

1 Communication

2 Comparison of variable and constant loading for 3 mesophilic food waste digestion in a long-term 4 experiment

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13 **Abstract:** Operators of commercial anaerobic digestion (AD) plants frequently note the challenge of
14 transferring research results to an industrial setting; especially in matching well-controlled
15 laboratory studies at a constant organic loading rate (OLR) with full-scale digesters subject to day-
16 to-day variation in loadings. This study compared the performance of food waste digesters at
17 fluctuating and constant OLR. In a long-term experiment over nearly three years, variable daily
18 OLR with a range as wide as 0 to 10.0 g VS L⁻¹ day⁻¹ (weekly average 5.0 g VS L⁻¹ day⁻¹) were applied
19 to one laboratory-scale digester, while a pair of control digesters was operated at a constant daily
20 loading of 5.0 g VS L⁻¹ day⁻¹. Different schemes of trace elements (TE) supplementation were also
21 tested to examine how they contributed to process stability. Variable loading had no adverse impact
22 on biogas production or operational stability when 11 TE species were dosed. When TE addition
23 was limited to cobalt and selenium, the stability of the variable-load digester was well maintained
24 for nearly 300 days before the experiment was terminated; while the control digesters required re-
25 supplementation with other TE species to reverse an accumulation of volatile fatty acids. This work
26 demonstrated that variation in daily OLR across quite a wide range of applied loadings is possible
27 with no adverse effects on methane production or stability of food waste digestion, giving
28 confidence in the transferability of research findings. The positive effect of variable OLR on TE
29 requirement requires further investigation considering its practical significance for AD industry.

30 **Keywords:** variable loading; food waste; anaerobic digestion; trace elements; cobalt; selenium;
31 process stability
32

33 1. Introduction

34 Food waste is a ubiquitous and energy-rich organic material with a high potential to be used in
35 anaerobic digestion (AD) for biogas production [1–3], but it can pose challenges with regard to
36 maintaining process stability and favourable long-term performance of the digester [4,5]. In single-
37 stage continuous stirred tank reactor (CSTR) digestion, which is the most common type of AD plant
38 operated in practice, mono-digestion of food waste without addition of stabilising agents is limited
39 to an organic loading rate (OLR) of up to 2.0 g VS L⁻¹ d⁻¹ (grams volatile solids per litre digester
40 working volume and day) [6–8], while even lower OLRs may be necessary to maintain a stable
41 process [9]. OLR is an important operational parameter and an indicator of the commercial capacity
42 of the digester; an elevated OLR is usually desirable as this means higher throughput of organic
43 material and therefore a higher volumetric methane production potential. Overloading or shock

44 loading may lead to instability and process failure, however, for two interlinked reasons: (1)
45 disturbance or breakdown of the delicate balance between production of volatile fatty acids (VFAs)
46 through hydrolysis, acidogenesis and acetogenesis on one hand and consumption of VFAs through
47 methanogenesis on the other hand [10]; and (2) washing out of slow-growing anaerobic microbial
48 biomass when hydraulic retention time is reduced as a result of overloading [11]. A disturbance in
49 the metabolic balance is typically accompanied by declining biogas and methane yields, VFA
50 accumulation and pH decrease [10,12], while overloading without imbalance may be indicated by a
51 simple drop in specific biogas and methane yields as the time available for feedstock degradation is
52 reduced [10,13]. AD of readily degradable organic material such as food waste usually suffers from
53 the first problem, and irreversible acidification may occur as a result of digester overload [5,14]. In
54 food waste AD, deficiency of trace elements (TEs) was identified as a major factor adversely affecting
55 process stability [15–17].

56 A common strategy to increase OLR and avoid process failure or inhibition is co-digestion of
57 food waste with other substrates [8,18–20]. As an alternative, TE supplementation was found to be
58 an effective strategy to ensure stable operation when using only food waste (mono-digestion) even
59 under an elevated OLR [21–23]. Banks et al. [15] found that with supplementation of TEs (Co, Se) the
60 OLR in mono-digestion of food waste could be increased to 5 g VS L⁻¹ d⁻¹. After supplementation of
61 deficient TEs (Co, Fe, Mo, Ni and Se), Voelklein et al. [6] observed the recovery of a strongly inhibited
62 food waste mono-digestion process (VFA accumulation was overcome), followed by stable digestion
63 at substantially higher loading rates of up to 5 g VS L⁻¹ d⁻¹. Jo et al. [7] successfully increased the OLR
64 of TE-supplemented food waste digestion to 5 g VS L⁻¹ d⁻¹ in a single-stage AD system, while a two-
65 stage system failed at this OLR with same TE supplementation. Based on these results, an OLR of 5
66 g VS L⁻¹ d⁻¹ is feasible for food waste mono-digestion in a single-stage CSTR system supplemented
67 with appropriate levels of TEs.

68 Zhang et al. [9] observed declining concentrations of Cobalt (Co), Molybdenum (Mo), Nickel
69 (Ni) and Iron (Fe) during long-term food waste mono-digestion, but supplementation of these TEs
70 did not achieve stable operation at elevated OLRs. At such elevated OLRs (5 g VS L⁻¹ d⁻¹), Cobalt (Co)
71 was reported as likely to become limiting due to its role either in acetate oxidation or in
72 hydrogenotrophic methanogenesis, otherwise Selenium (Se) was identified as the limiting TE [15].

73 Controlling for a constant OLR is common practice in scientific studies at laboratory scale, but
74 is not a simple task in full-scale AD plant. It requires constant monitoring of the feedstock
75 composition and advanced management of organic material flows during intermediate storage of
76 degradable substrates. In practice commercial digesters are therefore run with variable organic
77 loading rates, and this difference is often cited by industry as a reason why it may be challenging to
78 transfer research results or to match the performance achieved in well-controlled laboratory studies.
79 Research endeavours to date have provided some valuable evidence on the links between AD system
80 performance and transient or shock changes in organic loading. In a single-stage food waste digestion
81 process, Grimberg et al. [24] observed a lower methane yield under variable loading, but the methane
82 yield was higher in two-stage digestion; while this shows the possibility of reduced process
83 performance, it does not clarify the influential factors. Kim and Lee [25] found that pH control had a
84 crucial effect on the resilience and robustness of the microbial community under shock loading,
85 whereas the archaeal community was less affected. Ferguson et al. [26] demonstrated that multiple
86 changes in OLR, based on using different co-substrates, could increase the resilience of the process,
87 and suggested to use varying OLRs to manipulate the microbial community; however decreases in
88 biogas and methane production were observed, and their study operated at very short hydraulic
89 retention times (HRT) and did not consider food waste. Essentially, the findings from these
90 experiments provide some information on the response of anaerobic digesters to loading
91 perturbation and insights to the recovery patterns. To guide real-world digester operation, however,
92 better understanding is still needed of potential differences between results from carefully controlled
93 trials at constant OLR and those at variable loading, and also of factors affecting the performance of
94 variable loading digesters.

95 This work aimed to test the performance of food waste digestion under different variable
 96 loading patterns at relatively high average OLR of 5 g VS L⁻¹ d⁻¹, and to compare this with digesters
 97 at a constant OLR. Stability of the process was assessed by monitoring several indicators, including
 98 VFA concentrations and methane production. The response of the digestion process to trace elements
 99 limitation was also considered.

100 2. Materials and Methods

101 2.1. Materials

102 The batches of food waste used came from the waste transfer station operated by Veolia
 103 Environmental Services in Otterbourne, Hampshire, UK. Fresh food waste was transferred to the
 104 laboratory of University of Southampton in sealed plastic barrels; a total of nine batches of substrate
 105 were used in the study (Table 1). After manual removal of non-biodegradable materials, the feedstock
 106 was homogenised using a macerating grinder (S52/100 Waste Disposer, IMC Limited, Wrexham,
 107 UK). The macerated substrate was stored at -20°C and thawed before use. Table 1 summarises the
 108 substrate characteristics (macerated food waste) of each feedstock batch used during the long-term
 109 experiment over nearly 3 years.

110 **Table 1.** Characteristics of food waste used in this study (after manual removal of non-
 111 biodegradable components and maceration).

	Days in use	TS ¹ (g kg ⁻¹)	VS ¹ (g kg ⁻¹)	VS/TS (%)	TKN ¹ (g kg ⁻¹)	Trace elements ¹ (mg kg ⁻¹)				
						Co	Se	Ni	Mo	Fe
Batch 1	0–159	211.8	187.9	88.8	6.30	0.013	0.050	0.125	0.129	36.3
Batch 2	160–292	238.2	224.8	94.4	7.57	0.052	0.059	0.330	0.197	- ²
Batch 3	293–348	233.3	218.7	93.7	7.82	0.007	0.063	0.105	0.078	16.6
Batch 4	349–496	241.7	226.7	93.8	6.35	0.018	0.019	0.168	0.120	- ²
Batch 5	497–579	230.4	208.0	90.3	6.18	- ²	- ²	- ²	- ²	- ²
Batch 6	580–699	241.6	230.8	95.5	7.20	0.013	0.018	0.550	0.106	- ²
Batch 7	700–775	239.1	222.2	92.9	7.58	0.022	0.036	0.100	0.190	- ²
Batch 8	776–877	237.2	211.9	89.3	- ²	0.024	0.042	0.167	0.221	- ²
Batch 9	878–1068	249.7	232.2	93.0	- ²	- ²	- ²	- ²	- ²	- ²
Mean ³		236.3	218.5	92.4	6.89	0.022	0.039	0.233	0.149	31.2

112 ¹ Solids data are averages of duplicate analyses; all results are expressed on a fresh matter basis. ² Not
 113 measured. ³ Weighted average, considering the number of days the feedstock of each batch was used for the
 114 daily loading of the digesters.

115 The inoculum used came from digesters in the same laboratory which had previously been
 116 operated for more than 500 days on food waste obtained from the same source [27]. The inoculum
 117 had a pH of 7.69, a total ammonium nitrogen (TAN) content of 2.93 g L⁻¹, an intermediate/partial
 118 alkalinity (IA/PA) ratio of 0.34 and a total volatile fatty acids (VFA) content of <500 mg L⁻¹. Digesters
 119 labelled as F5&6 in the previous work of Jiang [27] were used as constant loading controls (CL1 and
 120 CL2) in this study, while the previously labelled digester F11 was used for variable loading (VL) in
 121 this work. Before use in this study, all three digesters had been operated at OLR 5.5 g VS L⁻¹ d⁻¹ for
 122 more than 150 days with F5&6 supplemented with Co, Se and Mo and F11 with 11 TEs, and these
 123 were the initial conditions for this study.

124 Eleven trace elements were used in this research, supplied in accordance with the strategies
 125 described in the results section. For this purpose, each individual trace element stock solution was
 126 made using the compounds listed in Table 2 and kept in the refrigerator when not in use. The dosing
 127 strength of each trace element was chosen based on the results of previous studies on trace elements
 128 supplementation during anaerobic digestion of food waste [15].

129 **Table 2.** Trace elements used in this study.

Element	Compound used	Supplementation strength in the digester (mg L ⁻¹ digester working volume)
Cobalt (Co)	CoCl ₂ ·6H ₂ O	1.0 until day 776, 0.3 from day 777 on ¹
Selenium (Se)	Na ₂ SeO ₃	0.2
Molybdenum (Mo)	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.2
Tungsten (W)	Na ₂ WO ₄ ·2H ₂ O	0.2
Nickel (Ni)	NiCl ₂ ·6H ₂ O	1.0
Iron (Fe)	FeCl ₂ ·4H ₂ O	5.0
Aluminium (Al)	AlCl ₃ ·6H ₂ O	0.1
Boron (B)	H ₃ BO ₃	0.1
Copper (Cu)	CuCl ₂ ·2H ₂ O	0.1
Manganese (Mn)	MnCl ₂ ·6H ₂ O	1.0
Zinc (Zn)	ZnCl ₂	0.2

130 ¹ The supplementation strength was reduced to study the resilience of the process under trace element
131 limitation (see Section 2.3 for details).

132 2.2. Description of digesters

133 Laboratory-scale CSTR digesters were used, each with a total volume of 5 L and a working
134 volume of 4 L. The digesters were constructed of PVC tube with gas-tight top and bottom plates. The
135 top plate was fitted with a gas outlet, a feed port sealed with a rubber bung, and a draught tube liquid
136 seal through which an asymmetric bar stirrer was inserted with a 40-rpm motor mounted directly on
137 the top plate. Temperature was controlled at 37.0 ± 0.5°C by circulating water from a thermostatically
138 controlled bath through a heating coil around the digesters.

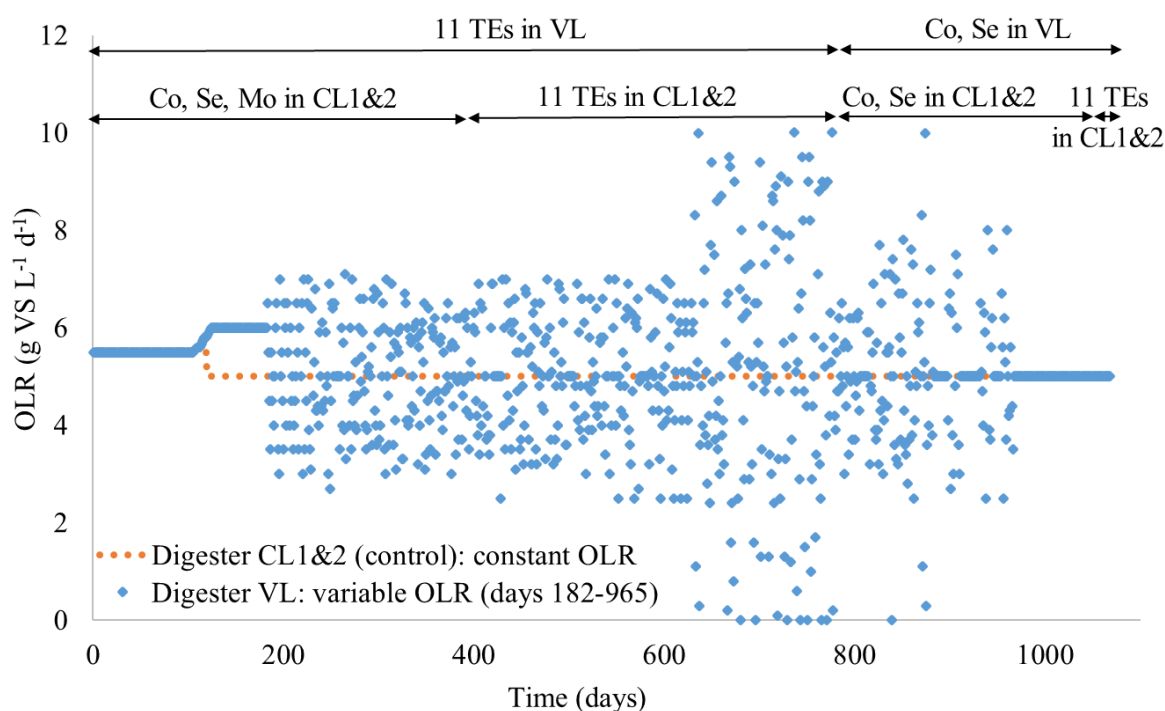
139 Digesters were fed daily throughout the whole experiment and digestate was removed twice a
140 week to maintain an approximately constant working volume. Semi-continuous operation was
141 achieved by removing digestate through the outlet port in the base plate before adding feed via the
142 inlet in the top plate.

143 2.3. Experimental Procedure

144 The experimental work was carried out as a long-term study over a period of 1068 days. Three
145 digesters were used. Digesters CL1 and CL2 were used as control and operated at constant loading,
146 while digester VL was exposed to variable OLR. The operational scheme of the whole work is plotted
147 in Figure 1. To establish a baseline, the digesters were first run in parallel for a period of 105 days to
148 confirm stable and repetitive operation at a constant loading of 5.5 g VS L⁻¹ d⁻¹ (organic loading per
149 digester working volume and day).

150 From day 105 on, the digesters were no longer subjected to the same OLR. The OLR of digester
151 VL was increased gradually over a period of 2 weeks to 6.0 g VS L⁻¹ d⁻¹, to provide some slight
152 additional acclimatisation to intermittent increases in loading. This steady OLR was maintained for
153 a period of 8 weeks (days 123–181); then from day 182 on, digester VL was operated with variable
154 loading, to investigate how digester performance was affected by load fluctuation. During the period
155 of variable loading the digester received a weekly average OLR of 5 g VS L⁻¹ d⁻¹. From day 182 to day
156 629 (64 weeks), the loading in S2 fluctuated from 2.5 to 7.5 g VS L⁻¹ d⁻¹. For the first 6 weeks of this
157 period, the daily loading was selected from values at increments of 0.5 g VS L⁻¹ d⁻¹. From day 225 to
158 day 629 the daily loading was obtained from a purpose-designed random number selection routine
159 working at increments of 0.1 g VS L⁻¹ d⁻¹. From day 630 to 965 (48 weeks), the range of loading
160 variation was increased to between 0 and 10 g VS L⁻¹ d⁻¹. For the first 25 weeks of this period, the daily
161 loading was obtained from the selection routine as before; while for the next 23 weeks, a pattern of a
162 period of variable loadings between 0–10 g VS L⁻¹ d⁻¹ followed by one or two weeks of constant
163 loading at 5 g VS L⁻¹ d⁻¹ was tested to increase the complexity of the load fluctuation. In contrast to
164 digester VL, after the initial phase of operation in parallel the OLR on control digesters CL1&2 was
165 reduced to 5.0 g VS L⁻¹ d⁻¹ on day 119 and kept at this level until day 1068. During the last 14 weeks

166 (days 966–1068), the OLR of digester VL was also set to this level and all digesters were again
 167 operated at equal OLR.
 168



169 **Figure 1.** Operational scheme of digesters used in this study, including organic loading rate (OLR)
 170 change and trace elements (TE) supplementations.

171 The control digesters CL1&2 were supplemented with Co, Se and Mo as the most essential trace
 172 elements [15] during the first 56 weeks, then with all eleven trace elements for a period of 55 weeks
 173 (days 392–776). In contrast, digester VL was supplemented with all eleven trace elements during
 174 these 111 weeks to ensure that TEs were not limiting when the OLR variation scheme was applied.
 175 From day 777 on, the TE supplementation was replaced in all digesters with a dosage of 0.3 mg L⁻¹
 176 Co and 0.2 mg L⁻¹ Se in order to investigate the resistance of each digester to TE limitation. Control
 177 digesters CL1&2 were supplemented with the eleven TEs from day 1056 on, to test whether the
 178 observed process inhibition could be reduced by this measure; this is described with the results and
 179 their discussion (Section 3).

180 2.4. Laboratory Analyses

181 Total solids (TS) and volatile solids (VS) were measured according to Standard Method 2540G
 182 [28]. pH was measured using a Jenway 3310 pH meter (Bibby Scientific Ltd, Stone, UK) with electrode
 183 and thermometer, calibrated in buffers at pH 4.0, 7.0 and 9.2 (Fisher Scientific, Loughborough, UK).
 184 Alkalinity was measured by titration with 0.25 N H₂SO₄ to endpoints of pH 5.75 and 4.3, allowing
 185 calculation of total alkalinity (TA), partial alkalinity (PA), and intermediate alkalinity (IA) [29]. Total
 186 Kjeldahl nitrogen (TKN) and total ammonium nitrogen (TAN) were determined using a Kjeltach
 187 block digester and ammonia by steam distillation unit according to the manufacturer's instructions
 188 (Büchi, Oldham, UK). Volatile fatty acids (VFA) were measured by Shimadzu GC-2010 gas
 189 chromatograph (Shimadzu, Milton Keynes, UK) equipped with a capillary column type SGE BP21
 190 and a flame ionization detector. The temperature at the injector and detector were maintained at 200
 191 and 250°C. Helium was used as the carrier gas at a flow rate of 190.8 mL min⁻¹.

192 2.5. Quantity and Composition of Biogas, Calculation of the Rolling Average Volumetric Methane
193 Production (VMP)

194 Gas production was measured using tipping-bucket gas counters, with collection in gas-
195 impermeable bags. Calibration was carried out by measuring the volume of biogas using the weight-
196 based water displacement method [30], and values are corrected to standard temperature and
197 pressure (273.15 K, 1 atm (1.01325 bar), STP). Carbon dioxide and methane concentrations were
198 monitored using a Varian star 3400 CX Gas Chromatograph (Varian, Oxford, UK) with argon as the
199 carrier gas.

200 The variation of the daily loading makes it difficult to directly compare the daily methane
201 production between variable and constant loading digesters. Therefore, the 28-days rolling average
202 volumetric methane production (VMP) was calculated, using the volumetric methane production of
203 the 28 days before the specific date. The rolling average approach creates a series of averages over
204 the studied time period. The rolling average VMP is reported in litres methane per litre active digester
205 volume and day ($\text{L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$).

206 **3. Results and Discussion**

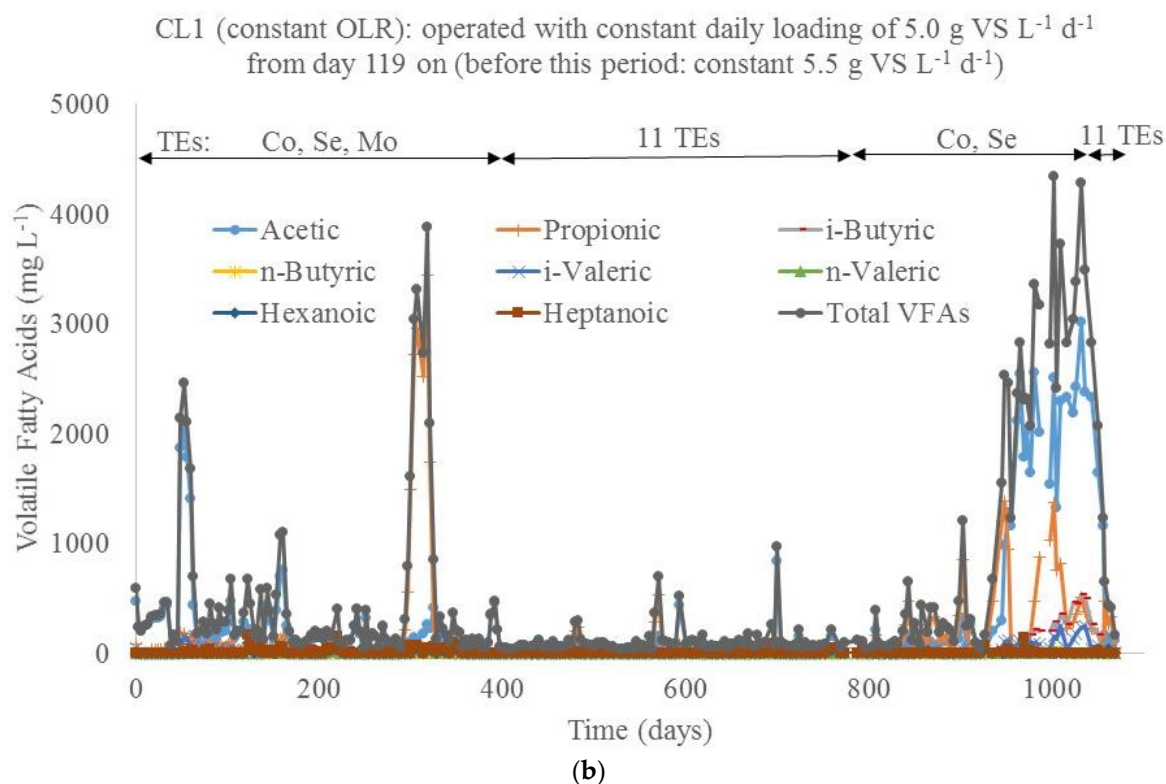
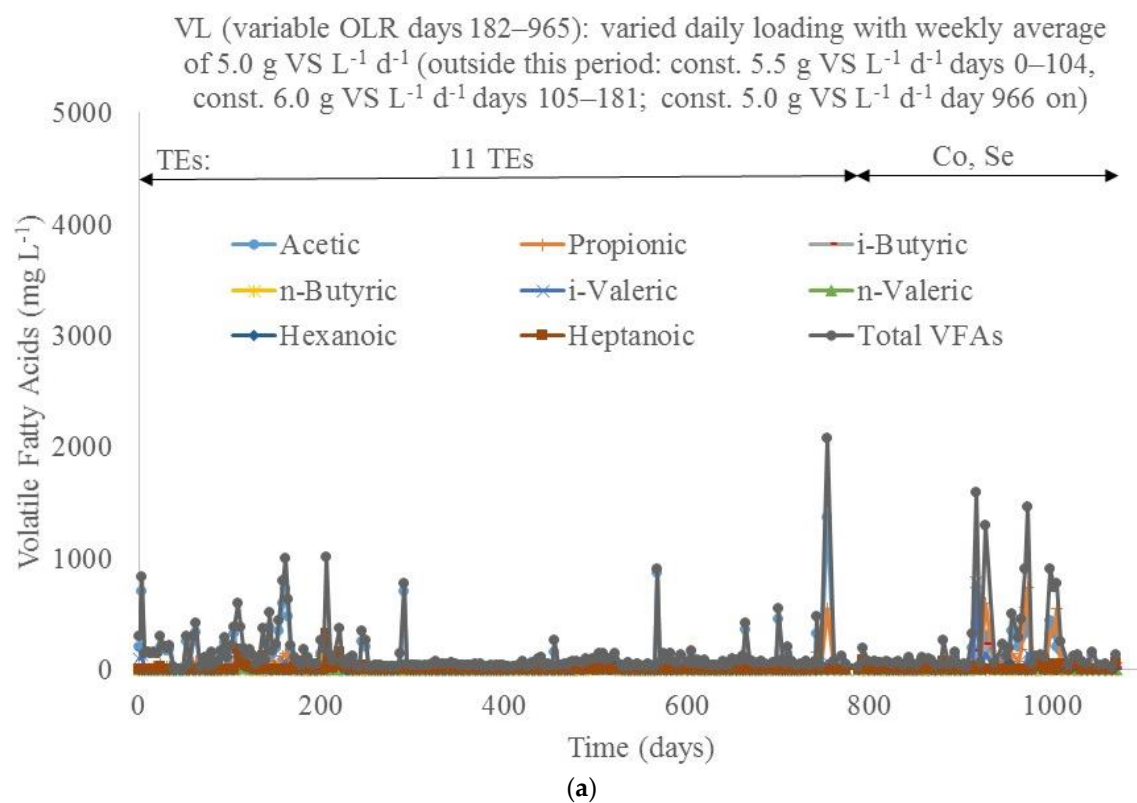
207 3.1. Process Stability under Variable and Constant Loading of Digester

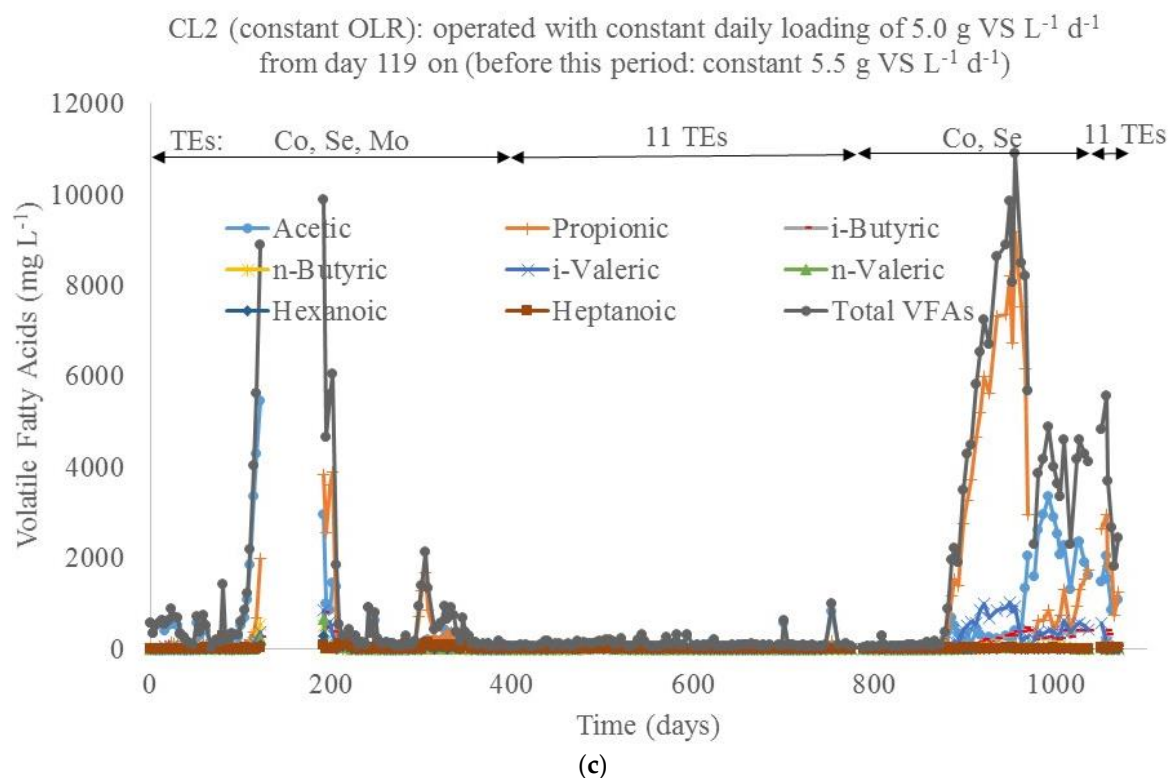
208 The profiles of volatile fatty acids, pH, alkalinity and ammonia provide information on the
209 stability of the digestion process. From day 105 on, the digesters were no longer operated at the same
210 OLR. In Figure 2 it can be seen that elevated VFA concentrations in CL1&2 were more frequent and
211 more pronounced than in digester VL. This pattern, however, did not occur during periods where all
212 eleven trace elements were supplemented; under operation with full TE addition, all digesters
213 showed low total VFA concentrations and no acids were accumulated. Acetic acid accounted for most
214 of the VFA when increased concentrations were observed, and it degraded very quickly. VFAs with
215 inhibitory impact, in particular propionic, iso-butyric and iso-valeric acid [9,15], were found only in
216 low concentrations less than 500 mg L^{-1} . This indicates that there was no risk of process instability
217 and confirms that with appropriate supplementation of trace elements, mono-digestion of food waste
218 is a stable process in long-term operation (here more than 110 weeks).

219 Most notably, from day 777 on when supplementation with the eleven TEs was stopped and
220 only Selenium and a reduced dosage of Cobalt were added, digester VL showed less VFA
221 accumulation compared to digesters CL1&2. A delay in reaction to this change in operation is visible
222 for all digesters, reflecting the time required for washing-out of the earlier dosages of the eleven TEs.
223 After day 970, some elevated acid concentrations were detected in digester VL, but this was a short-
224 term phenomenon only and observed concentrations were moderate (less than 1600 mg L^{-1}). In
225 contrast, VFAs started to accumulate in both CL1&2 seven weeks after dosage with the eleven TEs
226 had been stopped. From day 950 on, the concentration of total VFAs fluctuated at around $3000\text{--}4000$
227 mg L^{-1} in CL1 and $8000\text{--}10000 \text{ mg L}^{-1}$ in CL2. Acetic acid remained the dominant component in CL1.
228 However, propionic, iso-butyric and iso-valeric acid, reported to be most influential to inhibit mono-
229 digestion of food waste under trace element limitation [9,23], also occurred in high concentrations
230 with propionic acid even becoming the dominant species in CL2. It can be assumed that this
231 accumulation of acids was induced by trace element limitations. This assumption is confirmed by the
232 observation that, when supplementation with eleven TEs to digesters CL1&2 began again from day
233 1056 on, total VFA decreased very rapidly to below 500 mg L^{-1} (Figure 2).

234 Small differences in VFA accumulation were also observed early in the experimental period,
235 with VFA peaks seen in both steady OLR digesters at around days 290 and 330, which did not appear
236 in the variable load digester. The reason for this is unknown, but may be related to the different TE
237 supplementation at the time and in the period of operation preceding the start of this trial, when
238 digester VL was supplemented with 11 TE while CL1&2 received only Co, Se and Mo. These peaks
239 were transient, however, and were followed by a period of 520 days equivalent to around 12 HRT of
240 stable operation in all digesters. It is therefore considered unlikely that the earlier difference in TE

241 supplementation had a major effect on pattern of VFA accumulation in the final days (day 850–1068)
 242 of the trial.
 243

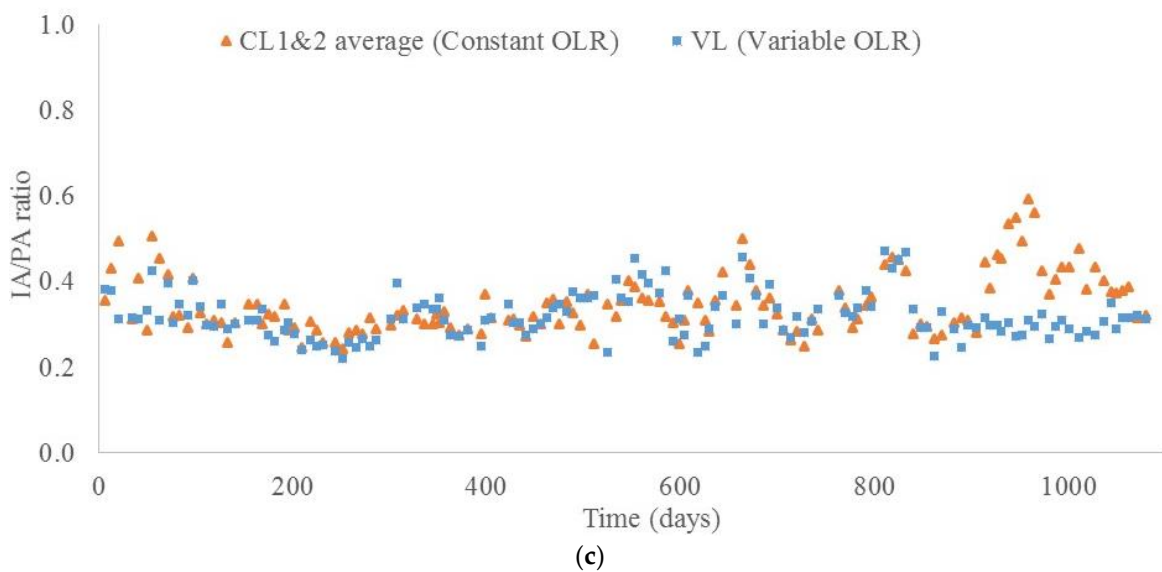
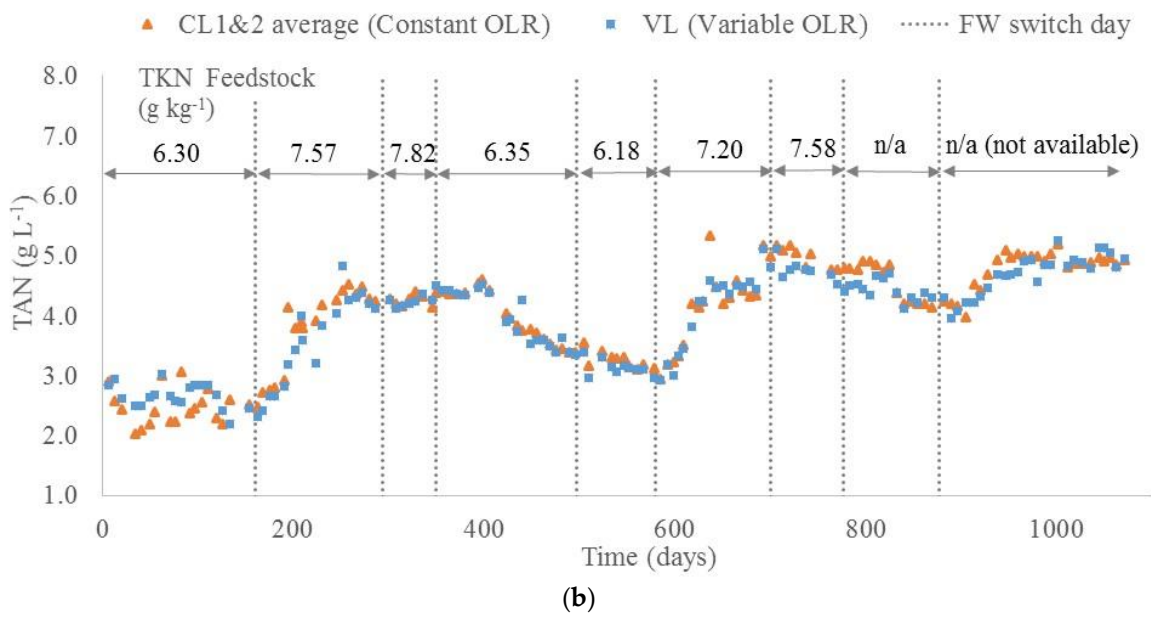
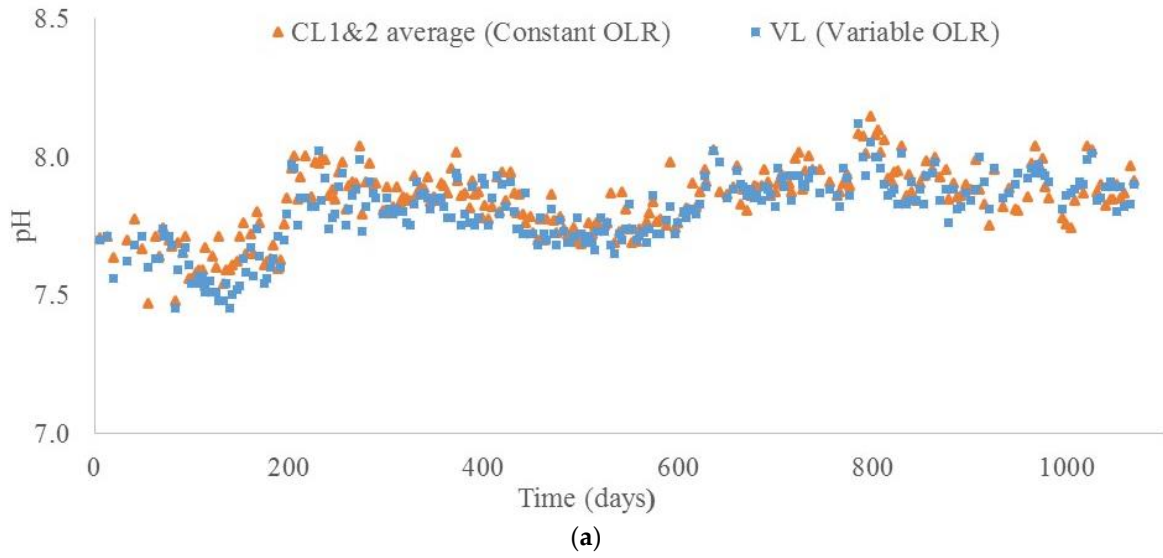




244 **Figure 2.** VFA profiles: (a) Digester VL with varying organic loading rate; (b) Control digester CL1;
 245 (c) Control digester CL2. (Note: 1. These digesters had the same constant OLR until day 105 and from
 246 day 966 on, but not always the same TE supplementation scheme; 2. The digester CL2 suffered from
 247 a severe operation problem on day 103: water in biogas counter was siphoned back to the digester
 248 causing disturbance and VFA increase for a period of 200 days. The VFA peak after that event was
 249 not displayed fully in Fig 2c due to its scale; 3. The y-axis scale of control digester CL2 is different
 250 from others due to its high VFA peak around day 1000.).

251
 252 The pH remained stable at around 7.80 ± 0.20 over the whole experimental period in all digesters,
 253 which is a favourable level for anaerobic digestion. Changes in pH were linked to varying TKN
 254 concentrations in the feedstock affecting the digester TAN content (Figure 3b), rather than to VFA
 255 accumulation. TAN concentrations in both digesters fluctuated between 2.0 and 5.0 g L⁻¹, with the
 256 fluctuations reflecting differences in the properties of food waste batches as shown in Table 1. As
 257 reviewed by Yenigün and Demirel [31], a wide range of ammonia concentrations have been reported
 258 to cause inhibition in mesophilic anaerobic digestion, from 2.8 to 6.0 g L⁻¹; however previous work
 259 with food waste of the same type has shown stable mesophilic operation at TAN concentrations
 260 similar to those found here [15], and thus ammonia concentrations in this study are unlikely to have
 261 significantly affected process stability or methane production.

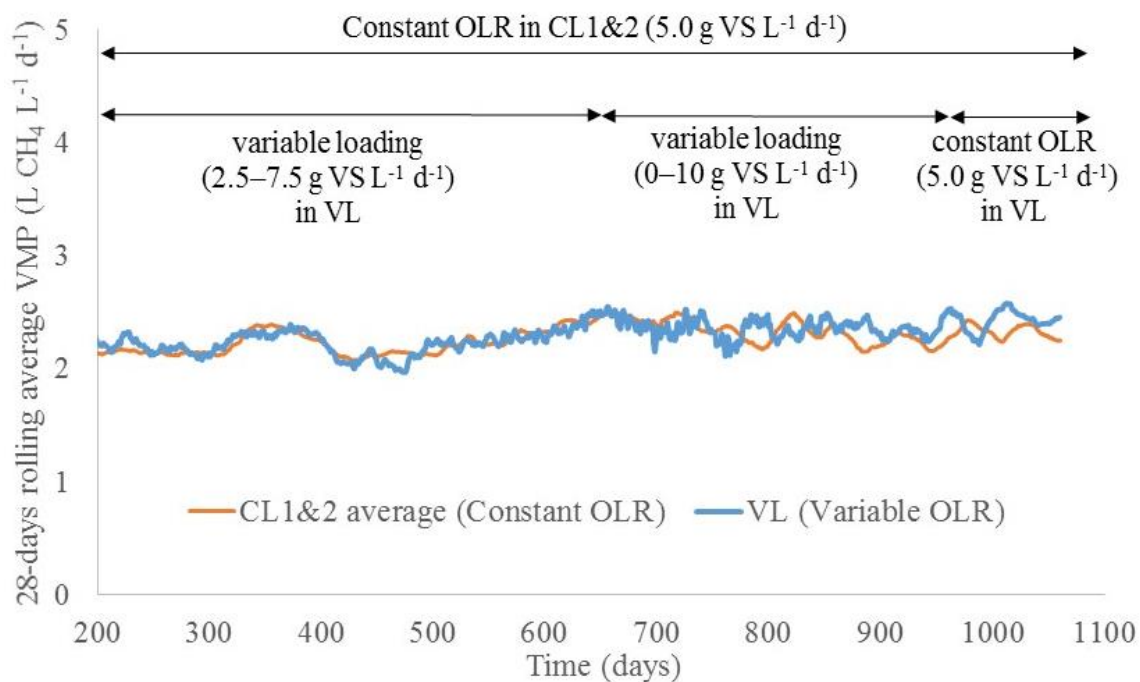
262 While total VFA concentrations in this study did not correlate with pH values or TAN
 263 concentrations, elevated concentrations of VFA corresponded to higher IA/PA ratios (Figure 3c). For
 264 CL1&2, this can be seen during the first months of the experiment, during the short period of slight
 265 VFA increase (mainly propionic acid) around day 307 and during the last months of the experiment.
 266 The IA:PA ratio remained at 0.30 ± 0.10 during the whole operation. As with the pH value, this
 267 parameter was located in an optimal range, indicating a favourable buffering capacity and good
 268 operational stability.
 269



270 **Figure 3.** Operational parameters of digester VL with variable OLR and the control with constant
 271 loading (CL1&2, average values): (a) pH of digester content (measured in digestate leaving the
 272 digester); (b) Ammonia of digester content (additionally, TKN of feedstock is provided); (c) Alkalinity
 273 of digester content.

274 3.2. Productivity Comparison between Variable and Constant Loading Food Waste Digesters

275 Biogas methane concentrations were stable at around $58\% \pm 2\%$ throughout the experimental
 276 period for all digesters. The comparison between digesters with constant and variable loading started
 277 from day 182; at this time, digester VL had shown stable operation at constant loadings (5.5 g VS L^{-1}
 278 d^{-1} , then $6.0 \text{ g VS L}^{-1} \text{ d}^{-1}$) for almost 200 days (26 weeks). When looking at the methane production
 279 during the comparison period (from day 182 until day 1068) (Figure 4), no obvious difference was
 280 found between VL and CL1&2 when all digesters were supplemented with the full spectrum of
 281 eleven TEs (days 392–776); in all cases, the volumetric methane production (VMP) fluctuated around
 282 an average of $2.27 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$. It is noteworthy that when larger loading variations ($0\text{--}10 \text{ g VS L}^{-1}$
 283 d^{-1}) were introduced in digester VL from day 632, the 28-day rolling average VMP showed stronger
 284 fluctuations. Nevertheless, in tendency the average methane production remained unchanged. When
 285 looking at the gas production from periods with different variable OLR, methane production was
 286 smoother at loading rates of $2.5\text{--}7.5 \text{ g VS L}^{-1} \text{ d}^{-1}$.
 287



288 **Figure 4.** 28-days rolling average volumetric methane production of digester VL and control digesters
 289 CL1&2. (Note: from day 777 on, the digesters were no longer supplemented with the full set of 11 TEs,
 290 but with Co and Se only).

291 A slight decrease in VMP was observed for CL1&2 in response to the TE limitation and
 292 consequent VFA accumulation during the last months of the work. During this period, where all
 293 digesters received as TE supplementation only Se and a reduced strength of Co, digester VL
 294 maintained stable performance, with a 28-days rolling average VMP above $2.25 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$. When
 295 shifting from the variable to a constant OLR on day 966, digester VL first responded with a slight
 296 decrease in methane production, but VMP gradually climbed back to around $2.34 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$. The
 297 reason for these fluctuations might be an adaptation need as a result of changed loading schemes
 298 [32].

299 The typical biochemical methane potential (BMP) of mixed food waste of the type used in this
300 study is 440–480 L CH₄ kg⁻¹ VS_{added} [17,33], with slightly lower values expected in single-stage semi-
301 continuous digestion. Gray et al. [34] reported a wide range of values between 305 and 530 L CH₄ kg⁻¹
302 TS_{added} for semi-continuous digestion depending on food waste composition. In this study, the
303 methane production of digester VL (approximately 456 L CH₄ kg⁻¹ VS_{added}, calculated from the
304 average VMP value) is well within the literature range, and close to the upper value in previous
305 reports, confirming the productive performance.

306 3.3. Discussion of Findings and Relevance to Commercial Food Waste Digestion

307 The above results suggest that day-to-day variability in organic loading, even over quite a wide
308 range of applied loadings, does not necessarily have a negative impact on mono-digestion of food
309 waste when compared to digesters operated at the same average OLR with a stable loading pattern,
310 and may even have some advantages. Based on patterns of VFA accumulation, digester VL was in
311 fact able to operate stably with a smaller range of TE than that needed by the digesters at steady OLR.
312 The reason for this difference is unknown. If the variation in loading was sufficient to bring about
313 shock changes in digester conditions, this might lead to die-off of a proportion of the microbial
314 population: cell lysis might then lead to release of trace elements, making these available for rapid
315 cycling. In practice, however, operating parameters gave no indication of any shock in the variable
316 loading digester, and if anything the pattern of total VFA concentrations indicates greater stability
317 than in the constant loading digesters. The variable loading digester also appeared to have a slightly
318 higher methane productivity in comparable operating periods (e.g. days 780–960), although
319 inevitable limitations of accuracy in gas measurement mean a greater number of replicates is needed
320 to provide statistical certainty. There is no obvious reason for this in terms of microbial kinetics.
321 Compared with the weekly cycle of fluctuating loading, the timescale for biological degradation of
322 food waste in a digester is relatively long, as indicated by typical batch biochemical methane
323 production curves for this type of material [35] and by continuing gas production after the cessation
324 of feeding e.g. in residual biogas production tests [36]. The average hydraulic retention time of 44
325 days is also relatively long, and in these conditions the range of fluctuation of daily loading is unlikely
326 to cause shock changes or long-term perturbation in gas production. This is also supported by the
327 stable operating parameters (pH, IA/PA) seen in all digesters. It may be the case that a more resilient
328 community of microorganisms can be developed from the digester with changing conditions. Food
329 waste digestion has been found to be dominated by hydrogenotrophic methanogens [7,15]. In
330 previous studies it has been reported that hydrogen-utilizing methanogens are more resistant to
331 environmental changes than the acetate-utilizing methanogens; hydrogen-utilizing methanogens
332 were more favourably impacted by substrate perturbation [37]. This characteristic enables the
333 hydrogen-utilizing methanogens to quickly adapt to changes in their environment [37,38]. In this
334 case, however, no evidence of any clear environmental changes was observed.

335 While the effect of shock or transient loading on anaerobic treatment has been previously
336 investigated, there has been little research on loading variations as tested in this study. The only
337 similar work found, which however was not carried out with food waste but with a glucose feed, also
338 confirmed that the anaerobic system was able to adapt to the periodic substrate perturbation and
339 better results could be achieved compared to constant loading; a long-term change in microbial
340 community was used to explain the good performance in the digester experiencing loading
341 perturbation [37]. This study did not analyse the composition of the microbial communities; instead,
342 it placed a focus on long-term operation and trace elements addition. It was shown that long-term
343 mono-digestion of food waste is a reliable and highly effective process if the digestion is supported
344 by the regular dosage of appropriate trace elements. Such dosage does not only avoid accumulation
345 of volatile acids with subsequent process instability, but it can also stabilise a digester that is already
346 affected by VFA accumulation. The results of this work also showed that in the event of trace element
347 limitations, mono-digestion of food waste may be less resilient in digesters subjected to constant
348 organic loading compared to a digester with varying organic load.

349 In the last stage of the experiment, re-supplementation of the full set of eleven TEs stimulated
350 the degradation of accumulating VFAs in CL1&2. Such accumulation had occurred despite the
351 addition of Cobalt and Selenium. Co and Se are the two TEs which were previously found to be
352 limiting for food waste mono-digestion, with their deficiency causing VFA accumulation [15]. The
353 results of this study suggest that either some other TE was limiting, or the reduced Co concentration
354 of 0.3 mg L⁻¹ in the final part of the trial was not sufficient for the OLR of 5 g VS L⁻¹ d⁻¹. This highlights
355 the need to consider a set of limiting factors and also points to the issue of co-limitation. The Liebig's
356 law of the minimum implies that there is a single limiting nutrient which controls the yield [39], but
357 this concept can be expanded to co-limitation due to the simultaneous scarcity of more than one
358 nutrient. This issue of multiple potentially limiting nutrients reflects the complexity of trace metal
359 functions at the physiological and ecological (environmental) levels, rather than at the biochemical
360 level [40]. This is illustrated by the results of this work in the context of anaerobic digestion systems,
361 where the extent of trace element deficiency was related to the variability or otherwise of the applied
362 OLR, and thus not only to the feedstock characteristics but also to the environmental parameters.

363 While in theory the continuous dosage of a full set of relevant trace elements might appear the
364 most favourable option to ensure stable food waste digestion, in practice other factors also need be
365 considered. In commercial plants, excessive TE supplementation and strict OLR control will increase
366 costs and management requirements. The findings of this study therefore are highly relevant for the
367 waste management industry. The results revealed that when applying a varying instead of constant
368 organic loading rate to a digester, the process was not disadvantaged by this operation pattern and
369 may even have been more robust and less susceptible to inhibition. When other trace elements had
370 been washed out, the addition of 0.3 mg L⁻¹ Co and 0.2 mg L⁻¹ Se was sufficient to maintain a stable
371 performance in the variable-loading digester, whereas the digesters with constant organic loading
372 showed elevated VFA concentrations and a slight reduction in methane productivity, and required
373 re-supplementation of the full spectrum of trace elements to achieve the same performance.
374 Operation of food waste digesters under varying loading rates can therefore be recommended.
375 Loading rates between 2.5 and 7.5 g VS L⁻¹ d⁻¹ can further be recommended.

376 Considering the relevance of these findings for commercial food waste AD, further research
377 should explore in detail the nature of the link between OLR fluctuations and reduced TE requirement.

378 4. Conclusions

379 This work confirmed that mono-digestion of food waste could be operated stably and reliably
380 with daily organic loading ranging widely from 0–10 g VS L⁻¹ d⁻¹ at a weekly average OLR of 5 g VS
381 L⁻¹ d⁻¹, and with very limited supplementation of essential trace elements, namely a dosage of 0.3 mg
382 L⁻¹ Co and 0.2 mg L⁻¹ Se. No VFA accumulation or methane loss were observed for such an operating
383 mode, whereas the control digesters operated at constant organic loading showed less stable
384 performance and higher risk of process inhibition. In particular, the digester with variable loading
385 had greater resilience to trace element limitations in this study. The results indicate that the day-to-
386 day-variation in organic loadings experienced in full-scale food waste digestion plant is unlikely to
387 have any negative effect on biogas production or operational stability when compared to that
388 achieved by operation at constant organic load; and thus the results also provide some confidence
389 that findings from well-controlled laboratory-scale trials are transferrable to commercial AD plants.
390 More studies are required to explore the theoretical basis for the difference in trace element
391 requirements under constant and variable loading, and to quantify its practical significance.

392 **Supplementary Material:** Data supporting this study are openly available from the University of Southampton
393 repository at <http://doi.org/10.5258/SOTON/xxxxx>.

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