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Strategies for the stable performance and rapid inhibition recovery of a thermophilic digester treating coffee wastes and the synergistic effects of microbes

Qian Li[a], Xinru Cheng[a], Chaosui Yuwen[a], Xiaohuan Yang[a], Wei Qiao[b], Yu-You Li[c,d], Xiaochang Wang[a,*]

[a] International Science and Technology Cooperation Center for Urban Alternative Water Resources Development, Key Laboratory of Northwest Water Resource, Environment and Ecology, MOE, Engineering Technology Research Center for Wastewater Treatment and Reuse, Shaanxi, Key Laboratory of Environmental Engineering, Shaanxi, Xi'an University of Architecture and Technology, No. 13 Yanta Road, Xi'an, 710055, China
[b] College of Engineering, China Agriculture University, Beijing, 10081, China
[c] Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai, 980-8579, Japan
[d] Graduate School of Environmental Studies, Tohoku University, Sendai, 980-8579, Japan

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ABSTRACT

A 200-day experiment was conducted to evaluate strategies for the stable performance and rapid inhibition recovery of a thermophilic reactor treating coffee wastes, waste activated sludge, and milk waste under a high organic loading rate (OLR) and the synergistic effects of microbes. Excessive accumulation of propionate was determined to be the main reason for the deterioration of the reactor, which resulted from reduced methanogenic activity. Supplementation of inoculum sludge led to the complete inhibition recovery of the reactor in fewer than 35 d by enhancing propionate degradation. The microelement and low concentration of sulfate ensured the stable performance of the reactor at OLR of 4.6 g-TS/L/d. The efficiency of organic matters and propionate degradation was also improved. The microbial community was analyzed by high-throughput sequencing targeting 16S rRNA to determine the relationship between microbial composition and reactor performance. Propionate markedly inhibited *Methanosarcina*, which dominated the stable state; meanwhile, *Methanobacterium* and *Methanothermobacter* were more tolerant and comprised more than 75% of total archaea at the inhibitory stage. The deterioration of the reactor led to a shift in the CH_4 generation pathway from acetoclastic to hydrogenotrophic. *Clostridiales* was responsible for organic degradation at stable stages; however, almost all of the genera of *Clostridiales* could not survive at stage II and were replaced by *Thermoanaerobacterales* and *Lactobacillales*.

1. Introduction

Thermophilic digestion is a preferred method for the reduction of volatile solids (VS) and inactivation of pathogens in organic waste treatment (Pender et al., 2004). The stability of CH_4 production from coffee grounds is difficult to achieve specifically under high organic loading rates (OLRs) because of high carbon content, high biodegradability, and toxic compounds (Dinsdale et al., 1997; Qiao et al., 2013). Co-digestion with waste activated sludge (WAS) characterized by high levels of nitrogen and low hydrolysis rate can overcome these disadvantages by balancing the C/N ratio and diluting the toxic compounds (Qiao et al., 2013; Xie et al., 2017; Zhao et al., 2016). However, excessive accumulation of volatile fatty acids (VFAs), which leads to sharp reductions in pH and methane yield (Yang et al., 2015) and results in system failure, remains an important issue. Failure of the anaerobic system caused by VFA accumulation may require a recovery time of more than one year, as reported by Rimkus et al. (1982). Therefore, effective strategies for immediate inhibition recovery and robust performance need to be established.

Among the VFAs, propionic acid is considered a "trouble maker". It can be converted into methane via syntrophic oxidation by cooperation between acetogens and H_2-utilizing methanogens only when a considerably low hydrogen partial pressure (10^{-6}–10^{-4}) is maintained (McCarty and Smith, 1986). Kim et al. (2002) indicated that supplementation of microelements (Ca, Fe, Ni, and Co) is required to ensure the effective removal of propionate in a thermophilic digester when

* Corresponding author.
E-mail address: xcwang@xauat.edu.cn (X. Wang).

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VFA is increased. Other studies suggest that the low concentration of sulfate contributes to the degradation of VFAs because it is necessary for the growth of some microorganisms (Speece, 1983). Sulfate-reducing bacteria (SRB) provide more advantages for VFA degradation, compared with acetogens (Wu et al., 1991). If COD/SO_4^{2-} is sufficiently higher, SRB do not compete with methanogens, thus ensuring the CH_4 yield. Therefore, these strategies can potentially help thermophilic digestion achieve a stable performance by overcoming the excessive accumulation of VFAs.

As a complex biochemical process, balancing between major bacteria and archaea may affect the stable performance and efficiency of an anaerobic system. High-throughput sequencing (HTS) based on the 16S rRNA gene has been widely used for microbial community analysis in recent years (Antwi et al., 2017; Jang et al., 2016; Zhang et al., 2015). HTS provides more sufficient data to cover the overall taxonomic composition. Variations in microbial communities during thermophilic co-digestion of coffee waste in stable, inhibitory, and stable states after the inhibition recovery of the reactor have not been investigated. The action of the microorganism that helped the reactor return to a stable performance is not clearly established.

Therefore, the current study aims to develop a multi-strategy for promoting thermophilic co-digestion of coffee grounds, coffee liquid, milk waste, and dewatered activated sludge toward inhibition recovery and for maintaining a stable performance by sulfate and microelement addition. The change in metabolic capacity and microbial community was analyzed to elucidate the action of microorganisms during deterioration and recovery in this long-term experiment.

2. Materials and methods

2.1. Preparation of feedstock and inoculum sludge

The feedstock used in this study consisted of coffee grounds, coffee liquid, milk waste, and dewatered WAS provided by Tokyo Gas Co., Ltd. In accordance with the design of the full-scale methane fermentation plant, the coffee grounds, coffee liquid, milk waste, and WAS were mixed at a ratio of 14.6:65.3:12.2:7.9, homogenized using a high-speed blender (WARING LBC-15, UAS) at 18,500 rpm for 30 min, and stored in a substrate tank at 4 °C. The co-substrate was characterized as follows: total solid (TS), 69.6 g/L; VS, 65.1 g/L; pH, 5.18; total COD, 120.0 g/L; and total VFA, 4.00 g/L.

2.2. Experimental setup and operation of the reactor

The experiment was conducted in a semi-continuous digester, which was operated at a working volume of 12 L. The feedstock was pumped from a substrate tank to a reactor by a peristaltic pump 8 times a day. The temperature of the reactor was controlled at 55 °C, using a water jacket and a thermostatically controlled water bath.

For a stable startup, the feedstock was intermittently fed into the reactor at a low OLR until a stable performance was attained. The long-term experiment was then divided into 3 stages: stage I, in which the OLR was maintained at 2.3 and then increased to 4.6 g-TS/L/d by shortening HRT from 30 d to 15 d; stage II, in which feeding was stopped, and 0.5 L new inoculum sludge from another robust thermophilic digester treating the same organic waste was added into the continuous flow stirred-tank reactor (CSTR) to promote the inhibition recovery of the reactor; stage III, in which Na_2SO_4 at the concentration of 500 mg/L and microelements at specific concentrations were added into feedstock. The concentrations of the microelements were as follows (in mg/L): Fe, 0.1; Co, 30; Ni, 23; B, 1.3; W, 0.31; Se < 0.01; Mo, 3.1; Mn, 20; Cu, 1.9; Zn, 37; Na, 1.7; K, 0.05; Ca, 0.13; Mg, 0.46; N, 0.45; P, 0.001; and S, 0.75. Meanwhile, feeding was restarted and then OLR was increased from 2.3 to 4.6 g-TS/L/d. To maintain a suitable pH, NH_4HCO_3 was added into the reactor when the pH dropped to lower than 6.8.

2.3. Determination of methanogenic kinetics of acetate and propionate

To compare the variation in the methanogenic capacity of microbes for acetate and propionate degradation at stage I and stage III, batch test was employed to obtain the kinetics of CH_4 production, such as lag time and maximum CH_4 production rate. Sodium acetate and sodium propionate at 4 different concentrations—1000, 2000, 3000, and 5000 mg/L—were used as substrates to determine the methanogenic kinetics of acetate and propionate, respectively. Both were added to evaluate the effects of loading rate on the kinetics of methanogenesis from acetate and propionate. Inoculum sludge was taken from the reactor at stage I (HRT of 15 d) and stage III (HRT of 15 d) to compare the methanogenic capacity before and after sulfate and trace element were added. Batch test was conducted in 120 mL serum bottles in accordance with a previous study (Li et al., 2015). The biogas produced from each bottle was analyzed using a syringe. After the produced biogas was measured, 0.4 mL biogas was taken from serum bottles to analyze the biogas composition.

The maximum reaction rate and lag time were determined by simulation using the modified Gompertz equation:

$$P = P_0 \cdot \exp\left\{-\exp\left[\frac{R_{max} \cdot e}{P_0} \cdot (t_0 - t_1) + 1\right]\right\} \quad (1)$$

where P is the accumulated CH_4 production (mL), P_0 is the CH_4 production potential (mL), R_{max} is the maximum CH_4 production rate (mL/d), t_0 is the lag time (days), and e = 2.718281828. The constants P_0 and t_0 were estimated by a non-linear fitting program using the OriginPro 8.5 software.

2.4. Chemical analysis

Daily biogas production of reactor was recorded using a wet gas meter. The components of the biogas (CH_4, CO_2, N_2, and H_2) were determined using a Shimadzu GC-8A gas chromatograph. The pH, COD, TS, VS, and VSS were determined using the Japan Standard Testing Method for Wastewater (JSWA (Japanese Standard Methods of the Examination of Wastewater), 1997) (Akizuki et al., 2013). VFAs were assayed with the use of an Agilent-6890 gas chromatograph. SO_4^{2-} was determined using a Thermo Scientific ICS-1100 ion chromatograph. H_2S was measured using hydrogen sulfide detecting tubes (Gastec, No. 4L).

2.5. Microbial analysis

2.5.1. DNA extraction

Three samples on Days 105 (stage I), 144 (stage II), and 216 (stage III) were selected to analyze the bacterial and archaeal communities. A 2 mL sludge sample was centrifuged at 13,000 rpm for 10 min, and the pellet was washed twice with phosphate-buffered saline by resuspension and centrifugation. DNA was then extracted using the PowerSoil® DNA Isolation Kit (MO BIO, USA) in accordance with the instructions provided by the manufacturer. The extracted DNA was stored at −20 °C until analysis.

2.5.2. Bacterial and archaeal community analysis

HTS targeting the 16S rRNA gene was employed to analyze the bacterial and archaeal communities (Antwi et al., 2017). Primer sets 341F and 806R were used for bacterial DNA amplification. PCR amplification was conducted using 50 μL PCR reaction mixtures consisting of 5 μL 10 × PCR buffer, 0.5 μL dNTP (10 mM each), 10 ng Genomic DNA, 0.5 μL of each primer (50 μM), 0.5 μL Platinum Taq (5U/μL), and H_2O added to 50 μL. The PCR protocol was as follows: (1) 94 °C for 3 min; (2) 5 cycles at 94 °C for 30 s, 45 °C for 20 s, 60 °C for 30 s; (3) 20 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s; (4) 72 °C for 10 min, and cooling at 10 °C. Meanwhile, nested PCR was conducted