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

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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 CH₄ 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 CH₄ 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(CH₄) was obtained with TEs supplementation: Ni*Co interaction produced the positive influences; and Co*Se produced negative effects. The optimum TEs configuration for CH₄ 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

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Introduction

Methanization involves anaerobic digestion (AD) of biomass to produce energy-rich methane (CH₄)(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 CH₄ (Appels et al., 2008; Mata-Alvarez, 2003). Weak VFA metabolism is a challenge in methanization and results in VFA accumulation and low CH₄ production (Tiantao et al., 2010).

VFA metabolism and CH₄ 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 (Halarinkar 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 CH₄ production. Commonly reported TEs used in co-supplementation during methanization include Nickel (Ni), Cobalt (Co), Selenium (Se), Tungsten (W), Molybdenum (Mo), and Iron (Fe).
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 CH₄ 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. CH₄ 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. CH₄ production (Nml) ($Y_{CH₄}$) was measured as...
the CH₄ 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,i}) 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-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-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.

\[
Y_i = \beta_0 + \beta_1 VFA + \beta_2 Ni + \beta_3 Co + \\
\beta_4 Se + \beta_5 Mo + \beta_6 VFA^2 + \beta_7 VFA \cdot Ni + \\
\beta_8 Ni \cdot Co + \beta_9 VFA \cdot Co + \beta_{10} Ni \cdot Co + \\
\beta_{11} VFA^2 + \beta_{12} VFA \cdot Se + \beta_{13} Ni \cdot Se + \\
\beta_{14} Co \cdot Se + \beta_{15} Se^2 + \beta_{16} VFA \cdot Mo + \\
\beta_{17} Ni \cdot Mo + \beta_{18} Co \cdot Mo + \beta_{19} Se \cdot Mo + \\
\beta_{20} Mo^2 + \varepsilon
\]  

(Eq. 1)

Where, \(Y_i\) is the predicted response (\(Y_{VFA-DR}\) or \(Y_{CH₄}\) production); \(\beta_0\) is the intercept calculated with the algorithm in JMP 10; and \(\beta_i\) 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 \(\ldots\) Xki are the factors Ni, Co, Se, Mo and VFA and all the interactions between any two of the factors; and \(\varepsilon\) 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 \(Y_{VFA-DR}\) 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 (\(Y_{VFA-DR}\) or \(Y_{CH₄}\)) is shown in Eq. 2a; and the desirability function (\(D_1\)) for co-maximizing \(Y_{VFA-DR}\) and \(Y_{CH₄}\) is shown in Eq. 2b.

\[
D = \frac{Y_i - A}{B - A} \cdot w
\]

(A ≤ Y_i ≤ B; D = 1, Y_i > B; D = 0, Y_i < A)  

(Eq. 2a)

\[
D_{1-k} = \left[ D_{e1} \cdot D_{e2} \ldots \cdot D_{ek} \right]^{1/k}
\]

(Eq. 2b)

Where \(Y_i\) is the response (relative \(Y_{VFA-DR}\) or \(Y_{CH₄}\) production) predicted with the RSM shown in Eq. 1; \(A, B\) are the lowest and highest values respectively of the relative \(Y_{VFA-DR}\) or \(Y_{CH₄}\) production; and \(w\) is the value that shows the importance of \(Y_{CH₄}\) production in relation to \(Y_{VFA-DR}\). \(D_{e1}, D_{e2}, D_{e3}\) are the individual desirability values of the responses (\(Y_{VFA-DR}\) 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. \(Y_{VFA-DR}\) 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 (R²) of 0.94, standardized R² 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,
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

| Treatment/Reactor Nr. | Treatment | VFA | Ni | Co | Se | Mo | RMP | 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|
| 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|
| R29                  | 107±5     | 0.09| 0.03| 0.00| 0.04| 1.00| 1.00|
| R30                  | 209±11    | 0.09| 0.03| 0.00| 0.04| 1.00| 1.00|

*Treatment/Reactor Nr.; *Volatile fatty acids (mmol/L); *Nickel (mg/L); *Cobalt (mg/L); *Selenium (mg/L); *Molybdenum (mg/L); *Relative methane production; *Relative volatile fatty acid degradation rate; Control reactor for low VFA level; Control reactor for medium VFA level; Control reactor for high VFA level.

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. production.
Optimum TEs configuration for thermophilic CH₄ production

Fig. 1a and 1b show the optimum TEs configuration that maximized thermophilic Yᵢ₄ production at different VFA levels. Fig. 1a shows the prediction profiles for the relative Yᵢ₄ 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 Yᵢ₄ 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 Yᵢ₄ 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 CH₄ 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 $Y_{VFA-DR}$:

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

Fig. 2a, b and c show the optimum TEs configurations for the maximization of $Y_{VFA-DR}$ 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 $Y_{VFA-DR}$ 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 $Y_{VFA-DR}$. Conversely, Se concentrations ≥ 0.5 mg/L show synergy with VFA concentration ≤ 55 mmol/L and enhance $Y_{VFA-DR}$.

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 $Y_{VFA-DR}$ was 1.28 or 28% increase in $Y_{VFA-DR}$ 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 $Y_{VFA-DR}$. Conversely, Se concentrations above 0 mg/L result in proportional increase in $Y_{VFA-DR}$.

Fig. 2c (high VFA level) shows the influence of the TEs on relative $Y_{VFA-DR}$ 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 $Y_{VFA-DR}$ (rel. $Y_{VFA-DR}$ 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 $Y_{VFA-DR}$. Se concentrations > 0 mg/L show synergy with VFA ≥ 200 mmol/L and result in increase in $Y_{VFA-DR}$.
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
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 CH₄ 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 CH₄ 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 Yᵥfa-dr and Yᵥch₄ 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 Yᵥfa-dr and Yᵥch₄ production. As a result of VFA*Co, VFA*Mo and Co*Mo, the optimum TEs configurations for enhanced Yᵥch₄ production and Yᵥfa-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 Yᵥch₄ 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 Yᵥfa-dr. This implies that for simultaneous optimization of thermophilic Yᵥfa-dr and Yᵥch₄ production, a decrease in Co (3.73 – 0.03 mg/L) concentration and Mo (1.24 – 0.21 mg/L) concentration as VFA increased (10 – 250 mmol/L) 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.

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