1 **Anaerobic digestion of autoclaved and untreated food waste**

2 Elina Tampio[a, *], Satu Ervasti[a], Teija Paavola[a, 1], Sonia Heaven[b], Charles Banks[b], Jukka

3 Rintala[a, 2]

4 [a]*Bioenergy and Environment, MTT Agrifood Research Finland, FI-31600 Jokioinen,*

5 *Finland*

6 [b]*University of Southampton, Faculty of Engineering and the Environment, Southampton*

7 *SO17 1BJ, UK*

8 **Abstract**

9 Anaerobic digestion of autoclaved (160 °C, 6.2 bar) and untreated source segregated

10 food waste (FW) was compared over 473 days in semi-continuously fed mesophilic

11 reactors with trace elements supplementation, at organic loading rates (OLRs) of 2, 3, 4

12 and 6 kgVolatile solids(VS)/m^{3}d. Methane yields at all OLR were 5-10 % higher for

13 untreated FW (maximum 0.483 ± 0.013 m^{3}CH$_4$/kgVS at 3 kgVS/m^{3}d) than autoclaved

14 FW (maximum 0.439 ± 0.020 m^{3}CH$_4$/kgVS at 4 kgVS/m^{3}d). The residual methane

15 potential of both digestates at all OLRs was less than 0.110 m^{3}CH$_4$/kgVS, indicating

16 efficient methanation in all cases. Use of acclimated inoculum allowed very rapid

17 increases in OLR. Reactors fed on autoclaved FW showed lower ammonium and

18 hydrogen sulphide concentrations, probably due to reduced protein hydrolysis as a

19 result of formation of Maillard compounds. In the current study this reduced

[*] Corresponding author. Tel.: +358 29 531 7800, E-mail address: elina.tampio@mtt.fi
[1] Present address: Biovakka Suomi Ltd, Autokatu 8, FI-20380 Turku, Finland
[2] Present address: Department of Chemistry and Bioengineering, Tampere University of Technology, FI-33101 Tampere, Finland.

20   biodegradability appears to outweigh any benefit due to thermal hydrolysis of ligno-

21   cellulosic components.

22   **Keywords**

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

24

25   **1. Introduction**

26   Anaerobic digestion is an efficient technique for the treatment of source

27   segregated biodegradable municipal wastes, e.g. biowastes and food waste (FW), as it

28   recovers energy in the form of biogas for use in combined heat and power (CHP) plants,

29   in vehicles and for grid injection; and also allows recycling of nutrients through

30   application of digestion residues in crop production. Both the Renewable Energy

31   directive (2009/28/EC, EU 2009) and the Landfill directive (99/31/EC, EU 1999) have

32   been strong drivers in promoting the use of anaerobic digestion for this application in

33   recent years.

34   Although co-digestion of FW with sewage sludge and animal manures has been

35   common practice, treatment of FW alone has often proved difficult (Banks et al. 2008,

36   Neiva Correia et al. 2008, Zhang et al. 2012). These difficulties have been attributed to

37   ammonia inhibition resulting from a high protein content (Gallert et al. 1998), and are

38   often indicated by accumulation of volatile fatty acids (VFA) (Banks et al. 2012). To

39   achieve stable anaerobic digestion with FW alone, organic loading rates (OLR) are

40   usually maintained at low values: 2.25 kgVS/m^{3}d at a hydraulic retention time (HRT) of

41   80 days in Banks et al. (2011) and from 1-4 kgVS/m^{3}d (HRT 14-30 days) as reported in

42 Cecchi et al. (2003). VFA accumulation at higher OLR has recently been linked to trace

43 element (TE) deficiencies (Banks et al. 2012). When supplemented with TE successful

44 FW digestion has been reported at OLRs of 5 kgVS/m^{3}d (Banks et al. 2012) and 6.64

45 kgVS/m^{3}d (Zhang and Jahng 2012).

46 Thermal and hydrothermal pre-treatments have been widely studied as a means of

47 hydrolysing recalcitrant components in a wide range of wastes to make them easier to

48 degrade (Papadimitriou 2010, Ren et al. 2006, Takashima and Tanaka 2008); these

49 techniques have also been used as pre-treatments before anaerobic digestion of mixed

50 biowastes (Lissens et al. 2004, Sawayama et al. 1997). One such hydrothermal

51 treatment is autoclaving, where water is used as a reagent at increased temperature and

52 pressure, to hydrolyse and solubilise sugars, starch, proteins and hemicellulose

53 (Papadimitriou 2010, Ren et al. 2006). Materials pre-treated by autoclaving under

54 various conditions have shown increased methane production in batch tests: digested

55 swine slurry autoclaved at 120 °C showed an increase in CH$_4$ yield of 115 % (Menardo

56 et al. 2011) and autoclaving of mixed kitchen garbage (175 °C, 40 bar, 1 hour)

57 increased CH$_4$ yield by 30 % (Sawayama et al. 1997). Improved methane production has

58 also been observed in continuously-stirred tank reactors (CSTRs) treating waste

59 activated sludge (WAS), with 12 % and 25 % increases after autoclaving at 135 °C and

60 190 °C, respectively (Bougrier et al. 2007).

61 In contrast, more aggressive thermal and hydrothermal pre-treatments at higher

62 temperatures (around 180 °C) have been reported to decrease biodegradability and

63 biogas production during anaerobic digestion of WAS and sewage sludge (Bougrier et

64 al. 2008, Pinnekamp 1989). This is believed to be related to the formation of complex

65 and inhibitory Maillard compounds, produced by reactions between amino acids and

66 carbohydrates (Bougrier et al. 2008, Takashima and Tanaka 2008). Maillard compounds

67 start to form at temperatures above 100 °C depending on the retention time (Müller

68 2001, Nursten 2005), while the formation of more complex compounds, such as

69 acrylamides and other vinylogous compounds, increases at higher temperatures (180 °C,

70 Stadler et al. 2004).

71   The aim of this study was to evaluate the anaerobic digestion of untreated and

72 autoclaved (160 °C, 6.2 bar) FW at a range of different OLRs (2, 3, 4 and 6

73 kgVS/m^{3}day) in semi-continuously fed intermittently-stirred mesophilic reactors. The

74 biochemical methane potential (BMP) of the feedstocks and the residual methane

75 potential (RMP) of the digestates were also assessed in batch assays.

76 **2. Materials and methods**

77 **2.1. Origin and characterization of FW and inocula**

78   The source segregated domestic FW used in the study was collected from the

79 South Shropshire Biowaste digestion plant in Ludlow, UK. Biodegradable bags used for

80 waste collection were removed and the FW material was mixed and divided into two

81 equal portions. One portion was pre-treated at 160 °C and 6.2 bars in a novel double-

82 auger autoclave (AeroThermal Group Ltd, UK) that provides improved mixing and

83 steam penetration; the other portion was left untreated. Both portions were then passed

84 through a macerating grinder (S52/010 Waste Disposer, IMC Limited, UK), packed into

85 35-litre plastic boxes (7 untreated and 8 autoclaved), frozen and shipped at - 20 °C to

86 MTT Agrifood Research, Finland.

87   At MTT the frozen material was chopped into smaller portions corresponding to

88 amounts required for weekly feeding of the digesters, and these smaller portions were

89   again stored at -20 °C. Each week portions of the autoclaved and untreated FW were

90   thawed and stored at 4 °C and used as daily feed. The pH, total solids (TS), volatile

91   solids (VS), ammonium nitrogen (NH_4-N), total Kjeldahl nitrogen (TKN), soluble

92   chemical oxygen demand (SCOD) and VFA content was determined for each new box

93   of feed.

94   The reactors were inoculated with digestate from a mesophilic CSTR digesting

95   mechanically dewatered sewage sludge (Biovakka Suomi Ltd, Turku, Finland) (Table

96   1). In the BMP assays inoculum was taken from an anaerobic digester treating

97   municipal and industrial biowastes (Envor Biotech Ltd, Forssa, Finland).

98   **2.2. Semi-continuous trials**

99   Four 11-litre stainless steel stirred tank reactors (STRs) (Metener Ltd, Finland)

100 were operated at 37 °C. Stirring (32 rpm) was semi-continuous with 5 seconds on and

101 60 seconds off. The reactors were fed manually five times a week through an inlet tube

102 which extended below the digestate surface, and which was also used for digestate

103 sampling. Digestate overflowed from the reactors by gravity through a u-tube trap to

104 prevent gas escape. Between days 1-195 hourly gas volume and methane content were

105 measured using an automatic system in which the produced biogas was collected into a

106 small (~220 ml) gas storage vessel on top of the reactor. From day 195 onwards, due to

107 break down of the automated system, gas volume was measured by water displacement

108 in a volume-calibrated cylindrical gas collector, after which the gas was collected in

109 aluminium gas bags.

110   Reactors were fed with untreated FW (R1) and autoclaved FW (R3). After 18

111 days acclimation period with reduced feeding the experiments started at an OLR of 2

112 kgVS/m^3day, corresponding to HRT of 117 and 94 days for R1 and R3 respectively. On

113 day 151, after 1.1 (R1) and 1.4 (R3) HRTs, the OLR was raised to 3 kgVS/m^3day and

114 after 1.3 (R1) and 1.7 (R3) HRTs to 4 kgVS/m^3day on day 256 (HRT 78 d and 58 d for

115 untreated, 63 d and 47 d for autoclaved FW, respectively).

116   On day 327 parallel reactors fed on untreated (R2) and autoclaved FW (R4) were

117 started at an OLR of 3 kgVS/m^3day, using 5.7 litres of digestate from R1 and R3

118 respectively as inoculum. After 2.8 and 3.4 HRTs in reactors R1 and R3 and 1.2 and 1.4

119 HRTs in reactors R2 and R4, the OLR in all four reactors was further increased to 6

120 kgVS/m^3day on day 418, with a corresponding decrease in HRT to 39 d and 31 d in the

121 untreated and autoclaved FW reactors. Most of the data presented below are taken from

122 reactors R1 and R3 due to the longer running period. During days 179-193 reactors R1

123 and R3 were once a week supplemented with 11 ml of a trace element (TE) solution

124 containing Se (0.2 mg/l) and Co (1.0 mg/l). From day 199 onwards all reactors were

125 given a weekly supplement of two TE solutions, one containing cation elements (mg/l):

126 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

127 (mg/l): Mo 0.2, Se 0.2 and W 0.2 (Banks et al. 2012). 1 ml of each of these TE solutions

128 was added for each kg of digestate removed from the reactors over the one-week period.

129   Grab samples of digestate (about 250 g) were taken every two weeks for analysis

130 of TS, VS, SCOD, NH_4-N, TKN, and samples for VFA analysis (about 50 g) were taken

131 once a week. Digestate pH was measured weekly. Larger volumes of digestate were

132 collected on days 130 (2 l), 214 (1 l), 287 (1 l) and 321 (1 l). After removal of these

133 larger samples, daily feeding of the reactors was adjusted to compensate for the reduced

134 volume until the normal operating level was restored.

2.3. Biochemical and residual methane potential assays

135

136   BMP and RMP assays were performed at 37 °C using automated testing

137   equipment (Bioprocess Control Ltd, Sweden). The assays were mixed mechanically (84

138   rpm) for one minute per hour. Carbon dioxide was absorbed by NaOH before the

139   automated gas volume measurement, which was based on liquid displacement. Assays

140   were conducted in duplicate or triplicate, each with a total liquid volume of 400 ml

141   (BMP) or 200 ml (RMP assays). The inoculum to substrate ratio in BMP assays was 1:1

142   on a VS basis. $NaHCO_3$ (3 g/l) was used as a buffer and if the pH was lower than 7.5 it

143   was adjusted to around 8 with 3 M NaOH. In RMP assays digestates from the STR

144   reactors were incubated without inoculum. The results are given as average values of

145   the triplicate or duplicate assays.

2.4. Analyses and calculations

146

147   TS and VS were determined according to SFS 3008 (Finnish Standard

148   Association 1990) and NH_4-N according to McCullough (1967). TKN was analysed by

149   a standard method (AOAC 1990) using a Foss Kjeltec 2400 Analyzer Unit (Foss

150   Tecator AB, Höganäs, Sweden), with Cu as a catalyst. For soluble COD analysis FW

151   samples were diluted 1:10 with distilled water, and agitated for 1 hour. Diluted FW and

152   raw digestate samples were centrifuged (2493 × g, 15 min) after which the supernatant

153   was further centrifuged (16168 × g, 10 min) and stored in a freezer, then thawed before

154   analysis according to SFS 5504 (Finnish Standards Association 2002). pH was

155   determined using a VWR pH100 pH-analyzer (VWR International). Iron concentration

156   was analysed according to Luh Huang and Schulte (1985) using inductively coupled

157 plasma emission spectrometry (ICP-OES) (Thermo Jarrel Ash Iris Advantage, Franklin,

158 USA).

159   Samples for VFA analysis were centrifuged ($1831 \times g$, 10 min) and filtered with

160 Chromafil GF/PET-20/25 filters. Concentrations of acetic, propionic, iso-butyric, n-

161 butyric, iso-valeric, valeric and caproic acids were determined using a HP 6890 gas

162 chromatograph with an HP 7683 autosampler (Hewlett-Packard, Little Falls, USA) and

163 GC ChemStation Rev. B.03.02 software. The GC was fitted with a 10 m x 0.53 mm x 1

164 µm HP-FFAP capillary column (Agilent Technologies, USA) and a flame ionisation

165 detector with helium as a carrier gas (9 ml/min). Oven temperatures were 60-78 °C (25

166 °C/min), isothermal 1 min, 150 °C (7.5 °C/min) and 25 °C/min to 180 °C with 3 min

167 final time. The injector and detector temperatures were 220 °C and 280 °C,

168 respectively.

169   From day 1 to 195 methane composition was determined automatically during

170 emptying of the gas storage vessel by infrared analysis (ExTox Gasmess-Systeme

171 GmbH, Germany). From day 195 to 314, gas composition was analysed using a portable

172 Combimass GA-m gas analyzer (Binder Engineering GmbH, Germany), and during

173 days 315-446 the infrared measuring equipment was used.

174   The reactor was fed for 5 days a week, but the OLR in kgVS/m^{3}day is expressed

175 as the average daily weight of substrate fed to the reactor over a one-week period. HRT

176 was calculated based on feedstock densities. All biogas and methane yields were

177 converted to STP conditions (0 °C, 100 kPa) according to the ideal gas law. Methane

178 yields in the RMP assays were calculated in two ways; by dividing the cumulative

179 methane production by the 1) VS of the added digestate and 2) by the VS of the feed of

180   the semi-continuous reactors at the time of digestate sampling. The latter enables direct

181   comparison of the methane yield in the RMP with that in the reactors. Free ammonia

182   (NH_3-N) concentrations were calculated according to Anthonisen et al. (1976):

183    $$NH_3\text{-}N=(NH_4\text{-}N\times10^{pH})/((K_b/K_w)+10^{pH}),\qquad\qquad(1)$$

184   where K_b is the ammonia ionisation constant and K_w the ionisation constant of water at

185   37 °C.

186   **3. Results and discussion**

187   **3.1. Material characterization**

188   The autoclaved FW appeared much darker than the untreated FW and had a

189   pleasant caramel odour. TS and VS in the autoclaved FW were both about 15 % lower

190   than in the untreated FW due to dilution by steam condensation during the autoclave

191   treatment (Table 1). TKN on fresh matter basis was lower in the autoclaved FW (6.8 ±

192   0.3 g N/kg) than in untreated FW (7.4 ± 0.3 g N/kg). The autoclaved FW had about 22

193   % higher NH_4-N and 16 % higher SCOD, indicating that autoclaving had solubilised

194   some organic nitrogen and carbon components. Total VFA concentrations were lower in

195   the autoclaved material (2.2 ± 0.2 g/l) than in the untreated FW (3.1 ± 0.6 g/l)

196   suggesting either that some VFA had volatilised during or after autoclaving, or that

197   some acidification of the untreated material had occurred.

198   Changes in the chemical composition of materials during autoclave treatment are

199   dependent on the temperature as well as the materials used. In this study autoclaving

200   conditions of 6.2 bars and 160 °C were used. Increased concentrations of NH_4-N and

201   solubilisation of carbohydrates have previously been reported after autoclave treatment

202   of dewatered sewage sludge (175 °C, 20 bar), with an increase from 2.6 to 3.2 g NH_4-

203   N/l (Inoue et al. 1996); temperatures above 90 °C have also been reported to increase

204   ammonia concentrations from 0.35 gN/l to 0.7 gN/l in WAS (Bougrier et al. 2008).

205   **3.2. BMP assay**

206   The 35-day BMP value for untreated FW was 0.501 ± 0.020 m^3CH_4/kgVS, while

207   that for autoclaved FW was 0.445 ± 0.001 m^3CH_4/kgVS (Figure 1, Table 1). The lower

208   methane yield of the autoclaved FW could be explained by Maillard reactions. Support

209   for the occurrence of these is given by the darkening in colour of the autoclaved FW and

210   the caramelised odour, while the increase in SCOD provides evidence of increased

211   solubilisation of carbon compounds. Similar phenomena have also been observed with

212   autoclaved WAS (Bougrier et al. 2008) and municipal solid waste (Takashima and

213   Tanaka 2008). In other studies higher methane yields have been reported after similar

214   thermal treatments (Lissens et al. 2004), but this can be attributed to the improved

215   availability of the ligno-cellulosic materials; and when these form a large proportion of

216   the waste the resulting increase may far exceed any decrease due to Maillard

217   compounds. In contrast where ligno-cellulosic content is low, as in this type of food

218   waste (Zhang et al. 2012) reductions in methane yield may result.

219   **3.3. Semi-continuous operation**

220   *3.3.1. Effect of loading rate on methane yields*

221   Process parameters from the whole experimental period (days 1-473) are shown in

222   Figure 2 and detailed results from the last four weeks of stable operation at each OLR

223   are presented in Table 2. Operation was considered stable when variations were < 0.2

224   units in pH, < 90 mg/l in VFA and < 1.8 % in CH_4.

225   Throughout the experimental period specific methane yields were 5-10 % higher

226   for untreated FW than for autoclaved FW. The methane yields at OLR 2 kgVS/m^{3}day

227   were on average 0.443 ± 0.038 and 0.373 ± 0.037 m^{3}CH$_4$/kgVS for untreated (R1) and

228   autoclaved FW (R3), respectively. The highest yield for untreated FW was observed at

229   OLR 3 kgVS/m^{3}day (0.483 ± 0.013 m^{3}CH$_4$/kgVS) while autoclaved FW produced the

230   highest yield at OLR 4 kgVS/m^{3}day (0.439 ± 0.020 m^{3}CH$_4$/kgVS). When the OLR was

231   further increased to 6 kgVS/m^{3}day methane yields decreased by 12 % and 11 % in

232   untreated FW and autoclaved FW, respectively. The specific methane yield for

233   autoclaved FW was lower at OLR 2 kgVS/m^{3}day than at higher OLRs, which could

234   possibly indicate some acclimatisation. This was not seen in the untreated FW where

235   the lowest specific methane yield occurred at OLR 6 kgVS/m^{3}day, which could indicate

236   retarded hydrolysis as no increased SCOD nor VFA was detected. At OLR 6

237   kgVS/m^{3}day the difference in methane yields between the parallel (R2 and R4) and

238   original (R1 and R3) reactors was < 7 % (Table 2).

239   In reactors R1 and R3 relatively long operating times were applied, to allow the

240   process to stabilise between incremental increases in OLR. Using this approach, stable

241   digestion of both autoclaved and untreated FW was achieved at the relatively high OLR

242   of 6 kgVS/m^{3}day. It was also shown, however, that when an inoculum acclimated to the

243   feedstocks was used in R2 and R4, the OLR could be rapidly increased without

244   operational disturbances such as VFA accumulation. The maximum loading rates

245   applied were similar to the 6.64 kgVS/m^{3}day achieved by Zhang and Jahng (2012) and

246   higher than the 5 kgVS/m^{3}day of Banks et al. (2012). Both of these long-term digestion

247   studies used trace elements supplementation, as did the present study.

248   As far as is known, this is the first study to report anaerobic digestion of

249   autoclaved food waste in a semi-continuously fed system. Methane yields of $0.483 \pm$

250   0.013 and 0.423 ± 0.002 $m^3CH_4/kgVS$ for the untreated and autoclaved FW at OLR 3

251    $kgVS/m^3day$ are in good agreement with previous studies, where a full-scale digester

252   fed on the same type of source-segregated household food waste at an average OLR of

253   2.5 $kgVS/m^3day$ yielded 0.402 $m^3CH_4/kgVS$ (Banks et al. 2011). Earlier pilot-scale

254   studies gave an average of 0.390 $m^3CH_4/kgVS$, but using a different source of source-

255   segregated domestic food waste at higher OLR (3.5 to 4 $kgVS/m^3day$), and without TE

256   supplementation (Banks et al. 2008). Laboratory-scale FW digestion with TE

257   supplementation was reported to yield 0.352-0.439 $m^3CH_4/kgVS$ at an OLR of 6.64

258    $kgVS/m^3day$ by Zhang and Jahng (2012); while in the study by Banks et al. (2012) the

259   methane yield for TE supplemented FW was 0.435 $m^3CH_4/kgVS$.

260   The maximum methane yields for untreated and autoclaved FW in the semi-

261   continuous trials were 0.483 ± 0.013 and 0.439 ± 0.020 $m^3CH_4/kgVS$ respectively.

262   These were slightly lower than the BMP values in each case. The results therefore

263   strongly indicate that even after long periods of operation no significant acclimatisation

264   that could improve the biodegradability of compounds produced in the autoclaving

265   process had taken place.

266   With mixed biowastes, the benefits of increased biogas production due to

267   improved degradation of ligno-cellulosic materials may outweigh any losses in

268   biodegradability as a result of formation of recalcitrant compounds during thermal

269   treatment. FW, however, has a relatively low ligno-cellulosic fibre content compared to

270   other municipal biowaste components (e.g. garden or yard waste, paper and card), and

271   in the present study the net effect of treatment was a reduction in specific methane yield.

272   This balance may however change with different autoclaving conditions, and in

273   particular a lowering of temperature may produce more favourable results.

274   *3.3.2. Digestion parameters*

275   Results for pH, VFA, TS, VS, SCOD, NH_4-N, TKN are presented in Table 2 and

276   Figure 3. pH in the untreated FW reactor remained around 7.8 throughout the

277   experimental period, while with autoclaved FW the pH decreased from pH 7.6 at OLR 2

278   kgVS/m^3day to 7.3 at OLR 6 kgVS/m^3day.

279   At an OLR of 2 kgVS/m^3day, total VFA concentration in both reactors remained

280   under 250 mg/l. When the OLR was increased to 3 kgVS/m^3day, VFA in the untreated

281   FW reactor increased to 2400 mg/l by day 153, and consisted mainly of acetic (about 85

282   %) and propionic acids (about 10 %). In the autoclaved FW reactor VFA concentration

283   showed smaller increases with peaks of 1500 mg/l on day 139 (consisting 98 % of

284   acetic acid) and 910 mg/l on day 160 (27 % acetic acid and 65 % propionic acid). The

285   relatively large samples (2 litres) taken from the reactors on day 130 could have

286   contributed to these increases in VFA concentration, but similar removals of digestate at

287   later stages in the experimental run did not have this effect. VFA concentrations reduced

288   to under 200 mg/l in both reactors by day 214, shortly after the introduction of trace

289   element additions of selenium and cobalt on day 179 and full TE supplementation on

290   day 199. This behaviour is consistent with previous reports of responses to TE

291   supplementation where the VFA increase was linked with the loss of electron transfer

292   interspecies during digestion (Banks et al. 2012).

293   TS, VS and TKN contents in both reactors gradually increased during the

294   experimental period, with TS increasing from under 70 to over 80 g/kg. Despite the

295   lower feedstock solids concentration, the solids content in the autoclaved FW reactor

296   was slightly higher than in the untreated FW up to the end of OLR 4 kgVS/m^{3}day. After

297   OLR was increased to 6 kgVS/m^{3}day there was an increase in solids concentrations in

298   the untreated FW reactor, which was not apparent with the autoclaved FW. The initial

299   TKN concentration in both reactors was 4.9 g N/kg and showed a similar increase to ~8

300   g N/kg by around day 200. TKN in the untreated FW reactor continued to increase until

301   around day 300 at which point it stabilised at ~9 g N/kg, whereas for the autoclaved FW

302   it remained at ~8 g N/kg. The differences in TKN reflected the differences in feedstock

303   concentrations. The increases in solids content were most likely associated with the

304   increase in loading, although it is possible that some accumulation was due to

305   stratification despite the intermittent mixing, as surplus digestate was discharged from

306   an overflow at the top of the reactor. Mass balance calculations affirmed, in the

307   beginning of OLR 4 kgVS/m^{3}day, that accumulation of TKN was taking place.

308   The SCOD concentration in both reactors increased from around 10 g/l to over 20

309   g/l during the first 300 days of operation, then stabilised in the autoclaved FW reactor.

310   In the untreated FW reactor the SCOD increased sharply to ~36 g/l for over 50 days

311   then decreased equally sharply in the end of the run: these variations did not correspond

312   to changes in OLR and were not accomplished with changes in methane yield nor

313   digestate VFA. Total VFA concentrations accounted for only 0.5-2 % of the SCOD. A

314   probable explanation for the general increase in SCOD in both reactors is an increase in

315   the quantity of soluble microbial products present in the digestate; this phenomenon has

316   previously been observed with solid substrates and at long retention times (Kuo et al.

317   1996, Rinćon et al. 2012).

318   *3.3.3. Ammonium and ammonia*

319   NH_4-N concentration in the untreated FW reactor increased during the first ~170

320   days from 2.4 (inoculum) to 4 g/kg and then showed a very gradual decrease to around

321   3 g/kg by the end of the experimental run. This decrease could be associated with the

322   increase in microbial biomass (Lindorfer et al. 2011) or in soluble microbial products

323   caused by the increasing OLR. In the autoclaved FW reactor, however, NH_4-N

324   decreased from 2.4 to about 1.2 g/kg by the end of the experimental period. The low

325   NH_4-N concentrations in the autoclaved FW reactor were probably mainly due to the

326   effect of autoclaving and the formation of Maillard compounds from the reaction of

327   proteins with carbohydrates (Bougrier et al. 2007, 2008). Free ammonia concentrations

328   in the reactors were calculated, but NH_3 remained below 0.30 g/kg in untreated FW and

329   below 0.10 g/kg in the autoclaved FW reactor.

330   The pH value in the untreated FW reactor rose to around 7.8 by day 55 and

331   remained relatively stable until the OLR was raised to 6 $kgVS/m^3day$, at which point it

332   fell very slightly. In the autoclaved reactor after a slight initial rise pH decreased during

333   the experimental run to a final value of around 7.3. These pH values reflect the relative

334   NH_4-N concentrations in each case, as NH_4-N provides buffering capacity (Procházka et

335   al. 2012). High NH_4-N concentration can also inhibit the digestion process, but this is

336   greatly dependent on the feedstock materials and acclimation times (Chen et al. 2008,

337   Procházka et al. 2012). In the present study, after TE supplementation was introduced,

338   there was no evidence of the VFA accumulation that is often associated with ammonia

339   toxicity, and the free ammonia concentrations were similar to those previously observed

340   in FW digestion (Zhang et al. 2012).

341   *3.3.4. Gas composition*

342   The biogas methane content in both autoclaved and untreated FW digesters was

343   similar and ranged between 55-63 % during the experiment, with an average of around

344   58% (Table 2, Figure 2). It did not appear to be affected by changes in applied OLR. In

345   contrast, in a study by Zhang and Jahng (2012) on FW digestion the methane content

346   was found to decrease from 53 % to 48 % as the OLR was gradually increased from

347   2.19 to 6.64 kgVS/m^{3}day.

348   Hydrogen sulphide concentration was monitored between days 166-313 while the

349   reactors were operated at OLR 3 and 4 kgVS/m^{3}day (Figure 4). H_2S concentrations at

350   OLR 3 kgVS/m^{3}day were < 100 ppm in the untreated FW reactor and < 75 ppm in the

351   autoclaved FW reactor. Shortly before the OLR was increased to 4 kgVS/m^{3}day the H_2S

352   concentration in the untreated FW reactor began to increase, and reached 480 ppm by

353   day 314 at which point monitoring ceased; while in the autoclaved FW reactor H_2S

354   content remained < 60 ppm. H_2S was also monitored at the OLR of 6 kgVS/m^{3}day

355   (days 448-473) and concentrations were 751 ± 182 ppm in the untreated FW reactors

356   (R1 and 2) compared to 63 ± 4 ppm in the autoclaved FW reactors (R3, R4).

357   In the autoclaved FW reactors H_2S concentrations remained low, probably due to

358   the effect of autoclaving on proteins in the food waste, which may have reduced the

359   availability of sulphur. The low H_2S concentration could also be due in part to

360   precipitation through the formation of iron sulphides. The iron content in the autoclaved

361   FW was 170 times higher than in the untreated FW (Table 1), possibly due to metal

362   contamination from the autoclaving apparatus. O'Flaherty et al. (1998) showed that

363   sulphate-reducing bacteria (SRBs) have an optimum pH slightly higher than that of

364   methanogenic archaea, and hence the higher pH in the untreated FW reactors may have

365 favoured the growth of SRBs causing increased H_2S concentrations. Decreasing HRT

366 will also give SRB an additional competitive advantage.

3.4. Residual methane potential assays

368   50-day RMP values were determined at the end of each period of reactor

369 operation at OLRs 2, 4 and 6 kgVS/m^{3}day (Table 3). The RMPs increased with the

370 increasing OLRs and decreasing HRTs from 0.069 ± 0.005 m^{3}CH$_4$/kgVS to $0.105 \pm$

371 0.002 m^{3}CH$_4$/kgVS with the untreated FW and from 0.063 ± 0.002m^{3}CH$_4$/kgVS to

372 0.095 ± 0.012 m^{3}CH$_4$/kgVS with the autoclaved FW (OLR 2 to 6 kgVS/m^{3}day).

373 However, RMPs after operation with OLR 4 kgVS/m^{3}day were 6 and 10 % lower in

374 untreated and autoclaved FW compared to OLR 2 kgVS/m^{3}day reflecting the highest

375 CH$_4$ yields obtained with OLRs 3 and 4 kgVS/m^{3}day in STRs. Also few days longer

376 storage time might have affected the RMPs after OLR 4 kgVS/m^{3}day allowing

377 materials to slightly degrade before the RMP start.

378   Overall, when results were calculated per VS of FWs fed to the STRs, RMP$_{original}$

379 increased total methane yield of the semi-continuous reactors by 2.9-4.7 % with the

380 untreated FW and by 4.3-5.2 % with the autoclaved FW (Table 3). The calculated total

381 methane yield with the untreated FW was, after OLRs 2, 4 and 6 kgVS/m^{3}day, 3.6-12.6

382 % lower than the BMP value (0.501 m^{3}CH$_4$/kgVS) being closest after OLR 4

383 kgVS/m^{3}day and thus reflecting the specific yields in STRs. Autoclaved FW showed

384 similar STR reflecting behavior but after OLR 4 kgVS/m^{3}day the calculated total

385 methane yield was 3.1 % higher than the BMP value (0.445 m^{3}CH$_4$/kgVS). The VS

386 removals were not cohesive with the calculated total methane yields, which could partly

387 be explained with deviations between samples. The results suggest that in both materials

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   Stable digestion of untreated and autoclaved FW was possible in TE-

392   supplemented mesophilic reactors at OLRs up to 6 kgVS/m^{3}d, with yields of 0.435 and

393   0.393 m^{3}CH$_4$/kgVS, respectively. Using an acclimated inoculum allowed rapid

394   increases in OLR without process disturbance. Untreated FW showed a higher specific

395   methane yield than autoclaved FW at all OLRs and in batch assays. This difference may

396   be due to the formation of Maillard compounds, with the resulting reduction in

397   biodegradability apparently outweighing any benefits from thermal hydrolysis of ligno-

398   cellulosic components under the autoclaving conditions used. Biogas H$_2$S

399   concentrations were much lower in reactors treating autoclaved FW.

406   **References**

407   Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition

408   of nitrification by ammonia and nitrous acid. J. Water Pollut. Control Fed. 48, 835-852.

409   AOAC, 1990. Official Methods of Analysis. Association of Official Analytical

410   Chemists, Inc., Arlington, VA. 1298 p.

411   Banks, C.J., Chessire, M., Stringfellow, A.S., 2008. A pilot-scale comparison of

412   mesophilic and thermophilic digestion of source segregated domestic food waste. Water

413   Sci. Technol. 58(7), 1475-1481.

414   Banks, C.J., Chesshire, M., Heaven, S., Arnold, R., 2011. Anaerobic digestion of

415   source-segregated domestic food waste: Performance assessment by mass and energy

416   balance. Bioresour. Technol. 102, 612-620.

417   Banks, C.J., Zhang, Y., Jiang, Y., Heaven, S., 2012. Trace element requirements

418   for stable food waste digestion at elevated ammonia concentrations. Bioresour. Technol.

419   104, 127-135.

420   Bougrier, C., Delgenès, J.P., Carrère, H., 2007. Impacts of thermal pre-treatments

421   on the semi-continuous anaerobic digestion of waste activated sludge. Biochem. Eng. J.

422   34, 20-27.

423   Bougrier, C., Delgenès, J.P., Carrère, H., 2008. Effects of thermal treatments on

424   five different waste activated sludge samples solubilisation, physical properties and

425   anaerobic digestion. Chem. Eng. J. 139, 236-244.

426   Cecci, F., Traverso, P., Pavan, P., Bolzonella, D., Innocenti, L., 2003.

427   Characteristics of the OFMSW and behavior of the anaerobic digestion process, in:

428   Mata-Alvarez, J. (Ed.), Biomethanization of the Organic Fraction of Municipal Solid

429   Waste. IWA Publishing, UK. pp. 141-180.

430   Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion

431   process: A review. Bioresour. Technol. 99, 4044-4064.

432   EU, 1999. Council Directive 1999/31/EC of 26 April 1999 on the landfill of

433   waste. Official Journal L 182, 16/07/1999 P. 0001-0019.

434   EU, 2009. Directive 2009/28/EC of the European Parliament and of the Council

435   of 23 April 2009 on the promotion of the use of energy from renewable sources and

436   amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official

437   Journal L 140, 05/06/2009 P. 0016-0062.

438   Finnish Standard Association, 1990. SFS 3008, Determination of total residue and

439   total fixed residue in water, sludge and sediment, Finnish Standard Association,

440   Helsinki, Finland.

441   Finnish Standard Association, 2002. SFS 5504, Determination of chemical

442   oxygen demand (CODCr) in water with closed tube method, oxidation with dichromate,

443   Finnish Standard Association, Helsinki, Finland.

444   Gallert, C., Bauer, S., Winter, J. 1998. Effect of ammonia on the anaerobic

445   degradation of protein by mesophilic and thermophilic biowaste population. Appl.

446   Microbiol. Biotechnol. 50, 495-501.

447   Inoue, S., Sawayama, S., Ogi, T., Yokoyama, S., 1996. Organic composition of

448   liquidized sewage sludge. Biomass Bioenergy 10(1), 37-40.

449   Kuo, W., Sneve, M.A., Parkin, G.F., 1996. Formation of soluble microbial

450   products during anaerobic treatment. Water Environ. Res. 68(3), 279-285.

451   Lindorfer, H., Ramhold, D., Frauz, B., 2011. Nutrient and trace element supply in

452   AD plants and effect of trace element application. Proc. International IWA Symposium

453   on Anaerobic Digestion of Solid Wastes and Energy Crops, Vienna 28 August - 1

454   September 2011.

455   Lissens, G., Thomsen, A.B., De Baere, L., Verstraete, W., Ahring, B.K., 2004.

456   Thermal wet oxidation improves anaerobic biodegradability of raw and digested

457   biowaste. Environ. Sci. Technol. 38, 3418-3424.

458   Luh Huang, C.Y., Schulte, E.E., 1985. Digestion of plant tissue for analysis by

459   ICP emission spectrometry. Commun. Soil Sci. Plant Anal. 16, 943-958.

460   McCullough, H., 1967. The determination of ammonia in whole blood by a direct

461   colorimetric method. Clin. Chim. Acta 17, 297-304.

462   Menardo, S., Balsari, P., Dinuccio, E., Gioelli, F., 2011. Thermal pre-treatment of

463   solid fraction from mechanically-separated raw and digested slurry to increase methane

464   yield. Bioresour. Technol. 102, 2026-2032.

465   Müller, J.A., 2001. Prospects and problems of sludge pre-treatment process.

466   Water Sci. Technol. 44,121-128.

467   Neiva Correia, C., Vaz, F., Torres, A., 2008. Anaerobic digestion of

468   biodegradable waste – operational and stability parameters for stability control. 5th

469   IWA International Symposium on AD of Solid Wastes and Energy Crops, Tunisia.

470   Nursten, H.E., 2005. The Maillard reaction: chemistry, biochemistry and

471   implications. Royal Society of Chemistry, Cambridge, UK.

472   O'Flaherty, V., Mahony, T., O'Kennedy, R., Colleran, E., 1998. Effect of pH on

473   growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic

474   and sulphate-reducing bacteria. Process Biochem. 33(5), 555-569.

475   Papadimitriou, E.K., 2010. Report: Hydrolysis of organic matter during

476   autoclaving of commingled household waste. Waste Manage. 30, 572-582.

477   Pinnekamp, J., 1989. Effect of thermal pretreatment of sewage sludge on

478   anaerobic digestion. Water Sci. Technol. 21(4-5), 97-108.

479   Procházka, J., Dolejš, P., Máca, J., Dohányos, M., 2012. Stability and inhibition

480   of anaerobic process caused by insufficiency or excess ammonia nitrogen.

481   Appl. Microbiol. Biotechnol. 93(1), 439-447.

482   Ren, L., Nie, Y., Liu, J., Jin, Y., Sun, L., 2006. Impact of hydrothermal process in

483   the nutrient ingredients of restaurant garbage. J. Environ. Sci. (China) 18(5): 1012-

484   1019.

485   Rincón, B., Heaven, S., Banks, C.J., Zhang, Y., 2012. Anaerobic digestion of

486   whole-crop winter wheat silage for renewable energy production. Energy Fuels 26,

487   2357-2364.

488   Sawayama, S., Inoue, S., Minowa, T., Tsukahara, K., Ogi, T., 1997.

489   Thermochemical liquidization and anaerobic treatment of kitchen garbage. J. Ferment.

490   Bioeng. 83(5), 451-455.

491   Stadler, R.H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S.,

492   Goldmann, T., Hau, J., Blank, I. 2004. In-depth mechanistic study on the formation of

493   acrylamide and other vinylogous compounds by the Maillard reaction. J. Agric. Food

494   Chem. 52, 5550-5558.

495   Takashima, M., Tanaka, Y., 2008. Comparison of thermo-oxidative treatments for

496   the anaerobic digestion of sewage sludge. J. Chem. Technol. Biotechnol. 83, 637-642.

497   Zhang, L., Jahng, D., 2012. Long-term anaerobic digestion of food waste

498   stabilized by trace elements. Waste Manage. 32, 1509-1515.

499   Zhang Y., Banks C. J., Heaven S., 2012. Anaerobic digestion of two

500   biodegradable municipal waste streams. J. Environ. Manage. 104, 166-174.

501

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

	Control FW	Autoclaved FW	Inoculum
pH	4.96 ± 0.16	5.01 ± 0.12	N/A
TS (g/kg)	247.5 ± 4.7	210.9 ± 18.6	77.3
VS (g/kg)	229.9 ± 4.5	194.6 ± 17.6	43.1
VS/TS (%)	92.9	92.3	55.8
SCOD (g/l)	98.2 ± 6.5	117.5 ± 10.3	11.9
TVFA (g/l)	3.1 ± 0.6	2.2 ± 0.2	2.4
TKN (g/kg)	7.4 ± 0.3	6.8 ± 0.3	4.9
NH_4-N (g/kg)	0.32 ± 0.12	0.41 ± 0.10	2.4
Fe (g/kg$_{TS}$)	0.13 ± 0.01	22.73 ± 12.54	N/A
SMP (m^{3}CH$_4$/kg$_{VS}$)	0.501 ± 0.020	0.445 ± 0.001	N/A
SMP (m^{3}CH$_4$/kg$_{TS}$)	0.462 ± 0.019	0.408 ± 0.001	N/A
SMP (m^{3}CH$_4$/kg$_{FM}$)	0.112 ± 0.005	0.084 ± 0.0001	N/A
Density (kg/l)	1.064 ± 0.0042	1.063 ± 0.0002	N/A

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

503

Table 2. Reactor characteristics during the last 4 weeks of each organic loading rate (OLR, kgVS/m^{3}d) periods.

OLR	Reactor	HRT (d)	Specific CH$_4$ yield (m^3/kgVS)	TS (g/kg)	VS (g/kg)	VS removal (%)	pH	TVFA (mg/l)	TKN (g/kg)	NH4-N (g/kg)	SCOD (g/l)
2	R1	117	0.443 ± 0.038	69.2 ± 1.7	44.5 ± 0.9	80.6	7.8 ± 0.13	267.5 ± 53.2	7.2 ± 0.1	3.8 ± 0.14	16.0 ± 2.9
	R3	94	0.373 ± 0.037	76.6 ± 2.3	55.6 ± 1.9	71.4	7.6 ± 0.04	132.5 ± 17.1	7.0 ± 0.3	2.1 ± 0.06	14.8 ± 0.1
3	R1	78	0.483 ± 0.013	71.1 ± 2.6	51.4 ± 2.5	77.7	7.8 ± 0.03	188.0 ± 71.9	8.4 ± 0.4	4.2 ± 0.15	15.6 ± 3.1
	R2		0.478 ± 0.009	69.8 ± 2.2	56.1 ± 9.1	75.6	7.8 ± 0.08	108.0 ± 17.9	8.9 ± 0.1	4.1 ± 0.14	23.0 ± 4.3
	R3	63	0.423 ± 0.002	84.0 ± 5.3	66.3 ± 4.4	65.9	7.5 ± 0.02	136.0 ± 26.1	8.2 ± 0.6	2.0 ± 0.05	17.6 ± 2.3
	R4		0.433 ± 0.009	76.4 ± 1.0	63.0 ± 1.1	67.6	7.5 ± 0.03	92.0 ± 23.9	7.9 ± 0.3	1.7 ± 0.03	19.7 ± 0.5
4	R1	58	0.465 ± 0.023	85.2 ± 5.6	64.2 ± 3.7	72.1	7.8 ± 0.07	112.0 ± 25.9	9.0 ± 0.1	3.5 ± 0.03	36.2 ± 0.6
	R3	47	0.439 ± 0.020	86.1 ± 2.6	69.9 ± 2.7	64.1	7.4 ± 0.06	90.0 ± 24.5	8.3 ± 0.5	1.3 ± 0.01	20.3 ± 0.5
6	R1	39	0.405 ± 0.006	102.1 ± 7.3	72.8 ± 4.1	68.3	7.7 ± 0.06	165.0 ± 42.0	9.4 ± 0.2	3.2 ± 0.08	28.3 ± 11.6
	R2		0.435 ± 0.008	90.3 ± 2.8	68.7 ± 2.8	70.1	7.7 ± 0.05	140.0 ± 54.8	9.4 ± 0.1	3.3 ± 0.05	25.9 ± 10.5
	R3	31	0.393 ± 0.044	85.7 ± 1.7	69.1 ± 1.4	64.5	7.2 ± 0.05	108.0 ± 35.6	7.8 ± 0.1	1.2 ± 0.07	18.2 ± 2.0
	R4		0.383 ± 0.013	88.3 ± 5.4	72.0 ± 3.1	63.0	7.3 ± 0.06	110.0 ± 21.6	8.2 ± 0.3	1.2 ± 0.13	18.7 ± 3.0

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/m^{3}day) 2, 4 and 6 in the stirred tank reactors (STRs).

OLR	Reactor	RMP (m^3/kgVS)	RMP$_{original}$ (m^3/kgVS$_{feed}$)[a]	Total CH$_4$ yield in STR+RMP (m^3/kgVS$_{feed}$)[a]	VS removal in STR+RMP (%)
2	R1	0.069 ± 0.005	0.013 ± 0.0009	0.456	85.1
	R3	0.063 ± 0.002	0.017 ± 0.0006	0.390	75.3
4	R1	0.065 ± 0.001	0.017 ± 0.0004	0.482	80.9
	R3	0.057 ± 0.002	0.020 ± 0.0006	0.459	67.4
6	R1	0.105 ± 0.002	0.032 ± 0.0005	0.437	76.9
	R3	0.095 ± 0.012	0.034 ± 0.0045	0.427	69.3

[a] Results calculated according to VS fed to STRs
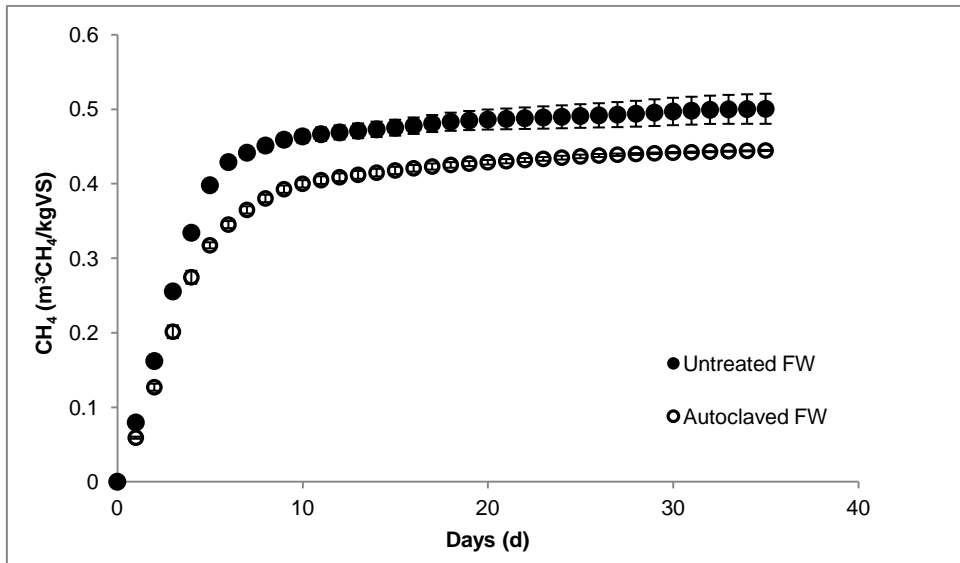
N=2-3

Fig. 1. Biochemical methane potential (BMP) and standard deviation of untreated and autoclaved food waste (FW) in 35-day assays.
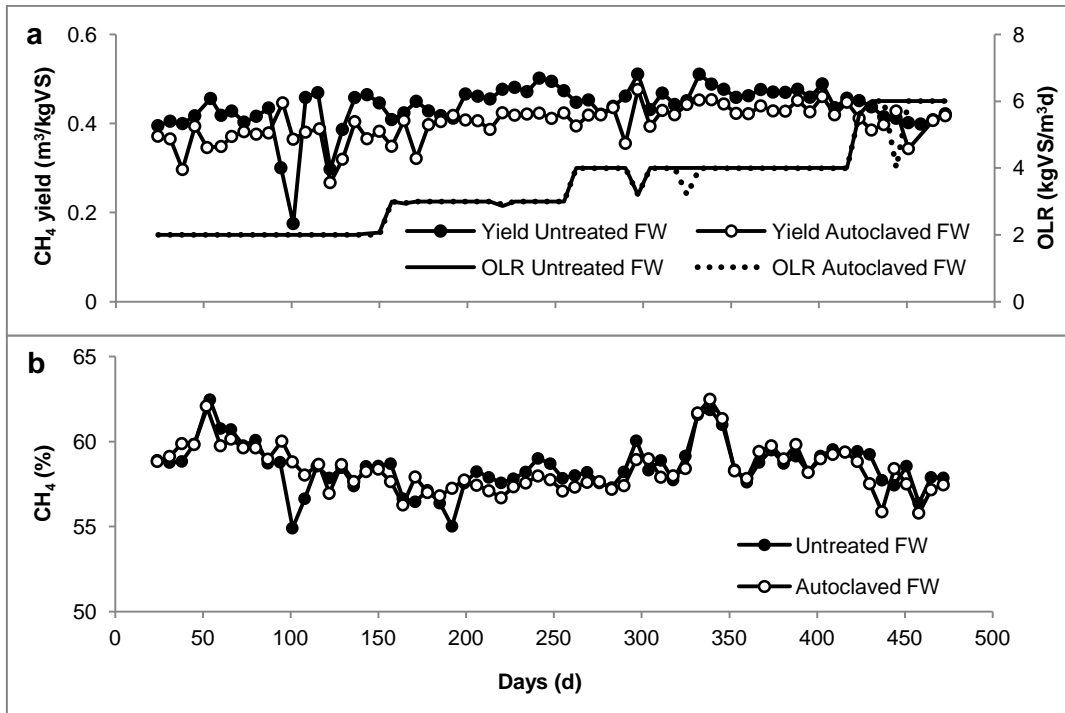
Fig. 2. Methane yields and contents in reactors treating untreated food waste (FW) and autoclaved FW during the semi-continuous operation.
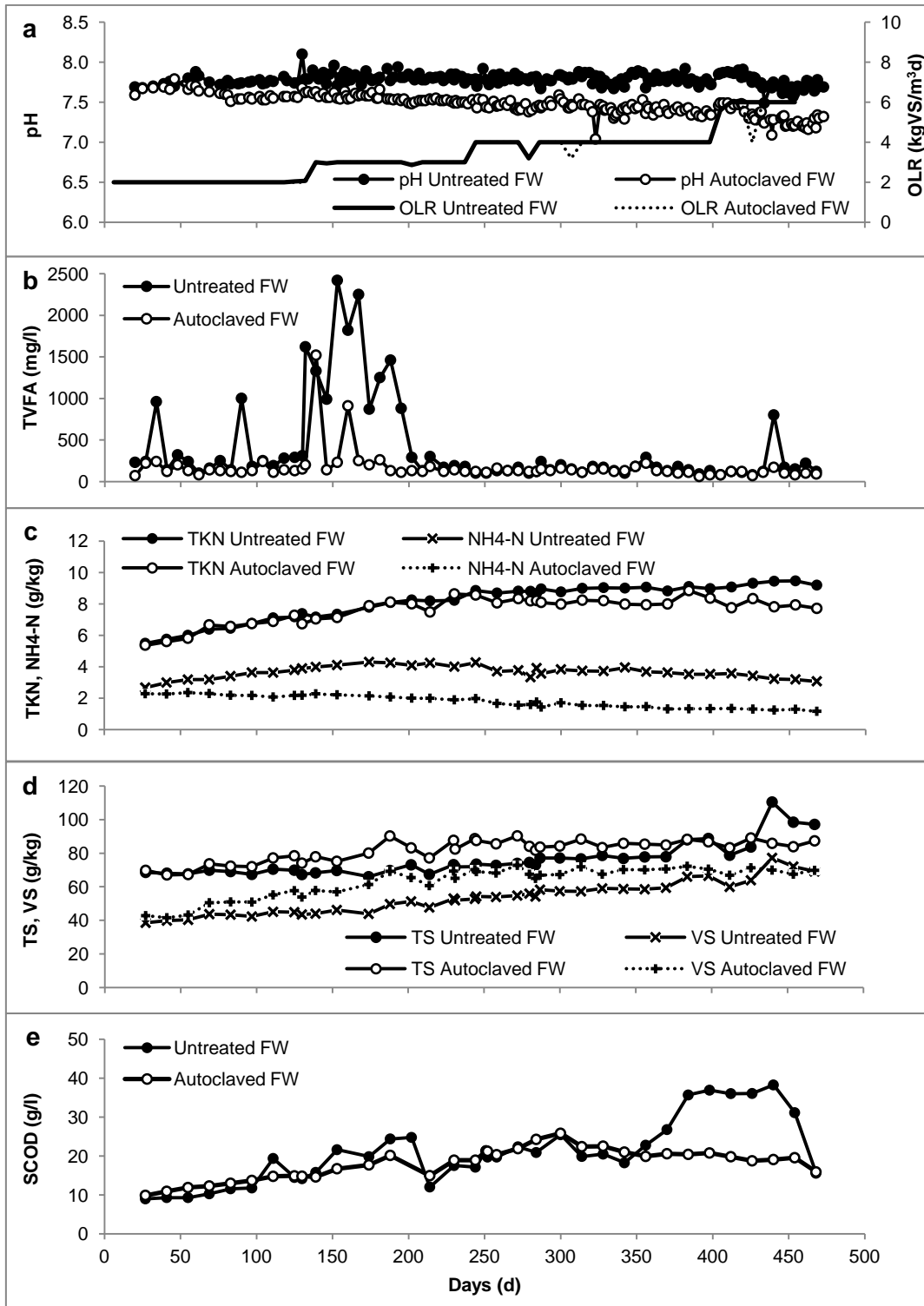
Fig. 3. Chemical characteristics (pH, TVFA, TKN, NH_4-N, TS, VS, SCOD) of untreated food waste (FW) and autoclaved FW reactor contents during the semi-continuous operation.
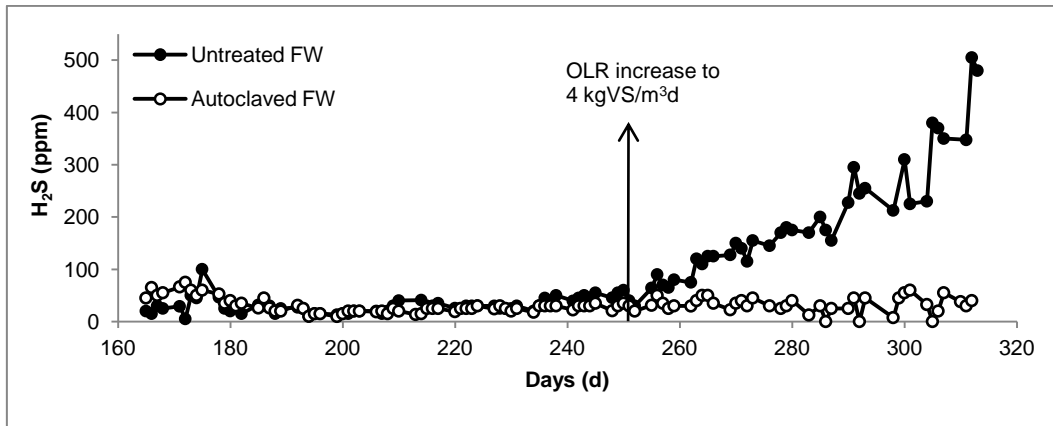
29

Fig. 4. H_2S contents in reactors treating untreated food waste (FW) and autoclaved FW during days 166-314.