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Responses of microbial capacity and community on the performance of mesophilic co-digestion of food waste and waste activated sludge in a high-frequency feeding CSTR

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ABSTRACT

To understand the relationship between microbes and digester performance of high-frequency feeding CSTR, which could achieve stable CH_4 production at high OLR by easing instantaneous feeding shock, attentions were paid on the variations of methanogenic capacity (MC) and microbial community with OLR increasing. Results showed that the MC for feedstock degradation could satisfy the need of effective conversion from feedstock to CH_4 when the OLR remained below 16.4 g-TS/L/d. Furthermore, the MC for acetate, propionate and butyrate degradation increased by 73.8%, 303%, and 164%, respectively, with OLR increasing from 3.03 g-TS/L/d 12.6 g-TS/L/d. The evolution of both bacterial and archaeal communities provided additional information on the adaptation of functional microbes to environmental factors. The significant increase of abundance of *Methanoculleus* and *Methanomassiliicoccus* likely promoted the utilization of H_2, thus facilitating syntrophic methanogenesis, and consequently ensuring efficient CH_4 production in stable stage.

1. Introduction

Anaerobic co-digestion is an advantageous technology for treating food waste (FW) and waste activated sludge (WAS), since it can facilitate stable and effective conversion from organic matter to renewable energy in the form of biogas via diluting toxic compounds, balancing nutrients, and improving synergistic effects of microorganisms (Dai et al., 2013; Iacovidou et al., 2012; Lo et al., 2010; Prabhu and Mutnuri, 2016). However, because anaerobic digestion (AD) is affected by several factors, stable and efficient CH_4 at expected high organic loading rate (OLR) is still difficult to be achieved even under mesophilic conditions (Dareioti and Kornaros, 2014; Liu et al., 2012; Méndez-Acosta et al., 2016; Shen et al., 2013).

OLR is a critical factor to determine the stability and efficiency of the AD system. Although low OLR can ensure stable CH_4 production, high OLR is still expected since more efficient CH_4 recovery could be achieved (Ziganshin et al., 2016). However, the significantly shorter doubling time of acidogenic bacteria compared to methanogenic archaea (Zhang et al., 2017a,b) aggravates the imbalance between acidogenesis and methanogenesis, which may result in excessive accumulation of volatile fatty acids (VFAs) at high OLR, consequently lowering system stability and efficiency. To overcome these drawbacks, a high-frequency feeding (HFF) system has been tested to ease the loading shock, thus achieving stable and efficient performance in our previous study (Li et al., 2017), but the role of microbes were not investigated.

As a complex biochemical process, the AD proceeds under the action of many microbial species, the dynamically metabolic balance between these acidogenic bacteria and methanogenic archaea determines the stable performance of AD. Therefore, many researchers focused on the characterization of microbial community structure or the evolution of microbial communities during the AD process, since an understanding of the microbial behaviors is necessary to improve the performance of the digester (Lee et al., 2016; Lin et al., 2012; Shin et al., 2010a). However, the microbial activity related to the CH_4 production and the responses to environmental changes are often overlooked.

The specific methanogenic activity (SMA) test is a common method to determine the anaerobic sludge activity in anaerobic processes (Ince et al., 1995; Isa et al., 1993; Jiménez et al., 2015). SMA values based on different substrates can reveal the activities of not only methanogens but also other microflora involved in CH_4 production (Liu et al., 2016). Our previous study found that the SMA of individual VFA could be facilitated by increasing OLR, based on the premise that the concentration of VFA was not overloading (Li et al., 2015b). Therefore, the possibility remained that SMA might increase with increasing OLR if

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microbes could be well acclimated. Nevertheless, the variation of SMA with increasing OLR and the relationship with CH_4 production has not been investigated during the co-digestion of FW and WAS.

In conclusion, both microbial activity and community probably respond to environmental changes and their synergetic effects might show a response on the performance of the digester. Therefore, to reveal the underlying reason for the stable performance of mesophilic co-digestion of FW and WAS in a HFF CSTR, the microbial activity using the same feedstock and solo VFA was tested when the operational condition was changed, combined with the analysis of microbial community.

2. Materials and methods

2.1. Feedstock and seed sludge

FW used in this study was synthesized according to the composition of FW in our campus cafeteria as shown in Supplementary material. WAS collected from a waste water treatment plant was completely mixed with FW at a ratio of 4:1 for higher CH_4 yield (Dai et al., 2013; Jang et al., 2016); then, the mixture was crushed using a blender, and diluted with tap water to obtain the feedstock with a TS content of 9.5%, which was sufficiently high to achieve high OLR. The seed sludge was taken from full-scale mesophilic digester treating sewage sludge. The physicochemical properties of the feedstock and seed sludge are listed in Table 1.

2.2. Reactor configuration and experimental procedure

A long-term experiment was conducted in a HFF CSTR with a working volume of 3 L as shown in Supplementary material. The temperature of the reactor was controlled at 35 °C using a water jacket connected to a thermostatically controlled water bath. From the substrate tank kept at 4 °C, the feedstock was intermittently sent to the CSTR at a feeding frequency of once per 15 min to eliminate the instantaneous loading shock. The feeding duration time of each stage was calculated according to the OLR, and the feeding pump was adjusted at the beginning of each stage to ensure that the volume of the feedstock coincided with the calculated value.

For a smooth start-up, the reactor pre-seeded with 3 L seed sludge was operated at a low OLR of 1.50 g-TS/L/d, which was gradually increased to 3.03 g-TS/L/d corresponding to a HRT of 30 days. NH_4HCO_3 solution was injected during the first seven days to maintain the pH at a suitable range. After the reactor reached steady state, OLR was gradually increased up to 18.8 g-TS/L/d, corresponding to a shortened HRT of 5 days. The feeding was stopped when the pH dropped below 6.6.

Table 1
Physicochemical properties of feedstock and seed sludge.

Parameter	Unit	Seed sludge	Feedstock
TS	g/L	20.9 ± 1.3	91.3 ± 3.2
VS	g/L	7.96 ± 0.52	79.7 ± 3.1
T-COD	g/L	21.3 ± 2.1	126.7 ± 5.6
S-COD	g/L	1.05 ± 0.33	45.3 ± 2.3
pH		7.54 ± 0.02	5.34 ± 0.38
Alkalinity	g-$CaCO_3$/L	2.23 ± 0.14	/
Acetic acid	g-COD/L	0.32 ± 0.11	0.98 ± 0.47
Propionic acid	g-COD/L	0.09 ± 0.02	0.03 ± 0.01
Butyric acid	g-COD/L	0.11 ± 0.04	/
Valeric acid	g-COD/L	0.08 ± 0.01	/
C	%	/	41.1 ± 0.9
H	%	/	7.61 ± 0.01
O	%	/	46.6 ± 1.2
N	%	/	4.52 ± 0.16
S	%	/	0.35 ± 0.03

2.3. Determination of methanogenic capacity

SMA test is widely used to determine the methanogenic activity of microbes. Volatile suspended solid (VSS) which is used to represent biomass in the calculation of SMA is not suitable to be used for the determination of methanogenic activity when using organic waste as substrate, since the inert part in feedstock also accounted for a part of VSS. Therefore, methanogenic capacity (MC) was introduced to represent the methanogenic activity without considering the effect of VSS in this study.

2.3.1. Feedstock as substrate

To find the relationship between MC of microbes for converting feedstock to CH_4 and performance of HFF CSTR, an SMA test was conducted in a 120 mL serum bottle after the reactor reached stable state at each OLR. The feedstock (same as used in long-term experiment) was mixed with 50 mL seed sludge, which was taken from the reactor before use at volumes of 1.67 mL, 2.51 mL, 3.34 mL, 5.01 mL, and 6.68 mL, coinciding with the real OLR of the reactor. The serums were purged by nitrogen gas for 2 min to remove the oxygen, and then put into a water bath at a temperature of 35 °C. After 5 min incubation, the headspace was vented to release pressure caused by thermal expansion. The MC was calculated via equation (1), based on per unit volume rather than VSS which was used in the calculation of SMA.

$$MC = R_{max} \cdot 24/1000/V \quad (1)$$

where MC is CH_4 production per L sludge in reactor per day (L/L/d), R_{max} is the maximum CH_4 production rate among different organic loadings (volumes of feedstock) (mL/h), for each organic loading the maximum CH_4 production rate is the slope of cumulative CH_4 production versus time graph according to Isa et al. (1993); V is the volume of seed sludge in the serum (mL). The variation of maximum CH_4 production rate along with organic loading increasing accorded with quadratic equation, therefore, R_{max} was obtained as the maximum value of simulated quadratic equation.

2.3.2. Acetate, propionate, and butyrate as substrate

To evaluate the MC of microbes for the degradation of mainly accumulated VFAs in different stages, individual acetate, propionate, and butyrate were used as solo substrates, respectively. The concentration of acetate/propionate/butyrate was 5000 mg-COD/L, which was sufficient for microbes achieving highest CH_4 production rate as reported in a previous study (Li et al., 2015b). The test procedure and MC calculation method were used as described in Section 2.3.1.

2.4. Physicochemical analysis

Biogas production of the reactor was recorded with a wet gas meter, while biogas production of the SMA test was analyzed via glass syringe. The biogas compositions (CH_4, CO_2, N_2, and H_2) were measured with a Shimadzu GC-8A gas chromatograph (Shimadzu, Japan). The pH, COD, total solid (TS), volatile solid (VS), volatile suspended solid (VSS), and ammonium nitrogen (NH_4^+) were determined with the Japan Standard Testing Method for Wastewater (JSWA, 1997). VFAs were assayed with an Agilent-6890 gas chromatograph (Agilent, USA). The total VFA (TVFA) was calculated by adding the COD of the individual VFAs. Total alkalinity (T-Alka) and bicarbonate alkalinity (B-Alka) were measured via titration with 0.1 N HCl according to Kafle and Kim (2011). The elemental compositions of C, H, O, N, and S were analyzed with a Vario EL III CHNS elemental analyzer (Elementar, German). Statistical analysis was conducted with IBM SPSS statistics 19.

2.5. Microbial community analysis

2.5.1. DNA extraction

Six samples were extracted from the reactor at different stages for