Utilization of high solid waste activated sludge from small facilities by anaerobic digestion and application as fertilizer

Taira Hidaka, Masato Nakamura, Fumiko Oritate and Fumitake Nishimura

ABSTRACT

Anaerobic co-digestion of sewage sludge with organic wastes has recently gained attention in small facilities. For small facilities, high solids sludge is suitable for transportation to a centralized co-digester, and direct utilization of the digested sludge as liquid fertilizer is recommended. Effects of high solid and hyperthermophilic pretreatment (80°C, 24 hr) on anaerobic digestion at low temperatures and utilization as fertilizer are investigated by anaerobic/aerobic digestion and paddy soil incubation experiments. The volatile solids (VS)/total solids (TS) ratio decreases to 0.57(-), and the VS removal rate is approximately 0.7 (-) after long-term aerobic digestion. This is possibly the limitation of biodegradation, even with pretreatment, within engineering time. Substrate TS of 16% (not diluted), 10% and 5% are compared. The effect of substrate TS on biogas production performance (0.2 – 0.3 NL/gVS-added) is not statistically observed. Laboratory-scale paddy soil incubation experiments are performed fed with anaerobically digested pretreated or not pretreated dewatered sludge as liquid fertilizer. Pretreatment promotes nitrogen mineralization before use as fertilizer, which is helpful to prevent an outflow of surplus ammonia to the environment. The effect of soil type on microbial communities is more significant than that of anaerobically digested sludge conditions.

Key words | ammonia, biodegradation, co-digestion, dewatered sludge, paddy soil, pretreatment

INTRODUCTION

In Japan, there are approximately 2,200 wastewater treatment plants (WWTPs), among which only 300 large or middle-scale WWTPs have anaerobic digesters (JSWA 2018). In local cities with decentralized small WWTPs, centralized anaerobic digestion at a WWTP that accepts more sludge and other waste biomass can serve as an energy hub for the region (Wang et al. 2014; Hidaka et al. 2015a). Around 1,000 small-scale WWTPs whose treatment capacity is less than 1,000 m³/d use oxidation ditch processes (ODs), and have no anaerobic digester. This is reasonable because sewage sludge from ODs generally has a low biogas production rate and is not suitable for anaerobic digestion. The concept of OD is to minimize sludge production by retaining solids for a longer time and enhancing self-degradation.

Anaerobic co-digestion of sewage sludge with organic wastes has recently gained attention for use in small facilities in Japan (Hidaka et al. 2015b; Gu et al. 2016). In Ishikawa Prefecture, Japan, there are two small-scale WWTPs with anaerobic co-digestion using (dewatered) sludge from ODs and waste biomass such as kitchen garbage. One WWTP in Suzu City and the other in Nakanoto Town adopted this system in 2007 and 2017, respectively. Contrary to WWTPs with pipeline networks for sludge in large cities (Gyobu et al. 2015), dewatering sewage sludge for less sludge volume is helpful for transporting sludge to a centralized WWTP. This means that high solids anaerobic digestion is promising (Li et al. 2015; Fürer et al. 2018; Tamaki et al. 2019). Direct anaerobic digestion of dewatered sludge is another candidate, although mixing is difficult. After anaerobic digestion, direct utilization of the digestate as liquid fertilizer at farmland near WWTPs is recommended (Nakamura et al. 2019), considering further processing costs to produce fertilizer like compost or crystallization.

Typically, anaerobic digestion is performed at mesophilic (around 35°C) or thermophilic (around 55°C) conditions by heating, but it can be operated at lower
temperatures (Fair & Moore 1954). Psychrophilic operation with less heating energy reduces operational cost, although longer retention time is required. In the anaerobic treatment of low-strength wastewater, heating energy is huge and psychrophilic operation is evaluated (Lettinga et al. 2001). Anaerobic digestion of sewage sludge without heating is under operation at several WWTPs in Japan (JSWA 2018). Pretreatment to increase the biodegradability of sludge can reduce the required retention time or lower the operating temperature while ensuring the same performance. Pretreatment by microwave for sewage sludge from ODs is reported (Togari et al. 2016), but its operational cost is not negligible. Among various pretreatment methods, hyperthermophilic pretreatment at 80 °C, which can be operated by using waste heat (Lee et al. 2009; Tamaki et al. 2019), is suitable for small facilities. The feasibility of the pretreatment at 80 °C is reported (Osumi & Tsubota 2010). This temperature range is much lower than that of well-known thermal hydrolysis systems such as Cambi (Kepp et al. 2000). In the case of direct utilization of digestate as fertilizer, heat treatment is essential for sterilization and prevention of unintended weed growth, which is completed by this pre-treatment. Post-treatment for solubilization of the digested sludge that is returned to the digester can be an option. However, this cannot ensure that all of the sludge is treated at 80 °C. A combination of pretreatment and psychrophilic digestion is a candidate for small facilities.

Co-digestion is practically expected because the main sources of biogas production are more biodegradable organic wastes such as kitchen garbage than sewage sludge from ODs. However, there have been few reports on the biodegradation characteristics of sewage sludge from ODs in long-term operation under various combined temperature conditions, and application of digestate as fertilizer in paddy fields. In this study, the effects of high solids and pretreatment on anaerobic digestion at low temperatures and utilization as fertilizer were investigated by anaerobic digestion and paddy soil cultivation experiments, including microbial community analysis. The limitation of biodegradation was investigated by a long-term aerobic digestion experiment.

**MATERIALS AND METHODS**

**Substrate**

Dewatered sewage sludge used in the experiments was periodically collected at WWTP-A in Japan. WWTP-A treats municipal domestic wastewater from a separate sewer system with OD operated at biochemical oxygen demand (BOD) – mixed liquor suspended solids (MLSS) loading of 0.02 kgBOD/(kgMLSS-d). The average total solids (TS) and volatile solids (VS) of the sludge were 15.6% and 12.8%, respectively. Anaerobically digested dewatered sludge from WWTP-A under the feeding TS of 10% was seeded at the beginning of the operation.

**Aerobic digestion**

Laboratory-scale continuous aerobic digestion experiments fed with the dewatered sludge at WWTP-A were performed in a batch mode for 450 d to evaluate the potential biodegradation performance of the dewatered sludge. To ensure the aerobic condition without drying the sludge, the dewatered sludge was diluted with ion-exchange water to TS 5%. A reactor with a working volume of 1 L was maintained in an aerobic state with aeration of 1 L/min. The temperature was set at 30 °C. During the experiment, the volume of the sludge decreased due to evaporation, and ion-exchange water was added to maintain the total volume of 1 L. Three runs were performed using sludge obtained at WWTP-A on different days; that is, each run started on different days.

**Anaerobic digestion**

**Continuous experiments and following batch operation**

Laboratory-scale continuous anaerobic digestion experiments fed with dewatered sludge from OD were performed for 320 d under a mesophilic (35 ± 1 °C) condition. An effect of the feeding TS on biogas production was evaluated by comparing the feeding substrate TS of 16% (not diluted), 10% and 5%. Laboratory-scale reactors with a working volume of 0.5 L were maintained in an anaerobic state without mechanical mixing in the case of the feeding TS 10% and 5%. The temperature was controlled with a hot water bath. An organic loading rate (OLR) of dewatered sludge was adjusted to 0.5–2.0 gVS/(L·d), which is within or lower than typical values of 1–3 gVS/(L·d) in Japan (JSWA 2015). In each reactor, the substrate was fed manually once or twice a week, and the digested sludge was removed approximately once a month. In the case of the feeding TS 16%, a reactor of 2 L was prepared. At the beginning of the experiment, the anaerobically digested sludge at the feeding TS of 10% in the previous experiment was seeded (0.5 L). The dewatered sludge was fed once or
twice a week manually, without withdrawing the sludge. This was because complete mixing and withdrawal were difficult with too high shear stress (Füreder et al. 2018). After the continuous experiment, the reactor of TS 16% was maintained without feeding the substrate like a batch operation under the same mesophilic condition, and the biogas production was monitored for 10 months.

**Hyperthermophilic pretreatment**

The effect of pretreatment (80 °C, 24 hr) on anaerobic digestion performance was investigated. For the pretreatment, the raw dewatered sludge diluted to TS 5% was placed in a vial having an effective volume of 100 mL and put in an oil bath at 80 °C for 24 hr. The dewatered sludge (T) and the pretreated dewatered sludge (TH) of TS 5% were anaerobically digested continuously at an OLR of 1 gVS/(L·d). The digestion temperatures were controlled at 35 ± 1 °C, 50 ± 1 °C, 25 ± 1 °C, and 15 ± 1 °C with water baths.

**Paddy soil incubation experiments**

Laboratory-scale paddy soil incubation experiments were performed fed with anaerobically digested sludge as liquid fertilizer (Nakamura et al. 2019). Dewatered sludge samples anaerobically digested at 15, 25, 30 and 35 °C with (TH) and without (T) hyperthermophilic pretreatment were incubated in 60-mL vials. Rice straw, which is usually fed into paddy fields. The vial was then covered with a butyl rubber stopper, its headspace air was replaced by N₂ gas, and it was incubated at 30 °C. Ammonia concentrations and microbial structures in the soil were analyzed after 84 days of incubation. The experiments were performed twice using the sludge obtained at WWTP-A on different days, which are identified as sludge I and sludge II.

**Analysis**

**Sludge and biogas analysis**

Standard methods (APHA-AWWA-WEF 2022) were used to analyze the pH, TS, and VS. Total chemical oxygen demand (TCOD) and soluble COD (SCOD) were analyzed using a spectrophotometer (DR2400, HACH, CO, USA), and COD regents (HR, HACH). Particulate COD (PCOD) was calculated by the difference between TCOD and SCOD. Ammonia was measured by an auto-analyzer (Bran + Luebbe, Norderstedt, Germany). Dissolved samples were prepared by filtration through a cellulose acetate filter (pore size = 0.45 μm). The carbon: hydrogen: nitrogen (CHN) mass ratio was measured at Organic Elemental Microanalysis Laboratory, Kyoto University, Japan. Gas volumes were measured by a glass syringe in the continuous experiments and by a respirometer (AER-800, Challenge Technology, AR, USA) in the batch experiments. All mentioned gas volumes are standardized to standard conditions of 101.3 kPa and 0 °C. Statistical analysis was performed using BellCurve for Excel (version 3.20, Social Survey Research Information Co., Ltd, Tokyo, Japan).

**Microbial community analysis**

Microbial communities of the anaerobically digested sludge were analyzed according to Hidaka et al. (2018). DNA was extracted with an Extrap Soil DNA Kit Plus ver. 2 (Nippon Steel and Sumikin Eco-Tech, Tokyo, Japan). The DNA concentration of purified extracts was measured using a PicoGreen dsDNA Assay Kit (Invitrogen, CA, USA). Total bacterial 16S rRNA gene copy numbers were quantified by quenching probe PCR (QProbe PCR) using Rotor-Gene Q (Qiagen, Hilden, Germany) (Tani et al. 2009). The primer pair Bac1055VF and Bac1539RF and the QProbe of modified Bac1115Probe were used (Ritalahti et al. 2006). The standard curve was constructed with the presentative strain of *Escherichia coli* K12 (ATCC 10798), DNA sequencing was conducted using MiSeq (Illumina Inc., San Diego, CA, USA). Reads with more than 97% sequence similarity were grouped into the same operational taxonomic unit (OTU), and OTU picking and cluster analysis was performed in QIME (Caporaso et al. 2010). OTUs were identified using the Greengene database (DeSantis et al. 2006), and the SILVA database project (2018) as a reference.

**RESULTS AND DISCUSSION**

**Aerobic digestion performance**

The time course of VS/TS ratios during the aerobic digestion experiment is shown in Figure 1. The TS and VS concentrations were affected by evaporation and addition of
ion-exchange water, and a decrease in the TS and VS concentrations is meaningless. Here, the VS/TS ratio was used to understand the biodegradation performance. Considering that an ash concentration that is the difference between TS and VS is constant during anaerobic digestion, a VS removal ratio was calculated by VS removed per initial VS. During the digestion, pH dropped to 3 with an increase in nitrate of more than 70 mgN/L. This pH drop naturally happened; that is, no acid reagent was added, and pH was not adjusted during the experiment.

The VS/TS ratio decreased rapidly during the first year, and then slowly degraded, and finally reached 0.57 ( ). The VS removal rate was calculated to be approximately 0.7 ( ) after the long-term aerobic digestion. This is possibly the limitation of biodegradation, even with pretreatment, within engineering time. The COD/VS ratio of 1.4–1.5 at the beginning is closed to a typical COD/VS of waste activated sludge of 1.4, assuming a bacterial composition of $\text{C}_3\text{H}_7\text{O}_2\text{N}$. Schaum et al. (2016) reported that the COD/VS ratio of sewage sludge was around 1.5 independent of the type of sewage sludge, and heating values were correlated with COD rather than VS. The COD/VS ratios after aerobic treatment decreased to approximately 1.0 ( ), which indicates the progress of oxidation and degradation of energy-containing organic material.

### Anaerobic digestion performance

Biogas production in the continuous anaerobic digestion experiments is summarized in Figure 2. The biogas production performance was 0.2–0.3 NL/gVS-added, regardless of the feeding substrate TS. The effect of substrate concentrations on biogas production performance was not statistically observed (t-test, $P \leq 0.05$). After the continuous experiment of TS16% (raw dewatered sludge), the biogas production was monitored without feeding (Figure 3). Biogas production continued for approximately 200 days.

Assuming a first-order reaction like ADM1 (Batstone et al. 2002), reaction rates were calculated. The first-order reaction rate constant was 0.05 (1/d) in the first month and decreased to 0.02 (1/d) in the following second to ninth months. These reaction rates are similar to those of the diluted sludge of TS 5% from the same WWTP-A. In anaerobic digestion of dewatered sludge, mixing is hardly possible. This might not be acceptable for an engineering reactor, but in terms

![Figure 1](image1.png) **Figure 1** | Change in sludge characteristics during aerobic digestion.

![Figure 2](image2.png) **Figure 2** | Biogas production under different substrate concentrations.

![Figure 3](image3.png) **Figure 3** | Biogas production from raw dewatered sludge of TS16% during batch operation.
of biological reaction, anaerobic digestion can proceed in a similar manner, even if raw dewatered sludge is accumulated. After 10 months of operation, biogas production was hardly observed, and the VS/TS ratio reached 0.68.

The effect of pretreatment (80 °C, 24 hr) on anaerobic digestion performance was investigated. As an index of biodegradation, VS/TS ratios after anaerobic digestion at different temperatures are summarized in Figure 4. The sludge used in this experiment corresponds to sludge II. The pretreatment upgraded digestion performance, and a lower VS/TS ratio was obtained with the pretreatment than that without pretreatment at each temperature condition. Biodegradation performance at lower temperatures was worse, but this can be overcome if enough retention time is ensured. Similar results were reported by Tamaki et al. (2019) under different operational conditions.

Behavior of ammonia

Ammonia is one of the key components when the sludge is used as fertilizer. An ammonia concentration of the digested sludge and the raw sludge is summarized in Figure 5(a). Both sludge I and sludge II were taken at WWTP-A but on different days. Different anaerobic digestion temperatures resulted in different VS/TS ratios of the digested sludge, which caused different ammonia releases. Pretreatment promoted nitrogen mineralization before use as fertilizer. This corresponds with previous researches, in which protein degradation was promoted by hyperthermophilic treatment (Lee et al. 2009). The total amount of ammonia release in anaerobic digestion and paddy soil cultivation is summarized in Figure 5(b). Pretreatment did not affect total ammonia release in anaerobic digestion and paddy soil. This is helpful to prevent an outflow of surplus ammonia to the environment, because the feeding amount of fertilizer is generally controlled by the amount of ammonia, and unintended ammonia release during paddy soil cultivation is reduced.

Microbial communities in paddy soil cultivation

The bacterial 16S rRNA gene copy numbers measured by QProbe PCR are summarized in Figure 6. The raw HC had $8 \times 10^9$ copies/mL, while the addition of rice straw
slightly increased the numbers. The addition of T35 resulted in $1.1 \times 10^{10}$ copies/mL. Although the methane production amount in HC + rice straw was higher than that in HC + T35, the latter had more bacterial gene copy numbers. This is possibly due to being derived from various bacteria cultivated in the previous anaerobic digestion (T35). On the other hand, BLS + T35 had $7 \times 10^9$ copies/mL, which was lower than that of HC + T35. This is possibly because the raw BLS had fewer copy numbers. Hidaka et al. (2018) reported $1-10 \times 10^9$ copies/mL in anaerobic digestion of sewage sludge from various types of wastewater treatment facilities. The results in the present study are in a similar range. In terms of bacterial gene copy numbers, the condition of paddy soil cultivation is similar to that in anaerobic digestion.

Microbial structure and the detected ratios on the phylum level are summarized in Table 1. Only items with the detected ratios of 1% or over are shown. Each sample had approximately 100,000 reads. Both Archaea and Bacteria were detected because the universal primers targeting both domains were used, but the detected ratio of Archaea was less than 3%. Here, the results in domain Bacteria were evaluated. On the phylum level, Chloroflexi, Firmicutes, and Proteobacteria were detected at more than 10%. In Chloroflexi, family Anaerolineaceae was deleted the most. In Proteobacteria, various species of more than 400 OTUs were detected.

In the acid fermentation step, various species in Firmicutes are often reported. Within the three samples of HC, the difference in the structure of family Clostridiaceae (class Clostridia, order Clostridiales) was obvious. Microbial structure and the ratios on the genus level in family Clostridiaceae are summarized in Table 2. The addition of rice straw increased *Clostridium* a lot.
The most detected species of *Clostridium* were *Clostridium* *swellfunianum* sp. (5.9%), and unknown species accounted for 4.6%. *Clostridium* is often reported to be detected in paddy fields, and this corresponds with the addition of rice straw. The results include some aerobic microbes, and they are totally different from the structures in anaerobic digestion of sewage sludge (Hidaka *et al.* 2018). The three samples of HC had similar structures to each other, while the sample of BLS had a totally different structure. The effect of long-term feeding of digested sludge on the microbial structure is not significant. The microbial structure in paddy soil is strongly affected by the original soil, rather than digested sludge containing different microbial structures.

**CONCLUSIONS**

Biodegradation characteristics of dewatered sewage sludge from OD, whose biodegradability is quite low, were compared under various conditions of aerobic/anaerobic, 16/10/5% feeding TS, mesophilic/psychrophilic temperatures, and with/without hyperthermophilic pretreatment by the digestion and paddy soil incubation experiments. The VS/TSS ratio of 0.57(-) and the VS removal rate of approximately 0.7 (-) after long-term aerobic digestion are possibly the limitation of biodegradation even with pretreatment, within engineering time. The effect of substrate concentrations on biogas production performance (0.2–0.3 NL/gVS-added) was not statistically observed. Pretreatment promoted nitrogen mineralization before using as fertilizer, which is helpful to prevent an outflow of surplus ammonia to the environment. The effect of soil type on microbial communities was more significant than that of previous anaerobic digestion conditions. The results proved that the hyperthermophilic pretreatment is preferable for anaerobic digestion in local cities with decentralized small WWTPs including OD with direct utilization of the digestate as fertilizer.

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