Evaluation of sludge properties in a pilot-scale UASB reactor for sewage treatment in a temperate region

ABSTRACT

In this study, continuous operation of a pilot-scale upflow anaerobic sludge blanket (UASB) reactor for sewage treatment was conducted for 630 days to investigate the physical and microbial characteristics of the retained sludge. The UASB reactor with a working volume of 20.2 m³ was operated at ambient temperature (16–29 °C) and seeded with digested sludge. After 180 days of operation, when the sewage temperature had dropped to 20 °C or lower, the removal efficiency of both total suspended solids (TSS) and total biochemical oxygen demand (BOD) deteriorated due to washout of retained sludge. At low temperature, the cellulose concentration of the UASB sludge increased owing to the rate limitation of the hydrolytic reaction of suspended solids in the sewage. However, after an improvement in sludge retention (settleability and concentration) in the UASB reactor, the process performance stabilized and gave sufficient results (68% of TSS removal, 75% of total BOD removal) at an hydraulic retention time (HRT) of 9.7 h. The methanogenic activity of the retained sludge significantly increased after day 246 due to the accumulation of Methanosaeta and Methanobacterium following the improvement in sludge retention in the UASB reactor. Acid-forming bacteria from phylum Bacteroidetes were detected at high frequency; thus, these bacteria may have an important role in suspended solids degradation.

Key words | microbial structure, sewage, sludge retention, suspended solids, UASB

INTRODUCTION

From past to present, anaerobic treatment technology has been an attractive way to treat wastewater because of its low energy consumption, and low construction and maintenance costs. In addition, the application of anaerobic technology, such as UASB (upflow anaerobic sludge blanket), to domestic sewage has become widespread in both tropical and subtropical regions, including India, Brazil, Colombia, Egypt and Mexico (Draaijer et al. 1992; Florencio et al. 2001; Sato et al. 2007; Heffernan et al. 2011). Domestic sewage is a low-strength, complex type of wastewater, characterized by (i) low chemical oxygen demand (COD) concentrations (100–600 mg/L), (ii) strong fluctuations in hydraulic and organic loading rates, (iii) relatively low temperatures in temperate regions (15–25 °C), and (iv) high fractions of organic suspended solids (SS) (50–60% of the total COD). In particular, the high concentration of suspended solids components, such as cellulose and protein, is a major problem for anaerobic digestion of the sewage. Because at low temperatures (lower than 20 °C) the fermentation process of biomass needs a longer solid retention time (SRT) (de Man et al. 1986), the accumulation of suspended solids inside the UASB reactor always leads to washout of the active biomass. As a result, the SRT decreases. This phenomenon leads to a decrease in methanogenic activity and process performance (Zeeman & Lettinga 1999).

Nevertheless, anaerobic treatment processes for domestic sewage are still most commonly operated as ‘black boxes’. In particular, only a few studies have described the
structure and function of the microbial populations in anaerobic treatments for domestic sewage (Gomec et al. 2008). In order to operate the UASB reactor stably at low temperature, therefore, further studies are needed.

In this study, a pilot-scale UASB reactor was operated in the conditions of a temperate region (sewage temperature, 16–29°C) for 630 days, in order to investigate the changes in physical and microbial properties of the retained sludge.

MATERIALS AND METHODS

Experimental setup of the UASB reactor

A pilot-scale UASB reactor was set up and operated at Kokubu-Hayato (Kirishima city, Kagoshima prefecture, Japan: 31.7°N) sewage treatment center. The process flow of the UASB reactor is shown in Figure 1. The reactor, with a working volume of 20.2 m³ (sectional area 4.2 m², height 5 m), was operated for 630 days at ambient temperature. Raw sewage was introduced to an auto-screen to remove large (>2.5 mm diameter) suspended solids and then fed to the UASB reactor. The temperature of sewage in Kokubu-Hayato was 16–29°C. On day 0 and day 54, the reactor was seeded with 10 m³ of mesophilic digested sludge each. It amounted to 199 kg total suspended solids (TSS) (144 kg volatile suspended solids (VSS)).

The hydraulic retention time of the UASB reactor ranged from 9.7 to 12.1 h. In order to maintain sufficient quality of the treated wastewater, a trickling filter system (Tandukar et al. 2007) using sponge media as a support material was installed as a post-treatment. In addition, in order to obtain detailed data at low temperature operation, another UASB reactor was operated on a semi-pilot scale (1 m³ volume) for sewage treatment at Nagaoka city’s sewage treatment center (37.48°N). The temperature of sewage in Nagaoka was 12–27°C during operation.

Analyses

Water quality analysis

The process performance of the UASB reactor was investigated by analyzing samples from the influent and effluent. The analysis included concentrations of TSS, VSS, biochemical oxygen demand (BOD) and COD, and biogas production and composition, among others. All analytical procedures were carried out according to APHA (1998). After sampling, sewage and effluent were homogenized for measurement of TSS and total BOD and total COD. A filtered sample (0.45 μm: GB 140 filter paper, ADVANTEC, Japan) was used for the analysis of soluble BOD and COD. Biogas composition was analyzed by TCD (thermal conductivity detector) gas chromatograph.

Physical properties of retained sludge

The UASB reactor had nine sampling ports every 50 cm from 25 cm of bottom portion. Most of the sludge bed existed up to 1–2 m height during operation, so the retained sludge from port No. 2 (75 cm from the bottom) of the UASB reactor was sampled occasionally for the analysis of physical properties such as sludge concentration (mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS)) and sludge settleability (sludge volume index (SVI)). Measurements of sludge concentration and SVI followed the standard method of APHA (1998). Moreover, the cellulose content of the retained sludge was measured according to Van Soest & McQueen (1973). The amount of the retained sludge calculated from the sludge profile data of the reactor was used to calculate SRT and the sludge loading.

Methanogenic activity of retained sludge

The methanogenic activity of the retained sludge was determined in duplicate at days 0 (seed), 85, 155, 246, 344, 477, 540 and 610 with 122-mL serum vial bottles (liquid phase 50 mL) according to Harada et al. (1994). For the measurement of methanogenic activity, the sludge sample was homogenized at 10,000 rpm for 30 seconds under anaerobic conditions. Final concentration of sludge in the vial bottles was 6 to 8 g VSS/L. The test substrates were acetate and
H₂/CO₂ (80:20%, v/v). For the measurement of hydrogenotrophic activity, the vial’s headspace was filled with H₂/CO₂ gas at 1.4 atm (142 kPa). All vials were incubated in a reciprocal-shaker (120 rpm) at 20–35 °C.

**Analysis of microbial community structure and population**

The microbial community structure of the retained sludge was investigated by 16S rDNA-targeted DGGE (denaturing gradient gel electrophoresis). The DNA was extracted from sludge samples by using an Isoil beads beating kit (Nippon Gene, Japan). The SS fraction of sewage at Kokubu-Hayato sewage treatment center and other UASB sludge (placed in Nagaoka city) were used to test as reference. PCR (polymerase chain reaction) was performed using a primer specific for amplifying either Domain Bacteria (341F, 534R) (Muyzer et al. 1996) or Domain Archaea (PARCH340F-GC, PARCH519R) (Ovreås et al. 1997). DGGE analysis was conducted by using a DCode™ gel electrophoresis system (Bio-rad, USA) at 60 °C for 3.5 h. Major bands containing DNA were excised, and the nucleotide sequences were determined by using a 3100 genetic analyzer (Applied Biosystems Japan Ltd). The sequences obtained were compared with the data stored in NCBI GenBank using BLAST.

Population changes of methanogens belonging to a major genus of methanogen, namely *Methanoseta, Methanobacterium* or *Methanospirillum*, were quantitatively monitored by SYBR Green I fluorescence real-time PCR with specific primer sets targeting 16S rRNA (Furukawa et al. 2009). Total RNA was extracted from the sludge sample (according to Kang et al. 2009), and then cDNA for real-time PCR was synthesized by reverse transcription reaction with random primers.

**RESULT AND DISCUSSION**

**Process performance of the UASB reactor**

Operation of the pilot-scale UASB reactor was started in May 2007 in the Kokubu-Hayato sewage treatment center. Figure 2 shows the process performance of the UASB reactor during 630 days of operation. The sewage temperature differed from 16 to 29 °C according to the season (Figure 2(a)). The average concentration of COD, BOD and TSS in the influent sewage was T-COD 402 mg/L.
of the UASB reactor was 0.41 kg BOD/m³/day. Therefore, during days 235 to 274, the HRT of the reactor was extended to 12.1 h. As a result, sludge settleability was improved after day 246. At this time, the sludge concentration and SVI of the retained sludge obtained from port No. 2 (75 cm from the bottom) of the reactor.

In the second winter season (around day 600), it was possible to keep the HRT of the reactor constant at 9.7 h due to the improvement in sludge retention (Figure 3).

The average biogas production rate in the later part of experiment (after day 397) was about 2.1 Nm³/day. The methane content of the biogas was 70%. At this stage, only 33% of the removed COD was recovered as biogas. It is expected that most of the methane was soluble in the effluent.

These results show that the organic loading for stable operation of the UASB reactor for sewage treatment was about 0.41 kg BOD/m³/day (1.0 kg COD/m³/day, 0.11 kg COD/kg TSS/day) in a temperate region. In addition, maintenance of a sufficient level of sludge concentration (over 20 g TSS/L of MLSS, see Figure 3) in the UASB reactor is very important for stable operation of the reactor at low temperatures.

Changes in sludge physical properties

The physical properties of the retained sludge were determined to investigate the behavior of the retained sludge during operation. Figure 3 shows the changes in sludge concentration and SVI of the retained sludge obtained from port No. 2 (75 cm from the bottom) of the reactor.

During the start-up period, serious washout of the retained sludge occurred due to low settleability of the seed sludge (SVI was about 210 mL/g, based on MLSS concentration). On day 54, therefore, the reactor was seeded again with mesopilic digested sludge. As a result, the MLSS concentration increased to 23 g TSS/L (MLVSS, 17 g VSS/L) on day 85, but it then decreased to half this level by day 155 owing to washout of flocculent sludge again. At this time, the SRT of the UASB reactor was 51 days based on the total amount of retained sludge determined from data of the MLSS profile.

In order to maintain the sludge retention and process performance, HRT was extended to 12.1 h during days 235 to 274. As a result, sludge settleability was improved after day 246. At this time, the sludge flock became larger in size as compared with the seed sludge (data not shown). An improvement in sludge settleability caused an increase in sludge concentration at the sludge bed. So, the HRT was reduced to 9.7 h again on day 275 to give appropriate organic loading and upflow velocity (0.5 m/h). Finally, the MLSS concentration of the sludge at port 2 reached 25–28 g TSS/L (MLVSS, 19–21 g VSS/L). At this time, the VSS/TSS ratio of the retained sludge was about 0.75. After 500 days of operation, the SVI decreased to 50 mL/g or less. As a result, the SRT increased to 111 days (at around day 600). The formation of a small amount of granular sludge was confirmed in the bottom portion of the sludge bed.
In Japan, suspended solids in sewage contain a significant amount of cellulose and protein derived from flush toilets. In this UASB experiment, a primary sedimentation tank was omitted to simplify the treatment process. Therefore, in order to clarify the behavior of solid organic compounds in the UASB reactor and its relation to sewage temperature, the cellulose concentration in the sludge bed was measured (Figure 4). On day 246, at a sewage temperature of 16.7 °C, 1.38 g/L of cellulose was present in the sludge bed (at port No. 2), but this concentration gradually decreased to 0.63 g/L (day 477) in accordance with the increase in sewage temperature. Afterwards, it was confirmed that cellulose accumulated again in the sludge bed due to the drop in sewage temperature. Cellulose is known to be a difficult compound to degrade anaerobically even at high temperature (Schwarz 2001). Therefore, degradation of cellulose was much affected by decreasing of sewage temperature.

From these observations, we concluded that keeping an HRT of 9.7 h is sufficient for maintaining sludge physical properties in a temperate region. In addition, biodegradation of organic suspended solids, such as cellulose, is the rate-limiting step in the anaerobic digestion of sewage at low temperature (less than 20 °C).

**Methanogenic activity of the retained sludge**

Figure 5 shows the methanogenic activity of the retained sludge determined at 20 °C. Until day 246, the methanogenic activity of the retained sludge remained at a low level, 0.017 to 0.032 g COD/g VSS/day for H₂/CO₂ and 0.005 to 0.014 g COD/g VSS/day for acetate.

In the first half of the operational phase of the UASB reactor, the sludge retention (settleability and concentration) was not sufficient. Therefore, the methanogenic activity of the retained sludge was clearly lower when the sewage temperature dropped below 20 °C (on day 246). After day 246, sludge retention improved in the UASB reactor, causing a continuous increase in the methanogenic activity of the retained sludge. Even in winter (around day 600, Figure 2(a)), the activity of the retained sludge maintained a sufficient level.

Finally, at day 610, the H₂/CO₂-fed activity and acetate-fed activity reached 0.11 and 0.03 g COD/g VSS/day respectively. These results show that the improvement in sludge retention in the UASB reactor caused an accumulation of methanogens in the retained sludge. As a result of good retention of methanogens, the effluent BOD of the UASB reactor stably remained at a low level even at low temperature (Figure 2(c)). The same tendency was observed for the activity of the retained sludge at 35 °C (data not shown).

During the 610-day operational phase of the UASB reactor, H₂/CO₂-fed activity was always prominent as compared with acetate-fed activity.

**Microbial structure of the retained sludge**

The microbial structure of the retained sludge was investigated by 16S rDNA targeted DGGE analysis. In addition, two reference samples, Nagaoka UASB sludge for sewage treatment and suspended solids of sewage (sewage SS) in Kokubu-Hayato sewage treatment center, were subjected to DGGE.

Figure 6 shows the DGGE profiles of the retained sludge with respect to the Archaea domain. In this figure, information on the related DNA sequence of major bands is presented. As a result of DGGE analysis with respect to the Archaea domain, genus *Methanosaeta* (bands 1 and 7) as an acetoclastic methanogen and genus *Methanobacterium* (bands 3, 5) as a hydrogenotrophic methanogen were detected in the Kokubu-Hayato UASB sludge. A DGGE band corresponding to genus *Methanospirillum* (band 6) was also detected. In the later stage of the operational
phase, bands corresponding to *Methanosaeta* sp. (band 1) and *Methanobacterium beijingense* (band 5) were prominent. In the Nagaoka UASB sludge, greater band intensity for *Methanobacterium formicicum* (band 8) was observed instead of *Methanobacterium beijingense*.

In a previous study, proliferation of genus *Methanosaeta* was observed in sewage treatment granular sludge at 13°C (Gomec et al. 2008). However, presence of genus *Methanobacterium* was not confirmed by FISH (fluorescence in situ hybridization) analysis of granular sludge.

Figure 7 shows the DGGE profiles of the retained sludge with respect to the bacteria domain. It indicates that the band patterns of sewage SS and the retained sludge were quite different. Also, major DGGE bands (bands 13, 17, 22) were common in the Kokubu-Hayato UASB and the Nagaoka UASB sludges. Interestingly, most of the major bands belonged to the phylum Bacteroidetes (bands 12, 13, 17, 19) and the phylum Firmicutes (bands 10, 20, 23). Both Bacteroidetes and Firmicutes are known as anaerobic acid-forming bacteria. In
mesophilic anaerobic digestion, a major proportion of the acid-forming bacteria is generally categorized as Firmicutes, such as genus *Clostridium*. However, DGGE bands related to Bacteroidetes accounted for the majority in the sewage treatment UASB sludge. The presence of Bacteroidetes-related bacteria has been confirmed in human feces (Lu *et al.* 2008) and water environments. Some of the Bacteroidetes-related bacteria (bands 1, 5, 6) were detected in sewage SS in this experiment. Therefore, it seems that Bacteroidetes proliferated significantly in the UASB sludge. Moreover, these bacteria may play an important role in acidification of the organic SS at ambient temperature.

Lastly, a band corresponding to Deltaproteobacteria (such as genus *Syntrophus*, a fatty acid-degrading bacteria, band 15) was detected.

Population dynamics of methanogens in the retained sludge

Population changes in the methanogens detected in DGGE were analyzed by real-time PCR. In Figure 8, the amount of 16S rRNA for each methanogen is shown as a copy number of rRNA per 1 g VSS of sludge sample.

Until day 155, the rRNA copy numbers of both *Methanosaeta* and *Methanobacterium* slightly increased. On the other hand, the rRNA copy number of genus *Methanospirillum* decreased. At low temperature, after day 246, the rRNA copy numbers of both *Methanosaeta* and *Methanobacterium* remained at a constant level (1.8–2.0 × 10^{10} copies/g VSS).

During operational days 246 to 477, the rRNA copy numbers of methanogens tended to decrease slightly. However, the MLVSS concentration of the sludge bed clearly increased after 155 days of operation (Figure 3); therefore, the total amount of rRNA derived from methanogens in the sludge bed was slightly higher at days 344 and 477. After day 477, when sludge retention improved significantly (Figure 3), the copy numbers of rRNA for both *Methanosaeta* and *Methanobacterium* increased, reaching a level of to 2.3–3.2 × 10^{11} copies/g VSS at day 610. This tendency was similar to the increase in methanogenic activity of the retained sludge (Figure 5).

The rRNA copy number of genus *Methanospirillum* decreased due to the drop in sewage temperature. Moreover, its rRNA copy number was clearly lower than that of genus *Methanosaeta* and genus *Methanobacterium*.

From these observations, we conclude that maintaining sufficient sludge retention in the UASB reactor is very important for accumulating methanogens in the retained sludge and thus achieving sufficient process performance of the UASB reactor for sewage treatment in temperate regions.

**CONCLUSIONS**

The UASB reactor for sewage treatment showed sufficient removal efficiency of BOD and TSS, even when operated at low temperature after the improvement in sludge retention (settleability and concentration). The organic loading for stable operation of the UASB reactor for sewage treatment was about 0.41 kg BOD/m^{3}/day (1.0 kg COD/m^{3}/day) in this study (sewage temperature 16–29 °C). Accumulation of cellulose, which is contained in sewage, was confirmed during the low-temperature season (less than 20 °C) in the sludge bed of the UASB reactor. Maintenance of a sufficient level of sludge retention (SRT 111 days) in the UASB reactor promoted an accumulation of the *Methanosaeta* and *Methanobacterium* in the retained sludge, which caused an increase in the methanogenic activity of the retained sludge. Bacteria from the phylum Bacteroidetes were detected at high frequency in the UASB sludge, and may have an important role in the acidification process.

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