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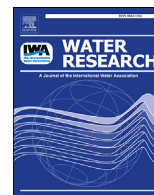

Effect of increased load of high-strength food wastewater in thermophilic and mesophilic anaerobic co-digestion of waste activated sludge on bacterial community structure

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A B S T R A C T

In recent years, anaerobic co-digestion (AcoD) has been widely used to improve reactor performance, especially methane production. In this study, we applied two different operating temperatures (thermophilic and mesophilic) and gradually increased the load of food wastewater (FWW) to investigate the bacterial communities during the AcoD of waste activated sludge (WAS) and FWW. As the load of FWW was increased, methane production rate (MPR; L CH_4/L d) and methane content (%) in both Thermophilic AcoD (TAcoD) and Mesophilic AcoD (MAcoD) increased significantly; the highest MPR and methane content in TAcoD (1.423 L CH_4/L d and 68.24%) and MAcoD (1.233 L CH_4/L d and 65.21%) were observed when the FWW mixing ratio was 75%. However, MPR and methane yield in both reactors decreased markedly and methane production in TAcoD ceased completely when only FWW was fed into the reactor, resulting from acidification of the reactor caused by accumulation of organic acids. Pyrosequencing analysis revealed a decrease in bacterial diversity in TAcoD and a markedly different composition of bacterial communities between TAcoD and MAcoD with an increase in FWW load. For example, Bacterial members belonging to two genera *Petrotoga* (assigned to phylum *Thermotogae*) and *Petrimonas* (assigned to phylum *Bacteroidetes*) became dominant in TAcoD and MAcoD with an increase in FWW load, respectively. In addition, quantitative real-time PCR (qPCR) results showed higher bacterial and archaeal populations (expressed as 16S rRNA gene concentration) in TAcoD than MAcoD with an increase in FWW load and showed maximum population when the FWW mixing ratio was 75% in both reactors. Collectively, this study demonstrated the dynamics of key bacterial communities in TAcoD and MAcoD, which were highly affected by the load of FWW.

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1. Introduction

Anaerobic digestion (AD) is a widely applied and mature technology for the treatment of the organic fraction of municipal solid waste (OFMSW), which provides the advantage of generating renewable energy in the form of methane (Li et al., 2011). Additionally, AD generates relatively low biomass and can be used for soil amendment (Abubaker et al., 2012). Recently, many researchers have focused on anaerobic co-digestion (AcoD) to overcome some drawbacks of mono-substrate digestion (Mata-Alvarez et al., 2014). The addition of a co-substrate can improve reactor stability and performance via dilution of inhibitory compounds and balancing micro- and macro-nutrients (Astals et al., 2013). Despite these advantages, numerous studies have reported negative effects of AcoD on methanogenesis, especially when adding carbon rich wastes as a co-substrate (Astals et al., 2012; Marañón et al., 2012; Robra et al., 2010). Therefore, it is important to select optimum

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co-substrates and mixing ratios to maximize synergisms between substrates.

Although most studies regarding AD have been conducted under mesophilic conditions, interest in thermophilic AD (TAD) has been raised mainly due to the insufficiently low biogas yield of mesophilic AD (MAD) (Peces et al., 2013). Recent studies have shown that TAD is more effective in producing methane and allowing higher organic loading rates (OLR) than MAD (Giuliano et al., 2013; Goberna et al., 2010). Also, TAD can achieve higher reduction of pathogens and antibiotic resistance genes (Ma et al., 2011; Watanabe et al., 1997). However, some researchers have pointed out that the high energy input necessary to maintain thermophilic conditions can offset any advantages as described above (De Baere, 2000; Li et al., 2011).

The key to successfully operating AD is the establishment of a microbial community delicately balanced between its two major domain bacteria and archaea. In general, bacteria are responsible for hydrolysis, acidogenesis and acetogenesis. According to previous studies, operating temperature and feedstock characteristics are the main factors influencing the bacterial community in AD. Guo et al. (2014) reported differences in bacterial communities between TAD and MAD of food waste (FW). More specifically, MAD showed higher bacterial diversity and predominantly acetic acid producing bacteria which are highly coupled with aceticlastic methanogen, especially *Methanosaeta*. Sundberg et al. (2013) found quite different microbial communities depending on feedstock characteristics. The author described that *Firmicutes* was the major phylum in AcoD of various substrates, while other phyla such as *Actinobacteria*, *Proteobacteria* and *Chloroflexi* were most prevalent in AD of sewage sludge.

Numerous molecular microbiology techniques based on 16s rRNA gene sequences such as fluorescence in situ hybridization (FISH), terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) and quantitative real-time polymerase chain reaction (qPCR) have been applied to elucidate microbial communities in target samples (Mata-Alvarez et al., 2014). Recently, pyrosequencing has also been widely used to determine microbial community structure in complex biological samples. This technique can provide high throughput of sequence data sufficient to cover the overall taxonomic composition. Furthermore, highthroughput sequencing of multiple samples is available in parallel using specific barcodes (Jang et al., 2014b). Sundberg et al. (2013) and Lee et al. (2012) reported the applicability of pyrosequencing in characterizing the highly-diverse microbial communities in full-scale AD treating various substrates under thermophilic and mesophilic conditions.

To the best knowledge of the authors, there have been no reports to date comparing the bacterial community in thermophilic AcoD (TAcoD) and mesophilic AcoD (MAcoD) of waste activated sludge (WAS) and high-strength food wastewater (FWW). In this study, pyrosequencing was used to investigate the bacterial community. More specifically, bacterial community shift in TAcoD and MAcoD of WAS and FWW was investigated as the load of FWW was gradually increased. Additionally, qPCR was also applied to quantify 16S rRNA gene copy numbers of total bacteria and archaea.

2. Materials and methods

2.1. Preparation of feedstock and seed sludge

The operation of TAcoD and MAcoD can be divided into five Runs (Fig. 1). For a stable start up, only WAS was fed into the reactors during Run I (day 1–60); the applied OLR was 2.83 kg COD/m^3 d. Subsequently the OLR of both reactors was gradually increased to 4.18 (Run II; day 61–120), to 5.62 (Run III; day 121–180), to 6.88 (Run IV; day 181–240) and to 8.21 (Run V; day 241–300) kg COD/m^3 d with a gradually increased FWW load (volume % of FWW in the feedstock mixture of WAS and FWW) in the following manner: 0% for Run I, 25% for Run II, 50% for Run III, 75% for Run IV and 100% for Run V (Fig. 1b).

The feedstock used in this study was collected from a municipal wastewater treatment plant (treats approximately 520,000 m^3/d of domestic wastewater) in Daegu, South Korea and a FWW storage tank in a FW recycling facility (treats approximately 180 ton/d of FW) in Pohang, South Korea. The FWW was filtered through a 1.0-mm sieve to remove inert materials, then distributed in 3-L bottles and stored at −25 °C until use. The seed sludge was obtained from a successfully-operated full-scale MAD plant in Daegu, South Korea.

The characteristics of WAS and FWW were assayed (Table S1); the main characteristics were as follows: pH 6.42 and 4.12, total solids (TS) 44.20 and 113.14 g/L, volatile solids (VS) 34.28 and 108.07 g/L, total chemical oxygen demand (TCOD) 56.58 and 164.25 g/L, soluble COD (SCOD) 6.48 and 105.57 g/L, total organic acids (TOA) 0.79 and 55.26 g COD/L, respectively.

2.2. Experimental setup and reactor operation

The experiment was conducted in lab-scale semi-continuous digesters (Fig. 1a), which were operated with a working volume of 5 L. To adapt seed sludge to thermophilic conditions, step wisely increased temperature (1 °C/d until reaching 55 °C) was used for TAcoD (de la Rubia et al., 2005). A constant 20-d hydraulic retention time (HRT) was used for both TAcoD and MAcoD (35 °C) over a period of 300 days, simulating that often used for AD. Feedstock was fed into the reactor four times a day using a peristaltic pump controlled by a timer and a relay.

2.3. Physico-chemical analytical methods

According to standard methods (APHA-AWWA-WEF, 1998), TS, VS, TCOD, SCOD and TOA were measured. The pH in the reactor and feedstock were continuously monitored using a pH meter (405-DPAS-SC-K85, METTLER TOLLEDO, Switzerland). Organic acids were quantified using a high performance liquid chromatograph (HPLC, Agilent Technology 1100 series, Agilent Inc., USA) equipped with an organic acid and alcohol analysis column (Aminex HPX-87H, BIORAD Inc., USA), a refractive index detector (RID), and a diode array detector (DAD). The quantified values were converted to g COD/L as previously described (Jang et al., 2014b). The total volume of biogas produced from the reactors was quantified using the water displacement method; biogas composition was determined using gas chromatography (Model 6890N, Agilent Inc., USA) equipped with a pulsed discharge detector (PDD).

2.4. qPCR and pyrosequencing analysis

Total genomic DNA used for qPCR and pyrosequencing was extracted and purified from seed sludge and samples during steady-state conditions for each Run using a Nucleo Spin® Soil kit (MACHEREY-NAGEL, Germany). The purified DNA was eluted with 50 μL of 1 × TE buffer and stored at −25 °C for further analysis. To quantify 16S rRNA gene copy numbers of total bacteria and archaea, qPCR amplification and fluorescence detection were conducted using an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, Forster City, USA) with universal primer sets and a representative strain reported in previous research (Jang et al., 2014a). All standards and samples were carried out in triplicate.

Hypervariable regions within bacterial 16s rRNA genes were amplified by PCR using universal primers: Bac27F (5′-adaptor A-Barcode-AC-GAG TTT GAT CMT GGC TCA G-3′)/Bac541R (5′-adaptor

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