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Temporal variation in methanogen communities of four different full-scale anaerobic digesters treating food waste-recycling wastewater

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HIGHLIGHTS

- *Methanoculleus* was dominant in the plug flow thermophilic digester.
- *Methanoculleus* and *Methanothermobacter* were dominant in the thermophilic CSTR.
- *Methanosaeta* was dominant in the UASB mesophilic digester.
- *Methanoculeus* and *Methanosaeta* were dominant in the mesophilic CSTR.

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ABSTRACT

Methanogen communities were investigated using 454 pyrosequencing in four different full-scale anaerobic digesters treating food waste-recycling wastewater. Seasonal samples were collected for 2 years, and 24 samples were available for microbial analysis from a plug flow thermophilic (PT) digester, a continuously-stirred tank thermophilic (CT) digester, an upflow anerobic sludge blanket mesophilic (UM) digester, and a continuously-stirred tank mesophilic (CM) digester. *Methanoculleus*, *Methanobacterium*, *Methanothermobacter*, and *Methanosaeta* were revealed to be key methanogens in full-scale anaerobic digestion process treating food waste-recycling wastewater. In the PT digester, *Methanoculleus* was dominant (96.8%). In the CT digester, *Methanoculleus* was dominant (95.4%) during the first year of operation, but the dominant genus was shifted to *Methanothermobacter* (98.5%) due to pH increase. In the UM digester, *Methanosaeta* was dominant (87.2%). In the CM digester, *Methanoculleus* was constantly dominant (74.8%) except during CM5 when *Methanosaeta* was dominant (62.6%) due to the low residual acetate concentration (0.1 g/L).

1. Introduction

Food waste-recycling wastewater (FRW) is produced by the process of recycling food wastes into animal feed or compost, and this process generated 3.4 million tons of FRW in 2011; this constituted 41% of the total annual food waste and wastewater generation in Korea (MoE, 2012). FRW is a high-strength organic wastewater, of which chemical oxygen demand (COD) is 60–300 g/L. For these reasons of high quantity and high organic material content, release of FRW into rivers or onto the ground could cause serious environmental damage. As a treatment of FRW, anaerobic digestion (AD) is a promising technology that can achieve organic waste reduction and sustainable energy generation simultaneously. Moreover, due to the high biodegradability and biogas potential of FRW, it is a more attractive substrate for anaerobic digesters compared to low-strength organic wastewaters (Shin et al., 2010). As a consequence, the Korean government has instituted a plan to install several full-scale anaerobic digestion plants to treat food waste and FRW in major cities in Korea by the end of 2014; currently seven full-scale AD plants treating FRW are being operated.

As the last stage of AD process, methanogenesis is performed by a wide variety of methanogens, which are sensitive to environmental conditions and various inhibitors. Because methanogenesis is generally regarded as the rate-limiting step in the overall AD process treating wastewater, appropriate control of the methanogenic phase has been a key factor in the successful operation of AD processes. Because the characteristics of FRW vary greatly for a variety of reasons including region of origin, seasonal change,

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and daily difference, the future composition of the FRW received at a full-scale AD system is almost impossible to predict. As a result, unpredictable fluctuations in substrate characteristics are often encountered. These fluctuations can stress the methanogenic communities, and eventually lead to instability of AD process and, sometimes, severe disturbances in process performance (Cho et al., 2013; Madsen et al., 2011). Therefore, an improved understanding of the structure and dynamics of the methanogen community in the anaerobic digester is thought to be of particular importance to obtain insight into the stability of the process and to allow future improvement by optimization of process operation.

The implementation of next-generation sequencing (NGS) technologies has initiated a new era in metagenomic analysis, enabling qualitative and quantitative analysis of the community of microorganisms in an AD system quickly and at low cost. 454 FLX Titanium pyrosequencing (Roche) is a commercially available NGS technology. The number of studies investigating microbial community structures in AD systems using 454 pyrosequencing is increasing rapidly, but still reference data for temporal profiling of anaerobic microbial communities are limited. Moreover, methanogen community structures in full-scale AD systems treating FRW have been seldom researched. Therefore, in this study, methanogen communities of four different full-scale anaerobic digesters treating FRW were analyzed using 454 pyrosequencing. To investigate temporal variation in methanogen communities, non-metric multidimensional scaling (NMS) and cluster analysis were performed on seasonal samples collected over a two-year period.

2. Methods

Four full-scale AD reactors in Korea were chosen for investigating the microbial community structures (Table 1): a thermophilic plug flow digester in Dalseong (PT), a thermophilic continuously-stirred tank reactor (CSTR) in Gwangju (CT), a mesophilic upflow anaerobic sludge blanket (UASB) digester in Songdo (UM), and a mesophilic CSTR at the Sudokwon Landfill Site (CM). Seasonal samples were collected from each digester over 2 years (Oct 2010–Jul 2012). All digesters were in stable operation with only minor changes in substrate composition and organic loading before the samples were collected. Some samples were not available for microbial analysis for various reasons (Table 1). Samples were collected from the effluent of PT but sampled directly from the other digesters. Pretreatment and DNA extraction were performed as previously described (Shin et al., 2010). The primers A-787f and B-1492r were used to amplify the target sequence of extracted genomic DNA (Quince et al., 2009). After purification, the 454 pyrosequencing analysis was performed at Macrogen (Seoul, Korea) following the manufacturer's instructions (454 Life Science, Branford, USA). The 16S rRNA gene sequences from the samples were sorted using trimBarcode.pl (Macrogen, South Korea). Low-quality reads (<Q20), short sequences (<270-bp) and potentially-chimeric sequences were discarded before further analysis.

pH and chemical oxygen demand (COD) were determined according to the procedures in Standard Methods (APHA-AWWA-WEF, 2005). Amounts of ionic compounds such as ammonium were measured using ion chromatography (790 Personal IC, Metrohm, Switzerland). Volatile fatty acids (VFAs, C2-C6) were identified using gas chromatography as previously described (Shin et al., 2010).

NMS ordination and unweighted pair group method with arithmetic means clustering analysis using Sorensen (Bray–Curtis) distance were conducted to visualize the similarity of methanogen communities within and among digesters and to group samples that share a similar methanogen community profile in a relatively quantitative manner (PC-ORD v.5.0, MjM software, Gleneden Beach, OR). Relative abundance of 16S rRNA gene sequence of methanogens at the genus level obtained by 454 pyrosequencing analysis was used as the data set. Closeness in the plot means that methanogen community structures are similar (Gardiner et al., 2009). Each main matrix was processed for ordination such that the stress (<10) and the instability ($<10^{-3}$) criteria were met (McCune et al., 2002).

3. Results and discussion

3.1. pyrosequencing analysis of methanogenic population profile in total

To monitor the temporal variability of methanogenic community structures within and among the digesters, seasonal samples were collected for 2 years. The 454 pyrosequencing of archaeal 16S rRNA gene amplicons from 24 samples (six samples from each of the anaerobic digesters observed) resulted in 133 317 non-chimeric sequence reads. In total, two phyla, four classes, six orders, nine families, and twenty genera were found in Archaea. Methanogens were the dominant (99.1%) archaea present. Non-methanogenic archaea (phylum *Crenarchaeota*, and classes *Halobacteria*, and *Thermoplasmata*) composed 0.9% of total archaeal sequence reads. The detected methanogens were from four orders (*Methanobacteriales* (MBT), *Methanocellales*, *Methanomicrobiales* (MMB) and

Table 1
Characteristics, operating conditions and sampling date of the four different full-scale reactors. (Absence of data due to a, Start-up period; b, Facility maintenance; c, Fail to analyze; d, total reads < 100).

Reactor name (location)		PT (Dalseong)	CT (Gwangju)	UM (Songdo)	CM (SLC)
Operating conditions					
Reactor style		Plug flow	2-Phase CSTR	UASB	2-Phase CSTR
Size (m^3)		290 × 2	2200	400 × 4	600
Temperature (°C)		55	55–62	35	35–37
HRT (d)		39	15.5–17.5	7–10	30
COD (g/L)		130–253	131–207	20–51	57–131
Sampling date					
1	2010	a	d	Oct. 07	a
2	2011	Jan. 28	Jan. 27	Jan. 27	Jan. 27
3		Apr. 06	Apr. 05	Apr. 04	Mar. 30
4		Jul. 13	d	b	Jul. 12
5		Oct. 13	Oct. 08	Oct. 07	Oct. 12
6	2012	Jan. 19	Jan. 18	Jan. 17	Jan. 17
7		c	Apr. 20	b	b
8		Jul. 15	Jul. 14	Jul. 13	Jul. 13