production is for human consumption, and the remaining 85% is baby maize husk with silk. The production of stalks and leaves after harvesting ears is 30 tonnes ha^{-1} (Bakshi *et al.*, 2016).

In India, maize is the third most important cereal crop, after rice and wheat. Baby maize cropping systems have not been studied as well as those for rice and wheat. Baby maize yield was 1.6-2.2 tonnes ha^{-1} without husk and stover , baby maize fodder yield was 25.5-31.7 tonnes ha^{-1}(Kumar and Bohra, 2014).

Figure 4. Baby maize stover from the Tropical Power biogas plant in Kenya (photo courtesy of Ms. Angie Bywater)

Fertilizer application can increase yield and reduce the growth period. Kumar and Bohra (2014) conducted field experiments to assess the effect of N, P, K, S and Zn supplementation on baby maize growth for 2 years. The research reported higher N, P, K, S and Zn dose reduced the 4-8 days to initiation of baby maize cob, increased the yield approximately 20%, boosted carbohydrates, starch, and sugars of baby maize. The chemical properties (pH, organic carbon, N, P, K, S, Zn) of soil after harvest; N concentration increased, Zn was almost same, K, S and P were decreased, however, these values did not differ significantly compared with the soil before the field experiments. The authors reported 187.5 kg N, 93.75 kg P_2O_5, 75 kg K_2O, 50 kg S ha^{-1} and Zn ha^{-1} had positive impacts to achieve higher productivity.

FAO reported baby maize stover can be utilised for animal feeding, and the stover is more acceptable and palatable as compared with conventional maize silage in sustainable manner (Wadhwa, 2013). There are some papers about baby maize stover as an animal feed. Devendra and Sevilla (2002) considered the availability of feed resources for livestock and their use in crop-animal systems in Asia. Feed resources have impacts in animal nutrition on production and animal health. The research considered four main categories (pastures, crop residues, agro-industrial by-products, non-conventional feed resources) of feed resources for small farms. The authors noted that cereal straw and stover were not traditional animal feed resources, however, there was potential to use them due to the substantial amounts available. The problems associated with these substrates were low digestibility, low crude protein, poor palatability and sheer bulk. Feeding of cereal straws alone caused poor productivity in animals. Chemical treatment of stover and supplementation to feeding (protein, mineral) could increase the nutrient value. These techniques were not adopted by farmers due to the failure of demonstrating cost-effectiveness in 2002.

Hiep (2003) evaluated baby maize production systems for human food consumption and stover ensiling systems for cattle feeding. In Vietnam, the demand for animal products has considerably increased due to economic development in recent decades. Vietnamese government programmes promoted maize for animal feed. The author noted there were also opportunities for using maize as human food consumption. The baby maize was harvested on day 64 after the planting day. The yield of baby maize was 2.4 tonnes ha^{-1} and baby maize stover was 27.6 tonnes ha^{-1}. The research reported ensiling had positive impacts to avoid mould appearance and preserve high moisture baby maize stover.

Nouala *et al.* (2004) assessed the major horticultural residues (baby maize stover, pea vines and bean vines) available as a ruminant feed in the Greater Banjul area, Gambia to address the problems of poor quality and quantity of ruminant feeds in tropical countries. Ruminant feeds were not always available and affordable for small farmers. On the other hand, the horticultural sector has grown significantly due to small-holding farmers located around city fringes. These farmers produced short-cycle crops and generated substantial residues round the year. Nouala reported that baby maize stover's crude protein was 5% TS which was approximately one third that of bean vines and pea vines. Therefore, these vines could be used as supplements to baby maize stover based rations. The baby maize stover chemical composition; TS was 33% wet weight (WW), VS was 93% TS, neutral detergent fibre (NDF) was 63.1%, acid detergent fibre (ADF) was 39.8%, crude protein was 5% TS.

In 2008 and 2009, Nouala used baby maize stover as a basal diet for cattle feeding in west Africa (Nouala *et al.*, 2008; Nouala *et al.*, 2009). The research aim in 2008 was to identify the rumen microbial community of cattle in Kenya (Nouala *et al.*, 2008). The research did not report the baby maize stover's chemical composition alone but showed the chemical composition of a mixture of baby maize stover and concentrate. The research aim in 2009 was to assess the potential of a novel cattle feed, the leaves of *Moringa oleifera Lam* which is a tree with a high crude protein content (Nouala *et al.*, 2009). In Africa, conventional concentrates as a supplement to poor quality roughage often not affordable or available to buy. The baby maize stovers were cut after harvesting baby maize. The harvesting was done 10 days after flowering. The stovers were left in the field for 2-3 days for wilting, and then the substrate was bailed, and sun-dried in open air. Before feeding to cattle, the maize stover was cut into 5-7 cm lengths. The research reported the baby maize stover's chemical composition was 92.4% organic matter, crude protein 6%, neutral detergent fibre 65.7% and acid detergent fibre 34.9% on dry matter basis.

Bakshi *et al.* (2016) considered vegetable wastes for animal feeding. The vegetable wastes were baby maize, cabbage, carrot, cauliflower, cucumber, jackfruit, peas, potato, sweet potato, sweet corn, tomato and radish leaves. Bakshi noted that increased future consumption of animal production affects climate change, food-fuel-feed competition, land degradation, water shortage, biodiversity loss and other environmental issues. The use of vegetable wastes may have positive impacts in a sustainable manner. The major problem of using these wastes as livestock feeding was their high moisture content and contaminants (e.g. pesticides). Drying and ensiling had positive impacts to enhance shelf life. These resources were not yet tapped.

The current research looked at the potential for baby maize stover as a substrate for AD as this is a novel source of waste biomass and little is known about its properties or how it might differ from other maize-related biomass. It looked at AD because there is a need for renewable energy both globally and in sub-Saharan Africa and other places where maize is grown; and also because AD potentially has some environmental advantages in allowing the return of nutrients and fibre to the soil, in contrast to e.g. combustion of dried agro-wastes. For instance, Malav *et al.* (2015) applied biogas slurry from agricultural waste treatment for growing baby maize because the disposal of the biogas slurry was a major concern for the environment in India. In the paper, no information is given on the biogas plant operating temperature, feeding history, single stage or two stage operation, or size. Only location of the plant was mentioned as being near the Indian Agriculture Research Institute; however, the chemical composition of the biogas slurry was checked before application to the field. The pH was 7.9 ± 0.16, total nitrogen 2.1 ± 0.16 %, total phosphorus 1.1 ± 0.07 %, potassium 0.98 ± 0.13 %, Fe 0.34 ± 0.03 ppm, Cu 0.004 ± 0.01 ppm, Mn 0.088 ± 0.01 ppm, Zn 0.023 ± 0.00 ppm. The biogas slurry was applied 7-10 days before sowing. This study checked six conditions; control, 100% recommended dose of fertilizer, 25% biogas slurry + 75% recommended dose of fertilizer, 50% biogas slurry + 50% recommended dose of fertilizer, 75% biogas slurry + 25% recommended dose of fertilizer, 100% biogas slurry. Each condition was tested with four replications and the research was conducted throughout six seasons from 2013 to 2014. The application of 50% biogas slurry + 50% recommended dose of fertilizer gave 20% more yield. The protein and total sugar content of the baby corn was increased up to approximately 100%. The biogas slurry helped the growth of baby maize.

This study reviewed papers related to baby maize stover, but it was difficult to find examples of the chemical composition. The reported studies concentrated on increasing yield, and on soil chemical composition. The literature values for baby maize stover are shown in Table 1, which also includes values for maize silage and maize stover for comparison. The TS and VS in baby maize stover are 24-33 % WW and 31-33 % WW which are within the lower range of that of maize silage. TS in maize stover was approximately 90 % WW which was much higher than that of maize silage and baby maize stover. The higher TS content may be because maize stover is typically harvested at a later growth stage than maize silage, and is not protected from drying, while the ensiling process may preserve moisture. The cellulose content in baby maize stover was 34.8 % TS which was slightly lower than the average of 38.3 % TS for maize stover and slightly greater than that of 29.5 % TS for maize silage. Hemicellulose in baby maize stover was 23.3-28.5 % which was similar to maize stover, but lower than the average for maize silage. The lowest lignin value of 3.2 % TS was in the single value for baby maize stover. Lignin in maize stover was on average greater than in maize silage but the value varied from 4.2 to 18 % TS. These varied values suggests physicochemical parameters depend on each plant.

No papers were found on anaerobic processing of baby maize stover. The reported methane yield of maize silage was 0.260-0.366 L CH_4 g^{-1} VS which was slightly higher than the range for maize stover of 0.134-0.348 L CH_4 g^{-1} VS. The lower methane yield of maize stover may be related to the absence of ensiling and the higher lignin content of maize stover. These parameters suggest that baby maize stover is maize derived substrate as maize silage and maize stover are, but baby maize stover has different physicochemical characteristics. The variations in value between the three maize-derived materials may arise from several factors including differences in the strains used in each case, the preferred growth stage at harvest in each case and how the material is handled after harvest, as well as natural variation from site to site and year to year. The range of variation and the limited amount of data for baby maize stover means there are no strong boundaries between the three materials, but baby maize stover may be slightly more similar to maize silage than to traditional maize stover. The difference may cause different anaerobic processing and biogas production. Baby maize stover is a novel substrate for anaerobic digestion, therefore, the optimum conditions for anaerobic processing of baby maize stover have not yet been identified. This work therefore assessed the process stability and net energy yield from the anaerobic processing of baby maize stover.

Table 1. Maize silage, maize stover and baby maize stover chemical composition and methane yield

	TS [% WW]	VS [% WW]	Cellulose [% TS]	Hemicellulose [% TS]	Lignin [% TS]	Methane yield [L CH_4 g^{-1} VS]	Reference
Maize silage	19.4-43.1	18.4-41.8	22.2-36.2	25.3-30.4	4.8-6.4	0.280 - 0.334	(Amon *et al.*, 2007)
	18.0-43.0	17.2-41.4	26.1-33.8	25.4-35.9	5.5-8.6	0.268 - 0.366	
	19.4-52.9	18.4-50.7	23.8-37.1	26.4-36.2	4.5-5.3	0.322	
	18.1-48.0	17.4-46.7	19.3-37.3	26.1-34.2	4.3-7.5	0.287 - 0.326	
Maize stover	94.7	88.0	35.7	27.8	4.2	0.188-0.248	Y. Li et al. (2017)
	94.0	87.4	41.2	28.1	8.7	0.141-0.257	Zhong et al. (2011)
	-	-	37.4	27.5	18	-	Sheehan (2003)
	86.0	81.1	41.4	28.2	18.3	0.206-0.348	Lizasoain et al. (2017)
	-	-	32	23	14	0.134-0.167	Strang (2017)
	92.4	-	39.0	25.2	4.6	-	Tirado-Gonzalez (2016)
	90.8	-	43.0	21.8	5.7	-	
	90.1	-	39.0	22.8	4.9	-	
	92.1	-	38.6	24.4	6.2	-	
	89.9	-	37.6	22.5	5.7	-	
	89.4	-	39.0	19.1	7.0	-	
	94.9	85.5	36.2	29.8	8.6	-	Tian (2015)
	-	-	43.6	27.8	6.9	-	Pang (2008)
Baby maize stover	24	23	34.8	28.5	3.2	-	Hiep (2003)
	33	31	-	23.3	-	-	Nouala (2004)
	-	-	-	30.8	-	-	Nouala (2009)
	-	-	-	23.3	-	-	Bakshi (2016)

2.4. Policy and drivers for renewables in Kenya

The republic of Kenya is on the equator on Africa's east coast. In 2016, the population was 48.46 million, in a land area of 580400 km^2 (IBRD, 2017). 47% of the land area, 273500 km^2 is utilised for agriculture (FAO, 2017b), which is Kenya's main industry (FAO, 2008). Maize is the second largest agricultural crop and 3.2 million tonnes of maize is produced per year (FAO, 2017b). Baby maize is generally counted as part of maize production and it is difficult to find separate figures for the amount of baby maize produced in Kenya.

The government of the Republic of Kenya set the Kenya vision 2030 which states that aims to be "a middle-income, newly-industrialising country offering a high quality of life to all its citizens in a secure environment" to eradicate poverty, illiteracy and diseases (MoEP, 2013). As part of Kenya vision 2030, the Ministry of Energy and Petroleum published a strategic plan 2013-2017 (MoEP, 2013). The strategic plan aimed to increase electricity connectivity from 30% to 75-80% of population. The lack access to electricity is serious problem in rural areas, where two-thirds of residents do not have electricity connectivity. This results in respiratory infections due to unsustainable use of traditional biomass and agriculture waste for cooking etc. Currently, Kenya's energy usage is 22% petroleum, 9% electricity, 1% others (coal and solar), and 68% traditional biomass (MoEP, 2013).

The target for new installed electricity is 5538 MW and approximately 42% (2318MW) of this target is expected to come from renewable energy. The renewable energy programmes included 24MW from hydro, 1646 MW from geothermal, 630 MW from wind and 18 MW from bio energy. Regarding bio-energy, a pilot project has been undertaken for electricity generation from municipal/industrial solid waste (MoEP, 2013). By 2013, the Kenyan government had already installed 6000 domestic biogas digesters with support from the Netherlands through the "Biogas initiative for Africa" due to Kenyan 2008-2012 strategic plan. The strategic plan 2013-2017 aimed to construct 3000 digesters for individual households, and 250 for the energy centres.

Tropical Power Ltd has built and opened the first and largest grid-connected biogas plant in Africa in 2015. The anaerobic digestion plant is located at Gorge Farm energy park, Naivasha, Kenya (TP, 2017). More details of the design and operation are given in chapter 5.

The company's publicity information states that "We were the first to introduce the BATCH Hydrolysis System and have ever since achieved biogas yields that lay up to 30% above the yields of single stage biogas plant." (TheSnowLeopardProjectsGmbH, 2017).

For this reason, in the current research a two stage AD process with thermophilic hydrolysis of baby maize stover was tested at laboratory scale in comparison with single stage operation in mesophilic and thermophilic conditions to identify the effects of the different operating conditions. In addition, the overall energy balance was assessed to determine which operating mode gave the highest net energy gain.

2.5. Trace elements

Anaerobic microorganisms generally consist of: carbon 45-55%, oxygen 20%, hydrogen 10%, nitrogen 6-14% and phosphorus 1-3% on dry weight basis (Todar; Banks, 2011). The amount of sulphur, sodium, potassium, calcium and magnesium depends on the microorganism strain, and each element can reach 1%. Trace elements are the remaining components comprising less than 1% (Todar; Banks, 2011). Trace elements are defined as "any chemical element that occurs in very small amount in organisms, but is essential for many physiological and biological process" (Zandvoort *et al.*, 2006).

Trace elements act as cofactors for essential enzymatic reactions in the cell. The growth of acetogens (Ljungdahl, 1986) and methanogens (Scherer *et al.*, 1983) is supported by trace elements. This study used municipal waste water biosolids as inoculum, and maize silage and baby maize stover as feedstocks. Numerous researcher have studied trace elements requirements in municipal wastewater biosolids and maize silage. There is no paper about trace element requirements for anaerobic processing of baby maize stover.

Municipal waste water biosolids

Zitomer *et al.* (2008) assessed the impacts of Fe, Ni and Co on digestion of sludge from 4 wastewater treatment plants which included mesophilic and thermophilic systems. The study carried out batch tests and acetate and propionate were used as substrate. 77% of digestate samples benefited from this trace element dosing and propionate and acetate utilisation improved by 50% and 35% respectively. The trace element dosing concentration of the samples were 25 mg Ni L^{-1}, 25 mg Co L^{-1} and 25 mg Fe L^{-1} of the digestion mix. Apart from in this 77% of samples, single trace element dose increased the methane production in some cases, but the three trace elements did not benefit in other cases. Thermophilic digestion showed higher acetate and propionate utilisation. The trace element dose increased methane production: 4 – 51 % for thermophilic digestion and 7 – 36% for mesophilic digestion.

Hinken *et al.* (2008) carried out batch tests to assess the influence of Fe, Ni and Co. The study used anaerobic digester sludge and maize silage. Anaerobic digestion of maize silage with trace elements dosing produced 35% higher biogas production than in the reference reactor. The trace element dosing to the sludge did not show improvement. Hinken reported that the amount of trace elements in the sludge was already sufficient.

Maize silage

Lebuhn *et al.* (2008) carried out anaerobic processing of maize silage in six CSTRs over a year. Even at the low organic loading rate of 2 g VS L^{-1} day^{-1}, the digesters showed acidification in 8 months. After supplementation with a cocktail of trace elements, the acidified reactors recovered and showed stable operation. Co dosing was recommended because it was the most limiting element. The recommended dose was 0.02-0.4 mg Co L^{-1}. The authors noted Se and Mo also should be provided. Agler *et al.* (2008) also reported Co was essential for anaerobic processing of maize silage under thermophilic conditions.

Ezebuiro and Körner (2017) studied the trace element requirements (Ni, Co, Se and Mo) for hydrolysis. The substrate was a maize silage-based feedstock which included 75% maize silage, 15% grass cutting, 5% wine residue, 2.5% cow dung from milking cows and 2.5% other bio wastes. This study was carried out as a batch test in 1 L glass reactors under mesophilic conditions. This study checked the trace elements concentration in inoculum and substrate, and then a VFA cocktail was added. The VFA cocktail concentrations were 10, 120, 200 mmol L^{-1}. The inoculum was digested sludge from wastewater treatment. The trace element concentrations in sludge were: Co 16.71 mg kg^{-1} TS, Mo 1.08 mg kg^{-1} TS, Ni 3.1 mg kg^{-1} TS, and the substrate Co 1.17 mg kg^{-1} TS, Mo 1.46 mg kg^{-1} TS, Ni 3.62 mg kg^{-1} TS. At VFA level 200 mmol, the trace element in the substrate could be less than the required amount for hydrolysis.

The concentration of TE in sewage sludge and other digestates, and TE supplementation strategies reported in the literature are shown in Table 2. The sewage sludge digestates were used as inoculum for anaerobic digestion and the concentrations of TE in the sewage sludge from different wastewater treatment plants were clearly different. For instance, the Mo concentration in a wastewater treatment plant in China was 0.12 mg L^{-1}, in Turkey was 2.9 mg L^{-1} and in Japan was 817-896 mg L^{-1} (Uemura, 2010; Lo *et al.*, 2012; Evranos and Demirel, 2015). TE concentration in digestate also depends on the substrate. Anaerobic digesters fed on food wastes showed higher Fe concentrations than that of digestate from maize silage (Pobeheim *et al.*, 2010a; Banks *et al.*, 2012).

The presence of TE in inoculum and substrate has effects on the TE requirements. This study used Fe, Ni, Co, Mo, Se and W because these trace elements are considered to have positive impacts and improve anaerobic digestion performance (Choong *et al.*, 2016). The TE supplementation recipe in this study followed the studies by Banks *et al.* (2012) and Jiang *et al.* (2012). These studies were carried out in the same research group and laboratory using the same source of inoculum, although with different substrates in the named studies. The main roles of these trace elements are as follows.

Table 2. Concentration of TE in digestate and TE supplementation in literatures

	TE in sewage sludge				TE in digestate		TE supplementation in literatures			
References	(Evranos and Demirel, 2015)	(Lo *et al.*, 2012)	(Uemura, 2010)		(Pobeheim *et al.*, 2010a)	(Banks *et al.*, 2012)	(Evranos and Demirel, 2015)	(Banks *et al.*, 2012)		(Jiang *et al.*, 2012)
Substrate	-	-	-		Maize silage	Food waste	Maize silage	Mix of propionic, acetic acid, glucose, starch and ammonia	Food waste	Vegetable waste
Operation	Wastewater treatment plant in Turkey	Wastewater treatment plant in China	Wastewater treatment plant in Japan, mesophilic	Wastewater treatment plant in Japan, thermophilic	Agricultural biogas plant	A laboratory digester	Batch, 37°C	Batch, 36°C	CSTR, 36°C	CSTR and batch, 35°C
Unit	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Co	4.34	0.33	3.52	3.49	ND	0.083	0.1, 0.5	1	1	1
Cu	47.21	6.12	-	-	0.48	5.75	-	-	0.1	0.1
Fe	9261.08	590.4	-	-	32.8	173.7	-	5	5	10
Mn	145	77.07	-	-	3.18	18.5	-	-	1	0.1
Mo	2.9	0.12	817	896	0.02	0.29	0.05, 0.25	0.2	0.2	0.1
Ni	4.78	1.89	0.133	0.22	0.06	2.9	0.1, 0.5	1	1	1
Se	-	-	-	-	0.03	0.05	-	0.2	0.2	0.1
W	-	-	-	-	-	<0.035	-	0.2	0.2	0.1
Zn	290.46	40.41	-	-	3	8.11	-	-	0.2	10

Fe

Fe has been shown to be a crucial trace element in anaerobic digestion. Fe has a large reduction capacity and contributes to anaerobic processes in many ways. Zandvoort reported that Fe had positive impacts on the methanol degradation rate and methanogenic activity (Zandvoort *et al.*, 2003). The research studied the impacts of Fe, Co and Ni in an upflow anaerobic sludge blanket (UASB), and the substrate was methanol. Only Fe had a significant effect. The role of Fe in methanogenesis is related to Formyl MF dehydrogenase, CO dehydrogenase (CODH), Acetyl-Coenzyme A (CoA) synthesis and hydrogenases (Banks, 2011). Fe and Ni make Fe-Ni-S cluster or Fe-S cluster which are subunits of these enzymes (Lindahl and Chang, 2001; Thauer *et al.*, 2010).

Ni

Ni is also an important trace element in the anaerobic digestion process. If carbon dioxide and hydrogen are the only energy source, numerous anaerobic micro-organisms rely on Ni (Kayhanian and Rich, 1995). The role of Ni in methanogenesis is related to CODH, methylreductase, hydrogenases and synthesis of F_{430} (Banks, 2011) (Diekert *et al.*, 1981). Most of Ni is utilised as a part of the coenzyme F_{430} and the CODH' component is a Ni protein and may support sulphur-reducing microorganisms. Every methanogen ever examined includes F_{430} (Thauer *et al.*, 1980; Diekert *et al.*, 1981; Hausinger, 1987; Kayhanian and Rich, 1995; Kida *et al.*, 2001). For instance, *M. thermoautotrophicum* includes F_{430} as a part of Methyl Coenzyme M reductase. Methyl Coenzyme M reductase has two genetically different isozymes which have two molecules of F_{430}, respectively (Craft *et al.*, 2004). The reductase is in acetoclastic and hydrogenotrophic methanogens (Banks, 2011).

Co

There is Co in specific enzymes and corrinoids (Oleszkiewicz and Sharma, 1990; Kayhanian and Rich, 1995; Kida *et al.*, 2001). Vitamin B12 is one of the corrinoids which contains Co ion to bind to coenzyme M methylase. Coenzyme M methylase is an essential enzyme for methane formation in acetoclastic and hydrogenotrophic. CODH also uses Co. The enzyme is also important in acetogenesis (Murakami and Ragsdale, 2000; Muller, 2003; Thauer *et al.*, 2008). The role of Co in methanogenesis is related to methyltransferase. Feng and Lo carried out AD with Co dose, the substrate was industrial food wastes (Feng *et al.*, 2010; Lo *et al.*, 2012; Amaral *et al.*, 2014). The Co dose had positive impacts to biogas production.

Se

Se is included in some anaerobic bacterial enzymes and bacterial nucleic acids. For instance, FDH contains selenium. Enzymes depending on Se are active at around pH 7 and may help VFA degradation (Stadtman, 1980; Kayhanian and Rich, 1995; Schattauer *et al.*, 2011).

Mo

Mo is in the enzyme formate dehydrogenase (FDH) (Banks, 2011). Mo also limits the formation of necessary sulphides and that may inhibit sulphate-reducing bacteria (Oleszkiewicz and Sharma, 1990). Mo dosing increased the biogas production from maize silage (Jarvis *et al.*, 1997; Pobeheim *et al.*, 2010b) and municipal solid waste (Lo *et al.*, 2012).

W

Formate dehydrogenase (FDH) contains W. Like Ni, Tungsten may help metabolize CO_2 and H_2 (Zellner *et al.*, 1987; Kayhanian and Rich, 1995). W dose has been reported to have positive impacts for propionic acid degradation (Reda *et al.*, 2008; Plugge *et al.*, 2009). Jiang carried out anaerobic digestion of vegetable wastes from Kenya in ten 5L CSTR under mesophilic condition and found that W helped to maintain digester stability (Jiang *et al.*, 2012).

2.5.1. Trace elements requirement for baby maize stover

The type of substrate affects the requirement for trace element supplementation. Some substrates (e.g. swine wastewater) are typically rich in the necessary micronutrients (Amaral *et al.*, 2014), while others require trace elements dosing (e.g. maize silage (Evranos and Demirel, 2015), wheat stillage (Schmidt *et al.*, 2014)). Without trace elements, even food-processing wastewaters which are highly biodegradable could not support proper methane fermentation (Speece, 1983). In anaerobic digesters treating food wastes, trace elements deficiency causes volatile fatty acids accumulation and the lack of Se and Mo in particular are related with propionic acid accumulation (Banks *et al.*, 2012). Baby maize stover is a novel feed stock to anaerobic digestion. It was not known whether trace elements dose were essential or not. If trace element dosing was needed, it was not sure which trace elements were needed. The purpose of trace element dosing was not to identify the essential trace elements for the baby maize stover, but to ensure stable operation.

2.6. Modelling for overall energy balance

2.6.1. Modelling

Various modelling approaches are widely applied to AD systems, ranging from life cycle assessment (Velásquez Piñas *et al.*) to tools such as anaerobic digestion model No 1 (ADM1).

Life cycle assessment can be defined as a "tool that can be used to evaluate the potential environmental impacts of a product, material, process, or activity. An LCA is a comprehensive assessment of a range of environmental impacts across the full life cycle of a product system, from materials acquisition to manufacturing, use, and final disposition" (EPA, 2017). Rehl carried out LCA of biogas digestate processing technologies (Rehl and Müller, 2011). In 2011, the demand for large-scale biogas plants increased rapidly with government subsidies in Germany. The study assessed whether the digestate should be disposed of or utilised to avoid overprovision of nutrients. Rehl found that the optimum digestate utilisation route depends on nitrogen emissions from the process. For instance, N_2O emission from bacterial decomposition of NH_3 during the composting process had negative impacts for global warming. In addition, fuel consumption for heating and processing were also important.

Turning to ADM1, this tool was published by the IWA Anaerobic Digestion Modelling task group in 2002. ADM1 is a mathematical model for biochemical and physicochemical processes in CSTR (Batstone *et al.*, 2002). In 2005, Blumensaat assessed two stage AD by using ADM1 (Blumensaat and Keller, 2005). The two stage system consisted of thermophilic digestion as first stage and mesophilic digestion as second stage. The study compared the modelling data and the experimental data from a pilot-scale plant (operating volume; thermophilic digestion 160 L and mesophilic digestion 800L). The research compared propionate simulated and measured in thermophilic and mesophilic reactors. The modelling tool could predict propionate concentration well in the thermophilic reactor, but real and modelled propionate concentration was not in good agreement in mesophilic conditions.

This research did not use these tools, LCA and ADM1, however, because the purpose of this study was to assess the baby maize stover as novel substrate for anaerobic digestion and determine the overall energy balance. LCA aims to assess the overall impacts on the environment while ADM1 simulates the biological reactions. This study started because there is biogas plant in Kenya which uses baby maize stover as substrate. There is growing awareness about global warming such as the Paris agreement in 2015; however, Kenya is not covered by this. In addition, this study was associated with a real commercial plant and the interest was toward to the energy production. This work had enough data for biological reaction because anaerobic processing of baby maize stover in

10 CSTR was carried out over 2 years. Therefore, this study's modelling was for overall energy balance.

2.6.2. Modelling for overall energy

All the energy inputs and outputs should be taken into consideration to determine the energy efficiency of renewable energy source. The system can be divided into three parts: 1. biomass production and transportation, 2. conversion of biomass into primary fuel source, 3. processing of the primary fuel source into usable energy (Salter and Banks, 2009).

Regarding 1. biomass production and transportation, crop production requires energy and values for the different operations involved have been reported in the literature (Richards, 2000; Audsley *et al.*, 2006). If an energy crop is used as a substrate, the energy for cultivation, sowing, crop maintenance and growth, harvesting and transportation should be considered (Salter and Banks, 2009). If the substrate is an agro-waste such as maize stover, only the energy for harvesting and transportation should be considered as the other stages will take place regardless of whether the crop residues are utilised. Even if the agro-waste is not used as biomass, it is harvested and left the field for the next planting. The energy utilisation difference between the use of agro-waste and non-use of agro-waste is thus not significant. This PhD study aimed to include any additional energy demand to assess the energy balance.

The biogas plant in Kenya used 2 forage harvesters and 4 tractors for harvesting and transportation. According to data from the Nebraska Tractor Test Laboratory, forage harvester fuel consumption averaged 14.7 L ha^{-1}, with the lowest consumption 1.9 L ha^{-1} and the highest was 18.7 L ha^{-1} (Grisso *et al.*, 2004). They also reported newer tractors generally show greater efficiency than old tractors. Tractor fuel consumption improved 10 -15% from 1980 to 2000. The Nebraska Tractor Test Laboratory did not assess the fuel consumption in Kenya and it was difficult to find good data for forage harvester in Kenya. The fuel consumption of tractor trailer depends on location and tractor model. Sharpe (2015) reported tractor fuel consumption in the USA, European Union (EU) and China. In USA, the fuel consumption was 0.353-0.406 L km^{-1}, in the EU 0.309 – 0.381 L km^{-1} and in China 0.435 – 0.470 L km^{-1}. The author noted that technology development and deployment due to mandatory tractor efficiency standards may contribute to higher efficiency.

Turning to 2. conversion of biomass into primary fuel source, anaerobic digesters require energy for heating and mixing. Outputs from this step are biogas and digestate. Theoretical energy requirements can be calculated and depend on the design of the digester and ancillary equipment.

The electricity requirement depends on the electrical equipment used in the anaerobic digestion plant. It is possible to estimate the heat requirement in the digestion process because heat requirement is related to heat losses through the walls of the digester and according to the following equations

"hl = UAΔT

where hl, heat loss (kJ s^{-1}); U, overall coefficient of heat transfer (W m^{-2} °C); A, cross-sectional area through which heat loss is occurring (m^2); ΔT, temperature drop across surface in question (Audsley *et al.*).

q = CQΔT

where q, heat required to raise feedstock to digester temperature (kJ s^{-1}); C, specific heat of the feedstock (kJ kg^{-1} $°C^{-1}$); Q, volume to be added (m^3); ΔT, temperature difference (Audsley *et al.*).

Total heat requirement for the process = hl + q." (Salter and Banks, 2009) .

This is a simplification as it does not take into account heat losses from other parts of the plant, such as pipework, external heat exchangers and pasteurisers etc; this is likely to be particularly significant in smaller plants. Nor does it include any heat recovery e.g. from using heat exchanges to allow the digestate to heat the incoming feed.

The World Metrological Organisation (WMO) has data for six locations in Kenya: Lodwar, Kitale, Wajir, Garissa, Dagoretti Corner and Mombasa International Airport. Dagoretti Corner is the closest to the Tropical Power Ltd biogas plant, and therefore this study used the data from this site. On the other hand, WMO climate normal data is based on averages from from 1961 to 1990 even though the data was accessed in 2017: the real current values may therefore be slightly different. Temperature and precipitation in Dagoretti Corner according to the WMO (WMO, 2017) is shown in Figure 5.

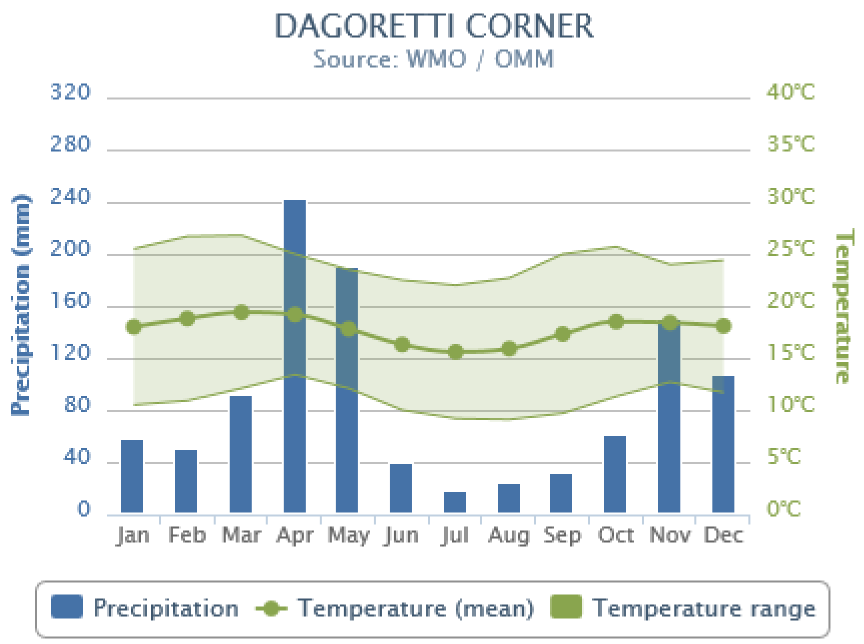


Figure 5. Temperature and precipitation in Dagoretti Corner, Kenya, adopted from 1961-1990, (WMO, 2017)

Turning to 3. processing of the primary fuel source into usable energy, there are three main ways to use biogas. 1. Burning biogas directly for heating, 2. Combined heat and power (CHP) unit to produce electricity and heat, 3. Upgrading biogas for injection into the gas grid or use as a vehicle fuel. Efficiency for burning biogas directly for heating is approximately is 85% (Salter and Banks, 2009). CHP's electrical efficiency is 33% and thermal efficiency is 45% (Appels *et al.*, 2011). Strzalka *et al.* (2017) also reported the average electrical efficiency is around 33% and the efficiency lies in the range of 20-40%. Efficient modern CHP manage up to 42% electrical efficiency, however, the total conversion efficiency is still around 85% with electrical efficiency around 30-40 % (Lantz, 2012). Biogas upgrading may require up to 0.75 kWh m^{-3} upgraded biogas (Murphy *et al.*, 2004).

In addition, digestate can be used as fertiliser and soil conditioner. If the digestate is used as fertiliser, energy for transport and spreading are included the energy balance. Berglund reported liquid phase digestate energy needs as: loading 2.5 MJ $tonne^{-1}$, transport 5 MJ $tonne^{-1}$, spreading 17 MJ $tonne^{-1}$. If the digestate is solid the energy requirements are loading 7 MJ $tonne^{-1}$, transport 7 MJ $tonne^{-1}$, spreading 14 MJ $tonne^{-1}$ (Berglund and Börjesson, 2006). Even if the digestate is not used as fertiliser or soil conditioner, transportation is needed to dispose of it. Any additional transport that would not be required if the crop waste was just left in the field thus has to be taken into account. This PhD project included the energy for digestate because this study aimed to include any additional energy demand to assess the energy balance.

Some researchers have worked on assessment of overall energy balances. Poschl *et al.* (2010) assessed the energy efficiency of single and co-digestion of multiple feedstock, different biogas utilisation and waste-stream management. The energy input to output ratio varied, single digestion was 10.5-64.0%, co-digestion was 34.1-55%. They depended on the energy requirement for feedstock supply. If the transportation distances were over 22 km for cattle manure and 425 km for municipal solid waste, the energy balance was negative.

Banks *et al.* (2011a) studied the mass and enery balance of a real anaerobic digester receiving domestic kitchen wastes. The digester was a 900 m^3 CSTR at 42^oC which was followed by pasteurisation tank and storage tank. The mass balance accounted for over 90% of substrates as gaseous or digestate products. Banks reported the potential recoverable energy was 405 kWh from a tonne of the substrate. For calculation of the recoverable energy, energy for CHP, parasitic energy, digestate use were considered.

Rupf *et al.* (2017) developed an optimal biogas system design model for Kenya region. This study considered inputs (energy and fertiliser requirement), feedstock (type, feeding amount and feeding rate), available construction material locally, sustainability criteria. They checked how much energy is needed for rural Kenyan households and calculated the optimum design. The calculated optimum design was 6 m^3 soil block single digester. The main feedstock was cattle manure, and other animal dung e.g. from pigs could be part of the feeding. Rupf reported the modelling tool could be used as a decision making tool for biogas technology.

This study focused on assessment of the energy balance (input and output energy). The Tropical Power biogas plant has already been built before the start of this research. Location, type of feeding, distance between the biogas plant and harvesting field, digester construction were decided. Consideration of choosing these factors were not required. This research carried out modelling of the overall energy for anaerobic processing of baby maize stover which used data from Tropical Power Ltd biogas plant. Furthermore, the modelling data and chemical experiments data were compared to consider overall energy balance.

2.7. Summary of the identified research gaps and research questions

No reports on the anaerobic digestion of baby maize stover were found in the scientific literature, although baby maize stover is currently being used at Tropical Power's biogas plant in Kenya. Baby maize stover is a maize-derived substrate similar to maize silage and maize stover, but the physicochemical parameters (TS, VS and fibre contents) of these materials are slightly different. While baby maize stover appears slightly more similar to maize silage than to maize stover in terms of its properties, it is not an ensiled substrate; and so comparison of the digestion performance of maize silage and baby maize stover may be interesting. The differences may cause different performance and energy output. Baby maize stover is a novel substrate, therefore, the optimum conditions for digestion have not been identified yet. The gaps in the literature review have been a lack of info on biogas production potential and performance of baby maize stover as a substrate for AD, and of the optimum conditions and trace element requirements for stable operation; and information on whether AD of this substrate is a sensible and feasible idea in terms of energy balance.

This study firstly assessed the anaerobic of processing of baby maize stover under mesophilic and thermophilic condition at typical OLR 3-4 g VS L^{-1} day^{-1}. This was to assess the anaerobic digestion behaviour, stable operation and biogas production. If problems happened, next trials were for to the respond the issues. From the laboratory experimental results, this study aimed to obtain better operational condition for this novel substrate, baby maize stover. Energy balance calculation was also conducted to assess the actual operation and better operation.

3. Materials and methods

Chapter 3 presents information on the materials and methods used in this study. The methods below make use of the standard descriptions being developed for use in the Bioenergy and Organic Resources Research Group at the University of Southampton. Sections 3.1 to 3.8 describe the analytical methodology, while Section 3.9 summarises the setup and objectives for each experimental trial.

3.1. General

Reagents

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

Water

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

Laboratory practice

All laboratory operations were carried out using good laboratory practice, and having first carried out the appropriate risk assessments and, where necessary, control of substances hazardous to health (COSHH) assessments. All equipment, laboratory apparatus, and analytical instruments were operated in accordance with the manufacturer's instructions unless noted. All glassware was washed using washing detergent followed by rinsing with tap water and DI water. The glassware used for acid digestion was soaked in a 10% nitric acid bath for a 24-hour period after which it was rinsed with ultra-pure water.

3.2. Feed stocks

Maize silage

Approximately 200 kg of ensiled fodder maize was provided by the Centre for Dairy Research (CEDAR) at the University of Reading, UK. The maize silage was delivered to the laboratory in Southampton on 24/11/2016 and was ground by passing through a macerating grinder (S52/010

Waste Disposer, IMC Ltd, UK), stored in freezer bags and frozen at -18 °C until use, when it was thawed at room temperature overnight then stored for up to 7 days at 4 °C.

Baby maize stover

Approximately 400 kg of baby maize stover was provided by Gorge Farm in Kenya. The baby maize stover was delivered to the laboratory in Southampton in two batches on 30/04/2015 (200 kg) and 15/12/2015 (200 kg), and was ground, stored in freezer bags and frozen at -18 °C until use, when it was thawed at room temperature overnight then stored for up to 7 days at 4 °C.

Inoculum

Unless noted, digester inoculum was taken from a mesophilic digester treating municipal wastewater biosolids at Millbrook Wastewater Treatment Plant (WWTP), in Southampton, UK operated by Southern Water Plc. Thermophilic digesters used an inoculum taken from this source, which was then acclimated to thermophilic conditions as described in the individual experimental methods in each case.

3.3. Gravimetric Analysis

Total Solids and Volatile Solids

TS and VS determination was based on Standard Method 2540 G (APHA, 2005). After thorough agitation, approximately 10 g of sample was transferred into a pre-weighed crucible by pipetting (digestate samples) or spatula (substrate samples). Samples were weighed to an accuracy of 10 g ± 0.001 g (Sartorius LC6215 balance, Sartorius AG, Gottingen Germany) and placed in an oven (LTE Scientific Ltd., Oldham UK) for drying overnight at 105 ± 1 °C. After drying the samples were transferred to a desiccator to cool for at least 40 minutes. Samples were then weighed again with the same balance, transferred to a muffle furnace (Carbolite Furnace 201, Carbolite, UK) and heated at 550 ± 10 °C for two hours. After this ashing step, samples were again cooled in a desiccator for at least one hour before weighing a third time.

After all analyses, crucibles were washed with detergent, rinsed with DI water, and stored in a desiccator until required for the next analysis. Crucibles were transferred from the oven to a desiccator for cooling to room temperature before each analysis. Total and volatile solids were calculated according to the following equations:

$$\% \text{ TS } = \frac{W_3 - W_1}{W_2 - W_1} \text{ x } 100$$ Equation 3.3.1

$$\% \text{ VS (on a wet weight basis) } = \frac{W_3 - W_4}{W_2 - W_1} \text{ x } 100$$ Equation 3.3.2

$$\% \text{ VS (on a TS basis) } = \frac{W_3 - W_4}{W_3 - W_1} \text{ x } 100$$ Equation 3.3.3

Where:

W_1 = weight of empty crucible [g]

W_2 = weight of crucible containing fresh sample [g]

W_3 = weight of crucible and sample after drying at 105 °C [g]

W_4 = weight of crucible and sample after heating to 550 °C [g]

3.4. Chemical and Electrochemical analysis

pH

pH was measured using a Jenway 3010 meter (Bibby Scientific Ltd, UK) with a combination glass electrode, calibrated in buffers at pH 4.7 and 9.2. The pH meter was temperature compensated and had a sensitivity of ±0.01 pH unit and accuracy of 0.01±0.005 pH units. Buffer solutions used for calibration at pH 4.7 and 9.2 were prepared from buffer tablets (Fisher Scientific, UK) according to the supplier's instructions. During measurements, the sample was stirred to ensure homogeneity. The pH probe was rinsed with DI water in between measurements and placed into a mild acid solution to avoid cross-contamination. Digestate samples were measured immediately after sampling to prevent changes in pH due to the loss of dissolved CO_2.

Alkalinity

Alkalinity was measured by titration based on Standard Method 2320B for Alkalinity (APHA, 2005). 2-5 g of digestate was added to 40 mL of DI water. Titration was done using a Schott Titroline Easy automatic digital titration burette system (Schott, Mainz, Germany), with the samples being magnetically stirred while the titration was carried out. A 0.25 N H_2SO_4 titrant was used to determine endpoints of pH 5.7 and 4.3 allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA) (Ripley *et al.*, 1986). PA is a measurement of bicarbonate buffering while IA is attributed to the buffering capacity of Volatile Fatty Acids (VFA). The pH probe was calibrated before titration using buffers as described before and washed with DI water between subsequent samples to avoid cross contamination. Alkalinity was calculated according to the following equations:

$$TA = \frac{(V_{4.0} + V_{4.3} + V_{5.7})\ x\ N\ x\ 50}{V} \qquad \text{Equation 3.4.1}$$

$$PA = \frac{V_{5.7}\ x\ N\ x\ 50}{V} \qquad \text{Equation 3.4.2}$$

$$IA = \frac{V_{4.3}\ x\ N\ x\ 50}{V_s} \qquad \text{Equation 3.4.3}$$

Where:

TA = total alkalinity [g $CaCO_3$ kg^{-1} WW]

PA = partial or bicarbonate alkalinity [g $CaCO_3$ kg^{-1} WW]

IA = intermediate or volatile fatty acid alkalinity [g $CaCO_3$ kg^{-1} WW]

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

$V_{4.3}$ and $V_{5.7}$ = volume of titrant used to endpoints 4.3 and 5.7 respectively [mL]

V = amount of sample [g WW]

Total Ammonia Nitrogen

Total ammonia nitrogen (TAN) analysis was based on Standard Method 4500-NH3 B and C (APHA, 2005). A sample aliquot of between 2-3 g was weighed into a digestion tube and 50 mL of DI water added. Blanks (50 mL DI water) and standards (containing 10 mL of 1000 mg L^{-1} NH_4Cl with 40 mL DI water) were also prepared in digestion tubes. 5 mL of 10 M sodium hydroxide (NaOH) was added to each digestion tube to raise the pH above 9.5 and the samples were distilled using a Büchi K-350 Distillation Unit (Büchi, UK). Erlenmeyer flasks previously filled with 25 mL of boric acid as an indicator were used to collect the distillate and progress of the distillation was indicated by a colour change from purple to green. The distillate was titrated manually with 0.25N H_2SO_4 using a digital titration system (Schott Titroline, Gerhardt UK Ltd) until an endpoint was reached as indicated by a colour change to purple at which point the volume of titrant added was recorded. Standards and blanks were distilled in the same way. The TAN concentration was calculated according to the following equation:

$$TAN = \frac{(A - B) \times 14.0 \times N}{V_s}$$ Equation 3.4.4

Where:

TAN = total ammonia nitrogen [g N kg^{-1} WW]

A = volume of titrant used to titrate the sample [mL]

B = volume of titrant used to titrate the blank [mL]

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

V_s = amount of sample [g WW]

Total Kjeldhal Nitrogen

Total Kjeldhal Nitrogen (TKN) analysis was carried out on duplicate samples alongside blanks and controls as follows: 3-5 g (weighed to ± 1 mg) of sample was placed in a glass digestion tube. Two Kjeltab Cu 3.5 catalyst tablets were added to facilitate acid digestion by lowering the activation energy of the reaction. 12 mL of low nitrogen concentrated H_2SO_4 was added carefully to each digestion tube and agitated gently to ensure that the entire sample was completely exposed to acid. The digestion tubes were then placed into the heating block with exhaust system using either a Foss Tecator 1007 Digestion System 6 (Foss Analytical, Hoganas Sweden) or a Büchi K-435 Digestion Unit (Büchi, UK) for approximately two hours until the solution colour became a clear blue-green. Both systems operated at 420 ± 5 ^{o}C and once the reaction was completed the tubes were cooled to around 50 ^{o}C and 40 mL of DI water slowly added to the digestion tube to prevent later crystallisation on further cooling. Samples, blanks and standards were then distilled and titrated as for Total ammonia nitrogen.

$$TKN = \frac{(A - B) \text{ x } 14.0 \text{ x } N \text{ x } 1000}{V_s}$$ Equation 3.4.5

Where:

TKN= total kjeldhal nitrogen [mg N kg^{-1} WW]

A = volume of titrant used to titrate the sample [mL]

B = volume of titrant used to titrate the blank [mL]

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

V = amount of sample [g WW]

3.5. Instrumental analysis

Gas Chromatograph (GC) determination of volatile fatty acids (VFA)

The method used was based on SCA (1979): Determination of Volatile Fatty Acids in Sewage sludge (1979). Samples were prepared for analysis by centrifugation at 14,000 revolutions per minute (rpm) (micro-centrifuge, various manufacturers) for 15 minutes. 0.9 mL of the supernatant was transferred by pipette to vials with 0.1 mL formic acid to give a final concentration of 10% formic acid. Where dilution was necessary, DI water was used and formic acid was added to give a concentration of 10% of the total volume for analysis. If the samples at this point were turbid they were centrifuged again at 14,000 rpm to obtain a clearer supernatant. The supernatant after acidification and centrifugation was transferred into the vials and loaded onto the GC auto-sampler ready for the VFA measurement.

A mixed acid standard solution containing acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids, at three dilutions to give individual acid concentrations of 50, 250 and 500 mg L^{-1} respectively, was used for calibration and also loaded onto the GC.

Quantification of the VFA was by a Shimadzu GC-2010 gas chromatograph (Shimadzu, Milton Keynes, UK) using a flame ionization detector and a capillary column type SGE BP-21. The carrier gas was helium at a flow of 190.8 mL min^{-1} and a split ratio of 100 to give a flow rate of 1.86 mL min^{-1} in the column and a 3.0 mL min^{-1} purge. The GC oven temperature was programmed to increase from 60 to 210 °C in 15 minutes with a final hold time of 3 minutes. The temperatures of injector and detector were 200 and 250°C, respectively.

Flame Atomic Absorption Spectrometry

Trace elements were measured on a Flame Atomic Absorption Spectrometer (AAnalyst 200, PerkinElmer, USA) operated according to the manufacturer's instructions using a hollow cathode lamp. The conditions used are shown in Table 3. Calibration solutions were prepared from a stock solution of Fe, Co and Ni (Fisher Scientific Standard solution, 1000 mg L^{-1} in HNO_3 for atomic spectroscopy) by dilution to the required concentration range using 12.5% nitric acid (HNO_3).

Approximately 1.5 g of sample was added to the digestion tube, with blanks prepared in parallel. 15 mL of 35-36% w/v HCl (Hydrochloric acid) was added, then after ~5 minutes 5 mL of 70% w/v HNO3 (Nitric acid) was added, and the tubes were gently agitated. The tubes were placed into the digestion block (Gerhardt Kjeldatherm), connected to the condenser system and left for 24 hours prior to heating. The acid digestion involved gradually increasing the temperature first to 100 ^{o}C and then to the final temperature of ~180 ^{o}C which was maintained for about 2 hours ± 10 min. After cooling, the mixtures were filtered (Filter paper No. 1 Qualitative 11 cm, Whatman, UK) into a 50- mL volumetric flask. Any remaining residue in the tube was washed out with ~5 ml of warm 12.5% v/v HNO_3 and transferred to the 50 mL flask, with up to 5 washes being performed. The volume was then made up to 50 mL with HNO_3 (12.5% v/v). Digested sample was then analysed for Fe, Co and Ni as described above and with additional dilution with 12.5% nitric acid (HNO_3) if required.

Some samples (maize silage, baby maize stover and digestate from laboratory experiments) were sent for analysis (NRM Ltd, UK).

Table 3. Parameters for each of element analysed

Metal	Wave length [nm]	Slit [nm]	Linear Range [mg L^{-1}]	Flame
Fe	248.3	0.2	6.0	Oxidizing / LEAN-BLUE
Co	240.7	0.2	7.0	Oxidizing / LEAN-BLUE
Ni	341.5	0.2	20.0	Oxidizing / LEAN-BLUE

3.6. Gas analysis

Gas composition

The gas produced during anaerobic digestion of wastes contains methane and carbon dioxide (CO_2) as its major components with minor quantities of water vapour, hydrogen, hydrogen sulphides, nitrogen, and oxygen and other trace components.

Methane and carbon dioxide

Biogas composition was quantified using a Varian Star 3400 CX gas chromatograph (Varian Ltd, Oxford, UK). The GC was fitted with a Hayesep C column and used either argon or helium as the carrier gas at a flow of 50 mL min^{-1} with a thermal conductivity detector. The biogas composition was compared with a standard gas containing 65 % CH_4 and 35% CO_2 (v/v) (BOC Ltd, UK) for calibration. A sample of 10 mL was taken from a gas-impermeable sampling bag used for sample collection and was injected into a gas sampling loop.

Gas volume

Gas bags. Unless noted, biogas was collected in gas-impermeable sampling bags. Gas bag volumes were measured using a weight-type water displacement gasometer (Walker *et al.*, 2009). The measurement procedure was as follows: the initial height of solution in the gasometer (h_1) was recorded before the collected gas was introduced into the column through the top valve. After the bag was empty, the final height (h_2) and the weight of water (m) were recorded, as well as the temperature (T) and pressure (P) in the room. This study used weight gasometer governing equation, with height gasometer governing equation to provide a check on any gross measurement errors. All gas volumes reported are corrected to standard temperature and pressure of 0^oC, 101.325 kPa as described by Walker (Walker *et al.*, 2009) according to the following equations;

Height Gasometer Governing Equation Equation 3.6.1

$$V_{stp} = \frac{T_{stp} A}{T_{atm} p_{stp}} \left(\left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g \left(h_{t2} - h_{c2} \right) \right) h_{c2} - \left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g \left(h_{t1} - h_{c1} \right) \right) h_{c1} \right)$$

Weight Gasometer Governing Equation Equation 3.6.2

$$V_{stp} = \frac{T_{stp} A}{T_{atm} P_{stp}} \left[\left(\left(P_{atn} - p_{H_2O}(T_{atm}) + \rho_b g \left(H - h_1 - \frac{m_b}{A\rho_b} \right) \right) \left(h_1 + \frac{m_b}{A\rho_b} \right) \right) - \left(p_{atn} - p_{H_2O}(T_{atm}) + \rho_b g (H - h_1) \right) h_1 \right]$$

Where:

V = gas volume [m^3]

P = pressure [Pa]

T = temperature [K]

H = total height of column [m]

h = distance to liquid surface from a datum [m]

A = cross-sectional area of gasometer [m^2]

m_b = mass of barrier solution [kg]

ρ = density pf barrier solution [kg m^{-3}]

g = gravitational acceleration [m s^{-2}]

1, 2,standard temperature and pressure [STP], atm, b,H20c subscripts refer to condition 1 (before addition of gas to column), condition 2 (after gas addition to column), standard temperature and pressure, atmospheric, barrier solution and water vapour, respectively.

Note: this calculation gives the volumes of dry biogas i.e. without water vapour

3.7. Specific analysis

Calorific value

Calorific value was quantified using a bomb calorimeter (CAL2k EC, Digital Data System Ltd, South Africa) according to the manufacturer's instructions. The sample was pre dried in an oven overnight at 105 ºC. Then, 0.4 g (weighed with an accuracy of 0.1 mg) was added to the crucible and placed in position in the calorimeter. A cotton firing thread was attached to the ignition wire and fed to the crucible. The bomb vessel was then assembled and pressurised, using the filling station, with oxygen until the pressure reached 3 MPa. The bomb was then placed in the calorimeter and fired and temperature changes logged on the computer. A blank was also run to account for the energy released in burning the fuse and a standard was run using benzoic acid (around 1 g with an accuracy of 0.1 mg) with an higher heat value (HHV) of 26.454 kJ g^{-1} TS.

Elemental Composition

Carbon, hydrogen and nitrogen contents of samples were determined using a FlashEA 1112 Elemental Analyser (Thermo Finnigan, Italy). Samples were air dried and milled to obtain a homogenous sample. Sub-samples of approximately 3-4 mg were weighed into standard weight tin disks using a five decimal place analytical scale (Radwig, XA110/X, Poland). These were placed in a combustion/reduction reactor held at 900°C then flash combusted in a gas flow temporarily enriched with oxygen resulting in a temperature greater than 1700 °C and the release of N_xO_x, CO_2, H_2O and SO_2 (depending on the composition of the sample). The gas mixture was then analysed by GC with the different components are measured by appropriate detectors. The working conditions of the elemental analyser were as described in the manufacturer's technical literature and method sheets. Standards used in this analysis were methionine, nicotinamide and birch leaf. The sample preparation was carried out by this study, then the samples were kindly analysed by Pilar Pascual-Hidalgo.

Capillary Suction Time (CST) Test

The CST test was carried out using a Triton-WRPL type 130, a type 319 Multi CST apparatus and paper (Triton Electronics Ltd, UK). 5 mL of the digestate sample was poured into the small circular tube which presses down on a piece of CST filter paper placed on the lower perspex block of the apparatus (Figure 6). Two electrodes placed at a standard distance from the central filling tube detect the presence of water in the CST filter paper. The CST is as the time taken for the water to travel along the paper between the first and second electrodes. The time interval depends on the resistance of the cake to giving up its water (Scholz, 2005). A digestate with a CST lower than 10 second is considered to have a good dewaterability.

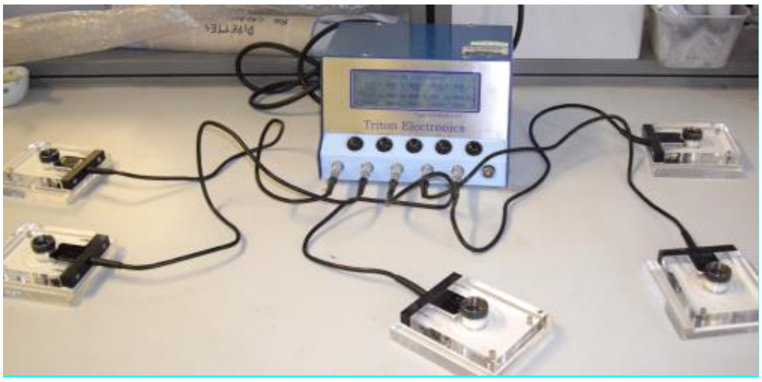
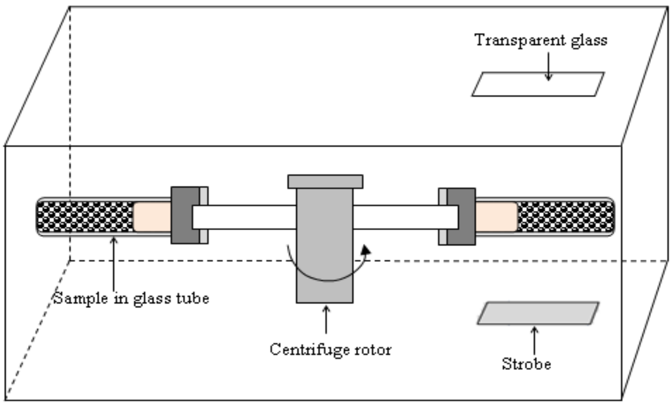


Figure 6. CST test apparatus

Frozen Image Centrifuge (FIC) Test

The FIC test was carried out using a Triton WRC model I6I centrifuge (Triton Electronics Ltd, UK) at maximum speed (1070 rpm), with supernatant height recorded against time. The time observations were from 10 min to 1 hr. This test uses a stroboscopic technique in which a 'frozen image' of the sample is generated which allowing changes in the solid liquid interface to be measured in real time without stopping the centrifuge. The mechanism operates by matching the frequency of the strobe light to the rotor speed of the centrifuge (Figure 7).



(a) Schematic of FIC apparatus (b) FIC

Figure 7. FIC test apparatus

Viscosity

The viscosity test was carried out using a Brookfield digital viscometer model DV-E (Brookfield engineering laboratories Ltd, USA) according to the manufacturer's instructions. The viscosity test was carried out immediately after sample collection because viscosity relied on temperature. The viscometer was provided with a set of four-six spindles. The DV-E had a spindle entry code number to calculate viscosity values, therefore, the two digit entry code for each spindle was used when spindle was changed. Spindle was inserted and centred in the test material until the fluid's level was at the immersion groove on the spindle's shaft. When % (torque) readings exceed 100 % (over range), the display changed to that shown "cP EEEE". In that case, reduction the speed or use a smaller size spindle to correct this condition was required. For maximum accuracy, flashing readings below 10 % was avoided. Viscosity analysis was conducted in duplicate. In this study, the following operation was conducted.

1. Sample collection
2. Mixing sample
3. Insert spindle
4. Reading the viscosity when the value settled

Determination of structural carbohydrates and lignin in biomass

Determination of structural carbohydrates and lignin in biomass followed the method by of the National Renewable Energy Laboratory (NREL) (Sluiter, 2008). Samples were freeze dried and 300 ± 10.0 mg were weighed for acid digestion. These acid solubilisation, gravimetric and HPLC analyses were used for determination of lignin, glucose and xylose.

3.8. Laboratory scale digesters

Description. The digesters used were of the continuously-stirred tank reactor (CSTR) type with a total volume of 5 litres, and were operated at a working volume of 3 – 4 L. A schematic drawing of a pair of digesters is shown in Figure 8. The Laboratory-scale digesters used in the research is shown in Figure 9. The digesters were constructed in polyvinyl chloride (PVC) with a top flange to which a top plate was secured using stainless steel bolts and wing nuts. A gas tight seal between the top plate and the digester flange was maintained using a closed pore neoprene gasket. The top plate was fitted with a gas outlet connector and a feed port sealed with a rubber bung. On the top plate a DC motor was mounted which coupled to the digester stirrer through a draught tube water gas seal, the draught tube itself being secured in a gas tight compression seal. Digestate was removed from the digester via a 15 mm diameter outlet port at the base of the digester. The contents of the digesters were continuously mixed by means of an asymmetric stirrer at 40 rpm. Temperature was maintained at 35 ^{o}C +/- 0.5; by water circulating through an external heating coil that surrounded the digesters.

When assembled, and before filling, each digester was tested for gas leaks by applying a positive pressure to the digester and submerging in water to ensure there was no gas escape when all ports were sealed. The digesters were connected to gas counters, which continuously measured gas production throughout the digestion period; the gas counters operated by the alternate filling and discharging of a calibrated cell which logged each discharge via a labjack (labjack ltd) computer interface (Walker et al, 2009). The calibration of each gas counter was checked twice a week by attaching a 10-litre gas collection bag (Tedlar SKC 232, SKC Ltd, Blandford Forum, UK) to the gas vent of gas counter.

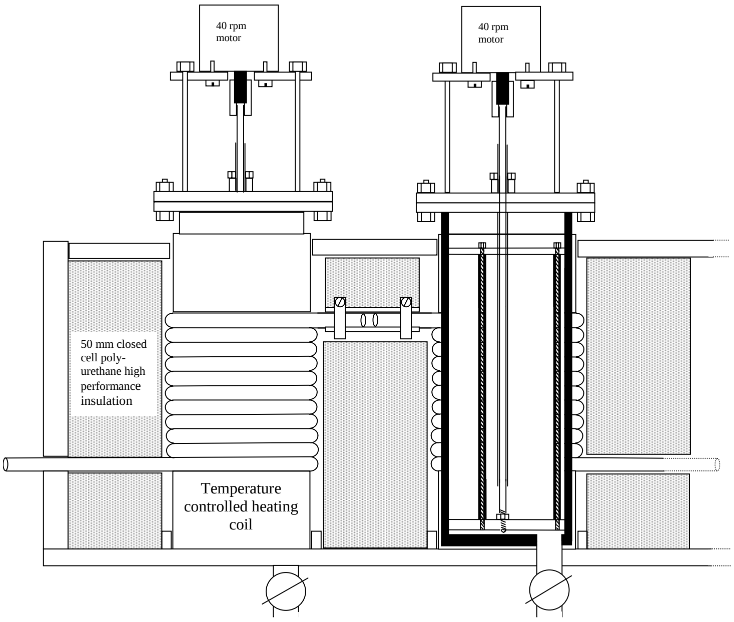
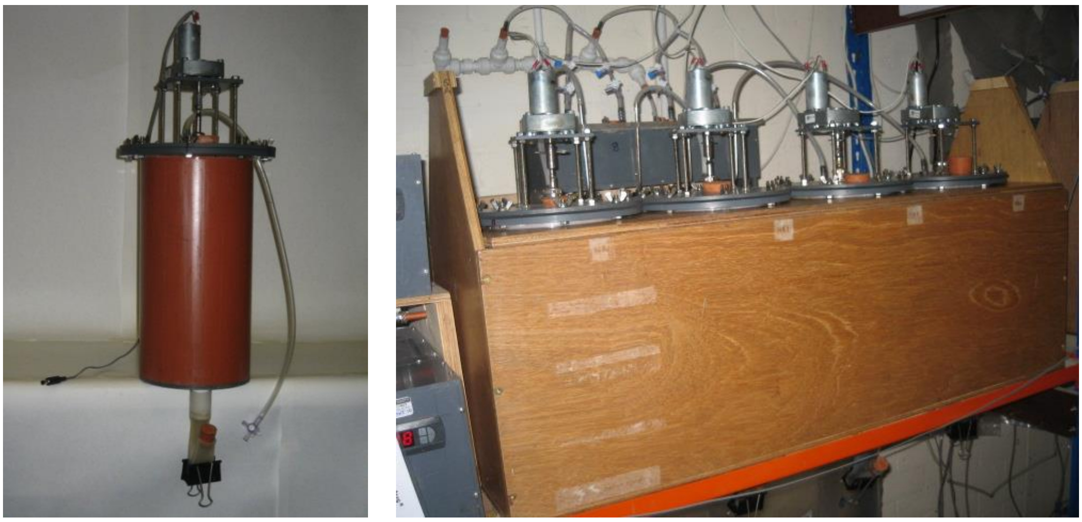


Figure 8. Schematic of 5 L CSTR digester



5 L digester

Set of 5 L digesters in temperature controlled box

Figure 9. Laboratory-scale digesters used in the research

Digester operation and calculations

The digesters were operated in a semi continuous mode i.e. fed daily with a specific amount of feedstock and digestate removed weekly to maintain a constant volume in the digester. The organic loading rate (OLR) [g VS L^{-1} day^{-1}] was determined according to equation 3.8.1.

$$OLR = \frac{mVS_{substrate}}{V_{reactor}}$$ Equation 3.8.1

Where:

m is the mass of substrate daily added to the reactor [g day^{-1}]

$VS_{substrate}$ is the volatile solid content of feedstock [% wet weight]

$V_{reactor}$ is the volume of reactor [L]

HRT [day] of the digester is expressed in equation 3.8.2.

$$HRT = \frac{V_{reactor}}{Q}$$ Equation 3.8.2

Where:

$V_{reactor}$ is the working volume of each reactor [mL]

Q is the daily flow of material (substrate added and digestate removed) through the reactor [mL day^{-1}]

The amount of substrate and digestate was measured in g but for ease of calculation it was assumed that both the substrate and digestate had a specific gravity of 1.0. Therefore, 1 g of substrate and digestate was considered to be equivalent to 1 mL.

The amount of substrate and digestate was measured in g but for ease of calculation it was assumed that both the substrate and digestate had a specific gravity of 1.0. Therefore, 1 g of substrate and digestate was considered to be equivalent to 1 mL.

Where reactors were operated at a series of different loading rates (OLR) for periods of less than 3 HRT, in some cases an equivalent HRT is quoted. Equivalent HRT is useful as a simple comparative indicator of the state of progression of a trial when reactors have had a complex feeding pattern (e.g. pauses in feeding due to VFA accumulation or other disturbances); although it does not give an accurate reflection of the changing status e.g. of materials being washed out of or accumulated in the reactor over time.

Equivalent HRT is calculated from the volume of the reactor divided by the total amount of feed added in the time period considered. HRT equivalent for total amount of substrate addition [-] is expressed in equation 3.8.3.

$$HRT\ equivalent\ for\ total\ amount\ of\ substrate\ addition = \frac{V\ feed}{V\ reactor}$$

Equation 3.8.3

V_{feed} is the total amount of added substrate [L]

$V_{reactor}$ is the working volume of each reactor [L]

The performance of digesters was monitored in terms of specific biogas and methane production and VS destruction which were calculated using equations 3.8.4, and 3.8.5.

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

Equation 3.8.4

V_{biogas} is the volume of biogas produced daily [L day^{-1}]

OLR is the organic loading rate [g VS L^{-1} day^{-1}]$V_{reactor}$ is the volume of reactor [L]

$$\text{Specific methane production} = \frac{V_{CH4}}{OLR \times V_{reactor}}$$

Equation 3.8.5

Where:

V_{CH4} is the volume of methane produced daily [L day^{-1}]

OLR is the organic loading rate [g VS L^{-1} day^{-1}]

$V_{reactor}$ is the volume of reactor [L]

BMP test

The BMP test was carried out using a bioreactor (CJC LABS Ltd, UK)(http://cjc-labs.com/products). The BMP apparatus consisted of glass reaction bottles each with a capacity of 550 mL and a working volume of 400 mL sealed with a rubber bung through which a stainless steel metal tube was inserted. The bottles were maintained at 35 ^{o}C in a temperature controlled water bath at 35^{o}C with each one connected from the stainless steel tube to a gas bag. The volume of biogas collected was corrected to a STP of 0°C and 101.325 kPa (Walker *et al.*, 2009). Biogas samples were taken from the gas bags and analysed for gas composition.

On the day of test, fresh inoculum from Millbrook WWTP and substrate were collected. Control reactors were filled with 400 mL inoculum whereas; the test reactors were filled with a mixture of inoculum and substrate in a ratio according to the experimental design. Temperature, pressure and gas volume was noted during working hours every hour in the first week, every 2-3 hours in the 2nd and 3rd week and twice a day over the remaining period of the test.

Pre-hydrolysis

Pre-hydrolysis was carried out by removing a quantity of digestate from the main digester each day and placing it in a 500 mL flask in a water bath at 55 ^{o}C for 24 hours.

The quantities of material to be added or removed from the main digester were aimed at achieving a TS content in the hydrolyser of < 10%, and were decided based on the result of a preliminary assessment of the solids content of the supernatant fraction of digestate after centrifugation. The following example is based on a working volume of 4 L, and OLR of 4 kg VS L day^{-1} and a digestate supernatant TS of ~ 4% (Figure 10a).

i. 400 mL of digestate (i.e. 10 % of digester volume) was removed from the test reactors every day.
ii. A proportion of this, sufficient to maintain the digester volume constant was wasted (e.g. slightly less than volume of daily feed for a test reactor)
iii. The remainder was placed in a centrifuge tube
iv. The centrifuged digestate was separated into 2 parts: supernatant (liquid) and precipitate (solid)
v. The precipitate went back to the CSTR
vi. The supernatant was placed in a 500 mL conical flask
vii. Baby maize stover was added to the conical flask with the supernatant

viii. The conical flask was sealed by a bung with an outlet tube connected to a gas bag, and placed in a shaking water bath at 55 ^oC for 24 hours

ix. After the removal of the next daily sample of digestate as in (i), the mixture of supernatant and baby maize stover in the flask was added to the CSTR

A modified pre-hydrolysis method was conducted from Trial 2-2 which did not include the centrifugation step (Figure 10b).

The weight of conical flask before and after pre-hydrolysis was noted and the weight loss was calculated using equation 3.8.6.

Weight loss [%] = $\frac{W3 - W1}{W2 - W1} \times 100$

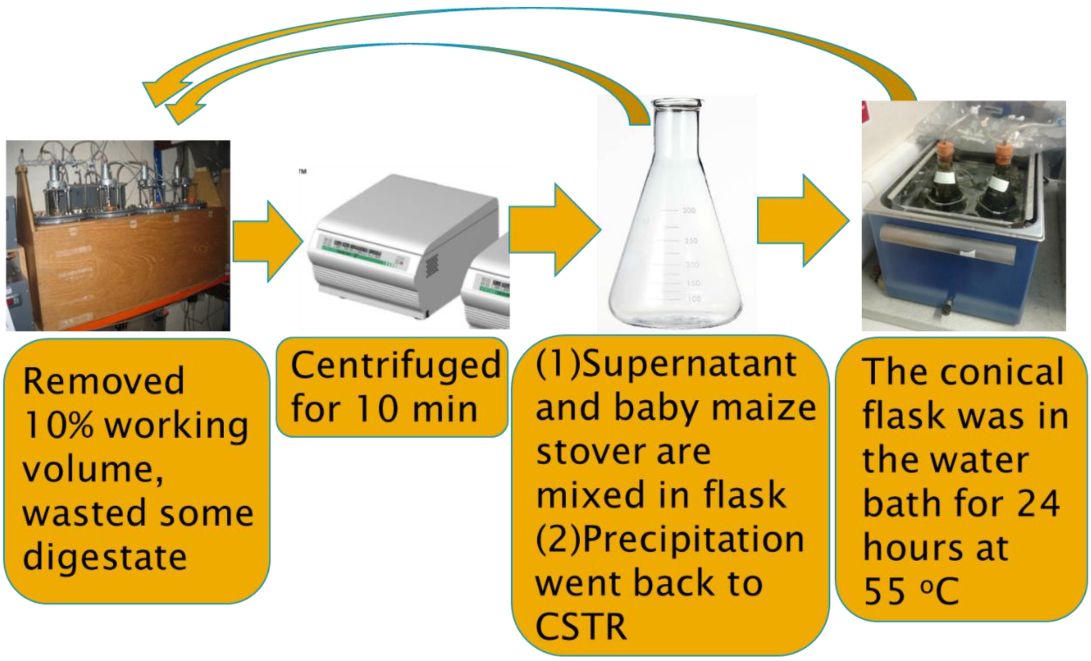
Equation 3.8.6

W1: conical flask weight [g]

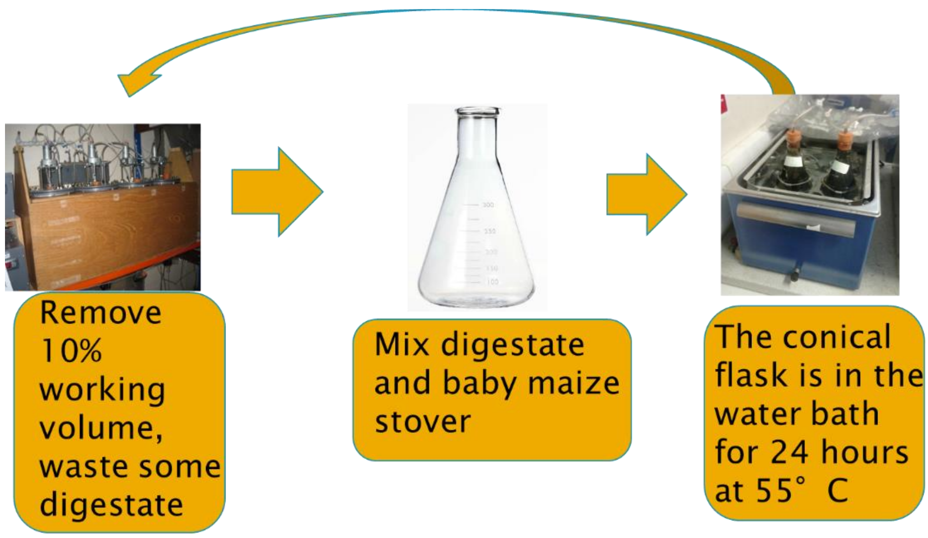
W2: conical flask with digestate before pre-hydrolysis [g]

W3: conical flask with digestate after pre-hydrolysis [g]

The digestate loss due to the process (i.e. wasted digestate, spillage) was noted and applied in calculation of the VS destruction in the main digesters.



(a) Pre-hydrolysis for Trial 1 and Trial 2-1



(b) Pre-hydrolysis for Trial 2-2 and Trial 2-3

Figure 10. Pre-hydrolysis

3.9. Summary of the objectives, trials and parameters of the different trials

Table 4 shows the aim of each trial and the experimental parameters. Trial 1 was for anaerobic processing of conventional substrate, maize silage, to establish a baseline for comparison with baby maize stover. Trial 2-1 was the first trial using baby maize stover and assessed the anaerobic digestion performance under mesophilic and thermophilic conditions, and with or without thermophilic pre-hydrolysis for the mesophilic digestion. Trial 2-1 showed some signs of instability and gas production from the 2-stage digesters was always less than that of single stage. It was considered that the instability and other features may be related to a lack of TE as the inoculum used was taken from Trial 1. Therefore, Trial 2-2 used fresh Millbrook digestate as inoculum. Trial 2-3 was to assess the effects of TE addition.

Table 4. Summary of trial objectives

	Objective	Substrate	Inoculum source	Temperature	TE additions	Pre-treatment
Trial 1	To establish the baseline if methane productivity for European fodder maize	Maize silage	D1-8: fresh Millbrook digestate	D1-4: 35 °C D5-8: 55 °C	D1-8: Fe 10 mg L^{-1}, Co 1 mg L^{-1}, Ni 1 mg L^{-1} from day 140 in D1-4 and 135 in D5-8	N/A
Trial 2-1	To assess the anaerobic digestion performance of baby maize stover at mesophilic and thermophilic temperatures in terms of digestion efficiency, stability and methane production potential	Baby maize stover	D1-4: mixture of digestate in D1-4 from Trial 1 D5-8: digestate from Trial 1 D9-10: 1 L fresh Millbrook digestate + 2 L mixture of digestate in D1-4 from Trial 1	D1-4: 35 °C D5-8: 55 °C D9-10: 35 °C	D1-10: Fe 10 mg L^{-1}, Co 1 mg L^{-1}, Ni 1 mg L^{-1}	D3-4: theremophilic digestion at 55 °C for 24 hours (Pre-hydrolysis included centrifuge step)
Trial 2-2	To determine the difference in gas production potential and stability of baby maize stover digestion in mesophilic conditions with and without a thermophilic pre-hydrolysis stage, using fresh Millbrook digestate as inoculum, not digestate from Trial 1	Baby maize stover	D1-4: fresh Millbrook digestate	D1-4: 35 °C	N/A	D3-4: theremophilic digestion at 55 °C for 24 hours (At the beginning, pre-hydrolysis included centrifusing step, and then, pre-hydrolysis omitted the centrifuge step)
Trial 2-3	To determine the effect of alternative TE supplementation strategies on the performance of thermophilic digestion (3 and 5 TE) and mesophilic digestion (5 TE and no TE), and a thermophilic pre-hydrolysis step before mesophilic digestion TE addition (5 TE), as indicated by gas production and operational stability	Baby maize stover	D1-4, 9-10: 2 L fresh Millbrook digestate + 1 L mixture of digestate in D9-10 from Trial 2-1 D5-8: mixture of digestate in D5-8 from Trial 2-1	D1-4: 35 °C D5-8: 55 °C D9-10: 35 °C	D1-4: Fe, Co, Ni, Se, Mo D5-6: Fe, Co, Ni D7-8: Fe, Co, Ni, Se, Mo D9-10: No TE (concentration: Fe 10 mg L^{-1}, Co 0.4 mg L^{-1}, Ni 1 mg L^{-1}, Se 0.2 mg L^{-1}, Mo 0.2 mg L^{-1}) *One-off W 0.2 mg L^{-1} dosing to D5-8	D3-4: theremophilic digestion at 55 °C for 24 hours (Pre-hydrolysis did not includ centrifuge step)

4. Results and discussion

Chapter 4 reports on the results of the laboratory experimental trials. It includes feedstock and inoculum characterisation, Trial 1, Trial 2-1, Trial 2-2, Trial 2-3, conical flask test for Trial 2-3, energy conversion efficiency and discussion. Trial 1 was for maize silage and Trial 2 was for baby maize stover.

4.1. Feedstock and inoculum characterisation

The feedstock and inoculum characteristics are described below.

Baby maize stover: This study used baby maize stover (Figure 11a) which was delivered by air freight from Gorge Farm, Naivasha, Kenya in 2 batches of around 200 kg each. During the experimental work the TS and VS contents were measured in triplicate every time a new storage bag was opened. The results are shown in Table 5 and Figure 12. The average TS was 19.5 ± 1.30 % WW and VS was 17.6 ± 1.22 % WW. The slope of the line of baby maize stover was 0.88 (Figure 12) which was slightly lower than that of the VS/TS 90.4 ± 0.37 % (Table 5). Nouala (Nouala *et al.*, 2004) reported values for baby maize stover of TS 33 % WW, VS 93 % TS. Hiep (2003) reported TS 24 % WW, VS 95 % TS. The baby maize stover from Kenya thus had a slightly higher moisture content than these literature values, and a slightly lower ratio of VS to TS.

The substrate characteristics are shown in Table 6. The carbon to nitrogen (C/N) ratio was around 43 which is outside the optimum for anaerobic digestion but within the typical range for conventional maize silage feedstocks (Amon *et al.*, 2007). The fibre analysis results were lignin 24.5 % TS, glucose 30.1 % TS, xylose 9.6 % TS, The analysed lignin value was much higher than the value of 3.2 % TS obtained by Hiep (2003). This may be related to the use of different baby maize stover: the lack of other reported values makes it difficult to assess whether this is simply. The farm location, growing method, season and harvesting time may all have effects on the fibre composition (Rincón *et al.*, 2016) and there are quite large variations in reported values for maize-derived materials. For maize stover, Tirado-González *et al.* (2016) reported a lignin content of 4.6 % TS but Lizasoain *et al.* (2017) found lignin was 18.3 % TS which was approximately 4 times higher.

It was difficult to find other literature values to compare with experimental results because baby maize stover is not a common substrate for biogas production. To check the data reliability, where possible cross checking was carried out. Trace element analysis (Fe, Co, Ni) was conducted at

University of Southampton and NRM Ltd. NRM reported Co was less than 1 mg kg^{-1} TS and the result from this study was less than 0.7 mg kg^{-1} TS. NRM's value for Ni was 1.0 mg kg^{-1} TS and this study was 1.0 mg kg^{-1} TS. NRM's value for Fe was 524 mg kg^{-1} TS and this study was 523 mg kg^{-1} TS. These values show reasonable agreement.

(a) Baby maize stover from Gorge Farm, Naivasha, Kenya

(b) Maize silage from CEDAR, showing signs of fungal attack and degradation

Figure 11. Images of baby maize stover and maize silage feedstocks as delivered to Southampton

Maize silage: Maize silage was obtained from the Centre for Daily Research (CEDAR) at the University of Reading, UK. On opening the bulk bags of maize silage for processing in the laboratory at Southampton it was realised that the quality was variable, with some material drier (e.g. that taken from the top of the pile) and some wetter, and some beginning to ferment (Figure 13b). No single container was available that was big enough to mix the whole batch in one load, however, and there were concerns that if mixing was delayed until the whole batch was ground, more of the material might ferment. The maize silage was therefore mixed as well as possible, consistent with the need for rapid processing; and it was accepted that some variation in parameters was likely for different bags used during feeding.

During the experimental work the TS and VS contents were measured in triplicate every time a new storage bag was opened. The results are shown in Table 5 and Figure 12. The maize silage showed more variation than the baby maize stover (Figure 12). Average TS was 39.1 ± 1.58 % WW and VS was 37.0 ± 1.99 % WW. The slope of the line of maize silage was 0.93 (Figure 12) which was within the range of the VS/TS 94.6 ± 3.63 % (Table 5). The literature values were: TS 18 - 52.9 % WW, VS 17.2 - 50.7 % WW (Amon *et al.*, 2007; Bauer *et al.*, 2010; Cornell, 2011; Evranos and Demirel, 2015). The experimental values were thus within the mid-range of the literature values. Cornell (2011) analysed maize silage at BORRG, University of Southampton where this study was carried out. The maize silage was TS - 33.7 % WW, VS - 32.2 % WW. This study's maize silage TS was approximately 5 % drier than that used in Cornell's study.

The substrate characteristics for the maize silage are shown in Table 6. The C/N ratio was around 37, again outside the preferred range (Mata-Alvarez, 2003). Fibre analysis results showed some difference from those of Amon *et al.* (2007). Results for the maize used were lignin 15.1 % TS, glucose 53.7 % TS, xylose 5.7 % but literature values were lignin 4.3 – 8.6 % TS, cellulose 19.3 – 37.3 % TS, hemicellulose 25.3 – 36.2 % TS. The difference in lignin content may be due to growth stage at harvest or to maize species.

TE analysis cross checking was also carried out as above. Co and Ni values were similar, but Fe was different. This study result was 130 mg kg^{-1} TS and the NRM value was 263 mg kg^{-1} TS. The maize silage was less homogeneous than the baby maize stover, and samples from different bags were used for TE analysis by NRM and at Southampton. The inhomogeneous substrate may have contributed to the different Fe concentration.

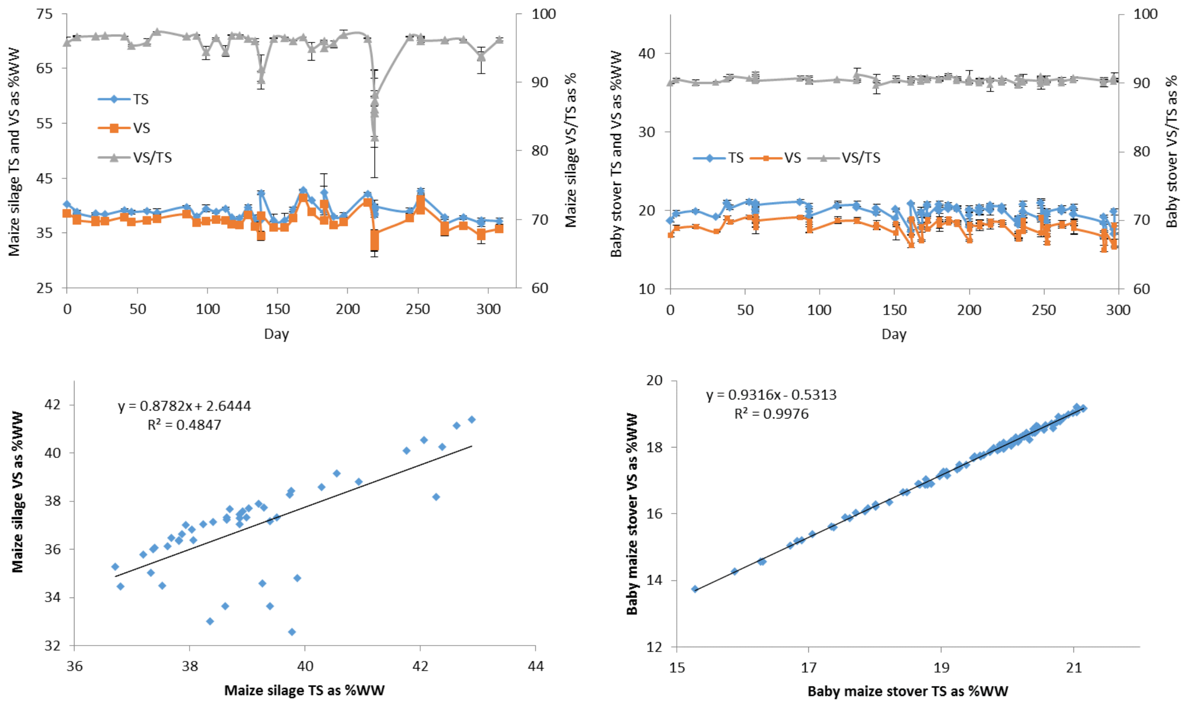


Figure 12. Substrate TS and VS content for maize silage and baby maize stover: triplicate samples taken every time a new bag is opened. Error bars indicate range

Table 5. Feedstock solids content: long-term averages from each bag used in this trial, triplicate samples taken every time a new bag is opened

	Maize silage	Baby maize stover
TS [% Wet Weight (WW)]	39.1 ± 1.58	19.5 ± 1.30
VS [% WW]	37.0 ± 1.99	17.6 ± 1.27
VS [% TS]	94.6 ± 3.63	90.4 ± 0.37

Table 6. Feedstock characteristics – maize silage and baby maize stover

	Unit	Maize silage	Baby maize stover
Lignin[a]	% TS	15.1	24.5
Glucose[a]	% TS	53.7	30.1
Xylose[a]	% TS	5.7	9.6
C[a]	% VS	48.9	51.6
H[a]	% VS	5.9	5.6
O[a]	% VS	43.8	41.5
N[a]	% VS	1.3	1.2
S[a]	% VS	0.1	0.1
HHV (Buswell)[a]	kJ g^{-1} VS	18.3	19.3
HHV(bomb calorimetry)[a]	kJ g^{-1} VS	20.7	22.1
TKN[a]	% TS	-	1.3
Total nitrogen (N)[b]	% TS	1.2	1.2
Total phosphorus (P)[b]	% TS	0.2	0.3
Total potassium (K)[b]	% TS	1.0	2.9
Total sulphur (S)[b]	% TS	0.1	0.1
Cobalt (Co)[a]	mg kg^{-1} TS	<0.7	<0.7
Cobalt (Co)[b]	mg kg^{-1} TS	<1	<1
Nickel (Ni)[a]	mg kg^{-1} TS	<0.8	1.04
Nickel (Ni)[b]	mg kg^{-1} TS	<1	1.01
Iron (Fe)[a]	mg kg^{-1} TS	130	523
Iron (Fe)[b]	mg kg^{-1} TS	263	524
Total copper (Cu)[b]	mg kg^{-1} TS	4.2	5.6
Total zinc (Zn)[b]	mg kg^{-1} TS	22.4	38.8
Calcium (Ca)[b]	mg kg^{-1} TS	2486	3140
Molybdenum (Mo)[b]	mg kg^{-1} TS	<1	2.4
Manganese (Mn)[b]	mg kg^{-1} TS	11.1	32.2
Selenium (Se)[b]	mg kg^{-1} TS	<0.09	<0.09
Tungsten (W)[b]	mg kg^{-1} TS	<0.1	<0.1
TS[b]	% WW	41	18.6

[a]: Analysis was carried out at University of Southampton
[b]: Samples were sent to NRM for analysis

Inoculum: The inoculum characteristics were measured on 03/12/2014 (Trial 1, D1-8), 10/6/2015 (Trial 2-1, D9&10), 3/3/2016 (Trial 2-2, D1-4) and 20/04/2016 (Trial 2-3, D1-4 and D9&10). The analysis was carried out twice or three times for each determination except for pH The fresh Millbrook inoculum had TS 4.1- 4.5 % WW, VS 2.8 – 3.0 % WW, ammonia 1.5 - 1.6 g N kg^{-1} WW, TA 7.3 – 7.7 g $CaCO_3$ kg^{-1}, PA 4.7 – 5.0 g $CaCO_3$ kg^{-1}, IA 2.4 – 2.9 g $CaCO_3$ kg^{-1} (Table 7).

Table 7. Inoculum characteristics before digestion trials

	03/12/2014	10/06/2015	03/03/2016	20/04/2016
Trial	Trial 1, D1-8	Trial 2-1, D9&10	Trial 2-2, D1-4	Trial 2-3, D1-4, D9&10
pH	8.22	8.25	7.48	7.48
TS [% WW]	4.1	4.2	4.4	4.5
VS [% WW]	2.8	3.0	2.9	3.0
Ammonia [g N kg^{-1}]	1.5	1.7	1.6	1.6
Total Alkalinity [g $CaCO_3$ kg^{-1}]	7.3	7.6	7.7	7.6
Partial Alkalinity [g $CaCO_3$ kg^{-1}]	4.9	5.1	5.0	4.7
Intermediate Alkalinity [g $CaCO_3$ kg^{-1}]	2.4	2.5	2.7	2.9
IA:PA	0.48	0.49	0.53	0.62

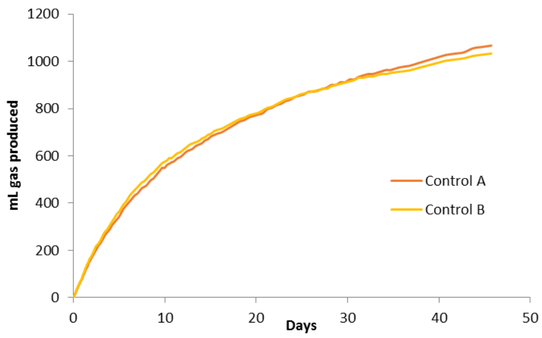
BMP test

A biochemical methane potential test was carried out on the baby maize stover by colleagues. Four 0.5-litre digesters of the type described in section 3.8 were used in this trial, two with baby maize stover and two as inoculum-only controls. Temperature was controlled at 35 ℃ in a water bath. The digesters were seeded with fresh inoculum from Millbrook WWTP. Table 8 provides the condition of the BMP trial.

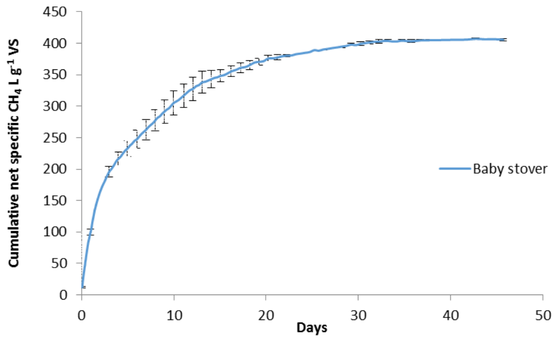
Table 8. BMP operational condition

	Inoculum	Substrate	VS content	Substrate	VS basis
	g WW	g WW	[% WW]	[g VS]	[I/S ratio]
Control 1	500	0.0	24.0	0.00	-
Control 2	500	0.0	24.0	0.00	-
Maize 1	500	17.4	178.4	3.10	3.87
Maize 2	500	17.4	178.4	3.10	3.87

Figure 13 shows the gas production during the BMP test. The final BMP was taken as the value obtained from gas bag measurements and was equal to 0.404 and 0.407 L CH_4 g^{-1} VS for the duplicate samples, giving an average value of 0.405 L CH_4 g^{-1} VS.



(a) Cumulative gas production – blanks controls



(b) Cumulative net specific methane production

Figure 13. Gas production in BMP test

4.2. Digestion Trial 1 – maize silage

The purpose of this trial was to establish a baseline value of methane productivity for European fodder maize in mesophilic and thermophilic conditions as organic loading rates typical of those used in commercial AD plant. In addition, mesophilic digestion and thermophilic digestion performance were compared.

As maize silage and baby maize stover were different substrate, the methane yield would be different. Based on physicochemical characteristics reported in the literature review, baby maize stover appeared to be more similar to maize silage than to maize stover, although baby maize stover is not ensiled. Maize silage was therefore selected as the preferred material for a baseline study to allow comparison with the behaviour of the baby maize stover. The trial with maize silage was carried out while awaiting the delivery of baby maize stover from Gorge Farm. It would have been interesting to carry out the same baseline trials with maize stover to allow comparison of all 3 substrates, but time constraints did not allow this.

4.2.1. Objectives and methodology

Objective. To establish the methane productivity and operational characteristics for digestion of ensiled European fodder maize in mesophilic and thermophilic conditions at organic loading rates typical of those used in commercial AD plant.

Methodology. Eight 5-litre digesters of the type described in section 3 were used in this trial. The digesters were seeded with fresh inoculum from Millbrook WWTP. Temperature was controlled at 35 ℃ (D1-D4) or 55 ℃ (D5-D8) by thermocirculators. D1-4 were initially fed with maize silage at an organic loading rate (OLR) of 3 g VS L^{-1} day^{-1}. Digesters D5-8 were not fed for 5 days to allow acclimatisation to the step change in temperature. Feeding on maize silage then began at an OLR of 0.5 g VS L^{-1} day^{-1} which was then steadily raised to 3 g VS L^{-1} day^{-1} by day 50. After 174 days and 145 days of operation respectively, the OLR on one pair of mesophilic (D3-4) and one pair of thermophilic (D5-6) digesters was gradually increased to 4 g VS L^{-1} day^{-1}. Feeding of the other pair of mesophilic digesters (D1-2) continued at 3 g VS L^{-1} day^{-1}, while feeding of the thermophilic digesters D7-8 on maize silage stopped on day 145 when they were transferred to the following digestion trial.

After day 140 for mesophilic and day 135 for thermophilic digesters, trace elements were added to the digesters to give additional concentrations in the digestate of 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1}, and weekly addition of these trace elements was started in proportion to the quantity of feed added to the digester, in order to maintain digestate TE concentrations.

For clarity and ease of understanding the results for thermophilic digestion are presented first, then those for mesophilic digestion, followed by a comparative discussion. A summary of the reactors' history (trace elements addition and other events) is given in Table 9 and 11 in the results sections.

4.2.2. Thermophilic digestion results

Operating parameters. Figure 16 shows the OLR, daily wet weight of feed added and hydraulic retention time for D5-8 during the experimental period. It can be seen that the target OLRs were successfully maintained (Figure 16a and b), while there were small variations in the daily feed (Figure 16c and e) and HRT due to variations in the solids content of different batches, as noted in section 4.1 (Figure 14). Since no water or other liquid was added to the digesters, the high solids content of the maize silage meant that the corresponding HRTs were also high, with average values of 123 and 77 days at OLR of 3 and 4 g VS L^{-1} day^{-1} respectively.

On day 24 (Table 9), 400 µL Goldcrest antifoam was dosed into each reactor. At this point, the mesophilic digesters had serious foaming issues, as described in section 4.2.3. The thermophilic digesters did not show foaming problems at this time, but were supplemented as a precautionary measure. On day 45, all of the digesters received a one-off dose of trace element solution to give an additional concentration in the digestate of Co 1 mg L^{-1} and Se 0.2 mg L^{-1} in response to the foaming issues in mesophilic digesters. Several researchers have reported that TE dosing had positive impacts to reduce foaming (Karlsson et al., 2012; Ortner et al., 2014; Suhartini et al., 2014).

Digester D5 experienced some mixing problems. The motor stopped several times on day 76, 81, 82, 87, 99. It was replaced on day 76 and cleaned again on day 82. On day 94, D5's stirrer broke and was replaced.

On day 145, feeding of D7&8 was stopped and these digesters were transferred to the next digestion trial. From day 146, D5&6 OLR increased from 3 to 4 g VS L^{-1} day^{-1} for over an 8-day period. D5&8 feeding ceased on day 301 because instabilities appeared.

HRT is expressed in units of days, on the basis of the calculation given in section 3.8 equation 3.8.2 and equation 3.8.3. Equivalent HRT is expressed as a ratio (no unit) (Table 10). D5&6 and D7&8 were operated for an equivalent total of 2.5 HRT and 1.0 HRT, respectively (Table 10).

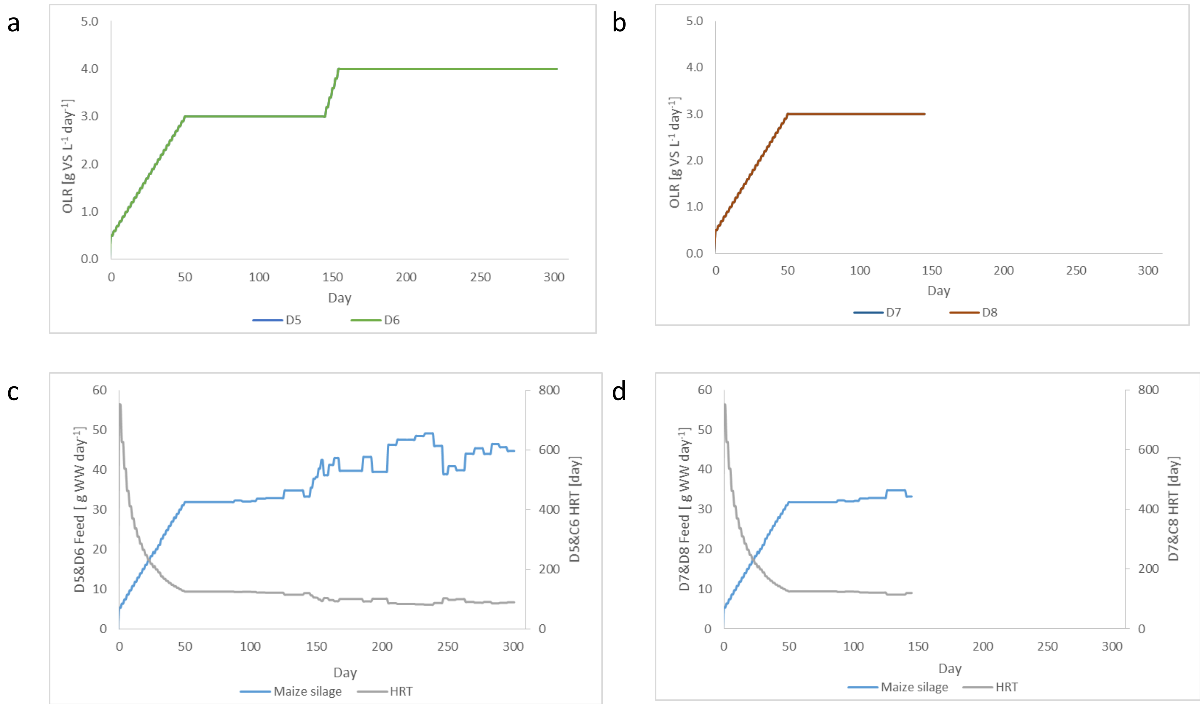


Figure 14. OLR, daily feed and HRT for thermophilic digesters D5-8 during digestion Trial 1 on maize silage

Table 9. Summary of reactor history for digesters D5-8 during digestion Trial 1 on maize silage

Day	Date	D5	D6	D7	D8
-6	03/12/2014	Set up			
-5	04/12/2014	Acclimatisation (Not fed)			
0	09/12/2014	Start Feed: raise OLR from 0 to 3 g VS L^{-1} day^{-1} for 50 days			
24	02/01/2015	Antifoam Goldcrest AF-530 400 µL/each reactor			
45	23/01/2015	Trace elements (4 mL) added; Co 1 mg L^{-1}+ Se 0.2 mg L^{-1}			
135	23/04/2015	Co 1 mg L^{-1}, Ni 1 mg L^{-1}, Fe 10 mg L^{-1} added for each reactor working volume			
143	01/05/2015	Weekly TE addition started to maintain TE concentrations			
145	03/05/2015			Ceased feeding	
146	04/05/2015	Increase OLR from 3 to 4 g VS L^{-1} day^{-1} for 8 days			
301	06/10/2015	Ceased feeding			

Table 10. Total amount of added substrate and equivalent HRT completed

	D5	D6	D7	D8
Amount of added substrate [g]	10088	10088	4092	4092
Equivalent HRT [-]	2.5	2.5	1.0	1.0

Biogas and methane production. Biogas and methane production and biogas methane content are shown in Figure 15. The thermophilic digesters responded well to the initial increase in OLR, with volumetric biogas production (VBP) and volumetric methane production (VMP) rising in proportion to the applied load (Figure 15a and b). On day 26, the feed to the thermophilic digesters (OLR 1.7 g VS L^{-1} day^{-1}) was accidentally swapped with that for the mesophilic digesters (OLR 3 g VS L^{-1} day^{-1}); therefore, gas production from the thermophilic digesters briefly increased around day 27.

Until day 15, the specific methane production was around 0.4-0.6 L CH_4 g^{-1} VS During this period, the OLR was less than 1.0 g VS L^{-1} day^{-1} so the micro-organisms may have been consuming some residual organic components in the inoculum, not only maize silage.

From day 125 to 147, biogas production in D6 was 0.5 L L^{-1} lower than in the other reactors (Figure 17a). The reason for this difference is not known.

Specific biogas production (SBP) and VBP in D5-8 (Figure 15a-d) showed some fluctuation but appeared to stabilize around 0.37 - 0.38 L CH_4 g^{-1} VS. From day 165 foaming appeared in all thermophilic digesters, making operation more difficult. On day 176 and 196, the gas outlet tube from D5 filled with digestate, and the gas volume on that day could not be measured. The outlet tube was replaced, and the gas counter was cleaned. After day 207, foaming was not observed. Biogas methane content appeared to increase slightly between day 35-145, and stabilised at around 57% by the end of the run (Figure 15e). This led to a corresponding small increase in specific methane production (SMP) over the same period, making it difficult to identify steady-state SMP values for D7&8.

The reason for occasional variability in gas production between duplicate reactors is not known, but may be linked to the variable quality of the feedstock and the occurrence of foaming. Apart from these variations, the duplicate pairs of digesters showed reasonably good similarity when operating under the same conditions.

Average gas production values during pseudo-steady state periods are given in Table 13 in the discussion section below, together with values for other monitoring parameters.

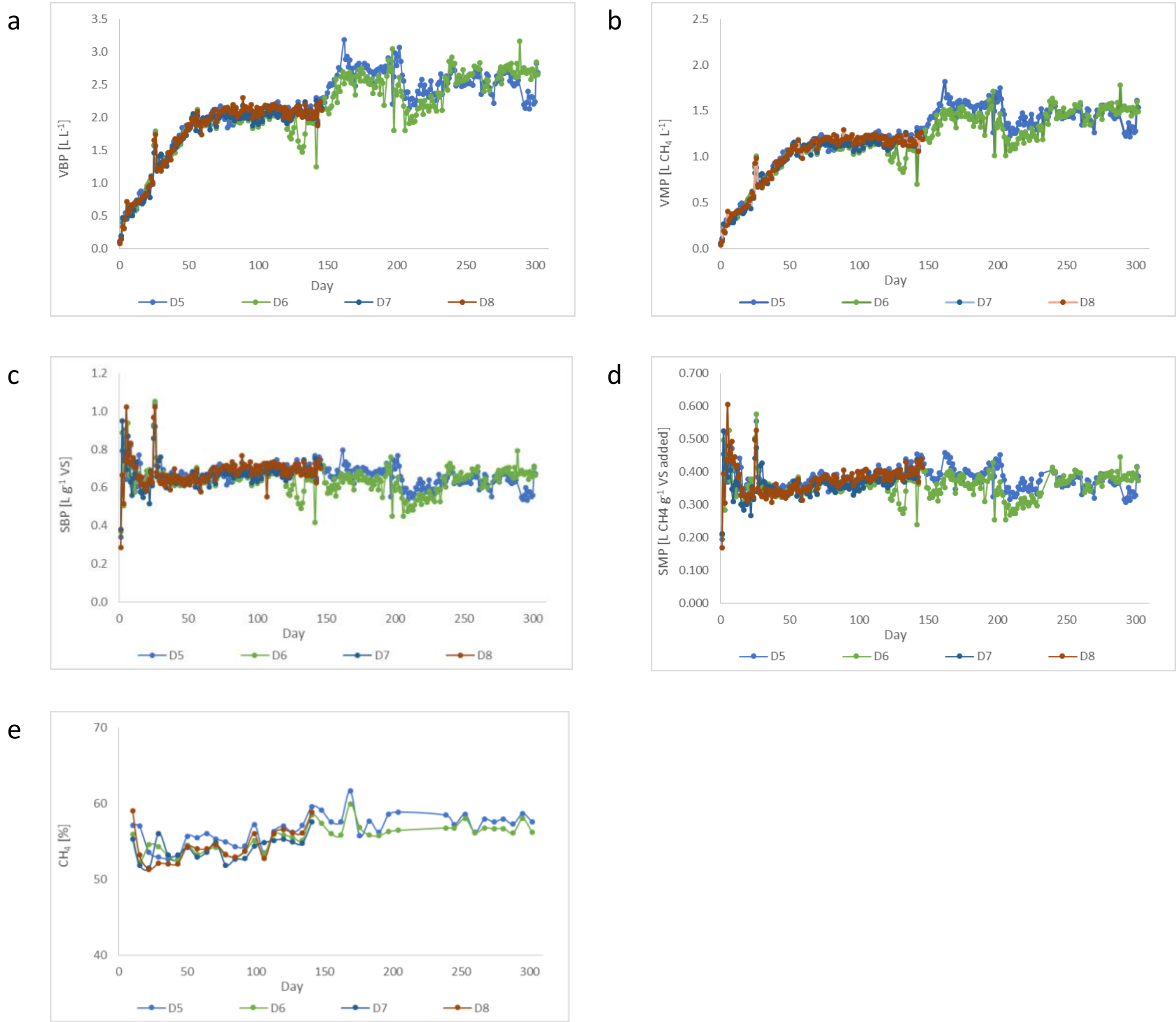


Figure 15. VBP, VMP, SBP, SMP and biogas methane content for thermophilic digesters D5-8 during digestion Trial 1 on maize silage

Operational stability. Figure 16 shows the values of monitoring parameters for operational stability during the experimental period.

pH in all digesters remained within a narrow range throughout, at an average of 7.6 ± 0.2 (Figure 16a). TAN concentrations fell from initial values of around 2.1 g N kg^{-1} in all digesters and stabilised at around 1.6 g N kg^{-1} in D5&6 near the end of the experimental period (Figure 16b).

There were some relatively sharp fluctuations in TA, PA and IA (Figure 16c - e) during the period when foaming occurred, which may have indicated sampling issues and/or minor instability.

As a result of these changes the IA/PA ratio showed some minor fluctuations (Figure 16f), but remained close to 0.5 indicating a reasonable degree of stability.

The cause of the slightly raised IA and IA/PA values at the start of the run was probably an initial peak in total VFA (Figure 16g), which reached around 3 g COD L^{-1} on day 1 but then rapidly decreased, with total VFA concentrations in all digesters stabilising at below 0.50 g COD L^{-1} after day 50. The initial peak was primarily acetic acid (Figure 16h) with some propionic acid also present, especially in D7; and was probably associated with the resumption of feeding after the step change to thermophilic conditions.

Taken together, these parameters indicated that the digesters adapted successfully to the thermophilic temperature regime and stabilised well.

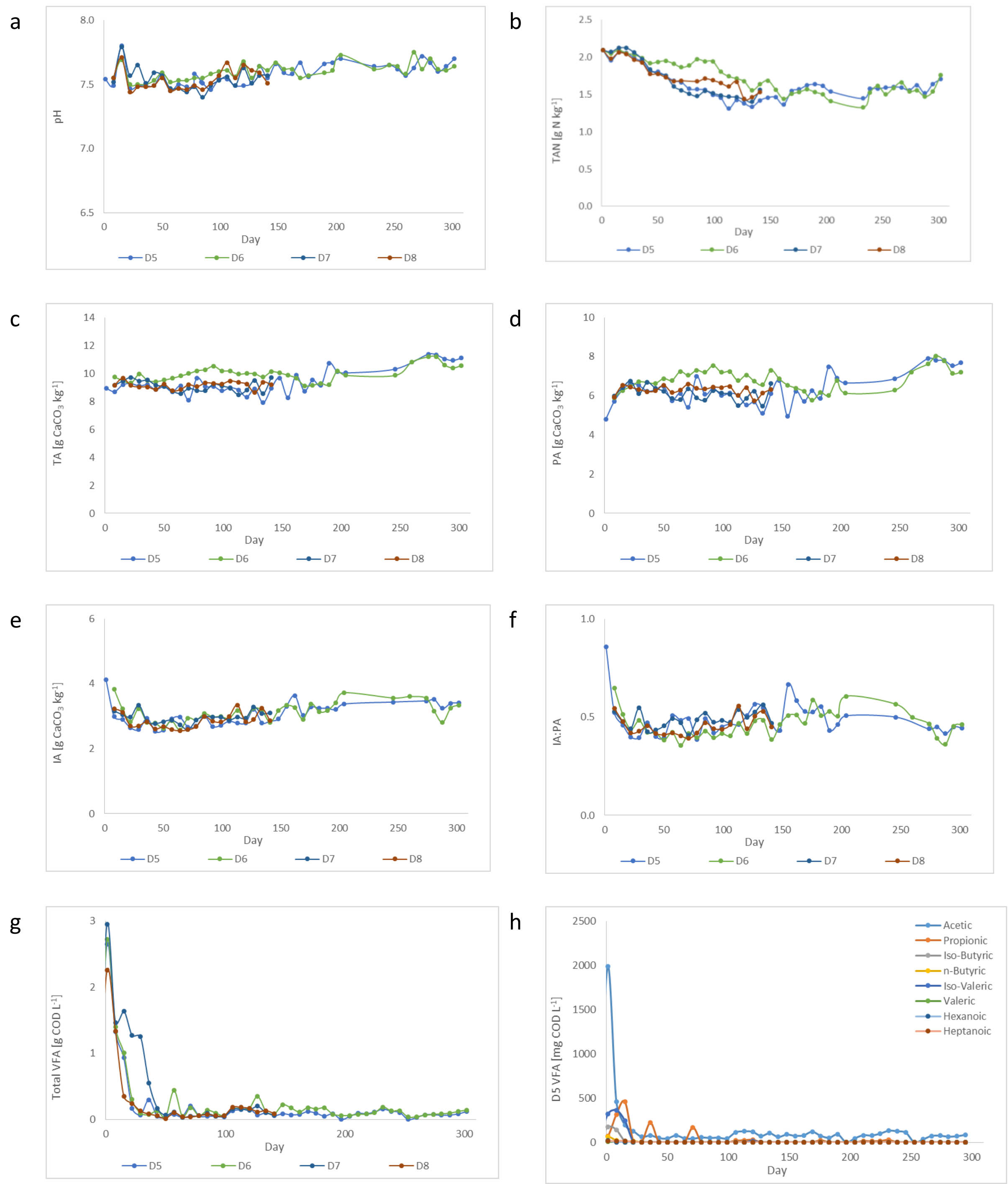


Figure 16. pH, TAN, Alkalinity and total VFA for thermophilic digesters D5-8 and VFA profile in during digestion Trial 1 on maize silage

Solids parameters. Figure 17 shows the solids parameters for the digesters. TS and VS rose steadily until near the end of the experimental period (Figure 17a and b), stabilising at around 11.4 % WW and 9.6 % WW respectively in digesters D5&6 by around day 280. In terms of the amount of total material added to D5&6, this was equivalent to 2.2 HRT. The VS/TS ratio (Figure 17c) stabilised slightly earlier than the TS and VS contents, at around 84%. Even using a mass balance approach, it was difficult to obtain consistent VS destruction values at the beginning of the run (Figure 17d). From day 43, VS destruction started to settle at around 80%, with minor fluctuations attributable to inhomogeneity between individual samples of digestate.

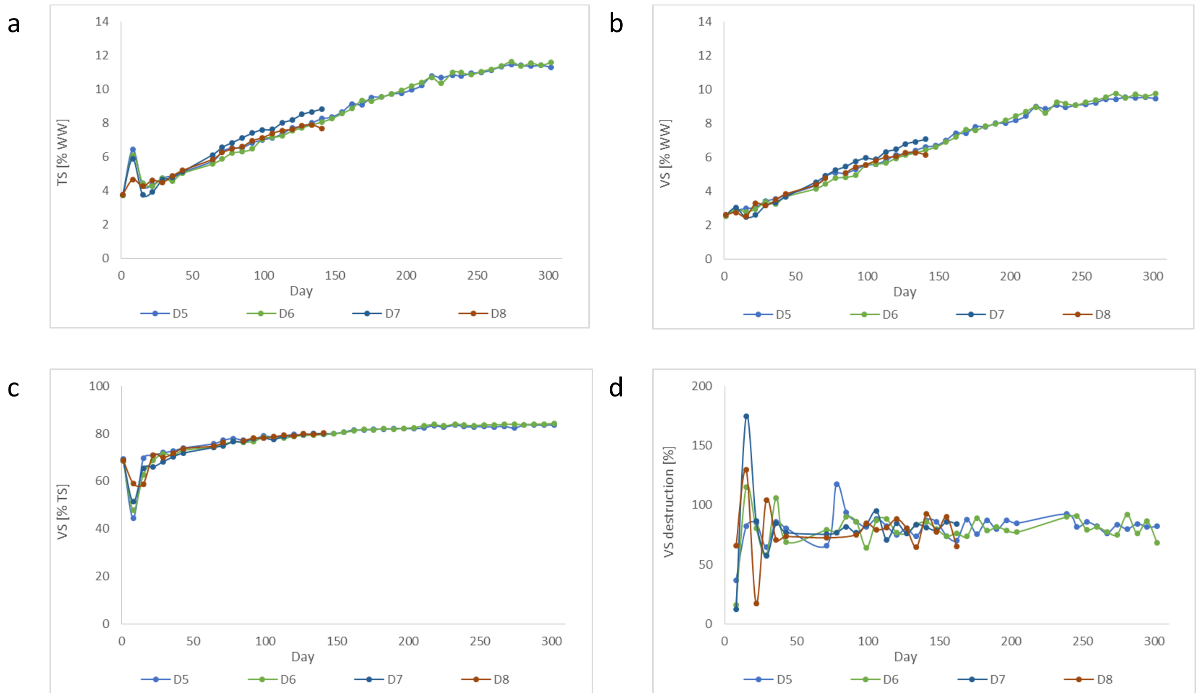


Figure 17. Digestate solids parameters for thermophilic digesters D5-8 during digestion Trial 1 on maize silage

Images of digestate samples taken on day 176 and results of CST and FIC are given in section 4.2.3 below with the results for the mesophilic digesters.

4.2.3. Mesophilic digestion results

Operating parameters. Figure 18 shows the OLR, daily wet weight of feed added and HRT for D1-4 during the experimental period. A summary of the reactors' history (trace elements addition, and other events) is shown in Table 11.

There were considerable deviations in the applied OLR both from the values originally planned at the start of the experiment, and between pairs of digesters under duplicate conditions (Figure 18a and b). These were made in response to changes in the monitoring parameters, for the reasons described below. These included an unplanned cold shock between days 14 to 17, when the thermocirculator turned itself off due to a lack of water (Table 11). Digesters D1-4 were not fed for 4 days after the error was identified, then the OLR was returned to 3 g VS L^{-1} day^{-1}. Changes in OLR affected the HRT in each digester as shown in Figure 18c-f.

From day 14, foaming started and was a severe problem for operation. The foaming digestate sometimes blocked the gas outlet tube, so that pressure built up inside the reactor leading to the release of digestate. Therefore, antifoam was dosed on day 29 (Table 11). From day 48 onwards OLRs in all four digesters were reduced to deal with the effects of instability (VFA accumulation and pH drop), including temporary cessation of feeding in the worst affected reactors D1 and D4. OLR was then gradually increased as shown in Figure 20. One-off dose of TE were also added on days 50 and 140 as shown in Table 11, with weekly dosing of TE solution introduced from day 148. By day 104, the OLR on all mesophilic digesters was back to 3 g VS L^{-1} day^{-1}; but there were further interruptions and reductions in feeding for D1-3 until day 138 when all four digesters were again at the target OLR. On day 175, the OLR on D3&4 was increased from 3 to 4 g VS L^{-1} day^{-1}, while D1&2 remained at 3 g VS L^{-1} day^{-1}. To reduce the problems associated with foaming, on day 182 the working volume of the reactors was decreased from 4 to 3 L by removal of digestate (on a weight basis), while the OLR was maintained by proportional reduction in the daily quantity of feed added.

There were number of problems with mixing, especially in D4 where the motor stopped intermittently on day 256-258, 261, 281-286, 292-298 and 305. The motor in D2 also stopped on day 279, 285-286. These may have been associated with changes in the digestate properties.

Digesters D1, D2, D3 and D4 were operated for the equivalent of 2.0, 2.1, 2.4 and 2.4 HRT, respectively (Table 12).

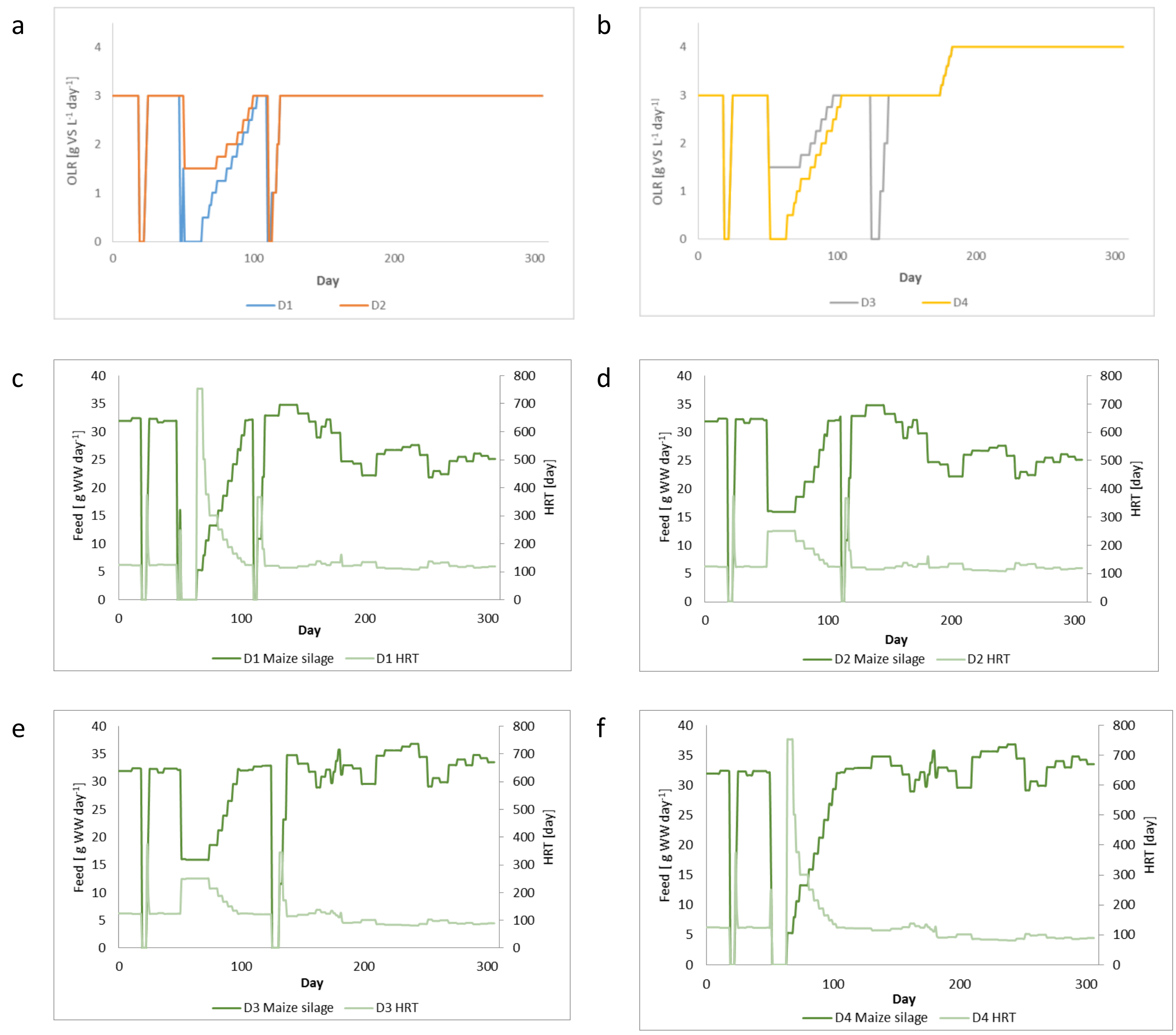


Figure 18. OLR, daily feed and HRT for mesophilic digesters D1-4 during digestion Trial 1 on maize silage

Table 11. Summary of reactor history for digesters D1-4 during digestion Trial 1 on maize silage

Day	Date	D1	D2	D3	D4
-1	03/12/2014	Set up			
0	04/12/2014	Start Feeding: OLR 3 g VS L^{-1} day^{-1}			
14	18/12/2014	Thermocirculator turned off for 4 days due to water shortage			
18	22/12/2014	Thermo circulator turned on			
19	23/12/2014	No feeding for 4 days			
23	27/12/2014	OLR back to 3 g VS L^{-1} day^{-1} for 3 days			
29	02/01/2015	Antifoam Goldcrest AF-530 added 400µL/each reactor			
48	21/01/2015	Not fed for 2 days			
50	23/01/2015	OLR back to 1.5 g VS L^{-1} day^{-1}			
50	23/01/2015	Co 1 mg L^{-1} + Se 0.2 mg L^{-1}; one-off dose for digestate			
51	24/01/2015	Not fed for 13 days	Decrease OLR from 3 to 1.5, OLR 1.5 maintained for 23 days		Decrease OLR from 3 to 1.5 g VS L^{-1} day^{-1}
52	25/01/2015				Not fed for 12 days
64	06/02/2015	OLR back to 3 g VS L^{-1} day^{-1} for 40 days			OLR back to 3 g VS L^{-1} day^{-1} for 40 days
74	16/02/2015		Increase OLR from 1.5 to 3 g VS L^{-1} day^{-1} for 27 days	Increase OLR from 1.5 to 3 g VS L^{-1} day^{-1} for 24 days	
110	24/03/2015	Not fed for 3 days			
111	25/03/2015		Not fed for 3 days		
113	27/03/2015	OLR back to 3 g VS L^{-1} day^{-1} for 7 days			
114	28/03/2015		OLR back to 3 g VS L^{-1} day^{-1} for 6 days		
125	08/04/2015			Not fed for 6 days	
131	14/04/2015			OLR back to 3 g VS L^{-1} day^{-1} for 7 days	
140	23/04/2015	One off dosing; Co 1 mg L^{-1}, Ni 1 mg L^{-1}, Fe 10 mg L^{-1} to give additional TE for digestate			
148	01/05/2015	Weekly TE addition started to maintain the TE concentrations (Fe, Co, Ni)			
175	28/05/2015			Increase OLR from 3 to 4 g VS L^{-1} day^{-1} for 8 days	
182	04/06/2015	Working volume changed from 4 to 3 [kg]			
307	07/10/2015	Feeding ceased			

Table 12. Total amount of added substrate and equivalent HRT

		D1	D2	D3	D4
Amount of added substrate [g]	4L working volume	4376	4882	4830	4709
	3L working volume	2748	2748	3661	3661
Equivalent HRT [-]		2.0	2.1	2.4	2.4

*Mesophilic digesters' working volume decreased from 4L to 3L on day 183

Operational stability. Digester operating parameters appeared to be quite stable for the first 5-6 weeks, despite the cold shock. The digesters showed signs of incipient foaming from around day 14, and by day 28 showed foaming which appeared likely to cause operational problems, so 400 µL of antifoam Goldcrest AF-530 (Goldcrest Chemicals, Basildon, UK) was added to each reactor on day 29. This reduced the foam without any other apparent effect.

By day 48, however, there was fall in pH in D1 and D4 (Figure 19a) accompanied by a rise in total VFA in all digesters, which was most pronounced in D1 and D4 (Figure 19b). TA and PA, which had been rising slightly (Figure19c and d), also fell in these digesters while IA rose (Figure 19e), leading to a peak in the IA/PA ratio for D1, D2 and D4 (Figure 21f). Similar phenomena, usually referred to as the "40-day bump" have been observed previously at around day 40-60 when adapting this inoculum to new feedstocks (Yirong *et al.*, 2017). D1 and D4 were therefore not fed until day 64 when the total VFA accumulation had been reduced, while the OLR on D2 and D3 was reduced to 1.5 g VS L^{-1} day^{-1}. Furthermore, trace elements were added to all four digesters on day 50 as noted above to give an additional TE concentration in the digestate of 0.2 mg Se L^{-1} and 1 mg Co L^{-1}. Once the digesters appeared to have stabilised, the OLR was then gradually increased again to its target value (Figure 19).

TAN concentrations in all digesters were rising at the start of the run (Figure 19g), but increased sharply in D1 and D4 after day 55, possibly indicating some die-off in the microbial population in these two digesters, which were the worst affected in terms of the rise in total VFA concentrations and fall in pH (Figure 19a and b).

After day 100, there were signs of further instability in the mesophilic digesters, especially D2 and D3, as indicated by increases in the total VFA concentration (Figure 19b), fluctuations in alkalinity leading to changes in IA/PA ratio (Figure 19f), and a fall in pH in D3 (Figure 19a). D2 and D3 both showed similar trends in behaviour in terms of the rise in VFA concentration and IA/PA ratio and the fall in pH; but the VFA peak of 7.3 g COD L^{-1} in D3 occurred around day 124, while in D2 the onset of instability was three weeks later with peak VFA of 4.9 g COD L^{-1} occurring around day 146. This type of variation, in which reactors operating under similar conditions show the onset of instability at slightly different times, was also observed by Yirong *et al.* (2015) who ascribed it to the “Anna Karenina principle” (Moore, 2001).

Figure 19. pH, total VFA, alkalinity and TAN for mesophilic digesters D1-4 during digestion Trial 1 on maize silage

In addition, the digesters were still suffering from foaming which sometimes blocked the gas outlets. Several researcher have reported TE dosing had positive impacts to reduce foaming (Karlsson et al., 2012; Ortner et al., 2014; Suhartini et al., 2014). Therefore to address these signs of instability, on day 140 a one-off dose of trace elements Co, Ni and Fe were added to reactors D1-4 to give additional concentrations in the digestate of 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1}. From day 148, weekly addition of trace elements Co, Ni and Fe was started in proportion to the quantity of feed added to the digester, in order to maintain digestate TE concentration. Despite this, however, the digesters still suffered with foaming, including an explosive loss of digestate from D1 on day 144 which led to a small reduction in digestate volume.

In the last 50 days of the run most of the operational parameters showed signs of stabilising, although there were short-term fluctuations especially in TAN and alkalinity. These may have been related to the texture and viscosity of the digestate. As the run progressed the mesophilic digestate became very thick and sticky with a resemblance to strawberry jam. For alkalinity and ammonia analysis, the digestate was mixed with water by a stirrer in a beaker. Sometimes, undiluted digestate was found in the bottom of the beaker after analysis. When this occurred, the analysis was carried out again, but the difficulty in diluting the digestate may have affected some results.

VFA profiles. VFA profiles of the individual digesters are shown in Figure 20. The peak in VFA concentrations between days 40-60 was mainly acetic acid, although small amounts of other VFA were seen in D1 and D4, which were the worst affected digesters (Figure 20a and d).

The second VFA peaks in D2 and D3 after day 100 included some propionic acid (Figure 20b and c). The propionic concentration in D2 reached 2127 mg COD L^{-1} on day 146. The highest propionic acid was 2549 mg COD L^{-1} on day 125 in D3 and this may be related to the dates of non-feeding which were days 111-113 and days 125-130 for D2 and D3 respectively. Earlier non-feeding may help to reduce the amount of VFA accumulation. The second peaks in D1 and D4 also included propionic acid but mainly consisted of acetic acid, and the concentration was less than D2 and D3.

This difference in the second peaks in D2&3 and D1&4 indicates that D2&3 were in poorer condition for stable operation, although no other parameters indicate this. The second peak results were opposite to those for the first peak, which showed greater VFA accumulation in D1&4 than D2&3. This may also be related to feeding. The cold shock and 40-day bump experience had more negative impacts on D1&4, therefore D1&4 were not fed. On the other hand, D2&3 were not as badly affected as D1&4, so the feeding amount was reduced to half amount.

From day 160, propionic acid concentrations fell to low values; but for the rest of the run these was a residual VFA concentration of ~500 mg COD L^{-1} in all digesters which mainly consisted of acetic acid.

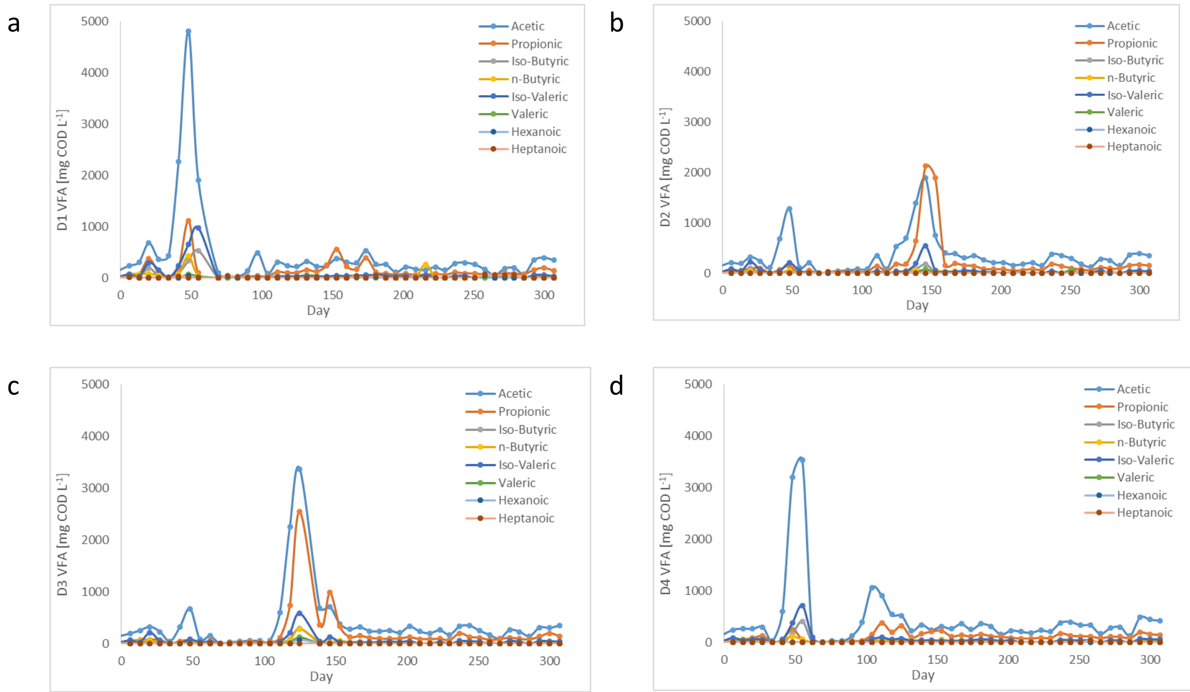


Figure 20. VFA profiles for D1-4 during digestion Trial 1 on maize silage

Biogas and methane production. Gas production parameters are shown in Figure 21. VBP and VMP (Figure 21a and b) broadly reflected the changes in applied OLR, as well as the intermittent periods of instability described above. Methane concentrations also followed the instability (Figure 21e).

From day 14 to 17, when the thermocirculator was not working, the temperature dropped and biogas production fell from 1.8 to 0.8 L L^{-1} day^{-1}. On day 18 when the thermocirculator was turned back on, gas production rose to 2.8 L L^{-1} for one day only. The following 4 days without feeding, due to the cold shock and foaming issues, led to a decrease in gas production. From day 23 the OLR was returned to 3 g VS L^{-1} day^{-1} over a 3-day period.

The SMP recovered. On day 29 antifoam was dosed and there was another small dip in gas production but by day 33 SMP had returned to around 0.32 L CH_4 g^{-1} VS which was almost same as before the cold shock.

Gas production started to fall in all digester at the onset of VFA accumulation, from about day 41 in D1 and D4 and day 52 in D2 and D3. It fell further as feeding was reduced in response to this accumulation, then rose again with the stepwise reintroduction of feeding in each digester. The increase in biogas methane content in D1 and D4 around day 57 (Figure 21e) reflects the consumption of accumulated VFA. The OLR was returned to 3 g VS L^{-1} day^{-1} over the following month in combination with checking of the stability parameters.

Between days 108 – 145 there were further significant dips in VBP and VMP in response to the changes in monitoring parameter values reported above, and the temporary reductions in OLR that were implemented to control them.

After day 145, the digesters appeared to be stable and had constant biogas production with only minor fluctuations. Therefore, the OLR of D3&4 was increased from 3 to 4 g VS L^{-1} day^{-1} to obtain gas data at OLR 4 g VS L^{-1} day^{-1}.

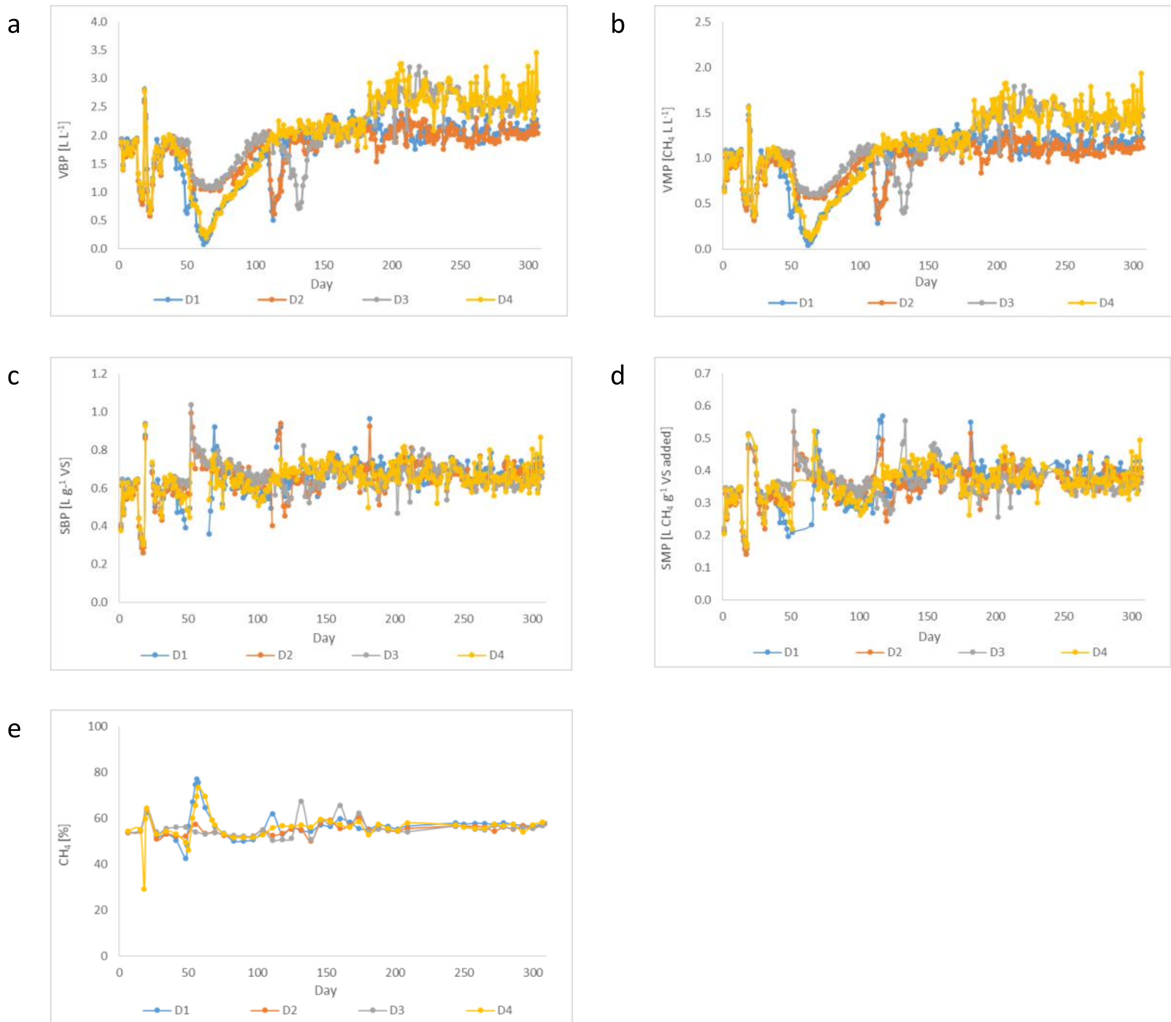


Figure 21. VBP, VMP, SBP, SMP and biogas methane content for mesophilic digesters D1-4 during digestion Trial 1 on maize silage

Despite this increase, D3 and D4 were stable foe the rest of the experimental period. At OLR 3 g VS L^{-1} day^{-1} SBP and SMP (Figure 21c and d) stabilised at 0.679 L g^{-1} VS and 0.385 L CH_4 g^{-1} VS, respectively. At OLR 4 g VS L^{-1} day^{-1}, SBP and SMP stabilised at 0.645 L g^{-1} VS and 0.361 L CH_4 g^{-1} VS. Average gas production values during pseudo-steady state periods are given in Table 15 with values for other monitoring parameters, in the discussion section at the end of this experiment.

Solids parameters. Figure 24 shows the solids parameters for this digestion trial. TS, VS and VS/TS ratio rose steadily during the trial (Figure 22a, b and c and appeared to stabilise in D3&4 after about day 287 which was equivalent to 2.2 HRT. The OLR on D3&4 was increased from 3 to 4 g VS L^{-1} day^{-1} from day 175, and the effect of this can be seen in the solids concentrations in the later part of the trial.

Even using a mass balance approach, it is generally difficult to obtain very consistent values for VS destruction during the initial stages of a run while the system is stabilising, and this was especially so in this case because of the short-term changes in OLR in response to digester operating conditions. By the end of the trial, however, VS destruction appeared to have stabilised at around 78.0%, with the digestate VS/TS ratio at 85.2% (Figure 22d).

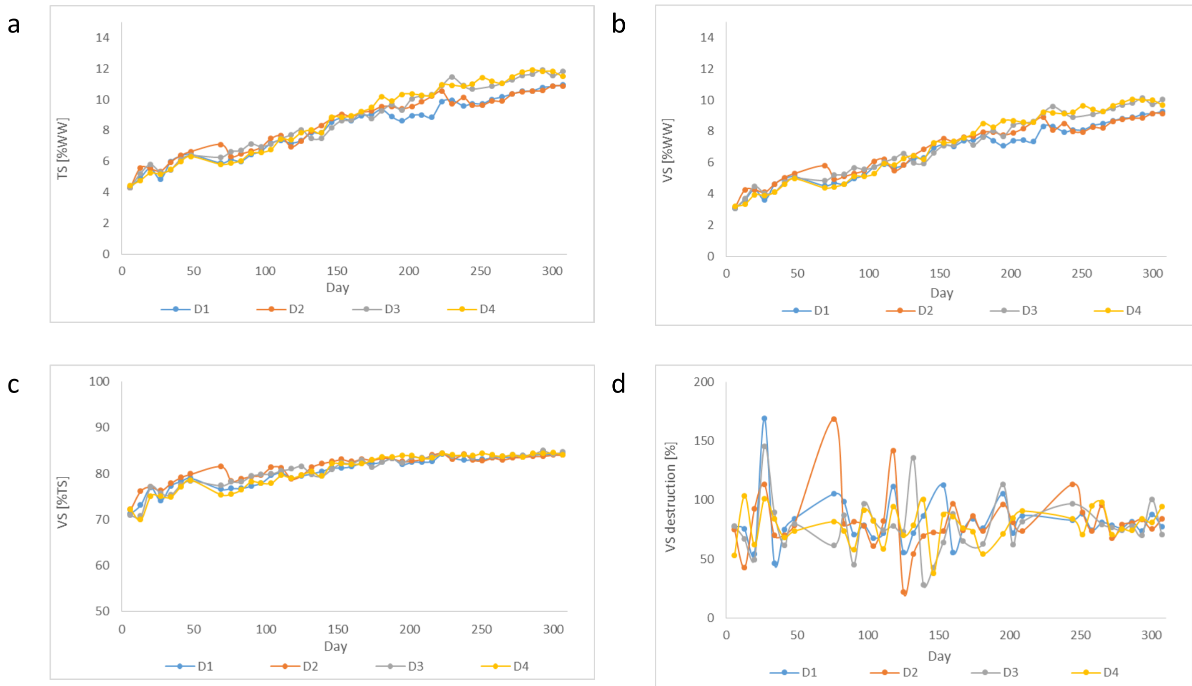


Figure 22. Digestate solids parameters for D1-4 during digestion Trial 1 on maize silage

Digestate appearance and dewaterbility parameters. Figure 23 shows the appearance of the digestate on 03/06/2015, corresponding to day 181 for mesophilic digesters, day 176 for thermophilic digesters of digestion Trial 1.

Figure 23. Digestate appearance at the end of the run, day 181 on digestion Trial 1

Dewaterability of D1-8 was assessed by FIC and CST. FIC was carried out on day 298 for D1-4, and day 293 for D5-8. CST was carried out at 02/10/2015 which was equivalent to day 302 for D1-4, and day 297 for D5-8. FIC analysis took place for 1 hour at RPM 850 but no separation was observed for any of the digestates. CST analysis was carried out for 24 hours but again not final values obtained (i.e. CST > 86400 seconds for all digestates).

Although viscosity was not measured in this trial, differences were observed in the appearance of the digestate (Figure 23). Thermophilic digestate was smoother in appearance with little or no undigested material visible even at OLR 4 g VS L^{-1} day^{-1}. In contrast, mesophilic digestate appeared much thicker and more viscous, and pieces of undigested material could be clearly seen even at OLR 3 g VS L^{-1} day^{-1}.

4.2.4. Discussion of result for thermophilic and mesophilic digestion of maize silage in digestion Trial 1

Table 13 shows the average values of monitoring parameters during pseudo steady-state periods in digestion Trial 1. As can be seen, there was some variation between supposedly duplicate reactors, mainly because of the operational issues discussed above; however, SMP values for the maize silage appeared to be around 0.385 L g^{-1} VS under assumed steady state conditions at OLR 3 g VS L^{-1} day^{-1} in both mesophilic and thermophilic conditions, and slightly lower at OLR 4 g VS L^{-1} in each case.

Different operating temperatures at same OLR

At OLR 3 g VS L^{-1} day^{-1}, there were no significant differences in SMP between mesophilic and thermophilic digestion. The thermophilic digesters showed lower total VFA concentrations. VS destruction was similar for both temperatures, at 80.0 % for mesophilic and 80.6% for thermophilic.

At OLR 4 g VS L^{-1} day^{-1}, the SMP of the mesophilic digesters was less than for thermophilic digestion. This may indicate the mesophilic digesters had started to struggle with degrading substrate for methane production. The thermophilic reactors stabilised at very low total VFA concentrations (< 200 mg L^{-1}) by the end of the experimental period, while the mesophilic reactors carried small residual VFA concentrations of around 350-720 mg COD L^{-1}. TAN concentrations were much lower in thermophilic than mesophilic digesters, at average values of around 1.6 and 2.0 g N kg^{-1} respectively (Table 13), although the TAN in D3 was a little below that in the other mesophilic digesters.

SMP seemed to decline at higher OLR in both temperature ranges, but it is difficult to be certain of this or of the causes. Although the digesters appeared to perform in a similar way in terms of SMP, VS destruction, the mesophilic temperature range presented some problems in terms of tendency to foaming, and the appearance of the digestate may also suggest that these reactors were nearer to their limit of stable operational OLR.

Difficulties in process at different temperatures

Foaming was the serious issue during Trial 1 and mesophilic digesters showed more foaming. Mesophilic digestate was more thick and viscous than that of thermophilic digestate. The difference in foaming behaviour between mesophilic and thermophilic digesters may be simply due to the temperature difference. The viscosity of the digestate is expected to be lower at higher temperature so it is easier for gas bubbles formed to escape from the digestate, and reduce any risk of bed expansion. On the other hand, the period of foaming and VFA accumulation appearance were slightly different. This may be related in part to the cold shock event in the mesophilic digesters. The 4-day experience had significant negative impacts and may have caused more foaming. Foaming is a serious problem in commercial digesters, however, so if this operational difference is a common problem it could be significant for other types of maize-based substrate.

Foaming issues have also been reported by many other researchers. Zábranská *et al.* (2002) used sewage sludge as feedstock and found mesophilic digestion showed more foaming issues than the thermophilic digestion. Suhartini *et al.* (2014) carried out anaerobic processing of sugar beet pulp under mesophilic and thermophilic conditions at OLR 4 g VS L^{-1} day^{-1} and 5 g VS L^{-1} day^{-1} and reported foaming was more severe at higher OLR and lower temperature. Thermophilic digesters showed noticeable foaming after 3 HRT at OLR 5 g VS L^{-1} day^{-1} but mesophilic digesters showed foaming issues at OLR 4 g VS L^{-1} day^{-1} for 0.6 HRT. Foaming issues have been attributed to the high cellulosic composition of sugar beet pulp (Stoppok and Buchholz, 1985). Maize is also fibrous material, so the foaming may be caused in part by the maize silage composition. Foaming is reported to be associated with VFA accumulation, and changes in OLR (Kougias *et al.*, 2013; Suhartini, 2014). Mesophilic digesters in trial 1 had the cold shock experience so the digesters experienced VFA accumulation and changes in OLR. These factors have contributed to the more serious foaming issues.

Overall, thermophilic digestion showed more stable operation, but there was no significant difference in gas production between thermophilic and mesophilic conditions. It should be noted that mesophilic digestion of this maize silage was particularly problematic in terms of its reaction to various changes. As already noted there is considerable past experience with maize digestion in the Environmental Laboratories at Southampton (Cysneiros *et al.*, 2008; Heaven *et al.*, 2008; Cornell, 2011; Cornell *et al.*, 2012) ; but this study showed particular sensitivity to the feedstock change and temperature shock, and very early onset of foaming. Although VFA accumulation at around 40-60 days has been previously observed with different substrates (Climenhaga and Banks, 2008; Zhang and Banks, 2012; Zhang *et al.*, 2012; Yirong *et al.*, 2015; Roberts *et al.*, 2016b; Yirong *et al.*, 2017), the 'bump' that occurred in D1, D2 and D3 after day 100 has not been seen before. This and other difficulties in achieving stable operation may have been due to the nature of the maize silage feedstock (section 4.1), which was both drier than that used previously and appeared to be in less good condition in terms of partial fermentation and fungal attack. As the anaerobic digestion of maize silage in Trial 1 had some issues, the methane yield and the energy recovery were not compared with baby maize stover in section 4.7. Although mesophilic digestion showed some problems at the beginning these CSTRs stabilised at the end, and thermophilic digestion operated stably. The methane yield, 0.385 g CH_4 g^{-1} VS in Trial 1 was within the range of typical literature values (Amon *et al.*, 2007; Evranos and Demirel, 2015). The data in Trial 1 concerning the difference between mesophilic and thermophilic digestion may be usable but the problematic nature of the substrate should be taken into consideration. Despite this, the trial did achieve its aim of providing some baseline data under mesophilic and thermophilic conditions for comparison with the novel baby stover feedstock, as well as testing the analytical and operating methods used. Although there were some difficulties achieving steady operation the SMP of the maize silage under pseudo steady state conditions corresponded to around 84 % of the theoretical methane potential of 0.459 L CH_4 g^{-1} VS based on the Buswell equation.

Table 13. Average values for reporting parameters during pseudo steady state periods in digestion Trial 1 (maize silage)

Parameter	Unit	D1[a]	D2[a]	D3[a]	D4[a]	Ave[a]	Ave[a]	D5[b]	D6[b]	D7[b]	D8[b]	D5[a]	D6[a]	Ave[b]	Ave[a]
Temp[c]		M	M	M	M	M	M	T	T	T	T	T	T	T	T
OLR	g VS L^{-1} day^{-1}	3	3	4	4	3	4	3	3	3	3	4	4	3	4
SBP	L g^{-1} VS	0.688	0.669	0.632	0.658	0.679	0.645	0.704	0.616	0.686	0.694	0.631	0.672	0.675	0.651
SMP	L g^{-1} VS	0.396	0.373	0.353	0.370	0.385	0.361	0.406	0.349	0.386	0.397	0.362	0.380	0.385	0.371
VBP	L L^{-1} day^{-1}	2.08	2.02	2.53	2.64	2.05	2.58	2.11	1.85	2.06	2.08	2.51	2.67	2.02	2.59
VMP	L L^{-1} day^{-1}	1.16	1.10	1.41	1.47	1.13	1.44	1.21	1.04	1.16	1.18	1.44	1.51	1.15	1.48
CH_4 content	% v/v	57.5	55.8	55.8	56.2	56.6	56.0	57.5	56.2	55.6	56.9	57.4	56.5	56.6	56.9
Digestate TS	%WW	10.5	10.5	11.5	11.7	10.5	11.6	–	–	–	–	11.3	11.4	–	11.4
Digestate VS	%WW	8.9	8.7	9.7	9.8	8.8	9.7	–	–	–	–	9.4	9.6	–	9.5
VS destruction	%VS	79.9	80.2	79.8	80.5	80.0	80.1	79.7	82.1	79.3	81.4	81.2	81.4	80.6	81.3
pH	–	7.50	7.58	7.57	7.58	7.54	7.57	7.55	7.62	7.57	7.59	7.64	7.65	7.58	7.64
TA	g $CaCO_3$ kg^{-1} WW	11.4	12.0	11.2	12.6	11.7	11.9	8.5	10.0	9.1	9.1	11.2	10.8	9.2	11.0
PA	g $CaCO_3$ kg^{-1} WW	8.1	8.5	7.8	9.0	8.3	8.4	5.6	6.9	6.0	6.2	7.8	7.6	6.2	7.7
IA	g $CaCO_3$ kg^{-1} WW	3.4	3.5	3.4	3.5	3.4	3.5	2.9	3.0	3.1	3.0	3.4	3.3	3.0	3.3
IA/PA ratio	–	0.42	0.41	0.44	0.39	0.41	0.41	0.52	0.44	0.51	0.48	0.44	0.43	0.49	0.44
TAN	g N kg^{-1} WW	2.00	2.16	1.83	2.14	2.08	1.98					1.58	1.57		1.57
Total VFA	g COD L^{-1}	0.330	0.372	0.348	0.426	0.351	0.387	0.109	0.179	0.136	0.139	0.062	0.075	0.141	0.069

[a] Average value for days 265-294

[b] Average value for days 122-151

[c] Digester operating temperature, M = mesophilic (i.e. 35 °C), T = thermophilic (i.e. 55 °C)

Not included as parameter did not appear to have reached a stable (i.e. pseudo steady state) value

4.3. Digestion Trial 2-1 – baby maize stover

The purpose of this trial was to assess the anaerobic digestion performance of baby maize stover at mesophilic and thermophilic temperatures in terms of the digestion efficiency, stability and methane production potential. In addition to comparing the digestion performance in mesophilic and thermophilic conditions, it was decided to include a further variant with a thermophilic pre-hydrolysis step before mesophilic digestion. This was included both to provide insight into any differences between the thermophilic and mesophilic performance, and because the Tropical Power AD plant then under commissioning in Kenya included two buffer tanks before the main digester, the first of which was to be maintained at thermophilic temperature.

4.3.1. Objectives and methodology

Objective. To assess the anaerobic digestion performance of baby maize stover at mesophilic and thermophilic temperatures in terms of digestion efficiency, stability and methane production potential; including comparison between mesophilic digestion with and without a thermophilic pre-hydrolysis stage.

Methodology. Ten 5-litre digesters of the type described in section 3.8 were used in this trial. Temperature was controlled at 35 ℃ (D1-D4, D9-D10) or 55 ℃ (D5-D8) by thermocirculators. A summary of the reactors' history (trace element addition, other events) is shown in Tables 14 (D5&6), 16 (D7&8), 19 (D9&10), and 21 (D1-4) in the results sections. D1-8 were initially used for digestion Trial 1, and the substrate was switched from maize silage to baby maize stover as follows:

D1-4: On day 0 of the current trial (day 308 of Trial 1) digestate from these 4 digesters was mixed and redistributed equally between them. This was done to ensure homogeneity of the initial inoculum, as the digesters had shown slightly different behaviour in the previous trial. Feeding on baby maize stover began on day 1 at an OLR of 3 g VS L^{-1} day^{-1} which was increased to 4 g VS L^{-1} day^{-1} from day 2. Weekly TE dosing (1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1} in the feed) was carried out in proportion to the added feed material to maintain TE concentrations.

D5&6: on day 0 of the current trial (day 302 of digestion Trial 1) the feed to these two digesters was switched to baby maize stover at an OLR of 4 g VS L^{-1} day^{-1}. This was done without mixing of the digestate, since the digesters had shown consistent behaviour in the previous trial. Due to concerns about the possible effect of this change in feedstock, the digesters were not fed on days 3 and 4, then the OLR was increased stepwise over the following week back to 4 g VS L^{-1} day^{-1}. Between days 149-158 the OLR was increased steadily to 6 g VS L^{-1} day^{-1}. Weekly TE dosing (1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1} in the feed) was carried out in proportion to the added feed material to maintain TE concentrations.

D7&8: On day 0 of the current trial (day 146 of digestion Trial 1) the feed to these digesters was switched to baby maize stover. As with digesters D5&6, this was done without mixing of the digestate since the two digesters had shown consistent behaviour in the previous trial. The OLR was reduced to 2 g VS L^{-1} day^{-1} for 12 days, then increased stepwise to 3 g VS L^{-1} day^{-1} over the following 9 days. The OLR was further increased from 3 to 4 g VS L^{-1} day^{-1} between days 170 and 176, and from 4 to 5 g VS L^{-1} day^{-1} between days 204-218. Weekly TE dosing (1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1} in the feed) was carried out in proportion to the added feed material to maintain TE concentrations.

D9&10: these digesters were inoculated with a mixture of Millbrook inoculum and digestate collected from D1-4 in previous trial over an 8-day period prior to start-up, at a ratio of 1:2 on a volume basis. The purpose of this was to take advantage of any acclimatisation to a similar type of feedstock that had occurred in the previous trial, and thus eliminate or minimise the so-called '40-day bump'. On day 0, a one-off dose of TE to give additional concentration 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1} in the digestate of was carried out. From day 7, weekly TE dosing started to maintain the concentration.

The results of Trial 2-1 are reported in the sequence: D5&6 (thermophilic CSTR), D7&8 (thermophilic CSTR), D9&10 (mesophilic CSTR), and D1-4 (mesophilic CSTR). These reactors' operational conditions were different, and therefore the description has been separated for clarity. A comparative discussion is included at the end of these sections.

4.3.2. Thermophilic digestion results

Results for D5&6 are presented separately from those for D7&8 as, although the two pairs of reactors both started with inoculum from the previous trial, the dates on which feeding with baby maize stover began were different, and therefore the initial conditions were also different (amount of added maize silage, operating history and OLR in trial 2-1). These differences may cause different anaerobic digestion behaviour. The behaviour of the two sets of reactors is compared after the results for individual pairs have been presented to assess the anaerobic digestion performance of baby maize stover under thermophilic condition.

4.3.2.1. Thermophilic digestion - D5&6

Operating parameters. Figure 24 shows the applied OLR, daily wet weight of feed added and hydraulic retention time for digesters D5&6, and Table 16 shows a summary of the operating history.

In general the target OLRs were successfully maintained during the experimental period without any major variations. The exceptions were at the start of the trial and around day 115. Both of the digesters showed foaming in response to the change in feedstock, and were therefore not fed on days 3 and 4. On day 3, 400 µL of Goldcrest antifoam was added to each digester and over the next 6 days feeding was raised stepwise back to the target OLR of 4 g VS L^{-1}. Intermittent dosing with antifoam continued as shown in Table 14 until the foaming ceased, with D5 requiring a longer period at a higher dosage of antifoam than D6. For more details on the foaming, see the following section.

The digesters were not fed on day 115 and 116, due to closure of the laboratory for a planned power shutdown, but digester temperatures were maintained using an external power supply. Feeding stopped on day 169 for D5 and day 177 for D6, shortly after the increase in OLR from 4 to 6 g VS L^{-1} day^{-1}. D5 and D6 were operated for the equivalent of 3.7 HRT and 4.0 HRT, respectively (Table 15).

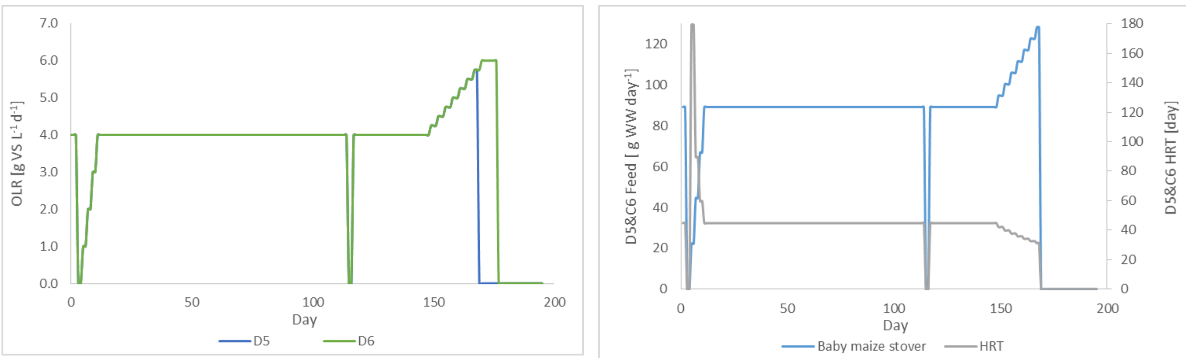


Figure 24. OLR, daily feed and HRT for D5&6 during digestion Trial 2-1 on baby maize stover

Table 14. Summary of history for thermophilic digesters D5-6 during Trial 2-1 on baby maize stover

<table>
<tr><th>Day</th><th>Start date</th><th>D5</th><th>D6</th></tr>
<tr><td>0</td><td>07/10/2015</td><td colspan="2">Feeding start
Weekly TE dosing start to maintain concentration; 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1}</td></tr>
<tr><td>3</td><td>10/10/2015</td><td colspan="2">Antifoam Goldcrest AF-530 400μL
Not fed for 2 days</td></tr>
<tr><td>13</td><td>20/10/2015</td><td>Antifoam Goldcrest AF-530 400 μL</td><td></td></tr>
<tr><td>19</td><td>26/10/2015</td><td colspan="2">Antifoam Goldcrest AF-530 400 μL</td></tr>
<tr><td>20-22</td><td>27/10/2015</td><td>Antifoam Goldcrest AF-530 100μl</td><td></td></tr>
<tr><td>23</td><td>30/10/2015</td><td>Antifoam Goldcrest AF-530 100μl</td><td>Antifoam Goldcrest AF-530 100 μL</td></tr>
<tr><td>24-27</td><td>31/10/2015</td><td>Antifoam Goldcrest AF-530 100 μL</td><td></td></tr>
<tr><td>28-29</td><td>04/11/2015</td><td>Antifoam Goldcrest AF-530 10 μL</td><td></td></tr>
<tr><td>30</td><td>06/11/2015</td><td>Antifoam Goldcrest AF-530 100 μL</td><td></td></tr>
<tr><td>31-34</td><td>07/11/2015</td><td>Antifoam Goldcrest AF-530 10μl</td><td></td></tr>
<tr><td>115</td><td>30/01/2016</td><td colspan="2">No fed for 2 days due to lab closure</td></tr>
<tr><td>117</td><td>01/02/2016</td><td colspan="2">No further dosing with TE solutions</td></tr>
<tr><td>149</td><td>04/03/2016</td><td colspan="2">OLR increase from 4 to 6 over 22 days</td></tr>
<tr><td>169</td><td>24/03/2016</td><td>Stop feeding</td><td></td></tr>
<tr><td>177</td><td>01/04/2016</td><td></td><td>Stop feeding</td></tr>
</table>

Table 15. Total amount of added substrate and equivalent HRT

	D5	D6
Amount of added substrate [g]	14887	15952
Equivalent HRT for added substrate [-]	3.7	4.0

Foaming. Foaming issues had quite negative impacts for stable operation. On the day after feeding with baby maize stover began, severe foaming and expansion occurred leading to some loss of digestate. Figure 25 shows foaming D5 on day 2. The expanding digestate came out through the stirrer draft tube, blocked the gas outlet and blew out the bung on the feeding port. An upside-down 100 mL plastic container without a lid was used instead of the D5 bung for the next few days (Figure 25a). The top of the digester was also surrounded with tissue to prevent the foamed digestate spilling continuously (Figure 25b). Even with the tissue, digestate sometimes climbed and a little spilled outside. Foaming in D6 was not as serious as in D5 but was also observed.

On day 3, 400 μL of Goldcrest antifoam was dosed to D5&6 and the digesters were not fed. From day 5 to day 9, the OLR was gradually returned to 3 g VS L^{-1} day^{-1}. On day 9, foaming in D5&6 was observed immediately after feeding but the volume was not as much as on day2. On day 13, the foaming digestate in D5 filled all the headspace and reached the feed bung, therefore D5 was dosed with 400 µL of Goldcrest antifoam again. Up to day 34, the amount of antifoam dosed into D5 and D6 was decided based on the degree of foaming and the amount needed to reduce it (Table 16).

From day 35 to 68, additional hand mixing of D5 digestate was carried out after feeding to disperse the foam because the foaming issues were reducing, but some foaming still remained. From day 62 to 68, this additional mixing was also carried out for D6 due to signs of foaming. After addition of the baby maize stover feed, the digestate was mixed using a 25 cm rod which was inserted at the top of the digester and stirred manually. This operation was carried out to ensure that the substrate was pushed down into digestate, and to break up the foam on the surface. After this, significant foaming was not seen. Up to day 68, D5&6 both received 5712 g WW of baby maize stover feed, equivalent to 1.4 HRT.

From day 60 to day 168, no significant foaming occurred. On day 168, the stirrer for D5 broke and the digestate expanded and came out of the reactor. The stirrer was replaced and it was observed that the digestate in D5 had separated into 2 layers, with the upper part containing more liquid, and the bottom more solid. These phenomena indicated that both bed expansion and foaming may have occurred. Foaming and separation was also observed next day. Therefore, D5 operation was stopped on day 169. D6 did not show such severe foaming as D5, but feeding was stopped on day 177.

(a) with 100 mL container as a lid

(b) without 100 mL container as a lid

Figure 25. Foaming in D5 on day 2, with tissue to retain foam

Operational stability. Figure 26 shows the monitoring parameters for operational stability during the experimental period.

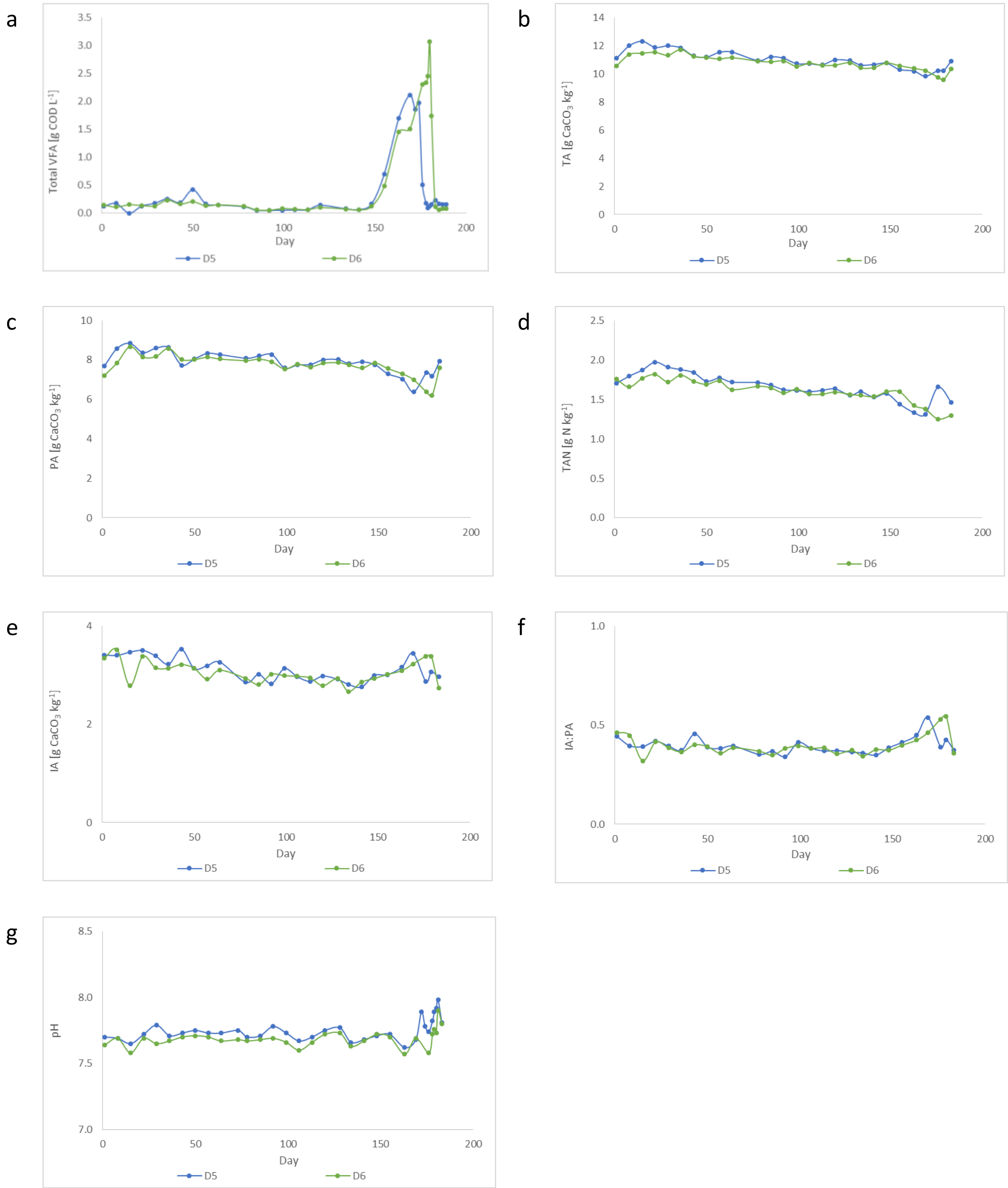


Figure 26. Total VFA, alkalinity, TAN concentration and pH in D5&6 during digestion Trial 2-1 on baby maize stover

Total VFA concentrations (Figure 28a) remained less than 0.5 g COD L^{-1} until day 155. TA, PA and TAN (Figure 28b, c and d) increased slightly during the first 15 days of operation, and during this period foaming occurred. The increase in TAN was especially evident in D5. TAN in D6 stabilised at around 1.7 g N kg^{-1} and did not increase as in D5. The difference between D5&6 was the severity of foaming. As seen in Figure 27, D5 showed more intense foaming. During these periods of foaming, the feed port was not sealed off with the bung and therefore the digestate in D5 was exposed to air. In addition, D5 received a great deal more anti-foam than D6 so it could be the anti-foam that was having the effect of killing selected microorganisms and thus leading to an increase in TAN. The increase in TAN may indicate some die-off of micro-organisms due to exposure to atmospheric oxygen, with protein from the microorganisms being degraded into ammonia. The presence of ammonia and bicarbonate at the same time can increase buffer capacity (Mata-Alvarez, 2003).

After that, these values settled around TA 10.7 g $CaCO_3$ kg^{-1}, PA 7.8 g $CaCO_3$ kg^{-1}, TAN 1.6 g N kg^{-1} at OLR 4 g VS L^{-1} day^{-1}. IA showed a long slow decline from 3.3 g $CaCO_3$ kg^{-1} to 2.8 g $CaCO_3$ kg^{-1} with minor fluctuations (Figure 27e). IA:PA ratio and pH also settled around 0.4 and 7.7 (Figure 27f and g).

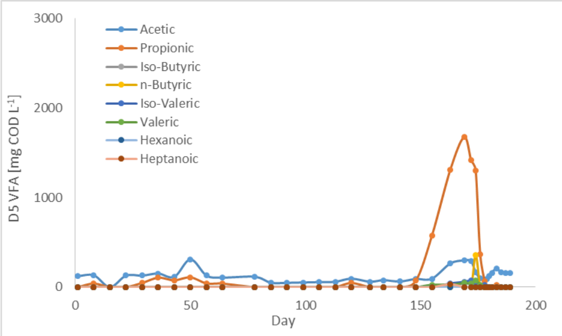
From day 155 just 1 week after the start of the OLR step wise increase from 4 to 6 g VS L^{-1} day^{-1} , VFA started to increase which was accompanied by slightly increase of IA and IA/PA. Feeding was stopped on day 169 for D5, and day 177 for D6, then the VFA accumulation rapidly disappeared.

The instability may be related to the TE status of the reactors. TE dosing ceased from day 117 as the TE solution ran out. Between day 118 and 141 each digester received 2053 g WW which was equivalent to 0.5 HRT. The proportion of reactor contents (and therefore TE) removed in 0.5 HRT is about 40%, so this could have been contributing factor.

Taken together these results suggested that the digesters had experienced some inhibition to methanogenesis, due to the increase in OLR and possibly also the cessation of TE dosing.

Average values obtained for monitoring parameters during periods of pseudo-steady state operation are given in Table 26, after the results for all pairs of digesters.

Volatile Fatty acids. Figure 27 shows the VFA profiles of the digesters. The very slight elevation of VFA noted above during the first part of the run mainly consisted of acetic acid (< 310 mg COD L^{-1}) which was present until around day 100, after which concentrations fell slightly until day 150. At this point a sharp increase in propionic acid concentrations occurred



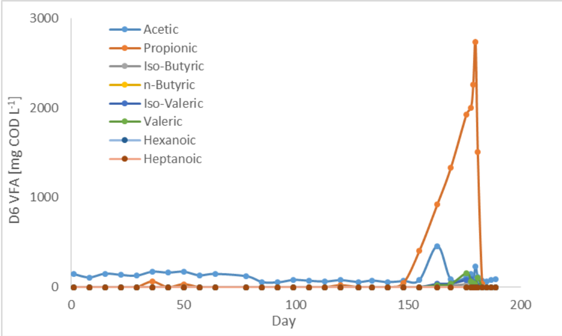


Figure 27. VFA profiles for D5&6 during digestion Trial 2-1 on baby maize stover

Biogas and methane production. Biogas and methane production and biogas methane content are shown in Figure 28. Gas production in D5 and D6 reflected the applied OLR, so production decreased around day 3-4 and day 115-116 when the digesters were not fed. VBP and VMP (Figure 30a and b) in D5&6 started to stabilise from day 30 to 148. Gas production in D5 slightly lower than D6 by day 100 which showed more foaming in the first month. During this period, SBP and SMP were also settled and SMP was around 0.31 L CH_4 g^{-1} VS. From day 149, SBP and SMP (Figure 28c and d) decreased because SBP and SMP did not respond well to the OLR increase.

Methane concentration showed some fluctuation and the value range was 53-63 % (Figure 28e). D5 methane concentration increased from 57 to 63 % after ceasing feeding as residual VFA were consumed.

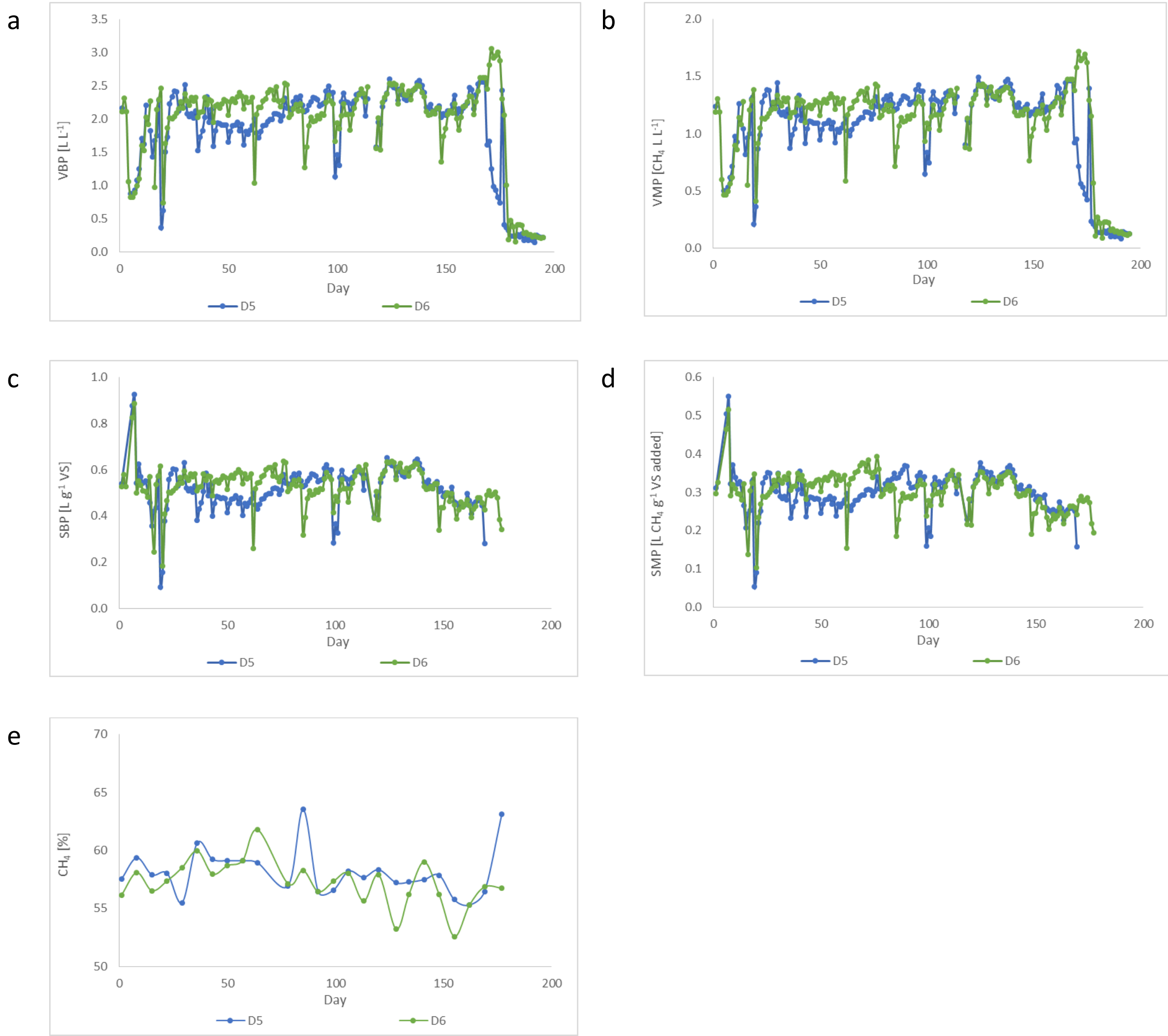


Figure 28. VBP, SBP, VMP and SMP for D5&6 during digestion Trial 2-1 on baby maize stover

Solids parameters. Figure 31 shows the solids parameters for the digesters. The TS and VS content in D5&6 declined steadily from the start of the run (Figure 29a and b), reflecting the change in feedstock properties from maize silage to the lower solids baby maize stover. The TS decreased from an initial value of around 11.6 % WW to stabilise at around 8.6 % WW by day 133, while the VS decreased from around 9.8 to 6.3 % WW. The VS/TS ratio (Figure 29c) also decreased from around 84 % at the start of the run to 74 % by day 120. VS destruction showed some variability (Figure 29d) but also appeared to be stabilising at around 70% towards the end of the run.

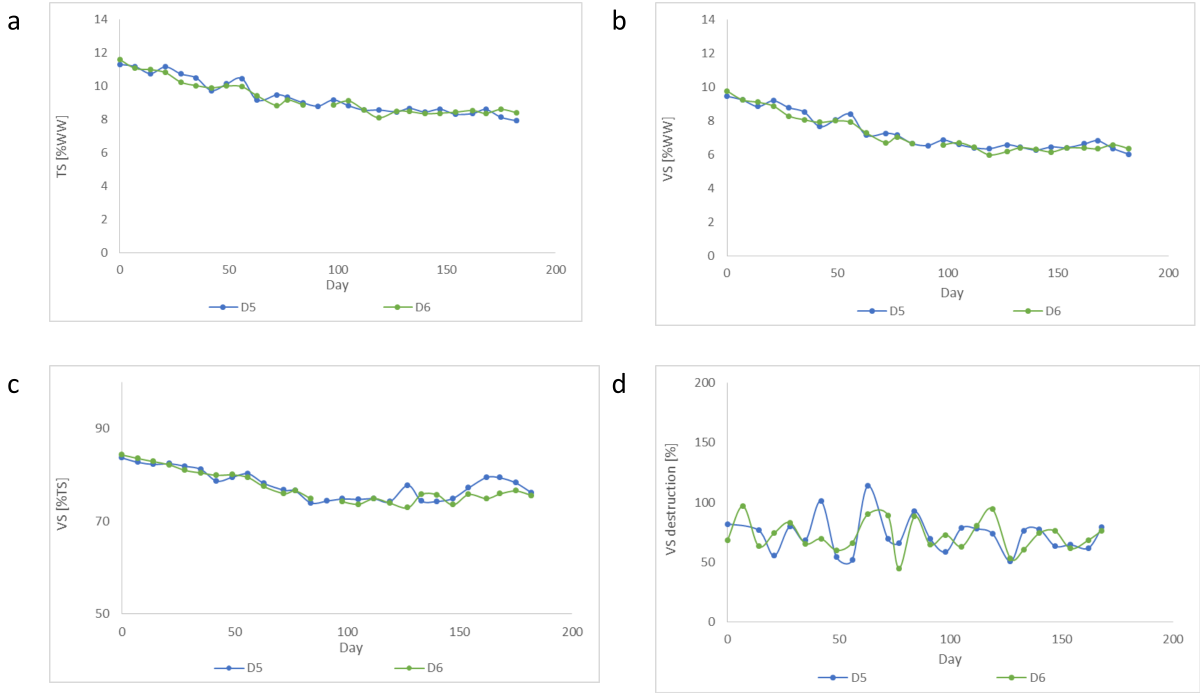


Figure 29. Digestate solids parameters for D5&6 during digestion Trial 2-1 on baby maize stover

The significance of these results is discussed in more detail below with the results for digesters D7&8.

4.3.2.2. Thermophilic digestion - D7&8

Operating parameters. These digesters were operated in parallel with D5&6, but feeding with baby maize stover started 157 days earlier. Figure 30 shows the OLR, daily wet weight of feed added and hydraulic retention time during the experimental period for thermophilic digesters D7-8 during digestion Trial 2-1 on baby maize stover and Table 16 shows a summary of the operating history.

In general target OLRs were successfully maintained, without any significant variations in the daily feed. At the beginning D7&8 were fed at OLR 2 g VS L^{-1} day^{-1} for 12 days then the OLR was gradually increased to 3 g VS L^{-1} day^{-1} by day 20. This step was carried out to allow acclimatisation to the new baby maize stover substrate; in the previous trial 1, D7&8 were fed with maize silage at OLR 3 g VS L^{-1} day^{-1}. Foaming in D7 and D8 was observed from day 18 to 60, but it was not as serious as in D5&6.

From day 170 to 176, the OLR was increased from 3 to 4 g VS L^{-1} day^{-1}. On day 182, some signs of foaming appeared in D7 and the digester was dosed with 100 µL Goldcrest antifoam. Antifoam was used on only this occasion. Between day 191 and 206, a small amount of foaming appeared in D7, and the digestate was therefore mixed with a 25 cm rod after feeding to reduce foaming.

Between day 204 and 218, it was decided to raise the OLR from 4 to 5 g VS L^{-1} day^{-1}, since at this point the results of the trial with D5&6 appeared to indicate that stable operation at OLR 4 g VS L^{-1} day^{-1} was possible. From day 212 to 224, again a small amount of foaming appeared in D7&8 so the surface was mixed after feeding. The foamed digestate did not fill the head space and did not block the gas outlet tubes in this period.

The digesters were not fed on day 270 and 271 due to the laboratory closure mentioned above, but operating temperatures were maintained. Feeding of both digesters was stopped on day 334 due to signs of instability. D7&8 were operated for the equivalent of 6.8 HRT (Table 17). Therefore, it was considered that this trial had enough data.

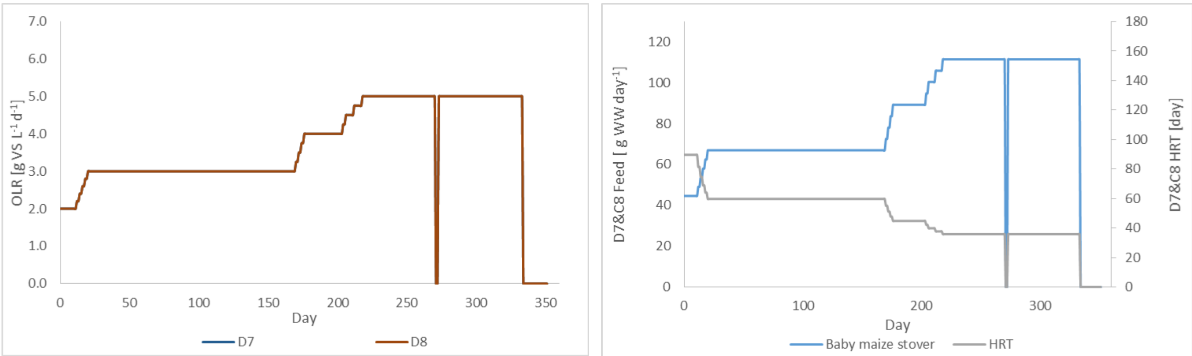


Figure 30. OLR, daily feed and HRT for D7&8 during digestion Trial 2-1 on baby maize stover

Table 16. Summary of history for thermophilic digesters D7-8 during digestion Trial 2-1 on baby maize stover

Day	Date	D7	D8
0	04/05/2015	Feeding started TE dose (Fe, Co, Ni) start to maintain concentration	
170	21/10/2015	Increase OLR from 3 to 4 g VS L^{-1} day^{-1} over 6days	
181	01/11/2015	Antifoam Goldcrest AF-530 100µL	
204	24/11/2015	OLR increase from 4 to 5 over 14 days	
271	30/01/2016	No fed for 2 days due to lab closure	
273	01/02/2016	No further dosing with TE solutions	
334	02/04/2016	Stop feeding	

Table 17. Total amount of added substrate and equivalent HRT

	D7	D8
Amount of added substrate [g]	27019	27019
Equivalent HRT for added substrate [-]	6.8	6.8

Operational stability. Figure 31 shows the monitoring parameters for operational stability during the experimental period.

Total VFA concentrations (Figure 31a) remained less than 0.5 g COD L^{-1} until day 304. TA, PA and TAN (Figure 31b, c and d) increased slightly during the first 15 days of operation, then, these values settled around TA 10.5 g $CaCO_3$ kg^{-1}, PA 7.7 g $CaCO_3$ kg^{-1}, TAN 1.6 g N kg^{-1} at OLR 3 g VS L^{-1} day^{-1}. IA, IA/PA and pH were also stable and settled around IA 3.3 g $CaCO_3$ kg^{-1} to 3.3 g $CaCO_3$ kg^{-1}, IA/PA 0.43 and pH 7.7. (Figure 31e, f and g).

From day 304, VFA started to increase which was accompanied by slightly increase of IA and IA/PA. Feeding was stopped on day 334, then the VFA accumulation rapidly disappeared.

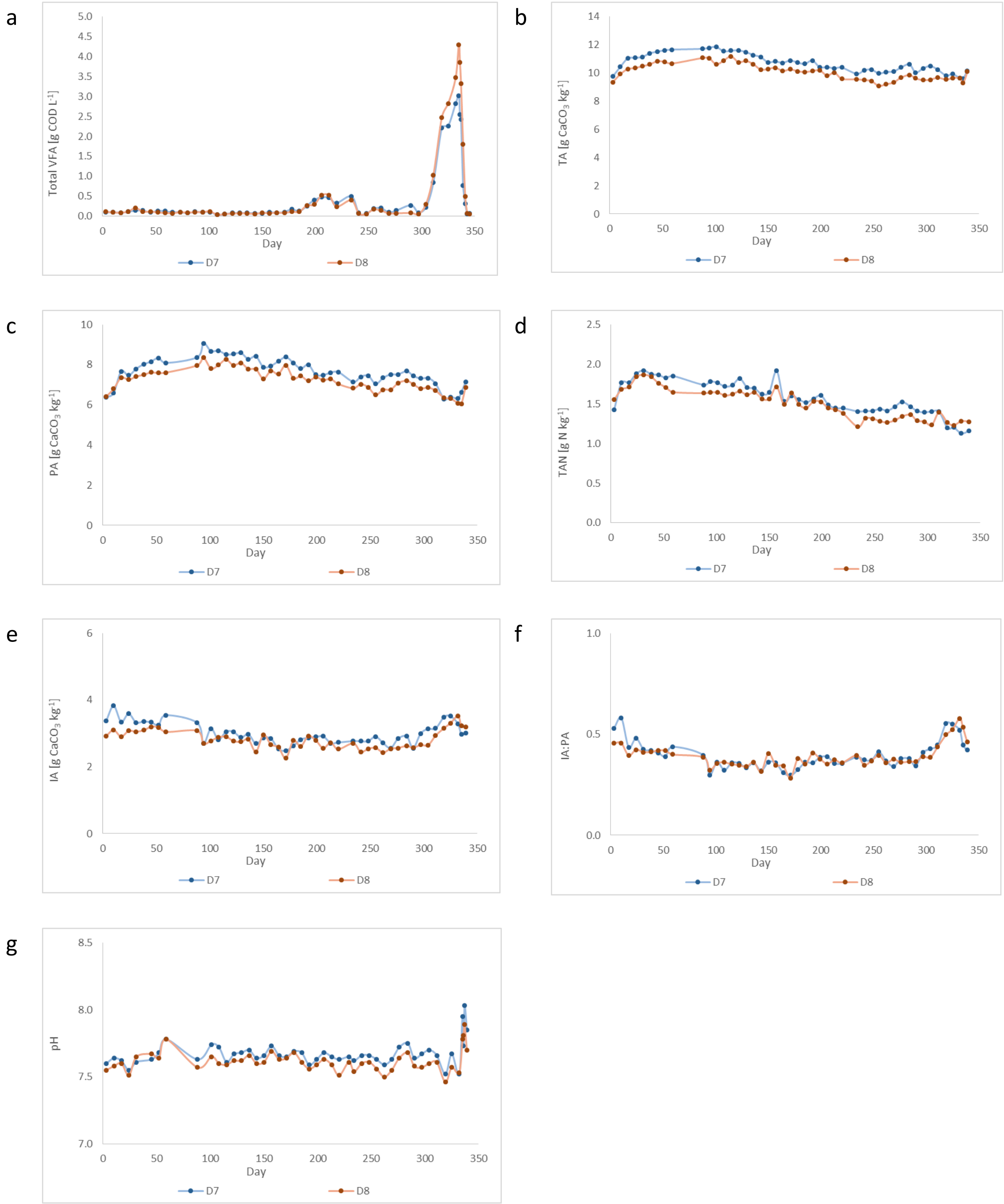


Figure 31. Total VFA, TAN, Alkalinity and pH in D7&8 during digestion Trial 2-1 on baby maize stover

The instability may be related to the TE status of the reactors. TE dosing ceased from day 273 as the TE solution ran out. Between day 273 and 304, each digester received 3459 g WW which was equivalent to 0.9 HRT, so this could have been contributing factor.

Taken together these results suggested that the digesters had experienced some inhibition to methanogenesis, due to the increase in OLR and possibly also the cessation of TE dosing.

These parameter values and trends were quite similar to those in D5&6, including the rapid but reversible VFA accumulation at the end of the run. These parameter values and trends were quite similar to those in D5&6, including the rapid but non-irreversible VFA accumulation at the end of the run. This was surprising at first sight, since unlike D5&6 these digesters were not subject to an increase in OLR during this period to which the VFA accumulation could be attributed. D7&8 had responded well to the earlier increase in OLR and were more stable. This difference is discussed in section 4.3.2 and 4.3.4.

Average values obtained for monitoring parameters during periods of pseudo-steady state operation are given in Table 24, after the results for all pairs of digesters.

Volatile Fatty acid profiles. Figure 32 shows the VFA profiles of the digesters. There were 2 peaks in this trial; the first was between day 192 and 241, and the second was from day 304 to 345.

The first peak was mainly acetic acid which increased to around 400 mg COD L^{-1}. Propionic was also found but it was less than 110 mg COD L^{-1}. This may have been related to the rise in OLR which was increased from day 170 to 176. 15 days after the start of the OLR increase, the first VFA peak appeared. In addition, the appearance of this first peak and of foaming occurred at the same time.

The second peak was mainly propionic acid which rose sharply to reach 2556 mg COD L^{-1} in D7 and 3041 mg COD L^{-1} in D8 on day 335. Acetic acid and small amounts of other acids were also detected. For instance, in D8 on day 335 these concentrations were; iso-butyric acid 149 mg COD L^{-1}, iso-valeric acid 64 mg COD L^{-1} and valeric acid 230 mg COD L^{-1}. Concentrations of all VFA species fell sharply from day 335 a few days after feeding ceased.

This pattern of VFA accumulation may be related to the trace element status of the inoculum. The inoculum came from the previous Trial 1 so the digestate had been in use for 486 days in Trial 1 and 2-1. D8 received 4092 g WW in Trial 1, 27019 g WW in Trial 2-1, giving a total of 31111 g WW which is equivalent to 7.8 HRT. Wash-out of some essential TE for microorganisms may therefore have occurred, even though the digesters received certain TE (Fe, Co, Ni) from the beginning of the run.

The cessation of TE dosing may also be related to this issue. TE dosing ceased on day 273 because the TE solution ran out.

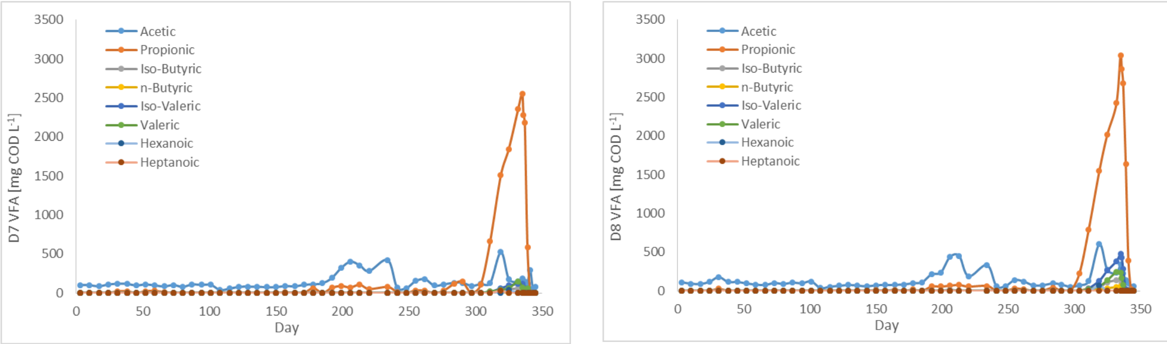


Figure 32. VFA profiles for D7&8 during digestion Trial 2-1 on baby maize stover

Biogas and methane production. Biogas and methane production and biogas methane content are shown in Figure 33.

In the previous Trial 1, the digesters were fed at OLR 3 g VS L^{-1} day^{-1} but at the start of the current trial OLR was decreased to 2 g VS L^{-1} day^{-1} to allow acclimatisation to the new feedstock. The initial SMP value was around 0.48 L CH_4 g^{-1} VS added which is higher than literature values for most maize derived substrates. This indicates that D7 and D8 were digesting leftover VS from Trial 1 as well as VS from Trial 2. From day 0 to day 14, the VBP (Figure 33a) decreased to around 0.3 L L^{-1} day^{-1} due to the OLR decrease.

From day 14-21 these digesters responded well to the introduction of the baby maize stover feedstock, with VBP and VMP (Figure 33a and b) rising in proportion to the initial increase in the applied load, then appearing to stabilise at around 1.8 L L^{-1} day^{-1} and 1.0 L CH_4 L^{-1} day^{-1}, respectively.

There was some disturbance in VBP and VMP during the next increases in OLR between days 171-178 and 204-218; then values appeared to stabilise again at around 2.7 L L^{-1} day^{-1} and 1.5 L CH_4 L^{-1} day^{-1} once the OLR reached 5 g VS L^{-1}, but with slightly higher day-to- day variation.

VBP and VMP decreased to around 2.3 L L^{-1} day^{-1} and 1.3 L CH_4 L^{-1} day^{-1} between days 298-320 when VFA accumulation appeared, then recovered between day 321-330 before feeding was stopped on day 334.

SBP and SMP (Figure 33c and d) appeared fairly stable from day to day, but showed a long slow decline over time. During the period of OLR increase from day 204 to 218, the values fluctuated slightly due to changes in methane concentration (Figure 33e).

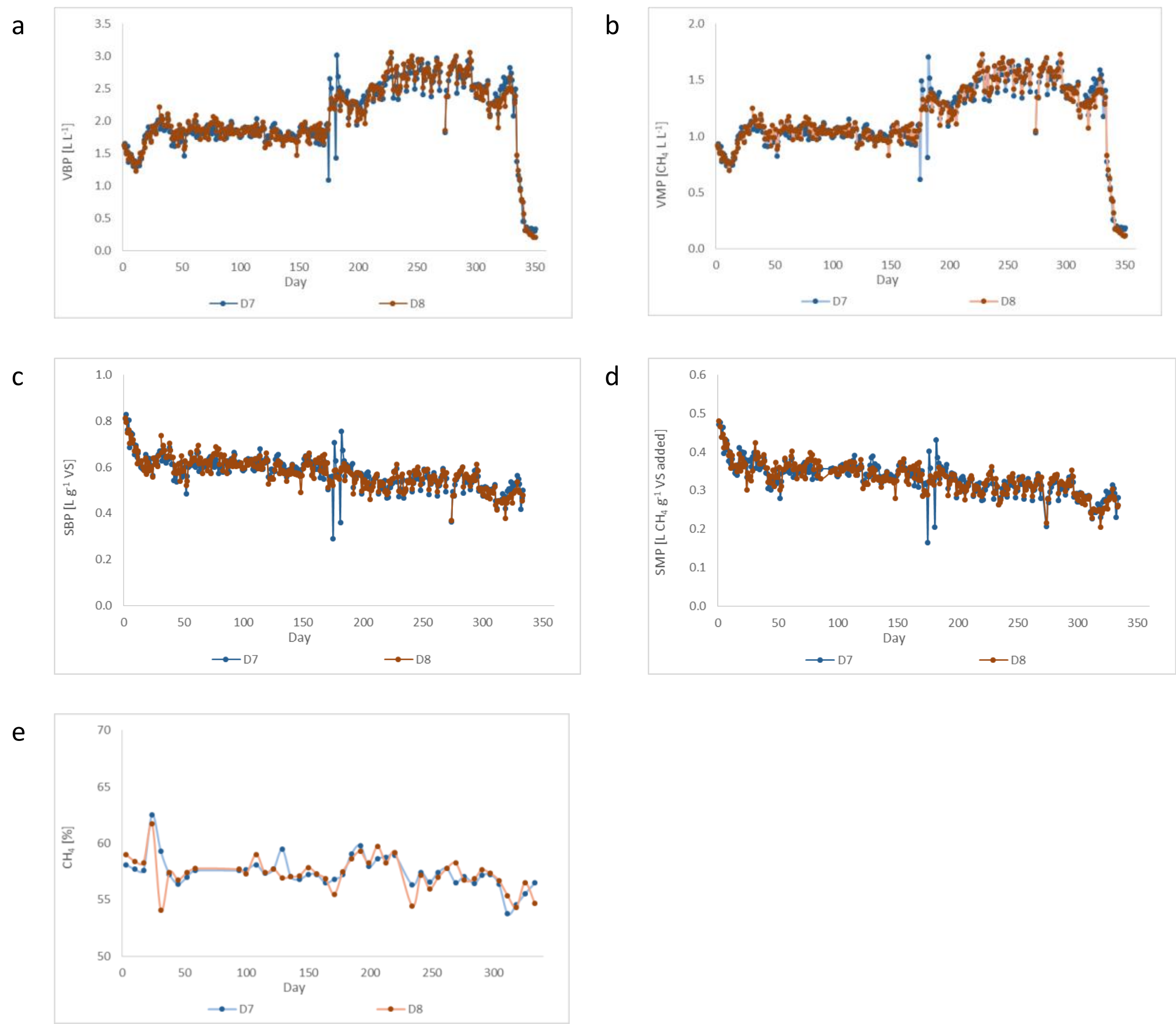


Figure 33. VBP, SBP, VMP and SMP for D7&8 during digestion Trial 2-1 on baby maize stover

Solids parameters. Figure 34 shows the solids parameters for the digesters.

TS and VS (Figure 34a and b) decreased until day 52, with the values in D7 which showed more foaming around 1 % greater than in D8.

Between day 63 and 84, TS and VS appeared to increase but there was no OLR increase around this period. During this period, digestate samples were frozen and kept in freezer and analysed later. The samples may not have been representative or homogeneous enough due to lack of mixing. If these data are removed, solids parameters continuously decreased until day 122.

From day 88 to 122, TS and VS decreased slightly and settled around 6.5 % WW and 4.8 % WW. The VS/TS ratio (Figure 34c) also decreased from 80 % TS to 72 % TS from day 1 to 122.

If it is assumed that TS and VS continuously decreased to day 122, this probably reflects the difference between maize silage and baby maize stover. Baby maize stover has a higher moisture content than maize silage. TS of maize silage and baby maize stover were 39.1 ± 1.66 % WW and 19.8 ± 1.03 % WW. VS of maize silage and baby maize stover were 37.5 ± 1.71 % WW and 17.9 ± 0.96 % WW.

On the other hand, TS and VS increased after day 122. At this point there had been no OLR increase for 100 days, therefore the increase was not related to a change in OLR increase. It could be a sign of stress indicating that the microorganisms could not digest TS and VS well.

The initial value for VS destruction (Figure 34d) was 78 %. This slowly declined and although there were considerable fluctuations the range of variation became smaller so that at the end of the experimental period, VS destruction was around 70%. Even in the absence of a period of OLR increase, the VS destruction decreased, which may also suggest the system was running out of some essential element.

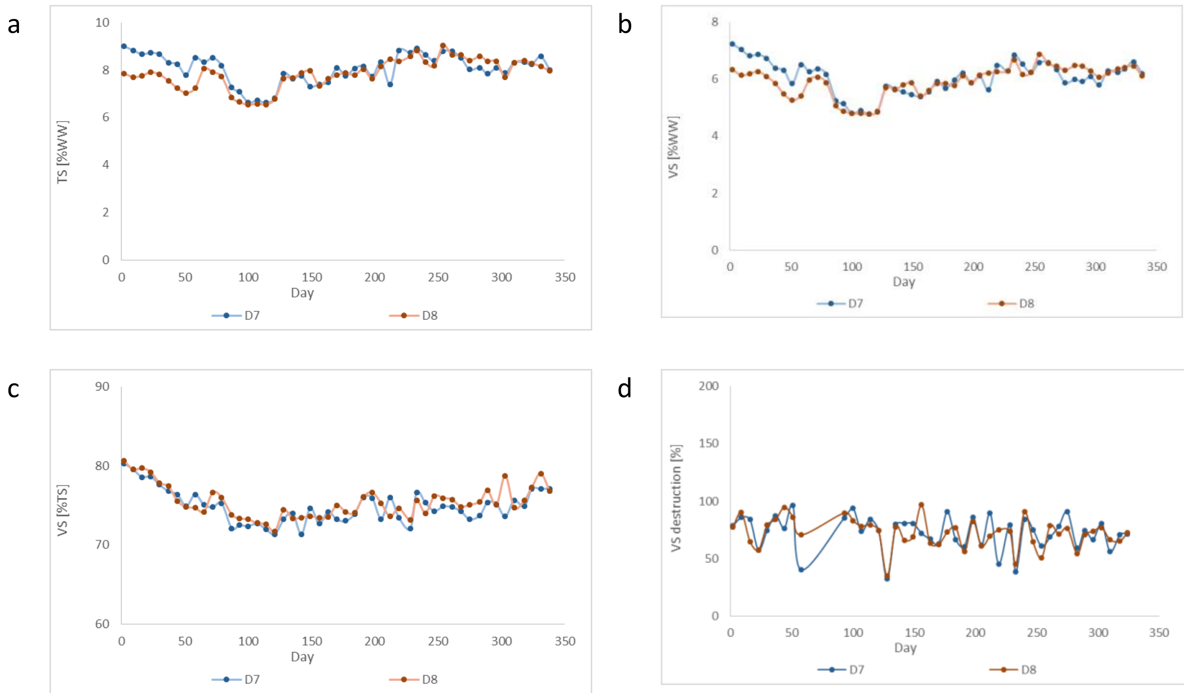


Figure 34. Digestate solids parameters for D7&8 during digestion Trial 2-1 on baby maize stover

Digestate appearance. The digestate appearance of D7&8 in Trial 2-1 and digestate in Trial 1 showed significant difference (Figure 35). The thermophilic digestion of baby maize stover was operated for just 30 days when photos were taken, and the inoculum was the viscous digestate from Trial 1, but it was clear that the thermophilic digestate was more thin than that of maize silage.

Figure 35. The digestate appearance of D7&8 on day 30

Average values for monitoring parameters during pseudo-steady state periods are given in Table 24 after the results for all pairs of digesters.

Discussion of thermophilic digestion results.

Table 18 shows the average values for reporting parameters in D5&6 and D7&8 during periods of pseudo steady-state operation. The slow declines in gas production noted above made it difficult to compare steady state values. At OLR 3 and 4 g VS L^{-1} day^{-1}, SBP and SMP were closely similar, at around 0.60 L g^{-1} VS and 0.340 L CH_4 g^{-1} VS respectively. VBP and VMP increased approximately in proportion to the OLR, from around 1.8 to 2.4 L L^{-1} day^{-1} and 1.0 to 1.4 L CH_4 L^{-1} day^{-1}. This indicated the system was not overloaded at OLR 3 and 4 g VS L^{-1} day^{-1}. At OLR 5 g VS L^{-1} day^{-1}, SBP and SMP appeared to be around 10 % lower. Overloading causes lower biogas production but OLR 5 g VS L^{-1} day^{-1} is not a particularly high loading. OLR of up to 8 g VS L^{-1} day^{-1} have been achieved with food waste, a highly degradable substrates, without loss of SMP (Song, 2016a). Mata-Alvarez (2003) noted 6 – 8 g VS L^{-1} day^{-1} was a reasonable upper limit for most feedstocks. The SMP decline at higher OLR may have been caused by other factors.

D5&6 and D7&8 both started to show increases in total VFA concentration at almost the same time; after day 146 (3.1 HRT) for D5&6 and day 303 (5.9 HRT) for D7&8, in both cases corresponding to day 455 since the digesters originally started running in digestion trial 1. The two sets of digesters operating under different conditions showed signs of stress at the same time. These digesters showed very similar trends in monitoring parameters, the sudden onset of VFA accumulation, process instability (e.g. a fall in gas production, and a rise in IA/PA ratio).

On the other hand, there was no temperature shock in D5-8 and no increase in OLR around this period in D7&8. There was only one change, which was ceasing TE supplementation. From the day TE addition ended to the day of VFA accumulation increase was 29 days in D5&6, 32 days in D7&8. These digesters received 2588 g WW (0.65 HRT) in D5&6, and 3459 g WW (0.86 HRT) in D7&8 during this period, corresponding to replacement of roughly 48 % and 57 % of the digester contents. The concentration of other TE may also have been close to the minimum acceptable amount in the thermophilic digesters because the digestate used was from Trial 1. In Trial 1, D5-8 were operated for 2.5, 2.5, 1.0 and 1.0 HRT, respectively. The total HRT before appearance of VFA accumulation was 5.6 HRT in D5&6 and 6.9 HRT in D7&8. A long slow decline in gas production, and increase in VS content and VS/TS were observed when there were no other obvious signs of stress. The digesters may thus have run out of some essential trace elements or nutrients, although the situation is made slightly more complex by the change in feedstocks at different times, as maize silage does not have the same TE content as baby maize stover. The difference is discussed in section 4.1.

In addition, the VFA peaks mainly consisted of propionic acid, with acetic acid at lower concentrations. Böck (2006) reported that high concentrations of propionic acid were difficult to recover and were attributed to the deficiency of trace elements Se, Mo and W. The evidence from this trial therefore suggested that a different TE dosing strategy may be required.

D5&6 and D7&8 showed significant differences in terms of foaming, although the inoculum for all of the digesters came directly from digestion trial 1. D7&8 started 155 days earlier and the OLR started at 2 g VS L^{-1} day^{-1}. From day 18 to 60, a small volume of foaming was observed but it did not disturb operation of these digesters. On the other hand, D5&6 feeding started at OLR 4 g VS L^{-1} day^{-1}. Foaming was observed on day 1 and digestate came out, blocking the gas outlet tube.

The difference in response may have been caused by the higher OLR, and/or the difference in digestate properties when the feedstock was changed. The initial OLR on D5&6 was double that on D7&8. On the other hand, OLR 4 g VS L^{-1} day^{-1} is not a very high OLR to cause serious foaming at the beginning of operation under thermophilic condition. Suhartini *et al.* (2014) confirmed significant foaming at OLR 5 g VS L^{-1} day^{-1} only after 3 HRT in thermophilic digesters. The substrate used in that study was also a lignocellulosic material, sugar beet pulp. OLR may therefore not be the main or only cause of the foaming. It was probably also partly due to change of substrate.

In digestion trial 1, D5-8 received maize silage from CEDAR as a substrate. The silage was drier and some parts were slightly fermented. At the time of the change in the feedstock D5&6 had received 10088 g of silage feed which was 2.5 times the amount fed to D7&8. This contributed to a more thick and viscous inoculum for D5&6. The dewaterability of the inoculum for D5&6 was greater than the maximum value measurable in the CST and FIC test. Stoppok and Buchholz (1985) reported high viscosity liquid attributed to foaming.

Based on all of these results taken together, it was decided to stop these digesters, even though the VFA accumulation that had occurred up to that point appeared to be recoverable; and to start a new thermophilic digestion experiment with a better-defined starting point.

Table 18. Average values for reporting parameters during pseudo steady state periods in digestion Trial 2-1 (Baby maize stover, thermophilic digesters only)

Parameter	Unit	D7[a]	D8[a]	D5[b]	D6[b]	D7[c]	D8[c]	Ave[a]	Ave[b]	Ave[c]
OLR	g VS L^{-1} day^{-1}	3	3	4	4	5	5	3	4	5
SBP	L g^{-1} VS	0.601	0.598	0.603	0.604	0.539	0.545	0.599	0.604	0.542
SMP	L g^{-1} VS	0.343	0.342	0.346	0.332	0.308	0.315	0.342	0.339	0.311
VBP	L L^{-1} day^{-1}	1.80	1.79	2.41	2.42	2.70	2.73	1.80	2.41	2.71
VMP	L L^{-1} day^{-1}	1.02	1.01	1.38	1.36	1.52	1.54	1.02	1.37	1.53
CH_4 content	% v/v	55.5	56.1	57.4	56.0	56.8	57.5	55.8	56.7	57.2
Digestate TS	%WW	7.4	7.6	8.5	8.4	8.3	8.2	7.5	8.5	8.2
Digestate VS	%WW	5.5	5.6	6.4	6.3	6.0	6.4	5.5	6.3	6.2
VS destruction	%VS	75.1	73.8	71.2	72.6	75.6	68.2	74.4	71.9	71.9
pH	–	7.7	7.6	7.7	7.7	7.7	7.6	7.7	7.7	7.6
TA	g $CaCO_3$ kg^{-1} WW	10.8	10.2	10.8	10.6	10.2	9.5	10.5	10.7	9.9
PA	g $CaCO_3$ kg^{-1} WW	7.9	7.5	7.9	7.7	7.5	7.0	7.7	7.8	7.2
IA	g $CaCO_3$ kg^{-1} WW	3.4	3.2	2.9	2.8	2.7	2.5	3.3	2.9	2.6
IA/PA ratio	–	0.43	0.43	0.36	0.37	0.36	0.37	0.43	0.36	0.36
TAN	g N kg^{-1} WW	1.68	1.59	1.58	1.56	1.46	1.31	1.63	1.57	1.39
Total VFA	g COD L^{-1}	0.21	0.17	0.09	0.07	0.17	0.08	0.19	0.08	0.12

[a] Average value for days 140-169
[b] Average value for days 120-139
[c] Average value for days 265-294

Note: these values are also shown in Table 24 below, which includes the values for the mesophilic digesters run in parallel, but are given here for ease of reference.

4.3.3. Mesophilic digestion results

As with the thermophilic reactors, result for D9&10 are presented separately from those for D1-4 since the inoculum and initial conditions in each case were different. The behaviour of the two sets of reactors is compared after the results for individual pairs have been presented.

4.3.3.1. D9&10

Operating parameters. Figure 36 shows the organic loading rate, daily wet weight of feed added and hydraulic retention time for D9-10 during the experimental period, while Table 19 summarises the history for mesophilic digesters D9&10 during digestion trial 2-1 on baby maize stover.

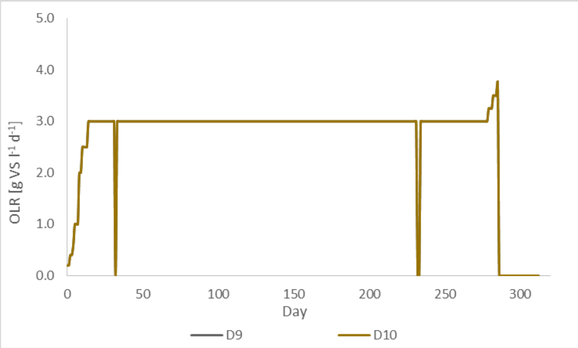
As the initial inoculum was a mixture of fresh Millbrook digestate and material from D1-4 digestion Trial 1, the digesters were not fed for the first 7 days to allow consumption of any residual feed. The OLR was then raised stepwise from 0.2 to the target 3.0 g VS L^{-1} day^{-1} over the next 15 days.

On day 33 feeding was forgotten, and on day 37 the thermocirculator turned itself off due to water shortage. On days 232 and 233, digesters were not fed because of the laboratory closure.

It can be seen that, apart from these events, the planned OLR and HRT were successfully maintained until day 278 which was equivalent to 3 HRT. Between day 279 and 286 the OLR was increased from 3 to 3.75 g VS L^{-1} day^{-1}; but for operational reasons discussed below, feeding was stopped on day 287.

D9&10 digestate appeared more thick and viscous towards to the end, so stirring was quite slow and the motors stopped quite often on day 286 and 287. The digesters did not show foaming so there was no antifoam supplementation. This may be related with lower OLR and/or different inoculum.

On day 0, trace elements Co, Ni and Fe were added to reactors D9&10 to give additional digestate concentrations of 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1}, and weekly addition of trace elements Co, Ni and Fe was started in proportion to the quantity of feed added to the digester, in order to maintain digestate TE concentrations. After day 234, the trace elements solution ran out so D9&10 did not receive any more TE supplementation. D9&10 were operated for 4.4 HRT (Table 20).



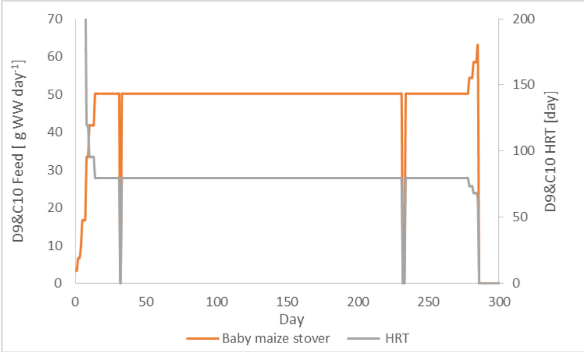


Figure 36. OLR, daily feed and HRT for D9&10 during digestion Trial 2-1 on baby maize stover

Table 19. Summary of history for mesophilic digesters D9&10 during digestion Trial 2-1 on baby maize stover

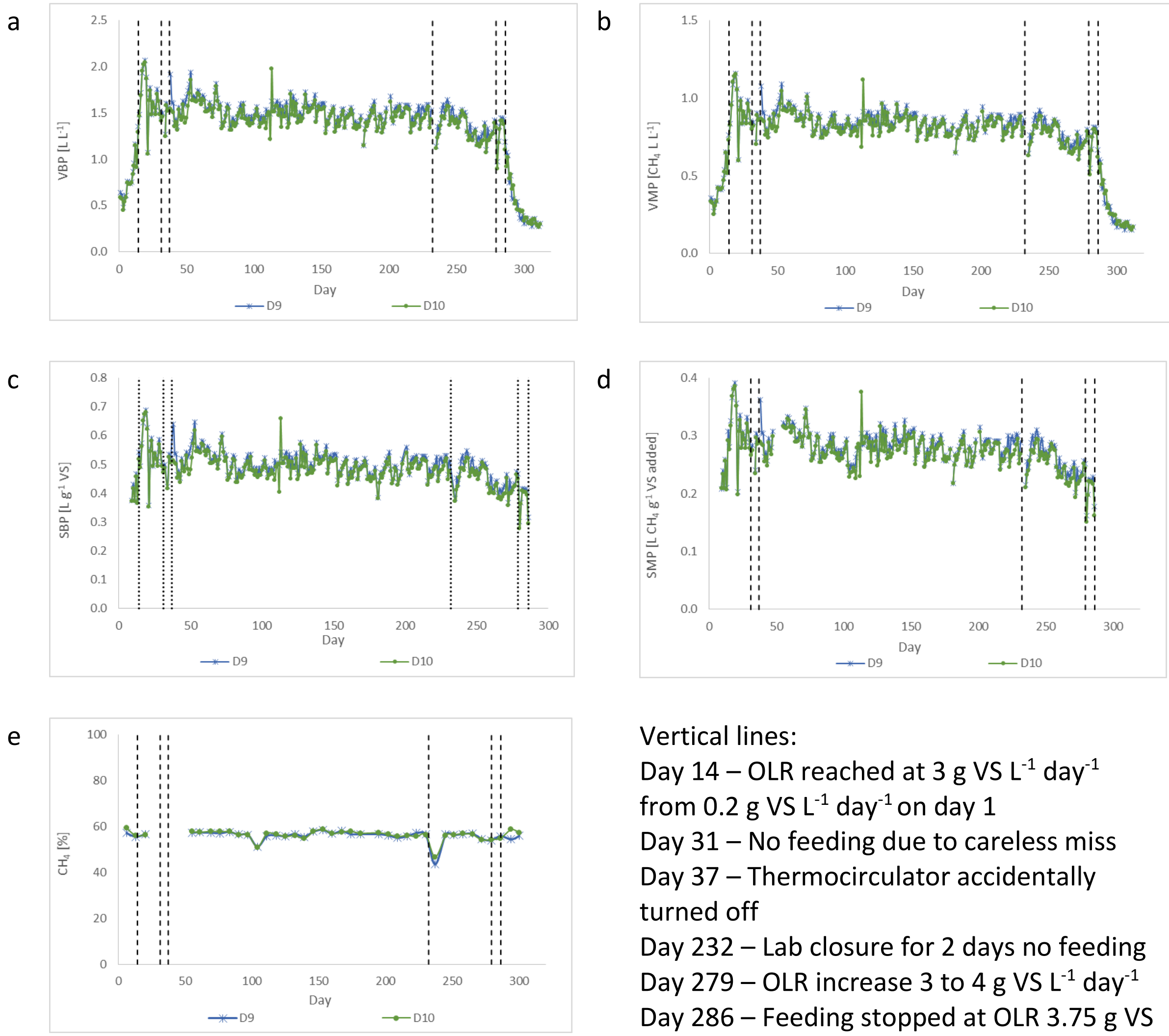
Day	Date	D9	D10
-8	04/06/2015	Mix Millbrook inoculum and digestate from D1-4 in Trial 1 (Millbrook:Trial 1 = 1:2 volume basis)	
0	12/06/2015	Start feeding; increase OLR from 0.2 to 3 for 15 days One off TE dose (Co 1 mg L^{-1}, Ni 1 mg L^{-1}, Fe 10 mg L^{-1}) for digestate	
7	19/06/2015	Weekly TE start to maintain concentration	
31	13/07/2015	No fed due to careless miss	
37	19/07/2015	Thermocirculator was turned off due to water shortage	
179	08/12/2015	The computer software for gas counter counting was changed	
232	30/01/2016	No fed for 2 days due to lab closure	
234	01/02/2016	No further dosing with TE solutions	
279	17/03/2016	OLR increase from 3 to 4 g VS L^{-1} day^{-1}	
286	24/03/2016	Stop feeding at OLR 3.75 g VS L^{-1} day^{-1}	

Table 20. Total amount of added substrate and equivalent HRT

	D9	D10
Amount of added substrate [g]	13065	13065
Equivalent HRT for added substrate [-]	4.4	4.4

Biogas and methane production. Figure 37 shows the specific and volumetric biogas and methane production and the biogas methane content during the experimental period.

The reactors responded rapidly to the initial increase in OLR. The OLR increase stopped on day 16, but gas production increased until day 20. From day 21 to around day 60, VBP (Figure 37a) fluctuated between 1.3 to 1.9 L L^{-1}. These fluctuations may be related to the non-feeding on day 32 and turning off of the thermocirculator on day 38.



Vertical lines:
Day 14 – OLR reached at 3 g VS L^{-1} day^{-1} from 0.2 g VS L^{-1} day^{-1} on day 1
Day 31 – No feeding due to careless miss
Day 37 – Thermocirculator accidentally turned off
Day 232 – Lab closure for 2 days no feeding
Day 279 – OLR increase 3 to 4 g VS L^{-1} day^{-1}
Day 286 – Feeding stopped at OLR 3.75 g VS L^{-1} day^{-1}

Figure 37. VBP, VMP, SMP and biogas methane content for D9&10 during digestion Trial 2-1 on baby maize stover

After day 38, gas production stabilised until day 258. During this period, the values were VBP 1.5 L L^{-1}, VMP 0.8 L CH_4 L^{-1}(Figure 37b), SBP 0.5 L g^{-1} VS (Figure 37c) and SMP 0.29 L CH_4 g^{-1} VS (Figure 37d). Gas production and methane yield decreased when feeding was interrupted for the lab closure on day 232 but recovered within 10 days.

After day 258, equivalent to 3.6 HRT, VBP, VMP and SMP all started to fall. SMP decreased from 0.29 to 0.21 L CH_4 g^{-1} VS added by day 268. There were some recovery between days 268 and days 279, although the methane concentration fell slightly (Figure 37e).

From day 280, after the OLR was increased from 3 to 4 g VS L^{-1} day^{-1}, the VBP and VMP increased slightly. On the other hand, the methane concentration remained slightly lower and the SMP fell. Feeding was stopped on day 284 due to signs of instability in the monitoring parameters as reported below.

Operational stability. Figure 38 shows the values of monitoring parameters for operational stability during the experimental period.

Total VFA (Figure 40a) showed slow increase with fluctuation up to day 272, but the concentrations was not high. TAN, alkalinity and pH decreased from the initial value by day 160, then settled around TAN 0.7 g N kg^{-1}, TA 7.1 g $CaCO_3$ kg^{-1}, PA 4.9 g $CaCO_3$ kg^{-1}, IA 2.2 g $CaCO_3$ kg^{-1}, and pH 7.2 at OLR 3 g VS L^{-1} day^{-1}. (Figure 38b, c, d, e and f). IA:PA ratio (Figure 38g) stabilised around 0.4.

From day 272, VFA started to increase which was accompanied by slightly increase of IA and IA/PA. Feeding was stopped on day 286, then the VFA accumulation rapidly disappeared.

The instability may be related to the TE status of the reactors. TE dosing ceased from day 234 as the TE solution ran out. Between day 234 and 272 each digester received 1908 g WW which was equivalent to 0.64 HRT, so this could have been contributing factor.

Although the accumulated VFA concentrations were not very high by day 272, digesters D9&10 appeared to be following a similar pattern to the thermophilic digesters D5-8. D5-8 and D9-10 used digestate from Trial 1 as inoculum. The difference was that the inoculum for D9&10 was mixture of 2 L digestate and 1 L fresh Millbrook inoculum. In digestion Trial 1, digesters showed signs of wash out of TE. For this reason feeding was stopped in order to undertake a revised experiment from a more consistent start-point.

Average values obtained for monitoring parameters during periods of pseudo-steady state operation are given in Table 26, after the results for all pairs of digesters.

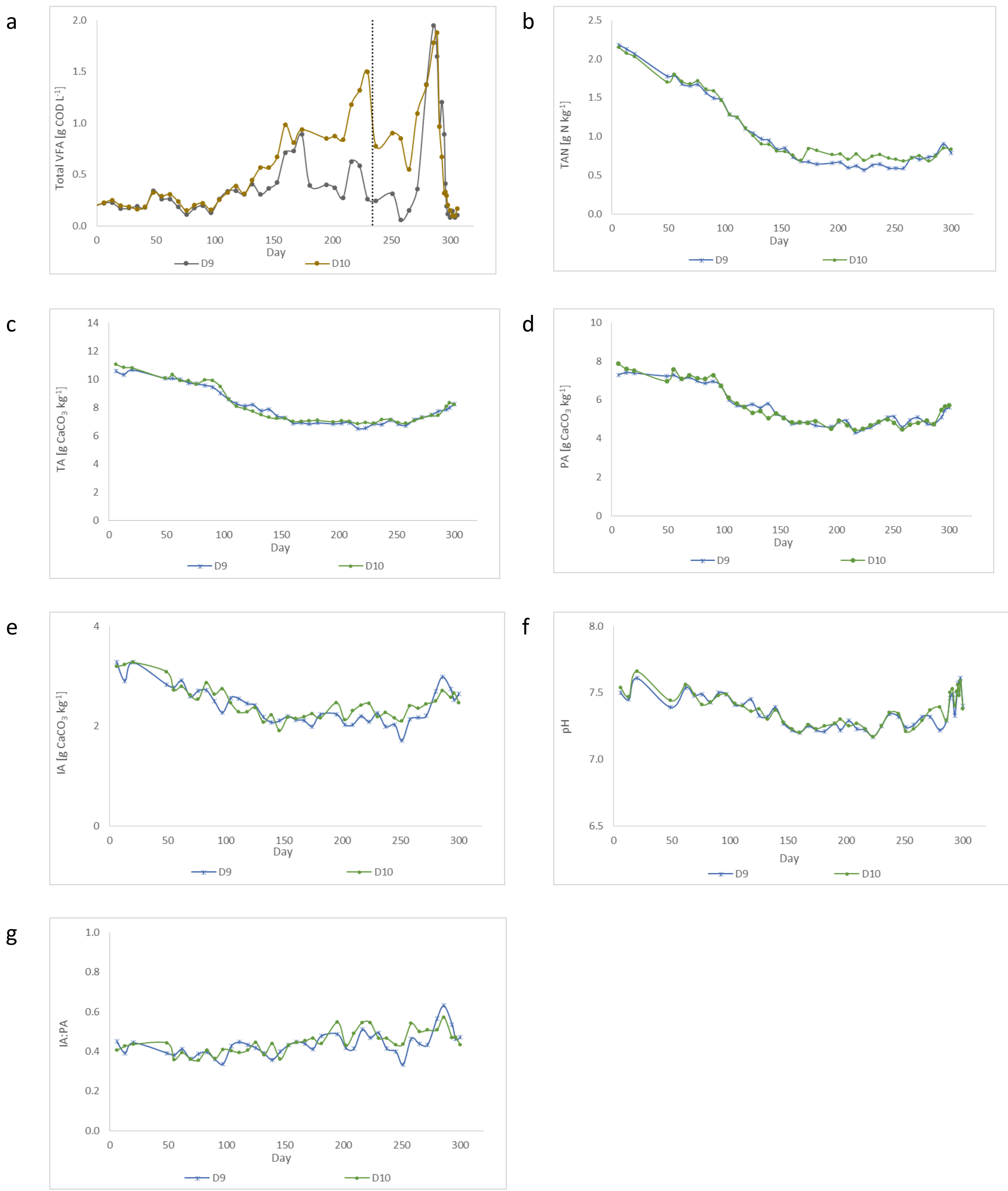


Figure 38. Total VFA, TAN, alkalinity and pH in D9&10 during digestion trial 2-1 on baby maize stover. Vertical lines indicate day 234; no further TE dosing.

Volatile Fatty acid profiles. Figure 39 shows the VFA profiles for D9&10.

The VFA mainly consisted acetic and propionic acid and remained less than 550 mg COD L^{-1} by day 160. From day 169, acetic acid tended to decrease but propionic acid kept increasing in D10. After day 280, propionic acid dominated the accumulation and small amount of other acids (iso-butyric and iso-valeric acid) were also detected in D9&10. Concentrations of all VFA species fell sharply from day 286 a few days after feeding ceased.

This pattern of VFA accumulation may be related to the trace element status of the inoculum. The 66 % inoculum came from the previous Trial 1. Wash-out of some essential TE for microorganisms may therefore have occurred, even though the digesters received certain TE (Fe, Co, Ni) from the beginning of the run.

The cessation of TE dosing may also be related to this issue. TE dosing ceased on day 273 because the TE solution ran out.

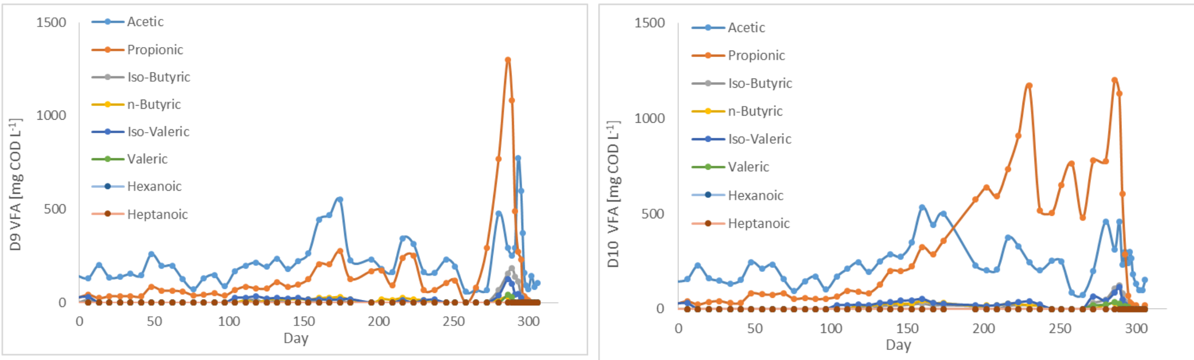


Figure 39. VFA profiles for D9&10 during digestion Trial 2-1 on baby maize stover

Solids parameters. Figure 40 shows the solids parameters for digesters D9&10.
TS and VS (Figure 40a and b) gradually increased until feeding ceased. TS increased from 6 to 10 % WW, while VS rose from 5 to 8 % WW. Between day 125 and 230, the values showed some fluctuation but stabilised after day 233 when the lab closure period happened. After stopping feeding, TS and VS decreased around 1 % WW in 2 weeks. TS and VS in D10 was slightly greater than in D9, and from day 180 the VS/TS ratio was slightly higher (Figure 40c). Based on visual inspection the digestate in D10 also appeared slightly thicker and more viscous. The visual appearance of the digestate may have indicated a change in properties, but not necessarily in solids content. VS destruction was also measured but varied widely.

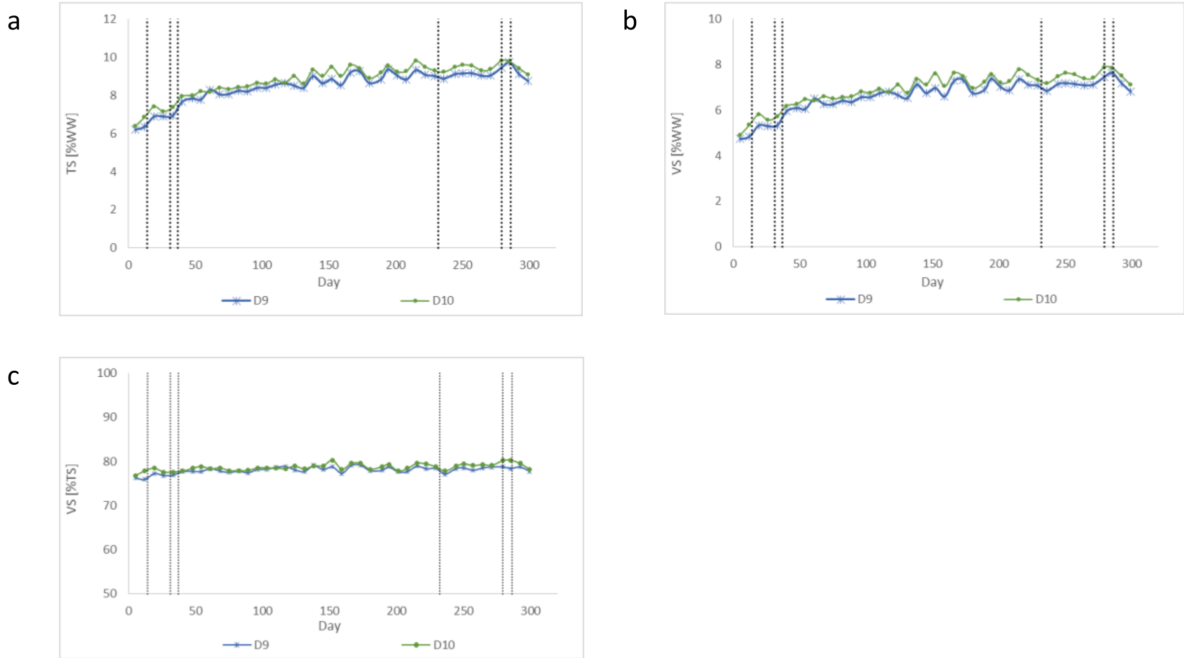


Figure 40. Digestate solids parameters for D9&10 during digestion trial 2-1 on baby maize stover

4.3.3.2. D1-4

Operating parameters. Figure 41 shows the organic loading rate, daily wet weight of feed added and hydraulic retention time for D1-4 during the experimental period. Table 21 shows the summary of history for mesophilic digesters D1-4 during digestion Trial 2-1 on baby maize stover.

These digesters were inoculated with 3 L of mixed digestate from D1-4 at the end of Trial 1, and were run with TE addition to maintain the additional concentration (1 mg Co L^{-1}, 1 mg Ni L^{-1}, 10 mg Fe L^{-1}) in digestate. The OLR was increased from 3 to 4 g VS L^{-1} day^{-1} on day 1 without apparent problems (i.e. foaming, VFA accumulation, etc).

Feeding of in D3 and D4 was interrupted by unexpected events: unintended feed break on day 69 – 70, repairs to the water bath used in pretreatment on day 97-98, and lab closure on day 114-115. During the lab closure period, D1 and D2 were also not fed. Except for these events, feeding of all digesters was carried out at OLR 4 g VS L^{-1} day^{-1} as planned.

D1's motor stopped on day 72, 74, 87, 89. The motor was changed on day 89, but stopped again on day 90.

From day 18, once the digesters appeared to be operating stably, thermophilic pretreatment was carried out for D3 and D4 as described in section 3.8. After day 97, D1-4 started to sho signs of instability. Feeding ceased on day 91 in D1, day 97 in D2, day 119 in D3 and day 94 in D4. D1, 2, 3, 4 were operated for the equivalent of 2.0, 2.2, 2.5 and 2.0 HRT, respectively (Table 22). Foaming was not observed at any point in this trial.

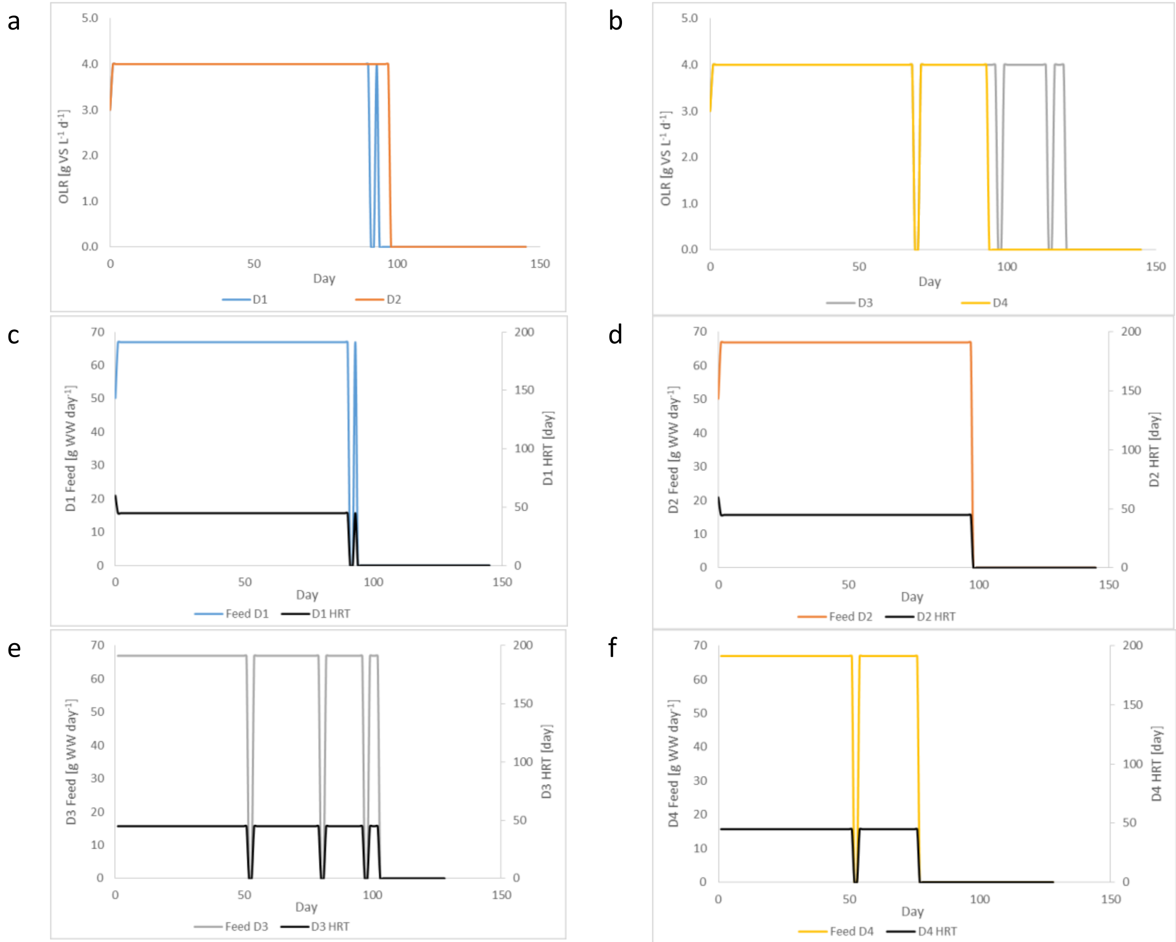


Figure 41. OLR, daily feed and HRT for D1-4 during digestion Trial 2-1 on baby maize stover

Table 21. Summary of history for mesophilic digesters D1-4 during digestion trial 2-1 on baby maize stover

<table>
<tr><th>Day</th><th>Date</th><th>D1</th><th>D2</th><th>D3</th><th>D4</th></tr>
<tr><td></td><td>07/10/2015</td><td colspan="4">Mixing and dividing digestate from D1-4 in trial 1</td></tr>
<tr><td>0</td><td>08/10/2015</td><td colspan="4">Feeding start: OLR at 3 g VS L^{-1} day^{-1}
Weekly TE dosing start to maintain concentration; 1 mg Co L^{-1}, 1 mg Ni L^{-1} and 10 mg Fe L^{-1}</td></tr>
<tr><td>1</td><td>09/10/2015</td><td colspan="4">OLR increase from 3 to 4 g VS L^{-1} day^{-1}</td></tr>
<tr><td>18</td><td>26/10/2015</td><td colspan="2"></td><td colspan="2">Pre-hydrolysis step start</td></tr>
<tr><td>43</td><td>20/11/2015</td><td colspan="4">Thermocirculator turned off overnight due to water shortage</td></tr>
<tr><td>69</td><td>16/12/2015</td><td colspan="2"></td><td colspan="2">Not fed for 2 days due to operator's sickness</td></tr>
<tr><td>72</td><td>19/12/2015</td><td>Motor tended to stop</td><td colspan="3"></td></tr>
<tr><td>74</td><td>21/12/2015</td><td>Motor stop</td><td colspan="3"></td></tr>
<tr><td>87</td><td>03/01/2016</td><td>Motor stop</td><td colspan="3"></td></tr>
<tr><td>89</td><td>05/01/2016</td><td>Motor stop and replaced</td><td colspan="3"></td></tr>
<tr><td>90</td><td>06/01/2016</td><td>Motor stop</td><td colspan="3"></td></tr>
<tr><td>91</td><td>07/01/2016</td><td>Ceased feeding</td><td colspan="3"></td></tr>
<tr><td>93</td><td>09/01/2016</td><td>Fed due to mistake</td><td colspan="3"></td></tr>
<tr><td>94</td><td>10/01/2016</td><td>Ceased feeding</td><td colspan="2"></td><td>Ceased feeding</td></tr>
<tr><td>97</td><td>13/01/2016</td><td></td><td>Ceased feeding</td><td>No fed for 2 days for repairing water bath</td><td></td></tr>
<tr><td>103</td><td>19/01/2016</td><td colspan="2">One-off TE dose: 0.1 mg L^{-1} (Se, Mo, W, Mn, Al, B, Zn and Cu)</td><td></td><td>One-off TE dose: 0.1 mg L^{-1} (Se, Mo, W, Mn, Al, B, Zn and Cu)</td></tr>
<tr><td>114</td><td>30/01/2016</td><td colspan="2"></td><td>No fed due to lab closure</td><td></td></tr>
<tr><td>119</td><td>04/02/2016</td><td colspan="2"></td><td>Ceased feeding</td><td></td></tr>
</table>

Table 22. Total amount of added substrate and equivalent HRT

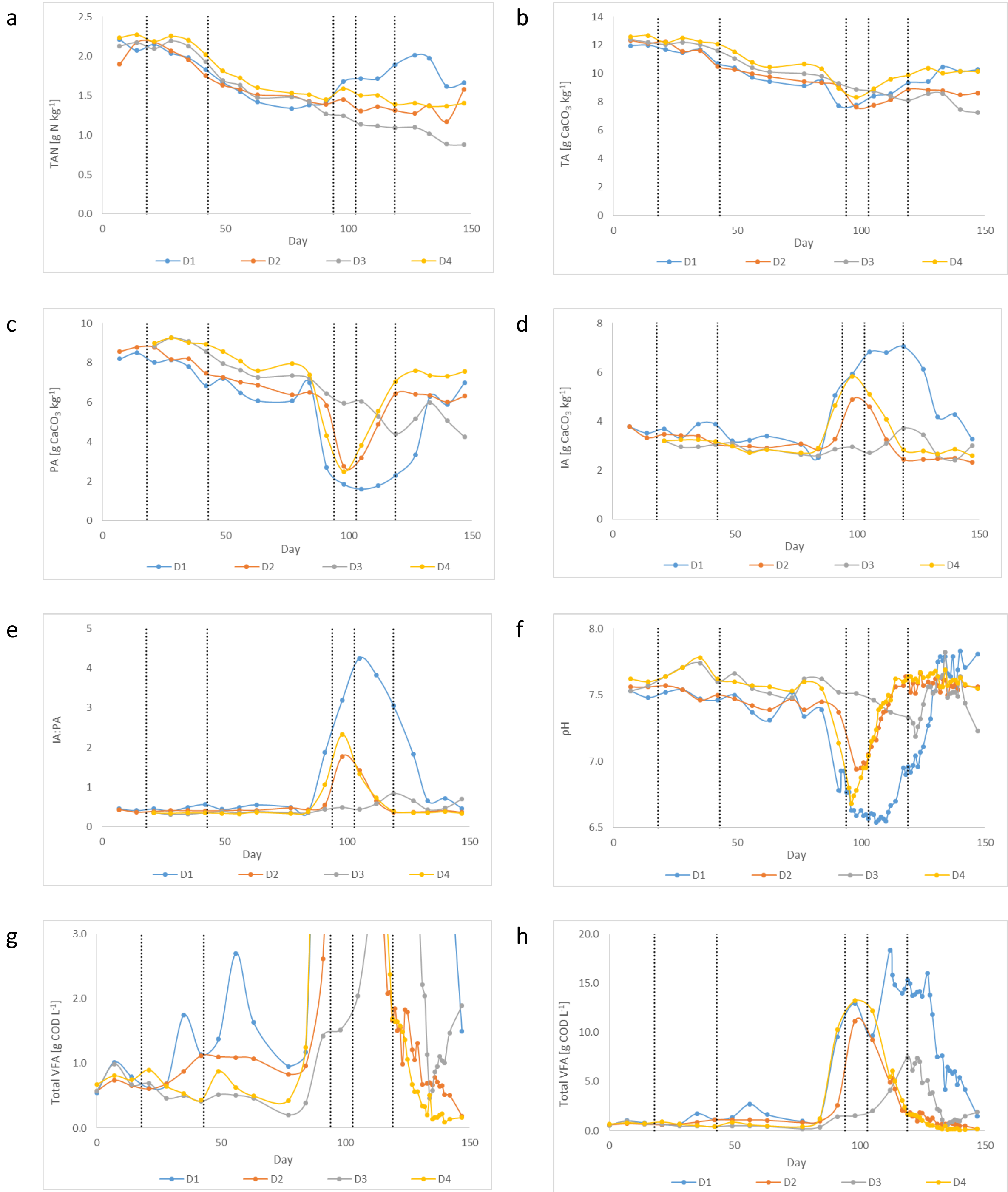
	D1	D2	D3	D4
Amount of added substrate [g]	6141	6543	7614	6141
Equivalent HRT for added substrate [-]	2.0	2.2	2.5	2.0

Operational stability. Figure 42 shows the monitoring parameters for operational stability during the experimental period.

For the first ~12 weeks of operation while the digesters adapted to the new feedstock and pre-treatment there were no dramatic changes in operating parameters. In this period TAN fell from around 2.2 to 1.4 g N kg^{-1} (Figure 42a) in all four digesters. In D3&4 after pre-treatment started there was a slight increase in TAN, perhaps due to improved hydrolysis and/or die-off of microorganisms in the recycled digestate during the thermophilic stage; then a decline in TAN which started around two weeks later than in D1&2. This was mirrored in the alkalinity with initial values of TA 12, PA 9 and IA 3.5 g $CaCO_3$ kg^{-1} falling to around 10, 7 and 2.7 g $CaCO_3$ kg^{-1} respectively by day 83 (Figure 44b-d). The TA and PA in digesters D3&4 with pre-hydrolysis was around 1 g $CaCO_3$ kg^{-1} higher than in D1&2 without pre-hydrolysis (Figure 42d), following the trend in TAN. This led to fairly stable IA/PA ratios of between 0.3-0.6 (Figure 42e), with values in D3&4 very slightly lower (i.e. more stable) than in D1&2. The initial pH in all digesters was around 7.5. After pre-treatment started, the pH in D3&4 increased until around day 35. It then began to fall, but remained slightly higher than in D1&2, again reflecting the higher alkalinity (Figure 42f). Total VFA concentrations were slightly elevated, especially in D1 where they reached 2.7 g COD L^{-1} around day 55 after the temperature shock caused by accidental turning off of the thermocirculator overnight; but this peak disappeared within 2 weeks, and average values for the period were only 0.5-1 g COD L^{-1} and around 0.5 g COD L^{-1} in D3&4 (Figure 42g). D1 appeared to have a slightly higher IA/PA ratio in this period and slightly less favourable values for other parameters than the other reactors, including its duplicate D2; but the differences were fairly small.

After day 83, however, there was a sharp rise in total VFA to 9-10 g COD L^{-1} in D1&2 and D4 and a slightly less marked increase in D3 (Figure 42g). Over the next few days feeding to D1&2 and D4 was briefly interrupted and/or stopped as shown in Figure 43 and Table 23. As a result total VFA in D2 and D4 fell in the 15-20 days after feeding ceased; but in D1, which had previously been the least stable digester, the total VFA concentration rose to a peak of more than 14 g COD L^{-1} by day 111 and only started to fall sharp after day 126, over 30 days after all feeding had stopped.

The changes in total VFA concentrations were reflected in all of the other monitoring parameters. IA rose sharply while PA fell, leading to a large increase in IA/PA ratios in D1&2 and D4 (Figure 42e). The IA/PA ratio in D2 and D4 fell after day 98 shortly after feeding ceased, but in D1 it continues to rise, reaching a maximum of around 4.3 on day 105. The VFA accumulation was sufficient to break the buffering capacity of D1&2 and D4, with pH falling to minimum values of 6.54, 6.94 and 6.68 respectively; recovery in pH only occurred when the VFA concentrations in each reactor began to fall. Low pH in this digester may have caused microbial die-off and cell lysis. While pH and alkalinity parameters had recovered by around day 147, no attempt was made to re-start feeding.



Vertical lines;
Day 18: Prehydrolysis step started for D3 and D4
Day 43: Thermocirculator turned off overnight
Day 94: Feeding of D1 and D4 ceased (D2 was on day 98)
Day 103: TE supplementation to D1, D2 and D4
Day 119: Feeding stopped of D3

Figure 42. TAN, alkalinity, pH and total VFA in D1-4 during digestion Trial 2-1 on baby maize stover

This behaviour of the digesters was unexpected. At the start of the trial it had been assumed that digesters D3&4 with pre-hydrolysis might show better gas production than D1&2 without pre-hydrolysis; or that pre-hydrolysis step might be too stringent and cause disruption of the microbial community and metabolic pathways to methane production. In fact all four digesters showed instability, with the disturbance most severe in D1 without pre-hydrolysis, least severe in D3 with pre-hydrolysis, and similar in D2 and D3. This suggested that the instability was due to some factor affecting all of the digesters.

Volatile Fatty acid profiles. Figure 43 shows the VFA profiles for D1-4. Concentrations of individual VFA species remained fairly low until day 84, although acetic acid were present in D1 and D2 from day 5 with peaks in propionic acid of up to 1.6 g COD L^{-1} after day 28 (Figure 43a and b). Concentrations of these acids in D3&4 were generally lower and tended to decline until day 84 (Figure 43c and d).

In D1 and D4, the initial increase after day 84 was mainly acetic acid, which by day 98 had reached 6.7 and 5.8 g COD L^{-1}, respectively. Propionic acid also increased, reaching a peak of 5.2 g COD L^{-1} in D4 on day 105. In D2 the rise in propionic acid occurred before that in acetic acid, but both acids peaked at around 4.8 g COD L^{-1} on day 98. In D3 the propionic acid also rose first but plateaued on day 98 at just over 1.0 g COD L^{-1} with correspondingly small rise in acetic acid to around 0.3 g COD L^{-1}. Other acids with longer carbon chains also started to appear from day 91, and broadly followed the trends in propionic acid concentration. The highest concentrations in D1 were on day 112 with acetic 9114 mg COD L^{-1}, propionic 5560 mg COD L^{-1}, iso-butyric 901 mg COD L^{-1}, n-butyric 1010 mg COD L^{-1}, iso-valeric 1443 mg COD L^{-1} and valeric 140 mg COD L^{-1}. The highest values in D2 were on day 98 with acetic 4758 mg COD L^{-1}, propionic 4779 mg COD L^{-1}, iso-butyric 495 mg COD L^{-1}, n-butyric 333 mg COD L^{-1}, iso-valeric 605 mg COD L^{-1} and valeric 143 mg COD L^{-1}.

The response of the digesters to the one-off TE supplementation on day 103 was interesting. D2 and D4 showed similar behaviour, with a fall in all species of VFA immediately after TE addition, although acetic and n-butyric acid concentrations may already have started to decline. In D1, however, there was a rise in all VFA species which then plateaued until around day 126 when acetic and n-butyric started to fall rapidly, followed 10-12 days later by the other VFA species, with iso-butyric the last to fall. This phenomenon of an increase in VFA after TE addition has been reported before (Song, 2016a) and may indicate that the TE are having a greater stimulating effect on acidogenesis that on methanogenesis (Jiang *et al.*, 2012; Song, 2016a).

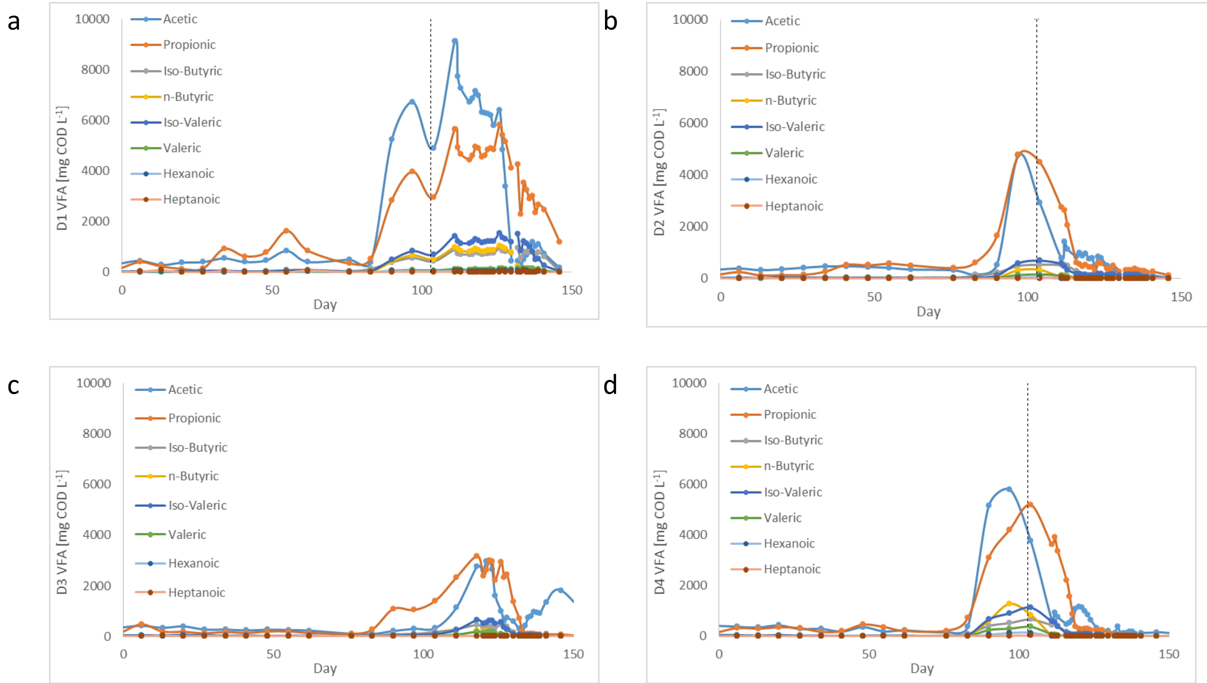


Figure 43. VFA profiles for D1-4 during digestion trial 2-1 on baby maize stover – all acids. Vertical line indicates one-off TE addition as in Table 21.

Biogas and methane production. Figure 44 shows the specific and volumetric biogas and methane production and the biogas methane content during the experimental period. Up to day 14, gas production increased, and then decreased to the end of the trial, but with different patterns of fluctuation in different digesters.

Before day 18 gas production in D1-4 was almost the same. When pre-treatment started, D3&4 started to show 10-20 % less VBP than that of D1&2. D1&2 showed greater fluctuations than D3&4 in VBP, VMP (Figure 44b) and SMP (Figure 44c). This was mainly due to the pattern of removal, as digestate was taken from these reactors once a week. The fall in gas production occurred the day after digestate was removed, with recovery over the next 6-7 days.

On day 44, it was observed that the thermocirculator was off and VBP was 67-69% of the previous day's value. The thermocirculator was turned on immediately and gas production recovered next day. After this event, gas production settled until day 69. On day 69, SMP was 0.31 L CH_4 g^{-1} VS in D1, 0.29 L CH_4 g^{-1} VS in D2, 0.24 L CH_4 g^{-1} VS in D3, 0.25 L CH_4 g^{-1} VS in D4. D3&4 experienced an unplanned break in feeding on day 69 and 70, and therefore gas production dropped during this period. Gas production in D1&2 and D4 started to decline from day 77 just before the VFA accumulation which started from day 84. The decrease in D3 appeared later, from day 112. The VBP in the worst affected digester D1 remained at 0.1 L L^{-1} from day 98 to day 126.

Methane concentration (Figure 44d) in D1-4 was around 60 % until day 77. After the start of pre-treatment, the value in D3&4 increased by 3 % but then returned to 60 % in the following 2 weeks. After day 77, methane concentrations in D1 and D4 dropped to 40 % when signs of instability appeared. D2 followed the decline one week later. D3 started to show fluctuation but did not decrease until day 140.

These data indicated that D3 and D4 reacted surprisingly well to the pre-treatment. D3&4 showed little disturbance, even though 10% of the digester volume was removed each day and subjected to thermophilic conditions for 24 hours. As pre-hydrolysis started on day 18, digesters D3&4 would have experienced the equivalent of 3 retention times of this operating regime by day 47. Most of the original digestate would have been exposed to these conditions, but the gas production appeared to stabilise in these digesters. There was a similar apparent stabilisation in D1&2; however, it seems that the digesters with pre-hydrolysis were able to adapt to this fairly harsh treatment without apparent difficulty.

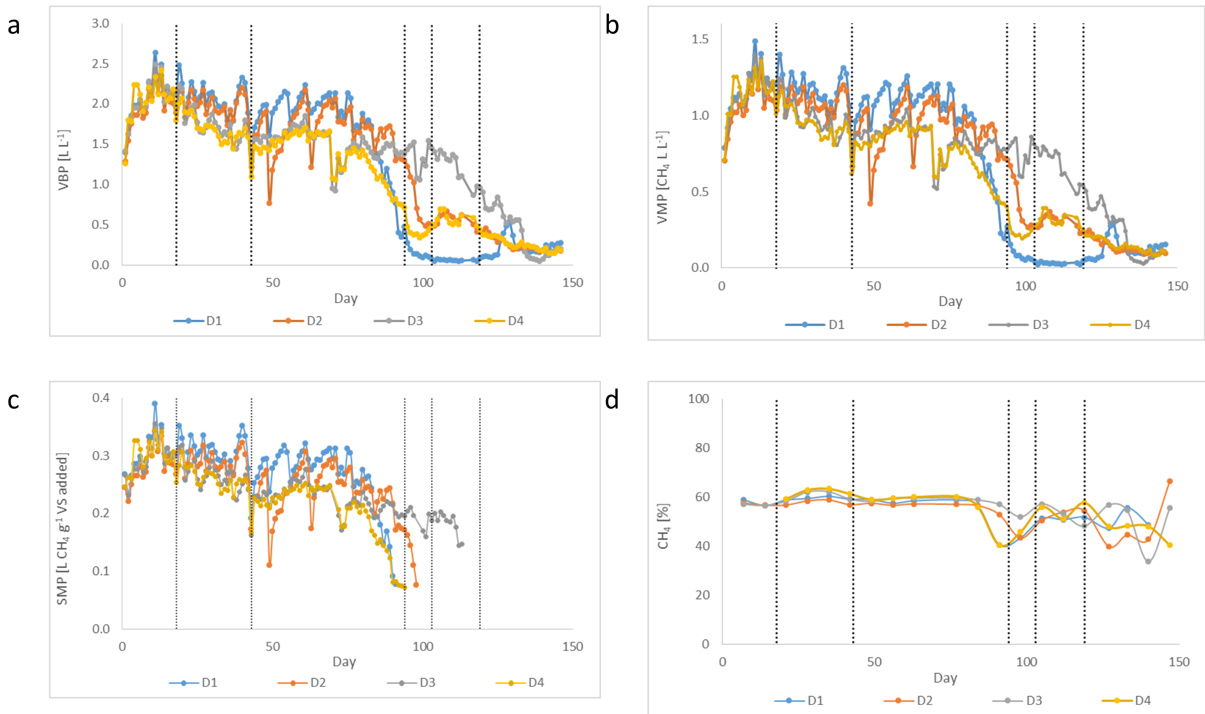


Figure 44. VBP, VMP and SMP for D1-4 during digestion Trial 2-1 on baby maize stover

Figure 45 shows the gas production from the pre-hydrolysis stage. SBP fluctuated but settled around 0.08 L g^{-1} VS added until day 68 (Figure 45a). Methane concentration was around 20 % with some fluctuation, so SMP was less than 0.03 L CH_4 g^{-1} VS (Figure 45b and c). Even when the SMP from pre-treatment was added to the SMP from main digesters, the two-stage system gas production was lower than D1&2 single stage digesters (Figure 45d).

Figure 45. SBP, SMP and gas composition for pre-hydrolysis experiment during digestion Trial 2-1 on baby maize stover. Pre-hydrolysis in D3&4 started on day 19.

Hydrogen production was observed. The concentration was around 8 % v/v and hydrogen yield remained less than 0.01 L H_2 g^{-1} VS (Figure 47e and f). Hydrogen can also be useful for energy production. On the other hand, the HHV of hydrogen is 11700 kJ m^{-3} SATP which is only 32.3 % of the HHV of CH_4 at 36264 kJ m^{-3} SATP (Dahlquist, 2013) . Even if a large volume of hydrogen is produced, the lower energy density means that it makes a relatively small contribution to the overall energy yield.

The weight loss for pre-hydrolysis digesters was also checked (Figure 47g). The method is described in section 3.8. Around 1 % WW of digestate was lost during pre-treatment. The 1 % loss may be caused by evaporating or substrate degradation.

In terms of SMP, pre-hydrolysis did not have any positive effects.

Solids parameters. Figure 48 shows the solids parameters for the digestion trial.

TS (Figure 48a), VS (Figure 48b) VS/TS ratio (Figure 48c) showed long slow decline throughout the trial. D1-4 showed almost same values until day 98 when instabilities happened. By day 98%, TS was around 10%, VS was around 8% and VS/TS ratio was around 80 %. Feeding was stopped on day 95 in D1&4, day 98 in D2 and day 120 in D3, and then solids parameter declined respectively.

VS destruction (Figure 48d) value fluctuated but D1&2 value was around 65 %, D3&4 was generally higher. On day 73, there was drop. On day 70, D1&2 showed instability sign, motor often stopped due to its thick digestate. At the period, instability in ammonia, alkalinity, pH and VFA was not confirmed yet. This may be foresight of instability.

The gas production from single-stage D1&2 was greater than that combined from pre-hydrolysis and main digesters D3&4. On the other hand, the VS destruction in D3&4 did not tie in with this because D3&4 showed greater VS destruction. That may be caused by thermophilic stage and/or digestate loss during pre-treatment.

Digestate weight 1% decreased after pre-treatment for 24 hours, and digestate may be lost during preparation for treatment. For pretreatment, removed digestate was centrifuged and then moved into conical flask. When digestate was transferred into new container, the weight was checked and recorded every action. These weight was reflected to calculation of VS destruction. Even checking these steps, some digestate may be lost without notice.

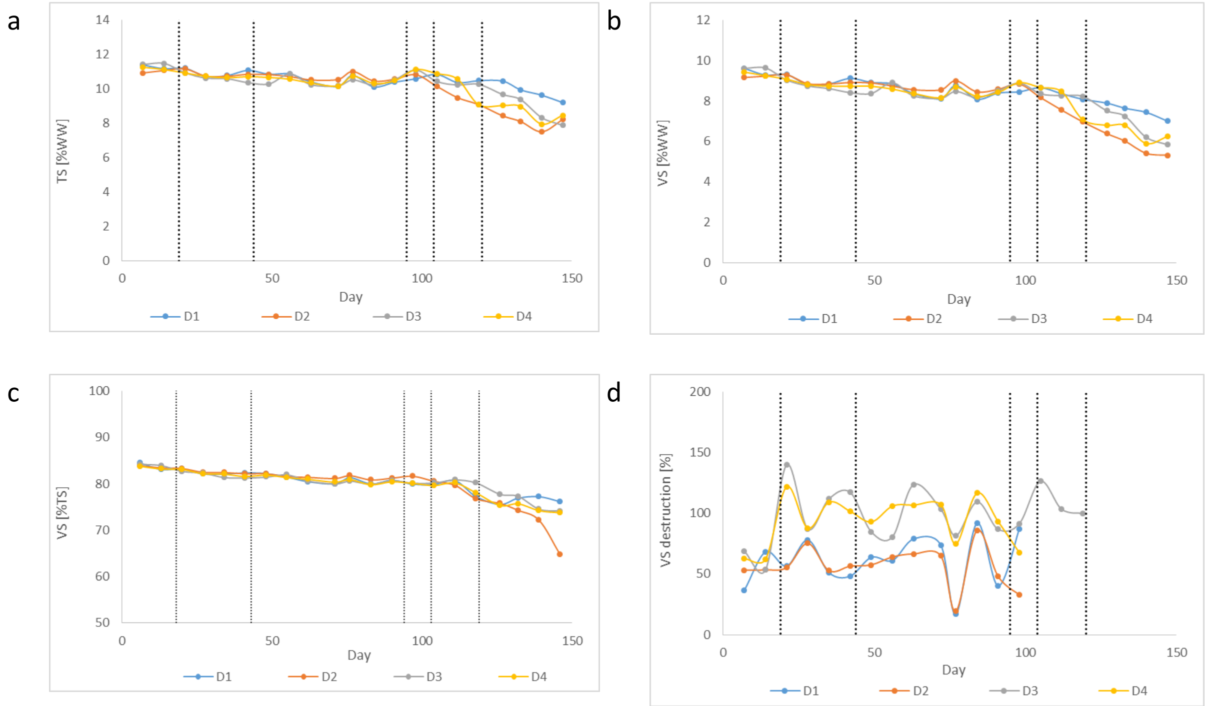


Figure 46. Solids data for D1-4 during digestion Trial 2-1 on baby maize stover

Digestate appearance. The digestate from D1&2 and D3&4 showed significant differences in appearance. D1&2 was more thick and viscous and undigested fibre was observed in the digestate. D3&4 was thinner and more watery. The difference was similar to that between mesophilic digestate and thermophilic digestate in Trial 1. Images of the digestates in Trial 1 and the thermophilic digestate in Trial 2-1 are shown in in Figure 37 in section 4.3.2. The digestate in Trial 2-1 was basically thinner than that of Trial 1 which may be attributable to the higher moisture content of baby maize stover. The main reason is most likely to be that the ones with pre-hydrolysis are exposed to thermophilic temperature at least for a proportion of the time. Therefore it seems likely that some component which causes the high viscosity in mesophilic digestion (e.g. extracellular polymer) is either not formed in thermophilic conditions or is broken down: it is not possible to say which. Stoyanova *et al.* (2014) compared the viscosity of digestate from single mesophilic and two stage (thermophilic-mesophilic) digestion. Stoyanova *et al.* (2014) also reported the viscosity of the digestate in the two stage showed less viscosity than that of the single stage. Stoyanova *et al.* (2014) mentioned the lower viscosity was caused by the pectin degradation. In this study, pectin degradation was not analysed as it was not included the original objective. It may be worth to check the pectin degradation in future work.

Discussion of results for mesophilic reactors in digestion Trial 2-1.

Table 23 shows the average values for reporting parameters in D1-4 and D9-10 during periods of pseudo steady-state operation. Even through the operating regimes were different, D1&2 and D3&4 showed VFA accumulation and other signs of failure at the same time, from around day 84. This may suggest depletion of TE, or accumulation of toxic compounds.

The possibility of TE wash-out is related to the long period of operation. The inoculum was a mixture of D1-4 digestate from Trial 1. In Trial 1, the amounts of maize silage added were 7124 g WW in D1, 7630 g WW in D2, 8491 g WW in D3, 8370 g WW in D4. The working volume was changed from 4 to 3 L during trial 1 so HRT were 2.0 in D1, 2.1 in D2, 2.4 in D3&4. In Trial 2-1, D1&2 received 6007 g WW (2.0 HRT), D3&4 received 5873 g WW (2.0 HRT) until day 84 when instabilities appeared. Total HRT in trial 1 and trial 2-1 were 4.0 in D1, 4.1 in D2, 4.4 in D3&4. The combined HRT were over 3 HRT so essential TE wash-out may be possible. The VFA accumulation consisted propionic acids which was at almost the same concentration as acetic acid, and other acids which were mainly iso-butyric, n-butyric and iso-valeric acids were also observed. Böck (2006) reported propionic acid accumulation was attributed to the deficiency of Se, Mo and W. Weekly TE supplementation (Fe, Co, Ni) in proportion to the added substrate was carried out but the dosing did not include Se, Mo and W.

It is also possible that toxic or inhibitory compounds present in the maize stover feedstock accumulated during Trial 1 and were therefore present in the inoculum at the start of trial 2-1 but this explanation seemed less likely, and no further steps were taken to investigate it.

D9&10's inoculum was mixture of 1 L fresh Millbrook digestate and 2 L mixed digestate from D1-4 in Trial 1. D9&10 showed stable operation for a longer period than D1-4, even though D9&10 used the digestate from trial 1 partly as inoculum. D9&10's steady state gas production was 0.278 L CH_4 g^{-1} VS which was larger than the pseudo-steady state value of 0.269 for D1&2 between day 55 and 84. D9&10 HRT was 4.4 HRT which was double that for D1-4. This may also support the possibility of TE wash out.

Table 23. Average values for reporting parameters during pseudo-steady state periods

Parameter	Unit	D1 [a]	D2 [a]	D3 [a]	D4 [a]	D9 [b]	D10 [b]	Ave [b]	Ave [a]	Ave [a]
		M	M	M + pre	M + pre	M	M	M	M	M + pre
OLR	g VS L^{-1} day^{-1}	4	4	4	4	3	3	3	4	4
SBP	L g^{-1} VS	0.483	0.450	0.388	0.372	0.503	0.486	0.495	0.467	0.380
SMP	L CH_4 g^{-1} VS	0.281	0.257	0.231	0.222	0.283	0.273	0.278	0.269	0.227
SMP from pre	L CH_4 g^{-1} VS	–	–	0.012	0.005	–	–	–	–	0.009
SHP from pre	L H_2 g^{-1} VS	–	–	0.006	0.006	–	–	–	–	0.006
VBP	L L^{-1} day^{-1}	1.93	1.80	1.51	1.46	1.51	1.46	1.48	1.87	1.48
VMP	L CH_4 L^{-1} day^{-1}	1.09	0.99	0.84	0.82	0.85	0.82	0.84	1.04	0.83
CH_4 content	% v/v	57.8	56.9	59.5	58.9	56.2	56.1	56.1	57.4	59.2
Digestate TS	%WW	10.5	10.6	10.4	10.4	9.1	9.4	9.2	10.6	10.4
Digestate VS	%WW	8.4	8.7	8.4	8.4	7.1	7.4	7.3	8.5	8.4
VS destruction	%VS	64.5	60.1	99.7	102.2	69.7	67.6	68.6	62.3	100.9
pH	–	7.4	7.4	7.6	7.6	7.2	7.2	7.2	7.4	7.6
TA	g $CaCO_3$ kg^{-1} WW	9.5	9.7	10.1	10.6	6.7	6.9	6.8	9.6	10.3
PA	g $CaCO_3$ kg^{-1} WW	6.4	6.7	7.4	7.8	4.6	4.6	4.6	6.5	7.6
IA	g $CaCO_3$ kg^{-1} WW	3.1	3.0	2.7	2.8	2.1	2.3	2.2	3.0	2.8
IA/PA ratio	–	0.48	0.44	0.37	0.36	0.46	0.50	0.48	0.46	0.37
TAN	g N kg^{-1} WW	1.42	1.50	1.50	1.59	0.62	0.74	0.68	1.46	1.55
Total VFA	g COD L^{-1}	0.32	0.24	0.31	0.29	0.42	1.14	0.78	0.28	0.30

Note: these values are also shown in Table 24 below, which includes the values for the thermophilic digesters run in parallel, but are given here for ease of reference. Table 24 does not include the values for D1-4 as these digesters did not complete 3 HRT.

4.3.4. Discussion of result for mesophilic and thermophilic conditions in digestion Trial 2-1

Table 24 summarises some of the key performance parameters obtained during periods of apparently stable operation. Because of the operational issues described above it is difficult or impossible to make a fully valid comparison of digestion performance with baby maize stover under mesophilic and thermophilic conditions. Nevertheless some tentative conclusions and hypotheses can be drawn from the data obtained.

All of the digesters fed on baby maize stover showed a decline in performance during long term operation, in terms of a prolonged slow reduction in SMP and methane content (D7&8 Fig 35, D9&10 Fig 39, D1&2 Fig 46) and/or a rise increase in VFA concentration (D5&6 Fig 28, D7&8 Fig 33, D9&10 Fig 40, D1&2 Fig 44). These phenomena appeared at similar points in pairs of digesters, or after similar equivalent HRT in pairs of digesters with different operating histories. VFA accumulation appeared almost same date even the history in pairs of digesters was different. VFA concentration in D5-8 and D9&10 accumulated after around one month when weekly TE dosing ceased (Fe, Co and Ni). During the one month, the equivalent HRT was 0.65 HRT in D5&6, 0.86 HRT in D7&8, 0.64 HRT in D9&10. That indicate the digesters may consist minimum amount of TE which came from weekly TE supplementation. The concentration of other TE may also have been close to the minimum acceptable amount in the digesters because the digestate used was from Trial 1.

The SMP for baby maize stover appeared to be significantly higher in thermophilic conditions than in mesophilic. In D9&10 under mesophilic conditions at OLR 3 g VS L^{-1} day^{-1} there was a period of apparently stable operation from day 200 onwards, where most parameters had stabilised and only limited VFA accumulation had occurred. The average SMP between days 210-239 was 0.278 L CH_4 g^{-1} VS day^{-1}. In thermophilic conditions pseudo steady state periods occurred in D7&8 at OLR 3 g VS L-1 day-1 between days 140-169 (corresponding to approximately 2.0 HRT at this OLR), in D5&6 at OLR 4 g VS L^{-1} day^{-1} between days 112-141 (approximately 2.4 HRT), and in D7&8 at OLR 5 g VS L^{-1} day^{-1} between days 265-294 (approximately 4.9 HRT). The corresponding values of SMP were 0.342, 0.332 and 0.311 L CH_4 g^{-1} VS. The experimental conditions applied mean it is difficult to say whether the apparent decline in SMP in thermophilic conditions is due to increasing OLR, progressive washout of a critical trace element (or accumulation of a toxic compound), or a combination of both. The results do suggest, however, that in this situation thermophilic digestion gave considerably higher gas production.

The difference in SMP values in mesophilic and thermophilic conditions is supported by the difference in digestate VS content and in estimated VS destruction, which stabilised at around 74.4 and 71.9% for thermophilic digestion at OLR 3 and 4 g VS L^{-1} day^{-1} compared to 68.6% for mesophilic digestion at OLR 3 g VS L^{-1} day^{-1}. These values correspond to an SMP per g VS destroyed of around 0.40 and 0.46 L CH4 g^{-1} $VS_{destroyed}$ day^{-1} for mesophilic and thermophilic digestion respectively, indicating significant differences in yield. There were also significant differences in the pseudo-steady state pH, alkalinity and TAN concentrations at the two operating temperatures, as well as the difference in visual appearance and foaming behaviour noted above. The lower TAN concentration in mesophilic conditions (0.68 g N kg^{-1} WW at OLR 3 g VS L^{-1} day^{-1}) compared to thermophilic (1.63 and 1.57 g N kg^{-1} WW at OLR 3 and 4 g VS L^{-1} day^{-1} respectively) could possibly indicate less effective breakdown of proteinaceous compounds in the feedstock, which might contribute to the lower SMP per g VS added and destroyed; or alternatively could indicate that nitrogen is being taken up by the biomass for formation of proteinaceous microbial products which could be linked to the greater propensity for foaming in mesophilic conditions, while also reducing the SMP. This is speculative, however: a number of complex factors may affect the relationship between TAN, TKN and biological fixed nitrogen concentrations in digestate (Lindorfer et al., 2012) and no specific measurements other than TAN concentration were taken in this experimental run.

It was difficult to assess the effect of the pre-hydrolysis stage on gas production because of the short duration of the experimental periods and the failure to reach an apparent steady state; but there was no evidence that the pre-hydrolysis gave any performance advantage and gas production appeared to be at best similar to that without the pre-hydrolysis step.

Because of the problems associated with the different starting conditions and unexpected near-simultaneous failure of the reactors in this digestion trial, it was therefore decided to carry out a second series of experiments from well-defined set of starting conditions.

The results of these previous digestion trials with baby maize stover suggested that the reactors may have been running out of trace elements and it was therefore decided to look at different trace element supplementation strategies. Table 25 shows the trace element contents of the maize silage and baby maize stover on a wet weight basis, i.e. approximately in proportion to their concentration in the digestate, though not all forms will be equally available. It can be seen that the TE contents are similar but maize silage appears to have more Co and Ni. Baby stover may have less selenium and tungsten but more molybdenum, though it is difficult to be sure since these values are apparently close to the detection limits of the methods used. On this basis it was decided to use two different TE mixes consisting of (the 3TE and 5TE mixture).

Table 24. Average values for reporting parameters during pseudo-steady state periods

Parameter	Unit	D9[a]	D10[a]	Ave[a]	D7[b]	D8[b]	D5[c]	D6[c]	D7[d]	D8[d]	Ave[b]	Ave[c]	Ave[d]
Operating temp	-	M	M	M	T	T	T	T	T	T	T	T	T
OLR	g VS L^{-1} day^{-1}	3	3	3	3	3	4	4	5	5	3	4	5
SBP	L g^{-1} VS	0.503	0.486	0.495	0.601	0.598	0.603	0.604	0.539	0.545	0.599	0.583	0.542
SMP	L g^{-1} VS	0.283	0.273	0.278	0.343	0.342	0.346	0.332	0.308	0.315	0.342	0.332	0.311
VBP	L L^{-1} day^{-1}	1.51	1.46	1.48	1.80	1.79	2.41	2.42	2.70	2.73	1.80	2.33	2.71
VMP	L L^{-1} day^{-1}	0.85	0.82	0.84	1.02	1.01	1.38	1.36	1.52	1.54	1.02	1.32	1.53
CH_4 content	% v/v	56.2	56.1	56.1	55.5	56.1	57.4	56.0	56.8	57.5	55.8	56.7	57.2
Digestate TS	%WW	9.1	9.4	9.2	7.4	7.6	8.5	8.4	8.3	8.2	7.5	8.5	8.2
Digestate VS	%WW	7.1	7.4	7.3	5.5	5.6	6.4	6.3	6.0	6.4	5.5	6.3	6.2
VS destruction	%VS	69.7	67.6	68.6	75.1	73.8	71.2	72.6			74.4	71.9	
pH	–	7.2	7.2	7.2	7.7	7.6	7.7	7.7	7.7	7.6	7.7	7.7	7.6
TA	g $CaCO_3$ kg^{-1} WW	6.7	6.9	6.8	10.8	10.2	10.8	10.6	10.2	9.5	10.5	10.7	9.9
PA	g $CaCO_3$ kg^{-1} WW	4.6	4.6	4.6	7.9	7.5	7.9	7.7	7.5	7.0	7.7	7.8	7.2
IA	g $CaCO_3$ kg^{-1} WW	2.1	2.3	2.2	3.4	3.2	2.9	2.8	2.7	2.5	3.3	2.9	2.6
IA/PA ratio	–	0.46	0.50	0.48	0.43	0.43	0.36	0.37	0.36	0.37	0.43	0.36	0.36
TAN	g N kg^{-1} WW	0.62	0.74	0.68	1.68	1.59	1.58	1.56	1.46	1.31	1.63	1.57	1.39
Total VFA	g COD L^{-1}	0.42	1.14	0.78	0.21	0.17	0.09	0.07	0.17	0.08	0.19	0.08	0.12

[a] Average value for days 210-239 (NB not last 30 days of operation)

[b] Average value for days 140-169 [c] Average value for days 120-139

[d] Average value for days 265-294 M= mesophilic, T = thermophilic

Table 25. Trace element content of maize silage and baby maize stover on a wet weight basis

	Unit	Maize silage	Baby maize stover
Cobalt (Co)	mg kg^{-1} WW	<0.4	<0.2
Nickel (Ni)	mg kg^{-1} WW	<0.4	0.19
Iron (Fe)	mg kg^{-1} WW	108	97
Total nitrogen (N)	% TS	1.2	1.2
Total phosphorus (P)	% TS	0.2	0.3
Total potassium (K)	% TS	1.0	2.9
Total copper (Cu)	mg kg^{-1} WW	1.71	1.04
Total zinc (Zn)	mg kg^{-1} WW	9.2	7.2
Total sulphur (S)	% TS	0.1	0.1
Calcium (Ca)	mg kg^{-1} WW	1019	584
Molybdenum (Mo)	mg kg^{-1} WW	<0.4	0.45
Manganese (Mn)	mg kg^{-1} WW	4.6	6.0
Selenium (Se)	mg kg^{-1} WW	<0.04	<0.02
Tungsten (W)	mg kg^{-1} WW	<0.04	<0.02
TS	% TS	41.0	18.6

4.4. Digestion Trial 2-2 – pre-treatment of baby maize stover

In Trial 2-1, the inoculum used for D1-4 was digestate from Trial 1. Digesters D3&4 with thermophilic pre-hydrolysis showed lower gas production than D1&2 without pre-hydrolysis, but all digesters showed VFA accumulation. The total operating time in Trial 1 and 2-1 was equivalent to 4.0, 4.3, 4.9 and 4.4 HRT in D1-4 respectively and it was suspected that washout of TE may have caused this instability. In this trial, fresh inoculum from Millbrook municipal wastewater treatment was used. Trace elements dosing was not carried out from the start of the trial, as the duration was expected to be short (i.e. just long enough to confirm whether gas production with pre-hydrolysis was lower that without even when using healthy digestate), and the inoculum was believed to contain sufficient TE for this purpose.

4.4.1. Objectives and methodology

Objective: To determine the difference in gas production potential and stability of baby maize stover digestion in mesophilic conditions with and without a thermophilic pre-hydrolysis stage, using fresh Millbrook digestate as inoculum.

Methodology: As before, D1&2 were operated as single-stage mesophilic digesters, D3&4 as mesophilic digesters with thermophilic pre-treatment. For the first 4 days, the method was that described in section 3.8. From day 5, the pre-hydrolysis method was modified. In the previous trial the pre-treatment used digestate supernatant obtained from whole digestate by centrifugation, but the modified method used whole digestate without any separation.

4.4.2. Mesophilic digestion results

Operating parameters. Figure 49 shows the organic loading rate, daily wet weight of feed added and hydraulic retention time for D1-4 during the experimental period. Table 26 shows a summary of the operating history of mesophilic digesters D1-4 during digestion Trial 2-2 on baby maize stover.

Feeding began on day 0 at OLR 3 g VS L^{-1} day^{-1} and continued for 20 days in total, equivalent to only 0.3 HRT (Table 27). Feeding was stopped on day 20 because gas production from D3&4 was always less than from D1&2, but all of the digesters were showing signs of instability.

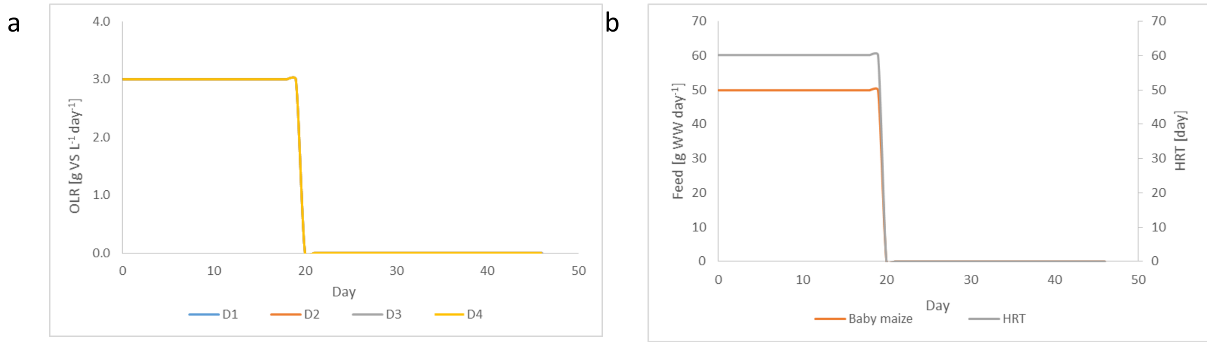


Figure 47. OLR, daily feed and HRT for D1-4 during digestion Trial 2-2 on baby maize stover

Table 26. Summary of operating history for mesophilic digesters D1-4 during digestion Trial 2-2

Day	Date	D1	D2	D3	D4
-2	02/03/2016	Set up			
0	04/03/2016	Feeding started at OLR 3			
5	09/03/2016	Pre-treatment method change : no centrifuge			
20	24/03/2016	Stop feeding			

Table 27. Total amount of added substrate and equivalent HRT

	D1-4
Amount of added substrate [g]	948
Equivalent HRT for added substrate [-]	0.3

Operational stability. Figure 48 shows the monitoring parameters for operational stability during the experiment.

Total VFA (Figure 48a) showed a rise from the initial value, which was less than 0.3 g COD L^{-1} on day 5. In D3 and D4 with pre-hydrolysis, total VFA concentrations reached a peak of 3.7 and 5.0 g COD L^{-1} respectively on day 22, just after feeding ceased; and then fell to less than 0.5 g COD L^{-1} by day 31. On the other hand, total VFA in D1 and D2 continuously increased until day 26, reaching peak values of 8.6 and 8.0 g COD L^{-1} respectively. Values in D1&2 started to fall sharply from day 29. Trends in pH reflected the degree of VFA accumulation, with the value falling to below 7 in D1&2 (Figure 48b).

TA (Figure 48c) remained steady at 8 g $CaCO_3$ kg $^{-1}$ in all digesters while feeding continued, but increased in D3&4 to 9.5 g $CaCO_3$ kg $^{-1}$ from day 26 just after feeding stopped. D1&2 did not show a rise until day 29, and reached same value as D3&4 on day 33. In PA, D1&2 seemed to be less stable (Figure 48d).

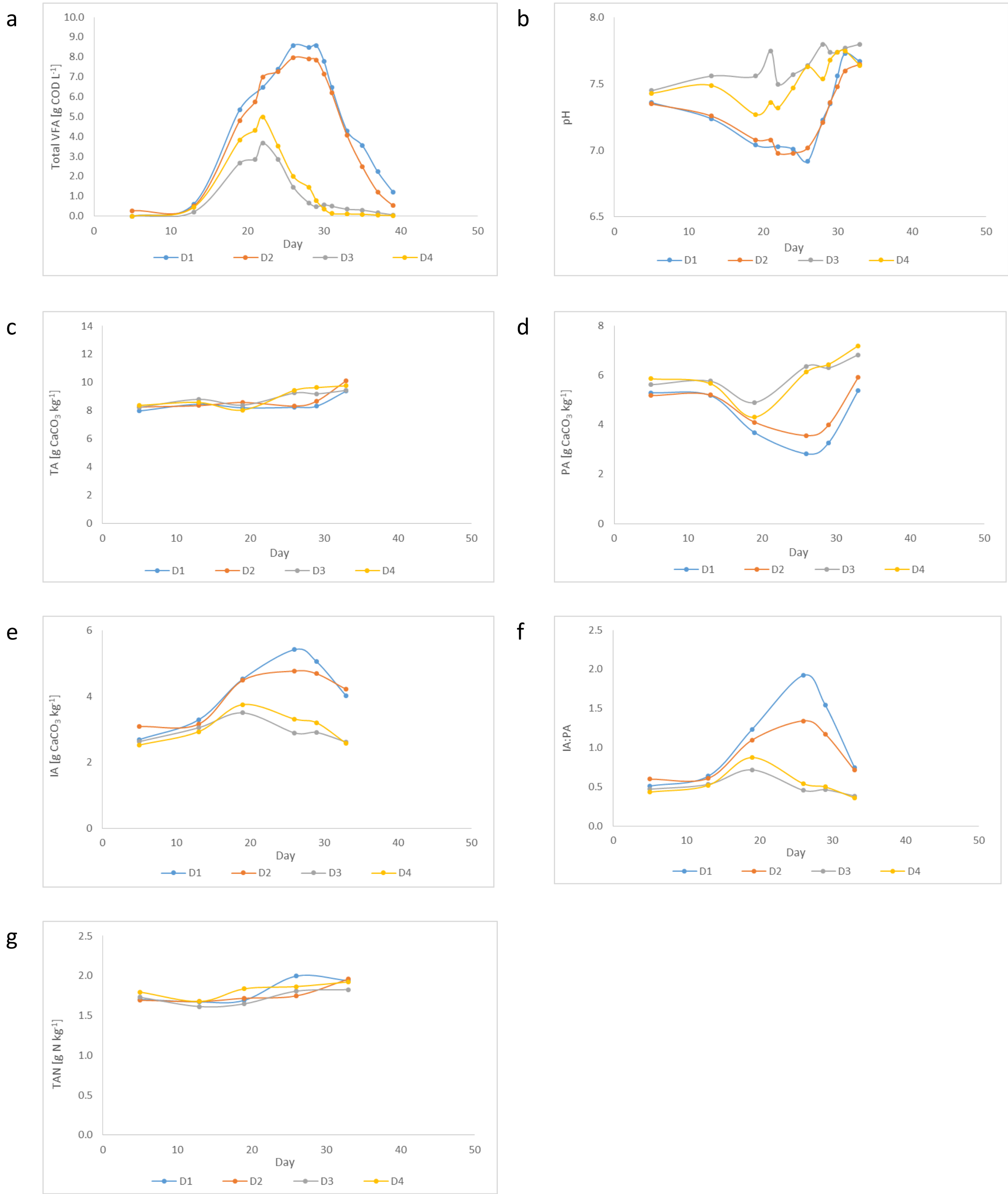


Figure 48. Total VFA, pH, alkalinity and TAN in D1-4 during digestion Trial 2-2 on baby maize stover

IA (Figure 48e) increased from its initial value, which was less than 3.1 g $CaCO_3$ kg^{-1}. The rise started from day 5 and followed the trend in VFA accumulation. D1&2 showed greater peaks than D3&4 and took more time to recover. The increase in IA values led to peaks in the IA:PA ratio (Figure 48f). Total VFA concentrations in D1&2 were not significantly different, but the IA:PA value in D1 was greater than that of D2, suggesting D1 was under slightly more stress.

TAN concentrations (Figure 48g) started from 1.7-1.8 g N kg^{-1} and showed a small increase. The final value on day 33 was 1.8-1.9 g N kg^{-1}.

Based on these parameters, digesters D1&2 without pre-hydrolysis appeared to show greater instability than D3&4 with pre-hydrolysis.

Volatile Fatty acid profiles. Figure 49 shows the VFA profiles for D1-4.

The peak in VFA concentrations after day 15 was mainly acetic and propionic acid, although small amounts of other VFA were seen especially in the single-stage digesters D1&2. As with the total VFA, concentrations of each VFA species in D1&2 were much greater than in D3&4 with pre-hydrolysis. The concentrations of all VFA species continued to rise for some time after feeding stopped, especially in D1&2.

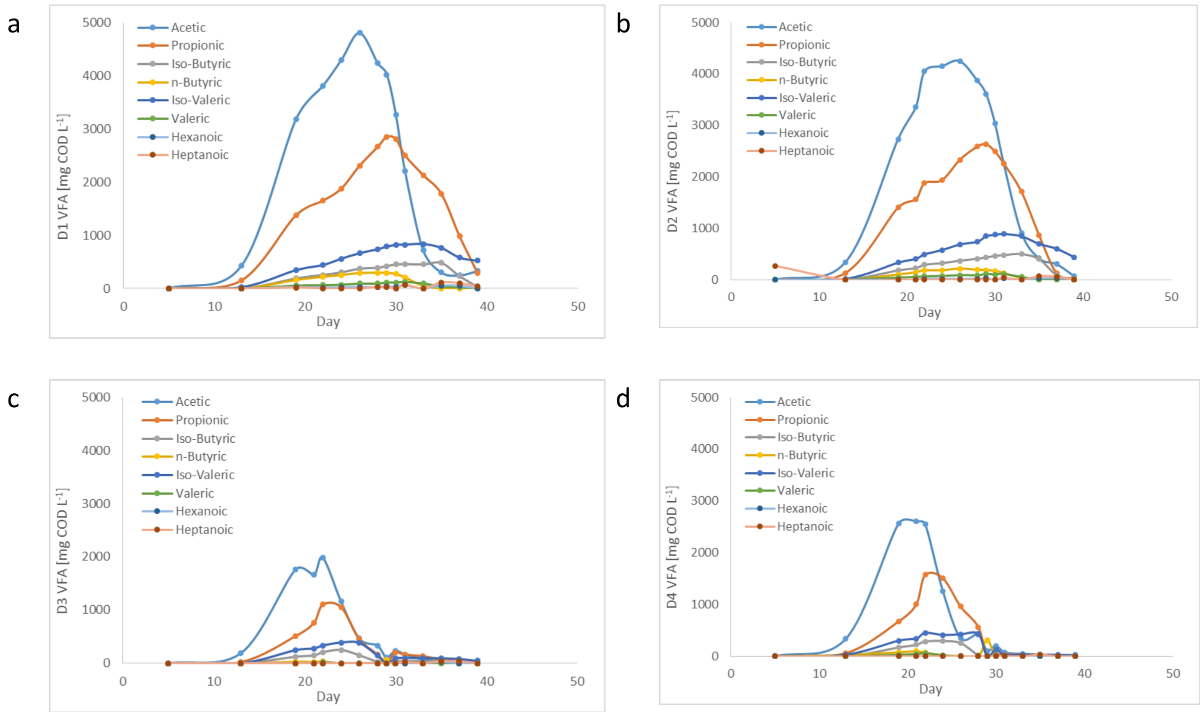


Figure 49. VFA profiles for D1-4 during digestion Trial 2-2 on baby maize stover

Biogas and methane production. Figure 50 shows the specific and volumetric biogas and methane production and the biogas methane content during the experimental period.

Biogas production from D1-4 increased from initial values (i.e. VBP 0.5 L L^{-1} and VMP 0.3 L CH_4 L^{-1}) until day 12 (Figure 50 a and b). The increase in gas production in D3&4 (with pre-treatment) lagged about 1 day behind that in D1&2 (no pre-treatment). This was due to the pre-treatment step itself, as the first day's feed for the reactors with pre-treatment went into the pre-hydrolysis reactor, so the main digesters only received feed one day later. SMP in D3&4 was also less than in D1&2, in part due to the production of some gas in the pre-hydrolysis stage (Figure 50c). From day 14, however, gas production in all reactors began to fall. VBP and VMP profiles were almost the same for each digester because the methane concentration (Figure 50d) was almost constant. Methane concentrations fluctuated when instabilities appeared.

Feeding was ceased on day 20 because D3&4 gas production was always less than D1&2, gas production continuously decreased from day 12 and instability signs started to appear.

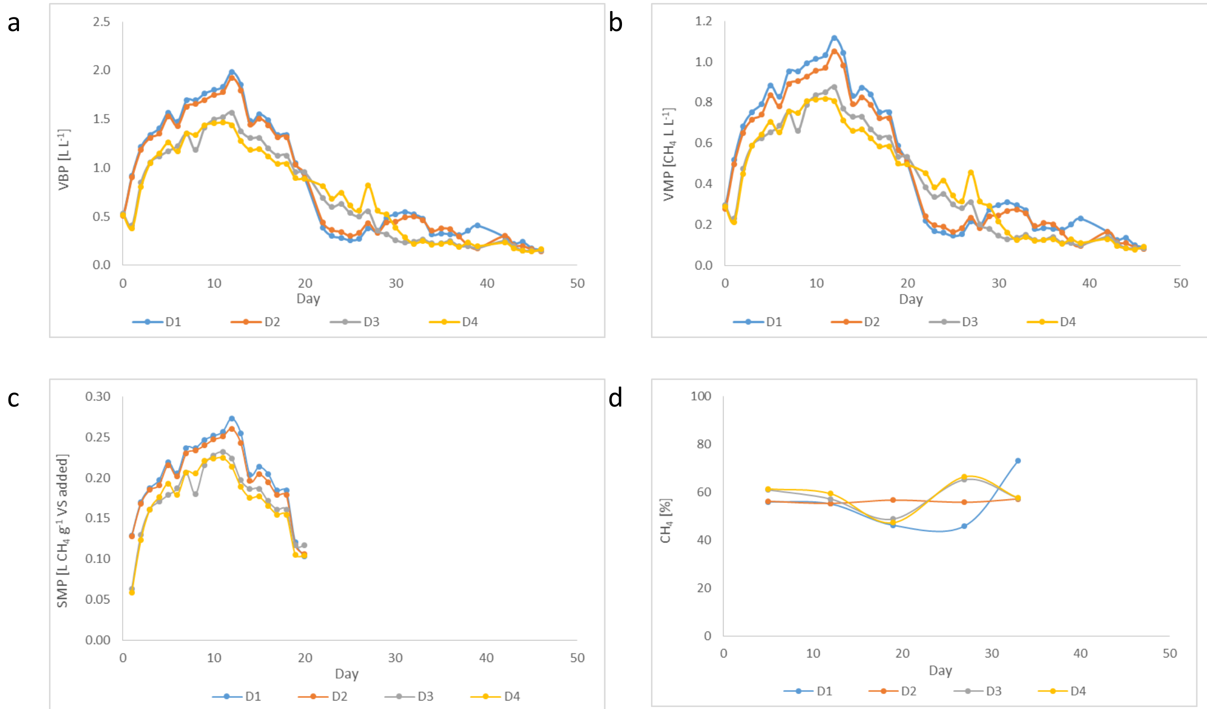


Figure 50. SBP, SMP for D1-4 during digestion Trial 2-2 on baby maize stover

The biogas production from the pre-hydrolysis step is shown in Figure 51. SBP slowly increased and settled around 0.10 L g^{-1} VS added from day 8 (Figure 51a). Methane concentration was around 30 % so SMP was less than 0.04 L CH_4 g^{-1} VS (Figure 51b and c). Even the SMP from pre-treatment was added to the SMP from main digesters, two stage system gas production was lower than that of D1&2 single stage digesters (Figure 51d).

Figure 51. SBP, SMP and weight loss for pre-treatment stage of D3-4 during digestion Trial 2-2 on baby maize stover

Hydrogen production was observed. The concentration was around 6 % and hydrogen yield remained less than 0.01 L H_2 g^{-1} VS (Figure 51e and f).Hydrogen can also be useful for energy production. Only a small amount of hydrogen was produced and the lower energy density meant that the contribution to the total energy was small.

The weight loss for pre-hydrolysis digesters was also checked (Figure 51g). The weight loss fluctuated but settled around 0.5 % WW of digestate was lost during pre-treatment. The 0.5 % loss may be caused by evaporating or substrate degradation.

In terms of SMP, pre-hydrolysis did not have any positive effects.

Solids parameters. Figure 52 shows the solids parameters for the digestion trial.

It is difficult to draw conclusions as the system had not reached steady state, but there were no major changes in TS (Figure 52a) and VS (Figure 52b) content during the trial. There was a small difference in VS/TS (Figure 52c) for the two sets of digesters by day 33. D3&4 recovered earlier than D1&2, which still had high VFA and low pH, were therefore better able to start digesting residual VS in the digestate.

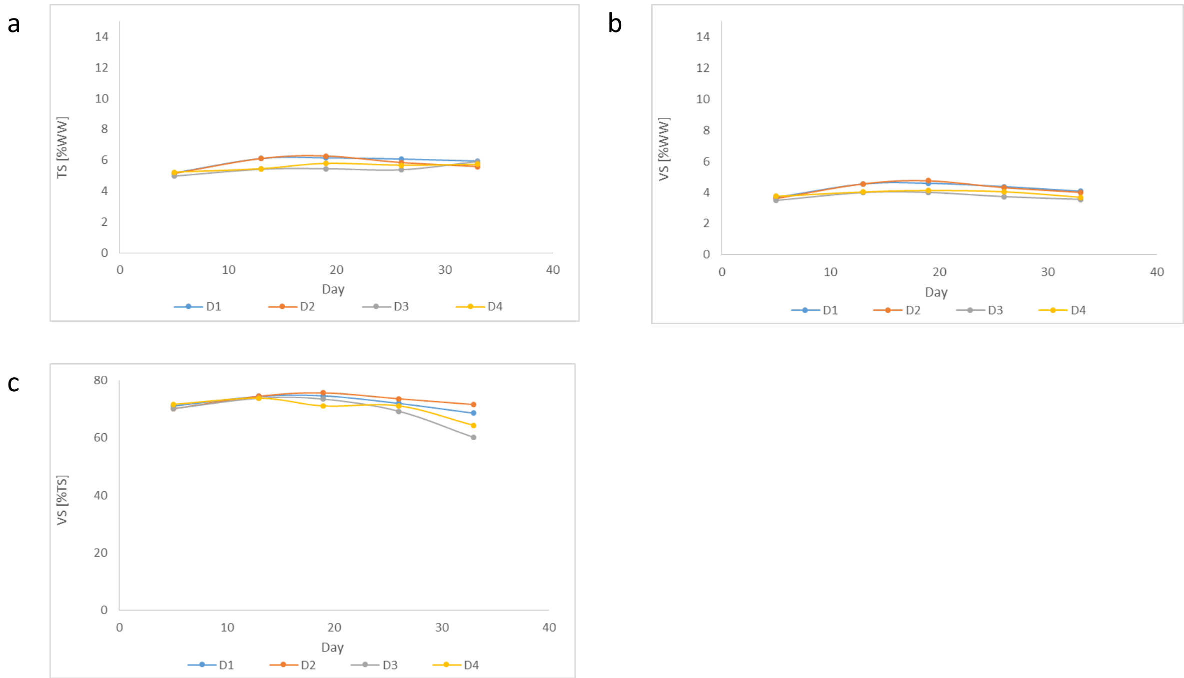


Figure 52. Solids data for D1-4 during digestion Trial 2-2 on baby maize stover

Digestate appearance. The appearance of the digestate in D1&2 and D3&4 showed a clear difference, as in Trial 2-1. D1&2 was more thick and viscous and D3&4 was thinner like thermophilic digestate.

Discussion of results for digestion Trial 2-2.

From day 5 to day 13, D1-4 seemed to be operating stably in terms of IA/PA ratio, pH and rising gas production; then operation was interrupted by the appearance of VFA peaks. The inoculum used in the trial was fresh Millbrook digestate. Although TE supplementation as not carried out, only minimal washout of trace elements would have occurred in this period. It seems more likely that the VFA increase was due to the change in feedstock to baby maize stover at relatively high OLR of 3 g VS L^{-1} day^{-1}, and was possibly also associated with an early start to the 40-day bump.

The set of digesters with pre-hydrolysis were less severely affected than the digesters without pre-hydrolysis. The thermophilic pre-hydrolysis step may have caused some inhibition of acidogenesis in the main digesters, leading to lower VFA concentrations; or some VFA could have been consumed in this stage.

D3&4's specific gas production was lower than D1&2, however, even including gas from the pre-hydrolysis step. Hydrogen was also observed during pre-treatment.

In Trial 2-1 D3&4 also showed lower overall gas production, but there were problems with stability of all digester after around 80 days of operation. The cause in this case was suspected to be washout of essential TE or nutrients. Therefore, Trial 2-2 used fresh Millbrook inoculum. Lower gas production from D3&4 was observed again, but the results were unsatisfactory because of the instability and short duration of the trial. It was therefore decided to compare performance with and without pre-hydrolysis again in the next trial, if possible under more stable operating conditions.

4.5. Digestion Trial 2-3 – baby maize stover

In Trial 1, the mesophilic digesters D1-4 showed rapid digestate volume expansion, foaming and VFA accumulation. TE dosing was started near the end of the trial to help stabilise the anaerobic digestion process. In Trial 2-1, Fe, Co, Ni, were added at the beginning and weekly TE dosing was carried out based on the amount of added substrate. Although the two trials are not directly comparable, as the substrates used (maize silage and baby maize stover) were different, operational performance in Trial 2-1 was more stable than in Trial 1. Ni and Mo in maize silage were less than detection limit but baby maize stover consisted 0.19 mg Ni kg^{-1} WW and 0.45 mg Mo kg^{-1} WW (Table 26). Trial 2-2 was carried out without TE dosing, for a one-month period only. The result of these trials thus indicated that TE dosing had some effect on the stability of anaerobic digestion of maize-derived substrates, but also suggested that there might be a requirement for other elements not present in the TE mix sed.

In Trial 2-3, it was therefore decided to supplement the digesters with different combinations of TE to assess the effects in mesophilic and thermophilic digestion. Mesophilic digesters were also operated with TE supplementation (Fe, Co, Ni, Se, Mo) with and without the thermophilic pre-hydrolysis treatment.

Furthermore, the inoculum for D1-4 in Trial 2-2 was fresh Millbrook digestate, and one possible explanation for the instability of both sets of digesters in this trial was lack of acclimatisation to the new feedstock at the applied OLR of 3 g VS L^{-1} day^{-1}. The inoculum used for D9-10 in Trial 2-1, however, included digestate from reactors that were fed on maize silage, so the digestate was at least partially acclimated to this type of material; and it did not show a strong reaction to the change in feedstock (i.e. little or no sign of a 40-day bump). To reduce or eliminate the effect of the feedstock change in the current trial, it was therefore decided to inoculate D1-4 with mixture of fresh Millbrook inoculum and digestate from D9&10 at the end of Trial 2-1.

The results of Trial 2-3 are reported in the following sequence to illustrate the effects of TE supplementation: D1&2 and D9&10 (mesophilic CSTR), D1-4 (mesophilic CSTR), and D5-8 (thermophilic CSTR). The set of D1&2 (CSTR with 5 TE) and D9&10 (CSTR without TE) are compared to assess the effect of the TE supplementation in mesophilic conditions. D1&2 (1 stage AD) is compared D3&4 (2 stage). The comparison of D5&6 (CSTR with 3 TE) and D7&8 (CSTR with 5 TE) is to assess the effect of TE supplementation in thermophilic conditions.

4.5.1. Objectives and methodology

Objective. To determine the effect of alternative TE supplementation strategies on the performance of thermophilic digestion (3 and 5 TE) and mesophilic digestion (5 TE and no TE), and a thermophilic pre-hydrolysis step before mesophilic digestion TE addition (5 TE), as indicated by gas production and operational stability.

Methodology. Ten 5-L digesters of the type described in section 3 were used in this trial. Temperature was controlled at 35 ℃ (D1-D4, D9-10) or 55 ℃ (D5-D8) by thermocirculators. All digesters apart from D9&10 were supplemented with TE to give known additional concentrations in the digestate on day 41, then weekly addition of trace elements was carried out to maintain the concentration based on the added substrate. TE dosages, OLR, HRT and temperature condition for each pair of reactors are shown in Table 28. Viscosity was measured from day 68 using a Hounsfield viscometer as described in section 3.7 because it is associated with possibility of foaming and VFA accumulation. Viscosity measurements were conducted in triplicate. TE concentration in inoculum was checked (Table 29).

D1-4, D9-10: reactors were inoculated with 3 L of a mixture of Millbrook inoculum and digestate collected from D9-10 from trial 2-1 at a ratio of 2:1 on a volume basis. The purpose of this was to take advantage of any acclimatisation to the baby maize stover feedstock that had occurred in the previous trial. Feeding on baby maize stover began on day 0 at an OLR of 2 g VS L^{-1} day^{-1} and was increased to 3 g VS L^{-1} day^{-1} from day 13.

D5-8: On day -2 of the current trial (corresponding to day 196 of the preceding trial D5-6, and day 352 for D7-8) digestate from these 4 digesters was mixed and redistributed equally between them. This was done to ensure homogeneity of inoculum, as the digesters had shown slightly different behaviour in the previous trial. The working volume was decreased from 4 L to 3 L to match the working volume in the mesophilic digesters. Feeding on baby maize stover began on day 0, two days after the reactor contents were mixed and redistributed, at an OLR of 0.5 g VS L^{-1} day^{-1} which was increased to 4 g VS L^{-1} day^{-1} from day 15.

The method used for the pre-hydrolysis step for D3&4 is described in section 3.8. In this trial the digestate used in pre-hydrolysis was not centrifuged.

Table 28. CSTR conditions

	Temperature [°C]	TE	HRT [kg L^{-1}]	OLR [g VS L^{-1} day^{-1}]	Pre-hydrolysis
D1-2	35	Co, Ni, Fe, Se, Mo	3.1	3-4	N/A
D3-4	35	Co, Ni, Fe, Se, Mo	2.1	3-4	Thermophilic
D5-6	55	Co, Ni, Fe	4.7	4	N/A
D7-8	55	Co, Ni, Fe, Se, Mo	4.7	4	N/A
D9-10	35	N/A	3.0	3-4	N/A

Note: Additional working concentrations of TE were 1 mg Co L^{-1}, 0.4 mg Ni L^{-1}, 10 mg Fe L^{-1}, 0.2 mg Se L^{-1}, 0.2 mg Mo L^{-1}.

Table 29. Inoculum characteristics

	[mg kg^{-1} WW]		[mg kg^{-1} TS]	
	D5-8	D1-4, D9-10	D5-8	D1-4, D9-10
P	371	1031	5175	18954
K	3913	1167	54645	21449
Cu	1	17	14	304
Zn	6	26	88.3	472
S	149	449	2078	8260
Ca	501	1363	6996	25046
Fe	80	1400	1119	25732
Mo	0.6	1.4	8.68	26.3
Mn	5.4	9.4	74.9	172
Ni	2.1	1.9	29.3	35.2
Se	0.11	0.22	1.52	4.09
Co	0.31	0.26	4.38	4.71
W	0.02	0.17	0.23	3.19
TS [% WW]	7.2	5.4		

The results of Trial 2-3 are reported in the following sequence: D1&2 and D9&10 (mesophilic CSTR) in section 4.5.2.1, D1-4 (mesophilic CSTR) in section 4.5.2.2, and D5-8 (thermophilic CSTR) in section 4.5.3. The two sets of digesters D1&2 (CSTR with 5 TE) and D9&10 (CSTR without TE) are compared to assess the absence of TE supplementation. The next section compares D1&2 (1 stage AD) and D3&4 (2 stage). Then D5&6 (CSTR with 3 TE) and D7&8 (CSTR with 5 TE) are compared to provide information on TE supplementation.

4.5.2. Mesophilic digestion results

Operating parameters. Figure 53 shows the organic loading rate, daily wet weight of feed added and hydraulic retention time for mesophilic digesters (D1-4, D9-10) during the experimental period. A summary of the reactors' history is given in Table 30. From day 0 to day 12, the digesters were fed at OLR 2 g VS L^{-1} day^{-1}. From day 13 to day 29, the OLR was increased from 2 to 3 g VS L^{-1} day^{-1}. Feeding was stopped between day 40 and 45 due to signs of instability, then the OLR was gradually returned to 3 g VS L^{-1} day^{-1}. From day 121 to day 136, the OLR was increased from 3 to 4 g VS L^{-1} day^{-1}. Feeding of D3&4 was stopped on day 136, which was equivalent to 2.1 HRT, because it was clear the performance of this pair of digesters was poor in comparison with the other digesters. D1 and 2 operated for 174 days, equivalent to 3.1 HRT. D9 and 10 operated for 173 days, equivalent to 3.0 HRT (Table 31).

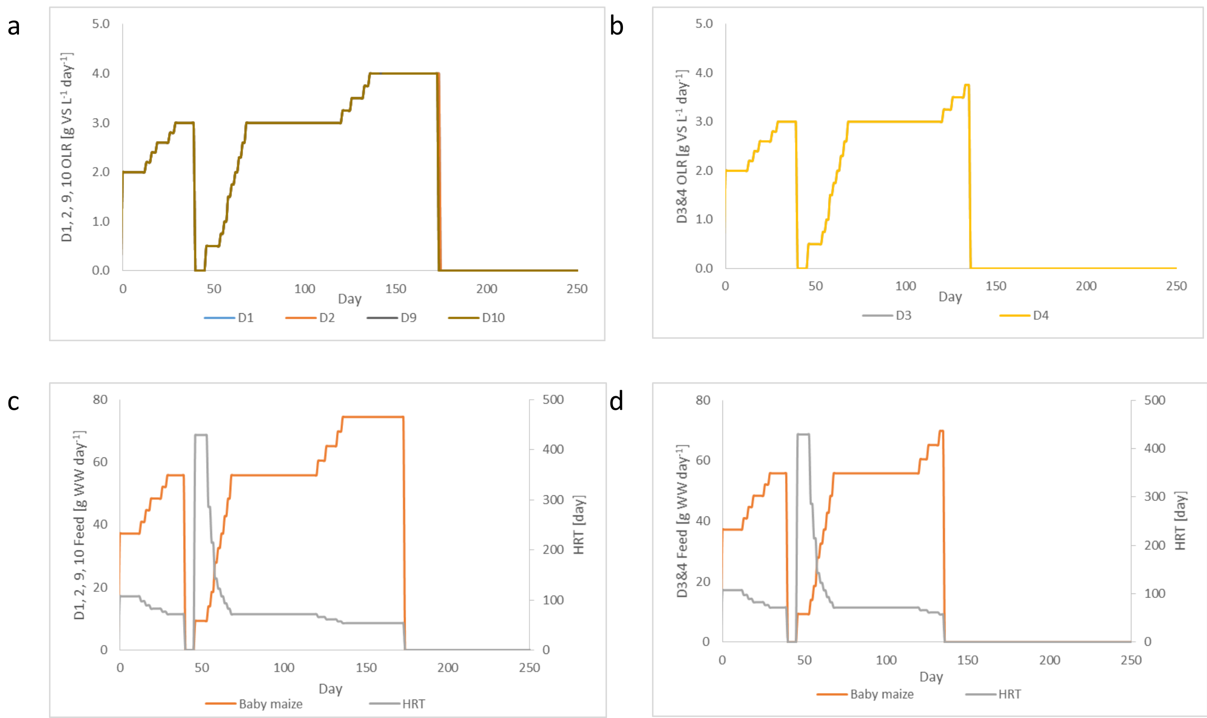


Figure 53. OLR, daily feed and HRT for D1-4, 9-10 during digestion

Table 30. Summary of reactors' history for mesophilic digesters D1-4, D9-10 during digestion trial 2-3 on baby maize stover

Day	Date	D1	D2	D3	D4	D9	D10
-2	20/04/2016	Inoculate with mixture of Millbrook and D9-10 digestate from trial 2-1					
0	22/04/2016	Feeding start: OLR at 2					
13	05/05/2016	OLR increase from 2 to 3 for 17 days					
40	01/06/2016	No feeding for 6 days due to decrease in gas production					
41	02/06/2016	5 TE(Fe (10), Ni (1.0), Co (0.4), Se (0.2), Mo (0.2)): dosing for digestate(3 mL) + weekly dosing start				No TE	
46	07/06/2016	Feeding start at OLR 0.5					
53	14/06/2016	OLR increase from 0.5 to 3 for 15 days					
121	21/08/2016	OLR increase from 3 to 4 for 16 days					
136	05/09/2016			Stopped feeding at OLR 3.75			
139	08/09/2016	Slow stirring observed				Slow stirring observed	
140	09/09/2016						Slow stirring observed
142	11/09/2016						Not fed for 1 day due to slow stirring
173	12/10/2016					Stopped feeding	
174	13/10/2016	Stopped feeding					
180	19/10/2016	Improved stirring observed				Improved stirring observed	

Table 31. Total amount of added substrate and equivalent HRT

	D1	D2	D3	D4	D9	D10
Amount of added substrate [g]	9205	9205	6299	6299	9131	9056
Equivalent HRT for added substrate [-]	3.1	3.1	2.1	2.1	3.0	3.0

4.5.2.1. D1&2 and D9&10 – effect of trace element addition

Biogas and methane production. Biogas and methane production are shown in Figure 54.

VBP and VMP in all of the digesters without pre-hydrolysis (i.e. D1&2, D9&10) showed a clear response to the increase in OLR between day 15 and 31 (Figure 54a and b). In this period, the SBP (Figure 56c) in these digesters was almost constant at around 0.6 L g^{-1} VS added, while SMP (Figure 56d) appeared to reduce slightly. Gas production in D9&10 without TE addition was very slightly lower than in D1&2, especially from about day 21 onwards (average SMP value day 21-42, 0.305 g VS L^{-1} day^{-1} in D1&2, 0.288 g VS L^{-1} day^{-1} in D9&10). By day 39 just before feeding was interrupted, the VMP and SMP had reached 1.9 L L^{-1} and 0.32 L CH_4 g^{-1} VS respectively.

After day 40 when feeding was temporarily reduced in response to signs of instability, there was a drop in VBP and VMP and a corresponding peak in SBP and SMP as production of biogas and methane continued from residual feed in the digesters. Once the increase in OLR started from day 48 onwards, VBP and VMP rose again in step with the rise in feeding. SBP and SMP also rose in this period as the gas production from slower-degrading fractions recovered from the deficit of feeding in the previous days. Gas production in D1&2 and D9&10 recovered to the value before feeding was stopped by day 78, and remained stable from day 79 to day 161 while showing quite significant day-to-day fluctuations. In this period, there was no apparent difference between digesters D1&2 and D9&10 with and without TE addition.

From day 162 both specific and volumetric gas production in D9 and D10 started to drop, falling to less than 0.6 L CH_4 L^{-1} by day 169, although D10 then showed some recovery back toward the performance of the other digesters. In D1&2 with TE addition, gas production remained stable until day 174 when feeding was stopped in all digesters.

Methane concentrations were in good agreement for all reactors by day 117, then D9&10 began to show more fluctuation and slightly less methane than D1&2.

Average values for gas production during periods of relatively stable operation are shown in Table 32 and 38.

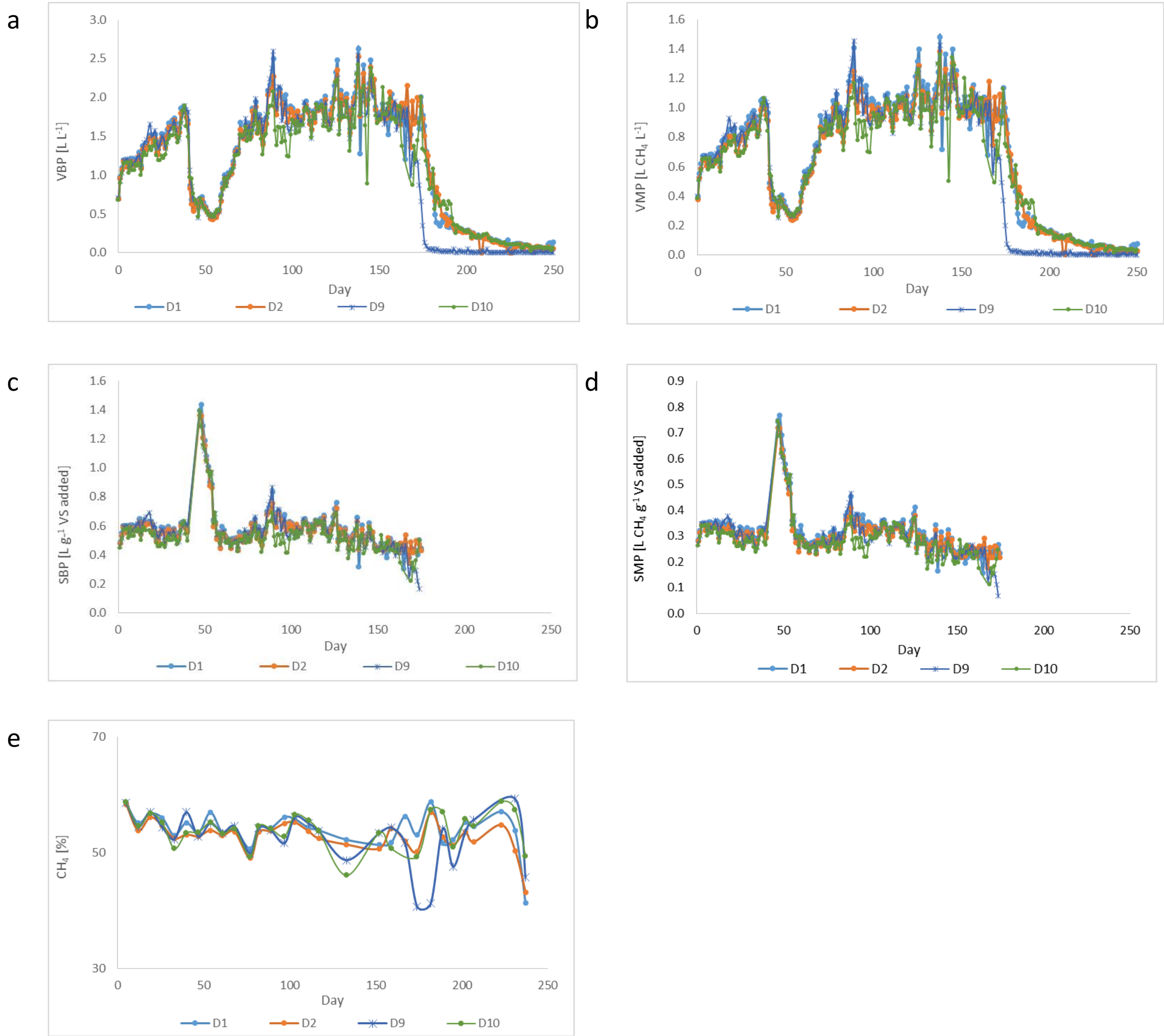


Figure 54. VBP, SBP, VMP and SMP for D1-2, 9-10 during digestion Trial 2-3 on baby maize stover

Operational stability. Figure 55 shows the monitoring parameters for operational stability during the experimental period.

VFA accumulation (Figure 55a) occurred on two occasions around day 40 and 180. The first of these appeared to be the typical 40 day bump, when the highest total VFA concentrations occurred in D9 and D10 without TE addition at 1.71 and 3.05 g COD L^{-1}, compared with only 0.33 and 0.39 g COD L^{-1} in D1 and D2. This indicated that addition of the 5TE mix was effective in promoting stable operation at this point. The use of a mixture of acclimated digestate and fresh Millbrook inoculum in combination with the reduction in OLR when the VFA peak appeared may have reduced the severity of the VFA accumulation at this time, but did not completely prevent it.

The second VFA peak started from around day 132, just before the end of the stepwise increase in OLR from day 121 to day 137. On day 174 when feeding was stopped, the total VFA concentrations in D9 and D10 were 20.1 and 10.9 g COD L^{-1}, respectively. The total VFA concentration in D10 fell over the next 2 weeks and stabilised at between 1-2 g COD L^{-1}, but in D9 it only reduced slightly to 16-17 g COD L^{-1} indicating that the disturbance in this digester was more severe. In contrast the total VFA in D1&2 with TE addition remained low until day 159, when it also started to increase sharply, reaching around 7 g COD L^{-1} by the time feeding was stopped. Concentrations then fell rapidly over the next 2 weeks, stabilising at below 0.1 g COD L^{-1}.

This difference in the time of onset of VFA accumulation, and to some extent of its severity, indicated that supplementation with the 5 TE mix was helpful in prolonging stable operation, but it was not sufficient to prevent instability. In both cases the onset of instability occurred close to 3 HRT. TE requirements in thermophilic digestion are less well understood and may differ from those in mesophilic conditions, but taken with the result of the thermophilic trial this may suggest these reactors were still running out of an essential element not present in the 5 TE mix.

Other monitoring parameters also showed signs of instability. TA (Figure 55b) remained fairly stable until day 132, at an average of around 8.6-8.8 g $CaCO_3$ kg^{-1} in D2, D9 and D10 and around 8.3 g $CaCO_3$ kg^{-1} in D1; then declined to between 6.5-7.5 g $CaCO_3$ kg^{-1} when VFA accumulation appeared. From day 181, TA started to recover to the previous the value except in D9.

PA declined from day 132 to between 2.7-3.4 g $CaCO_3$ kg^{-1} in D1&2 and D10 before recovering due to the cessation of feeding (Figure 55c), but the value in D9 fell to below 1.0 g $CaCO_3$ kg^{-1} and did not recover. IA (Figure 55d) was stable at around 2.7 g $CaCO_3$ kg^{-1} in all digesters until day 152, then showed some fluctuation in all digesters. IA in D9 showed a strong rise to over 6.4 g $CaCO_3$ kg^{-1}. This led to increases in the IA/PA ratio (Figure 55e) to above 1.0 in all digesters, and a catastrophic increase to above 10 in D9. The pH (Figure 55f) reflected these changes in alkalinity, with values falling during the period of disturbance, but remaining above 7.0 in all digesters except D9. D9's pH was below 6. TAN concentrations (Figure 55g) in all digesters decreased slowly, from around 1.5 g N kg^{-1} at the start of the experimental period to between 0.32 – 0.36 g N kg^{-1} in D1&2 and D10 during the period of disturbance, and slightly higher in D9. TAN increased after feeding was stopped, which may have been caused by degradation of some of the microbial biomass.

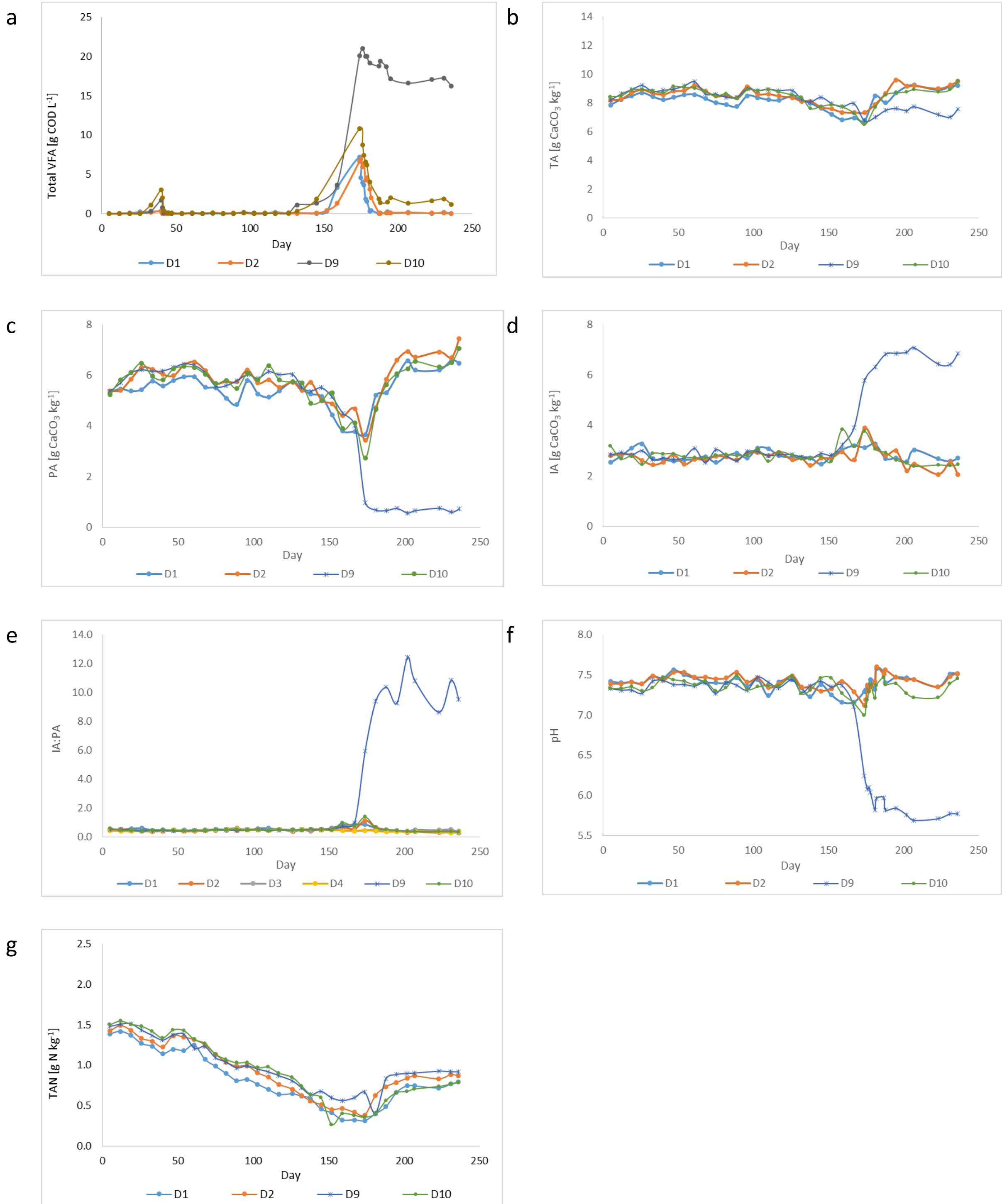


Figure 55. VFA, pH, alkalinity, TAN in D1-2, 9-10 during digestion Trial 2-3 on baby maize stover

Volatile Fatty acid profiles. Figure 56 shows the VFA profiles for D1&2 and D9&10. As mentioned above, there were two VFA peaks at around day 40 and day 170. The first peak in D1&2 with TE addition consisted only of acetic acid and was less than 0.4 g COD L^{-1}. In D9&10 without TE the peak consisted of propionic and acetic acid accumulations of 0.9-1.0 mg COD L^{-1} and 0.4-1.5 mg COD L^{-1}, respectively. This also supports the view that the addition of TE was useful in promoting stability at the start of the run. In the second VFA peak, propionic acid accumulation was greater than acetic acid in all digesters and made up the majority of the VFA. Slightly elevated concentrations of n-butyric, iso-valeric, iso-butyric and valeric acid were also present in all digesters on day 174 when feeding stopped. These declined rapidly in D1&2, but remained present in D9&10 as long as monitoring continued.

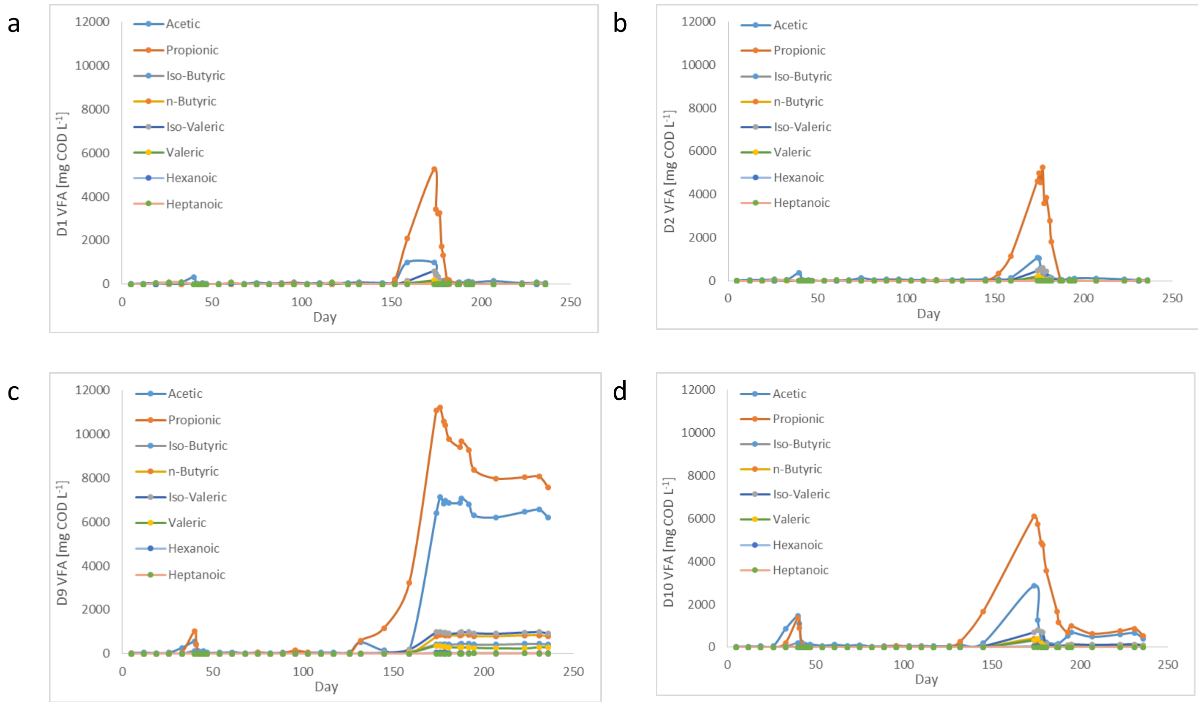
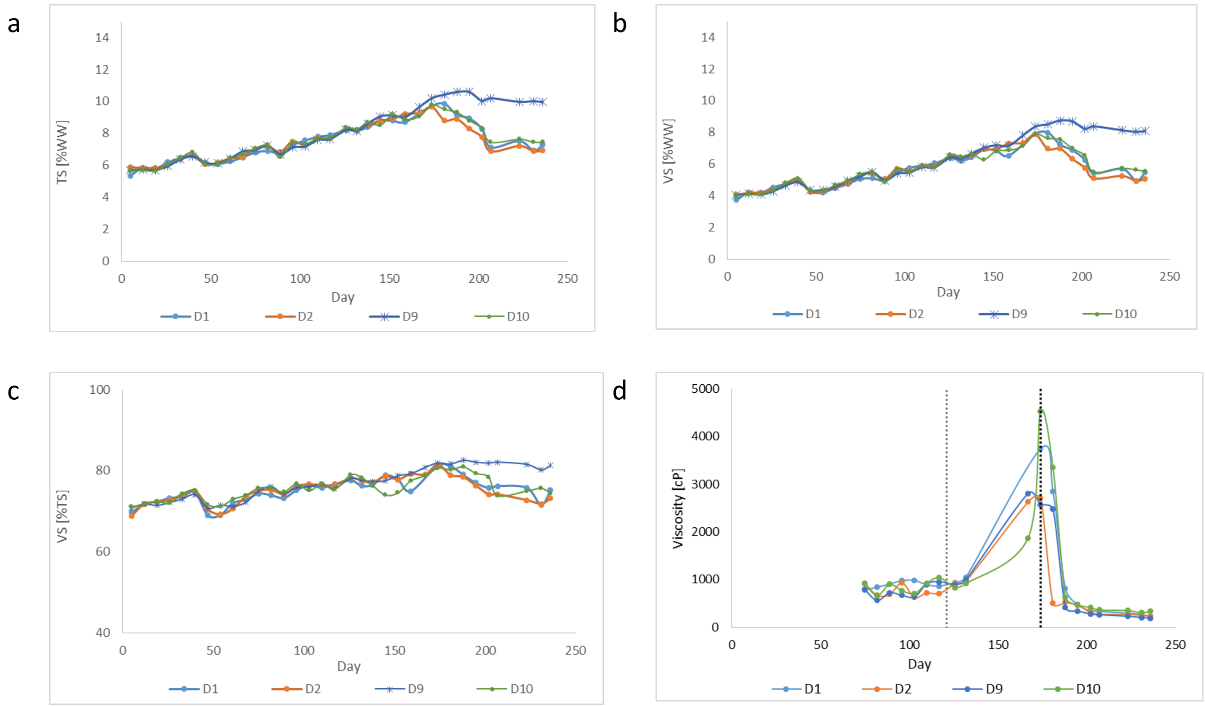


Figure 56. VFA profiles for D1-2, 9-10 during digestion Trial 2-3 on baby maize stover

Solids parameters. Figure 57 shows the solids parameters for digesters D1&2 and D9&10. TS, VS and VS/TS were similar in all digesters and all increased through the digestion trial. The solids parameters then decreased after feeding was stopped, apart from in D9. D9 experienced severe VFA accumulation and did not show a decrease in solids content. This may indicate that the pH or VFA concentration remained low enough to cause some inhibition of hydrolytic activity.

Viscosity was measured from day 75 and settled at around 700-1000 cP. From day 132 when VFA accumulation appeared, viscosity started to increase. During this period, when the OLR increased from 3 to 4 g VS L^{-1} day^{-1}, more undigested fibrous materials were observed, and the digestate was more thick and viscous. That may indicate that the digesters were starting to struggle with digesting the substrate.

The increase in viscosity may be a result of the accumulation of undigested fibrous materials in the digestate. Slow stirring was observed and stirring sometimes stopped altogether due to the thick digestate. The VFA accumulation may be related to the insufficient mixing and/or higher OLR. On day 174 when feeding was stopped, the viscosity in the digesters was 3745 cP in D1, 2707 cP in D2, 2576 cP in D9, 4527 cP in D10. After feeding ceased, the viscosity dropped to below 900 cP on day 188, then settled around 300 cP from around day 200. This indicates the viscosity increase was associated with VFA accumulation.



Vertical line (d):

Day 121: OLR increase from 3 to 4 g VS L^{-1} day^{-1} for 16 days

Day 174: Feeding stopeed

Figure 57. Digestate solids parameters for D1-2, 9-10 during digestion Trial 2-3 on baby maize stover

Discussion of results for mesophilic TE comparison. Table 34 shows the monitoring parameter values for D1&2 and D9&10 pseudo-steady state periods.

D1&2 received 5 TE (Fe, Co, Ni, Se, Mo) while D9&10 did not receive any trace element supplementation. The SMP values for D9&10 were 0.311 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} and 0.236 L CH_4 g^{-1} VS at OLR 4 g VS L^{-1} day^{-1} which were 5-6 % less than that of D1&2. At OLR 4 g VS L^{-1} day^{-1}, D9&10 showed 1-2% higher digestate TS and VS than that of D1&2 suggesting that the ability of the micro-organisms to degrade the substrate was reduced.

VFA accumulation around day 40 and 180 clearly showed significant differences between D1&2 and D9&10. The 40-day bump in D1&2 consisted only of acetic acid which was less than 0.4 g COD. The acetic acid in D9&10 was greater than D1&2 and propionic acid was also present in similar concentrations to the acetic acid. The second peak was much higher than the first peak, and had reached 6.6 g COD L^{-1} in D1&2, 20.1 g COD L^{-1} in D9 and 10.9 g COD L^{-1} in D10 on day 174 when feeding stopped. These accumulations quickly decreased in D1&2 but remained present in D9&10 while monitoring continued.

Alkalinity measurements also supported the difference in VFA production. During the second VFA peak, the IA:PA ratio increased. Peak values in D9&10 were greater than in that of D1&2. After feeding ceased, the IA:PA ratio recovered quickly but recovery in D9 was observed by end of the Trial 2-3.

Taken together, these data indicate the 5 TE supplementation helped stable operation and greater biogas production.

One more noteworthy piece of data was the increase of viscosity after the OLR increase from 3 to 4 g VS L^{-1} day^{-1}. The greatest viscosity was observed on day 174, and this rapidly decreased after feeding stopped. The order of highest viscosity was D10>D1>D2>D9. Viscosity seemed not to be affected as strongly by the 5 TE dose as other parameters were.

It was difficult to find literature describing the relationship between OLR and viscosity, but some papers report a link between high viscosity and foaming. Stoppok and Buchholz (1985) reported that foaming in a digester treating sugar beet pulp was caused by the high viscosity of the fluid-substrate mixture and high cellulosic composition of substrate. Suhartini *et al.* (2014) reported that thermophilic digestion was more resistant to foaming and that may be related to lower viscosity. Bartek *et al.* (2015) reported rapid volume expansion was a result of gas bubble hold up in digestate due to inadequate mixing. When the mixing stopped, the digestate could behave like a solid because gas bubbles would remain stationary in suspension and continue to grow.

Similar situation might happen in D1&2 and D9&10. TS and VS increased until feeding stopped so undigested substrate was accumulated. After the OLR increase from 3 to 4 g VS L^{-1} day^{-1}, the amount of residual undigested substrate was accelerated. Slow and/or stopped mixing were observed. The fibrous digestate and inadequate mixing may catch more gas bubbles and the digestate may show rapid volume expansion. That may cause higher viscosity.

Overall, it is clear that 5 TE supplementation (Fe, Co, Ni, Se, Mo) allowed greater methane yield and more stable operation, but the digesters showed signs of instabilities at OLR 4 g VS L^{-1} day^{-1}. OLR 4 g VS L^{-1} day^{-1} is not high OLR for maize derived AD (Cornell, 2011). That may indicate other TE requirement.

Table 32. Monitoring parameter values for mesophilic digestion with and without TE addition during pseudo-steady state periods in Trial 2-3

Parameter	Unit	D1 [a]	D2 [a]	D1 [b]	D2 [b]	D9[a]	D10[a]	D9[b]	D10[b]	Ave [a]	Ave [a]	Ave [b]	Ave [b]
		M + 5TE	M + 5TE	M + 5TE	M + 5TE	M	M	M	M	M	M + 5TE	M	M + 5TE
OLR	g VS L^{-1} day^{-1}	3	3	4	4	3	3	4	4	3	3	4	4
SBP	L g^{-1} VS	0.621	0.600	0.475	0.487	0.591	0.549	0.471	0.476	0.570	0.610	0.474	0.481
SMP	L CH_4 g^{-1} VS	0.341	0.326	0.247	0.253	0.321	0.301	0.241	0.232	0.311	0.333	0.236	0.250
VBP	L L^{-1} day^{-1}	1.86	1.80	1.90	1.95	1.77	1.65	1.88	1.87	1.71	1.83	1.88	1.93
VMP	L CH_4 L^{-1} day^{-1}	1.05	0.99	1.07	1.07	1.00	0.93	1.06	1.05	0.96	1.02	1.06	1.07
CH_4 content	% v/v	55.0	54.1	51.5	52.4	54.1	54.6	53.8	52.0	54.4	54.6	52.9	52.0
Digestate TS	%WW	7.6	7.5	7.4	7.6	7.4	7.5	9.0	8.8	7.5	7.6	8.9	7.5
Digestate VS	%WW	5.8	5.8	5.1	5.3	5.6	5.7	7.0	6.7	5.7	5.8	6.8	5.2
VS destruction	%VS	76.0	76.4	77.0	78.2	76.0	76.0	78.2	75.6	76.0	76.2	76.9	77.6
Viscosity	cP	929	746	1050	969	793	859	1001	914	826	837	958	1010
pH	–	7.36	7.40	7.26	7.35	7.38	7.35	7.38	7.38	7.4	7.4	7.4	7.3
TA	g $CaCO_3$ kg^{-1} WW	8.3	8.7	7.4	7.7	8.9	8.9	8.0	7.7	8.9	8.5	7.9	7.6
PA	g $CaCO_3$ kg^{-1} WW	5.4	5.8	5.2	5.8	6.0	6.0	5.1	4.8	6.0	5.6	5.0	5.5
IA	g $CaCO_3$ kg^{-1} WW	2.9	2.9	2.7	2.7	2.9	2.8	2.9	3.0	2.9	2.9	2.9	2.7
IA/PA ratio	–	0.54	0.50	0.61	0.55	0.48	0.47	0.57	0.64	0.48	0.52	0.61	0.58
TAN	g N kg^{-1} WW	0.73	0.88	0.45	0.50	0.93	0.97	0.62	0.48	0.95	0.81	0.55	0.47
Total VFA	g L^{-1}	0.11	0.08	1.25	0.63	0.11	0.09	2.54	1.90	0.10	0.10	2.22	0.94

[a] Average value for days 91-120

[b] Average value for days 137-166

4.5.2.2. D1&2 and D3&4 effect of thermophilic pre-hydrolysis

Note : results for D1&2 shown above are repeated here for ease of comparison.

Biogas and methane production. Biogas and methane production are shown in Figure 58. During the period from day 40 onwards while feeding was reduced and then re-introduced, gas production in D3&4 followed similar trends as in D1&2, but again the gas production in D3&4 were lower than that of D1&2 (Figure 58a, b and c). After day 107 it reached a plateau with an SMP of around 0.194 L CH_4 g^{-1} VS added, i.e. about 58 % the total in the other digesters, until feeding was discontinued on day 136 (Figure 58d). It was therefore clear that gas production from the main digesters D3&4 with the pre-hydrolysis stage was considerably less than from D1&2, and also from D9&10 (without TE). Feeding of D3&4 was stopped on day 136 after which residual gas production slowly declined.

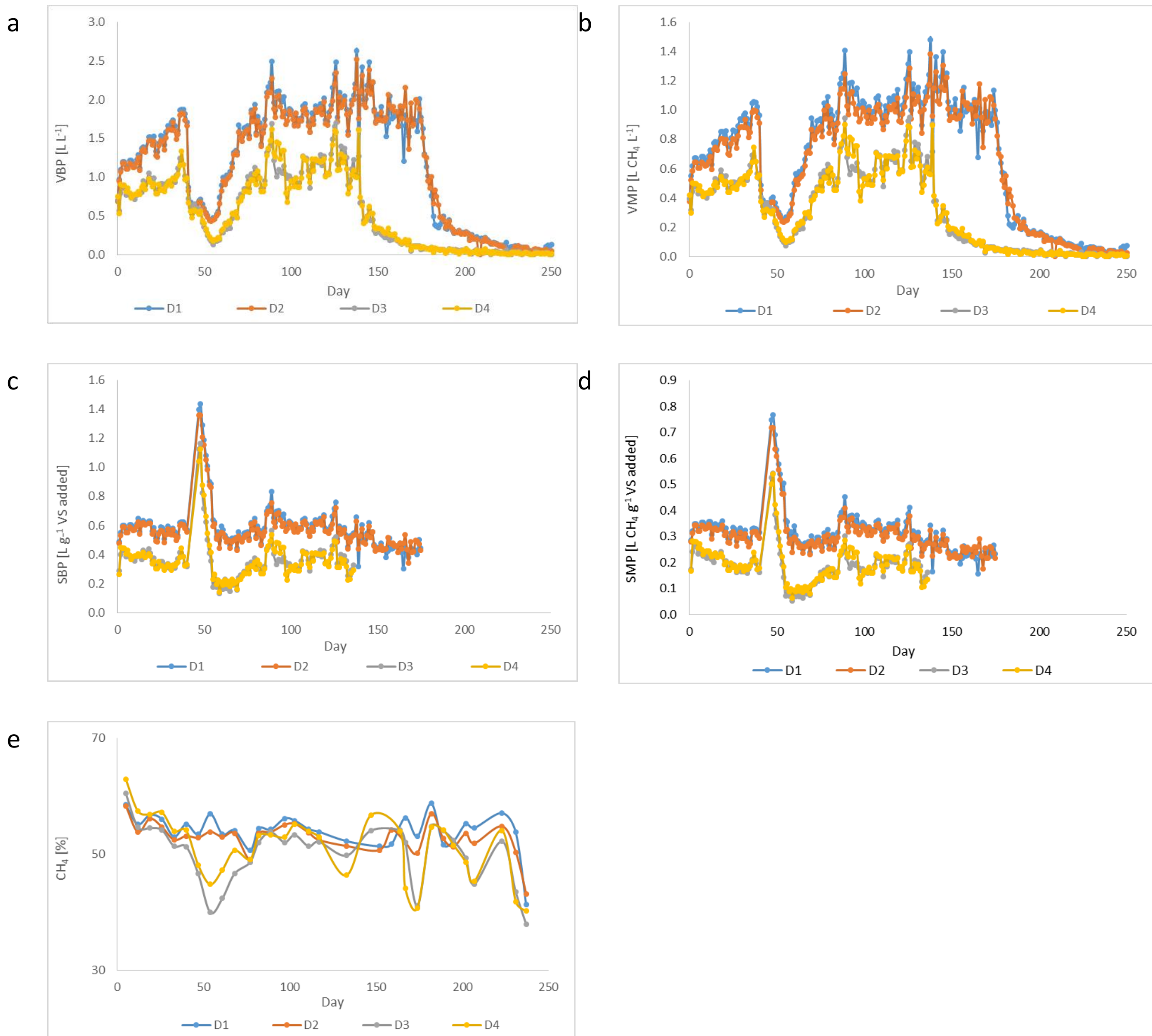


Figure 58. VBP, SBP, VMP and SMP for D1-4 during digestion Trial 2-3 on baby maize stover

Figure 58e shows the methane concentration of the biogas from D1-4. Fluctuations in biogas composition after day 159 were associated with changes in feeding and reactor parameters described below.

Gas production from pre-hydrolysis step. Figure 59 shows the gas production from the pre-hydrolysis step. SBP slowly increased and settled around 0.20 L g^{-1} VS added from day 60 (Figure 59a). Methane concentration was around 50 % so SMP settled 0.093 L CH_4 g^{-1} VS (Figure 59b and c). Even the SMP from pre-treatment was added to the SMP from main digesters, two stage system gas production was lower than D1&2 single stage digesters (Figure 59d).

Hydrogen production was observed. The concentration was from 0 to 3 % and hydrogen yield remained less than 0.009 L H_2 g^{-1} VS (Figure 59e and f). The contribution of the hydrogen to the overall energy present in the form of gaseous energy products was therefore small.

The weight loss for pre-hydrolysis digesters was also checked (Figure 59g). The weight loss fluctuated but settled around 1 % WW of digestate was lost during pre-treatment. The 1 % loss may be caused by evaporating or substrate degradation.

In terms of SMP, pre-hydrolysis did not have any positive effects.

Figure 59. SBP, SMP, SHP for pre-hydrolysis step during digestion Trial 2-3 on baby maize stover

Operational stability. Figure 60 shows the monitoring parameters for operational stability during the experimental period.

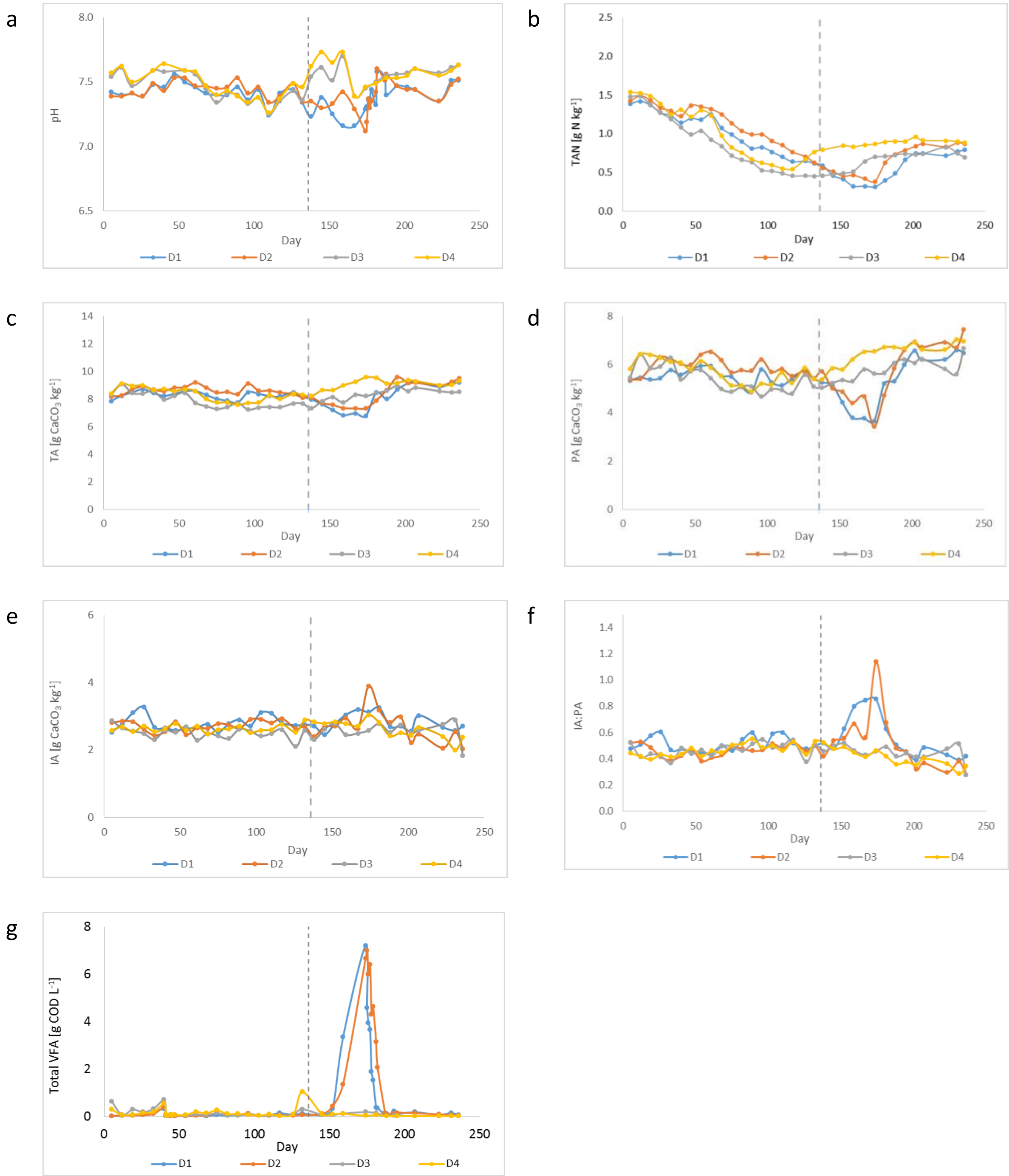


Figure 60. pH, TAN, alkalinity and total VFA in D1-4 during digestion Trial 2-3 on baby maize stover.

There was little difference between the values of monitoring parameters for D3&4 and D1&2 up to the time when feeding of D3&4 stopped on day 136. pH was initially slightly higher in D3&4 (Figure 60a), but had equalised by day 175. TAN was similar in all digesters until day 33, then started to diverge slightly, with values in D1&2 on average around 0.25 g N kg^{-1} higher than in D3&4 (Figure 60b). This could possibly indicate either a lower degree of breakdown of organic N in the feedstock in D3&4, or a higher degree of uptake into microbial biomass; or some other mechanism for reduction of TAN such as losses in biogas in the first stage hydrolysis reactors. Alkalinity parameters (Figure 60c-f) showed no consistent differences between the two pairs of digesters, while the 40-day peak concentrations of total VFA (Figure 62g) in D3&4 were 0.70 and 0.54 g COD L^{-1}, i.e. only marginally higher than in D1&2.

Volatile Fatty acid profiles. Figure 61 shows the VFA profiles for D1-4. These confirm the very low VFA concentrations in both sets of digesters during most of the run. D3&4 (Figure 61c and d) showed a very slight elevation in acetic acid concentrations (< 0.15 g COD L^{-1}) in the first 30 days of the run, and the presence of propionic acid in concentration of up to 0.40 g COD L^{-1} at day 40, which was not seen in D1&2 (Figure 61a and b). Small increases in propionic and acetic acid around day 132 may have been a response to the increase in OLR, but reduced immediately once feeding ceased.

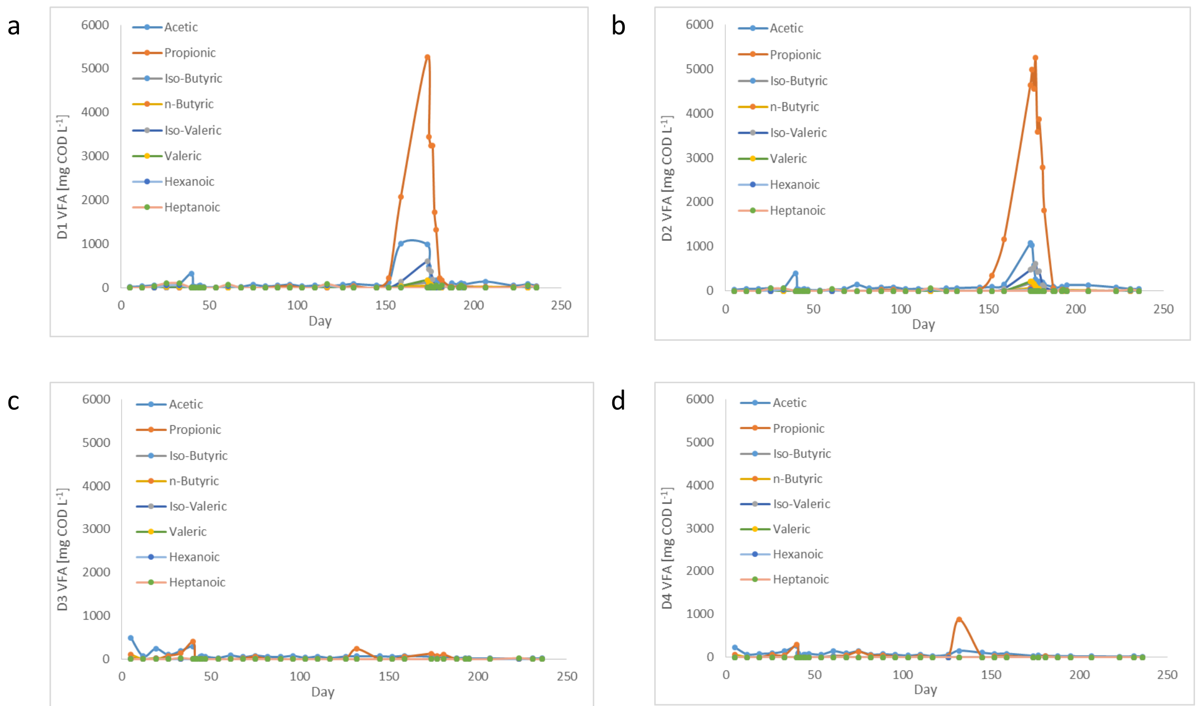


Figure 61. VFA profiles for D1-4 during digestion trial 2-3 on baby maize stover (Note change of y-axis scale from previous results for D1&2 in Figure 58).

Solids parameters. Figure 62 shows the solids parameters for digesters D1-4. TS, VS and VS/TS increased by the time feeding stopped. From day 89, there was a divergence in the TS and VS content, with values in D3&4 around 0.5 % WW lower than in D1&2 by the time feeding of D3&4 was stopped. This lower solids content could indicate that additional hydrolysis was occurring in the thermophilic pre-treatment phase, but this was not translated into higher specific methane yields most of time.

The VS/TS ratio in the two pairs of digesters remained similar, but there was a significant difference in the viscosity, with average values of 854 cP and 296 cP for D1&2 and D3&4 from day 75 until feeding of D3&4 stopped on day 136.

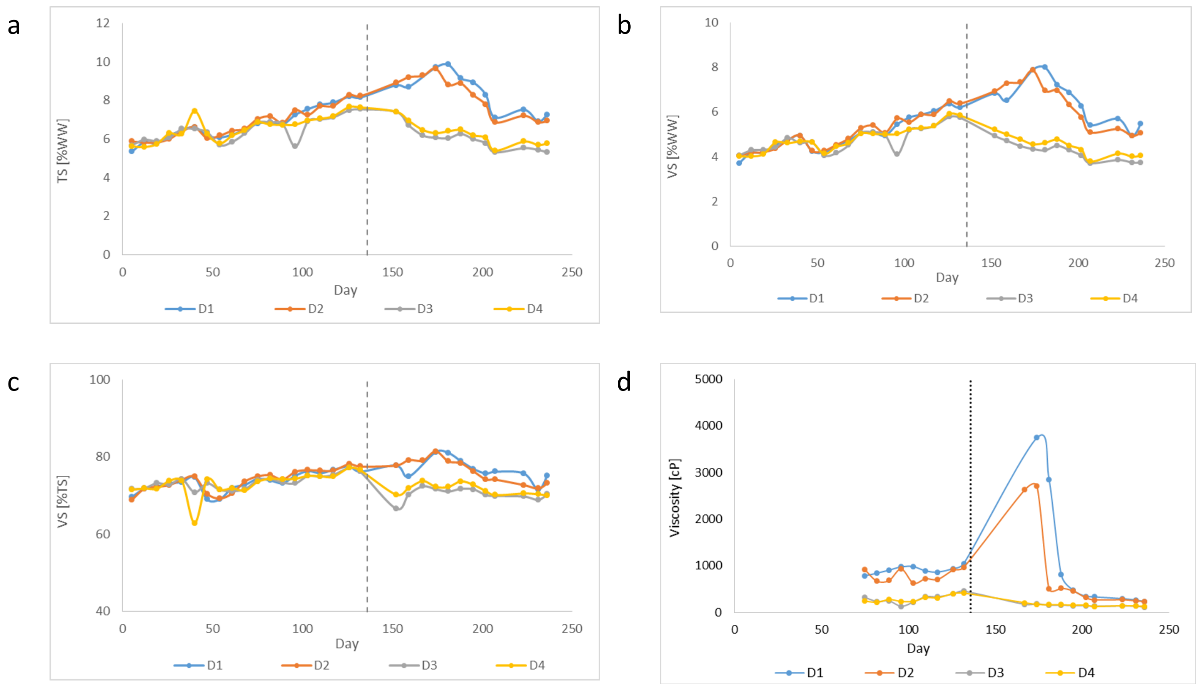


Figure 62. Digestate solids parameters and viscosity for D1-4 during digestion Trial 2-3 on baby maize stover

Discussion of result for mesophilic pre-hydrolysis comparison. The average values during pseudo steady state periods are shown in Table 35. D1-4 received 5 TE. D1&2 were without pre-hydrolysis and D3&4 were with pre-hydrolysis.

There was a significant difference in SMP between D3&4 with pre-hydrolysis (0.194 L CH_4 g^{-1} VS) and D1&2 without (0.333 L CH_4 g^{-1} VS). Even when the gas production from pre-hydrolysis (SMP 0.093 L CH_4 g^{-1} VS, SHP 0.001 L H_2 g^{-1} VS) was included, D3&4 could not cover the SMP difference.

The SMP from the pre-hydrolysis stage in Trial 2-3 was significantly higher than in Trial 2-1 and 2-2 (Table 36) and the weight loss after pre-hydrolysis was correlated with gas production. This indicates that 5TE supplementation helped methanogen activities.

A clear difference was also observed in viscosity which was 837 cP in D1&2, and 265 cP in D3&4. This phenomena was also observed by Stoyanova *et al.* (2014)who compared viscosity in a single mesophilic digester and in two stage AD (first stage thermophilic, second stage mesophilic). The lower viscosity in the two-stage system was attributed to pectin degradation during pre-hydrolysis. Toğrul and Arslan (2003) reported that higher cellulose concentration and lower temperature caused higher viscosity.

Taken together, the two stage system helped reduce viscosity but caused lower gas production. In terms of SMP, the two stage system did not have any positive effects. Other modes of operation of pre-hydrolysis are possible: for example some plants may operate by retaining a proportion of the feed in the pre-hydrolysis tank to provide a good inoculum of hydrolytic organisms adapted to the conditions; but this option was not investigated in the current work due to time constraints.

Table 33. Monitoring parameter values from mesophilic digestion with and without pre-hydrolysis during pseudo-steady state periods in Trial 2-3

Parameter	Unit	D1 [a]	D2 [a]	D3 [a]	D4 [a]	Ave [a]	Ave [a]
		M + 5TE	M + 5TE	M + pre	M + pre	M + 5TE	M + pre
OLR	g VS L^{-1} day^{-1}	3	3	3	3	3	3
SBP	L g^{-1} VS	0.621	0.600	0.361	0.369	0.610	0.365
SMP	L CH_4 g^{-1} VS	0.341	0.326	0.190	0.198	0.333	0.194
SMP from pre	L CH_4 g^{-1} VS	–	–	0.093	0.094	–	0.093
SHP from pre	L H_2 g^{-1} VS	–	–	0.001	0.000	–	0.001
VBP	L L^{-1} day^{-1}	1.86	1.80	1.08	1.11	1.83	1.09
VMP	L CH_4 L^{-1} day^{-1}	1.05	0.99	0.60	0.62	1.02	0.61
CH_4 content	% v/v	55.0	54.1	52.2	53.7	54.6	53.0
Digestate TS	%WW	7.6	7.5	6.7	7.0	7.6	6.8
Digestate VS	%WW	5.8	5.8	5.0	5.2	5.8	5.1
VS destruction	%VS	76.0	76.4	74.7	74.9	76.2	74.8
Viscosity	cP	929	746	255	275	837	265
pH	–	7.4	7.4	7.33	7.34	7.4	7.3
TA	g $CaCO_3$ kg^{-1} WW	8.3	8.7	7.4	7.9	8.5	7.7
PA	g $CaCO_3$ kg^{-1} WW	5.4	5.8	4.8	5.3	5.6	5.1
IA	g $CaCO_3$ kg^{-1} WW	2.9	2.9	2.5	2.6	2.9	2.6
IA/PA ratio	–	0.54	0.50	0.52	0.49	0.52	0.51
TAN	g N kg^{-1} WW	0.73	0.88	0.50	0.58	0.81	0.54
Total VFA	g COD L^{-1}	0.11	0.08	0.05	0.06	0.10	0.05

[a] Average value for days 91-120

Table 34. SMP and SHP from pre-hydrolysis in Trial 2-1, 2-2 and 2-3

		Trial 2-1	Trial 2-2	Trial 2-3
OLR	g VS L^{-1} day^{-1}	4	3	3
SMP from pre	L CH_4 g^{-1} VS	0.005	0.018	0.093
SHP from pre	L H_2 g^{-1} VS	0.011	0.007	0.001
TE		3TE	–	5TE
Inoculum		D3-4 from Trial 1	Fresh Millbrook digestate	Mixture of 1L D9-10 in Trial 2-1 and 2L fresh Millbrook digestate

4.5.3. Thermophilic digestion results

Operating parameters. Figure 63 shows the OLR, daily wet weight of feed added and the hydraulic retention time for digesters D5-8. A summary of the reactors' history (trace elements addition, other events) is shown in Table 35. From start-up onwards the target OLRs were successfully maintained with only minor variations. During this trial, the digesters were not fed on day 40 due to an unintended break in feeding, and on day 81 due to VFA accumulation. Additional trace element supplementation was carried out on day 81, 84 and 118 as shown in Table 35. On day 143, the OLR on D7 only was decreased from 4 to 2 g VS L^{-1} day^{-1} for one day as the biogas production on the day before was half the normal amount. This may have been a false alarm due to leaking gas. Feeding of all reactors was stopped for 2 days on day 223 then resumed on day 226 for one day before finally ceasing. D5-8 were operated for the equivalent of 4.7 HRT, respectively (Table 36).

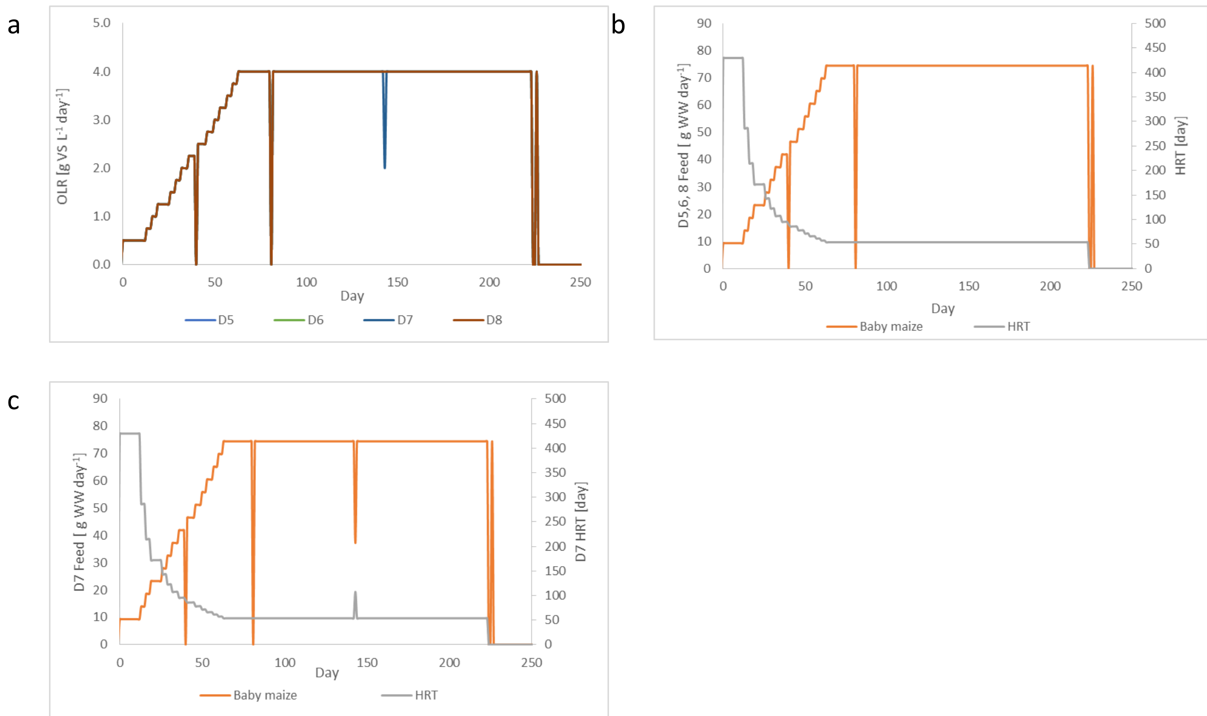


Figure 63. OLR, daily feed and HRT for D5-8 during digestion Trial 2-3 on baby maize stover

Table 35. Summary of history for thermophilic digesters D5-8 during digestion Trial 2-3 on baby maize stover

Day	Date	D5	D6	D7	D8
-2	20/04/2016	Mixed D5-8 from trial 2-1 and separated equally. Working volume decrease from 4 L to 3 L			
0	22/04/2016	Feeding start: OLR at 0.5			
13	05/05/2016	OLR increase from 0.5 to 4 for 50 days			
40	01/06/2016	Not fed for 1 day due to absence			
41	02/06/2016	3TE(Fe (10), Ni (1.0), Co (0.4)): dosing for digestate(3 mL) + weekly dosing start		5TE(Fe (10), Ni (1.0), Co (0.4), Se (0.2), Mo (0.2)): dosing for digestate(3 mL) + weekly dosing start	
81	12/07/2016	Not fed for 1 day due to VFA accumulation			
		3TE(Fe (10), Ni (1.0), Co (0.4)): one-off dose for digestate (3 mL)		3TE(Fe (10), Ni (1.0), Co (0.4)): one-off dose for digestate (3 mL)	
84	15/07/2016		W(0.2): one off doe for digestate (3 mL)		W(0.2): one off dose for digestate (3 mL)
118	18/08/2016	W(0.2): one off doe for digestate (3 mL)		W(0.2): one off doe for digestate (3 mL)	
143	12/09/2016			50 % fed due to low gas production	
223	01/12/2016	Stopped feeding for 2 days			
226	04/12/2016	Fed digesters 1 day			
227	05/12/2016	Stopped feeding			

Table 36. Total amount of added substrate and equivalent HRT for D5-8 during digestion Trial 2-3 on baby maize stover

	D5	D6	D7	D8
Amount of added substrate [g]	14128	14128	14091	14128
Equivalent HRT for added substrate [-]	4.7	4.7	4.7	4.7

Operational stability. Figure 64 shows the monitoring parameters for operational stability during the experimental period. On day 41 a one-off dose of TE was added to the digesters as shown in Table 29 (D5&6 = 3 TE: Fe, Co and Ni; D7&8 = 5 TE: Fe, Co, Ni, Se and Mo) and weekly TE dosing was begun to maintain the TE concentration.

Total VFA concentrations in all four digesters remained fairly low (average < 0.2 g L^{-1}) until day 61. From day 61 onwards, however, VFA concentrations rose sharply in all four digesters over the next 2 weeks (Figure 66 a and b), reaching around 3 g COD L^{-1} in D5, D6 and D8 and 2 g COD L^{-1} in D7.

On day 81 a one-off 3 mL dose of the 3TE mix was added to D5 (3TE) and D7 (5TE) to bring the additional concentration in the digestate up by a further 10 mg Fe L^{-1}, 1 mg Ni L^{-1} and 0.4 mg Co L^{-1}. These additional TE concentrations were then allowed to wash out over the remainder of the operating period, but weekly dosing at the original concentration was continued in proportion to the amount of feedstock added.

The immediate response in both D5 and D7 was a further rise in VFA concentrations to around 4.7 g COD L^{-1} in D5 (3TE addition) and 3.3 g COD L^{-1} in D7 (5TE addition) (Figure 64a). Total VFA concentration in D5 then fluctuated around 5 g COD L^{-1} until day 118, while that in D7 gradually decreased but remained above 1.7 g COD L^{-1}. On day 118 both digesters were supplemented with a one-off dose of tungsten to bring the additional concentration in the digestate up by 0.2 g W L^{-1}. In D5 the VFA concentration fell sharply, declining to 0.36 g COD L^{-1} by day 152 and stabilising below 0.1 g COD L^{-1}. In D7 where the initial concentration was lower the rate of fall was slightly less sharp, but total VFA also stabilised below 0.1 g COD L^{-1} from day 152 until the end of the run.

Digesters D6 and D8 did not receive any additional supplementation with the 3TE mix, but on day 84 they were given a one-off dose of tungsten to raise the additional concentration in the digestate by 0.2 g W L^{-1}. This was also allowed to wash out over the following weeks, while the previous regular TE dosing in each reactor continued. In response to the one-off dose, both digesters showed a relatively short-term increase in total VFA (Figure 64b), which was more noticeable in D6 (3TE) where the VFA concentration had previously been falling. Total VFA in D6 reached 3.2 g COD L^{-1} on day 90 then began to fall steadily, stabilising at < 0.1 g COD L^{-1} by day 152. In D8 (%TE) the peak VFA concentration was 5.4 g COD L^{-1} on day 90. From day 95 this fell sharply and reached values of < 0.1 g COD L^{-1} shortly after D6.

Despite these increases in VFA, the other monitoring parameters remained relatively stable. TAN fell from an initial value of 1.5 g N kg^{-1} to stabilise after day 89 at around 1.0 g N kg^{-1}, apart from a brief downward excursion in D7 between days 145-174 (Figure 64c). TA declined from around 11.5 g

$CaCO_3$ kg^{-1} at the start of the run until day 89 (Figure 64d), then increased slightly to stabilise at around 10 g $CaCO_3$ kg^{-1} in all digesters while feeding continued, with a small dip in D7 matching the fall in TAN in the same period. PA followed a similar trend to TA, with a small decrease in D5 while total VFA concentrations had plateaued or were falling (Figure 64e). IA was stable until day 61, at around 3 g $CaCO_3$ kg^{-1}. Over the next 4-5 weeks it increased to a slightly different degree in each digester, reflecting the accumulation of VFA in each case, before starting to decline (Figure 64f). By the end of the run IA was around 2.5 g $CaCO_3$ kg^{-1}.

These changes in IA and PA led to a rise in IA/PA ratio from 0.4 up to 0.7 during the period of higher VFA concentrations, indicating some instability; the ratio then returned to around 0.4 (Figure 64g). pH fell slightly in all digesters in the first few weeks of operation, increased briefly from day 132-159, but then stabilised at around 7.5 until the end of feeding (Figure 64h). These parameters indicated that the additional TE dosing interventions undertaken to prevent further VFA accumulation were successful in preventing any serious instability.

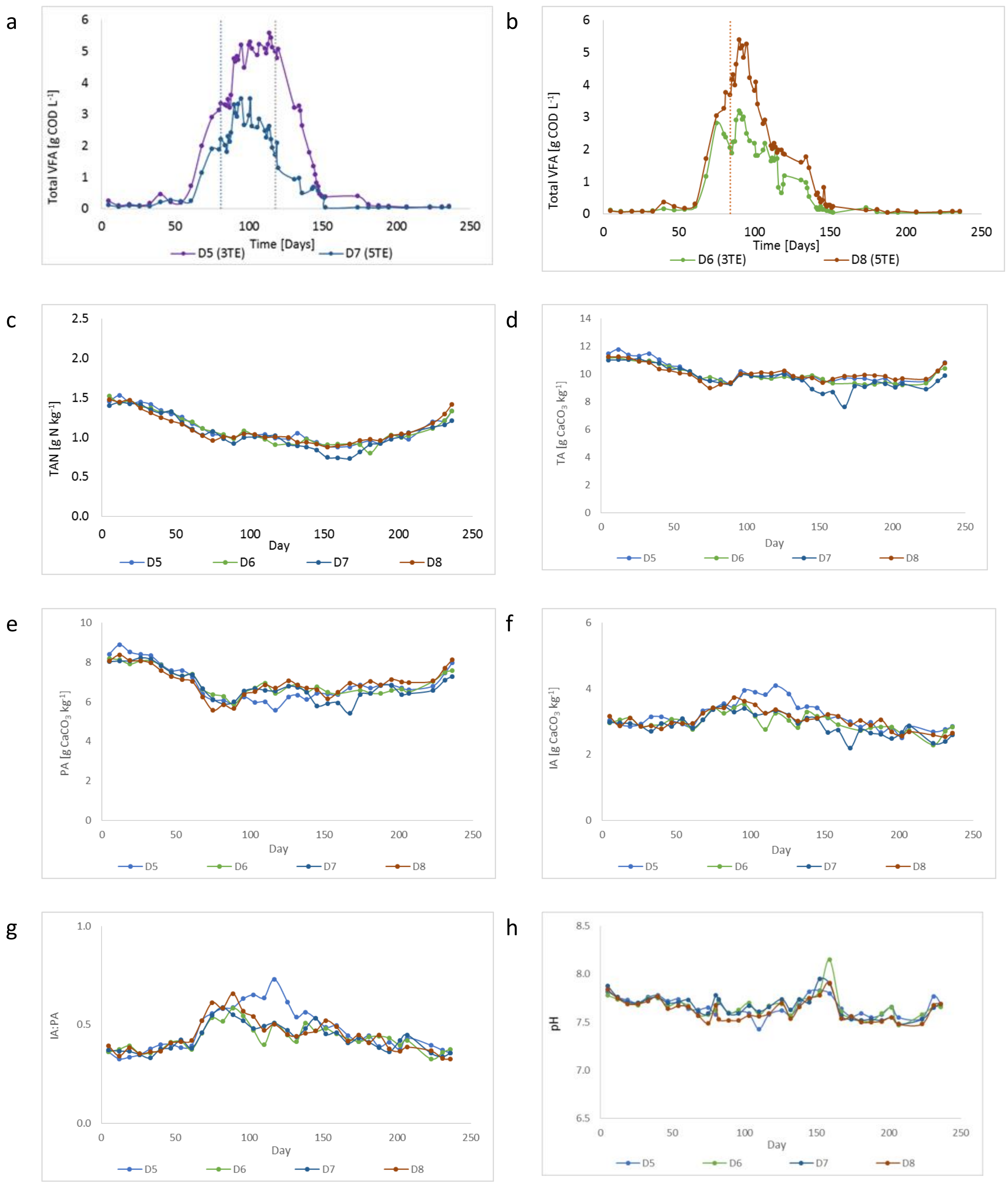


Figure 64. Total VFA, pH, alkalinity and TAN concentrations in D5-8 during digestion trial 2-3 on baby maize stover.

Vertical dotted lines indicated in Table 35 (D5 and D7 3TE on day 81, W on day 118, D6 and D8 W on day 84).

Volatile Fatty acids. Figure 65 shows the VFA profile of the digesters. Acetic acid concentrations were slightly elevated in all digesters until day 61, with a small transient peak at day 40 which also appeared in the propionic acid concentrations in D5, D7 and D8. From day 61 on there was a sharp rise in propionic acid concentrations accompanied by a slower increase in acetic acid.

Addition of the one-off dose of 3TE to D5 and D7 on day 81 caused a brief fall in propionic acid concentrations by day 85 (Figure 65a and b), followed by an increase to 2.5 and 1.7 g L^{-1}, respectively. The initial small fall in propionic acid was matched by a slight rise in acetic acid, which stabilised at around 0.8 and 0.5 g COD L^{-1} in D5 and D7 respectively. At the same time, small amounts of some longer chain VFA appeared, including iso-valeric, iso-butyric and valeric, and n-butyric in order of concentration. The maximum iso-valeric concentration observed was around 0.25 g COD L^{-1} in D5 (3TE). There was a slow decline in propionic acid in D7 between day 100-116, leading to the fall in total VFA in this digester; but apart from this, concentrations of all the VFA species mentioned remained approximately steady until the one-off dose of tungsten was added on day 118. This led to a sharp fall in propionic acid and longer chain VFA in both D5 and D7, followed shortly after by a decrease in acetic acid concentrations. For the remainder of the run, only acetic acid was present at low concentrations (~ 80 mg COD L^{-1}).

In D6 and D8 after the addition of the one-off dose of tungsten on day 84 there was a sharp rise in propionic acid concentration, of around 1.1 g COD L^{-1} in both reactors (Figure 65c and d). Acetic acid concentrations also rose slightly until they reached plateaus of around 0.5 and 0.9 g COD L^{-1} in D6 and D8, respectively. There were also small increases in iso-valeric, iso-butyric, valeric and n-butyric. From day 90 the propionic acid concentration in both reactors fell, sharply at first and then more slowly after it reached about 1.1 g COD L^{-1}. Concentrations of other VFA also fell, with acetic acid the last to reduce. As with D5 and D7, for the last 50 days only acetic acid was present in low concentrations.

Clearly the addition of tungsten alone had a big effect on the VFA accumulation, producing a rapid reduction in propionic acid in D6 and D8. The concentration in D5 (3TE) only began to fall after tungsten addition; the effect in D7 with 5TE plus additional 3TE supplementation was similar but slightly less clear, as propionic acid was already falling slightly when the tungsten was added.

The rise in VFA after the addition of either more 5TE or tungsten may indicate that the hydrolytic or acidogenic population were lacking trace elements, and were able to respond more quickly to the supplementation than the methanogenic population. This phenomena was also observed by Jiang *et al.* (2012) and Song (2016a). In the study by Jiang *et al.* (2012), an increase in acetic and propionic acid after W dosing was also observed. The increase was maintained for 2 weeks and then dropped.

This phenomenon occurred in only one digester in a duplicate pair, however, and Jiang did not discuss the possible causes for the increase.

In the study by Song (2016a), reported Co had positive effects on propionic and acetic acid degradation because Co-depending enzymes were required for propionic acid oxidation and the following methanogenesis. Insufficient Se caused the remaining acid accumulation as Se is required for syntrophic acetic acid oxidisation. In this study, VFA in D7 (regular 5TE and one off 3TE) started to decrease slightly before the W supplementation, perhaps due to the presence of Se and Co.

The fall in propionic acid indicates unblocking of a metabolic pathway. W has been reported to take an active role in propionate degradation (Böck, 2006; Reda *et al.*, 2008; Plugge *et al.*, 2009). Jiang *et al.* (2012) noted W dosing had positive effects for reducing propionic and acetic acid accumulation, pH recovery and slightly higher methane yield. W is a component of FDH, and can assist the metabolism of methanogens growing on CO_2 and H_2 (Zellner *et al.*, 1987; Zellner and Winter, 1987). This indicate W is also important for methanogenesis. On the other hand, W is not included in most of the commonly used TE recipes, and only limited studies have been carried out on the effect of W dosing (Kayhanian and Rich, 1995; Jiang *et al.*, 2012).

It is also possible that the propionate-degrading microorganisms were short of W. Gallert and Winter (2008) noted that the activity of propionate degrading microorganisms was most important to degrade propionate acid accumulation. Xiao *et al.* (2015) reported adenosine triphosphate (ATP) reduction rate was higher when propionic acid accumulation (4585 mg L^{-1}) was degraded. Zamanzadeh *et al.* (2013) also reported higher propionic acid accumulation in thermophilic digestion was attributed to low affinities of propionate-degrading microorganisms. The higher propionic acid accumulation in D5-8, Trial 2-3 may indicate the propionate-degrading microorganisms activity or population were poor. W dosing may help to increase ATP.

The propionate-degrading microorganisms were reported as growing slowly and as being sensitive to pH (Pind *et al.*, 2003). The optimal pH range was 6.8 – 8.5 (Boone and Xun, 1987) but pH in D5-8 remained over 7.5 in Trial 2-3. The propionate degradation was thus not inhibited by pH.

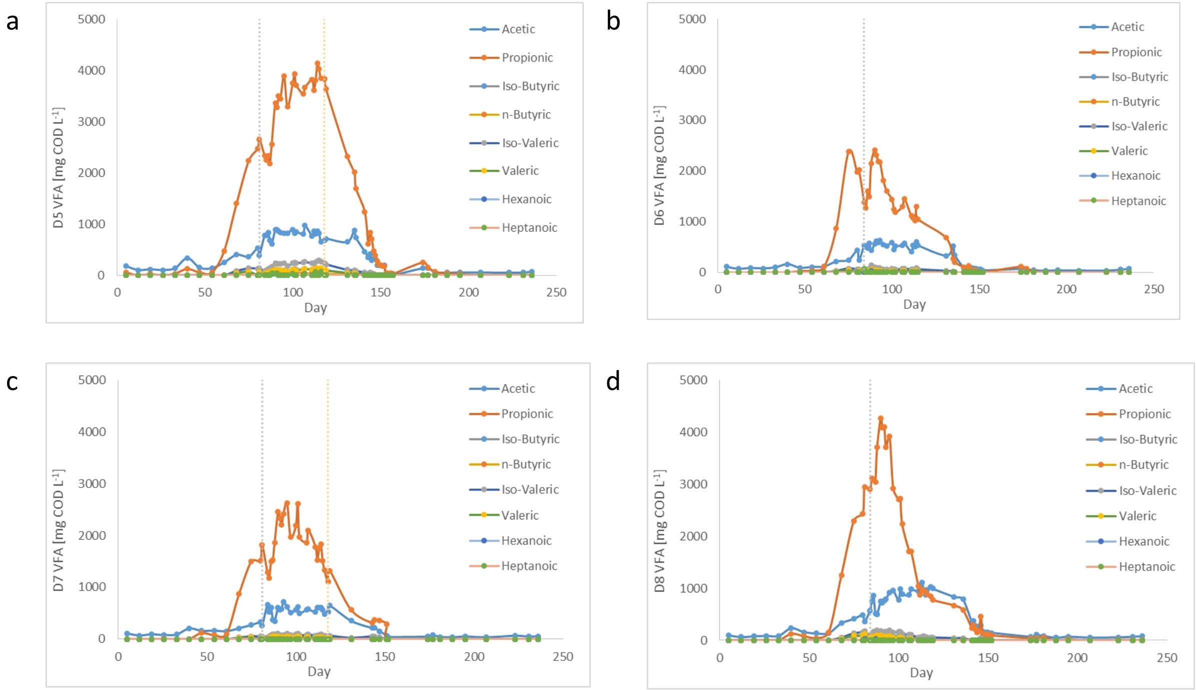


Figure 65. VFA profiles for D5-8 during digestion Trial 2-3 on baby maize stover

Biogas and methane production. Biogas and methane production and biogas methane content are shown in Figure 66. The thermophilic digesters responded well to the increase in OLR between day 13-62, with VBP and VMP rising in proportion to the applied load. On day 40, D5-8 were not fed due to an unintended feed break. On day 81, the digesters were not fed due to VFA accumulation, and therefore gas production decreased on day 41 and day 82. The duplicate pairs of digesters showed good similarity. Biogas methane content appeared to decrease slightly on day 77, and stabilised at around 54 % by the end of the run. Average gas production values during pseudo-steady state periods are given in Table 40 with values for other monitoring parameters.

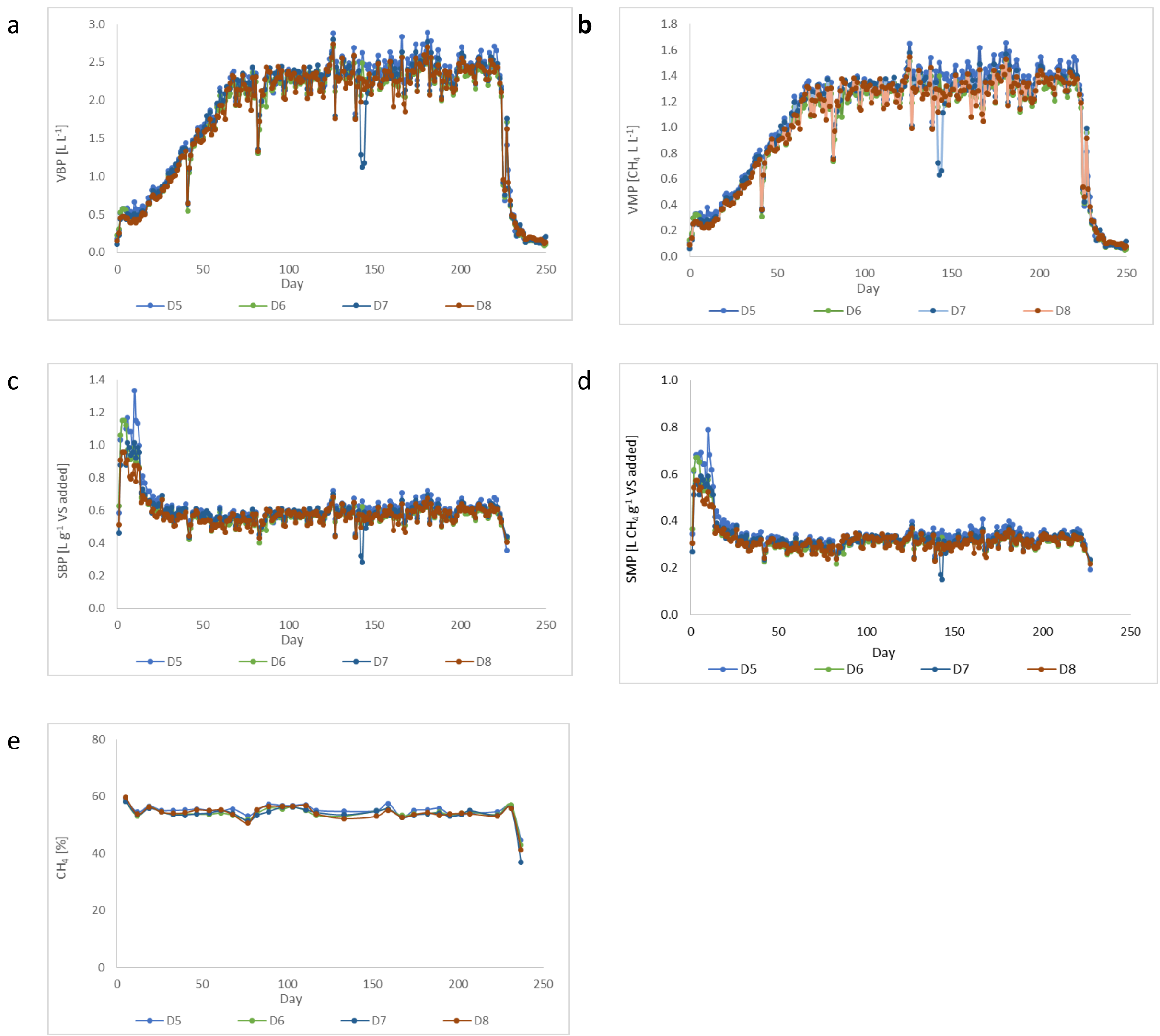


Figure 66. VBP, SBP, VMP and SMP for D5-8 during digestion Trial 2-3 on baby maize stover

Solids parameters and viscosity. Figure 67 shows the solids parameters for the digester. The TS and VS content in D5-8 increased slightly from the start of the run, and stabilised by around day 80. The VS/TS ratio fluctuated and the value in D5&6 was less than in D7&8 until day 61 when VFA accumulation appeared. VS destruction showed some variability but appeared to be stabilising at around 70%. The TS and VS decreased after day 227 reflecting the cessation of feeding. To compare with D5-8 in Trial 2-1, the difference was the solids parameter in Trial 2-2 did not decrease constantly as in Trial 2-1. This indicates the digestate in Trial 2-3 worked well for anaerobic processing of baby maize stover.

Viscosity analysis started from day 75. Viscosity in D7&8 was slightly greater than in D5&6 but was always less than 500 cP.

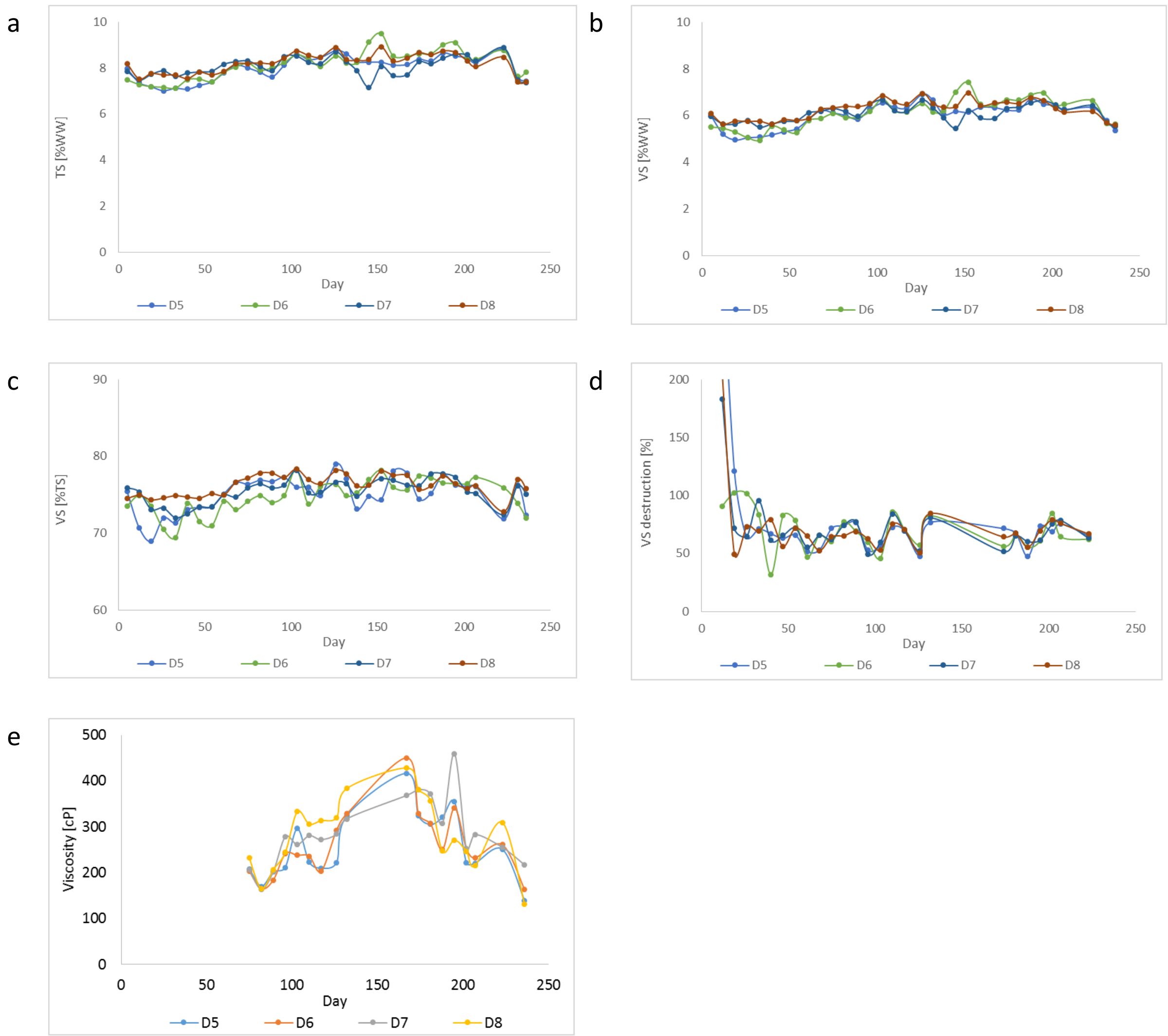


Figure 67. Digestate solids parameters for D5-8 during digestion trial 2-3 on baby maize stover

Discussion of results for thermophilic digesters. Table 37 shows the monitoring parameter values for thermophilic digestion during pseudo-steady state periods.

The noteworthy VFA accumulation happened from day 61. To respond to this, one-off TE dosing was carried out. On day 81, D5&7 received Fe, Co and Ni. On day 84, D6&8 received W. On day 118, D5&7 received W. Each digester showed a different pattern of VFA accumulation due to additional TE dose.

Adding TE (either 3TE mix or W) first made the VFA increase. This may indicate hydrolytic or acidogenic microorganisms were lacking TE, and responded more quickly than the methanogenic population. This phenomenon was also observed by Song (2016a) and Jiang *et al.* (2012).

With addition of W only the VFA then fell. With addition of 3TE, VFA concentrations plateaued and only fell after W was added. Concentrations in D7 which received 5TE weekly and one off 3TE dosing started to decrease slightly before the W dose. Se and Co syntrophic reaction was reported by Song (2016a). The dose of 5 TE and 3 TE helped the degradation of propionic.

A decrease in VFA concentrations after W dosing was also reported by Jiang *et al.* (2012). Facchin *et al.* (2013) also reported W supplementation increased methane yield. W is component of FDH so it is possible to aid acetogenesis and hydrogenotrophic methanogenesis (Zellner and Winter, 1987; Kayhanian and Rich, 1995). On the other hand, the commonly used TE mix recipe did not include W, and the effects of W have not been extensively studied (Jiang *et al.*, 2012; Facchin *et al.*, 2013).

The VFA accumulation was dominated by propionic acid which indicates propionate-degrading microorganisms population or activity were limited. The reported propionate microorganisms are *Syntrophobacter wolini* (Boone and Bryant, 1980), *Smithella propionica* (Liu *et al.*, 1999), *Desulfobulbus* (Kremer and Hansen, 1988) and *Desulfacinum hydrothermale* (Sievert and Kuever, 2000). The degradation of accumulated propionic acids depends on these microorganisms (Gallert and Winter, 2008). These microorganisms grow slowly and are sensitive to pH (Pind *et al.*, 2003). The optimal pH range is 6.8 – 8.5 (Boone and Xun, 1987) but pH in D5-8 remained over 7.5 in Trial 2-3. Thus the propionate degradation was not prohibited by pH.

Xiao *et al.* (2015) reported ATP reduction rate was higher when higher propionic acid accumulation (4585 mg L^{-1}) was degraded. Zamanzadeh *et al.* (2013) also reported higher propionic acid accumulation in thermophilic digestion was attributed to low affinities of propionate-degrading microorganisms. Insufficient ATP or affinities may cause the limited propionate degrading microorganisms activity.

It is clear that W supplementation had positive effects to degrade VFA accumulation. There was no significant difference between the pair of digesters in terms of gas production, alkalinity, ammonia, TS, VS and VFA. The trial completed 4.7 HRT with stable operation restored after W addition.

Table 37. Monitoring parameter values for thermophilic digestion pseudo-steady state periods in Trial 2-3

Parameter	Unit	D5 [c]	D6 [c]	D7 [c]	D8 [c]	Ave [a]	Ave [a]
		T + 3TE	T + 3TE	T + 5TE	T + 5TE	T + 3TE	T + 5TE
OLR	g VS L^{-1} day^{-1}	4	4	4	4	4	4
SBP	L g^{-1} VS	0.602	0.590	0.603	0.594	0.596	0.599
SMP	L CH_4 g^{-1} VS	0.325	0.320	0.327	0.320	0.323	0.324
VBP	L L^{-1} day^{-1}	2.41	2.36	2.41	2.38	2.38	2.39
VMP	L CH_4 L^{-1} day^{-1}	1.38	1.33	1.36	1.34	1.35	1.35
CH_4 content	% v/v	53.8	54.1	53.9	53.9	54.0	53.9
Digestate TS	%WW	8.4	8.6	8.5	8.3	8.5	8.4
Digestate VS	%WW	6.4	6.6	6.4	6.4	6.5	6.4
VS destruction	%VS	76.1	76.7	75.9	76.1	76.4	76.0
Viscosity	cP	262	272	313	261	267	287
pH	–	7.60	7.57	7.52	7.51	7.6	7.5
TA	g $CaCO_3$ kg^{-1} WW	9.4	9.3	9.2	9.7	9.4	9.5
PA	g $CaCO_3$ kg^{-1} WW	6.7	6.6	6.5	7.0	6.6	6.8
IA	g $CaCO_3$ kg^{-1} WW	2.7	2.7	2.7	2.6	2.7	2.7
IA/PA ratio	–	0.41	0.42	0.41	0.38	0.41	0.39
TAN	g N kg^{-1} WW	1.01	1.02	1.01	1.04	1.01	1.03
Total VFA	g COD L^{-1}	0.07	0.04	0.04	0.07	0.05	0.06

[c] Average value for days 193-222

4.5.4. Discussion of all results from Trial 2-3

Table 38 summarises average values for some of the key performance parameter obtained during periods of apparently stable operation for all parts of Trial 2-3.

SMP in thermophilic digesters were 0.324-0.325 L CH_4 g^{-1} VS. SMP in mesophilic digesters with pre-hydrolysis showed the lowest value of 0.194 L CH_4 g^{-1} VS. Even when the SMP from pre-hydrolysis was added to that from the main digester, the total was only 0.287 L CH_4 g^{-1} VS which was the lowest pseudo-steady value achieved at OLR 3 g VS L^{-1} day^{-1}. SMP in mesophilic digesters without TE was 0.311 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} and 0.236 L CH_4 g^{-1} VS at OLR 4 g VS L^{-1} day^{-1}, with the latter value clearly reflecting the onset of VFA accumulation and instability. In contrast, in mesophilic digesters with 5TE supplementation the SMP value was 0.333 L CH_4 g^{-1} VS which was close to that of thermophilic digesters. The 5 TE (Fe, Co, Ni, Se, Mo) supplementation clearly increased SMP in mesophilic digesters and delayed the onset of VFA accumulation, but was not enough to ensure stable operation.

VFA accumulation was observed in both mesophilic and thermophilic digesters. The 3TE dose caused an increase in VFA accumulation but W dose had positive effects for VFA degradation. That indicates these digesters were lacking in W for anaerobic processing of baby maize stover, or in some other trace element for which W can act as a substitute. Very few studies on the requirement for W have been reported in the literature, but the fact that Jiang *et al.* (2012) also found tungsten had a stimulatory effect in digestion of crops from this location may indicate that there is a particular deficiency of specific trace elements in this region. This should be taken into account in operation of large-scale AD plants on local agro-wastes, especially in planning the TE addition strategy or if there is any sign of a decline in digestion performance.

Viscosity was much greater in the mesophilic digesters than in the thermophilic digesters (Figure 68). The higher viscosity in mesophilic digesters indicates the potential for gas hold up due to accumulation of undigested fibrous material in digestate, although no foaming was seen in this trial. TE addition in both mesophilic and thermophilic digesters less foaming was also observed by Karlsson *et al.* (2012).

The viscosity of mesophilic digesters with pre-hydrolysis was lower than in single mesophilic digesters, and almost the same as that of the thermophilic digestate. Stoyanova *et al.* (2014) also reported the viscosity of digestate from two stage AD was lower than from single stage and that lower viscosity was due to pectin degradation.

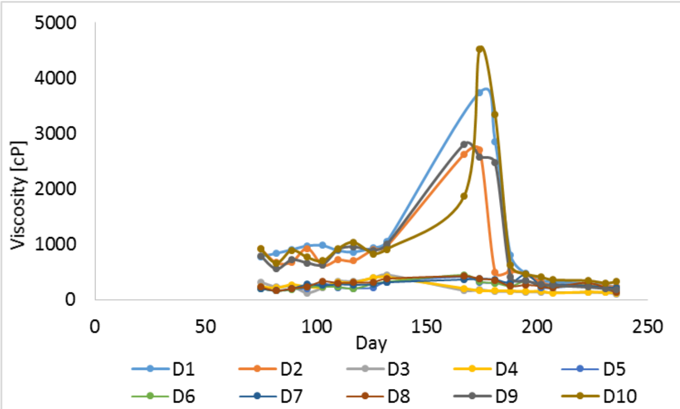


Figure 68. Viscosity in all digesters

A difference between mesophilic and thermophilic digestion was also observed in ammonia concentration. Thermophilic digesters showed greater TAN 1.0-1.03 g N kg^{-1} than mesophilic digesters. The lowest value was found in the mesophilic digesters with pre-hydrolysis 0.54 g N kg^{-1}. TAN concentration is influenced by a number of factors including degree of substrate degradation, biomass uptake, OLR and HRT (Roberts *et al.*, 2016a), and the reason for this difference is not known.

Trial 2-3 showed more stable operation than Trial 2-1 and 2-2, especially in the mesophilic digesters. In Trial 2-1 and 2-2, it was not possible to start directly at an OLR of 3 g VS L^{-1} day^{-1} with Millbrook inoculum as was done in Trial 1. The OLR on Millbrook AD plant was possibly close to 3 g VS L^{-1} day^{-1} but the feedstock (municipal wastewater biosolids or sewage sludge) was very different from maize-derived substrates. Sewage sludge is much less degradable than maize silage or baby maize stover. Although the applied OLR was similar the actual OLR in terms of g VS destroyed was therefore probably higher, and this may also suggest the need for acclimatisation.

Taken together, 5 TE (Fe, Co, Ni, Se, Mo) and W supplementation helped greater methane yield and stability. Without TE supplementation, SMP in mesophilic digesters was substantially less than that of thermophilic digesters. 5TE supplementation brought mesophilic digestion SMP up to the value in thermophilic digesters. W dosing caused VFA degradation in thermophilic digesters: it would be interesting to know if it has a similar effect in mesophilic digestion but this was not tested in the current trial. In terms of SMP, pre-hydrolysis did not have any positive effects. Acclimatisation to the feedstock was essential for anaerobic processing of baby maize stover.

Table 38. Average values for reporting parameters during pseudo-steady state periods in digestion Trial 2-3 with baby maize stover

Parameter	Unit	M + pre[a]	M [a]	M + 5TE[a]	M [b]	T + 3TE [b]	T + 5TE [b]
OLR	g VS L^{-1} day^{-1}	3	3	3	4	4	4
SBP	L g^{-1} VS	0.365	0.570	0.610	0.474	0.596	0.599
SMP	L CH_4 g^{-1} VS	0.194	0.311	0.333	0.236	0.323	0.324
SMP from pre	L CH_4 g^{-1} VS	0.093	–	–	–	–	–
SHP from pre	L H_2 g^{-1} VS	0.001	–	–	–	–	–
VBP	L L^{-1} day^{-1}	1.09	1.71	1.83	1.88	2.38	2.39
VMP	L CH_4 L^{-1} day^{-1}	0.61	0.96	1.02	1.05	1.35	1.35
CH_4 content	% v/v	53.0	54.4	54.6	52.9	54.0	53.9
Digestate TS	%WW	6.8	7.5	7.6	8.9	8.5	8.4
Digestate VS	%WW	5.1	5.7	5.8	6.8	6.5	6.4
VS destruction	%VS	74.8	76.0	76.2	76.9	76.4	76.0
Viscosity	cP	265	826	837	958	267	287
pH	–	7.3	7.4	7.4	7.4	7.6	7.5
TA	g $CaCO_3$ kg^{-1} WW	7.7	8.9	8.5	7.9	9.4	9.5
PA	g $CaCO_3$ kg^{-1} WW	5.1	6.0	5.6	5.0	6.6	6.8
IA	g $CaCO_3$ kg^{-1} WW	2.6	2.9	2.9	2.9	2.7	2.7
IA/PA ratio	–	0.51	0.48	0.52	0.61	0.41	0.39
TAN	g N kg^{-1} WW	0.54	0.95	0.81	0.55	1.01	1.03
Total VFA	g COD L^{-1}	0.05	0.10	0.10	2.22	0.05	0.06

[a] Average value for days 91-120

[b] Average value for days 137-166

[c] Average value for days 193-222

4.6. Conical flask test for digestion Trial 2-3

In Trial 2-3, it seemed that additional TE dosing (Co, Fe, Ni, W) had positive impacts on VFA accumulation. The history of each pair of digesters was not exactly the same (Table 39), and therefore the results were not available in duplicate making it more difficult to draw firm conclusions. In this trial, batch samples of digestate was spiked with acetate and propionate and changes in VFA concentration were observed. The TE dose impacts was assessed by the decrease of acetate and propionate.

Table 39. Summary of trace elements dosing history of thermophilic digesters in Trial 2-3

		D5	D6	D7	D8
Regular dose		Fe, Co, Ni	Fe, Co, Ni	Fe, Co, Ni, Se, Mo	Fe, Co, Ni, Se, Mo
One off dose	Day 81	Fe, Co, Ni		Fe, Co, Ni	
	Day 84		W		W
	Day 118	W		W	

4.6.1. Objectives and methodology

Objective. The aim of this trial was to assess the impacts of TE dosing to digestate form the thermophilic digesters from Trial 2-3

Methodology. The tests were carried out in an orbital shaking incubator at a thermophilic (55 ± 1 ºC) temperature and arranged as follows: 12× 250-ml Erlenmeyer flasks were used each filled with 200 mL of VFA-supplemented digestate liquor taken from digesters D5-8 after the end of Trial 2-3. The inoculum digestate was also spiked with sodium acetate and sodium propionate. On day 0, 200 mL of digestate was spiked with 2 mL of 400 g L^{-1} sodium acetate or sodium propionate to make the concentration of each acid up to 4000 mg L^{-1}. In COD unit, it was assumed propionic acid 6047 mg COD L^{-1}, 4263 mg COD L^{-1}. Tests were carried out in triplicate.

The headspace of each flask was flushed with a carbon dioxide and nitrogen (20:80) mixture (BOC, UK) before the flasks were sealed with rubber bungs with an outlet connection to a 1-L gas sampling bag (Tedlar, SKC Ltd, UK) to collect generated gas, maintain the system at ambient pressure and keep it under anaerobic conditions. The flasks were then randomised and incubated in an orbital incubator (Weiss-Gallenkamp, UK) at a constant temperature of 55 °C and agitated at 60 RPM. Digestate in each flask was regularly sampled from the flasks and analysed at certain intervals to monitor the VFA degradation until all VFAs were consumed. After sampling, the flasks are flushed with carbon dioxide and nitrogen (20:80) and sealed again before being returned to the incubator. The experiment continued until all VFAs in flasks were depleted.

4.6.2. Results

First trial

The first trial compared digestate from D5 and D6. The digestate was collected on day 283 in Trial 2-3. D5 and D6 had received Fe, Co and Ni weekly to maintain the TE concentration, in proportion to the added substrate. Additional one-off TE dosing was carried out due to VFA accumulation. D5 received a one-off 10 mg L^{-1} Fe, 0.4 mg L^{-1}Co, and 1 mg L^{-1} Ni on day 81, 0.2 mg L^{-1} W on day 118. D6 received 0.2 mg L^{-1} W on day 84 (Table 41).

Figure 69 shows the results of batch flask trials, VFA degradation.

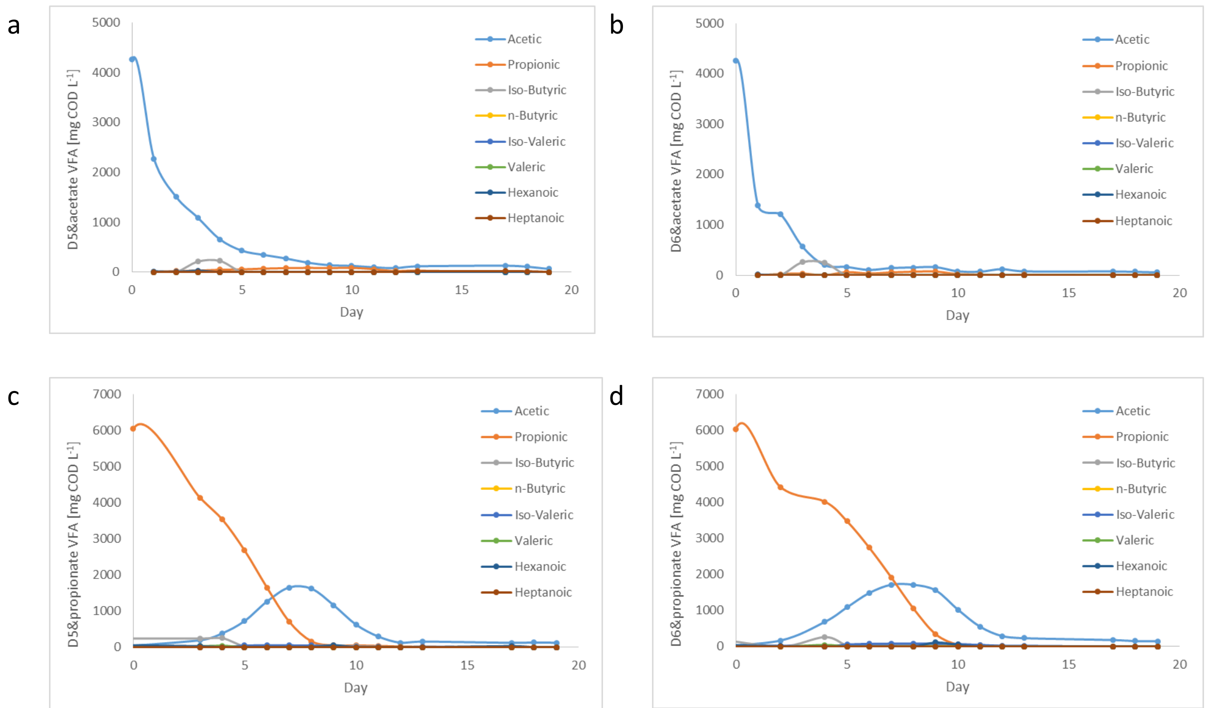


Figure 69. VFA profiles in D5&6 with sodium acetate (a and b) and propionate (c and d) for digestate samples takes on day 283

The concentration of the spiked acetate decreased rapidly in both D5 and D6 (Figure 69a and b), but faster in D6 which received the one-off dose of W earlier than D5. A small increase in iso-butyric acid was observed in the flask tests for both reactors on day 3 and 4.

Turning to sodium propionate, D5 and D6's propionic concentration decreased which was accompanied by acetic acid accumulation (Figure 69c and d). The difference between D5 and D6 was D6's acetic acid concentration rose more quickly after sodium propionate dose.

Second trial

The second trial compared digestates from D5-8. The digestates were collected on day 342 in trial 2-3. D5&6 had received Fe, Co and Ni and D7&8 received Fe, Co, Ni, Se, Mo weekly to maintain the TE concentration in proportion to the added substrate. Additional one-off TE dosing was carried out due to VFA accumulation. D5&7 receive additional Fe, Co, and Ni on day 81, and W on day 118. D6&8 received only W on day 84.

Figure 70 (acetate dose) and 71 (propionate dose) show the results of batch flask trials, VFA degradation. The digestate were spiked with sodium acetate and sodium propionate, however, dose amount was 10% of first trial due to a mistake in making up the concentrated solution. Only D5 and D6 were spiked with the expected sodium acetate.

Regarding sodium acetate dose, it might be difficult to compare D5&6 (Figure 70a and b) and D7&8 (Figure 70c and d) because dose amount was different. Acetic acid in D5&6 dropped to around 1000 mg COD L^{-1} on day 2 then disappeared on day 7. This phenomena was also observed in D7&8.

Turning to sodium propionate, propionic acid in D6&D8 quickly disappeared within 6 days. In contrast, D5&7 took 8 days and increase of acetic acid was observed which was not shown in D6&8 (Figure 71a-d). D6&8 received W 35 days earlier than D5&7 in Trial 2-3 and VFA accumulation fell only after W dosing. D5&7 experienced higher VFA accumulation for a longer time than D6&8, which may have negative effects on the microbial population, leading to imbalance of AD process in D5&7. That indicates earlier W has positive effects for propionic acid degradation.

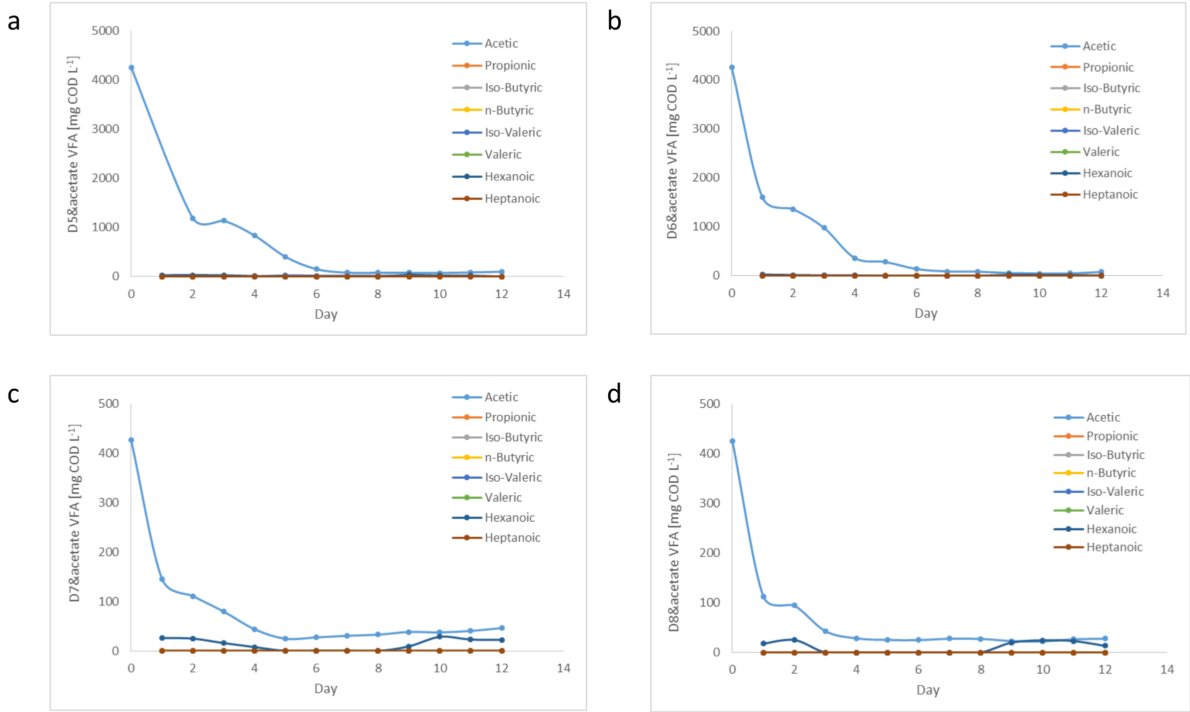


Figure 70. VFA profiles in D5-8 with sodium acetate

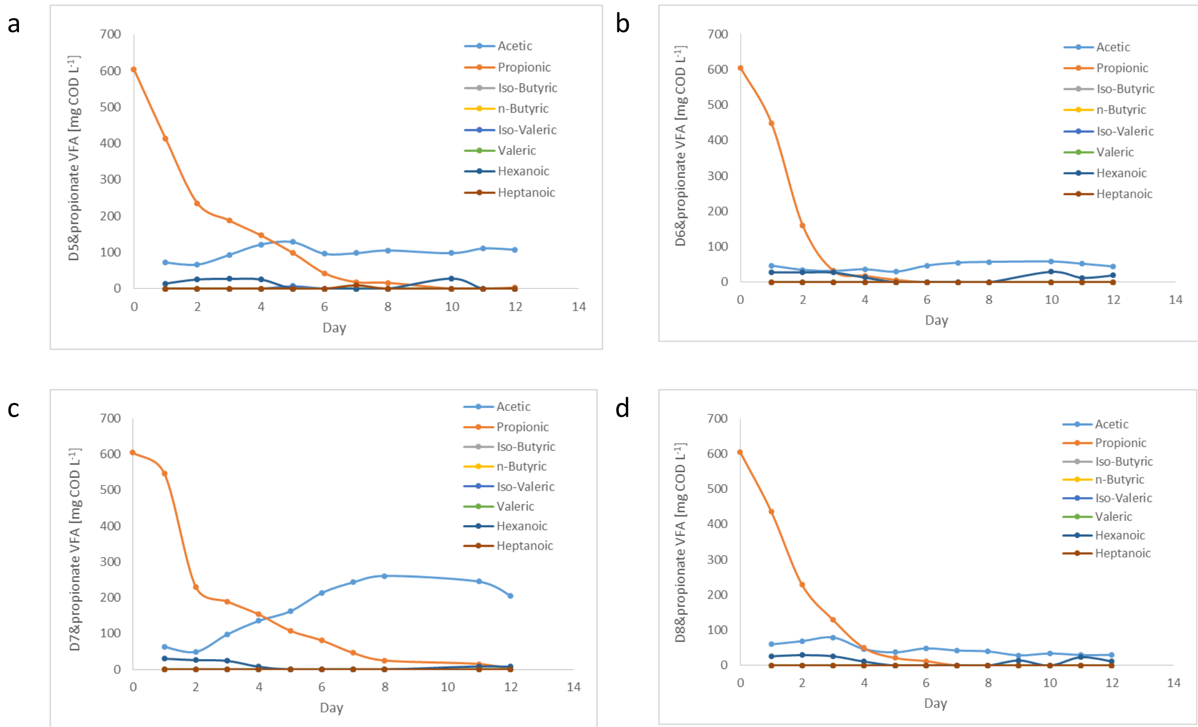


Figure 71. D5-8 with propionate

4.6.3. Discussion

In first trial, the acetic acid concentration in D6 fell more rapidly than in D5. In second trial, the digestate with sodium propionate dosing, D5&7 and D6&8 showed some differences. D5&D7 showed a higher acetic acid concentration and took more time to degrade propionic acid than D6&8. D6&8 received W 35 days earlier.

To discuss first and second trial, it seems earlier W dose might be effective quick VFA degradation, however, data from first trial and second trial were slightly different. This may be related to the date on which the digestate was collected. First trial collected the digestate on day 283, second trial was day 342. Microorganisms change the digestate slightly every day as their nature. Each sample was triplicate and showed similar results.

Jiang *et al.* (2012) reported W supplementation caused VFA degradation and slightly higher methane production and Facchin *et al.* (2013) noted W dosing increased methane yield. W is a component of FDH. FDH is enzyme to degrade formate, not propionic acid. W may work not only for FDH but also other enzymes, acidogens and methanogens. Only limited studies have been conducted the effects of W supplementation so the chemical reaction is not understood.

To sum up, batch assays for D6&8 responded better than D5&7 as they had a shorter period of VFA accumulation which is likely to have disturbed the microbial population.

4.7. Energy conversion efficiency

Measured calorific value and theoretical methane potential value are shown in Table 40 to compare with the SMP results and VS destruction. Measured calorific value was calculated from the bomb calorimetry analysis. Theoretical value was calculated from the Buswell equation and the Boie equation. BMP test was conducted. The experiment results are from Trial 2-3, Table 40. The greatest calorific value (CV) was found in measured CV by bomb calorimetry analysis, second greatest was theoretical methane potential, third greatest was BMP and then experimental results. This indicates the analysis was conducted successfully.

Mesophilic digestion with baby maize stover showed significant differences in performance in different operating conditions as noted above. The theoretical methane potential energy recovery of the mesophilic digestion with pre-hydrolysis (Buswell) was only 40.1 %. Measured CV recovery was slightly lower 35 % and lowest VS destruction 74.8 % was also observed. The energy recovery in single mesophilic digestion without TE was 64.3 % which was greater than that of two stage, but could not achieve the value in thermophilic digestion 66.7-66.9 %. The energy recovery in mesophilic digester was 68.9 % which was close to the value in thermophilic digesters. This results indicate that appropriate TE supplementation could increase the energy recovery in mesophilic digestion.

Table 40. Energy recovery value for methane production

	g VS L^{-1} day^{-1}	L CH_4 g^{-1} VS	kJ g^{-1} VS	% measured CV	% ThMP(Boie)	% ThMP(Buswell)	% BMP	% VS destruction
Measured CV (HHV)[a]	–	0.554	22.1	–	–	–	–	–
ThMP(Boie)[b]	–	0.505	20.1	–	–	–	–	–
ThMP(Buswell)[b]	–	0.484	19.3	–	–	–	–	–
BMP[c]	–	0.405	16.1	–	–	–	–	–
SMP mesophilic + pre[d]	3	0.194	7.7	35.0	38.4	40.1	47.9	74.8
SMP mesophilic without TE [d]	3	0.311	12.4	56.2	61.7	64.3	76.8	76.0
SMP mesophilic with 5 TE[d]	3	0.333	13.3	60.2	66.1	68.9	82.3	76.2
SMP mesophilic without TE[d]	4	0.236	9.4	42.7	46.9	48.9	58.4	76.9
SMP thermophilic with 3TE[d]	4	0.323	12.9	58.3	64.0	66.7	79.7	76.4
SMP thermophilic with 5 TE[d]	4	0.324	12.9	58.5	64.2	66.9	80.0	76.0

[a] Measured CV was obtained by bomb calorimetry analysis

[b] Theoretical methane potential (ThMP) calculated from the Buswell equation or Boie equation

[c] Measured SMP in BMP test

[d] Measured SMP during stable operation in Trial 2-3; the values from Table 40

4.8. Discussion for result of experimental work

This study conducted mesophilic digestion and thermophilic digestion for anaerobic processing of baby maize stover. In Trial 2-1, digesters received 3 TE (Fe, Co, Ni), and the SMP in thermophilic digestion was much higher than in mesophilic digestion so it seemed that the higher temperature was better for higher methane yield. In contrast, in Trial 2-3, the SMP in mesophilic digesters with 5 TE (Fe, Co, Ni, Se, Mo) supplementation was about equal to that of thermophilic digesters with 3 TE and 5 TE. When digesters received appropriate TE, SMP could overcome the temperature difference. In this case, thermophilic digestion did not appear to have any advantages in terms of gas production.

Mesophilic digestion and mesophilic digestion with thermophilic pre-hydrolysis was also compared. Digestion trial carried out in Trial 2-1, 2-2, 2-3. Every trial, operating condition was changed and higher SMP was obtained but the 2 stage system always showed less SMP than single stage. Hydrogen and methane production were observed from pre-hydrolysis step but these production could not cover the SMP difference.

These digestion trials indicate the requirement of TE for baby maize stover. In Trial 2-3, 5 TE was helpful for gas production in mesophilic conditions but could not prevent VFA accumulation. In thermophilic conditions there was no strong difference between 3 TE and 5 TE supplementation in terms of gas production, and increasing the dose of 3TE had some effect on VFA accumulation, but W addition was necessary to cause a fall in accumulated VFA. Se and W were at very low concentrations or not detected in baby maize stover so these TE supplementation were more essential.

Maize stover is a suitable substrate for AD. The SMP from Trial 2-3 were 0.333 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} under mesophilic condition, 0.324 L CH_4 g^{-1} VS at OLR 4 g VS L^{-1} day^{-1} under thermophilic condition. The SMP value is higher than that of maize stover in literatures; 0.188-0.248 L CH_4 g^{-1} VS (Li *et al.*, 2017), 0.141-0.257 L CH_4 g^{-1} VS (Zhong *et al.*, 2011), 0.134-0.167 L CH_4 g^{-1} VS (Strang *et al.*, 2017), 0.124 L CH_4 g^{-1} VS (Brown *et al.*, 2012). The SMP value was almost same to the SMP of maize silage; 0.280-0.334, 0.268-0.336, 0.322, 0.287-0.326 L CH_4 g^{-1} VS (Amon *et al.*, 2007), 0.345 L CH_4 g^{-1} VS (Bauer *et al.*, 2010), 0.331 L CH_4 g^{-1} VS (Cornell, 2011), 0.139-0.429 L CH_4 g^{-1} VS (Evranos and Demirel, 2015).

FAO reported baby maize stover could be utilised for animal feeding as stover was acceptable and palatable in sustainable manner, and a substantial amount was available (Wadhwa, 2013) but this technique was not adopted by farmers due to the failure to demonstrate cost-effectiveness (Devendra and Sevilla, 2002). Devendra and Sevilla reported the weaknesses of the baby maize stover for animal feeding were low digestibility, low crude protein which caused poor productivity in animals. Therefore, farmers had to buy concentrations or supplementations to increase nutrition of feeding. Nouala studied supplementation and used baby maize stover as basic animal feed in Africa (Nouala *et al.*, 2004; Nouala *et al.*, 2008; Nouala *et al.*, 2009). These studies indicate the use of baby maize stover alone for animal feeding was difficult, especially in developing countries in Africa.

The government of Kenya has supports and increase in the number of AD plants and tried to generate more energy from renewable sources (MoEP, 2013). If baby maize stover was used for AD, bioenergy and digestate as fertiliser could be obtained. The digestate from biogas plant increased yield and nutritional quality of baby maize (Malav *et al.*, 2015). The baby maize stover is a suitable substrate for AD and may work especially in Africa.

5. Modelling

Chapter 5 consists of 7 sections. Section 5.1 provides a background to the work carried out. Section 5.2 provides basic information about the biogas plant in Kenya, and sets sub-objectives to achieve the main objectives given in section 1.3.2. Sections 5.3 to 5.7 cover the 5 sub objectives. Section 5.3 is on harvesting, 5.4 is about the digester, 5.5 is about digestate application, and 5.6 provides energy balance calculations for harvesting, digester and digestate application. 5.7 is for rationalisation of the plant design to overcome the issues identified in sections 5.3 to 5.6. Section 5.9 summarises the conclusions of the work in the context of the issues presented in the introduction.

5.1. Introduction

As mentioned in the literature review, there is an increasingly widespread view that crops should not be grown for energy production only. In some cases the debate has extended to the economics and ethics of production of high-value crops for export in low income and developing countries and the sustainability of large-scale agriculture of this type (Swinnen and Maertens, 2007; Ashraf *et al.*, 2009). This research did not examine ethical, ecological or economic issues, but the following general and specific points can be made in relation to the scenario modelled in this chapter:

- low income countries need ways to increase incomes and so in the present world economic system, the growth of high value export crops can be seen as beneficial to local economies and people

- more specifically, in the Naivasha area of Kenya no crops can be grown without irrigation. Irrigation is expensive in both energy and financial terms, and therefore only high value crops are economically justifiable. Baby maize also has the advantage of relatively low water demand (REUTERS, 2017)

- in the Naivasha area, as in much of Kenya and elsewhere in sub-Saharan Africa, soils are poor and crops cannot be grown without fertiliser; this is also expensive, so again only high value crops can be justified

- anaerobic digestion of baby maize stover allows return of digestate to the soil as a fertiliser and soil conditioner, thus reducing the need for external fertiliser inputs. Although part of the crop is exported, local use of digestate for land application also helps to move the system towards a closed loop system

- this type of farm typically employs 500 or more workers in a region with a shortage of employment

For these reasons organisations in Kenya and elsewhere in sub-Saharan Africa have tried to promote cultivation of baby maize (Kuzablog, 2016; Africa, undated), and this means it is likely that baby maize production will continue and that baby maize stover will be available as an agro-waste.

The idea of using baby maize stover as an animal feed is attractive but, as noted in the literature review, trials indicate it may not be a good source of animal nutrition and will require expensive supplements that local farmers cannot afford. The pattern found in this part of Kenya and elsewhere of relatively large commercial farms rather than smallholdings is also well suited to setting up and operating anaerobic digesters to process baby maize stover: this would not work at very small scale without cooperation between many farmers.

These points are qualitative, and are not direct results of the modelling or laboratory studies. The work in this chapter cannot answer broader ethical or economic questions: but it does provide data and results that can be used as a basis for deciding whether to build biogas plants and to encourage anaerobic digestion of baby maize stover for renewable energy production in Kenya.

In this section of the thesis, the results of the laboratory experimental work were combined with data obtained from Gorge Farm in Naivasha, Kenya to evaluate the overall energy balance of the system and to use this to provide insight onto whether anaerobic digestion of baby maize stover is a good option for Kenya. Data for the period August 2015 – July 2016 on crop production, number of hectares of harvested crop production, digester loading rates, diesel consumption, produced biogas volume, methane concentration, CHP run time, generated electricity, sold electricity was supplied by Tropical Power from Gorge Farm site records. Other data used in modelling were obtained from the literature or the experimental results.

5.2. Basic information on the Tropical Power biogas plant in Kenya

Tropical Power Ltd built and opened the first and largest grid-connected biogas plant in Africa in 2015. The anaerobic digestion plant is located at Gorge Farm energy park, Naivasha, Kenya at a longitude of 36.37208 E (36° 22' 20" E) latitude -0.84905 S (0° 50' 57" S) and grid location of KI89ED (http://qthlocator.free.fr/). Gorge Farm is a 700 ha vegetable farm which is owned and operated by Vegpro Group Ltd (VG) (www.vegpro-group.com). VG is the largest fresh-produce exporter in East Africa. Tropical Power has a partnership with VG, in which Tropical Power uses agro-wastes from VG for the biogas plant, and gives digestate as a natural fertiliser and soil conditioner to VG. The generated electricity is sold to Kenya Power and Lighting Company (KPLC) and VG.

Baby maize is grown at Gorge Farm all year round. The farm has 11 circles which are divided into 16 sectors or blocks (Figure 72a), each of 2.5 ha. The baby maize is planted in the blocks. As each block becomes ripe, the edible baby maize cobs are hand-picked by employees of VG. Not all of the cobs become ripe at the same time, so it can take several days for a block to be completely picked. The baby maize takes 86 to 95 days to grow to the point of harvest, depending on variety. The residues such as baby maize stalks and leaves are then available for the biogas plant. Once VG is satisfied that all of the edible corn has been picked, the block is signed off and handed over to Tropical Power. Tropical Power goes in with a tractor and forage harvester, which cuts and shreds the stalks, and blows the shredded waste into a trailer. The trailer is then driven back to the biogas site (Figure 72b). Tropical Power uses 2 forage harvesters and 4 tractors for harvesting and delivery from the harvesting place to the biogas plant.

(a) View of Gorge Farm and the biogas plant (original image Google Earth)

(b) View of the biogas plant showing CHP units (front left) and main digester (back right) (Photo courtesy of Ms Angela Bywater)

Figure 72. View of the biogas plant at Gorge Farm

The biogas plant is adjacent to the farmed area (Figure 72a). The longest straight-line distance from the biogas plant to where baby maize stover is harvested is 3.4 km, and the shortest distance is 0.5 km.

A simple schematic of the design and original process flow is shown in Figure 73. The biogas plant has 2 hydrolysers (H1 and H2) maintained at 55 °C and a main digester at 43 °C. Each hydrolyser volume is 760 m^3, the digester volume is 5655 m^3, and the recyclate store volume is 760 m^3 (Table 41). The plant was originally designed assuming a feed input of 45420 tonnes WW $year^{-1}$ which is equal to 124.44 tonnes WW day^{-1}. The expected TS of baby maize stover from VG was 32.5%. The biogas plant has capacity to produce 2.4 MW electrical output.

The process flowchart for the biogas plant in during the period for which Tropical Power provided operating data was different from the original. Hydrolyser 1 (H1) was not used because the mixing achieved was insufficient and substrate was floating. H1 was therefore bypassed and baby maize stover was fed directly to hydrolyser 2 (H2) and digester (DG).

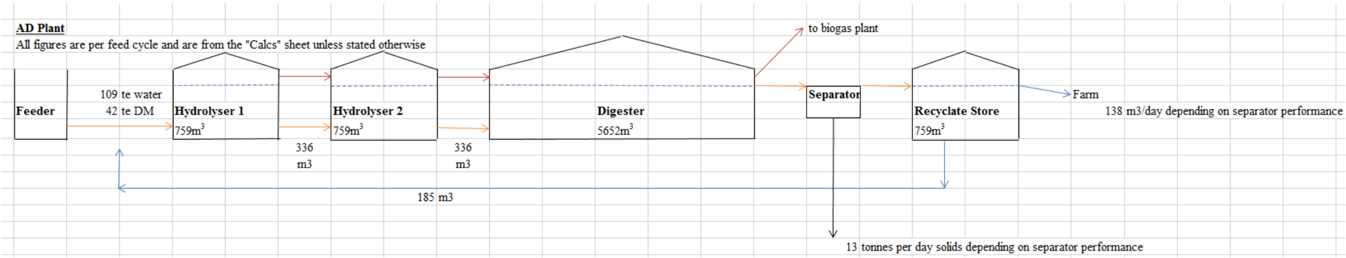


Figure 73. The biogas plant design (data from Tropical Power Ltd)

Table 41. Size of hydrolysis tank, digester and recyclate store

	Hydrolysis tank	Digester	Recyclate store
Number of units	2	1	1
Diameter [m]	11	30	11
Height [m]	8	8	8
Volume [m^3]	760	5655	760

To carry out an assessment and an energy balance for the Tropical Power plant, the following sub-objectives were set to achieve the main objective of the modelling work

- **i.** To establish the energy input for feedstock collection and transport
- **ii.** To determine energy output of biogas plant
- **iii.** To determine the energy input for digestate transport and application
- **iv.** To determine the energy balance for the plant based on the information in (i), (ii) and (iii) above
- **v.** To rationalise the plant design based on actual feedstock availability

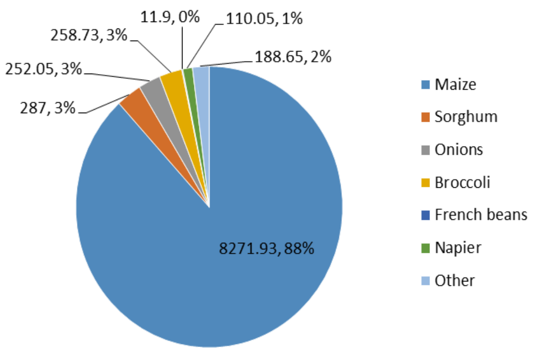
5.3. Sub-objective 1 - Energy input for feedstock collection and transport

In Section 5.3 the energy input for feedstock collection and transport is assessed and calculated. Tropical Power provided the data on the feeding amount and the fuel consumption for harvesting and transport. The data was collected in Kenya so the location and operating environment were correct, but the accuracy of the data collection and any factors that might have affected it were not known. Therefore, the actual data were compared with literature values to provide some cross checking and validation.

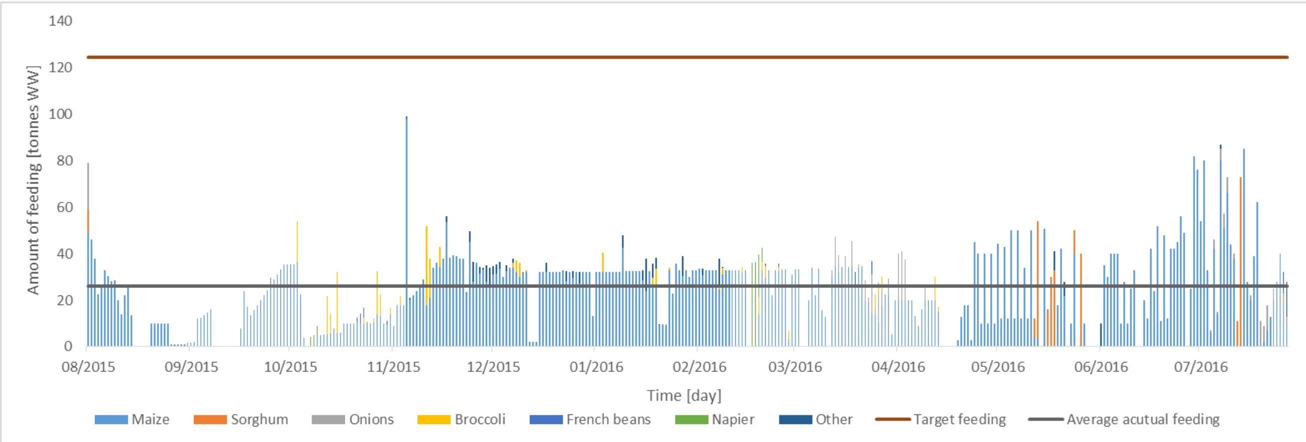
5.3.1. Defining the control time period

The control time period for the plant was defined as August 2015 to July 2016 because of the availability of detailed data on digester feeding, fuel consumption, produced biogas and electricity from Tropical Power for this period.

The biogas plant was originally built for anaerobic processing of baby maize stover, and was mainly fed on baby maize stover in this period. The plant sometimes received sorghum, onions, broccoli, French beans, and napier grass, but the amounts of these materials were very small compared with the quantity of baby maize stover which made up 88% of total feeding (Figure 74a). Even with these additions of other feedstocks, however, the biogas plant did not achieve the target feeding amount of 124 tonnes WW day^{-1}. The actual average feeding was 26 tonnes day^{-1} which was only 21% of the target (Figure 74b).



(a) Proportion of each feedstock type based on wet weight



(b) Feedstock amounts against time

Figure 74. Total amount of feeding to Tropical Power's biogas plant in Kenya from August 2015 to July 2016

5.3.2. Estimate diesel usage in harvesting and transporting material to plant

Values obtained from Tropical Power for the amount of crop harvested and the fuel consumption in crop transport are presented in Table 42.

Table 42. Number of hectares of baby maize harvested, tonnes delivered to the biogas plant and fuel consumption

	Number of hectares harvested baby maize [ha]	tonnes delivered [tonnes WW]	Diesel consumption [L]
Aug-15	14.4	459.9	1548
Sep-15	9.3	298.9	1509
Oct-15	13.5	431.1	2258
Nov-15	27.7	887.7	3814
Dec-15	27.6	883.1	3231
Jan-16	27.8	890.1	4366
Feb-16	25.2	805.4	2777
Mar-16	22.5	720.5	2770
Apr-16	14.2	453.0	2607
May-16	19.7	631.5	2135
Jun-16	23.3	745.8	2907
Jul-16	33.3	1065.0	4291
Total	258.5	8271.9	34213

As the data on fuel consumption was not gathered first hand in the project, and its accuracy was not known, literature values were also used to estimate diesel consumption for comparison with the values reported by Tropical Power. Firstly, diesel usage in harvesting was calculated and then diesel usage in transportation was calculated.

Harvesting:

Regarding the diesel fuel consumption, a forage harvester uses 0.2-2.0 gallons acre^{-1} with an average of 1.57 gallons acre^{-1} (Grisso *et al.*, 2004), equivalent to 1.9-18.7 L ha^{-1} and 14.7 L ha^{-1}, respectively. Turning to the tractors, in 2015 Tropical Power carried out small-scale trials to check fuel consumption with and without a load of 10 tonnes. The diesel consumption of a tractor loaded with 10 tonnes was estimated as 0.50 L km^{-1} on tarmac roads and 0.56 L km^{-1} on rough roads. The diesel consumption of the tractor with an empty tanker was 0.64 L km^{-1} on rough roads. The farm roads are not surfaced, and therefore this modelling used the diesel fuel consumption on a rough road.

For instance, the estimate for August 2015 was

Forage harvester

14.4 ha month^{-1} × 14.7 L ha^{-1} = 211.0 L month^{-1}

Turning to the tractor fuel consumption, the fuel consumption given by Tropical Power was based on a different unit (L km^{-1}) from that used for the forage harvester (L ha^{-1}). Therefore, the following assumption was made. The tractor follows the forage harvester to collect the baby maize stover. The width of the forage harvester is 2 m, so the estimated distance travelled by the tractor per month is 14.4×10^4 m^2 $month^{-1}$ ÷ 2 m = 72 km $month^{-1}$

On the outward journey from the biogas plant the tractor is unloaded, but on the return journey the tractor is full of baby maize. The fuel consumption was therefore calculated from the average of consumption with and without loading.

72 km $month^{-1}$ × (0.64 + 0.56) ÷ 2) L km^{-1} = 43.1 L $month^{-1}$

The calculated fuel consumption for harvesting is shown in Table 43.

Transportation:
The capacity of the tractor trailer is 10 tonnes and the tractor can carry a maximum load of 7 tonnes of baby maize stover on each trip. It was assumed that each trip carried 80% of maximum load or 5.6 tonnes.

459.9 tonnes $month^{-1}$ ÷ (80 % × 7 tonnes $trip^{-1}$) = 82 trips $month^{-1}$

The average straight-line distance between the fields and the biogas plant is 1.95 km ((3.4 + 0.5) km ÷ 2). This is not the actual distance travelled because vehicles have to travel on the farm tracks. For instance, if the field to be harvested was the one indicated by a red circle in Figure 75, the straight-line distance (yellow allow) clearly crosses the cultivation area, while the vehicles would run on the farm tracks (orange arrows). A correction factor was applied to take this into account. This factor was based on the assumption that the straight-line distance was the hypotenuse of a right-angled triangle, and the distance to be travelled could be represented on average by a triangle with angles 30°, 60°, 90° and sides in the ratio 2:1: $\sqrt{3}$. Therefore, the distance between the biogas plant and the harvesting field for vehicles was estimated as

Distance travelled by vehicles = (average straight-line distance) × ((1+$\sqrt{3}$) ÷ 2)

Figure 75. Gorge Farm's farm tracks (original image Google Earth)

Fuel consumption for the forage harvester was calculated because the set of a harvester and a tractor went together for harvesting and transportation from same parking area. Tropical Power's operation required the fuel for the harvester and the tractor. On the other hand, the harvester did not transport the baby maize stover, therefore the fuel consumption for the forage harvester operation between the parking place and the harvesting field was added into the harvesting category (Table 43). The fuel consumption was quoted on an areal basis, but could be estimated by multiplying the distance travelled by the width of the harvester (2 m). The distance travelled therefore corresponds to

$(1.95 \times 10^3$ m single trip$^{-1}) \times ((1+\sqrt{3}) \div 2) \times 2$ m = 0.53 ha single trip^{-1}

The fuel consumption for the forage harvester is

82 trips month^{-1} × 0.53 ha single trip^{-1} × 2 × 14.7 L ha^{-1} = 1284.8 L month^{-1}

Fuel consumption for the tractor: the tractor goes to the field with an empty tanker firstly

82 trips month^{-1} × ((1.95 × 10^3 m single trip^{-1}) × ((1+$\sqrt{3}$) ÷ 2)) m single trip^{-1} × 0.64 L km^{-1} = 140.0 L month^{-1}

On its return the tractor is loaded with 5.6 tonnes of baby maize stover

82 trips month^{-1} × ((1.95 × 10^3 m single trip^{-1}) × ((1+$\sqrt{3}$) ÷ 2)) km single trip^{-1} × 0.56 L km^{-1} = 122.5 L month^{-1}

The calculated fuel consumption for transportation is shown in Table 43. The results were compared with the literature values in section 5.3.3.

Table 43. Calculated fuel consumption for harvesting

	Harvesting		Moving	
	Forage harvester [L month^{-1}]	Tractor [L month^{-1}]	Forage harvester [L month^{-1}]	Total [L month^{-1}]
Aug-15	211.0	43.1	1284.8	1538.9
Sep-15	137.2	28.0	835.1	1000.2
Oct-15	197.8	40.4	1204.4	1442.6
Nov-15	407.3	83.2	2480.1	2970.6
Dec-15	405.2	82.8	2467.2	2955.2
Jan-16	408.4	83.4	2486.7	2978.6
Feb-16	369.6	75.5	2250.1	2695.2
Mar-16	330.6	67.5	2012.9	2411.1
Apr-16	207.9	42.5	1265.6	1515.9
May-16	289.8	59.2	1764.3	2113.3
Jun-16	342.2	69.9	2083.6	2495.8
Jul-16	488.7	99.8	2975.4	3563.9
Total	3795.7	775.5	23110.2	27681.4

Table 44. Calculated fuel consumption for transportation

	Tractor		
	Empty [L month^{-1}]	Loaded [L month^{-1}]	Total [L month^{-1}]
Aug-15	140.0	122.5	262.5
Sep-15	91.0	79.6	170.6
Oct-15	131.2	114.8	246.1
Nov-15	270.2	236.5	506.7
Dec-15	268.8	235.2	504.1
Jan-16	271.0	237.1	508.1
Feb-16	245.2	214.5	459.7
Mar-16	219.3	191.9	411.3
Apr-16	137.9	120.7	258.6
May-16	192.2	168.2	360.5
Jun-16	227.0	198.7	425.7
Jul-16	324.2	283.7	607.9
Total	2518.2	2203.4	4721.6

The total calculated fuel consumption of harvesting and transportation for August 2015 is thus 262.5 + 1538.9 = 1801.4 L month^{-1} = 58.1 L day^{-1}

The calculated results are shown in Table 45. The results were compared with the literature values in section 5.3.3.

Table 45. Total calculated fuel consumption for harvesting and transportation

	Total of harvesting and transportation					
	[L month^{-1}]			[L day^{-1}]		
	Forage harvester	Tractor	Total	Forage harvester	Tractor	Total
Aug-15	1495.8	305.6	1801.4	48.3	9.9	58.1
Sep-15	972.2	198.6	1170.9	32.4	6.6	39.0
Oct-15	1402.2	286.5	1688.7	45.2	9.2	54.5
Nov-15	2887.4	589.9	3477.3	96.2	19.7	115.9
Dec-15	2872.4	586.9	3459.3	92.7	18.9	111.6
Jan-16	2895.1	591.5	3486.6	93.4	19.1	112.5
Feb-16	2619.7	535.2	3154.9	90.3	18.5	108.8
Mar-16	2343.6	478.8	2822.4	75.6	15.4	91.0
Apr-16	1473.5	301.0	1774.5	49.1	10.0	59.2
May-16	2054.1	419.7	2473.7	66.3	13.5	79.8
Jun-16	2425.8	495.6	2921.5	80.9	16.5	97.4
Jul-16	3464.1	707.7	4171.9	111.7	22.8	134.6
Total	26905.9	5497.1	32403.1	882.1	180.2	1062.3

The calculated data were compared with actual fuel consumption as reported by Tropical Power (Table 46). The calculated values were not exactly the same as the actual fuel consumption. In August 2015, for example, the calculated value was 1801 L but actual fuel consumption was 1548 L, i.e. a difference of 253 L. The ratio (calculated value / actual value) was determined for each month and the range was from 68 to 116 %, but the annual average was 95 %. This indicated both that the fuel consumption data provided by Tropical Power was reasonably accurate, and that the methods used for estimation gave reasonably good results. The results suggest that in cases where local data are not available the literature values and assumptions made here could be used for estimation of fuel usage.

The variation between monthly figures may be related to harvesting or other features of the cultivation process. The amount harvested was different every month and this may have caused other differences in operation or record keeping that were not taken account of in the calculations.

Table 46. Difference between calculated value and actual fuel consumption

	Calculated fuel consumption [L month^{-1}]	Actual fuel consumption [L month^{-1}]	Difference [L month^{-1}]	Ratio: (calculation / actual)[%]
Aug-15	1801	1548	-253	116.4
Sep-15	1171	1509	338	77.6
Oct-15	1689	2258	570	74.8
Nov-15	3477	3814	337	91.2
Dec-15	3459	3231	-229	107.1
Jan-16	3487	4366	879	79.9
Feb-16	3155	2777	-378	113.6
Mar-16	2822	2770	-53	101.9
Apr-16	1775	2607	832	68.1
May-16	2474	2135	-338	115.8
Jun-16	2921	2907	-14	100.5
Jul-16	4172	4291	119	97.2
Total	32403	34213	1810	94.7

5.3.3. Translating energy use into standard units of MJ to check against literature values for baby maize harvesting and tractor transport

For the comparison with literature values, the diesel fuel consumption was translated into standard units of MJ using a conversion factor of 39 MJ L^{-1} (WNA, 2016). Baby maize stover wet tonnes harvested in the table above was converted to tonnes of dry matter to compare with literature values (MJ tonnes^{-1} TS) for harvesting. The TS percentage was 21.3% which was average value obtained from Tropical Power data during control period. Transportation was converted into MJ tonnes^{-1} WW km^{-1}.

Calculations for harvesting and transportation in August, 2015 are presented in detail, and were repeated for other months.

Harvesting

This calculation did not include the fuel consumption for the forage harvester moving between the biogas plant and the harvesting field, because this operation is not directly involved in the harvesting process.

254.1 L diesel month^{-1} × 39 MJ L^{-1}diesel ÷ 459.9 tonnes WW month^{-1} × (21.3 TS % WW ÷ 100) = 101 MJ tonnes^{-1} TS

Transportation

Mileage is

(459.9 tonnes WW month^{-1} ÷ (80 % × 7 tonnes trip^{-1}) × ((1.95 × 10^{3} m single trip^{-1}) × ((1+$\sqrt{3}$) ÷ 2)) km single trip^{-1} = 219 km

The value in the unit, MJ tonnes^{-1} WW km^{-1} was

122.5 L diesel month^{-1} × 39 MJ L^{-1}diesel ÷ 459.9 tonnes WW month^{-1} ÷ 219 km = 0.05 MJ tonnes^{-1} WW km^{-1}

Table 47 shows the calculated results and literature values for stover production and energy consumption. Table 48 is for literature values.

Table 47. Modelling data in MJ

	Harvesting [MJ tonnes^{-1} TS]	Transportation	
		Mileage [km]	Calculated results [MJ tonnes^{-1} WW km^{-1}]
Aug-15	101	219	0.05
Sep-15	101	142	0.07
Oct-15	101	205	0.05
Nov-15	101	422	0.02
Dec-15	101	420	0.02
Jan-16	101	423	0.02
Feb-16	101	383	0.03
Mar-16	101	343	0.03
Apr-16	101	215	0.05
May-16	101	300	0.03
Jun-16	101	355	0.03
Jul-16	101	507	0.02

Table 48. Literature values for harvesting, transportation and fuel consumption

	Harvesting [MJ tonne^{-1} TS]	Transportation [MJ tonnes^{-1} WW km^{-1}]	Fuel consumption	Reference
Straw harvesting	280			(Berglund and Börjesson, 2006)
Tops and leaves of sugar beet harvesting	540			
Straw harvesting	140			(Poschl *et al.*, 2010)
Straw		2.9		(Berglund and Börjesson, 2006)
Ley crops, tops and leaves of sugar beet		1.1		
Straw		6.9		(Poschl *et al.*, 2010)
Forage harvester in Nebraska, USA [L ha^{-1}]			14.7	(Grisso *et al.*, 2004)
Forage harvester in Iowa, USA [L ha^{-1}]			17.5	
Tractor trailer in USA [L km^{-1}]			0.35-0.41	(Sharpe, 2015)
Tractor trailer in EU [L km^{-1}]			0.31-0.38	
Tractor trailer in China [L km^{-1}]			0.44-0.47	

Berglund and Börjesson (2006) and Poschl *et al.* (2010) reported 280 and 140 MJ tonnes^{-1} TS, respectively for straw harvesting. The calculated results were less than these values, at 101 MJ tonnes^{-1} TS. This is may reflect actual differences in harvesting methods. Berglund includes transport and bailing while Poschl includes only fuel consumption during bailing operations. In this research harvesting consumption consists of fuel used in operations by a forage harvester and tractor. Poschl also reported on the energy consumption in harvesting grass silage which was 732 MJ tonnes^{-1} TS. This high value may be due to the higher moisture content of the grass silage, or to the inclusion of other steps in harvesting of grass for silage. When compared to the results for straw, however, the calculated results for baby maize stover seem acceptable despite being slightly lower.

Turning to transportation, the fuel consumption of the tractor and forage harvester was calculated as around 0.02 – 0.07 MJ tonnes^{-1} WW km^{-1} which is much lower than literature values of 1.1-6.9 MJ tonnes^{-1} WW km^{-1} (Berglund and Börjesson, 2006; Poschl *et al.*, 2010). Poschl's value includes the loading process and considers the empty return, but he did not include a forage harvester. If only tractor fuel consumption is considered, the calculated results would decrease. In contrast, the calculated fuel consumption and actual fuel consumption were in good agreement as noted above, with a ratio between the calculated to actual fuel consumption of 95 %. This indicates the operation in Kenya was genuinely slightly different from that reported by Poschl *et al.* (2010) and Berglund and Börjesson (2006). The difference between the calculated results and literature values may be due in part to small differences in assumptions, however, and in general the literature data for straw was in reasonable agreement with the baby maize stover results.

5.4. Sub-objective 2 – Energy output of the biogas plant

Section 5.4 assessed the energy output for the biogas plant in Kenya. Methane yield and electricity conversion were calculated to check the reliability of the data. The heating requirement for feedstock and heat loss was calculated. The requirement was compared with the heat produced in the CHP to check the balance.

5.4.1. The energy output of the biogas plant

The energy output of the biogas plant (biogas consumed, estimated methane volume, produced electricity and CHP run time) was provided by Tropical Power and is shown in Table 49. Tropical Power reported the typical methane concentration in the biogas as 52%. The estimated methane volume is calculated by multiplying the biogas volume and the methane concentration. The biogas volume is calculated from the % change in the volume in the gas storage dome over the day. The gas storage dome is of the double membrane type and a constant positive pressure of +3.5 mbar (0.35 kPa) is applied between the two membranes. The gas level indicator is attached to the top of the inner membrane and it provides an indicative value of the gas volume by measuring the rise and fall of the inner membrane. More information on the double membrane gas storage is given on the website (http://baur-folien.com/docs/3d_2schl_tld.php). The gas volume calculations did not take into account the location of the biogas plant, which is at an altitude of around 1900 m so atmospheric pressure is only around 80 % of that at sea level. Taking into account both the difference in altitude and the internal positive pressure in the gas storage membrane the gas volume is likely to be around 1.25 times larger than at 101.325 kPa.

The exported energy and parasitic energy are shown in Table 49. While there is some variation between months, the average value for parasitic energy of around 15% of generated electricity appears reasonable compared to estimates reported in the literature (Salter and Banks, 2009).

Table 49. Energy output, exported energy and parasitic energy from the biogas plant in Kenya

				Exported energy [kWh]				
	Biogas consumed [m^3]	Estimated methane volume [m^3]	Generated electricity [kWh]	VG sales	KPLC sales	Total	Parasitic energy [kWh]	[%]
Aug-15	69259	36015	114276	109225	0	109225	25140	22.0
Sep-15	67746	35228	94992	105822	0	105822	22520	23.7
Oct-15	98198	51063	175594	141028	0	141028	21680	12.3
Nov-15	108810	56581	200836	65118	0	65118	13240	6.6
Dec-15	118563	61653	218663	73879	0	73879	9680	4.4
Jan-16	152522	79311	242090	187502	21390	208892	20700	8.6
Feb-16	142796	74254	257841	348806	63306	412112	43020	16.7
Mar-16	144724	75256	243447	386524	34949	421473	49160	20.2
Apr-16	74488	38734	128520	262311	27613	289924	50040	38.9
May-16	142996	74358	249293	144246	88326	232572	22360	9.0
Jun-16	143081	74402	242581	165354	75134	240488	71094	29.3
Jul-16	201073	104558	357371	253661	66019	319680	47126	13.2
Total	1464255	761413	2525504	3621	2243476	376737	2620213	15.7

5.4.2. Checking of results for tonnage processed and expected methane yield

The output data was checked as follows. Firstly, the volume of biogas consumed by the CHP was divided by the feeding amount. For instance, for August 2015,

69259 m^3 month^{-1} ÷ 459.9 tonnes WW month^{-1} = 151 m^3 tonne^{-1} WW

Secondly, the methane yield was calculated. According to Tropical Power, the gas concentration was checked automatically by a sensor every 2 hours on a daily basis and the average biogas methane content recorded in the digester tank was 52% CH_4. The methane concentration in the thermophilic digesters in Trial 2-3 was around 53-54 %, which is slightly higher than the value reported by Tropical Power.

Tropical Power did not have VS data, therefore, VS data from the laboratory results from the UK was applied. The average VS content of the baby maize stover was taken as 18% WW.

151 m^3 tonnes^{-1} WW ÷ 10^3 × (52 % CH_4 ÷ 100) ÷ (18 VS % WW ÷ 100) = 0.435 L CH_4 g^{-1} VS

If this value is corrected for the difference in altitude, the specific CH_4 production would be

0.435 L CH_4 g^{-1} VS ÷ 1.25 = 0.348 L CH_4 g^{-1} VS (or 0.350 L CH_4 g^{-1} VS if the pressure in the gas membrane is also considered)

0.348 L CH_4 g^{-1} VS is reasonably close to the experimental value in Trial 2-1, of 0.342 L CH_4 g^{-1} VS added at OLR 3 g VS L^{-1} day^{-1} under thermophilic conditions. The range of calculated values for specific methane production is 0.283-0.526 L CH_4 g^{-1} VS added (Table 50). The upper value is much higher than the experimental value. The incorrect sizing of the Tropical Power plant for the available feedstock means it was operating at very long retention times and low organic loading rates. In the laboratory experiments, methane yield decreased slightly with increasing OLR. If the digester working volume is taken as 5655 m^3 this equates to an OLR of 0.47 g VS L^{-1} day^{-1} and a HRT of over 350 days, which may therefore have resulted in a slightly higher specific methane yield.

$$459.9 \text{ tonnes WW month}^{-1} \times 10^3 \times (18 \text{ VS \% WW} \div 100) \div 5655 \text{ m}^3 \div 31 \text{ days month}^{-1}$$
$$= 0.47 \text{ g VS L}^{-1} \text{ day}^{-1}$$

Even taking this into account, the calculated methane yield in September and October in 2015 and May, June and July in 2016 were much higher than literature value for similar materials e.g. 0.134-0.348 L CH_4 g^{-1} VS added for maize stover (Zhong *et al.*, 2011; Li *et al.*, 2017; Lizasoain *et al.*, 2017; Strang *et al.*, 2017). The methane yield for pure cellulose is 0.412 L CH_4 g^{-1} VS added, and as baby maize stover is mainly cellulose it is not likely to be much higher than this; only proteins and fats have much higher specific methane yields (Angelidaki and Sanders, 2004) . The maximum theoretical methane yield for the baby maize stover digested in Southampton based on the Buswell equation was 0.459 L CH_4 g^{-1} VS.

Some of the monthly variation may be caused by the Tropical Power's method of biogas volume calculation. They did not use a flow meter so the biogas volume data was mainly indicative. Tropical Power makes its profit by selling electricity, therefore the biogas volume data is not considered as important as the generated electricity data. The use of indicative data may contribute to the very high methane yield in certain months. Furthermore, Tropical Power assumed a biogas methane concentration of 52% every month, but this may not be accurate. The variation in specific gas production values for each month could also reflect variable feeding patterns as shown in Figure 74, since not all of the feed may produce gas in the month when it was fed to the digester. It is also possible that the plant was accumulating VFA at certain times and then converting the accumulated VFA at others, leading to variations in monthly gas yield. According to Tropical Power's data the average specific methane yield over the whole control period was around 0.42 L CH_4 g^{-1} VS, which is below the maximum value from the Buswell equation and close to the theoretical value for cellulose.

Turning to electricity conversion, the CHP efficiency can be calculated from

Electricity conversion [%] = (Total generated electricity [kWh] × 3.6 [MJ kWh^{-1}]) ÷ (((Estimated methane volume [m^3]) ÷ 1.25) × (35.814 [MJ m^{-3}])

The calorific value of CH_4 was taken as 35.814 MJ m^{-3} and 1 kWh is 3.6 MJ. The calculated value for the electrical conversion efficiency was therefore 34-45 % (Table 50). The annual average was 41 %. In practice typical CHP conversion efficiencies are around 35 to 42 %, so the calculated conversion efficiency seems reasonable.

Table 50. Consumed biogas, methane yield and calculated CHP electricity conversion efficiency

	Methane yield [L CH_4 g^{-1} VS]	Electricity conversion [%]
Aug-15	0.348	40
Sep-15	0.524	34
Oct-15	0.526	43
Nov-15	0.283	45
Dec-15	0.310	45
Jan-16	0.396	38
Feb-16	0.410	44
Mar-16	0.464	41
Apr-16	0.380	42
May-16	0.523	42
Jun-16	0.443	41
Jul-16	0.436	43
Average	0.420	41

5.4.3. Estimation of energy required to heat feedstock and maintain digester temperature

Heating for feedstock

The energy for heating to bring feedstock material up to digester temperature was calculated.

Heating was carried out in the second hydrolyser (H2) and the main digester (DG). The target operating temperatures for H2 and DG were 55°C and 43°C, respectively. The temperature in Kenya was obtained from WMO data (WMO, 2017) for Dagoretti corner as the closest location to the biogas plant (Table 51).

Table 51. Average monthly temperature in Dagoretti corner, Kenya (WMO, 2017)

	Mean temperature [°C]
January	18
February	18.8
March	19.4
April	19.2
May	17.8
June	16.3
July	15.6
August	15.9
September	17.3
October	18.5
November	18.4
December	18.1
Average	17.8

In August 2015, the biogas plant received 459.9 tonnes of baby maize stover. The substrate was fed to H2 and DG directly. The total volume of H2 is 760 m^3 and of DG is 5655 m^3. The working volume is likely to be about 80% of the total volume, but for the purposes of estimating heat energy requirements loss it was assumed that the whole external area of the tank was subject to heat loss. The ratio of volumes is thus

H2 760 m^3 ÷ (5655 + 760) m^3 × 100 = 11.8%

DG 100 % - 11.8 % = 88.2%

It was assumed that the feeding amount for each tank was based on this ratio. To calculate the energy required to heat the feedstock, it was assumed that the feedstock temperature is the average monthly temperature in Kenya. Specific heat capacity is 4.18 MJ $tonne^{-1}$ $°C^{-1}$.

Hydrolysis tank

(55 – 15.9) °C × (459.9 tonnes $month^{-1}$ × 11.8% ÷ 100%) × 4.18 MJ $tonne^{-1}$ $°C^{-1}$ = 8.9 GJ $month^{-1}$

Digester

(43 – 15.9) °C × (459.9 tonnes $month^{-1}$ × 88.2% ÷ 100%) × 4.18 MJ $tonne^{-1}$ $°C^{-1}$ = 45.9 GJ $month^{-1}$

Total

8.9 + 45.9 = 54.8 GJ $month^{-1}$

54.8 GJ month^{-1} ÷ (365 days year^{-1} ÷ 12 month year^{-1}) = 1.8 GJ day^{-1}

In normal operation the feedstock would pass through the hydrolysis tank into the digester at 55 ^{o}C not at 15.9 ^{o}C; therefore, it actually will warm the digester. The operation was changed to direct feeding during the control period, however, because the biogas plant had some performance issues and Tropical Power was trying to improve its operation. It was therefore assumed that the feedstock had to be heated to the operating temperature for both tanks. There may also be some heat losses in pipework and some heat recovery in heat exchangers etc, but these were not taken into account in the current calculations.

Regarding the rest of the control period, the calculation results are shown in Table 52.

Table 52. Feedstock heating requirements

	Monthly [GJ month^{-1}]			Daily [GJ day^{-1}]		
	H2	DG	Total	H2	DG	Total
Aug-15	8.9	45.9	54.8	0.3	1.5	1.8
Sep-15	5.6	28.3	33.9	0.2	0.9	1.1
Oct-15	7.8	38.9	46.7	0.3	1.3	1.5
Nov-15	16.1	80.5	96.6	0.5	2.7	3.2
Dec-15	16.1	81.0	97.2	0.5	2.6	3.1
Jan-16	16.3	82.0	98.3	0.5	2.6	3.2
Feb-16	14.4	71.8	86.3	0.5	2.5	3.0
Mar-16	12.7	62.7	75.4	0.4	2.0	2.4
Apr-16	8.0	39.7	47.8	0.3	1.3	1.6
May-16	11.6	58.6	70.3	0.4	1.9	2.3
Jun-16	14.3	73.4	87.7	0.5	2.4	2.9
Jul-16	20.8	107.5	128.3	0.7	3.5	4.1
Total	152.7	770.4	923.1	5.0	25.2	30.3

Maintaining digester temperature (heat loss)

Heat loss occurs from the walls, floor and roof of digester. Therefore, heat is needed to maintain the digester temperature. Heat losses can be calculated from the following equation

Heat loss (hl) = UAΔT

U = overall coefficient of heat transfer [W m^{-2} ^{o}C^{-1}]

A = cross-sectional area through which heat loss is occurring [m^{2}]

ΔT = temperature drop across surface in question [^{o}C]

The biogas plant in Kenya is a concrete digester which is insulated with styrene foam (Figure 76). The digester's roof is a membrane and the hydrolyser's roof is concrete.

Figure 76. Section of digester wall showing concrete, insulation and cladding (Photo courtesy of Ms Angie Bywater)

Regarding the overall coefficient of heat transfer, the following assumptions were applied (Table 53). The ambient temperature is shown in Table 53. The size of H2 and DG is shown in Table 41.

Table 53. Structure and the overall coefficient of heat transfer (Salter and Banks, 2009; Banks *et al.*, 2011b)

Structure	U [W m^{-2} $^{o}C^{-1}$]
Concrete wall with 50mm insulation	0.43
Concrete floor (in contact with dry earth)	1.7
Fixed concrete cover with insulation	1.4
Membrane with insulation	1.0

Therefore, the calculation for heat losses in August 2015 is as follows,

Walls:

H2 – 0.43 W m^{-2} $^{o}C^{-1}$ × (8 m × π × 11 m) × (55 – 15.9) ^{o}C = 4.6 kW

DG – 0.43 W m^{-2} $^{o}C^{-1}$ × (8 m × π × 30 m) × (43 – 15.9) ^{o}C = 8.8 kW

Floor:

H2 – 1.7 W m^{-2} $^oC^{-1}$ × ((11 m ÷ 2)^2 × π) × (55 - 15.9) ^{o}C = 6.3 kW

DG – 1.7 W m^{-2} $^oC^{-1}$ × ((30 m ÷ 2)^2 × π) × (43 - 15.9) ^{o}C = 32.6 kW

Roof

H2 – 1.0 W m^{-2} $^oC^{-1}$ × ((11 m ÷ 2)^2 × π) × (55 - 15.9) ^{o}C = 3.7 kW

DG – 1.4 W m^{-2} $^oC^{-1}$ × (4 × π × (30 m ÷ 2)^2) ÷ 2 × (43 - 15.9) ^{o}C = 53.6 kW

Total

H2 – 4.6 + 6.3 + 3.7 = 14.7 kW

DG – 8.8 + 32.6 + 53.6 = 95.0 kW

1 kWh = 3.6 × 10^6 J, the results in J are

H2 – 14.7 kW × (3.6× 10^6) J × 24 hours day^{-1}= 1.3 GJ day^{-1}

DG – 95.0 kW × (3.6× 10^6) J × 24 hours day^{-1} = 8.2 GJ day^{-1}

Total – 1.3 GJ day^{-1} + 8.2 GJ day^{-1} = 9.5 GJ day^{-1}

The results of the calculation for the rest of the control period are shown in Table 54.

Table 54. Heat loss calculation results

	Wall [kW]		Floor [kW]		Roof [kW]		Each total [kW]		Total [kW]
	H2	DG	H2	DG	H2	DG	H2	DG	
Aug-15	4.6	8.8	6.3	32.6	3.7	53.6	14.7	95.0	109.7
Sep-15	4.5	8.3	6.1	30.9	3.6	50.9	14.2	90.1	104.2
Oct-15	4.3	7.9	5.9	29.4	3.5	48.5	13.7	85.9	99.6
Nov-15	4.4	8.0	5.9	29.6	3.5	48.7	13.7	86.2	100.0
Dec-15	4.4	8.1	6.0	29.9	3.5	49.3	13.9	87.3	101.1
Jan-16	4.4	8.1	6.0	30.0	3.5	49.5	13.9	87.6	101.5
Feb-16	4.3	7.8	5.8	29.1	3.4	47.9	13.6	84.8	98.4
Mar-16	4.2	7.7	5.8	28.4	3.4	46.7	13.4	82.7	96.1
Apr-16	4.3	7.7	5.8	28.6	3.4	47.1	13.4	83.4	96.9
May-16	4.4	8.2	6.0	30.3	3.5	49.9	14.0	88.3	102.3
Jun-16	4.6	8.7	6.3	32.1	3.7	52.8	14.5	93.6	108.1
Jul-16	4.7	8.9	6.4	32.9	3.7	54.2	14.8	96.0	110.8
	Wall [GJ day^{-1}]		Floor [GJ day^{-1}]		Roof [GJ day^{-1}]		Each total [GJ day^{-1}]		Total [GJ day^{-1}]
	H2	DG	H2	DG	H2	DG	H2	DG	
Aug-15	0.4	0.8	0.5	2.8	0.3	4.6	1.3	8.2	9.5
Sep-15	0.4	0.7	0.5	2.7	0.3	4.4	1.2	7.8	9.0
Oct-15	0.4	0.7	0.5	2.5	0.3	4.2	1.2	7.4	8.6
Nov-15	0.4	0.7	0.5	2.6	0.3	4.2	1.2	7.4	8.6
Dec-15	0.4	0.7	0.5	2.6	0.3	4.3	1.2	7.5	8.7
Jan-16	0.4	0.7	0.5	2.6	0.3	4.3	1.2	7.6	8.8
Feb-16	0.4	0.7	0.5	2.5	0.3	4.1	1.2	7.3	8.5
Mar-16	0.4	0.7	0.5	2.5	0.3	4.0	1.2	7.1	8.3
Apr-16	0.4	0.7	0.5	2.5	0.3	4.1	1.2	7.2	8.4
May-16	0.4	0.7	0.5	2.6	0.3	4.3	1.2	7.6	8.8
Jun-16	0.4	0.7	0.5	2.8	0.3	4.6	1.3	8.1	9.3
Jul-16	0.4	0.8	0.5	2.8	0.3	4.7	1.3	8.3	9.6

5.4.4. Comparing the energy requirement for heating and the heat produced in the CHP

The heat produced in the CHP was calculated as follows

In August 2015, 114276 kWh electricity was generated. If an electrical conversion efficiency of 35% is assumed and an overall conversion efficiency to useful energy of 85 % with 15 % non-recoverable losses (Salter and Banks, 2009), the estimated heat production is 19.0 GJ day^{-1}.

The energy needed for heating in August 2015 was 1.8 GJ day^{-1} for heating feedstock and 9.5 GJ day^{-1} for heat losses, giving a total of 11.2 GJ day^{-1}. This corresponds to 58.9 % of the produced heat, leaving a total of 7.7 GJ day^{-1} available for other purposes. The results of calculations for the whole control period are shown in Table 55. In other months during the control period the proportion of the heat generated that was required to run the plant ranged from 25-66%, and averaged around one third of the heat available. This value probably reflects the fact that the digester is oversized and therefore loses more heat than necessary. The data was compared with the literature value. Banks *et al.* (2011) reported the parasitic heat requirement of the recoverable heat which was 30 %. The percentage which was calculated from the total in this work was 32.1 %. The calculated results were not significantly different from the literature value.

Data provided by Tropical Power on the actual temperature in hydrolysis and digester is shown in Table 56. As noted above, Hydrolysis tank 1 was not used, and only hydrolysis tank 2 and digester were in use. Hydrolysis tank 2 successfully achieved the target temperature of 55 ^{o}C. The digester temperature was approximately 4 ^{o}C lower than the target temperature of 43^{o}C, meaning it was effectively operating in mesophilic conditions with a thermophilic pre-hydrolysis step as in the laboratory experiment in Trial 2, 2-1 and 2-2.

Table 55. Heat produced in the CHP and required energy for heating

	Heat produced in the CHP [GJ day^{-1}]	Energy for heating [GJ day^{-1}]			Difference [GJ day^{-1}]	Requirement/produced heat [%]
		Feedstock	Heat loss	Total		
Aug-15	19.0	1.8	9.5	11.2	7.7	59.3
Sep-15	16.3	1.1	9.0	10.1	6.1	62.2
Oct-15	29.1	1.5	8.6	10.1	19.0	34.7
Nov-15	34.4	3.2	8.6	11.9	22.6	34.4
Dec-15	36.3	3.1	8.7	11.9	24.4	32.7
Jan-16	40.2	3.2	8.8	11.9	28.2	29.7
Feb-16	45.7	3.0	8.5	11.5	34.2	25.1
Mar-16	40.4	2.4	8.3	10.7	29.7	26.6
Apr-16	22.0	1.6	8.4	10.0	12.1	45.2
May-16	41.4	2.3	8.8	11.1	30.3	26.9
Jun-16	41.6	2.9	9.3	12.3	29.3	29.5
Jul-16	59.3	4.1	9.6	13.7	45.6	23.1
Total	425.6	30.3	106.2	136.4	289.2	32.1

Table 56. Actual temperature profile in the biogas plant during control period

	H1 [°C]	H2 [°C]	DG [°C]	FS [°C]
Aug-15	21.7	49.2	42.2	38.2
Sep-15	21.8	54.1	41.8	33.9
Oct-15	21.6	55.4	39.5	30.0
Nov-15	21.8	55	38.2	32.7
Dec-15	21.8	55.3	38.6	37.4
Jan-16	22.4	55	39.3	35.1
Feb-16	21.3	54.7	40.1	35.1
Mar-16	22.4	53.1	41.5	38.2
Apr-16	30.2	53.1	42.4	31.2
May-16	44.5	55.1	41.7	37.7
Jun-16	42.5	48.4	41.4	40.6
Jul-16	43	55.2	42.5	40.4

H1: hydrolysis tank 1
H2: hydrolysis tank 2
DG: digester tank
FS: final storage tank

5.5. Sub-objective 3 - Energy input for digestate transport and application

In Section 5.5 the energy requirement for the digestate transport and application is calculated. There were no records of the number of trailers leaving the site or the number of hectares on which the digestate was applied as a fertiliser, because Tropical Power did not apply the digestate to land itself. Therefore, the calculations in this section were based on assumptions using data from the laboratory experiments and on information provided by Tropical Power. The amount of digestate used for fertiliser, number of tractor trips, and fuel consumption for tractor and spreading were estimated.

All digestate (liquid and solid) removed from the biogas plant was given to VG. VG comes to the biogas plant and collects the digestate for application in a bowser-type trailer. The digestate is sprayed on the farm land by a tanker with capacity 10,400 L. Based on the information used for Tropical Power it is assumed the tractor can carry 5.6 tonnes trip^{-1}. According to Tropical Power, their tractor trailer for transportation of baby maize stover also has 10 tonnes capacity and can carry maximum 7 tonnes trip^{-1}. A value of 5.6 tonnes trip^{-1} which is 80 % of maximum loading was close to actual fuel consumption.

Estimation of digestate removal in September 2015 was based on the fact that biogas plant received 459.9 tonnes WW month^{-1} in August 2015. The feedstock VS content is 18 % WW and the laboratory experiments in Trial 2-1 indicated that VS destruction under thermophilic digestion is 74.4 % VS. The amount of digestate for removal is therefore

459.9 tonnes WW month^{-1} – (459.9 tonnes WW month^{-1} **×** **(**18 VS % WW ÷ 100) **×** **(**74.4 % VS ÷ 100))
= 398.3 tonnes WW month^{-1}

It was assumed the number of hectares used for digestate disposal was same as the number of hectares of baby maize harvested. In September 2015, this was 14.4 ha month^{-1}.

Transportation between the biogas plant and the farm was calculated from the number of trips:
401.9 tonnes WW month^{-1} ÷ 5.6 tonnes trip^{-1} = 72 trips month^{-1}

The energy requirement for transportation was taken as 2.5 MJ tonne^{-1} km^{-1} which includes empty return transport, and the energy requirement for spreading was taken as 0.5 GJ ha^{-1} (Berglund and Börjesson, 2006)

2.5 MJ $tonne^{-1}$ km^{-1} × 398.3 tonnes WW $month^{-1}$ × (1.95 km one way trip × 2) × 72 trips $month^{-1}$ =

276.2 GJ $month^{-1}$

Spreading digestate

14.4 ha $month^{-1}$ × 0.5 GJ ha^{-1} = 7.2 GJ $month^{-1}$

The total is

276.2 + 7.2 = 283.4 GJ $month^{-1}$ = 9.4 GJ day^{-1}

The results of the calculations for the rest of the control period are shown in Table 57.

Table 57. Energy input for digestate application and transportation

	Baby maize harvested in previous month		Estimated digestate for fertiliser [tonne $month^{-1}$]	No. of trips [times]	Transport [GJ $month^{-1}$]	Spreading [GJ $month^{-1}$]	Total [GJ $month^{-1}$]	Total [GJ day^{-1}]
	Area [ha $month^{-1}$]	Weight [tonne $month^{-1}$]						
Aug-15								
Sep-15	14.4	459.9	398.3	71	276.2	7.2	283.4	9.4
Oct-15	9.3	298.9	258.9	46	116.7	4.7	121.3	4.0
Nov-15	13.5	431.1	373.4	67	242.7	6.7	249.4	8.3
Dec-15	27.7	887.7	768.8	137	1029.1	13.9	1043.0	33.6
Jan-16	27.6	883.1	764.8	137	1018.5	13.8	1032.3	33.3
Feb-16	27.8	890.1	770.9	138	1034.6	13.9	1048.5	33.8
Mar-16	25.2	805.4	697.5	125	847.1	12.6	859.7	27.7
Apr-16	22.5	720.5	624.0	111	678.0	11.3	689.2	22.2
May-16	14.2	453.0	392.3	70	268.0	7.1	275.1	8.9
Jun-16	19.7	631.5	546.9	98	520.8	9.9	530.7	17.1
Jul-16	23.3	745.8	645.9	115	726.4	11.7	738.1	23.8
Aug-16	33.3	1065.0	922.4	165	1481.3	16.6	1497.9	48.3
Total	258.5	8271.9	7164.2	1279.3	8239.3	129.2	8368.6	270.7
Average	21.5	689.3	597.0	106.6	686.6	10.8	697.4	22.6

5.6. Sub-objective 4 –Energy balance for the plant

Section 5.6 assessed the operating energy balance for the biogas plant in Kenya. The results from section 5.3 to section 5.5 (harvesting, feedstock transportation, energy output, heat requirement and digestate application) were used to calculate the balance.

The energy balance based on the above calculations is shown in Table 58. To allow comparison of the calculated results, all values are given in GJ month^{-1}. Assumed fuel consumption from sub-objective 1 was in L month^{-1}, so diesel used was multiplied by a conversion factor of 39 MJ L^{-1}. Heating loss from objective 2 was in GJ day^{-1} so a factor of day month^{-1} was applied. The unit of generated electricity was in kWh and produced heat in CHP was in GJ day^{-1} therefore they were also converted into GJ month^{-1}. Energy balance was calculated in two ways: firstly, including the energy for digestate application, and secondly without the energy for digestate application because the actual operation by Tropical Power in Kenya did not include this since VG came to the biogas plant and collected the digestate as fertiliser.

1. Energy balance with digestate application

Energy balance [GJ month^{-1}] = Output – (Input + Heating + Application)

Energy balance [%] = Energy balance ÷ Output × 100

2. Energy balance without digestate application

Energy balance [GJ month^{-1}] = Output – (Input + Heating)

Energy balance [%] = Energy balance ÷ Output × 100

Table 58. Overall energy balance

									Output [GJ month^{-1}]			
	Input [GJ month^{-1}]			Heating [GJ month^{-1}]		Application [GJ month^{-1}]			With digestate application		Without digestate application	
	Harvesting	Transportation	Loss	Feedstock	Application	Transportation	Generated electricity	Produced heat	Net energy [GJ month^{-1}]	[%]	Net energy [GJ month^{-1}]	[%]
Aug-15	60.0	10.2	293.7	54.8			411.4	587.7				
Sep-15	39.0	6.7	270.2	33.9	7.2	276.2	342.0	488.5	197.4	23.8	473.6	57.0
Oct-15	56.3	9.6	266.7	46.7	4.7	116.7	632.1	903.1	1034.6	67.4	1151.2	75.0
Nov-15	115.9	19.8	259.1	96.6	6.7	242.7	723.0	1032.9	1015.1	57.8	1257.9	71.6
Dec-15	115.3	19.7	270.9	97.2	13.9	1029.1	787.2	1124.6	365.8	19.1	1394.9	73.0
Jan-16	116.2	19.8	271.9	98.3	13.8	1018.5	871.5	1245.0	578.1	27.3	1596.6	75.4
Feb-16	105.1	17.9	246.6	86.3	13.9	1034.6	928.2	1326.0	749.9	33.3	1784.5	79.2
Mar-16	94.0	16.0	257.4	75.4	12.6	847.1	876.4	1252.0	825.9	38.8	1673.1	78.6
Apr-16	59.1	10.1	251.1	47.8	11.3	678.0	462.7	661.0	66.4	5.9	744.3	66.2
May-16	82.4	14.1	274.0	70.3	7.1	268.0	897.5	1282.1	1463.7	67.2	1731.7	79.5
Jun-16	97.3	16.6	280.2	87.7	9.9	520.8	873.3	1247.6	1108.3	52.3	1629.1	76.8
Jul-16	139.0	23.7	296.9	128.3	11.7	726.4	1286.5	1837.9	1798.5	57.6	2524.9	80.8
Total	1079.6	184.1	3238.6	923.1	112.6	6758.1	9091.8	12988.3	9203.7	41.7	15961.8	72.3
Average	90.0	15.3	269.9	76.9	10.2	614.4	757.7	1082.4	836.7	40.9	1451.1	73.9

If digestate application is included as an energy input, the output is greater than the input. The energy balance varied from 6-67 % due to monthly yield amounts and differences in digestate application, with an average value across the monitoring period of 40.9%. Without the energy for digestate application, the energy output was greater than input and the annual average was 73.9 %. As expected, these values for the energy balance were always positive.

5.6.1. Discussion of limitations in the energy balance and data

The above calculations for the operating energy balance relied on a number of assumptions which may be incorrect. The fuel consumption values used in calculation for sub-objective 1 may be not correct, although actual and estimated values showed good agreement in the long term. This modelling used the data from Grisso *et al.* (2004) of 14.7 L ha^{-1} for a forage harvester and data from Tropical Power of 0.56-0.64 L km^{-1} for a tractor. Grisso's paper also reported the forage harvester fuel consumption is 17.5 L ha^{-1} in Iowa, USA. This study used 14.7 L ha^{-1} because Grisso's research was carried out in Nebraska and the research in Iowa did not clearly explain the assumptions made. The fuel consumption depends on location and tractor type (Grisso *et al.*, 2004; Sharpe, 2015), so forage harvester fuel consumption in Kenya may be different. Regarding the fuel consumption for the tractor trailer, this was obtained from Tropical Power. In 2015, Tropical Power carried out small-scale trials to check fuel consumption with and without a load of 10 tonnes at Gorge Farm in Kenya. The fuel consumption was 0.56-0.64 L km^{-1} which was greater than the literature value reported by Sharpe (2015); 0.35-0.41 L km^{-1} in USA, 0.31-0.38 L km^{-1} in EU, 0.44-0.47 L km^{-1} in China. The tractor at Gorge Farm consumed more fuel than the reported values for the USA, EU and China. On the other hand, the small-scale trial by Tropical Power was not based on a large amount of data. The trial consisted of 3 trial runs: the first was on a tarmac road loaded with 10 tonnes, the second for rough roads with an empty tanker, and the third for rough road loaded with 10 tonnes. Each trial was not duplicated, but run just once. Thus the data are from actual fuel consumption in Kenya but their validity should be considered carefully.

In sub-objective 2, output data from the biogas plant was checked. The calculated methane yield was 0.283 – 0.526 L CH_4 g^{-1} VS during the control period, with an average of around 0.42 L CH_4 g^{-1} VS. Some of the monthly SMP values are much higher than literature values for similar materials such as maize silage (Amon *et al.*, 2007; Bauer *et al.*, 2010; Cornell, 2011; Evranos and Demirel, 2015) and maize stover (Zhong *et al.*, 2011; Brown *et al.*, 2012; Tian *et al.*, 2015; Li *et al.*, 2017; Lizasoain *et al.*, 2017). On the other hand, the calculated CHP electrical conversion efficiency was 34-45%. The literature values were around 32-45% (Appels *et al.*, 2011; Lantz, 2012; Strzalka *et al.*, 2017). This indicates conversion efficiency is acceptable but methane yield does not meet the literature value. This may be caused by the methane concentration and biogas volume measuring method.

The plant checks methane concentration in the digester every 2 hours automatically but Tropical Power always used 52% as the methane concentration for the whole year. Tropical Power reported that 52% was the average methane concentration. The amount of feeding was not consistent (Table 44) and that may affect microorganism growth, population and methane concentration.

Tropical Power measured biogas volume by recording the increase in gas volume in the gas storage dome (membrane) by looking at the % change over a day. The accuracy of this approach is not known. The biogas plant is located at an altitude of 1900 m. High altitude affects the pressure calculation. As noted above, according to MIDE (2017), the pressure at around 1900 m is only around 80% of that at sea level. If the gas volume is not corrected, it would be 1.25 times larger than at 101.325 kPa.

In objective 3, the energy requirement for digestate application was considered. The literature values came from Berglund and Börjesson (2006). Berglund's research was carried out in Sweden so, as with the tractor, the fuel consumption in Kenya may be different. This study should have used local fuel consumption data from Kenya but it was difficult to find reported values. The assumption was that the tractor carried a load of 7 tonnes of digestate every trip and the distance was 1.95 km one way which was same as the assumption in objective 1. The energy for transportation was the highest value than other energy requirement: input and heating. This study used only one literature value so more literature values are needed. In the overall operating energy balance for Tropical Power, this digestate application energy is not needed because VG came to the biogas plant and collected the digestate as fertiliser. This process is not included in the process at the biogas plant.

In objective 4, the energy balance for energy input, output and digestate application was calculated. The balance was positive and the method adopted could be used in planning new systems or assessing the performance of other plants. Because the Tropical Power plant is oversized for its current feedstock, however, the actual values obtained may not be representative for other plants. The energy balance also did not include energy for CHP or heat loss from pipes etc. Therefore the "AD assessment tool" which considers some of these parameters was used to check the energy balance in objective 5.

5.7. Sub-objective 5 - Rationalise design based on feedstock availability

In this section, a rationalised design based on actual feedstock availability was assessed. The results from sections 5.3 and 5.6 suggested the energy balance of the biogas plant was positive; however, there were potential points of improvement for better operation (i.e. matching feedstock availability and digester size). The operating energy balances under mesophilic and thermophilic conditions were compared as this parameter was compared in the laboratory studies section.

In section 5.7, firstly, the feedstock availability, digester volume, OLR and HRT were checked. Secondly, the information was applied to two scenarios: single tank mesophilic and single tank thermophilic for calculation of heating energy and methane production. The scenarios were calculated by hand and using the AD assessment tool which was developed at Southampton with funding from a range of sources. The purpose of this was to compare the overall energy balance of mesophilic and thermophilic digestion, based on data from the laboratory studies scaled to an appropriate size for the operation in Kenya. Finally, the scenario was compared to the biogas plant in Kenya.

5.7.1. Required working volume

Regarding feedstock availability, the biogas plant received 8271.9 tonnes year^{-1} from April 2015 to July 2016. The value per day is

8271.9 tonnes WW year^{-1} ÷ 365 days = 22.7 tonnes day^{-1}

According to Tropical Power's original biogas plant design, the HRT is 16 days. The required volume of a digester at this HRT is

22.7 tonnes day^{-1} × 16 days = 363 m^{3}

Therefore, OLR is

22.7 tonnes WW day^{-1} × 0.18 tonnes VS tonne^{-1} WW ÷ 363 m^{3} = 11 kg VS m^{-3} day^{-1}

The OLR of 11 g VS L^{-1} day^{-1} is too high for a conventional single-stage digester, however, and such high loadings may cause rapid volume expansion and VFA accumulation. In this study, laboratory experiments showed the highest methane yield at OLR 3 g VS L^{-1} day^{-1}. The digester volume was therefore calculated for this condition.

Working volume is

22.7 tonnes WW day^{-1} × 0.18 tonnes VS $tonne^{-1}$ WW ÷ 3 kg VS m^{-3} day^{-1} = 1360 m^3

HRT is

1360 ÷ 22.7 = 60 days

5.7.2. Hand calculation of heating energy and methane production

General

As mentioned in the previous section, the required digester working volume is 1360 m^3, daily feeding is 22.7 tonnes WW, OLR is 3 kg VS m^{-3} day^{-1}, and HRT is 60 days. The methane yields from Tropical Power were not used for sub-objective 5 because, as noted in section 5.4, the values from the plant were too high in comparison with literature values. The specific methane yield was taken from the result of the laboratory experiments, as these were monitored more accurately than the Tropical Power plant. This laboratory data is also slightly more conservative than the data from the plant and therefore using it provides a robust basis for estimating of the anaerobic digestion energy production from baby maize stover in other plants. The temperature in Kenya was applied.

The purpose of this step was to compare mesophilic and thermophilic digestion in commercial plant. Therefore, this part focused on heating and energy output. If the amount of feeding and the digester volume are same, the energy for harvesting, transportation, and mixing should be the same. The modelling conditions are shown in Table 59. The methane yield for mesophilic conditions comes from Trial 2-3, for mesophilic digesters with 5 TE. The methane yield of thermophilic digestion comes from Trial 2-1. Regarding heat loss, the calculation followed the method used in sub-objective 2.

The required height and width of digester for the heat loss calculation was calculated and is given in Table 60. The heat loss and methane output are shown in Table 61.

Table 59. Modelling condition

	Scenario 1	Scenario 2
	single tank mesophilic	single tank thermophilic
Location	Kenya	
Working volume [m^3]	1360	
HRT [day]	60	
OLR [g VS L^{-1} day^{-1}]	3	
Feeding [tonne WW day^{-1}]	22.7	
Feeding [tonne VS day^{-1}]	4.1	
Digester construction	Concrete	
Digester roof	Membrane	
Temperature [°C]	35	55
Methane yield [L CH_4 g^{-1} VS_{added}]	0.333	0.342

Heating of feedstock and heat loss

Scenario 1

Heating energy for substrate

In January, the temperature in Kenya is 18 °C

(35-18) °C × 22.7 tonnes WW day^{-1} × 4.18 MJ $tonne^{-1}$ $°C^{-1}$ = 1.6 GJ day^{-1}

Heat loss

Regarding diameter and height, the calculation was as follows. The shape of digester is cylindrical and the working volume is 1360 m^3. Working volume is usually around 80 % of digester volume. The digester total volume is thus

1360 ÷ 0.8 = 1700 m^3

The aspect ratio (height : diameter) of Tropical Power is

Height : Diameter = 8 : 30

Diameter [m] = 3.75 height [m]

Diameter is x

$\pi \times r^2 \times h$ = volume

$\pi \times (0.5\ x\ [m])^2 \times (x \div 3.75\ [m]) = 1700\ m^3$

X = 20.1 m

Height is

20.1 m ÷ 3.75 = 5.4 m

Table 60. Digester size

Working volume [m^3]	1360
Digester volume [m^3]	1700
Tropical Power' digester diameter to height ratio	3.75
Height [m]	5.4
Diameter [m]	20.1

The heat loss calculation in January for scenario 1 is as follows

Walls

$0.43\ W\ m^{-2}\ {}^oC^{-1} \times (\ 5.4\ m \times \pi \times 20.1\ m\ ) \times (\ 35 - 18\ )\ {}^oC \times 3.6\ MJ\ kWh^{-1} \times 24\ hours\ day^{-1} = 0.2\ GJ\ day^{-1}$

Floor

$1.7\ W\ m^{-2}\ {}^oC^{-1} \times ((5.4\ m \div 2)^2 \times \pi\ ) \times (\ 35 - 18\ )\ {}^oC \times 3.6\ MJ\ kWh^{-1} \times 24\ hours\ day^{-1} = 0.8\ GJ\ day^{-1}$

Roof

$1.0\ W\ m^{-2}\ {}^oC^{-1} \times (\ 4 \times \pi \times (20.1\ m \div 2)^2\ ) \div 2 \times (\ 35 - 18\ )\ {}^oC \times 3.6\ MJ\ kWh^{-1} \times 24\ hours\ day^{-1} = 0.9\ GJ\ day^{-1}$

Total

$0.2 + 0.8 + 0.9 = 1.9\ GJ\ day^{-1}$

The calculated results for scenario 1 and scenario 2 are shown in Table 63.

Table 61. Requirement of heating energy

(a) Scenario 1 (single mesophilic digester)

	Heating of feedstock [GJ day^{-1}]	Heat Loss [GJ day^{-1}]				Total heat requirement [GJ day^{-1}]	Average [GJ day^{-1}]
		Walls	Floor	Roof	Total		
January	1.6	0.2	0.8	0.9	1.9	3.5	3.6
February	1.5	0.2	0.8	0.9	1.8	3.4	
March	1.5	0.2	0.7	0.9	1.8	3.3	
April	1.5	0.2	0.7	0.9	1.8	3.3	
May	1.6	0.2	0.8	0.9	2.0	3.6	
June	1.8	0.2	0.9	1.0	2.1	3.9	
July	1.8	0.2	0.9	1.1	2.2	4.0	
August	1.8	0.2	0.9	1.0	2.2	4.0	
September	1.7	0.2	0.8	1.0	2.0	3.7	
October	1.6	0.2	0.8	0.9	1.9	3.4	
November	1.6	0.2	0.8	0.9	1.9	3.5	
December	1.6	0.2	0.8	0.9	1.9	3.5	

(b) Scenario 2 (single thermophilic digester)

	Heating of feedstock [GJ day^{-1}]	Heat Loss [GJ day^{-1}]				Total heat requirement [GJ day^{-1}]	Average [GJ day^{-1}]
		Walls	Floor	Roof	Total		
January	3.5	0.5	1.7	2.0	4.2	7.7	7.8
February	3.4	0.5	1.7	2.0	4.1	7.6	
March	3.4	0.4	1.7	2.0	4.1	7.4	
April	3.4	0.4	1.7	2.0	4.1	7.5	
May	3.5	0.5	1.7	2.0	4.2	7.8	
June	3.7	0.5	1.8	2.1	4.4	8.1	
July	3.7	0.5	1.8	2.2	4.5	8.2	
August	3.7	0.5	1.8	2.1	4.5	8.2	
September	3.6	0.5	1.8	2.1	4.3	7.9	
October	3.5	0.5	1.7	2.0	4.2	7.6	
November	3.5	0.5	1.7	2.0	4.2	7.6	
December	3.5	0.5	1.7	2.0	4.2	7.7	

Produced methane

Under mesophilic conditions, the methane yield was 0.333 L CH_4 g^{-1} VS and daily feeding is 4.1 tonnes VS day^{-1}

0.333 L CH_4 g^{-1} × 4.1 tonnes VS day^{-1} × 10^6 = 1358410 L CH_4 day^{-1}

CH_4 calorific value is 39.84 MJ m^{-3}

1358410 L CH_4 day^{-1} × 10^{-3} × 39.84 MJ m^{-3} = 54.1 GJ day^{-1}

Scenario 2 was calculated using the same methods and the results are shown in Table 64. The heat requirement for raising temperature of feedstock and for replacing heat losses are similar in scale in this case and represent about 6.7 % in scenario 1 and 14.0 % in scenario 2 of the total energy produced (methane production). If required the digester heat losses could be reduced by increasing the insulation. If there is no alternative economic use for the heat, however, increasing the insulation will improve the calculated energy balance but not the income from AD.

Table 62. Methane production

	Scenario 1 (mesophilic)	Scenario 2 (thermophilic)
Methane [L CH_4 day^{-1}]	1358410	1395123
Methane [GJ day^{-1}]	54.1	55.6

Energy balance

The operating energy balance (output-input) was calculated and is shown in Table 63. The energy output in scenario 2 was 55.6 GJ day^{-1} which was 1.5 GJ day^{-1} or about 2.7% greater than that of scenario 1. In contrast, the energy balance in scenario 1 was around 2.8 GJ day^{-1} or 5.8% greater than scenario 2 as thermophilic digestion required more energy for heating. Taking all factors together, mesophilic digestion is better for overall energy balance. This difference is not very large in absolute terms, even though the plant is oversized and so the effect of differences in heat loss may be magnified. This finding is similar to that reported by Zhang et al. (2017) who modelled mesophilic and thermophilic digestion of food waste with and without dilution using the AD modelling tool and noted that the differences in net energy production were not large except at very small plant sizes. On the other hand a difference of 5-6% in net energy production will be commercially significant to a plant operator. Heat transfer is strongly affected by insulation and so even in Kenya it may make sense to improve the digester insulation and decrease energy losses. The heat requirement is around 7% of total energy produced for mesophilic conditions and more than twice that at around 14% in thermophilic conditions.

This modelling did not include transportation, energy for CHP, therefore, the AD assessment tool was used to assess these impacts for the overall energy balance.

Table 63. Energy balance

	Scenario 1 (mesophilic)	Scenario 2 (thermophilic)
Energy input		
Heat requirement [GJ day^{-1}]	3.6	7.8
Energy output		
Methane [GJ day^{-1}]	54.1	55.6
Energy balance		
Output - Input [GJ day^{-1}]	50.5	47.8

This energy balance deliberately did not include the embodied energy as this is not critical for a farm or other organisation when deciding whether to construct a digester: in this case the important information is the net amount of energy (biogas, electricity etc) produced which can be exported and sold. The income from the sale then pays for construction of the plant; but the farmer does not pay for the embodied energy separately. This study started because the biogas plant in Kenya was already built and beginning operation on a novel substrate. The sponsor, Tropical Power, was primarily interested in the amount of generated electricity. Therefore, this modelling was made for the actual commercial biogas plant. For organisations such as government agencies, however, consideration of the embodied energy as part of the overall energy balance the embodied energy may be important, as policy-makers may need to assess the net benefit to the whole economy as well as the feasibility of operation at the scale of a single farm.

5.7.3. Calculation by AD assessment tool

The calculation was also carried out using the AD assessment tool developed by the Bioenergy and Organic Resources Group at the University of Southampton. The assumptions made for scenarios 1 and 2 were the same as in the above manual calculations; however, the AD assessment tool requires more input data for calculation. The data and the assumptions made are shown in Table 64. The AD assessment tool considered transportation, utilization of digestate as fertiliser, parasitic energy,

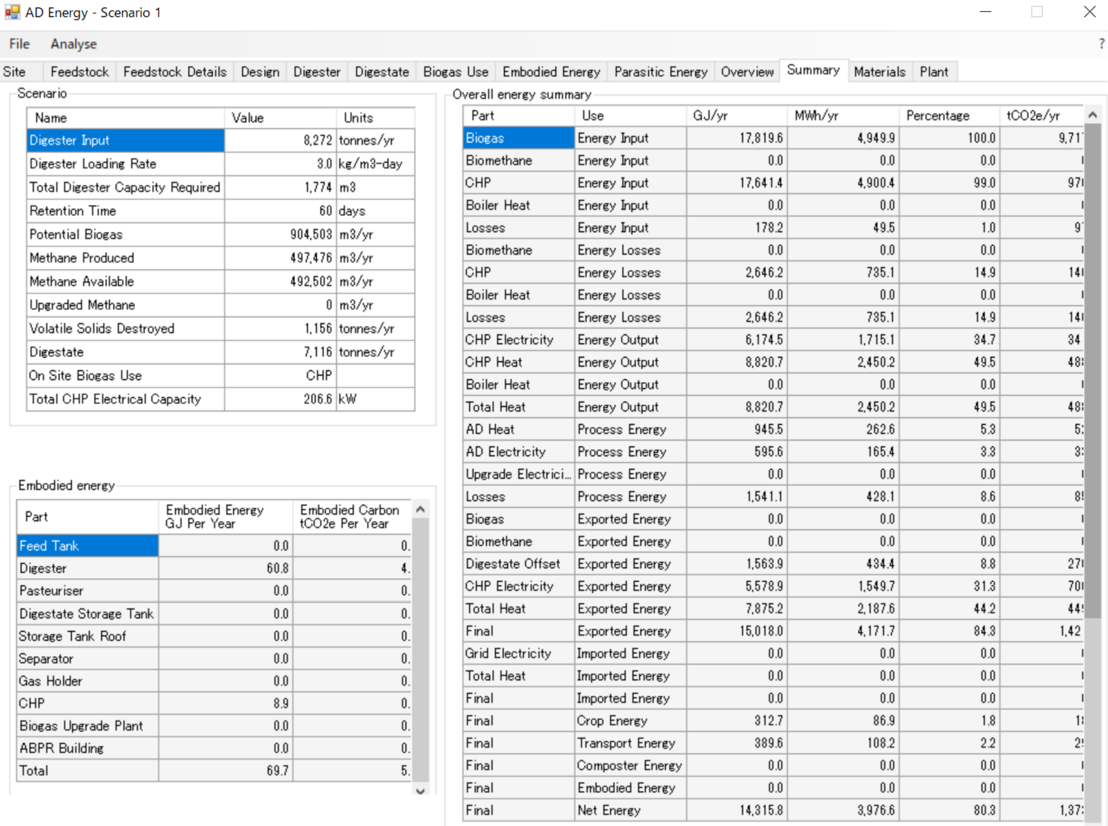
energy output (methane, electricity and heat), process loss, heating requirement, CHP and net energy.

Calculated results are shown in Figure 77. Figure 77 includes 4 screenshots from AD assessment tool. Figure 77a and b are the summary tab which gives the final summary of the main fields within the embodied energy, parasitic energy and overview tabs, based on data from the other tabs. Figures 78c and d are the overview tab which shows the resultant data in a format taking into account the energy produced, the energy used and any applicable losses. Detailed information on the AD assessment tool and the basis for the calculations used is given in the user manual (http://www.bioenergy.soton.ac.uk/AD_software_tool.htm).

According to the AD assessment tool, the net energy in scenario 1 was 14315.8 GJ year^{-1} and in scenario 2 was 13934.2 GJ year^{-1} (Figure 77a and b). Even though the methane yield in scenario 1 was lower than that of scenario 2, scenario 1 could produce more net energy due to the greater requirement for energy for heating in Scenario 2. Scenario 1 required 6.6 % of total input energy for heating which was less than half that needed for scenario 2, at 14.7 % (Figure 77c and d). The net energy in scenario 1 was 80.3 % and in scenario 2 was 76.1 %. Even if the energy for the CHP was considered, mesophilic digestion showed greater net energy than thermophilic digestion.

Table 64. Information for the AD assessment tool

	Scenario 1	Scenario 2	Reference
TS [% WW]	21		Tropical power data
VS [% WW]	18		UK lab data
VS [% TS]	86		
Methane yield [L CH_4 g^{-1} VS added]	0.333	0.342	UK lab data
N [g kg^{-1} TS]	12		NRM analysis data
P [g kg^{-1} TS]	3		
K [g kg^{-1} TS]	29		
Production Energy [MJ $tonne^{-1}$]	37.8		AD assessment tool's "Maize Corn - Straw" data
Production [kg CO_2 e $tonne^{-1}$]	2.2		
Para energy [kWh $tonne^{-1}$]	20		
Biogas CH_4 [%]	55		
Production Fixed C	0.5		
Feeding [tonnes WW $year^{-1}$]	8272		Tropical power data
Transport type	Tractor & trailer		
Distance [km]	2.7		
OLR [kg VS m^{-3} day^{-1}]	3		
Temperature [°C]	35	55	
Height to width ratio	0.27		Tropical power data
On site biogas Use	CHP (no biogas upgrade)		



AD Energy - Scenario 1

File Analyse

Site | Feedstock | Feedstock Details | Design | Digester | Digestate | Biogas Use | Embodied Energy | Parasitic Energy | Overview | Summary | Materials | Plant

Scenario

Name	Value	Units
Digester Input	8,272	tonnes/yr
Digester Loading Rate	3.0	kg/m3-day
Total Digester Capacity Required	1,774	m3
Retention Time	60	days
Potential Biogas	904,503	m3/yr
Methane Produced	497,476	m3/yr
Methane Available	492,502	m3/yr
Upgraded Methane	0	m3/yr
Volatile Solids Destroyed	1,156	tonnes/yr
Digestate	7,116	tonnes/yr
On Site Biogas Use	CHP	
Total CHP Electrical Capacity	206.6	kW

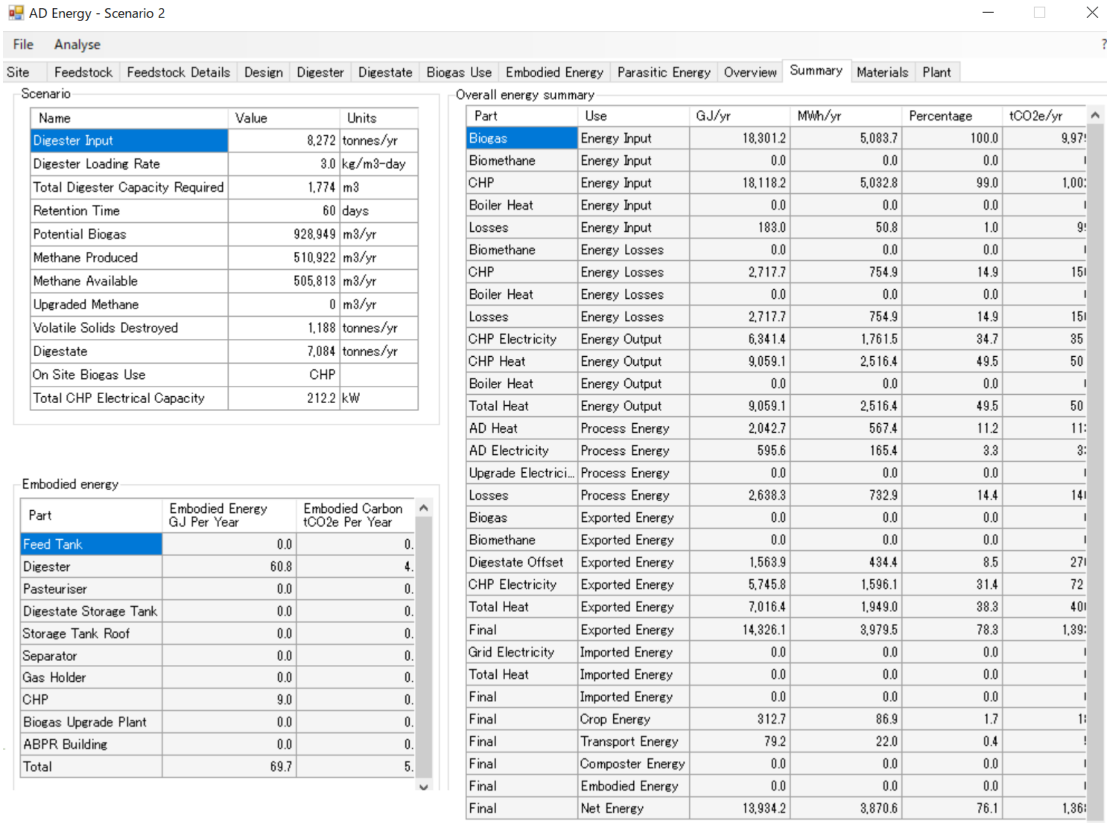
Embodied energy

Part	Embodied Energy GJ Per Year	Embodied Carbon tCO2e Per Year
Feed Tank	0.0	0.
Digester	60.8	4.
Pasteuriser	0.0	0.
Digestate Storage Tank	0.0	0.
Storage Tank Roof	0.0	0.
Separator	0.0	0.
Gas Holder	0.0	0.
CHP	8.9	0.
Biogas Upgrade Plant	0.0	0.
ABPR Building	0.0	0.
Total	69.7	5.

Overall energy summary

Part	Use	GJ/yr	MWh/yr	Percentage	tCO2e/yr
Biogas	Energy Input	17,819.6	4,949.9	100.0	9,71
Biomethane	Energy Input	0.0	0.0	0.0	
CHP	Energy Input	17,641.4	4,900.4	99.0	97
Boiler Heat	Energy Input	0.0	0.0	0.0	
Losses	Energy Input	178.2	49.5	1.0	9
Biomethane	Energy Losses	0.0	0.0	0.0	
CHP	Energy Losses	2,646.2	735.1	14.9	14
Boiler Heat	Energy Losses	0.0	0.0	0.0	
Losses	Energy Losses	2,646.2	735.1	14.9	14
CHP Electricity	Energy Output	6,174.5	1,715.1	34.7	34
CHP Heat	Energy Output	8,820.7	2,450.2	49.5	48
Boiler Heat	Energy Output	0.0	0.0	0.0	
Total Heat	Energy Output	8,820.7	2,450.2	49.5	48
AD Heat	Process Energy	945.5	262.6	5.3	5
AD Electricity	Process Energy	595.6	165.4	3.3	3
Upgrade Electrici...	Process Energy	0.0	0.0	0.0	
Losses	Process Energy	1,541.1	428.1	8.6	8
Biogas	Exported Energy	0.0	0.0	0.0	
Biomethane	Exported Energy	0.0	0.0	0.0	
Digestate Offset	Exported Energy	1,563.9	434.4	8.8	27
CHP Electricity	Exported Energy	5,578.9	1,549.7	31.3	70
Total Heat	Exported Energy	7,875.2	2,187.6	44.2	44
Final	Exported Energy	15,018.0	4,171.7	84.3	1,42
Grid Electricity	Imported Energy	0.0	0.0	0.0	
Total Heat	Imported Energy	0.0	0.0	0.0	
Final	Imported Energy	0.0	0.0	0.0	
Final	Crop Energy	312.7	86.9	1.8	1
Final	Transport Energy	389.6	108.2	2.2	2
Final	Composter Energy	0.0	0.0	0.0	
Final	Embodied Energy	0.0	0.0	0.0	
Final	Net Energy	14,315.8	3,976.6	80.3	1,37

(a) Scenario 1: summary tab



AD Energy - Scenario 2

File Analyse

Site | Feedstock | Feedstock Details | Design | Digester | Digestate | Biogas Use | Embodied Energy | Parasitic Energy | Overview | Summary | Materials | Plant

Scenario

Name	Value	Units
Digester Input	8,272	tonnes/yr
Digester Loading Rate	3.0	kg/m3-day
Total Digester Capacity Required	1,774	m3
Retention Time	60	days
Potential Biogas	928,949	m3/yr
Methane Produced	510,922	m3/yr
Methane Available	505,813	m3/yr
Upgraded Methane	0	m3/yr
Volatile Solids Destroyed	1,188	tonnes/yr
Digestate	7,084	tonnes/yr
On Site Biogas Use	CHP	
Total CHP Electrical Capacity	212.2	kW

Embodied energy

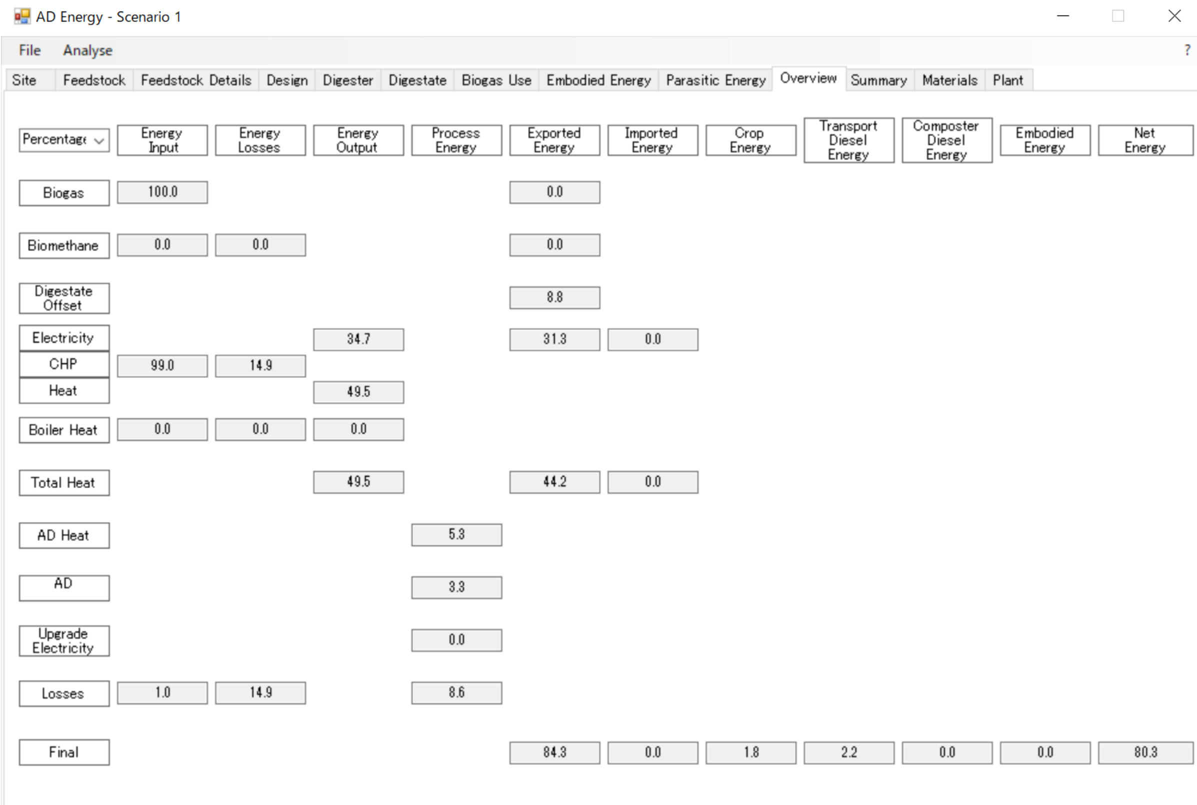
Part	Embodied Energy GJ Per Year	Embodied Carbon tCO2e Per Year
Feed Tank	0.0	0.
Digester	60.8	4.
Pasteuriser	0.0	0.
Digestate Storage Tank	0.0	0.
Storage Tank Roof	0.0	0.
Separator	0.0	0.
Gas Holder	0.0	0.
CHP	9.0	0.
Biogas Upgrade Plant	0.0	0.
ABPR Building	0.0	0.
Total	69.7	5.

Overall energy summary

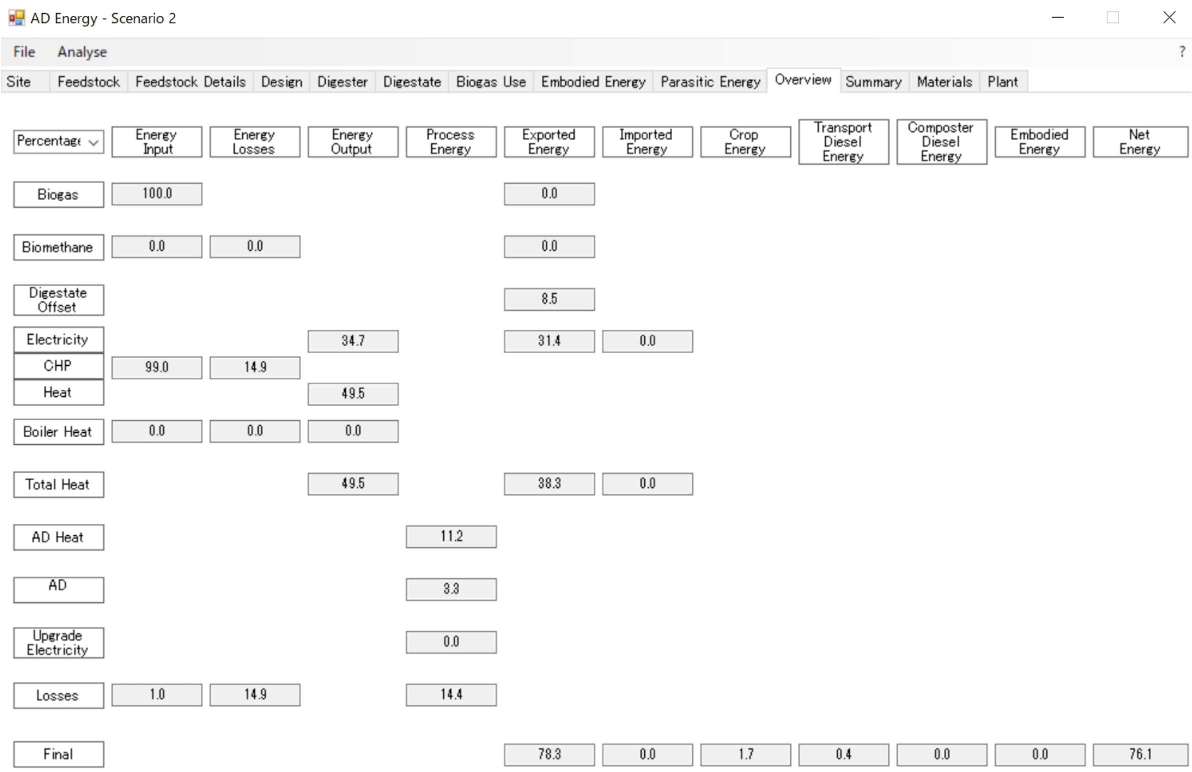
Part	Use	GJ/yr	MWh/yr	Percentage	tCO2e/yr
Biogas	Energy Input	18,301.2	5,083.7	100.0	9,97
Biomethane	Energy Input	0.0	0.0	0.0	
CHP	Energy Input	18,118.2	5,032.8	99.0	1,00
Boiler Heat	Energy Input	0.0	0.0	0.0	
Losses	Energy Input	183.0	50.8	1.0	9
Biomethane	Energy Losses	0.0	0.0	0.0	
CHP	Energy Losses	2,717.7	754.9	14.9	15
Boiler Heat	Energy Losses	0.0	0.0	0.0	
Losses	Energy Losses	2,717.7	754.9	14.9	15
CHP Electricity	Energy Output	6,341.4	1,761.5	34.7	35
CHP Heat	Energy Output	9,059.1	2,516.4	49.5	50
Boiler Heat	Energy Output	0.0	0.0	0.0	
Total Heat	Energy Output	9,059.1	2,516.4	49.5	50
AD Heat	Process Energy	2,042.7	567.4	11.2	11
AD Electricity	Process Energy	595.6	165.4	3.3	3
Upgrade Electrici...	Process Energy	0.0	0.0	0.0	
Losses	Process Energy	2,638.3	732.9	14.4	14
Biogas	Exported Energy	0.0	0.0	0.0	
Biomethane	Exported Energy	0.0	0.0	0.0	
Digestate Offset	Exported Energy	1,563.9	434.4	8.5	27
CHP Electricity	Exported Energy	5,745.8	1,596.1	31.4	72
Total Heat	Exported Energy	7,016.4	1,949.0	38.3	40
Final	Exported Energy	14,326.1	3,979.5	78.3	1,39
Grid Electricity	Imported Energy	0.0	0.0	0.0	
Total Heat	Imported Energy	0.0	0.0	0.0	
Final	Imported Energy	0.0	0.0	0.0	
Final	Crop Energy	312.7	86.9	1.7	1
Final	Transport Energy	79.2	22.0	0.4	
Final	Composter Energy	0.0	0.0	0.0	
Final	Embodied Energy	0.0	0.0	0.0	
Final	Net Energy	13,934.2	3,870.6	76.1	1,36

(b) Scenario 2: summary tab

Figure 77. Calculated results by the AD assessment tool



(c) Scenario 1; overview



(d) Scenario 2; overview

Figure 77 continued. Calculated results by the AD assessment tool

The results for the AD modelling tool are broadly similar to those for the manual modelling, (e.g. in absolute output (electricity and heat), heat loss, heat demand as a percentage of usable energy, and the relationship of the net energy between scenario 1 and scenario 2). Table 65 shows the comparison of results of the hand calculation and the AD assessment tool. For instance, the electricity output from hand calculation were 6914 GJ $year^{-1}$ in scenario 1, 7101 GJ $year^{-1}$ in scenario 2. The electricity output from AD assessment tool were bit less than these values; 6175 GJ $year^{-1}$ in scenario 1 and 6341 GJ $year^{-1}$ in scenario 2. These phenomenon were observed in other values. This may be caused by the complicated modelling system of AD assessment tool. The hand calculation considered only heating requirement and energy output. On the other hand, the AD assessment tool included more various data (e.g. process losses, percent methane lost upgrading in CHP, transportation, digestate utilisation, electricity requirement for parasitic energy etc). In fact, the net energy in hand calculation was 84-92 % which was approximately 10 % greater than that of AD assessment tool.

The percentage required for parasitic heat energy demand per produced heat in hand calculation were 13 % in scenario 1, 28% in scenario 2. In AD assessment tool, the results were 11 % in scenario 1, 23 % in scenario 2. The results from the AD assessment tool were less than that of the hand calculation. These results were less than the literature value 20-30 % (Banks *et al*., 2011).

The percentage of the parasitic electricity demand of produced electricity were 9-10 %. The literature values were 30 % (Banks *et al*., 2011) and 29 % (Havukainen *et al*., 2014). The calculated results were one third of the literature values. The parasitic energy demand of digestion plants depends on each plant and the demands are significantly different (Havukainen *et al*., 2014). Laaber assessed the 27 biogas plants in Austria and the demand of the produced biogas energy was 3 % in 2007 (Havukainen *et al*., 2014). The calculated results were within the range of the literature values.

The parasitic energy was always less than that of produced heat and electricity. Although there are minor difference between the results of the hand calculation and the AD assessment tool, these modelling showed same results. Scenario 1 (mesophilic condition with TE supplementation) was greater than scenario 2 (thermophilic condition) in terms of the net energy balance.

Electricity is useful for its economic value (sold to electricity company) and in some situations may also provide independence from the grid if this is subject to power cuts etc. It is more difficult to find an economic use for the heat, however, it may be worth considering bottling and sale of biogas (with or without upgrading) as an alternative to electricity generation. These data could be used for assessment of the economic and financial feasibility of the plant.

Table 65. Comparison the results of hand calculation and AD assessment tool

		Hand calculation		AD assessment tool	
Scenario		1	2	1	2
Energy input[GJ year^{-1}]					
Parasitic energy	Heat requirement	1312	2836	946	2043
	Electricity			596	596
Energy output [GJ year^{-1}]					
CHP	Heat	9877	10144	8821	9059
	Electricity	6914	7101	6175	6341
	Heat loss	2963	3043	2646	2718
Energy balance [%]					
	Net energy	92	84	80	76
Parasitic energy requirement of produced energy [%]					
	Heat	13	28	11	23
	Electricity			10	9

Note: these values are also shown or came from Table 63 (hand calculation) and Figure 77 (AD assessment tool) above, but are given here for ease of reference.

5.7.4. Comparison of results with actual biogas plant

Feeding amount

Feeding amount yearly average of baby maize stover is 22.7 tonne WW day-^{1}. According to Tropical Power's original AD plant process flow diagram, the expected amount of baby maize stover to feed to the biogas plant was 124.4 tonne WW day^{-1}. The actual feeding was thus only 18.2 % of target feeding.

Digester size

The calculated digester volume required is 1700 m^3 which includes head space. The actual digester volume is 5655 m^3, so the required volume is only around 30 % of the actual volume. The required working volume of 1360 m^3 is less than the volume of the two hydrolysers (volume 760 + 760 = 1520 m^3). This would require operating the hydrolysers at around 90 % of their overall volume, which may not be possible in practice; or alternatively the working volume could be reduced with a small increase in OLR. If the feeding amount is around 8271.9 tonne year^{-1} as in 2015-2016 as, taking the main digester out of service and using the hydrolysers as digesters may be a good option for efficient operation.

Mesophilic versus thermophilic digestion

Modelling showed the heat requirements for mesophilic and thermophilic digestion were 4.4 and 8.6 GJ day^{-1}, respectively. The energy production for mesophilic and thermophilic digestion was 54.1 and 55.6 GJ day^{-1}, respectively. Mesophilic digestion showed greater net energy than thermophilic digestion. This calculation did not consider the energy requirement for CHP and other parameters so the evaluation was carried out by AD assessment tool.

As the results in Figure 77 show, although the modelling is based on slightly different assumptions, the output also show that mesophilic operation is more favourable in terms of net energy and thus helps to confirm the above results.

5.8. Discussion and conclusions

Actual annual data from a real commercial biogas plant in Kenya was assessed. Assumptions were made to cover the missing data, and the reliability of data was checked where possible by calculation and comparison with literature values. Rationalisation was carried out as a basis for improved operation.

The data from Tropical Power showed differences between actual and expected values. The actual feeding amount was 21 % of the target feeding. The actual fuel consumption and assumed fuel consumption for harvesting and feedstock transportation showed reasonable agreement; however, the results did not match exactly with literature values. This may be caused by the different operating conditions, substrate and location and shows the importance of local datasets.

The reported specific methane yield was too high for gas production from a conventional agro-waste. This may have been caused by the biogas volume measuring method. The electricity conversion in CHP was acceptable. Tropical Power sells the electricity to the electricity company in Kenya, therefore, the data was more reliable. The study highlights the difference in data reliability between industry and academic studies.

Rationalisation was carried out to overcome the arising issues (i.e. feedstock availability). Mesophilic and thermophilic conditions were compared as considered in the laboratory studies. Rationalisation was carried out by hand calculation and software. Even though more complex factors are considered in the AD modelling tool software, the results were similar. Scenario 1 (mesophilic digestion with TE supplementation) showed a better energy balance than that of scenario 2 (thermophilic digestion). If suitable TE supplementation is not used for the anaerobic processing of baby maize stover, the energy balance may change as shown in the laboratory experiments in section 4.5.4. TE chemicals represent a cost which may have negative impacts in terms of profitability. This study did not consider the financial aspects of plant operation, or the embodied energy in TE. Even thermophilic digestion required TE dosing (especially W) in trial 2-3, however, and if the amounts of TE required are similar in both cases then mesophilic digestion will still be better than thermophilic digestion in terms of the net energy balance.

The overall results of the modelling showed that, as expected, the net energy production from anaerobic digestion of baby maize stover was positive in all cases, and also allowed quantification of some aspects of this energy output. The following conclusions could be made:

- The methods and values for calculation of energy used in harvesting and transportation of the baby maize stover feedstock were successfully validated against the data from the Tropical Power plant, and could therefore be use elsewhere in Kenya and similar locations where no data are available. The results also suggested that the energy use in harvesting at Gorge Farm of around 101 MJ tonne^{-1} TS was slightly lower than typical values reported for straw materials in Europe, highlighting the importance of local data –objective 1

- Although the Tropical Power plant is incorrectly sized for the currently available mass of feedstock, the results indicated the same or better performance than the laboratory studies with respect to specific methane production. The high average specific methane yield of 0.42 L CH_4 g^{-1} VS apparently achieved by the plant may have been caused by the method of biogas volume measurement. Therefore, the amount of generated electricity was used in determining the energy output of the biogas plant –objective 2

- In the case of the Tropical Power plant, until the amount of feedstock available increases a better solution would be to operate the two hydrolysers as digesters – objective 5

- The laboratory trials were better monitored than the Tropical Power plant and the data from them is based on actual rather than estimated gas production values. This data is also slightly more conservative than the data from the Tropical Power plant: using it therefore provided a robust basis for estimating the net anaerobic digestion energy production from baby maize stover in other plants – sub-objectives 2 and 5.

- The percentage of the energy input that was available as electricity and heat were 35 % and 50 % respectively. The percentage required for parasitic heat energy demand per produced heat in hand calculation were 13 % in scenario 1, 28% in scenario 2. In AD assessment tool, the results were 11 % in scenario 1, 23 % in scenario 2. The results of give specific values for net energy output that can be used in planning new systems or assessing the performance of other plants. – objectives 4 and 5

- When considered in context of the situation in Naivasha and Kenya the results indicate that using baby maize stover as an anaerobic digestion feedstock is a good option – objectives 1-5

- The same modelling tools can be used in assessment at other specific locations in Kenya – objectives 1-5

Thus the results of modelling, in conjunction with the broader context described in the introduction to this chapter, provide a rational basis for saying that the use of baby maize stover as an AD feedstock in Kenya is justified and is likely to be a good option. The biogas produced could also be used directly or after upgrading as a fuel source for cooking or transportation, rather than converted to electricity: this was not modelled in the current work but could be done in future studies

6. Conclusions

The overall aim of this research was to determine the suitability of baby maize stover as an anaerobic digestion feedstock in terms of its energy yield and any operating requirements for stable performance. The work was based on practical experiments and theoretical modelling.

The conclusions are organised in the sequence: summary of key results for each trial and issues, general conclusions and future work.

6.1. Summary of key results

Trial 1

- Mesophilic digestion of maize silage showed rapid volume expansion and foaming. This may have been related to the rather poor quality of maize silage and to a cold shock experience, but digestion finally stabilised and gave reasonable gas production 0.385 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} in mesophilic and thermophilic digesters.
- Mesophilic digestion and thermophilic digestion of maize silage did not show significant differences in terms of specific biogas or methane production.
- Thermophilic digestate appeared considerably less viscous than mesophilic digestate.

Trial 2-1

- In periods of pseudo steady operation, SMP in mesophilic digesters seemed to be significantly lower than in thermophilic digesters (0.278 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} in mesophilic digesters and 0.342 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} in thermophilic digesters).
- Stable anaerobic processing of baby maize stover needed more than 3 TE (Fe, Co, Ni as above)
    - Mesophilic digesters failed around 150 days even though they were receiving regular 3 TE supplementation
    - D9&10 were operated stably for twice as many equivalent HRT as the other mesophilic digesters. Only inoculum of D9&10 partially consisted fresh Millbrook digestate which came from wastewater treatment plant.
    - D5-10, six digesters operation failed at approximately the same time even though their inoculum, operating history and temperature range was different. The failure happened after TE dosing ran out about 30 days, equivalent to replacing around 60 % of reactor volume.

- Because of the complex history of these digesters, it was hard to determine whether lack of TE had more effect on mesophilic or thermophilic digestion; however, 3 TE dosing was insufficient for mesophilic digestion.

- Thermophilic pre-hydrolysis did not appear to offer any increase in total gas production. Hydrogen and methane production were observed during pre-hydrolysis. Even if this gas production were considered, SMP in two stage was less than that of single stage.
- Digestate from reactors with a thermophilic pre-hydrolysis stage was less viscous in appearance than from single-stage mesophilic reactors
- Little or no foaming was observed in mesophilic digesters
- Foaming was a problem in thermophilic digesters at start of the trial. This may have been due to the strategy for switching feedstock at high load.

Trial 2-2

- All four digesters operation failed within one month
- The digesters were overloaded so results are unreliable, however, gas production with pre-hydrolysis was still lower than that of single stage
- Digesters with pre-hydrolysis showed lower VFA accumulation which may be just less successful at hydrolysing

Trial 2-3

- SMP in mesophilic digesters with 5TE was very similar to that of thermophilic digesters (0.333 L CH_4 g^{-1} VS at OLR 3 g VS L^{-1} day^{-1} in mesophilic digesters and 0.324 L CH_4 g^{-1} VS at OLR 4 g VS L^{-1} day^{-1} in thermophilic digesters)
- With 5 TE, gas production from pre-hydrolysis also improved but was still not better than single stage
- Even 5 TE conducted, mesophilic digestion still failed after ~150 days.
- Mesophilic digesters without TE failed very slightly earlier and VFA worse. 40-day bump in mesophilic digesters with 5 TE were less than that of mesophilic digesters without TE.
- After giving an additional dose of 3 TE to either digesters with 3TE or 5TE, VFA values in the digesters increased. This may stimulated hydrolytic and acidogenic organisms.
- After adding W only to thermophilic digesters with 3 TE, VFA increased but then fell.
- After adding W only to 5 TE digester, VFA fell
- VFA in digesters with 3 TE and 5 TE that had received additional 3 TE only fell after adding W.

- 5 TE appears to be helpful but W also essential at least in thermophilic digestion as Jiang *et al.* (2012)

Modelling

- The biogas plant in Kenya received only 21 % of target feeding
- The required working volume of digester was 1360 m^3 which was 24 % of actual digester volume.
- The required working volume 1360 m^3 was less than that of total hydrolyser volume 1520 m^3. The hydrolysers have capacity as digester.
- Modelling assumption was similar to actual operation; the calculated fuel consumption for substrate harvesting and collection were 95 % of actual fuel consumption.
- The results of hand calculation and AD assessment tool indicate the overall energy balance in single mesophilic digester was greater than that of single thermophilic digester.
- The data from modelling confirm that baby maize stover produces a net positive energy output when used as an anaerobic digestion substrate, and provides information on which the design of future systems can be based

Issues

Regarding Trial 1, the aim was to create a baseline for trial 2; however, the maize silage quality as a substrate was poor. Some parts of the maize silage were already fermented when it arrived. Mesophilic digestion experienced rapid volume expansion and cold shock experience, reducing the reliability of these results as a baseline.

Turning to Trial 2-1, 2-2, 2-3, mesophilic digestion with pre-hydrolysis was carried out 3 times and all of these trials failed within 3HRT. During experiments, inoculum and TE dose were changed to improve the performance. The reason for failure in Trial 2-1 might be related to the pre-hydrolysis procedure. The substrate was mixed with supernatant from digestate because the actual biogas plant has a separator. All of the mixture of separated digestate plus feed was then returned to the reactor each day. A better operating mode might be to leave a proportion of this material as an inoculum allowing it to adapt to the conditions in the pre-hydrolysis reactor (i.e. thermophilic and primarily intended for hydrolysis so lower pH acceptable).

It seemed TE dosing had positive effects for stable operation and higher methane yield. W dosing caused VFA degradation in thermophilic digesters in Trial 2-3: it would be interesting to know if it has a similar effect in mesophilic digestion but this was not tested in the current trial.

Moving to modelling, some data from Tropical Power were not dependable (e.g. biogas volume) and this might be related to their method of measurement. They did not have the same chemical analytical facilities as were available in the laboratory at Southampton. The biogas plant is in Kenya and longitude, latitude and altitude were different to those in Southampton. The ambient temperature in Kenya was applied in the software for modelling but pressure or other factors might have impacts.

6.2. General conclusions

- In this study, the two stage system with thermophilic pre-hydrolysis did not have any positive effects in terms of increasing the SMP from baby maize stover.
- Acclimatisation of inoculum (e.g. through mixing of fresh Millbrook digestate and digestate from previous trial) was essential for stable start-up. Where digestate acclimated to another maize-derived substrate is not available, a reduced OLR should be applied and at the start and then slowly increased to the target OLR.
- 5 TE and W supplementation helped produce higher methane yields and stable operation.
    - SMP in mesophilic digesters with 3 TE (10 mg Fe L^{-1}, 1mg Co L^{-1}, 1 mg Ni L^{-1}) was significantly lower than that of thermophilic digesters. Interestingly this was not the case in the baseline substrate of conventional maize silage.
    - SMP in mesophilic digesters with 5 TE (10 mg Fe L^{-1}, 1mg Co L^{-1}, 0.4 mg Ni L^{-1}, 0.2 mg Se L^{-1}, 0.2 mg Mo L^{-1}) was close to that of thermophilic digesters.
    - Even with 5 TE supplementation, mesophilic digestion still failed after ~150 days.
    - W dosing (0.2 mg W L^{-1}) helped to decrease VFA accumulation in thermophilic digesters and allow stable operation.
- AD of baby maize stover with 5 TE is feasible at OLR of 3 kg VS m^{-3} day^{-1} with methane production of 0.333 L kg^{-1} VS under mesophilic condition.
- AD of baby maize stover with 5 TE is feasible at OLR of 4 kg VS m^{-3} day^{-1} with methane production of 0.324 L kg^{-1} VS under thermophilic conditions. Thermophilic digestion thus does not appear to offer any advantage over mesophilic digestion when adequate trace element supplementation is available.
- Digestate viscosity in thermophilic digesters and in mesophilic digesters with pre-hydrolysis was lower than that of single-stage mesophilic digesters.
- Tropical Power Ltd's biogas plant in Kenya was much larger than required for the initial feed amount.

- In terms of overall energy balance, mesophilic digestion was performed better than thermophilic digestion.

6.3. Future work

- Further work on pre-treatment to explore if the pre-treatment was to explore whether alternative operating modes such as partial retention of inoculum in the hydrolysis phase would be more effective in increasing overall specific methane yields.
- Further work to determine the essential trace elements for baby maize stover under mesophilic condition, and to confirm that long-term stable operation if possible with W addition. The optimum TE dosing strength of these limiting elements should also be tested.
- Further work on data collection and analysis from Tropical Power Ltd to provide a detailed basis for re-design of the plant. It was originally intended to visit and gather first-hand data on the AD plant and on operations at Gorge Farm. Unfortunately in addition to time constraints at the end of the laboratory work a planned visit to Kenya had to be cancelled due to political unrest at the time of the 2017 elections.

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