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Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project

Michihiko Ike [a], Daisuke Inoue [a], Tomoki Miyano [a], Tong Tong Liu [a], Kazunari Sei [a], Satoshi Soda [a,*], Shiro Kadoshin [b]

[a] Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan
[b] Kyotango Recycling Resources Factory, Amita Co. Ltd., 301-1 Kikoritani, Yayoicho, Funaki-koaza, Kyotango, Kyoto 627-0143, Japan

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ABSTRACT

The microbial community in a full-scale anaerobic digester (2300 m^3) treating industrial food waste in the Kyoto Eco-Energy Project was analyzed using terminal restriction fragment length polymorphism for eubacterial and archaeal 16S rRNA genes. Both thermophilic and mesophilic sludge of treated swine waste were seeded to the digestion tank. During the 150-day startup period, coffee grounds as a main food waste, along with potato, kelp and boiled beans, tofu, bean curd lees, and deep-fried bean curd were fed to the digestion process step-by-step (max. 40 t/d). Finally, the methane yield reached 360 m^3/t-feed with 40 days' retention time, although temporary accumulation of propionate was observed. Eubacterial communities that formed in the thermophilic digestion tank differed greatly from both thermophilic and mesophilic types of seed sludge. Results suggest that the *Actinomyces*/*Thermomonospora* and *Ralstonia*/*Shewanella* were contributors for hydrolyzation and degradation of food waste into volatile fatty acids. Acetate-utilizing methanogens, *Methanosaeta*, were dominant in seed sludges of both types, but they decreased drastically during processing in the digestion tank. *Methanosarcina* and *Methanobrevibacter*/*Methanobacterium* were, respectively, possible main contributors for methane production from acetate and H_2 plus CO_2.

1. Introduction

Kyoto prefecture in Japan is promoting the Kyoto Eco-Energy Project (KEEP) for reducing fossil fuel consumption (Nakamura, 2006). Supported by the New Energy and Industrial Technology Development Organization (NEDO), Japan, KEEP is done in Kyotango city and at several private enterprises. An anaerobic digestion process has been used for economical recovery of methane gas from food wastes in KEEP since 2005. Organic waste collected from food-processing plants is treated in an anaerobic digestion tank (2300 m^3). Methane produced from food waste is used for power generation by fuel cells (250 kW) and gas engines (80 kW × 5).

The startup is generally considered as the most critical step in the operation of anaerobic digestion processes. At high loadings, such as during startup, reduced intermediates (propionate, butyrate, lactate, and acetate) accumulate because hydrogenotrophs fail to consume hydrogen produced during the fermentation and acetogenesis processes. The presence of lipids in food wastes might also cause volatile fatty acid (VFA) accumulation through hydrolysis of triglycerides. The accumulation of VFAs can engender a drop in pH and inhibit methanogenesis. However, once an anaerobic digester has been started up successfully, it is expected to run without much attention as long as operational conditions are not changed drastically. For starting up of anaerobic digestion processes, the microbiological aspects of the process must be studied to elucidate the biochemical reactions and the key controlling microorganisms such as acidogens, acetogens, and methanogens.

Until recently, such studies were difficult because of the lack of adequate tools to monitor the microorganisms. Advances in molecular biology have enabled rapid characterization of anaerobic digestion processes. However, none has characterized changes in the microbial community structure during the starting up of full-scale anaerobic digestion plants designed for industrial food wastes. Few studies of the archaea (methanogen) community have investigated the digestion performance of laboratory-scale plants treating simulated municipal solid waste and biosolids (Griffin et al., 1998), municipal solid waste and sewage sludge (McMahon et al., 2001, 2004), and waste-activated sludge (Kobayashi et al., 2009). In addition, to archaea, eubacteria including acidogens and acetogens should be monitored and controlled for the successful startup of anaerobic digestion processes. Angenent et al. (2002) elucidated the major route of methane production through a

* Corresponding author. Tel.: +81 (0)6 6879 7673; fax: +81 (0)6 6879 7675.
E-mail address: soda@see.eng.osaka-u.ac.jp (S. Soda).

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syntrophic relation between acetate-oxidizing bacteria and H_2-utilizing methanogens using 16S rRNA gene probes during the starting up of a full-scale anaerobic digester (600 m^3) treating swine waste. Cheon et al. (2008) applied the random cloning method to a full-scale digester (1100 m^3) treating garbage during startup, but the relation between the microbial community and the digestion performance was not discussed. Such reports describing microorganisms in full-scale reactors with qualitative and quantitative changes of substrates during startup are scarce.

In this study, the microbial community in the full-scale anaerobic digester treating industrial food waste during startup in KEEP was characterized. During the startup period of 150 days, the anaerobic digester treated coffee grounds as a main food waste, along with potato, kelp (kombu) and boiled beans, tofu (traditional oriental soy bean cake), bean curd lees (a byproduct of tofu production), and deep-fried bean curd, added step-by-step. Microbial community analyses were executed by terminal restriction fragment length polymorphism (T-RFLP) analysis for eubacterial and archaeal 16S rRNA genes.

2. Methods

2.1. Anaerobic digestion process

The food waste treatment in KEEP started in September 2005. Food waste was fed to mixers and stocked in a stock tank (150 m^3) before entering a digestion tank. The 2300 m^3 gas-stirring type digestion tank (BIMA; Obayashi Corp., Tokyo) was operated at 30 °C on days 0–15 and at 54 °C after day 16. The configuration of a digestion tank of the same type is described elsewhere (Ogawa et al., 2003, 2005). On day 1, the digestion tank was filled with 600 m^3 water. As seed microorganisms, thermophilic sludge and mesophilic sludge collected from cow and pig manure treatment plants (Ogawa et al., 2003, 2005) were inoculated into the digestion tank step-by-step on days 1–36. Samples for chemical analyses were collected from the storage tank of food waste, the center tube, and the main chamber of the BIMA digestion tank. The schematic flow of the food waste treatment process in KEEP is provided in Supplementary materials.

2.2. Food waste

The startup period was divided into four phases: phase I (days 1–35), phase II (days 36–63), phase III (days 64–84), and phase IV (days 85–), according to types of food waste. Coffee grounds were the main food waste; thereafter, different types of food waste were increasingly fed to the process step-by-step. In phase I, only coffee grounds were treated. In phase II, potato waste was treated with coffee grounds. In phase III, kelp and boiled beans were also added. Finally, deep-fried bean curd and tofu waste were also added to the anaerobic digestion process. Typical compositions of food wastes treated in this study are provided in Supplementary materials.

2.3. Analytical procedures

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the main chamber of the digestion tank were measured according to standard methods (American Public Health Association (APHA), 1998). Methane in biogas collected from the gas holder was measured using a TCD gas chromatograph (GC14B; Shimadzu Corp., Kyoto). The VFA concentrations in the anaerobic digestion process were measured using an FID gas chromatograph (GC-8APF; Shimadzu Corp.). The total VFA concentration in this study was defined as the sum of acetate, propionate, butyrate, and valerate concentrations.

For T-RFLP analyses, the MLSS concentration of sludge collected from the digestion tank was diluted to 5000 mg/l. Then 0.5 ml of the sample was washed twice with 5 mg/l sodium tripolyphosphate buffer by centrifugation (8500*g* for 15 min, 4 °C). The sludge pellet was suspended in 0.5 ml sterilized pure water. The DNA in the sample was extracted using ISOIL for Beads Beating (Nippon Gene Co. Ltd., Tokyo) and purified using MagExtractor-PCR&Gel Clean up (Toyobo Co. Ltd., Tokyo) according to the manufacturer's instructions. Using the 27f and 1392r bacterial primer pair, PCR targeting eubacterial 16S rRNA genes was performed (Jeffery et al., 2007). The 5′-end of the forward primer was labeled with 6-carboxyfluorescein (FAM) for T-RFLP analysis. Then PCR was performed using one denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 3 min, with final extension at 72 °C for 10 min. Then PCR targeting archaeal 16S rRNA genes was performed using the A109f and A912r archaeal primer pair (Lueders and Friedrich, 2000). The 5′-end of the reverse primer was labeled with FAM for T-RFLP analysis. Subsequently, PCR was performed using one denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1.5 min, with final extension at 72 °C for 6 min. Amplicons from duplicate PCR reaction mixtures were filtered using Montage PCR (Qiagen Inc., Chatsworth, CA, USA). The filtrate was digested for 5 h separately with *Hha*I, *Hae*III, or *Msp*I at 37 °C for eubacterial 16S rRNA genes or with *Taq*I at 65 °C or *Hha*I at 37 °C for archaeal 16S rRNA genes. A mixture of digested products with HiDi formamide and GeneScan 2500 TAMRA Size Standard was denatured at 95 °C for 3 min. The fluorescently labeled T-RFs obtained in this manner were separated using capillary electrophoresis (ABI Prism 310 Genetic Analyzer; Applied Biosystems, CA, USA) to determine the number and size of T-RFs obtained from each sample. Fragment analyses were conducted using software (GeneScanTM Ver. 3.7; Applied Biosystems, CA). The 16S rRNA gene sequences of the domains Bacteria and Archaea used for *in silico* analysis were downloaded from http://mica.ibest.uidaho.edu (Conrad et al., 2007).

3. Results and discussion

3.1. Biogas production from food waste

The seed microorganisms and the inoculum size are important factors for the anaerobic digestion process during startup. Thermophilic sludge and mesophilic sludge of 895 and 470 t-wet in all were seeded, respectively, into the digestion tank step-by-step on days 1–36 and days 8–36.

Fig. 1 presents the feeding rates of food waste, total VFA concentration, and the biogas production rate in the anaerobic digestion process. During phase I (days 1–35), the feeding rate of coffee grounds increased gradually to 10 t/d. The operational temperature for the anaerobic digestion process was changed from 30 °C to 54 °C on day 16. It was expected that the thermophilic digester performs better, has a shorter startup period, and is more stable than the mesophilic digester. In phase II (days 36–63), potato waste was treated with coffee grounds every 4–7 days. The working volume of the anaerobic digestion tank increased gradually from 600 m^3 on day 1 to reach full working volume on day 45. The biogas production started, but its rate fluctuated from 300 to 1800 m^3/d. During phase III (days 64–84), kelp, bean curd lees, and boiled beans were also fed to the anaerobic digester almost every day. The biogas production rate increased steadily from 500 to 2000 m^3/d. In phase IV (days 85–), various food wastes