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RESEARCH ARTICLE

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Anaerobic digestion of chicken manure: Influence of trace element supplementation

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In this study, anaerobic digestion of nitrogen-rich chicken (egg-laying hen) manure at different trace element (TE) mix doses and different total ammonia nitrogen (TAN) concentrations was investigated in batch digestion experiments. With respect to non-supplemented TE sets, addition of TE mixture containing 1 mg/L Ni, 1 mg/L Co, 0.2 mg/L Mo, 0.2 mg/L Se, 0.2 mg/L W, and 5 mg/L Fe at TAN concentrations of 3000 mg/L and 4000 mg/L, cumulative CH_4 production and CH_4 production rate improved by 7–8% and 5–6%, respectively. The results revealed that at a very high TAN concentration of 6000 mg/L, the effect of TE addition was significantly high and the cumulative CH_4 production and production rate were increased by 20 and 39.5%, respectively. Therefore, it is concluded that at elevated TAN concentrations the CH_4 production that was stimulated by TE supplementation was presumably occurred through syntrophic acetate oxidation.

KEYWORDS

ammonia, anaerobic monodigestion, trace element, syntrophic acetate oxidation

1 | INTRODUCTION

Chicken manure (CM) contains high amounts of biodegradable organic matter. Hence, anaerobic digestion (AD) is a favorable option to treat and stabilize the organic matter in CM along with biogas production. Biogas can be converted to heat and electricity and also used as a vehicle fuel and injected to the natural gas grid after upgrading [1]. Organic nitrogen in CM, which abundantly exists in the form of undigested protein, is hydrolyzed to polypeptides and amino acids. As a result of amino acids fermentation ammonia is generated in CM digesters [2]. Although ammonium at low concentration is required for the growth of microorganisms and provides enough buffering capacity for anaerobic consortia, free ammonia molecule may pass through the cell membrane and causes inhibition of methanogens [3]. Therefore, in practice, ammonia inhibition is experienced as the major problem in AD of CM [4]. To alleviate ammonia inhibition in AD, stripping, zeolite adsorption, membrane separation, struvite precipitation, dilution, and codigestion have been applied in number of studies [5]. Although these processes mitigate the ammonia inhibition, technical and economic viability of the process are the major challenges when applying these technologies [6]. Therefore, the objectives of recent scientific papers are to develop reliable methods for industrial-scale anaerobic digesters.

Westerholm et al. [7] have reported that stable CH_4 production at high TAN concentrations is possible without significant volatile fatty acid accumulation in ammonia-adapted anaerobic digesters. They explain the adaptation process with the inhibition of acetoclastic methanogens and relocation of the metabolic pathway to syntrophic acetate oxidation (SAO) followed by hydrogenotrophic CH_4 production, which is more resistant to ammonia inhibition. If the essential TEs are lacking, SAO does not occur efficiently at elevated TAN levels causing accumulation of volatile fatty acids during AD [8,9]. The redox enzyme, formate dehydrogenase, plays an important role in SAO, and its effectiveness depends on the availability of TE such as Se, Co, Mo, and W [3,9–11]. In addition, TE, such as Co, Mo, Ni, Se, W, and Fe, are the essential nutrients for the growth of hydrogenotrophic methanogens [10,12, 13].

The optimum supplementation of TE is critical for microbial reproduction and metabolic activities in AD. Hence,

deficiency of TE may seriously affect the microbial activities [14]. On the other hand, excess TE supplementation may be toxic for anaerobic consortia [9] and high concentrations of TE can limit the use of digestate in agricultural purposes and cause environmental pollution [14]. The suggested TE concentration values for stable AD in the literature vary over a wide range [15]. Moreover, the bioavailability of TEs in AD is another concern that must be considered when reporting the required concentrations. By applying chemical sequential extraction techniques, bioavailability might be investigated [16]. For instance, Fuentes et al. [17] have reported that in addition to water-soluble fraction, exchangeable fraction of TEs is also bioavailable for metabolic activity. Generally, to increase bioavailability and stimulate methanogens in AD, and hence, increase CH_4 production rate, TEs are supplemented at concentrations slightly above the stimulatory levels [14].

In the past, researchers assumed that manures contain relatively high amounts of TEs and, therefore, did not consider any TE deficiency as a problem in AD of manures [15,18]. However, recent findings contradict this assumption. We found that the addition of Se in AD of CM increased the activity and number of hydrogenotrophic *Methanoculleus bourgensis* at high TAN concentration of 7200 mg/L and 54% higher CH_4 yield was achieved [6]. Moreover, it was found that after starting the addition of TE mix containing Co, Ni, Mo, W, and Se in CM digester at high TAN concentration of 5000 mg/L, another hydrogenotrophic methanogen, *Methanobrevibacter* became dominant and CH_4 yield increased by 59% and reached to 0.32 ± 0.01 L/gVS [11]. Although CM lacks some TE essential for anaerobic microorganisms, to our knowledge, limited studies have been carried out on TE requirements for AD of CM at elevated TAN levels. In a recent study, TEs necessary for stable CH_4 production from food waste was investigated broadly and Se and Co were found to be critical at high TAN concentrations [10].

In this study, the influence of TE supplementation on CH_4 production from CM digestion was investigated with batch digestion experiments.

2 | MATERIALS AND METHODS

2.1 | Substrate and inoculum

The inoculum used in the experiments was taken from a 16 liters laboratory scale mesophilic anaerobic CM digester. Details and operational conditions of lab-scale anaerobic digester were given in our previous study [19]. CM used in this study was collected from different parts of egg-laying hen farms located in Afyonkarahisar, Turkey. To ensure that samples used for batch experiments are homogenized and representative, CM was completely mixed. Total solids (TSs), volatile solids (VSs), total Kjeldahl nitrogen (TKN), and total sulfur contents of CM and inoculum were determined immediately after their arrival in our laboratory. CM was stored in a refrigerator at 4 ± 2°C during the study. Characterization of CM and inoculum are given in Table 1.

TABLE 1 Characteristics of CM and inoculum

Parameter	CM	Inoculum
TS (% wet weight)	25.3	2.5
VS (% wet weight)	17.93	1.3
TKN (g/kg of TS)	47.4	1.25
Total sulfur (g/kg of TS)	0.64	–

2.2 | Experimental plan

Two batch CM digestion experiments were performed in this study.

In Experiment 1, along with control set, the effect of increase in TEs concentrations from one-fold (1 mg/L Ni, 1 mg/L Co, 0.2 mg/L Mo, 0.2 mg/L Se, 0.2 mg/L W, and 5 mg/L Fe) to 50-fold on CH_4 production potential and rate was examined at constant TAN concentration of 3000 mg/L.

In the second experiment (Experiment 2), the effect of one-fold TE addition, on biogas production performance of CM was investigated at increasing TAN concentrations (from 420 mg/L to 6000 mg/L). Experimental plan is summarized in Table 2.

The inoculum/substrate ratio and the TS content were kept at 2 and 0.6%, respectively, in all experiments by using the dilution solution containing 2.5 g KH_2P0_4, 1.0 g K_2HPO_4, and 0.1 g $MgCl_2$ per 1.0 L tap water (pH = 6.7). The dilution solution was prepared as described by Valcke and Verstraete [20] without using NH_4Cl and $Na_2S{\cdot}7H_2O$. Higher TS contents were not tested because of difficulties in mixing of test bottles. Hence, to adjust the TAN concentration of test bottles to the desired levels, NH_4Cl was added externally.

Experiments were conducted in 250 mL glass bottles with 100 mL active volume in triplicates. Inoculum and substrate were transferred to the test bottles under anaerobic conditions and then the bottles were sealed with special caps and located on multimagnetic stirrers (2MAG, Mix15). Experiments were performed in an incubator (WTW, TS606/4-i) at 36 ± 0.5°C. The biogas production was monitored with a multitube water displacement setup and recorded daily as described by Inanc et al. [21]. A schema of experimental setup is shown in Figure 1.

The TE solution was prepared from the reagent grade salts of $NiCl_2{\cdot}6H_2O$ (Merck, Darmstadt, Germany), $CoCl_2{\cdot}6H_2O$ (Merck), $Na_2MoO_4{\cdot}2H_2O$ (Merck), Na_2SeO_3 (Sigma-Aldrich, St. Louis, MI), $Na_2WO_4{\cdot}2H_2O$ (Sigma-Aldrich), and $FeCl_3{\cdot}6H_2O$ (Merck).

TABLE 2 Experimental plan

	Experiment 1											Experiment 2			
Test #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**1**	**2**	**3**	**4**
TE doses	No TE	One-fold[a]	Two-fold	Three-fold	Four-fold	Five-fold	10-fold	20-fold	30-fold	40-fold	50-fold	Control (No TE)			
												One-fold			
TAN mg/L	3000											420	3000	4000	6000
I/S ratio VS/VS	2														
TS%	0.6														

[a]One-fold TE: 1 mg/L Ni, 1 mg/L Co, 0.2 mg/L Mo, 0.2 mg/L Se, 0.2 mg/L W, and 5 mg/L Fe.
I/S = inoculum/substrate.

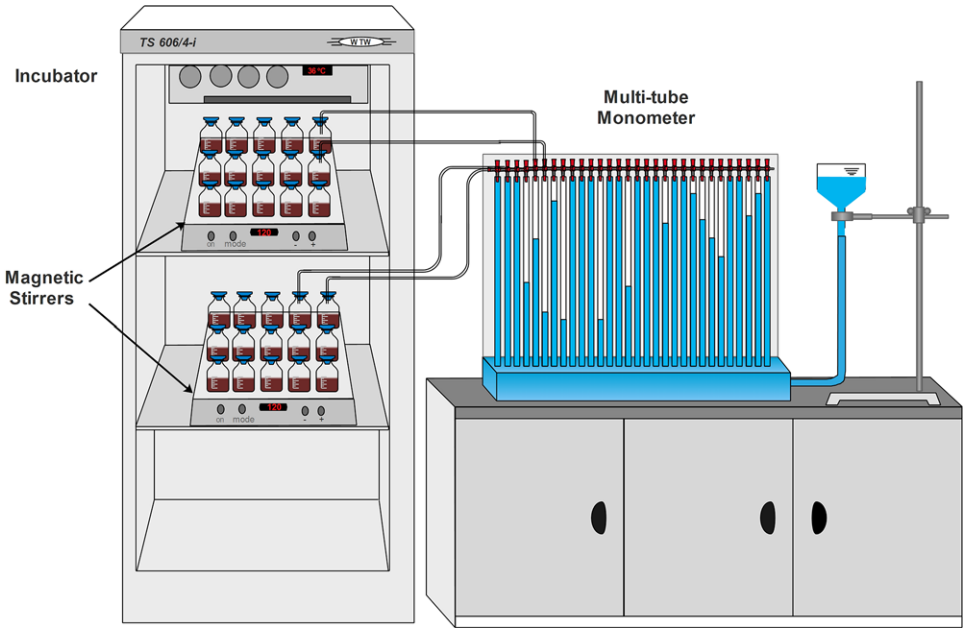

FIGURE 1 Experimental setup

2.3 | Analytical methods

pH was measured using a pH meter (Eutech, PCD 6500, Singapore), alkalinity was determined with titration Method 2320 B. TS and VS were quantified using methods 2540 B and 2540 E, respectively. TKN was measured using method 4500 N_{org} B [22]. The CH_4 content of the biogas produced in each bottle was analyzed twice during biomethane potential (BMP) tests using a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan) equipped with thermal conductivity detector according to the method of Reddy et al. [23].

Total TE and the portion in the liquid fraction of digestate and CM were determined by microwave-assisted acid leaching technique [24]. For total TE analyses, 0.5 g of dry sample was grounded and acid digested with concentrated nitric acid in a digestion vessel by using a microwave oven (CEM, MARS-5, Matthews, NC) as described in our previous study [6]. For TE in the liquid fraction, the sample was first centrifuged at 6000 rpm for 10 min and then for its supernatant the same acid digestion method was applied. The total Ni, Mo, Se, W, Co, and Fe contents of acid digested samples were then determined by ICP-MS (Agilent 7700, Santa Clara, CA).

2.4 | Data analysis

After batch digestion experiments, maximum CH_4 production rate (R_m, mL CH_4/gVS/d), lag-phase time (λ, day), and CH_4 production potential (P, mL CH_4/gVS) were determined by using modified Gompertz model (Equation (1)) [25].

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where M is the cumulative CH_4 production at time t (d) and e is a mathematical constant (2.718).

To estimate the soluble fraction of TEs under anaerobic conditions and to reveal the equilibrium mass distribution among the solid phase, dissolved and adsorbed species, chemical equilibrium software Visual MINTEQ 3.0 was used. The solubility is highly dependent on precipitation/dissolution/complexation equilibrium that can be predicted according to these equilibrium reactions. Therefore,

Visual MINTEQ 3.0 was run for one-fold (1 mg/L Ni, 1 mg/L Co, 0.2 mg/L Mo, 0.2 mg/L Se, 0.2 mg/L W, and 5 mg/L Fe) TE-supplemented test of Experiment 1 [26]. The speciation was predicted based on one-fold TEs, the ion composition of the dilution solution (2.5 g KH_2P0_4, 1.0 g K_2HPO_4, and 0.1 g $MgCl_2$ per 1.0 L tap water) used, pH value and TAN concentration of the CM, and inoculum and dilution solution mixture.

Adsorption was predicted based on the surface complexation model and the simulation was made according to the predefined database of HFO-Dzomback & Morel, available in Visual MINTEQ 3.0.

3 | RESULTS AND DISCUSSION

3.1 | TE contents of CM and batch tests

Considering the TE examined in AD studies, Co, Se, Mo, W, Ni, and Fe were selected as target TEs and analyzed in CM used in batch AD experiments. Banks et al. [10] particularly showed the need of Co and Se at higher ammonia levels in the food waste digesters to prevent propionic acid accumulation. Besides, it is known that Ni, Co, and Fe are also considered essential nutrients for methanogens and their deficiencies may result in process failure in anaerobic digesters [3,13].

The TE analysis results are listed in Table 3 along with the typical TE contents of CMs for comparison. The results showed that CMs used in this study contain total TE concentrations below or close to the lower limit of values reported by Bolan et al. [27]. This difference may be related with dissimilarities between the nutritional quality of the poultry feed and weight, age, and egg production rate of chickens [27].

The concentrations of Co, Se, and Ni in the CM were in the range, but of Mo were fairly below the typical values reported by Bolan et al. [27]. Among the six elements analyzed, W was found to be the rarest with an average concentration of 0.022 ± 0.008 mg/kg TS. Although W is one of the valuable elements in commercial organic fertilizers, there is very limited data about the W content of poultry manure in the literature.

The Co, Se, W, Mo, Ni, and Fe concentrations in experiments are given in Table 4. It is obvious that in the control set, the concentrations of Se and W were lower than the values recommended for stable AD [15].

TABLE 3 Total TE analyses results of raw CM used in experiments

Elements	CM mg/kg TS	Bolan et al. [27]
Co	1.93 ± 0.11	2–8
Se	0.57 ± 0.05	0.38–1.23
W	0.022 ± 0.008	–
Mo	2.21 ± 0.30	5–7.7
Ni	5.22 ± 0.92	2.46–15
Fe	606 ± 62	–

3.2 | Biomethane potential of batch digestion experiments

Experiment 1 was performed to investigate the effect of increasing TEs concentration on biogas production from CM at moderately high TAN concentration (3000 mg/L). In addition, the optimum TEs concentrations were determined according to CH_4 production potential, rate, and lag phase in relation to concentration of TE in each batch assay. Therefore, along with the control set, 10 different doses were examined by increasing the concentrations in TE mix from one- to 50-fold.

The results showed that compared to nonsupplemented set, the addition of TEs up to five-fold increased the CH_4 production rate by 34% and recorded as 22.4 ± 1.3 mL CH_4/gVS/d.

Increase in TEs concentration up to 10-fold did not significantly results in a reduction in CH_4 production potential and rate. Using modified Gompertz model, CH_4 production potential and rate were predicted as 235 ± 36 mL CH_4/gVS and 21.3 ± 3.1 mL CH_4/gVS/d, between one- and 10-fold TE concentrations, respectively. Only at 10-fold TE concentrations, lag phase slightly increased to 12 days (Figure 2 and Supporting Information Figure 1).

After 10-fold inversely proportional to increase in TEs concentration, both CH_4 production potential and rate decreased considerably. At 20-fold, they dropped to 182 ± 6 mL CH_4/gVS and 12.5 ± 0.7 mL CH_4/gVS/d, respectively. With increase in TEs concentration up to 50-fold they decreased even more and fell down to 55 ± 9 mL CH_4/gVS and 7.0 ± 2.3 mL CH_4/gVS/d, respectively (Figure 2).

Although lower than one-fold TE mix was not tested in this study, the results indicated that one-fold or lower TE mix is adequate and there is no need to supplement more than one-fold considering their costs and the potential use of digestate as biofertilizer in agriculture [28].

While assessing the effects of TE supplementation on AD, chemical speciation and consequently bioavailability of TEs in the digester have to be considered. It is known that speciation of TEs is a function of their concentrations and the amounts of organic and inorganic ligands with which TEs may react [29].

Dissolved TE in AD existed in the forms of free ions and soluble complexes [16]. Free ion is commonly used as the major bioavailable species for the predictions of TE availability [30]. However, in anaerobic digesters bioavailability of organic or inorganic complexes of TEs may increase via uptake of hydrophilic complexes through a ligand transporter system or passive diffusion of lipophilic complexes [16].

In AD process with considering the variation of the major ions, namely NH_4^+, Mg^{2+}, PO_4^{3-}, organic and inorganic

TABLE 4 Total TE concentrations (mg/L) in experiments

	Control set	Experiment 1 (with one- to 50-fold TE suppl.)	Recommended concentrations for AD [15]
Co	0.016	1.02–50.8	0.003–20
Se	0.005	0.2–10.2	0.008–0.79
W	0.0002	0.2–10.0	0.018–18.3
Mo	0.02	0.22–11.0	0.005–50
Ni	0.021	1.02–51.0	0.005–30
Fe	4.092	9.09–446.4	0.28–200

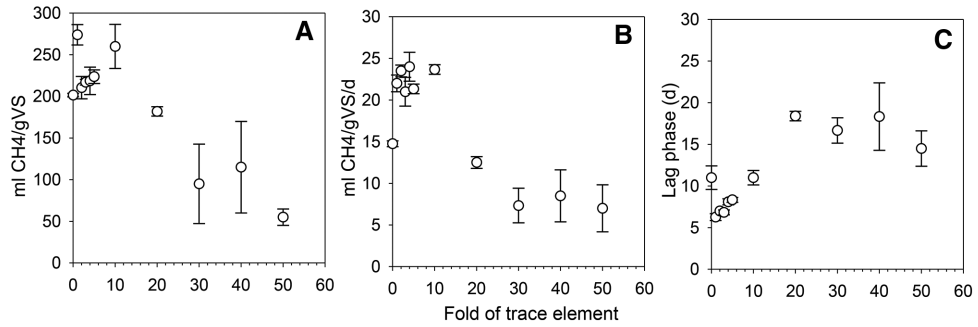

FIGURE 2 CH_4 production potential (A), rate (B), and lag phase (C) at 3000 mg/L of TAN in relation to concentrations of TEs from 0- to 50-fold (one-fold: 1 mg/L Co; 1 mg/L Ni; 0.2 mg/L Mo; 0.2 mg/L Se; 0.2 mg/L W, and 5 mg/L Fe)

compounds may result in precipitation of TEs. Therefore, some portion of the TEs supplemented to batch bottles might not be bioavailable for anaerobic consortia.

Since maintaining anaerobic conditions during the sampling is not easy and may fail to determine the bioavailable fraction of TEs [16], in this study only fractions in the sludge liquid phase and pellet were determined. For this purpose, at the end of BMP test, mixed liquor samples were taken from one-fold TE supplemented set and analyzed for the portion in the liquid and solid phases.

In addition, the species of TEs were estimated by Visual MINTEQ 3.0. The model-based speciation outcomes and ICP–MS results are presented in Figure 3.

The ICP–MS results showed that in addition to Fe, a significant portion of the Co supplemented to bottles was in the pellet form and seems not to be bioavailable for anaerobic microorganisms (Figure 3A). Likewise, Gustavsson et al. [31] reported that only 10–20% of the total Co concentration was in dissolved form during AD of stillage. The results exhibited that in addition to Fe and Co, more than 80% of Se, above 40% of Ni and W, and about 65% of the Mo added to BMP bottles were not in the supernatant.

Besides, Visual MINTEQ predicted that Fe, Co, and Ni were completely precipitated as $CoFe_2O_4$, Fe_2O_3, $CoFe_2O_4$, and NiS. Moreover, it estimated that 94% of W, 42% of Mo, and 12.6% of Se were adsorbed in the forms of WO_4^{2-}, MoO_4^{2-}, and $HSeO_3^-$. The remaining portions of the aforementioned metals were predicted to be soluble under the conditions defined in the model (See Supporting Information Table S1).

In Experiment 2, the effect of TEs supplementation on biogas production was investigated at 420, 3000, 4000, and 6000 mg/L of TAN concentrations. The cumulative CH_4 production profiles are presented in Figure 4. The highest CH_4 production rate of 41.0 ± 1.7 mL/gVS/d was achieved at 420 mg/L of TAN as expected (Figure 4A). In accordance with the results of similar studies in the literature [4,32–34], CH_4 production rates decreased to 25.5 ± 0.8, 11.5 ± 0.2, and 5.1 ± 0.1 mL/gVS/d as TAN concentration increased to 3000, 4000, and 6000 mg/L, respectively (Figure 4). Likewise, Bujoczek et al. [4] have performed a batch screening assay to investigate the AD of CM and experienced a comparable decrease in CH_4 production rate from 9.2 mL/gVS/d at 4000 mg/L of TAN to 5.9 mL/gVS/d at 6500 mg/L.

Although TE supplementation at 420 mg/L of TAN has increased the cumulative CH_4 production slightly by 3%, there is no clear difference between the CH_4 production rates of sets with and without TE supplementation. At 3000 mg/L and 4000 mg/L of TAN, addition of TEs improved the CH_4 production rate from 23.8 ± 0.8 and 11.0 ± 0.2 mL/gVS/d to 25.1 ± 0.7 and 11.7 ± 0.3 mL/gVS/d, respectively. On the other hand, at 6000 mg/L of TAN, the CH_4 production rate of TE supplemented set was visibly higher (39.5%) than that of nonsupplemented one. A similar tendency was observed in cumulative CH_4 production. With TE supplementation, cumulative CH_4 production increased by 7–8% at TAN of 3000 and 4000 mg/L. The improvement with TE supplementation exceeded 20% at 6000 mg/L. These results clearly show that TE mix consisting of Co, Se, W, Mo, Ni, and Fe is more

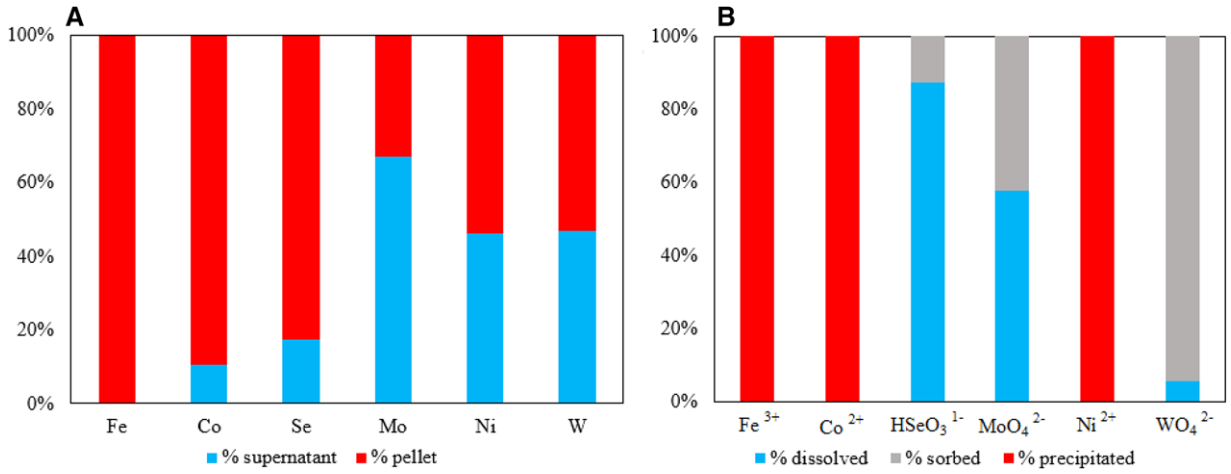

FIGURE 3 ICP-MS analyses of TEs in supernatant and pellet (A), visual MINTEQ prediction of TEs in soluble, precipitated, and adsorbed species (B)

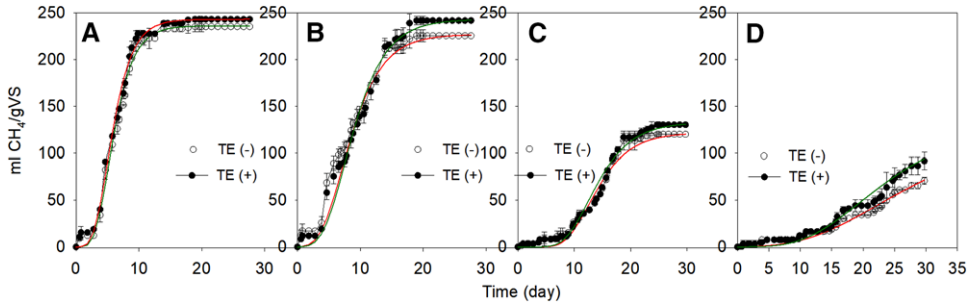

FIGURE 4 Cumulative CH_4 production at TAN of (A) 420 mg/L, (B) 3000 mg/L, (C) 4000 mg/L, and (D) 6000 mg/L. The lines represent Gompertz fitting curves

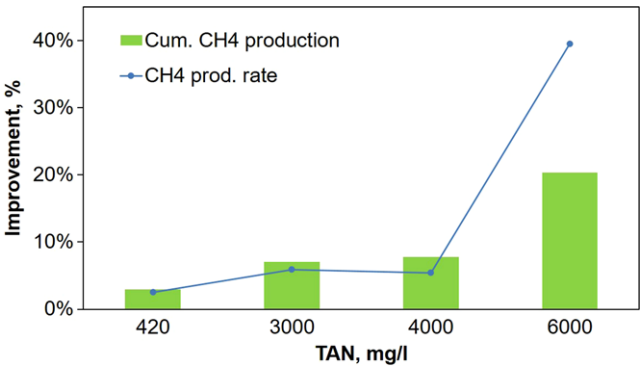

FIGURE 5 Improvement in cumulative CH_4 production and production rate with addition of TEs

effective on CH_4 production at elevated TAN concentrations (Figure 5).

Hydrogenotrophic methanogens are more resistant to elevated TAN concentrations than acetoclastic ones and become dominant in anaerobic digesters as TAN concentrations increase [5,35–37]. The results of metagenomics analyses of our previous studies showed that *M. bourgensis* and *Methanobrevibacter*, hydrogenotrophic methanogens, were dominant when the TAN concentration was higher than 5000 mg/L [3,6,11]. Generally, acetate is first oxidized to CO_2 by a proton-reducing bacterium and then CO_2 is reduced to CH_4 by hydrogen and/or formate consuming methanogens at high TAN concentrations. This reaction is known as SAO and thermodynamically favorable only if the end product H_2 is consumed and kept at very low levels [15,38]. Anaerobic formate oxidation is catalyzed by formate dehydrogenase and this enzyme is highly dependent on Se, W, and Mo [39]. Moreover, Co, Ni, and Fe are the important cofactors for the coenzyme M methyl transferase and carbon monoxide dehydrogenase [16]. All these enzymes are known to be active on the syntrophic association with hydrogenotrophic methanogens [11]. Therefore, it is concluded that the enhanced CH_4 production achieved in sets with TE supplementation at high TAN levels was related to the stimulation of SAO.

4 | CONCLUDING REMARKS

Increasing TE concentrations from one-fold to 10-fold at moderately TAN level did not affect the CH_4 production. However, over 10-fold TE supplementation resulted in considerable decrease in both CH_4 production rate and cumulative

CH_4 production. Hence, supplementation of TE mix containing 1 mg/L Co, 1 mg/L Ni, 0.2 mg/L Mo, 0.2 mg/L Se, 0.2 mg/L W, and 5 mg/L Fe was speculated to be enough.

TE supplementation did not enhance the biogas production at low TAN concentrations of 420 mg/L, while significant improvement especially in CH_4 production rate was observed at high TAN concentrations of 6000 mg/L. It is concluded that TE supplementation enhanced SAO and hydrogenotrophic CH_4 production at higher TAN concentrations (> 4000 mg/L).

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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