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Selective simplification and reinforcement of microbial community in autothermal thermophilic aerobic digestion to enhancing stabilization process of sewage sludge by conditioning with ferric nitrate

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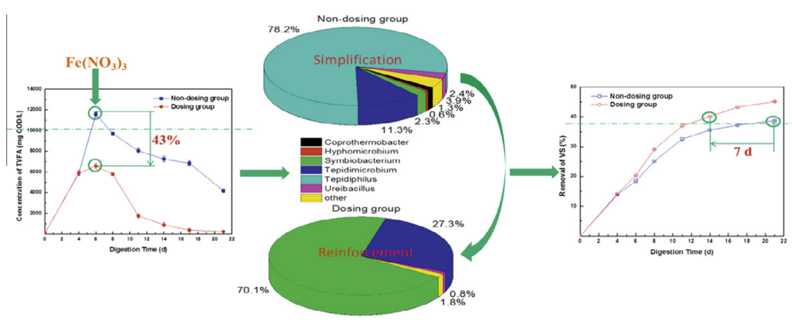
HIGHLIGHTS

- The dosing group achieved disinhibition of VFA and change of individual VFA order.
- Phylotypes were selectively simplified and reinforced with ferric nitrate.
- Multiple biodegradable substances in DOM fractions were achieved with ferric nitrate.
- Higher stabilizing level was acquired with $Fe(NO_3)_3$ as SCOD/TCOD and TCOD decreased.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of ferric nitrate on microbial community and enhancement of stabilization process for sewage sludge was investigated in autothermal thermophilic aerobic digestion. The disinhibition of volatile fatty acids (VFA) was obtained with alteration of individual VFA concentration order. Bacterial taxonomic identification by 454 high-throughput pyrosequencing found the dominant phylum *Proteobacteria* in non-dosing group was converted to phylum *Firmicutes* in dosing group after ferric nitrate added and simplification of bacteria phylotypes was achieved. The preponderant *Tepidiphilus* sp. vanished, and *Symbiobacterium* sp. and *Tepidimicrobium* sp. were the most advantageous phylotypes with conditioning of ferric nitrate. Consequently, biodegradable substances in dissolved organic matters increased, which contributed to the favorable environment for microbial metabolism and resulted in acceleration of sludge stabilization. Ultimately, higher stabilization level was achieved as ratio of soluble chemical oxygen demand to total chemical oxygen demand (TCOD) decreased while TCOD reduced as well in dosing group comparing to non-dosing group.

1. Introduction

Sewage sludge produced in wastewater treatment processes usually contains pathogens, heavy metals and abundant organic substances, which impose tremendous stress on environment. Autothermal thermophilic aerobic digestion (ATAD) is an advanced thermophilic aerobic digestion process. The ATAD technology possesses high efficiencies of pathogens inactivation and volatile solids (VS) removal. But it requires limited land scale and simple control (Layden et al., 2007). Hence, it has been well adopted by numerous small and medium-sized wastewater treatment plants (WWTPs) in Europe and North America since early 1970s (Liu et al., 2011). Nevertheless, some inherent defects emerged in practical operations, e.g. ammonia inhibition (Yuan et al., 2014), volatile fatty acids (VFA) inhibition (Jin et al., 2015a). Especially

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for the latter, substantial amounts of VFA generate during the process of self-heating and lysis of microbes in ATAD system. This is due to the insufficient aeration while high TS content of 5–7% (Chen et al., 2008). Thus, it incurs the inhibition of VFA to the dominant Gram-positive bacteria inside the system (Hayes et al., 2011).

Similar phenomena have also been found in anaerobic sludge digestion systems with high TS and VS level at thermophilic temperature. Thermophilic fermentation of sludge with TS of 14.1% (w/w) and VS 8.9% (w/w) accumulated VFA level 10-fold more comparing to mesophilic fermentation. The abundances of *Clostridiaceae*, *Microthrixaceae* and *Thermotogaceae* increased, which could promote either hydrolysis or acidification (Hao and Wang, 2014). However, inhibition showed up during fermentation of waste activated sludge pretreated by a thermal hydrolysis process when the VFA concentration came to 17 ± 1 g COD_{VFA} L^{-1} (Pratt et al., 2012). Acetate acid was found approximately 50% as inhibitory as the other organic acids (COD basis) (Pratt et al., 2012). Moreover, significant inhibition appeared when the propionic acid concentration increased to 900 mg/L with the concentration of methanogenic archaea decreased from 6×10^7 to $0.6–1 \times 10^7$ mL^{-1} (Wang et al., 2009). In addition, their activity would not recover (Wang et al., 2009). In view of the above-mentioned facts, $FeCl_3$ was found to contribute to disinhibition of excessive VFA in the thermophilic anaerobic digestion process (Yu et al., 2015). Enriched *Coprothermobacter* and *Methanosarcina* were found for proteins fermentation and methanogenesis with the values of 18.7% and 63.2%, respectively (Yu et al., 2015).

Iron salts or iron, widely used in wastewater treatment for odor control and sufficient phosphorus removal, has been extensively devoted to sludge digestion. Lots of interests have also been attracted to the mechanism of interaction between iron salts or iron and microbes. In addition to the utilization of $FeCl_3$ in improvement of thermophilic anaerobic digestion process as mentioned above, zero-valent iron (ZVI) was a popular option for enhancing methane production and sulfate reduction in anaerobic granular sludge groups (Liu et al., 2015). In anaerobic digestion of sludge after pretreated by the heat or alkali process, the acetoclastic methanogenesis and the H_2-utilizing methanogenes could be enhanced with the addition of ZVI (Zhang et al., 2015). Furthermore, the addition of Fe^0 powder was applied in acidogenic group to enhance the anaerobic conversion of propionate to acetate (Meng et al., 2013). The abundance of microbial communities, especially propionate-utilizing bacteria and homoacetogenic bacteria were found increased by adding Fe^0 powder (Meng et al., 2013). Additionally, some study has revealed that the iron ions Fe(III) and Fe(II) could be coordinated to the DNA (Ambroż et al., 2004). The iron ions Fe(III) and Fe(II) could played the roles of electron and hole scavengers, respectively in bioorganic systems as well (Ambroż et al., 2004). On the other hand, iron could also play an essential metabolic role as a component of a diverse group of metalloproteins from the Hyperthermophilic Archaeon *Archaeoglobus fulgidus* (Chiu et al., 2001). Moreover, iron serve as an energy source in catabolic iron metabolism in microbes (Chiu et al., 2001). Two primary pathways for iron uptake in fungi and how the prevention of ferric iron from 'rusting out' has been put forward (Kosman, 2013). Activations of redox inert and coordinately stable ferric complex so as to make it a substrate for iron metabolic pathways in aerobes were clarified (Kosman, 2013).

In consideration of the inevitable over-production of VFA in initial stage of ATAD process for sludge and the original superiority of iron salts in microbes, coupling ferric nitrate coordination with ATAD technology was introduced. It has been certified to be effective in disinhibition of VFA and promoting stabilization of sludge in ATAD process (Jin et al., 2015a). In recent years, the thermal treatment of sludge has been popular as a pretreatment for high production of VFA or as a fast reducing treatment of sludge. The dominant bacterial in thermophilic aerobic digestion, especially the ATAD were *Firmicutes* and *Deinococcus-Thermus* families (Kim et al., 2014). However, a marked decrease in the relative abundance of a large part of bacterial taxa such as *Proteobacteria* was also found (Kim et al., 2014). Moreover, enriched *Coprothermobacter* was found for proteins fermentation (Yu et al., 2015), and *Bacteroides*- and *Clostridium*-related microorganisms were suggested to be mainly responsible for the hydrolysis and VFAs production, respectively (Seon et al., 2014). However, considerable work still remains to characterize the relationship between stabilization processes of sludge substances and microbial community in ATAD system conditioned by ferric nitrate, if a methodology is expected to be set up. Therefore, 454 high-throughput pyrosequencing of the 16S rRNA gene was utilized in clarification of the microbial community structure during the stabilization process in ATAD system for sewage sludge in this study.

2. Methods

2.1. Startup of the digestion process

Sewage sludge used in this study was collected from the secondary sedimentation tank of Minhang municipal wastewater treatment plant (Shanghai, China), in which anaerobic–anoxic–oxic process was applied. The sludge sampled was screened to get rid of particles of size greater than 0.5 mm. After that, it was went through centrifugation at 2200*g* for 3 min to acquire concentration of total solid (TS) between 5% and 6% (Liu et al., 2011). The main properties of raw sludge were presented in Table 1.

The profile of the group was tempered glass cylinder of 200 mm $(D) \times 400$ mm (H). The available volume was 4 L to contain sludge. The double-deck cylinder body was designed to connected to a water bath for maintaining a constant digestion reaction temperature. The temperature of digestion reaction was set to raise from 35 °C to 55 °C for simulating the start-up process of one stage ATAD (Liu et al., 2010). The temperature was controlled by the water bath at a rate of 5 °C per day. Then the temperature of the digestion reaction held at 55 °C for 17 days until the end. Aeration was supported at a rate of 0.13 L/min to cultivate a micro-aerobic condition (Chu et al., 1996). Meanwhile a constant stirring rate of 120 resolutions was provided (Xu et al., 2013).

The complete digestion reaction process sustained 21 days. $Fe(NO_3)_3$ were dosed in digesters of group R2 on 6th day (Jin et al., 2015c) with optimal dosage of reducing 1000 mg/L of acetic acid (Jin et al., 2015b). But the digesters of group R1 were not fed. The decreased amount of sludge by sampling before 6th day was taken into account when the chemical reagent was added into the group. $Fe(NO_3)_3$ was put into digester 6 h before sampling on 6th day to acquired adequately reaction between chemical reagent and sludge. Microbial communities were analyzed in sludge sampled at the end of the digestion processes. All experiments were conducted triplicated, and all of indicators were determined in triplicate with the standard deviations achieved.

2.2. Chemical analysis

VS and TS were measured according to the Standard Methods (APHA et al., 2005) with values brought in by chemical reagents subtracted. The SCOD in the supernatant and TCOD were analyzed on the basis of Standard Methods (APHA et al., 2005). The sampled sludge was subjected to centrifugation at 12,000*g* for 5 min, following with filtration through a 0.45 μm mixed cellulose ester membrane. Then the filtrate mixed with 3% H_3PO_4 (to keep the pH of the filtrate staying at approximately 4.0) was injected into a Shimadzu GC-2010 gas chromatograph with a flame ionization