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Original Research Paper

Effect of Trace Elements Supplement on Anaerobic Fermentation of Food Waste

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ABSTRACT

Laboratory scale semi-continuous stirred tank reactors fed with food waste were used to investigate the effects of trace elements Co, Ni and chelating agent EDTA supplements on the biogas process. It was observed that the addition of Co, Ni and combination of the two elements increased the biogas yield compared to the control reactor by 15.0%, 30.8% and 39.5% respectively. The VFA, pH, methane content and TS content in the digesters were also stabilized. Ni was found to play a more important role in anaerobic digestion of food waste than Co. The combination Co-Ni added reactor performed better than those with individual elements regarding total biogas production and process stability. Addition of EDTA known to improve metal bioavailability showed that Co with EDTA supplement moderately affected the performance compared to Co only reactor, whereas EDTA with Ni addition showed no improvement.

INTRODUCTION

With rapid population growth and economic development, the rising amount of food waste coming from household, restaurants and canteens has accounted for over 50% of the municipal solid wastes (MSW) in many cities of China (Zhang et al. 2014). Due to its high water content and easy decay nature, the conventional treatments of food waste such as landfill and incineration become neither economic nor environmental friendly (Zhang & Jahng 2012).

Anaerobic digestion has been considered as an environmentally beneficial and energy-efficient waste disposal process (Weiland 2010), which converts organic waste into biogas, a biofuel with high calorific value and producing only carbon dioxide and water when combusted (Holm-Nielsen et al. 2009). Food waste is characterized as high biodegradability and nutrient contents , therefore suitable for anaerobic treatment (Zhang et al. 2011). The estimated biochemical methane potential (BMP) of the food waste is approximately 0.44-0.48 L CH_4/g VS (Zhang et al. 2011). However, food waste based anaerobic reactors are often found to suffer with VFA accumulation and low pH levels, the instable operation could finally end up with process failure. This is likely due to high contents of carbohydrate and fat in the substrate. Trace elements such as Co and Ni supplements are found to be beneficial for performance of anaerobic digesters, in avoiding or abating volatile fatty acids (VFA) accumulation, accelerating the turnover of substrate and stimulating the methane-producing bacteria (Patidar & Tare 2004, Patidar & Tare 2006, Feng et al. 2010). This could be correlated to the critical roles of these elements in enzymatic activity, energy conservation, membrane stability and nutrient transport (Scherer & Sahm 1981, Patidar & Tare 2004). For instance, Ni is present in cofactor F430, a component of methyl-coenzyme M reductase complex, which catalyzes the formation of methane from methyl-coenzyme M. Ni is also contained in factor F420, a hydrogenase and CO dehydrogenase. Co is found in methyl-H_4SPT, which is the coenzyme M methyl-transferase complex of the methanogens (Sauer & Thauer 2000, Patidar & Tare 2004, Pobeheim et al. 2010a, Pobeheim et al. 2010b). Nevertheless, these essential trace elements are often found insufficient when compared the bioavailable part with the total content in the digester (Oleszkiewicz & Sharma 1990). Despite lots of works done in relevant area, there are few studies using food waste as single substrate in continuous digester and on intermediates degradation during the process. Moreover, there are also knowledge gaps regarding bioavailability of trace element addition for food waste based digesters. Bioavailability to some extent depends on the solubility. The presence of precipitator like sulfide could decrease the solubility of trace elements, and by adding chelating agent and forming soluble complexes, the metal solubility could be improved (Garrabrants & Kosson 2000, Jansen et al. 2007).

The aim of this paper is to study the effects of chelating agent EDTA and various concentrations of Co and Ni additions in CSTR feed with food waste, evaluating process improvement of single and combination of the two elements, as well as the degradation patterns of important intermediate compounds in the process.

MATERIALS AND METHODS

Collection and preparation of inoculum and substrate: The inoculum was from a biogas plant on pig farm, and had been domesticated with food waste through several batch experiments, then left with no feeding till no significant gas production before seeding. Food waste was collected from canteen of South China Agricultural University, then disintegrated and homogenized in a blender. Prior to use, the substrate was weighed and stored in bottles, kept in refrigerator at -18°C. The characteristics of inoculum and food waste are listed in Table 1.

Experimental design: The experiment was carried out in tank reactors with working volume of 2 L. Each reactor was connected to a gas gathering system base on saturated brine displacement. The reactors were placed in the electricity-heated thermostatic water bath at 37±1°C. The OLR was set to 2.5g/L/d and the HRT was 25 days. The feed and withdrawal was performed with syringes through a rubber tube in the reactor. Stirring was done manually three times a day.

Before any trace elements were added, the reactors had been operating for one month to avoid fluctuation in the start-up phase. The design of the experiment included six reactors with EDTA and low, middle and high concentrations of Co and Ni as given in Table 2. The reactors were working under constant trace elements concentrations as designed by adding the same amount of elements in feed as in withdraw.

Analytical methods: TS and VS were determined using standard techniques (APHA 1998). pH was measured by pH a meter (PHS-3C, Shanghai REX instrument Factory). Elementary analysis was performed by Vario EL element analyser (ElementarAnalysensysteme GmbH). Concentrations of trace elements were measured by ICP-AES. Gas samples analysis was performed on an Agilent 6890 (Agilent Technologies, USA). The liquid samples were centrifuged at 12000 rpm for 20 minutes and filtered with a 0.22 µm membrane filter for VFA analysis. VFA including formic, lactic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acid were measured by HPLC with a refractive index (RI) detector, 1% phosphoric acid as mobile phase.

Calculation of theoretical methane potential: Calculation of theoretical methane potential follows the Buswell equation (Buswell & Muller 1955):

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow$$

$$\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + cNH_3 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2$$

$$\text{Theoretical } CH_4 = \frac{1000 \times 24.5 \times (\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8})}{12n + a + 16b + 14c} \frac{mL\ CH_4}{gVS}$$

$$\text{Theoretical } CO_2 = \frac{1000 \times 24.5 \times (\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8})}{12n + a + 16b + 14c} \frac{mL\ CO_2}{gVS}$$

RESULTS

Process performance of reactors in the presence of different trace elements: Fig. 1 and Table 3 show the performance of reactors supplied with no trace elements (R0), individual elements (RCo, RNi) and combined elements (RCoNi). It was found that the anaerobic digestion process of control reactor R0 was unstable and unsustainable, both biogas production and methane content exhibited sharp decrease from around 4000 mL/d to 0 and over 50% to 30%, and the lowest biogas yield of 518.3 mL/gVS was obtained, accounting only 41.5% of the theoretical yield. Also, significant accumulation of volatile fatty acids (VFAs) up to 8215 mg/L , low pH of 4.72 and increasing TS content from around 2% to 2.9% were observed. This was in accordance with previous studies indicating the easy failure of food waste based single-stage anaerobic digestion (Zhang et al. 2012, Zhang et al. 2014). Unlike R0, reactors with trace elements supplements displayed improved performances in different extent, i.e. 15.0% improvement for biogas yield with Co addition, 30.8% with Ni, and 39.5% with the combination addition. Although the Co-added reactor also failed followed the faith of control, the failure trend was slightly abated: the sharp decease of biogas production and pH happened few days later than R0, and the final VFA concentration and TS content were 3612.2 mg/L and 2.3% respectively, comparable lower than R0. And an improved biogas yield of 595.9 mL/gVS was obtained. Similarly, RNi showed an abated failure trend, no complete process collapse happened during the operation period, also the biogas yield was considerably higher than R0 and RCo (677.8 mL/gVS). The highest biogas yield was found in reactor fed with combined Co and Ni, which was most stable reactor among all. At the end of digestion, the pH in RCoNi remained above 7 and biogas production was around 4000mL/day at OLR of 2.5gVS/L/day; at the same time, no observation of significant VFA accumulation (504.4mg/L) and TS content increase (2.18%). These results indicated that supplements of trace

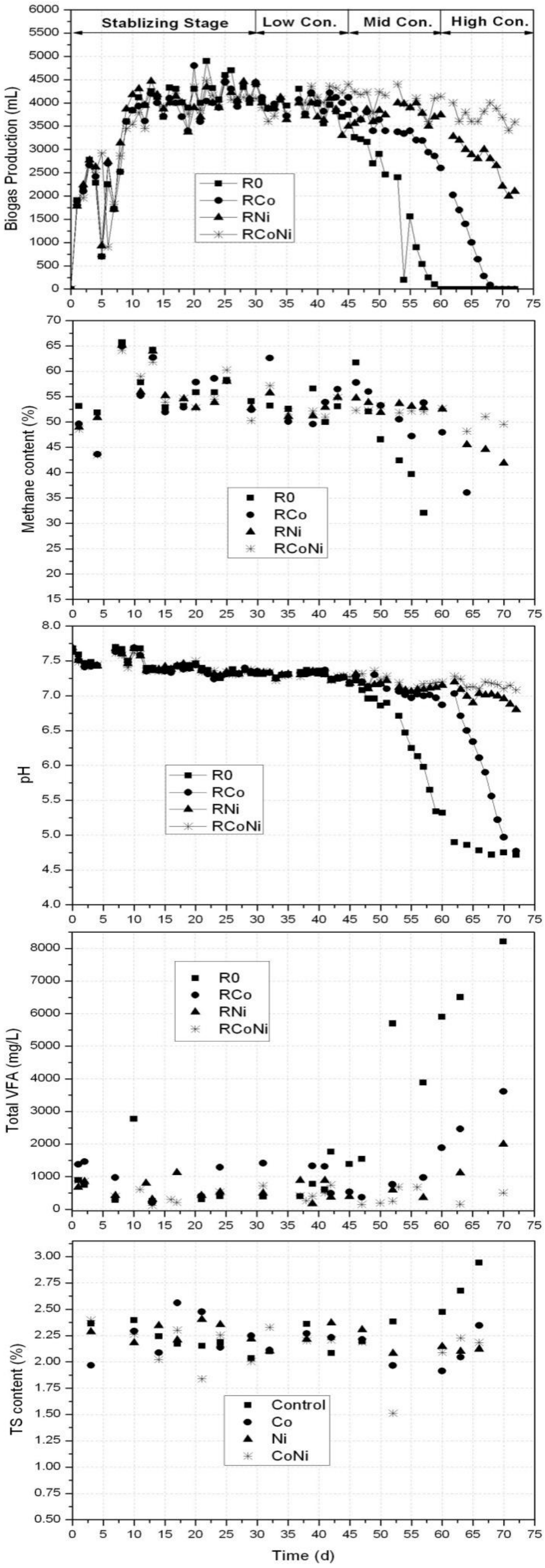

Fig. 1: Anaerobic digestion performance of reactors in the presence of different trace elements.

Table 1: Main characteristics of substrates and inoculum.

Characteristic	Unit	Inoculum	Food waste
pH	-	8.00	4.30
C/N	-	-	19.0
Total solids (TS)	%	1.80	18.0
Volatile solids (VS)	%TS	61.1	93.2
[C]	%TS	-	52.2
[H]	%TS	-	8.10
[O]	%TS	-	30.1
[N]	%TS	-	2.70
[S]	%TS	-	0.14
Carbohydrate	%TS	-	39.0
Total sugar	%TS	-	9.10
Protein	%TS	-	17.0
Fat	%TS	-	35.0
Crude fiber	%TS	-	3.30
Calorific value	MJ/kg TS	-	251
Co	mg/L	nd[a]	nd
Ni	mg/L	nd	nd

[a] Lower than detection limit, and the detection limit was 0.05mg/L.

elements Co and Ni positively affect the anaerobic digestion process of food waste, which agreed well with the conclusion from previous studies. Pobeheim et al. (2011) observed that in semi-continuous anaerobic fermentations of a defined model substrate for maize, when Ni concentrations below 0.1 mg/kg (fresh mass) at OLR above 2.6 g organic dry mass/L/day and Co concentrations below 0.02 mg/kg, the deficiency of Ni and Co had a negative impact on the system stability and methane production. Gonzalez-Gil et al. (1999) found that increased concentrations of Ni and Co in the anaerobic processes accelerated methane production rate and reduced temporary decrease rate in gas production. Patidar & Tare (2004) also reported that supplement of Ni, Co and Fe in an up-flow anaerobic sludge bed system fed with synthetic sulphate-containing wastewater could restore the process and improve the sulphate and COD removal. The stimulation effects of these two trace elements could be due to their instrumental roles in methanogenesis. Cofactor F430 with Ni catalyzes the formation of methane from methyl-coenzyme M, and Ni is essential component in factor F420, hydrogenase and CO dehydrogenase. Co is included in methyl-H_4SPT, a coenzyme M methyl-transferase complex of the methanogens (Sauer & Thauer 2000, Patidar & Tare 2004, Pobeheim et al. 2010a, Pobeheim et al. 2010b, Pobeheim et al. 2011). In this study, it was also found that the combination Co-Ni added reactor performed better than those with individual elements regarding total biogas production and process stability. Murray & Van Den Berg (1981) demonstrated that Co combined with Ni increased the conversion of acetate more in comparison to when these elements were added individually.

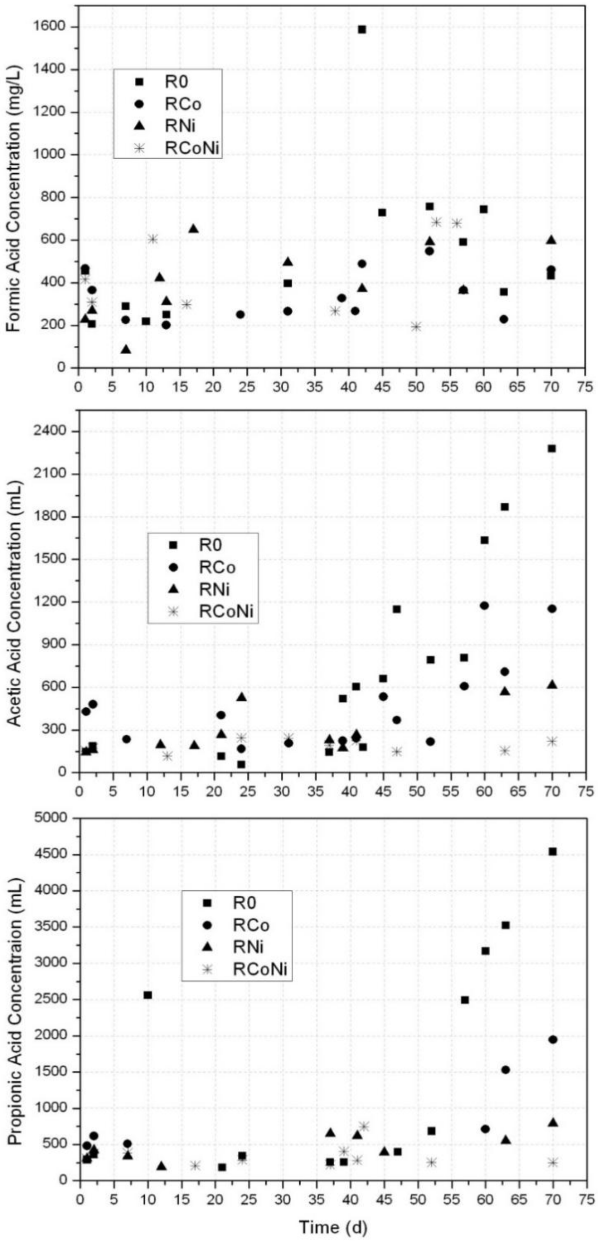

Fig. 2: Dominant VFA concentrations in reactors.

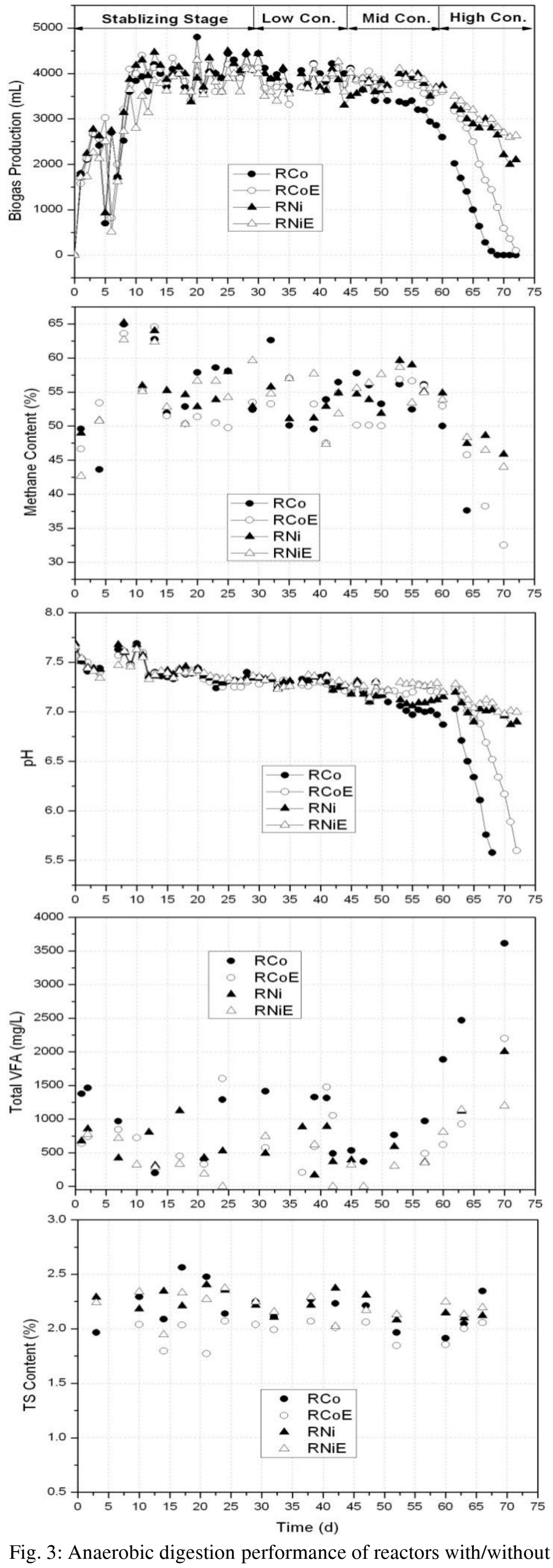

Fig. 3: Anaerobic digestion performance of reactors with/without EDTA addition.

RCo and RNi were set to investigate the effects of individual elements Co and Ni. As summarized in Table 3 and Fig. 1, although both Co and Ni added reactors were able to stabilize the digestion process in different extent, the stable operation duration for RNi was moderately extended compared with that with Co added reactor. Similar results were reported by Pobeheim et al. (2010a), in batch digestion of maize silage and a defined model substrate, the only Ni and mixture of trace elements additions gave an increase of methane production by up to 30%, whereas Co only enhanced

Table 2: Experimental design.

No.	Trace elements	EDTA(μmol/L)	Low (mg/L)	Middle (mg/L)	High (mg/L)
R0	Control	0	0	0	0
RCo	Co	0	0.05	0.50	5.00
RNi	Ni	0	0.10	1.00	10.0
RCo-Ni	Co and Ni	0	0.05 Co + 0.10 Ni	0.5 Co + 1 Ni	5 Co + 10 Ni
RCo-E	Co + EDTA	10	0.05	0.50	5.00
RNi-E	Ni + EDTA	10	0.10	1.00	10.0

Table 3: Performance parameters in anaerobic digestion of food waste.

Reactor	Theoretical Biogas Yield (mL/gVS)	Actual Biogas Yield (mL/gVS)	Y_{actual}/Y_{The}(%)	Theoretical Methane Content(%)	Average Methane Content (%)
R0		518.3	41.5		54.9
RCo		595.9	47.7		54.5
RNi	1249	677.8	54.3	55.7	53.6
RCoNi		723.1	57.9		53.5
RCoE		639.0	51.2		52.9
RNiE		657.5	52.6		54.1

the production by up to 15%. Aresta et al. (2003) also observed that Ni was more effective than Co in increasing methane/carbon dioxide ratio. These differences could be due to the important role of Ni as part of the active site in hydrogen consuming hydrogenases (Albracht 1994). Ni supplement could improve activity of the hydrogen consuming hydrogenases hence reduce the hydrogen amount in the digester and accordingly increase the CH_4/CO_2 ratio (Aresta et al. 2003). Aresta's study also suggested that after the start of the fermentation, there was no variation of hydrogen when adding Co (Aresta et al. 2003). This could be the main reason for spectacular effect of Ni added reactor. Contrary result reported by Zhang & Deokjin (2011) indicated there were no noticeable differences regarding process performance between Ni supplement reactor and control reactor. This could be due to the high Ni content in the substrate and inoculum (0.18mg/L and 0.518mg/L on wet basis respectively) compared with the Ni concentrations in this study (lower than 0.05mg/L for both substrate and inoculum).

In this experiment, the added concentrations of trace elements were designed to increase from 0.05mg/L to 5mg/L for Co and 0.1mg/L to 10mg/L for Ni. It was found no significant positive effects on process performance when higher concentrations of trace elements were added. Similar results were reported by Pobeheim et al. (2011), which suggested that an increase of Ni and Co beyond 0.6 and 0.05 mg/kg fresh mass did not further enhance biogas production.

The dominant VFA in all reactors were formic, acetic acid and propionic acid. The pH in R0 and RCo were dropping accordingly with the VFA increase. R0 and RCo were found significant propionate acid accumulation of 4541.2 mg/L and 1945.4mg/L in the final stage, and also high level of acetic acid concentrations of 2280.2mg/L and 1152.2 mg/L respectively. However, the total acid and individual acid levels in RCo were much lower than that in R0, suggesting that Co supplement was moderately effective in avoiding VFAs accumulation in the anaerobic digester. The individual VFA levels showed less variation in the Ni received reactors RNi and RCoNi. The acetic and propionate acid concentrations slightly increased for RNi to 615.3 mg/L and 791.2mg/L in the end; and no considerable accumulation for RCoNi with the final concentrations of 219.7mg/L and 248.7mg/L. This result indicated that Ni was more effective than Co in improving the turnover of VFAs to methane and avoiding toxic effects of VFAs.

There were studies demonstrating that when only carbohydrates were degraded, the partial pressure of hydrogen increased more easily, and the higher partial pressure of hydrogen would inhibit the degradation of propionic acid (Pind et al. 2003, Deublein & Steinhauser 2011). It was also suggested by Ahring et al. (1995) that acetate concentrations higher than 13 mM (780mg/L) indicated process imbalance. Deublein & Steinhauser (2011) thought that when pH was lower than 7, the inhibition concentration for acetic acid could be up to 1000 mg/L. In this experiment, the acetic acid in R0 and RCo reached over 2000 mg/L and 1000mg/L, which thus show a considerable imbalance in the reactor. Another VFA indicator, the ratio of propionate/acetate, was found that if it exceeded 1.4 for the continuous stirred-tank reactor system, there could be imbalance in reactor (Ahring et al. 1995). It is observed in the experiment that the ratio in R0 and RCo exceeded 1.4 to 3.0 and 2.1 in the later stage of the fermentation, suggesting a gradual crash of the system. Regarding formic acid, normally formic acid is easily degradable and not considered as an indicator for the system stability (Dinsdale et al. 2000).

Only low concentrations of butyric and isobutyric acid were found in the reactors, which was unusual but explainable. The substrate used here was dominated by carbohydrates, which produce acetic and butyric acid when degraded. However, the formation of acetic acid produces twice as much as ATP than the formation of butyric acid, which could be the reason that much less butyric acid appeared in the reactors (Deublein & Steinhauser 2011). Propionic acid was the last one to increase among the main VFA observed. Since propionic acid was considered as a better indicator among the others, in this experiment, it seems that the propionic acid was less sensitive than acetic acid to system instability (Varel et al. 1977, Kaspar & Wuhrmann 1978). This might be because that the conversion of acetic acid to hydrogen and carbon dioxide is quite sensitive to hydrogen partial pressure. When the pressure increases, this conversion could be inhibited and bring on accumulation of acetic acid (Deublein & Steinhauser 2011).

Comparison of anaerobic digestion with/without EDTA addition: Table 3 depicts a moderately positive effect on biogas production when adding EDTA together with Co: higher biogas yield of 639.0mg/L for RCoE compared that 595.9mg/L for RCo. In Fig. 3, it was found that although the EDTA-Co reactor demonstrated a sharp methane production decrease from around 4000mL/d to 100mL/d, the crash happened few days later than that in only Co added reactor. Thus, the EDTA seemed to delay the failure that the Co-reactor with no EDTA experienced. A delay in the pH decrease also occurred with the EDTA-Co reactor, and the final VFAs concentration of 2200.3mg/L in reactor with EDTA added was considerably lower than the Co only one (3612.2mg/L). On the other hand, no significant improvements were observed for EDTA-Ni treatments. The effects of EDTA addition with Ni on biogas production, methane content, pH and VFA stabilization were not as considerable as for Co with EDTA addition.

According to study from Wu & Steinhauser 2008), the stable constant Kr of EDTA with Ni is 18.6 which is higher than that of Co, 16.2. It means that the chelation of EDTA with Ni is stronger than with Co. Strong chelation does not always improve the bioavailability of the metals, since a too strong chelation disable the binding ability of metal to the active site on the surface of cells, and is therefore affecting the absorptivity negatively (Wu 2008). That could be the reason that the EDTA with Ni did not perform as well as that with Co.

CONCLUSION

In this study, it was found that the trace elements Ni and Co were effective to improve anaerobic biogas production and process stability. Addition of these elements stabilized the VFA, pH, methane and TS content in the digesters. For individual elements, it was observed that Ni played a more important role in anaerobic digestion of present substrate and reactor set up than Co. This was confirmed by the experiment, where the Ni and Co mixture added reactor performed better than those with individual elements added. EDTA addition with Co was found moderately effective compared to the Co only reactor, whereas EDTA with Ni supplement showed no improvement.

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