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Variations of organic matters and microbial community in thermophilic anaerobic digestion of waste activated sludge with the addition of ferric salts

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HIGHLIGHTS

- $FeCl_3$ was in favor of the increase of available materials for methanogenesis.
- Accumulation of non-biodegradable materials was achieved with $Fe(NO_3)_3$ additive.
- Variations of DOM was assessed by EEM fluorescence spectroscopy and FRI technique.
- Large shifts of bacteria and archaea community structure were observed in reactors.
- *Coprothermobacter* and *Methanosarcina* can be enriched with $FeCl_3$ additive.

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ABSTRACT

Ferric salts will influence the thermophilic anaerobic digestion of waste activated sludge (WAS). $FeCl_3$ was found to contribute to the anaerobic digestion process with a cumulative biogas production of 357 mL/gVS, 79.6% higher than that in the control group, and $Fe_2(SO_4)_3$ had no distinct impact, while $Fe(NO_3)_3$ inhibited the methanogenesis process. A favorable balance between the release of organic matters from WAS and consumption rate was established after dosing $FeCl_3$ from the perspective of variations of soluble COD, volatile fatty acids (VFAs) and the dissolved organic matters (DOM) assessed by EEM fluorescence spectroscopy and fluorescence regional integration (FRI) technique. Conversely, the system with $Fe(NO_3)_3$ achieved an unsuitable substrates environment. Pyrosequencing revealed that the anaerobic digestion system with $FeCl_3$ enriched *Coprothermobacter* for proteins fermentation and *Methanosarcina* for methanogenesis with the values of 18.7% and 63.2%, respectively, while that with the supplementation of $Fe(NO_3)_3$ obtained the lowest relative abundance.

1. Introduction

Waste activated sludge (WAS) is the troublesome problem for municipal wastewater treatment plant due to the huge production, potentially environmental risk and high cost for disposal (Zhen et al., 2013). The rapid increasing energy demand, along with growing concerns for environmental protection, has fostered the search for alternative energy sources. Production of biogas, mainly composed of CH_4 and CO_2, from high-concentration wastewater, municipal solid waste (MSW) and WAS via anaerobic digestion (AD) is a viable option for the generation of bioenergy resources (Baek et al., 2014), and especially stabilizing WAS by AD process attracted more and more attentions for the energy recovery. However, some limitations attributed to the slow growth rate of anaerobic microbes involved in the AD process, for example, longer retention time and unstable status resulting from the accumulation of volatile fatty acids (VFAs) (Ahring, 2003). AD process is a series of biological reactions performed by various microbial groups, and therefore its performance basically depends on the harmonized activity of the microbes involved, especially in the thermophilic digestion with a fast hydrolysis and acidification rate (Nasr et al., 2012). This suggests that approaches for the stable operation are necessary for fundamentally improving AD performance (Bouallagui et al., 2009).

Supplementation of trace metals (i.e. micronutrients) to biogas reactors has recently become a common practice for the stable operation of AD process and efficient biogas production

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(Lindorfer et al., 2012). Trace metals serve an important function in the growth of methanogens and are essential co-factors of enzymes, such as methyl-coenzyme M, carbon monoxide dehydrogenase (CODH), and coenzyme M methyl-transferase, which are involved in the anaerobic degradation of WAS (Zhang et al., 2014a). In particular, Co and Ni are common additives to biogas process due to their vital role in the metabolic machinery of a wide range of anaerobic microorganisms active in the conversion of complex organic substrates to biogas (Gustavsson et al., 2013).

Fe is another alternative and largely supplement to biogas reactors treating sulfur-rich substrates for the removal of biogenic sulfide (Zhang and Jahng, 2012; Schmidt et al., 2013). Fe^{3+} removes sulfide in two steps, namely sulfide oxidation to elemental sulfur by Fe^{3+}, which is reduced to Fe^{2+}, and sulfide precipitation of FeS (Firer et al., 2008). Although the effects of ferric salts on AD were primarily investigated, most studies focused on the sulfur control, the response relationship among the ferric salts, variations of the main organic matters in the supernatant and microbial communities (bacteria and archaea) was not established to date. Besides, the availability of trace metals for microbial uptake and growth depends on the metal speciation, which in turn is controlled by the reactor conditions (such as substrates and temperature), and the knowledge of effects of different ferric salts on the thermophilic anaerobic digestion of WAS was still limited.

To clarify the effects of ferric salts in sludge anaerobic digestion system, a comparative study of the potential influence with the addition of $Fe(NO_3)_3$, $Fe_2(SO_4)_3$ and $FeCl_3$ was conducted under the thermophilic conditions from the perspective of variations of substrates environment. Aside from measuring the composition of soluble COD and VFAs, the dissolved organic matters (DOM) species and content of the fermentation liquid was also examined using three-dimensional excitation–emission matrix (EEM), and fluorescence regional integration (FRI) technique was applied for the microbial metabolism analysis. High-throughput sequencing technology was also applied to quantitatively characterize the shift of microbial communities responsible for anaerobic digestion performance. Also, the fate and bioavailability of Fe was assessed in terms of the enhanced biogas production with the supplementation of $FeCl_3$.

2. Methods

2.1. Characteristics of sludge and inoculum

WAS used in this experiment was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant (MWWTP) in Shanghai, China, where wastewater was treated by the anaerobic-anoxic-aerobic process with a capacity of 50,000 m^3/d. The sludge obtained was screened with a 1.0-mm mesh to eliminate large particles and hair before thickening to required solid concentrations. Then the pretreated samples were stored at 4 °C for further analyses. The inoculum (seed sludge) was collected directly from a long-term continuous lab-scale anaerobic bioreactor in our lab. The main characteristics of WAS and seed sludge are given in Table 1.

2.2. Batch experiments

Batch experiments were carried out in double-walled cylindrical vessels with 6 L working volume as shown in Fig. S1(a). Oxygen was removed from the headspace by the injection of nitrogen gas (99.99%) for 5 min after loading the sludge. All the reactors maintained at a thermophilic digestion temperature of 55 ± 2 °C by water circulation, equipped with stainless-steel stirrers for mixing the contents. The biogas was measured using a calibrated sampling syringe. All samples from the reactors were analyzed in triplicate.

Table 1
Characteristics of WAS and seed sludge used in experiments.[a]

Parameters	WAS	Seed sludge
pH	6.32–6.40	6.87–6.90
TS (g/L)	39.5–39.9	68.7–69.2
VS (g/L)	29.0–29.3	49.6–52.2
TCOD (mg/L)	32,570–37,640	84,576–87,260
SCOD (mg/L)	124.0–535.6	16,240–18,440
Soluble proteins (mg/L)	31.4.18–35.8	428.6–492.4
Soluble carbohydrates (mg/L)	1.51–2.04	276.10–266.38
Fe (%)	1.72–1.91	1.83–1.94

SCOD: soluble chemical oxygen demand. The content (%) of Fe was in dry sludge solid.
[a] TS: total solid; VS: volatile solid; TCOD: total chemical oxygen demand.

After the experimental startup and digesting for 3 days, three ferric salts i.e. $Fe(NO_3)_3$, $Fe_2(SO_4)_3$ and $FeCl_3$ were dosed into the reactors (referred to as R2, R3 and R3, respectively) in the form of ferric salts solutions (50 mL). Aim to investigate the effects of different ferric salts on the thermophilic anaerobic digestion of WAS, a fixed dosage of 200 mg/L $Fe(NO_3)_3$, $Fe_2(SO_4)_3$ and $FeCl_3$ was applied, which based on the concentration of iron ion (Zhang et al., 2012). The control group (R1) was also carried out under the same operation conditions without ferric salts (Fig. S1). No alkalinity or buffering agent was added into the system, and the pH value was not adjusted during the entire process.

2.3. Analytical methods

Sludge samples collected from the reactors were analyzed for pH, ORP, total solids (TS) and volatile solids (VS). DOM extraction: The sludge was centrifuged at 12,000 rpm for 5 min and then a subsequent filtration through 0.45 μm microfiber filter paper was carried out for the corresponding supernatant. TS, VS, soluble chemical oxygen demand (SCOD), soluble proteins and soluble carbohydrates were determined according to Standard Methods (APHA et al., 2005).The pH and ORP were measured by a pH meter (pHs-3C, Leici Co. Ltd., Shanghai) and an ORP meter (ORP-502, Ruosull Technology Co., Ltd., Shanghai), respectively. VFAs (including acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid) were analyzed by a gas chromatograph (GC-2010, Shimadzu) with a chromatographic column (DB-FFAP: 30 m × 0.25 mm × 0.25 mm) and a flame ionization detector (FID) (Yu et al., 2014a). The sludge solids were pretreated by microwave digestion system (ETHOS ONE, MILESTONE) with a mixture of $HNO_3/H_2O_2/HF$ followed by neutralization with H_3BO_3. Then the concentration of iron ion in the sludge and supernatant was analyzed with atomic absorption spectrometry method (spectrometer contrAA, Analytik Jena). The contents of C, H, N and S in WAS were measured by an elemental analyzer (Vario Macro Cube, Elementar) with sulfanilamide ($C_6H_8O_2N_2S$) as reference material.

2.4. EEM fluorescence spectra and fluorescence regional integration (FRI) analysis

DOM was measured by EEM fluorescence spectroscopy in a luminescence spectrometry (F-7000 spectrophotometer, Hitachi, Japan). The EEM spectra were collected with corresponding scanning emission spectra from 250 nm to 600 nm at 5 nm increments by varying the excitation wavelength from 200 nm to 500 nm at 5 nm sampling intervals. The excitation and emission slits were maintained at 5 nm and the scanning speed was set at 1200 nm/min. The spectrum of deionized (DI) water was recorded as the blank and Raleigh scattering was subtracted according to Chen et al. (2003).

The FRI technique was adopted for the quantitative analyses of EEM spectra of DOM (Chen et al., 2003), and EEM peaks were