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DOI: [10.1016/j.wasman.2013.10.024]
Anaerobic digestion of autoclaved and untreated food waste

Elina Tampio\textsuperscript{a, }, Satu Ervasti\textsuperscript{a}, Teija Paavola\textsuperscript{a, 1}, Sonia Heaven\textsuperscript{b}, Charles Banks\textsuperscript{b}, Jukka Rintala\textsuperscript{a, 2}

\textsuperscript{a}Bioenergy and Environment, MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland

\textsuperscript{b}University of Southampton, Faculty of Engineering and the Environment, Southampton SO17 1BJ, UK

Abstract

Anaerobic digestion of autoclaved (160 °C, 6.2 bar) and untreated source segregated food waste (FW) was compared over 473 days in semi-continuously fed mesophilic reactors with trace elements supplementation, at organic loading rates (OLRs) of 2, 3, 4 and 6 kg Volatile solids(VS)/m\textsuperscript{3}d. Methane yields at all OLR were 5-10 % higher for untreated FW (maximum 0.483 ± 0.013 m\textsuperscript{3}CH\textsubscript{4}/kgVS at 3 kgVS/m\textsuperscript{3}d) than autoclaved FW (maximum 0.439 ± 0.020 m\textsuperscript{3}CH\textsubscript{4}/kgVS at 4 kgVS/m\textsuperscript{3}d). The residual methane potential of both digestates at all OLRs was less than 0.110 m\textsuperscript{3}CH\textsubscript{4}/kgVS, indicating efficient methanation in all cases. Use of acclimated inoculum allowed very rapid increases in OLR. Reactors fed on autoclaved FW showed lower ammonium and hydrogen sulphide concentrations, probably due to reduced protein hydrolysis as a result of formation of Maillard compounds. In the current study this reduced

\textsuperscript{}Corresponding author. Tel.: +358 29 531 7800, E-mail address: elina.tampio@mtt.fi

\textsuperscript{1}Present address: Biovakka Suomi Ltd, Autokatu 8, FI-20380 Turku, Finland

\textsuperscript{2}Present address: Department of Chemistry and Bioengineering, Tampere University of Technology, FI-33101 Tampere, Finland.
biodegradability appears to outweigh any benefit due to thermal hydrolysis of ligno-cellulosic components.

Keywords

Food waste, anaerobic digestion, autoclave treatment, organic loading rate, nitrogen

1. Introduction

Anaerobic digestion is an efficient technique for the treatment of source segregated biodegradable municipal wastes, e.g. biowastes and food waste (FW), as it recovers energy in the form of biogas for use in combined heat and power (CHP) plants, in vehicles and for grid injection; and also allows recycling of nutrients through application of digestion residues in crop production. Both the Renewable Energy directive (2009/28/EC, EU 2009) and the Landfill directive (99/31/EC, EU 1999) have been strong drivers in promoting the use of anaerobic digestion for this application in recent years.

Although co-digestion of FW with sewage sludge and animal manures has been common practice, treatment of FW alone has often proved difficult (Banks et al. 2008, Neiva Correia et al. 2008, Zhang et al. 2012). These difficulties have been attributed to ammonia inhibition resulting from a high protein content (Gallert et al. 1998), and are often indicated by accumulation of volatile fatty acids (VFA) (Banks et al. 2012). To achieve stable anaerobic digestion with FW alone, organic loading rates (OLR) are usually maintained at low values: 2.25 kgVS/m$^3$d at a hydraulic retention time (HRT) of 80 days in Banks et al. (2011) and from 1-4 kgVS/m$^3$d (HRT 14-30 days) as reported in
Cecchi et al. (2003). VFA accumulation at higher OLR has recently been linked to trace element (TE) deficiencies (Banks et al. 2012). When supplemented with TE successful FW digestion has been reported at OLRs of 5 kgVS/m³d (Banks et al. 2012) and 6.64 kgVS/m³d (Zhang and Jahng 2012).

Thermal and hydrothermal pre-treatments have been widely studied as a means of hydrolysing recalcitrant components in a wide range of wastes to make them easier to degrade (Papadimitriou 2010, Ren et al. 2006, Takashima and Tanaka 2008); these techniques have also been used as pre-treatments before anaerobic digestion of mixed biowastes (Lissens et al. 2004, Sawayama et al. 1997). One such hydrothermal treatment is autoclaving, where water is used as a reagent at increased temperature and pressure, to hydrolyse and solubilise sugars, starch, proteins and hemicellulose (Papadimitriou 2010, Ren et al. 2006). Materials pre-treated by autoclaving under various conditions have shown increased methane production in batch tests: digested swine slurry autoclaved at 120 °C showed an increase in CH₄ yield of 115 % (Menardo et al. 2011) and autoclaving of mixed kitchen garbage (175 °C, 40 bar, 1 hour) increased CH₄ yield by 30 % (Sawayama et al. 1997). Improved methane production has also been observed in continuously-stirred tank reactors (CSTRs) treating waste activated sludge (WAS), with 12 % and 25 % increases after autoclaving at 135 °C and 190 °C, respectively (Bougrier et al. 2007).

In contrast, more aggressive thermal and hydrothermal pre-treatments at higher temperatures (around 180 °C) have been reported to decrease biodegradability and biogas production during anaerobic digestion of WAS and sewage sludge (Bougrier et al. 2008, Pinnekamp 1989). This is believed to be related to the formation of complex and inhibitory Maillard compounds, produced by reactions between amino acids and
carbohydrates (Bougrier et al. 2008, Takashima and Tanaka 2008). Maillard compounds start to form at temperatures above 100 °C depending on the retention time (Müller 2001, Nursten 2005), while the formation of more complex compounds, such as acrylamides and other vinylogous compounds, increases at higher temperatures (180 °C, Stadler et al. 2004).

The aim of this study was to evaluate the anaerobic digestion of untreated and autoclaved (160 °C, 6.2 bar) FW at a range of different OLRs (2, 3, 4 and 6 kgVS/m³ day) in semi-continuously fed intermittently-stirred mesophilic reactors. The biochemical methane potential (BMP) of the feedstocks and the residual methane potential (RMP) of the digestates were also assessed in batch assays.

2. Materials and methods

2.1. Origin and characterization of FW and inocula

The source segregated domestic FW used in the study was collected from the South Shropshire Biowaste digestion plant in Ludlow, UK. Biodegradable bags used for waste collection were removed and the FW material was mixed and divided into two equal portions. One portion was pre-treated at 160 °C and 6.2 bars in a novel double-auger autoclave (AeroThermal Group Ltd, UK) that provides improved mixing and steam penetration; the other portion was left untreated. Both portions were then passed through a macerating grinder (S52/010 Waste Disposer, IMC Limited, UK), packed into 35-litre plastic boxes (7 untreated and 8 autoclaved), frozen and shipped at -20 °C to MTT Agrifood Research, Finland.

At MTT the frozen material was chopped into smaller portions corresponding to amounts required for weekly feeding of the digesters, and these smaller portions were
again stored at -20 °C. Each week portions of the autoclaved and untreated FW were
thawed and stored at 4 °C and used as daily feed. The pH, total solids (TS), volatile
solids (VS), ammonium nitrogen (NH$_4$-N), total Kjeldahl nitrogen (TKN), soluble
chemical oxygen demand (SCOD) and VFA content was determined for each new box
of feed.

The reactors were inoculated with digestate from a mesophilic CSTR digesting
mechanically dewatered sewage sludge (Biovakka Suomi Ltd, Turku, Finland) (Table
1). In the BMP assays inoculum was taken from an anaerobic digester treating
municipal and industrial biowastes (Envor Biotech Ltd, Forssa, Finland).

2.2. Semi-continuous trials

Four 11-litre stainless steel stirred tank reactors (STRs) (Metener Ltd, Finland)
were operated at 37 °C. Stirring (32 rpm) was semi-continuous with 5 seconds on and
60 seconds off. The reactors were fed manually five times a week through an inlet tube
which extended below the digestate surface, and which was also used for digestate
sampling. Digestate overflowed from the reactors by gravity through a u-tube trap to
prevent gas escape. Between days 1-195 hourly gas volume and methane content were
measured using an automatic system in which the produced biogas was collected into a
small (~220 ml) gas storage vessel on top of the reactor. From day 195 onwards, due to
break down of the automated system, gas volume was measured by water displacement
in a volume-calibrated cylindrical gas collector, after which the gas was collected in
aluminium gas bags.

Reactors were fed with untreated FW (R1) and autoclaved FW (R3). After 18
days acclimation period with reduced feeding the experiments started at an OLR of 2
kgVS/m$^3$ day, corresponding to HRT of 117 and 94 days for R1 and R3 respectively. On
day 151, after 1.1 (R1) and 1.4 (R3) HRTs, the OLR was raised to 3 kgVS/m$^3$ day and
after 1.3 (R1) and 1.7 (R3) HRTs to 4 kgVS/m$^3$ day on day 256 (HRT 78 d and 58 d for
untreated, 63 d and 47 d for autoclaved FW, respectively).

On day 327 parallel reactors fed on untreated (R2) and autoclaved FW (R4) were
started at an OLR of 3 kgVS/m$^3$ day, using 5.7 litres of digestate from R1 and R3
respectively as inoculum. After 2.8 and 3.4 HRTs in reactors R1 and R3 and 1.2 and 1.4
HRTs in reactors R2 and R4, the OLR in all four reactors was further increased to 6
kgVS/m$^3$ day on day 418, with a corresponding decrease in HRT to 39 d and 31 d in the
untreated and autoclaved FW reactors. Most of the data presented below are taken from
reactors R1 and R3 due to the longer running period. During days 179-193 reactors R1
and R3 were once a week supplemented with 11 ml of a trace element (TE) solution
containing Se (0.2 mg/l) and Co (1.0 mg/l). From day 199 onwards all reactors were
given a weekly supplement of two TE solutions, one containing cation elements (mg/l):
Al 0.1 , B 0.1, Co 1.0, Cu 0.1, Fe 5.0, Mn 1.0, Ni 1.0, Zn 0.2; and the other oxyanions
(mg/l): Mo 0.2, Se 0.2 and W 0.2 (Banks et al. 2012). 1 ml of each of these TE solutions
was added for each kg of digestate removed from the reactors over the one-week period.

Grab samples of digestate (about 250 g) were taken every two weeks for analysis
of TS, VS, SCOD, NH$_4$-N, TKN, and samples for VFA analysis (about 50 g) were taken
once a week. Digestate pH was measured weekly. Larger volumes of digestate were
collected on days 130 (2 l), 214 (1 l), 287 (1 l) and 321 (1 l). After removal of these
larger samples, daily feeding of the reactors was adjusted to compensate for the reduced
volume until the normal operating level was restored.
2.3. Biochemical and residual methane potential assays

BMP and RMP assays were performed at 37 °C using automated testing equipment (Bioprocess Control Ltd, Sweden). The assays were mixed mechanically (84 rpm) for one minute per hour. Carbon dioxide was absorbed by NaOH before the automated gas volume measurement, which was based on liquid displacement. Assays were conducted in duplicate or triplicate, each with a total liquid volume of 400 ml (BMP) or 200 ml (RMP assays). The inoculum to substrate ratio in BMP assays was 1:1 on a VS basis. NaHCO₃ (3 g/l) was used as a buffer and if the pH was lower than 7.5 it was adjusted to around 8 with 3 M NaOH. In RMP assays digestates from the STR reactors were incubated without inoculum. The results are given as average values of the triplicate or duplicate assays.

2.4. Analyses and calculations

TS and VS were determined according to SFS 3008 (Finnish Standard Association 1990) and NH₄-N according to McCullough (1967). TKN was analysed by a standard method (AOAC 1990) using a Foss Kjeltec 2400 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden), with Cu as a catalyst. For soluble COD analysis FW samples were diluted 1:10 with distilled water, and agitated for 1 hour. Diluted FW and raw digestate samples were centrifuged (2493 × g, 15 min) after which the supernatant was further centrifuged (16168 × g, 10 min) and stored in a freezer, then thawed before analysis according to SFS 5504 (Finnish Standards Association 2002). pH was determined using a VWR pH100 pH-analyzer (VWR International). Iron concentration was analysed according to Luh Huang and Schulte (1985) using inductively coupled
plasma emission spectrometry (ICP-OES) (Thermo Jarrel Ash Iris Advantage, Franklin, USA).

Samples for VFA analysis were centrifuged ($1831 \times g$, 10 min) and filtered with Chromafil GF/PET-20/25 filters. Concentrations of acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric and caproic acids were determined using a HP 6890 gas chromatograph with an HP 7683 autosampler (Hewlett-Packard, Little Falls, USA) and GC ChemStation Rev. B.03.02 software. The GC was fitted with a 10 m x 0.53 mm x 1 $\mu$m HP-FFAP capillary column (Agilent Technologies, USA) and a flame ionisation detector with helium as a carrier gas (9 ml/min). Oven temperatures were 60-78 °C (25 °C/min), isothermal 1 min, 150 °C (7.5 °C/min) and 25 °C/min to 180 °C with 3 min final time. The injector and detector temperatures were 220 °C and 280 °C, respectively.

From day 1 to 195 methane composition was determined automatically during emptying of the gas storage vessel by infrared analysis (ExTox Gasmess-Systeme GmbH, Germany). From day 195 to 314, gas composition was analysed using a portable Combimass GA-m gas analyzer (Binder Engineering GmbH, Germany), and during days 315-446 the infrared measuring equipment was used.

The reactor was fed for 5 days a week, but the OLR in kgVS/m$^3$day is expressed as the average daily weight of substrate fed to the reactor over a one-week period. HRT was calculated based on feedstock densities. All biogas and methane yields were converted to STP conditions (0 °C, 100 kPa) according to the ideal gas law. Methane yields in the RMP assays were calculated in two ways; by dividing the cumulative methane production by the 1) VS of the added digestate and 2) by the VS of the feed of
the semi-continuous reactors at the time of digestate sampling. The latter enables direct comparison of the methane yield in the RMP with that in the reactors. Free ammonia (NH$_3$-N) concentrations were calculated according to Anthonisen et al. (1976):

\[
\text{NH}_3\text{-N} = (\text{NH}_4\text{-N} \times 10^{\text{pH}})/((K_b/K_w) + 10^{\text{pH}}),
\]

(1)

where $K_b$ is the ammonia ionisation constant and $K_w$ the ionisation constant of water at 37 °C.

3. Results and discussion

3.1. Material characterization

The autoclaved FW appeared much darker than the untreated FW and had a pleasant caramel odour. TS and VS in the autoclaved FW were both about 15 % lower than in the untreated FW due to dilution by steam condensation during the autoclave treatment (Table 1). TKN on fresh matter basis was lower in the autoclaved FW (6.8 ± 0.3 g N/kg) than in untreated FW (7.4 ± 0.3 g N/kg). The autoclaved FW had about 22 % higher NH$_4$-N and 16 % higher SCOD, indicating that autoclaving had solubilised some organic nitrogen and carbon components. Total VFA concentrations were lower in the autoclaved material (2.2 ± 0.2 g/l) than in the untreated FW (3.1 ± 0.6 g/l) suggesting either that some VFA had volatilised during or after autoclaving, or that some acidification of the untreated material had occurred.

Changes in the chemical composition of materials during autoclave treatment are dependent on the temperature as well as the materials used. In this study autoclaving conditions of 6.2 bars and 160 °C were used. Increased concentrations of NH$_4$-N and solubilisation of carbohydrates have previously been reported after autoclave treatment.
of dewatered sewage sludge (175 °C, 20 bar), with an increase from 2.6 to 3.2 g NH₄-N/l (Inoue et al. 1996); temperatures above 90 °C have also been reported to increase ammonia concentrations from 0.35 gN/l to 0.7 gN/l in WAS (Bougrier et al. 2008).

3.2. BMP assay

The 35-day BMP value for untreated FW was 0.501 ± 0.020 m³CH₄/kgVS, while that for autoclaved FW was 0.445 ± 0.001 m³CH₄/kgVS (Figure 1, Table 1). The lower methane yield of the autoclaved FW could be explained by Maillard reactions. Support for the occurrence of these is given by the darkening in colour of the autoclaved FW and the caramelised odour, while the increase in SCOD provides evidence of increased solubilisation of carbon compounds. Similar phenomena have also been observed with autoclaved WAS (Bougrier et al. 2008) and municipal solid waste (Takashima and Tanaka 2008). In other studies higher methane yields have been reported after similar thermal treatments (Lissens et al. 2004), but this can be attributed to the improved availability of the ligno-cellulosic materials; and when these form a large proportion of the waste the resulting increase may far exceed any decrease due to Maillard compounds. In contrast where ligno-cellulosic content is low, as in this type of food waste (Zhang et al. 2012) reductions in methane yield may result.

3.3. Semi-continuous operation

3.3.1. Effect of loading rate on methane yields

Process parameters from the whole experimental period (days 1-473) are shown in Figure 2 and detailed results from the last four weeks of stable operation at each OLR are presented in Table 2. Operation was considered stable when variations were < 0.2 units in pH, < 90 mg/l in VFA and < 1.8 % in CH₄.
Throughout the experimental period specific methane yields were 5-10 % higher for untreated FW than for autoclaved FW. The methane yields at OLR 2 kgVS/m³ day were on average $0.443 \pm 0.038$ and $0.373 \pm 0.037$ m³CH₄/kgVS for untreated (R1) and autoclaved FW (R3), respectively. The highest yield for untreated FW was observed at OLR 3 kgVS/m³ day ($0.483 \pm 0.013$ m³CH₄/kgVS) while autoclaved FW produced the highest yield at OLR 4 kgVS/m³ day ($0.439 \pm 0.020$ m³CH₄/kgVS). When the OLR was further increased to 6 kgVS/m³ day methane yields decreased by 12 % and 11 % in untreated FW and autoclaved FW, respectively. The specific methane yield for autoclaved FW was lower at OLR 2 kgVS/m³ day than at higher OLRs, which could possibly indicate some acclimatisation. This was not seen in the untreated FW where the lowest specific methane yield occurred at OLR 6 kgVS/m³ day, which could indicate retarded hydrolysis as no increased SCOD nor VFA was detected. At OLR 6 kgVS/m³ day the difference in methane yields between the parallel (R2 and R4) and original (R1 and R3) reactors was < 7 % (Table 2).

In reactors R1 and R3 relatively long operating times were applied, to allow the process to stabilise between incremental increases in OLR. Using this approach, stable digestion of both autoclaved and untreated FW was achieved at the relatively high OLR of 6 kgVS/m³ day. It was also shown, however, that when an inoculum acclimated to the feedstocks was used in R2 and R4, the OLR could be rapidly increased without operational disturbances such as VFA accumulation. The maximum loading rates applied were similar to the 6.64 kgVS/m³ day achieved by Zhang and Jahng (2012) and higher than the 5 kgVS/m³ day of Banks et al. (2012). Both of these long-term digestion studies used trace elements supplementation, as did the present study.
As far as is known, this is the first study to report anaerobic digestion of autoclaved food waste in a semi-continuously fed system. Methane yields of 0.483 ± 0.013 and 0.423 ± 0.002 m³CH₄/kgVS for the untreated and autoclaved FW at OLR 3 kgVS/m³/day are in good agreement with previous studies, where a full-scale digester fed on the same type of source-segregated household food waste at an average OLR of 2.5 kgVS/m³/day yielded 0.402 m³CH₄/kgVS (Banks et al. 2011). Earlier pilot-scale studies gave an average of 0.390 m³CH₄/kgVS, but using a different source of source-segregated domestic food waste at higher OLR (3.5 to 4 kgVS/m³/day), and without TE supplementation (Banks et al. 2008). Laboratory-scale FW digestion with TE supplementation was reported to yield 0.352-0.439 m³CH₄/kgVS at an OLR of 6.64 kgVS/m³/day by Zhang and Jahng (2012); while in the study by Banks et al. (2012) the methane yield for TE supplemented FW was 0.435 m³CH₄/kgVS.

The maximum methane yields for untreated and autoclaved FW in the semi-continuous trials were 0.483 ± 0.013 and 0.439 ± 0.020 m³CH₄/kgVS respectively. These were slightly lower than the BMP values in each case. The results therefore strongly indicate that even after long periods of operation no significant acclimatisation that could improve the biodegradability of compounds produced in the autoclaving process had taken place.

With mixed biowastes, the benefits of increased biogas production due to improved degradation of ligno-cellulosic materials may outweigh any losses in biodegradability as a result of formation of recalcitrant compounds during thermal treatment. FW, however, has a relatively low ligno-cellulosic fibre content compared to other municipal biowaste components (e.g. garden or yard waste, paper and card), and in the present study the net effect of treatment was a reduction in specific methane yield.
This balance may however change with different autoclaving conditions, and in particular a lowering of temperature may produce more favourable results.

### 3.3.2. Digestion parameters

Results for pH, VFA, TS, VS, SCOD, NH$_4$-N, TKN are presented in Table 2 and Figure 3. pH in the untreated FW reactor remained around 7.8 throughout the experimental period, while with autoclaved FW the pH decreased from pH 7.6 at OLR 2 kgVS/m$^3$ day to 7.3 at OLR 6 kgVS/m$^3$ day.

At an OLR of 2 kgVS/m$^3$ day, total VFA concentration in both reactors remained under 250 mg/l. When the OLR was increased to 3 kgVS/m$^3$ day, VFA in the untreated FW reactor increased to 2400 mg/l by day 153, and consisted mainly of acetic (about 85%) and propionic acids (about 10%). In the autoclaved FW reactor VFA concentration showed smaller increases with peaks of 1500 mg/l on day 139 (consisting 98% of acetic acid) and 910 mg/l on day 160 (27% acetic acid and 65% propionic acid). The relatively large samples (2 litres) taken from the reactors on day 130 could have contributed to these increases in VFA concentration, but similar removals of digestate at later stages in the experimental run did not have this effect. VFA concentrations reduced to under 200 mg/l in both reactors by day 214, shortly after the introduction of trace element additions of selenium and cobalt on day 179 and full TE supplementation on day 199. This behaviour is consistent with previous reports of responses to TE supplementation where the VFA increase was linked with the loss of electron transfer interspecies during digestion (Banks et al. 2012).

TS, VS and TKN contents in both reactors gradually increased during the experimental period, with TS increasing from under 70 to over 80 g/kg. Despite the
lower feedstock solids concentration, the solids content in the autoclaved FW reactor
was slightly higher than in the untreated FW up to the end of OLR 4 kgVS/m$^3$day. After
OLR was increased to 6 kgVS/m$^3$day there was an increase in solids concentrations in
the untreated FW reactor, which was not apparent with the autoclaved FW. The initial
TKN concentration in both reactors was 4.9 g N/kg and showed a similar increase to ~8
g N/kg by around day 200. TKN in the untreated FW reactor continued to increase until
around day 300 at which point it stabilised at ~9 g N/kg, whereas for the autoclaved FW
it remained at ~8 g N/kg. The differences in TKN reflected the differences in feedstock
concentrations. The increases in solids content were most likely associated with the
increase in loading, although it is possible that some accumulation was due to
stratification despite the intermittent mixing, as surplus digestate was discharged from
an overflow at the top of the reactor. Mass balance calculations affirmed, in the
beginning of OLR 4 kgVS/m$^3$day, that accumulation of TKN was taking place.

The SCOD concentration in both reactors increased from around 10 g/l to over 20
g/l during the first 300 days of operation, then stabilised in the autoclaved FW reactor.
In the untreated FW reactor the SCOD increased sharply to ~36 g/l for over 50 days
then decreased equally sharply in the end of the run: these variations did not correspond
to changes in OLR and were not accomplished with changes in methane yield nor
digestate VFA. Total VFA concentrations accounted for only 0.5-2 % of the SCOD. A
probable explanation for the general increase in SCOD in both reactors is an increase in
the quantity of soluble microbial products present in the digestate; this phenomenon has
previously been observed with solid substrates and at long retention times (Kuo et al.

3.3.3. Ammonium and ammonia
NH₄-N concentration in the untreated FW reactor increased during the first ~170 days from 2.4 (inoculum) to 4 g/kg and then showed a very gradual decrease to around 3 g/kg by the end of the experimental run. This decrease could be associated with the increase in microbial biomass (Lindorfer et al. 2011) or in soluble microbial products caused by the increasing OLR. In the autoclaved FW reactor, however, NH₄-N decreased from 2.4 to about 1.2 g/kg by the end of the experimental period. The low NH₄-N concentrations in the autoclaved FW reactor were probably mainly due to the effect of autoclaving and the formation of Maillard compounds from the reaction of proteins with carbohydrates (Bougrier et al. 2007, 2008). Free ammonia concentrations in the reactors were calculated, but NH₃ remained below 0.30 g/kg in untreated FW and below 0.10 g/kg in the autoclaved FW reactor.

The pH value in the untreated FW reactor rose to around 7.8 by day 55 and remained relatively stable until the OLR was raised to 6 kgVS/m³day, at which point it fell very slightly. In the autoclaved reactor after a slight initial rise pH decreased during the experimental run to a final value of around 7.3. These pH values reflect the relative NH₄-N concentrations in each case, as NH₄-N provides buffering capacity (Procházka et al. 2012). High NH₄-N concentration can also inhibit the digestion process, but this is greatly dependent on the feedstock materials and acclimation times (Chen et al. 2008, Procházka et al. 2012). In the present study, after TE supplementation was introduced, there was no evidence of the VFA accumulation that is often associated with ammonia toxicity, and the free ammonia concentrations were similar to those previously observed in FW digestion (Zhang et al. 2012).

3.3.4. Gas composition
The biogas methane content in both autoclaved and untreated FW digesters was similar and ranged between 55-63% during the experiment, with an average of around 58% (Table 2, Figure 2). It did not appear to be affected by changes in applied OLR. In contrast, in a study by Zhang and Jahng (2012) on FW digestion the methane content was found to decrease from 53% to 48% as the OLR was gradually increased from 2.19 to 6.64 kgVS/m$^3$ day.

Hydrogen sulphide concentration was monitored between days 166-313 while the reactors were operated at OLR 3 and 4 kgVS/m$^3$ day (Figure 4). H$_2$S concentrations at OLR 3 kgVS/m$^3$ day were < 100 ppm in the untreated FW reactor and < 75 ppm in the autoclaved FW reactor. Shortly before the OLR was increased to 4 kgVS/m$^3$ day the H$_2$S concentration in the untreated FW reactor began to increase, and reached 480 ppm by day 314 at which point monitoring ceased; while in the autoclaved FW reactor H$_2$S content remained < 60 ppm. H$_2$S was also monitored at the OLR of 6 kgVS/m$^3$ day (days 448-473) and concentrations were 751 ± 182 ppm in the untreated FW reactors (R1 and 2) compared to 63 ± 4 ppm in the autoclaved FW reactors (R3, R4).

In the autoclaved FW reactors H$_2$S concentrations remained low, probably due to the effect of autoclaving on proteins in the food waste, which may have reduced the availability of sulphur. The low H$_2$S concentration could also be due in part to precipitation through the formation of iron sulphides. The iron content in the autoclaved FW was 170 times higher than in the untreated FW (Table 1), possibly due to metal contamination from the autoclaving apparatus. O’Flaherty et al. (1998) showed that sulphate-reducing bacteria (SRBs) have an optimum pH slightly higher than that of methanogenic archaea, and hence the higher pH in the untreated FW reactors may have
favoured the growth of SRBs causing increased H$_2$S concentrations. Decreasing HRT will also give SRB an additional competitive advantage.

3.4. Residual methane potential assays

50-day RMP values were determined at the end of each period of reactor operation at OLRs 2, 4 and 6 kgVS/m$^3$ day (Table 3). The RMPs increased with the increasing OLRs and decreasing HRTs from 0.069 ± 0.005 m$^3$CH$_4$/kgVS to 0.105 ± 0.002 m$^3$CH$_4$/kgVS to 0.095 ± 0.012 m$^3$CH$_4$/kgVS with the autoclaved FW (OLR 2 to 6 kgVS/m$^3$ day).

However, RMPs after operation with OLR 4 kgVS/m$^3$ day were 6 and 10 % lower in untreated and autoclaved FW compared to OLR 2 kgVS/m$^3$ day reflecting the highest CH$_4$ yields obtained with OLRs 3 and 4 kgVS/m$^3$ day in STRs. Also few days longer storage time might have affected the RMPs after OLR 4 kgVS/m$^3$ day allowing materials to slightly degrade before the RMP start.

Overall, when results were calculated per VS of FWs fed to the STRs, RMP$_{original}$ increased total methane yield of the semi-continuous reactors by 2.9-4.7 % with the untreated FW and by 4.3-5.2 % with the autoclaved FW (Table 3). The calculated total methane yield with the untreated FW was, after OLRs 2, 4 and 6 kgVS/m$^3$ day, 3.6-12.6 % lower than the BMP value (0.501 m$^3$CH$_4$/kgVS) being closest after OLR 4 kgVS/m$^3$ day and thus reflecting the specific yields in STRs. Autoclaved FW showed similar STR reflecting behavior but after OLR 4 kgVS/m$^3$ day the calculated total methane yield was 3.1 % higher than the BMP value (0.445 m$^3$CH$_4$/kgVS). The VS removals were not cohesive with the calculated total methane yields, which could partly be explained with deviations between samples. The results suggest that in both materials

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388 there was still a small part of biodegradable material after semi-continuous reactors and
389 the amount increased with the increasing OLRs and decreasing HRTs.

390 **4. Conclusions**
391
392 Stable digestion of untreated and autoclaved FW was possible in TE-
393 supplemented mesophilic reactors at OLRs up to 6 kgVS/m$^3$d, with yields of 0.435 and
394 0.393 m$^3$CH$_4$/kgVS, respectively. Using an acclimated inoculum allowed rapid
395 increases in OLR without process disturbance. Untreated FW showed a higher specific
396 methane yield than autoclaved FW at all OLRs and in batch assays. This difference may
397 be due to the formation of Maillard compounds, with the resulting reduction in
398 biodegradability apparently outweighing any benefits from thermal hydrolysis of ligno-
399 cellulosic components under the autoclaving conditions used. Biogas H$_2$S
400 concentrations were much lower in reactors treating autoclaved FW.

400 **Acknowledgements**
401
402 This work was funded by the EU FP7 Valorisation of Food Waste to Biogas
403 (VALORGAS) project (241334). The authors are grateful to Aerothermal Group for
404 autoclaving, to BiogenGreenfinch Ltd for providing the food waste and to Biovakka
405 Suomi Ltd and Envor Biotech Ltd for providing the inoculums. We also wish to thank
406 the MTT laboratory staff for their excellent work.

406 **References**
407
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acrylamide and other vinylogous compounds by the Maillard reaction. J. Agric. Food Chem. 52, 5550-5558.


Table 1. Characteristics of untreated food waste (FW), autoclaved FW and inoculum.

<table>
<thead>
<tr>
<th></th>
<th>Control FW</th>
<th>Autoclaved FW</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.96 ± 0.16</td>
<td>5.01 ± 0.12</td>
<td>N/A</td>
</tr>
<tr>
<td>TS (g/kg)</td>
<td>247.5 ± 4.7</td>
<td>210.9 ± 18.6</td>
<td>77.3</td>
</tr>
<tr>
<td>VS (g/kg)</td>
<td>229.9 ± 4.5</td>
<td>194.6 ± 17.6</td>
<td>43.1</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>92.9</td>
<td>92.3</td>
<td>55.8</td>
</tr>
<tr>
<td>SCOD (g/l)</td>
<td>98.2 ± 6.5</td>
<td>117.5 ± 10.3</td>
<td>11.9</td>
</tr>
<tr>
<td>TVFA (g/l)</td>
<td>3.1 ± 0.6</td>
<td>2.2 ± 0.2</td>
<td>2.4</td>
</tr>
<tr>
<td>TKN (g/kg)</td>
<td>7.4 ± 0.3</td>
<td>6.8 ± 0.3</td>
<td>4.9</td>
</tr>
<tr>
<td>NH₄-N (g/kg)</td>
<td>0.32 ± 0.12</td>
<td>0.41 ± 0.10</td>
<td>2.4</td>
</tr>
<tr>
<td>Fe (g/kgTS)</td>
<td>0.13 ± 0.01</td>
<td>22.73 ± 12.54</td>
<td>N/A</td>
</tr>
<tr>
<td>SMP (m³CH₄/kgVS)</td>
<td>0.501 ± 0.020</td>
<td>0.445 ± 0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>SMP (m³CH₄/kgTS)</td>
<td>0.462 ± 0.019</td>
<td>0.408 ± 0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>SMP (m³CH₄/kgFM)</td>
<td>0.112 ± 0.005</td>
<td>0.084 ± 0.0001</td>
<td>N/A</td>
</tr>
<tr>
<td>Density (kg/l)</td>
<td>1.064 ± 0.0042</td>
<td>1.063 ± 0.0002</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N=24 for pH, N=8 for TS, VS, SCOD, TVFA, TKN, NH₄-N, N=2 for specific methane potentials (SMPs) and Fe, N=3 for density
N/A, not available
Table 2. Reactor characteristics during the last 4 weeks of each organic loading rate (OLR, kgVS/m\(^3\)d) periods.

<table>
<thead>
<tr>
<th>OLR</th>
<th>Reactor</th>
<th>HRT (d)</th>
<th>Specific CH(_4) yield (m(^3)/kgVS)</th>
<th>TS (g/kg)</th>
<th>VS (g/kg)</th>
<th>VS removal (%)</th>
<th>pH</th>
<th>TVFA (mg/l)</th>
<th>TKN (g/kg)</th>
<th>NH(_4)-N (g/kg)</th>
<th>SCOD (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>R1</td>
<td>117</td>
<td>0.443 ± 0.038</td>
<td>69.2 ± 1.7</td>
<td>44.5 ± 0.9</td>
<td>80.6</td>
<td>7.8 ± 0.13</td>
<td>267.5 ± 53.2</td>
<td>7.2 ± 0.1</td>
<td>3.8 ± 0.14</td>
<td>16.0 ± 2.9</td>
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<tr>
<td></td>
<td>R3</td>
<td>94</td>
<td>0.373 ± 0.037</td>
<td>76.6 ± 2.3</td>
<td>55.6 ± 1.9</td>
<td>71.4</td>
<td>7.6 ± 0.04</td>
<td>132.5 ± 17.1</td>
<td>7.0 ± 0.3</td>
<td>2.1 ± 0.06</td>
<td>14.8 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>R1</td>
<td>78</td>
<td>0.483 ± 0.013</td>
<td>71.1 ± 2.6</td>
<td>51.4 ± 2.5</td>
<td>77.7</td>
<td>7.8 ± 0.03</td>
<td>188.0 ± 71.9</td>
<td>8.4 ± 0.4</td>
<td>4.2 ± 0.15</td>
<td>15.6 ± 3.1</td>
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<tr>
<td></td>
<td>R2</td>
<td></td>
<td>0.478 ± 0.009</td>
<td>69.8 ± 2.2</td>
<td>56.1 ± 9.1</td>
<td>75.6</td>
<td>7.8 ± 0.08</td>
<td>108.0 ± 17.9</td>
<td>8.9 ± 0.1</td>
<td>4.1 ± 0.14</td>
<td>23.0 ± 4.3</td>
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<tr>
<td></td>
<td>R3</td>
<td>63</td>
<td>0.423 ± 0.002</td>
<td>84.0 ± 5.3</td>
<td>66.3 ± 4.4</td>
<td>65.9</td>
<td>7.5 ± 0.02</td>
<td>136.0 ± 26.1</td>
<td>8.2 ± 0.6</td>
<td>2.0 ± 0.05</td>
<td>17.6 ± 2.3</td>
</tr>
<tr>
<td>4</td>
<td>R1</td>
<td>58</td>
<td>0.465 ± 0.023</td>
<td>85.2 ± 5.6</td>
<td>64.2 ± 3.7</td>
<td>72.1</td>
<td>7.8 ± 0.07</td>
<td>112.0 ± 25.9</td>
<td>9.0 ± 0.1</td>
<td>3.5 ± 0.03</td>
<td>36.2 ± 0.6</td>
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<tr>
<td></td>
<td>R3</td>
<td>47</td>
<td>0.439 ± 0.020</td>
<td>86.1 ± 2.6</td>
<td>69.9 ± 2.7</td>
<td>64.1</td>
<td>7.4 ± 0.06</td>
<td>90.0 ± 24.5</td>
<td>8.3 ± 0.5</td>
<td>1.3 ± 0.01</td>
<td>20.3 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>R1</td>
<td>39</td>
<td>0.405 ± 0.006</td>
<td>102.1 ± 7.3</td>
<td>72.8 ± 4.1</td>
<td>68.3</td>
<td>7.7 ± 0.06</td>
<td>165.0 ± 42.0</td>
<td>9.4 ± 0.2</td>
<td>3.2 ± 0.08</td>
<td>28.3 ± 11.6</td>
</tr>
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</tr>
<tr>
<td></td>
<td>R2</td>
<td></td>
<td>0.435 ± 0.008</td>
<td>90.3 ± 2.8</td>
<td>68.7 ± 2.8</td>
<td>70.1</td>
<td>7.7 ± 0.05</td>
<td>140.0 ± 54.8</td>
<td>9.4 ± 0.1</td>
<td>3.3 ± 0.05</td>
<td>25.9 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>31</td>
<td>0.393 ± 0.044</td>
<td>85.7 ± 1.7</td>
<td>69.1 ± 1.4</td>
<td>64.5</td>
<td>7.2 ± 0.05</td>
<td>108.0 ± 35.6</td>
<td>7.8 ± 0.1</td>
<td>1.2 ± 0.07</td>
<td>18.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td></td>
<td>0.383 ± 0.013</td>
<td>88.3 ± 5.4</td>
<td>72.0 ± 3.1</td>
<td>63.0</td>
<td>7.3 ± 0.06</td>
<td>110.0 ± 21.6</td>
<td>8.2 ± 0.3</td>
<td>1.2 ± 0.13</td>
<td>18.7 ± 3.0</td>
</tr>
</tbody>
</table>

N/A, not available
N=2-5, for pH N=15
Table 3. Residual methane potentials (RMPs), total methane yield and VS removals of food waste digestates after organic loading rates (OLRs, kgVS/m³day) 2, 4 and 6 in the stirred tank reactors (STRs).

<table>
<thead>
<tr>
<th>OLR</th>
<th>Reactor</th>
<th>RMP (m³/kgVS)</th>
<th>RMP&lt;sub&gt;original&lt;/sub&gt; (m³/kgVS&lt;sub&gt;feed&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total CH₄ yield in STR+RMP (m³/kgVS&lt;sub&gt;feed&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VS removal in STR+RMP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>R1</td>
<td>0.069 ± 0.005</td>
<td>0.013 ± 0.0009</td>
<td>0.456</td>
<td>85.1</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>0.063 ± 0.002</td>
<td>0.017 ± 0.0006</td>
<td>0.390</td>
<td>75.3</td>
</tr>
<tr>
<td>4</td>
<td>R1</td>
<td>0.065 ± 0.001</td>
<td>0.017 ± 0.0004</td>
<td>0.482</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>0.057 ± 0.002</td>
<td>0.020 ± 0.0006</td>
<td>0.459</td>
<td>67.4</td>
</tr>
<tr>
<td>6</td>
<td>R1</td>
<td>0.105 ± 0.002</td>
<td>0.032 ± 0.0005</td>
<td>0.437</td>
<td>76.9</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>0.095 ± 0.012</td>
<td>0.034 ± 0.0045</td>
<td>0.427</td>
<td>69.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results calculated according to VS fed to STRs
N=2-3
Fig. 1. Biochemical methane potential (BMP) and standard deviation of untreated and autoclaved food waste (FW) in 35-day assays.
Fig. 2. Methane yields and contents in reactors treating untreated food waste (FW) and autoclaved FW during the semi-continuous operation.
Fig. 3. Chemical characteristics (pH, TVFA, TKN, NH₄-N, TS, VS, SCOD) of untreated food waste (FW) and autoclaved FW reactor contents during the semi-continuous operation.
Fig. 4. H$_2$S contents in reactors treating untreated food waste (FW) and autoclaved FW during days 166-314.