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Effect of Fe⁰ addition on volatile fatty acids evolution on anaerobic digestion at high organic loading rates

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ABSTRACT

Excessive acidification frequently occurs in the anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) at high organic loading rates (OLR), due to the accumulation of non-acetic volatile fatty acids (VFA). In this study, the performance of Fe⁰ in enhancing various VFA production and metabolism was investigated. The butyric acid concentration in a high OLR reactor with Fe⁰ addition decreased from 7200 to 0 mg/L after a short lag phase, and the total VFA (TVFA) concentration also decreased substantially. The corresponding dominant acidogenesis type also changed from butyric type to propionic type fermentation. Furthermore, the CH₄ yield of the reactor with added Fe⁰ was approximately 595 ml CH₄/g VS_{added}, which was an increase of 41.7% compared with the biochemical methane potential (BMP) test results in controls without added ZVI. A microbial diversity analysis, using high throughput sequencing, showed that *Methanofollis* and *Methanosarcina* were dominant in terms of the archaeal structures of the Fe⁰ reactor at the butyric converting stage; however, *Methanosaeta* was predominant in the reactor during the control BMP test. These results suggested that Fe⁰ can convert non-acetic VFA to acetic VFA and improve the CH₄ yield by enhancing the activity of methanogens.

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1. Introduction

Hydrolysis and acidogenesis are key steps in the anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW). The macromolecules in OFMSW, such as carbohydrates, proteins, and lipids, are dissolved and hydrolysed first and then further degraded by acidogens, forming volatile fatty acids (VFAs), including acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, as well as hydrogen. Acetic acid is a good substrate for methanogens, but other non-acetic VFAs cannot be utilized directly for methane (CH₄) production and therefore must be converted to acetic acid. Generally, both the hydrolysis and acidogenesis processes have a short duration, and during this period, the pH in the reactor would drop to a certain level (such as 6.5), and then would recover to a favorable level (6.6–7.4) for methanogens, due to the buffering capacity of the increasing alkalinity (ALK) (Zhong et al., 2012). However, in the AD of OFMSW at high organic loading rates (OLRs), due to the fast rate of hydrolysis and acidogenesis processes, pH declines substantially and is irreversible (Izumi et al., 2010; Ma et al., 2011), which has a negative effect on microbial activity. This phenomenon is called excessive acidification. The

excessive acidification results in the failure of methanogenesis and the emission of some acidic malodorants (Kong et al., 2016a).

Excessive acidification is caused mainly by the high concentrations of non-acetic VFAs. In a normal AD system without acidification risk, the ratio of VFA to ALK should lower than 0.3. Nagao et al. (2012) discovered that the main VFAs were acetic and propionic acids when the total VFA (TVFA) concentration was less than 5000 mg/L. In contrast, butyric acid was dominant in AD reactors when the TVFA concentration was greater than 5000 mg/L. Furthermore, the accumulated butyric acid could not be degraded further. Gadhe et al. (2014) evaluated hydrogen production during the AD of ultrasonic pretreated food waste and found that the ratios of the butyric to acetic acid concentrations were high in all reactors, with the highest hydrogen yield observed at a concentration ratio of butyric to acetic (H_{Bu}/H_{Ac}) of 2.2 and a pH of 5.5.

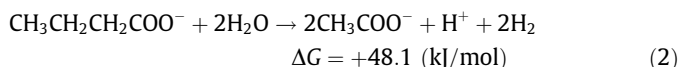
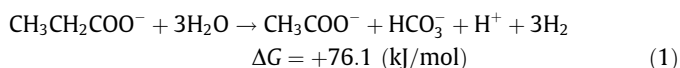
The most widely used method to relieve excessive acidification at present is to add alkalinity sources, such as sodium hydroxide, to buffer excessive VFAs in the system. In addition, alkaline pretreatment (Janke et al., 2016) and the precipitation of VFAs via the addition of metal ions (Jin et al., 2015) have been reported to be effective in reducing VFA concentrations. However, some problems exist when using these methods. They increase the OFMSW disposal costs, especially when overdosing occurs, and the dissolved alkalinity sources would be wasted and discharged with the efflu-

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ent. Furthermore, the salinity and metal ion concentration of mixtures in reactors can be increased, which leads to further difficulties in the disposal of the digested effluent.

Zero valent iron (ZVI) is a reductive material that donates electrons for methanogens producing CH_4 , reduces the oxidation-reduction potential (ORP) of the system, and increases methanogenic activity (Zhen et al., 2015; Xiao et al., 2013; Zhang et al., 2015). The substrates used in most studies were activated sludge or synthetic wastewater, with a single carbon source, while few studies have focused on OFMSW, which is more complicated substrate and it is much easier to hydrolyse and acidify due to rich biodegradable fractions. A good performance of ZVI in inhibiting excessive acidification in the AD of OFMSW at high OLRs was reported in our previous study (Kong et al., 2016a). Another important effect of ZVI is to enhance the conversion of other VFA types to acetic acid. In traditional AD reactors, the conversions of butyric and propionic to acetic were blocked due to the high Gibbs energy, as shown in Eqs. (1) and (2), which resulted in their accumulation constantly.



Meng et al. (2013) evaluated ZVI effect based on the use of sodium propionate as the sole carbon substrate, and the results showed that the Gibbs value of the conversion could be reduced by 8–10.2%, with a clear abundance of propionate-utilizing bacteria and homoacetogenic bacteria. An enhancement of the conversion of butyric or propionic acid to acetic acid was also discovered in our recent study (Kong et al., 2016a). Yet, Meng's research were conducted in a specific condition. The substrate was sole and the organic acid generated was only propionic and acetic, not mixing VFAs. Thus, the role of ZVI in mixed VFAs metabolizing was unclear. Again, in our earlier study, although it was a mixed VFAs system, some mechanisms involved were still not fully explained because of lacking of enough sampling points and evolution analysis for VFAs profile. In addition, few reports studied the microbial groups during the stage of excessive acidification alleviation.

In this study, three AD reactor types (at high OLR with or without ZVI and at low OLR without ZVI) were established to treat OFMSW. The performance of ZVI on enhancing CH_4 yield and on promoting VFAs production and metabolism were assessed. Furthermore, the variation of acidogenesis type in acidogenic stage were determined through calculating the relationship among changes of VFAs concentration and biogas production. A high throughput technique was used for 16s rRNA sequencing to determine the microbial community structures and the diversity of Bacteria and Archaea at key reaction stages in the different reactors. Combining the function of predominant microbial groups at the stage of excessive acidification alleviation in reactor with ZVI addition, the study further verified the mechanism of ZVI improving AD performance.

2. Methods and materials

2.1. Substrate and inoculum

The artificial OFMSW comprised mainly of cabbage (60%), fruit peels (15%), rice (15%), tofu (5%) and pork (5%), and they were crushed and mixed manually to ensure the homogeneity of the substrate for each reactor. The inoculum in this study were diges-

tate from a full-scale food waste AD treatment plant, and its CH_4 potential was low and could be neglected (Kong et al., 2016a).

2.2. Zero valent iron

Commercial ZVI (purity 98%, 0.2 mm diameter, and $0.05 \text{ m}^2/\text{g}$ Brunauer-Emmett-Teller surface area, Aladdin Brand, Shanghai, China) was washed to remove the surface oxides or other impurities using 10% diluted hydrochloric acid for 2 min, then rinsed with deionized water acetone, and finally dried at 105°C , under N_2 gas (Wu et al., 2015).

2.3. Reactor design

The Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control, Sweden), with a total volume of 0.5 L, was used for the batch reactors (Kong et al., 2016b). Some improvements to the AMPTS II were made for more convenient sampling. Both biogas and digestate sampling ports were established on each reactor, and the digestates were sampled from the liquid sampling port using a syringe.

Three reactor types were operated at mesophilic conditions (35°C), and the total solid content of the mixture in the reactor was adjusted to 6%. Six replicates were conducted for each reactor type to ensure the accuracy of the results. Due to much higher volatile solid (VS) content of OFMSW than inoculum sludge, the OLRs of different reactors could be adjusted through changing the inoculum to substrate ratios (ISRs). In the AD reactor with ZVI added at a high OLR (H_ZVI), the ISR was 2, and the corresponding OLR was $30 \text{ g VS}_{\text{substrate}}/\text{L}$. According to our previous study, the optimal ZVI dosage was 0.4 g/gVS (Kong et al., 2016a). The first control reactor without ZVI (H_Control) had the same ISR and OLR as H_ZVI (as shown in Table 1). At day 60 after the experiment stated, the methanogenic process was recovered in the two reactors (H_Control-1), but another four reactors (H_Control-2) were in an excessive acidified state, with no CH_4 production until the reaction finished. In the H_Control-2 reactors, the pressure in the reactor was maintained in a negative state, and only few digestate were sampled for analysis, because large amounts of acid gases (e.g., CO_2 and H_2S) would be absorbed by non- CH_4 gas adsorption units of AMPTS II. The liquid mixtures from H_Control-2 reactors were only monitored at the end of the reactor operation. Another control reactor was operated at a low OLR with an ISR of 0.5 and without the addition of ZVI (L_Control), which was the optimal condition for biochemical methane potential (BMP) testing of the substrate adopted in most studies (Browne et al., 2014; Wang et al., 2015). The corresponding OLR was $11.5 \text{ gVS}_{\text{substrate}}/\text{L}$.

2.4. Analytical methods

2.4.1. Digestate and biogas analysis

The total solids (TS), volatile solids (VS), pH, soluble chemical oxygen demand (SCOD), ALK, as well as VFA concentrations of the digestate were monitored as described in a previous study (Kong et al., 2016a). The composition of the biogas was analysed by gas chromatography (GC) (GC2014: Shimadzu, Kyoto, Tokyo), using a thermal conductive detector and packed column (TDX-01, $2 \text{ m} \times 2 \text{ mm}$), and the carrier gas was nitrogen. The temperatures of the oven, injector port, and detector were 70, 130, and 130°C , respectively.

2.4.2. DNA extraction and amplicon generation

DNA was extracted from digestate samples from the three types of reactors using the PowerFecal DNA Isolation Kit (Code No. 12830-50: Anbiosci Tech Ltd, Shenzhen, China). The V4 region of

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