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NITROGEN CONTROL IN SOURCE SEGREGATED DOMESTIC FOOD WASTE ANAEROBIC DIGESTION USING STRIPPING TECHNOLOGIES

by

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ABSTRACT

FACULTY OF ENGINEERING AND THE ENVIRONMENT

Doctor of Philosophy

NITROGEN CONTROL IN SOURCE SEGREGATED DOMESTIC FOOD WASTE ANAEROBIC DIGESTION USING STRIPPING TECHNOLOGIES

Alba Serna-Maza

Anaerobic digestion of source segregated domestic food waste (SS-DFW) offers a sustainable management route for reclaiming potential energy in the form of a fuel gas, and nutrients which can be recycled back to land. However, the biochemical characteristics of SS-DFW can lead to free ammonia nitrogen (FAN) concentrations that are inhibitory to the digestion process causing unstable operation and in some cases complete process failure, particularly in thermophilic systems.

With the purpose of reducing the total ammoniacal nitrogen (TAN) in the digester, side-stream and *in situ* biogas stripping technologies were tested.

Mesophilic and thermophilic temperatures were evaluated under moderate and complete biogas mixing rates (0.4 l min^{-1} – 2.6 l min^{-1}) in a batch system. Laboratory investigations showed that TAN reductions in an *in situ* bubbling reactor with moderate and complete gas mixing rates were non-existent at mesophilic temperatures and minimal at thermophilic temperatures. For this reason, it is unlikely that *in situ* biogas stripping would be adequate to prevent TAN concentrations greater than 2500 mg N l^{-1} in a food waste digester and thus will not mitigate ammonia inhibition in a thermophilic system.

Semi-continuous trials carried out on SS-DFW in laboratory-scale digesters, fed daily at an organic loading rate (OLR) of 2 kg VS m^{-3} day^{-1} and coupled to stripping columns at low bleed rates (2 – 3.5 % digester volume per day treated in the stripping process) were effective in reducing ammonia concentrations to below thermophilic toxic levels (TAN concentration of 2500 – 3500 mg N l^{-1}). The experiments also confirmed that removal of a proportion of the digester contents and their exposure to thermophilic conditions with pH adjustment to 10 had no adverse effects on performance in terms of biogas production (0.83 ± 0.03 l g^{-1} VS without stripping, 0.84 ± 0.05 l g^{-1} VS with stripping) or VS destruction (81.8 % without stripping, 88.5 % with stripping). The process required high pH and temperature (≥70 ºC) to achieve a TAN concentration below the toxic threshold for thermophilic digestion, and it is unlikely that stripping at 55 ºC and pH 10 would achieve the target reduction.

The research showed the way forward for the application of side-stream stripping to prevent the build-up of ammonia under thermophilic conditions, if the digester is started up with a non-inhibitory FAN concentration in the inoculum.

Keywords: Anaerobic digestion; source segregated domestic food waste; ammonia removal; *in situ* stripping; side-stream stripping

Contents

List of tables

List of figures

DECLARATION OF AUTHORSHIP

I, Alba Serna-Maza 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.

Nitrogen control in source segregated domestic food waste anaerobic digestion using stripping technologies

I confirm that:

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Signed: .....

Date: .....

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Definitions and Abbreviations

AD	Anaerobic Digestion
ATP	Adenosine Triphosphate
C/N	Carbon to Nitrogen Ratio
COSSH	Control of Substances Hazardous to Health
CSTR	Continuously Stirred Tank Reactor
DI	Deionised Water
EU	European Union
EPS	Extracellular Polymeric Substances
FAN	Free (unionized) Ammonia Nitrogen (NH_3)
FISH	Fluorescent In-Situ Hybridisation
FW	Food Waste
HRT	Hydraulic Retention Time
H_t	Total Height
IA	Intermediate Alkalinity
LCFA	Long Chain Fatty Acid
MAP	Magnesium Ammonium Phosphate
MC	Mesophilic conditions
MSW	Municipal Solid Waste
MS-OFMSW	Mechanically Sorted Organic Fraction of Municipal Solid Waste
OFMSW	Organic Fraction of Municipal Solid Waste
OHPA	Obligate Hydrogen-Producing Acetogens
OLR	Organic Loading Rate
PA	Partial Alkalinity
RPM	Revolutions Per Minute
RT	Retention Time
SBP	Specific Biogas Production
SMP	Specific Methane Production
SRT	Solids Retention Time

SS-OFMSW	Source Segregated Organic Fraction of Municipal Solid Waste
SS-DFW	Source Segregated Domestic Food Waste
STP	Standard Temperature and Pressure
t	Stripping Time Constant
TA	Total Alkalinity
TAN	Total Ammoniacal Nitrogen
TC	Thermophilic Conditions
TE	Trace Element
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
UASB	Upflow Anaerobic Sludge Bed
VS	Volatile Solids
VFA	Volatile Fatty Acid
ΔpH	Trans-membrane pH
ΔΨ	Trans-membrane Electrical Gradient
$\Delta_{\mu_H^+}$	Proton Motive Force

1. Introduction

It is estimated that 89 million tonnes of food waste are generated in the EU27 each year by households, manufacturing and other sectors, not including agricultural food waste or fish catches returned to the sea. This equates to 179 kg per capita and year, varying hugely between different countries. Most of the household waste generated in Europe is currently landfilled, but this involves a loss of potential resources and may increase the generation of greenhouse gas emissions due to the release of methane when the food waste is degraded (Service and Commission, 2010).

To avoid the loss of this potential energy the member states of the European Union are required by the Council Directive (1999/31/EC) to reduce the quantity of biodegradable waste going to landfill. The final target was set in 2016, by which time biodegradable municipal waste going to landfill must be reduced to 35 % (by weight) of the total amount produced in 1995. The strategy to achieve this target should include measures such as recycling, composting, valorisation or materials/energy recovery. Unless these actions are taken, food waste would be expected to increase 40 % by 2020 (Development, 2011).

Source segregated municipal solid waste (SS-OFMSW) is generally associated with high methane yields (0.43 – 0.63 STP m^3 CH_4 kg^{-1} VS) (Hansen et al., 2007; Bernstad et al., 2013; Zhang et al., 2012a; Banks and Zhang, 2010) and good digestate quality, whereas mechanically sorted municipal solid waste (MS-OFMSW) usually has lower methane yields (0.11 – 0.36 STP m^3 CH_4 kg^{-1} VS) (Hartmann and Ahring, 2006; Zhang et al., 2012a; Banks and Zhang, 2010). In addition MS-OFMSW digestate has a greater degree of contamination by plastics and inert materials, preventing land spreading (Braber, 1995); consequently, MS-OFMSW digestate often needs to be either landfilled or incinerated. For this reason, source segregation is preferable to mechanical separation whenever possible.

The source segregation, separate collection and subsequent anaerobic digestion (AD) of source segregated domestic food waste (SS-DFW) can help to reduce the OFMSW for disposal and, in some cases, help governments to meet

the targets of the EU Landfill directive (1999/31/EC). Importantly, anaerobic digestion also offers a method of reclaiming potential energy in the waste in the form of a fuel gas (Mata-Alvarez et al., 2000; Chen et al., 2008; Hall and Howe, 2012; Verena et al., 2012), and opens up a route by which nutrients can be recycled back to land (Jokela and Rintala, 2003; Arthurson, 2009; Lukehurst et al., 2010; Fuente et al., 2012). This has advantages even compared to incineration for energy recovery, as the high moisture content of food waste negates much of the energy gain and in thermal processing many nutrients are lost (Braber, 1995; Hartmann and Ahring, 2006). Digestion may therefore offer a more sustainable route to resource recovery compared to other waste treatment technologies that are less suited to dealing with this high moisture fraction.

1.1 Background

In order to maximize the economic efficiency of the digestion process and the biogas productivity, process imbalances during digestion need to be avoided (McCarty, 1964). Macro and micro nutrient concentrations, toxic compounds, inoculum acclimation to the operational conditions and critical process control parameters are all factors that need to be considered in order to achieve this (Chen et al., 2008).

Although anaerobic digestion offers numerous benefits (Seghezzo et al., 1998), AD of food waste can present difficulties, mainly due to its high protein content. Ammoniacal nitrogen is released by the biological degradation of nitrogenous matter which, although essential for the growth of anaerobic microorganisms, can lead to free ammonia concentrations that are inhibitory to the digestion process. The ammonia inhibits the methanogenic *Archaea*, in particular the acetoclastic methanogens (Angelidaki and Ahring, 1993b; Kayhanian, 1999; Liu and Sung, 2002; Chen et al., 2008; Schnürer and Nordberg, 2008; Prochazka et al., 2012). The result is operational instability caused by volatile fatty acid (VFA) accumulation, decrease in biogas production, and in the worst cases failure of digestion (Koster and Lettinga, 1984; Angelidaki and Ahring, 1993b, 1994; Poggi-Varaldo et al., 1997; Hansen et al., 1998; Niu et al., 2013b; Niu et al., 2013c).

These problems have been largely resolved at mesophilic temperatures through stimulation of the hydrogenotrophic metabolic pathway by the addition of selenium and cobalt, both of which are commonly deficient in food waste (Climenhaga and Banks, 2008). This strategy has allowed stable digestion of food waste at high OLR (> 5 kg VS m^{-3} day^{-1}) and total ammoniacal nitrogen (TAN) concentrations > 6 g N l^{-1} (Banks et al., 2012). Recent work in the FP7 VALORGAS project has shown that SS-DFW digestion can operate at OLR up to 8 g VS l^{-1} day^{-1} with addition TE supplementation; and at TAN concentrations of up to 8 g N l^{-1} in a TE-supplemented digester treating urea-added SS-DFW (VALORGAS D4-6, 2013).

A comparison between different digestion strategies for the OFMSW (wet, semidry, and dry) has reported higher biogas yields in thermophilic AD processes when compared to mesophilic; the difference in biogas yield between the two operational temperatures decreases when the OLR is increased (Hartmann and Ahring, 2006). At temperatures in the thermophilic range, however, the TAN toxic threshold is reduced as the equilibrium moves towards free ammonia, and under these conditions trace element additions have not been successful in overcoming the associated problem of VFA accumulation as the methanogenic/acetogenic syntrophy breaks down (Yirong et al., 2013a). Yirong et al. (2013a) compared mesophilic and thermophilic digestion of SS-DFW without water addition into the system and found failure symptoms in the thermophilic range when TAN concentration reached 3.5 g N l^{-1}. Similar inhibition limits are reported by other authors digesting manures and other wastes (Angelidaki and Ahring, 1993b, 1994; Borja et al., 1996; Hansen et al., 1998; Angelidaki et al., 2006b; Nielsen and Ahring, 2007; Niu et al., 2013c).

To solve the operational problems found during the thermophilic digestion of food waste one approach is to reduce the TAN concentration in the digester by substrate dilution (Kayhanian, 1999; Chen et al., 2008; Neiva Correia et al., 2008, VALORGAS D4-6, 2013) but this has both resource and energy implications. Co-digestion with a carbon rich substrate to increase the C/N ratio to reach optimum N (Kayhanian, 1999) is also possible, but depends on the availability of a suitable low nitrogen co-substrate.

Other possible solutions involve reducing the ammonia in the digester or its feed by biological or physicochemical methods (i.e. ammonia stripping). The use of stripping techniques makes possible to reduce the ammoniacal nitrogen concentration in the digester, and improve the performance by avoiding ammonia inhibition and VFA accumulation (Siegrist et al., 2005; Belostotskiy et al., 2013). Additionally, nitrogen can be recovered as ammonium sulphate, an important nitrogen fertiliser source (Gowariker et al., 2009), while the use of nitrogen-reduced digestate may allow a higher application rate in nitrogen-vulnerable zones under the Nitrates directive (91/676/EEC). Ammonia stripping has been trialled using a range of approaches, including with or without solids/liquid separation and using air, nitrogen, steam and biogas as the stripping agent (Jiang et al., 2014; Ledda et al., 2013; Jiang et al., 2013; Zeng et al., 2006; Nielsen et al., 2012). Side-stream and *in situ* biogas stripping configurations are particularly attractive as these offer a simple 'bolt-on' concept that could be used with existing anaerobic digestion process designs (Walker et al., 2011). To date, however, no study has conclusively proved that these practices can successfully reduce TAN concentration below inhibitory limits in thermophilic food waste digestion without detrimental effects on the digestion process in the longer term; and there is a corresponding lack of reliable information on design parameters and operating protocols for implementation of these systems.

1.2 Aims and objectives

1.2.1 Aims

The aim of this research is to determine whether biogas stripping techniques can be successfully used to control the total ammoniacal nitrogen concentration in a digester treating source segregated domestic food waste to below inhibitory concentrations, without detrimental long-term effects on the digestion process, and to identify suitable operating parameters for the stripping process.

1.2.2 Objectives

The following objectives were identified as necessary to achieve the above aim.

- To carry out semi-continuous digestion of source segregated domestic food waste in a continuously stirred tank reactor (CSTR) for the provision of fresh digestate samples from a stable and well-operated digester with a known history running under conditions typical of full-scale plants.
- To assess the feasibility of decreasing the TAN in a digester to below the toxic threshold using *in situ* biogas bubbling at typical gas mixing rates.
- To conduct batch stripping tests at different temperatures and pH conditions and low biogas bubbling rates to determine the ammonia removal kinetics of fresh food waste digestate.
- To operate semi-continuously fed digesters coupled with stripping columns to evaluate the capability of the side-stream system to control TAN concentration while also gauging the long term effects of the stripping process on digester operation and performance.
- To conduct a nitrogen mass balance to confirm whether the side-stream stripping strategy would succeed in maintaining a safe TAN concentration in a digester fed on source separated food waste at different organic loading rates.

Although the processes being developed here are primarily intended for use with thermophilic digestion systems, the experiments used mesophilic conditions as the starting point as these allow operation at a high initial TAN concentration in the digester, in order to demonstrate an effective TAN reduction produced by the side-stream process operating at a low bleed rate.

2. Literature review

2.1 Principles of anaerobic digestion

2.1.1 Degradation route

In the absence of oxygen microorganisms breakdown complex organic matter in a series of syntrophic chemical reactions to form methane, carbon dioxide, hydrogen and hydrogen sulphide as well as other soluble compounds. Fig. 1 shows the general degradation route of particulate organic matter to biogas in the AD process.

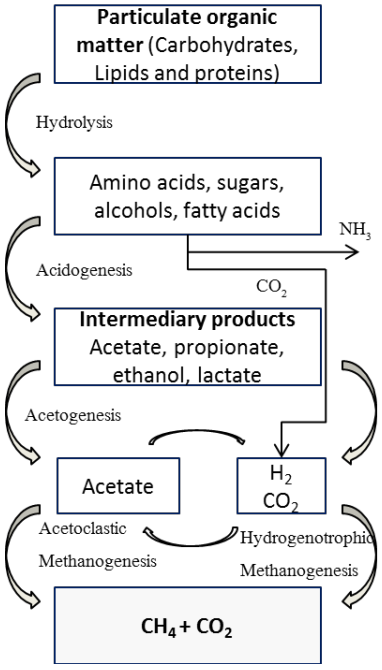


Fig. 1. Anaerobic conversion of particulate organic matter to biogas (Modified Demirel and Scherer (2008))

Hydrolysis:

This is the first essential stage in the anaerobic degradation of complex biopolymers by hydrolytic microorganisms; since methanogenic *Archaea* and acetogenic bacteria are incapable of utilizing complex polymeric substrates directly, a number of extracellular hydrolytic enzymes (protease, lipase, cellulase, pectinase, amylase, chitinase, etc) initiate the solubilisation into monomers. Hydrolysis products include monosaccharides, amino acids and fatty acids (Mara and Horan, 2003). Hydrolysis becomes rate limiting in a CSTR

when the HRT is very low and it is matched to SRT, due to a lack of time to hydrolyse the solids (Batstone and Jensen, 2011).

Acidogenesis:

During acidogenesis the hydrolysed monomers are fermented to produce several intermediate products, such as organic acids (VFA and LCFA), hydrogen, and carbon dioxide. Acidogens provide important substrates for acetogens and methanogens. This is considered to be the fastest step (Mata-Alvarez, 2003) and the least susceptible to inhibition in the anaerobic digestion process (Koster and Lettinga, 1988; Robbins et al., 1989).

Acetogenesis:

Acetogenic bacteria produce acetate, carbon dioxide and hydrogen that will be further metabolized by methanogens in the final stage of anaerobic digestion. There are two distinct groups of acetogenic bacteria, i.e. the obligate hydrogen-producing acetogens (OHPA) or proton-reducing acetogens, and the homoacetogens (Mara and Horan, 2003).

The first group degrades VFA, alcohols and LCFA to produce acetic acid, carbon dioxide and hydrogen. These species are essential in the β-oxidation of LCFA produced in the hydrolysis of lipid, and are also involved in the anaerobic degradation of aromatic compounds. These microorganisms are only capable of growth in environments that maintain a low concentration of the metabolic products. Methanogens are inhibited by fatty acids (substrates of the OHPA) and the OHPA are inhibited by hydrogen (a substrate of the methanogens) (Hattori, 2008). Hence, the syntrophic relationships between hydrogen-consuming species (e.g. methanogens and sulphate-reducing bacteria) and OHPA in an anaerobic digester are held in a fairly fragile state of equilibrium, and any small perturbation in the concentration of either of these substrates may lead to inhibitory effects of both groups (Mara and Horan, 2003).

The second group of acetogenic bacteria (homoacetogens) utilizes hydrogen and carbon dioxide to generate acetate. Nevertheless, this pathway is considered a minor role for methanogenic purposes (Mara and Horan, 2003). The acetate-oxidizing bacteria can also accomplish the reverse reaction (Demirel and Scherer, 2008). In terms of methane production this trajectory is

vital in those reactors with the acetoclastic methanogenic population inhibited or suppressed, e.g. at high FAN concentration (Schnürer and Nordberg, 2008) or at psychrophilic temperatures (McKeown et al., 2009; O'Reilly et al., 2009).

Methanogenesis:

This is the last stage in the conversion into biogas from acetate, hydrogen and carbon dioxide; it is often considered the slowest step in the AD process. Methanogens are strict anaerobes and are the key microorganisms, since without them, the ultimate breakdown of an organic material would not take place due to the accumulation of the end-products of the acid-producing bacteria (Mara and Horan, 2003). Methanogenesis is the critical phase of the anaerobic process due to the lower tolerance to environmental stress, the high substrate specificity (acetate, hydrogen and carbon dioxide) and the low growth rate of methanogenic *Archaea*. Therefore, it is important to control the inhibitory factors to optimize the AD performance (Koster and Lettinga, 1988).

Methanogens are classified with regard to the substrate utilised in the methane generation into two groups: acetoclastic and hydrogenotrophic methanogens.

The acetate-utilizing methanogens (acetoclastic) can cleave acetate into methane and carbon dioxide and can also accomplish the reverse reaction (Demirel and Scherer, 2008). The syntrophic acetate oxidation pathway is a two-step reaction. This pathway is initialised with acetate oxidation: both methyl and carboxyl groups of acetate are oxidized to carbon dioxide and hydrogen by syntrophic acetate-oxidising bacteria, followed by methanation of those products by hydrogenotrophic methanogens (Schnürer et al., 1994; Hattori, 2008). The hydrogen-utilizing methanogens are considered to be slow growers, therefore under non stress conditions the main acetate degradation route is by acetoclastic methanogenesis. The dominance of this pathway can be altered by the presence of ammonia since hydrogenotrophic methanogens have a higher tolerance for this substance (Angelidaki and Ahring, 1993b).

Acetate oxidation activity is determined by measuring the production of $^{14}CH_4$ and $^{14}CO_2$ when labelled [2-^{14}C] sodium acetate is used in an incubation process. Labelled methane is exclusively formed when acetoclastic methanogens degrade acetate. In the syntrophic acetate oxidation pathway, however, both carbon atoms of acetate are converted to carbon dioxide and

only part of the carbon dioxide is subsequently reduced to methane (Table 1). Therefore, an increase in the $^{14}CO_2$:$^{14}CH_4$ ratio indicates a proliferation of the syntrophic acetate-oxidising pathway (Karakashev et al., 2006). Microbial ecology evaluation with fluorescent *in situ* hybridization and PCR temporal temperature gradient gel electrophoresis together with labelled [2-^{14}C] sodium acetate analysis conducted by Karakashev et al. (2006) on mesophilic and thermophilic full-scale digesters fed on manure and wastewater sewage sludge indicated that $^{14}CO_2$:$^{14}CH_4$ ratios below 0.1 were dominated by *Methanosaetaceae* and low levels of acetate oxidation, while $^{14}CO_2$:$^{14}CH_4$ ratio above 1 had high levels of acetate oxidation with populations dominated by other methanogenic *Archaea* and without *Methanosaetaceae*.

Table 1. Reactions involved in acetate and hydrogen metabolism

(Hattori, 2008)

Process	Reaction	ΔG^0 (kJ mol^{-1})
(1) Acetoclastic methanogenesis	$^*CH_3COO^- + H_2O \rightarrow {}^*CH_4 + HCO_3^-$	-31
(2) Syntrophic acetate oxidation	$^*CH_3COO^- + 4H_2O \rightarrow H^*CO_3^- + 4H_2 + HCO_3^- + H^+$	104.6
(3) H_2-consuming methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-135.6
(4) Sum (2) + (3)	$^*CH_3COO^- + H_2O \rightarrow H^*CO_3^- + CH_4$	-31
(5) H_2-consuming acetogenesis	$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	-104.6

* methyl group carbon of acetate (labelled carbon)

100 % of the labelled carbon it is converted to CH_4 by reaction 1 or HCO_3^- by reaction 4 or the syntrophic acetate oxidation pathway.

2.1.2 Environmental factors

The final performance of an anaerobic reactor does not only depend on the structure of the microbial population. It is also influenced by operational and environmental factors, nutrients and inhibitors present in the media as well as correct inoculation.

2.1.2.1 Temperature

Anaerobic digesters are very sensitive to temperature. Changes in temperature have both biochemical and physicochemical implications. When temperature increases, reaction rates are increased according to the Arrhenius equation, as are microbial activity and yields. Temperature changes also induce shifts in reaction pathways due to modifications in the free energy of reaction.

These biochemical impacts occur in any temperature range, but three temperature ranges have been differentiated based on dominant microbial groups, i.e. psychrophilic (10 – 30 ºC), mesophilic (30 – 40 ºC) and thermophilic (40 – 70 ºC) (Batstone and Jensen, 2011).

With regard to the physicochemical impacts, when temperature is increased the volumetric gas production, gas transfer rates and water-vapour fraction in the gas phase also increase. On the other hand there is a decrease in gas solubility: this effect presents a challenge for psychrophilic systems, since at low operational temperatures a significant part of the produced methane is dissolved in the treated effluent (Lettinga et al., 2001; Bandara et al., 2011; Smith et al., 2012; Smith et al., 2013). An increase in temperature modifies the solubility of solids and the equilibrium constants (e.g. free ammonia to total ammoniacal nitrogen ratio) (Batstone and Jensen, 2011), and decreases the viscosity in the reactor which reduces the energy required for mixing (Lettinga et al., 2001).

As temperature increases from mesophilic (MC) to thermophilic conditions (TC) AD is boosted with enhanced hydrolysis and increased solid destruction rates. This permits a reduction in digester size or an increase in organic loading rates (Zeeman et al., 1985; Harris and Dague, 1993; Khanal, 2008; Chi et al., 2010; Ge et al., 2011; Suhartini et al., 2014). Thermophilic temperatures increase pathogen reduction, and allow a higher degree of waste stabilization and a reduction in foaming (Zeeman et al., 1985; Krugel et al., 1998; Song et al., 2004; Chen et al., 2008; Suhartini et al., 2014). Biogas production (Cecchi et al., 1991; Banks et al., 2008) and dewaterability may also be improved by TC (Mata-Alvarez, 2003; Suhartini et al., 2014). On the other hand thermophilic systems have some disadvantages. The system is more susceptible to ammonia inhibition which stops methanogenesis but not acidogenesis, increasing the VFA concentration and thus decreasing the effluent quality. Although the specific biogas production may be higher under thermophilic conditions, this increase in energy production needs to be balanced with a higher heating energy cost. Special attention needs to be given in the start-up period to allow a gradual acclimation of the biomass to the new substrate, temperature conditions, loading rate and hydraulic retention time (HRT), since poor start-up periods will prolong the acclimation time (Angelidaki et al.,

2006a). Angelidaki et al. (2006a) recommended inoculating with at least 10 – 15 % of the final operation volume and gradually increasing OLR (0.5 – 4.3 kg VS m^{-3} day^{-1}) to achieve rapid process stabilization.

2.1.2.2 pH and alkalinity

The production of carbon dioxide results in the production of carbonic acid and alkalinity due to the bicarbonate-carbon dioxide equilibrium. The ammonia produced in the degradation of proteinaceous matter dissolves in water with carbon dioxide to form ammonium bicarbonate (Georgacakis, 1982). Both equilibriums are pH dependent. Sufficient alkalinity is essential to maintain the pH value in the optimum range (6.8 to 8).

The carbonate system is described by the following equations (equations 1 and 2) (Manahan, 2000).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \overset{K_{a1}}{\leftrightarrow} H^+ + HCO_3^- \overset{K_{a2}}{\leftrightarrow} 2H^+ + CO_3^{2-} \quad (1)$$

$$NH_3 + H_2O + CO_2 \leftrightarrow NH_4HCO_3 \quad (2)$$

$K_{a1} = 4.45 \times 10^{-7} \rightarrow pK_{a1} = 6.35$

$K_{a2} = 4.69 \times 10^{-11} \rightarrow pK_{a2} = 10.33$

The following diagram (Fig. 2) shows the predominant carbonic species as a function of pH (Manahan, 2000).

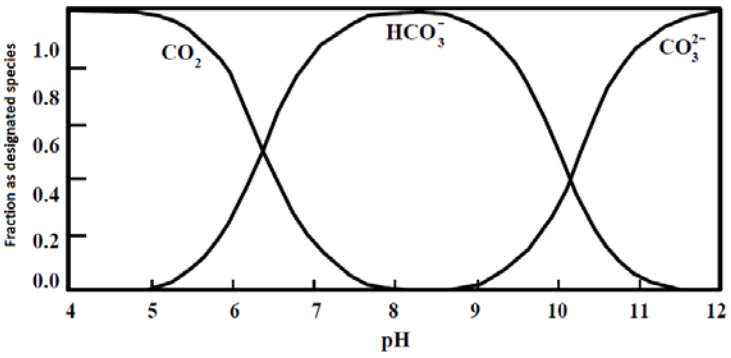


Fig. 2. Diagram of carbonic species in water

Optimal TAN concentration guarantees adequate buffer capacity which maintains a healthy pH and allows the system to recover after a VFA shock caused by acetogenic-methanogenic imbalance (Prochazka et al., 2012). To avoid acidification of the reactor alkalinity is destroyed and it is not returned

until methane is produced. An example of how the buffer system works when glucose is degraded it is shown in the following equations (3 to 5).

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (3)$$

$$3CH_3COOH + 3NH_4HCO_3 \rightarrow 3CH_4COONH_4 + 3H_2O + 3CO_2 \quad (4)$$

$$3CH_4COONH_4 + 3H_2O \rightarrow 3CH_4 + 3NH_4HCO_3 \quad (5)$$

Some authors have previously reported that the interaction between VFA, pH and FAN lead to an inhibited steady state characterised by stable process with a low methane yield (Angelidaki and Ahring, 1993b; Hansen et al., 1998).

It is generally acknowledged that changes in VFA concentration are one of the most important parameters for digestion control, since they are indicative of process instability (McCarty, 1964). The VFA concentration is altered accordingly when hydraulic loading, organic loading or temperature changes are applied to the reactor, indicating microorganism imbalances (Ahring et al., 1995). If the end products of the acid oxidising bacteria (formic acid or hydrogen) accumulate in the reactor, these organisms stop working. Formic acid accumulation will cease propionic acid degradation, in the same way as hydrogen accumulation will stop higher all chain-length VFA being degraded increasing the VFA concentration (VALORGAS D4.1, 2013). To avoid a loss in energy production this reactor behaviour needs to be avoided.

In other to understand the interactions between different groups of microorganisms (acidogenic-acetogenic-methanogenic) accurate short-chain fatty acids results are required. These are obtained by gas chromatographic analysis. In full-scale plants, however, this analysis is sometimes not implemented since it implies high costs. pH and alkalinity, as previously discussed, can be indicators of VFA concentration. Although it is more accessible and simple to determine the pH of the digester, this only gives an indication of what has already happened in the digester, and may be too late for control measures. Furthermore, in well buffered systems pH changes are small even when the process is stressed, e.g. in bioreactors with high ammonia loads (Angelidaki and Ahring, 1994). On the other hand, titration-based methodologies to determine alkalinity are considered a cheap alternative to indicate VFA concentrations in an anaerobic digester. Changes in alkalinity

indicate what is happening in the digester and corrective measures can be applied to stabilise the process (Deublein and Steinhauser, 2008). The main species that contribute to the proton accepting capacity in anaerobic digesters are the carbonate/bicarbonate system and the non-protonated forms of VFAs. Robust anaerobic reactors show low IA/PA ratios, typically below 0.3 – 0.5 (Neiva Correia et al., 2008; Ferrer et al., 2010; Martín-González et al., 2013).

2.1.2.3 Nutrients

For optimal digestion performance microorganisms need many nutrients for growth and metabolism. These can be categorised into macro-nutrients and micro-nutrients depending on the amount needed.

Carbon, nitrogen, phosphorus, potassium and sulphur are included in macro-nutrients and these elements are needed in substantial quantities (Kayhanian and Rich, 1995; Mata-Alvarez, 2003). However, special attention needs to be given to the amount of nitrogen in the feedstock. High C/N ratios lead to a nitrogen deficiency in the digester and low ratios may raise FAN concentration to inhibitory concentrations. This topic is discussed in section 2.3.5.

Micro-elements are required only in trace amounts and can become inhibitory or toxic to the anaerobic digestion process when present at high concentrations. The enzyme system of the methanogenic *Archaea* has micronutrient requirements that are different from those of microorganisms (Speece, 1983). There are many essential nutrients such as calcium, sodium, cobalt, iron, nickel, magnesium, tungsten, copper, iron, molybdenum, selenium and zinc (Kayhanian and Rich, 1995; Mata-Alvarez, 2003). Micronutrient deficiencies in anaerobic digesters have often been mistaken for symptoms of toxicity.

In some cases substrate trace element concentration is not sufficient for the metabolic processes under determinate operational conditions. By external trace element supplementation to the digester the shortage of micro-elements can be diminished and proper degradation of substrate with good operation of the enzyme system is ensured (Kim et al., 2002; Uemura, 2010; Banks et al., 2012).

2.1.2.4 Inhibitors

With some exceptions, methanogens are usually considered the most sensitive microorganisms of the consortium (Speece, 1983; Batstone and Jensen, 2011).

The list of inhibitory compounds is large and includes ammonia, sulphide, light metal ions, heavy metals, organics (e.g. LCFAs, pharmaceuticals), solvents, detergents and disinfectants (Chen et al., 2008). Adaptation of microorganisms to inhibitory substances and dilution or elimination of the toxicant can improve the anaerobic digestion of the material (Chen et al., 2008).

2.2 Ammonia inhibition of anaerobic digestion

Protein and urea contained in organic matter release ammoniacal nitrogen during anaerobic degradation; which, although essential for the growth of anaerobic microorganisms (Jokela and Rintala, 2003), can lead to free ammonia concentrations that are inhibitory to the digestion process (Chen et al., 2008). The quantity of ammonia produced from a substrate can be estimated theoretically by stoichiometry (equation 6) (Kayhanian, 1999; Chen et al., 2008).

$$C_aH_bO_cN_d + \frac{4a-b-2c+3d}{4} H_2O \rightarrow \frac{4a+b-2c-3d}{8} CH_4 + \frac{4a-b+2c+3d}{8} CO_2 + dNH_3 \quad (6)$$

Ammonia is present in the digester in the ionized (NH_4^+) (ammonium ion) and in the free ammonia (NH_3) forms establishing equilibrium as in any aqueous solution (equation 7). The fraction of FAN relative to TAN concentration is temperature and pH dependent. Based on the dissociation constant of ammonium in water it is possible to calculate the FAN concentration, as indicated by the following equations (equations 7 and 8) (Angelidaki and Ahring, 1993b; Hansen et al., 1998; Calli et al., 2005; Cuetos et al., 2008; Hafner and Bisogni, 2009).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad (7)$$

$$FAN\ (mg\ N\ l^{-1}) = \frac{TAN}{1+\frac{[H^+]}{K_a}} = \frac{TAN(mg\ N\ l^{-1})}{1+10^{(pKa-pH)}} = \frac{TAN(mg\ N\ l^{-1})}{1+10^{\left(0.09018+\frac{2729.92}{T\ (K)}-pH\right)}} \quad (8)$$

An increase in temperature leads to a decrease in pK_a which in turns increases FAN concentration as indicated by equation 8. The same trend in FAN concentration is originated by an increase in pH (Le Chatelier's principle).

Fig. 3 illustrates the important role that pH and temperature play in the ammonia-ammonium equilibrium. When pH is increased from 7 to 8 the FAN percentage is 9 times greater at mesophilic (36 ºC) temperature and 7.5 times at thermophilic (55 ºC). In a typical pH found in food waste AD (pH 8) (Banks et al., 2012; Cho et al., 2013; Serna-Maza et al., 2014) FAN:TAN ratio increases from 11 % to 28 % when temperature is increased from mesophilic to thermophilic conditions.

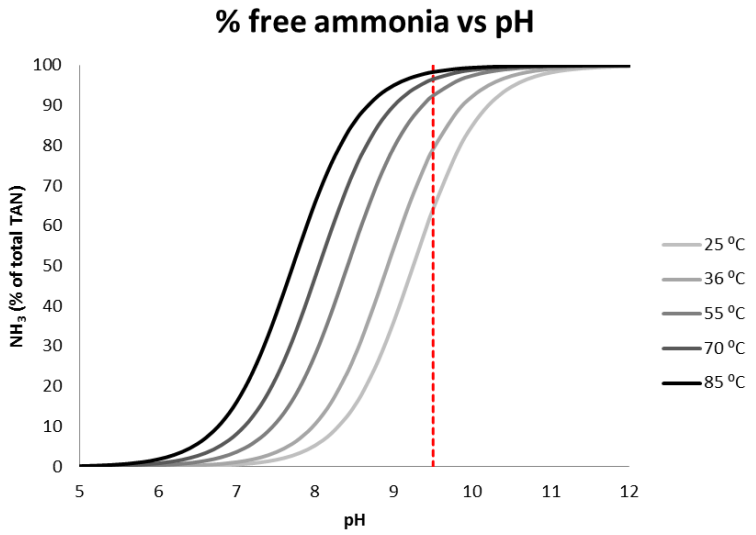


Fig. 3. FAN (% of TAN) at different temperatures and pH values

2.2.1 Impact of ammonia toxicity to methanogenesis pathway in anaerobic digestion

It is agreed that among all the anaerobes, the methanogens are the least tolerant to ammonia and the most likely to be inhibited (Koster and Lettinga, 1988; Robbins et al., 1989). Koster and Lettinga (1988) studied the methanogenic activity of a mesophilic UASB reactor digesting potato juice at increasing TAN concentrations. This investigation reported a decrease in

methanogenic activity of 56.5 % at TAN concentration of 4051 – 5734 mg N l^{-1}, while the acidogenic population was not affected and VFA production remained unchanged. Due to the syntrophic imbalance between acidogenic bacteria and methanogens, VFA accumulation in un-buffered systems results in pH-drop and total failure of the AD process. The same observation was made by Robbins et al. (1989) digesting cattle manure at 37 ºC at different TAN concentrations (850 - 5100 mg N l^{-1}).

Nevertheless, conflicting information can be found in the literature in regard to acetoclastic and hydrogenotrophic methanogens. In early studies it was thought that ammonia was inhibiting hydrogenotrophic methanogens specifically, whereas acetoclastics were not affected by this chemical species. Specific growth rates of acetate utilizing methanogens were not altered up to 5000 mg N l^{-1} in thermophilic conditions in a study conducted by Wiegant and Zeeman (1986). It was hypothesized that intermediate compounds accumulated due to a cessation in hydrogen consumption, leading to propionate degradation suppression and inhibition of acetoclastic methanogens.

In contrast, recent studies have extensively proved that ammonia inhibits the methanogenic *Archaea*, in particular the acetoclastic methanogens, under both mesophilic and thermophilic conditions; and thus the AD system under high TAN concentration relies on the syntrophic association of hydrogen-generating and hydrogen-consuming microbes (Angelidaki and Ahring, 1993b; Borja et al., 1996; Kayhanian, 1999; Angenent et al., 2002; Liu and Sung, 2002; Chen et al., 2008; Schnürer and Nordberg, 2008; Prochazka et al., 2012; Niu et al., 2013c; Williams et al., 2013).

Angelidaki and Ahring (1993b) analysed the specific methanogenic activity when cattle manure was digested at a thermophilic temperature and different ammonia levels in continuously fed reactors. The inhibitory effect of ammonia was found at 2 g N l^{-1} for acetoclastic methanogens and at 3.5 g N l^{-1} for hydrogenotrophic methanogens. The specific growth rate was halved at 3.5 and 7 g N l^{-1} for acetoclastic and hydrogenotrophic methanogens respectively.

Schnürer and Nordberg (2008) used labelled [2-^{14}C] sodium acetate analysis to determine the major methanogenic pathway of two reactors treating diluted SS-

OFMSW at a mesophilic temperature. One of the reactors was supplemented with egg albumin to increase the TAN concentration. The bioreactor running at a low TAN concentration (0.65 – 0.9 g N l^{-1}) showed a $^{14}CO_2$:$^{14}CH_4$ ratio between 0.5 – 0.8, indicating that the main methanogenic pathway was acetoclastic. The N increased reactor had acetoclastic as the main acetate degradation route between TAN concentrations of 0.8 and 3.3 g N l^{-1}. At 5.5 g N l^{-1} the methanogenic mechanism clearly shifted to syntrophic acetate oxidation ($^{14}CO_2$:$^{14}CH_4$ ratio above 2). Similar results were found by Jiang et al. (2012), who detected a higher quantity of ^{14}C labelled carbon dioxide in the biogas ($^{14}CO_2$:$^{14}CH_4$ ratio 1.94 - 3.05) when analysing SS-DFW anaerobic digestate at the same operational temperature with high ammonia concentration (5-6 g N l^{-1}), which suggests that the digestion of this feedstock adopts a syntrophic methanogenic pathway as the major route for methane production.

Although it is now generally accepted that ammonia toxicity threshold for acetoclastic methanogens is lower than that for hydrogen consuming methanogens under both mesophilic and thermophilic conditions, investigation has not yet shed much light on the possible reasons for this difference. Some authors, however, have found a clear difference in the relative growth rate profile under different TAN concentrations in the growing media (Poggi-Varaldo et al., 1991; Borja et al., 1996). Ammonia toxicity showed a sigmoidal pattern for the acetoclastic populations, i.e. a three stage profile with an initial linear decrease in the growth rate, followed by a plateau and a final linear inhibition stage with increasing TAN. This pattern could indicate that two inhibition mechanisms are present acting at different concentrations. In contrast, hydrogenotrophic populations presented a linear inhibition pattern.

Due to the relatively slow growth rate of the hydrogen utilizing methanogens (doubling times of 9-78 days and 1.5-3 days respectively for mesophilic and thermophilic temperatures) compared to acetoclastic organisms (8-36 h for *Methanosarcina* sp. and 1-9 days for *Methanosaeta* sp.) Schnürer and Nordberg (2008); Ek et al. (2011) and Westerholm et al. (2013) recommended that reactors highly loaded with ammonia should be run at long HRT in order to exceed the microbial doubling time, avoid washout of the population, and

obtain a stable process. The decrease in acetogenic activity linked to increasing FAN induces VFA accumulation, a decrease in pH and may cause process failure (Schnürer et al., 1999).

2.2.2 Mechanisms of ammonia inhibition

Studies conducted in pure cultures have shown that high TAN concentration inhibits the methanogens through two mechanisms associated with the two main soluble inorganic nitrogen forms in the anaerobic process, i.e. FAN and ammonium ion (Sprott et al., 1984, 1985; Sprott and Patel, 1986; Wittmann et al., 1995; Kayhanian, 1999).

2.2.2.1 Free ammonia nitrogen

The main cause of inhibition is the hydrophobic ammonia molecule which diffuses into the cell, inducing proton imbalance and proton motive force ($\Delta_{\mu_H}{}^{+}$) across the cell membrane. $\Delta_{\mu_H}{}^{+}$ is integrated by trans-membrane pH (ΔpH) and electrical gradient (ΔΨ) and is the origin of a chain reaction, i.e. potassium depletion, change in cytoplasmic pH, and increase in maintenance energy requirement (Kadam and Boone, 1996).

Microorganisms can modify the ΔpH maintaining a $\Delta_{\mu_H}{}^{+}$ constant by altering ΔΨ through the cation transport systems. Some acidophilic bacteria preserve their internal pH near neutrality by capturing K^+. The cells develop a reversed ΔΨ (interior positive) providing sufficient $\Delta_{\mu_H}{}^{+}$ to make ATP. For bacteria growing in alkaline media (as is normally the case for high TAN concentrations), reversed ΔpH imparts negative energy to the $\Delta_{\mu_H}{}^{+}$. To allow ATP synthesis ΔΨ must be very strong and bigger than the reversed ΔpH. Cells achieve this by cation extrusion (Ni et al., 1994). Ni et al. (1994) demonstrated that the methanogen *Methanolobus taylorii* GS-16 balanced the decreased $\Delta_{\mu_H}{}^{+}$ energy by reversed ΔpH, increasing ΔΨ when K^+ is discharged to the cell-exterior and losing the hosmeostatic and osmotic regulation capability of the cells.

Cytoplasmic membrane is no barrier for FAN flux under high external TAN concentrations; which closely equalise the internal FAN level to the external one (Muller et al., 2006). Intracellular FAN shifts to the AI form, absorbing protons in the process to maintain the equilibrium; ultimately this phenomenon modifies the intracellular pH. Kadam and Boone (1996) studied

the adaptation to ammonia toxicity under different external pH values (7.0 to 9.5) of three members of the family *Methanosarcinaceae,* i.e. *Methanolobus bombayensis, Methanolobus taylorii,* and *Methanohalophilus zhilinaeae*. Reversed membrane pH gradients (ΔpH) in the range of -0.4 to -0.9 pH units were found at all pH values tested. In all cases the cytosol pH was more acidic than the external pH; internal-cell pH was near neutrality even with external pH above 7. The internal ammonium ion concentration depends on the external FAN concentration, therefore on environmental TAN, pH and temperature. Since the intracellular pH is lower than that of the media, the ammonium ion concentration in the cell will be larger. Kadam and Boone (1996) proved that elevated cytosolic ammonium ion concentration was coupled with greater ΔpH and lower concentrations of the major cytosolic cation (K^+). In a study conducted by Sprott et al. (1984) it was proved that high external TAN concentration (NH_4OH, various NH_4^+ salts or methylamine addition) caused 98 % of the cytoplasmic K^+ to escape from *Methanospirillum hungatei* using the H^+/K^+ cross-membrane antiport system. The experiment also proved that the release of K^+ is associated with ammonia uptake, and higher K^+ efflux was connected to external alkaline pH; as expected, since the FAN is the active specie. Thus, when a cell suffers from ammonia inhibition ammonium ion accounts for a considerable fraction of the intracellular cations and the cell wastes energy to counterbalance the up-taken FAN proton by pumping K^+ to the exterior, causing K^+ deficiency.

Although Sprott et al. (1985) did not account for any cytoplasmic Mg^{2+} lost during the ammonia toxicity assays, Kadam and Boone (1996) suggested that other essential cations found in the cytosol such as Mg^{2+} and Na^+ are affected by ammonia in the same way as K^+.

In a different study Sprott and Patel (1986) evaluated ammonia toxicity in terms of methanogenesis inhibition and cation exchange in pure cultures of different methanogens. The heterogeneity between these organisms was evident: it was found that *M. Smithii* and *Methanobacterium* strain G2R were more resistant, whereas *M. Bryantii*, *M. Arboriphilus* and especially acetoclastic organisms, i.e. *M. Concelii*, *M. Hungatei* and *M. Barkeri* were highly sensitive to ammonia. A similar conclusion was obtained by Kadam and Boone (1996) when adaptation to ammonia was examined for three members of

Methanosarcinaceae, i.e. *Methanolobus bombayensis, Methanolobus taylorii* and *Methanohalophilus zhilinaeae*. The activities of three ammonia assimilating cytosolic enzymes (glutamate dehydrogenase, glutamine synthetase and alanine dehydrogenase) were tested in three methanogen species of the family *Methanosarcinaceae* with increasing TAN concentration in the growth media in a study conducted by Kadam and Boone (1996). Direct inhibition of glumanine synthetase activity by un-ionized ammonia was found; different responses were determined for the other enzymes which correlated to the ammonia tolerances of the different microorganisms. This work indicates the importance of cytosolic enzyme systems in ammonia inhibition.

2.2.2.2 Ammonium ion

Ammonium ion displaces divalent ions (Ca^{2+}/Mg^{2+}), essential for the methane synthesizing enzyme system, from the cell surface. Sprott (1985) found that K^+ depleted cells lost the ability to re-accumulate the ion; however, the external addition of Mg^{2+} recovered methanogenic activity in *Methanospillum hungatei*. Cells washed free of ammonia after ammonia shock and cytoplasmic K^+ depletion which were exposed to Ca^{2+}, Mg^{2+} and Mn^{2+} showed improved methanogenic activity and Rb^+ transport to the cytoplasm until a concentration similar to that before K^+ diminution was reached, but did not recuperate K^+. This finding showed the importance of this second inhibition model, since enhancement of CH_4 synthesis occurred even in low loaded K^+ cells. On the other hand, other divalent ions, i.e. Fe^{2+}, Co^{2+} and Ni^{2+} (chlorides) were ineffective in this function. Therefore, it is reasonable to consider that micro-nutrient deficiency can also be caused by high ammonia levels, and methanogenic activity can be stimulated from the outer face of the cytoplasmic membrane by trace element external addition.

The proposed mechanisms of ammonia toxicity in methanogenic *Archaea* based on cytoclasmic ammonium accumulation, cation depletion, trans-membrane pH gradient, and inactivation of the methane synthesizing enzyme system are represented in Fig. 4.

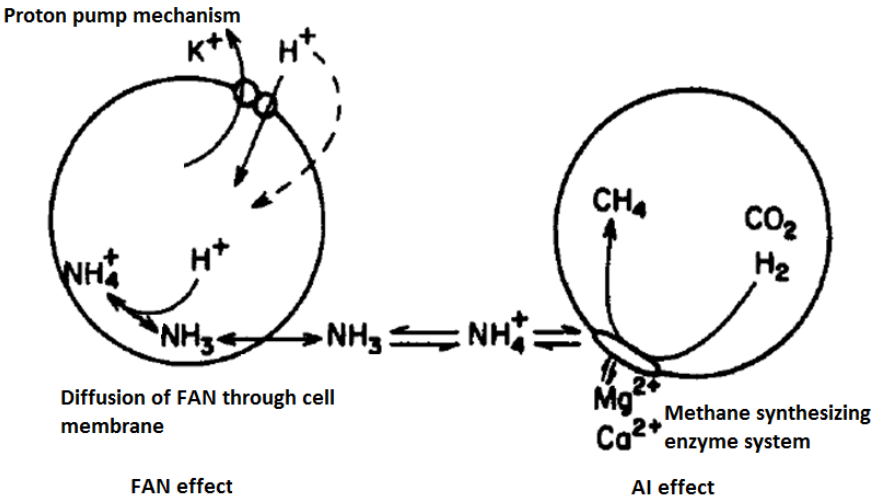


Fig. 4. Proposed mechanisms of ammonia inhibition in methanogenic *Archaea* (adapted from Sprott and Patel (1986))

The toxicity caused by either of the two ammonia inhibition mechanisms hypothesized has a synergistic effect exerted by high pH and TAN (Kadam and Boone, 1996). FAN is more detrimental to methanogenic activity, however, as was demonstrated by Kadam and Boone (1996). Higher specific growth rates were determined for *Methanolobus bombayensis, Methanolobus taylorii* and *Methanohalophilus zhilinaeae* at the same TAN concentration under lower environmental pH values.

2.2.3 Inhibition levels

Nitrogen is an essential nutrient for anaerobic microorganisms. Ammonium ions obtained by protein degradation are employed to synthesise amino acids, proteins and nucleic acids in microbial growth (Nelson and Cox, 2005). McCarty (1964) and Liu and Sung (2002) reported beneficial TAN concentration for the anaerobic process of 50 - 200 mg N l^{-1}. Prochazka et al. (2012) found low methane yield, loss of biomass, loss of acetoclastic methanogenic activity and low buffer capacity unable to recover the system from VFA shock at TAN concentrations lower than 500 mg N l^{-1}. The minimum nutrient C:N:P:S ratio required for methane generation is 600:15:5:3 (Fricke et al., 2007).

As noted in section 2.2.2 the FAN fraction is considered the major inhibitive specie. FAN-ammonium ion equilibrium is highly affected by temperature and pH (equation 8), with the FAN fraction increasing with temperature and pH. Accordingly, in mesophilic reactors higher TAN concentrations are tolerated in comparison to thermophilic systems. Fig. 5 and Fig. 6 show the FAN

concentration obtained at different TAN concentrations, mesophilic and thermophilic temperatures, and high pH close to 8, characteristic values found in digesters treating food waste and cattle manure (Borja et al., 1996; Banks et al., 2012). In these reactors, an increase in pH from 7.6 to 8.1 leads to a more than 2-fold increase in FAN concentration. The effect of temperature increase can also be seen: a FAN of 700 mg N l^{-1} is obtained at TAN concentrations of 5500 mg N l^{-1} or 2500 mg N l^{-1} respectively for the mesophilic and thermophilic systems at pH 8.

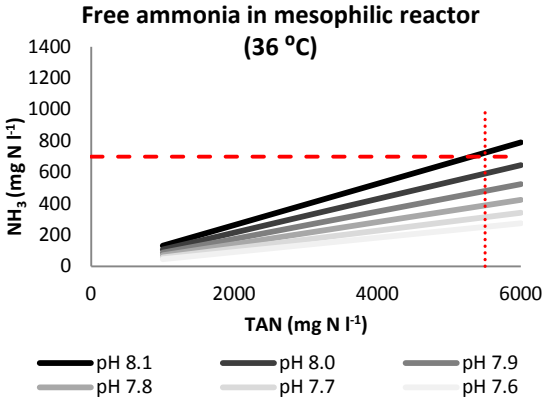


Fig. 5. FAN vs. TAN at different pH values in a mesophilic reactor

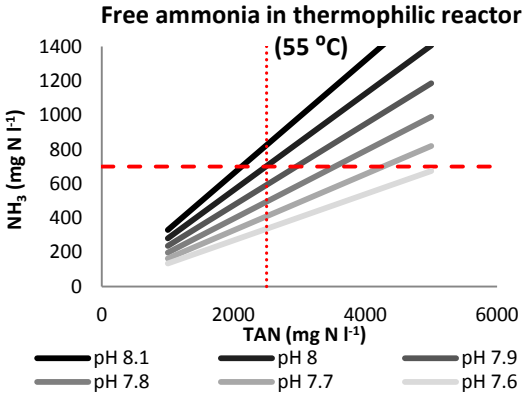


Fig. 6. FAN vs. TAN at different pH values in a thermophilic reactor

In addition to the higher FAN fraction, proteins attain a greater degradation at thermophilic than at mesophilic conditions, leading to higher final TAN concentrations (Gallert and Winter, 1997; Gallert et al., 1998; Chi et al., 2010; Ge et al., 2011; Nordell et al., 2013; Suhartini et al., 2014). Gallert and Winter (1997) reported that ⅓ of the TKN present in source-segregated household waste was converted to TAN during the mesophilic digestion, and ½ in the thermophilic digestion. The same authors in a different study demonstrated that peptone and SS-OFMSW produced more ammonia at thermophilic temperatures; however, generation was faster at mesophilic temperatures (Gallert et al., 1998).

Inhibition limits (FAN and TAN) reported in different literature studies in continuously fed digesters are summarised in Table 2 and Table 3. Substantial divergences can be found caused by the use of different substrates, inoculum, start-up methodology, acclimation periods, HRT, SRT, reactor configuration, temperature and pH conditions in the digester (Angelidaki and Ahring, 1994; Chen et al., 2008).

Angelidaki and Ahring (1993b) clarified that although the growth of methanogenic *Archaea* was affected from a TAN concentration of 2 g N l^{-1} when cattle manure is anaerobically digested at thermophilic temperatures, a TAN concentration of 4 g N l^{-1} was needed to affect the reactor performance, reducing the biogas production to 75 % of the non-inhibited reactor. After prolonged exposure to high TAN 5 - 6 g N l^{-1} the reactor showed an inhibited-steady state, characterized by higher VFA concentration (3 g l^{-1}) than the reactors with lower ammonia stress which decreased the pH and the FAN level stabilizing the system with a 75 % of the biogas potential.

In a different study, Angelidaki and Ahring (1994) increased the temperature from 55 ºC to 65 ºC in two digesters at two different TAN concentrations (2.5 and 6 g N l^{-1}); this resulted in a decrease in the biogas yield in both cases. The opposite strategy was also applied, a decrease in temperature (from 55 ºC to 46 ºC) in a reactor with high TAN (6 g N l^{-1}) and resulted in a relief in inhibition, a decrease in VFA concentration and an increase in biogas yield. It appears evident that the cause of these changes in reactor performance is FAN. A plot of VFA concentration and biogas as a function of FAN indicates that a FAN concentration of 0.6 – 0.8 g N l^{-1} was harmful for the anaerobic system.

Hansen et al. (1998) investigated swine manure degradation under thermophilic conditions and at different ammonia concentrations (3.1 – 8.1 g N l^{-1}) in batch experiments. The apparent specific growth rate showed a four-stage curve when plotted against FAN. The first stage with a constant and maximum growth rate (0.150 day^{-1}) was found up to a FAN concentration of 1.1 g N l^{-1}; between 1.1-1.2 g N l^{-1} growth rate relative to the maximum value decreased to 0.67. In the third stage, between FAN concentrations 1.2 – 1.3 g N l^{-1}, a constant relative growth rate of 0.67 was found. In the last stage the value decreased as low as 0.18 at a FAN concentration of 1.8 g N l^{-1}. Therefore, a threshold FAN concentration of 1.2 g N l^{-1} (corresponding to TAN 3.1 – 4.1 g N l^{-1}) was reported in this study. However, it is important to highlight that FAN concentrations below 1.1 g N l^{-1} were not tested. In the same study H_2/CO_2 was used as substrate to conduct an ammonia inhibition test on hydrogenotrophic methanogens in thermophilic temperatures and at three TAN concentrations (3.3. 4.8, and 5.8 g N l^{-1}). It was found that hydrogen utilizing methanogenic *Archaea* were inhibited at a FAN concentration above 1.2 g N l^{-1}.

Table 2. TAN and FAN inhibition limits at thermophilic temperatures in continuously fed reactors

Reference	Substrate	ExtN	T °C	R	Inoc.	OLR kg_{VS} $m^{-3}day^{-1}$ (Kg_{COD} m^{-3})	pH	TAN mg N l^{-1} ($^{+}$)	FAN mg N l^{-1}	$CH_{4\ after\ inhibition}$: CH_4
Sung and Liu (2003)	synthetic wastewater	NH_4Cl	55	CSTR	AC	(4)	6.92	3050	96*	0.68
							6.71	4920	97*	0.61
							6.4	5770	56*	0.36
							NR	8000-13000^	-	0
Zeeman et al. (1985)	cattle manure	NH_4Cl	50	CSTR	AC	NR	NR	1700	NR	1
Vermeulen et al. (1993)	kitchen waste (no garden/paper)	no	55	CSTR	AC	5.2	8.2	3500	1340*	1
Kayhanian and Hardy (1994)	OFMSW	no	54-60	CSTR	NR	6.5	6.9	1000	28-40	1
							NR	2500	-	0
Kayhanian (1994)	OFMSW	no	54-60	CSTR	NR	6.5	NR	2500 (1200)	NR	0
Angelidaki and Ahring (1993b)	cattle manure	no	55	CSTR	NR	NR	7.9	2500	550*	1
		NH_4Cl						4000	900*	0.75
Angelidaki and Ahring (1994)	cattle manure	no	55	CSTR	AC	RT 15 days	8.0	2500	700	1
		NH_4Cl					7.8	6000	1190	0.6
Kayhanian (1999)	MS-OFMSW	no	45	CSTR	NR	NR	7.2	1200	45	NR
Borja et al. (1996)	cattle manure	no	55	UASB	AC	NR	7.9	3000	590	1
		NH_4Cl	55				7.9	5000	995	0.25
Yirong et al. (2013a)	SS-DFW	no	55	CSTR	AC	2	8	3000	843*	1
Yirong et al. (2013b)	low N synthetic SS-DFW	CH_4N_2O					8	3000	843*	1
Angelidaki et al. (2006b)	SS-OFMSW	NH_4HCO_3	55	CSTR	AC	3.2	8.4	3500	877	NR
Nielsen and Ahring (2007)	diluted cattle manure; VS 4.5%	NH_4Cl	53	CSTR	AC	2.8	7.6	3000	380-600	1
Hejnfelt and Angelidaki (2009)	$co\text{-}dig_1$	no	55	CSTR	AC	control 1.9; 3.9	8.23	3370	1330	0
	$co\text{-}dig_2$					control 1.9; 2.6	8.1	3320	1080	0.28

Table 3. TAN and FAN inhibition limits in continuously fed reactors at different temperatures

Reference	Substrate	ExtN	T °C	R	Inoc.	OLR kg_{VS} $m^{-3}day^{-1}$ (Kg_{COD} m^{-3})	pH	TAN mg N l^{-1} ($^{+}$)	FAN mg N l^{-1}	$CH_{4\ after\ inhibition}$: CH_4
Pitk et al. (2013)	co-dig_3	no	37	CSTR	NAC	3.55	8.1	2795	548 ± 33.9	1
						3.55	8.2	2993	645 ± 41	0.91
						4.54	8.4	3700	1035.5 ± 115.3	0.85
Hansen et al. (1998)	swine manure	no	37	CSTR	AC	RT 15 days	8.06	5900	750	1
			45				8.15	6000	1400	0.72
			55				7.97	6000	1600	0.53
			60				8.15	6100	2600	0.57
Niu et al. (2013b)	chicken manure diluted (44% to 10 %TS)	NH_4Cl	35	CSTR	NR	0.21 kg l^{-1} day^{-1}	7.5-8	15000 (8000)	1500 (800*)	0
Niu et al. (2013a, c)		no	55				7.5-8	4000 (2500-3000)	2000 (700-850*)	0.72
Gallert and Winter (1997)	SS-OFMSW	no	37	CSTR	AC	1-9.5	7.6	3000-3700	220-280	0.50
			50				7.6	3400-3500	680-690	0.50
Nordell et al. (2013)	co-dig_4	no	55	CSTR	AC	4.7-6.5	8.1	2500	860	0.99
			38				7.6	2000	100	1
		CH_4N_2O	38				8.1	5600	750	0.99
Angelidaki and Ahring (1994)	cattle manure	no	40	CSTR	AC	RT 15 days	8	2500	340*	1
		NH_4Cl	40				7.8	6000	540*	0.75
		no	55				8	2500	700*	1
		NH_4Cl	55				7.8	6000	1190*	0.6
		no	64				8.2	2500	1270*	0.4
		NH_4Cl	64				7.6	6000	1240*	0.15

ExtN: external nitrogen addition; **R**: reactor configuration; **Inoc**: Inoculum; * Calculated equation (8) when FAN was not provided by the author in the study but TAN and pH were; $^{+}$ beginning of VFA accumulation; ^ batch experiment; **synthetic wastewater**: non-fat dry milk; **co-dig_1**: 5% slaughterhouse waste and manure. Control - manure; **co-dig_2**: 20% slaughterhouse waste and manure. Control - manure; **co-dig_3**: sewage sludge and sterilized solid slaughterhouse waste (2.5 - 10 %); **co-dig_4**: OFMSW (59 - 82 % OLR) slaughterhouse (13 - 18 % OLR) and glycerol (0 - 28 % OLR); **NAC**: non acclimated; **AC**: acclimated; **NR**: non reported

Yirong et al. (2013a) compared mesophilic and thermophilic digestion of SS-DFW without water addition into the system and found failure symptoms in the thermophilic system when the TAN concentration reached 3.5 g N l^{-1}. The digestion of low N (1.45 % of dry weight) synthetic food waste conducted in a different study succeed at thermophilic temperatures; external addition of urea established a critical TAN of 2.5 – 3.5 g N l^{-1} (Yirong et al., 2013b; VALORGAS D4-6, 2013). Stimulation of the hydrogenotrophic metabolic pathway by the addition of selenium and cobalt allowed stable dry digestion of food waste at mesophilic temperatures, high OLR (> 5 kg VS m^{-3} day^{-1}) and TAN concentrations > 6 g N l^{-1} (Banks et al., 2012). At temperatures in the thermophilic range the toxic threshold is reduced as the equilibrium moves towards free ammonia, and under these conditions trace element additions were not successful in overcoming the associated problem of VFA accumulation as the methanogenic/acetogenic syntrophy breaks down (Yirong et al., 2013a).

Considering the higher ultimate ammonia TAN and FAN concentrations with no signs of inhibition in the digestion system obtained in previous investigations (Table 2 and Table 3) it is possible to conclude that TAN levels of 8000 mg N l^{-1} or FAN 800 mg N l^{-1} and TAN levels of 2500 – 3500 mg N l^{-1} or FAN 550 – 877 mg N l^{-1} suppress methanogenic activity in mesophilic and thermophilic systems respectively.

Industry produces large quantities of high-N waste streams which could be exploited in AD due to their high methane potential. Table 4 summarises the N content of different high-N feedstocks (manures, OFMSW, slaughterhouse waste) reported in the literature. From the characteristics of a feedstock it is possible to determine whether it is a good candidate for AD as a mono-substrate under TC or MC, since it is possible to estimate the approximate final TAN concentration in the digester, and thus whether the biological treatment will lead to ammonia inhibition.

Table 4. High-N feedstock

Feedstock	Reference	Total N (average ± std)	Units	TS (average ± std)	Units	Total N ($g\ N\ l^{-1}_{FM}$)
ChM	Belostotskiy et al. (2013)	27.7-33.4	$g\ N\ kg^{-1}_{FM}$	42.6 - 53.7	$\%_{FM}$	27.7-33.4*
	Niu et al. (2013b) and (2013b)	6.45 ± 0.81	$g\ N\ l^{-1}_{FM}$	11.2 ± 0.53	$g\ l^{-1}$	6.5+
	Abouelenien et al. (2010)	87	$g\ N\ kg^{-1}_{TS}$	25	$\%_{FM}$	21.8*
PW	Zhang et al. (2011a)	7.6	$g\ N\ l^{-1}_{FM}$	5.95	$g\ l^{-1}$	7.6+
PSW	Nakakubo et al. (2008)	5.2	$g\ N\ l^{-1}_{FM}$	5.9	%	5.2+
		6.7	$g\ N\ l^{-1}_{FM}$	22.1	%	6.7+
CoM	Kaparaju and Rintala (2008)	3.5	$g\ N\ l^{-1}_{FM}$	8.7	$\%_{FM}$	3.5
	(1993b)	3.7	$g\ N\ l^{-1}_{FM}$	5.9	$\%_{FM}$	3.7
	Zhang et al. (2012b)	35.0 ± 0.5	$g\ kg^{-1}_{TS}$	9.31 ± 0.14	$\%_{FM}$	3.3
	Castrillon et al. (2013)	1.8 ± 0.3	$g\ N\ kg^{-1}_{FM}$	9.8 ± 0.1	$g\ kg^{-1}$	1.8
SS-OFMSW		3.2 ± 0.4	$g\ N\ kg^{-1}_{FM}$	23.2 ± 1.1	$g\ kg^{-1}$	3.2
MG	Yabu et al. (2011)	6.3	$g\ N\ kg^{-1}_{FM}$	22	$\%_{FM}$	6.3+
SS-OFMSW	Angelidaki et al. (2006 a and b)	6.5	$g\ N\ kg^{-1}_{FM}$	30	$\%_{FM}$	6.5+
	Zhang et al. (2012b)	34.2 ± 0.4	$g\ N\ kg^{-1}_{TS}$	23.74 ± 0.08	$\%_{FM}$	8.1+
	Zhang et al. (2012a)	3.44 ± 0.04	$\%_{TS}$	23.74 ± 0.08	$\%_{FM}$	8.2+
MS-OFMSW		1.32 ± 0.08	$\%_{TS}$	52.83 ± 0.63	$\%_{FM}$	6.9+
	Cecchi et al. (1991)	2.2	$\%_{TS}$	76.3	$\%_{FM}$	16.8*
DWAS	Nakashimada et al. (2008)	77	$g\ N\ kg^{-1}_{TS}$	17	$\%_{FM}$	13.1*
PigS	Bayr et al. (2012a)	15	$g\ N\ kg^{-1}_{FM}$	32	$\%_{FM}$	15*
PoS	Salminen and Rintala (2002)	24.3	$g\ N\ kg^{-1}_{FM}$	26	$\%_{FM}$	24.3*
PoS	Bayr et al. (2012b)	26.3	$g\ N\ kg^{-1}_{FM}$	38.2	$\%_{FM}$	26.3*
PigS		15.8	$g\ N\ kg^{-1}_{FM}$	31.8	$\%_{FM}$	15.8*
BoS		5.6	$g\ N\ kg^{-1}_{FM}$	53.2	$\%_{FM}$	5.6+

SSSW	Pitk et al. (2013)	59.8 ± 6.1	g N kg^{-1}_{TS}	96 ± 1.3	$\%_{FM}$	57.4*
SW	Siegrist et al. (2005)	20	g N l^{-1}_{FM}	NR	-	20*

ChM: Chicken manure
PW: Piggery wastewater
PSW: Solid fraction of piggery wastewater
CoM: Cow manure
SS-OFMSW: Source-segregated organic fraction of municipal solid waste
MG: Model garbage
MR-OFMSW: Mechanically-recovered organic fraction of municipal solid waste
DWAS: Dehydrated waste activated sludge
PigS: Pig slaughterhouse waste
PoS: Poultry slaughterhouse waste
BoS: Bovine slaughterhouse waste
SW: Slaughterhouse wastes
SSSW: Sterilized solid slaughterhouse waste
* above free ammonia inhibition threshold at MC and TC
+ above free ammonia inhibition threshold at TC

As a general rule of thumb, it is possible to conclude that chicken manure and slaughterhouse wastes may be inhibited by ammonia under both TC and MC, while pig and OFMSW can be digested at mesophilic temperatures (applying the previous inhibition thresholds). However, specific characteristics of moisture and total N need to be taken into consideration for each feedstock (Table 4). As an example of this rule, Niu et al. (2013 b and c) conducted CSTR digestion of chicken manure (TKN: 6.5 g N l^{-1}) in TC and MC. Ammonia toxicity was only found at thermophilic temperatures, since TAN in the digester reached 6000 mg N l^{-1}; while at mesophilic temperature and a TAN concentration of 5000 mg N l^{-1} the digestion proceeded efficiently.

Since the TAN concentration in the digester depends only on the stoichiometry (as stated on equation 6) alleviation of the inhibited state cannot be obtained by reduction in the OLR. A different strategy needs to be adopted to decrease the FAN concentration below the inhibition threshold and to obtain an efficient digester performance. The different tactics to diminish TAN concentration in a bioreactor are discussed in the following section.

2.3 Ammonia inhibition mitigation methods

2.3.1 Acclimation of microflora

Extensive research has proven that methanogens become less sensitive to TAN and pH changes after acclimation by a moderate increase in the exposure to TAN surpassing the initial inhibitory concentrations, on condition that the ultimate inhibition level is not exceeded (Hashimoto, 1986; Koster and Lettinga, 1988; Calli et al., 2005).

Koster and Lettinga (1988) found initial inhibition in a UASB reactor treating potato juice at 30 ºC at TAN concentrations of 1900-2000 mg N l^{-1}, after an adaptation period methanogenesis was recovered to give a maximum tolerable ammonia concentration 6.2 times higher than the initial value. van Velsen (1979) used two inocula acclimated to different ammonia concentrations (815 mg N l^{-1} and 2420 mg N l^{-1}) to study the adaptation of methanogens to high TAN concentration (5000 mg N l^{-1}) at 30 ± 2 ºC. The low-ammonia acclimated inoculum showed methane activity even at concentrations as high as 5000 mg N l^{-1}, although a lag phase in the methane production was found for this seeding material. The same behaviour, i.e. initial inhibition returning to a steady acclimated state, was found by Robbins et al. (1989) when dairy cattle manure slurry was digested at 37 ºC and different TAN concentrations 0.85 - 5.1 g N l^{-1}, and by Sossa et al. (2004) where a maximum adaptation period of 36 h was determined at the maximum FAN concentration evaluated (848.8 mg N l^{-1}) in a methanogenic activity test.

Specific methanogenic activity batch tests using three inocula acclimated to TAN concentrations of 400, 1200 and 3050 mg N l^{-1} and TC were conducted by Liu and Sung (2002). In the study a sharp linear decrease in activity was found with increasing TAN when inoculum acclimated to 400 mg N l^{-1} was used. However, for inoculum acclimated to 1200 and 3050 mg N l^{-1} the activity remained almost constant up to 2500 mg N l^{-1} TAN; at higher TAN concentrations it decreased abruptly.

2.3.2 Temperature and pH control

An increase in temperature generates a positive effect on microbial metabolic growth rate and a rise in FAN concentration as previously stated (equation 8). If toxic levels are approached the result is operational instability, and in the worst cases digestion failure (Angelidaki and Ahring, 1994). A decrease in temperature temporarily alleviates ammonia inhibition but the microbial population might be altered. Angelidaki and Ahring (1994) suggest that the optimum temperature of a digester treating N-rich matter will differ from that under low ammonia loads.

FAN concentration can also be corrected by modifying the pH and the ammonia-ammonium equilibrium. Ho and Ho (2012) conducted thermophilic batch experiments reducing piggery wastewater (TAN: 2104-2111 mg N l^{-1}, FAN: 916-920 mg N l^{-1}) pH from 8.3 to 7.5, 7.0 and 6.5 by adding concentrated hydrochloric acid. The greatest methane production (3.4-fold when compared to the control without pH modification) coupled with 58 % VFA reduction was found when pH was reduced to 6.5. Initially, FAN concentration decreased to 24 mg N l^{-1}, but at the end of the batch trial pH increased to 7.8 and FAN accordingly built up to 425 mg N l^{-1}. Although methane production was enhanced in all the trials, foaming appeared due to the CO_2 release during pH reduction, and acetate and propionate accumulated when the initial pH was reduced to 7.5 and 7; suggesting that the acetoclastic methanogens and propionate-degrading acetogenic bacteria were inhibited. The drawbacks found in this study indicate that this approach would not solve the long term problems associated with ammonia inhibition.

This investigation does not consider temperature or pH control as a strategy to mitigate ammonia inhibition since the major problem when food waste is digested resides in the thermophilic range, and these methodologies could originate a new source of microorganism upset; furthermore nitrogen cannot be recovered.

2.3.3 Substrate dilution

In some studies high-N feedstocks were diluted with water to decrease the TS content and to avoid ammonia inhibition (Pechan et al., 1987; Gallert et al.,

1998; Angelidaki et al., 2006b; Hejnfelt and Angelidaki, 2009; Ek et al., 2011; Nagao et al., 2012; Belostotskiy et al., 2013). The large water requirement makes this process unattractive for large industrial plants, however, especially in water-constrained regions; this approach has both resource and energy implications since the volume of waste to be treated by the dewatering process and the reactor size are increased considerably (Kayhanian, 1999; Chen et al., 2008; Li et al., 2011). Furthermore, a shift in the methanogenic pathway from the hydrogenotrophic to acetoclastic route at high dilution rates has been reported by Shigematsu et al. (2004), suggesting potential operational complications.

An increase of TAN concentration in the reactor is predicted when process water is recirculated to the digester; however, this accumulation can be eluded when air stripping is applied. Angelidaki et al. (2006b) implemented three strategies to allow stable digestion of SS-OFMSW in thermophilic reactors, i.e. water dilution, recirculation of process water obtained by centrifugation (10000 rpm for 10 min) of reactor effluent with and without ammonia stripping. Similar reactor performances were achieved by water and stripped water dilution, whereas an 'inhibited steady-state' was reached when non-stripped process waster was returned to the reactor.

Some studies have investigated reactor dilution with fresh water to temporarily mitigate ammonia inhibition. Inhibitory concentrations were avoided in thermophilic digestion of OFMSW by dilution with fresh water; nevertheless, this method may pose a problem in the short term, since solid concentration and active biomass is decreased too shifting into a wet process (Kayhanian, 1999). Four biomass dilution strategies were evaluated by Nielsen and Angelidaki (2008) in four thermophilic CSTRs highly loaded with TAN (9 – 11 g N l^{-1}) by NH_4Cl addition, i.e. reduction of TAN concentration by continuous feed (manure), water dilution (50 % reactor volume), 50 % of reactor dilution using digestate collected during stable operation, 50 % dilution adding undiluted fresh manure. All the strategies reached a safe TAN concentration after 40 days of operation and gave a similar methane yield in the end. The addition of digested manure gave a more balanced recovery with lower VFA fluctuation; however, fresh manure obtained the highest biogas production during the recovery.

In the past, most full-scale anaerobic digesters applied wet processes (< 20% TS in the feed). Now dry (> 20% TS in the feed) or semi-dry (~ 20% TS in the feed) is becoming increasingly prevalent to reduce the reactor volume, heating energy, and the amount of digestate for disposal; therefore the reported net energy gain from dry AD is more favourable than the wet process (Bolzonella et al., 2003; Hartmann and Ahring, 2006; Karthikeyan and Visvanathan, 2012). 72 % of new anaerobic digestion plants installed in Europe between 2005 and 2010 adopted the so-called dry technology (Karthikeyan and Visvanathan, 2012). This study employs semi-dry processes, thus dilution to reduce TAN concentration in the digester is not considered as a strategy.

2.3.4 Trace element supplementation

The importance of the bioavailability of inorganic nutrients in food waste AD has been proven in numerous studies. Trace element supplementation at MC has been shown to stimulate microbial growth, resulting in an enhanced digestion performance with an immediate reduction in VFAs and increase in VS destruction (Kim et al., 2002; Climenhaga and Banks, 2008; Uemura, 2010; Banks et al., 2012; Zhang and Jahng, 2012; Williams et al., 2013). Stable digestion of food waste at high OLR (> 5 kg VS m^{-3} day^{-1}) and TAN concentrations greater than 6 g N l^{-1} has been achieved by stimulation of the hydrogenotrophic metabolic pathway when selenium and cobalt were added to the mesophilic reactor (Banks et al., 2012). Similar reactors that were not supplemented with trace metals showed VFA accumulation at OLR of 2 kg VS m^{-3} day^{-1} and total failure of the digestion occurred at OLR of 3 kg VS m^{-3} day^{-1}.

Thermophilic systems are considered to require more trace elements than mesophilic ones due to greater rates of nutrients assimilation by the biomass and/or less bioavailability under higher temperature conditions (Uemura, 2010; Takashima et al., 2011). Yirong et al. (2013a) applied four times the trace element supplementation effective in a mesophilic reactor as a corrective action in a thermophilic reactor treating SS-DFW with VFA accumulation due to ammonia toxicity (3.5 g N l^{-1}). A reduction in VFA concentration was clear for acetic acid, n-butyric, valeric and hexanoic acids; however, propionic, iso-butyric and iso-valeric acids continued to accumulate which eventually drove the system to failure when the TAN concentration reached 5 g N l^{-1} (Yirong et

al., 2013b). The same trend in VFA reduction was found when Ca, Fe, Ni, and Co were supplemented during the start-up period of thermophilic reactors treating diluted dog-food (Kim et al., 2002).

Difficulties in operating food waste reactors at thermophilic temperatures with matched HRT and SRT could be attributed to the antagonistic effect of a dearth of trace elements and free ammonia toxicity, and under these conditions trace element additions have not succeed in overcoming the associated problem of VFA accumulation as the methanogenic/acetogenic syntrophy breaks down.

2.3.5 C/N ratio adjustment

Kayhanian (1999) found the C/N ratio in feedstock should be kept between 22-35 for stable digester operation at thermophilic temperatures and recommended choosing a ratio of 27-32 to optimize the digestion of biodegradable OFMSW. However, high VFA levels were found by Obuli et al. (2012) when food waste and vegetable waste was digested at TC with a C/N ratio of 27 even at low OLR 0.65 – 1.6 kg VS m^{-3} day^{-1}. An increase of C/N ratio to 32 significantly decreased TAN and VFA concentration giving a higher pH in the reactor (7.75) and allowing higher OLR (4 – 7.3 kg VS m^{-3} day^{-1}). Hills (1979) found the maximum gas production when dairy manure was digested at MC and C/N of 25. Therefore, temperature and substrate C/N ratio play an important role in co-digestion.

A C/N ratio of 14-16 (considering all the C present in the feedstock) was found in different SS-DFW characterized in United Kingdom (Zhang et al., 2010); similar ratios were determined by Castrillon et al. (2013), Han and Shin (2004) and Zhang et al. (2007). Wise selection of the substrates to be employed in an anaerobic digester can not only adjust the C/N ratio to approach the optimum value, but can also help to optimise buffering capacity, rheological properties and moisture, supply missing nutrients and minimize toxic or inhibitory compounds in the digester (Kayhanian and Rich, 1995; Mata-Alvarez et al., 2000; Zhang et al., 2011b; Karthikeyan and Visvanathan, 2012).

The co-digestion of food waste with cattle slurry (FW:CS ratio 40:60) and with card packaging (FW:CP ratio 78.4:21.6) in a mesophilic digester at OLR 4 kg VS m^{-3} day^{-1} stabilised the anaerobic digestion of SS-DFW as mono-substrate that

was suffering from VFA levels of 7000 mg l^{-1} (Zhang et al., 2012b). Co-digestion allowed higher OLR and gave stability to the system by reducing the TAN in the bioreactor and free ammonia inhibition.

Resch et al. (2011) used glycerine and starch to adjust the carbon content in synthetic animal by-products digestion. The fast hydrolysis of the co-substrates in a reactor with an already inhibited methanogenic population led to VFA accumulation and a decrease in COD degradation, which in the end aggravated the inhibition state. Consequently, the usage of an easy degradable carbon source to balance the C/N ratio is not recommended in an already inhibited reactor.

It has been proven that co-digestion to increase the C/N ratio enhances the AD of high nitrogen substrates when it is used cautiously but depends on the supply of a low nitrogen co-substrate. Reducing the ammonia in the digester or its feed are also possible solutions.

2.3.6 N removal

Biological processes such as nitrification/denitrification and anaerobic ammonia oxidization (anammox) transform soluble nitrogen into nitrogen gas, incurring a loss of ammonia-nitrogen fertilizer value (Jordening and Winter, 2005). Furthermore, the first method consumes biodegradable carbon, decreasing the energy potential of the waste. The main complication associated with the use of anammox bacteria is their low growth rate leading to poor kinetics and performance.

Anaerobic digestion of food waste without dilution reaches TAN concentrations of 5-7 g N l^{-1}; Mulder (2003) suggests that physicochemical methods may be cost-effective when TAN concentrations are over 5 g N l^{-1} while biological processes could be inhibited. Physicochemical methods include technologies such as gas stripping, vacuum evaporation, chemical precipitation, membrane contactor and inorganic adsorbent (zeolite or clay minerals) (Angelidaki and Ahring, 1993a; Tada et al., 2005; Ho and Ho, 2012).

Vacuum evaporation is a process where a liquid is boiled under negative pressure. This technology has not been broadly applied in the digestate management field. Chiumenti et al. (2013) tested vacuum evaporation (35 ºC

and -98 kPa) on the liquid fraction (screw-press separated with 1 mm screen) of swine manure, corn silage and other biomass digestates (TAN concentration of 3.5 - 4.6 g N l^{-1}) with and without acidification by means of sulphuric acid addition, with one and two evaporation phases. In the experiment carried out without acid addition 78.2 % of the total nitrogen was removed from the digestate and recovered as condensate (79.8 % total mass). When a single evaporation phase was applied at a reduced pH of 3.5, 99.2% of the total nitrogen was maintained in the liquid as concentrate (20.2 % of the initial mass). Finally, after two evaporation phases with pH adjusted to 5.0, 97.5 % of the nitrogen was found in the final concentrate accounting for 5.6 % of the initial total mass. Therefore, the substantial mass reduction increases the fertilizer potential of this concentrate.

Lauterböck et al. (2012) used hollow fibre membranes directly submerged in a mesophilic reactor treating slaughterhouse wastes characterised by low TS content < 3.7 % and high TKN concentrations 7.4 – 10.4 g N l^{-1} (OLR 0.7 - 2.8 kg COD m^{-3} day^{-1}). The driving force to remove nitrogen from the reactor was provided by the difference in ammonia concentration between the digestate and the diluted sulphuric acid that flows in the membrane interior. A clear difference in performance was found between the membrane reactor and the control. In the membrane reactor the TAN, pH and VFA concentration were reduced to 1.2 – 3 g N l^{-1}, 7.9 and 2000 mg l^{-1} respectively, which in turn caused a reduction in the FAN concentration of 70 % and enhanced the production of biogas. In the reference reactor TAN concentration reached 7 g N l^{-1} and VFA had maximum peaks of 9000 mg l^{-1}. Operation of this system would be problematic, however, due to membrane fouling aggravated by the higher TS content of food waste digestate. In addition, a higher H_2S concentration in the biogas was determined in the reactor coupled with a membrane, possibly due to sulphuric acid transfer, which could upset the AD process as well as leading to increased costs for gas treatment.

Magnesium ammonium phosphate (MAP $MgNH_4PO_4 \cdot 6H_2O$, commonly known as struvite) is attractive as a fertilizer as when applied to land it releases nitrogen and phosphorus slowly, not burning the crop (Uludag-Demirer et al., 2008). Precipitation of struvite by addition of phosphoric acid, when the P amount in the wastewater is not sufficient, and of MgO, $MgCl_2$ or any other Mg source

(Mg:P:N of 1.3:1:1) to the anaerobically digested effluent is a possible nitrogen removal route under certain operational conditions. The salt is soluble in acid solution, therefore the pH needs to be increased to 9.5 in order to precipitate (Siegrist, 1996). Suspended solids decrease the process efficiency, and for this reason the amount of solids needs to be reduced using methods such as flocculation, which involves polyelectrolyte addition. SS-DFW digestate supernatant obtained good N and P removal efficiencies with MgO, phosphoric acid and NaOH supplementation to the minimum molar ratio (VALORGAS D4-7, 2013). Siegrist (1996) reported that 9.5 kg of 75 % H_3PO_4, 4.0 kg of MgO and 4 kg of 30 % NaOH were required to remove 1 kg of NH_4-N; whereas for stripping at pH 10 and temperatures 10 – 22 ºC, 24 kg of 30 % NaOH and 9.6 kg H_2SO_4 were needed to obtain 12 kg of 40 % $(NH_4)_2SO_4$ solution. In the same study the operating and investment costs of the physicochemical and denitrification processes were compared. The cheapest solution in order to reduce ammonium from the digester supernatant is nitrification and denitrification in a biological step with an external carbon source. Although the physicochemical methods are more expensive, these methods have the potential to recover nitrogen as a bio-fertilizer, do not consume biodegradable carbon and do not depend on nitrification inhibition. Between the physicochemical methods, air stripping at ambient temperature was found to be almost 30 % cheaper than MAP precipitation due to the need for chemical supplementation, dewatering and drying costs to reclaim the precipitate as fertilizer.

Of methods that can remove and sequester ammonia nitrogen from the effluent of AD as a bio-nitrogen resource, stripping has the greatest potential for application in different configurations with the objective of decreasing TAN concentration in a bioreactor. The stripping technology and its application to AD are explained in further detail in the next section.

2.3.7 Stripping

2.3.7.1 Introduction

Stripping or desorption is a unit operation where the transfer of a dissolved component from a liquid to a gas phase takes place. This process can be

classified as a physical or reversible/irreversible reaction (Kohl, 1987; Perry and Green, 1999; Richardson et al., 2002).

In physical stripping, the desorbed component is more soluble in the gas than in the liquid state where is initially found. During desorption the stripped compound does not react chemically with the gas phase. The equilibrium concentration in the gas phase is primarily a function of partial pressure in the gas phase, concentration in the liquid phase and the equilibrium constant of the specific compound (Kohl, 1987). Ammonia stripping using biogas as the gas phase is an example of this type of system, although in this case the amount of soluble ammonia in the liquid is influenced by the ammonia-ammonium equilibrium. By selecting adequate operational conditions the % FAN can be increased as shown in Fig. 3; high temperatures and pH values favour ammonia removal. The utilization of the heat produced e.g. in a combined heat and power (CHP) unit at an AD plant for heating the digestate in the stripping column up to 70 ºC is feasible, since the water in the cycle is normally heated to 90 – 130 ºC and flows back to the unit at 70 – 110 ºC (Deublein and Steinhauser, 2008).

Reversible/irreversible-reaction stripping is characterized by a chemical reaction between the gaseous component being desorbed and a component in the gas phase, to form a different compound. The absorption of ammonia by sulphuric acid solution is an example of an irreversible reaction. In this case ammonium sulphate is formed when ammonia is bubbled through sulphuric acid (Kohl, 1987).

In order to maximize the mass transfer in the stripping process, the contact surface area between the gas and the liquid can be increased by applying techniques such as dispersing the gas into a continuous liquid phase (e.g. tray columns and bubbling columns), allotting the liquid stream into small films that flow through a continuous gas phase (e.g. packed columns), or breaking the liquid into discrete droplets while gas is flowing (e.g. spray contactors). Other more complicated devices have been designed to remove ammonia from digested sludge. In the patented device invented by Stultz and Bice (1997) a thin film of alkaline digestate (minimum pH 9) is created in the interior of a hollow stripping column, and the stripping gas flows through the film removing ammonia. The design includes a liquid-agitated area to release gas at

the bottom of the apparatus. Removal of nitrogen can also be carried out using an evaporator where the liquid is heated to boil below atmospheric pressure (Bonde, 2008; Chiumenti et al., 2013).

Numerous types of stripping apparatus have been designed with different applications: handling slurries is considered a challenge, however, since packing or trays might plug. In this study bubbling column technology was selected, since spray contactors and bubble columns are recommended to avoid operational problems with slurries (Kohl, 1987). This basically involves a simple vessel filled with liquid through which a gas is bubbled. This configuration has low maintenance costs and high heat transfer rates (Kohl, 1987).

The second step in the design of the stripping system is the selection of the gas that will carry the stripped ammonia. In a study where nitrogen was used as the stripping gas, this led to the removal of carbonates from the digestate in the form of carbon dioxide and a pH increase from 8.3 to 9.3 (Zhang et al., 2010). Air has the same effect on the carbonate system (Zeng et al., 2006; Campos et al., 2013). Although pH increment is a beneficial consequence that increases N removal in the liquid by increasing the % FAN, if this stripped liquor is returned to the digester it may cause alkalinity deficiency and inability to buffer VFA increases. The possibility of recycling part of the exhaust gas (CO_2) from the biofuel combustion to the anaerobic digesters was studied as a mode to reduce the net CO_2 emissions, and possibly increase methane production since some studies claim that part of the CO_2 can be reduced to methane (McTavish and Offerman, 2009; Bajon et al., 2014); a similar effect would be expected when biogas is recycled. Nevertheless, CO_2 is not found to be deficient in the anaerobic digester liquor; in fact, it is saturated. Therefore, an external addition of CO_2 would not boost the methane production via the hydrogenotrophic pathway.

Biogas was therefore selected to remove N from digestate because is produced on site in anaerobic digestion plants, and to reduce the possible risk of carbonates deficiency in the anaerobic digester.

2.3.7.2 Physicochemical effects of biogas stripping

For effective gas stripping of ammonia it may be necessary to change the temperature and/or pH of the waste to be treated.

A temperature increase in digestate leads to a decrease in CO_2 solubility (Fig. 7) (equation 10).

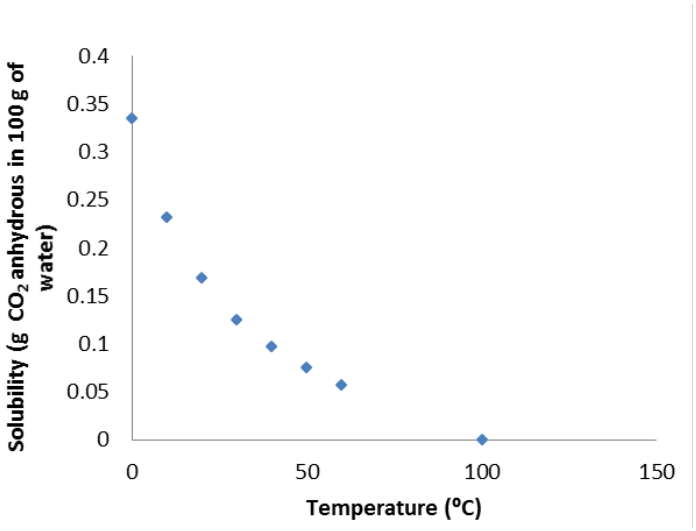


Fig. 7. Solubility of CO_2 in water at various temperatures

(Perry and Green, 1999)

A different effect of the thermal pre-treatment is the possible degradation enhancement of the organic nitrogen-containing materials into the ammoniacal form when the temperature is increased (equation 11). During the AD process proteins are hydrolysed through multiple stages into volatile fatty acids, carbon dioxides, hydrogen gas, ammonium and reduced sulphur (Jokela and Rintala, 2003). However, not all the nitrogen contained in SS-DFW is converted to TAN during the AD process (Gallert and Winter, 1997).

Previous studies on thermal-hydrolysis at low temperature (55 ºC – 95 ºC) have assessed the biomass solubilisation in terms of VS or COD solubilisation ratio for feedstocks (sludge, press mud, microalgal biogas). It was found that the effect was not as pronounced as under extreme temperatures; and more material was solubilised at higher temperature (Prorot et al., 2011; Lopez Gonzalez et al., 2013; Passos et al., 2013).

Interestingly, González-Fernández et al. (2012) studied the effect of thermal pre-treatment at two temperatures (70 and 90 ºC) on *Scenedesmus* biomass in

terms of solubilisation of organic matter (COD and VFA) and ammonium for 3 hours and its subsequent effect on anaerobic digestion. Similar solubilisation profiles were obtained for both temperatures in the treatment period. However, big differences in biodegradability were obtained during the anaerobic digestion. While raw and pre-treated at 70 ºC microalgae attained 22 and 24 % anaerobic biodegradability, microalgae pre-treated at 90 ºC achieved 48 %. The author emphasised that the slightly higher COD solubilised at 90 ºC was not directly responsible of this increase but the damage caused in the microalgae cell wall. With regard to TAN profile during the pre-treatment stage this one stabilized after 90 and 30 min to values around 70 and 60 g N l^{-1} for 70 ºC and 90 ºC respectively, while the TAN concentration in biomass without pre-treatment remained constant at 15 g N l^{-1}. Therefore, different temperatures applied as a pre-treatment to the biomass originated different ammonium releasing rates, with a higher rate at the higher temperature, but similar final TAN concentration when stabilized after 90 min. The small difference found in the analysis could be caused by greater ammonia volatilisation produced at higher temperature.

Furthermore, some evidences that stripping could improve the sanitation effect due to the hyper-thermophilic treatment are found in the bibliography. Hartmann and Ahring (2005) conducted thermophilic digestion of SS-OFMSW (15-days HRT) and hyper-thermophilic post-treatment (1-day HRT, 68 ºC). In the study, the number of colony-forming units was reduced by 4 after the thermophilic reactor and the following treatment reduced it in 1 more order of magnitude.

Due to the removal of free ammonia from the digester by the stripping process (equation 9) the ammonia-ammonium equilibrium tries to re-establish itself by deprotonation of ammonium (equation 12). Alkalinity is destroyed in this reaction (equation 2).

The carbonate system (equation 13) buffers the pH changes caused by the previous reactions (Zeng et al., 2006), and by the increase in VFA concentration if this occurs. pH is increased when CO_2 is removed from the liquid; this phenomenon was demonstrated with anaerobically digested effluent with a pH increase from 7.5 to 9 – 9.5 using air (flow of 2.5 l min^{-1} l^{-1})

(Lei et al., 2007); steam (Zeng et al., 2006) and nitrogen (Zhang et al., 2010). Conversely, pH decreases when biogas is injected into ammonia stripped effluent since CO_2 is adsorbed, following the contrary direction in the bicarbonate equilibrium and purifying the biogas (Lei et al., 2007).

Finally, the addition of CaO to digestate to increase the pH creates slaked lime; when digestate is alkalinized part of the bicarbonate is converted to carbonate (Fig. 2). In the stripping column when biogas is bubbled through in the presence of slaked lime, the carbon dioxide reacts chemically to produce insoluble carbonate salts, increasing the CH_4 concentration in the gas (Sánchez-Hernández et al., 2013) in accordance with equation 14. This precipitate may introduce operational problems in the stripping column such as blockages.

The pH may increase or decrease during the stripping process depending on which equilibrium prevails in regards to generating or consuming protons.

$$\mathrm{NH_3(aq)} \leftrightarrow \mathrm{NH_3(g)}\ ammonia\ desorption \quad (9)$$

$$CO_2(aq) \rightarrow CO_2(g)\ desorption\ effect\ of\ increasing\ temperature \quad (10)$$

$$N_{org} \xrightarrow{originates} NH_3(\mathrm{aq}) + {NH_4}^+(\mathrm{aq})\ thermal\ hydrolysis \quad (11)$$

$$N\mathrm{H_3}\text{removed: } {\mathrm{NH_4}}^+(\mathrm{aq}) \rightarrow \mathrm{H^+} + \mathrm{NH_3(aq)} \quad (12)$$

$$\mathrm{CO_2} + \mathrm{H_2O} \leftrightarrow \mathrm{H_2CO_3} \leftrightarrow \mathrm{H^+} + {\mathrm{HCO_3}}^- \leftrightarrow 2\mathrm{H^+} + {\mathrm{CO_3}}^{2-} \quad (13)$$

$$\mathrm{CaO} + \mathrm{H_2O} \rightarrow \mathrm{Ca(OH)_2}; \mathrm{CO_2} + \mathrm{Ca(OH)_2} \rightarrow \mathrm{CaCO_3} \downarrow + \mathrm{H_2O}\ \text{pH increased with lime}$$

(McCarty, 1964) (14)

These effects are clear in the steam stripping experiment carried out by Zeng et al. (2006) using anaerobically digested cattle manure with ammonium concentrations increased from 910 to 45000 mg N l^{-1} and non-adjusted pH (8.2 – 8.5). At low TAN concentrations the pH increased to 10 due to carbonates removal and low free ammonia discharge; whereas in the case of extremely high TAN concentrations (>18000 mg N l^{-1}) the pH decreased to 7, since the ammonia removal had a bigger effect than the loss of carbonates. In those cases where pH increases to values close to 10 there is no need to add any

chemical compound for pH control since the alkalinity has the same effect naturally during the stripping process.

Since gas-liquid mass transfer often represents the limiting step, equilibrium data are needed to optimise the gradient and mass driving force. Henry's Law is commonly used to describe the gas-liquid equilibrium. This law states that the solubility of a gas in a liquid is directly proportional to its partial pressure in the gas phase (equation 15), and is true for dilute concentration of gases. Normally, solubility falls with a rise of temperature, therefore the efficiency of the stripping process is boosted when the temperature increases.

$$P_a = H \cdot C_a \qquad (15)$$

Where P_a is the partial pressure of the solute in the gas phase (kPa), H is Henry's proportionality constant (kPa mol^{-1} l) and C_a is the concentration of solute in the liquid phase (mol l^{-1}).

Figures 8-10 show the gas-liquid equilibrium of ammonia in water at different temperatures. Linearity between P_a and X_a (solute molar fraction in the liquid phase) can be observed at low concentration (Fig. 9). Fig. 10 shows H constants at different temperatures; an increase in H with temperature can be seen (Perry and Green, 1999). H values calculated with data from Perry and Green (1999) are comparable to those reported by Sander (1999).

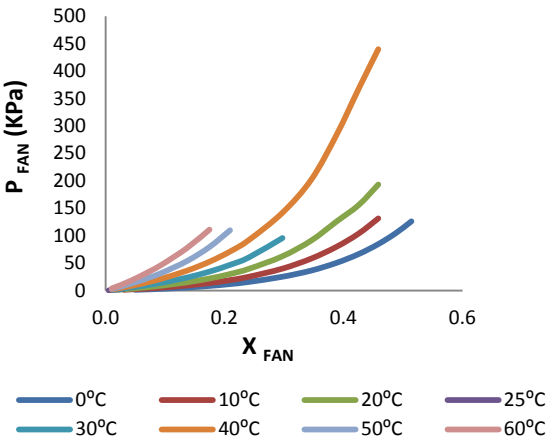


Fig. 8. Ammonia liquid-gas equilibrium data (Perry and Green, 1999)

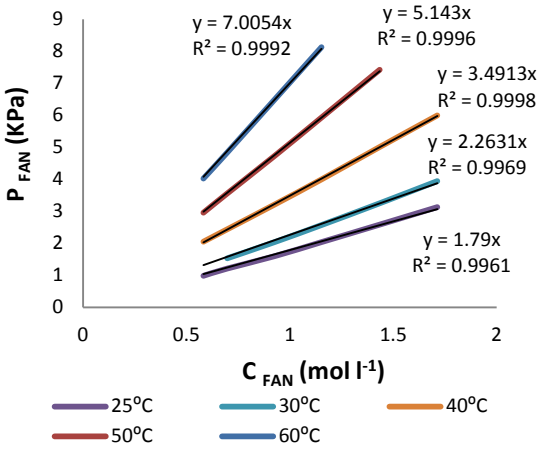


Fig. 9. Liquid-gas ammonia equilibrium. Linear range

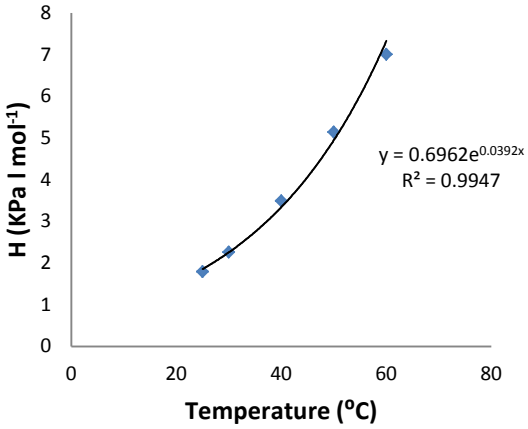


Fig. 10. Ammonia Henry constant as a function of temperature

2.3.7.3 Stripping configurations

Gas stripping has been chosen as the most suitable method to remove ammonia in the current work since the system can be integrated into the AD process using existing technology and infrastructure, while recovering nitrogen as a by-product. There are four possible configurations to integrate the unit stripping operation to the AD process: *in situ*, where the ammonia is stripped continuously from the digester while biogas is produced; post-hydrolysis, where removal is performed after an initial anaerobic hydrolysis stage to break the N_{org} of the feedstock into TAN, and the low-nitrogen feedstock goes to the methanogenic phase afterwards; side-stream, where nitrogen is removed from the digestate in a semi-continuous process, and the low-nitrogen digestate is returned to the reactor to decrease TAN concentration; and post-digestion included as a pasteurisation step, similar to side-stream stripping but in this case low nitrogen digestate is not returned to the reactor (Walker et al., (2011).

By using these techniques a wider range of high-N feedstocks (Table 4), food waste (domestic and commercial), abattoir waste and some animal manures, may become candidates for anaerobic digestion as a single substrate under both mesophilic and thermophilic temperatures, recovering a valuable fertilizer.

Pre-digestion

Ammonia stripping of the feedstock (pre-digestion) can only be applied in resources with a high TAN/TKN ratio. In the specific case of SS-DFW it is not possible to locate a stripping stage in this configuration since the TAN/TKN

ratio is as low as 7 % (determined in food waste collected in UK – unpublished data); therefore, a pre-hydrolysis stage is needed in this case. Slaughterhouse wastes cannot be stripped of nitrogen before pre-treatment, since this kind of waste is also characterised by low TAN/TKN ratios; e.g. poultry waste 1.6 % (Salminen and Rintala, 2002) - 2.7 % (Bayr et al., 2012b), swine waste 7.0 % (Bayr et al., 2012b), bovine waste 4.3 % (Bayr et al., 2012b), pig waste 6.7 % (Bayr et al., 2012a) and sterilized solid slaughterhouse waste 14.5 % (Pitk et al., 2013). On the other hand, feedstocks with a more favourable ratio that allows ammonia stripping previous digestion are chicken manure 12.1 % (Abouelenien et al., 2010) - 59.7 % (Niu et al., 2013b), pig wastewates 65.1 % (Zhang et al., 2011a), and cow manure 40.0 % (Kaparaju and Rintala, 2008) - 67.6 % (Angelidaki and Ahring, 1993b).

Some of the above studies focused on the optimization of the stripping process, whereas other authors also examined the effects of the stripping treatment on the AD performance.

Liao et al. (1995) studied the effects of air-to-swine-manure (TAN: 694 – 2198 mg N l^{-1}, 0.66 - 3.78 % TS) ratio and pH on stripping efficiency at ambient temperatures (13, 20, 22 ºC). At aeration flow rates of 0.38 - 0.53 l_{air} min^{-1} l^{-1}_{manure} and pH values of 11.2-11.5, 140 hours were needed to achieve 90 % of TAN reduction, whereas the same reduction was obtained after 10 h, 15 h and 30 h at higher flows (9, 6.5 and 4.5 l_{air} min^{-1} l^{-1}_{manure}). When the same comparison was made at lower pH (9.3 - 9.5), low air flows needed 225 hours to give 80 % TAN reduction and 16, 18 and 40 h were needed to achieve the same reduction at 9, 6.5 and 4.5 l_{air} min^{-1} l^{-1}_{manure}. Therefore, pH and gas rate have a strong influence on the required treatment duration and efficiency. The authors recommended adjusting the pH of liquids being treated by stripping technologies to 10, a value that achieved good ammonia removal rates while minimizing the risk of problems caused by an excess of lime.

Guštin and Marinšek-Logar (2011) studied the effect of pH (8.5 to 11.0), temperature (30, 40, 50, 60 and 70 ºC) and air-to-manure ratio (416, 833, 1250, 1875, 2100) in the continuous stripping of anaerobic digested centrate from an AD plant co-digesting pig slurry, glycerine, oil food processing waste and slaughterhouse waste. It was observed that pH had a strong effect on N removal, but only up to pH 10. It was also found that temperature had the

smallest effect on N removal under the extremely high gas to manure ratios selected.

Zhang and Jahng (2010) claimed that the type of alkali used in the stripping process and the initial pH value directly influence the ammonia removal rate. Ammonia air stripping was conducted on pig manure (TKN: 7.6 g N l^{-1}, TAN: 4.95 g N l^{-1}) at 1 l min^{-1} l^{-1}_{manure}, 37 ºC and pH 9.5 and 10.0 using NaOH, KOH and CaO as alkaline agents. The TAN concentrations achieved at the end of the run were 1.42 – 3.44 g N l^{-1}. In the study it is noted that the higher the pH at the start of the experiment, the higher removal rate achieved. Lime was considered a less efficient alkaline agent than sodium hydroxide and potassium hydroxide, due to the higher residual TAN concentration achieved at the end of the test. Nevertheless, in a semi-continuous digestion experiment inhibitory effects were reported when sodium hydroxide or potassium hydroxide was used for pH alteration in the physical treatment. Inhibitory effects were shown in the form of low methane yield, which at the end of the experiment matched that of the control reactor without N removal and with a high VFA concentration. When treated manure with lime pH adjustment was digested the performance of the anaerobic reactor was enhanced, i.e. the VFA concentration accumulated in the system before the application of stripping decreased, and methane production increased (to double that of the control), as did organics removal. It was also shown in a batch AD test with NaCl, KCl and $CaCl_2$ additions that K^+ and Na^+ toxicity was the cause of the inhibition. A similar observation was made by Zhang et al. (2011a). In this study air stripping was used under different conditions (37 ºC, 1 - 10 l_{air} min^{-1} l^{-1}, and pH 7.2 - 11.0 with NaOH addition) as a pre-treatment for piggery wastewater in mesophilic anaerobic digestion. In batch conditions it was found that the methane yield decreased as the ammonia concentration increased when treated or untreated waste was digested. Higher methane yields were associated with higher pH values and higher bubbling rates (2-4 l_{air} min^{-1} l^{-1}) in the stripping process; however, a decrease in the biogas production was observed at the highest air flow (10 l_{air} min^{-1} l^{-1}) due to the aerobic degradation of organics. Semi-continuous digestion trials showed enhanced methane production from the nitrogen-reduced feedstock; however, a negative effect on the digestion performance was found even at low TAN concentrations caused by Na^+ accumulation in the reactor due to pH adjustment to 10 and 11 during

the stripping process. The optimal pH in the stripping column to avoid Na^+ inhibition in the reactor and to enhance methane production in comparison to the untreated waste was 9.5.

Kleybocker et al. (2012) observed that CaO was more capable of stabilizing the anaerobic process than NaOH, due to the formation of aggregates containing insoluble salts of calcium. The authors recommended the addition of CaO, as less time is needed to stabilize the process.

Therefore, when the target is to improve reactor performance; the alkali used should be cautiously selected to avoid cation toxicity, as well as taking into account its effectiveness in removal of nitrogen from the feedstock.

Niu et al. (2013b and c) studied the performance and microbial community shift of a reactor fed on low nitrogen (stripped) chicken manure with a TKN 3590 mg N l^{-1} and diluted chicken manure (TKN 6450 mg N l^{-1}) under TC and MC. For low nitrogen chicken manure the biogas yield reached 0.35 - 0.4 l g^{-1} VS at mesophilic temperatures and 0.35 l g^{-1} VS at thermophilic. Under both operational conditions (TAN 3000 and 3500 mg N l^{-1} for the mesophilic and thermophilic reactors, respectively) no major signs of inhibition were found, although at the higher temperature VFA accumulation was present (< 3 g l^{-1}). In the second phase diluted chicken manure from 40 % TS to 10 % TS was used and TAN concentration gradually increased to 6000 mg N l^{-1}. At mesophilic temperature biogas production reduced to 80 % of the value obtained in phase 1 due to a slight increase of VFA to 2 g l^{-1}; however no major signs of ammonia inhibition were found. When the TAN concentration was increased to 10000 mg N l^{-1} by addition of NH_4HCO_3, VFA levels as high as 18000 mg l^{-1} were found with a decrease in biogas production to 0.1 – 0 l g^{-1} VS, indication that without dilution of the substrate some other control technique would be required (Niu et al., 2013b). In the thermophilic experiment severe deterioration of the reactor performance was found: VFA concentrations rose to 30000 mg l^{-1} and biogas yield reduced to 1/3 of the maximum achieved in phase 1 with stripped chicken manure. Therefore the tactic of removing N before digestion was found effective in controlling ammonia inhibition in thermophilic chicken manure digestion.

The performance of a mesophilic UASB reactor treating poultry litter leachate (TAN 4000 – 6000 mg N l^{-1}, 1-2 % TS) was successfully enhanced when ammonia was removed from the feedstock using air stripping. Higher OLR (18.2 kg COD m^{-3} day^{-1} with low nitrogen and 13.6 kg COD m^{-3} day^{-1} with the untreated feedstock) could be applied when the low nitrogen feedstock was used and exhibited good COD reduction (96 %) and biogas production yields (0.26 m^3 CH_4 Kg^{-1} $COD_{reduced}$) (Gangagni Rao et al., 2008).

A comparison of the stripping efficiency was conducted for fresh pig slurry (TAN: 3.39 g N kg^{-1}; pH: 7.5; TS: 5.3 %; TA: 13.4 g $CaCO_3$; VFA: 10.84 g acetate kg^{-1}), anaerobically digested pig slurry (TAN: 3.68 g N kg^{-1}; pH: 8.4; TS: 3.2 %; TA: 14.5 g $CaCO_3$; VFA: 0.24 g acetate kg^{-1}), and synthetic manure solution (TAN: 3.5 g N kg^{-1}, 15 g $CaCO_3$) by Bonmati and Flotats (2003). This study showed that the nitrogen removal depends greatly on the nature of the liquid being treated. Extreme conditions (80 ºC and pH 11.5) were needed to achieve good TAN removal rates with fresh manure; whereas unadjusted pH was suffice for digested waste and for synthetic manure at the same temperature. During the stripping of fresh pig slurry at pH 11 98.8 % reduction was achieved. At non-modified pH and pH 9.5, TAN removal efficiency decreased to 65 - 69 % after 4 hours due to a pronounced fall in pH caused by a loss of buffering capacity and a high VFA concentration. With regard to removal rates the highest was found for the synthetic manure and increased with pH. Digested sludge had the second highest rate at unmodified pH and 9.5, but the performance did not improve when the pH was increased to 11.5. The slowest rate at unmodified pH and 9.5 was for fresh pig slurry; at pH 11.5 the removal rate was boosted to become the second fastest. Batch anaerobic experiments were carried out in mesophilic conditions using pig manure untreated (TAN: 3.24 g N kg^{-1}), stripped at non adjusted pH (TAN: 2.4 g N kg^{-1}), and stripped with pH increased to 9.5 (TAN: 2.15g N kg^{-1}) and 11.5 (TAN: 1.18 g N kg^{-1}). No improvement in methane generation was found when the stripped substrates were digested in batch trials; nevertheless, the high substrate to inoculum ratio (54 g substrate - 6 g inoculum) used in the experiment gave a high initial pH (8.5 – 9.9) when compared to the control digesting raw substrate with no stripping (pH 7.7) which was in the normal range for methanogenesis. Therefore, the recommendation of using stripping only as a post-treatment for pig manure AD could have been induced by a poor experimental set up.

Overall the literature clearly indicates that the anaerobic digestion of manure can be enhanced by pre-digestion stripping processes. However, Laureni et al. (2013) recommend a previous anaerobic digestion stage in order to reduce the total organic matter and increase the ammonia stripping efficiency.

Post-hydrolysis

Ammonia removal after the first stage of fermentation can be conducted outside the fermenter, in a stripping column where the pH and/or temperature can be modified to increase the nitrogen removal, or in the fermenter. Most previous studies in this field (lab scales size) have used an external stripping apparatus.

A steam stripping unit after a first fermenter was used for the treatment of simulated animal by-products (dog food). The experimental work focused on the effect of different TKN reduction set points (4.0 g N kg^{-1} and 5.5 g N kg^{-1}), HRT (30 and 40 days) and OLR (1.8 to 3.71 kg COD m^{-3} day^{-1}) on the subsequent mesophilic methanogenic stage. Reactor performance was compared to reference reactors at the same OLR and HRT without N control, and to reactors with the C/N ratio adjusted via starch or glycerine addition. In the investigation, the highest COD degradation (60.3 %) and biogas production (293 Nm^3 $tonne^{-1}_{COD}$) was achieved when the TKN in the inlet stream was set to 4.0 g N kg^{-1}, corresponding to a TAN concentration in the reactor of 3.45 g N l^{-1}. A 25 % reduction in HRT decreased the COD degradation (to 53.0 %), as well as the methane production, but VFA concentrations remained below 2 g l^{-1}; however, the performance was still better than that of the reference reactor under the same conditions. When the OLR was increased while maintaining the HRT, deterioration in the digestion become apparent due to VFA accumulation and pH decrease to 7.37; therefore, the highest OLR to obtain a good digester performance with N adjustment to 4.0 g N kg^{-1} was 2.90 kg_{COD} m^{-3} day^{-1}. When the TKN set point was fixed at 5.5 g kg^{-1} better degradation of VFA was accomplished when compared to the untreated feedstock, but not as complete as it was for a lower TKN set point, indicating there was still some ammonia inhibition present in the system (Resch et al., 2011). The reduction of TKN to 4.0 g N kg^{-1} prior to AD was recommended to increase COD degradation, and to allow increased OLR.

Zhang et al. (2010) implemented a mesophilic pre-hydrolysis stage before the methanogenic phase for food waste at low retention times (2 – 10 days). Only 15 % of the total nitrogen was hydrolysed in the study, due to a pH decrease in the reactor to 3.9 - 4.7 (without pH control). At such a low pH values hydrolysis can become partially inhibited, which may explain the low concentrations of ammonia in the reactors. Even though a higher % of total nitrogen could be solubilised under different operational conditions, e.g. higher retention times or thermophilic temperature, pH increase in the stripping stage would be needed to maximise the N removal. Yabu et al. (2011) achieved ~50 % of total nitrogen conversion to TAN for synthetic household food waste (TS: 22 %, TKN: 6300 mg N kg^{-1}) with 12 days of SRT, thermophilic temperatures and pH adjusted to 7 – 8 using $Ca(OH)_2$. In the next step of the process 77 % of the TAN or 38 % of the TKN was removed by air stripping at 85 ºC, 2 l min^{-1} $l^{-1}_{digestate}$ and pH 11 using $Ca(OH)_2$. The treated feedstock was digested at thermophilic temperature over 180 days with OLR increasing from 2 to 5 kg VS m^{-3} day^{-1} and with a final TAN concentration close to 2000 g N kg^{-1}, without VFA accumulation and with high biogas production yields (0.68 - 0.8 N m^{-3} kg^{-1} VS). The application of a post-hydrolysis stripping stage improved the performance of synthetic food waste digestion, avoiding free ammonia inhibitive concentration in the thermophilic reactor. The same system was used successfully by Nakashimada et al. (2008) treating dehydrated waste-activated sludge.

A different alternative is to accomplish the hydrolysis at the same time as ammonia is removed from the reactor. Abouelenien et al. (2010) treated chicken manure (TKN: 87 g N kg^{-1}_{TS}, TS: 25 %) in a batch ammonia fermenter by bubbling biogas at 55 ºC (1 l min^{-1} $l^{-1}_{digestate}$) to decrease TAN concentration. After 5 days of treatment 49.7 % of TKN was converted to TAN and 55 % of TAN was removed from the reactor; at the end of the batch test (10 days) 91.4 % of the total nitrogen was converted to TAN. The main difficulty of this method was the pH decrease (although initially controlled to 9.5 using NaOH) caused by accumulation of VFAs which would impede N removal in a continuous process.

This configuration is advantageous because hydrolysis and methane production can be optimised in different reactors, removing the toxic ammonia before the methanogens can be affected. However, some comparisons between

single and two-phase systems treating fruit and vegetable wastes showed difficulties in maintaining the separation in the two phase system and worse performance compared to the single phase system, especially at high OLR (Mtz-Viturtia et al., 1994). Hartmann and Ahring (2006) concluded that a two-phase system can be an advantageous configuration to treat wastes containing high fractions of recalcitrant organic matter, while if the organic matter is easily degradable the system can suffer from overloading. In order to avoid possible long run operational problems that may appear in the two-phase system where some additional critical variables needs to be considered, post-hydrolysis tests were not considered in this study.

In situ

The application of this configuration, where the ammonia is stripped continuously from the digester while biogas is produced, has the advantage of removing ammonia while it is being produced by hydrolysis. However, pH and temperature alteration to favour nitrogen transfer from the liquid phase to the gas phase is not recommended since methanogenic conditions can be disturbed.

Nielsen et al. (2013) claimed it was possible to remove a substantial amount of ammonia from a pig manure digester by recirculating biogas through the upper 30 cm of the reactor. Although the method is intended to be used with biogas as stripper gas, nitrogen was used to obtain mass transfer coefficients at different flow rates (4 – 48.8 l min^{-1} for 15 min) in a 206-L (133-L working volume) pig manure digester (TAN concentration of 2.8 g N l^{-1} increased with NH_4Cl addition simulating the typical concentration of an ammonia inhibited process) without new feedstock addition. A modified version of ADM1 was used to simulate the TAN reduction in a reactor with (HRT of 15 days) and without feeding and at a bubbling flow rate of 25 l min^{-1}. 25 % reduction in TAN was predicted in the fed system over 15 days; however, temperature in the digester during the experiment and modelling were omitted in this publication, this is an essential parameter in the AD process with or without *in situ* ammonia stripping. Temperature affects the molecular flow of ammonia per cross sectional area and the ammonium dissociation equilibrium constant which in turn affects the ammonia mass transfer rate into the bubbles. In this case it has also influenced the selection of the experimental initial TAN

concentration to determine the mass transfer rate into the bubbles. From this TAN concentration (2.8 g N l^{-1}) it could be inferred that the reactor was kept at thermophilic temperature, since it is reported that it is an inhibitive concentration and that level would not pose a challenge for AD at mesophilic conditions; however, this is just speculation. It would have been interesting if the reported study had provided with a simulation at a critical initial TAN concentration (>5 g N l^{-1}) in the reactor, in order to consider if the *in situ* configuration could succeed in decreasing the concentration to below inhibitive levels in a thermophilic reactor.

Abouelenien et al. (2010) compared the results of ammonia stripping using *in situ* biogas injection at the bottom of the reactor (1 l_{biogas} min^{-1} $l^{-1}_{reactor}$) for low nitrogen chicken manure (21.4 % TKN of the untreated chicken manure) and a 1:1 mixture of chicken manure (TKN: 87 g Kg^{-1} TS, TS: 25%) and the low nitrogen chicken manure in thermophilic semi-batch culture. New feedstock was added only when the acetate concentration was < 3 mmol kg^{-1}. For the low nitrogen feedstock 9 feeding-cycles were conducted in 30 days, and for the mixed substrate 5 feeding-cycles in 22 days, indicating better degradation of the low nitrogen substrate. In the experiment, TAN was maintained below 2 g N kg^{-1} in both reactors, but methane production (195 ml g^{-1} VS) was 24.2 % higher when low nitrogen chicken manure was used as mono-substrate. Post-hydrolysis and methane fermentation with *in situ* stripping in both stages was thus shown to maintain TAN below the thermophilic ammonia inhibition threshold with satisfactory methane production in semi-batch cultures for dry chicken manure.

Walker et al. (2011) modelled a mesophilic gas-mixed anaerobic reactor with *in situ* ammonia stripping treating food waste at different substrate OLR (1 – 6 kg VS m^{-3} day^{-1}). In the simulation the ammonia removal time constant representing TAN removal in the digestate (595 hours) was obtained in a batch stripping experiment conducted with food waste digestate at 35 ºC and a bubbling rate of 0.375 l min^{-1} $l^{-1}_{digestate}$. The simulated TAN concentration in the reactor when steady state was reached was < 2.5 g N l^{-1} at all OLR.

Jiang et al. (2013) used *in situ* gas stripping to decrease the ammonia concentration of a thermophilic anaerobic reactor treating diluted (1:1) distillery residue (TS 144.4 g Kg^{-1}, Total N 6.1 %, C/N 9.0) arising from

bioethanol production from kitchen waste. The study compared recirculation of ammonia-free biogas (0.25 l_{biogas} min^{-1} $l^{-1}_{reactor}$) to the headspace of the digester and to the liquid phase. At an OLR of 4 kg VS m^{-3} day^{-1} TAN stabilized at 3500 mg N l^{-1} in the headspace recirculated system and 2200 mg N l^{-1} in the liquid recirculated configuration. Significantly lower VFA concentration, higher biogas yield and more stable reactor performance was achieved in the liquid recirculated configuration.

However, stability in the anaerobic reactors might be affected by the mixing strategy: adequate mixing provides good contact between the microbial population and enzymes, whereas inadequate mixing will result in stratification, formation of a floating layer of solids and dead zones. In the case of high solids digestion Stroot et al. (2001) found that vigorous continuous mixing originated instability in the system suppressing good performance in the digester. Intense mixing discomposes the structure of microbial flocks, which disturbs the syntrophic relationships between organisms.

Different references show comparable typical mixing flow rates; the configuration of the digester (depth of tank, type of diffuser, or diffuser location) has a strong dependence on the mixing effectiveness. Turovskiy and Mathai (2006) recommend a flow of 0.005–0.007 m^3 m^{-3} min^{-1} when a confined external gas recirculation mixing system is used. Agency (1989) suggests 0.0152-0.0365 m^3 m^{-2} tank cross section min^{-1}. Rico et al. (2011) used 0.005 – 0.011 $m^3 m^{-3} min^{-1}$ when the effect of mixing on biogas production was studied. All the recommendations give mixings flows in the same range as those used in this study (Table 5; Perry and Green, 1999).

Table 5. Degrees of agitation in a tank

Air rate (depth 2.7m)	**Air rate (depth 0.9m)**	**Degree of agitation**
$m^3 m^{-2}_{\text{tank cross section}} min^{-1}$	$m^3 m^{-2}_{\text{tank cross section}} min^{-1}$	
0.0033	0.0066	Moderate
0.0066	0.0132	Complete
0.016	0.032	Violent

The use of a single reactor system where both biogas generation and the nitrogen removal take place using the lowest efficient biogas recycle flow could reduce the initial investment cost of building facilities and running costs. The literature clearly indicates that this technique may succeed in maintaining TAN

concentration in a biodigester below the inhibition threshold for mesophilic and TC without extra heating or chemical costs required. For this reason the *in situ* configuration is considered in this study.

Post-digestion and side-stream

In this configuration the nitrogen removal stage is performed after anaerobic digestion. Side-stream and post-digestion treatment have similar features: the only difference is that in post-digestion the stripping is included e.g. as a pasteurisation step (when the temperature is increased in the stripping column) with no return of the low nitrogen digestate to the reactor. Therefore, only side-stream stripping can be used as a nitrogen control technology in AD.

In order to reduce the management costs of distribution and land application of cattle and swine digested manure (TKN: 3678 from cattle and 3351 for swine), the N-Free ® process was applied as a post-digestion treatment. This process used a series of solid-liquid separation units (screw press, flocculation, and centrifugation), membranes (ultrafiltration and reverse osmosis) and zeolites to generate a clean liquid stream (49 % of the initial volume of manure) that can be discharged into the water bodies. In addition, 12-32 % of the initial mass is obtained as a solid fraction rich in organics, N and P, and an ammonium sulphate fertilizer (1.8 m^3 of ammonium sulphate per 100 m^3 of digestate treated) by cold air stripping of the concentrate stream of a reverse osmosis unit (Ledda et al., 2013).

A side-stream process was tested for the membrane-separated liquid fraction from a mesophilic digester (SRT 30 - 40 days) treating slaughterhouse wastes. The liquid fraction was stripped at 65 ºC and pH 8.5 - 9 with NaOH addition (Siegrist et al., 2005). A TAN concentration of 4300 mg l^{-1} was obtained (71 % lower than the theoretical value without intervention), with high COD removals (90 - 95 %) even when some VFA accumulation was present in the system (3 – 12 g l^{-1}). Side-stream stripping applied to the liquid fraction of chicken manure digestate after filtration at 80 ºC, 600 mbar without pH adjustment also maintained free ammonia concentration below the mesophilic inhibition threshold (chicken manure characteristics TS: 42.6 – 53.7 %; TKN: 27.7 – 33.4 g kg^{-1}_{FM}). Specific biogas production ranged between 0.6 – 0.4 l N g^{-1} VS and VS removal ratio decreased from 60 % to 30 % with an increase in OLR from 2.25

to 4 kg VS m^{-3} day^{-1} (Belostotskiy et al., 2013). A similar stripping system was used by Nie et al. (2014) to control the ammonia concentration in an anaerobic digester fed on chicken manure at 40 ºC. A specific biogas yield of 0.39 l g^{-1} VS was achieved controlling the FAN concentration at 0.77 g l^{-1} at an OLR of 5.3 kg VS m^{-3} day^{-1}.

De la Rubia et al. (2010) conducted preliminary stripping studies with stored food waste digestate (TAN: 7170 mg N l^{-1}, TS: 5.8 %, VFA: 9.8 g l^{-1}) using biogas as stripping medium, at different temperatures (35, 55, 70 ºC), and flow rates (0.125, 0.250, 0.375 l_{biogas} min^{-1} $l^{-1}_{digestate}$), with unmodified pH. Low TAN removal rates were found in these experimental conditions. Greater removal rates were found by Walker et al. (2011) using the same type of digestate under the same conditions with or without pH increase. In the same study a mesophilic anaerobic reactor was simulated treating SS-DFW at different OLR (1-6 kg VS m^{-3} day^{-1}) and different percentages of reactor volume per day (10 % – 1 %) treated by a side stream stripping column. In the model the ammonia removal time constant (17.7 hours) was obtained in a batch stripping experiment at 70 ºC with non-modified pH and a biogas bubbling rate of 0.375 l min^{-1} $l^{-1}_{digestate}$. In the scenario where 2.5 % of the reactor was treated per day TAN reductions of 66.6 %, 58.3 %, 50.0 % and 41.7 % respectively were achieved in steady state for OLR of 2, 3, 4, 5 and 6 kg VS m^{-3} day^{-1}. Therefore, this stripping configuration may be capable of achieving TAN concentrations below the inhibition threshold for MC and TC.

The same approach to control de ammonia level in the reactor and to enhance the AD of food waste was taking by Zhang et al. (2010). In this study mesophilic digesters were fed on food waste at an OLR of 2 kg VS m^{-3} day^{-1} and 10 % of the digester content was placed into the corresponding stripping column on a daily basis. The columns run at mesophilic and thermophilic temperatures, unmodified pH and at a bubbling rate of 0.375 l l^{-1} min^{-1}. Surprisingly TAN concentration in the reactors remained unaltered (5 g N l^{-1}) after 100 days of operation. The conclusion was that the stripping process did not succeed in removing the expected TAN. Indeed, the operational temperature in the stripping columns was not optimal to achieve high removal rates. Under these stripping conditions a small partition of the TAN goes to the FAN fraction (less than 10 %). To increase the FAN to TAN ratio in the liquid

phase and the ammonia concentration gradient in the gas-liquid phase a higher temperature and/or pH are recommended (Table 6, Fig. 11).

Ammonia stripping has barely been used in industrial biogas plants since, so far, it has not been entirely proved as a tool to increase stability in the reactors. Weiss et al. (2009) applied this technology to a process water stream (TAN: 5095 – 5374 mg N l^{-1}, TS: 3.4 - 3.8 %) before its addition to the bio-waste to be treated in two 3300 m^3 thermophilic CSTRs. Air was injected into a stripping column with a height of 6 m and 0.5 m internal diameter after pH adjustment to 10.5 - 11.0 with NaOH addition. 80 % of the TAN was removed from the water, improving not only the quantity of biogas but also the quality (higher CH_4 content). Even though the application of this technology produced an increase of 35 % in methane production in the system, some improvements are needed to make this technique attractive to large-scale plants due to the considerable flow of gas used in this experiment (2000 m^3 h^{-1}) compared to the water stream flow treated (3 m^3 h^{-1}).

Table 6. Temperature and pH conditions needed to achieve certain % FAN to TAN

25 ºC	36 ºC	55 ºC	70 ºC	85 ºC	FAN %
9.2	9	8.4	8	7.8	**50**
9.4	9.2	8.6	8.2	8	**60**
9.6	9.4	8.8	8.4	8.2	**70**
9.8	9.6	9	8.6	8.4	**80**
10.2	9.8	9.4	9	8.6	**90**
10.6	10.2	9.6	9.2	9	**95**

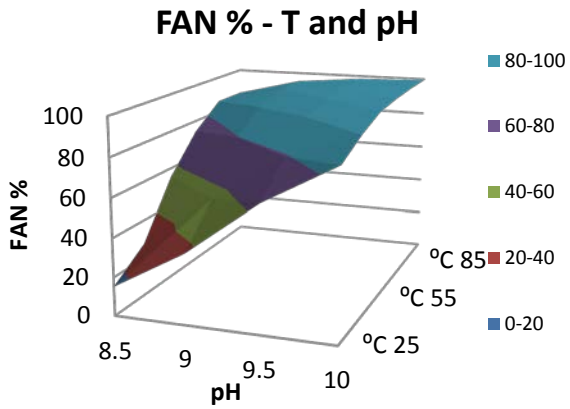


Fig. 11. % FAN to TAN under different temperature and pH conditions

In the current research, the experimental work was designed to allow assessment of biogas side-stream and *in situ* stripping technologies as a basis for recommending the most appropriate ammonia removal strategy for practical use.

2.3.7.4 Nitrogen recovery: reasons and methodology

Nitrogen is an essential element for organisms, with living cells containing up to 14 % nitrogen in crucial components such as proteins and DNA (Mara and Horan, 2003).

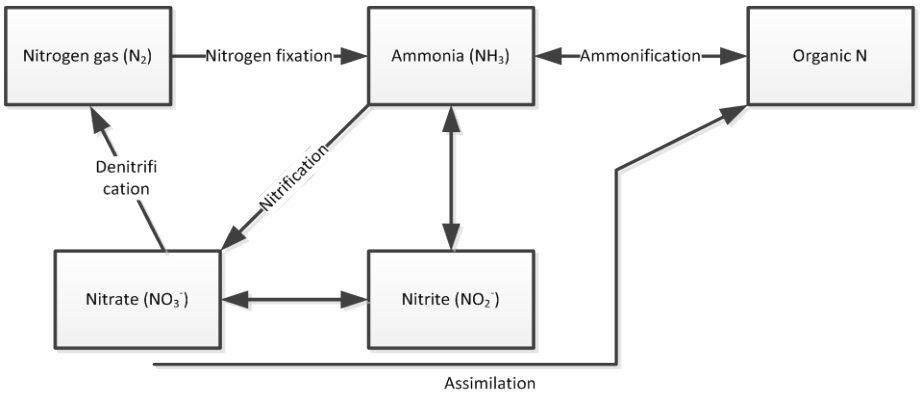


Fig. 12. The biological nitrogen cycle

The fundamental pathways to favour plant growth in agriculture are nitrogen fixation and assimilation. Although nitrogen gas makes up around 79 % of the atmosphere, only a select group of microorganisms is capable of fixing nitrogen directly into a biologically usable form as it is chemically inert. Intense cultivation practices can locally distort the natural balance of nitrogen; therefore, nitrogenous fertilizers are used to improve productivity.

Ammonium sulphate (21 % N) is an important nitrogen and sulphur fertilizer source. Moreover, it is a quick-acting fertilizer, resistant to leaching as it is adsorbed on the soil, then slowly released and used in plant growth (Gowariker et al., 2009).

The world nitrogen fertilizer demand is expected to increase at an annual rate of 1.7 % from 105.3 million tonnes in 2011 to 112.9 million tonnes in 2015 (Food and agriculture organization of the United Nations, 2011). Ammonium sulphate production has increased from 16691 thousand tonnes of product in 1999 to 21075 thousand tonnes in 2010 (IFA, 2010). 90 % of the ammonium sulphate demand in 2001 was used directly as fertilizer or processed as multicomponent mixtures; the remaining 10 % was used in the chemical industries (OECD SIDS, 2004). The high TAN concentration traps used to remove ammonia from the stripping biogas could thus generate fertilizer as a valuable by-product.

Ammonium sulphate has been traditionally produced as a by-product of synthetic-fibre intermediates manufacture and coke oven process. It can also be synthesised by combining anhydrous ammonia and sulphuric acid in a reactor. (Hofmann et al. 2009). Once the ammonium sulphate solution has been generated crystallization is used as a convenient method to purify the compound because crystal form facilitates the packaging and storage of the final product. Ammonium sulphate crystals are formed using a water evaporator to obtain a supersaturated solution. A centrifuge is used afterwards to separate the crystals from the liquor. A settling tank can be used to reduce the liquid load on the centrifuge and decrease the operating cost. The saturated liquor is returned to the diluted ammonium sulphate brine of the evaporator. The crystals are fed to either a fluidised-bed or a rotary drum dryer (Hofmann et al., 2009; Myerson, 2001).

Other biological or physico-chemical methods could be applied to recover the stripped ammonia. A nitrification bio-filter converts the stripped ammonia into nitrogenous compounds: the nitrate compounds can be collected and converted in nitrogen-rich compost (Burke, 2006; Posmanik et al., 2013). The capability to upgrade the biogas quality using the biogas produced in AD to strip ammonia out of digestate and the N-rich gas to precipitate insoluble ammonium carbonates and bicarbonates increasing the energy content of the final biogas line has also been also explored (Burke, 2010).

3. Methodology

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 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, COSSH assessments. All equipment, laboratory apparatus, and analytical instruments were operated in accordance with the manufacturer's instructions. All glassware was washed using washing detergent followed by rinsing with tap water and deionised water. The glassware used for the acid digestion was soaked in a 10 % nitric acid bath for a 24 hour period after which the glassware was rinsed with Milli-Q water.

3.2 Monitoring and analytical methods

3.2.1 Total and Volatile Solids

Substrate and digestate total solids (TS) and volatile solids (VS) determination was based on Standard Method 2540 G (APHA, 2005). After thorough homogenisation, sample was transferred into a weighed crucible by pouring (digestate samples) or spatula (substrate samples). Samples were weighed to a sensitivity of ± 0.1 mg (Sartorius LC6215 balance, Sartorius AG, Gottingen Germany) and placed in a fan-assisted oven (Heraeus Function Line series, UK) for drying overnight at 105 ± 1 ^{0}C. After drying the samples were transferred to

a desiccator to cool for at least 40 minutes. Samples were then weighed again with the same balance, transferred to a muffle furnace (Carbolite Furnace 201, Carbolite, UK) and heated to 550 ± 10 ^{0}C for two hours. After this ashing step, samples were again cooled to ambient temperature in a desiccator before weighing a third time.

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

$$\%\, TS\ = \frac{W_3 - W_1}{W_2 - W_1} x\ 100 \tag{16}$$

$$\%\, VS\ (on\ a\ wet\ weight\ basis) = \frac{W_3 - W_4}{W_2 - W_1} x\ 100 \tag{17}$$

$$\%\, VS\ (on\ a\ TS\ basis) = \frac{W_3 - W_4}{W_3 - W_1} x\ 100 \tag{18}$$

Where W_1 is weight of empty crucible (g); W_2 is weight of crucible containing fresh sample (g); W_3 is weight of crucible and sample after drying at 105 ºC (g) and W_4 is weight of crucible and sample after heating to 550 ºC (g).

3.2.2 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 solution used for calibration was prepared from buffer tablets (Fisher Scientific, UK) according to the supplier's instructions. During measurements, the sample was stirred to ensure homogeneity. In addition, the pH probe was rinsed with deionised 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.

3.2.3 Alkalinity

Alkalinity of liquid samples was measured as mg $CaCO_3$ l^{-1} by titration based on Standard Method 2320B for Alkalinity (APHA, 2005). Digestate was sieved to obtain a homogenous sample and 2-5 g of this was added to 40 ml of deionised water. Titration was done using a Schott Titroline Easy automatic digital titration burette system (Schott, Mainz, Germany) fitted with a combination glass electrode calibrated in buffer solutions at pH 4, 7 (Fisher Scientific, UK), 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, 4.3 and 4.0, 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 deionised water between subsequent samples to avoid cross contamination. Alkalinity was calculated according to the following equations:

$$TA = \frac{(V_{4.0} + V_{4.3} + V_{5.7})\ x\ N\ x\ 50000}{V} \quad (19)$$

$$PA = \frac{V_{5.7}\ x\ N\ x\ 50000}{V} \quad (20)$$

$$IA = \frac{V_{4.3}\ x\ N\ x\ 50000}{V} \quad (21)$$

Where $V_{4.0}$, $V_{4.3}$ and $V_{5.7}$ are the volumes of titrant used to endpoints 4.0, 4.3 and 5.7 respectively (ml); N is normality of titrant (H_2SO_4) and V is volume of sample (ml).

3.2.4 Total ammonia nitrogen

Total ammonia nitrogen (TAN) analysis was based on Standard Method 4500-NH_3 B and C (APHA, 2005). A sample aliquot of between 2-3 g was weighed (i201, My Weigh Europe, Huckelhoven Germany) into a digestion tube and 50 ml of deionised water added. Blanks (50 ml deionised water) and standards (containing 10 ml of 1000 mg l^{-1} NH_4Cl with 40 ml deionised 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 either a Foss Tecator Kjeltec system 1002 distillation unit (Foss Tecator A-B, Hoganas, Sweden) or 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:

$$TAN = \frac{(A - B)\ x\ 14.0\ x\ N\ x\ 1000}{W_S} \qquad (22)$$

Where TAN is total ammonia nitrogen (mg N kg^{-1}); A is volume of titrant used to titrate the sample (ml); B is volume of titrant used to titrate the blank (ml); N is normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant and W_s is wet weight of sample (kg).

An ion selective electrode (Jenway 924 328) was used to determine low TAN concentration in aqueous solutions. The response of the electrode is Nernstian up to a concentration of 0.1 M ammonia. Ammonium chloride solutions were used as calibration standards. The detection limit is established by the water purity used in the calibration curve.

3.2.5 Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) is the sum of N_{org} and TAN (ammonia and ammonium) and was determined after acid digestion by steam distillation and titration.

Total Kjeldahl Nitrogen (TKN) analysis was carried out on duplicate or triplicate samples alongside blanks and controls as follows: 0.1-1.0 g of dry sample or 2.5-4.0 g of digestate (weighed to ± 1 mg) was placed in a glass digestion tube. Two Kjeldahl Cu 3.5 catalyst tablets (Copper Kjeltabs, 3.5 g, FOSS) 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 ºC and once the reaction was completed the tubes were cooled to around 50 ºC and 40 ml of deionised 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 using a BÜCHI Distillation Unit K-350 with NaOH addition, followed by collection of the distillate in boric acid indicator and titration with 0.25 N H_2SO_4.

$$TKN = \frac{(A - B)\ x\ 14.0\ x\ N\ x\ 1000}{W_S} \qquad (23)$$

Where TKN is total ammonia nitrogen (mg kg^{-1} wet weight); A is volume of titrant used to titrate the sample (ml); B is volume of titrant used to titrate the blank (ml); N is normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant and W_s is wet weight of sample (kg).

3.2.6 Volatile Fatty Acids

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 13,000 rpm (micro-centrifuge, various manufacturers) for 30 minutes. The supernatant was diluted with deionised water as appropriate to obtain a maximum acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic concentration of 500 mg l^{-1} and the final formic acid composition was adjusted to 10 % vol. The diluted sample was centrifuged 30 minutes at 13,000 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 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 Shimazdu 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 ^{0}C in 15 minutes with a final hold time of 3 minutes. The temperatures of injector and detector were 200 and 250 ^{0}C, respectively.

Total VFA concentration is reported as sum of the single compounds (acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids).

3.2.7 Trace metals extraction and analysis

Analysis was carried out using duplicate samples and blanks. Samples with high solids contents, e.g. food waste, were air dried and ground using a centrifuge mill with a 0.5 mm mesh sieve (Glen Creston LTD type ZM-1, UK). Acid digestion was based on EPA method 3010 A. Approximately 1-2 g of fresh sample or 0.5 – 1.0 g of dried samples 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 HNO_3 (Nitric acid) was added, and the tubes were gently agitated. The tubes were placed into the digestion block (Gerhardt Kheldatherm), connected to the condenser system and left for 24 hours prior to heating. The acid digestion involved gradually increasing the temperature first to 100 ºC and then to the final temperature of ~180 ºC 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) when ambient temperature was reached. The filtrate was then transferred into a PET bottle and sent for analysis by ICP-MS (Severn Trent Services, Coventry, UK).

3.2.8 [2-^{14}C] Sodium acetate labelled analysis to determine the methanogenic pathway

The metabolic pathway for methanogenesis was determined by labelled [2-^{14}C] sodium acetate analysis on duplicate samples (Jiang, 2012). Each 15 g sample of digestate was mixed with anaerobic medium in the ratio of 1:2 and 0.15 ml of $^{14}CH_3COONa$ solution with a specific activity of 10 kBq ml^{-1} was added (MP biomedical, Solon, OH, USA). The mixture was incubated in 119 ml crimp top serum bottles at 37 ºC for 48 hours. At the end of the incubation process the sample/medium mixture was acidified with 2 ml of 1mM H_2SO_4 and sparged using N_2 and O_2 gas mix (9:1 on a volume basis). The CO_2 and CH_4 produced were first passed through 20 ml 5M NaOH before CH_4 was oxidised to CO_2 in a tube furnace consisting of a heating block within which was embedded a quartz tube (6.2 mm OD, 4 mm ID, 180 mm length, H. Baumbach & Co Ltd, Suffolk, UK) packed with copper (II) oxide. The operating temperature was regulated at 800 ± 5 ºC using a temperature controller (Omega DP7004, Manchester, UK). The sparge gas then carried the CO_2 generated from CH_4 to a second CO_2 trap filled with 20 ml 1M NaOH. After absorption, 1 ml of each alkali trap and 1 ml of the centrifuged sample/medium mixture were added into 15 ml Gold Star multi-purpose liquid scintillation cocktail (Meridian

Biotechnologies Ltd, Surry, UK) and counted in a Beckman Coulter LS6500 scintillation counter.

3.2.9 Capillarity Suction Time

To assess the digestate resistance to filtration Triton-WRPL type 130, a type 319 Multi CST apparatus was used (Fig. 13). 5 ml of the digestate sample was poured into the small circular tube which presses down on a piece of CST filter paper (Triton Electronics Ltd, UK) placed on the lower perspex block of the apparatus. Two electrodes detect the presence of water in the CST filter paper and these are placed at a standard distance from the central filling tube (radial interval of electrodes of 7mm). 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.

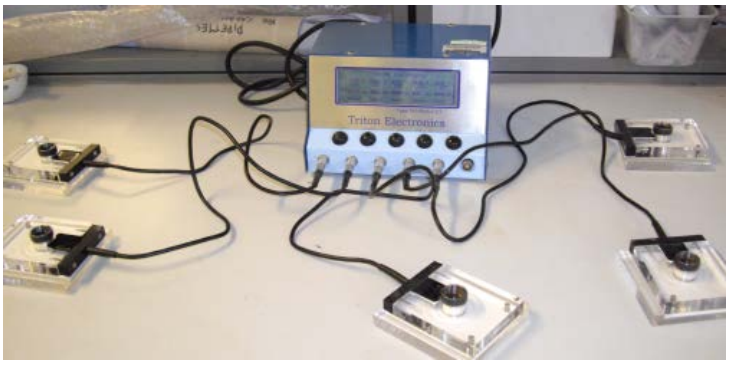


Fig. 13. Capillary suction time apparatus

3.2.10 Frozen Image Centrifuge Test

The Frozen Image Centrifuge (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 techniques in which a 'frozen image' of the sample is generated which when observed allows 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 (Fig. 14).

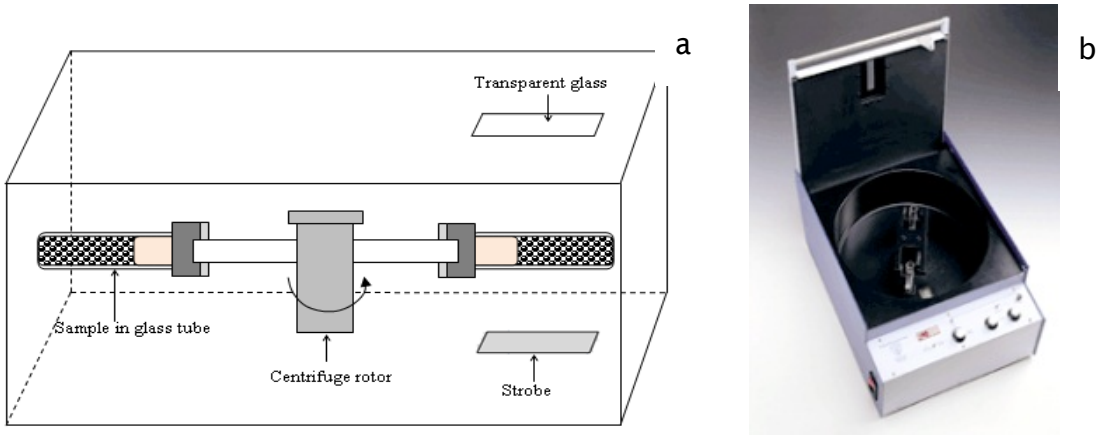


Fig. 14. Visualisation of FIC test

3.2.11 Gas composition

Biogas composition (CH_4 and CO_2) was quantified using a Varian Star 3400 CX gas chromatograph (Varian Ltd, Oxford, UK). The GC was fitted with a packed stainless steel SUPELCO 80/100 mesh porapack-Q column (Hayesep C) and used either argon or helium as the carrier gas at a flow of 25 ml min^{-1} with a thermal conductivity detector. The biogas composition was compared with a standard gas containing 65 % CH_4 and 35 % CO_2 (v/v) (BOC) for calibration. A sample of 10 ml was injected into a gas sampling loop.

3.2.12 Gas volume

Gas bag volumes were measured using a weight-type water displacement gasometer (Walker et al., 2009). In this device the biogas flows from a gas-bag to a column under vacuum generated by water displaced into a balance which allows volume determination. 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. Gas yields and volumes were corrected to standard temperature and pressure (0°C and 101325 Pa). Volume was calculated as described by Walker et al. (2009) according to the following equations:

Height Gasometer Governing Equation (24)

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

Weight Gasometer Governing Equation (25)

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

Where V is gas volume (m^3); P is pressure (Pa); T is temperature (K); H is total height of column (m); h is distance to liquid surface from a datum (m); A is cross-sectional area of gasometer (m^2); m_b is mass of barrier solution (kg); ρ is density pf barrier solution (kg m^{-3}); *g* is gravitational acceleration (m s^{-2}) and *1, 2, stp, atm, b, t, c* 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, collection trough and column respectively.

Digester biogas production was measured using continuous gas flow meters (Walker et al., 2009).

3.2.13 Rotameter and pump calibration

The rotameters were calibrated by collecting biogas pumped over a fixed time in a gas-impermeable bag, then accurately measuring the volume using a weight gasometer (equation 24 and 25) (Walker et al., 2009).

The peristaltic pump used in the batch ammonia stripping experiments was calibrated to provide 0.250 l min^{-1} at room conditions collecting the displaced gas in a sampling bag during a certain period of time at different pumping rates. A weight gasometer was used for measuring the gas volume (equation 24 and 25) (Walker et al., 2009).

3.3 Materials

3.3.1 Feedstocks

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

Some researchers have studied the effect of freezing/thawing on solid hydrolysis and anaerobic digestion performance of kitchen waste (Ma et al., 2011) and food waste (Liu et al., 2008; Stabnikova et al., 2008). Freezing organic matter is considered as a physical pre-treatment that leads to formation of intracellular ice crystals causing cell membrane damage (cell disruption). Liu et al. (2008) used two batch two-phase digesters to compare the digestion of frozen/thawed food waste to unfrozen food waste. The pre-treatment was able to alter the characteristics and structure of substrates favouring solubilisation, and hence the specific and rate of methane production. The total duration of the experiment, however, was only 12 days. Ma et al. (2011) compared batch methane production in thermophilic one-phase reactors for 30 days. Treated and untreated kitchen waste provided equivalent biogas yields (0.35-0.38 l g^{-1} $COD_{removed}$). It was stated that satisfactory performance in a continuous reactor could be achieved with frozen material at a higher OLR of 4 kg VS m^{-3} day^{-1} compared with 3 kg VS m^{-3} day^{-1} in the non-frozen control. The current research employs one-phase CSTRs at lower OLR than those operated by Ma et al. (2011), and thus higher retention times (approximately 100 days). In addition the substrate SS-DFW is easily hydrolysable and contains a fairly high proportion of material that has already been cooked and/or frozen (only 36 % of fresh vegetables and fruit was found

by WRAP, 2009 in the total household food waste produced in UK). Therefore, the effect of any substrate cell disruption caused by freezing on the biogas yield obtained is expected to be minor.

3.3.2 Trace element solution

The trace element (TE) solutions used, one composed of cations and the other oxyanions (see Table 7) were based on a modified TE recipe developed by University of Southampton (Banks et al., (2012). TE were supplemented by weekly addition of the two solutions at a rate of 0.5 ml of each solution for every 1 kg of food waste added to give a steady state minimum concentration of TE in the digester.

Table 7. Concentration of trace elements in stock solution

Trace element	Compound used	Element concentration in the working condition ($mg\ l^{-1}$)	Compound concentration in stock solution ($g\ l^{-1}$)
Cation			
Aluminium (Al)	$AlCl_3 \cdot 6H_2O$	0.1	1.790
Boron (B)	H_3BO_3	0.1	1.144
Cobalt (Co)	$CoCl_2 \cdot 6H_2O$	1.0	8.076
Copper (Cu)	$CuCl_2 \cdot 2H_2O$	0.1	0.536
Iron (Fe)	$FeCl_2 \cdot 4H_2O$	5.0	35.6
Manganese (Mn)	$MnCl_2 \cdot 4H_2O$	1.0	7.204
Nickel (Ni)	$NiCl_2 \cdot 6H_2O$	1.0	8.100
Zinc (Zn)	$ZnCl_2$	0.2	0.834
Oxyanion			
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.2	0.736
Selenium (Se)	Na_2SeO_3	0.2	0.876
Tungsten (W)	$Na_2WO_4 \cdot 2H_2O$	0.2	0.718

3.4 Equipment

Two types of digesters and two types of stripping columns were used in the research.

3.4.1 75-L CSTR digesters

The continuously-stirred tank reactor (CSTR) digesters used had a total volume of 100-L and a working volume of 75-L, and was constructed from 40 cm inner

diameter PVC pipe sealed at its top and bottom with plates incorporating feed and drainage ports. Digester temperature was controlled at 36 ± 1 ºC by recirculating water from a thermostatic bath through an internal heating coil. The digesters were sealed from the outside atmosphere by a draught tube through which an offset bar stirrer was inserted to allow low speed mixing at 26 rpm by means of geared motors (Parvalux, UK). Biogas production was measured using continuous gas flow meters (Walker et al., 2009). The configuration of the system is shown in Fig. 15.

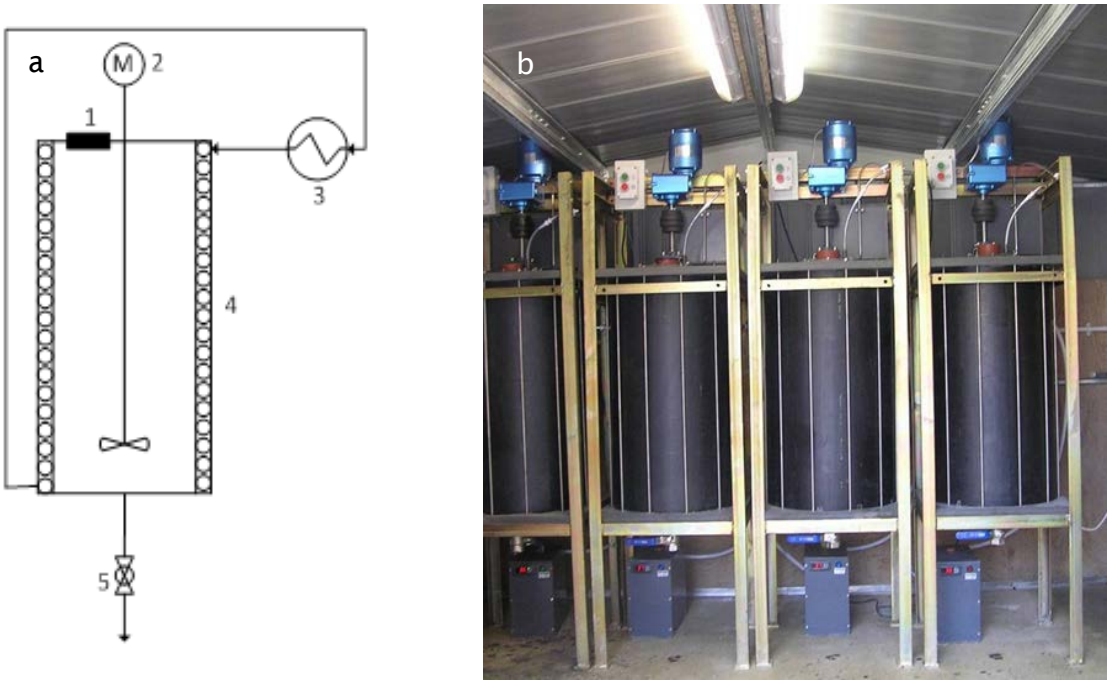


Fig. 15. 75-L CSTR digesters. a) Schematic showing b) four digesters image
(1) NB port, (2) motor to propel the stirrer, (3) heater, (4) heating coil, (5) digestate outlet

For trials of *in situ* gas stripping one 75-L digester was modified by the addition of eight open-ended standpipe spargers (6 mm external diameter) installed at the bottom of the reactor at spacings of approximately 8.5 cm. The configuration of the system is shown in Fig. 16. Biogas flow was regulated using two rotameters (Key Instrument, air range 4-50 l min^{-1} and 0.4-5 l min^{-1}) installed in the reactor biogas line and in the bypass line, respectively. The reactor flow meter was calibrated to operational conditions (i.e. gas type). After passing through a condensate trap, the stripping gas was bubbled through a deionised water trap and a 0.25 N H_2SO_4 trap to capture any ammonia removed from the reactor. The ammonia-free biogas was then pumped back into the reactor in a closed loop.

The headspace of the reactor, which was initially full of air, was flushed using the biogas generated overnight by two 75-L food waste anaerobic digesters (section 4.1). The gas line was bled by flushing from a Tedlar bag (SKC, Blandford Forum, UK) filled with standard biogas (65.12 % CH_4 and 34.88 % CO_2 (v/v), BOC Ltd) to remove the remaining air from the system (mainly from the ammonia traps). At the end of the flushing process the bag was kept in place to mitigate pressure changes and gas leakages in the system. Biogas composition was monitored during the experiment, and if this indicated the presence of air the flushing process was repeated.

a

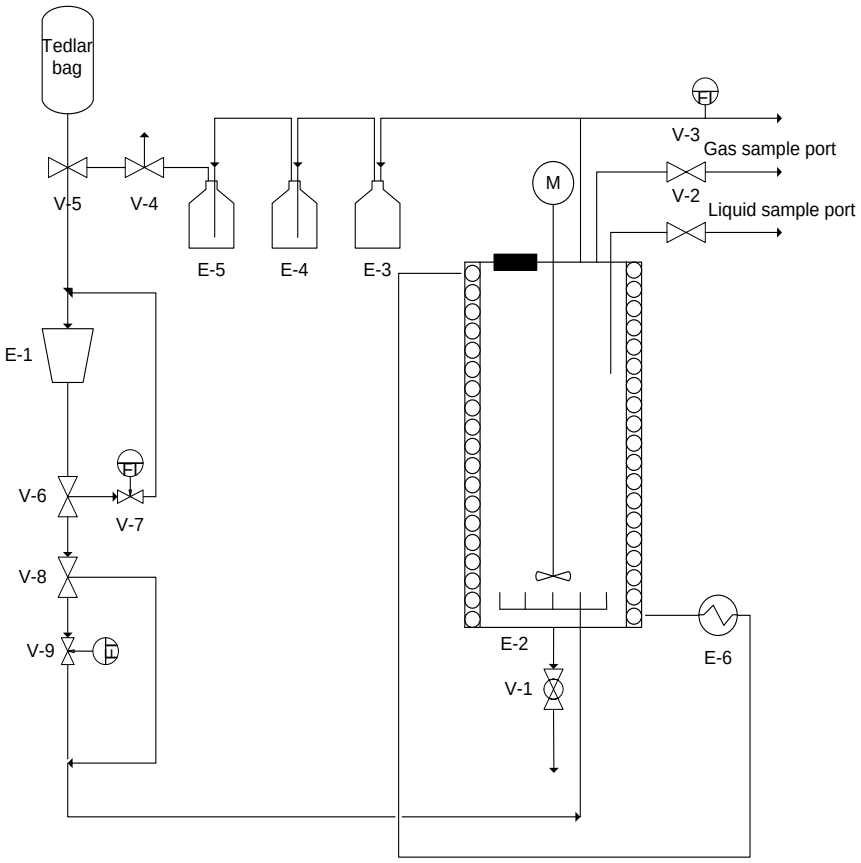


Fig. 16. a) Schematic drawing of CSTR *in situ* stripping reactor b) experimental rig
E-1 Gas diaphragm pump KNF N828-KNE, E-2 CSTR reactor 100-L total volume, E-3 Condensate trap, E-4 Water trap, E-5 Acid trap, E-6 Heater

3.4.2 35-L CSTR digesters

The four 35-L CSTR digesters used were of the same basic design as the 75-L digesters. Each digester had a total volume of 40-L and a working volume of 35-L, and was constructed from 36 cm inner diameter PVC pipe. Temperature in each digester was continuously monitored using LM35DZ temperature sensors. Low speed mixing at 30 rpm was provided by geared motors (Parvalux, UK). Fig. 17 shows the system configuration.

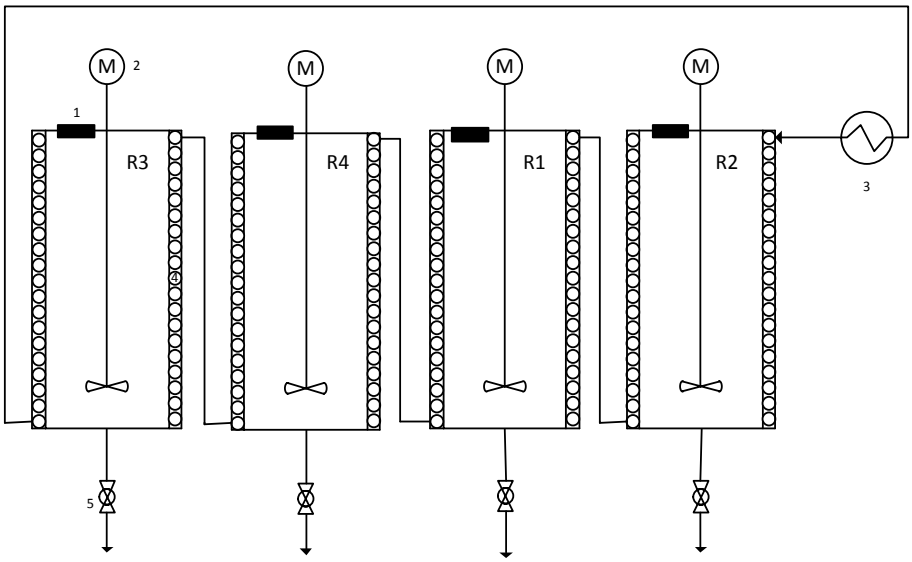


Fig. 17. Schematic drawing of CSTR reactor for semi-continuous anaerobic digestion of food waste
(1) NB port, (2) motor to propel the stirrer, (3) heater, (4) heating coil, (5) digestate outlet

3.4.3 Side-stream stripping columns

Three of the 35-L digesters (Fig. 17) were coupled to stripping columns to remove ammonia in a semi-batch process. The stripping columns were made from stainless steel tube with a height of 56 cm and 10 cm internal diameter. Temperature was controlled using externally mounted thermostatically-controlled electrical heating mats (Non Adhesive Wire Wound Heater 104 Dia x 200 P 230V 200W; Holroyd, UK). Biogas was recirculated through the columns using a diaphragm pump (A.1F17N1.C06VDC; Parker, UK). The flow was adjusted using a rotameter set to 0.15 l min^{-1} $l^{-1}_{digestate}$ and the recirculated biogas entered the stripping column through a sintered-glass diffuser. The biogas leaving the column was passed through traps to remove ammonia: this was achieved by provision of a condensate trap followed by bubbling through deionised water and then through 0.25 N H_2SO_4 before recirculation to the stripping columns. Flow meters were calibrated to operational conditions (gas type). After each batch fill with digestate and replenishment of the ammonia traps the system was first flushed with biogas for 15 min to remove any air before switching to biogas. Fig. 18a shows a process flow diagram of the biogas stripping apparatus, and Fig. 18b shows a picture of the bubbles generated in a stripping column full of water at the operational flow rate.

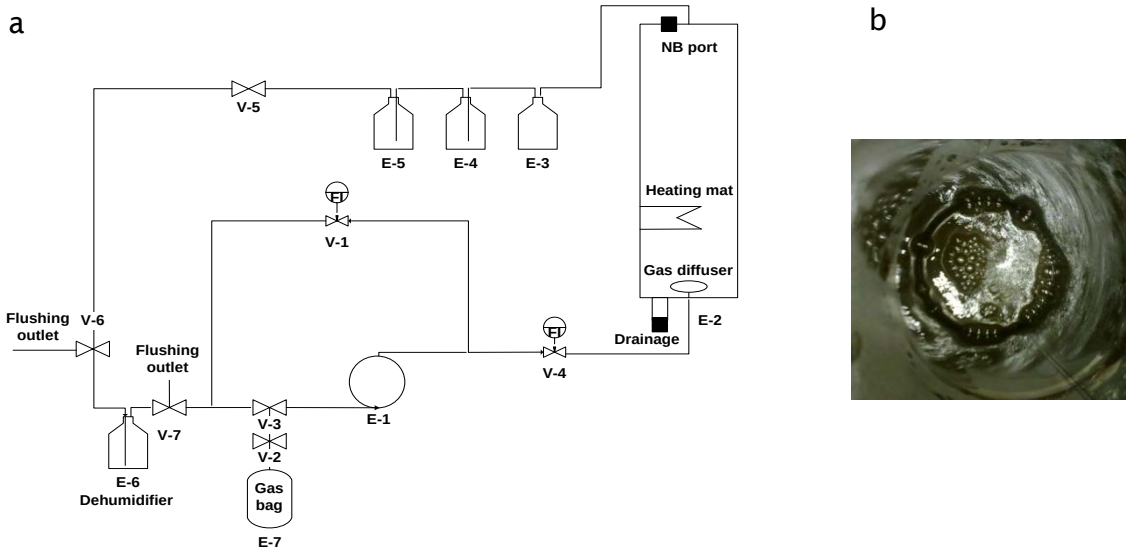


Fig. 18. a) Details of experimental set-up: Process flow diagram for stripping column E-1 diaphragm pump, E-2 stripping column, E-3 condensate trap, E-4 water trap, E-5 0.25N H_2SO_4 trap, E-6 dehumidifier, E-7 gas bag b) Bubbles produced (flow: 0.15 l min^{-1} $l^{-1}_{digestate}$) in water

Digestate samples were taken from the stripping columns via a tubular sampling port installed at the top of the columns (Fig. 19) to determine the N removal kinetics and compare it to the batch experiments.

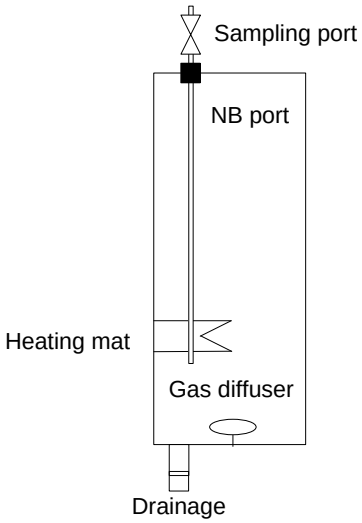


Fig. 19. Stripping column with sampling port

A schematic diagram and a picture of the overall digester/stripping column coupled process are shown in Fig. 20.

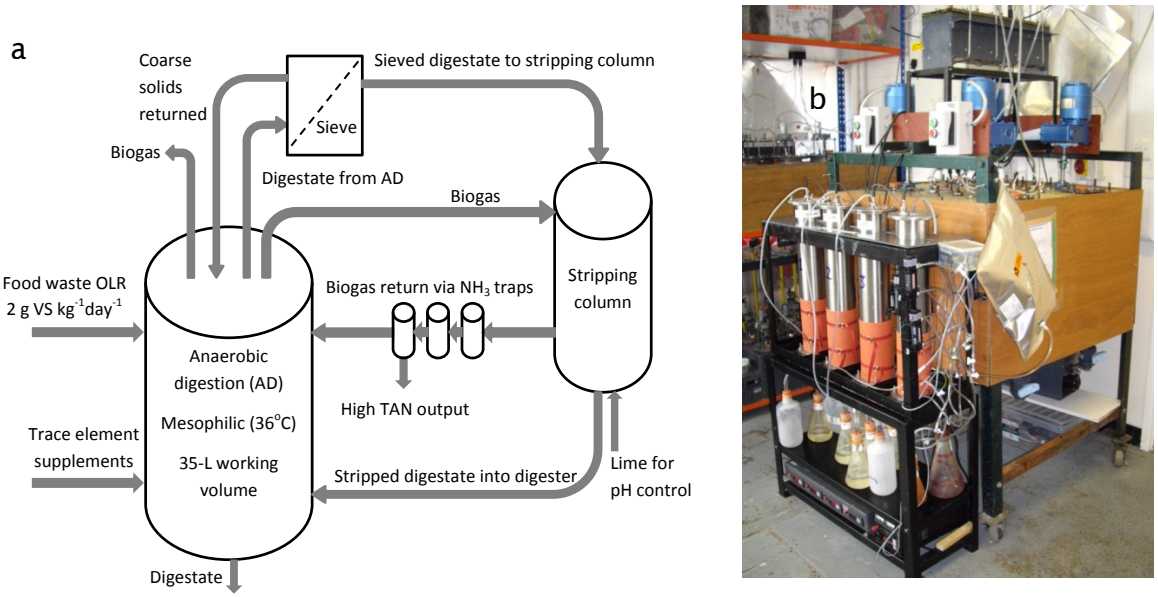


Fig. 20. a) Details of experimental set-up: Schematic of the coupled process b) Side-stream stripping rig

3.4.4 Batch stripping columns

The batch stripping column was a 56 cm high and 10 cm inner diameter glass column with a temperature controlled (Techne Circulator C-85A) water jacket. A peristaltic pump (Watson Marlow Sci-Q 323) was used to pump biogas through sieved digestate (1 mm mesh) via a diffuser located at the bottom of the column. A Tedlar bag initially full of standard biogas (65.12 % (v/v) CH_4 and 34.88 % (v/v) CO_2 BOC) was connected to the system to replace the air initially present in the gas loop with standard biogas, and then to act as a gas reservoir mitigating any subsequent volume changes in the system. The experimental set-up is shown in Fig. 21.

Initial flushing to remove air from the system was carried out for 15 min at a pumping rate of 0.380 l min^{-1}. After this, the gas loop was closed to the ambient air and the peristaltic pump rate was set at the desired gas flow rate for the experiment (0.250 l min^{-1}). Biogas was initially bubbled through a condensate trap, a deionised water trap, a 0.25 N H_2SO_4 acid trap and second deionised water trap in order to remove the ammonia and allow reuse of the same biogas in a closed loop. After 5 experimental trials it was verified that the biogas was free of ammonia after the second acid trap, and the number of traps was reduced to two (water and acid). TAN concentrations in the ammonia traps were determined at the end of each experiment. The experimental procedure for batch ammonia stripping experiments was based on Walker et al. (2011).

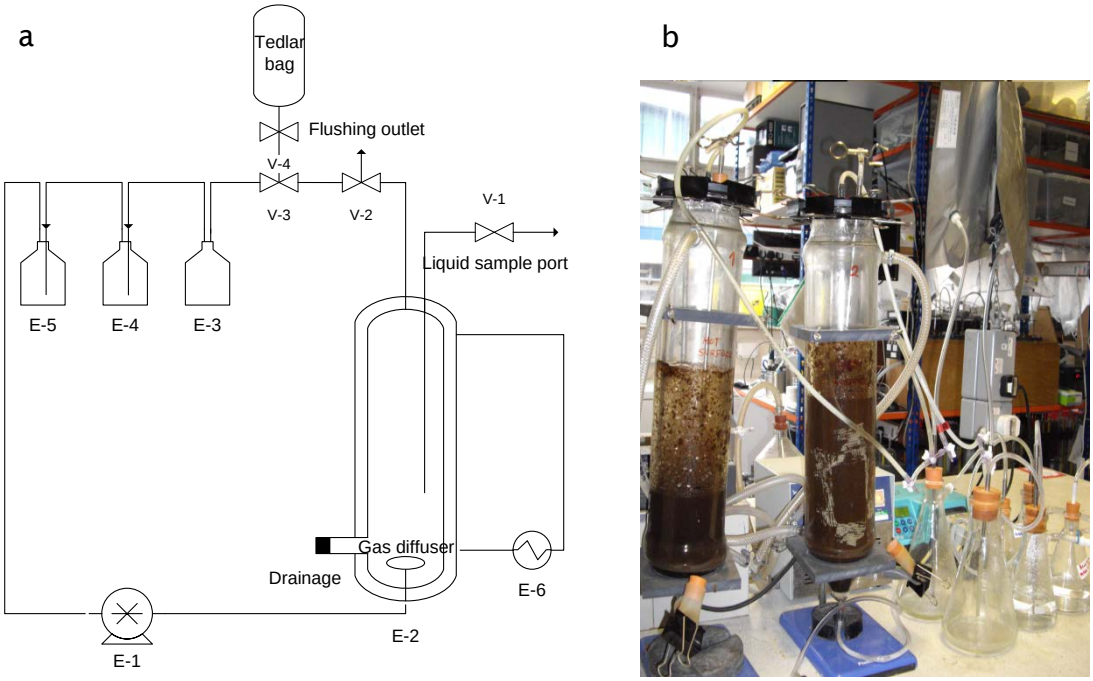


Fig. 21. a) Diagram of batch ammonia stripping system
E-1 Peristaltic pump WATSON MARLOW Sci-Q 323, E-2 Bubbling column with heating jacket, inner diameter = 10 cm, H_t = 56 cm, E-3 Condensate trap, E-4 Water trap, E-5 Acid trap, E-6 Heater TECHNE circulator C-85A b) experimental rig picture

3.5 Experimental plan

The work involved experimental studies at laboratory scale to investigate the potential for biogas stripping of ammonia, as an approach that may allow thermophilic anaerobic digestion of source segregated domestic food waste. The trials operated under a range of temperature regimes, % of the digester volume treated and pH control strategies. The experimental work carried out was as follows:

- Semi-continuous digestion trial of source segregated domestic food waste in a continuously stirred tank reactor (CSTR) to provide fresh digestate samples from a stable and well-operated digester with a known history running under conditions typical of full-scale plants. At the end of the trial the acclimated digestate was used to inoculate digesters coupled to stripping columns (section 4.4).

- *In situ* biogas stripping trial to assess the feasibility of decreasing the TAN in a digester to below the toxic threshold at typical gas mixing rates and mesophilic and thermophilic temperatures.
- Batch stripping tests under different temperature (35 ºC, 55 ºC and 70 ºC) and pH conditions (unadjusted and pH increased to 10) at low biogas bubbling rates (0.125 and 0.250 l min^{-1} l^{-1}) to determine the ammonia removal kinetics of fresh food waste digestate. These trials set the experimental conditions of AD with side-stream stripping.
- Operation of semi-continuous digesters coupled with stripping columns to evaluate the capability of a side-stream biogas stripping system to control TAN concentration while also gauging the long term effects of the stripping process on digester operation and performance.

Full details of the experiments are given in Chapter 4.

3.6 Calculation methods

3.6.1 VS destruction

VS destruction was calculated using a mass balance approach in two ways: i) based on the mass and VS concentration of food waste added to and digestate removed from the digester (equation 26); and ii) based on the mass and VS content of the food waste added, the VS of the digestate removed, and the mass of biogas produced in the digester (equation 27). The mass of biogas removed was calculated from the average gas volume and gas composition in terms of % CH_4 and CO_2 (ignoring water vapour and other gases).

$$VS\ destruction = \frac{VS_{fw\ in} \cdot Mass_{fw\ in} - VS_{digestate\ out} \cdot Mass_{digestate\ out}}{VS_{fw\ in} \cdot Mass_{fw\ in}} . 100 \quad (26)$$

$$VS\ destruction = \frac{VS_{fw\ in} \cdot Mass_{fw\ in} - VS_{digestate\ out} \cdot \left(Mass_{fw\ in} - Mass_{biogas\ out}\right)}{VS_{fw\ in} \cdot Mass_{fw\ in}} . 100 \quad (27)$$

Where $VS_{fw\ in}$ and $VS_{digestate\ out}$ are the volatile solids concentration for the food waste added to and the digestate removed from the digester, respectively (g VS kg^{-1} wet weight); $Mass_{fw\ in}$ refers to mass of food waste added to the digester

(kg); $Mass_{digestate\ out}$ and $Mass_{biogas\ out}$ refer to mass of digestate and biogas removed from the digester (kg).

In a digester with side-stream stripping the mass lost from the digestate due to evaporation and captured in traps during the stripping process also needs to be taken into account. In a side-stream stripping process VS destruction can be calculated by equation 26 and equation 28.

$$VS\ destruction = \frac{VS_{fw\ in} \cdot Mass_{fw\ in} - VS_{digestate\ out} \cdot \left(Mass_{fw\ in} - Mass_{biogas\ out} - Mass_{lost\ traps}\right)}{VS_{fw\ in} \cdot Mass_{fw\ in}} . 100 \quad (28)$$

Where $Mass_{lost\ traps}$ refers to the mass of digestate evaporated in the stripping process and captured in the traps.

3.6.2 Ammonia removal rate

In order to evaluate the effectiveness of this technique for removing ammonia from digestate and to allow comparison with previous studies, the concept of the time constant was used (Walker et al., 2011). The TAN concentration profile obtained for each stripping condition was fitted to an exponential curve (1st order kinetic) as it is stated in equation 29.

$$C = C_o \cdot e^{\frac{-t}{\tau}} \quad (29)$$

Where C is ammonia concentration (mg N kg^{-1} wet basis); C_0 is the initial ammonia concentration (mg N kg^{-1} wet basis), t is time (hours) and τ is the ammonia removal time constant (hours).

3.6.3 Ammonia stripping efficiency

The efficiency with which the stripping gas is used can be calculated based on the difference between the ammonia concentration in the biogas after stripping and the theoretical concentration at equilibrium, according to equation 30.

$$E = \left(1 - \frac{H_{equilibrium} - H_{stripping}}{H_{equilibrium}}\right) \cdot 100 \quad (30)$$

Where E is the efficiency or effectiveness of the biogas in the stripping column (%); $H_{equilibrium}$ is the proportionality constant relating the solubility of ammonia in water to its partial pressure in the gas phase (in diluted gases) and is obtained from Henry's law (equation 31) (kPa kg mol^{-1} N) (Perry and Green, 1999); $H_{stripping}$ is the experimental proportionality constant (equation 32) calculated following the concept of Henry's law from the batch ammonia stripping results (kPa kg mol^{-1} N).

$$H_{equilibrium} = \frac{P_{NH3}}{C_{FAN}} \quad (31)$$

$$H_{stripping} = \frac{P'_{NH3}}{C'_{FAN}} \quad (32)$$

Where C_{FAN} is the concentration of the solute (free ammonia, FAN) in the liquid phase (mol N kg^{-1}); C'_{FAN} is the experimental concentration of the solute (free ammonia, FAN) in the liquid phase (mol N kg^{-1}); P_{NH3} is the partial pressure (kPa) of the solute (ammonia) in the gas phase in equilibrium with a certain C_{FAN}; P'_{NH3} is experimental partial pressure of ammonia in the biogas phase during the stripping experiments (kPa), and is defined according to equation 33 (Perry and Green, 1999).

$$P'_{NH_3} = P_T \cdot Y_{NH_3} = P_T \cdot \frac{\dot{mol}_{NH_3}}{\dot{mol}_T} = \frac{(-1)\cdot 101.325}{14000\cdot \dot{mol}_T} \frac{\Delta(mass_{column}\cdot C_{TAN})}{\Delta t} \quad (33)$$

Where P_T is the total pressure or the sum of the partial pressures of all the gas components (kPa); Y_{NH3} is the mole fraction of ammonia in the gas; $\dot{mol}_{NH_3}$ and $\dot{mol}_T$ are the ammonia (mol N hour^{-1}) and total gas mol flow (mol hour^{-1}), respectively; $mass_{column}$ is the mass of digestate in the stripping column (kg); C_{TAN} is the digestate total ammoniacal nitrogen concentration in the stripping column (mg N kg^{-1}); t is time (hours) and $\frac{\Delta(mass_{column}\cdot C_{TAN})}{\Delta t}$ indicates the rate of total ammoniacal nitrogen change in the stripping column (mg hour^{-1}).

The experimental $Mass_{column}$ data was fitted using the initial and final mass of digestate in the stripping column to the following linear equation (equation 34).

$$Mass_{column} = -C \cdot t + D \quad (34)$$

Where C and D are experimentally-obtained coefficients.

The TAN concentration was considered to decrease exponentially (equation 35).

$$C_{TAN} = A \cdot e^{-B \cdot t} \quad (35)$$

Where *A* and *B* are experimentally-obtained coefficients.

P'_{NH3} in the stripping column is calculated from equation 36.

$$P'_{NH_3} = K_1 \cdot \frac{\Delta}{\Delta t}\left(A \cdot e^{-B \cdot t} \cdot (-C \cdot t + D)\right) = K_1 \cdot (-B \cdot A \cdot e^{-B \cdot t} \cdot (-C \cdot t + D) - C \cdot A \cdot e^{-B \cdot t}) \quad (36)$$

Where $K_1 = constant = \frac{(-1) \cdot 101.325}{14000 \cdot \dot{mol}_T}$

Experimental values for digestate FAN concentration (C_{FAN}') in the stripping column (mol N kg^{-1}) are needed to allow determination of $H_{stripping}$. The experimental FAN decreases exponentially with time (equation 37).

$$C_{FAN'} = \frac{A' \cdot e^{-B' \cdot t}}{14000} \quad (37)$$

Where *A'* and *B'* are experimentally-obtained coefficients for C_{FAN}'.

3.6.4 N mass balance

3.6.4.1 N mass balance: Batch stripping column

A nitrogen balance to the batch stripping column can be done applying the following equations (38 and 39).

$$N_{dig\ start} = N_{dig\ end} + N_{traps} + N_{samples} \quad (38)$$

Where $N_{dig\ start}$ is the mass of nitrogen in the digestate at the start of the batch experiment (g); $N_{dig\ end}$ is the mass of nitrogen in the digestate at the end of the batch experiment (g); N_{traps} is the mass of nitrogen in the ammonia traps (g) and $N_{samples}$ is the mass of nitrogen sampled from the experiment as digestate (g).

$$Mass_{dig\ in} \cdot TKN_{dig\ in} = Mass_{dig\ out} \cdot TKN_{dig\ out} + Mass_{cond} \cdot TAN_{cond} + Mass_{water} \cdot TAN_{water} + Mass_{acid} \cdot TAN_{acid} + N_{samples} \quad (39)$$

Where $Mass_{dig\ in}$ and $TKN_{dig\ in}$ refer to mass (kg) and TKN (mg N kg^{-1}) of digestate at the start of the experiment; $Mass_{dig\ out}$ and $TKN_{dig\ out}$ refer to mass (kg) and TKN (mg N kg^{-1}) of digestate at the end of the experiment; $Mass_{cond}$ and TAN_{cond} refer to mass (kg) and TAN (mg N kg^{-1}) of condensate trap at the end of the experiment; $Mass_{water}$ and TAN_{water} refer to mass (kg) and TAN (mg N kg^{-1}) of water trap at the end of the experiment; $Mass_{acid}$ and TAN_{acid} refer to mass (kg) and TAN (mg N kg^{-1}) of water trap at the end of the experiment; and $N_{samples}$ is the mass of nitrogen sampled from the experiment as digestate (g) (this term has not been estimated in the calculation of the N mass balance).

The mass of unrecovered matter from the columns can be determined applying equation 40.

$$Unrecovered\ matter = Mass_{dig\ in} - Mass_{dig\ out} - Mass_{sample} - Mass\ gain_{traps} - Solid_{recovered} \quad (40)$$

Where $Mass_{sample}$ is the mass of digestate taken from the column in the experiment (~0.007 kg per sample); $Mass\ gain_{traps}$ is the mass increase measured at the end of the experiment in the condensate, water and acid ammonia traps (kg); and $Solid_{recovered}$ is the mass of solid in stripping column at the end of the experiment (kg).

3.6.4.2 N mass balance: Continuous digester

Control reactor

A nitrogen mass balance to the steady-state of the control reactor (Fig. 22) can be done applying the following equation (41).

$$Mass_{fw} \cdot TKN_{fw} = Mass_{dig\ out} \cdot TKN_{reactor} \quad (41)$$

Where $Mass_{fw}$ is the mass of food waste added to the digester (kg); $TKN_{no\ stripping}$ is the TKN concentration in the food waste added to the digester (mg N kg^{-1}); $Mass_{dig\ out}$ is the mass of digestate removed from the digester (kg); $TKN_{reactor}$ is the steady state TKN concentration in the control digester (mg N kg^{-1}).

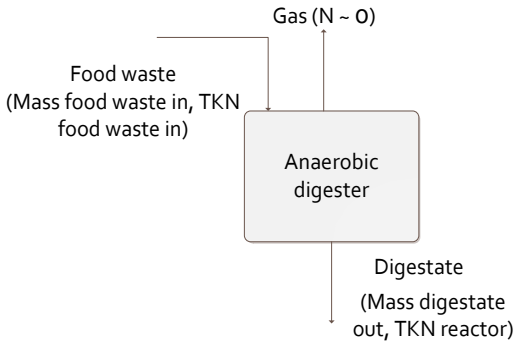


Fig. 22. N streams in the control reactor

Side-stream stripping system

A mass balance for the side-stream stripping procedure (Fig. 23) can be done analysing the steady-state or in a time dependant model. In both cases the following assumptions were made:

- food waste has a typical VS content of 210 g VS kg^{-1} wet weight (average value found in SS-DFW used in 35-L CSTRs, Table 31); however, VS content ranged between 200 to 230 g VS kg^{-1} wet weight.

- the TAN concentration achieved after complete hydrolysis of food waste in the digester is constant; in this research 5155 mg N kg^{-1}, similar to the average obtained when no stripping was applied to the digesters.

- the stripping column works 24 hours per day.

- the time constants shown in Table 8 were used (experimentally-obtained coefficients).

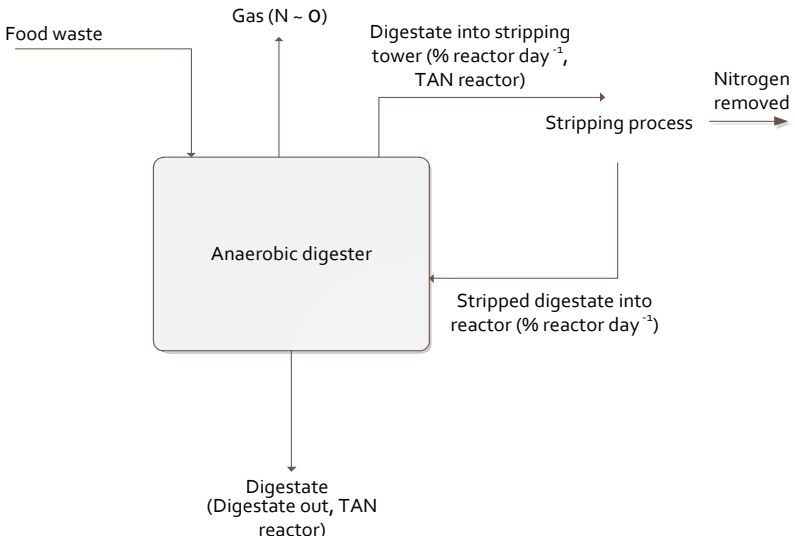


Fig. 23. N streams in the reactors coupled with side-stream columns

Table 8. Time constants used in N mass balance

	τ (hours)
70 ºC unadjusted pH	155.1*
70 ºC pH 10	71.1*
85 ºC unadjusted pH	122.5*
85 ºC pH 10	27.1*
55 ºC pH 10	168.0+

* obtained in the N removal kinetic study during continuous experiments (Table 37)
+ average from batch experiments

A simplified mass balance for nitrogen in steady state conditions in the digester is shown in equations 42 to 46:

$$Mass_{dig\ out\ no\ stripping} \cdot TAN_{no\ stripping} = Mass_{dig\ out} \cdot TAN_{reactor\ stripped} + N_{removed} \quad (42)$$

Where $Mass_{dig\ out\ no\ stripping}$ is the mass of digestate removed from the digester without stripping (kg); $TAN_{no\ stripping}$ is the TAN concentration in a food waste digester without stripping (5155 mg N kg^{-1}); $Mass_{dig\ out}$ is the mass of digestate removed from the digester (kg) (equation 43); $TAN_{reactor\ stripped}$ is the steady state TAN concentration in a digester with stripping (mg N kg^{-1}) and $N_{removed}$ is the nitrogen removed by the stripping process (mg) (equation 44).

$$Mass_{dig\ out} = Mass_{fw} - Mass_{biogas} - Mass_{lost\ in\ stripping\ column} \quad (43)$$

Where $Mass_{biogas}$ is the mass of biogas produced by the digester (kg) and $Mass_{lost\ in\ stripping\ column}$ is the mass of digestate evaporated in the stripping process (experimentally-obtained average values are shown in Table 9) (kg).

Table 9. Average mass increase in ammonia traps (experimental values)

Conditions	Average (g day^{-1})	Max (g day^{-1})	Min (g day^{-1})
70 ºC	22	58	6
55 ºC	8	12	3
85 ºC	37	75	12

$$N_{removed} = M_{reactor} \cdot \frac{\%vol\ to\ stripping}{100} \cdot TAN_{reactor\ stripped} \cdot \left(1 - e^{\frac{-t}{\tau}}\right) \quad (44)$$

Where $M_{reactor}$ is the total mass of digestate in the digester (kg); $\%_{vol\ to\ stripping}$ is the percentage of digester that is treated in the stripping column per day (%); t is the time for which the stripping column works (hours) and τ is the experimental time constant (hours) (Table 8).

The concentration in the stripped digestate is calculated using equation 45.

$$TAN_S = TAN \cdot e^{\frac{-t}{\tau}} \quad (45)$$

Where TAN_s is the total ammoniacal concentration reached after stripping digestate with a certain TAN concentration (mg N kg^{-1}).

The steady state TAN concentration in a digester coupled to a stripping column ($TAN_{reactor\ stripped}$) can be calculated using equation 46.

$$TAN_{reactor\ stripped} = \frac{Mass_{dig\ out\ no\ stripping} \cdot TAN_{no\ stripping}}{Mass_{dig\ out} + V_{reactor} \cdot \frac{\%vol\ to\ stripping}{100} \cdot \left(1 - e^{\frac{-t}{\tau}}\right)} \quad (46)$$

The same analysis can be done in a time dependent system by considering daily addition of food waste to the digester, digestate removed from the digester in the wastage line and nitrogen removed in the stripping process. TAN concentration in the digester at time t can be calculated using equation 47.

$$TAN(t) = TAN(t-1) \cdot \left(1 - \frac{\%_{vol\ to\ stripping}}{100} - \frac{1}{HRT}\right) + TAN_{no\ stripping} \cdot \frac{1}{HRT} + \frac{\%_{vol\ to\ stripping}}{100} \cdot TAN(t-1) \cdot e^{\frac{-t}{\tau}} \quad (47)$$

Where *TAN (t)* is the total ammoniacal concentration in the digester at time t (mg N kg^{-1}); *TAN (t-1)* is the total ammoniacal concentration in the digester at time t-1 (mg N kg^{-1}) and HRT is the hydraulic retention time (d).

4. Results and discussion

Overview

The experimental work involved trials at laboratory scale to investigate the potential for biogas stripping of ammonia, as an approach that may allow thermophilic anaerobic digestion of source segregated domestic food waste without water addition. The experimental overview is shown below.

- Semi-continuous digestion of SS-DFW in two 75-L CSTRs (section 4.1) was conducted for 462 days to provide fresh digestate samples to carry out batch ammonia stripping experiments (section 4.3) from a stable and well-operated digester. At the end of the trial the acclimated digestate was used to inoculate digesters coupled to stripping columns (section 4.4).
- *In situ* biogas stripping trials (section 4.2) were conducted to assess the feasibility of decreasing the TAN in a digester to below the toxic threshold at typical gas mixing rates and mesophilic and thermophilic temperatures. This experiment was conducted in parallel with the digestion of SS-DFW in 75-L CSTRs (section 4.1).
- Batch stripping tests (section 4.3) were conducted under different temperature (35 ºC, 55 ºC and 70 ºC) and pH conditions (unadjusted and pH increased to 10) at low biogas bubbling rates (0.125 and 0.250 l min^{-1} l^{-1}) to determine the ammonia removal kinetics of fresh food waste digestate originated from the 75-L CSTRs (section 4.1). The aim of these trials is to set the experimental conditions of AD with side-stream ammonia stripping (section 4.4).
- Operation of three 35-L mesophilic semi-continuous digesters coupled with stripping columns (section 4.4) to evaluate the capability of a side-stream biogas stripping system to control TAN concentration while also gauging the long term effects of the stripping process on digester operation and performance.

4.1 75-L CSTR digesters for anaerobic digestion of food waste

Objective. To carry out semi-continuous digestion of source segregated domestic food waste in a continuously stirred tank reactor (CSTR) for the provision of fresh digestate samples from a stable and well-operated digester with a known history running under conditions typical of full-scale plants.

4.1.1 Methodology for food waste anaerobic digestion trial

Two digesters of the type described in section 3.4.1 were inoculated with 75 litres of digestate from a commercial AD plant (Biocycle digester operated by BiogenGreenfinch, Ludlow) treating SS-DFW. The inoculum characteristics are shown in Table 10. The digesters were fed on source segregated food waste on a daily basis at an OLR 2 kg VS m^{-3} day^{-1}; TS and VS values of the food waste batches used are shown in Table 11. Digestate was removed twice a week and samples were analysed for pH, alkalinity, TAN, TS/VS, VFA and TKN when steady state was reached. Gas produced in was collected for 30 min in gas-impermeable sampling bag five hours after feeding the reactor to determine its composition at least fortnightly. The trial ran for 462 days equivalent to almost four retention times.

Table 10 shows a small discrepancy on the Fe and Ni concentrations although same inoculum source was used in both reactors. Trace element extraction was conducted in house, and it is unlikely that samples were contaminated since accurate and repetitive results were obtained for Co, Mo and Se. A reasonable cause of these differences is analytical error during the analysis conducted by the external company carrying out the ICP-MS analysis. These differences were not spotted on time to repeat the analysis. Nevertheless, in this case both concentrations are below critical toxic levels (Ahring and Westermann, 1983; Mudhoo and Kumar, 2013) and therefore the AD performance should not be influenced by the analytical error here reported.

Table 10. Characteristics of inoculum from Biocycle mesophilic digester

	R_1	R_2
pH	8.40 ± 0.04	8.34 ± 0.04
TA g l^{-1}	16.98 ± 0.06	16.89 ± 0.06
PA g l^{-1}	12.86 ± 0.03	12.81 ± 0.03
IA g l^{-1}	3.59 ± 0.09	3.46 ± 0.09
TAN g N l^{-1}	3.28 ± 0.09	3.42 ± 0.09
TKN g N l^{-1}	5.17 ± 0.02	5.09 ± 0.04
TS g kg^{-1}	35.5 ± 0.7	36.5 ± 0.7
VS g kg^{-1}	23.9 ± 0.5	24.7 ± 0.5
VFA (100% acetic) **mg l^{-1}**	130 ± 5	125 ± 5
Co mg l^{-1}	1.51	1.60
Fe mg l^{-1}	62.31	105.18
Mo mg l^{-1}	0.54	0.50
Ni mg l^{-1}	2.36	4.10
Se mg l^{-1}	0.08	0.09

Trace element supplementation. Following the recommendations of Banks et al. (2012), trace element supplementation (Al, B, Co, Cu, Fe, Mn, Ni, Zn, Mo, Se, W, see section 3.3.2.) was done from day 49 of operation to favour stable anaerobic digestion of the food waste, due to the lack of these essential elements in the food waste itself (Climenhaga and Banks, 2008; Zhang and Jahng, 2012).

Table 11. Total and volatile solids of the food waste batches used in the experiment

Food waste batch	Start (feeding day)	End (feeding day)	TS (g kg^{-1})	Standard deviation (g kg^{-1})	VS (g kg^{-1})	Standard deviation (g kg^{-1})
1	0	142	258.9	0.1	240.0	0.3
2	143	190	235.2	1.0	217.4	0.6
3	191	235	237.6	3.8	219.1	3.4
4	236	265	249.2	5.8	231.0	6.3
5	266	323	232.4	1.7	219.5	2.5
6	324	456	237.3	2.5	223.9	2.3
7	457	462	246.2	2.4	228.1	4.4

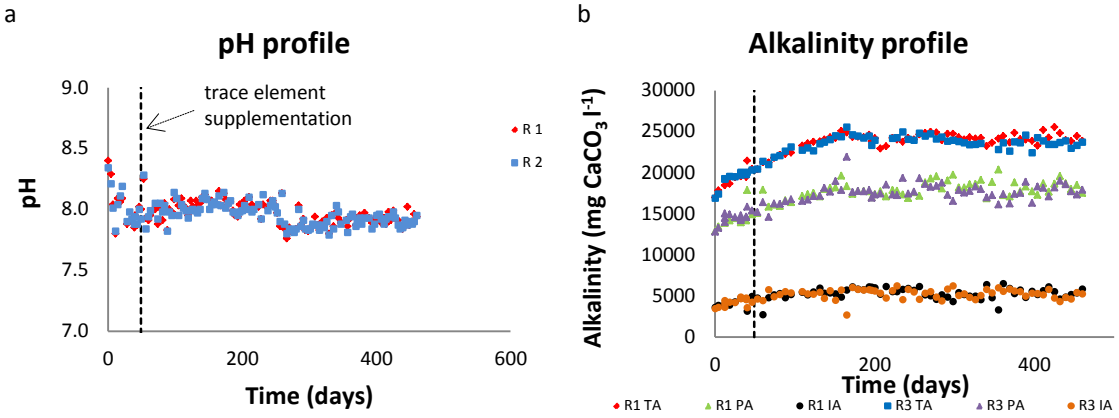
4.1.2 Results of food waste anaerobic digestion trial

Average values for performance and monitoring parameters during the steady state period are shown in Table 12 and Fig. 24. The digesters showed good performance throughout the operating period. Specific biogas production was stable with values of 0.83 ± 0.04 l g^{-1} VS for digester 1 and 0.85 ± 0.04 l g^{-1} VS for digester 2 and average methane concentration was in the range of 52.3 - 57.9 %.

Table 12. Food waste bioreactors characteristics (average at steady state)

	R_1				R_2			
	Average	Standard deviation %	max	min	Average	Standard deviation %	max	min
pH	7.91	0.7	8.03	7.80	7.89	0.7	8.03	7.79
TA g l^{-1}	24.2	2.2	25.5	23.2	23.6	2.1	24.5	22.4
PA g l^{-1}	18.4	4.7	20.4	16.8	17.6	4.5	18.9	16.1
IA g l^{-1}	5.2	12.9	6.5	3.3	5.2	9.6	6.2	4.3
***TKN mg N l^{-1}**	8780	0.4	8800	8750	8720	1.0	8780	8660
TAN mg N l^{-1}	4907	1.2	5020	4800	4808	1.5	4964	4680
TS g kg^{-1}	65.84	1.1	67.26	64.48	66.68	1.4	68.04	63.84
VS g kg^{-1}	48.00	1.3	49.24	46.82	48.51	1.5	49.68	46.94
VFA mg l^{-1}	152	-	422	31	143	-	328	35

* At the end of the experiment



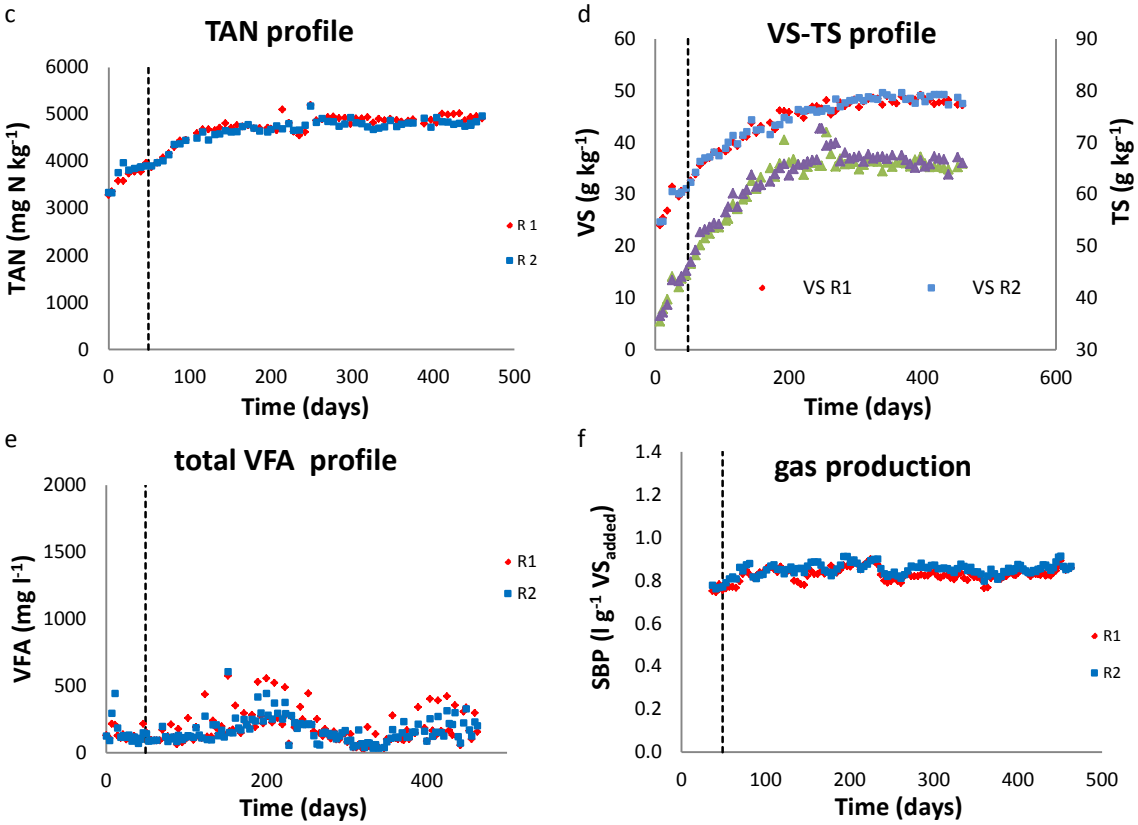


Fig. 24. 75-L CSTR reactors performance a) pH profile b) alkalinity profiles c) TAN profiles d) TS and VS profiles e) VFA profile f) specific biogas production

The stable operation of these digesters, albeit at a fairly low OLR, provided further confirmation of the effectiveness of the trace element supplementation in preventing VFA accumulation when the results obtained in this study are compared to results obtained by Climenhaga and Banks (2008) where catering waste was treated at an OLR of 1.45 kg VS m^{-3} day^{-1}. In the study, system failure and/or high VFA concentration was found at different HRT in anaerobic digesters without trace element supplementation. Trace element concentrations in the digestate at the end of the trial are shown in Table 13.

Table 13. Trace elements concentration in digesters at the end of the semi-continuous trial

	R_1	R_2
Co mg l^{-1}	1.21	1.15
Fe mg l^{-1}	65.84	32.77
Mo mg l^{-1}	0.43	0.35
Ni mg l^{-1}	1.99	1.66
Se mg l^{-1}	0.26	0.24

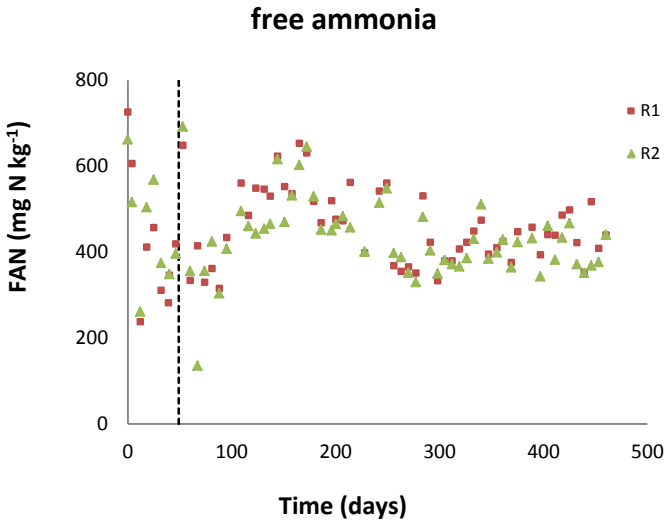


Fig. 25. Free ammonia profile in 75-L CSTR reactors

The free ammonia profile is shown in Fig. 25. The average value in the steady state period was 420 mg N kg^{-1} and digesters did not exhibit any inhibition response such as VFA accumulation or a decrease in the biogas production at any point in the experiment. At an average HRT of 114 days 55 % of the TKN was converted to TAN. The volatile solids destruction under steady state conditions was 84 %.

Similar VS destruction rates, methane yields and composition were obtained in lab scale digesters (Banks et al., 2012; Yirong et al., 2013a) and full scale plants under similar conditions and feedstock (VALORGAS D4.2, 2013).

4.1.3 Conclusion

This trial successfully provided of fresh digestate from a stable and well-operated digester with similar characteristics to that from large-scale AD plant operating on same feedstock to conduct batch ammonia stripping experiments and acclimated inoculum for use in subsequent digestion trials.

4.2 *In situ* stripping

Objective. To determine whether effective ammonia removal could be achieved in a simplified system representing a biogas-mixed digester, as a first step towards deciding whether the same approach could be adopted in a semi-continuous digester fed on food waste.

4.2.1 Methodology for *in situ* stripping experiments

The experiment was carried out in a 75-L CSTR digester modified to allow gas mixing and recirculation, as described in section 3.4.1.

A synthetic digestate was used with a TAN concentration of 5 g N kg^{-1}, similar to that found in digesters operating on real food waste (OLR 2 kg VS m^{-3} day^{-1}). The components of the synthetic digestate were water, urea $CO(NH_2)_2$ and a small amount (~3 litres) of food waste digestate (section 4.1) to provide an inoculum able to hydrolyse the urea to ammonia (Garrido et al., 2001). The TAN concentration was adjusted at the beginning of the experiment and no new nitrogen source was added to the reactor in the course of the study.

Ammonia removal was evaluated at mesophilic and thermophilic temperatures using different biogas recirculation rates (0.4 l min^{-1} – 2.6 l min^{-1}, Table 14). These were chosen based on the recommendations of Perry and Green (1999) (Table 5, Section 2.3.7.3) to represent moderate and complete mixing rates which would allow a good distribution of the food in a digester. Despite the fact that higher N removal rates would be achieved at higher bubbling rates these were not chosen to avoid upsetting the anaerobic system.

Table 14. Experimental degrees of agitation

Biogas flow (l min^{-1})	**Biogas flow** (m^3 m^{-2} $_{tank\ cross\ section}$ min^{-1})	**Degree of agitation**
0.4	0.003	Moderate
1.1	0.008	Moderate
2.4	0.019	Complete
2.6	0.021	Complete

Batch stripping tests were also carried out on the synthetic digestate and real food waste digestate to determine whether the performance of the process was the same or was modified by changes in the nature of the digestate under the same initial TAN concentrations. The batch stripping tests were carried out using the apparatus described in section 3.4.4 at 55 ºC and biogas recirculation rates of 0.250 l min^{-1} l^{-1} and 0.125 l min^{-1} l^{-1} with synthetic digestate (TAN: 3.9 and 4.7 g N kg^{-1}, TA: 14 g $CaCO_3$ l^{-1}, PA: 12 g $CaCO_3$ l^{-1}, IA: 2 g $CaCO_3$ l^{-1}); and sieved fresh food waste digestate taken on day 400 and 415 from the 75-L digestion trial described in section 4.1 (TAN: ~ 5 g N kg^{-1}, TS value of 6.60 %, TA: 25 g $CaCO_3$ l^{-1}, PA: 18 g $CaCO_3$ l^{-1}, IA: 5 g $CaCO_3$ l^{-1}).

4.2.2 Results of *in situ* stripping experiments

4.2.2.1 *In situ* stripping at mesophilic temperature

Fig. 26 shows the biogas flow rate and the total ammoniacal concentration profile of the reactor during the mesophilic stripping experiment. Table 15 summarises the results at 35 ºC.

The pH value (8.21 - 8.31) and the alkalinity profile remained constant in the course of the experiment. TAN removal was almost insignificant at the tested flows, as can be seen in Fig. 26.

It can be seen that the time constant at a biogas flow rate of 1.1 l min^{-1} was 10000, very high but still lower than the value of 50000 at a flow rate 2.6 l min^{-1} (Table 15). Low ammonia removal implies a high time constant. The results obtained were therefore unexpected, since low gas flow rates are normally associated with higher time constants, as shown in previous research (Zhang et al., 2010; Walker et al., 2011). This behaviour may have been due in part to the fact that, as a result of mechanical problems, a high flow rate of around 2.3 l min^{-1} was applied for a period of 62 hours (see Fig. 26), which was considerably above the intended value of 1.1 l min^{-1}. In addition, it is difficult to fit exponential approximation to the flat TAN concentration profiles obtained in this experiment, this originated the high values of time constant; in the mesophilic *in situ* study exponential approximation was conducted with comparative purposes.

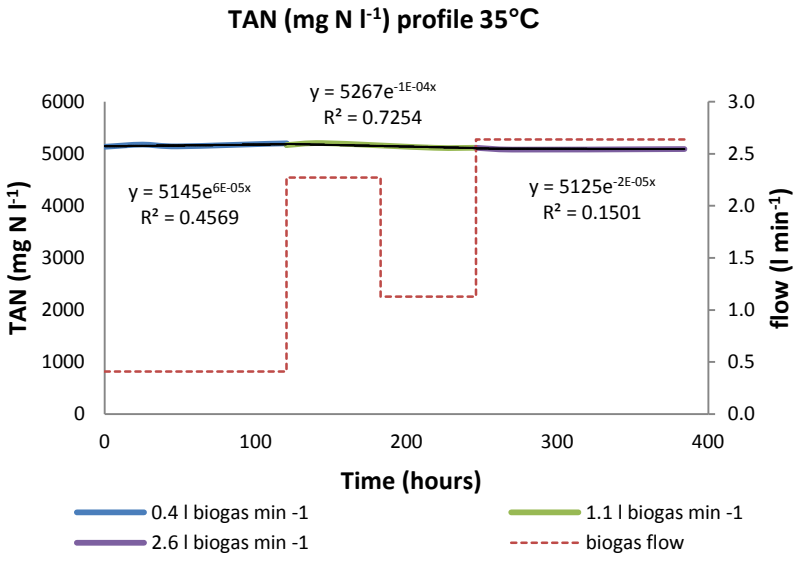


Fig. 26. Ammonia removal profile in 35 ºC *in situ* ammonia stripping

Table 15. Ammonia stripping results summary at 35 ºC

Biogas flow (l min^{-1})	Time (hours)	TAN $_{start}$ (mg N l^{-1})	TAN removal (%)	Time constant (hours)
0.4	144	5136	-	-
1.1	102	5207	1.73	10000
2.6	138	5117	0.48	50000

Mesophilic nitrogen balance. Table 16 shows the initial and final mass and the final TAN concentration in the ammonia traps at the end of the *in situ* ammonia stripping experiment at 35 ºC. These traps were only removed at the end of the whole experiment; solutions were not regenerated each time the flow was increased.

Table 16. Mass and TAN concentration in the traps at the end of the 35°C *in situ* ammonia stripping

	Mass $_{start}$ (kg)	Mass $_{end}$ (kg)	TAN (mg N l^{-1})
condensate*	0	0.524	7825
water trap*	1.019	0.999	1114
acid trap*	1.004	1.007	20

*Traps only removed at end of the whole experiment.

An adjustment was made for the N loss in the biogas which escaped from the system via the gas counter, bypassing the ammonia traps (Fig. 27). The total amount of biogas circulated through the ammonia traps was calculated by subtracting the total biogas which escaped to the atmosphere from the total biogas pumped through the reactor (see Fig. 16a). The N loss via the gas counter was then estimated based on the total nitrogen found in the ammonia traps.

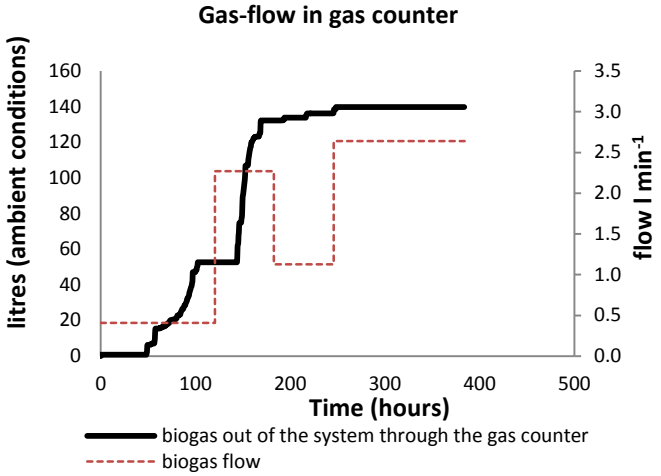


Fig. 27. Gas flow out of the system through the gas counter in 35 ºC *in situ* stripping

Table 17 shows the nitrogen balance at the end of the experiment at 35 ^{0}C. Including the adjustment for the loss of nitrogen in the biogas, the amount of unaccounted-for nitrogen in the system was as low as 0.3 % of the initial TAN or 1.2 g of N.

Table 17. Ammoniacal N balance to 35 ^{o}C *in situ* ammonia stripping at the end of the experiment

TAN in traps	g	5.2
TAN start digestate	g	385.2
TAN end digestate	g	378.8
TAN stripped (adding nitrogen lost in gas-outlet)	g	5.25
N loss*	%	0.31
N loss*	g	1.2

Replenishment of ammonia traps at the end of the experiment

* N loss: Unaccounted-for ammoniacal nitrogen. Calculated by a mass balance (TAN start digestate - TAN end digestate -TAN stripped)

4.2.2.2 *In situ* stripping at thermophilic temperature

Fig. 28 shows the biogas flow rate and the TAN concentration profile in the stripping reactor in the course of the thermophilic experiment, while Table 18 summarises the results at 55 ^{o}C. Nitrogen removal increased when biogas flow rate increased to 1.1 l min^{-1}; at higher flows no further improvement was found within the experimental range studied. The pH value (8.47 - 8.65) and alkalinity profile remained constant during the stripping experiment.

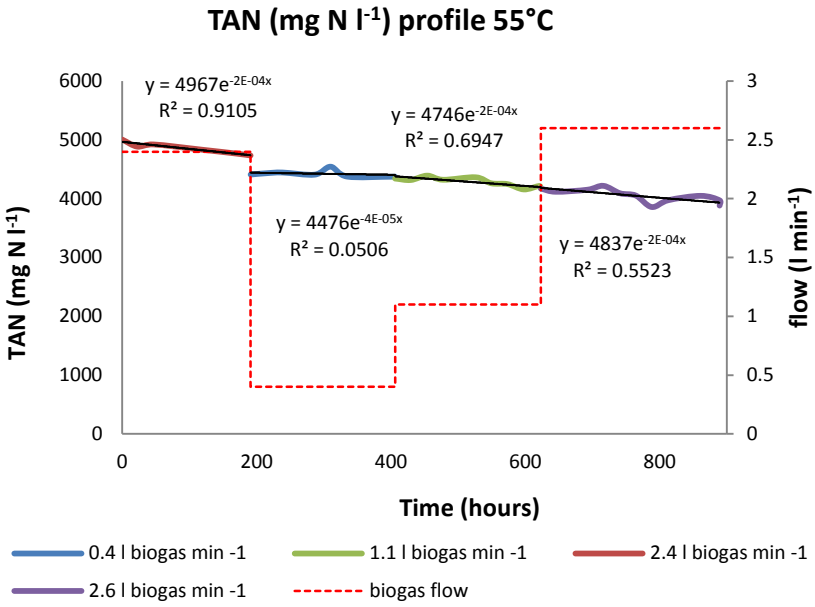


Fig. 28. Ammonia removal profile in 55 ^{o}C *in situ* ammonia stripping

Table 18. Ammonia stripping results summary at 55 ^{o}C

Biogas flow (l min^{-1})	Time (hours)	TAN $_{start}$ (mg N l^{-1})	TAN removal (%)	Time constant (hours)
0.4	215	4411	0.8	25000
1.1	217	4343	4.0	5000
2.4	191	5005	5.4	5000
2.6	266	4171	6.9	5000

Replenishment of ammonia traps for each flow rate analysed

Thermophilic nitrogen balance. Table 19 shows the initial and final mass and the final TAN concentration in the ammonia traps at the end of each of the experiments at different flow rates in the *in situ* ammonia stripping at 55 ºC.

Table 19. Mass and TAN concentration in the traps in 55 ^{o}C *in situ* ammonia stripping

	Biogas flow = 0.4 l min^{-1}			**Biogas flow = 1.1 l min^{-1}**		
	Mass $_{start}$ (kg)	**Mass $_{end}$ (kg)**	**TAN (mg N l^{-1})**	**Mass $_{start}$ (kg)**	**Mass $_{end}$ (kg)**	**TAN (mg N l^{-1})**
condensate*	0	0.223	24520	0	0.441	18506
water trap*	0.805	0.807	1622	0.809	0.804	1140
acid trap*	0.493	0.493	22	0.496	0.499	14
	Biogas flow = 2.4 l min^{-1}			**Biogas flow = 2.6 l min^{-1}**		
	Mass $_{start}$ (kg)	**Mass $_{end}$ (kg)**	**TAN (mg N l^{-1})**	**Mass $_{start}$ (kg)**	**Mass $_{end}$ (kg)**	**TAN (mg N l^{-1})**
condensate*	0	1.11	18972	0	1.336	18760
water trap*	1023	1.024	2857	0.803	0.826	5466
acid trap*	488	0.499	121	0.499	0.503	489

Ammonia traps regenerated every time the flow was increased

* N loss: Unaccounted-for ammoniacal nitrogen. Calculated by a mass balance (TAN start digestate - TAN end digestate -TAN stripped)

From the gas counter results, the flow of biogas released to the environment through the gas counter was 0 for 0.4 l min^{-1}, 1.1 l min^{-1} and 2.6 l min^{-1} experiments; correction for N loss was not required in this case. For 2.4 l min^{-1} the flow profile is as shown in Fig. 29 and the N loss via the gas counter was estimated as previously explained in section 4.2.2.1.

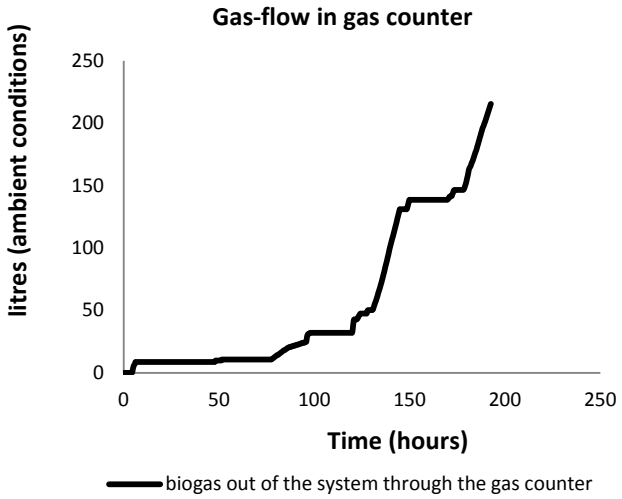


Fig. 29. Gas flow out of the system through the gas counter in 55 ºC *in situ* stripping, 2.4 l min^{-1}

Table 20 shows the nitrogen balance at the end of the experiment. Under these conditions the uncounted-for N was higher than in the *in situ* mesophilic trial. At the beginning of the mesophilic trial the volume of liquid in the reactor was measured accurately, but in the thermophilic trial which followed on immediately after the liquid volume was estimated by a mass balance, and this could be a reason for the increase in unaccounted-for N.

Table 20. Ammoniacal N balance to 55 ºC *in situ* ammonia stripping

		Biogas flow: 0.4 l min^{-1}	**Biogas flow: 1.1 l min^{-1}**
TAN in traps	g	6.8	9.1
TAN start digestate	g	330.8	324.6
TAN end digestate	g	327.0	307.3
TAN stripped (adding nitrogen lost in gas-outlet)	g	6.8	9.1
N loss*	%	0.91	2.54
N loss*	g	-3.0	8.2
		Biogas flow: 2.4 l min^{-1}	**Biogas flow: 2.6 l min^{-1}**
TAN in traps	g	24.0	29.8
TAN start digestate	g	375.4	307.3
TAN end digestate	g	349.6	281.0
TAN stripped (adding nitrogen lost in gas-outlet)	g	24.2	29.8
N loss*	%	0.42	1.15
N loss*	g	1.6	-3.5

4.2.2.3 Results of batch stripping test for synthetic and real digestates

The results of the batch stripping tests for synthetic and real digestates are presented in Table 21, Fig. 30 and Fig. 31, and show that ammonia was more easily removed from the synthetic digestate (time constant 625 at gas flow rate 0.125 l min^{-1}) than from real food waste digestate (time constant 1250 at 0.125 l min^{-1}).

Table 21. Time constant comparison between real and synthetic digestate using batch stripping process

Digestate	Time constant Flow: 0.250 l min^{-1} l^{-1}	Time constant Flow: 0.125 l min^{-1} l^{-1}
Real	909	1250
Synthetic	278	625

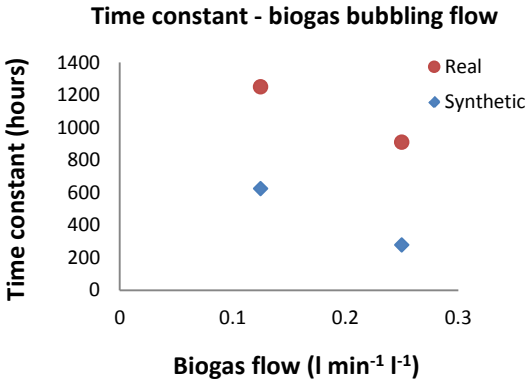


Fig. 30. Time constant of real and synthetic digestate at different biogas flows

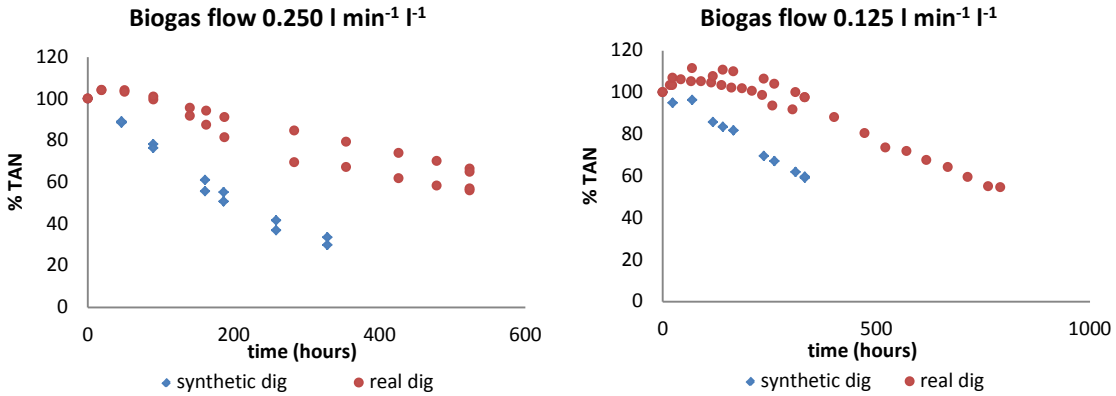


Fig. 31. Comparison of stripping process between synthetic and real digestate at 0.25 l min^{-1} l^{-1} and 0.125 l min^{-1} l^{-1} biogas flow rates

Walker et al. (2011) reported a time constant of 699.6 for a digestate collected from a commercial AD plant fed mainly on source segregated domestic food waste (total solids content 5.50 %), stripped under the same conditions as used

here and at a gas flow rate of 0.125 l min^{-1}. This value is reasonably close to that for the synthetic digestate, although in theory the real digestate should be a closer match in terms of its properties. In the study by Walker et al. (2011) consistently different nitrogen removal rates were found for commercial food waste digestate from two different sources (digestate 1: TS = 5.50 %; TAN = 8000 mg N kg^{-1}; pH 8.5-9.3; digestate 2: TS = 3.14 %; TAN = 6000 mg N l^{-1}; pH = 8.1-8.2) during stripping with biogas at 70 ºC and at different flow rates, especially with unadjusted pH. These digestates had, however, been stored prior to testing. It is not yet clear which factors promote higher removal since digestates may have similar TAN concentrations but different characteristics, e.g. solids content, VFA concentration, alkalinity.

Laureni et al. (2013) found a clear increase in stripping efficiency at lower organic matter contents for pig manure and digestate (50 ºC, air to slurry ratio 444). In the same study digestate was stored for 2 and 6 months. TS, COD, VFA and alkalinity decreased during storage, while ammonia stripping efficiency improved. The ability of the organic matter to bind cations such as ammonium reduces the amount of strippable ammonia in the system, and this was thought to be the reason for the change in efficiency with solids content.

In a study by Campos et al. (2013) air stripping was carried out on two synthetic solutions, i.e. ammonium chloride and ammonium bicarbonate at TAN concentrations of 2000 mg N l^{-1} at 60 ºC, unadjusted pH and 0.8 l_{air} min^{-1} $l^{-1}_{solution}$. Those that contained high ammonia and alkalinity achieved higher ammonia removal due to carbonate desorption, whereas those solutions with no alkalinity required alkali addition to reach low TAN levels (Campos et al., 2013).

The results therefore confirm once again that ammonia stripping is a strongly digestate-dependent process, and show that the rate of removal in the *in situ* process trialled is likely to be lower for not stored digestate from a semi-continuous digester treating real food waste in stable conditions than for the synthetic digestate used.

4.2.3 Conclusions

Simulation based on the results of previous experimental research (Walker et al., 2011) had identified *in situ* ammonia stripping as a promising technique for food waste digestion, with good nitrogen removal rates measured at TC and high biogas flows (0.375 l min^{-1} $l^{-1}_{digestate}$). To simulate this in the current work flows of 0.095 and 0.224 $m^3 m^{-2}_{tank\ cross\ section}$ min^{-1} would be needed in the laboratory stripping column and 75-L digester respectively, corresponding to extremely violent mixing in both cases (>0.032 $m^3 m^{-2}_{tank\ cross\ section}$ min^{-1}, Perry and Green (1999)). The current investigations have shown, however, that the reduction in TAN concentration in the *in situ* bubbling reactor is non-existent at mesophilic temperatures and small at thermophilic temperatures when moderate and complete gas mixing rates are used. For this reason it is possible to infer that *in situ* stripping is not a feasible technique at large scale in mesophilic conditions and with low gas recirculation flows; and although it may be possible at thermophilic conditions, violent mixing rates would be required. *In situ* stripping is not an appropriate solution to prevent ammonia inhibition of food waste digestion in a thermophilic full scale plant.

4.3 Batch ammonia stripping experiments

Objective: The main objective of this work was to gather data on the ammonia stripping performance parameters of fresh food waste digestate as a basis for the design of a pilot-scale system linked to an anaerobic digestion process (side-stream process). To achieve this, the following sub-objectives were set:

- To determine the relative suitability of alternative chemical compounds for adjusting and maintaining pH.

- To assess the performance of ammonia biogas stripping at different conditions of temperature, pH and biogas flow using fresh food waste digestate from well-run digesters with a known operating history.

- To assess whether the thermal-alkaline stripping treatment promotes the hydrolysis of particulate organic matter into soluble organic matter, leading to further degradation of organic nitrogen-containing materials into the ammoniacal form.

4.3.1 Methodology

The digestate used in the stripping experiments was collected from the wastage line of the 75-L digesters fed on 2 g VS kg^{-1} d^{-1} of food waste as described in section 4.1 above. Digestate from a mesophilic digester was used as stable operation of thermophilic digesters at these TAN concentrations cannot be achieved (Yirong et al., 2013a).

Batch stripping experiments were carried out using the apparatus described in section 3.4.4 until ammonia removal in the digestate was not found. The experiments were run at three different temperatures: 35 ºC and 55 ºC, as these are considered optimal for mesophilic and thermophilic microorganisms; and 70 ºC as this corresponds to pasteurisation temperature. Trials were carried out at the natural pH of the food waste digestate and at pH 10 to displace the ammonium-ammonia equilibrium and favour the release of ammonia from the liquid phase. An initial trial was carried out to compare the suitability of CaO, $Ca(OH)_2$ and NaOH as agents for increasing the pH of the digestate prior to stripping to 10. Then, stripping efficiency with pH control by NaOH and CaO addition was examined. The behaviour during the process and the final removal was compared to results from previous research in the field (De la Rubia et al., 2010; Zhang et al., 2010; Walker et al., 2011).

To assess whether the thermal-alkaline stripping treatment promotes the degradation of the organic nitrogen-containing materials into the ammoniacal form, the batch ammonia stripping equipment described in section 3.4.4 was used without stripping at 70 ºC with and without pH increase to 10 using CaO. 2 kg of sieved (1 mm mesh) food waste digestate collected from the wastage line of the 75-L digesters (section 4.1) was placed into the batch stripping column and heated to 70 ºC without biogas bubbling for 4 days. Digestate TAN, TKN, pH and VFA concentration were monitored.

4.3.2 Results of comparison of alkaline compounds for pH control

Initial tests. To determine which chemical compound is more effective for adjusting and maintaining pH an initial experiment was carried out in which doses of lime (CaO) or slaked lime ($Ca(OH)_2$) were added to 100 g of fresh food

waste digestate, and pH was monitored over time without any stripping. The results are shown in Fig. 32.

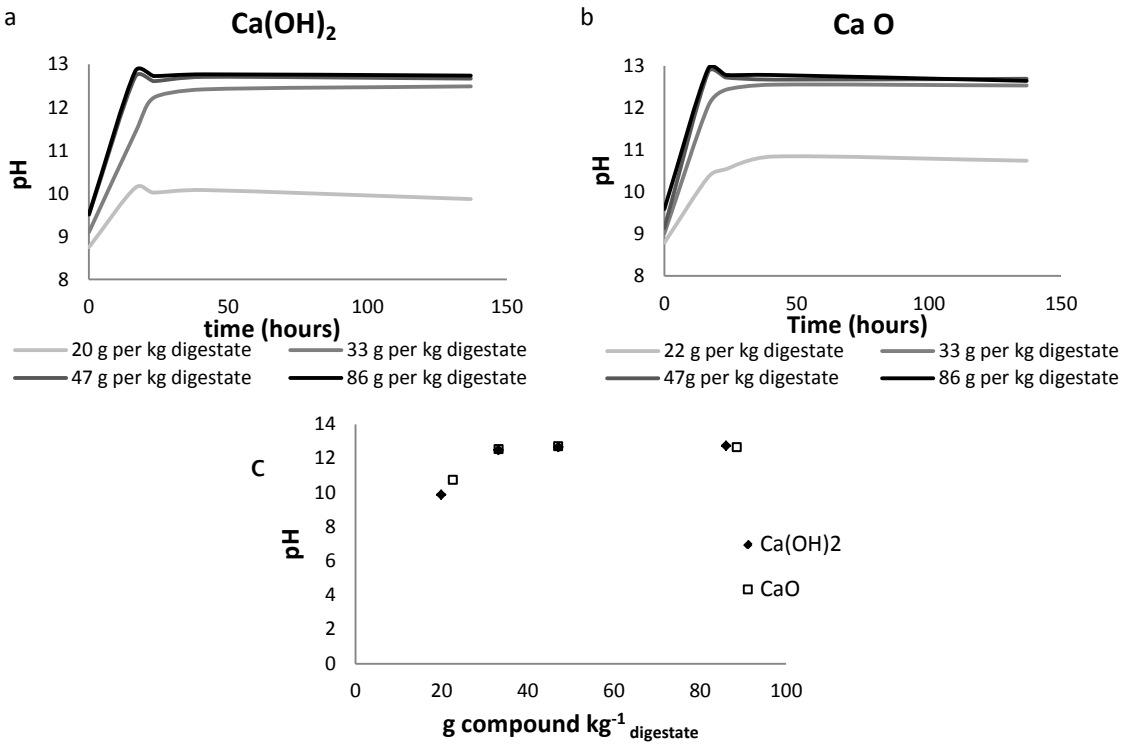


Fig. 32. a) pH evolution with time at different lime dosages, b) pH evolution with time at different slaked lime dosages, c) Final pH comparison at different dosages of lime and slaked lime

Both compounds were able to achieve the required final pH after 23 hours and to maintain it over time. Since CaO is easier to handle, and as the same dosages of both compounds were needed to reach a certain pH, CaO was selected in preference to $Ca(OH)_2$.

Lime is considered less inhibitory than sodium and potassium hydroxide to AD, but also less efficient as an alkaline agent (Zhang and Jahng, 2010; Kleybocker et al., 2012). The initial test carried out on lime and slaked lime was therefore subsequently repeated for sodium hydroxide, using a fresh sample of digestate from the same digester under the same operating conditions. The results are shown in Fig. 33. It can be seen that 33.8 ml of a 10 M solution of NaOH (13.6 g) were needed per kg of sieved food waste digestate to increase pH to 10, compared to 20 g of CaO. Both CaO and NaOH were subsequently tested in batch stripping trials.

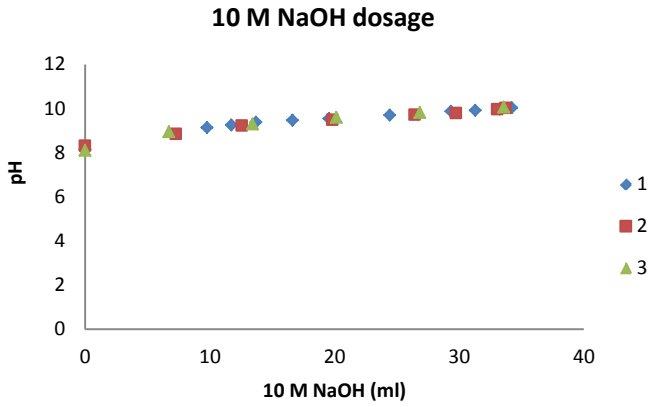


Fig. 33. NaOH 10 M dosage to achieve pH 10

Other authors have reported various different alkali dosages needed to raise pH depending on the nature of the material to be treated, e.g. to increase pH to 12 using $Ca(OH)_2$ 12.5 g l^{-1} were needed in the case of pig excreta and kitchen waste digestate (Lei et al., 2007) and 8 g l^{-1} for landfill leachates (Ozturk et al., 2003).

Batch stripping tests. Based on the results of the initial tests, batch stripping tests were carried out with CaO and NaOH. The results are shown in Fig. 34 and in the overall summary in Table 22 below. It can be seen that at 70 ºC the stripping performance with NaOH was slightly better, especially between 40-100 hours. At 35 ºC there was little difference between the two alkaline agents.

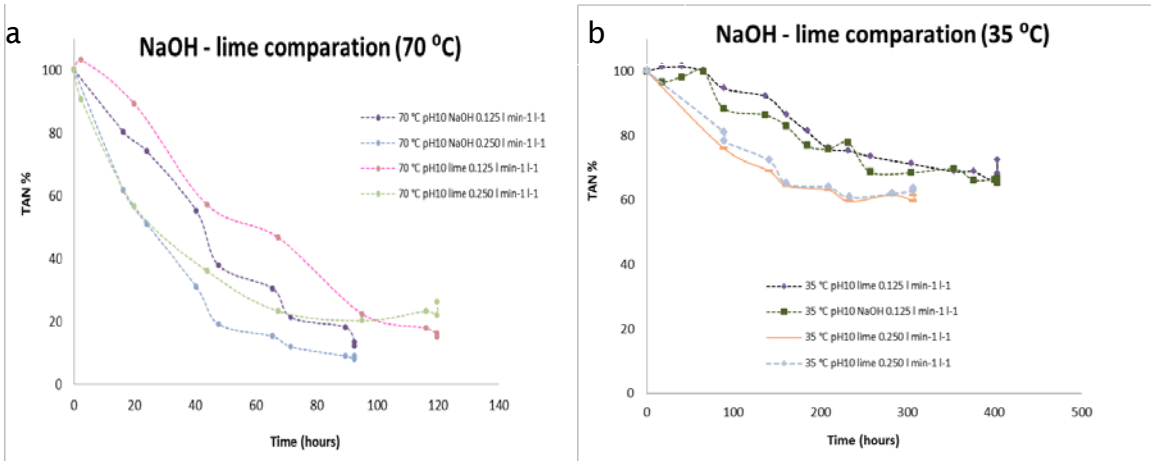


Fig. 34. NaOH and lime comparison as alkaline substances in ammonia stripping experiments. a) 35 ºC b) 70 ºC

From Fig. 34 it can be deduced that the behaviour of both substances is similar when used in ammonia stripping columns, and the alkaline agent can therefore

be selected based on operational factors. When CaO was used, long mixing times were needed to favour the hydration reaction where calcium hydroxide is generated. Moreover, when CO_2 present in the biogas reacts with $Ca(OH)_2$ insoluble carbonate salts are formed that may introduce operational problems in the stripping column such as solids blockage. Calcium hydroxide or lime is widely used to increase pH before stripping treatments, however, because of its low cost and the potential for phosphorus removal (K. C. Cheung, 1995). The Ca, N and P present in the resulting sludge can also be useful for soil amendment (Lei et al., 2007). Although sodium hydroxide addition was an easy and quick method to increase pH, foaming problems occurred during the stripping assays, making it necessary to add antifoaming agent. Inhibition by the Na^+ cation has been observed within a short period during air stripping of pig manure (Zhang and Jahng, 2010; Zhang et al., 2011a). In the current application a proportion of the digester contents is treated in a stripping column and returned to the digester and this may be a good reason to select CaO for pH control even though the dosage needed is 1.5 higher than with NaOH.

4.3.3 Overview of results of batch stripping experiments

Table 22 shows the experimental conditions used in the batch stripping experiments carried out on fresh food waste digestates, and the main results including the time constant achieved. TAN concentration profiles for digestate in the stripping column are shown in Fig. 35.

Table 22. Ammonia stripping experiments summary

Run	T °C	initial pH		flow l min^{-1} l^{-1}	TAN start mg l^{-1}	TAN end mg l^{-1}	TAN % removal	hours	τ (h)	R^2	equation
1	35		8.27	0.125	4650	4780	-	383	-	-	no stripped
2	55		8.04	0.125	4730	2440	48.4	836	1111	0.95	$y = 5374e^{-0.0009x}$
3	55		7.90	0.250	4930	3180	35.2	524	1111	0.98	$y = 5242e^{-0.0009x}$
4	55	n/a	7.90	0.250	4930	2790	43.2	524	833	0.98	$y = 5216e^{-0.0012x}$
5	70		8.30	0.125	4560	1890	58.5	243	222	0.98	$y = 4522e^{-0.0045x}$
6	70		7.90	0.250	4900	2880	57.0	142	161	0.92	$y = 4383e^{-0.0062x}$
7	70		7.90	0.125	4900	2370	52	234	278	0.94	$y = 5090e^{-0.0036x}$
8	35	10 (NaOH)	10.01	0.125	4020	2740	31.9	403	909	0.93	$y = 4111e^{-0.0011x}$
9	35		9.72	0.125	4380	2930	33.0	403	909	0.94	$y = 4342e^{-0.0011x}$
10	35	10 (CaO)	9.69	0.250	4770	2940	38.3	307	714	0.82	$y = 4248e^{-0.0014x}$
11	35		9.69	0.250	4770	3030	36.4	307	714	0.82	$y = 4316e^{-0.0014x}$
12	55		9.98	0.125	4120	470	88.6	427	189	0.96	$y = 4148e^{-0.0053x}$
13	55	10 (NaOH)	9.95	0.250	4350	330	92.5	382	147	0.98	$y = 4622e^{-0.0068x}$
14	70		9.99	0.125	3460	420	87.8	93	44	0.97	$y = 4.039e^{-0.0226x}$
15	70		9.99	0.250	3460	280	92.1	93	37	0.98	$y = 3147e^{-0.0267x}$
16	70	10 (CaO)	9.76	0.125	4180	620	85.1	120	62	0.98	$y = 4709e^{-0.0162x}$
17	70		9.88	0.250	4240	850	79.9	120	57	0.95	$y = 3742e^{-0.0175x}$

n/a: not adjusted

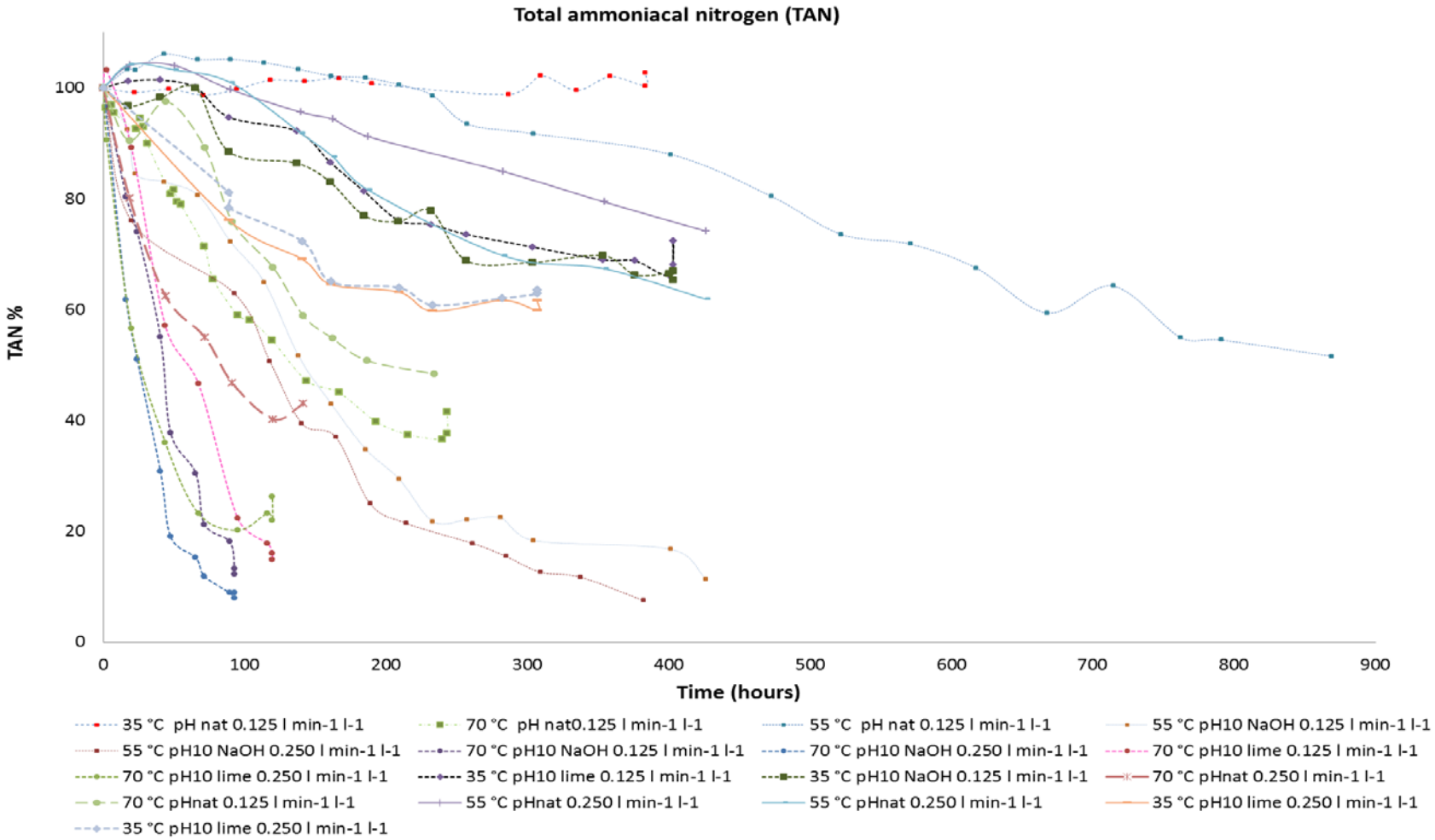


Fig. 35. TAN removal profile (%)

TAN removal. At unadjusted pH and mesophilic temperatures no ammonia removal was found; at thermophilic temperatures an average removal of 1.7 % N per day was measured. The biogas flow rate used in the batch stripping columns corresponds to a violent gas mixing rate (0.032 $m^3 m^{-2}_{\text{tank cross section}} min^{-1}$ according to Table 5). Therefore, it is confirmed that *in situ* stripping using violent biogas mixing rates under mesophilic and thermophilic temperatures would not succeed decreasing the TAN in the digester below inhibition limits.

Interestingly, the TAN concentration profile of the stripping conducted at 55 ºC and non-modified pH (run 4) shows an initial increase with a maximum peak found after 35 - 43 hours of experiment. This indicates that there is some generation of ammoniacal nitrogen from N_{org}, and that initially the TAN production is higher than the removal produced by the treatment. To confirm this hypothesis a hydrolysis experiment was subsequently conducted where digestate was subjected to temperature/pH increase without stripping (see section 4.3.6).

VFA. Initial and final total VFA concentrations in the batch stripping experiments are shown in Fig. 36. In general, the total VFA concentration increased progressively with time during the stripping process. In the case of runs 10 and 11 at 35 ºC an initial VFA increase was observed after pH control with lime, but this was not a general trend. The increase in VFA concentrations is likely to be influenced by many factors, e.g. length of the stripping experiment, temperature, pH adjustment; but although no clear pattern was found it is likely that some particulate organic matter is being hydrolysed into soluble organics due to the stripping conditions. Increases in VFA caused by thermal hydrolysis of fresh OFMSW at high temperatures (65 - 70 ºC) and digestion under thermophilic conditions have been previously reported (Rintala and Ahring, 1994; Hartmann and Ahring, 2005).

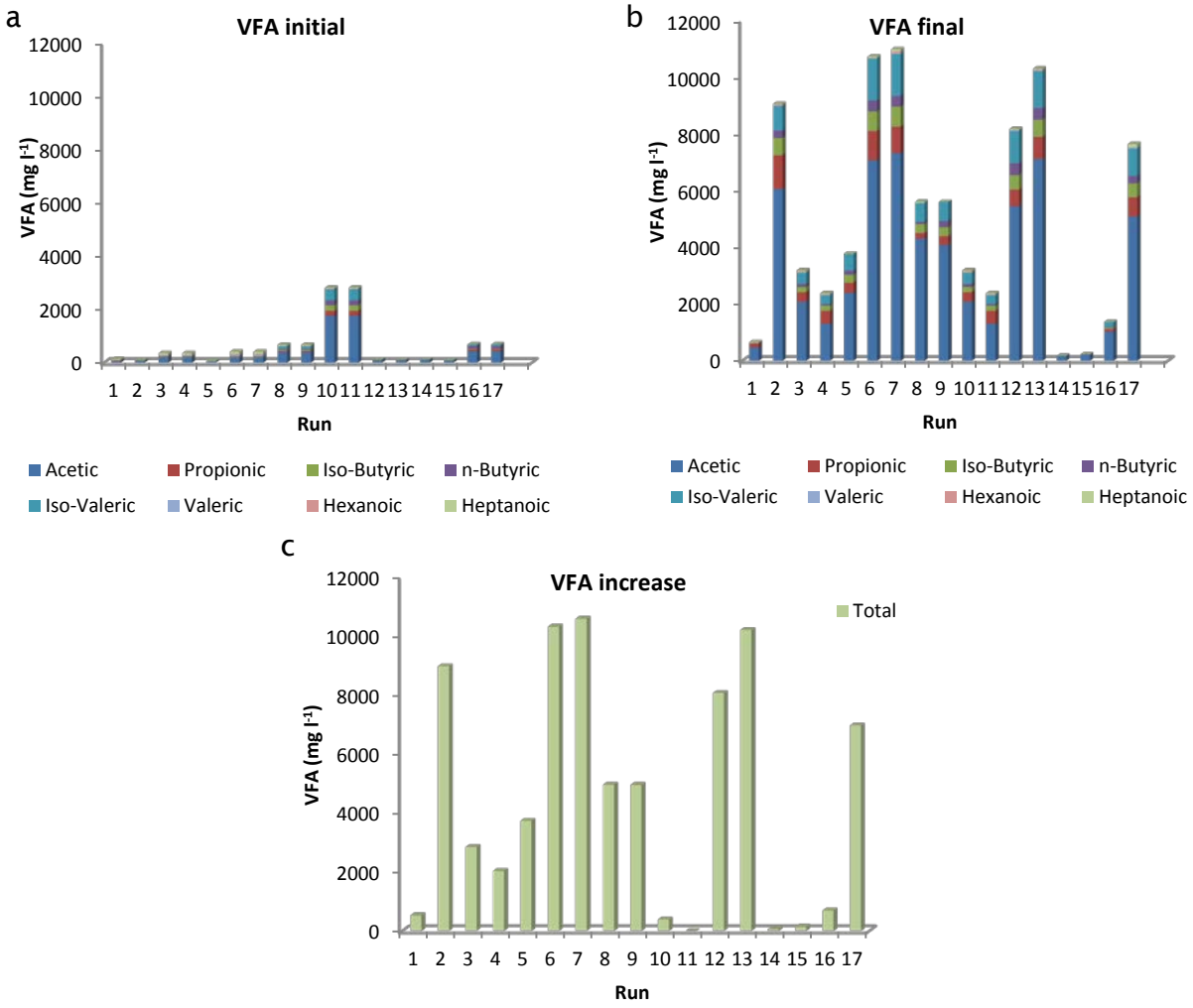


Fig. 36. a) VFA concentration at the beginning of the stripping experiment b) VFA concentration at the end of the stripping experiment c) VFA increase in the stripping experiments

pH. The pH profiles (Fig. 37) showed different tendencies in experiments with and without initial pH control. When alkali was not added there was an initial increase in pH of 0.3 - 0.5, probably caused by equilibration of carbonates and CO_2 volatilization. The decrease in CO_2 solubility caused by the temperature increase could be observed during the experimental work as an initial generation of foam that decreased with time. The pH then gradually decreased to 0.2 – 0.6 units lower than the original value with biogas stripping. In the specific case of 35 ºC without pH adjustment no N removal was found, and pH remained constant during the 383-hour experimental run. Sánchez-Hernández et al. (2013) reported a pH decrease from 7.5 - 7.3 to 7.1 - 6.9 after 150 minutes of biogas stripping at ambient temperature; the change in pH was

matched by an increase in CH_4 composition, indicating that part of the CO_2 was dissolved in the liquid.

When alkali was added to increase pH at the beginning of the experiment, the final pH value in all of the experimental runs decreased by 0.6 – 1.7. This is likely to be due to the removal of FAN which changes the ammonia-ammonium equilibrium to produce more dissolved gas and some protons, leading to a slight decrease in pH since the carbonate system is buffering pH changes.

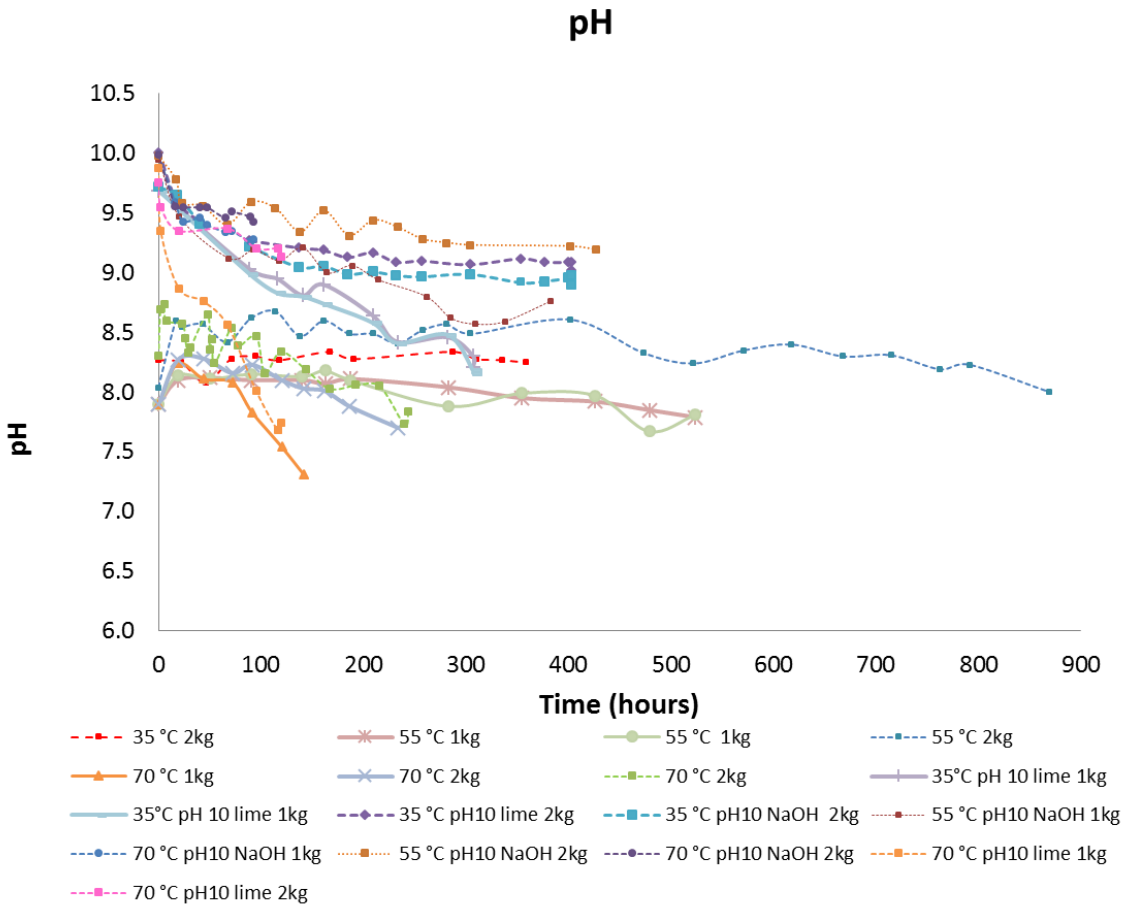


Fig. 37. pH profile of the digestate

Alkalinity. A clear decrease in alkalinity (Fig. 38) was found in those experiments without pH adjustment (except in run 1at 35 ºC). This is accounted for by carbonate destruction, which is promoted by FAN removal, increase in VFA concentration and precipitation of salts.

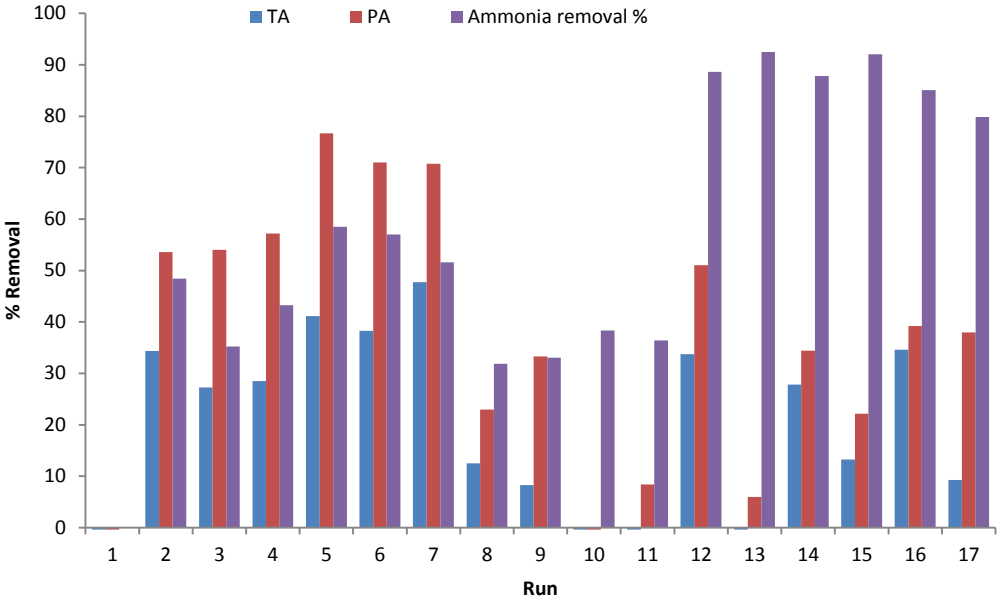


Fig. 38. Alkalinity and nitrogen removal during stripping

4.3.3.1 Nitrogen mass balances

Fig. 39 and Table 23 and Table 24 show the TAN, TKN and N_{org} concentration at the beginning and at the end of each run. In the stripping process part of the N_{org} in the digestate is broken down: the proportion varied between stripping conditions, with the lowest breakdown (7.3 - 9.4 %) found at 35 ºC, unadjusted and modified pH (runs 1, 10 and 11), while the highest (34.9 %) was found at 55 ºC, non-modified pH (run 2).

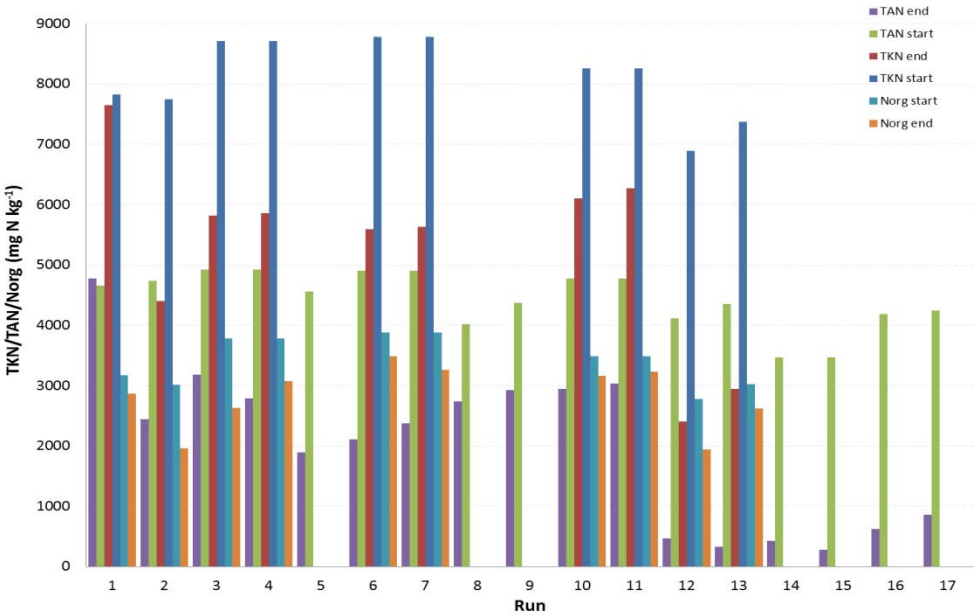


Fig. 39. Digestate TAN, TKN and N_{org} concentration at the beginning and end of the stripping process

Table 23. Digestate characteristics at the beginning of the the experiment

	Start experiment					
N exp	**Digestate^ mass kg**	**TAN start (mg N kg^{-1})**	**TKN start (mg N kg^{-1})**	**N_{org} (mg N kg^{-1})**	**N samples***	**N % removed+ increase pH**
1	1.997	4654	7820	3166		-
2	1.991	4732	7747	3015	16	-
3	0.999	4925	8706	3781	12	-
4	1.009	4925	8706	3781	12	-
5	2.005	4561	-	-	23	-
6	0.998	4904	8779	3875	6	-
7	2.004	4904	8779	3875	9	-
8	2.027	4018	-	-	15	17.4
9	2.085	4376	-	-	15	10.0
10	1.000	4771	8259	3488	8	21.3
11	1.004	4771	8259	3488	8	21.3
12	2.004	4116	6893	2777	16	12.5
13	1.002	4349	7370	3022	7	9.6
14	1.987	3463	-	-	8	24.5
15	0.999	3463	-	-	8	24.5
16	1.987	4183	-	-	7	13.1
17	0.993	4241	-	-	7	11.9

^ Initial digestate amount in the column

* Total number of samples taken from the batch experiment

+ N loss during the pH increase procedure

Table 24. Digestate characteristics at the end of the the experiment

	End experiment						
N exp	**Solid*** **(kg)**	**Digestate^ mass (kg)**	**TAN end (mg N kg^{-1})**	**TKN end (mg N kg^{-1})**	**N_{org} (mg N kg^{-1})**	**N in traps+ (g)**	**Unrecovered matter- (kg)**
1	-	1.785	4779	7648	2869	0.58	0.054
2	0.2635	1.094	2440	4403	1963	6.99	0.012
3	0	0.683	3184	5815	2630	3.37	0.036
4	0	0.578	2792	5860	3069	3.92	0.090
5	-	1.142	1893	-	-	7.84	0.114
6	0	0.570	2109	5594	3486	4.41	0.051
7	0	1.370	2374	5633	3259	8.26	0.075
8	0	1.841	2737	-	-	4.48	0.046
9	0.1387	1.677	2929	-	-	4.41	0.096
10	0	0.882	2941	6101	3160	2.30	0.039
11	0	0.876	3034	6267	3233	2.16	0.047
12	0.0215	1.600	467	2407	1940	9.53	0.082
13	0.0125	0.582	325	2945	2620	4.84	0.067
14	-	1.744	421	-	-	7.20	0.061
15	-	0.798	275	-	-	3.97	0.030
16	-	1.687	624	-	-	7.40	0.046
17	-	0.659	854	-	-	4.07	0.059

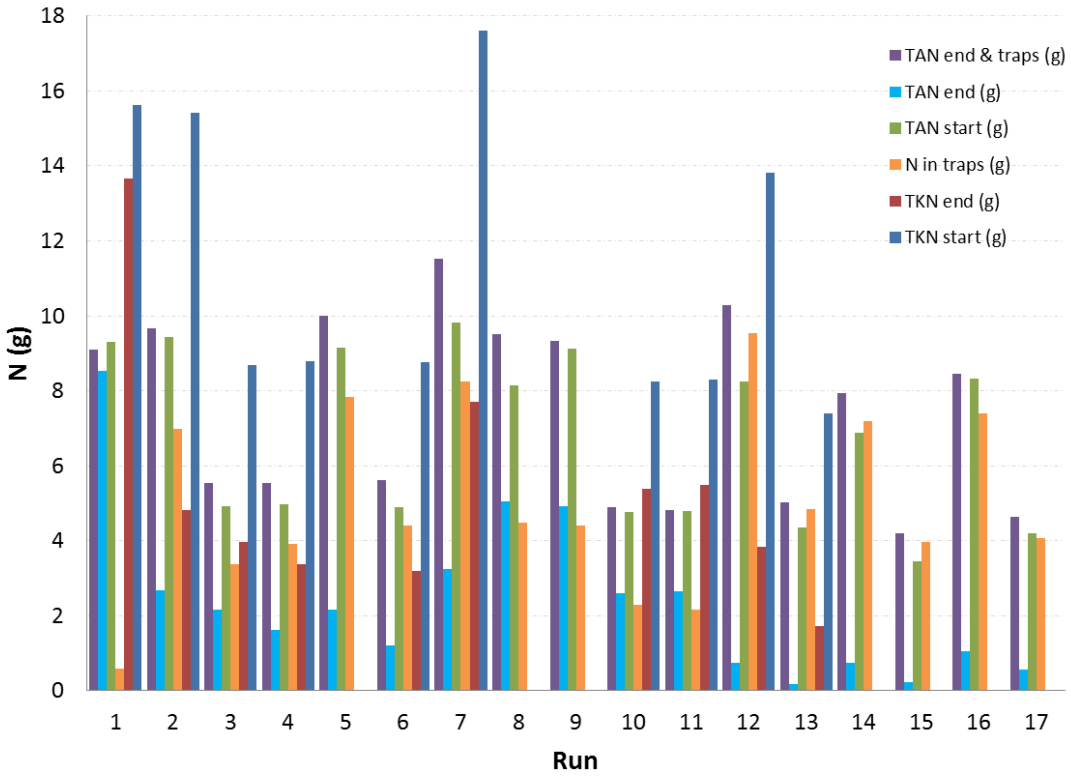
* Mass of solid found in the column at end of run
^ Mass of digestate in the column at end of run
+ Total mass of N found in the condensate, water trap and acid traps
- Mass unrecovered from the experiment, calculated value from mass balance: $Unrecovered\ matter = Mass_{dig\ in} - Mass_{dig\ out} - Mass_{sample} - Mass\ gain_{traps} - Solid_{recovered}$

Nitrogen mass balances (see section 3.6.4 equations 38 and 39) were calculated for each run and the results are shown in Fig. 40 and Table 25. In most of the experimental runs the TKN concentration in the digestate at the start of the experiment was higher than sum of the TKN in the digestate at the end and the nitrogen found in the traps. The main source of error in this balance is due to unrecovered material, including unaccounted-for N in the mass of digestate which remains attached to the column (see section 3.6.4 equation 40) and in samples removed for analysis.

On average the unrecovered digestate was estimated at 59 g (Table 24). If the lost mass is added to the final mass of digestate the % N loss is reduced by 0.3 - 6.0 %. The amount of missing nitrogen could be further reduced if the volume

sampled from the columns is added. Some of the lost mass could also be associated with crystallization of nitrogen compounds inside the tubes in the stripping rig: this would further decrease the unaccounted-for nitrogen. A secondary source of error is the nitrogen loss during the air-flushing period or in gas leaks. This is considered to be small, however, as the maximum mass of nitrogen present in the gas in the first 15 min of stripping was only 48 and 40 mg N (run 14 and 16 respectively); while a leak of 10 l of gas, which is unusual, would remove 253 and 216 mg of N.

Bonmati and Flotats (2003) reported similar problems with unrecovered nitrogen (6 % to 16 %) during air stripping of ammonia from pig slurry. Laureni et al. (2013) attributed N losses (3 % to 55 %) and overestimations (5 % to 15 %) to manure and digestate attached to the filling material in the stripping column. These studies, however, carried out a TAN balance, and not a TKN balance which is more accurate due to breakdown of N_{org}.



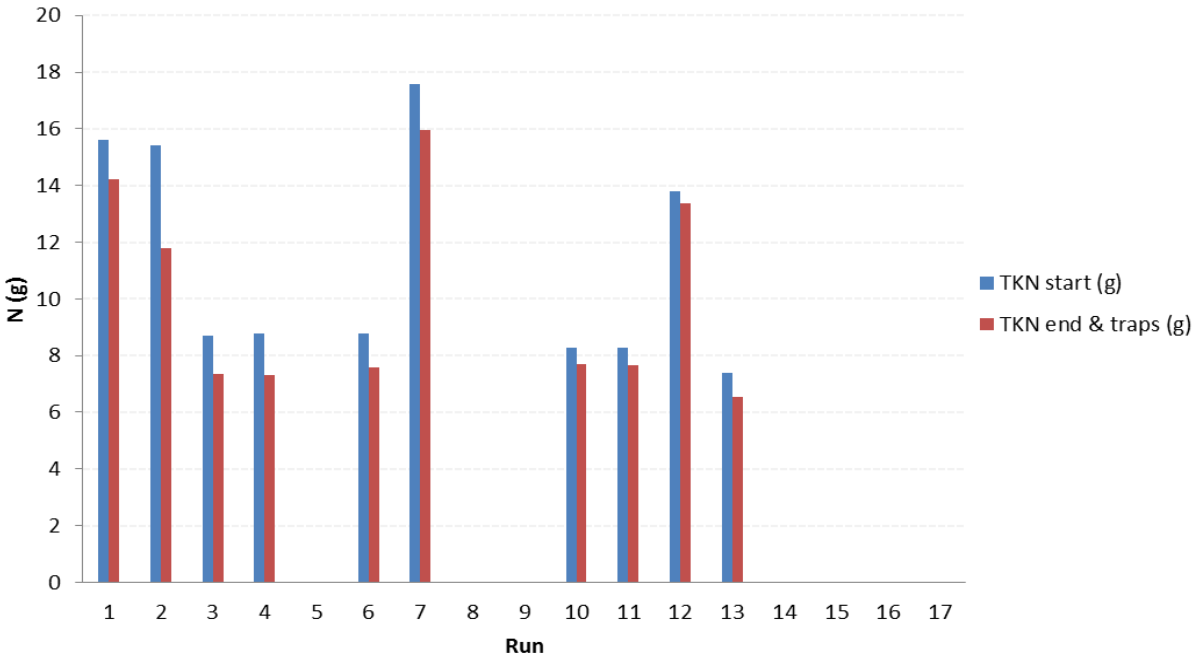


Fig. 40. Nitrogen mass balance in the stripping column

Table 25. Nitrogen balance

N exp	TAN start (g)	TAN end (g)	TAN end & traps (g)	TKN start (g)	TKN end (g)	TKN end & traps (g)	N lost (g)	N lost (%)	Mass lost (%)
1	9.3	8.5	9.1	15.6	13.7	14.23	1.39	8.9	2.7
2	9.4	2.7	9.7	15.4	4.8	11.81	3.62	23.4	0.6
3	4.9	2.2	5.5	8.7	4.0	7.34	1.36	15.6	3.6
4	5.0	1.6	5.5	8.8	3.4	7.31	1.48	16.8	8.9
5	9.1	2.2	10.0						5.7
6	4.9	1.2	5.6	8.8	3.2	7.60	1.16	13.3	5.1
7	9.8	3.3	11.5	17.6	7.7	15.98	1.62	9.2	3.7
8	8.1	5.0	9.5						2.3
9	9.1	4.9	9.3						4.6
10	4.8	2.6	4.9	8.3	5.4	7.68	0.58	6.9	3.9
11	4.8	2.7	4.8	8.3	5.5	7.65	0.64	7.7	4.7
12	8.2	0.7	10.3	13.8	3.9	13.38	0.43	3.1	4.1
13	4.4	0.2	5.0	7.4	1.7	6.55	0.83	11.3	6.7
14	6.9	0.7	7.9						3.1
15	3.5	0.2	4.2						3.0
16	8.3	1.1	8.5						2.3
17	4.2	0.6	4.6						5.9

4.3.3.2 Comparison of time constants.

Table 26 compares the time constants obtained in this study using fresh food waste digestate (TS = 6.60 %; TAN = 4560 - 4925 mg N kg^{-1}; pH 7.9-8.3) from stable well-run digesters with the results of a previous study conducted by Walker et al. (2011) that used two sources of food waste digestate both of which had been stored prior to testing (digestate 1: TS = 5.50 %; TAN = 8000 mg N kg^{-1}; pH 8.5-9.3; digestate 2: TS = 3.14 %; TAN = 6000 mg N l^{-1}; pH = 8.1-8.2). From Table 26, it can be seen that the time constants found in the current research were from 1.6 to 5.7 times larger than those determined in the previous work under the same experimental conditions; it therefore appears that it is more difficult to strip ammonia from fresh food waste digestate than from stored digestate.

A previous stripping study (50 ºC, air to slurry ratio 444) in pig manure and digestate conducted by Laureni et al. (2013) found a clear increase in stripping efficiency at lower organic matter contents. Digestate storage (2 and 6 months) decreased TS, COD, VFA and alkalinity and improved ammonia stripping efficiency. The ability of the organic matter to bind cations such as ammonium reduces the amount of strippable ammonia in the system, and this was thought to be the reason for the change in efficiency with solids content.

Table 26. Time constant comparison with previous research on the field

Walker et al. (2011)	**Flow: 0.125 l min^{-1} l^{-1}**				**Flow: 0.250 l min^{-1} l^{-1}**			
	exp 1	exp 2	exp 3	**av.**	exp 1	exp 2	exp 3	**av.**
70 ºC pH 10	*dig 1* - 45	*dig 1* - 36	-	41	*dig 1* - 28	*dig 1* - 15	*dig 1* - 15	19
70 ºC n/a pH	*dig 1* - 31	*dig 1* - 32	*dig 2* - 64	42	*dig 2* - 67	*dig 1* - 22	-	45
55 ºC pH 10	no data				no data			
55 ºC n/a pH	*dig 1* - 700	-	-	700	no data			
This study								
70 ºC pH 10	93	120	-	107	37	57	-	47
70 ºC n/a pH	243	234	-	239	161	-	-	161
55 ºC pH 10	427	-	-	427	147	-	-	147
55 ºC n/a pH	1111	-	-	1111	1111	833	-	972

av: average; exp: experiment; n/a: not adjusted; *dig 1*: digestate 1; *dig 2*: digestate 2

The solid effect can be observed in the stripping experiments conducted on digestate used in this study and digestate 2 used by Walker et al. (2011). However, it is not yet clear which factors promote higher removal since those

digestates have similar TAN concentrations but different characteristics, e.g. solids content, VFA concentration and alkalinity.

4.3.4 Analysis of the effects of temperature, pH, biogas flow analysis

The effect on the time constant of changing the gas flow rate, stripping temperature and pH was analysed (Fig. 41, Fig. 42, Fig. 43 and Table 27). The results showed that doubling the gas flow rate does not induce a similar increase in ammonia removal for this flow range. When the temperature in the stripping column is decreased, however, the time constant increases sharply (Fig. 43) with an exponential relationship.

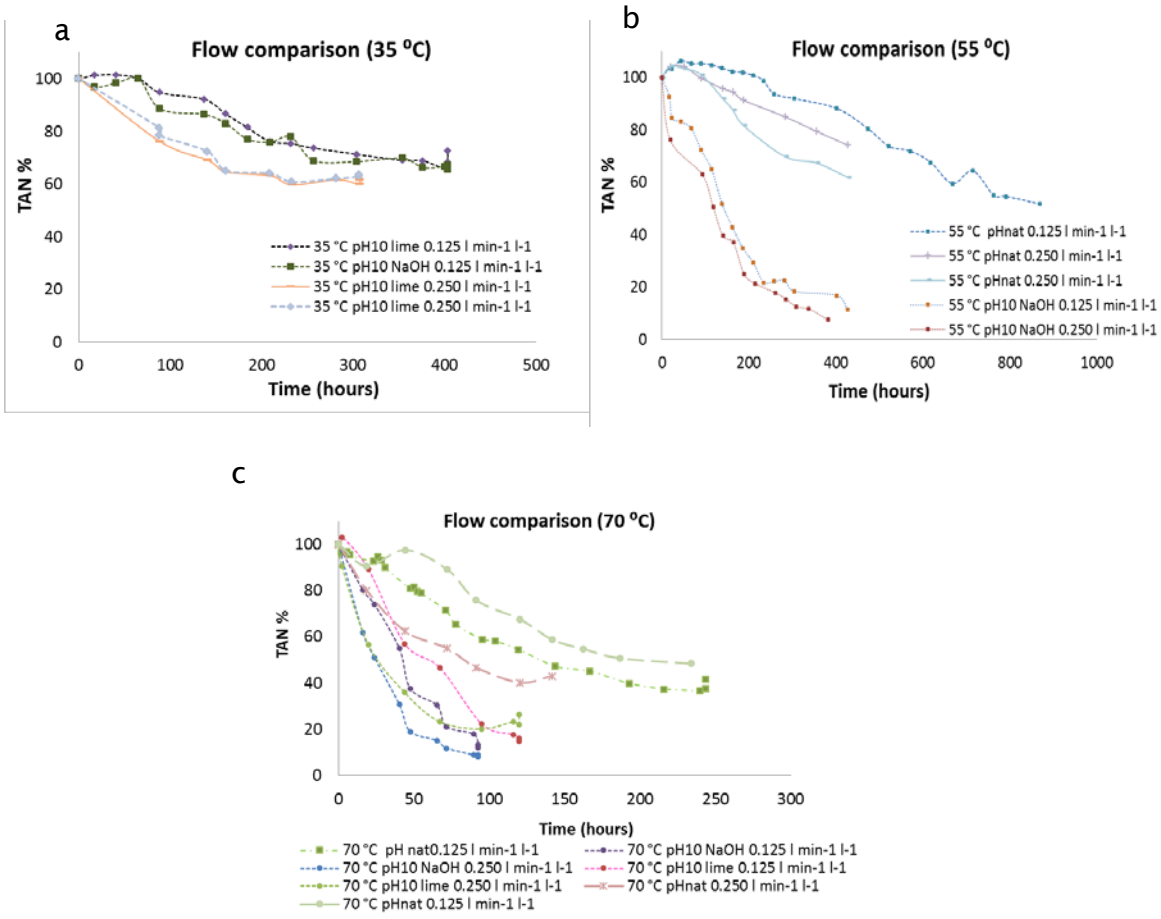


Fig. 41. Flow comparison in ammonia stripping experiments a) TAN removal profile 35 °C b) TAN removal profile 55 °C c) TAN removal profile 70 °C

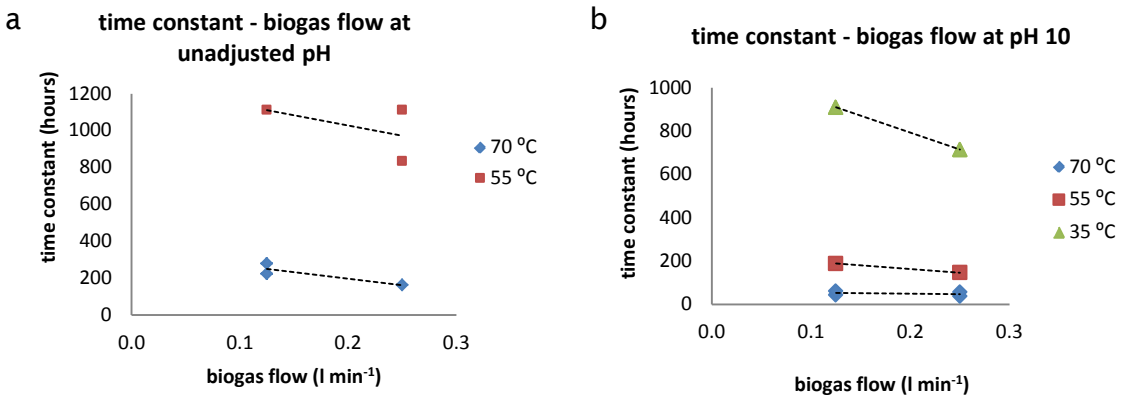


Fig. 42. pH comparison a) unadjusted pH time constant b) pH 10 time constant

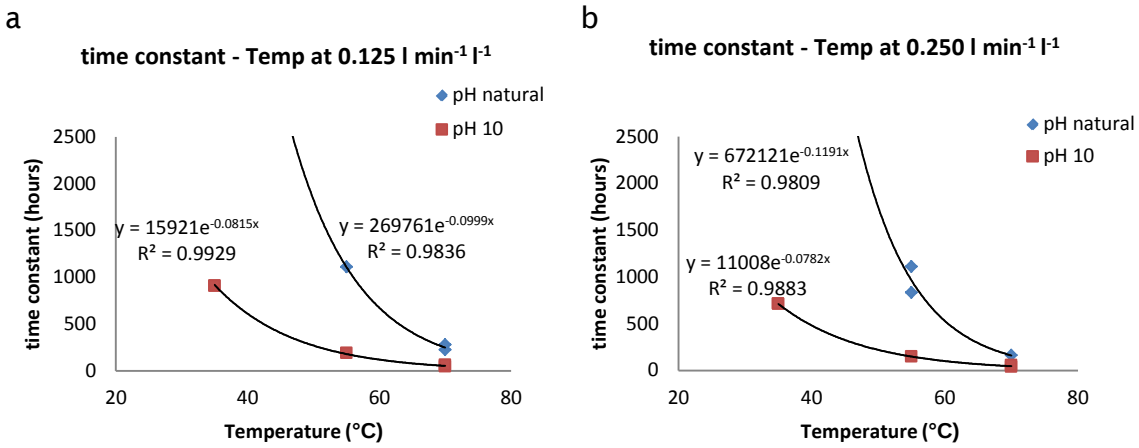


Fig. 43. Time constant relationship with temperature a) gas flow rate 0.125 l min^{-1} l^{-1} b) gas flow rate 0.250 l min^{-1} l^{-1}

Table 27. Time constant comparison under different variable changes

* τ_{low} : τ_{high}				
Temperature increased				
	0.125 l min^{-1} l^{-1}		**0.250 l min^{-1} l^{-1}**	
	pH n/a	**pH 10**	**pH n/a**	**pH 10**
35 °C to 55 °C	-	4.8	-	4.9
55 °C to 70 °C	4.4	3.6	6	3.1
pH increased (pH n/a to pH 10)				
	0.125 l min^{-1} l^{-1}		**0.250 l min^{-1} l^{-1}**	
35 °C	-		-	
55 °C	5.9		6.6	
70 °C	4.7		3.4	
Flow increased (0.125 l min^{-1} l^{-1} to 0.250 l min^{-1} l^{-1})				
	pH n/a		**pH 10**	
35 °C	-		1.3	
55 °C	1.1		1.3	
70 °C	1.6		1.1	

pH n/a: pH not adjusted

4.3.4.1 Conclusions

Summarising, ammonia removal in fresh food waste digestate appears to follow the same trends in relation to pH, temperature and stripping gas flow rate as widely reported for other materials by other authors. Ammonia removal generally increases with an increase in any of the other three variables. Under the moderate biogas flow rates used in this study, however, the biggest improvement in nitrogen removal; is associated with increases in pH and temperature as indicated in Table 27 and these are therefore the most important factors to control as both have a strong influence on the ammonia-ammonium equilibrium.

The results of the batch trials showed that ammonia removal rates were low at 35 ºC not modified pH and pH 10 (runs 1, 8 to 11), and 55 ºC not modified pH (runs 2 to 4). The amount of ammonia removed was greater at 55 ºC pH 10 and 70 ºC pH 10 and unadjusted pH. In order to maximize the ammonia removal in the stripping column and reduce TAN concentration below inhibitory concentration in a thermophilic digester these conditions were selected for evaluation in a side-stream process.

4.3.5 Ammonia stripping efficiency

Using the concept of Henry's law a relationship can be obtained between the FAN concentration in the liquid phase and the ammonia concentration expressed as partial pressure in the gas phase at each point in the experiment (see section 3.6.3 equations 31 and 32). The E value (equation 30) for each experiment shows the efficiency of the experimental conditions based on $H_{stripping}$ and $H_{equilibrium}$.

For all of the experimental runs, when P_{NH3} was plotted against C_{FAN} to allow determination of $H_{stripping}$ (equation 32), a linear relationship passing through the origin was found. At 35 ºC and pH 10 (run 8 to 11), however, the linear relationship had a positive intercept, due to the low driving force for ammonia removal represented by the low value of $H_{stripping}$ obtained (Table 28).

Table 28. H values and experimental constants for H calculation.

Exp no.	A	B	R^2	A´	B´	R^2	C	D	$H_{stripping}$
1									no stripped
2	5374	0.0009	0.9492	3117	0.0013	0.7445	0.000468	1.991	0.750
3	5242	0.0009	0.9791	1833	0.0020	0.7721	0.000604	0.999	0.891
4	5216	0.0012	0.9767	1867	0.0024	0.8310	0.000823	1.009	1.157
5	4522	0.0045	0.9746	3581	0.0070	0.9725	0.003546	2.005	3.244
6	4383	0.0062	0.9223	2811	0.0140	0.9235	0.003023	0.998	3.329
7	5090	0.0036	0.9402	3118	0.0055	0.8310	0.00271	2.004	3.276
8	4111	0.0011	0.9297	3278	0.0022	0.9208	0.00046	2.027	0.400
9	4342	0.0011	0.9351	3181	0.0022	0.8880	0.001012	2.085	0.585
10	4415	0.0016	0.8511	3918	0.0067	0.9550	0.000384	1.000	0.162
11	4316	0.0014	0.8233	3759	0.0068	0.9686	0.000415	1.004	0.150
12	4148	0.0053	0.9582	3943	0.0055	0.9611	0.000947	2.004	2.165
13	4622	0.0068	0.9775	4435	0.0080	0.9856	0.001098	1.002	1.517
14	4039	0.0226	0.9725	3957	0.0228	0.9738	0.002622	1.987	9.023
15	3147	0.0267	0.9799	3083	0.0271	0.9802	0.002169	0.999	5.356
16	4709	0.0162	0.9843	4542	0.0162	0.9836	0.002507	1.987	6.612
17	3742	0.0175	0.9539	3412	0.0194	0.9209	0.002791	0.993	4.136

The ammonia concentration of the biogas generated by the 75-L digesters in section 4.1 above was analysed. The proportionality constant ($H_{digester}$) that relates the solubility of ammonia in water to its partial pressure in the gas was calculated following the concept of Henry's law. The result showed good agreement with the stripping experiments performed at natural pH and 35 ºC (Fig. 44a). In both cases equilibrium is not reached.

The $H_{stripping}$ value was also calculated for the synthetic digestate based on the results of the comparative trial with real digestate (55 ºC 0.250 l min^{-1} l^{-1} and 0.125 l min^{-1} l^{-1}, Fig. 31). The results in Table 29 show a larger $H_{stripping}$ than for the real food waste digestate, corresponding to a more efficient use of the energy applied in the stripping system.

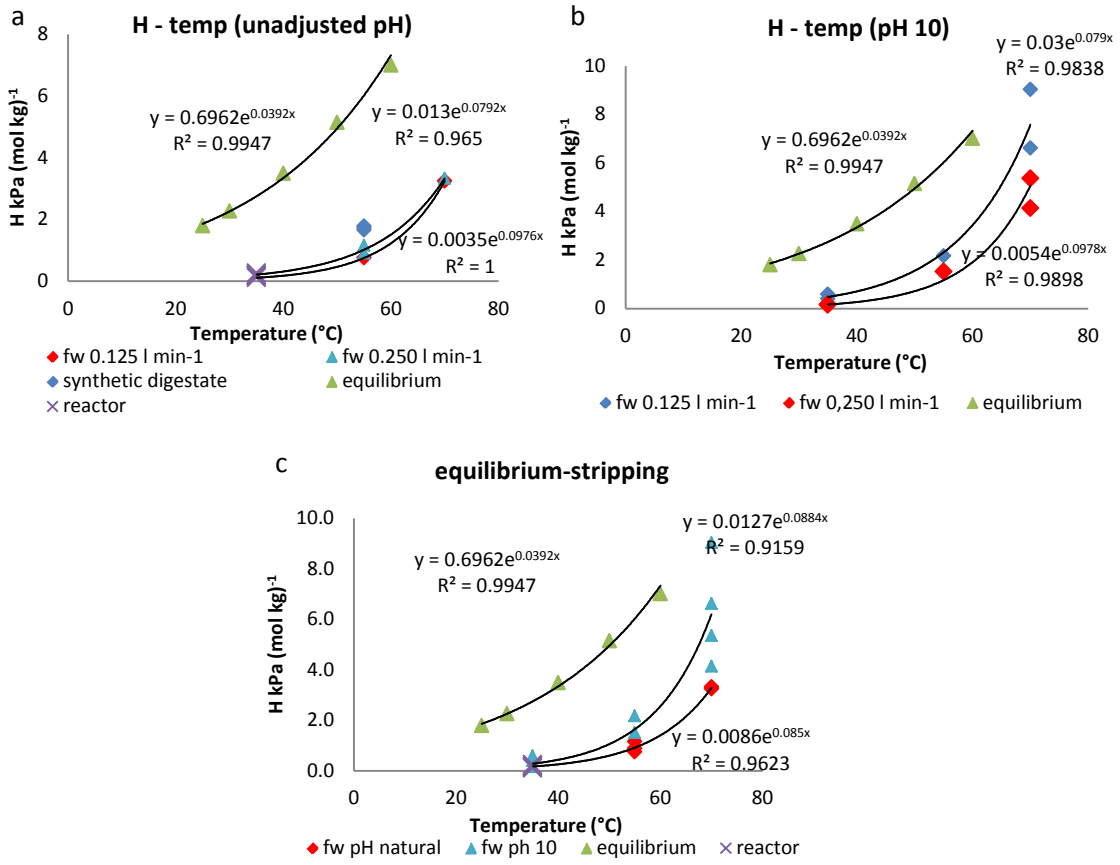


Fig. 44. H values vs. temperature a) unadjusted pH, flow comparison b) pH 10, flow comparison c) effect of temperature on H values

Table 29. Summary of ammonia stripping experiments for synthetic digestate and $H_{stripping}$ calculated values

Flow l min^{-1} l^{-1}	pH start	TAN start mg l^{-1}	TAN end mg l^{-1}	Ammonia removal %	T hours	Time constant hours	R^2	Equation	$H_{stripping}$
0.250	8.18	3880	1300	67	328	294	0.997	$y = 4009e^{-0.0034x}$	1.649
0.250	8.18	3880	1160	70	328	256	0.996	$y = 4034e^{-0.0039x}$	1.775
0.125	8.03	4700	2800	40	333	63	0.982	$y = 4835e^{-0.0016x}$	1.730

T: total experimental time
Equation: TAN concentration profile (equation 29)

At unadjusted pH an increase in the flow does not increase the $H_{stripping}$ value (Fig. 44a). When the pH was adjusted to 10, however, an increase in the flow leads to a decrease in the $H_{stripping}$ value (Fig. 44b), indicating that the biogas is used in a less efficient manner. Fig. 44c presents a comparison of non-

modified and adjusted pH: from this it can be concluded that an increase in pH would lead to a rise in $H_{stripping}$. The most efficient ammonia stripping condition tested is 70 ºC and pH 10 (Table 30, Fig. 45).

Table 30. E (%) comparison of stripping process at different conditions (pH, temperature)

	equation	R^2	E 35 ºC	E 55 ºC	E 70 ºC
pH 10	$y = 0.0127e^{0.0884x}$	0.916	9	27	75
pH n/a	$y = 0.0086e^{0.0850x}$	0.962	-	15	40

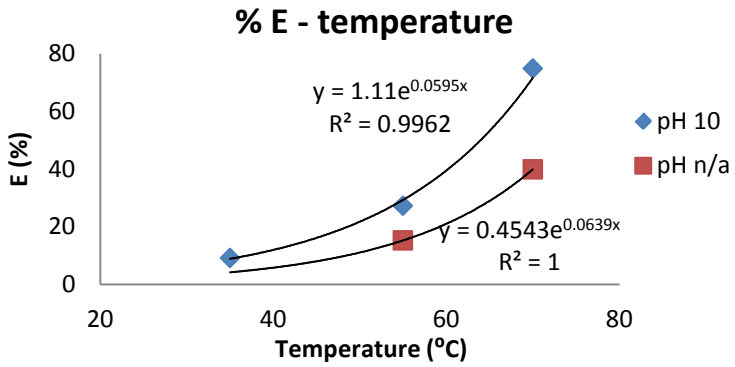


Fig. 45. E values vs temperature with and without pH adjustment

4.3.6 Hydrolysis experiment

To investigate the reasons for the initial increase in TAN concentration and the 'lag' in TAN removal observed over the first 35-43 hours in the stripping experiment at 55 ºC with non-modified pH (run 2, 3 and 4), and the VFA increases found in some of the runs (Fig. 36), 4-day batch tests were carried out in which food waste digestate was subjected to a temperature increase to 70 ºC, with and without pH increase, but without gas stripping. The experiment was repeated on two digestates from the control reactor (section 4.4) with similar characteristics (H1/H2-digestate 1-day 408 and H3/H4-digestate 2-day 431). Two consecutive acid traps of approximately 25 ml and 50 ml were used to avoid any nitrogen losses from the gas escaping during the initial temperature increase.

Digestate TAN, TKN, pH and VFA concentration were monitored. The results are shown in Fig. 46.

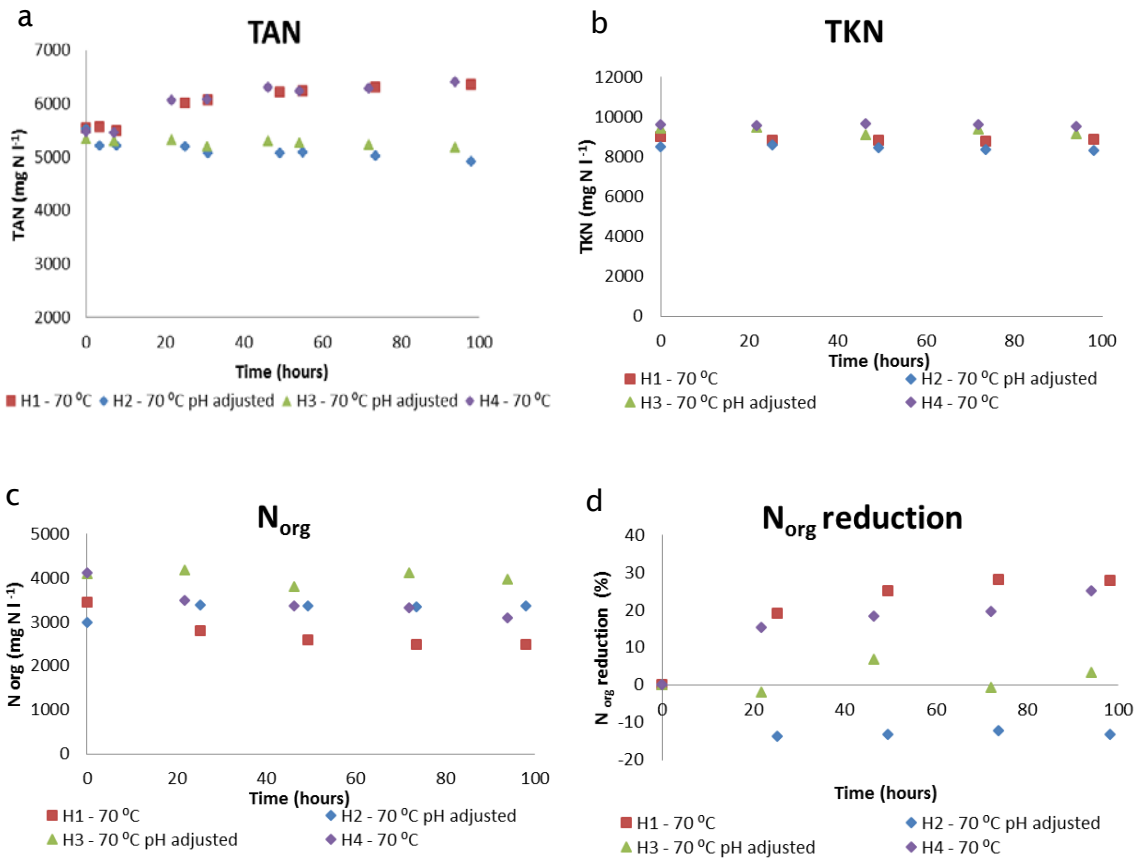
Heating to 70 ºC without pH increase (run H1 and H4) had a clear effect on the nitrogenous material: the TAN concentration increased and N_{org} decreased, with

the TKN concentration remaining constant during the experiment. There was a decrease in N_{org} of 15.3-19.0 % and 25.1-27.8 % after 22-25 and 94-98 hours, respectively. The pH showed an initial increase due to CO_2 desorption caused by the rise in temperature, then decreased slowly (range 8.37 - 8.07) due to ammonium de-protonation caused by the TAN increase. A mass balance calculation showed the unaccounted-for total nitrogen to be less than 1.6 %. The VFA concentration increased rapidly from 140 - 340 mg l^{-1} to 6300 - 6600 mg l^{-1}, which correlated with a decrease in TS from 6.3 % to 5.6 % and in VS from 4.4 % to 3.7 %.

At 70 ºC with increased pH (H2 and H3), the TAN profiles showed no increase. This may be due to volatilization of a small amount of free ammonia when the digestate is maintained at high temperature, and to a lesser extent during sample storage before analysis, leading to a lower TAN measurement which in turn affects the calculation of N_{org} and gives a slight negative value of N_{org} reduction (Fig. 46 d). At 70 ºC with pH 8.2 and 9.6, 58.8 % and 97.3 % respectively of the total TAN is in the form of free ammonia. At 4 ºC during storage in the refrigerator, 1.8 % and 31.5 % of the TAN is present as FAN at pH 8.2 and 9. The effect of volatilisation can be seen in the initial TKN values: in all cases the TKN before pH increase (H1 and H4) was greater than after the pH increase (H2 and H3) for the same digestate sample. Bonmati and Flotats (2003) also reported that pH adjustment produced a decrease in TKN and TAN concentration for digested and fresh pig slurry, and attributed this to volatilisation promoted by high temperatures. The VFA concentration in the hydrolysis experiment with increased pH showed only a very small change from 180-260 mg l^{-1} to 560 mg l^{-1}. The TS content at the end of the experiment was higher than that in the original sieved digestate due to the addition of lime (initial TS 6.3 % and final TS 9.1 %); the VS destruction rate was lower than in the hydrolysis experiment without pH adjustment (initial VS 4.4 % and final VS 4.1 %). The unaccounted-for nitrogen in the mass balance was 2.4 - 3.2 %, greater than in the experiments without pH adjustment. This is likely to be due to the fact that the higher pH promotes the escape of free ammonia into the gas phase, leading to higher losses and inaccuracy.

In all the experiments conducted the total amount of nitrogen found in the traps was lower than 4 mg, showing that this was not a route by which the element left the system.

The results of the hydrolysis experiments at 70 ºC without pH increase suggest that the increase in TAN concentration and subsequent 'lag' in removal at 55 ºC without pH modification is in fact due to further production of TAN in the stripping columns, by thermally-promoted alkaline hydrolysis of organic nitrogen-containing materials. The ammonia released then contributes to the TAN concentration in the column.



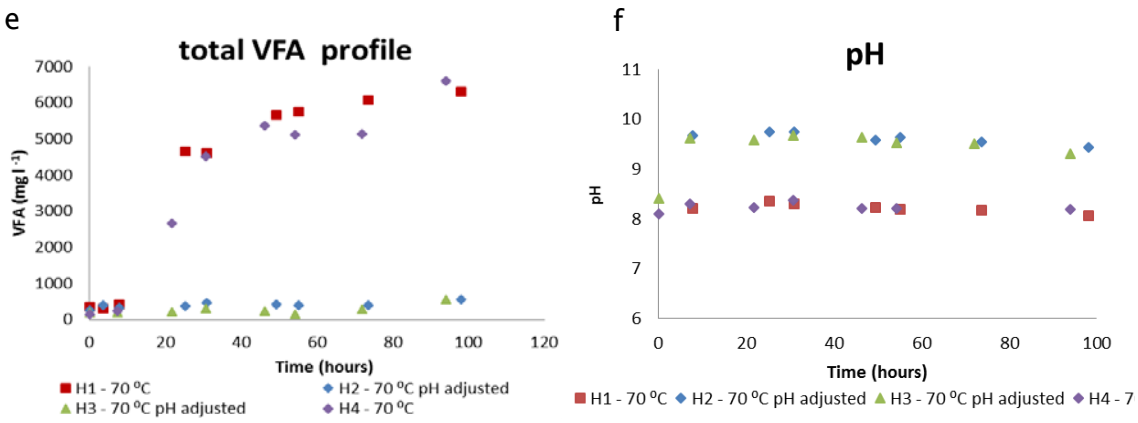


Fig. 46. Hydrolysis results obtained at 70 °C a) TAN profile b) TKN profile c) N_{org} profile d) N_{org} reduction e) VFA profile f) pH profile

4.4 Side-stream stripping

Objective: The main objective of this work was to evaluate the capability of a side-stream biogas stripping system to control TAN concentration while also gauging the long term effects of the stripping process on digester operation and performance. To achieve this, the following sub-objectives were set:

- To compare digestion performance of reactors with and without side-stream stripping.

- To compare data on ammonia stripping kinetics with batch trials.

- To assess changes in dominant methanogenic pathway caused by the side-stream stripping process.

- To determine whether the dewatering characteristics of the digestate were modified by the thermal-alkaline side-stream stripping technique.

- To conduct a nitrogen mass balance to compare TAN concentration in steady state with experimental results and to assess whether the side-stream stripping strategy would succeed in maintaining a safe TAN concentration in a digester fed on source separated food waste at different organic loading rates.

4.4.1 Methodology

The experiments were carried out in the 35-L digesters described in section 3.4.2. Inoculum was taken from the 75-L digesters used in the trial in section

4.1 after stable operation for more than 4 HRT. Daily feeding on source segregated food waste continued as before, at an OLR of 2 kg VS m^{-3} day^{-1} with trace element supplementation following the recommendation of Banks et al. (2012). The characteristics of original inoculum and of the different batches of food waste used in the experiment are shown Table 31. Digestate was removed twice a week and the digesters were monitored for pH, TAN, alkalinity, solids, biogas production, and volatile fatty acids. Gas produced in the 35-L reactors was collected for 1.5 hours in gas-impermeable sampling bag five hours after feeding the reactor to determine its composition at least fortnightly.

Table 31. Inoculum and feedstock characteristics

Inoculum characteristics					
		Average	**Standard deviation**	**max**	**min**
pH		7.9	-	7.91	7.89
TA	g l^{-1}	23.9	0.4	24.2	23.6
PA	g l^{-1}	18	0.5	18.4	17.6
IA	g l^{-1}	5.2	0	5.2	5.2
TAN	g N kg^{-1}	4.86	0.07	4.91	4.81
TKN	g kg^{-1}	8.75	0.04	8.78	8.72
TS	g kg^{-1}	66.3	0.6	66.7	65.8
VS	g kg^{-1}	48.3	0.4	48.5	48
VFA	mg l^{-1}	148	6	152	143

Characteristics of the food waste batches					
N	**Start** (feeding day)	**End** (feeding day)	**TS** (g kg^{-1})	**VS** (g kg^{-1})	**TKN** (% dry)
1	0	55	246.2 ± 2.4	228.1 ± 1.4	-
2	56	70	232.7 ± 3.8	211.8 ± 1.8	-
3	71	162	218.6 ± 6.2	202.9 ± 5.9	3.7 ± 0.5
4	163	227	209.8 ± 0.9	183.4 ± 0.3	3.6 ± 0.1
5	228	270	218.6 ± 6.2	202.9 ± 5.9	3.7 ± 0.5
6	271	334	239.8 ± 4.7	218.2 ± 4.3	3.5 ± 0.1
7	335	403	229.3 ± 1.2	208.1 ± 2.4	3.0 ± 0.1
8	404	423	249.1 ± 3.9	232.2 ± 3.8	3.2 ± 0.1

4.4.1.1 Phase 1: Establishing a digestion baseline

After inoculation the digesters were initially operated for 122 days (1.14 HRT) in order to establish a performance and stability baseline.

4.4.1.2 Phase 2: Ammonia removal by side-stream stripping

A stripping column (or pair of stripping columns) was used in conjunction with a single digester and both the digester and stripping system were operated in semi-continuous mode. Feeding of the digesters and digestate removal continued as in phase 1 but an additional portion of digestate, equivalent to 6 % of the digester volume, was removed, sieved through a 1 mm mesh, and the liquor placed in the stripping column. The solids separated by sieving were immediately returned to the digester. After stripping for the required interval the treated liquor was returned to the digester from which it had been taken, with any volume loss compensated for by returning digestate from the wastage line. The conditions used in the stripping trials are detailed in Table 32.

Table 32. Conditions used in side-stream stripping experiments

	Days 0 - 122	**Days 123 - 260**	**Days 261 - 311**	**Days 312 - 325**	**Days 326 - 361**	**Days 361 - 423**
R_1	Phase 1, no stripping	C_1 T: 70 °C, pH n/a, RT: 4 day, SP: 1.5% day^{-1}	as before	as before	C_1 T: 70 °C, pH 10, RT: 3 day, SP: 2% day^{-1}	as before
R_2	Phase 1, no stripping	C_2 T: 70 °C, pH 10, RT: 3 day, SP: 2% day^{-1}	C_2 T: 70 °C, pH n/a, RT: 4 day; C_4 T: 70 °C, pH n/a, RT: 4 day, SP: 3% day^{-1}	C_2 T: 70 °C, pH 10, RT: 3 day; C_4 T: 70 °C, pH n/a, RT: 4 day, SP: 3.5% day^{-1}	as before	as before
R_3	Phase 1, no stripping	C_3 T: 55 °C, pH 10, RT: 5 day, SP: 1.2% day^{-1}	C_3 T: 85 °C, pH n/a, RT: 3 day, SP: 2% day^{-1}	as before	as before	C_3 T: 85 °C, pH 10, RT: 2 day, SP: 3% day^{-1}
R_4	Phase 1, no stripping	Control, no stripping column				

R_1 - R_4 = anaerobic reactor 1 to 4; C_1 - C_4 = stripping column 1 to 4; T = temperature; RT = retention time; SP = reactor portion stripped per day; n/a = not adjusted

Where the pH in the stripping column was adjusted this was done by adding lime at 18.6 - 21.4 g CaO kg^{-1} of digestate (wet weight) to obtain a pH value around 10.

Success was measured in terms of TAN removal from the coupled digester as well as in showing that no inhibition of the digestion process occurred as a result of the stripping process.

4.4.2 Results

4.4.2.1 Phase 1: Baseline performance and stability assessment

All four digesters showed good performance over the first 122 days (1.14 HRT) despite a TAN concentration of 5.1 g N kg^{-1} (Fig. 47) and free ammonia around 500 mg N kg^{-1} (Fig. 48). No VFA accumulation was detected (Fig. 49), the IA/PA ratio was less than 0.3 (Fig. 50) (Ripley et al., 1986), and VS destruction rates (Fig. 51) were 82.3, 83.6, 83.5 and 83.8 % in digesters 1 - 4 respectively. Specific biogas production was stable with values of 0.84 ± 0.03, 0.83 ± 0.03, 0.83 ± 0.04 and 0.82 ± 0.04 l g^{-1} VS (Fig. 52) and methane concentration between 55 - 61 %.

Average digestate characteristics are shown in Table 33. No noticeable upset was associated with the start-up of the digesters, but this was not surprising as the inoculum was taken from digesters being fed on the same substrate at the same OLR and receiving the same trace element supplementation.

Table 33. Digestate characteristics without side-stream stripping (average day 0 to 122)

		R_1	R_2	R_3	R_4
pH		7.98 ± 0.07	7.96 ± 0.06	7.94 ± 0.07	7.93 ± 0.06
TA	g l^{-1}	25.1 ± 0.9	25.0 ± 1.0	24.8 ± 0.9	25.0 ± 1.1
PA	g l^{-1}	18.6 ± 0.8	18.0 ± 1.0	18.4 ± 0.7	18.9 ± 0.9
IA	g l^{-1}	5.8 ± 0.4	5.7 ± 0.6	5.7 ± 0.5	5.3 ± 0.8
TAN	g N kg^{-1}	5.1 ± 0.01	5.1 ± 0.01	5.1 ± 0.01	5.1 ± 0.01
TKN	g N kg^{-1}	8.75 ± 0.04	8.75 ± 0.04	8.75 ± 0.04	8.75 ± 0.04
TS	g kg^{-1}	64.5 ± 1.1	64.4 ± 1.4	65.5 ± 1.9	64.3 ± 0.9
VS	g kg^{-1}	47.4 ± 0.6	47.4 ± 1.0	47.9 ± 1.1	47.1 ± 0.6
VFA	mg l^{-1}	270 ± 100	260 ± 100	270 ± 80	290 ± 120

4.4.2.2 Phase 2: Side-stream ammonia stripping

Side-stream stripping was coupled to the digesters between days 123 and 423, equal to 3 HRT based on food waste input and more than 4 retention times

(RT) based on the internal HRT, i.e. taking into account the stripped digestate liquor returned to the digester.

Digesters performance

Fig. 47 to Fig. 55 show the performance of the reactors with (days 123- 423) and without (days 0 - 122) side-stream stripping.

Throughout the experimental period the control digester R4 was run without side-stream stripping and continued to show stable performance similar to that in the baseline period with a TAN concentration in the digester > 5.0 g N l^{-1}.

The performance and the stability of digesters R_1-R_3 did not appear to be affected by any of the measures introduced in the stripping columns. There was no major change in specific biogas production (Fig. 52) which remained stable with average values of 0.84 ± 0.05, 0.84 ± 0.04, 0.85 ± 0.05 and 0.83 ± 0.04 l g^{-1} VS during the side-stream stripping period (days 123-423). The measured methane concentration also remained steady at around 58 %. VFA concentrations remained below 400 mg l^{-1} (Fig. 49), although changes were seen in the alkalinity parameters (Fig. 53) depending on the treatment imposed.

Ammonia removal. The purpose of the side-stream stripping was to reduce the TAN concentrations in the stripped digesters, and the experiments tested the effectiveness of this under a number of different conditions. Changes in TAN for the different operational periods spanning days 123-423 can be seen in Fig. 47. Between days 123-260 the removal of TAN in digester R1 coupled to the stripping column operated at 70 ^{0}C without pH adjustment was very similar to that in digester R2, which was operated at the same temperature but with the pH adjusted to 10. Operation at a temperature of 55 ^{0}C and pH 10 gave a lower TAN removal, apparently indicating that temperature was the main factor governing the stripping process. During the first period (days 123-260) the stripping columns were operated with stripping gas connected to a biogas reservoir common to all the columns. During the second period (days 261-311) the stripping gas lines were separated, giving each column its own independent reservoir. As pH control had not appeared to be critical to TAN removal in the first period the addition of lime to the stripping column coupled to digester R_2 was also stopped. As temperature seemed to be the most

important stripping criterion this was increased to 85 ºC in the stripping column coupled to digester R_3. In an attempt to increase the rate of removal of TAN, a larger volume of digestate was removed from digester R_2 and loaded into two stripping columns working under the same conditions.

The results of these changes were surprising, in that digester TAN concentrations started to increase in R_1 and R_2, and there was no apparent improvement in the rate of TAN removal in R_3 despite the 30 ºC increase in temperature. It was concluded that separating the gas stripping lines had caused this change, possibly due to preventing the precipitation of CO_2 and the enhancement of CH_4 content in the common stripping gas. In the previous experimental period this precipitation reaction resulted in a pH rise in the column without pH adjustment by lime addition, and this led to the incorrect conclusion that pH was of secondary significance compared to temperature. To demonstrate this, pH adjustment was reintroduced to one of the stripping columns coupled to digester R_2 on day 312. This resulted in an immediate reversal in the trend of TAN accumulation in the digester when compared to R_1, where stripping continued without pH adjustment but on an independent biogas recirculation loop. On day 326 pH adjustment was reintroduced in the stripping column coupled to digester R_1, and again a reversal in the trend of TAN in the digester was seen almost immediately (Fig. 47).

On day 362 pH adjustment to the stripping column operating at 85 ºC was introduced and the RT reduced to 2 days; this immediately increased the TAN removal rate to the highest level seen throughout the experimental trial.

To reduce the TAN to a point where it would be unlikely to inhibit a thermophilic food waste digester requires a concentration of ≤ 2500 mg N l^{-1}. To achieve this in practice a side-stream striping process using both high temperature and pH adjustment would be necessary: this is borne out by the performance of digester R_3 which was coupled to a column operated at 55 ºC and pH 10, but only showed an overall 21.0 % reduction in TAN compared to the control when operated over a 137-day period. Digester R_2, which had the longest operational period at high temperature and pH (128 days), showed an overall TAN reduction of 48.2 %, and the potential for even greater removal exists when using a higher temperature of 85 ºC.

The use of side-stream stripping not only reduced digester TAN but also digester N_{org} content by between 20 - 33 % of the control value (Fig. 54), with the greatest reduction corresponding to the high temperature and pH stripping conditions. This is caused by additional thermally-mediated alkaline hydrolysis of organic nitrogen-containing materials that have been carried over from the digester to the stripping columns. The ammonia released then contributes to the TAN removed in the column.

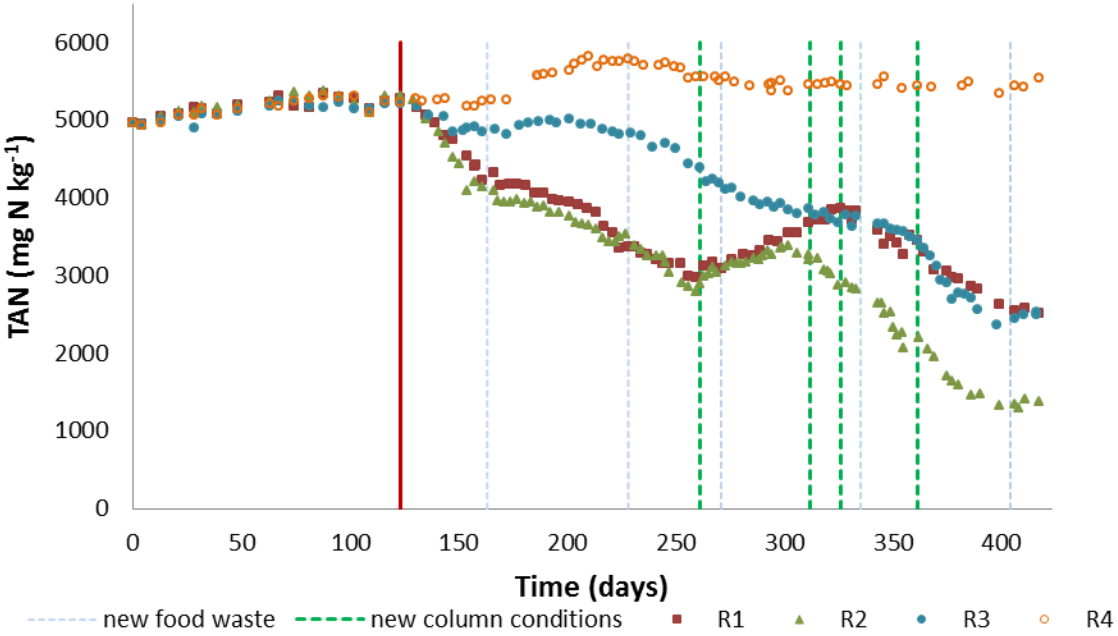


Fig. 47. Total ammoniacal nitrogen

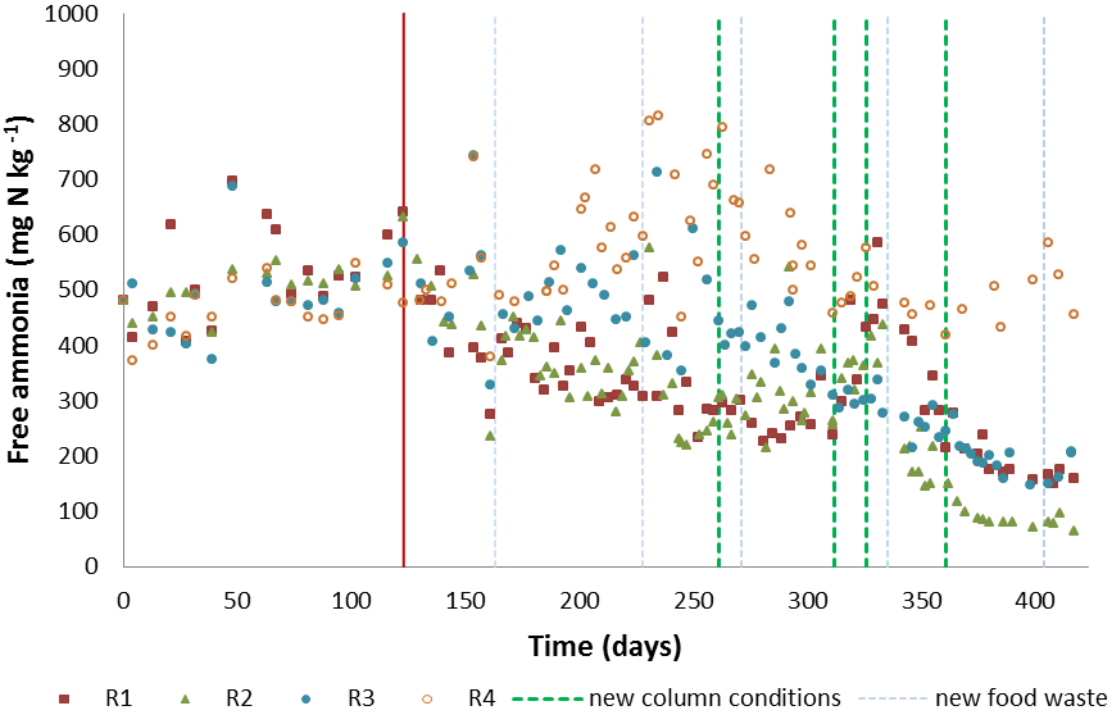


Fig. 48. Free ammonia

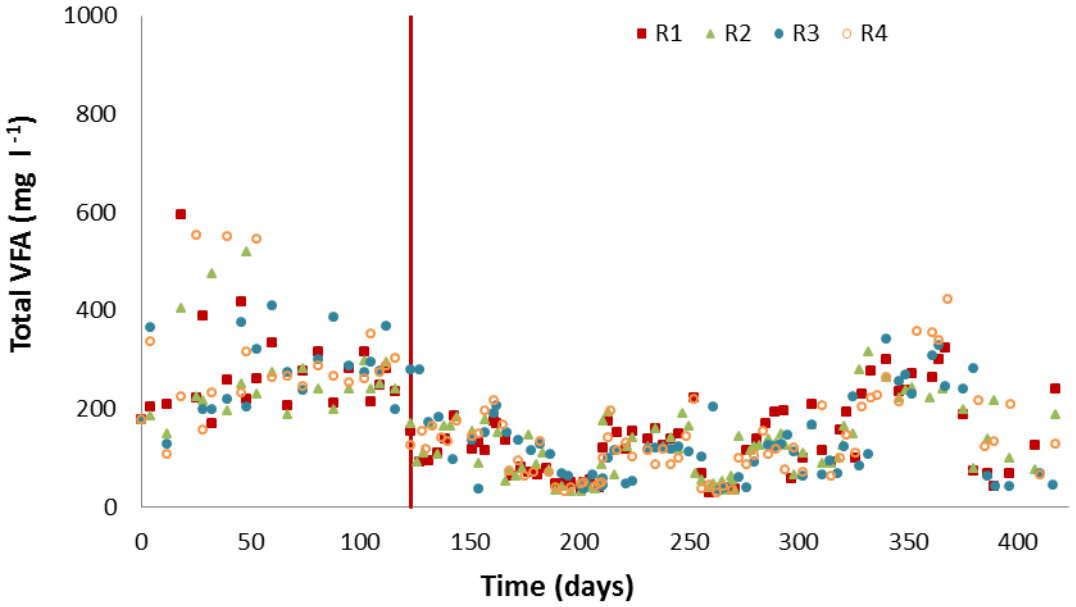


Fig. 49. Total VFA

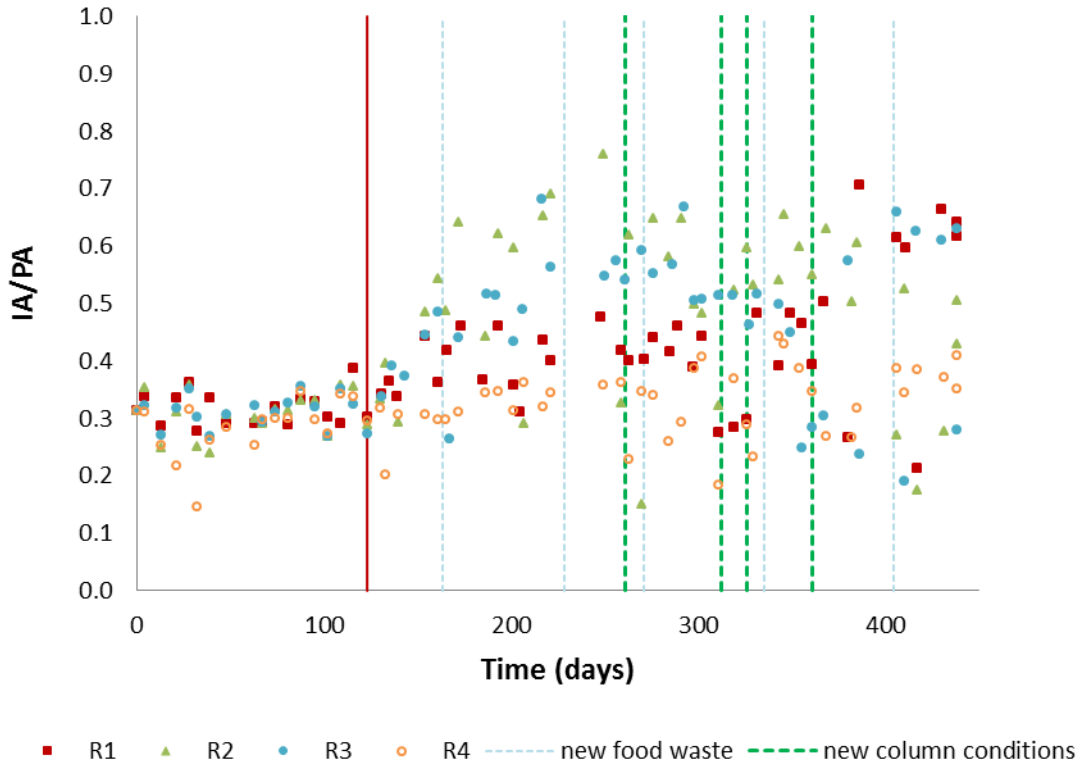
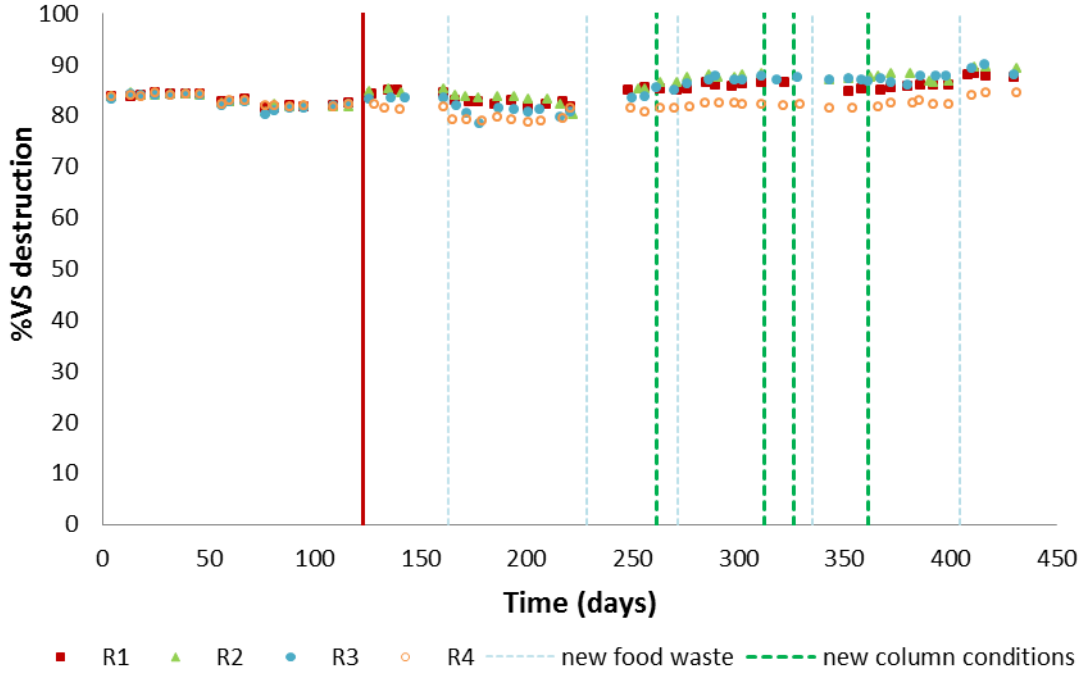


Fig. 50. IA/PA ratio

a



b

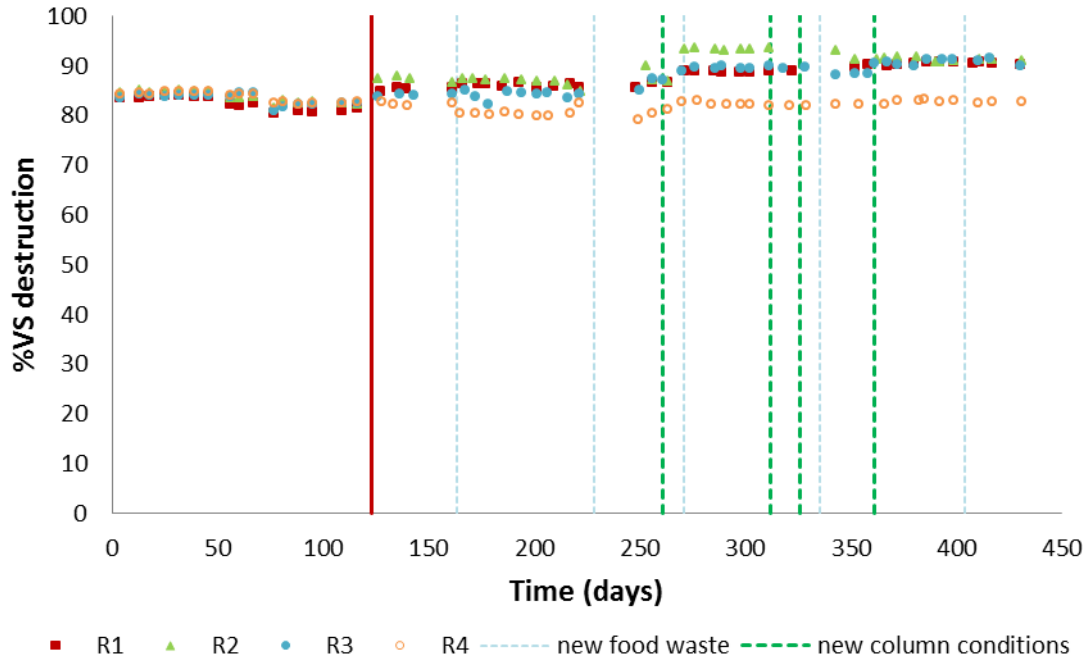


Fig. 51. Weekly VS destruction rate.
a) Calculated using biogas production b) Calculated using experimental outlet mass of digestate

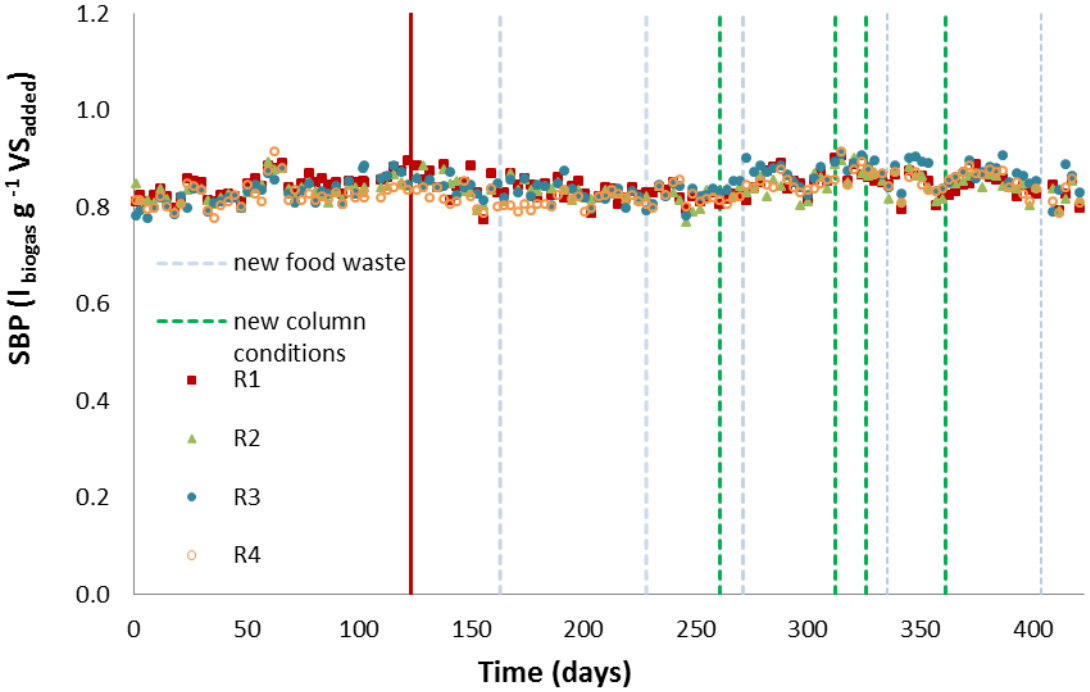


Fig. 52. Specific biogas production

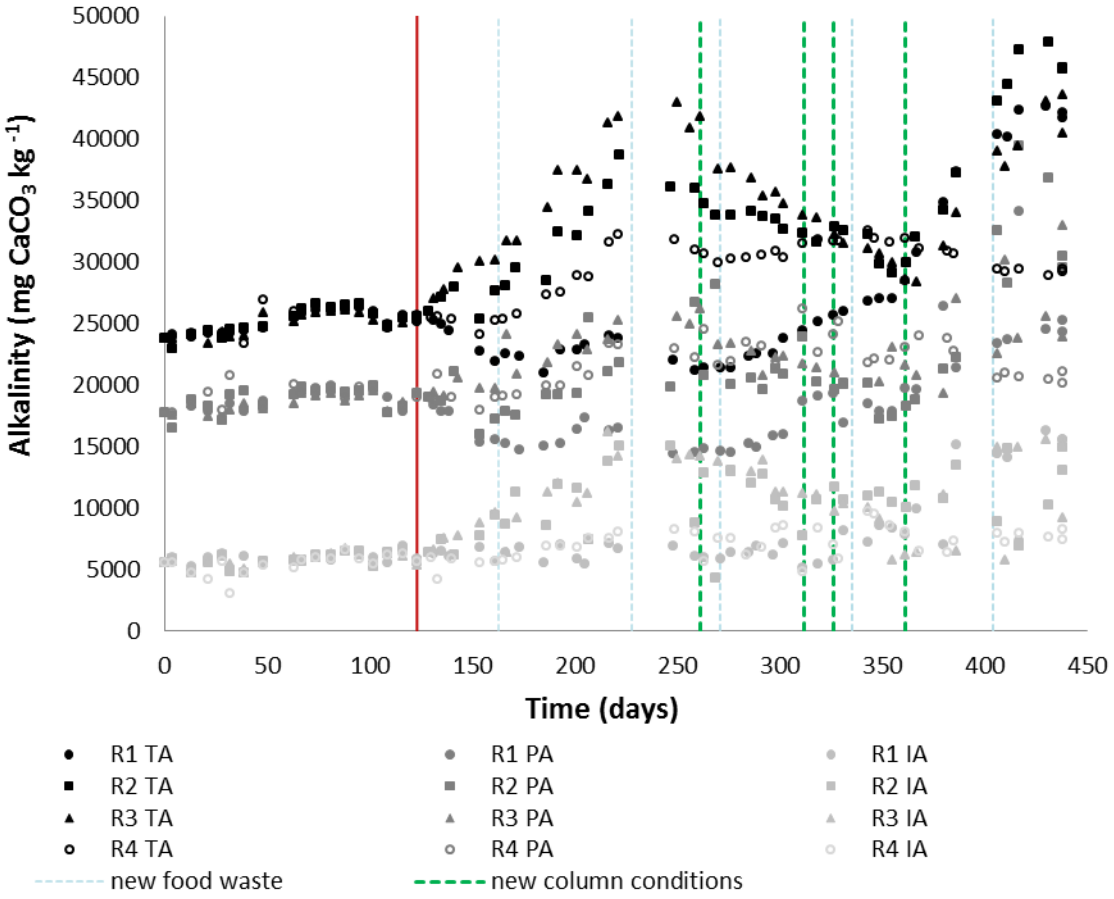


Fig. 53. Alkalinity

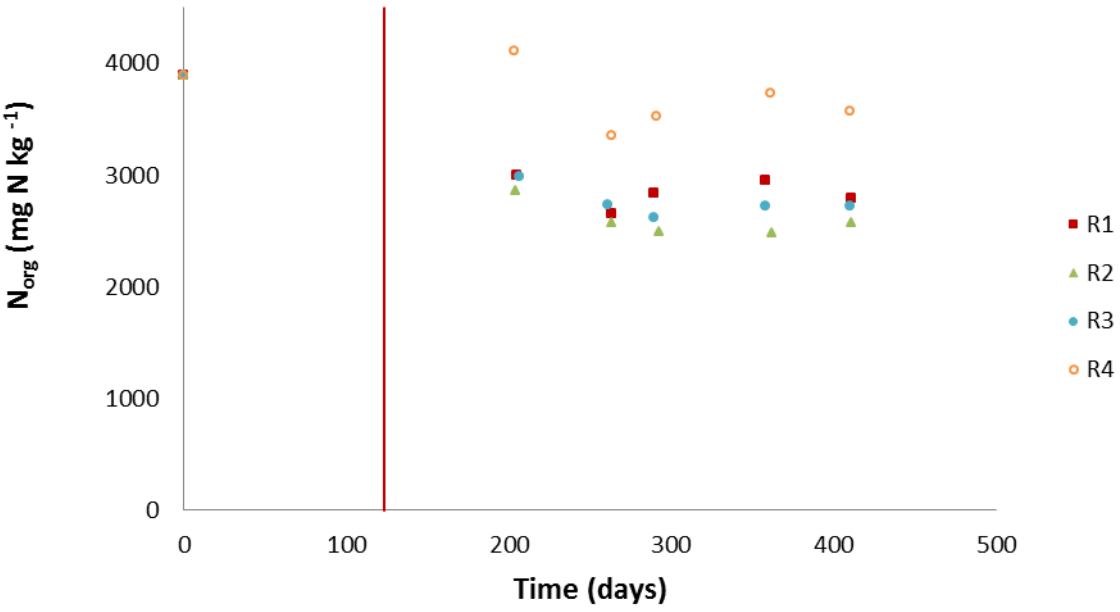


Fig. 54. Organic nitrogen

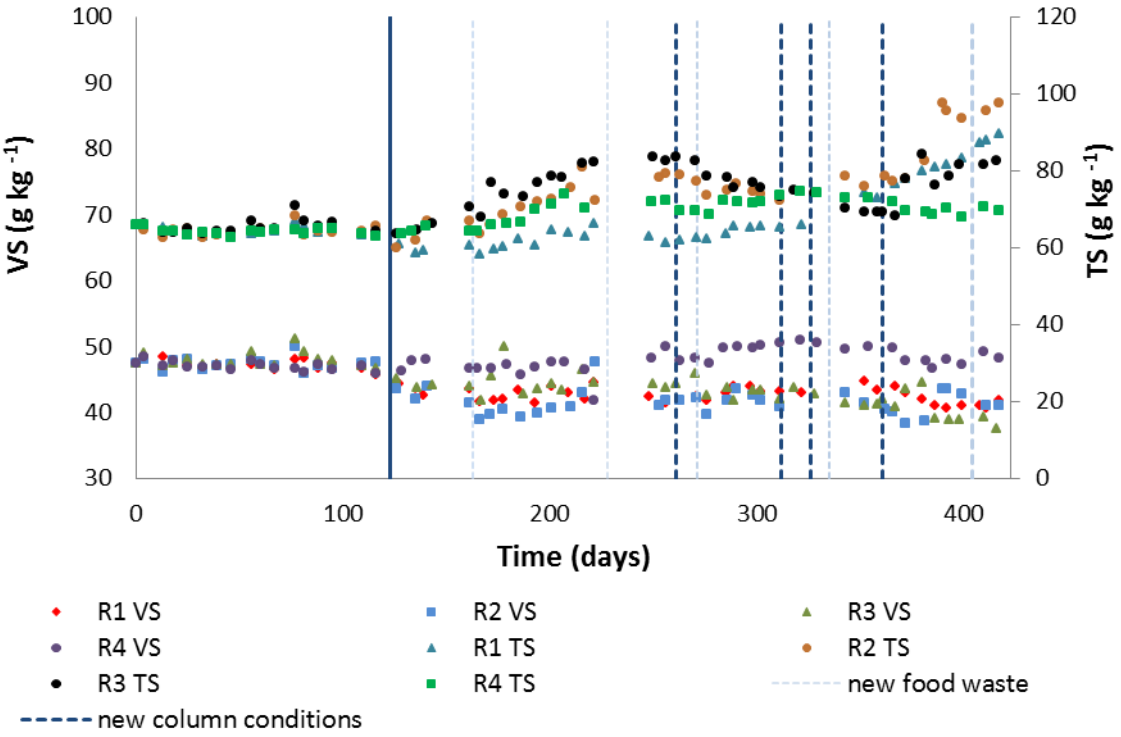


Fig. 55. Total and volatile solids

Alkalinity. The bicarbonate alkalinity (PA) profile (Fig. 53) shows a sharp increase due to the addition of lime. The CaO reacts with the CO_2 present in the stripping biogas and precipitates as calcium carbonate. The alkalinity in digester R_1 between days 123 and 260 is lower than the control digester as NH_4^+ is also lost from the system. Fig. 53 also shows that the IA in digester R_1 remained the same as in the control digester R_4 when there was no pH adjustment in the stripping column (days 123-260), whereas in digesters R_2 and R_3 it increased. An increase in the IA normally indicates a change in the concentration of VFA; however, this is not the case here as there was no indication of this occurring (Fig. 49). Increases in the IA/PA ratio show potential instability of the system and stable digesters typically have IA/PA ratios around 0.3 (Neiva Correia et al., 2008). During the baseline assessment (phase 1) and the control reactor IA/PA ratio fluctuated around 0.3. When coupled to the stripping columns the IA/PA ratio increased for all stripping conditions, with average values of 0.4 for stripping columns at 70 ºC without pH adjustment and 0.5 when pH is modified. IA/PA ratio for all the reactors remained below 0.8 (Fig. 50), not an uncommon value in stable digesters with high alkalinity.

It is clear that the changes in alkalinity-related parameters are brought about by the conditions in the stripping columns, including the addition of lime to control pH which in turn removes CO_2 from solution. The removal of ammonia will also change the alkalinity and buffering capacity of the digesters. These changes did not, however, appear to effect the overall productivity of the system as measured by specific biogas production nor its stability as assessed directly by the concentration of VFA rather than by a change in the IA/PA ratio.

TS and VS. An increase in TS concentration was seen in digesters coupled to stripping columns in which the pH had been increased by the use of lime. This was the case between days 123 and 260 in digester R_2 and R_3 where the TS was 9.5-15.7 % higher than in the control (Fig. 55). A similar observation was made from day 326 of operation for digesters R_1 and R_2 and from day 361 for R_3. A corresponding decrease in TS occurred between days 261-312 in digesters R_2 and R_3 when the pH in the stripping column was not increased: in both cases TS decreased until it reached that of the control digester. The TS concentration in digester R_1 between days 123-260 was 14.5 % lower than the control. It is postulated that this may be due to the high temperature in the stripping reactor accelerating or improving the hydrolytic conversion. Evidence to support this comes from the observed slight decrease of VS in the reactors with side-stream stripping under all stripping conditions (Fig. 55). It is thought that part of the VS of the liquor placed in the stripping columns is converted to VFA; in addition some of the N_{org} may also be broken down to ammoniacal nitrogen. This hypothesis also offers an explanation for the observation that there is no increase in TS and VS concentration over the duration of the stripping period, as might have been expected since water is lost from the stripping column as condensate. Without additional water production through improved hydrolysis both the TS and VS would be expected to rise.

VS destruction during the stripping period was calculated using the methods in section 3.6.1. To estimate the mass removed from a digester with side-stream stripping the mass of digestate lost due to evaporation during the stripping process needs to be taken into consideration. The experimental mass changes in traps in the stripping column at different stripping temperatures are shown in Table 34.

Table 34. Experimental data of mass gain in traps per day (average per day)

Conditions	Average (g)	Max (g)	Min (g)
70 °C	22	58	6
55 °C	8	12	3
85 °C	37	75	12

Table 35 shows the average VS destruction during the stripping period. Both methods showed good agreement for the digester without stripping (R_4) (Table 35 and Fig. 51). The difference for digesters coupled to stripping columns was larger, even after correction for digestate loss; nonetheless the variation was < 4 %. All digesters coupled to stripping reactors experienced higher VS destruction rates than the control digester (in the range of 3.5 - 8 %), but the methane yield did not increase in proportion to the improved VS reduction. Similar results were found by Hartmann and Ahring (2005) in a combined system involving a thermophilic digester and hyper-thermophilic treatment at 68 ºC, where VS reduction increased by 6 - 7 % when compared to the control without post-treatment but without any improvement in CH_4 yield. In the study by Hartmann and Ahring (2005) the gas produced during the treatment was determined and included in the final production, whereas in the stripping process carried out in the current work this was not measured.

Table 35. VS destruction %

	R_1	R_2	R_3	R_4
M_{exp}	88.0	89.8	87.7	81.8
M_{cal}	85.0	86.0	85.1	81.5

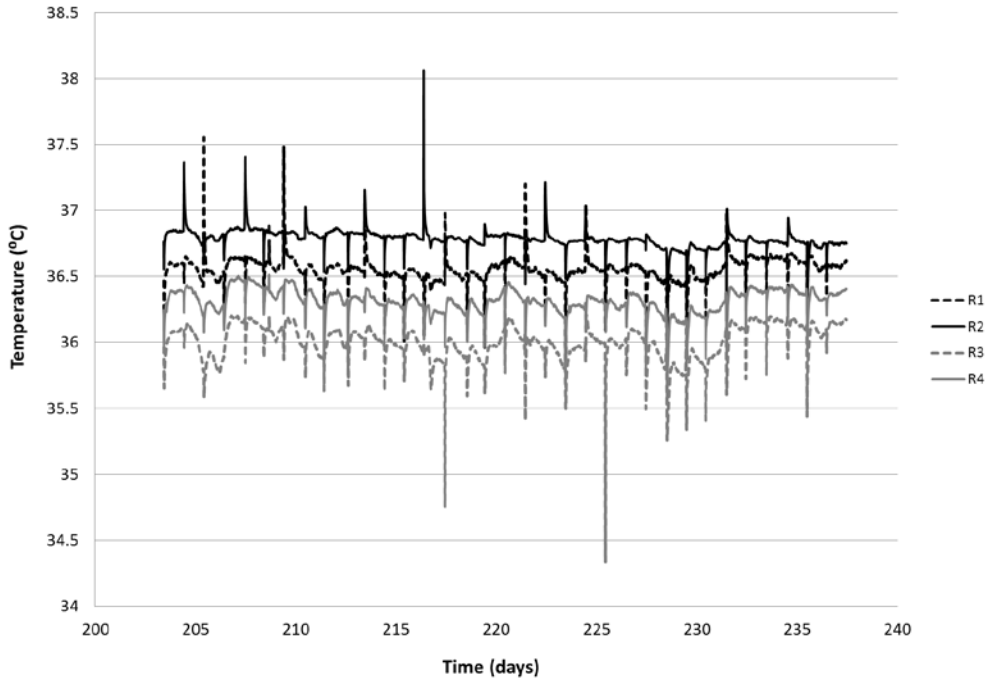
M_{exp}: determined using experimental mass of digestate
M_{cal}: digestate outlet calculated using experimental biogas production

Conclusion. The results showed that side-stream stripping was effective in reducing the total ammonia nitrogen in mesophilic food waste digestate, starting from a relatively high concentration that would have been toxic under thermophilic conditions. Removal of a proportion of the digester contents and exposure of them to thermophilic conditions with pH adjustment had no adverse effect on performance in terms of gas production or VS destruction. The research thus shows the way forward to the application of this technique in preventing the build-up of ammonia to toxic levels in thermophilic conditions and/or with feedstocks containing an even higher proportion of

organic nitrogen, if the digester is initially set up with a low-nitrogen inoculum. This process requires high pH and temperature (≥70 ºC) to achieve high TAN reduction. The potential to control the nitrogen content also opens up the possibility of creating 'designer digestates' in which the balance of nutrients is tailored to the soil type and crop needs; while the extracted ammonia is itself a valuable fertiliser product for application during crop growth (Gowariker et al., 2009).

Short-term effects of return of stripped digestate

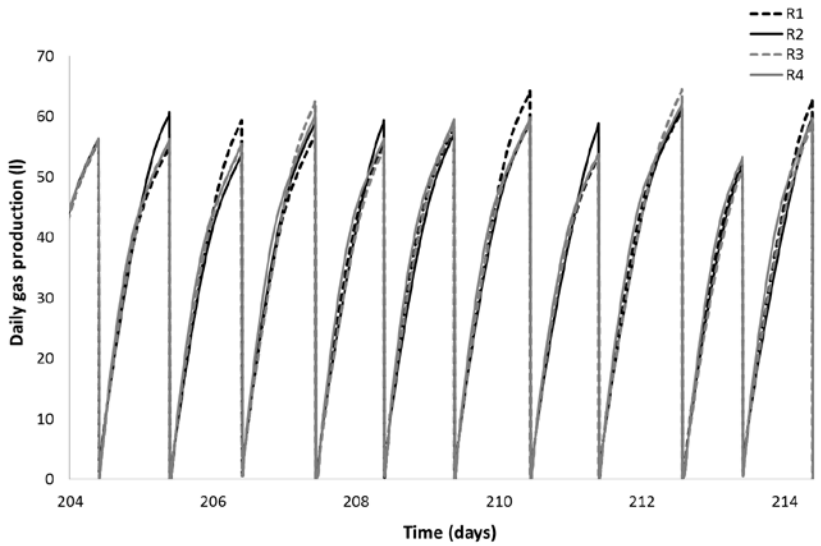
Reactor temperature was logged during the experiment, and a typical temperature profile for a period in which stripped digestate was returned to the digester is shown in Fig. 56. Temperature in all the digesters was controlled between 35.5 ºC and 37.0 ºC, and showed a brief decrease after digester feeding and a brief increase when the stripped digestate was returned to the digester. These instabilities were mitigated in a short period of time. The reactors were connected in series to the thermo-circulator, and this explains the slight difference in temperature ($T_{R2} > T_{R1} > T_{R4} > T_{R3}$).



Day	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	
Column		2	1	3	2		1	2	3		1,2			2,3	1		2	
Day	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	
Column		1,3	2		1,2,3			2	1	3		1,2			3,2	1		

Fig. 56. Reactor temperature profile and calendar showing column replenishment dates

With regard to biogas production, an increase in the daily volume produced was observed on days when the stripped digestate was returned to the digester (Fig. 57). This may have been caused by the VFA produced during stripping, or by a higher hydrolysis rate produced by a brief local rise in temperature when the stripped-digestate is returned.



Day	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215
Column	1, 2, 3			2	1	3	2		1	2	3		1, 2		

Fig. 57. Daily gas production with higher peaks corresponding to days when stripped digestate is returned

A difference in the biogas production rate was also observed when the stripped digestate was returned to the digester (Fig. 58). Biogas production rates after feeding were initially smaller on days when digestate was returned to the digester; after 8-9 hours, however, the production rate increased significantly. In phase 1 without stripping, all the digesters had a closely similar biogas production rate profile to that of R_4 during the stripping period (phase 2) (results not shown).

Changes in total VFA concentration after feeding and returning the stripped digestate to the digesters were analysed over a 2.5 to 8-hour period after feeding. Although the stripped digestate in some cases had a high VFA concentration, the final VFA concentration in the digester did not reach

problematic levels (Fig. 59), due to the dilution factor. It is therefore unlikely that the initial decrease in biogas production rate was caused by high initial VFA concentrations, and it may be due to re-solubilisation of CO_2 into the stripped digestate.

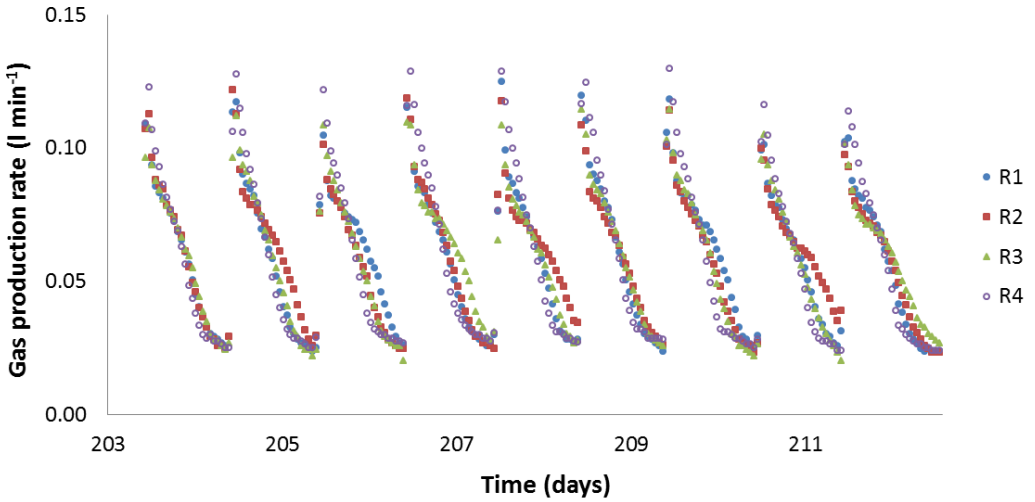


Fig. 58. Gas production rate with changes corresponding to days when stripped digestate is returned

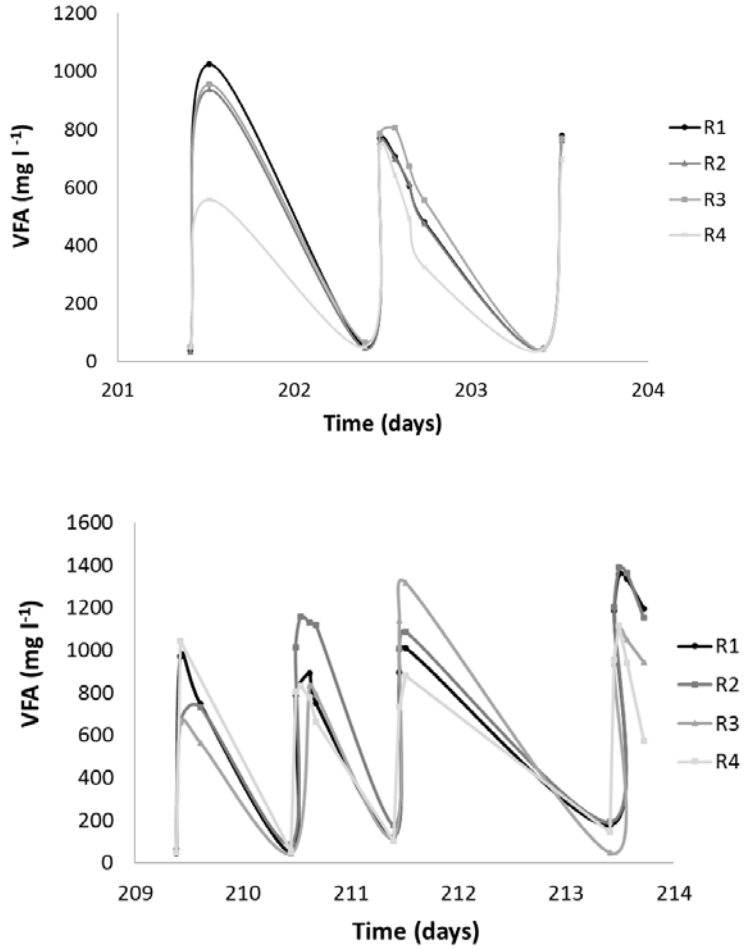


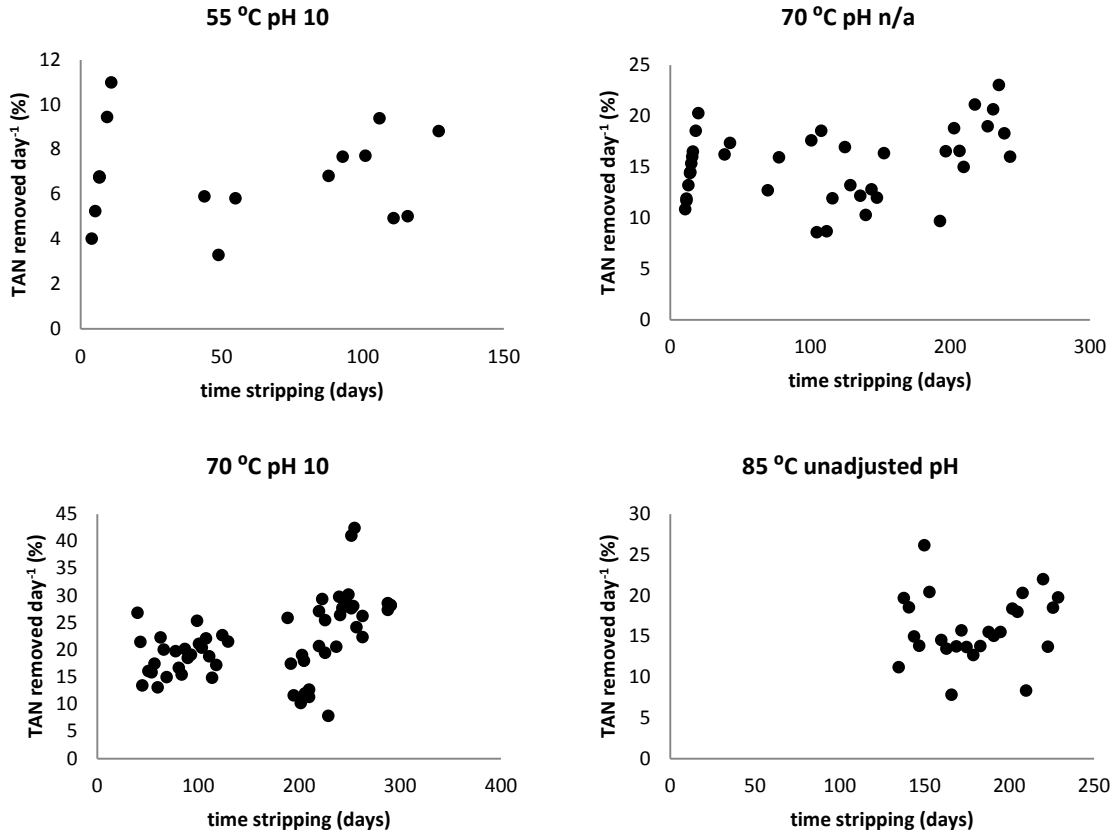
Fig. 59. VFA profile in the reactors after feeding (day 213 missing data)

Stripping columns

To determine the actual TAN removal in the stripping columns themselves, the TAN concentration was measured at the start and end of the stripping process for each of the stripping column conditions used. The average results are shown as % TAN decrease in Table 36; Fig. 60 shows the development of the average TAN removal with time during the side-stream process. These results confirm that both pH and temperature are important controlling factors, and as both increase so does the % TAN removal, with the highest value achieved at 85 ºC with pH 10.

Table 36. TAN concentration (average) decreased per day

	% TAN decrease day^{-1}
55 ºC pH 10	6.8
70 ºC unadjusted pH	15.4
70 ºC pH 10	21.1
85 ºC unadjusted pH	16.4
85 ºC pH 10	32.4



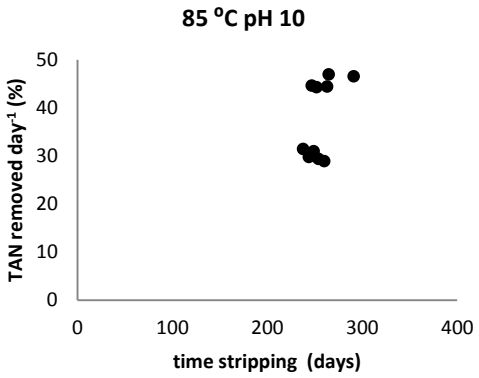


Fig. 60. TAN concentration (average) decreased per day in side-stream

Fig. 61 shows the results of a kinetic study of N removal in the stripping columns carried out on day 318 to 386 in which samples were removed from the stripping columns via a tubular sampling port installed at the top of the columns (see section 3.4.3). The results are shown in Table 37. The trend found in the time constant in different conditions agreed well with the TAN % decreased per day.

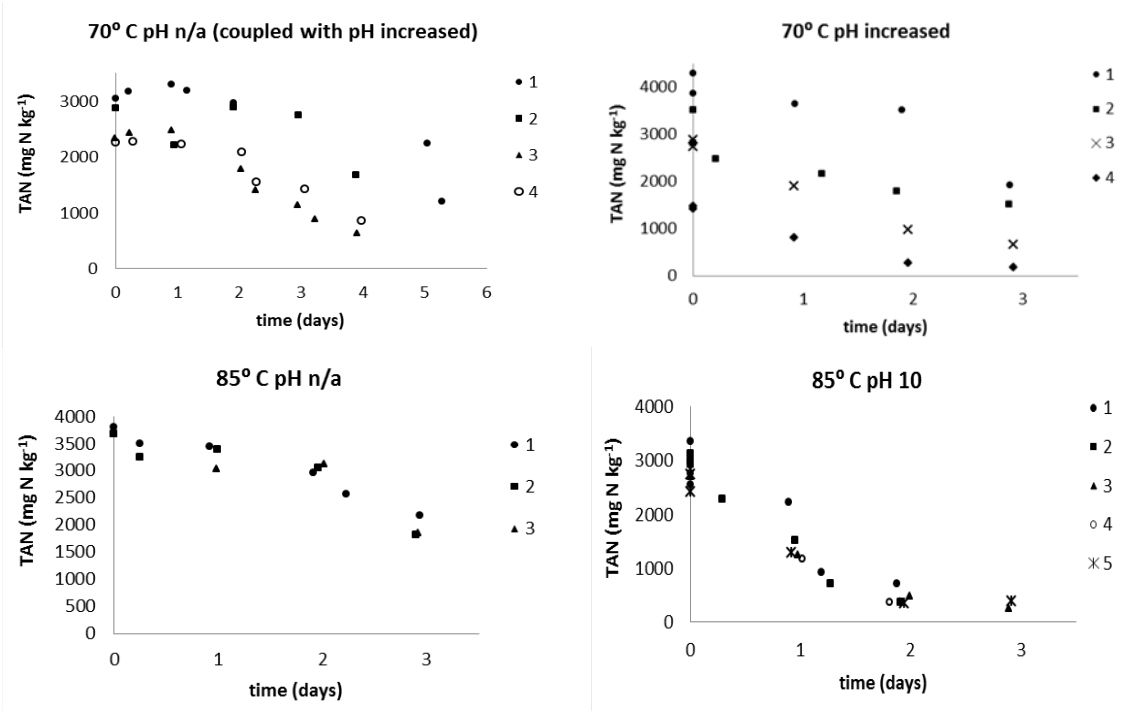


Fig. 61. N removal kinetic study: TAN profiles in stripping columns

Table 37. Time constants

	τ (hours)
70 unadjusted pH	155.1
70 pH 10	71.1
85 unadjusted pH	122.5
85 pH 10	27.1

In the batch stripping experiment (section 4.3) a time constant of 250 hours was determined for 70 ºC, 0.125 l biogas min^{-1} l^{-1}, and unadjusted pH (run 5 and 7). It is evident that sharing the same gas line in the side-stream stripping experiment has noticeably improved ammonia removal.

A sharp decrease in the biogas CO_2 concentration (from 36 % to 0 – 20 %) was observed where pH was increased using lime (batch stripping experiments and side-stream stripping) due to the carbonation reaction.

Digestate solids separation and dewaterability results

Dewatering of digestate is a common procedure in industrial wastewater treatment plants to reduce the volume by eliminating water, and thus reduce transportation costs. To achieve this volume reduction evaporation beds, vacuum filters, belt and filter presses, centrifuges and other industrial drying processes are employed.

The higher the VS concentration and the more colloidal the sludge or digestate, the more difficult it is to dewater. This property can be improved by conditioning before thickening and dewatering. Mineral chemicals such as iron salts and lime are used for this purpose to neutralize the colloidal surface charge by oppositely charged organic polymers or inorganic chemicals. Conditioning increases the particle size and decreases the bound water. Lime as a conditioning agent is only used in conjunction with iron salts on filter press applications; it is also used after dewatering to stabilize sludge (Turovskiy and Mathai, 2006).

The CST results show values greater than 6 hours for all digestates (digesters coupled to stripping columns and control) which indicates the poor dewaterability of the food waste digestate when filtering techniques are used. Since the CST time was not altered by the thermal-alkaline treatment, there is no improvement in filterability associated with the side-stripping operation.

The appearance of the samples at the end of the FIC analysis is shown in Fig. 62. A significant difference can be seen in the amount of supernatant for the digesters coupled to stripping columns and the control (R_4). The supernatant to total volume ratio was 60 % in digestate collected from the digesters with stripping and 10 % in the control, where no stripping is applied. The result indicates that the flocs break releasing water more easily in those digesters with side-stream stripping.

As can be seen from Fig. 63, the appearance of the digestate is not modified by the side-stream stripping process. The only apparent difference was in digestate from digester R_2, which had a lighter colour. Table 38 shows the digestate characteristics on the date when the picture was taken.

Table 38. TS, VS and TAN of digestate when Fig. 63 was taken

	TS g kg^{-1}	VS g kg^{-1}	TAN g N kg^{-1}
R_1	77.8 ± 0.1	43.0 ± 0.2	3.0
R_2	78.0 ± 1.8	38.3 ± 0.7	1.9
R_3	73.2 ± 6.7	43.6 ± 3.0	2.9
R_4	69.4 ± 1.0	47.8 ± 0.7	5.4

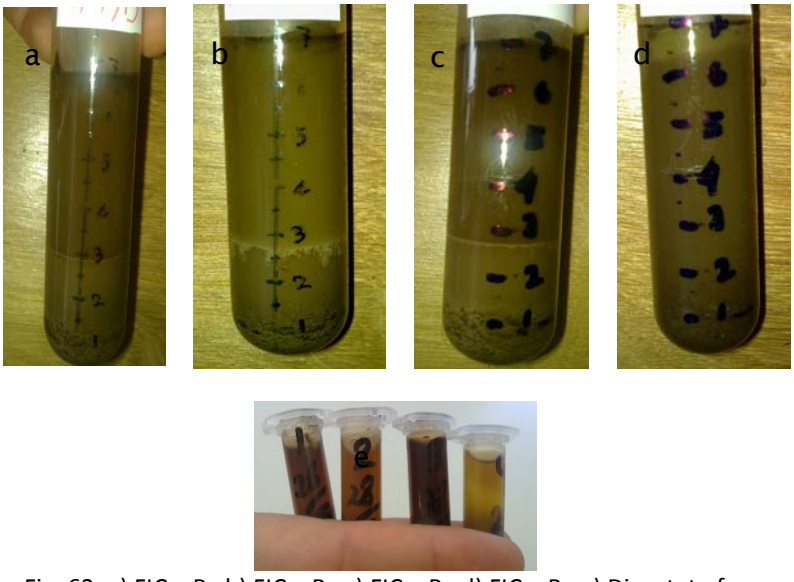


Fig. 62. a) FIC – R_1 b) FIC – R_2 c) FIC – R_3 d) FIC – R_4 e) Digestate from digester 1, 2, 3 and 4 centrifuged for 30 min at 13000 rpm (VWR; Galaxy 16DH)

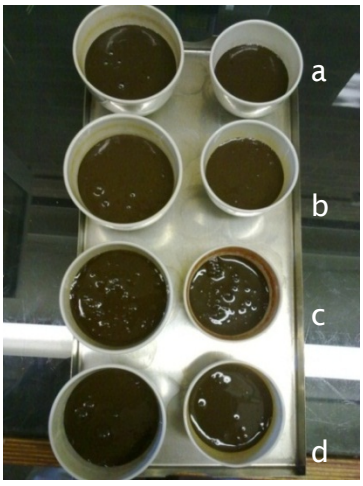


Fig. 63. Stripped and unstripped digestates
The picture shows samples taken on 4/5/13, 249 days after side-stream stripping began a) R1 70 °C, unadjusted pH, 1 column, 4 days in column, b) R2 T1:70 °C, pH 10, 3 days. T2:70 °C, unadjusted pH, 4 days, c) R3 85 °C, pH 10,1 column, 2 days in column, d) R4 control

^{14}C assay for dominant methanogenic pathway

At the end of the experimental period the ^{14}C labelling assay carried out by Dr Ying Jiang showed an average $^{14}CO_2$:$^{14}CH_4$ ratio of 4.40 for the control reactor (TAN 5600 ± 70 mg N kg^{-1}) (Table 39).

This ratio indicates the dominant methanogenic pathway was via syntrophic acetate-oxidising bacteria. The same result was found by Jiang (2012), who detected a higher quantity of ^{14}C labelled carbon dioxide in the biogas when analysing food waste anaerobic digestate with high ammonia concentration (5 - 6 g N l^{-1}). The ratio in the ammonia-stripped digester R_2 (TAN 1770 ± 20 mg N kg^{-1}) was 0.38, however, indicating that the acetoclastic route was now predominant in this case, even though the original inoculum for both digesters was the same and came from a digester in long-term operation on food waste. Schnürer and Nordberg (2008) showed a similar $^{14}CO_2$:$^{14}CH_4$ ratio between 0.5 - 0.8 for feedstock of diluted food waste with a low TAN concentration (0.65 - 0.9 g N l^{-1}), indicating that the main methanogenic pathway was acetoclastic. They also supplemented a reactor with egg albumin to increase the TAN concentration, and found that at 5.5 g N l^{-1} the mechanism had clearly shifted to syntrophic acetate oxidation ($^{14}CO_2$:$^{14}CH_4$ ratio above 2). The same technique applied on 11 digesters characterised by different VFA and salts concentrations (ammonium, Na^+ and K^+) exhibited increasing degree of syntrophic acetate oxidation when salts and VFA concentration followed the same trend (Schnürer et al., 1999).

Therefore, the current result confirms that even after long-term operation on food waste (123 days without stripping and 300 days with side-stream stripping) the acetoclastic population can be recovered when TAN concentration is decreased by side-stream stripping.

Table 39. Results from ^{14}C labelling experiment

Sample		^{14}C kBq	Count Eff.[a]	Total ^{14}C recovered kBq	% Rec[b]	$^{14}CO_2$:$^{14}CH_4$	TAN (FA) mg N kg^{-1}
R_2 1	Sludge	0.67	84.11				
	CO_2	0.22	95.19	1.40	93%	**0.42**	
	CH_4	0.51	95.16				1770 ± 20 (99 ± 1)
R_2 2	Sludge	0.69	83.21				
	CO_2	0.17	95.18	1.37	91%	**0.34**	
	CH_4	0.51	95.15				
R_4 (control) 1	Sludge	1.00	87.59				
	CO_2	0.26	94.92	1.31	87%	**4.43**	
	CH_4	0.06	95.05				5600 ± 70 (500 ± 6)
R_4 (control) 2	Sludge	1.00	87.57				
	CO_2	0.27	95.07	1.32	88%	**4.37**	
	CH_4	0.06	95.15				

[a] Counting efficiency determined by scintillation counting
[b] Recovery rate including kBq recovered from sample/medium mixture, 5M NaOH trap and 1M NaOH trap. 1.50 kBq was the initial dose in the anaerobic medium

The ^{14}C labelling assay does not show whether the microbial population structure itself is different in the control and ammonia-stripped digester, but simply indicates that the metabolic route has changed. *Methanosarcina* is able to utilise both the acetoclastic and the hydrogenotrophic pathways (Garcia et al., 2000). At the end of the experimental work Fluorescence In-Situ Hybridization (FISH) (Daims et al., 2005) was conducted by Dr Louise Byfield to compare the microbial populations of the control digester and R_2 the reactor showing the lowest TAN concentration when side-stream stripping was applied. However, the technique did not obtain positive FISH signals with EUB338, EUB338+, ARC915, MX825, MS1414, hMS1395, hMS1480, MG1200, MB1174 and MC1109 as oligonucleotide probes and *Bacteria*, *Archaea* (*Methanosaetaceae, Methanosarcinaceae, Methanomicrobiales, Methanobacteriales and Methanococcales*) as targeted groups. FISH techniques showed similar results when used in similar digestates by Yirong (VALORGAS D4-4, 2013).

The lack of positive FISH signals does not mean that the target organisms are not present in the sample. There are several reasons for a false negative result, e.g. target cells per volume of sample or rRNA concentration in the target cells are below the detection limits, probe target site inaccessibility, target cells might have a cell impermeable to the labelled oligonucleotide probes or microorganisms grow within a not translucent material and abiotic material contained in samples can also complicate the detection (Daims et al., 2005). In this specific case it is believed that the complex high-molecular-weight mixture of extracellular polymeric substances (EPS) excreted by the microorganisms which characterised the highly dense, thick and with low dewaterability properties in the digestate originated the false negative result in the FISH analysis.

4.4.2.3 Digester N mass balance

Control reactor

The nitrogen mass balance for the control reactor at steady-state was calculated using the methods in section 3.6.4.2. Table 40 shows the nitrogen balance calculated using two different values for outlet digestate mass: experimental and calculated via a mass balance based on experimental data for food waste mass added to the reactor and biogas yield production. The difference between the amount of nitrogen that goes into the reactor and the quantity that leaves the reactor in the liquid effluent (nitrogen in the gas stream can be disregarded) varies between 0.8 and 17.5 %. Differences for some of the food waste batches may be due to the short time that a specific food waste was used as a feedstock (64, 42, 63, 68 and 19 days for the batches shown in Table 40), in comparison to the HRT (average value of 105 days), meaning that the reactor did not reach the specific steady state for a particular feedstock.

Table 40. Nitrogen mass balance to control reactor (OLR 2 kg VS m^{-3} day^{-1})

	Food waste				Digestate			Experimental		Calculated	
N	TS $g\ kg^{-1}$	TKN $\%_{d.b}$	$Mass_{FW}$ g	N_{in} g	TKN $mg_{N}\ kg^{-1}$	$Mass_{dig\ out\ EXP}$ g	$Mass_{dig\ out\ CAL}$ g	N_{out} g	Diff %	N_{out} g	Diff %
4	209.8	3.59	380	2.86	9120	293	309	2.67	6.5	2.82	1.3
5	218.6	3.71	340	2.76	8910	269	278	2.40	12.9	2.47	10.3
6	239.8	3.53	320	2.71	8984	249	251	2.23	17.5	2.25	16.8
7	229.3	3.01	336	2.32	9185	250	258	2.30	0.8	2.37	2.2
8	249.1	3.23	300	2.41	9002	250	226	2.25	6.7	2.03	15.8

N: Food waste batch number (Table 31); d.b.: dry basis; N_{in}: TKN into the reactor; N_{out}: TKN out of the reactor in the digestate; $Mass_{dig\ out\ EXP}$: Experimental mass of digestate out of the reactor; $Mass_{dig\ out\ CAL}$: Calculated mass of digestate out of the reactor; Diff: Difference between the amounts of TKN in and out of the reactor

Side-stream stripping system

The nitrogen mass balances for the side-stripping system at steady-state and in a time dependant system were calculated using the methods in section 3.6.4.2 (Table 41 and Table 42), showing similar results.

There is some disagreement between the experimental TAN concentration in the digester and the calculated concentration (Table 41). All the TAN concentration measured in the digesters in steady state show significantly lower TAN concentration than those calculated.

The difference between the calculated and measured TAN concentrations may be due in part to N_{org} destruction during the stripping procedure; when this loss is taken into account, however, there are still some differences (Table 43).

Table 42 shows the final calculated TAN concentration in an anaerobic reactor coupled to ammonia stripping at different stripping conditions and digester OLR. This calculation was done using the assumptions stated in section 3.6.4.2. However, some researchers have found that reactor operation parameters such as TE supplementation or OLR applied can modify the TAN/TKN partition ratio. Jiang et al. (2012) reported an unexpected decrease in TAN concentration (see Table 44) when the loading rate increased from 4 to 5 kg VS m^3 day^{-1} although the TKN in the feedstock and in all digestates showed very similar values. A relationship between TE supplementation and biological fixed nitrogen which may represent increased microbial biomass in the digestate have been found by Lindorfer et al. (2011). These differences in TAN/TKN ratio in anaerobic reactors may indicate altered ammonium uptake

by the microbial biomass. Experimental data of TAN concentration reached after complete hydrolysis in a reactor without nitrogen control need to be used in order to predict the final TAN concentration in the side-stream stripped reactor at different OLR (Table 44).

Table 41. Experimental and calculated TAN concentration (OLR 2 kg VS m^{-3} day^{-1})

Conditions	Exp	Calc in steady	Calc (t)	Differ %
55 ^{o}C, pH 10, 1.2 %	3797	4402	4411	14
70 ^{o}C, pH n/a, 1.5 %	2880	4192	4199	31
70 ^{o}C, pH 10, 2 %	2600	3203	3207	19
70 ^{o}C, pH 10, 3.5 %	1375	2494	2495	45
85 ^{o}C, pH n/a, 2 %	3500	3732	3740	6
85 ^{o}C, pH 10, 3 %	2497*	1785	1785	-

Exp: experimental data (mg N kg^{-1}) * Last TAN measurement not in steady state; Calc in steady: calculated, steady state conditions (mg N kg^{-1}); Calc (t): calculated, time dependent when steady state is reached. Final TAN concentration (mg N kg^{-1}); Differ %: difference between Calc (t) and Exp; n/a: not adjusted

Table 42. Final calculated TAN concentration (mg N kg^{-1}) and % reduction at different OLR (kg VS m^{-3} day^{-1}) and stripping conditions

	1		2		3		4		5		6	
OLR	TAN	R %	TAN	R %	TAN	R %	TAN	R %	TAN	R %	TAN	R %
1	2367	54	1655	68	3634	30	3007	42	1080	79	3947	23
2	3207	38	2495	52	4199	19	3740	27	1785	65	4411	14
3	3666	29	3013	42	4470	13	4111	20	2282	56	4628	10
4	3951	23	3362	35	4623	10	4330	16	2651	49	4749	8
5	4144	20	3613	30	4720	8	4473	13	2937	43	4825	6
6	4284	17	3613	30	4787	7	4574	11	3163	39	4877	5

1: 2 % reactor 70 ^{o}C pH 10
2: 3.5 % reactor 70 ^{o}C pH 10
3: 1.5 % reactor 70 ^{o}C pH n/a
4: 2 % reactor 85 ^{o}C pH n/a
5: 2 % reactor 85 ^{o}C pH 10
6: 1.2 % reactor 55 ^{o}C pH 10
TAN: mg N l^{-1}
R: TAN reduction when side-stream stripping is compared to control %

Table 43. TAN concentration calculated (OLR 2 kg VS m^{-3} day^{-1})

conditions	N_{org} removed %	Cal in steady	diff
55 ^{o}C pH 10 EXP 1.2 %	18.3	3679	-3.2
70 ^{o}C pH n/a EXP 1.5 %	20.6	3351	14
70 ^{o}C pH 10 EXP 2 %	23.2	2490	-4.4
70 ^{o}C pH 10 EXP 3.5 %	28.0	1859	26
85 ^{o}C pH n/a EXP 2 %	27.0	2879	-21.6
85 ^{o}C pH 10 EXP 3 %	23.9	1376	-

N_{org} removed %: experimental data from side-stream stripping
diff: difference from experimental result

Table 44. TAN/TKN ratio at different OLR

Reference	Feedstock	OLR kg VS m^3	TAN g N kg^{-1}	TKN g N kg^{-1}	TAN/TKN
Jiang et al. (2012)	SS-DFW	1.8	6.14	10.29	59.7
This study 75-L CSTR	SS-DFW	2	4.91	8.78	55.9
This study 75-L CSTR	SS-DFW	2	4.8	8.8	54.5
This study 35-L CSTR (control)	SS-DFW	2	4.83-5.56	8.75-9.18	0.55-0.62
Jiang et al. (2012)	SS-DFW	5	5.22	10.42	50.1
	SS-DFW	5	5.07	10.16	49.9
	SS-DFW	5	4.64	10.43	44.5
	SS-DFW	5	4.31	10.41	41.4
	SS-DFW	4	5.57	10.75	51.8
	SS-DFW	4	6.17	10.4	59.3
	SS-DFW	5	4.69	10.52	44.6
	SS-DFW	5	4.61	10.75	42.9
Zhang et al. (2012b)	SS-DFW	2	5	8.21	60.9
	SS-DFW+CS	2	1.5	3.43	43.7
	SS-DFW+CP	2	2.3	4.41	52.2
Obuli et al. (2012)	food, fruit and vegetable, green and paper waste (C/N 27)	0.65	2.7	Not reported	77
		1.6	3		
		2.6	2.7		
	food, fruit and vegetable, green and paper waste (C/N 32)	4	2.4		40
		10.65	1.9		
		4.35	2.2		
		7.7	1.8		
		7.3	1.8		

CS: cattle slurry; CP: card packaging

These results indicate that TAN profiles alone cannot give an accurate N balance for the side-stream system: TKN profiles are needed in order to develop an accurate model capable of estimating the final concentration under different OLR. It should also be noted that AD is a complex biologically-mediated process in which physico-chemical factors may cause changes in population density and/or nitrogen uptake by the microbial biomass in the digestate, and consequently to the TAN/TKN partition (Lindorfer et al., 2012) and the assumption of simple first order relationships between ammonia removal and TAN concentration present in the CSTR may not hold.

5. Conclusions and further work

5.1 Conclusions

The following main conclusions can be drawn from the work carried out:

- Laboratory investigations using synthetic digestate (TAN: 5 g N kg^{-1}) in the 75-L in situ bubbling reactor have shown that the reduction in TAN concentration is non-existent at mesophilic temperatures and small at thermophilic temperatures when moderate and complete gas mixing rates are used.

- Batch ammonia stripping experiments conducted on fresh food waste digestate at a violent mixing flow rate (0.125 l min^{-1} $l^{-1}_{digestate}$) with unadjusted pH at 35 ºC achieved no ammonia removal after 16 days of treatment; at a temperature of 55 ºC 48.3 % TAN was removed in 34 days. The low N removal rate achieved by the physicochemical method at 55 ºC would not be sufficient to prevent TAN concentrations greater than 2500 mg N kg^{-1} in a reactor treating food waste without additional measures. Therefore, in situ biogas stripping is not an appropriate solution to prevent ammonia inhibition in a thermophilic or mesophilic full scale anaerobic plant.

- Batch ammonia stripping experiments conducted with fresh food waste digestate and two alkali agents (sodium hydroxide and calcium oxide) gave similar ammonia removal rates (run 8 using NaOH and 9 using lime at 35 ºC and 0.125 l min^{-1} $l^{-1}_{digestate}$ provided an ammonia removal time constant of 909 in both cases; run 14 using NaOH and 16 using lime at 70 ºC and 0.125 l min^{-1} $l^{-1}_{digestate}$ had time constants of 44 in the first experiment and 62 in the second experiment, values that provides similar ammonia removal profiles; run 15 using NaOH and 17 using lime at 70 ºC and 0.250 l min^{-1} $l^{-1}_{digestate}$ delivered time constants of 37 and 57) and pH profiles, both were therefore equally efficient and suitable for the stripping process. The usage of lime is recommended when the digestate

is returned to the digester, however, to avoid the risk of inhibition by Na^+.

- Comparison between batch ammonia stripping experiments conducted with fresh, stored food waste digestate and synthetic digestate showed that ammonia stripping is a strongly digestate-dependent process. The ammonia removal rate is likely to be lower for not stored digestate from a semi-continuous digester treating real food waste in stable conditions than for the synthetic or stored digestate.

- The highest ammonia removal rates achieved in batch ammonia stripping experiments conducted with fresh food waste digestate established the conditions to be used in a side-stream stripping process. These conditions were 55 ºC pH 10 and 70 ºC pH 10 and unadjusted pH.

- Semi-continuous trials carried out in 35-L digesters fed on source segregated domestic food waste (OLR 2 kg VS m^{-3} day^{-1}) and coupled to stripping columns at low bleeding rate (2.0 - 3.5 % digester volume per day treated in the stripping process) were successful in reducing ammonia concentrations to below the toxic levels for thermophilic or mesophilic operation.

- The side-stream biogas stripping process requires high pH and temperature to achieve high TAN reduction in the anaerobic digester, and it is unlikely that stripping at 55 ºC and pH 10 would achieve the target reduction. This could, however, be achieved at ≥70 ºC.

- Side-stream stripping of ammonia by thermal alkaline treatment at the bleed rate used in these experiments showed no adverse effect on performance or stability of the digestion process in terms of gas production or VS destruction.

- Organic nitrogen destruction was improved by thermally-mediated alkaline hydrolysis in the stripping column. N_{org} of the stripped reactors was 20 - 33 % inferior than the control value in the reactor with no treatment applied.

- CO_2 precipitation as $CaCO_3$ marginally increased digestate pH and enhanced ammonia stripping in systems where the pH was not modified.

- A radioisotope experiment showed that the methanogenic population was able to adapt to different ammonia concentrations. In food waste digesters at high ammonia concentrations (TAN 5600 ± 70 mg N kg^{-1}) the dominant methanogenic pathway was via syntrophic acetate-oxidising bacteria; while in the ammonia-stripped digester R_2 (TAN 1770 ± 20 mg N kg^{-1}) the acetoclastic route was recovered and predominant.

- Although FISH is a very useful technique to reveal the population structure in digesters with no need to culture the microbes. In the current study, FISH showed limitations due to the lack of positive FISH signals caused by the complex EPS excreted by the microorganisms when digesting the food waste.

5.2 Further work

The laboratory experiments accomplished as part of this work were terminated due to time limitations, and thus it was not possible to further investigate the behaviour of a thermophilic reactor when it is coupled to biogas stripping columns. However, the research has shown great potential for the application of this technique in preventing the build-up of ammonia in thermophilic conditions, if the digester is initially set up with a low-nitrogen inoculum. Further work should focus on testing the process with thermophilic digesters and at increased OLR (> 2 kg VS m^{-3} day^{-1}). These trials together with an economic study of the process will provide definitive information about the scope for application of side-stream stripping to anaerobic digestion of food waste and other high-nitrogen wastes and on its economic feasibility.

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