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Seasonal monitoring of bacteria and archaea in a full-scale thermophilic anaerobic digester treating food waste-recycling wastewater: Correlations between microbial community characteristics and process variables

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HIGHLIGHTS

- Na^+ and lipid could affect COD removal and bacteria community.
- Na^+ and lipid could affect archaeal quantity but not its community structure.
- *Gelria* and *Cardiocoprobacter* were negatively correlated with Na^+ and lipid.
- NH_3 had no correlation with COD removal or with total microbial populations.
- *Methanoculleus*, *Methanobacterium*, *Tepidanaerobacter* responded differently to NH_3.

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ABSTRACT

Microbial population size, community structure, and diversity, and the correlations of these characteristics with process variables were investigated in samples taken seasonally over two years from a full-scale thermophilic anaerobic digester treating food waste-recycling wastewater (FRW). The organic component of the FRW consisted of carbohydrate (35% of volatile solids), protein (34%) and lipid (30%). The chemical oxygen demand (COD) removal efficiency of the anaerobic digestion (AD) system negatively correlated with Na^+ (2.9–7.7 g/L) and lipid (3.3–22.8 g/L) concentrations, which varied significantly over the two years. *Tepidanaerobacter*, *Anaerobaculum*, *Defluviitoga*, *Keratinibaculum*, *Gelria*, *Tepidimicrobium*, *Caldicoprobacter*, *Bacillus*, and *Syntrophaceticus* were the major bacterial genera, and *Methanoculleus* and *Methanobacterium* were the major archaeal genera. Concentrations of Na^+ and lipid in the digester were negatively correlated with total bacterial and archaeal populations determined by real-time quantitative PCR. These concentrations could also significantly affect the bacterial community structure (e.g., negative correlations with *Gelria*), but not archaeal community structure. Lipid concentration was negatively correlated with bacterial diversity, but was not correlated with archaeal diversity. Ammonia concentration in the digester (2.0–4.3 g N/L) had no significant correlation with COD removal or total bacterial/archaeal populations, but could significantly affect both bacterial and archaeal community structures, including syntrophic acetate-oxidizing bacteria and hydrogenotrophic methanogens. These results indicate that Na^+, lipid and ammonia are among the key parameters that affect the process performance of a thermophilic AD system treating FRW and/or the microbial communities in it.

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1. Introduction

Food waste is one of the three largest components by weight of the organic waste stream in Korea; 8.4 million tons of food waste and wastewater (14.3% of total organic waste generation) was generated in 2011 [1]. In Korea, food waste has been selectively collected among household wastes by law since 2003, and > 95% is recycled as animal feed or compost. During the recycling process, 3.4 million tons of food waste-recycling wastewater (FRW, also referred to as food waste leachate or food wastewater) have been generated annually, and constituted 41% of the total annual food waste and wastewater generation in 2011 [1]. FRW is a high-strength organic wastewater, that has 48–200 g chemical oxygen demand (COD)/L [2]. Due to the large quantity and the high organic material content, significant environmental effects are anticipated if FRW is released into the ecosystem without adequate treatment.

Anaerobic digestion (AD) has been considered as a treatment option that can reduce the quantity of organic waste, and simultaneously generate CH_4, which can be used as a fuel. The Korean government has invested considerable money to install full-scale anaerobic digesters in major cities in Korea to treat food waste and/or FRW; at present, twenty full-scale AD plants are being operated.

AD consists of four sequential biochemical processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Process imbalance between acidogenesis and methanogenesis may occur (i.e., the total concentration of volatile fatty acids (VFAs) [TVFA] produced by acidogens exceeds the amount of VFAs consumed by methanogens) as a result of hydraulic or organic overloading, the presence of compounds that inhibit anaerobes, or changes in process conditions and influent substrate [3]. The content of major organic components (i.e., carbohydrate, protein, and lipid) would also affect the stability and efficiency of AD because they are degraded into various metabolites via different biochemical pathways [4]. FRW is reported to contain high contents of lipid (30.5 g/L; 37% of volatile solids (VS)), protein (24.6 g/L; 30% of VS), and Na^+ (2.1 g/L for FRW, 6.9 g/L for food waste), so potentially-inhibitory compounds such as long chain fatty acids (specified as lipid), ammonia, and Na^+ could be primary concerns in AD of FRW [2,5,6]. Furthermore, in a full-scale AD system, the characteristics of the feedstock can vary unpredictably; such fluctuations may cause imbalance between acidogenesis and methanogenesis, and can change the microbial community structure as well as process stability and performance [7,8]. In fact, substrate utilizations and inhibitory effects are correlated with growth of specific microorganisms and to specific shifts in the microbial communities during variations of process performance, but the natures and degrees of these correlations remain unknown. In general, process functionality (and metabolic rate) is higher but microbial diversity is lower in thermophilic AD than in mesophilic AD, so stable operation may be difficult to attain in thermophilic AD systems [9,10]. Thus, understanding of correlations within and among process variables and microorganisms in a thermophilic AD system would provide new insight into the interactions that affect the stability of the process.

Many studies have used high-throughput sequencing methods to quantify microbial community structures in AD systems, but reference data for profiling of anaerobic microbial communities on various types of organic waste are limited [11]. Moreover, variations of microbial communities in a full-scale thermophilic AD system treating FRW in the presence of variations in inhibitory effects (e.g., by Na^+ and lipid) have not been reported to the best of our knowledge. Therefore, in this study, two-year variations (covering four seasons) of microbial communities of a full-scale thermophilic anaerobic digester treating FRW were analyzed using 454 pyrosequencing. Non-metric multidimensional scaling was conducted to investigate temporal variation in microbial community structure over this period. Correlations within and among process variables and microbial communities in the AD system were determined using correlation tests and visualized using redundancy analysis.

2. Materials and methods

2.1. Full-scale anaerobic digester

A full-scale anaerobic digester (working volume 2200 m^3) fed with FRW in city of Gwangju, Korea was chosen for investigation of microbial community structures. The digester is a thermophilic (58.5 °C) continuously-stirred tank reactor (CSTR), and so it is abbreviated as TC. Hydraulic retention time of the digester varied from 15.5 d to 17.5 d.

2.2. Sampling and DNA extraction

The organic and inorganic composition of FRW varies widely over seasons [2], so the effects of this variation may affect process variables and microbial communities in the thermophilic AD system treating FRW. To consider its temporal variations, eight seasonal samples were collected from October, 2010 to July, 2012. The samples were numbered chronologically: TC1 = Oct 2010; TC2 = Jan 2011; TC3 = Apr 2011; TC4 = Jul 2011; TC5 = Oct 2011; TC6 = Jan 2011; TC7 = Apr 2012; TC8 = Jul 2012). Samples were collected from the influent pipe and directly from the digester, and stored in an ice box as soon as possible.

Total genomic DNA was extracted from the TC samples by using an automated nucleic acid extractor (Magtration System 6GC, 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 the supernatant was decanted. Then the pellet was washed twice in three steps: (1) the pellet was added with 100 μl of deionized distilled water and resuspended; (2) the suspension was centrifuged; (3) 100 μl of supernatant was decanted. Finally, the pellet was gently suspended and applied to an automated nucleic acid extractor (Magtration System 6GC, PSS Co., Japan). The pellets including the extracted DNA were eluted with 100 μl of Tris–HCl buffer (pH 8.0) and stored at −20 °C until further analyzed.

2.3. Physicochemical analysis

A gas chromatograph (6890 Plus, Agilent, Palo Alto, CA) with an HP Innowax capillary column and flame ionization detector was used to measure VFAs and ethanol. The pH, chemical oxygen demand (COD) and VS concentrations were determined according to the procedures in Standard Methods [12]. Carbohydrate concentration was measured using the phenol–sulfuric acid method [13]. Protein concentration and total ammonia nitrogen [ammonia] were determined using the Kjeldahl method [12]. Lipid concentrations [lipid] were measured by gravimetric analysis after extraction of lipid using chloroform: methanol (1:2 v/v) [14]. Sodium ion concentration [Na^+] and chloride ion concentration were measured using ion chromatography (790 Personal IC, Metrohm, Switzerland). All analyses were performed in duplicate.

2.3.1. Pyrosequencing analysis

Several fusion primers were designed under the guidance of Macrogen (Seoul, Korea) and synthesized by Bioneer (Daejeon, Korea). The fusion primers consisted of adapter A or B and the oligonucleotide for amplifying the target sequence. The combined primers A-787f (5′-ATTAG ATACC CNGGT AG-3′) and B-1492r