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A comparative study of thermophilic and mesophilic anaerobic co-digestion of food waste and wheat straw: Process stability and microbial community structure shifts

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ABSTRACT

Renewable energy recovery from organic solid waste via anaerobic digestion is a promising way to provide sustainable energy supply and eliminate environmental pollution. However, poor efficiency and operational problems hinder its wide application of anaerobic digestion. The effects of two key parameters, i.e. temperature and substrate characteristics on process stability and microbial community structure were studied using two lab-scale anaerobic reactors under thermophilic and mesophilic conditions. Both the reactors were fed with food waste (FW) and wheat straw (WS). The organic loading rates (OLRs) were maintained at a constant level of 3 kg VS/(m^3·d). Five different FW:WS substrate ratios were utilized in different operational phases. The synergetic effects of co-digestion improved the stability and performance of the reactors. When FW was mono-digested, both reactors were unstable. The mesophilic reactor eventually failed due to volatile fatty acid accumulation. The thermophilic reactor had better performance compared to mesophilic one. The biogas production rate of the thermophilic reactor was 4.9–14.8% higher than that of mesophilic reactor throughout the experiment. The shifts in microbial community structures throughout the experiment in both thermophilic and mesophilic reactors were investigated. With increasing FW proportions, bacteria belonging to the phylum Thermotogae became predominant in the thermophilic reactor, while the phylum Bacteroidetes was predominant in the mesophilic reactor. The genus *Methanosarcina* was the predominant methanogen in the thermophilic reactor, while the genus *Methanothrix* remained predominant in the mesophilic reactor. The methanogenesis pathway shifted from acetoclastic to hydrogenotrophic when the mesophilic reactor experienced perturbations. Moreover, the population of lignocellulose-degrading microorganisms in the thermophilic reactor was higher than those in mesophilic reactor, which explained the better performance of the thermophilic reactor.

1. Introduction

Anaerobic digestion (AD) has drawn wide attention as one of the most promising technologies for the treatment of high organic content waste and the recovery of renewable energy via biogas production in the last decade. Two typical bio-wastes, food waste (FW) and wheat straw (WS), greatly contribute to the high organic content in municipal solid waste and agricultural waste, respectively. About 90 million tons of FW is produced every year in China and it causes environmental pollution and threatens public health when inappropriately treated (Zhang et al., 2014). The total annual WS production is more than 130 million tons and most WS is discarded as environmental pollutants or even set on fire, causing serious air pollution (Chi et al., 2017; Shi et al., 2014). FW and WS has high organic matter contents and are suitable substrates for AD. Based on literature, if aforementioned FW and WS is properly treating by AD, the annual methane production potential can be up to 70 billion m^3, which is equivalent to 30.3% of annual demand for natural gas in China (Rajendran et al., 2014; Zhang, 2014). However, the efficiency of AD utilizing FW or WS as sole substrate is limited. Various operational problems such as ammonia inhibition, volatile fatty acid (VFA) accumulation, and low methane yield still exist and hinder the wide application of AD (Capson-Tojo et al., 2016; Li et al., 2017).

Abbreviations: AD, Anaerobic digestion; FW, Food waste; WS, Wheat straw; OLRs, organic loading rates; TS, Total solid; VS, Volatile solid; TAD, Thermophilic anaerobic digestion; MAD, Mesophilic anaerobic digestion; VFA, Volatile fatty acid.

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The AD process depends on a delicate balance between different groups of microorganisms to improve the stability and efficiency of the process. Microbial diversity and activities can be influenced by process parameters that in turn affect the overall performance of the reactor (De Vrieze and Verstraete, 2016; Lin et al., 2016). Understanding the microbial community structure and its shifts can therefore contribute to revealing how environmental factors affect digester performance (Fitamo et al., 2017). In the past decades, high-throughput sequencing technology based on the Illumina platform (MiSeq) is developed. It provides sufficient data to uncover the overall taxonomic composition of microbial communities, and to reveal the underlying mechanisms(Cabezas et al., 2015).

Substrate compositions and temperature are usually considered two of the key parameters that regulate the stability and performance of this process (Pagés-Díaz et al., 2015; Wang et al., 2014). Substrate compositions are usually addressed as C/N ratio of the substrate. The digestion of low C/N ratio substrates, such as FW, usually experiences ammonia inhibition, which is toxic to methanogens and leads to low biogas production (Ariunbaatar et al., 2015; Shi et al., 2017). High C/N ratio substrates, such as WS, have high content of cellulosic material and are not easily degraded, leading to low efficiency (Prochazka et al., 2012; Romero-Güiza et al., 2017). Anaerobic co-digestion of two or more substrates has been widely studied recently to overcome the drawbacks of digesting single substrates (Li et al., 2015; Mata-Alvarez et al., 2014). The addition of WS as a co-substrate of FW increases the C/N ratio and thus can alleviate ammonia inhibition to improve stability and performance.

Temperature is an important environmental parameter that affects how microorganisms live. AD was usually performed under mesophilic (30–40 °C) and thermophilic (at 50–60 °C) conditions. Previous studies showed that thermophilic AD (TAD) had the advantages of high biogas production, high organic loading rates (OLRs), and lower pathogen and virus content than mesophilic AD (MAD) (Guo et al., 2014). However, TAD required greater energy input to maintain its temperature. Several previous studies showed that MAD performance was more stable than that of TAD because TAD was more prone to perturbations and inhibition (from ammonia, lipids, etc.) (Guo et al., 2014; Jang et al., 2016).

Previous studies mainly focused on comparing the efficiency of the AD of certain substrates at a certain operating temperature, and some of the findings were contradictory. Besides, how the microbial community respond to different operational conditions and affect the process performance still remains little understood. A comprehensive study was thus required to compare performance at different temperatures with different substrate compositions, and to reveal the underlying mechanisms how the substrates composition and temperature affect the process performance and stability. The aim of the present study is to investigate the effects of temperature and substrate characteristics on process stability and microbial community shifts. Microbial communities in the reactors were investigated using Illumina platform (MiSeq) high-throughput sequencing. This work aimed to reveal the key microorganisms that are related to process performance and lignocellulose degradation at different temperatures. This study would be useful for the selection of optimal operation conditions and improving the efficiency of AD.

2. Materials and methods

2.1. Substrates and inocula

Raw FW was collected from students' restaurants at Tsinghua University, Beijing, China. The FW mainly consisted of cooked food leftovers such as rice, meat, tofu, vegetables, fats and oil. The raw FW was pretreated and homogenized into slurry using a food grinder after impurities such as bones, paper, and plastics were manually removed. The FW was stored at 4 °C before use. WS was collected from a farm located in Henan Province and dried at room temperature. The WS was grinded using a shredding machine and sieved through a 20-mesh screen before use. The total solid (TS) of WS was diluted to about 25% by adding fresh water. Thus, the volatile solid (VS) of FW and WS mixture was relatively stable in different phases. The characteristics of the substrates are summarized in Table 1. The inocula for a thermophilic reactor were taken from a full-scale thermophilic anaerobic digester that had been treating municipal sludge at 45–50 °C. The total solid (TS) percentage was 5.2 ± 0.5%, and the volatile solid (VS) percentage was 2.4 ± 1.1%. The inocula for a mesophilic reactor were taken from a full-scale mesophilic anaerobic digester that had been treating starch wastewater at 30–35 °C, and the TS and VS proportions were 5.7 ± 0.4 and 2.6 ± 0.7%, respectively.

2.2. Experimental set-up and reactor operation

Two identical lab-scale continuous stirred tank reactors (CSTRs) were prepared and then operated for 210 days. The total volume of each reactor was 6 L, and the working volumes were 4 L. The two reactors (R1, R2) were kept at 55 ± 1 and 35 ± 1 °C, respectively, using thermostatic water jackets (Fig. S1). The reactors were intermittently mixed using a mechanical stirrer (60 r/min). The substrates were fed into and the effluents were drawn from the reactors once per day. The organic loading rate (OLR) of both reactors was consistently 3 kg VS/(m^3·d). The experiment was divided into five phases (I-V) in which the proportion of FW (based on VS content) was 0, 50, 80, 90, and 100%, respectively. The experiment began with only WS as substrate in Phase I, and the FW proportion (base on VS content) then increased stepwise in Phases II-V. The operational conditions of both reactors are summarized in Table S1.

2.3. Physico-chemical analytical methods

TS, VS, protein, fat, cellulose, hemi-cellulose, and lignin contents of the samples were determined according to standard methods (APHA, 2005). The elemental compositions of substrates were analyzed using an elemental analyzer (CE-440, EAI CO., USA). Ammonia was first distilled (KDN-1, INESA CO., China) and then measured by titration (T860, Hanon CO., China) according to standard methods. Samples were centrifuged at 15,000 rpm for 20 min, and the supernatants were then filtered through a 0.45-μm membrane. VFA concentrations were then determined using a

Table 1
Solid and elemental contents of food waste and wheat straw.

Parameter		FW	WS
TS (%)		25.94 ± 1.12	95.94 ± 0.89
VS (%)		24.59 ± 0.84	94.91 ± 0.12
Elementary analysis	C/%	51.1 ± 1.4	42.7 ± 1.0
	H/%	7.4 ± 0.7	5.7 ± 0.3
	O/%	37.0 ± 1.6	36.8 ± 0.9
	N/%	3.4 ± 0.3	1.0 ± 0.1
C/N ratio		17.5 ± 1.5	42.2 ± 0.9
Total	C, H, O, N/%	98.9	86.2
Organic compounds	Protein/%	15.1 ± 0.8	2.7 ± 0.3
	Fats/%	10.6 ± 0.8	1.9 ± 0.1
	Cellulose/%	17.7 ± 0.8	45.0 ± 2.3
	Hemi-cellulose/%	21.3 ± 1.2	22.6 ± 1.1
	Lignin/%	9.0 ± 0.9	20.7 ± 1.8

TS: Total solids; VS: volatile solids; FW: food waste; WS: wheat straw.

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