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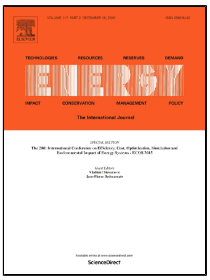

Role of trace elements in single and two-stage digestion of food waste at high organic loading rates

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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

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

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Abstract

This study investigated trace element deficiency and supplementation in mono-digestion of food waste. A single-stage system was contrasted to a two-stage system (hydrolysis followed by methanogenisis). Initial hydrolysis is beneficial as it releases hydrogen sulphide (H_2S), while the prevailing pH prevents an associated H_2S induced precipitation of trace elements (TE). Stable digestion took place without TE supplementation until an organic loading rate (OLR) of 2.0 g VS L^{-1} d^{-1}; this was followed by severe instability at an OLR of 2.5 g VS L^{-1} d^{-1} in both systems. A major accumulation of volatile fatty acids (VFA) inhibited methanogenic activity. A gradual deterioration of pH, VFA/TIC (ratio of VFA to alkalinity) and specific methane yields provoked reactor failure. The benefit of enhanced TE availability in the two-stage system was not apparent due to the complete absence of essential TE in the feed stock. Supplementation of deficient TE Co, Fe, Mo, Ni and Se induced recovery, reflected by an immediate improvement of VFA/TIC and VFA concentrations in both systems. Specific methane yields were restored and maintained at initial levels. At a 16 day retention time, elevated loading rates as high as 5 g VS L^{-1} d^{-1} allowed stable digestion with TE supplementation.

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

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1 Introduction

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

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

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

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

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

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

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

2 Materials and Methods

2.1 Design of experiment

The experiment investigated the impact of trace element depletion and subsequent supplementation in different reactor configurations; a duplicate two-stage system (M1 & M2) and a conventional single-stage reactor (M3). The reactors were tested with the same substrate (source segregated food waste) with stepwise increasing organic 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.

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

2.3 Analytical methods

VFA/TIC was measured using the Nordmann-method (Nordmann, 1977). This parameter indicates the ratio of volatile fatty acids to buffering capacity. The concentrations of individual volatile fatty acids were analysed with gas chromatography (Hewlett Packard HP6890) using a Nukol™ fused silica capillary column (30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (FID). Hydrogen was used as a carrier gas. All metal elements except selenium were analysed according to DIN EN ISO 11885 with inductively coupled plasma optical emission spectrometry (ICP-OES); selenium was determined according to DIN EN ISO 17294-2 (E29) with inductively coupled plasma mass spectrometry (ICP-MS). Biogas composition was analysed for CH_4, CO_2, H_2, O_2 and N_2 using a Hewlett Packard HP6890 gas chromatograph equipped with a Hayesep R packed GC column (3 m x 2 mm, mesh range of 80-100) and a thermal conductivity detector (TCD). Argon was used as carrier 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 g VS L^{-1} 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 $CoCl_2 \cdot 6H_2O$, Fe as $FeCL_3 \cdot 6H_2O$, Mo as
209 $H_{24}Mo_7N_6O_{24} \cdot 4H_2O$, Ni as $Cl_2Ni \cdot 6H_2O$ 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).

3.2 Single-stage reactor performance

3.2.1 Process performance until reactor failure

Figure 1 shows the performance of the single-stage reactor M3 during the 360 day operation period. After an initial commissioning period of two hydraulic retention times (equivalent to 32 days), the reactor was set at an OLR of 2 g VS L^{-1} d^{-1} and the SMY stabilised at 324.5 ±25.5 L CH_4 kg VS^{-1}. The pH and VFA/TIC values showed a minor deterioration towards the end of OLR 2 g VS L^{-1} d^{-1}. This phenomenon was explained with a decrease in measured TIC values, provoking reduced buffer capacity, raising the ratio of VFA/TIC and lowering the pH values. However, low VFA/TIC values of on average 0.21 indicated stable conditions during the overall steady state period at OLR 2 g VS L^{-1} d^{-1} (Table 2), as VFA/TIC ratios below 0.4 are associated with stable reactor performance (Drosg, 2013). Low VFA levels of 0.3 g L^{-1} (Table 2), and constant SMY, further strengthened the conclusion of stable reactor conditions. A further increase in loading rate to an OLR of 2.5 g VS L^{-1} d^{-1} was immediately accompanied by a subtle increase of VFA/TIC, enhanced VFA and declining pH. However, a decrease of the key reactor performance SMY was only gradually observed. After a continuous drop in gas production over the period of 3 HRTs, a significant deterioration of process parameters (pH, VFA/TIC, VFA) caused a distinct drop in SMY (Figure 1). After 3.5 HRTs at an OLR of 2.5 g VS L^{-1} d^{-1} the methane content decreased to 30.5 vol.-% and the pH dropped by 1 unit to 5.4 in only 5 days, emphasising the dynamic development in the final stage of failure. The acid consuming acetoclastic methanogens could not keep pace with the rising levels of total VFA (4.32 g L^{-1}) and were further inhibited by this accumulation. At the peak of reactor failure (day 133) SMY fell to levels as low as 82.7 L CH_4 kg VS^{-1} and 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 CH_4 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 CH_4 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

3.3 Two-stage reactor performance

3.3.1 Process performance until reactor failure

Figure 2 & 3 show the reactor performances of methane reactors (M1 & M2) deployed in a two-stage system. The experiments commenced with a 3 HRT starting period to acclimatise the microorganisms to food waste digestion. Thereafter, the OLR was brought to 2 g VS L^{-1} d^{-1} until a steady state was reached after 3 HRTs. The SMY for M1 and M2 settled at 392 ± 12.6 and 419 ± 23.2 L CH_4 kg VS^{-1} respectively. Low levels of VFA/TIC and pH indicated stable biological conditions. As the OLR was increased in M1 and M2 to 2.5 g VS L^{-1} d^{-1}, the SMY dropped acclimatising to the higher load. This was to be expected and from day 84 onwards the reactors temporarily appeared to recover, indicated by lower VFA/TIC and pH improvements after the initial deterioration. However, the advance of the experiment revealed a massive VFA/TIC increase and pH drop. A SMY reduction to levels as low as one third (M1) and a half (M2) of SMY as compared to that at an OLR of 2 g VS L^{-1} d^{-1} was identified. The magnitude and the dynamic change of process parameters exceeded previous observations significantly. As a consequence, the initial performance of M1 could not be re-obtained. M2 remained at unsteady levels (VFA/TIC, pH) for longer whilst showing a temporary gain in SMY, before ultimately being unable to cope with the loading. The higher level of Ni and Mo in the digestate of M2 as compared to M1 (Table 1) might have initially mitigated and delayed the final break down. In the final stage of reactor failure M1 (day 96-108) and M2 (day 132-144) pH values dropped as far as 6.69 (M1) and 6.92 (M2) whilst VFA/TIC analysis ultimately peaked at 1.42 and 1.34 respectively. A major accumulation of VFA in the range of 4.98 (M1) and 3.44 g L^{-1} (M2), dominated by acetic and propionic acid, reinforced the theory of an inhibition of

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

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

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

3.3.2 Recovery after trace element addition

After the reactors failed, trace element supplementation as recorded in Table 1 was started for M1 and M2 on day 108 and 144 respectively. As the reactor failure of M1 was more severe than M2, feeding was suspended for 3 days and pH was raised with sodium hydroxide to levels before failure. After 6 days (0.5 HRT) pH and VFA/TIC in M1 indicated stable fermentation and matched the results at OLR 2 g VS L^{-1} d^{-1} again. The SMY quickly reached 371.1 ± 5.5 L CH_4 kg VS^{-1} and corresponded with results before supplementation of trace elements. M2 neither received an alkaline solution for pH stabilisation nor a feeding stop. Therefore, the pH only gradually increased over time and VFA/TIC recovery to levels below 0.5 experienced a minor delay of 12 days. The OLR of M1 and M2 was further increased until an OLR of 5 g VS L^{-1} d^{-1} was reached whilst maintaining an HRT over the two stages of 16 days. Constant low VFA/TIC and VFA levels were observed with a gradual increase corresponding to rising OLR. The

SMY ranged between 373.9 ± 10.9 and 413.9 ± 22.6 L CH_4 kg VS^{-1} corresponding with values achieved before trace element addition. Stable fermentation conditions were restored and maintained after trace element supplementation, confirming the failure was induced by a deficiency of essential trace elements. Table 3 summarises the performance characteristics of each steady state.

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

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

All reactors were subjected to the same overall conditions in terms of loading rate, retention time and temperature. Figure 4 illustrates and compares the steady state key performance parameters VFA, VFA/TIC and SMY of the second stage of the two-stage systems (M1 & M2) with the one-stage reactor (M3). Without any addition of trace elements an elevated SMY of 392 ± 12.6 and 419 ± 23.2 L CH_4 kg VS^{-1} was obtained at an initial OLR of 2 g VS L^{-1} d^{-1} in the two-stage reactors M1 & M2 respectively, as opposed to 324 ±25.5 L CH_4 kg VS^{-1} for M3. The superior gas yields in the two-stage digestion is a result of the upstream hydrolysis and is further described in Voelklein et al. (2016).

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

(Drosg, 2013). Similar observations of unstable reactor behaviour at low OLR have been reported by Climenhaga and Banks (2008), Gustavsson et al. (2011), Nordell et al. (2015) and Zhang et al. (2015). The VFA spectrum of M1 and M2 was dominated by 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} respectively) with minor shares of longer chained fatty acids (C_4-C_6). In contrast the share of 1.88 g L^{-1} of propionic acid exceeded the share of 1.25 g L^{-1} of acetic acid in the single-stage reactor M3. The accumulation of VFA is an associated consequence of trace element deficiency (Banks et al., 2012; Nordell et al., 2015; Pobeheim et al., 2011).

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

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

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

The gap in SMY between the one and two-stage remained after stabilizing the reactors with trace elements. The 16 day BMP performance of 471.94 L CH_4 kg VS^{-1} was never reached regardless of the elimination of nutrient deficiency. This is a result of fully mixed continuous stirred tank reactors causing fresh matter to leave the reactor prior to complete digestion. The shorter the retention time the more significant this effect becomes. The gas yields further confirmed the observed conclusion that trace element addition had negligible impact on SMY (at a fixed HRT of 16 days), yet is essential for a stable fermentation with low VFA levels after exceeding a threshold OLR of 2.0 g VS L^{-1} d^{-1}. The positive effects of trace element addition are in line with studies conducted by Banks et al. (2012); Gustavsson et al. (2011); Nges et al. (2012); Qiang et al. (2012); Zhang and Jahng (2012); Zhang et al. (2015).

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

4 Conclusion

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

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487 **List of Figures and Tables**

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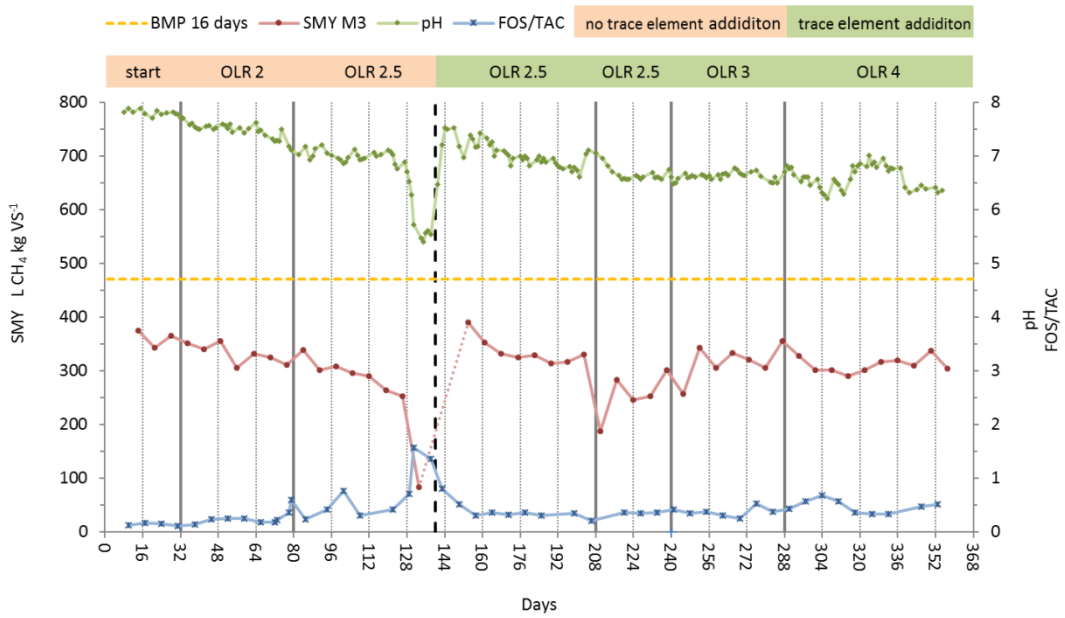
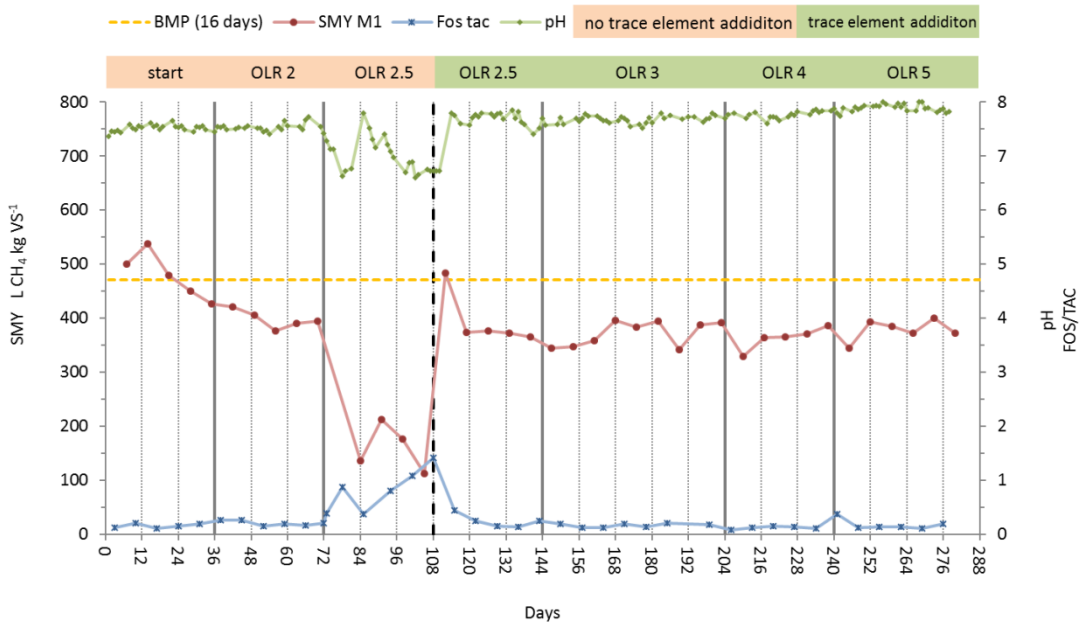


Dotted line for SMY M3 (day 133-154) represents feeding stop for 14 days to facilitate recovery after reactor failure (calculation of SMY not applicable); stabilisation of pH with NaOH and commencement of trace element supplementation on day 140; experimental difficulties (day 208-240) with gas measuring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

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



517

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

519 supplementation.

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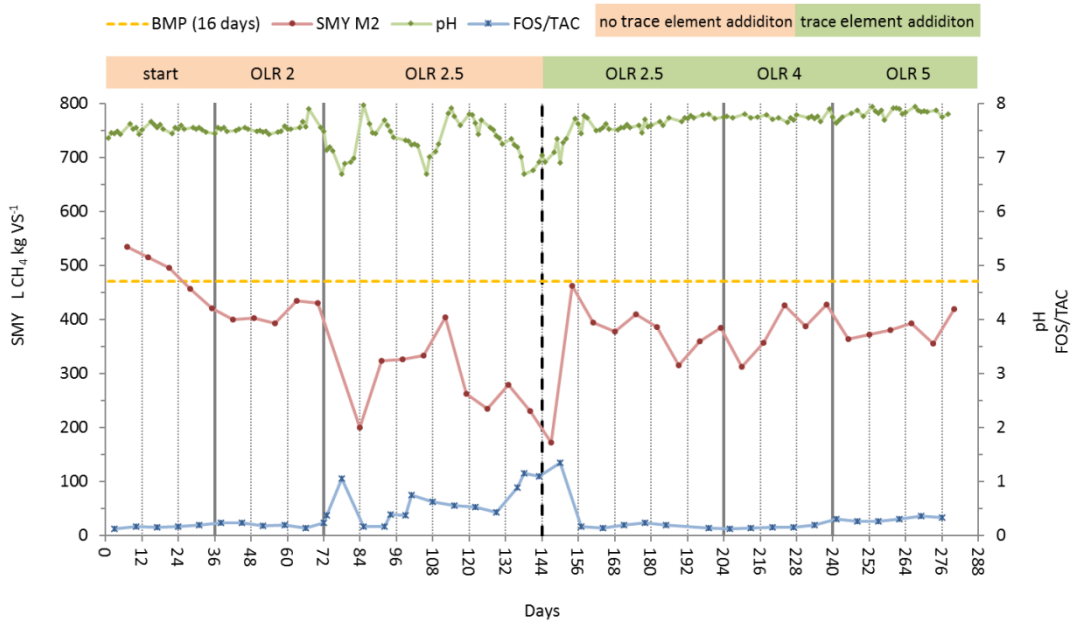

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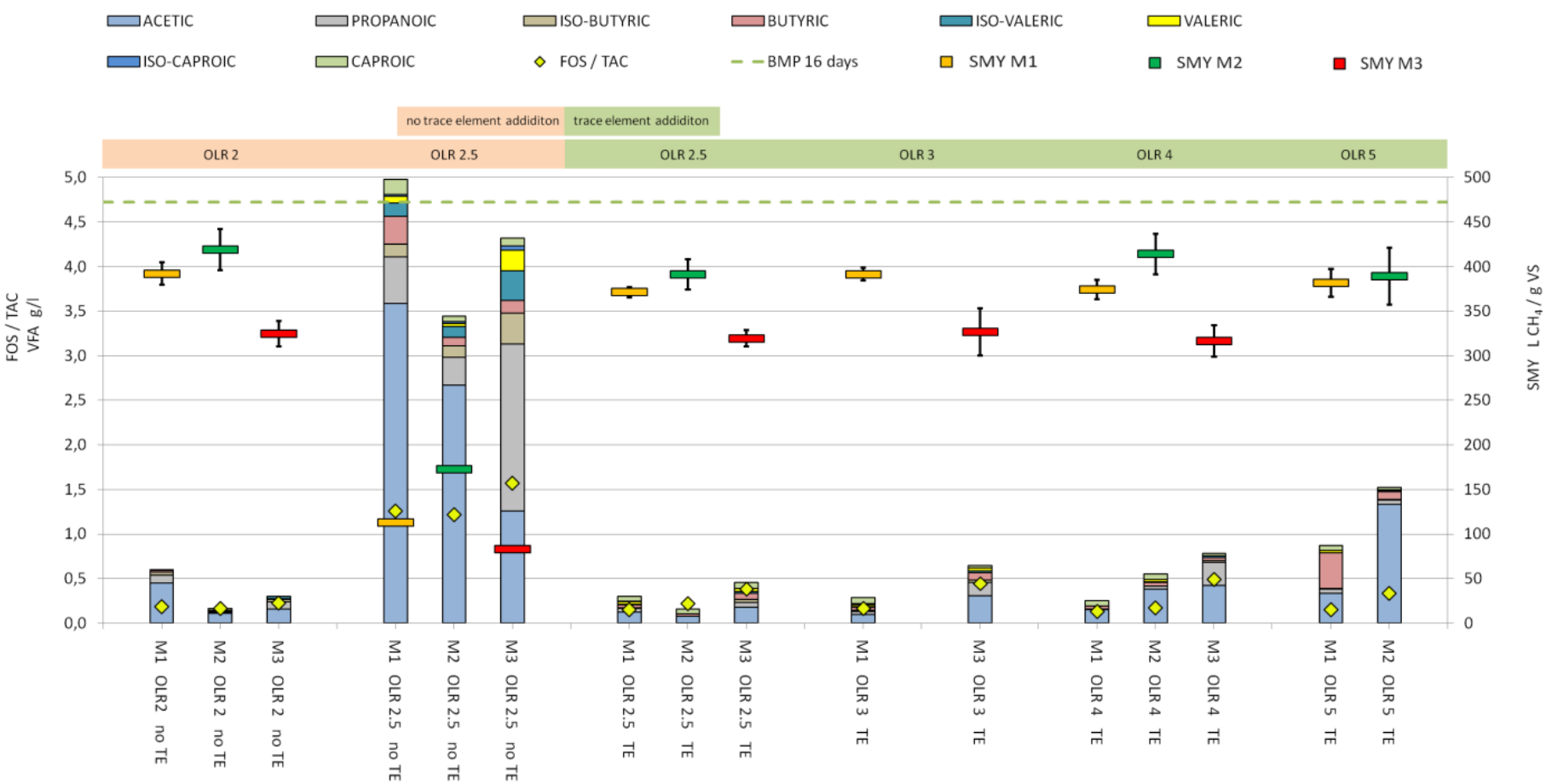
522 OLR 3 was omitted to match reactor M2 with M1.

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

524 supplementation.

525



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

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

Table 1

Trace element levels in food waste, in digestate at reactor failure, reported range of nutrients added in literature and nutrients added to feed 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]	-	-

[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 reasons); ww: wet weight.

Table 2

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

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

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.