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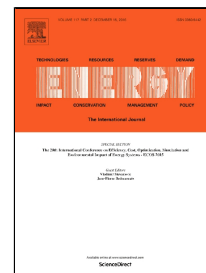
Role of trace elements in single and two-stage digestion of food waste at high organic loading rates

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Highlights

- Food waste lacks essential trace elements (TE) for single and multi-stage digestion
- Two-stage digestion did not show any better resilience to TE deficiency
- Failure occurred at loading rates in excess of 2 g VS L⁻¹ d⁻¹ at 16 days retention
- Addition of Co, Fe, Mo, Ni and Se enabled stable digestion at increased loading
- No additional gain in SMY was noted with trace element addition

1 **Role of trace elements in single and two-stage digestion of food waste at high**
2 **organic loading rates**

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6 **Abstract**

7 This study investigated trace element deficiency and supplementation in mono-
8 digestion of food waste. A single-stage system was contrasted to a two-stage system
9 (hydrolysis followed by methanogenesis). Initial hydrolysis is beneficial as it releases
10 hydrogen sulphide (H₂S), while the prevailing pH prevents an associated H₂S induced
11 precipitation of trace elements (TE). Stable digestion took place without TE
12 supplementation until an organic loading rate (OLR) of 2.0 g VS L⁻¹ d⁻¹; this was
13 followed by severe instability at an OLR of 2.5 g VS L⁻¹ d⁻¹ in both systems. A major
14 accumulation of volatile fatty acids (VFA) inhibited methanogenic activity. A gradual
15 deterioration of pH, VFA/TIC (ratio of VFA to alkalinity) and specific methane yields
16 provoked reactor failure. The benefit of enhanced TE availability in the two-stage
17 system was not apparent due to the complete absence of essential TE in the feed
18 stock. Supplementation of deficient TE Co, Fe, Mo, Ni and Se induced recovery,
19 reflected by an immediate improvement of VFA/TIC and VFA concentrations in both
20 systems. Specific methane yields were restored and maintained at initial levels. At a 16
21 day retention time, elevated loading rates as high as 5 g VS L⁻¹ d⁻¹ allowed stable
22 digestion with TE supplementation.

23

24 **Keywords:** biogas; two-stage digestion; food waste; trace elements; high performance.

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27 1 Introduction

28 Anaerobic digestion (AD) has become one of the dominant treatment technologies for
29 all kinds of wet organic wastes. In particular, source segregated food waste is a very
30 suitable substrate for AD due to its high biodegradability and volatile solids (VS)
31 content (Browne et al., 2014). A sufficient level of all macro- and micro-nutrients is a
32 vital prerequisite for key enzymes and microbes associated with stable
33 methanogenesis (Demirel & Scherer, 2011; Drosch, 2013; Kida et al., 2001). All essential
34 macro-nutrients, such as calcium (Ca), magnesium (Mg), nitrogen (N), phosphorus (P),
35 potassium (K), sodium (Na) and sulphur (S), are available in food waste. However,
36 mono digestion of food waste is challenging due to a lack of a sufficient level of micro-
37 nutrients (or trace elements) such as cobalt (Co), iron (Fe), nickel (Ni), molybdenum
38 (Mo) and selenium (Se) (Banks et al., 2012; Moestedt et al., 2015; Nges et al., 2012).
39 Recent studies have reported a deficiency in trace elements in single-stage digestion of
40 crop and waste based substrates (Banks et al., 2012; Karlsson et al., 2012; Wall et al.,
41 2014; Zhang & Jahng, 2012). If the substrate is deficient in nutrients, the process
42 performance diminishes or even fails (Drosch, 2013; Gustavsson et al., 2011; Schmidt et
43 al., 2014; Zhang & Jahng, 2012).

44 In an analysis of full scale biogas plants Lemmer et al. (2010) attributed a 10-50%
45 performance reduction per unit reactor volume to digester systems with insufficient
46 trace elements. The accessibility of trace elements is constrained by its bioavailability
47 (Karlsson et al., 2012; Ortner et al., 2015). In order to be available for methanogenic
48 archaea, trace elements have to be soluble and neither be fixed in precipitated
49 compounds (such as sulphates, sulphides, or carbonates) nor adsorbed. Ortner et al.

50 (2014) established that 30-70% of present trace elements were not bioavailable to the
51 microbial community.

52 The advantages of two-stage digestion systems potentially facilitate an increased
53 resilience towards a deficiency of trace elements. The spatial separation with different
54 pH in the two stages provides optimum conditions for individual anaerobic digestion
55 phases. The substrate is initially broken down into macro-molecules and liquid
56 fermentation products in the first reactor (Voelklein et al., 2016). Firstly, this is
57 associated with superior performances in terms of methane yields and process stability
58 as compared to the single stage system (Chen et al., 2015; Luo et al., 2011; Voelklein et
59 al., 2016). Secondly, the high degree of initial substrate acidification and degradation
60 (Voelklein et al., 2016) releases major sulphur contents as hydrogen sulphide (H_2S) into
61 the first reactor. The pK_a for the first dissociation of H_2S is 6.99 (Waechter, 2012). The
62 low pH of approx. 5 causes the hydrogen sulphide to be mainly present in its very
63 volatile state of H_2S , rather than in its more soluble conjugate base, the bisulfide ion
64 HS^- at more neutral pH (Waechter, 2012). As hydrogen sulphide is known to precipitate
65 trace metals (Gustavsson et al., 2011; Karlsson et al., 2012), the upstream release
66 potentially improves the bioavailability of these decisive elements. In addition, the
67 actual load of sulphur entering the downstream methane reactor at neutral pH is
68 diminished and limits the associated precipitation of trace elements. In contrast, in a
69 single stage reactor at neutral pH (without upstream treatment), approximately 50% of
70 hydrogen sulphide is available as bisulfide ion HS^- (pK_a 6.99) to potentially precipitate
71 trace elements (Waechter, 2012).

72

73 A general recommendation on optimal nutrient concentrations remains challenging.
74 The microbial community involved in the biogas process is composed of a huge variety
75 of microorganisms with differing nutrient requirements. In addition, bioavailability and
76 feedstock concentration of trace metals, temperature, loading and associated growth
77 rate of microbes determine the demand of nutrient supplementation (Ortner et al.,
78 2014; Uemura, 2010; Zhang et al., 2003). However, addition of deficient elements
79 proved to be vital in stabilizing the digestion process and overcoming biological
80 limitations (Demirel & Scherer, 2011; Karlsson et al., 2012; Nges et al., 2012; Pobeheim
81 et al., 2011; Qiang et al., 2012; Ward et al., 2008). Banks et al. (2012) established a
82 minimum trace element level for Co (0.22 mg L^{-1}) and Se (0.16 mg L^{-1}) in digestion of
83 food waste from the UK. In their study of trace element requirements for stable food
84 waste digestion at elevated ammonia concentrations, supplementation at levels of Co
85 (1.0 mg L^{-1}), Fe (5.0 mg L^{-1}), Mo (0.2 mg L^{-1}), Ni (1.0 mg L^{-1}), Se (0.2 mg L^{-1}) and tungsten
86 (W) (0.2 mg L^{-1}) were required (Banks et al., 2012). Zhang and Jahng (2012) reported
87 addition of Co (2 mg L^{-1}), Ni (10 mg L^{-1}), Mo (5 mg L^{-1}) and Fe (100 mg L^{-1}) in digestion
88 of food waste in Korea. A study by Zhang et al. (2015) described stable fermentation of
89 food waste at loading rates as high as $5.0 \text{ g VS L}^{-1} \text{ d}^{-1}$ while supplementing Co (1 mg L^{-1}),
90 Ni (1 mg L^{-1}), Se (0.2 mg L^{-1}) and Fe (5 mg L^{-1}). Gustavsson et al. (2011) suggested
91 supplementation of Co (0.5 mg L^{-1}), Ni (0.3 mg L^{-1}) and Fe (0.5 g L^{-1}) for digestion of
92 wheat stillage; addition of Se and W produced no effect. Sole supplementation of Ni,
93 Mo or Co proved to be insufficient (Moestedt et al., 2015; Zhang & Jahng, 2012).
94 The addition of trace elements to sustain stable fermentation ranged between 0.05-10
95 mg L^{-1} for Co, 5-500 mg L^{-1} for Fe, 0.0272-5 mg L^{-1} for Mo, 0.035-10 mg L^{-1} for Ni and
96 0.056-0.2 mg L^{-1} for Se (Banks et al., 2012; Gustavsson et al., 2011; Lemmer et al.,

97 2010; Moestedt et al., 2015; Nordell et al., 2015; Pobeheim et al., 2011; Qiang et al.,
98 2012; Zhang & Jahng, 2012; Zhang et al., 2012; Zhang et al., 2015). Further trace
99 metals such as manganese (Mn), tungsten and zinc (Zn) are rarely supplemented and
100 usually not considered to be deficient for digestion. Overdosing of trace metals
101 reduces enzyme and microbial activity (Lemmer et al., 2010).

102 The key role of trace element addition and its microbiological impact in anaerobic
103 digestion has been of major interest in recent studies. Long-term studies have
104 assessed conditions provoking reactor failure and subsequent reactor recovery after
105 trace element supplementation. However, research evaluating the implications of
106 trace element deficiency in two-stage systems is not to be found. This study expands
107 upon previous work on increasing loading rates in mono-digestion of food waste in
108 two-stage digestion (Voelklein et al., 2016). The objective in this work is to assess the
109 effect of trace elements on mono-digestion of source segregated food waste in single
110 and two-stage systems. The emphasis is not to analyse optimal concentrations of trace
111 elements, but to determine the impact of trace element deficiency and its response
112 after supplementation.

113

114 **2 Materials and Methods**

115 **2.1 Design of experiment**

116 The experiment investigated the impact of trace element depletion and subsequent
117 supplementation in different reactor configurations; a duplicate two-stage system (M1
118 & M2) and a conventional single-stage reactor (M3). The reactors were tested with the
119 same substrate (source segregated food waste) with stepwise increasing organic
120 loading rates. The reactors were fed once per day. The input substrate of the first

121 stage displaced a certain amount of effluent being introduced into the second stage.
122 Samples for analysis were obtained on a weekly basis from substrate, effluent stage
123 one and stage two. Biological parameters such as pH, VFA, VFA/TIC (ratio of volatile
124 fatty acids and total inorganic carbon) and specific methane yield (SMY) were assessed
125 as indicators of reactor stability and performance. The single- and two stage
126 experiments were performed at mesophilic conditions (38 °C) using 5 L continuous
127 stirred tank reactors (CSTRs) with vertically mounted stirrers. The actual working
128 volume for the first stage hydrolysis reactors was 1.35 L. The working volume was 4 L
129 for the subsequent methane reactors. The reactor volume of the single stage system
130 corresponded to the 4 L methane reactor volume of the two-stage system.

131

132 The hydraulic retention time (HRT) in the two-stage system was fixed at 4 days in the
133 upstream hydrolysis reactor and 12 days in the downstream methane reactor. This
134 matched the 16 day retention time of the single-stage reactor M3. The retention time
135 was achieved by diluting the substrate with specified amounts of water. The
136 experiment was commenced with an initial acclimatisation phase of 20 days. After
137 reaching steady state conditions (after at least 3 HRTs) the organic loading rate of M1
138 and M2 was increased gradually from 2 to 5 g VS L⁻¹ d⁻¹. The loading rate for the single-
139 stage reactor (M3) was increased from 2 to 4 g VS L⁻¹ d⁻¹.

140

141 **2.2 Inoculum and substrate**

142 The inoculum was obtained from a single-stage digester fed grass silage and food
143 waste. The source segregated food waste was obtained from a local waste
144 management company collecting food waste from major catering premises.

145 Approximately 80 kg of food waste was first manually screened and non-biodegradable
146 contaminants like bones and plastics were removed. The residual food waste was
147 subsequently shredded in a mechanical meat mincer to a pasty consistence with
148 particle size between 0.5 to 5mm. It was stored at a temperature of -20 °C until fed to
149 the anaerobic reactors. A total solids (TS) content of $24.63 \pm 0.72\%$ with a share of
150 $94.29 \pm 0.64\%$ present as volatile was determined. The pH yielded in 5.1 ± 0.05 with a
151 C:N ratio of 14.86. The physical and chemical characteristics of the substrate were
152 analysed and are further described in Voelklein et al. (2016).

153

154 **2.3 Analytical methods**

155 VFA/TIC was measured using the Nordmann-method (Nordmann, 1977). This
156 parameter indicates the ratio of volatile fatty acids to buffering capacity. The
157 concentrations of individual volatile fatty acids were analysed with gas
158 chromatography (Hewlett Packard HP6890) using a Nukol™ fused silica capillary
159 column (30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (FID). Hydrogen
160 was used as a carrier gas. All metal elements except selenium were analysed according
161 to DIN EN ISO 11885 with inductively coupled plasma optical emission spectrometry
162 (ICP-OES); selenium was determined according to DIN EN ISO 17294-2 (E29) with
163 inductively coupled plasma mass spectrometry (ICP-MS). Biogas composition was
164 analysed for CH₄, CO₂, H₂, O₂ and N₂ using a Hewlett Packard HP6890 gas
165 chromatograph equipped with a Hayesep R packed GC column (3 m x 2 mm, mesh
166 range of 80-100) and a thermal conductivity detector (TCD). Argon was used as carrier
167 gas. Certified gas standards were employed for the standardization of hydrogen,

168 methane and carbon dioxide. The utilised analytical methods are further described in
169 Voelklein et al. (2016).

170

171 **2.4 Recognising reactor failure and corrective measures**

172 The reason for reactor failure can be found mainly in organic overload, inadequate
173 mixing, enhanced dry solids content of digestate in the reactor, temperature changes,
174 ammonia inhibition, inhibitory substances in the feed stock or undersupply of trace
175 elements (Drosg, 2013). Close process monitoring allows identification of changes in
176 parameters such as pH, VFA/TIC, VFA, hydrogen concentration, biogas quality and
177 quantity. The reactor specific interpretation and comparison of those parameters
178 allows establishment of a characteristic baseline and immediate recognition when
179 deviating from the norm. Strategies to counteract depend on the initial circumstances
180 causing reactor failure. Pathways to recovery include for a reduction/cessation of
181 feedstock, elevation of pH, dilution with water or digestate, supplementation of
182 deficient nutrients and are always accompanied with close process monitoring.

183

184 **3 Results**

185 **3.1 Nutrient supplementation**

186 The food waste contained trace element metals Cu, Fe, Ni, Mn and Zn in the range of
187 0.42-31.5 mg L⁻¹ (Table 1). Some of the key trace elements for anaerobic digestion
188 (such as Co, Mo, Ni and Se) were undersupplied and partly below the detection limit. A
189 similar trace element spectrum in food waste was also found in other studies (Banks et
190 al., 2012; Qiang et al., 2012; Zhang & Jahng, 2012). The low concentrations in the

191 substrate were further reflected by the decreased values found in the effluent of the
192 reactors once they became critically unstable (Table 1).

193

194 **Table 1** Trace element levels in food waste, in digestate at reactor failure, reported
195 range of nutrients added in literature and nutrients added to feed stock.

196

197 The experiment commenced at a low OLR of $2 \text{ g VS L}^{-1} \text{ d}^{-1}$ without any nutrient
198 addition. Once the experiment became critically unstable, trace element
199 supplementation commenced. The trace elements added to the feedstock of the
200 methane reactors were designed to contain the deficient elements Co, Fe, Mo, Ni and
201 Se according to Table 1. The level of trace elements in the feedstock and trace element
202 solution consequently determines the concentration of trace elements in the
203 digestate, with a minor increase due to conversion of solid matter into gas. The
204 selected concentrations for supplementation in this experiment followed levels most
205 frequently applied and recommended in literature (Banks et al., 2012; Gustavsson et
206 al., 2011; Zhang & Jahng, 2012; Zhang et al., 2015). Thus 1 mg L^{-1} Co, 160 mg L^{-1} Fe, 0.2
207 mg L^{-1} Mo, 1 mg L^{-1} Ni and 0.2 mg L^{-1} Se were added to the feedstock (Table 1). In the
208 present study Co was added in the form of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, Fe as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, Mo as
209 $\text{H}_24\text{Mo}_7\text{N}_6\text{O}_{24} \cdot 4\text{H}_2\text{O}$, Ni as $\text{Cl}_2\text{Ni} \cdot 6\text{H}_2\text{O}$ and Se as Na_2SeO_3 . Trace elements were
210 introduced in the single stage reactor and the methane reactor of the two-stage
211 system. Adequate amounts of Fe were added to precipitate emerging hydrogen
212 sulphate to iron sulphur compounds. The bioavailability of supplemented trace
213 elements in dissolved form was sufficient for the methanogenic archaea (Gustavsson
214 et al., 2013; Ortner et al., 2015).

215 3.2 Single-stage reactor performance

216 3.2.1 Process performance until reactor failure

217 Figure 1 shows the performance of the single-stage reactor M3 during the 360 day
218 operation period. After an initial commissioning period of two hydraulic retention
219 times (equivalent to 32 days), the reactor was set at an OLR of 2 g VS L⁻¹ d⁻¹ and the
220 SMY stabilised at 324.5 ±25.5 L CH₄ kg VS⁻¹. The pH and VFA/TIC values showed a minor
221 deterioration towards the end of OLR 2 g VS L⁻¹ d⁻¹. This phenomenon was explained
222 with a decrease in measured TIC values, provoking reduced buffer capacity, raising the
223 ratio of VFA/TIC and lowering the pH values. However, low VFA/TIC values of on
224 average 0.21 indicated stable conditions during the overall steady state period at OLR
225 2 g VS L⁻¹ d⁻¹ (Table 2), as VFA/TIC ratios below 0.4 are associated with stable reactor
226 performance (Drosg, 2013). Low VFA levels of 0.3 g L⁻¹ (Table 2), and constant SMY,
227 further strengthened the conclusion of stable reactor conditions. A further increase in
228 loading rate to an OLR of 2.5 g VS L⁻¹ d⁻¹ was immediately accompanied by a subtle
229 increase of VFA/TIC, enhanced VFA and declining pH. However, a decrease of the key
230 reactor performance SMY was only gradually observed. After a continuous drop in gas
231 production over the period of 3 HRTs, a significant deterioration of process parameters
232 (pH, VFA/TIC, VFA) caused a distinct drop in SMY (Figure 1). After 3.5 HRTs at an OLR of
233 2.5 g VS L⁻¹ d⁻¹ the methane content decreased to 30.5 vol.-% and the pH dropped by 1
234 unit to 5.4 in only 5 days, emphasising the dynamic development in the final stage of
235 failure. The acid consuming acetoclastic methanogens could not keep pace with the
236 rising levels of total VFA (4.32 g L⁻¹) and were further inhibited by this accumulation. At
237 the peak of reactor failure (day 133) SMY fell to levels as low as 82.7 L CH₄ kg VS⁻¹ and
238 a VFA/TIC value of 1.57 clearly emphasised the irreversible state, exceeding stable

239 VFA/TIC levels of below 0.4 (Drosg, 2013). This development was attributed to major
240 trace element depletion as confirmed by laboratory analysis in Table 1.

241

242 **Fig. 1.** Single-stage reactor performance before and after trace element
243 supplementation.

244

245 **3.2.2 Recovery after trace element addition**

246 After severe reactor failure on day 133, it was decided to stop feeding (Figure 1). In
247 order to facilitate microbiological recovery, on day 140 the pH was adjusted to neutral
248 levels with sodium hydroxide and trace element supplementation was initiated (Table
249 1). After VFA/TIC levels dropped and a distinct improvement in gas quality and
250 production were observed (day 147), it was decided to recommence feeding. The OLR
251 of 2.5 g VS L⁻¹ d⁻¹ was further maintained for 4 HRTs. Neither an increase in VFA/TIC,
252 nor a significant reduction in pH was determined. The SMY reached a plateau of 319.3
253 ± 9.1 L CH₄ kg VS⁻¹ and regained the levels achieved before reactor failure. The
254 experiment continued with trace element addition for another 160 days with elevating
255 OLRs. The SMY remained at 326.6 ± 26.2 and 316.4 ± 17.9 L CH₄ kg VS⁻¹ at an OLR of 3
256 and 4 g VS L⁻¹ d⁻¹ respectively. The reactor performed at healthy conditions with only a
257 minor rise in VFA and VFA/TIC. However, pH never reached the initial values of 7 again
258 which was attributed to the gradually enhanced OLR. Table 2 summarises the
259 performance characteristics of each steady state.

260

261 **Table 2** Performance characteristics of single-stage reactor M3 at each steady state.

262

263 3.3 Two-stage reactor performance

264 3.3.1 Process performance until reactor failure

265 Figure 2 & 3 show the reactor performances of methane reactors (M1 & M2) deployed
266 in a two-stage system. The experiments commenced with a 3 HRT starting period to
267 acclimatise the microorganisms to food waste digestion. Thereafter, the OLR was
268 brought to $2 \text{ g VS L}^{-1} \text{ d}^{-1}$ until a steady state was reached after 3 HRTs. The SMY for M1
269 and M2 settled at 392 ± 12.6 and $419 \pm 23.2 \text{ L CH}_4 \text{ kg VS}^{-1}$ respectively. Low levels of
270 VFA/TIC and pH indicated stable biological conditions. As the OLR was increased in M1
271 and M2 to $2.5 \text{ g VS L}^{-1} \text{ d}^{-1}$, the SMY dropped acclimatising to the higher load. This was
272 to be expected and from day 84 onwards the reactors temporarily appeared to
273 recover, indicated by lower VFA/TIC and pH improvements after the initial
274 deterioration. However, the advance of the experiment revealed a massive VFA/TIC
275 increase and pH drop. A SMY reduction to levels as low as one third (M1) and a half
276 (M2) of SMY as compared to that at an OLR of $2 \text{ g VS L}^{-1} \text{ d}^{-1}$ was identified. The
277 magnitude and the dynamic change of process parameters exceeded previous
278 observations significantly. As a consequence, the initial performance of M1 could not
279 be re-obtained. M2 remained at unsteady levels (VFA/TIC, pH) for longer whilst
280 showing a temporary gain in SMY, before ultimately being unable to cope with the
281 loading. The higher level of Ni and Mo in the digestate of M2 as compared to M1
282 (Table 1) might have initially mitigated and delayed the final break down. In the final
283 stage of reactor failure M1 (day 96-108) and M2 (day 132-144) pH values dropped as
284 far as 6.69 (M1) and 6.92 (M2) whilst VFA/TIC analysis ultimately peaked at 1.42 and
285 1.34 respectively. A major accumulation of VFA in the range of 4.98 (M1) and 3.44 g L^{-1}
286 (M2), dominated by acetic and propionic acid, reinforced the theory of an inhibition of

287 the acetoclastic pathway in methanogenesis. Subsequent reactor failure was
288 attributed to major trace element depletion as confirmed by laboratory analysis in
289 Table 1.

290

291 **Fig. 2.** Two-stage reactor performance (M1) before and after trace element
292 supplementation.

293

294 **Fig. 3.** Two-stage reactor performance (M2) before and after trace element
295 supplementation.

296

297 **3.3.2 Recovery after trace element addition**

298 After the reactors failed, trace element supplementation as recorded in Table 1 was
299 started for M1 and M2 on day 108 and 144 respectively. As the reactor failure of M1
300 was more severe than M2, feeding was suspended for 3 days and pH was raised with
301 sodium hydroxide to levels before failure. After 6 days (0.5 HRT) pH and VFA/TIC in M1
302 indicated stable fermentation and matched the results at OLR 2 g VS L⁻¹ d⁻¹ again. The
303 SMY quickly reached 371.1 ± 5.5 L CH₄ kg VS⁻¹ and corresponded with results before
304 supplementation of trace elements. M2 neither received an alkaline solution for pH
305 stabilisation nor a feeding stop. Therefore, the pH only gradually increased over time
306 and VFA/TIC recovery to levels below 0.5 experienced a minor delay of 12 days. The
307 OLR of M1 and M2 was further increased until an OLR of 5 g VS L⁻¹ d⁻¹ was reached
308 whilst maintaining an HRT over the two stages of 16 days. Constant low VFA/TIC and
309 VFA levels were observed with a gradual increase corresponding to rising OLR. The

310 SMY ranged between 373.9 ± 10.9 and 413.9 ± 22.6 L CH₄ kg VS⁻¹ corresponding with
311 values achieved before trace element addition. Stable fermentation conditions were
312 restored and maintained after trace element supplementation, confirming the failure
313 was induced by a deficiency of essential trace elements. Table 3 summarises the
314 performance characteristics of each steady state.

315

316 **Table 3** Performance characteristics of second stage of two-stage reactors, M2 & M3
317 at each steady state.

318

319 **3.4 Impact and comparison of trace element supplementation on single and** 320 **two-stage digestion**

321 All reactors were subjected to the same overall conditions in terms of loading rate,
322 retention time and temperature. Figure 4 illustrates and compares the steady state key
323 performance parameters VFA, VFA/TIC and SMY of the second stage of the two-stage
324 systems (M1 & M2) with the one-stage reactor (M3). Without any addition of trace
325 elements an elevated SMY of 392 ± 12.6 and 419 ± 23.2 L CH₄ kg VS⁻¹ was obtained at
326 an initial OLR of $2 \text{ g VS L}^{-1} \text{ d}^{-1}$ in the two-stage reactors M1 & M2 respectively, as
327 opposed to 324 ± 25.5 L CH₄ kg VS⁻¹ for M3. The superior gas yields in the two-stage
328 digestion is a result of the upstream hydrolysis and is further described in Voelklein et
329 al. (2016).

330

331 After increasing the OLR to $2.5 \text{ g VS L}^{-1} \text{ d}^{-1}$ the VFA/TIC level in all reactors (M1, M2 &
332 M3) severely deteriorated by up to one order of magnitude to levels of 1.34-1.57. The
333 reactors failed and significantly exceeded levels of fermentation considered stable

334 (Drosg, 2013). Similar observations of unstable reactor behaviour at low OLR have
335 been reported by Climenhaga and Banks (2008), Gustavsson et al. (2011), Nordell et al.
336 (2015) and Zhang et al. (2015). The VFA spectrum of M1 and M2 was dominated by
337 acetic (3.59 g L^{-1} and 2.67 g L^{-1} respectively) and propionic acid (0.52 g L^{-1} and 0.31 g L^{-1}
338 respectively) with minor shares of longer chained fatty acids ($\text{C}_4\text{-C}_6$). In contrast the
339 share of 1.88 g L^{-1} of propionic acid exceeded the share of 1.25 g L^{-1} of acetic acid in
340 the single-stage reactor M3. The accumulation of VFA is an associated consequence of
341 trace element deficiency (Banks et al., 2012; Nordell et al., 2015; Pobeheim et al.,
342 2011).

343 The severe drop in SMY caused by reactor failure ultimately affected all reactors to the
344 same extend regardless of the reactor configuration. The initial acidification and break
345 down of substrate in the upstream reactor of the two-stage system resulted in a
346 prevailing pH of approx. 5 (Voelklein et al., 2016). This allowed part of the hydrogen
347 sulphide to be released and present as H_2S (gas), not resulting in precipitation and
348 potential deficiency of trace elements. A more robust and beneficial behaviour of the
349 two-stage process in respect of its upstream hydrolytic pre-treatment was not
350 observed, due to the complete lack of some trace elements in the feedstock (Table 1).
351 Therefore, the two-stage system did not show any better resilience to nutrient
352 deficiency in general, neither towards the potential advantage of reduced precipitation
353 nor to enhanced availability of trace elements.

354

355 However, the hypothesis of increased bioavailability in a two-stage system remains, as
356 an absence of trace element Co, Mb and Se can not be compensated by increased
357 bioavailability. The trace element supplementation after failure stimulated

358 methanogenic activity in all reactors, triggering a reduction in VFA and subsequently
359 sustained low VFA/TIC values. This is in line with observations made in studies
360 assessing the long term effects of trace element supplementation (Karlsson et al.,
361 2012; Nges et al., 2012; Pobeheim et al., 2011). The dynamic response in both reactor
362 configurations restored and enabled SMY levels comparable to the experimental
363 period before failure. Immediate beneficial effects after addition of deficient nutrients
364 were also obtained by Moestedt et al. (2015), Nordell et al. (2015), Qiang et al. (2012)
365 and Zhang et al. (2015).

366 The gap in SMY between the one and two-stage remained after stabilizing the reactors
367 with trace elements. The 16 day BMP performance of $471.94 \text{ L CH}_4 \text{ kg VS}^{-1}$ was never
368 reached regardless of the elimination of nutrient deficiency. This is a result of fully
369 mixed continuous stirred tank reactors causing fresh matter to leave the reactor prior
370 to complete digestion. The shorter the retention time the more significant this effect
371 becomes. The gas yields further confirmed the observed conclusion that trace element
372 addition had negligible impact on SMY (at a fixed HRT of 16 days), yet is essential for a
373 stable fermentation with low VFA levels after exceeding a threshold OLR of 2.0 g VS L^{-1}
374 d^{-1} . The positive effects of trace element addition are in line with studies conducted by
375 Banks et al. (2012); Gustavsson et al. (2011); Nges et al. (2012); Qiang et al. (2012);
376 Zhang and Jahng (2012); Zhang et al. (2015).

377

378 **Fig. 4.** Performance comparison of single and two-stage digestion at steady state with
379 and without trace element supplementation.

380

381 4 Conclusion

382 Food waste lacked essential nutrients causing instable single and two-stage reactor
383 performance after exceeding a threshold OLR of $2.0 \text{ g VS L}^{-1} \text{ d}^{-1}$. The break down was
384 characterised by pH, VFA/TIC, VFA and CH_4 concentrations far beyond stable limits and
385 a reduction in SMY. TE addition of Co, Fe, Mo, Ni and Se restored a stable process and
386 allowed increased loading rates. TE addition did not increase SMY beyond levels at
387 initial stable digestion. The two-stage system incorporating hydrolytic pre-treatment
388 showed improved SMY than the single-stage system but did not show any better
389 resilience to nutrient deficiency.

390

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400

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485 trace metals for methane producing bacteria. *Biomass Bioenergy* 25, 427-433.

486

487 **List of Figures and Tables**

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497

498 **Table 1** Trace element levels in food waste, in digestate at reactor failure, reported

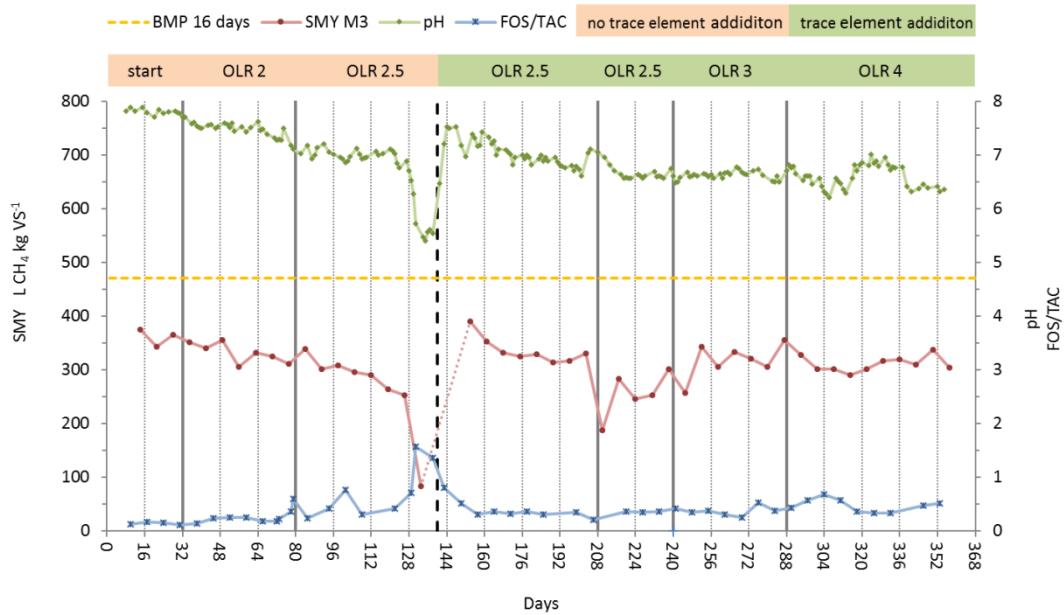
499 range of nutrients added in literature and nutrients added to feed stock.

500 **Table 2** Performance characteristics of single-stage reactor M3 at each steady state.

501 **Table 3** Performance characteristics of second stage of two-stage reactors, M2 & M3

502 at each steady state.

503



504
 505 Dotted line for SMY M3 (day 133-154) represents feeding stop for 14 days to facilitate recovery after
 506 reactor failure (calculation of SMY not applicable); stabilisation of pH with NaOH and commencement of
 507 trace element supplementation on day 140; experimental difficulties (day 208-240) with gas measuring
 508 equipment.
 509

510 **Fig. 1.** Single-stage reactor performance before and after trace element
 511 supplementation.

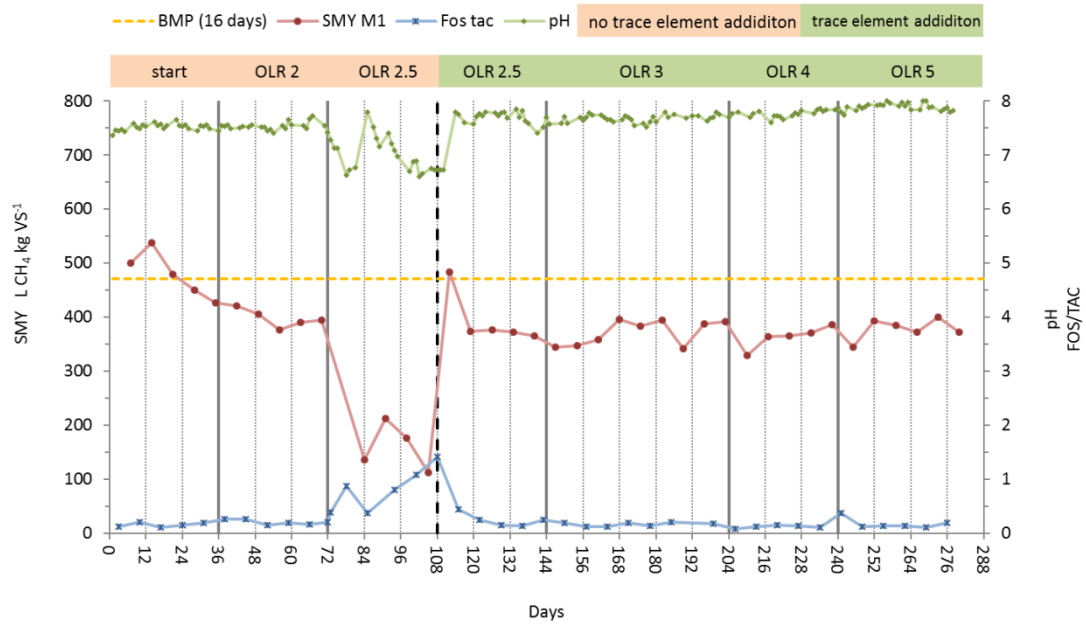
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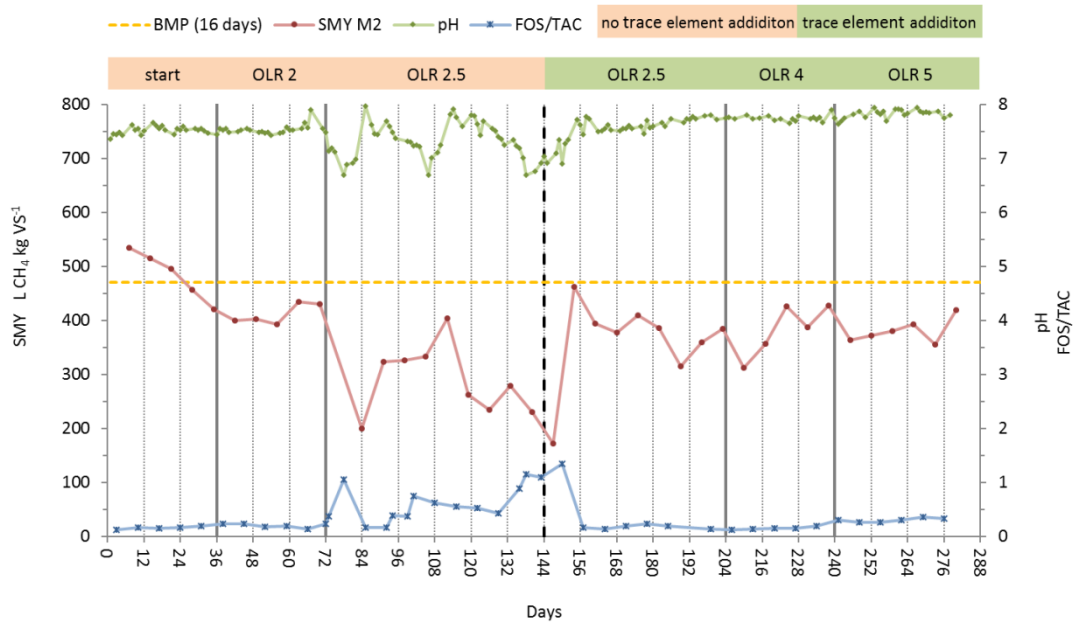
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518 **Fig. 2.** Two-stage reactor performance (M1) before and after trace element
 519 supplementation.

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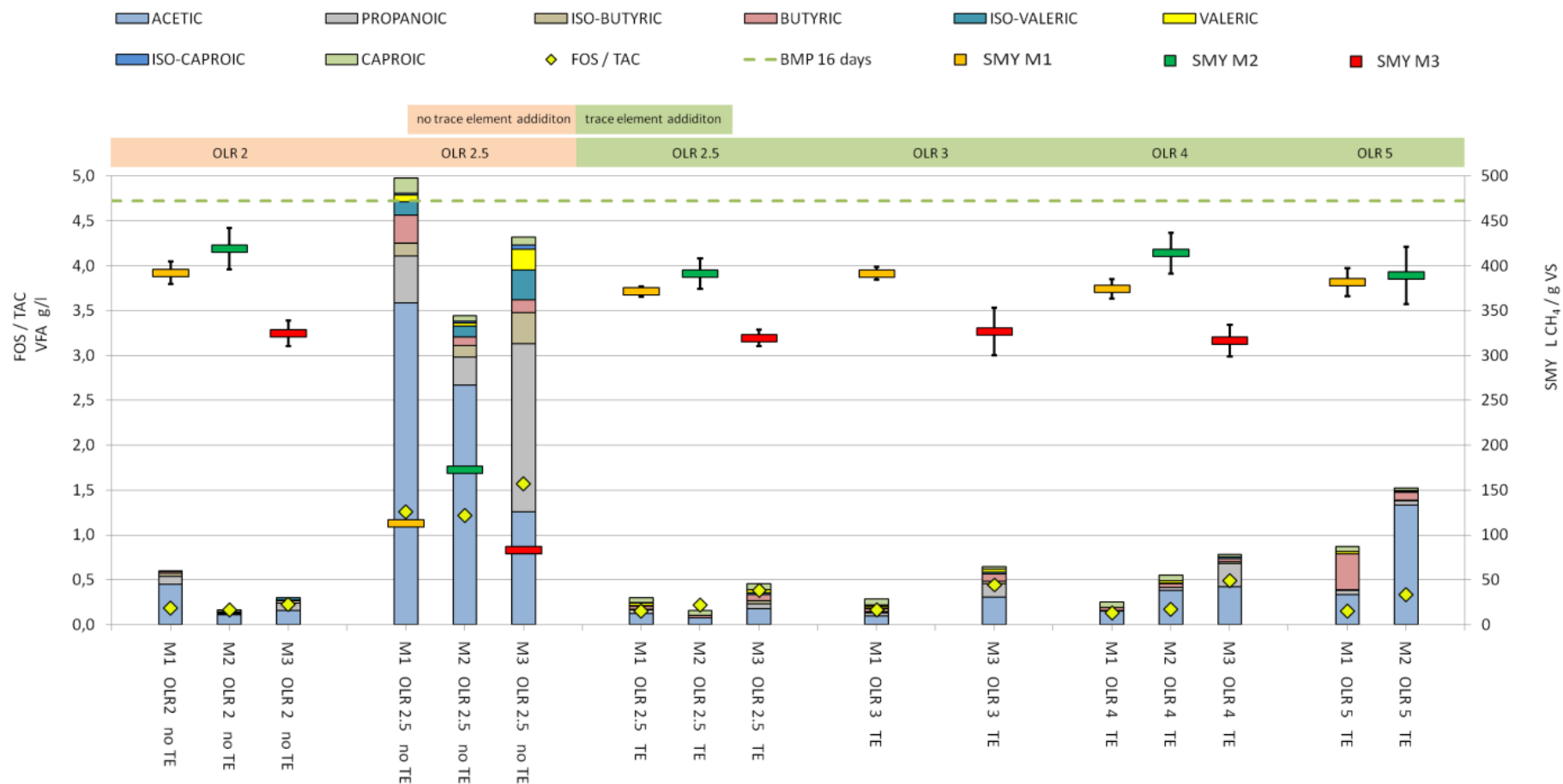
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522 OLR 3 was omitted to match reactor M2 with M1.

523 **Fig. 3.** Two-stage reactor performance (M2) before and after trace element

524 supplementation.

525



526
 527 Gas yields at OLR 2.5 no TE without error bars as values only represent the final state of reactor failure.
 528

529 **Fig. 4.** Performance comparison of single and two-stage digestion at steady state with and without trace element supplementation.

530 **Table 1**531 Trace element levels in food waste, in digestate at reactor failure, reported range of nutrients added in literature and nutrients added to feed
532 stock.

Element	Unit	Food waste	M1 ^b	M2 ^b	M3 ^b	Nutrients added in literature	Nutrients added to feed stock
Iron (Fe)	mg L ⁻¹ ww	31.5	21.6	25.6	19.7	5-500	160
Manganese (Mn)	mg L ⁻¹ ww	6.9	0.87	0.86	1.6	-	-
Zinc (Zn)	mg L ⁻¹ ww	7.3	0.83	0.84	1.6	-	-
Copper (Cu)	mg L ⁻¹ ww	1.3	0.78	1.0	1.2	-	-
Nickel (Ni)	mg L ⁻¹ ww	0.42	0.039	0.32	0.75	0.035-10	1
Molybdenum (Mo)	mg L ⁻¹ ww	< LD ^a	0.028	0.043	0.092	0.0272-5	0.2
Cobalt (Co)	mg L ⁻¹ ww	< LD ^a	0.019	< LD ^a	0.019	0.05-10	1
Selenium (Se)	mg L ⁻¹ ww	< LD ^a	< LD ^a	< LD ^a	< LD ^a	0.056-0.2	0.2
Cadmium (Cd)	mg L ⁻¹ ww	< LD ^a	< LD ^a	< LD ^a	< LD ^a	-	-

533 ^a <LD, lower than detection limit of 0.5 mg kg⁻¹ dry solids; ^b at OLR 2.5 g VS L⁻¹ d⁻¹ after reactor failure; mg L⁻¹ corresponds to mg kg⁻¹ (density neglected for comparison
534 reasons); ww: wet weight.

535 **Table 2**

536 Performance characteristics of single-stage reactor M3 at each steady state.

		M3	M3 ^a	M3	M3	M3
Trace element addition		no	no	yes	yes	yes
OLR	g VS L ⁻¹ d ⁻¹	2	2.5	2.5	3	4
HRT	days	16	16	16	16	16
pH		7 ±0.1	5.4	6.6 ±0.1	6.5 ±0.3	6.6 ±0.3
VFA/TIC		0.21 ±0.02	1.57	0.38 ±0.04	0.45 ±0.11	0.49 ±0.03
Acetate	g L ⁻¹	0.16 ±0.07	1.25	0.18 ±0.02	0.31 ±0.11	0.42 ±0.04
Propionate	g L ⁻¹	0.08 ±0.04	1.88	0.06 ±0.01	0.15 ±0.09	0.26 ±0.01
Iso-Butyrate	g L ⁻¹	0.03 ±0.03	0.35	0.03 ±0.01	0.03 ±0.01	0.02 ±0.01
Butyrate	g L ⁻¹	0.01 ±0.01	0.14	0.07 ±0.01	0.08 ±0.02	0.04 ±0.01
Iso-Valerate	g L ⁻¹	0.02 ±0.04	0.33	0.02 ±0.01	0.03 ±0.01	0.02 ±0.01
Valerate	g L ⁻¹	0.01 ±0.01	0.23	0.03 ±0.01	0.03 ±0.01	0.01 ±0.01
Iso-Caproate	g L ⁻¹	0.01 ±0.01	0.04	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01
Caproate	g L ⁻¹	0.01 ±0.01	0.09	0.07 ±0.01	0.03 ±0.02	0.02 ±0.01
Total VFA	g L ⁻¹	0.3 ±0.18	4.32	0.46 ±0.01	0.64 ±0.23	0.78 ±0.05
Methane concentration	Vol.-%	55.3 ±1.8	30.5	55 ±0.8	54.9 ±1	55.8 ±1
Methane yield	L CH ₄ kg VS ⁻¹	324.5 ±25.5	82.7	319.3 ±9.1	326.6 ±26.2	316.4 ±17.9

537 ^a no standard deviation applied as values only represent the final state of reactor failure.

538

Table 3

Performance characteristics of second stage of two-stage reactors, M1 & M2 at each steady state.

		M1	M2	M1 ^a	M2 ^a	M1	M2	M1	M1	M2	M1	M2
Trace element addition		no	no	no	no	yes	yes	yes	yes	yes	yes	yes
OLR	g VS L ⁻¹ d ⁻¹	2.0	2.0	2.5	2.5	2.5	2.5	3.0	4.0	4.0	5.0	5.0
HRT	days	12	12	12	12	12	12	12	12	12	12	12
pH		7.5 ±0.1	7.5 ±0.1	6.69	6.92	7.5 ±0.1	7.5 ±0.1	7.6 ±0.1	7.7 ±0.1	7.7 ±0.1	7.9 ±0.1	7.9 ±0.1
VFA/TIC		0.17 ±0.03	0.16 ±0.01	1.42	1.34	0.15 ±0.01	0.22 ±0.04	0.17 ±0.04	0.13 ±0.03	0.17 ±0.03	0.15 ±0.06	0.34 ±0.02
Acetate	g L ⁻¹	0.45 ±0.1	0.11 ±0.09	3.59	2.67	0.12 ±0.03	0.08 ±0.02	0.09 ±0.01	0.15 ±0.09	0.38 ±0.33	0.33 ±0.1	1.33 ±0.2
Propionate	g L ⁻¹	0.09 ±0.01	0.01 ±0.02	0.52	0.31	0.04 ±0.01	0.01 ±0.01	0.04 ±0.01	0.01 ±0.01	0.04 ±0.01	0.05 ±0.01	0.05 ±0.02
Iso-Butyrate	g L ⁻¹	0.04 ±0.01	0.01 ±0.01	0.14	0.13	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01
Butyrate	g L ⁻¹	0.02 ±0.01	0.01 ±0.01	0.31	0.10	0.04 ±0.01	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.04 ±0.01	0.4 ±0.09	0.09 ±0.07
Iso-Valerate	g L ⁻¹	0.01 ±0.01	0.01 ±0.01	0.15	0.12	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.02
Valerate	g L ⁻¹	0.01 ±0.01	0.01 ±0.01	0.07	0.04	0.02 ±0.02	0.01 ±0.01	0.02 ±0.02	0.01 ±0.01	0.03 ±0.01	0.03 ±0.01	0.01 ±0.02
Iso-Caproate	g L ⁻¹	0.01 ±0.01	0.01 ±0.01	0.02	0.02	0.01 ±0.01	0.01 ±0.01	0.02 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01
Caproate	g L ⁻¹	0.01 ±0.01	0.02 ±0.01	0.17	0.06	0.06 ±0.05	0.05 ±0.01	0.06 ±0.03	0.06 ±0.01	0.07 ±0.01	0.05 ±0.02	0.02 ±0.03
Total VFA	g L ⁻¹	0.6 ±0.1	0.16 ±0.14	4.98	3.44	0.3 ±0.17	0.16 ±0.03	0.29 ±0.11	0.25 ±0.1	0.55 ±0.36	0.87 ±0.23	1.52 ±0.29
Methane concentration	Vol.-%	68.6 ±2.5	69.5 ±1.9	55.40	61.20	74.3 ±1.5	68.5 ±1.1	72.8 ±0.3	69.2 ±1.1	66.7 ±1.5	70.2 ±0.8	67.6 ±2.9
Methane yield	L CH ₄ kg VS ⁻¹	392 ±12.6	419 ±23.2	112.60	172.40	371.1 ±5.5	391.2 ±16.7	391.4 ±7.2	373.9 ±10.9	413.9 ±22.6	381.7 ±15.5	389.2 ±31.8

^a no standard deviation applied as values only represent the final state of reactor failure.