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Bacteria and archaea communities in full-scale thermophilic and mesophilic anaerobic digesters treating food wastewater: Key process parameters and microbial indicators of process instability

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ABSTRACT

In this study, four different mesophilic and thermophilic full-scale anaerobic digesters treating food wastewater (FWW) were monitored for 1–2 years in order to investigate: 1) microbial communities underpinning anaerobic digestion of FWW, 2) significant factors shaping microbial community structures, and 3) potential microbial indicators of process instability. Twenty-seven bacterial genera were identified as abundant bacteria underpinning the anaerobic digestion of FWW. *Methanosaeta harundinacea*, *M. concilii*, *Methanoculleus bourgensis*, *M. thermophilus*, and *Methanobacterium beijingense* were revealed as dominant methanogens. Bacterial community structures were clearly differentiated by digesters; archaeal community structures of each digester were dominated by one or two methanogen species. Temperature, ammonia, propionate, Na^+, and acetate in the digester were significant factors shaping microbial community structures. The total microbial populations, microbial diversity, and specific bacteria genera showed potential as indicators of process instability in the anaerobic digestion of FWW.

1. Introduction

Food waste constitutes one of the three largest components of the organic waste disposed in South Korea; 8.4 million tons of food waste and wastewater was generated in 2011 (MoE, 2012). More than 90% of food waste generated in South Korea has been recycled into animal feed and compost, resulting in 3.4 million tons of food wastewater (FWW, also referred to as food waste leachate or food waste-recycling wastewater) per year (MoE, 2012). Owing to the high biogas potential of FWW (562 mL CH_4/g volatile-solids$_{added}$), anaerobic digestion (AD) has been considered as an attractive option to replace conventional treatment methods (Shin et al., 2015). Various types of AD process have been applied to full-scale systems in South Korea, including continuously stirred tank reactor (CSTR), upflow anaerobic sludge blanket (UASB), and plug-flow with different operating temperatures; at present, 20 full-scale AD plants treating food waste and/or FWW are being operated to treat 0.9 million tonnes of FWW/yr (Lee et al., 2014, 2016).

The AD process consists of the following four biochemical steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The first three steps are carried out by hydrolytic bacteria, acidogenic bacteria, and acetogenic bacteria, respectively, while methanogenesis is performed by methanogenic archaea (Demirel and Scherer, 2008). Hydrolysis and methanogenesis are commonly regarded as rate-limiting step of AD process depending on the nature of the substrate (Ma et al., 2013; Speece, 1996). Hydrolysis is limited in AD of suspended organic solids, whereas methanogenesis is limited in AD of soluble organic matter. Because acidogens and methanogens have different growing nature and sensitivity to environmental changes such as changes of temperature and potential inhibitory compounds, effective control or monitoring of the microbial communities underpinning these subsequent steps is required for successful operation of AD systems (Carballa et al., 2015; Hori et al., 2006; Speece, 1996; Wilson et al., 2013).

FWW is energy-dense waste, but its AD possess is associated with some concerns: 1) rich Na^+, lipid, and protein contents, and 2) unpredictable and large variations of FWW characteristics (Lee et al., 2009, 2016; Shin et al., 2015). This results in high loading of potential inhibitory compounds such as Na^+, ammonia, lipid, and volatile fatty acids (VFAs) in the digester with high variations over time. For these reasons, full-scale anaerobic digesters treating such complex organic wastewater often exhibit process instability with dynamic shifts of microbial community structures (Cho et al., 2013; Koo et al., 2017; Lee

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et al., 2016; Zhang et al., 2015). This explains why the concepts of microbial management and microbial indicators are receiving increasing attention; there is a need for an improved understanding of the structure and dynamics of the microbial communities in relation to different process conditions and their contributions in the different process stages to achieve effective management of the AD process and to pursue future improvement by optimizing process operation (Carballa et al., 2015; De Vrieze et al., 2016; Li et al., 2016).

Many studies have used high-throughput sequencing methods to elucidate microbial community structures in AD systems, but the relationships among process parameters, process performance, and microbial communities remain poorly understood (Li et al., 2016). Moreover, it has seldom been reported which factors are significantly shaping and changing microbial communities in different full-scale AD systems treating FWW. Therefore, in this study, process performance, process parameters, and microbial communities were monitored during 1–2 years of operation of four different full-scale mesophilic and thermophilic full-scale digesters treating FWW, in order to investigate: 1) microbial communities underpinning AD of FWW, 2) significant factors shaping microbial community structures, and 3) potential microbial indicators of process instability.

2. Materials and methods

2.1. Sampling and DNA extraction

To investigate the microbial profiles of full-scale AD systems treating FWW, four different anaerobic digesters were chosen: a thermophilic plug flow (TP) digester, a thermophilic CSTR (TC) digester, a mesophilic UASB (MU) digester, and a mesophilic CSTR (MC) digester. Four to five samples were collected from each digester over 1–2 years (Table 1). Four samples were collected during the period of stable performance (> 70% chemical oxygen demand (COD) removal efficiency) from each of the four digesters, and one sample from each of the TP, TC, and MU digesters (i.e., MU3, TP2, TC2) was collected during the period of low performance (< 60% COD removal efficiency) in order to investigate the relationships between microbial profiles and process performance of the digesters.

Influent FWW samples were collected from the influent pipes, and digester samples were collected directly from the digesters (except the TP digester, whose samples were collected from the effluent pipe). The collected samples were stored at 4 °C, and all physico-chemical analysis and DNA extraction were conducted within two days. Total DNA was extracted from the 19 different digester samples using an automated nucleic acid extractor (Magtration System 12GC, PSS, Chiba, Japan). Before extraction of genomic DNA, 200 µl of sample was centrifuged at 16,000*g* for 10 min, and 100 µl of supernatant was decanted. Then the pellet was washed twice in three steps: 1) the pellet was supplemented with 100 µl of deionized distilled water and resuspended; 2) the suspension was centrifuged; and 3) 100 µl of supernatant was decanted. Finally, the pellet was gently suspended and applied to the automated nucleic acid extractor. The pellet including the extracted DNA was eluted with 100 µl of Tris-HCl buffer (pH 8.0) and stored at −20 °C until further analysis.

Table 1
Characteristics, operating conditions and sampling date of the four different full-scale anaerobic digesters. (Sampling date: the date when influent and digester samples were collected).

Digester name	MU	MC	TP	TC
Digester configuration and operating conditions				
Digester type	UASB	CSTR	Plug-flow	CSTR
Size (m^3)	400 × 4	600	290 × 2	2200
Temperature (°C)	35	36	50	57.5
HRT (d)	7–10	30	39	15.5–17.5
Sampling date				
1st sample	OCT, 2010	OCT, 2011	JAN, 2011	JAN, 2011
2nd sample	APR, 2011	JAN, 2012	APR, 2011	APR, 2011
3rd sample	JUL, 2011	APR, 2012	JUL, 2011	OCT, 2011
4th sample	JAN, 2012	JUL, 2012	OCT, 2011	JAN, 2012
5th sample	JUL, 2012	–	JUL, 2012	JUL, 2012

Acronyms: CSTR, continuously stirred tank reactor; UASB, upflow anaerobic sludge blanket.

2.2. High-throughput sequencing analysis

To reveal the bacterial communities in the digesters, bacterial 16S rRNA genes were sequenced using the Ion PGM™ System (Life Technologies) in accordance with the manufacturer's instructions. For each DNA sample, the V3 hypervariable region of the bacterial 16S rRNA gene was amplified with Ion 16S Metagenomics™ kit (Life Technologies) and barcode-labeled with the Ion Plus fragment library kit (Life Technologies). For clonal amplification of the library, emulsion PCR was performed using the Ion PGM™ Hi-Q™ template kit (Life Technologies) and the OneTouch™ 2 instrument (Life Technologies). The template-positive ion sphere particles enriched by the Ion OneTouch™ ES (Life Technologies) were loaded on the 316™ chip using the Ion PGM™ Hi-Q™ sequencing kit, and sequenced in the Ion PGM™ sequencer (Life Technologies) operated by the Torrent Suite™ software (version 4.4.2). The obtained valid sequences, verified by the Ion Reporter™ software, were clustered with 97% sequence similarity as operational taxonomic units (OTUs) using UPARSE (usearch version v7.0.1090). A total of 634 406 rigid sequences were grouped into 1 454 OTUs, and these OTUs were analyzed using the SILVA database.

To reveal the archaeal communities in the digesters, 454 pyrosequencing of the archaeal 16S rRNA genes was performed at Macrogen (Seoul, South Korea) following the manufacturer's instructions (454 Life Science, Branford, CT, USA) as previously described (Lee et al., 2016). A total of 42 981 rigid sequences were grouped into 36 OTUs, which were analyzed using the SILVA database and the NCBI BLAST database. From the SILVA database, most archaeal OTUs (occupying 99% archaeal sequence reads) were classified as methanogen genera. For further analysis to the species level, NCBI BLAST was applied to estimate the closest species of each OTU.

2.3. Real-time quantitative polymerase chain reaction analysis

Real-time quantitative polymerase chain reaction (QPCR) (LightCycler 480, Roche) was used to quantify the populations (16S rRNA gene copies) of total bacteria and total archaea in the digesters. TaqMan probe-primer sets were used: for bacteria, BAC338F (5′-ACTCC TACGG GAGGC AG-3′), BAC516F (5′-TGCCA GCAGC CGCGG TAATA C-3′), and BAC805R (5′-GACTA CCAGG GTATC TAATC C-3′); for archaea, ARC787F (5′-ATTAG ATACC CSBGT AGTCC-3′), ARC915F (5′-AGGAA TTGGC GGGGG AGCAC-3′), and ARC1059R (5′-GCCAT GCACC WCCTC T-3′) (Yu et al., 2005).

2.4. Physicochemical analysis

A gas chromatograph (6890 Plus, Agilent, Palo Alto, CA, USA) with an HP Innowax capillary column and a flame ionization detector was used to measure VFAs and ethanol. The pH and COD concentration were determined according to the Standard Methods (APHA-AWWA-WEF, 2005). Carbohydrate concentration was measured using the phenol-sulfuric acid method (DuBois et al., 1956). Protein concentration and total ammonia nitrogen concentration [TAN] were determined using the Kjeldahl method (APHA-AWWA-WEF, 2005). Lipid concentrations [lipid] were measured by gravimetric analysis after extraction of lipid using chloroform: methanol (1:2 v/v) (Bligh and Dyer, 1959). Cations (including Na^+ concentration [Na^+]) were measured using ion chromatography (790 Personal IC; Metrohm, Herisau, Switzerland). All analyses were performed in duplicate.