Nig. J. Biotech. Vol. 35 (2018) 53-61
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v35i1.7

Dynamics and the interaction effects of trace elements and volatile fatty acids concentrations on methanization processes during thermophilic anaerobic digestion

Ezebuiro N. C.
Hamburg University of Technology, Eissendorferstrasse 42, 21073
Hamburg-Harburg, Germany

ABSTRACT

Volatile fatty acids (VFA) and trace elements (TEs) influence methane production and VFA metabolism in positive and negative ways during methanization. There is insufficient knowledge on why the interactions could go either way and this has generated unpleasant consequences for biogas operators. To fill the knowledge gap, statistically designed thermophilic batch experiments were conducted with VFA mixture as substrate to investigate the dynamics and interaction effects of TEs including Nickel (Ni), Cobalt (Co), Selenium (Se) and Molybdenum (Mo) on the methanization processes of CH_4 production and VFA degradation rate. Response surface model and desirability functions were used to determine TEs and VFA interaction effects and the dynamics of the optimum TEs configurations for CH_4 production and VFA degradation rate at different VFA concentrations. The results showed that TEs supplementation influenced Y_{CH4} production by -14% to 11%: whereas the positive interaction effects of VFA and TEs were by Co*Mo, VFA*Se and VFA*Mo; the interaction effects of Se*Mo and VFA*Co were negative. -15% to 45% increase in $Y_{VFA\text{-}DR}$ was obtained with TEs supplementation: Ni*Co interaction produced the positive influences; and Co*Se produced negative effects. The optimum TEs configuration for CH_4 production and VFA degradation rate varied with changes in VFA concentrations.

KEYWORDS: Thermophilic methanization, trace elements, VFA metabolism, methane production, trace elements synergism, trace elements interaction
n.c.ezebuiro@nabda.gov.ng

Introduction

Methanization involves anaerobic digestion (AD) of biomass to produce energy-rich methane (Ch_4)(Appels et al., 2008). Common substrates used for methanization include grease trap residue and wastewater; bio-waste from restaurants and households; and agricultural residues. Irrespective of the substrates used, volatile fatty acids (VFA) including formate, acetate, butyrate and propionate are produced as intermediate products in the acidification stage of AD. During acetogenesis and methanogenesis, VFA are metabolized to produce Ch_4 (Appels et al., 2008; Mata-Alvarez, 2003). Weak VFA metabolism is a challenge in methanization and results in VFA accumulation and low CH_4 production (Tiantao et al., 2010) .

VFA metabolism and CH_4 production could be improved by supplementing trace elements (TEs) to a methanization digester or performing methanization at thermophilic temperature(Appels et al., 2008; Mata-Alvarez, 2003). TEs are micro-nutrients that influence the expression and dominance of VFA degradation pathways, rate of VFA metabolism and methane production (Halarnkar and Blomquist, 1989; Osuna et al., 2003; Pobeheim et al., 2011). TEs are widely reported in literature as being associated with the enzymology of the acetyl CoA pathway, a dominant pathway during VFA metabolism and CH_4 production. Commonly reported TEs used in co-supplementation during methanization include Nickel (Ni), Cobalt (Co), Selenium (Se), Tungsten (W), Molybdenum (Mo), and Iron (Fe) .

(Gustavsson et al., 2011; Hinken et al., 2008; Pobeheim et al., 2010; Zitomer et al., 2008).

The challenge with the use of TEs for enhancing methanization is that the metallo-enzymes (MEs) whose biocatalytic potentials are enhanced by TEs supplementation are temperature and TEs specific. For example, anaerobic MEs such as Carbon monoxide dehydrogenase/Acetyl CoA synthase (CODH/ACS) complex and methyl transferase (MeTr) contain TEs in their active sites and are dysfunctional without TEs or when in operation outside the optimum temperature range (Dobbek et al., 2001; Menon and Ragsdale, 1999; Svetlitchnyi et al., 2001) **.** This study is embarked upon because limited knowledge about the interaction effects of TEs, VFA and thermophilic temperature remains an operational impediment to the use of TEs for optimization of thermophilic methanization in the biogas industry. While detailed studies have granted insight on these effects during mesophilic operation (Ezebuiro and Koerner, 2017), insight is lacking for thermophilic methanization. Hence, the objectives of this study are to determine the following under thermophilic conditions:

a) Relationship between TEs and VFA concentrations including the individual and interaction effects of Ni, Co, Se, Mo and VFA concentrations that influence VFA degradation rate (Y_{VFA-DR}) and CH_4 production (Y_{CH4}); and
b) Dynamics of the Optimum TEs configuration for co-optimization of Y_{VFA-DR} and Y_{CH4} production.

Materials and Methods

The materials and methods adopted for the realization of the objectives afore mentioned are the same as those published for the mesophilic investigation(Ezebuiro and Koerner, 2017). Highlights of the published materials and methods, and statistical procedures that are pertinent to this paper are provided subsequently. The inoculum adaptation and duration of the experiment have not been published earlier and are presented.

Experimental design and test system

The experimental design included five factors consisting of Ni, Co, Se, Mo and VFA mixture, which were expressed in three levels namely low, medium and high concentrations (Ezebuiro, 2014; Ezebuiro and Koerner, 2017). 30 implementable experimental runs were selected from the 243 possible experimental runs following earlier reported statistical procedures and assumptions (SAS Institute Inc., 2012a). The 30 implementable experimental runs were grouped as follows: 3 control units (R7, R29 and R30) and 27 treatment units (R1 – R27). The control units contained the inoculum, basic nutrient medium and VFA mixture comprising sodium salts of acetic-, propionic- and butyric acids**.** The treatment units contained varying TEs configurations including Ni, Co, Se and Mo in addition to the composition of the control units. The experimental test systems (ETS) have been described in (Ezebuiro and Koerner, 2017) and were designed based on a standardized procedure(Verein Deutscher Ingenieure, 2006). The reaction vessels comprised 1L glass reactors. To implement the experimental runs, the 1L glass reactors were loaded with Ni, Co, Se, Mo and VFA concentrations as shown in Table 1. The experimental runs were run in duplicate and the details of the start-up have been reported (Ezebuiro, 2014; Ezebuiro and Koerner, 2017).

Sample collection and analyses

Liquid (suspension) and gas samples were collected once in 3 or 4 days during the period of the experiment. The standard methods used for analyses include total VFA concentration (DIN 38414 – 19); pH (DIN 38404 – 5); and biogas volume . CH_4 concentration was measured using a GeoTech 5000 gas analyser, Geotechnical Instrument, UK, Ltd.

The experimental responses

The VFA degradation rate (Y_{VFA-DR}) was measured as the change in the concentration of the total VFA on every third day. The average value of the 3 highest VFA degradation rates for each reactor was taken to represent its VFA degradation rate. CH_4 production (Nml) (Y_{CH4}) was measured as

the CH_4 produced every day under standard conditions . For direct comparison of the results from the treatments with those from the controls, the relative values ($Y_{relative,\ r}$) of the responses (Y_i) were determined as the ratio of the treatment value for a response to the control value for the same response. Relative value greater than 1 was considered beneficial; relative value equal to 1 was equivalent to the control; and relative value less than 1 was inhibitory or non-beneficial.

Determination of the optimum TEs configuration for thermophilic $Y_{VFA\text{-}DR}$ and Y_{CH4} production

Response surface methodology (RSM) was used to model the relationships between the five experimental factors including the concentrations of Ni, Co, Se, Mo and VFA; and the two experimental responses (Y_i) including $Y_{VFA\text{-}DR}$ and Y_{CH4} production. Details of the RSM procedure can be found in the statistical software (JMP 10) used for the experimental design and analyses (SAS Institute Inc., 2012b). Eq. 1 is the JMP 10-generated RSM for the factors Ni, Co, Se, Mo and VFA.

$$Y_i = \beta0 + \beta1VFA + \beta2Ni + \beta3Co + \beta4Se + \beta5Mo + \beta6VFA\text{^}2 + \beta7VFA * Ni + \beta8Ni\text{^}2 + \beta9VFA * Co + \beta10Ni * Co + \beta11Co\text{^}2 + \beta12VFA * Se + \beta13Ni * Se + \beta14Co * Se + \beta15Se\text{^}2 + \beta16VFA * Mo + \beta17Ni * Mo + \beta18Co * Mo + \beta19Se * Mo + \beta20Mo\text{^}2 + \varepsilon \quad \text{Eq. 1)}$$

Where, Yi is the predicted response ($Y_{VFA\text{-}DR}$ or Y_{CH4} production); β0 is the intercept calculated with the algorithm in JMP 10; and βi … βk are the estimated coefficients of regression (CR) for the main effects and interactions of the factors Ni, Co, Se, Mo and VFA as calculated with the algorithm in JMP 10. X1i … Xki are the factors Ni, Co, Se, Mo and VFA and all the interactions between any two of the factors; and εi is the prediction error for the responses, as calculated with the algorithm in JMP 10.

Desirability function was used to determine the proportions of increase or decrease in $Y_{VFA\text{-}DR}$ and Y_{CH4} production that were achievable as a result of the interactions of Ni, Co, Se, Mo and VFA concentrations. It was also used to derive the optimum factor setting for a response or set of responses (Douglas, 2001; John, 2013; SAS Institute Inc., 2012c).The desirability function (D) for maximizing single response ($Y_{VFA\text{-}DR}$ or Y_{CH4}) is shown in Eq. 2a; and the desirability function ($D_{1\text{-}k}$) for co-maximizing $Y_{VFA\text{-}DR}$ and Y_{CH4} is shown in Eq. 2b. Details of the desirability functions can be found in the JMP 10 documentation(SAS Institute Inc., 2012b).

$$D = ((Yi - A) / (B - A)) * w \quad \text{(Eq. 2a)}$$
$$A \leq Yi \leq B;\ D = 1, Yi > B;\ D = 0, Yi < A$$

$$D_{1-K} = [D_{e1} * D_{f2} \ldots * D_{zk}]^{1/k} \quad \text{(Eq. 2b)}$$

Where Yi is the response (relative $Y_{VFA\text{-}DR}$ or Y_{CH4} production) predicted with the RSM shown in Eq. 1; A, B are the lowest and highest values respectively of the relative $Y_{VFA\text{-}DR}$ or Y_{CH4} production; and w is the value that shows the importance of Y_{CH4} production in relation to $Y_{VFA\text{-}DR}$. D_{e1}, D_{f2}, D_{zk} are the individual desirability values of the responses ($Y_{VFA\text{-}DR}$ and Y_{CH4} production); and *k* is the number of responses that are co-optimized.

Inoculum adaptation and experimental duration

The inoculum was the source of microbes for the experiments. A thermophilic inoculum was prepared by adapting a mesophilic inoculum as earlier described (Ahn and Forster, 2002). The test system for the adaptation of the inoculum from mesophilic to thermophilic condition was adopted from a standardized procedure (Verein Deutscher Ingenieure, 2006). The duration of the investigation was 39 days in the high and medium VFA levels and 20 days in the low VFA level.

Results

Interaction effects of TEs and VFA on thermophilic Y_{CH4} production

The output of the experimental design, rel. Y_{CH4} production and rel. $Y_{VFA\text{-}DR}$ for methanization at 55°C are shown subsequently in Table 1. The statistics for the RSM prediction of the relative Y_{CH4} production has a coefficient of determination (R^2) of 0.94, standardized R^2 of 0.81 and a fit error of 0.03. The model terms and the associated significance (p>0.05) in order of decreasing magnitude include Ni*Ni (0.0002), Co*Mo (0.0015), Ni (0.0031) VFA*Se (0.0064), VFA*Mo (0.0185), Se (0.0224), Co (0.0226), VFA*Co (0.0299) and Se*Mo (0.0397). The significant main factors for Y_{CH4} production include Ni, Co and Se concentrations. Similarly,

extreme concentrations of Ni, Co and Mo (Ni*Ni, Co*Co and Mo*Mo respectively); interactions of VFA with Se, Mo and Co (VFA*Se, VFA*Mo and VFA*Co); interaction of Co with Mo (Co*Mo); and interaction of Se with Mo (Se*Mo).

Positive influences were induced by Ni, Co*Mo, VFA*Se, and VFA*Mo. Negative influences originated from Co and Se, and from Ni*Ni, VFA*Co, and Se*Mo. Eq. 3 shows the final model for the significant factors that influence thermophilic Y_{CH4} production.

Table 1: Output of the design of experiment involving the factors Ni, Co, Se, Mo and VFA, and the relative values of the experimental responses at 55°C due to the influence of the factors

[a]Treatment	[b]VFA	[c]Ni	[d]Co	[e]Se	[f]Mo	[g]RMP	[h]RVDR
R1	55±3	0.09	0.03	0.00	1.24	0.95±0.03	1.23±0.03
R2		0.09	0.03	0.98	0.04	0.94±0.06	1.08±0.06
R3		0.09	1.88	0.00	0.64	0.94±0.06	1.02±0.07
R4		0.09	3.73	0.49	1.24	0.89±0.05	0.98±0.05
R5		1.05	3.73	0.00	0.04	1.03±0.07	1.27±0.09
R6		1.05	3.73	0.98	0.64	1.00±0.04	1.19±0.05
[i]R7		0.09	0.03	0.00	0.04	1.00	1.00
R8		2.01	0.03	0.98	1.24	0.81±0.02	1.19±0.03
R9		2.01	1.88	0.98	0.04	0.88±0.05	0.98±0.06
R10		2.01	3.73	0.00	1.24	0.98±0.07	1.28±0.09
R11	107±5	0.09	1.88	0.98	1.24	0.83±0.03	0.93±0.04
R12		0.09	3.73	0.49	0.04	0.90±0.05	0.94±0.05
R13		1.05	0.03	0.49	0.64	1.00±0.07	1.13±0.08
R14		1.05	1.88	0.00	0.64	1.02±0.07	1.06±0.07
R15		1.05	1.88	0.49	1.24	1.02±0.05	1.01±0.05
R16		1.05	1.88	0.98	0.64	0.98±0.03	1.07±0.03
R17		2.01	3.73	0.49	0.64	0.96±0.04	1.09±0.04
R18	209±11	0.09	0.03	0.00	0.64	0.96±0.05	1.08±0.05
R19		0.09	0.03	0.98	1.24	0.86±0.06	1.07±0.07
R20		0.09	1.88	0.49	0.64	0.92±0.05	1.04±0.06
R21		0.09	3.73	0.00	1.24	0.93±0.05	0.99±0.05
R22		0.09	3.73	0.98	0.04	0.84±0.04	0.85±0.04
R23		1.05	1.88	0.49	0.04	0.97±0.03	1.03±0.03
R24		1.05	1.88	0.49	0.64	0.99±0.06	0.99±0.06
R25		2.01	0.03	0.00	1.24	0.95±0.06	0.98±0.06
R26		2.01	0.03	0.98	0.04	0.99±0.05	1.06±0.05
R27		2.01	3.73	0.00	0.04	0.76±0.02	1.03±0.03
R28		2.01	3.73	0.98	1.24	0.97±0.06	0.98±0.06
[j]R29	107±5	0.09	0.03	0.00	0.04	1.00	1.00
[k]R30	209±11	0.09	0.03	0.00	0.04	1.00	1.00

[a]Treatment/Reactor Nr.; [b]Volatile fatty acids (mmol/L); [c]Nickel (mg/L); [d]Cobalt (mg/L); [e]Selenium (mg/L); [f]Molybdenum (mg/L); [g]Relative methane production; [h]Relative volatile fatty acid degradation rate; [i]Control reactor for low VFA level; [j]Control reactor for medium VFA level; [k]Control reactor for high VFA level.

Optimum TEs configuration for thermophilic CH_4 production

Fig. 1a and 1b show the optimum TEs configuration that maximized thermophilic Y_{CH4} production at different VFA levels. Fig. 1a shows the prediction profiles for the relative Y_{CH4} production and the influences of the TEs in the low VFA levels. The profiles also apply to the medium VFA level (125 mmol/L, not shown). The range of beneficial TE is 0.2 – 1.5 mg/L Ni. The optimum TE configuration is 0.9 mg/L Ni and resulted in a relative Y_{CH4} production of 1.11 (low VFA level) and 1.09 (medium VFA level). The optimum TEs configuration produced process efficiency of 99% (desirability of 0.99) in both low and medium VFA levels. The VFA profile suggests that 0.2 – 1.5 mg/L Ni could maintain the desirability of 0.99 up to VFA concentration of 175 mmol/L. The Ni profile suggests that changes outside the beneficial range of Ni would result in decline in Y_{CH4} production. The profiles for Co, Se and Mo suggest that concentrations up to 0.3 mg/L Co, 0.5 mg/L Se and 0.6 mg/L Mo are tolerable for 20 – 175 mmol/L VFA concentration.

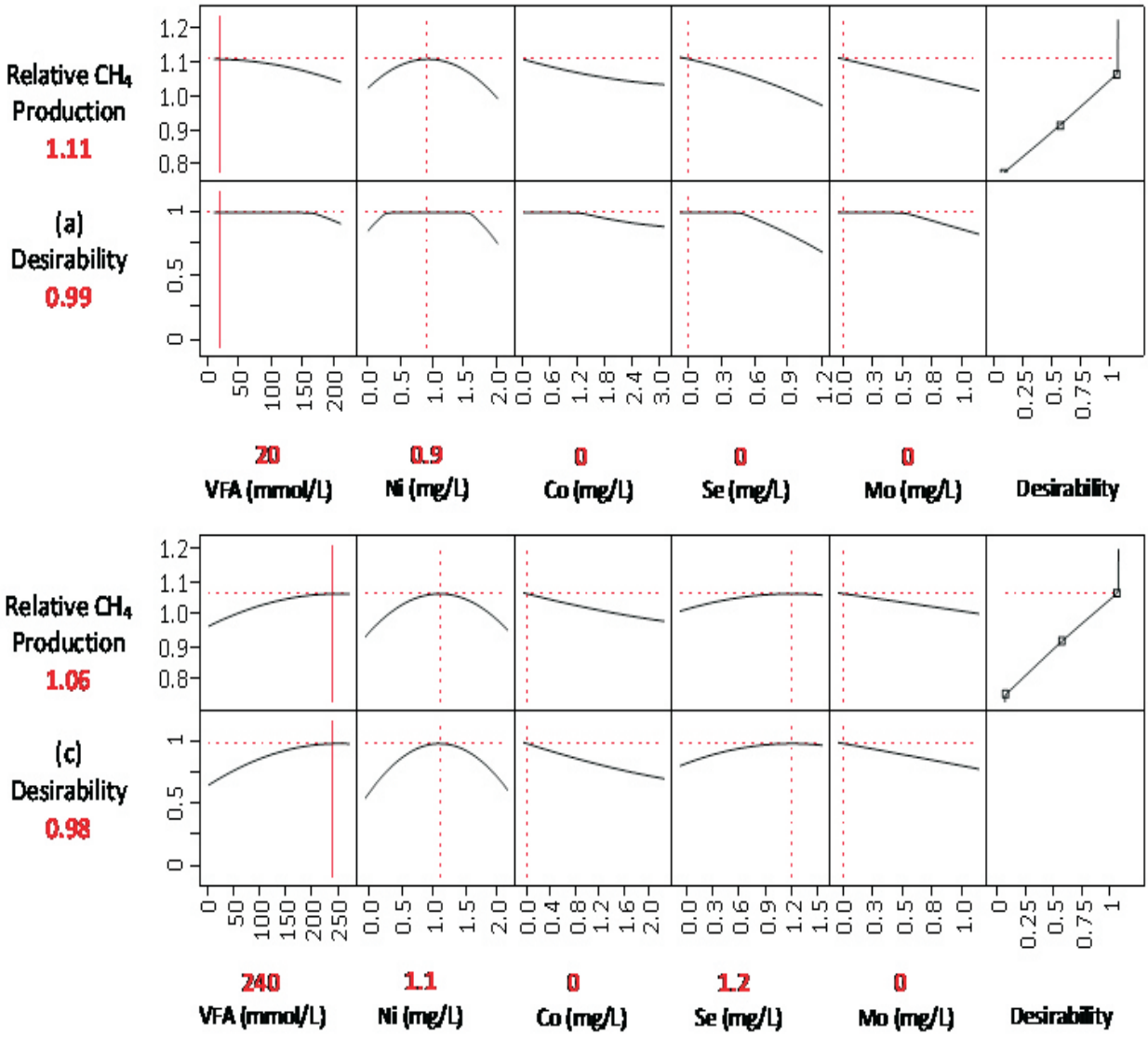

Figure 1: Profile and optimum TEs configuration for CH_4 production at 55°C due to Ni, Co, Se and Mo supplementation to different VFA concentrations (mmol/L) **(a)** 20 **(b)** 240

Fig. 1b (high VFA level) shows the relative Y_{CH4} production and the influence of the TEs in the high VFA level. The ranges of beneficial TEs are 0.5 – 1.5 mg/L Ni and 0.6 – 1.5 mg/L Se. The desirability profiles suggest that the TEs configuration of 1.1 mg/L Ni and 1.2 mg/L Se is optimum for VFA concentrations ≥ 200 mmol/L. The TEs profiles for relative Y_{CH4} production suggest that Ni concentrations > 1.5 mg/L would result in significant decline in Y_{CH4} production; concentrations of Co and Mo > 0 mg/L are antagonistic to Y_{CH4} but up to 1.5 mg/L Se is tolerable.

Interaction effects of TEs and VFA on thermophilic VFA degradation rate

The statistics for the RSM fit shows that the prediction has a coefficient of determination (R^2) of 0.88; standardized R^2 of 0.61; and a fit error of 0.062. The significant terms (p>0.05) include VFA (0.0079), Ni*Co (0.0213), Ni*Ni (0.0213), Co*Co (0.0228) and Co*Se (0.0330) and Ni (0.0202). Positive influences were induced by Ni, Ni*Co and Co*Co. Negative influences were induced by VFA, Ni*Ni and Co*Se. Eq. 4 shows the final model for the significant terms that influence thermophilic $Y_{VFA\text{-}DR}$.

$$Y_{VFA-DR} = 1.0869 - 0.0006\,VFAs + 0.0259\,Ni * Co - 0.0991\,Ni^2 + 0.0244\,Co^2 - 0.0462\,Co * Se + 0.0395\,Ni + 0.0619 \quad \text{(Eq. 4)}$$

Fig. 2a, b and c show the optimum TEs configurations for the maximization of $Y_{VFA\text{-}DR}$ in the low, medium and high VFA levels. In Fig. 2a (low VFA level), the ranges of the beneficial TEs are 0.8 - 1.6 mg/L Ni; 0 - 2.0 mg/L Se; and 0.4 - 1.3 mg/L Mo. The resultant relative $Y_{VFA\text{-}DR}$ is 1.32, which corresponds to 32% improvement in VFA metabolism and an associated process efficiency of 99% (desirability of 0.99). Whereas changes in Mo concentrations are weakly influential, Ni concentrations outside 0.8 - 1.6 mg/L and Co concentration > 0 mg/L show antagonism with VFA concentration ≤ 55 mmol/L and induce decline in $Y_{VFA\text{-}DR}$. Conversely, Se concentrations ≥ 0.5 mg/L show synergy with VFA concentration ≤ 55 mmol/L and enhance $Y_{VFA\text{-}DR}$.

In Fig. 2b (medium VFA level), the ranges of beneficial TEs are 0.8 - 1.6 mg/L Ni; 0 - 2.0 mg/L Se; and 0.4 - 1.3 mg/L Mo. The relative $Y_{VFA\text{-}DR}$ was 1.28 or 28% increase in $Y_{VFA\text{-}DR}$ with process efficiency of 94%. Whereas Mo concentrations outside 0.4 - 1.3 mg/L are weakly influential, Ni concentrations outside 0.8 - 1.6 mg/L and Co concentrations > 0 mg/L show antagonism with VFA ≤ 125 mmol/L and induce decline in $Y_{VFA\text{-}DR}$. Conversely, Se concentrations above 0 mg/L result in proportional increase in $Y_{VFA\text{-}DR}$.

Fig. 2c (high VFA level) shows the influence of the TEs on relative $Y_{VFA\text{-}DR}$ in the high VFA level. The ranges of the beneficial TEs are 0.2 - 2.0 mg/L Ni; 0 - 2.0 mg/L Se; and 0.2 - 1.2 mg/L Mo. The optimum TEs configuration resulted in 38% improvement in $Y_{VFA\text{-}DR}$ (rel. $Y_{VFA\text{-}DR}$ of 1.38) and process efficiency of 99%. Whereas changes in Mo concentration are weakly influential, Ni concentrations outside 0.2 - 2.0 mg/L and Co concentrations > 0.5 mg/L are antagonistic to VFA ≥ 200 mmol/L and induce decline in $Y_{VFA\text{-}DR}$. Se concentrations > 0 mg/L show synergy with VFA ≥200 mmol/L and result in increase in $Y_{VFA\text{-}DR}$.

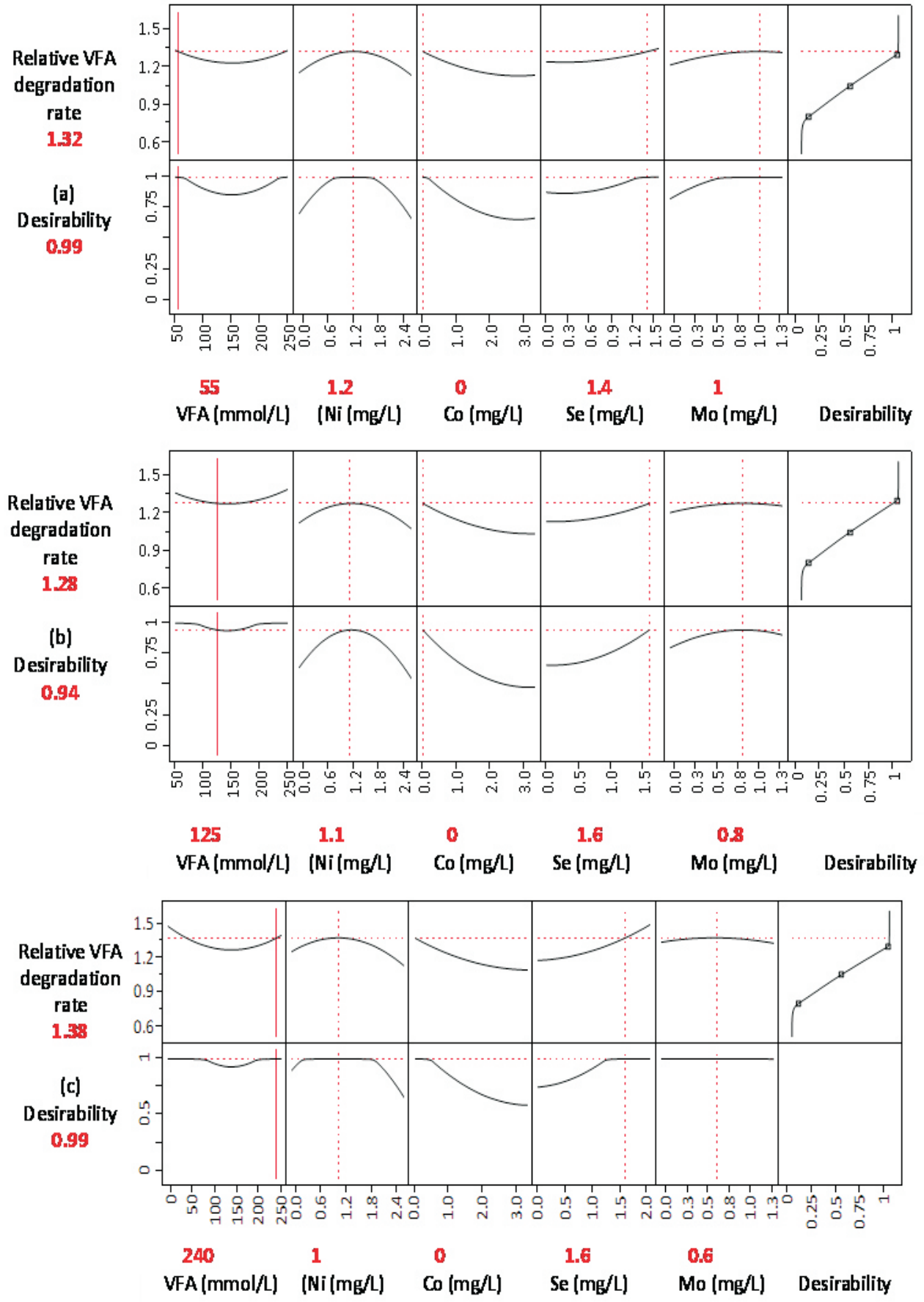

Figure 2: Profile and optimum TEs configuration for VFA degradation at 55°C due to Ni, Co, Se and Mo supplementation to different VFA concentrations (mmol/L) **(a)** 55 **(b)** 125 **(c)** 240

TEs configurations for simultaneous optimization of thermophilic Y_{CH4} production and Y_{VFA-DR}

The influence of TEs on Y_{CH4} production and Y_{VFA-DR} were co-evaluated to derive the TEs configurations that simultaneously maximize both responses. Table 2 shows the output of the multi-response optimization in the different levels of VFA.

Table 2: Optimum TEs configuration for co-optimization of VFA degradation rate and CH_4 production at 55°C for VFA levels between 10 and 250 mmol/L

VFA (mmol/L)	Ni (mg/L)	Co (mg/L)	Se (mg/L)	Mo (mg/L)	Relative VFA degradation rate	Relative CH_4 production	Desirability
10	1.14	3.73	0.00	1.24	1.44	1.08	1.00
50	1.25	3.73	0.00	1.16	1.32	1.07	1.00
100	1.23	3.73	0.00	1.06	1.21	1.05	0.89
150	1.08	0.03	1.24	0.17	1.17	1.03	0.81
200	1.05	0.03	1.24	0.20	1.21	1.04	0.88
250	1.03	0.03	1.24	0.21	1.30	1.05	0.99

Discussion

The effects of any TE during thermophilic methanization are more obvious when considered in relation to the interaction effects with VFA (VFA*TE) and with another TE (TE-TE). The interactions of VFA with Se, Mo and Co (VFA*Se, VFA*Mo VFA*Co); and interactions of Ni, Co, Se, Mo (Ni*Co, Se*Mo and Co*Se) particularly provided the necessary insight on the use of TEs for the enhancement of Y_{VFA-DR} and Y_{CH4} production during thermophilic methanization. Changes in VFA*Co, VFA*Mo, VFA*Se, Se*Mo, Co*Se, Co*Mo and Ni*Co shaped the optimum TEs requirements for co-optimization of Y_{VFA-DR} and Y_{CH4} production. As a result of VFA*Co, VFA*Mo and Co*Mo, the optimum TEs configurations for enhanced Y_{CH4} production and Y_{VFA-DR} require decreasing Co (3.73 – 0.03 mg/L) and Mo (1.24 – 0.21 mg/L) concentration as VFA increased (10 – 250 mmol/L). Conversely, the requirement for Se increased (0 – 1.24 mg/L) in response to the interaction effects of VFA*Se, Se*Mo and Co*Se following VFA increase (10 - 250 mmol/L).

The dynamics of the interaction effects between Co, Se, Mo and VFA are consistent with VFA*Co and Se*Mo having significant negative influence on Y_{CH4} production. Specifically, the Se*Mo effect necessitated a decrease in Mo concentration with increase in Se concentration (Table 2). This implies that based on the Se*Mo and VFA*Se effects, Mo bears an inverse relationship with Se and VFA concentrations during thermophilic methanization. Hence, in a TEs mixture that targets thermophilic methanization enhancement at a VFA concentration range of 20 – 250 mmol/L, either Se or Mo should be included in the TEs mixture but not both. Furthermore, as shown in Fig. 2a, b and c, Co*Se bears a significant negative influence on Y_{VFA-DR}. This implies that for simultaneous optimization of thermophilic Y_{VFA-DR} and Y_{CH4} production, a decrease in Co (3.73 - 0.03 mg/L) concentration is required for an increase in Se (0 - 1.24 mg/L) so as to reverse the negative interaction from Co*Se. This is evident in the Co and Se concentration dynamics in the VFA levels of 10 – 100 mmol/L and 150 – 250 mmol/L in Table 2.

Acknowledgement

The scholarship to enable the author to study and conduct this research at the Hamburg University of Technology (TUHH), Germany, was granted by the German Academic Exchange Programme (DAAD). The author is thankful to TUHH for funding the laboratory analyses during this investigation. The author is also thankful to the Herrling Group Hamburg for providing the inoculum used for the investigations. PD Dr. habil. Ina Körner and Prof. An-Ping Zeng are highly appreciated for supervising and co-supervising the author during the investigations.

References–

Ahn, J. H., Forster, C. F. (2002). The effect of temperature variations on the performance of mesophilic and thermophilic anaerobic filters treating a simulated papermill wastewater. Process Biochem. 37, 589594. doi:10.1016/S0032-9592(01)00245-X

Appels, L., Baeyens, J., Degrève, J., Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755781. doi:10.1016/j.pecs.2008.06.002

Dobbek, H., Svetlitchnyi, V., Gremer, L., Huber, R., Meyer, O. (2001). Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. Science 293, 12811285. doi:10.1126/science.1061500

Douglas, C. M. (2001). Introduction to statistical control, 4th ed. John Wiley and Sons Inc.

Ezebuiro, N. C. (2014). Optimization of Anaerobic Digestion: Influence of Trace Elements on Methanization Processes. (Band 87). ISBN 978-3942768-122, Hamburg, TUHH-GFEU.

Ezebuiro, N. C., Koerner, I. (2017). Characterisation of anaerobic digestion substrates regarding trace elements and determination of the influence of trace elements on the hydrolysis and acidification phases during the methanization of a maize silage-based feedstock. J. Environ. Chem. Eng. 5, 341351. doi:10.1016/j.jece.2016.11.032

Gustavsson, J., Svensson, B. H., Karlsson, A. (2011). The feasibility of trace element supplementation for stable operation of wheat stillage-fed biogas tank reactors. Water Sci. Technol. 64, 320325. doi:10.2166/wst.2011.633

Halarnkar, P. P., Blomquist, G. J. (1989). Comparative aspects of propionate metabolism. Comp. Biochem. Physiol. 92, 22731.

Hinken, L., Urban, I., Haun, E., Urban, I., Weichgrebe, D., Rosenwinkel, K. H. (2008). The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests. Water Sci. Technol. 58, 14531459. doi:10.2166/wst.2008.491

John, B. (2013). Applications of desirability function for optimizing the performance characteristics of carbonitrided bushes. Int. J. Ind. Eng. Comput. 4, 305314.

Mata-Alvarez, J. (2003). Fundamentals of the anaerobic digestion process, in: Mata-Alvarez, J. (Ed.), Biomethanization of the Organic Fraction of Municipal Solid Waste. IWA Publishing, UK, pp. 119.

Menon, S., Ragsdale, S. W. (1999). The role of an iron-sulphur cluster in an enzymatic methylation reaction. Methylation of CO dehydrogenase/acetyl-CoA synthase by the methylated corrinoid iron-sulphur protein. J. Biol. Chem. 274, 115138. doi:10.1074/jbc.274.17.11513.

Osuna, M. B., Zandvoort, M. H., Iza, J. M., Lettinga, G., Lens, P. N. L. (2003). Effects of trace element addition on volatile fatty acid conversions in anaerobic granular sludge reactors. Environ. Technol. 24, 573587. doi:10.1080/09593330309385592

Pobeheim, H., Munk, B., Johansson, J., Guebitz, G. M. (2010). Influence of trace elements on methane formation from a synthetic model substrate for maize silage. Bioresour. Technol. 101, 836839. doi:10.1016/j.biortech.2009.08.076

Pobeheim, H., Munk, B., Lindorfer, H., Guebitz, G. M. (2011). Impact of nickel and cobalt on biogas production and process stability during semi-continuous anaerobic fermentation of a model substrate for maize silage. Water Res. 45, 781787. doi:10.1016/j.watres.2010.09.001

SAS Institute Inc. (2012a). JMP 10 Design of Experiments Guide. Cary NC: SAS Institute Inc.

SAS Institute Inc. (2012b). JMP 10 Fitting Linear Models. Cary NC: SAS Institute Inc.

SAS Institute Inc. (2012c). JMP 10 Multivariate methods. Cary NC: SAS Institute Inc.

Svetlitchnyi, V., Peschel, C., Acker, G., Meyer, O. (2001). Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium Carboxydothermus hydrogenoformans. J. Bacteriol. 183, 51345144. doi:10.1128/JB.183.17.5134-5144.2001

Tiantao, Z., Lijie, Z., Youcai, Z. (2010). Study on the inhibition of methane production from anaerobic digestion of biodegradable solid waste. Waste Manag Res 28, 347354. doi:0734242X09351180

Verein Deutscher Ingenieure 4630 (2006). Fermentation of organic materials, characterisation of the substrate, sampling, collection of material data, fermentation tests. Germany.

Zitomer, D. H., Johnson, C. C., Speece, R. E. (2008). Metal Stimulation and Municipal Digester Thermophilic/Mesophilic Activity. J. Environ. Eng. 134, 4247. doi:10.1061/(ASCE)0733-9372(2008)134:1(42)