Original Article

Anaerobic Digestion of Oxidation Ditch Sludge at Low Temperatures with Hyperthermophilic Pretreatment

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ABSTRACT

Centralized anaerobic digestion of dewatered sludge from small scale wastewater treatment plants and other waste biomass such as kitchen garbage is a promising energy recovery technology. Anaerobic digestion at lower temperatures reduces operational cost, while hyperthermophilic pretreatment upgrades anaerobic digestion performance. The applicability of dewatered sewage sludge from an oxidation ditch process to anaerobic digestion was investigated by continuous and batch experiments under combined different temperature conditions. Dewatered sewage sludge was diluted to total solids 5% before feeding. When the organic loading rate was 0.94 kg volatile solids (VS)/(m³·d), biogas production from dewatered sewage sludge at a mesophilic condition (35°C) was 0.20 NL/gVS-added. Under anaerobic digestion conditions at 25, 30 and 35°C with hyperthermophilic pretreatment at 80°C for 24 h, biogas production was higher than 0.20 NL/gVS-added. Effect of different temperatures on digestate characteristics and microbial communities were revealed. An ammonia concentration of the digested sludge with pretreatment was higher than that of the digested sludge without pretreatment. These results proved that the combination of pretreatment and psychrophilic anaerobic digestion is applicable for sludge management in local cities with decentralized small facilities.

Keywords: biogas, dewatered sludge, heat treatment, psychrophilic digestion, small scale plant

INTRODUCTION

In Japan, there are approximately 2,200 wastewater treatment plants (WWTPs), among which only 300 large or middle scale WWTPs have anaerobic digesters [1]. 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 of the region [2–4]. 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 digestion. 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 from ODs with organic wastes has recently gained attention for use in small facilities in Japan. For example, in Ishikawa Prefecture, Japan, there are two WWTPs with anaerobic co-digestion

using sewage sludge from ODs and other waste biomass such as kitchen garbage. One WWTP in Suzu City adopted it in 2007 and the other in Nakanoto Town in 2017. The latter is characterized by the new anaerobic co-digester that is fed with dewatered sewage sludge of 11 surrounding WWTPs, among which five WWTPs use ODs. If this practice will be successful, this concept is expected to spread all over Japan. Contrary to WWTPs with pipeline networks for sludge in large cities [5], 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 [3,4].

For small facilities, simpler reactor configurations that have low operating cost requirements are preferable. Typically anaerobic digestion of sewage sludge is performed at mesophilic (around 35°C) or thermophilic (around 55°C) conditions by heating, but it can be operated at lower temperatures. In anaerobic treatment of low-strength wastewater, heating energy is huge and psychrophilic operation is

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Table 1 Characteristics of sludge.

	TS (%)	VS (%)	VS/TS (–)	COD (g/kg)
raw dewatered sludge	15.6	12.8	0.82	135
inoculum for the batch experiment	3.2	2.4	0.74	28.4

Table 2 Reactor configurations.

Digestion Temperature (°C)	Without pretreatment	With pretreatment
15	A15	AH15
25	A25	AH25
30	A30	AH30
35	A35	AH35

evaluated [6]. Anaerobic digestion of sewage sludge without heating is under operation at several WWTPs in Japan [1]. Psychrophilic operation with less heating energy reduces operational cost, although longer retention time is required [7]. Pretreatment to increase biodegradability of sewage sludge can reduce required retention time or lower the operational temperature for obtaining the same performance. Posttreatment for solubilization of the digested sludge which is returned to the digester can be an option. However, it is reported that posttreatment did not enhance biogas production [8]. Combination of pretreatment and psychrophilic digestion is a candidate operation for small facilities.

Pretreatment by microwave for sewage sludge from ODs is reported [9], but its operational cost is not negligible. Among various pretreatment methods, hyperthermophilic pretreatment at 80°C [10] is suitable for small facilities. In case of direct utilization of digested sludge as fertilizer, heat treatment at 50°C or over is essential for sterilization and prevention of unintended weed growth, and the hyperthermophilic pretreatment effectively eliminates pathogens [11]. This can be completed by the hyperthermophilic pretreatment. Co-digestion is practically assumed, because main sources of biogas production is more biodegradable organic wastes such as kitchen garbage than sewage sludge from ODs. However, there have been few reports on anaerobic digestion of sewage sludge from ODs in long-term operation under combined various temperature conditions. In this study, effect of hyperthermophilic pretreatment on anaerobic digestion at low temperatures for dewatered sewage sludge from OD was investigated by continuous and batch experiments to reveal digestion characteristics under combined various temperature conditions.

MATERIALS AND METHODS

Preparation of Sewage Sludge

Characteristics of dewatered sewage sludge used in the experiments are summarized in **Table 1**. The sludge was 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). Average total solids (TS) and volatile solids (VS) of the sludge were 15.6% and 12.8%, respectively. The effect of the feeding TS on biogas production was also evaluated, and the TS of 10% and 5% resulted in the similar biogas production performance [7]. In the present study, the digestate TS would be higher at lower digestion temperatures, which might affect digestion performance. Therefore, the sludge was diluted to TS 5% by ion-exchanged water before feeding to anaerobic reactors. The maximum TS of sewage sludge treated by practical anaerobic digestion is currently approximately 5% in Japan [1].

Continuous Experiment

Eight series of continuous experiments were performed (**Table 2**). Laboratory-scale reactors with a working volume of 0.2 L were maintained in an anaerobic state without mechanical mixing. Difference in biogas production performance by anaerobic digestion experiments of dewatered sludge from WWTP-A with and without mechanical mixing was not observed under the low loading condition [7]. The temperatures were controlled at $35 \pm 1^\circ\text{C}$, $30 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$, and $15 \pm 1^\circ\text{C}$ with water baths. The raw dewatered sludge (A) or pretreated dewatered sludge (AH) were fed. The hyperthermophilic pretreatment of the substrate was carried out as follows: the raw dewatered sludge diluted to TS 5% was

Table 3 Conditions of continuous operation.

operation period	Term0 day 0 – 36	Term1 day 37 – 119	Term2 day 120 – 175
OLR (kgVS/(m ³ ·d))	0.38 – 0.94	0.94	0.47

placed in a vial having an effective volume of 100 mL and put in an oil bath at 80°C for 24 h.

Anaerobically digested dewatered sludge from WWTP-A under the feeding TS of 10% [7] was seeded at the beginning of operation. Operational conditions in the continuous experiment is summarized in **Table 3**. An organic loading rate (OLR) was gradually increased from 0.38 to 0.94 kgVS/(m³·d) for start-up in Term 0. The diluted raw dewatered sludge was fed into A and AH reactors in Term 0. The diluted raw dewatered sludge was fed into A reactors and the pre-treated diluted dewatered sludge was fed into AH reactors in both Terms 1 and 2. OLRs were adjusted to 0.94 kgVS/(m³·d) and 0.47 kgVS/(m³·d) in Terms 1 and 2, respectively, which were within or lower than typical values of 0.6–1.6 kgVS/(m³·d) in case of no mixing [12]. The corresponding hydraulic retention times (HRTs) in Terms 1 and 2 were 46.7 d and 93.4 d, respectively. In each reactor, the digested sludge was withdrawn and the substrate was fed manually twice or once a week in Terms 1 and 2, respectively. Produced biogas volume was measured upon each substrate feeding.

Effect of Pretreatment Temperature on Solubilization and Biogas Production

The raw dewatered sludge diluted to TS 5% was pretreated at 70, 80, and 90°C for 24 h by the same procedure described above. Solubilization performance was evaluated in terms of TS, VS and chemical oxygen demand (COD).

Four series of batch experiments were performed with the pretreated sludge at 70, 80, and 90°C. Glass bottles with a working volume of 0.3 L were maintained in an anaerobic state using a shaking incubator for 27 d. The temperature was set at 30±1°C, which is lower than that for typical mesophilic operation. This was to evaluate the possibility of lowering the digestion temperature for pretreated substrate. For each bottle, inoculum of 100 g-wet and raw dewatered sludge of 10 g-wet were added. The inoculum was anaerobically digested sewage sludge from the continuous experiment (**Table 1**). The substrate/inoculum mass ratio (S/I) was 0.5 on a VS base. The batch experiment included a control using only inoculum sludge.

Sludge and Biogas Analysis

Standard methods were used to analyze the pH, TS, and VS [13]. Total COD (TCOD) and soluble COD (SCOD) were analyzed using a spectrophotometer (DR2400, HACH, Loveland, USA), and COD reagents (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. Viscosity was measured with a viscometer (LVDV1M, EKO Instruments Co., Ltd., Tokyo, Japan). Dewaterability was evaluated by capillary suction time (CST) (CST-1, Central Kagaku Corp., Tokyo, Japan). High polymer coagulant was added at a ratio of 1.3%TS before CST analysis. Gas volumes were measured by a glass syringe in the continuous experiments and by a respirometer (AER-800, Challenge Technology, Springdale, USA) in the batch experiments. All mentioned gas volumes are standardized to standard conditions of 101.3 kPa and 0°C.

The nitrogen degradation was calculated by equation (1).

$$\text{Nitrogen degradation (\%)} = (N_{out} - N_s) / N_{in} \times 100 \quad (1)$$

N_{out} : Ammonia concentration of digested sludge (gN/kg)

N_s : Ammonia concentration of substrate sludge (gN/kg)

N_{in} : Total nitrogen concentration of substrate sludge (gN/kg)

The VS removal ratio was calculated by equation (2).

$$\text{VS removal (\%)} = (V_{in} - V_{out}) / V_{in} \times 100 \quad (2)$$

V_{out} : VS of digested sludge (%)

V_{in} : VS of substrate sludge (%)

The COD solubilization performance in the pretreatment was calculated by equation (3).

$$\text{COD solubilization (\%)} = (C_T - C_{in}) / P_{in} \times 100 \quad (3)$$

C_T : SCOD of pretreated sludge at temperature T (= 70, 80, 90°C) (g/kg)

C_{in} : SCOD of substrate sludge (g/kg)

P_{in} : PCOD of substrate sludge (g/kg)

Microbial Community Analysis

Microbial communities of the anaerobically digested sludge were analyzed according to Hidaka *et al.* [14]. DNA

Fig. 1 Time course of TS in the continuous experiment.

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, Carlsbad, USA). Total bacterial 16S rRNA gene copy numbers were quantified by quenching probe PCR (QProbe PCR) using a Rotor-Gene Q (Qiagen, Hilden, Germany) [15]. The primer pair Bac1055YF and Bac1392R and a QProbe of modified Bac1115Probe were used [16]. 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, USA) for analysis. Reads with more than 97% sequence similarity were grouped into the same operational taxonomic unit (OTU), and OTU picking and a cluster analysis were performed in QIIME [17]. OTUs were identified using the Greengene database [18], and SILVA ribosomal RNA database project [19] as a reference.

RESULTS AND DISCUSSION

Continuous Experiment

TS concentration was stable at approximately 3.5–4.0%, after increase from the seed sludge (Fig. 1). The trend of VS concentration was similar to that of TS. The VS/TS ratio can be used as an indicator for VS removal. It was lower under higher digestion temperatures, i.e. it was 0.78 and 0.73 in A15 and A35, respectively, on day 115 in Term 1. It was lower with pretreatment, regardless of digestion temperature. It was 0.75 and 0.71 in AH15 and AH35, respectively on day 115 in Term 1.

Biogas production performance is summarized in Fig. 2. It was calculated by assuming that accumulated biogas production volume and fed VS amount were linearly correlated. The numbers of data in Terms 1 and 2 were 23, and 8, respectively, and stable biogas production was observed. At each digestion temperature, pretreatment enhanced biogas production. Biogas production at AH25 (0.22 NL/gVS-added), AH30 (0.23 NL/gVS-added) and AH35 (0.28 NL/gVS-added) was higher than that at A35 (0.20 NL/gVS-added) in Term 1. The biogas production at A15 was low and the enhancement was significant at AH15. Biogas production in Term 2 was lower than that in Term 1. This was possibly due to varying characteristics of substrate sludge. The biogas production at AH35 was the highest, but the input energy is also the highest. If sufficient reactor volume and sufficient retention time are available by high solid anaerobic digestion at small scale facilities, lower temperature operation, which requires less heating energy, can ensure equivalent biogas production, with appropriate pretreatment if necessary.

The ammonia concentration in the digestate was approxi-

Fig. 2 Biogas production performance in the continuous experiment.

Fig. 3 Relationship between nitrogen degradation and VS removal in the continuous experiment.

mately 1–2 gN/L, which is lower than that of inhibition level for mesophilic operation [20]. Relationship between nitrogen degradation and VS removal is shown in **Fig. 3**. Using a measured C, H and N containing ratio of the dewatered sludge obtained at WWTP-A on dry solids (DS) basis of 39.0, 5.8 and 7.2%DS, respectively [7], and specific gravity of sludge of 1.0 (kg/L), the total nitrogen concentration of the dewatered sludge at TS 5% is calculated to be 3.7 gN/L. At all temperatures, nitrogen degradation of the pretreated dewatered sludge was higher than that of the raw dewatered sludge. This is possibly due to higher protein degradation performance by pretreatment. Lee *et al.* [10] reported that 80°C pretreatment enhanced protein degradation, which resulted in release of ammonia. For each sludge conditions, the nitrogen degradation and the VS removal correlated. Using this relationship, an ammonia concentration of digested sludge can be estimated depending on a substrate nitrogen concentration and VS removal performance for preventing ammonia inhibition.

Viscosity of the digested dewatered sludge was measured on day 168 (**Fig. 4**). High viscosity requires more energy for mixing [21]. At each digestion temperature, viscosity with pretreatment (AH) was higher than that without pretreatment (A). This implies release of high-viscosity substance oriented from coagulants and extracellular polymeric substances by the pretreatment. Particularly in AH15, the produced substance might not be degraded in anaerobic digestion. Without pretreatment, viscosity in A15 was lower. This is possibly due to low biodegradation in anaerobic digestion. Pretreatment could increase required energy for agitation, and simple operation without mechanical mixing can reduce

Fig. 4 Viscosity of the digested dewatered sludge in the continuous experiment.

Fig. 5 CST of the digested dewatered sludge in the continuous experiment.

operational energy, if OLR is lower.

CST was analyzed on day 175 (**Fig. 5**). Each sample was measured two, three, or four times. Dewaterability of the digested sludge was not deteriorated obviously by lower temperature digestion. Pretreatment seemed to increase CST under all temperature conditions. This is also possibly due to release of high-viscosity substance by the pretreatment. In this study, dewatering conditions such as coagulant addition were not modified for each sample, but practically dewatering conditions are adjusted on site, and dewaterability is possibly improved more.

Fig. 6 Biogas production rate of pretreated sludge at different temperatures in the batch experiment.

Effect of Pretreatment Temperature on Solubilization and Biogas Production

Solubilization performance of COD in the batch experiment was 30, 32 and 40% after pretreatment at 70, 80 and 90°C for 24 h, respectively. Change in total COD concentrations and VS/TS ratios during the pretreatment was not observed. This indicated that no methane or hydrogen was produced during the pretreatment.

Biogas production rate in the batch experiment is summarized in **Fig. 6**. Regardless of the pretreatment temperature conditions, the rate was higher during 1–3 d. This means that most components were slowly biodegradable even with pretreatment, compared with waste activated sludge from conventional activated sludge processes [22]. The accumulated biogas amount after the 27-d experiment was 0.18, 0.20 and 0.20 NL/gVS-added for pretreatment at 70, 80 and 90°C, respectively. Although the COD solubilization was higher at 90°C, this did not result in higher biogas production. This might be due to production of melanoids by Maillard reactions [23]. These results show that the pretreatment temperature of 70–80°C is more appropriate than that of 90°C under this experimental condition.

Microbial Community Analysis

Bacterial gene copy numbers were compared on day 88 (**Fig. 7**). Hidaka *et al.* [24] compared bacterial gene copy numbers in anaerobically digested sewage sludge from ODS under various conditions and reported 1×10^9 – 4×10^9 copies/mL. The results of the present research are within this range. The gene copy numbers at 15°C were lower than those

Fig. 7 Bacterial gene copy numbers in substrate and digested sludge.

at 35°C, but in total the effect of digestion temperature and pretreatment on gene copy numbers was not significant.

The microbial community structure was compared on day 88. Approximately 70,000–100,000 sequence reads per sample were analyzed. **Table 4** summarizes the predominant phyla, and the detailed family-level analyses in Firmicutes. On the basis of phylum, Bacteroidetes and Proteobacteria decreased after digestion from the substrate. In general, the degradation ratio was lower at 15°C than at 35°C, and higher with pretreatment than without pretreatment. They are possibly related to degradation of bacteria from waste activated sludge. Firmicutes, which are related to acid fermentation, increased after digestion. Among Firmicutes, class Clostridia were detected the most. Family Clostridiaceae increased with pretreatment at both 15 and 35°C. Family Bacillaceae increased at 35°C, in particular with pretreatment. They are possibly related to enhancement of acid fermentation by the pretreatment.

Feasibility Analysis in Terms of Energy Balance

The calorific value of biogas generated by anaerobic digestion with solubilization treatment and the input energy required for heating were roughly estimated using the results obtained in the continuous experiment under a certain condition. The heating and heat-retaining energy were calculated according to Japan Sewage Works Association (JSWA) [25]. Two operation modes were compared: no-heated digestion with hyperthermophilic pretreatment, and mesophilic digestion followed by thermal treatment at 55°C for inactivation of weed seeds and pathogens. In both modes, the input energy is larger than the calorific value of biogas produced due to

Table 4 (a) Microbial structure and the ratios detected (%). Phylum level

<phylum>	A(substrate)	A35	AH35	AH25	A15	AH15
Acidobacteria	1.8	0.1	0	0	0.3	0
Actinobacteria	1.2	0.8	0.5	0.5	1.4	0.7
Bacteroidetes	45.8	14.1	21.9	25.4	27.5	24.3
Chloroflexi	1.7	0.8	0.8	0.7	1.8	1.4
Elusimicrobia	1.4	0.2	0	0	0.7	0.1
Firmicutes	4.5	22.7	32.8	32.3	26.1	39.7
Planctomycetes	3.1	1.1	0.2	0.3	2.3	0.6
Proteobacteria	28.3	6.7	4.2	10.5	30	23.4
Synergistetes	0	2	3.5	0.9	0.1	0.2
Others	10.8	50	35.1	28.1	8.4	8.4

Table 4 (b) Microbial structure and the ratios detected (%). Class, order and family levels of Firmicutes

<class>	<order>	<family>	A(substrate)	A35	AH35	AH25	A15	AH15
Bacilli	Bacillales	Bacillaceae	0	2.3	6.3	0.3	0	0
Clostridia	Clostridiales	Clostridiaceae	2.7	3.2	5.4	5.7	9	11.7
Clostridia	Clostridiales	Ruminococcaceae	0	0.8	1	2.9	4.2	3.8
Clostridia	Clostridiales	Syntrophomonadaceae	0	0.4	0.8	1.5	0.7	0.2
Clostridia	Clostridiales		0.2	3.5	2.7	4	2.2	3.9
Clostridia	Unclassified Clostridiales		0	2.7	6.7	12.2	7.2	14.9
Clostridia			0.1	2.6	2.4	1.2	0.5	0.5
Others			1.5	7.2	7.5	4.5	2.3	4.7

the low biogas production performance of the sludge from ODs. However, the former mode requires less input energy than the latter. Although it is necessary to evaluate the detail under co-digestion conditions, low-temperature digestion with hyperthermophilic pretreatment is shown to be a promising candidate to improve the energy balance.

CONCLUSIONS

Anaerobic digestion characteristics of sewage sludge from OD, whose biodegradability is quite low, were proved under the combined mesophilic, psychrophilic, and hyperthermophilic temperature conditions by the continuous and batch experiments. The biogas production performance of anaerobic digestion at low temperatures with hyperthermophilic pretreatment was equivalent or superior to that of typical mesophilic operation. Effect of different temperatures on digestate characteristics and microbial communities were revealed. The results proved that the combination of pretreatment and psychrophilic anaerobic digestion is applicable for sludge management in local cities with decentralized small WWTPs, including OD.

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