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A pilot scale two-stage anaerobic digester treating food waste leachate (FWL): Performance and microbial structure analysis using pyrosequencing

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ABSTRACT

Food waste leachate (FWL) from the food waste recycling facilities in Korea is a serious environmental problem. Much research was done on anaerobic digestion of FWL in a lab-scale; however, there is little information on a large scale anaerobic digestion system (ADS). In this study, a two-phase ADS in a pilot scale was operated using FWL and the ADS performance and microbial structure dynamics using pyrosequencing were investigated. The ADS was operated for 136 days using FWL containing a high concentration of volatile fatty acid (12,435 ± 2203 mg/L), exhibiting volatile acid (VS) removal efficiency of 74–89% and CH_4 yield of 0.39–0.85 Nm^3/kg of reduced VS. The microbial structure at 76, 101, and 132 days indicated the methanogen population shift from acetoclastic methanogens (*Methanosarcina* and *Methanosaeta*) to hydrogenotrophic methanogens (*Methanobacterium* and *Methanoculleus*). The bacterial community also shifted to the taxa syntrophically related with hydrogenotrophic methanogens (*Clostridia*). The statistical analysis revealed the positive correlation of VS removal efficiency with *Methanosarcina*, but the negative correlation with *Methanobacterium*. The results presented here suggest that acetoclastic methanogens and their associated bacteria were more efficient for VS removal in the pilot scale ADS system, providing useful information for FWL treatment in a large scale ADS.

1. Introduction

Increasing municipal waste has become one of the major environmental concerns in modern societies. Especially, in South Korea, daily disposed food waste is approximately 13,000 ton per day, accounting for over 27% of total municipal solid waste [1]. Food waste was generally dumped with other municipal solid waste or discharged to the ocean; however, landfilling of food waste has been prohibited since 2005 and the marine disposal of organic waste such as food waste has been banned since 2012 in South Korea according to London dumping convention [1,2]. To solve food waste problem, the strict food waste management policies have been enforced to collect food waste separately from other waste so that food waste can be recycled as a source of livestock feed or fertilizer [1,3]; however, a great amount of organic-rich food waste leachate (FWL) is generated as secondary wastewater from the food waste recycling processes [1]. Because FWL has been also banned to discharge in the ocean since 2012, a biological anaerobic digestion system (ADS) using FWL can provide solutions to both waste treatment and energy by producing methane [1,2,4,5].

In general, a series of anaerobic reaction occurs during anaerobic digestion by four groups of microorganisms responsible for hydrolysis, acidogenesis, acetogenesis, and methanogenesis [6]. Methane is produced through hydrogen oxidation ($H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) by hydrogenotrophic methanogens or through the acetoclastic cleavage of acetate ($CH_3COO^- + H^+ \rightarrow CO_2 + CH_4$) by acetoclastic methanogens. A two-phase system which physically separates hydrolysis/acidogenesis in an acidifying reactor and acetogenesis/methanogenesis in a methanogenic reactor is known to be

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effective because an independent kinetic control of each reactor can be maintained [5]. Because the performance and the stability of anaerobic digestion are strongly related with the microbial community structure, there have been many studies on the characterization of microbial community structure in ADS during the treatment of food waste and FWL [1,3,7–10]; however, those results were obtained from lab-scale anaerobic digesters with relatively low organic contents to stabilize anaerobic digestion processes. More importantly, considering that food waste recycling facilities in South Korea are operated throughout the country in a large scale, anaerobic digestion performance and microbial community in a large scale ADS using organic-rich FWL from the commercial food waste recycling facilities should be investigated.

Denaturing Gradient Gel Electrophoresis (DGGE) and clone library were the most commonly used technique to investigate the microbial community [11]. However, it is often difficult to investigate a complex microbial community using DGGE methods because of too many bands to identify and co-migration of different sequences. Indeed, Shin et al. [7] and Lee et al. [11] reported that no *Methanobacteriales*-related band was detected using DGGE, while 16S rRNA gene proportion of this order accounted for up to 19% using real-time PCR, indicating limitation of DGGE. Also, it is very laborious and expensive to analyze the microbial community quantitatively with clone library.

Recently, pyrosequencing technology, a high-throughput sequencing method, has been widely used in the field of microbial ecology with advantages over conventional methods [3,12]. Currently, this technology can generate up to 1,200,000 reads in single run with an average sequence read of 400 bp in length, making a massive and powerful analysis of complex microbial communities possible.

In this study, the organic-rich FWL generated from a livestock feed production facility using food waste was utilized as an influent for a pilot scale of ADS. A modified two-phase ADS with acidogenic and methanogenic reactors was operated over 130 days without failure and the microbial community structure of the ADS was analyzed to investigate the relationship between the microbial community and the physiological profiles in the ADS. Here we used 454 pyrosequencing technology and bioinformatics to quantitatively analyze the microbial communities in the ADS for both the methanogenic and the bacterial community. The results presented herein could provide information for the treatment of FWL in a large scale of ADS through not only environmentally friendly but also renewable processes by producing methane.

2. Materials and methods

2.1. ADS reactor operation and performance analysis

The FWL used in this study was obtained at regular intervals from a commercial livestock feed production facility utilizing food waste in Yeoncheon, South Korea. Activated sludge from a municipal wastewater treatment facility (Yeoncheon, Korea) was used as an inoculum. The ADS used in this study was a modified two-phase anaerobic digester with an additional stabilizing reactor. The acidified FWL was transferred to the methanogenic reactor, and then the digested FWL was transferred to the stabilizing reactor for further digestion. The methanogenic reactor was adapted from the waste treatment system of GBU mbH (Bensheim, Germany) (Fig. S1). It consisted of two separated fermentation chambers in which mixing was automatically achieved between chambers by a water head difference caused by biogas production. The acidifying reactor and the stabilizing reactor were continuously mixed using an impeller at 68 and 720 rpm, respectively. The working volume of the acidifying reactor, the methanogenic reactor, and the stabilizing reactor were 5.5 m^3, 50 m^3, and 20 m^3, respectively. The hydraulic retention time (HRT) of the acidifying reactor and the methanogenic reactor was set to 3 and 27 days, respectively, because the acidification was much faster than the methanogenic process. The total biogas produced from both the methanogenic reactor and the stabilizing reactor was collected in the head space of stabilizing reactor. The volatile solid (VS) was regarded as organic carbon and the organic loading rate (OLR) to the methanogenic reactor was 2.36 ± 0.15 (kg VS/m^3 d) during 151 days of operation. OLR was stopped after 151 days because biogas production had rapidly deceased for some reason. One liter of the sample used for analyses was obtained through the sampling ports of each reactor and was analyzed in duplicate. The microbial samples for the methanogenic reactor were taken from the main fermentation chamber (Fig. S1).

The pH of the sludge samples was measured using an Orion-502A pH meter (Thermoscientific, USA). The TS and VS of the sludge samples were measured according to the standard methods for the examination of water and wastewater from American Public Health Association (APHA). The concentration of VFA was determined using a GC-FID (GC7890A; Agilent, USA) equipped with HP-INNOWAX capillary column (Agilent, USA). Nitrogen was used as a carrier gas. The samples were centrifuged, and the supernatants were filtered using a syringe filter (0.22 μm). The filtrate was diluted with distilled water and used to determine the concentration of VFAs. The content of methane in the biogas was determined using a Biogas 905 gas analyzer (ADOS, Germany).

2.2. Pyrosequencing and processing of the reads

The samples for the microbial community analysis in the methanogenic and stabilizing reactor were obtained approximately once a month, specifically at 76, 101, and 132 days, as indicated in the following sample names: MA_{76}, MA_{101}, and MA_{132} for archaeal communities in the methanogenic reactor; MB_{76}, MB_{101}, and MB_{132} for bacterial communities in the methanogenic reactor; SA_{76}, SA_{101}, and SA_{132} for archaeal communities in the stabilizing reactor; SB_{76}, SB_{101}, and SB_{132} for bacterial communities in the stabilizing reactor.

Total genomic DNA was extracted from samples using an i-genomic BYF DNA extraction kit (Intron biotechnology, Korea), according to the manufacturer's instructions. The 16S rRNA genes were amplified using bar-coded universal primers for each sample. The primer sequences are as follows: bacterial universal (27F: AGA GTT TGA TCM TGG CTC AG, 518r: WTT ACC GCG GCT GCT GG) and archaeal universal (arc112F: GCT CAG TAA CAC GTG G, arc516r: GGT DTT ACC GCG GCK GCT G) for bacterial and archaeal 16S rRNA gene amplification, respectively. The PCR reactions were conducted in a thermocycler (SC200; Kyratec, Australia) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s, and elongation at 72 °C for 1 min. The amplified 16S rRNA gene was purified using a resin spin column, and 1 g of each amplified product was mixed and subjected to pyrosequencing using a 454 Genome Sequencer FLX Titanium (Roche, Basel, Swiss) at Chunlab (Seoul, Korea). Raw nucleotide sequence reads from the samples were sorted and processed, as previously described [12]. Raw nucleotide sequence reads were sorted by specific barcode sequences of each sample, then non-16S rRNA reads and reads without primer were removed from barcode-sorted sequences. The chimera sequences of selected reads were removed using MOTHUR with Bellerophon methods [13]. Each sequence read was assigned to a taxonomic group using Eztaxon-extended database, as previously described [12]. The ratio of each taxon in a specific sample was defined as the number of assigned sequence reads of specific taxon divided by the number of total sequence reads. Sequences in this study were deposited in the NCBI short-read archive under the accession number SRA023676.

2.3. Estimation of species richness and diversity

The samples were normalized by generating random subsets from each sample. Each random subset contained 1022 randomly selected archaeal sequence reads or 2892 randomly selected bacterial sequence reads from each sample. The diversity indices (ACE and Chao1) for each sample were calculated using MOTHUR [13]. The OTUs and estimators were calculated in different clustering distances; 0.01 (strain), 0.03 (species), and 0.06 (genus). The representative sequence of archaeal community was determined in the clustering distance of 0.03. The alignment and phylogenetic analysis was carried out as previously described [12].

2.4. Statistics

The correlation efficiency (r) and p-value (p) of the correlation between microorganisms and other factors were calculated using R package. p-Values less than 0.05 and 0.01 were regarded as statically significant and highly significant, respectively. Any r^2 values more than 0.5 and 0.85 were regarded as strongly correlated and very strongly correlated, respectively [14].

3. Results and discussion

3.1. ADS performance

As organic loading rate was gradually increased to 2.2 kg VS/m^3 d during 25 days of operation (a start-up period), pH in the methanogenic reactor dropped from 7.3 to 6.5 and then increased to 7.2. This pH drop at the initial start-up period possibly occurred because a buffering capacity in the methanogenic reactor was not build up enough to neutralize acidic effluent (pH 3.96 ± 0.30) from the acidifying reactor. Biogas production and CH_4