



# A comparative study on the process efficiencies and microbial community structures of six full-scale wet and semi-dry anaerobic digesters treating food wastes



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## ABSTRACT

The purpose of this study was to investigate the effect of different types of food wastes on the process efficiency and microbial community structures in full-scale anaerobic digesters and to identify parameters that affect these criteria. Six full-scale anaerobic digesters were investigated; three were operated under “wet” condition (total solids TS  $\leq$  10%), and three were run under “semi-dry” condition (10%  $\leq$  TS  $\leq$  20%). Removal efficiency of volatile solids was much higher in the wet digesters (75.2  $\pm$  3.8%) than in the semi-dry digesters (42.6  $\pm$  5.5%). The bacterial and archaeal communities were distinctly characterized by families Porphyromonadaceae, Sphingobacteriaceae, Syntrophomonadaceae, and Methanobacteriaceae in the wet digesters; and of Clostridiaceae, Patulibacteraceae, Pseudonocardiaceae, Lachnospiraceae, Rikenellaceae, and Methanomicrobiaceae in the semi-dry digesters. The discriminant parameters identified were TS content of influent, concentration of total ammonia nitrogen and the ratio of soluble chemical oxygen demand (COD) to COD in the digester.

## 1. Introduction

Approximately 5 million tonnes (Mt) of food waste (FW) is generated annually in South Korea and more than 90% of it is collected separately from other municipal solid wastes (MOE., 2013); 85.5% of the FW collected is recycled at public or private facilities to produce animal feed or fertilizer, and the rest is treated by anaerobic digestion (3.5%), co-treatment with sewage in wastewater treatment plants (2.4%), and landfill or incineration (8.4%). To meet the quality standards of feedstuffs or fertilizers, various unit operations such as screening, crushing, washing, evaporation, and filtration process should be conducted (Lee et al., 2009). However, recycling produces more than 3 Mt annually of food waste-recycling wastewater (FRW), which consists of food residues and leachates (MOE., 2015). Accordingly, a total of 8 Mt of food wastes (FWs) including FW and FRW is generated in South Korea every year. Because FWs commonly have low pH, high salt concentration, and high organic strength, disposal of FRW can cause serious environmental effects (Lee et al., 2009; Shin et al., 2015). Traditionally, most FRW has been dumped into the ocean, but this action has been prohibited since 2013 in accordance with London Convention and Protocol in 1996 on the prevention of marine pollution by dumping of wastes. Eventually, the FWs must be treated on land in an environmentally sound manner.

Anaerobic digestion (AD) is a promising treatment technology for FWs, because it can reduce FWs to a small quantity of innocuous digestate, while simultaneously generating combustible gas (including CH<sub>4</sub>). Furthermore, FWs have high biodegradability and energy production potential, and therefore are more attractive feedstocks for AD than are low-strength organics (Shin et al., 2015). Therefore, a plan to construct full-scale anaerobic digesters for treatment of FW and/or FRW has been instituted in South Korea, with 20 full-scale AD plants currently being operated: 15 for treatment of FRW and five for treatment of FW, including one for diluted FW (DFW). Thus, the FWs fed to the AD plants can be mainly divided into two types: FRW and FW. FRW is derived from FW, but may have different characteristics from FW, because water is added during recycling to reduce the salt concentration in FW to < 1% to meet government law. For this reason, moisture content is higher in FRW (84.1–96.0%) than in FW (61.3–87.1%) (Shin et al., 2015; Uçkun Kiran et al., 2014).

The moisture content of substrate affects the microbial behavior and mass transfer limitations in anaerobic systems (Bollon et al., 2013; Lay et al., 1997), so many researchers have claimed that total solids (TS) content affects the efficiency and microbial structures of anaerobic digesters. Decrease of moisture content from 96 to 90% (i.e., increase of

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TS content from 4 to 10%) can decrease the methanogenic activity from 100 to 53% (Lay et al., 1997). As TS increases from 10% to 25%, CH<sub>4</sub> production decreases; methanogenesis can be strongly suppressed at TS > 30% (Abbassi-Guendouz et al., 2012); genus *Clostridium* becomes dominant and archaeal community structures change (Abbassi-Guendouz et al., 2013). In contrast, increase in the TS content from 5% to 20% has been associated with increase in reduction of volatile solids (VS) and in CH<sub>4</sub> yield in a lab-scale continuous anaerobic digester treating FW; the species composition of bacterial and archaeal communities also shifted (Yi et al., 2014). The mixed observations of the effects of TS content suggest that this parameter is not the only factor that affects the AD efficiency and microbial structures, so additional study to identify other influential parameters is required.

Therefore, the aim of this study was to investigate the effect of different types of FWs that have different TS content on the process efficiency and microbial community structures in full-scale AD plants, and to identify the parameters that affect process efficiency and microbial structures.

## 2. Materials and methods

### 2.1. Full-scale AD plants and sampling

Six anaerobic digesters located in South Korea (Gimhae, Incheon, Goyang, Seoul, Daegu, and Busan) are full-scale plants that treat FWs (Table 1). All digesters are semi-continuously stirred tank reactors (CSTR); they operated stably over the sampling period under mesophilic condition. In Goyang only, FW gathered was diluted to 1.9 times its original volume with water before it was fed to the digester. Digesters W1 and W2 treat FRW, and D1 treats DFW; they were operated with various hydraulic retention times  $20 \leq \text{HRT} \leq 36$  d. Digesters F1, F2, and F3 treat FW; they were operated with  $30 \leq \text{HRT} \leq 40$  d. Samples were taken from the influent and the digesters and the first and second samples collected in August and October 2016 were marked “a” and “b”, respectively, with digesters name. Influent samples were taken from the pipes that convey mixed substrate into the digester, and digester samples were collected from pipelines that circulate digestate. Samples of 100–200 mL were collected in duplicate sterile plastic containers, stored in a mobile refrigerator at 4 °C, transported to the laboratory within 24 h, and mixed with equal volumes (100 mL) of samples in plastic containers (i.e., total 200 mL). Daily biogas production and CH<sub>4</sub> composition were obtained from digester-operating companies before and after 3 d of the sampling date; the average value was used. Methane yield was calculated as daily methane production divided by VS added to the digester.

**Table 1**

The operational parameters and sampling date of the six different full-scale digesters.

Feedstock	FRW	FRW	DFW	FW	FW	FW
Digester name	W1	W2	D1	F1	F2	F3
Location	Gimhae	Incheon	Goyang	Seoul	Daegu	Busan
<i>Operational parameter</i>						
Reactor type	Semi-CSTR	Semi-CSTR	Semi-CSTR	Semi-CSTR	Semi-CSTR	Semi-CSTR
Size (m <sup>3</sup> )	2500	18,100	11,166	2950	2400	6000
HRT (d)	25–27	30–36	21–25	30–40	30–40	30–40
Daily Biogas production (Nm <sup>3</sup> /d)	10,405 ± 430	48,339 ± 2,624	12,607 ± 1,563	10,101 ± 929	7,737 ± 954	10,004 ± 138
<i>Sampling date</i>						
a	2016 Aug. 08	Aug. 16	Aug. 16	Aug. 17	Aug. 12	Aug. 09
b	2016 Oct. 04	Oct. 10	Oct. 10	Oct. 12	Oct. 08	Oct. 06

**Acronyms:** CSTR, continuously stirred tank reactor; FRW, food waste-recycling wastewater; DFW, diluted food waste; FW, food waste; “W1 and W2”, digesters treating food waste-recycling wastewater; “D1”, digester treating diluted food waste; “F1, F2, and F3”, digesters treating food waste; “Nm<sup>3</sup>/d”, normalized to STP condition; “a”, the first sampling round; “b”, the second sampling round.

### 2.2. Physicochemical analysis

TS, VS, and chemical oxygen demand (COD) were measured using Standard Methods (APHA-AWWA-WEF, 2005). Soluble COD (SCOD) was measured in samples filtered through GF/C filters. Volatile fatty acid (VFA; C<sub>2</sub>–C<sub>6</sub>) concentrations were quantified using a gas chromatograph (6890 plus, Agilent, Palo Alto, CA) equipped with an Innovax capillary column (HP-INNOWAX, Agilent technologies, USA) and a flame ionization detector. The carrier gas was He at a flow rate of 1.7 mL/min with a split ratio of 10:1. Injector temperature was 230 °C and detector temperature was 250 °C. Oven temperature was programmed to increase from 60 °C to 120 °C at 20 °C/min, then to 205 °C at 10 °C/min, then held for 2 min. The carbohydrate concentration was quantified using the phenol-sulfuric acid method (Dubois et al., 1956). Total Kjeldahl nitrogen (TKN) and NH<sub>3</sub>-N concentrations were measured using the Kjeldahl method (APHA-AWWA-WEF, 2005). Organic nitrogen was estimated as the difference between TKN and NH<sub>3</sub>-N; for 1 g of organic nitrogen, 6.25 g of protein was assumed. Lipid concentration was analyzed using the gravimetric method following extraction of lipids by solvent (chloroform: methanol, 1:2 v/v) (Bligh & Dyer, 1959). All physicochemical analyses were conducted in duplicate.

### 2.3. DNA extraction and pyrosequencing

Immediately after return to the laboratory, 0.2 mL of each digester sample was centrifuged twice at 12,000 g for 10 min; the supernatant was removed each time to minimize levels of potential PCR inhibitors and DNA from cell debris (Shin et al., 2010). An automated nucleic acid extractor (Magtration System 6GC, Precision System Science, Chiba, Japan) was used to extract DNA from the pelleted samples. The purified DNA was eluted with nuclease-free water and stored at –20 °C until use.

To reveal the microbial community structures in the six AD plants, 16S rRNA genes were sequenced using the Ion PGM™ System (Life technologies) according to the manufacturer’s instructions. For each DNA sample, the V4 and V5 hypervariable regions for the bacterial and archaeal 16S rRNA gene were amplified using an Ion 16S Metagenomics™ kit, then barcode-labeled using the Ion Plus fragment library kit. For clonal amplification of the library, emulsion PCR was performed using the Ion PGM™ Hi-Q™ template kit and the OneTouch™ 2 instrument. The template-positive Ion Sphere Particles enriched by the Ion OneTouch™ ES were loaded on the 316™ chip using the Ion PGM™ Hi-Q™ sequencing kit, and sequenced in the Ion PGM™ sequencer operated by Torrent Suite™ software (version 4.4.2). Obtained sequence reads were analyzed using Ion Reporter™ software with the default

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