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Characterization of start-up performance and archaeal community shifts during anaerobic self-degradation of waste-activated sludge

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ABSTRACT

Successful start-up strategy for anaerobic digestion of waste-activated sludge using internal inoculum and relationship between the shift of methanogenic community and the digester performance during start-up was investigated. Combination of TS control of inoculum and batch operation during early days enabled the successful start-up operation without serious volatile fatty acid accumulation, followed by the stable continuous operation. However, the propionate degradation was rate-limiting step during the batch operation. The results of real-time quantitative polymerase chain reaction analysis suggested that there was a correlation between the population of the genus *Methanosarcina* and the methane production rate coupled with acetate consumption during batch operation, and the results of terminal-restriction fragment length polymorphism (T-RFLP) revealed that the increasing intensity of T-RF peaks of hydrogenotrophic methanogens was associated with a decrease in the level of C3-acids.

1. Introduction

Start-up is a critical step in the operation of anaerobic digestion, and it requires much attention so that the reactor is kept in good condition. Some researches focused on the start-up of anaerobic digestion revealed that the inoculum type is an important factor for a successful start-up (Griffin et al., 1998; McMahon et al., 2004; Forster-Carneiro et al., 2007). It is well recognized that digested sludge taken from another working digester is a good inoculum for a stable start-up of anaerobic digestion. In many cases, however, start-up relies on the internal inoculum inside the substrate by cultivating microorganisms throughout the anaerobic self-degradation because it is difficult, especially for small-scale biogas plants in rural areas, to obtain inoculum from another digester. It has been reported that start-up without external inoculums caused significant volatile fatty acids (VFAs) accumulation and took longer for the organic loading rate to be raised during the early days of operation (Stroot et al., 2001; McMahon et al., 2004), which makes the process less attractive economically. Therefore, there is a need for a strategy to enhance the start-up of anaerobic digestion from internal inoculum for optimum, stable operating conditions.

From the viewpoint of microbial ecology, it is possible that the methanogenic archaeal community in the inoculum affects the evolution of the digester operation, as has been discussed in various reports (Griffin et al., 1998; McMahon et al., 2001, 2004). In general, methanogenesis is an important and often rate-limiting step in the operation because methanogenic *Archaea* are strictly sensitive and slow growing. Thus, achieving a stable and rapid start-up may depend on the formation of the active methanogenic community. Recently, there have been a number of studies investigating the relationship between the reactor performance and the methanogenic community during start-up (Griffin et al., 1998; McMahon et al., 2001; Angenent et al., 2002). In these investigations, the reactor was seeded with digested sludge, cattle manure or with internal inoculums in municipal solid wastes. The reactor seeded with digested sludge, which had a higher level of *Methanosaeta* 16S rRNA than other inoculums, performed well during start-up, whereas digesters seeded with other inoculums resulted in a problematic amount of accumulation of VFA (McMahon et al., 2001; Stroot et al., 2001). These results revealed the importance of the methanogenic community in the inoculums for start-up operation. Even so, there has been considerably less interest in the internal microorganisms in the substrate than in the microorganisms in the major external inoculum, such as digested sludge or cattle manure.

In our study, our focus is centered on the start-up from waste-activated sludge (WAS). No research is yet to be done both on the successful start-up strategy using continuous system without

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external inoculum and on the relationship between the shift of the microbial community and the digester performance during start-up from WAS although it has been suggested as an alternative inoculum to digested sludge (Wu et al., 1987; Noyola and Moreno, 1994; Kim and Speece, 2002). The existing researches focused on start-up strategy dealt with dilution, mixing condition and feeding (Stroot et al., 2001; Vedrenne et al., 2008), which could be used for different aims. Start-up from WAS would particularly encounter the problem of absence of methanogen, which can lead to VFA accumulation and pH decrease. In this study, start-up operation using TS control of inoculum WAS and batch operation during early days was investigated in order to reduce the VFA accumulation level. The aim of this study was to evaluate our start-up strategy from WAS considering both digester performance and methanogenic archaeal community during start-up.

2. Methods

2.1. Experiment 1: batch test

Three serum bottles (B1, B2, and B3) were used for the experiment on self-degradation of WAS. The WAS used in experiments 1 and 2 was taken from a wastewater treatment plant in Miyagi, Japan. The characteristics of WAS used in this study are summarized in Table 1. B1 was filled with 80 mL of WAS of 45 g/L as total solids (TS). B2 was filled with 80 mL of WAS diluted with 23 g/L as TS with tap water. B3 was filled with 80 mL of WAS diluted with 15 g/L as TS with tap water. The headspace was replaced with nitrogen gas (purged for 2 min), followed by measurement of gas composition inside the bottles by a gas chromatograph to ensure anaerobic condition. Each bottle was incubated at 35 °C in a shaking incubator with a shaking speed of 90 rpm (BT 200, Yamato Scientific Co., Ltd., Japan).

2.2. Experiment 2: completely stirred tank reactor operation

A completely stirred tank reactor (CSTR) with a working volume of 5 L was used in this study. The reactor was maintained at 35 °C using a water circulator. The liquid in the reactor was continuously mixed with a motorized mixer (1400 rpm). pH measurement of digested sludge obtained from the digester was performed with a pH meter (Toa, HM-30 V) every week. The reactor was filled with WAS diluted with 15 g/L as TS with tap water. During the first 37 days of operation, the reactors were operated in batch mode without pH control. After day 37, fresh WAS was fed to the reactor every 6 h by a time-controlled pump from a feed tank maintained at 4 °C. The characteristics of WAS used in this experiment are listed out in Table 1. The hydraulic retention time (HRT) was gradually shortened from 100 days to 50 days to 30 days. Each digester was considered to be in a steady-state condition because the standard deviation of the pH, TS and alkalinity was below 5% during days 100–150.

Table 1
Characteristics of WAS used in this study.

Parameter	Waste-activated sludge		
	Inoculum (batch test B1)	Inoculum (reactor)	Substrate
pH	5.95	6.14	5.82 ± 0.03
Alkalinity (mg/L as $CaCO_3$)	1380	460	1244 ± 80
TS (g/L)	45.4	15.2	38.7 ± 1.6
VS (g/L)	34.1	11.9	29.6 ± 1.6
T-COD (g/L)	54.3	14.9	48.5 ± 2.2
S-COD (g/L)	–	1.8	8.7 ± 0.2
NH_4^+–N (mg/L)	790	216	246 ± 32
Total VFA (mg/L as acetate)	1870	408	2536 ± 371

2.3. Chemical analysis

A supernatant for the measurement of alkalinity, VFA and ammonium was prepared by centrifuging digested sludge samples at 3000 rpm for 20 min and filtering through a 0.45 μm pore-size filter, and that for soluble COD_{Cr} was obtained by centrifuging digested sludge samples at 3000 rpm for 20 min. Alkalinity as $CaCO_3$, total solids (TS) and volatile solids (VS) were measured in accordance with the testing method for wastewater (JSWA, 1997), and COD_{Cr} with APHA Standard Methods (APHA, 1995). Biogas production was measured with a wet gas meter (Shinagawa, W-NK). The composition of biogas obtained from the digester using a microsyringe was detected by a gas chromatograph (Shimadzu, GC-8A) equipped with thermal conductivity detector (TCD) and a stainless steel column packed with Porapack Q. The temperatures of the detector and column were maintained at 100 °C and 70 °C, respectively. VFAs were measured by the gas chromatograph (Agilent, 6890) with a flame ionization detector (FID) and 30 m column (Shimadzu, DB-WAXetr). Ammonium concentration was determined by capillary electrophoresis (I.D., 75 mm; UV detector 210 nm) (Ohtsuka, Photal CAPI-3200).

2.4. Molecular analysis

2.4.1. Polymerase chain reaction amplification, cloning and sequencing of 16S rRNA gene

The samples used in this experiment were WAS as inoculum and the digested sludge taken from the reactor on day 30 and day 150, respectively. Genomic DNA was extracted from the sludge with an Ultra Clean Soil DNA Isolation Kit (MO-BIO). Amplification of DNA was performed with the primers A109F (Grosskopf et al., 1998) and ARC1059R (Yu et al., 2005) targeted at the 16S rRNA gene of *Archaea*. Thirty thermal cycles of the polymerase chain reaction (PCR), which consisted of 30 s of denaturing at 94 °C, 40 s of annealing at 50 °C and 2 min of extraction at 72 °C, were performed. PCR products were cloned with a TOPO TA Cloning kit (invitrogen). Cloned DNA fragments obtained from randomly selected recombinants served as templates for sequencing analysis by the TAKARA BIO dragon genomics center (Yokkaichi, Japan). Operational Taxonomic Units (OTUs) were classified according to *Hae* III cleavage patterns. Representative sequences were searched for using the BLAST program (Altschul et al., 1997) to affiliate clone sequences with particular genus. Sequences determined in this analysis were deposited at GenBank with the accession numbers.

2.4.2. Terminal-restriction fragment length polymorphism

The analysis of the 16S rRNA gene-based terminal-restriction fragment length polymorphism (T-RFLP) was performed according to a protocol (Gruntzig et al., 2002). Community DNA extracted from the sludge obtained from the reactor on days 0 (inoculum), 5, 11, 14, 20, 30 and 150 was respectively followed by the PCR amplification with domain *Archaea*-specific primers; Beckman D4-labeled A109F and ARC1059R. Twenty-five thermal cycles of PCR were performed: 30 s of denaturing at 94 °C, 40 s of annealing at 50 °C and 2 min of extraction at 72 °C. The amplified PCR products were purified with the Minelute PCR purification kit (QIAGEN), and digested with two restriction nucleases (TaqI and HindIII). After ethanol precipitation, the digested PCR products were electrophoresed using a CEQ8000 sequencer (BECKMAN) at 60 °C for 65 min with a CEQ size standard 600 (BECKMAN). T-RF peaks were detected by the Beckman D4.