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Research article

Long-term influence of trace element deficiency on anaerobic mono-digestion of chicken manure

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ABSTRACT

Recent findings showed that some trace elements essential for anaerobic digestion might be deficient in chicken (laying hens) manure. In this study, the long-term influence of trace element deficiency on anaerobic mono-digestion of chicken manure was investigated. Three bench-scale anaerobic reactors were operated with or without trace element supplementation. As trace element, only Se or a mix containing Co, Mo, Ni, Se, and W was added to the reactors. The results revealed that in anaerobic digestion of chicken manure at total ammonium nitrogen concentrations over 6000 mg L^{-1}, Se supplementation was critical but not sufficient alone for long-term stable CH_4 production. Addition of a mix consisting of Co, Mo, Ni, Se and W resulted in a more stable digestion performance. Daily trace element mix supplementation promoted the hydrogenotrophic *Methanoculleus bourgensis*, which is an ammonia tolerant methanogen. The decrease in the relative abundance of *Methanoculleus* detected after termination of trace element addition and resulted in accumulation of acetate and propionate that followed by a significant decrease in CH_4 production.

1. Introduction

With the intensification of animal protein products and development of large-scale chicken farming, significant amounts of manure generated throughout the world (Lynch et al., 2013). Chicken (egg-laying hen) manure contains excreta including feces and urine which collected on the conveyor belts (Mitchell and Donald, 1993; Molaey et al., 2018a). This manure contains high amount of plant nutrients such as nitrogen, phosphorous, potassium and calcium that generally used on land as an organic fertilizer (Kelleher et al., 2002). However, over-application can lead to nitrate leaching into groundwater, phosphorus runoff into surface water, pathogen releasing, greenhouse gas emissions, crop toxicity and human health effect (Bayrakdar et al., 2017a; Kelleher et al., 2002). Hence, inappropriate management of this large amount of bio-waste rises a concern in agricultural sectors which try to use sustainable alternatives for disposal (Santos Dalólio et al., 2017). On the other hand, chicken manure contains high amount of easily biodegradable organic matter, hence; anaerobic digestion is considered as an alternative method to stabilize the waste and recover the profitable bioenergy (Nie et al., 2015; Niu et al., 2015).

Identification of the factors influencing microbial community dynamics, diversity and activity in anaerobic digestion provides information related to degradation process. Among different process parameters,ammonia has been reported as an important factor affecting microbial ecology (Molaey et al., 2018a, 2018b), which may result in irreversible failure of anaerobic reactors.

As a result of biological degradation of nitrogen-rich chicken manure, mostly in the forms of proteins and urea, free ammonia and ammonium ion are generated (Bayrakdar et al., 2017a; Bolan et al., 2010). However, although low levels of ammonium are necessary for optimum growth of anaerobic consortia and for establishment enough pH buffering capacity (Kayhanian, 1999); free ammonia molecules may diffuse through cell membrane causing in proton imbalance and/or potassium deficiency (Chen et al., 2008; Shi et al., 2017).

It is reported that at total ammonia nitrogen (TAN) concentrations greater than 3000 mg L^{-1}, inhibition of the anaerobic process occurs independent of operating pH (Tao et al., 2017) and exceeding 4000 mg L^{-1} severe inhibition of methanogens occurred (Procházka et al., 2012). Thus ammonia inhibition is reported as the major obstacle in the anaerobic digestion of chicken manure (Bayrakdar et al., 2017a; Molaey et al., 2018a).

Dissection of microbial communities may reveal the causes of reactor instability and thus introduce optimal environmental conditions to key microbial communities indispensable for stable anaerobic

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degradation process (Molaey et al., 2018a).

Whether trace element addition could induce the anaerobic digestion efficiency at high TAN concentrations, is still a hot topic of debate in the literature. Only a few studies have focused on the dynamics of anaerobic microbiota corresponding to the addition of trace element (Feng et al., 2010; Molaey et al., 2018a, 2018b; Plugge et al., 2009; Westerholm et al., 2015; Wintsche et al., 2016). Plugge et al. (2009) reported that trace element deficiency may depress the syntrophic acetate oxidation at high TAN concentrations and trace elements such as Co, Mo, Ni, Se and W are the most important nutrients for the enzymes active on the syntrophic methane production pathway (Molaey et al., 2018b).

Contrary to the results reported by Schattauer et al. (2011), in our previous study, we found that the trace elements essential for anaerobic digestion such as W, Se, and Co might be deficient in chicken manure especially in laying hens excreta (Molaey et al., 2018a). Therefore, this study aimed to reveal the long-term influence of trace element addition and deficiency in anaerobic mono-digestion of chicken manure and define the relationship between the trace elements and methanogenic population at high TAN concentrations ($> 6000\ mg\ L^{-1}$).

2. Materials and methods

2.1. Chicken manure feedstock

Chicken manures used in this study were collected from a biogas plant operated with egg-laying hen manure located in Afyonkarahisar province of Turkey. They were transferred to the laboratory in 30 L airtight plastic drums and stored in a refrigerator at 4 °C until use. The average total solid (TS), volatile solids (VS) and total Kjeldahl nitrogen (TKN) concentrations of chicken manure samples are given in Table 1.

2.2. Anaerobic reactors and operation conditions

Three 1.3-L anaerobic digesters with 0.8-L active volume were operated feeding with chicken manure daily. Before starting this experiment, the reactors were operated for about 9 months similarly and supplemented with different trace elements. The results of that study were presented elsewhere (Molaey et al., 2018a).

As in the last part of the former study, R1 was operated as a control here without trace element supplementation (Table 2). R2 was supplemented with only $0.2\ mg\ L^{-1}$ of Se and R3 with a mix containing $1\ mg\ L^{-1}$ of Co, $1\ mg\ L^{-1}$ of Ni, $0.2\ mg\ L^{-1}$ of Mo, $0.2\ mg\ L^{-1}$ of Se and $0.2\ mg\ L^{-1}$ of W. After day 110, trace element addition was terminated in R3. The type and concentration of trace elements were selected based on a previous study reported by Banks et al. (2012). As a source of trace elements, the reagent grade salts of $NiCl_2.6H_2O$, $CoCl_2.6H_2O$, $Na_2MoO_4.2H_2O$, Na_2SeO_3 and $Na_2WO_4.2H_2O$ were used.

Reactors were placed in an incubator (WTW, TS606/4-i) at 36 °C ± 1 °C and continuously stirred on an orbital shaker (Biosan, PSU-20i). They were fed manually once a day after withdrawal of digestate. During the withdrawal of digestate, the reactors were agitated vigorously on a magnetic stirrer (Velp-AGE) and digestate was taken using a 60 mL syringe. After sampling, they were fed using the same syringe according to the applied organic loading rate (OLR) of $3.65\ g\ L^{-1}\ d^{-1}$ based on VS. The routine digestate quality and metagenomics analyses were performed by using the samples selected among the digestate withdrawn daily from the reactors. Throughout the study, the hydraulic retention time (HRT) was kept constant at 30 days.

The biogas was collected in aluminum foil bags and the volume of produced biogas measured daily with water displacement method.

Table 2
Trace element (TE) addition schedule and durations.

Reactor	In the former study (Molaey et al., 2018a)	In this study
R1	No TE (the last 34 days)	No TE (Day 0–211)
R2	Only Se (the last 34 days)	Only Se (Day 0–211)
R3	TE-mix (262 days)	TE-mix (Day 0–110)
		No TE (Day 110–260)

2.3. Analytical methods

The digestate samples taken daily were analyzed for total alkalinity, TKN, TS and VS two times a week according to standard methods (APHA-AWWA-WEF, 2005). TAN concentration was determined with nesslerization method using a spectrophotometer (WTW 6100, Germany). Free ammonia nitrogen (FAN) concentration was calculated as described in Calli et al. (2005). pH was measured using a pH meter (Eutech, PCD 6500). Volatile Fatty Acids (VFAs) and biogas composition were analyzed using two separate gas chromatographs (Shimadzu GC-2014) equipped with flame ionization detector and thermal conductivity detector, respectively. In both analyses, the methods described by Bayrakdar et al. (2017a) were used.

For soluble trace element analyses, digestate samples were first centrifuged and the supernatant was acid digested in a microwave (CEM, MARS-5). Finally, Ni, Mo, Se, W and Co concentrations were determined by ICP-MS (Agilent 7700).

2.4. DNA extraction, amplification, sequencing and processing of the reads

To investigate the influence of trace element addition on methanogenic population dynamics, two digestate samples were taken from each reactor for metagenome analysis. From R1 and R2 samples were taken on days 5 and 85 and from R3 were taken on days 85 and 209 (Fig. 2). Because the amount of genomic DNA extracted was not sufficient, the sample taken from R1 on day 85 could not be analyzed.

The archaeal community of the samples taken from the reactors was assessed using metagenomics approach based on next-generation DNA sequencing. The DNA was extracted using a powerSoil DNA Isolation Kit (FastDNA SPIN, MP Biomedicals) following the manufacturer's instructions. For the microbial community analysis, polymerase chain reaction (PCR) amplification was performed with 16S rRNA barcoded primers: 349F GYGCASCAGKCGMGAAW and 806R GGACTACVSGGGTATCTAAT. The thermal profile of the archaeal PCR was conducted under the following conditions: initial denaturation for 3 min at 95° C followed by 28 cycles of denaturation at 94° C for 30 s, annealing at 53° C for 40 s, and elongation at 72° C for 1 min. The amplified 16S rRNA amplicons were purified using beads kit (AMPure, Agencourt Bioscience Corporation) and then sequenced with Ion PCMTM platform in a private laboratory. Finally, operational taxonomic units (OTUs)

Table 1
Characteristics of chicken manures used in this study (the numbers in parenthesis indicates in which days the chicken manure was used).

Parameter	CM-1(0–72)	CM-2(73–112)	CM-3(113–166)	CM-4(167–233)
TS (g/kg CM)	309.3 ± 2.9	275 ± 0.7	258.2 ± 4.9	261 ± 2.7
VS (g/Kg CM)	214 ± 2	190.9 ± 0.8	173.2 ± 4.6	177.3 ± 1.6
TKN (g/kg TS)	47.6 ± 0.128	48.7 ± 2.079	47.21 ± 1.57	46.8 ± 1.5

CM: Chicken manure.