Start-up of full-scale anaerobic digesters treating municipal solid waste


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Abstract

Raising organic loading rate, and the behavior of dissolved CODcr (D-CODcr), VFA and specific methanogen activity, were investigated through a laboratory experiment for the start-up of a sludge recycling center. Moreover, application for MPN-PCR methods using a gene as a direct technique to measure the quantity of methanogen was attempted. It was recognized that specific methanogen activity depends on the quantity of methanogen, and that gas production does not reflect the condition of methane fermentation. The methane fermentation condition was checked through the specific methanogen activity and analysis of D-CODcr. The target organic loading rate was reached in the short period of about 30 days, and rapid start-up was successfully attained for a full-scale anaerobic digester.

Keywords

Full-scale digester; MPN-PCR; municipal solid waste; night soil; specific methanogen activity; start-up

Introduction

Recently, methane fermentation, a technology that recycles energy from waste, has attracted attention in Japan as well as in other parts of the world. A “sludge recycling center,” which recycles methane from municipal solid waste (MSW) and night soil, is one example. For the full-scale digester, the start-up of methane fermentation requires a long period, usually about three months. Therefore, laboratory experiment of start-up and load rise incorporation, and evaluation of the fermentation condition aimed at establishing a rapid start-up method was carried out.

On the other hand, long-period start-up results from not directly monitoring the amount of methanogen in the continually operating methane fermentation equipment. If there is a correlation between the condition of the methane fermentation equipment and the amount of methanogen, the organic loading rate can be determined owing to stable operation.

Then, the application for MPN-PCR methods was attempted, using a gene as a direct technique for acting as a quantity of methanogen monitor. This study investigates the behavior of specific methanogen activity, biogas production rate, and the number of acetate-utilizing methanogen in the bench-top scale digester using the MPN-PCR method. Based on these results, the operation of the Ikoma Sludge Recycling Center (Eco-Park 21) has begun, and the rapid start-up has been successful.

Methods

Digester

The continuously mixed (100–200 rpm), 3 L bench-top fermenter of 2 L working volume operated in semi-continuous mode for a retention time of 15 days. The digester was
maintained at a steady thermophilic condition (55°C). The organic loading rate of No. 1 digester, and the start-up experiment, began with 1.15 kgVS/m³/day, and increased weekly by 35%. The rate of No. 2 digester, and the correlation between condition of methane fermentation and amount of methanogen experiment began with 2.0 kg VS/m³/day, and gradually increased as shown in Figure 2. The feed consisted of simulated municipal solid waste (MSW) and night soil. The simulated MSW was prepared by collecting food waste from several local restaurants. MSW was used as feedstock after adjusting it to 15% total solid (TS). The night soil was from the Ikoma night soil treatment plant in Japan.

Standard methods (APHA, 1995) were used to measure CODcr and TS, volatile solids (VS) and pH. The concentrations of volatile fatty acids (VFA) were determined by a gas chromatograph equipped with an FID. To measure the specific methanogen activity (SMA) of digester sludge, a series of batch experiments were performed at 55°C on the basis of an early reported method (Miller and Wolin, 1974).

**DNA extraction and purification**

Samples (500 µl) were collected from the digester, immediately concentrated by centrifugation at 15,000 g for 10 min at 4°C, washed twice with 1.5 ml of 0.9 M phosphate buffer solution. The pellets (about 100 µl volume) were resuspended in 200 µl of lysis buffer (2%[w/v] sodium dodecyl sulfate, 300 mM NaCl, 100 mM Tris-HCl, 20 mM EDTA, pH 8.0) and vortexed to homogenize the pellets, and incubated at 55°C for 30 min. Nucleic acids were extracted twice by phenol-chloroform-isoamyl alcohol, and precipitated with isopropanol. Purification was performed with Genomic-tip 20/G (Qiagen, Valencia, CA). The concentrations of the purified nucleic acids were measured spectrophotometrically.

**MPN-PCR**

The number 16S rRNA genes of Methanosarcinaceae (MS) per ml-sludge in the digester can be estimated using most probable number PCR (MPN-PCR) (Picard et al., 1992; Degrand and Bardin, 1995). 16S rDNA specific sequence of MS amplified from serially diluted (1:10) samples in triplicate using a Air Thermo Cycler 1605 (Idaho Technology, Idaho Falls, ID). The reaction mixtures (10 µl in glass capillary tubings) were composed of 1 fg–1 ng purified DNA, 0.2 µM of each primer, 2 mM MgCl₂, 0.2 unit Platinum Taq Polymerase (Invitrogen, Carlsbad, CA), 50 mM Tris (pH 8.5), 20 mM KCl, 500 µM each dNTP and 500 µg/ml BSA. The cycling condition was 10 cycles at (94°C, 0 s; 60°C, 0 s; 73°C, 18 s) followed by 60 cycles at (94°C, 0 s; 57°C, 0 s; 73°C, 18 s). The sequence of primers targeting the 16S rRNA gene (Raskin et al., 1994) was as follows: ARCG34F (5′-AGG AA T TGG CGG GGG AGC AC-3′) and MS1414R (5′-CTC ACC CAT ACC TCA CTC GGG-3′) (TaKaRa Bio, Otsu, Japan). PCR amplicons (552 bp) were checked by gel electrophoresis. The MPN of 16S rRNA genes of MS per ng-total DNA was determined by a Cochran’s table (Cochran, 1950). The number of 16S rRNA genes of MS per ml-sludge was computed by multiplying the MPN by the ng-total DNA extracted per ml-sludge.

**Results and discussion**

**Bench-top scale**

The results of No. 1 digester, organic loading rate, gas production, and behavior of dissolved CODcr (D-CODcr) and VFA are shown in Figure 1. As depicted in the figure, there was favorable gas production during the experiment. The gas production was mostly in agreement with the organic loading rate until the 22nd day, when the organic loading rate reached 3.2 kgVS/m³/day. Although the gas production did not change from the 22nd to the 26th day, D-CODcr and VFA increased drastically. Finally, gas production fell from the
38th day. This indicates that the condition of methane fermentation is unstable and gas production does not reflect the condition of methane fermentation. However, D-CODcr and VFA could check the condition of methane fermentation. From the above results, an organic loading rate rise starting from 1 kgVS/m^3/day and increasing by 35% weekly to reach 2.2 kgVS/m^3/day (about 60% of target load) seems feasible by detecting D-CODcr and VFA to check the condition of methane fermentation.

On the other hand, D-CODcr and VFA did not reflect the ability of the methane fermentation equipment. A method for measuring the ability of methane fermentation owing to the definition of the loading organic rate is necessary. Applying MPN-PCR methods using the gene as a direct technique to measure the quantity of methanogen was then tried.

The results of No. 2 digester, organic loading rate, gas production and behavior of D-CODcr and VFA are shown in Figure 2. In addition, the behavior of 16S rRNA genes of MS per ml-sludge and SMA are shown in Figure 2.

From the 36th to the 63rd day, the average organic loading rate in a week was raised from 1.6 to 3.8 kgVS/m^3/day. D-CODcr went up gradually during the operation. However, it did not result in a rapid reduction of gas production as with the results of No. 1. From the above results, in a start-up operation, although a system failure when there is a rapid rise in D-CODcr is possible, a gentle rise is not problematic.

Gas production differs considerably day-by-day because loading and extraction are carried out 5 days a week. With the full-scale digester, since there is a holiday, a performing operation with the above load change is assumed. For this reason, it is difficult to judge the quantity of methanogen by the amount of gas produced. Moreover, gas production seems to bear no correlation with the number of 16S rRNA genes of MS. However, it seems with SMA, there is a correlation with the number of 16S rRNA genes of MS (Figure 2).

During methane fermentation in this experiment, measuring the number 16S rRNA genes of MS and SMA is effective. Furthermore, it is possible to substitute simple SMA

![Figure 1](https://iwaponline.com/wst/article-pdf/48/4/249/423423/249.pdf)

**Figure 1** Profile of OLR, gas production, D-CODcr and VFA of No. 1 digester

![Figure 2](https://iwaponline.com/wst/article-pdf/48/4/249/423423/249.pdf)

**Figure 2** Profile of OLR, gas production, D-CODcr, VFA, the number of 16S rRNA genes of MS and SMA of No. 2 digester

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measurement for monitoring change in acetate-utilizing methanogen. From the results of SMA measurement, it is also possible to calculate the load that can be applied. Using this technique, one does not have to be too careful with the rise operation of the load, start-up time can be reduced and system failure by fault load can be avoided.

**Full-scale digester**

The results of the organic loading rate, gas production, and the measurement of D-CODcr and SMA are shown in Figure 3. The organic loading rate of 2.8 kgVS/m³/d was reached in 30 days. Gas production was about 200 m³/day, comparable to the predicted amount, and methane fermentation was carried out smoothly. D-CODcr during operation remained at around 1,000 mg/L, and fermentation proceeded smoothly. SMA reached a maximum of 1.7 m³CH₄/m³-digested sludge/day, and the decomposition of organic matter reached 34.8%. From the above results, it can be concluded that rapid start-up was successful.

**Conclusions**

Raising the organic loading rate and evaluating the fermentation conditions were investigated through laboratory experiments for the start-up of a sludge-recycling center. Although D-CODcr and VFA were useful in the evaluation of methane fermentation conditions, they did not reflect the ability of methane fermentation. Specific methane activity correlates with the number of 16S rRNA genes of MS using the MPN-PCR method. From the results of SMA measurement, it is also possible to calculate the load that can be applied. The target organic loading rate (2.8 kgVS/m³/day) can be reached in the short period of about 30 days, and rapid start-up can be successfully attained for full-scale anaerobic digesters by measuring SMA and D-CODcr.

**References**


