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Effects of molybdenum, selenium and manganese supplementation on the performance of anaerobic digestion and the characteristics of bacterial community in acidogenic stage

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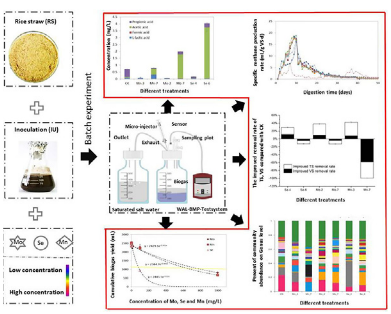
GRAPHICAL ABSTRACT



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ABSTRACT

The addition of trace elements to aid anaerobic digestion has already been widely studied. However, the effects of rare trace elements on anaerobic digestion remain unclear. In this study, the effects of Mo, Se and Mn on anaerobic digestion of rice straw were explored. The results showed the methane yield increased by 59.3%, 47.1% and 48.9% in the first 10 days following addition of Mo (0.01 mg/L), Se (0.1 mg/L) and Mn (1.0 mg/L), respectively. Toxic effects and the accumulation of volatile fatty acids (VFAs) were observed when the Se, Mo and Mn concentrations were greater than 100, 1000 and 1000 mg/L, respectively. The half-maximal inhibitory concentrations (IC_{50}) for Se, Mn and Mo were 79.9 mg/L, 773.9 mg/L and 792.3 mg/L, respectively. The addition of trace elements has changed the bacterial structure of the bacteria, which in turn has affected the digestion performance.

1. Introduction

A large amount of agricultural waste is produced in China every year, the main types of waste are wheat straw, corn stover, and rice straw (RS); these account for approximately 25%, 28%, and 47% of total crop residue, respectively (Chen, 2016). The disposal of this waste has caused serious environmental pollution issues. Anaerobic digestion is often used to treat various organic wastes to produce bioenergy. However, because of the high C/N of crop straw, anaerobic systems often become instable due to the accumulation of volatile fatty acids (VFAs), which can be alleviated via co-digestion with livestock manure (Thamsiriroj et al., 2012; Cai et al., 2017). But this approach has increased the difficulty of follow-up processing because of the additional digestate produced.

Trace elements play an important role in microbial metabolism and are widely used as additives in anaerobic digestion to alleviate the

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accumulation of VFAs. Fe, Co and Ni are the most important trace elements in this field and have already been studied extensively (Cai et al., 2018; Khatri et al., 2015; Shakeri Yekta et al.,2014; Yu et al., 2015). However, the roles of other rare elements, such as Mo, Se and Mn, remain unclear. The effects of minimum and excessive concentrations of Mo, Se and Mn on the microbial activity and performance of anaerobic digestion have not yet been explored (Romero-Güiza et al., 2016).

Previous studies have demonstrated Se is not required in the acetoclastic methanogenesis pathway (Deppenmeier, 2002). However, Se is considered to be extensively involved in the hydrogenotrophic methanogenic pathway because selenoproteins, which include formylmethanofuran dehydrogenase, formate dehydrogenase and hydrogenase, are found in this pathway (Jones et al., 1979; Vorholt et al., 1997). Banks et al. (2012) has reported that, after the addition of Se, the degradation of VFAs significantly improved in the anaerobic digestion of food waste with a high ammoniacal nitrogen concentration. Furthermore, two studies have reported that Mn plays an important role in the stabilization of methyltransferase and redox reactions (Fisher et al., 1973; Perry and Silver, 1982). In addition, Mo is closely involved in formylmethanofuran dehydrogenase (hydrogenotrophic methanogens) and formate dehydrogenase (syntrophic oxidizing bacteria and hydrogenotrophic methanogens) (Jiang et al., 2017). Although Mo is considered to be chemically analogous with W in enzyme formation (Kletzin and Adams, 1996), Mo cannot be replaced by other trace elements for any methanogenic species. Researchers have suggested that clarification of the rare trace elements (Mo, Se and Mn) required in the anaerobic digestion of these microorganisms is important (Thanh et al., 2016). Finally, to prevent overdosing, the inhibitory concentrations (IC_{50}) of these elements need to be established for anaerobic digestion.

A large number of reports have confirmed that the addition of trace elements can change the archaeal community structure (Neumann and Scherer, 2011; Cai et al., 2018; Munk and Lebuhn, 2014; Milan et al., 2010). Cai et al. (2018) has found the number of archaea significantly increases when 50 mg/L Fe^{2+} is added. Neumann and Scherer (2011) has reported that trace element mixtures can significantly change the archaeal community structure and increase the number of hydrogen-producing methane bacteria by 20%. In addition, Stock and Rother (2009) has shown bacterial metabolism requires the participation of trace elements. However, how indigenous bacterial communities respond to the addition of trace elements remains unknown. Bacteria are primarily involved in the acidification stage of anaerobic digestion, which is considered a rate-determining step (Yu et al., 2016). Therefore, exploring the effects of the addition of Mn, Mo and Se on bacterial community structure is valuable.

Batch experiments were designed in this study. Se, Mn and Mo were added to the anaerobic digestion of rice straw to determine the effects of these trace elements at different concentrations on the performance of anaerobic digestion. The biogas and methane yields, VFA and methane content and total solid (TS) and volatile solid (VS) removal rates were used as indicators. A first-order kinetic model and the modified Gompertz model were used to fit and predict related indicators, and the IC_{50} values were calculated from the results. We hypothesize bacterial community structures will respond to the addition of Mn, Mo and Se.

2. Materials and methods

2.1. Rice straw and inoculum

The substrate used in the current study was rice straw, which was obtained from the Shangzhuang experimental station at China Agriculture University. After the rice straw was harvested and air-dried, the dried rice straw was pulverized with a high-speed grinder and screened (1 mm) to ensure homogeneity of the substrate. The pulverized rice straw was stored at −20 °C until needed for use. The inoculum used in this study was obtained from a biogas plant that used cow manure as a substrate for the previous 2 years. Prior to use, the inoculum was incubated in glucose solution (1.0 g/L) for two weeks at 35 ± 1 °C to ensure microbial metabolic activity. Table 1 shows the characteristics of the inoculum and rice straw.

Table 1
Chemical characteristics of the inoculum and raw material.

Parameter	Inoculum (Solid)	Inoculum* (Liquid)	Rice straw
Mn (mg/kg TS)	237.85 (4.85)	0.087 (0.002)	169.50 (7.67)
Mo (mg/kg TS)	8.93 (6.68)	NA	0.59 (0.2)
Se (mg/kg TS)	0.14 (0.18)	0.007 (0.005)	0.19 (0.20)
Cellulose (% TS)	ND	ND	36.50 (0.05)
Hemi-cellulose (% TS)	ND	ND	24.65 (0.96)
Lignin (% TS)	ND	ND	2.08 (0.56)
C (mg/kg TS)	3,15,800 (0)	NA	4,59,300 (6680)
N (mg/kg TS)	15,820 (650)	6,27,000 (20,000)	6910 (180)
C/N	19.96 (0.77)	ND	66.43 (1.44)
pH	ND	7.8 (0.1)	ND
TS (% FW)	3.37 (0.23)	ND	92.80 (0.10)
VS (% TS)	58.71 (0.55)	ND	88.43 (0.38)

Note: Values are expressed as mean and figures in parentheses are standard deviations (n = 3).
* Trace elements concentration unit of inoculum (Liquid) is mg/L. NA: Beyond the detection limits. FW: fresh matter, TS: total solid, VS: volatile solid, ND: Not determined.

2.2. Chemicals

The sources of Mn, Mo and Se were $MnCl_2·4H_2O$, $Na_2MoO_4·2H_2O$ and $Na_2SeO_4·10H_2O$ (99% purity), respectively. First, different concentrations of Mn, Mo and Se (0.01, 0.1, 1, 10, 100, 1000 and 10,000 mg/L) were prepared in 10 mL plastic tubes. These concentrations were then added to the reactors. The same volume of physiological saline water was used as a control. Table 2 shows the specific concentrations of the trace elements added.

2.3. Experimental set-up

In this study, batch experiments were designed and performed in 500-mL reactors (with a working volume of 200 mL) equipped with a rubber stopper. First, rice straw (8 g, TS content = 92.8%) and inoculum (200 mL, TS content = 3.3%) were loaded into each reactor. Second, all reactors were flushed with N_2 (99.999% pure) for 2 min to ensure anaerobic conditions. Finally, the contents of the reactors were fermented at 35 ± 1 °C in a temperature-controlled room.

All treatment conditions were performed in sextuplicate. Every day, a 300-μL gas sample was taken to determine the CH_4 and CO_2 content. Biogas was removed using an exhaust bottle, which was filled with 75% saturated sodium chloride solution. To prevent sampling from interference, three reactors were stopped and removed from the temperature-controlled room after the biogas pressure peaked and used as three replicates. Samples from the three reactors were used to determine the concentrations of VFAs (lactic acid, formic acid, acetic acid and propionic acid) and the TS and VS content and the bacterial community structure via high-throughput sequencing. For the convenience of analysis, two treatments were selected for each trace element according to the digestion performance. For example, Mn-3 and Mn-7 were selected for Mn treatments.

2.4. Analytical methods

2.4.1. Determination of the basic properties of materials

The total nitrogen (TN), total carbon (TC), TS and VS content was measured using standard methods (APHA, 2005). The lignocellulose content in the rice straw was determined using a fiber analyzer