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Investigation of the impact of trace elements on anaerobic volatile fatty acid degradation using a fractional factorial experimental design

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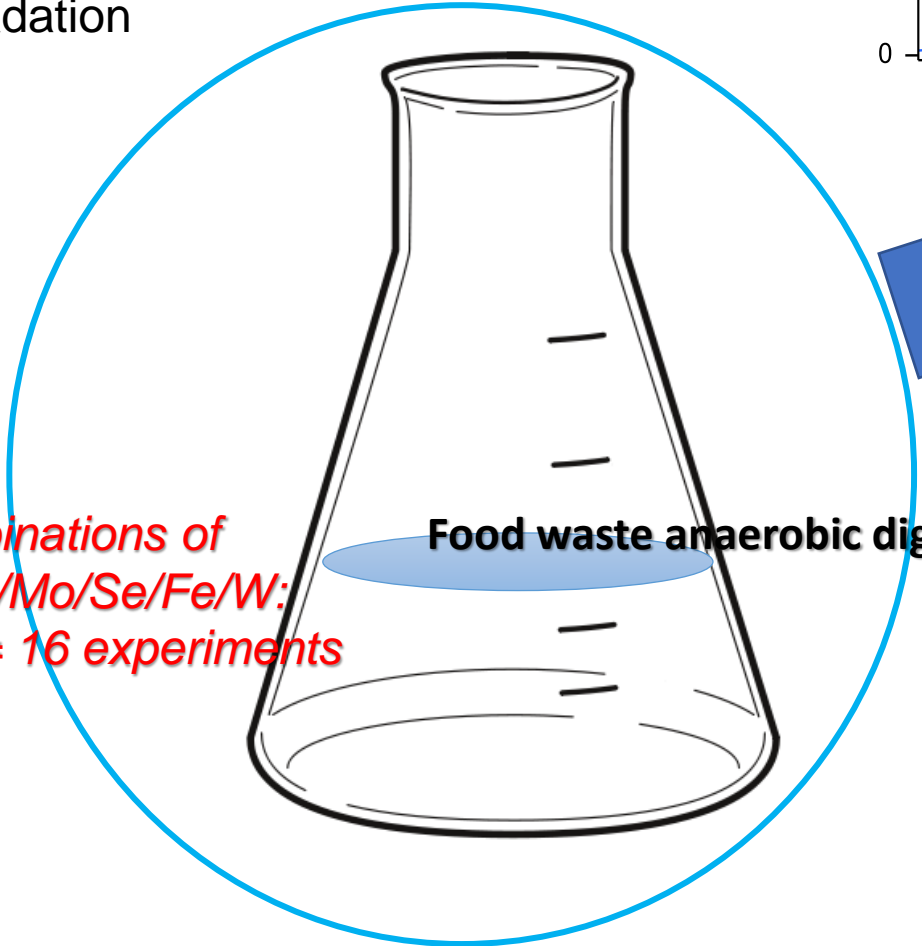
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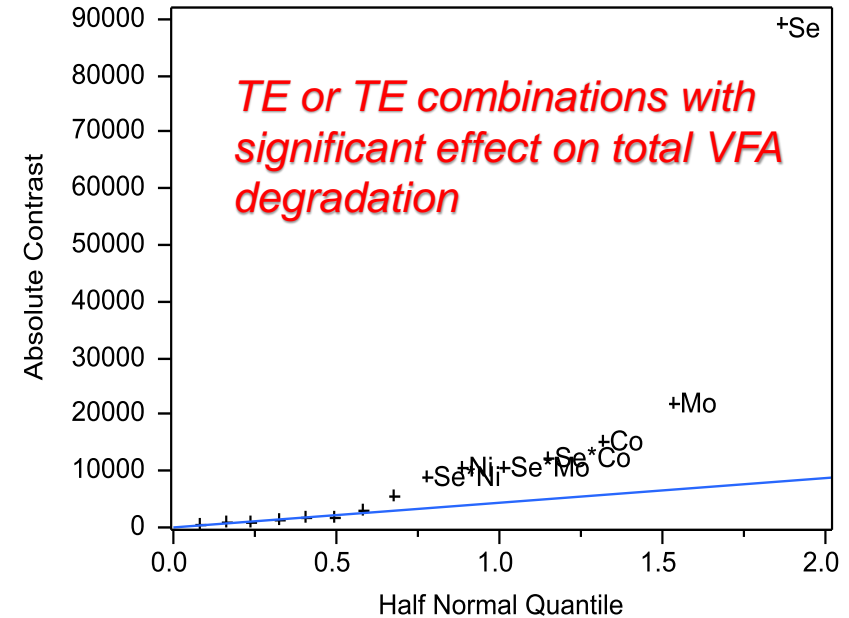
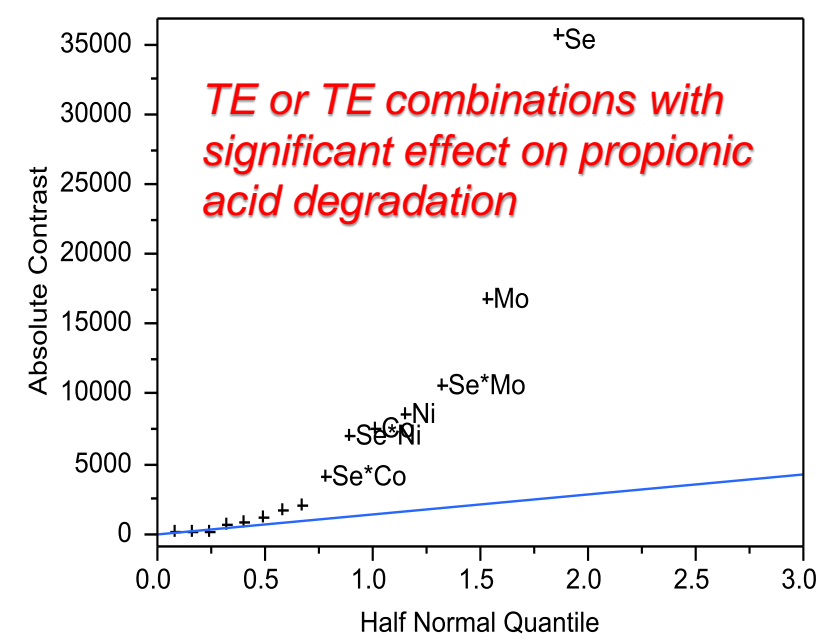
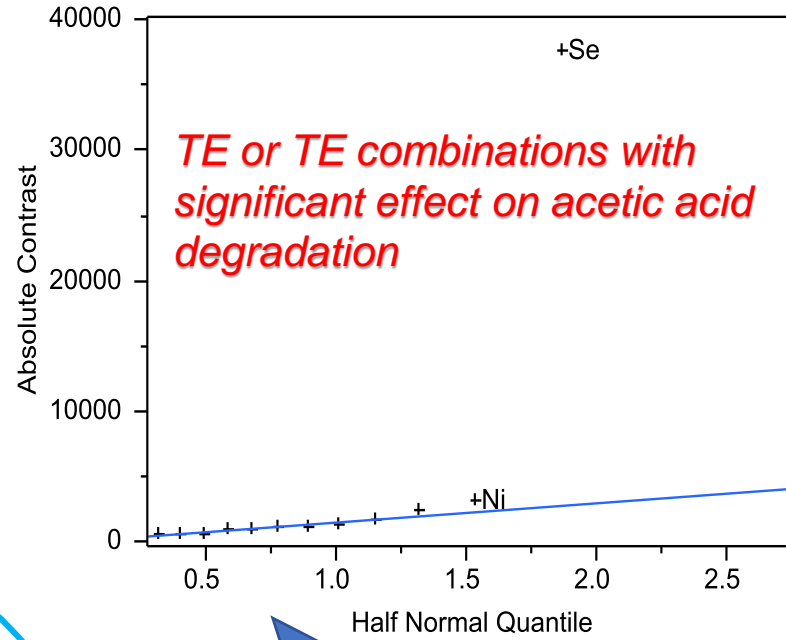
# DoE: 2-level Fractional factorial design

Investigate impact of six trace elements (TE) and their combination effect on VFA degradation



*Combinations of Co/Ni/Mo/Se/Fe/W:  $2^{6-2} = 16$  experiments*

**Food waste anaerobic digestate**



1 **Investigation of the Impact of Trace Elements on Anaerobic Volatile Fatty Acid**  
2 **Degradation Using a Fractional Factorial Experimental Design**

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10 **ABSTRACT**

11 The requirement of trace elements (TE) in anaerobic digestion process is widely  
12 documented. However, little is understood regarding the specific requirement of  
13 elements and their critical concentrations under different operating conditions such  
14 as substrate characterisation and temperature.

15 In this study, a flask batch trial using fractional factorial design is conducted to  
16 investigate volatile fatty acids (VFA) anaerobic degradation rate under the influence  
17 of the individual and combined effect of six TEs (Co, Ni, Mo, Se, Fe and W). The  
18 experiment inoculated with food waste digestate, spiked with sodium acetate and  
19 sodium propionate both to 10 g/l. This is followed by the addition of a selection of  
20 the six elements in accordance with a  $2^{6-2}$  fractional factorial principle. The  
21 experiment is conducted in duplicate and the degradation of VFA is regularly  
22 monitored.

23 Factorial effect analysis on the experimental results reveals that within these  
24 experimental conditions, Se has a key role in promoting the degradation rates of both  
25 acetic and propionic acids; Mo and Co are found to have a modest effect on  
26 increasing propionic acid degradation rate. It is also revealed that Ni shows some  
27 inhibitory effects on VFA degradation, possibly due to its toxicity. Additionally,  
28 regression coefficients for the main and second order effects are calculated to  
29 establish regression models for VFA degradation.

30 **Key Words:** Anaerobic digestion; Volatile fatty acids (VFA) degradation; Trace  
31 elements; Fractional factorial design

32

### 33 **1. Introduction**

34 Anaerobic digestion (AD) of organic fraction of municipal solid waste (OFMSW) is  
35 an attractive technology for sustainable waste treatment and renewable energy  
36 production. In Europe, the installed AD capacity has increased significantly. The  
37 annual AD capacity grow from 4 million tons in 2006 (De Baere, 2006) to ~6 million  
38 tons by the end of 2010 (De Baere and Mattheeuws, 2010), reflecting the growing  
39 popularity of the technology.

40 There is great potential for AD technology to generate both economic and  
41 environmental benefits. However, its application for certain organic waste streams has  
42 been considered intractable (Speece, 1983). This is largely due to lack of  
43 understanding of the complicated biological and biochemical reactions involved in the  
44 process, as well as the trace element (TE) requirements to maintain normal  
45 microbiological functions.

46 Whilst TEs are required only at low concentration compared to macronutrients such  
47 as C, N and P, they have a critical role in methanogenesis enzymatic activity (Demirel  
48 and Scherer, 2011; Kida et al., 2001; Osuna et al., 2003) and microbial respiration  
49 processes (Zandvoort et al., 2006). According to a number of studies and reviews  
50 (Diekert et al., 1980; Fermoso et al., 2009; Gonzalez-Gil et al., 1999; Speece et al.,  
51 1983; Zhang et al., 2003), cobalt, iron and nickel are required for both acetoclastic  
52 and hydrogenotrophic methanogenesis pathways. Cobalt is the major constituent of  
53 corrinoids which act as key cofactors in both methanogenesis pathways (Muller, 2003;  
54 Murakami and Ragsdale, 2000; Thauer et al., 2008). Iron and nickel form the centre  
55 parts of the Ni-Fe-S cluster and Fe-S cluster. These are important subunits of enzymes  
56 such as hydrogenase and acetyl-CoA synthase involved in methanogenesis pathways  
57 (Lindahl and Chang, 2001; Thauer et al., 2010). Nickel also forms the central site of  
58 Methyl-Coenzyme M reductase – the key enzyme in the final step of all the  
59 methanogenesis (Ermler et al., 1997).

60 Selenium, molybdenum and tungsten are also reported to be required in  
61 methanogenesis (Fermoso et al., 2009). Based on an extensive literature survey of  
62 biochemical reactions involved in methanogenesis pathways, there is no suggestion of  
63 a requirement for selenium in the acetoclastic methanogenesis pathway.  
64 (Deppenmeier, 2002; Ferry, 1999, 1992a.; Hille et al., 2014; Kryukov and Gladyshev,  
65 2004; Müller et al., 2010; Muller, 2003; Murakami and Ragsdale, 2000; Rother and  
66 Krzycki, 2010; Stock and Rother, 2009; Thauer et al., 2008). Whilst selenium is  
67 understood to be involved extensively in the hydrogenotrophic methanogenic pathway  
68 as it forms various selenoproteins, including hydrogenase, formate dehydrogenase  
69 (Jones et al., 1979) and formyl-methanofuran dehydrogenase (Vorholt et al., 1997). It  
70 is reported in a number studies that hydrogenotrophic methanogens become the

71 dominant microbial population in digesters treating high ammonia feedstocks, such as  
72 food waste (Angelidaki and Ahring, 1993; Banks et al., 2012;; Schnürer and Nordberg,  
73 2008). This can result an increased dependence on hydrogenotrophic methanogenic  
74 route in these digesters, hence the increased requirements of Se to form key enzymes.  
75 Banks et al. (2012) observed a significant improvement of volatile fatty acid  
76 degradation when Se was added to digesters treating food waste with an elevated  
77 ammoniacal nitrogen concentration.

78 Molybdenum and tungsten are closely involved in formate dehydrogenase (in  
79 hydrogenotrophic methanogens and syntrophic oxidising bacteria) and formyl-  
80 methanofuran dehydrogenase (in hydrogenotrophic methanogens) ( Hille et al., 2014;  
81 Johnson et al., 1996; Romão, 2009). Although these two elements are considered to  
82 be chemically analogous in enzyme formation (Kletzin and Adams, 1996), their  
83 requirements for each methanogen species cannot be replaced by each other. The  
84 stimulatory effect of W on the growth of methanogens *Methanococcus vannielii* was  
85 first reported in the late 1970s (Jones and Stadtman, 1977). When formate was used as  
86 the carbon source, the growth of *M. vannielii* was significantly enhanced by the  
87 addition of W, but not by Mo. However, the growth stimulation by W was not  
88 observed when the organism was fed with H<sub>2</sub> and CO<sub>2</sub>, which suggested the  
89 involvement of a W-containing formate dehydrogenase (FDH). In a lab scale  
90 digestion trial, an improvement of performance was reported by Jiang et al. (2012)  
91 following the addition of W to anaerobic digesters treating vegetable waste.

92 Combinations of key TEs were reported to have synergistic or antagonistic effects to  
93 the methanogenesis. Patidar and Tare (2006) report that addition of Fe/Co, Fe/Ni/Zn  
94 and Ni/Zn/Co combinations lead to maximum total methanogenic activity in a batch

95 study; however addition of Ni affects Co and Zn uptake in methanogens, due to  
96 antagonistic effect of metal ions. Feng et al. (2010) investigated the effects of  
97 additions of Co, Ni/Mo/B and Se/W on the biogas production and the associated  
98 anaerobic microbial community. The study revealed that the highest methane  
99 production occurs at high Se/W concentrations in combination with a low level of Co.  
100 However, the influence of the trace metal additions on the microbial community  
101 composition was not significant.

102 It is clear that TE addition can benefit the operation of many lab and commercial scale  
103 anaerobic digesters with TE deficiency. However, baseline TE concentration  
104 feedstocks as well as microbial community structure can significantly influence the  
105 TE requirement in anaerobic digesters. To date, it remains a challenge to determine  
106 whether a digester is deficient of TE particularly to identify exact TE species required.  
107 The currently practice of TE supplementation in many commercial AD plants tends to  
108 be a full addition of all trace elements, many of which are not required and even  
109 potentially harmful to their AD processes. It is therefore important for the operators to  
110 understand the specific requirement of each digester, in order to supplement only  
111 those elements required for improved performance and reduction of operational cost.

112 Significant work has been carried out to investigate the effects of TE and their  
113 combinations on VFA degradation (Feng et al., 2010; Worm et al., 2009). Feng et al.  
114 (2010) demonstrated advanced statistical methods can be a useful tool to interpret the  
115 impact of TE on anaerobic digestion process efficiency. However, to the authors' best  
116 knowledge, to date no study has applied factorial design method to investigate the  
117 effects of multiple elements simultaneously. Factorial design is widely applied in  
118 scientific investigations to identify the key factors and/or combinations of factors

119 influencing the process. In this study, the effects of six TEs (Co, Ni, Se, Mo, Fe and  
120 W) on the volatile fatty acids degradation rate were studied. In order to differentiate  
121 the effects of each element and/or element combination, a design of experiment (DoE)  
122 approach of 2-level fractional factorial design was adopted. Regression model has  
123 also been developed to correlate the response with each factor.

## 124 **2. MATERIALS AND METHODS**

### 125 **2.1 Food waste digestate inoculum**

126 Inoculum used in this study was acquired from a 5-litre lab scale CSTR mesophilic  
127 (37 °C) anaerobic digester fed with food waste. The digester had been in operation for  
128 over a year and reached its stable state, i.e. stable VFA concentration, ammonia  
129 nitrogen level and daily biogas production in the past 2 months. During the entire  
130 period of its operation, no TE supplementation regime was adopted. Before starting  
131 this study, acetate and propionate concentrations in digestate inoculum were both  
132 increased to 10 g/l by addition of sodium acetate and sodium propionate (Reagent  
133 grade, Fisher Chemical, UK). D-glucose and starch were added as substrates, both at  
134 concentrations of 4 g/l in the digestate. The background ammonia nitrogen  
135 concentration in the inoculum was high at 6.5 g/l.

### 136 **2.2 Analytical methods**

137 The baseline TEs concentrations in digestate inoculum were determined using ICP-  
138 MS or ICP-OES at a UKAS accredited commercial laboratory (Severn Trent Services,  
139 Coventry, UK) after in-house hydrochloric – nitric acid digestion in accordance with  
140 US EPA standard method 3010A. Baseline TEs concentrations in the inoculum have  
141 been determined and the results are shown in Table 1.



142 Volatile fatty acid (VFA) concentrations were quantified in a Shimadzu 2010 gas  
143 chromatograph (Shimadzu, Milton Keynes, UK) using a method described in Jiang et  
144 al. (2012). Samples were acidified using formic acid. Three standard solutions  
145 containing 50, 250 and 500 mg l<sup>-1</sup> of acetic, propionic, iso-butyric, n-butyric, iso-  
146 valeric, valeric, hexanoic and heptanoic acids were used for VFA calibration.

### 147 **2.3 Trace elements supplementation and VFAs degradation flask trial**

148 The batch experiment was set up in conical flasks each with 250 ml capacity. Each  
149 flask was inoculated with 200 ml digestate. Working solutions of six trace elements  
150 (Co, Ni, Mo, Se, Fe and W) were prepared using CoCl<sub>2</sub>, NiCl<sub>2</sub>, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>,  
151 Na<sub>2</sub>SeO<sub>3</sub>, FeCl<sub>2</sub> and Na<sub>2</sub>WO<sub>4</sub> (Analytical grade, Fisher Chemical, UK). Addition of  
152 these trace element solutions into each flask followed the pattern as shown in Table 2,  
153 with strings of plus (+) and minus (-) signs denoting with and without addition of a  
154 particular element. There are in total 16 TE addition combinations including a control  
155 where no TE was added. When TE solutions are added, the concentrations of  
156 elements increases by 1 mg/l, 1 mg/l, 0.2 mg/l, 0.2 mg/l, 5 mg/l and 0.2 mg/l for Co,  
157 Ni, Mo, Se, Fe and W, respectively. To ensure data quality, this experiment was  
158 conducted in duplicate, i.e. 32 total flasks.

159 Flask headspaces were flushed with carbon dioxide and nitrogen (20:80) mixture gas  
160 (BOC, UK). Each flask was then sealed with rubber bungs with an outlet connected to  
161 a 1-litre Tedlar bag to maintain an ambient pressure in the headspace. All flasks were  
162 randomised and placed in an orbital incubator (Weiss-Gallenkamp, UK) set at 36°C  
163 and 60RPM.

164 Digestate samples in flasks were routinely sampled and analysed for VFA  
165 concentrations in order to plot VFA degradation curves over the duration of the

166 experiment. 1 ml of the digestate was withdrawn from each flask using hand pipette  
167 with cut-off pipette tip. After sampling, the headspace of each flask was flushed with  
168 CO<sub>2</sub>/N<sub>2</sub> (20:80) and then sealed again before returning to the incubator. The flask  
169 experiment has continued for ~90 days until all VFAs in the flasks were depleted.

170 Similar to the approach adopted by Olaisen et al. (2002), the numerical integrals of  
171 each VFAs degradation curves, assigned as the Degradation Index (*DI*), are calculated  
172 according to the trapezoidal rule (Equation 1).

$$173 \quad DI = \int_0^{\infty} f(t)dt \approx \sum_{i=0}^n (t_{i+1} - t_i) \times (C_{i+1} + C_i)/2 \quad (\text{Equation 1})$$

174 Where: *t* is the time in days; *C* is the measured value of VFA concentration.

175 *DI* enables the quantification of VFA degradation efficiency based on the degradation  
176 curve; a smaller *DI* number indicates a more efficient degradation. The calculated *DI*  
177 numbers were used as experimental responses in the following statistical and  
178 modelling analysis.

## 179 **2.4 Statistical method**

### 180 *2.4.1 Fractional factorial design and data analysis*

181 The experiment was designed to expose the key TEs which are the most effective on  
182 improving anaerobic degradation of VFAs, whilst understanding possible synergistic  
183 effects from combinations of elements. Six trace elements were selected as factors to  
184 be investigated in this 2-level factorial experiment design. Due to practical difficulties,  
185 it is not feasible to run a 2-level full factorial experiment, as this requires 64 (=2<sup>6</sup>)  
186 flasks to be prepared for a single experiment. As a result, a 2<sup>6-2</sup> fractional factorial  
187 design, which is ¼ of the full factorial design with a resolution of IV was adopted.  
188 The chosen design reveals all the main effects. However it cannot distinguish fully  
189 the 2-factor interaction due to effect aliasing (Wu and Hamada, 2009). The

190 experimental arrangement of this  $2^{6-2}$  subset (fraction) of a full factorial design was  
 191 generated using the SAS JMP statistical suite (SAS Institute Inc. Cary, North Carolina,  
 192 USA).

193 The average independent effect of each TE (factor), known as the main effect (*ME*) is  
 194 calculated as follows:

195 For any factor (*A*), the main effect  $ME(A) = \bar{z}(A+) - \bar{z}(A-)$  (Equation 2)

196 Where  $\bar{z}(A+)$  is the average value of all observations (VFA concentrations) when  
 197 factor (*A*) is present (+ level).

198 Similarly,  $\bar{z}(A-)$  is the average value of all observation when factor (*A*) is not present  
 199 (level) of factor (*A*), i.e. without addition of the particular TE. Higher order factorial  
 200 effects can be calculated in the in a similar way.

#### 201 2.4.2 Regression Model

202 Factorial effects in a  $2^k$  factorial design can be estimated using a multiple regression  
 203 model (Wu and Hamada, 2009). For the current experiment design which contains 16  
 204 observations, the model can be expressed as:

$$205 \quad z_i = \beta_0 + \sum_{i=1}^{16} \beta_i x_i + \sum \sum_{j < i} \beta_{ij} x_i x_j \quad (\text{Equation 3})$$

206 Where:  $\beta_0$ ,  $\beta_i$  and  $\beta_{ij}$  are the regression coefficients which are estimated using Least  
 207 squares method.  $z_i$  is the experiment response (*DI*).  $x_i$  and  $x_j$  are the variables (i.e. the  
 208 factors in the experiment, coded with +/-).

### 209 3. RESULTS AND DISCUSSION

#### 210 3.4 Trace elements flask trial

211 A reasonable repeatability of the experiment was achieved as indicated by the error  
 212 bar shown the range of the result from the duplicated experiment (Figure 1). Clear  
 213 divergence in degradation rate was observed amongst the sample groups where  
 214 different TE supplementation regimes were adopted. Under each TE addition

215 combinations, the VFAs degradation indexes were calculated based on VFA  
216 concentration results using Equation 1. The *DI* results are summarised in Table 3.  
217 Notably, in samples where selenium was present, the acetate and propionate  
218 degradations were significantly stimulated in comparison with selenium deprived  
219 groups.  
220 On day 28, acetic acid concentration in all samples was observed to be degraded  
221 below 100 mg/l. However, this is followed by an increase of acetic acid  
222 concentrations in some samples as indicated by the bumps on the curves in Figure 1a  
223 & 1b around day 30. This increase of acetic acid is caused by acetic acid produced  
224 from propionate degradation as the timing coincided with an increased propionate  
225 degradation rate in those samples during this period as shown in Figure 1c & 1d. The  
226 fractional factorial design significantly reduced the experimental efforts normally  
227 required to understand complex TE requirement in AD process. Due to the simplicity  
228 of the experimental and analytical requirement, this method can therefore be adopted  
229 by AD operators to assess the deficiency of TE in digesters and provide critical  
230 information on specific TE requirement. This enables a more targeted element  
231 supplementation regime to avoid a full TE addition, therefore significantly reduce  
232 operational costs.

### 233 **3.5 Analysis of factorial effects**

234 The factorial effects of each individual element (Se, Mo, Co, Ni, W, Fe) and 2-factor  
235 combinations in contributing to VFA degradation rate is calculated according to  
236 Equation 2 and summarised in Table 4. The '-' signage indicates the effect contribute  
237 negatively to the *DI*. In other words, when a factorial effect is negative, that factor  
238 results a decrease in *DI* (an improved VFA degradation).

239 A high resolution factorial design (Resolution IV) was adopted in this study. This  
240 design ensures main effect of each individual factor is clear from confounding.  
241 However, higher order effects are affected by confounding and less likely to be  
242 important according to effect hierarchy principle (Wu and Hamada, 2009). For these  
243 reasons, this study only considers effects of individual elements and 2-factor  
244 combinations.

245 The factorial effects are illustrated in main effect plots and half- normal probability  
246 plots in Figure 2. In main effect plots, each factor is represented by a line connecting  
247 the average values of all observations at the high (+) level and low (-) level; the  
248 vertical height of each line indicates the main effect. In half- normal probability plots,  
249 factors of more significance are found at the upper right corner away from the 'near-  
250 zero' line and the significance is quantified as Lenth's t-ratio shown in Table 4.

251 The factorial effect of Se suggests it significantly improves the degradation for both  
252 acetic and propionic acids. Mo and Co show a moderate effect on improving  
253 propionic degradation rate; whereas Ni shows some negative effects on both acetic  
254 and propionic acids degradation as also indicated by its factorial effects (Table 4).

255 The inhibitory effect of Ni during anaerobic digestion processes were widely reported  
256 in previous literatures (Ahring and Westermann, 1983; Lin, 1993, 1992). Lin (1992)  
257 observed a moderate inhibitory effect from Ni in a batch experiment under mesophilic  
258 condition. The concentrations of Ni to inhibit 50% of the acetic and propionic acid  
259 degradation are reported to be 89 and 226 mg/l, respectively. In current study, the  
260 concentration of Ni at 1.3 mg/l is considerably lower than the reported values which  
261 are considered to have significant inhibitory effects. However, it is possible that the

262 Ni inhibition starts at low concentration and only can be revealed with statistical  
263 analysis.

264 Combination effects of two elements show no significant effect on acetic acid  
265 degradation as indicated by p-value (Table 4). For propionic acid, however, Se/Mo  
266 combination shows significant effect on improving the degradation of the acid,  
267 whereas Mo/W combination exhibits an inhibitory effect. However, the effect of  
268 Se/Mo is confounded with Co/W and Ni/Fe due to alias effect caused by the intrinsic  
269 inadequacy of a fractional design. Therefore, the improved propionic acid degradation  
270 can equally be caused by Co/W and Ni/Fe combinations. Further experiment is  
271 needed to clarify this ambiguity. For the same reason, the inhibitory effect of Mo/W is  
272 confounded with Se/Co. However, in a previous semi-continuous food waste  
273 digestion experiment which provided the inoculum for the study, it was confirmed  
274 that Co was beneficial to the degradation of VFA including propionic acid at the  
275 presence of selenium (Banks et al., 2012). This clearly suggests the confounding  
276 factorial effect of Se/Co can be dismissed in the current study, thus leaving the W/Mo  
277 effect as evident. Indeed, the antagonistic effects between W and Mo for some  
278 methanogens were reported in a number of studies (Kletzin and Adams, 1996; May et  
279 al., 1988; Zellner and Winter, 1987). The studies suggest that W replaces Mo as the  
280 centre of formate dehydrogenase (FDH), a key enzyme involved in propionic acid  
281 degradation, and consequently deactivates the enzyme (May et al., 1988).

282 The factorial analysis reveals a strong influence of Se on the VFA degradation,  
283 suggesting a specific requirement for Se to improve VFA, particularly acetate and  
284 propionate degradation in this type of digestate. Previous study (Banks et al., 2012)  
285 confirmed the foodwaste digestate used as inoculum in this study adopt

286 hydrogenotrophic methanogenesis as the principle methanogenic route due to the  
 287 elevated ammoniacal nitrogen level. This explains the significant effect of Se found in  
 288 this study, as it is understood based on wide range of literature evidence (Ferry, 1999,  
 289 1990; Jones et al., 1979; Vorholt et al., 1997) that Se involves significantly in the  
 290 syntrophic methanogenic pathway, but not in the acetoclastic pathway. In addition, as  
 291 reported by Worm et al. (2011, 2009), Se and Mo are involved in formate  
 292 dehydrogenases and hydrogenases required for propionate degradation, hence the  
 293 improved propionate degradation overserved in the presence of these two elements in  
 294 the factorial analysis.

### 295 **3.6 Regression models for VFA degradation indexes (DI)**

296 Regression coefficients for factorial effects of the individual elements and 2-factor  
 297 combinations were calculated using the least squares method and summarised in  
 298 Table 5. Using these coefficients, regression models correlates factorial effects with  
 299 degradation index for acetic acid ( $DI_{HAc}$ ), propionic acid ( $DI_{HPr}$ ) and total VFA  
 300 ( $DI_{Total}$ ) can be established according to Equation 3 as follows:

$$\begin{aligned}
 301 \quad DI_{HAc} = & 125728.25 - 33687.5X_{Se} - 1642.9X_{Mo} - 9.4X_{Co} + 5027.2X_{Ni} - 2940.6X_W \\
 302 \quad & + 3383X_{Fe} + 1008.8X_{Se}X_{Mo} - 1685.4X_WX_{Mo} - 3156.7X_{Mo}X_{Co} + 2849.6X_{Se}X_{Ni} \\
 303 \quad & + 3379.1X_{Mo}X_{Ni} - 1991.9X_{Co}X_{Ni} - 3692.1X_{Ni}X_W \quad \text{(Equation 4)}
 \end{aligned}$$

$$\begin{aligned}
 304 \quad DI_{HPr} = & 203360.9 - 40051.1X_{Se} - 18208.0X_{Mo} - 11276.6X_{Co} + 9196.4X_{Ni} - 1808.9X_W - \\
 305 \quad & 1580.0X_{Fe} - 9316.2X_{Se}X_{Mo} + 7821.2X_WX_{Mo} - 863.7X_{Mo}X_{Co} + 6341.9X_{Se}X_{Ni} - \\
 306 \quad & 1000.2X_{Mo}X_{Ni} + 550.1X_{Co}X_{Ni} - 1504.0X_{Ni}X_W \quad \text{(Equation 5)}
 \end{aligned}$$

$$\begin{aligned}
307 \quad DI_{Total} &= 392720.7 - 89005.3X_{Se} - 21657.1X_{Mo} - 15040.2X_{Co} + 10573.0X_{Ni} - 5443.6X_W - \\
308 \quad & 2883.7X_{Fe} - 10644.4X_{Se}X_{Mo} + 12260.4X_WX_{Mo} - 893.9X_{Mo}X_{Co} + 8807.8X_{Se}X_{Ni} \\
309 \quad & + 1258.5X_{Mo}X_{Ni} + 927.7X_{Co}X_{Ni} - 1540.0X_{Ni}X_W \quad \text{(Equation 6)}
\end{aligned}$$

310 Insignificant factorial effects at less than 5% significance level, i.e. adjusted p-  
311 value >0.05 (Table 4) can be ignored to simplify the above models, where the  
312 equations then become:

$$313 \quad DI_{HAc} = 125728.25 - 33687.5X_{Se} \quad \text{(Equation 7)}$$

$$\begin{aligned}
314 \quad DI_{HPr} &= 203360.9 - 40051.1X_{Se} - 18208.0X_{Mo} - 11276.6X_{Co} + 9196.4X_{Ni} - 9316.2X_{Se}X_{Mo} \\
315 \quad & + 7821.2X_WX_{Mo} \quad \text{(Equation 8)}
\end{aligned}$$

$$316 \quad DI_{Total} = 392720.7 - 89005.3X_{Se} - 21657.1X_{Mo} \quad \text{(Equation 9)}$$

317 Analysis of variance (ANOVA) is then used to evaluate the adequacy of the empirical  
318 models. The results show coefficients of determination ( $R^2$ ) for acetic, propionic and  
319 total VFA model are 0.9976, 0.9998 and 0.9998, respectively. There are significantly  
320 high F-ratios for acetic, propionic and total VFA models (65.27, 949.25 and 614.63,  
321 respectively) indicating a large values of model sum of squares (SS). All three models  
322 have sufficiently low (<5%) p-values indicating small probability of error for SS.  
323 Both F-ratios and p-values strongly support the robustness of the regression models  
324 developed under the experimental conditions in this study.

#### 325 **4. Conclusion**

326 This study applied a fractional factorial design in batch experiments to explore the  
327 impact of six trace elements (Co, Ni, Mo, Se, Fe and W) on VFA degradation rates in  
328 foodwaste digestate. The factorial analysis of the results shows a significant influence



329 from Se in improving the VFA degradation. This is in agreement with previous  
330 knowledge of the involvement of Se in hydrogenotrophic methanogenesis which is  
331 typically the main methane forming route in foodwaste digesters.  
332 The factorial results also reveal Ni shows slight inhibitory effect to VFA degradation  
333 and W/Mo combination inhibits propionic acid degradation, likely due the  
334 antagonistic effect of the two elements.  
335 The fractional factorial method has introduced an efficient experimental approach to  
336 identifying the deficient elements in anaerobic digesters, whilst offering guidance on a  
337 tailored TE supplementation recipe for digesters operating under different conditions.

338

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487

**Table 1.** Baseline TE concentrations in the inoculum

<b>Element</b>	<b>Baseline concentration (mg/l)</b>
Fe	103
Co	0.035
Mn	14.2
Al	42.8
Zn	7.94
Mo	0.13
Cu	2.23
Ni	0.304
Se	0.061
W	<0.1

**Table 2.** Experimental design matrix

Flasks	Co	Ni	Mo	Se	Fe	W
1, 2	-	-	-	-	-	-
3, 4	-	-	-	+	+	+
5, 6	-	-	+	-	+	+
7, 8	-	-	+	+	-	-
9, 10	-	+	-	-	+	-
11, 12	-	+	-	+	-	+
13, 14	-	+	+	-	-	+
15, 16	-	+	+	+	+	-
17, 18	+	-	-	-	-	+
19, 20	+	-	-	+	+	-
21, 22	+	-	+	-	+	-
23, 24	+	-	+	+	-	+
25, 26	+	+	-	-	+	+
27, 28	+	+	-	+	-	-
29, 30	+	+	+	-	-	-
31, 32	+	+	+	+	+	+

**Table 3.** Summary of VFA degradation indexes

Flasks	TE Combinations	<i>DI(Ave)</i>		
		Acetic	Propionic	Total
1, 2	Control	153,014	267,184	527,562
3, 4	Se/Fe/W	84,583	174,707	315,682
5, 6	Mo/Fe/W	155,723	249,938	488,264
7, 8	Mo/Se	81,555	127,735	260,954
9, 10	Ni/Fe	160,765	277,510	521,616
11, 12	Ni/Se/W	85,390	208,527	349,237
13, 14	Ni/Mo/W	161,457	255,408	498,665
15, 16	Ni /Mo/Se/Fe	123,414	156,092	300,108
17, 18	Co/W	163,569	232,874	462,669
19, 20	Co/Se/Fe	88,583	170,724	314,340
21, 22	Co/Mo/Fe	156,647	212,234	441,349
23, 24	Co/Mo/Se/W	81,935	117,920	246,362
25, 26	Co/Ni/Fe/Se	157,778	231,647	459,108
27, 28	Co/Ni/Se	99,000	209,378	364,809
29, 30	Co/Ni/Mo	166,373	220,501	454,576
31, 32	Co/Ni/Mo/Se/Fe/W	91,865	141,395	278,231

**Table 4.** Summary of factorial effects

Term	Aliases	Acetic acid				Propionic acid				Total VFA			
		Factorial effect	Lenth t-Ratio	Individual p-Value	Adjusted p-Value	Factorial effect	Lenth t-Ratio	Individual p-Value	Adjusted p-Value	Factorial effect	Lenth t-Ratio	Individual p-Value	Adjusted p-Value
Se	Mo/Co/W, Mo/Ni/Fe	-67375	-12.19	<0.0001*	<0.0008*	-80102	-26.69	<0.0001*	<0.0001*	-178011	-20.58	0.0001*	0.0002*
Mo	Se/Co/W, Se/Ni/Fe	-3286	0.59	0.59	1.00	-36416	-12.14	0.0002*	0.0008*	-43314	-5.01	0.0035*	0.02*
Co	Se/Mo/W, Ni/W/Fe	-19	-0.00	1.00	1.00	-22553	-7.52	0.0005*	0.0052*	-30080	-3.48	0.01*	0.09
Ni	Se/Mo/Fe, Co/W/Fe	10054	1.82	0.09	0.59	18393	6.13	0.0019*	0.01*	21146	2.44	0.03*	0.26
W	Se/Mo/Co, Co/Ni/Fe	-5881	-1.06	0.28	0.99	-3618	-1.21	0.22	0.95	-10887	-1.26	0.20	0.93
Fe	Se/Mo/Ni, Co/Ni/W	3383	0.61	0.58	1.00	-3160	-1.05	0.27	0.99	-5767	-0.67	0.50	1.00
Se/Mo	Co/W, Ni/Fe	2018	0.37	0.74	1.00	-18632	-6.21	0.0019*	0.01*	-21289	-2.46	0.03*	0.26
Mo/W	Se/Co	-3371	-0.61	0.58	1.00	15642	5.21	0.0028*	0.02*	24521	2.83	0.02*	0.20
Mo/Co	Se/W	-6313	-1.14	0.25	0.97	-1727	-0.58	0.60	1.00	-1788	-0.21	0.85	1.00
Se/Ni	Mo/Fe	5699	1.03	0.29	0.99	12684	4.23	0.0064*	0.05	17616	2.04	0.06	0.45
Mo/Ni	Se/Fe	6758	1.22	0.21	0.95	-2000	-0.67	0.50	1.00	2517	0.29	0.79	1.00
Co/Ni	W/Fe	-3984	-0.72	0.45	1.00	1100	-0.37	0.74	1.00	1855	0.21	0.84	1.00
Ni/W	Co/Fe	-7384	-1.34	0.18	0.90	-3008	-1.00	0.30	0.99	-3080	-0.36	0.74	1.00

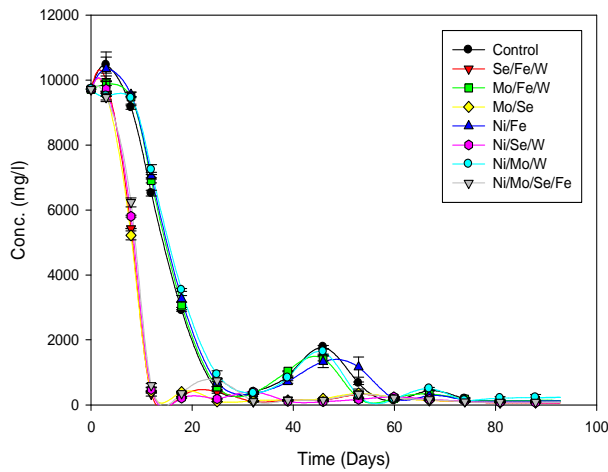
Note: p-Values marked with “\*” denote the factor being significant (<0.05)



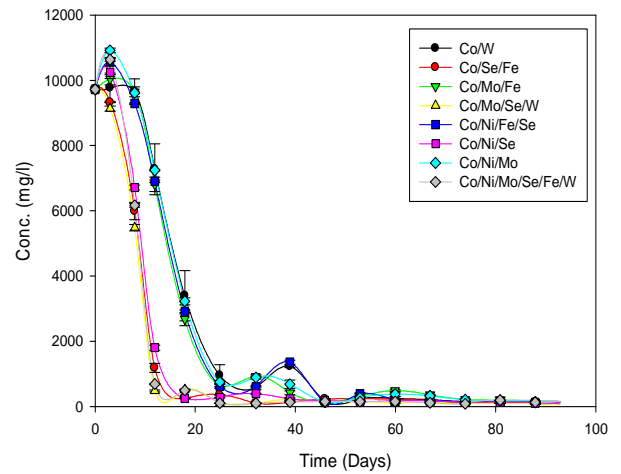
**Table 5.** Coefficients of the empirical regression models for acetic, propionic and total VFA degradation index (*DI*)

Term	Acetic Acid			Propionic Acid			Total VFA		
	Coefficient	t Ratio	Prob> t	Coefficient	t Ratio	Prob> t	Coefficient	t Ratio	Prob> t
Constant	125728.25	104.63	<0.0001	203360.9	466.45	<0.0001	392720.7	367.71	<0.0001
Se	-33687.5	28.03	0.0013	-40051.1	91.86	0.0001	-89005.3	83.34	0.0001
Mo	-1642.9	-1.37	0.3049	-18208.0	41.76	0.0006	-21657.1	20.28	0.0024
Co	-9.4	0.01	0.9945	-11276.6	25.87	0.0015	-15040.2	14.08	0.0050
Ni	5027.2	-4.18	0.0527	9196.4	-21.09	0.0022	10573.0	-9.90	0.0100
W	-2940.6	2.45	0.1342	-1808.9	4.15	0.0535	-5443.6	5.10	0.0364
Fe	1691.5	-1.41	0.2945	-1580.0	3.62	0.0684	-2883.7	2.70	0.1142
Se/Mo	1008.8	0.84	0.4895	-9316.2	-21.37	0.0022	-10644.4	-9.97	0.0099
Se/Co	-1685.4	-1.40	0.2958	7821.2	17.94	0.0031	12260.4	11.48	0.0075
Mo/Co	-3156.7	-2.63	0.1195	-863.7	-1.98	0.1861	-893.9	-0.84	0.4907
Se/Ni	2849.6	2.37	0.1411	6341.9	14.55	0.0047	8807.8	8.25	0.0144
Mo/Ni	3379.1	2.81	0.1066	-1000.2	-2.29	0.1487	1258.5	1.18	0.3599
Co/Ni	-1991.9	-1.66	0.2392	550.1	-1.26	0.3343	927.7	0.87	0.4766
Ni/W	-3692.1	-3.07	0.0916	-1504.0	-3.45	0.0747	-1540.0	-1.44	0.2861

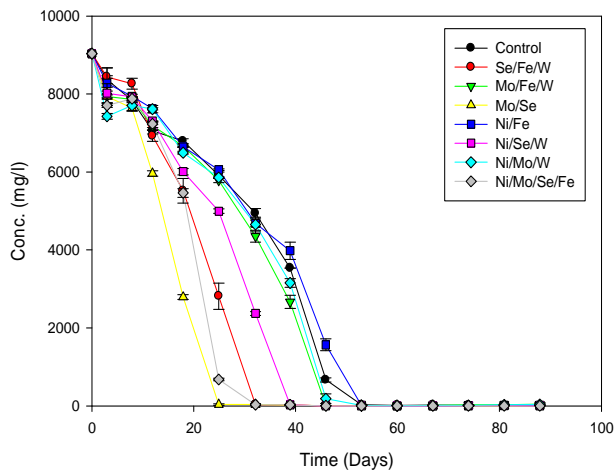
Note: Prob>|t|: Probability value of getting an even greater t-statistic (in absolute value), less than 0.05 are considered as significant evidence that the parameter is not zero



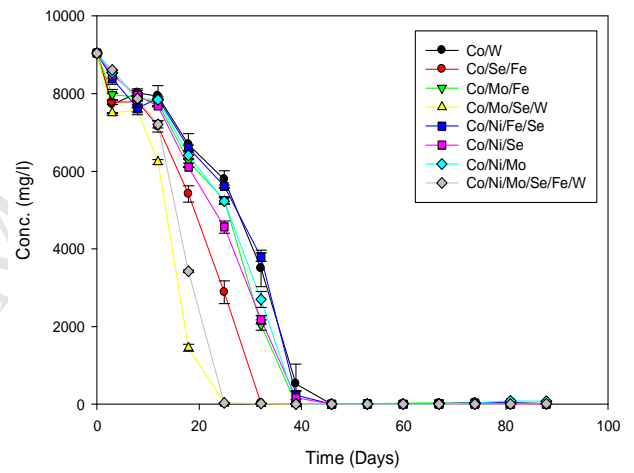
a) Sample 1-16 (Acetic acid)



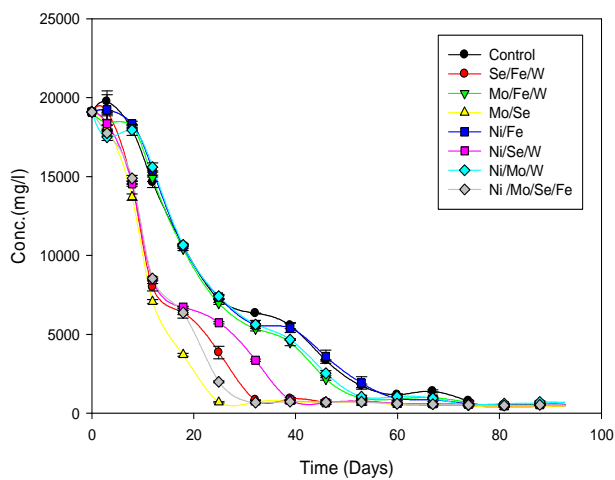
b) Sample 17-32 (Acetic acid)



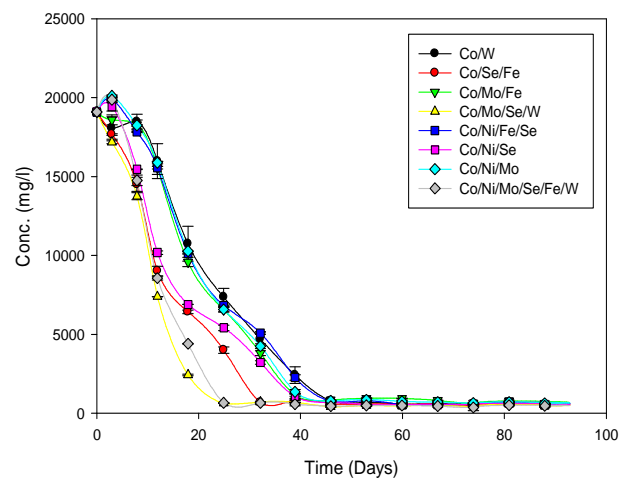
c) Sample 1-16 (Propionic acid)



d) Sample 17-32 (Propionic acid)



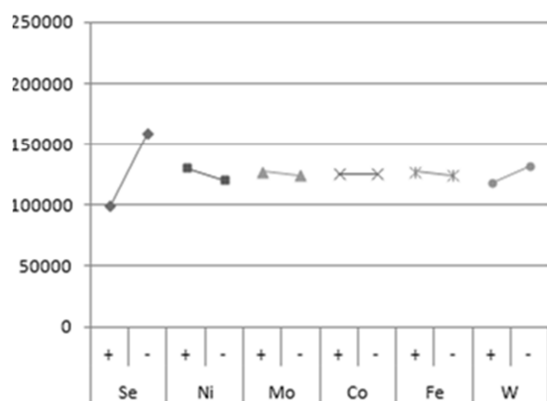
e) Sample 1-16 (Total VFA)



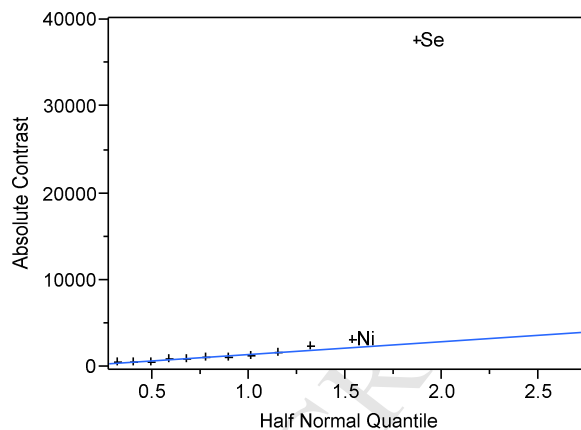
f) Sample 17-32 (Total VFA)

**Figure 1.** VFA degradation curves

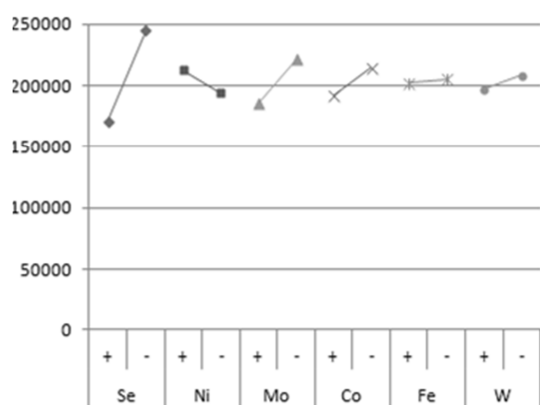
Note: Error bar indicate the range of results from duplicated experiment



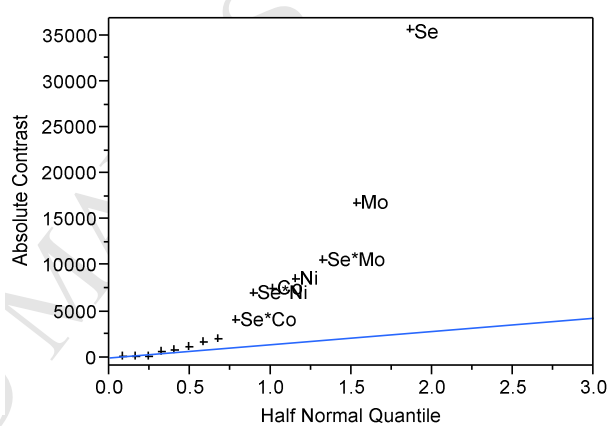
a) Main effects plot of acetic degradation



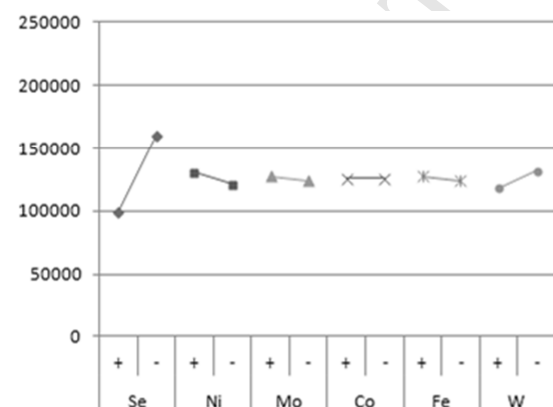
b) Half-normal plot of effects to acetic degradation



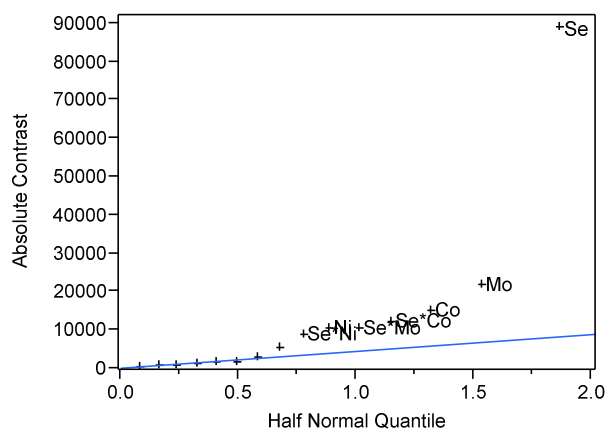
c) Main effects plot of propionic degradation



d) Half-normal plot of effects to propionic degradation



e) Main effects plot of total VFA degradation



f) Half-normal plot of effects to total VFA degradation

**Figure 2.** Main effects plot and Half normal probability plot of standardised effects for VFA degradation

**Highlights**

- A fractional factorial design of experiment to study effects of TE on VFA degradation
- Method enables to determine specific TEs requirement in a given AD plant
- Se has the most significant effect on improving VFA degradation in foodwaste digestate
- Using numerical integral to quantify VFA degradation performance over a given time