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Research paper

Anaerobic digestion of chicken manure: Mitigating process inhibition at high ammonia concentrations by selenium supplementation

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ABSTRACT

In this study, the anaerobic digestion of nitrogen-rich chicken manure from egg-laying hens was investigated via long-term continuous experiments with and without the addition of different trace elements. With trace element supplementation, a CH_4 yield of 0.26 ± 0.03 $m^3\ kg^{-1}$ of volatile solids (VS) added was achieved at an organic loading rate (OLR) of 3.62 kg $m^{-3}\ day^{-1}$ based on VS and total ammonia nitrogen (TAN) content greater than 7200 g m^{-3}. Selenium (Se) was identified as the critical trace element for the stable anaerobic digestion of chicken manure. The dominant methanogen in the reactors was the hydrogenotrophic methanogen *Methanoculleus bourgensis*. Therefore, we concluded that at elevated TAN concentrations, the CH_4 production stimulated by Se supplementation likely occurred through syntrophic acetate oxidation. Without trace element supplementation, severe acetic and propionic acid accumulation occurred, causing the CH_4 yield to decrease below 0.12 $m^3\ kg^{-1}$ of VS added.

1. Introduction

The rising demand for animal protein has led to intensive and mechanized chicken farming operations. These farms provide meat and eggs at much lower cost than traditional ways [1,2]. With increasing intensive chicken farming, large amounts of waste are being produced. Broiler (meat-chicken) litter contains excreta including faeces and urine, and bedding materials such as wood shavings, straw and peanut hulls; however, egg-laying hen manure (caged layer manure) is usually contains excreta and collected on conveyor belts [3]. Both type of waste contains feathers and some amount of wasted feed.

Chicken manure from layers is among the most valuable manures containing considerable amounts of major plant nutrients nitrogen, phosphorus and potassium [4]. However, its excess application to soil as fertilizer causes serious environmental concerns. Nitrate leaching into groundwater, phosphorus run off into surface water bodies, release of pathogenic microorganisms and greenhouse gasses are the main problems encountered if the chicken manure is used without a proper treatment [5,6].

Anaerobic digestion (AD) is one of the favourable options to stabilise the organic matter in chicken manure along with the biogas production [5,7]. In this way, the biogas produced may be converted to heat and electricity [5] and the digestate may be used as a sustainable agricultural fertilizer [8]. The organic nitrogen in chicken manure, which exists in the form of undigested protein and uric acid, is hydrolysed to ammonia during the AD process [8,9].

Ammonium is the primary source of nitrogen used in microbial cell growth. Whilst ammonium is beneficial to microorganisms up to a certain level, increased ammonia concentrations inhibit the anaerobic digestion (AD) and methane production in particular [10]. To alleviate the ammonia inhibition in AD process; acclimation of methanogenic biomass to high ammonia concentrations [11], controlling the digester pH [12] and temperature [13], removal of ammonium from the digester by means of struvite precipitation [14], zeolite adsorption [15,16] stripping [17] and membrane separation have been applied in number of studies [18]. Although these processes mitigate the ammonia inhibition, in practice, they make the AD process difficult to operate [19]. Therefore, ammonia inhibition is the major problem encountered in AD of nitrogen rich organic wastes [10,20].

In some recent studies, trace element supplementation was investigated to minimize the operational cost in AD of nitrogen rich substrate by operating the digester at higher loading rates [21,22]. The stability of AD process and the CH_4 production at high TAN concentrations were reported to improve with the addition of trace

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elements especially selenium (Se), cobalt (Co) and tungsten (W) to anaerobic food waste digesters [21,23]. Westerholm et al. [24] reported that at high TAN concentrations the CH_4 production is achieved via syntrophic acetate oxidation (SAO) followed by hydrogenotrophic methanogenesis. If the essential trace elements are lacking, SAO does not occur efficiently at elevated TAN levels, causing volatile fatty acids (VFAs) accumulation during AD [25,26]. The redox-enzyme, formate dehydrogenase (FDH) plays an important role in SAO and its effectiveness depends on the availability of trace elements such as Se, Mo and W [21,26–28].

Although some recent findings contradict with it [29], chicken manure is generally assumed to contain sufficient amounts of trace elements for AD. Accordingly, in former studies, trace element deficiency was not considered a problem in AD of chicken manure. Although the influence of trace element supplementation on the performance of food waste digesters was studied intensively, there is lack of knowledge about how the trace element supplementation affects the stability and CH_4 yield in AD of chicken manure at high organic loading rates and TAN levels.

Therefore, the main challenge is to develop a reliable and easy method to counteract the ammonia inhibition in AD of nitrogen-rich organic wastes. In this study, the enhancement effect of trace element supplementation on AD of chicken manure from egg-laying hens was studied for the first time by operating four laboratory-scale continuously fed anaerobic reactors in parallel for 262 days with and without supplementation of different trace elements.

2. Methods

2.1. Methanogenic inoculum and chicken (egg-laying hen) manure

The inocula used in the continuous digestion experiments were taken from a 16-L laboratory-scale mesophilic anaerobic digester fed with raw chicken manure and spent poppy straw. The details of the lab-scale anaerobic digester are given elsewhere [5,30]. Chicken manure was collected from egg-laying hen farm located in Afyonkarahisar, Turkey at four different times and was stored in 30 L airtight barrels at 4 °C ± 1 during the study. After arrival in the laboratory, dry matter (DM) of chicken manure, volatile solids (VS), total Kjeldahl nitrogen (TKN) and sulphur contents of the samples were analysed according to APHA standard methods [31].

The average DM, VS, TKN concentrations, total sulphur contents of the chicken manure and the methanogenic inoculum used in this study are given in Table 1. The exceptionally high total sulphur content of CM-III was attributed to the broken eggs in the sample.

Before using CM-III in experiments, the shells of broken eggs were removed, but the yolks and whites remained in the manure.

2.2. Long-run continuous digestion experiments

Four 1.3-L anaerobic reactors with active volumes of 0.8 L were operated for 262 days with daily feeding. The digesters were incubated at 36 °C ± 1 °C (WTW, TS606/4-i) and mixed continuously on an orbital shaker (Biosan, PSU-20i). The hydraulic retention time was kept constant at 30 days throughout the experiments and OLR, which represents the amount of VS loaded per unit volume of reactor per day, was increased stepwise from 2.5 kg m^{-3} day^{-1} to 3.62 kg m^{-3} day^{-1}.

The biogas produced was collected in aluminium foil bags. Digestate withdrawal and feeding were performed daily through the port shown in Fig. 1. The volumes of biogas collected in the gas bags were measured every day with a weight type gasometer.

The anaerobic reactors were operated with and without addition of different trace elements. The trace elements supplied, their concentrations and the supplementation regimen are depicted in Fig. 2. The stock solutions of trace elements containing 1250 g m^{-3} of Ni^{+2} as $NiCl_2{\cdot}6H_2O$, 1250 g m^{-3} of Co^{+2} as $CoCl_2{\cdot}6H_2O$, 250 g m^{-3} of Mo^{+6} as $Na_2MoO_4{\cdot}2H_2O$, 250 g m^{-3} of Se^{+4} as Na_2SeO_3 and 250 g m^{-3} of W^{+6} as $Na_2WO_4{\cdot}2H_2O$ were prepared in distilled water.

2.3. Analytical methods

The digestate withdrawn from the continuous digesters was analysed for pH every two days and for alkalinity, DM, VS, TKN and TAN once a week according to standard methods [31]. Free ammonia nitrogen (FAN) was calculated using the formula given by Hansen et al. [32]. VFAs in the digestate were analysed twice a week using a gas chromatograph (Shimadzu GC-2014) equipped with a flame ionisation detector. The details of the VFA analyses are described elsewhere [5]. The CO_2, H_2S and CH_4 contents of the biogas samples taken from the gas bags were analysed using another gas chromatograph (Shimadzu GC-2014) equipped with a thermal conductivity detector according to the method of Reddy et al. [33]. The unionised and total dissolved sulphide concentrations in the reactors were estimated based on the partial pressure of H_2S gas according to the calculations described by Sürmeli et al. [34]. Total trace element analyses of chicken manure and digestate samples were determined by microwave-assisted acid leaching technique [35]. 0.5 g of dry sample was ground and acid digested with concentrated nitric acid in a digestion vessel by using a temperature controlled microwave oven (CEM, MARS-5). Temperature was ramped to 200 °C in 15 min and held for 20 min at 200 °C. Digestion vessels were rinsed with ultra-pure water. The content of digestion vessel transferred to the volumetric flasks and volume was filled to 100 cm^3. The total Ni, Mo, Se, W, Co and Fe contents were then determined by ICP-MS (Agilent 7700).

2.4. Metagenomic analysis of the methanogenic archaeal community

The distributions of methanogenic archaea in the digestate samples taken on day 256 from R1 and on days 184 and 214 from R4 were investigated using a metagenomics approach based on next-generation DNA sequencing.

DNA was extracted using an isolation kit (FastDNA SPIN, MP Biomedicals) according to the manufacturer's instructions. PCR amplification was performed with 16S rRNA universal archaeal primer set (349F: GYG CAS CAG KCG MGA AW and 806R: GGA CTA CVS GGG TAT CTA AT) and using PCR master mix (HotStar Taq Plus Master Mix, Qiangen) under the following conditions: 94 °C for 3 min; followed by 28 cycles of 94 °C for 30 s; 53 °C for 40 s and 72 °C for 1 min.

After PCR, all amplicons were diluted to the same concentration and cleaned up using a purification kit (Agencourt AMPure, Beckman

Table 1
Characteristics of raw chicken manure (CM) and methanogenic inoculum.

Parameter	CM-I	CM-II	CM-III	CM-IV	Inoculum
DM (g kg^{-1} of wet weight)	253.4 ± 4	314 ± 1	287 ± 2	293 ± 10	25 ± 7
VS (g kg^{-1} of wet weight)	179.3 ± 15	199.9 ± 12	202.7 ± 17	204.1 ± 30	13 ± 2
TKN (g kg^{-1} of DM)	47.4 ± 2	54.4 ± 3	49.2 ± 2	47.62 ± 2	1.25 ± 0.04
Total Sulphur (g kg^{-1} of DM)	0.64	–	25	0.60	–

CM: Chicken manure, DM: Dry matter, VS: Volatile solids, TKN: Total Kjeldahl nitrogen.