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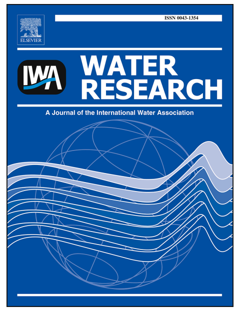

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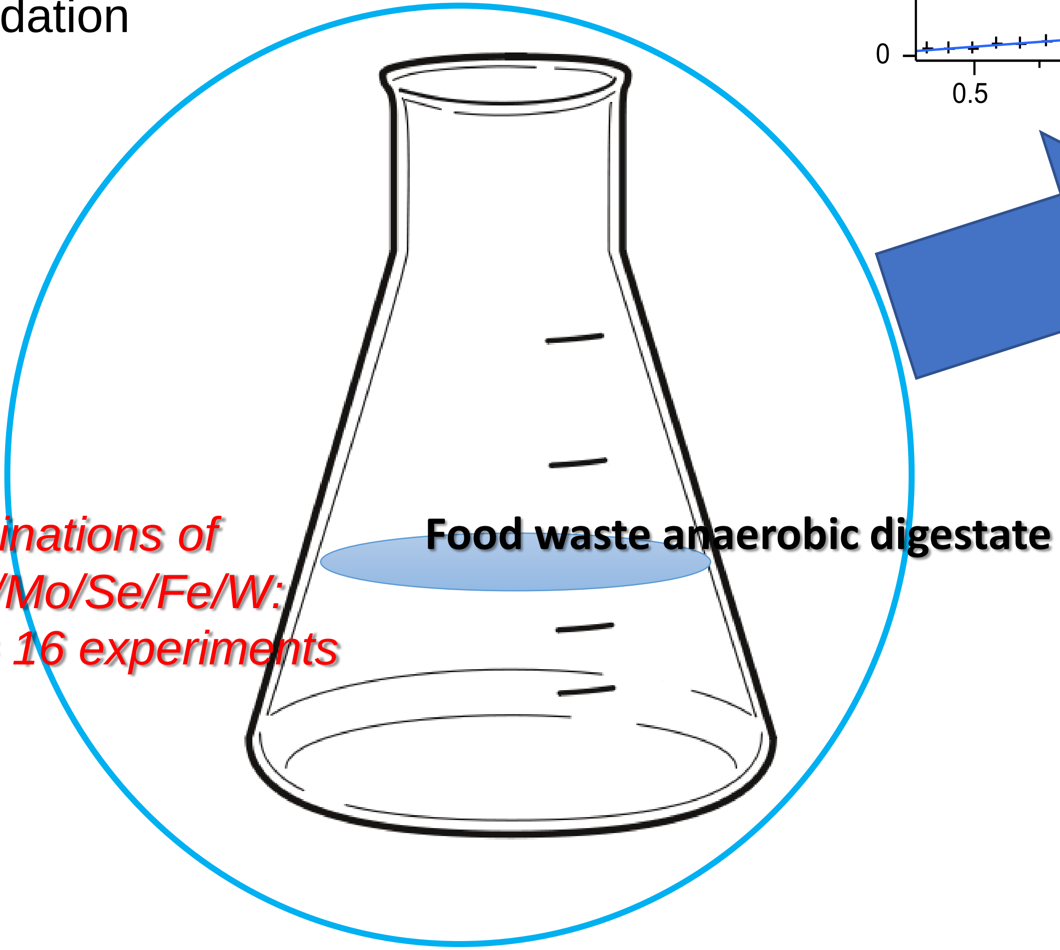
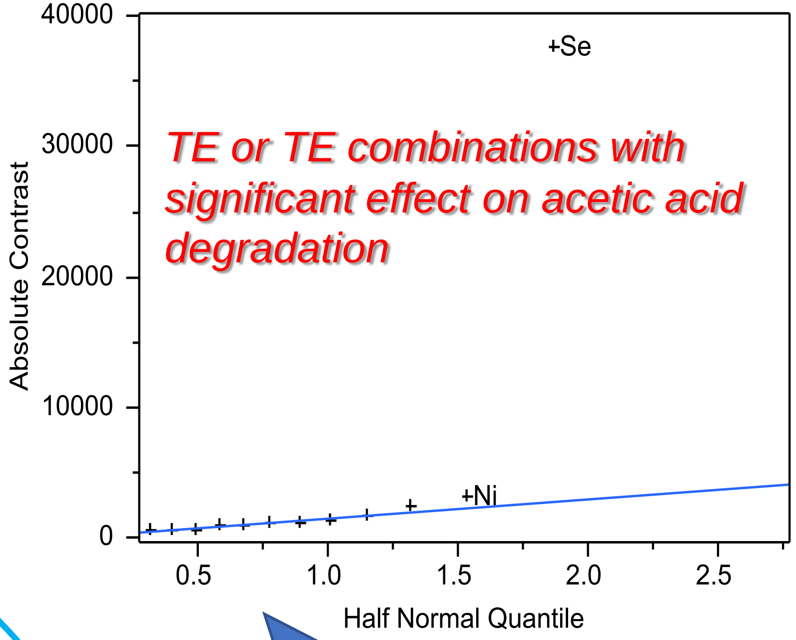
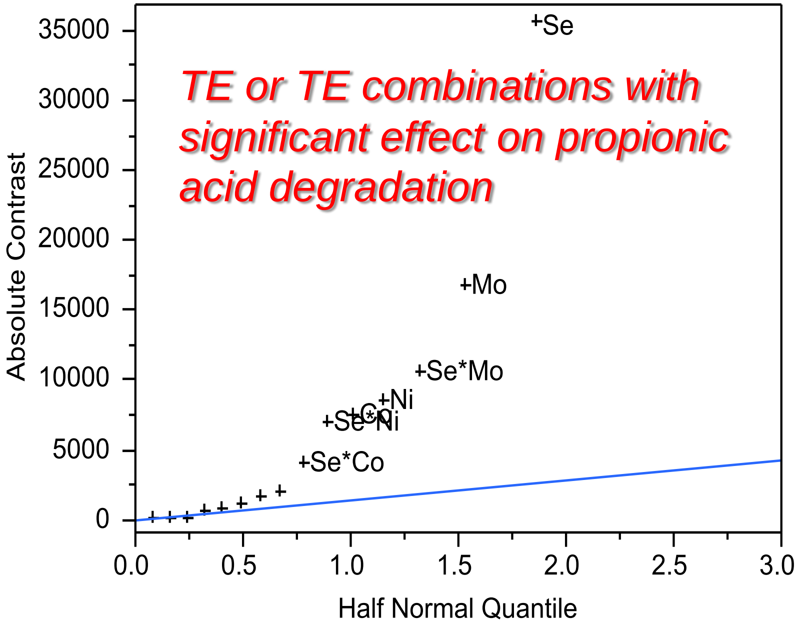
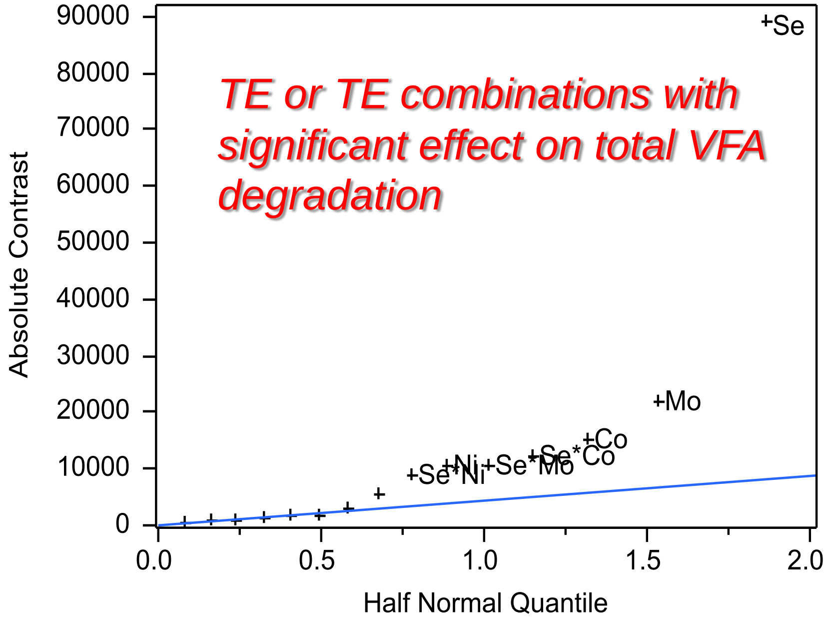
DoE: 2-level Fractional factorial design

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









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

Ying Jiang[1]*, Yue Zhang[2], Charles Banks[2], Sonia Heaven[2], Philip Longhurst[1]

1. Centre for Bioenergy & Resource Management, School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK

2. Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, UK

*Corresponding author: Tel.: +44 (0)1234 750111 ext. 2822;

Email: Y.Jiang@Cranfield.ac.uk

ABSTRACT

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

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

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

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

1. Introduction

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

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

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

Selenium, molybdenum and tungsten are also reported to be required in methanogenesis (Fermoso et al., 2009). Based on an extensive literature survey of biochemical reactions involved in methanogenesis pathways, there is no suggestion of a requirement for selenium in the acetoclastic methanogenesis pathway. (Deppenmeier, 2002; Ferry, 1999, 1992a,; Hille et al., 2014; Kryukov and Gladyshev, 2004; Müller et al., 2010; Muller, 2003; Murakami and Ragsdale, 2000; Rother and Krzycki, 2010; Stock and Rother, 2009; Thauer et al., 2008). Whilst selenium is understood to be involved extensively in the hydrogenotrophic methanogenic pathway as it forms various selenoproteins, including hydrogenase, formate dehydrogenase (Jones et al., 1979) and formyl-methanofuran dehydrogenase (Vorholt et al., 1997). It is reported in a number studies that hydrogenotrophic methanogens become the dominant microbial population in digesters treating high ammonia feedstocks, such as food waste (Angelidaki and Ahring, 1993; Banks et al., 2012;; Schnürer and Nordberg, 2008). This can result an increased dependence on hydrogenotrophic methanogenic route in these digesters, hence the increased requirements of Se to form key enzymes. Banks et al. (2012) observed a significant improvement of volatile fatty acid degradation when Se was added to digesters treating food waste with an elevated ammoniacal nitrogen concentration.

Molybdenum and tungsten are closely involved in formate dehydrogenase (in hydrogenotrophic methanogens and syntrophic oxidising bacteria) and formyl-methanofuran dehydrogenase (in hydrogenotrophic methanogens) (Hille et al., 2014; Johnson et al., 1996; Romão, 2009). Although these two elements are considered to be chemically analogous in enzyme formation (Kletzin and Adams, 1996), their requirements for each methanogen species cannot be replaced by each other. The stimulatory effect of W on the growth of methanogens *Methanococcus vannielii* was first reported in the late 1970s (Jones and Stadtman, 1977). When formate was used as the carbon source, the growth of *M. vannielii* was significantly enhanced by the addition of W, but not by Mo. However, the growth stimulation by W was not observed when the organism was fed with H_2 and CO_2, which suggested the involvement of a W-containing formate dehydrogenase (FDH). In a lab scale digestion trial, an improvement of performance was reported by Jiang et al. (2012) following the addition of W to anaerobic digesters treating vegetable waste.

Combinations of key TEs were reported to have synergistic or antagonistic effects to the methanogenesis. Patidar and Tare (2006) report that addition of Fe/Co, Fe/Ni/Zn and Ni/Zn/Co combinations lead to maximum total methanogenic activity in a batch study; however addition of Ni affects Co and Zn uptake in methanogens, due to antagonistic effect of metal ions. Feng et al. (2010) investigated the effects of additions of Co, Ni/Mo/B and Se/W on the biogas production and the associated anaerobic microbial community. The study revealed that the highest methane production occurs at high Se/W concentrations in combination with a low level of Co. However, the influence of the trace metal additions on the microbial community composition was not significant.

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

Significant work has been carried out to investigate the effects of TE and their combinations on VFA degradation (Feng et al., 2010; Worm et al., 2009). Feng et al. (2010) demonstrated advanced statistical methods can be a useful tool to interpret the impact of TE on anaerobic digestion process efficiency. However, to the authors' best knowledge, to date no study has applied factorial design method to investigate the effects of multiple elements simultaneously. Factorial design is widely applied in scientific investigations to identify the key factors and/or combinations of factors influencing the process. In this study, the effects of six TEs (Co, Ni, Se, Mo, Fe and W) on the volatile fatty acids degradation rate were studied. In order to differentiate the effects of each element and/or element combination, a design of experiment (DoE) approach of 2-level fractional factorial design was adopted. Regression model has also been developed to correlate the response with each factor.

2. MATERIALS AND METHODS

2.1 Food waste digestate inoculum

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

2.2 Analytical methods

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

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

2.3 Trace elements supplementation and VFAs degradation flask trial

The batch experiment was set up in conical flasks each with 250 ml capacity. Each flask was inoculated with 200 ml digestate. Working solutions of six trace elements (Co, Ni, Mo, Se, Fe and W) were prepared using $CoCl_2$, $NiCl_2$, $(NH_4)_6Mo_7O_{24}$, Na_2SeO_3, $FeCl_2$ and Na_2WO_4 (Analytical grade, Fisher Chemical, UK). Addition of these trace element solutions into each flask followed the pattern as shown in Table 2, with strings of plus (+) and minus (-) signs denoting with and without addition of a particular element. There are in total 16 TE addition combinations including a control where no TE was added. When TE solutions are added, the concentrations of 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, Ni, Mo, Se, Fe and W, respectively. To ensure data quality, this experiment was conducted in duplicate, i.e. 32 total flasks.

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

Digestate samples in flasks were routinely sampled and analysed for VFA concentrations in order to plot VFA degradation curves over the duration of the experiment. 1 ml of the digestate was withdrawn from each flask using hand pipette with cut-off pipette tip. After sampling, the headspace of each flask was flushed with CO_2/N_2 (20:80) and then sealed again before returning to the incubator. The flask experiment has continued for ~90 days until all VFAs in the flasks were depleted.

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

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

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

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

2.4 Statistical method

2.4.1Fractional factorial design and data analysis

The experiment was designed to expose the key TEs which are the most effective on improving anaerobic degradation of VFAs, whilst understanding possible synergistic effects from combinations of elements. Six trace elements were selected as factors to be investigated in this 2-level factorial experiment design. Due to practical difficulties, it is not feasible to run a 2-level full factorial experiment, as this requires 64 ($=2^6$) flasks to be prepared for a single experiment. As a result, a 2^{6-2} fractional factorial design, which is ¼ of the full factorial design with a resolution of IV was adopted. The chosen design reveals all the main effects. However it cannot distinguish fully the 2-factor interaction due to effect aliasing (Wu and Hamada, 2009). The experimental arrangement of this 2^{6-2} subset (fraction) of a full factorial design was generated using the SAS JMP statistical suite (SAS Institute Inc. Cary, North Carolina, USA).

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

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

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

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

2.4.2 Regression Model

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

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

Where: β_0, β_i *and* β_{ij} are the regression coefficients which are estimated using Least squares method. z_i is the experiment response (*DI*). x_i and x_j are the variables (i.e. the factors in the experiment, coded with +/-).

3. RESULTS AND DISCUSSION

3.4 Trace elements flask trial

A reasonable repeatability of the experiment was achieved as indicated by the error bar shown the range of the result from the duplicated experiment (Figure 1). Clear divergence in degradation rate was observed amongst the sample groups where different TE supplementation regimes were adopted. Under each TE addition combinations, the VFAs degradation indexes were calculated based on VFA concentration results using Equation 1. The *DI* results are summarised in Table 3.

Notably, in samples where selenium was present, the acetate and propionate degradations were significantly stimulated in comparison with selenium deprived groups.

On day 28, acetic acid concentration in all samples was observed to be degraded below 100 mg/l. However, this is followed by an increase of acetic acid concentrations in some samples as indicated by the bumps on the curves in Figure 1a & 1b around day 30. This increase of acetic acid is caused by acetic acid produced from propionate degradation as the timing coincided with an increased propionate degradation rate in those samples during this period as shown in Figure 1c & 1d. The fractional factorial design significantly reduced the experimental efforts normally required to understand complex TE requirement in AD process. Due to the simplicity of the experimental and analytical requirement, this method can therefore be adopted by AD operators to assess the deficiency of TE in digesters and provide critical information on specific TE requirement. This enables a more targeted element supplementation regime to avoid a full TE addition, therefore significantly reduce operational costs.

3.5 Analysis of factorial effects

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

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

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

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

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

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

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

The factorial analysis reveals a strong influence of Se on the VFA degradation, suggesting a specific requirement for Se to improve VFA, particularly acetate and propionate degradation in this type of digestate. Previous study (Banks et al., 2012) confirmed the foodwaste digestate used as inoculum in this study adopt hydrogenotrophic methanogenesis as the principle methanogenic route due to the elevated ammoniacal nitrogen level. This explains the significant effect of Se found in this study, as it is understood based on wide range of literature evidence (Ferry, 1999, 1990; Jones et al., 1979; Vorholt et al., 1997) that Se involves significantly in the syntrophic methanogenic pathway, but not in the acetoclastic pathway. In addition, as reported by Worm et al. (2011, 2009), Se and Mo are involved in formate dehydrogenases and hydrogenases required for propionate degradation, hence the improved propionate degradation overserved in the presence of these two elements in the factorial analysis.

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

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

$$DI_{HAc} = 125728.25-33687.5X_{Se}-1642.9X_{Mo}- 9.4X_{Co} +5027.2X_{Ni} -2940.6X_{W} +3383X_{Fe}+1008.8X_{Se}X_{Mo} -1685.4X_{W}X_{Mo} -3156.7X_{Mo}X_{Co} +2849.6X_{Se}X_{Ni} +3379.1X_{Mo}X_{Ni} -1991.9X_{Co}X_{Ni} -3692.1 X_{Ni}X_{W} \quad \text{(Equation 4)}$$

$$DI_{HPr} = 203360.9- 40051.1X_{Se} -18208.0X_{Mo} - 11276.6X_{Co} +9196.4X_{Ni} - 1808.9X_{W} - 1580.0X_{Fe} - 9316.2X_{Se}X_{Mo} +7821.2 X_{W}X_{Mo} - 863.7X_{Mo}X_{Co} + 6341.9X_{Se}X_{Ni} - 1000.2X_{Mo}X_{Ni} + 550.1X_{Co}X_{Ni} - 1504.0X_{Ni}X_{W} \quad \text{(Equation 5)}$$

$$DI_{Total} = 392720.7 - 89005.3X_{Se} - 21657.1X_{Mo} - 15040.2X_{Co} + 10573.0X_{Ni} - 5443.6X_{W} - 2883.7X_{Fe} - 10644.4X_{Se}X_{Mo} + 12260.4X_{W}X_{Mo} - 893.9X_{Mo}X_{Co} + 8807.8X_{Se}X_{Ni} + 1258.5X_{Mo}X_{Ni} + 927.7X_{Co}X_{Ni} - 1540.0X_{Ni}X_{W} \quad \text{(Equation 6)}$$

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

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

$$DI_{HPr} = 203360.9 - 40051.1X_{Se} - 18208.0X_{Mo} - 11276.6X_{Co} + 9196.4X_{Ni} - 9316.2X_{Se}X_{Mo} + 7821.2X_{W}X_{Mo} \quad \text{(Equation 8)}$$

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

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

4. Conclusion

This study applied a fractional factorial design in batch experiments to explore the impact of six trace elements (Co, Ni, Mo, Se, Fe and W) on VFA degradation rates in foodwaste digestate. The factorial analysis of the results shows a significant influence from Se in improving the VFA degradation. This is in agreement with previous knowledge of the involvement of Se in hydrogenotrophic methanogenesis which is typically the main methane forming route in foodwaste digesters.

The factorial results also reveal Ni shows slight inhibitory effect to VFA degradation and W/Mo combination inhibits propionic acid degradation, likely due the antagonistic effect of the two elements.

The fractional factorial method has introduced an efficient experimental approach to identifying the deficient elements in anaerobic digesters, whilst offering guidance on a tailored TE supplementation recipe for digesters operating under different conditions.

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Table 1. Baseline TE concentrations in the inoculum

Element	Baseline concentration (mg/l)
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	*DI(Ave)*		
		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

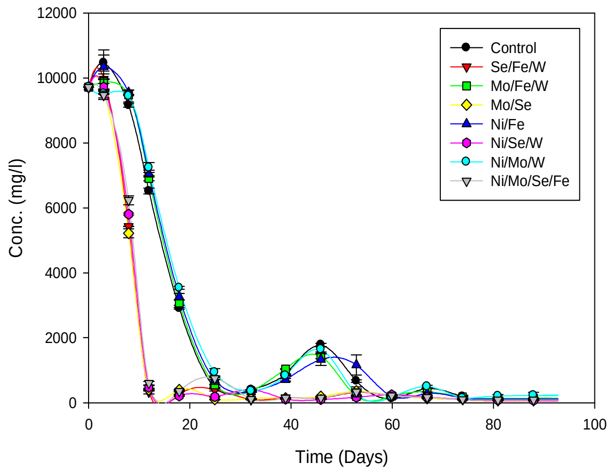
		Acetic acid				Propionic acid				Total VFA			
Term	**Aliases**	**Factorial effect**	**Lenth t-Ratio**	**Individual p-Value**	**Adjusted p-Value**	**Factorial effect**	**Lenth t-Ratio**	**Individual p-Value**	**Adjusted p-Value**	**Factorial effect**	**Lenth t-Ratio**	**Individual p-Value**	**Adjusted 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)

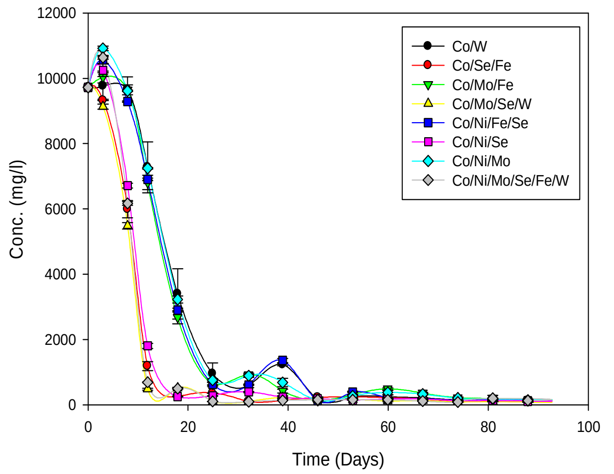
Table 5. Coefficients of the empirical regression models for acetic, propionic and total VFA degradation index (*DI*)

	Acetic Acid			Propionic Acid			Total VFA		
Term	**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

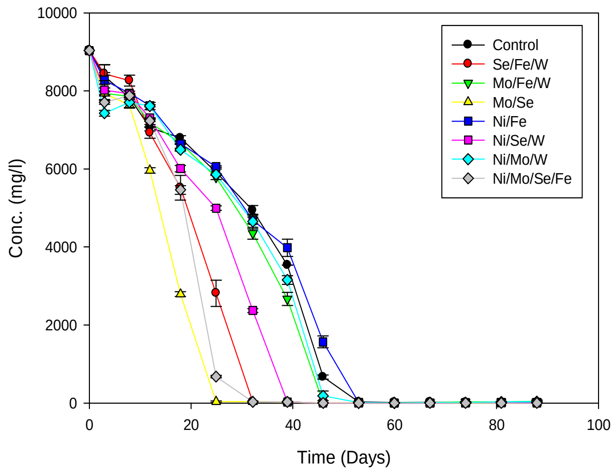
Note: Prob>|t|: Probability value of getting an even greater t-statistic (in absolute value), less than 0.05 are considered as significant evidence that the parameter is not zero



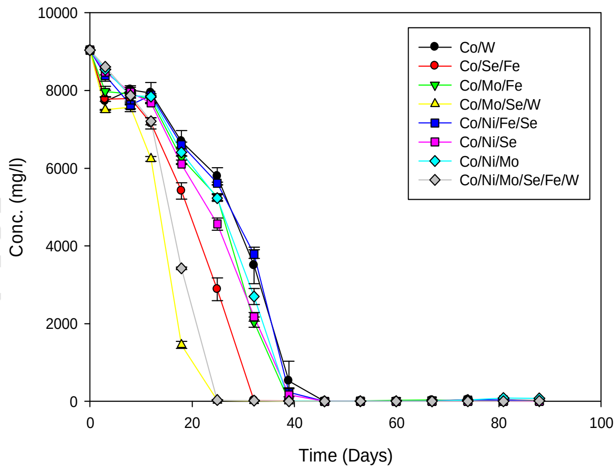
a) Sample 1-16 (Acetic acid)



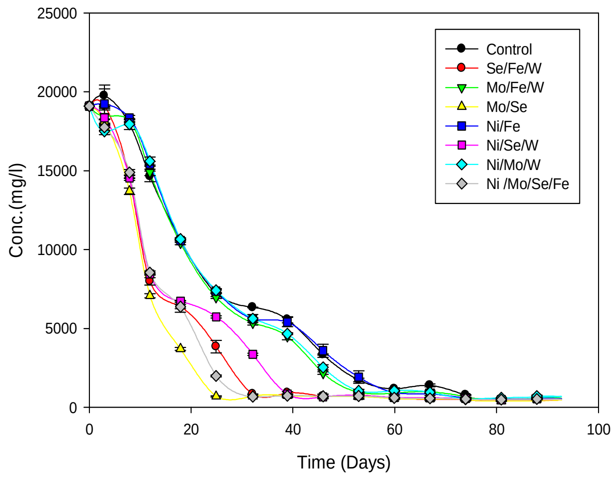
b) Sample 17-32 (Acetic acid)



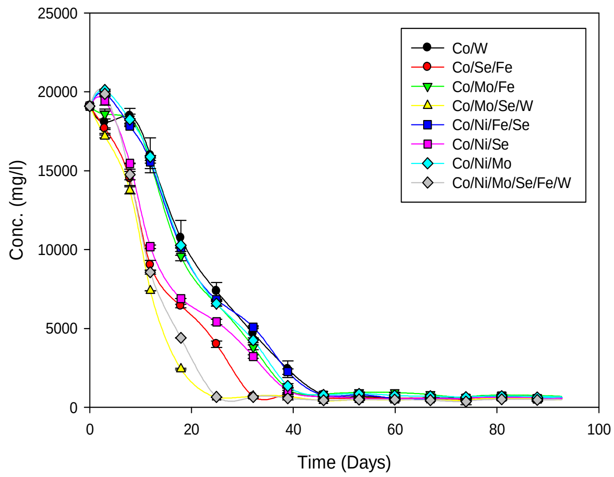
c) Sample 1-16 (Propionic acid)



d) Sample 17-32 (Propionic acid)



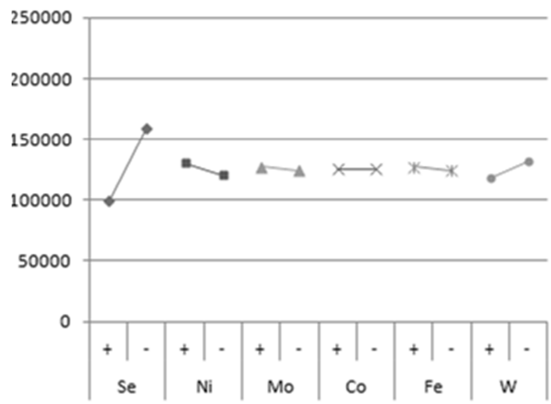
e) Sample 1-16 (Total VFA)



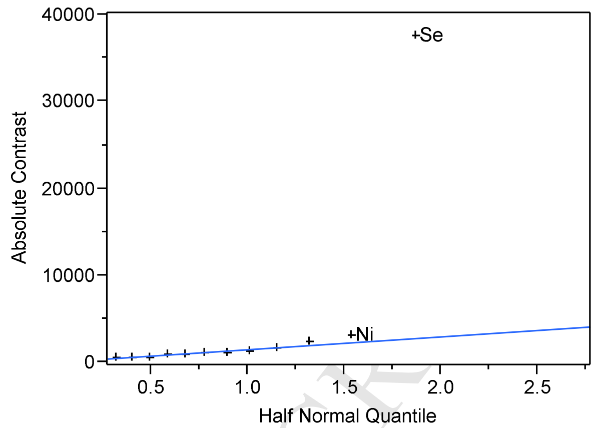
f) Sample 17-32 (Total VFA)

Figure 1. VFA degradation curves

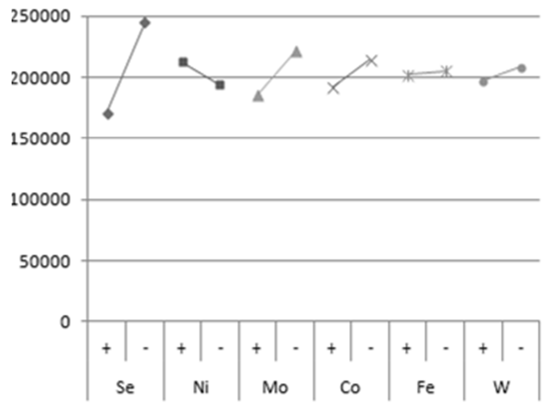
Note: Error bar indicate the range of results from duplicated experiment



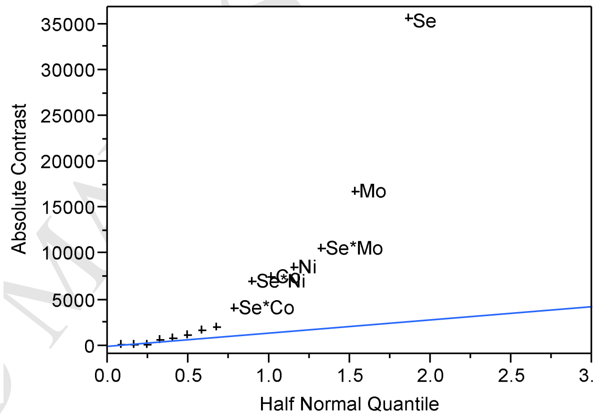
a) Main effects plot of acetic degradation



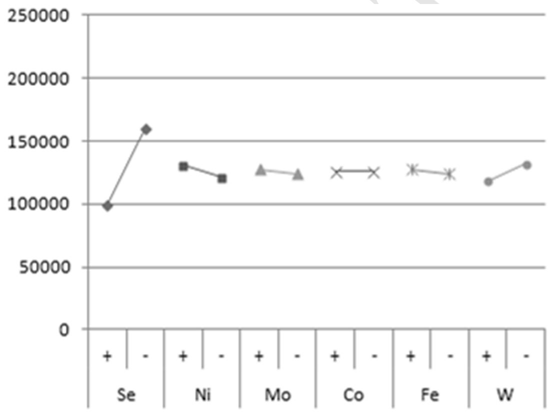
b) Half-normal plot of effects to acetic degradation



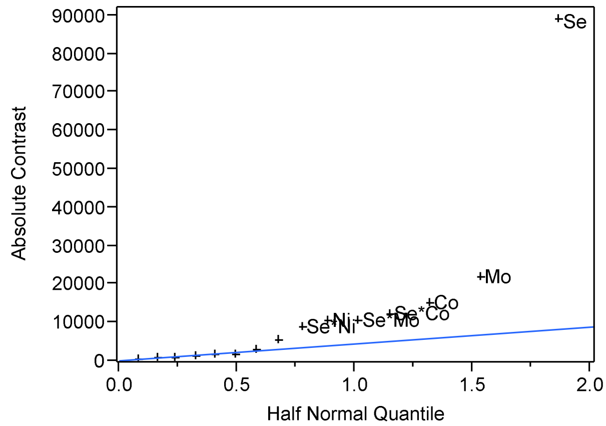
c) Main effects plot of propionic degradation



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 probablilty 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