1 **Performance evaluation of the sulfur-redox-reaction–activated up-flow anaerobic sludge blanket**
2 **and down-flow hanging sponge anaerobic/anoxic sequencing batch reactor system for municipal**
3 **sewage treatment**

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20

1 **Abstract**

2 A sulfur–redox–reaction–activated up-flow anaerobic sludge blanket (UASB) and down-flow hanging
3 sponge (DHS) system, combined with an anaerobic/anoxic sequencing batch reactor (A₂SBR), has been
4 used for municipal sewage treatment for over 2 years. The present system achieved a removal rate of $95 \pm$
5 14% for BOD, ~~$71 \pm 54\%$ for SS~~, $74 \pm 22\%$ for total nitrogen, and $78 \pm 25\%$ for total phosphorus,
6 including low water temperature conditions. Sludge conversion rates during the operational period were
7 0.016 and $0.218 \text{ g-VSS} \cdot \text{g-COD-removed}^{-1}$ for the UASB, and DHS, respectively, which are similar to a
8 conventional UASB–DHS system, ~~which is not used of sulfur–redox–reaction~~, for sewage treatment.
9 Using the sulfur-redox reaction made advanced treatment of municipal wastewater with minimal sludge
10 generation possible, even in winter. Furthermore, the occurrence of a unique phenomenon, known as the
11 anaerobic sulfur oxidation reaction, was confirmed in the UASB reactor under the ~~winter season influent~~
12 ~~wastewater temperature of $\leq 17^\circ\text{C}$~~ .

13

14 **Keywords:** phosphorus removal, denitrification, low excess sludge, anaerobic sulfur oxidation

15

1. Introduction

Conventional activated sludge treatment, commonly used to treat municipal wastewater, causes problems such as excessive generation of sludge and involves consumption of a large amount of energy. In contrast, the anaerobic biological treatment process, used mainly to treat wastewaters with medium-to-high chemical oxygen demand (COD) concentrations has a number of advantages such as low sludge production, low power consumption, and energy recovery as methane gas (Narihiro & Sekiguchi, 2007; van Lier et al., 2008). In particular, the up-flow anaerobic sludge blanket (UASB) based method has been successfully used for the treatment of industrial wastewater in medium-to-high temperature ranges (Frankin, 2001; Onodera et al., 2013c; Pattanauwat et al., 2013). In recent years, the UASB method has been applied to the treatment of sewage in tropical and subtropical countries such as India, Brazil, Colombia, Egypt, and Mexico (Alaerts et al., 1993; Seghezzo et al., 1998). However, only the anaerobic biological treatment method has been applied to sewage treatment, and thus, removal of organics, suspended solids (SS), and nutrient salts remains inadequate. Therefore, application of a post-treatment process is necessary to improve the treated water to a quality acceptable for discharge.

Several post-treatment units have been developed and applied to the UASB process (Kassab et al., 2010; Khan et al., 2011). One of the more promising post-treatment units is the down-flow hanging sponge (DHS) process, which is similar to a conventional trickling filter, except for the sponge media (Machdar et al., 1997; Okubo et al., 2015; Onodera et al., 2014; Tawfik et al., 2006). Recently, our research group developed an advanced wastewater treatment system comprising a UASB–DHS–anaerobic/anoxic sequencing batch reactor (A₂SBR) system in which the DHS method and the A₂SBR

are combined and then applied after implementation of the UASB method (Hatamoto et al., 2015; Maharjan et al., 2014). This system achieves the basic removal of organics and SS during the initial UASB process; this is then followed by decomposition of the organics and nitrification by the DHS method, nitrogen and phosphorus are finally removed by the A₂SBR, in which including use of denitrifying polyphosphate-accumulating organisms (DPAOs). Thus, this system improves the treated water to a level that meets or even exceeds the quality standards of discharge water.

Treatment of sewage by the UASB method in low-temperature environments creates problems such as reduction in the removal rate of organics and SS. The cause of such problems is the decrease in activities of the methanogenic archaea, which are the final decomposer of organics during the anaerobic treatment, associated with reduction in the water temperature. The reduced performance of treatment by the UASB method leads to decline in the performance of the DHS method in terms of removal of organics and nitrification, causing a system-wide decline in treatment performance. In an attempt to overcome these problems, the implementation of a sulfur-redox-reaction-activated UASB and aerobic post-treatment system has been reported (Sumino et al., 2007; Takahashi et al., 2011b). Such a system focuses on using sulfate-reducing bacteria as an alternative to the methanogenic archaea for the removal of the organics. The results of continuous treatment of sewage using the sulfur-redox reaction-activated UASB–DHS system showed that removal rates of $95 \pm 1\%$, $79 \pm 7\%$, and $38 \pm 8\%$ were achieved for biochemical oxygen demand (BOD), SS, and for total nitrogen, respectively, under the following conditions: hydraulic retention time (HRT) of 12 h, treatment water temperature of 7.0 ± 2.8 °C, and influent sulfate concentration of $140 \text{ mg-S} \cdot \text{L}^{-1}$ (Takahashi et al., 2011b).

This study combined the A₂SBR with the sulfur-redox reaction-activated UASB-DHS system and examined its performance in continuous treatment of wastewater, based on the amount of sludge generated. In addition, the unique sulfur dynamics of the UASB reactor were also investigated.

2. Materials and Methods

2.1 Reactor setup and operational conditions

The dimensions of the UASB used in this experiment were as follows: height, 4.7 m; inner column diameter, 0.56 m; and total volume, 1,178 L. The total volume of the sponge of the DHS was 454 L and its filling rate was 53%. As the sponge carrier for the DHS, cubic sponges (33 mm) were inserted into plastic net rings (φ33 mm; G 3.2. type)(Tanaka et al., 2012) and loaded into the reactor. Details of the process configuration have been described elsewhere (Onodera et al., 2013a). The A₂SBR was 0.86 m in height and 0.5 m in diameter, with a total volume of 200 L. A schematic diagram of UASB-DHS-A₂SBR system is shown in supplementary Fig. S1.

The complete UASB-DHS-A₂SBR system was installed in a sewage treatment center and was operated continuously under ambient conditions (influent wastewater temperature: 9–28 °C). For the sewage supply, separated sewage was temporarily stored in a tank after passing through a coarse screen. On the 178th day after the start of the operation, sodium sulfate was added to activate the microorganisms of the sulfur-redox cycle. About 500 L of mesophilic sewage digestion sludge was used for the seed sludge in the UASB and about 50 L of active sludge was used in the A₂SBR. Seed was not used for the DHS. The HRTs of the reactors were as follows: 8 h for the UASB, 3.1 h for the DHS, and 12 h for the

1 A₂SBR.

2 For the A₂SBR, a period of 6 h was set for one anaerobic/anoxic cycle. Anaerobic conditions
3 were maintained for 90 min from the start of the cycle. Following the period of anaerobic conditions, 50
4 L of UASB–DHS-treated water was added into the reactor and stirred and mixed for 270 min under
5 anoxic conditions. Then, the mixing was halted and sedimentation of the sludge conducted for 60 min,
6 following which 50 L of supernatant from the reactor was discharged as the A₂SBR-treated water. The
7 concentration of sludge in the A₂SBR was adjusted to about 2,000 mg-SS·L⁻¹ by continuous removal of
8 excess sludge. A mixed solution of acetic acid and sodium acetate was used as the added organics to the
9 A₂SBR at an amount of COD/P ratio against an inflow of phosphorus concentration of 25 g-COD/g-P.
10 The pH of the A₂SBR was maintained at 7.4 ± 0.3. Details of the A₂SBR configuration and operational
11 conditions have been described elsewhere (Hatamoto et al., 2015) .

12

13 2.2 Analytical method

14 Samples of the sewage and UASB effluent were obtained by 24-h composite sampling and
15 stored in tanks at 4 °C. Samples of the DHS and A₂SBR effluents were obtained by grab sampling.
16 Sampled water that passed through glass-fiber filter paper with 0.4-μm size particle retention (GB-140,
17 ADVANTEC) was used to analyze the soluble components. The analyzed items were SS, volatile
18 suspended solids (VSS), BOD, COD_{Cr}, ammonium, nitrate, nitrite, total Kjeldahl nitrogen (TKN), total
19 phosphorus (TP), sulfide, and sulfate. The values of COD, TKN, and TP were analyzed using a
20 water-quality analyzer (DR-2800, HACH). A high-performance liquid chromatograph (LC-20-ADsp,

Shimadzu) was used to measure the concentrations of sulfate, ammonium, nitrate, and nitrite. The total nitrogen (TN) concentration was calculated from the individual values measured for each type of nitrogen. Analyses of, both, the water quality in the vertical direction of the UASB reactor and the sludge samples were performed regularly. Each sample was collected from a tube installed in the UASB reactor. Samples for the measurement of sulfide concentration were collected while ensuring that air did not enter the vials, following which the solid and liquid were separated by centrifugation. Each sample was quickly transported to the laboratory, and the appropriate measurements were taken immediately. Other water-quality experiments were performed according to APHA (2005).

3. Results and Discussion

3.1 Treatment performance of the UASB–DHS–A₂SBR system

Time courses of COD, BOD, and SS concentrations that reflect the treatment performance of the UASB–DHS–A₂SBR system (UASB–DHS operation days 1–980, and A₂SBR operation days 700–980) are shown in Fig.1. A summary of the treatment performance of the UASB–DHS–A₂SBR system is also shown in Table 1. The period when the influent sewage temperature was ≥ 17 °C was considered summer, and the period when it was < 17 °C was considered winter. The mean total BOD concentration in summer was 227 ± 115 mg·L⁻¹ in the influent sewage, 88 ± 48 mg·L⁻¹ in the UASB-treated water, 8 ± 8 mg·L⁻¹ in the DHS-treated water, and 8 ± 10 mg·L⁻¹ in the A₂SBR-treated water (Table 1). In contrast, the mean BOD concentration in winter was 185 ± 107 mg·L⁻¹ in the influent sewage, 99 ± 39 mg·L⁻¹ in the UASB-treated water, 15 ± 11 mg·L⁻¹ in the DHS-treated water, and 9 ± 7 mg·L⁻¹ in the

1 A₂SBR-treated water (Table 1). Compared with summer, the influent BOD concentration in winter
2 decreased by about 10%; however, the BOD concentration of the UASB-treated water increased by about
3 10%. In addition, the BOD concentration of the DHS-treated water doubled in winter. However, the
4 A₂SBR-treated water showed no significant difference between summer and winter. Considered as a
5 single system, the treatment performances in summer and winter were similar. The mean SS
6 concentration in summer was $97 \pm 57 \text{ mg}\cdot\text{L}^{-1}$ for the influent sewage, $40 \pm 30 \text{ mg}\cdot\text{L}^{-1}$ for the
7 UASB-treated water, $20 \pm 25 \text{ mg}\cdot\text{L}^{-1}$ for the DHS-treated water, and $18 \pm 28 \text{ mg}\cdot\text{L}^{-1}$ for the
8 A₂SBR-treated water. In winter, the mean SS concentration was $80 \pm 32 \text{ mg}\cdot\text{L}^{-1}$ for the influent sewage,
9 $35 \pm 19 \text{ mg}\cdot\text{L}^{-1}$ for the UASB-treated water, $23 \pm 15 \text{ mg}\cdot\text{L}^{-1}$ for the DHS-treated water, and 23 ± 53
10 $\text{mg}\cdot\text{L}^{-1}$ for the A₂SBR-treated water. Both in summer and winter, the final treated water had similar SS
11 concentrations. This system exhibited stable organics and SS removal performance in both summer and
12 winter, and the treatment performance was comparable with that reported previously for a system that
13 used the sulfur-redox process (Takahashi et al., 2011b).

14 In summer, the mean ammonium concentration was 26 ± 7 and $8 \pm 11 \text{ mg-N}\cdot\text{L}^{-1}$ for the UASB
15 and DHS, respectively, and the nitrate nitrogen was 0 and $16 \pm 8 \text{ mg-N}\cdot\text{L}^{-1}$ for the UASB and DHS,
16 respectively. In contrast, in winter, the mean ammonia nitrogen was 21 ± 8 and $11 \pm 7 \text{ mg-N}\cdot\text{L}^{-1}$ for the
17 UASB and DHS, respectively, and the nitrate nitrogen was 0 and $6 \pm 5 \text{ mg-N}\cdot\text{L}^{-1}$ for the UASB and DHS,
18 respectively. A nitrification reaction was confirmed during both summer and winter; however, because the
19 total nitrogen concentration in the DHS-treated water decreased by 30% and 28% during summer and
20 winter, respectively, compared with the UASB-treated water, it is likely that the denitrification reaction

occurred in the DHS reactor. Denitrification was confirmed in A₂SBR and total nitrogen removal rate of $74 \pm 22\%$ was achieved in whole system. The mean total phosphorus concentration in winter was $3.2 \pm 1.0 \text{ mg-P}\cdot\text{L}^{-1}$ in the influent wastewater and $3.0 \pm 1.0 \text{ mg-P}\cdot\text{L}^{-1}$ in the DHS-treated water, indicating that hardly any phosphorus removal occurred in the UASB–DHS system. Conversely, the A₂SBR-treated water contained only $0.9 \pm 0.5 \text{ mg-P}\cdot\text{L}^{-1}$; thus, a phosphorus removal rate of $78 \pm 25\%$ was achieved. The mechanism of phosphorus and nitrogen removal of A₂SBR is action of DPAOs, and their activities have not been hindered by the influent sulfate used in this study (c.a. $50 \text{ mg-S}\cdot\text{L}^{-1}$).

The water quality of the UASB–DHS-treated water (influent of A₂SBR) in this research was similar to that reported previously for sewage treatment employing the UASB–DHS system (Onodera et al., 2014; Tanaka et al., 2012). The treatment performance of the A₂SBR was favorable regardless of the season. Furthermore, the quality of the treated water obtained from the UASB–DHS–A₂SBR system mostly achieved effluent water quality for a nitrogen and phosphorus concentration of $\leq 10 \text{ mg-N}\cdot\text{L}^{-1}$ and $\leq 1.0 \text{ mg-P}\cdot\text{L}^{-1}$, respectively. Therefore, using a combination of the DHS and A₂SBR after treatment by the UASB can achieve advanced treatment of sewage in both summer and winter. Although the effluent water quality is satisfactory, further study is required for reduction of relatively long HRT of the system.

3.2 COD balance in the process

Figure 2 shows the COD balance of the influent and discharge of the UASB, DHS, and A₂SBR during summer and winter (calculated by setting the influent COD of each system to 100%). The COD balance of the UASB discharge in summer was 27% for particulate COD, 26% for soluble COD, 18% for

sulfate reduction, 12% for methane gas, and 17% for dissolved methane. The COD balance of the UASB discharge in winter was 26% for particulate COD, 36% for soluble COD, 9% for sulfate reduction, 2% for methane gas, 15% for dissolved methane, and 2% unknown. This 2% unknown is believed to include the sludge and sulfide accumulated in the UASB reactor. The COD in the methane gas and the dissolved methane in the winter UASB discharge were less than that in summer. Sumino et al. (2007) examined the activities of methanogenic archaea and sulfate-reducing bacteria in the retained sludge in a wastewater treatment UASB. They reported that methane production activity decreased when the influent wastewater temperature was about 10 °C but that sulfate reduction activity was maintained at a high level (Sumino et al., 2007). Therefore, the decrease in generation of methane gas and dissolved methane is assumed to occur because of the decreased activities of methanogenic archaea caused by decrease in the water temperature. Based on the COD balance of the DHS, the excess sludge of the DHS was 2% of the influent COD in both summer and winter.

3.3 Sludge production in the UASB–DHS–A₂SBR system

The sludge concentration in the UASB increased gradually during the operational period. The organic loading rate decreased with increase in, both, the retained sludge and the operational time. Ultimately, it was maintained at about 0.05 g-COD·g-VS⁻¹·day⁻¹ at 200 days or more, which corresponded to 0.9–1.1 kg-COD·m⁻³·day⁻¹. The sludge conversion rate of the UASB was 0.016 g-VSS·g-COD-removed⁻¹ during the entire operational period. This conversion rate is comparable with that of other sewage-treating UASBs (Takahashi et al., 2011a). A typical sludge production rate for a

1 UASB treating domestic sewage has been reported to be 0.03–0.2 kg SS/kg COD removed⁻¹ (Lettinga et
2 al., 1983). The sludge conversion rate in the UASB presented in this study, in which the sulfur-redox
3 cycle was activated, was relatively low in comparison with that in previous reports.

4 The average concentration of the retained sludge in the DHS after day 600 was 11
5 g-VS·L-sponge⁻¹ (15 g-TS·L-sponge⁻¹), which is lower than in previous reports (Onodera et al., 2013a).
6 This indicated that the retained sludge concentration in the DHS sponge carrier of the presented system
7 did not reach saturation. The average sludge production rate in the DHS was 0.218 g-VSS·g-COD
8 removed⁻¹ over the entire operational period. This value is almost the same as that reported for other
9 DHSs (i.e., 0.18 g-SS·g-COD removed⁻¹) and lower than that of other UASB post-treatment processes
10 using biofilters and rotating disk devices (i.e., 0.370 g-TS·g-COD removed⁻¹ and 0.290–0.330
11 g-VSS·g-BOD removed⁻¹, respectively) (Goncalves et al., 1998). The average VS/TS ratio of the retained
12 sludge was 0.72 ± 0.05; hence, sludge production of the UASB–DHS system with the activated
13 sulfur-redox cycle is comparable with that of a conventional UASB–DHS system.

14 The sludge conversion rate of the A₂SBR in summer and winter was 0.179 and 0.225
15 g-VSS·g-COD-removed⁻¹, respectively. These values are almost within the range of other reports for the
16 simultaneous removal of nitrogen and phosphorous using an anaerobic/anoxic cycle, i.e., 0.23–0.28
17 g-VSS·g-COD-removed⁻¹ (Kuba et al., 1996).

18

19 **3.4 Sulfur concentration profile in the UASB reactor**

20 The average sulfide concentration in the UASB treated water was 33 ± 13 and 18 ± 11

1 $\text{mg-S}\cdot\text{L}^{-1}$ in summer and winter, respectively, and the average sulfate concentration was 12 ± 23 and $52 \pm$
2 $33 \text{ mg-S}\cdot\text{L}^{-1}$ in summer and winter, respectively. There was a 73% and 26% reduction in influent sulfate
3 in the UASB in summer and winter, respectively, indicating that part of the organic matter was
4 decomposed by the sulfate-reducing bacteria. Previously, the anaerobic sulfur oxidation phenomenon,
5 whereby the sulfide generated by the sewage treatment UASB is re-oxidized to sulfate by the addition of
6 sodium sulfate in the UASB (where there are no electron acceptors such as oxygen, nitrate, and nitrite),
7 has been reported (Ono et al., 2011). Therefore, the water quality in this study was analyzed in the vertical
8 direction in the UASB reactor. Figure 3 shows the water-quality profile of the UASB in the vertical
9 direction. The profile on day 826 (summer: influent wastewater temperature of $26.8\text{ }^{\circ}\text{C}$) shows that the
10 influent sulfate concentration of $44 \text{ mg-S}\cdot\text{L}^{-1}$ was reduced to $6 \text{ mg-S}\cdot\text{L}^{-1}$ at a height of 1.0 m in the
11 UASB; however, the profile does not show any further fluctuations before discharge ($\pm 1 \text{ mg-S}\cdot\text{L}^{-1}$).
12 During the summer, when the influent wastewater temperature was high, the anaerobic sulfur oxidation
13 reaction could not be confirmed. However, on day 980 of profile (winter: influent wastewater temperature
14 of $10.0\text{ }^{\circ}\text{C}$), the influent sulfate concentration of $81 \text{ mg-S}\cdot\text{L}^{-1}$ reduced to $3 \text{ mg-S}\cdot\text{L}^{-1}$ at a height of 2.0 m
15 in the UASB. Subsequently, the sulfide generated during the sulfate reduction was oxidized to sulfate, and
16 the sulfate concentration in the UASB-treated water became $73 \text{ mg-S}\cdot\text{L}^{-1}$. Thus, it was confirmed that the
17 anaerobic sulfur oxidation reaction occurred when the influent wastewater temperature was low during
18 winter. In the profiles produced in this study, the ORP values at the positions at which anaerobic sulfur
19 oxidation was confirmed were all -300 mV or less and the pH ranged from 6.9 to 7.3. In addition, the
20 soluble COD concentration increased at those positions at which anaerobic sulfur oxidation occurred.

1 Anaerobic sulfur oxidation in the UASB reactor leads to reduction in the sulfide load in the subsequent
2 DHS. A previous report stated that in a DHS reactor the influent sulfide and organics are oxidized first,
3 followed by the dissolved methane, and finally the ammonia (Hatamoto et al., 2011). To achieve a stable
4 nitrification performance in the DHS reactor, it is effective to ensure that sulfide is oxidized to sulfate in
5 the previous step, i.e., in the UASB, thus reducing the load on the DHS.

6

7 **4. Conclusions**

8 A sulfur-redox-reaction-activated UASB–DHS plus A₂SBR system exhibited stable treatment
9 performance, even at low water temperatures, and that the removal rates of BOD, total nitrogen, and total
10 phosphorus were $95 \pm 14\%$, $74 \pm 22\%$, and $78 \pm 25\%$, respectively. In addition, the occurrence of the
11 anaerobic sulfur oxidation reaction was confirmed in the UASB reactor under conditions in which the
12 influent wastewater temperature was $<17\text{ }^{\circ}\text{C}$, which could reduce the sulfide load on the DHS reactor.
13 Thus, it has been demonstrated that the system could be suitable for the low temperature treatment of
14 municipal sewage.

15

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References

- Alaerts, G.J., Veenstra, S., Bentvelsen, M., Vanduijl, L.A. 1993. Feasibility of Anaerobic Sewage-Treatment in Sanitation Strategies in Developing-Countries. *Water Sci. Technol.*, **27** (1), 179-186.
- APHA. 2005. *Standard Methods for the Examination of Water and Wastewater*. 21th ed. APHA/AWWA/WEF.
- Frankin, R.J. 2001. Full-scale experiences with anaerobic treatment of industrial wastewater. *Water Sci. Technol.*, **44** (8), 1-6.
- Goncalves, R.F., de Araujo, V.L., Chernicharo, C.A.L. 1998. Association of a UASB reactor and a submerged aerated biofilter for domestic sewage treatment. *Water Sci. Technol.*, **38** (8-9), 189-195.
- Hatamoto, M., Miyauchi, T., Kindaichi, T., Ozaki, N., Ohashi, A. 2011. Dissolved methane oxidation and competition for oxygen in down-flow hanging sponge reactor for post-treatment of anaerobic wastewater treatment. *Bioresour. Technol.*, **102** (22), 10299-10304.
- Hatamoto, M., Saito, Y., Dehama, K., Nakahara, N., Kuroda, K., Takahashi, M., Yamaguchi, T. 2015. Microbial community structure of a simultaneous nitrogen and phosphorus removal reactor following treatment in a UASB-DHS system. *Water Sci. Technol.*, **71** (3), 454-61.
- Kassab, G., Halalsheh, M., Klapwijk, A., Fayyad, M., van Lier, J.B. 2010. Sequential anaerobic-aerobic treatment for domestic wastewater - a review. *Bioresour. Technol.*, **101** (10), 3299-310.
- Khan, A.A., Gaur, R.Z., Tyagi, V.K., Khursheed, A., Lew, B., Mehrotra, I., Kazmi, A.A. 2011. Sustainable options of post treatment of UASB effluent treating sewage: A review. *Resour. Conserv. Recy.*, **55** (12), 1232-1251.
- Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J. 1996. Phosphorus and nitrogen removal with minimal COD requirement by integration of denitrifying dephosphatation and nitrification in a two-sludge system. *Water Res.*, **30** (7), 1702-1710.
- Lettinga, G., Roersma, R., Grin, P. 1983. Anaerobic treatment of raw domestic sewage at ambient temperatures using a granular bed UASB reactor. *Biotechnol. Bioeng.*, **25** (7), 1701-1723.
- Machdar, I., Harada, H., Ohashi, A., Sekiguchi, Y., Okui, H., Ueki, K. 1997. A novel and cost-effective sewage treatment system consisting of UASB pre-treatment and aerobic post-treatment units for developing countries. *Wat. Sci. Technol.*, **36** (12), 189-197.
- Maharjan, N., Dehama, K., Ohtsuki, K., Saito, Y., Miyaoka, Y., Tshering, T., Nakamura, A., Hatamoto, M., Yamaguchi, T. 2014. An Integrated System of UASB-DHS-A₂SBR for Effective Removal of Organic Matter and Nutrients from Municipal Wastewater. *J. Water Environ. Technol.*, **12** (5), 421-429.
- Narihiro, T., Sekiguchi, Y. 2007. Microbial communities in anaerobic digestion processes for waste and wastewater treatment: a microbiological update. *Curr. Opin Biotech.*, **18** (3), 273-278.
- Okubo, T., Onodera, T., Uemura, S., Yamaguchi, T., Ohashi, A., Harada, H. 2015. On-site evaluation of the performance of a full-scale down-flow hanging sponge reactor as a post-treatment process of

an up-flow anaerobic sludge blanket reactor for treating sewage in India. *Bioresour. Technol.*, **194**, 156-164.

Ono, S., Takahashi, M., Hatamoto, M., Kawakami, S., Yamaguchi, T., Yamazaki, S., Araki, N., Harada, H. 2011. Performance of a pilot- scale sewage treatment by UASB and DHS reactor combined system enhancing sulfate-reducing reaction. *The 4th IWA-ASPIRE Conference & Exhibition (IWA-ASPIRE 2011)*, Tokyo. pp. 307, No. 14-22-7.

Onodera, T., Matsunaga, K., Kubota, K., Taniguchi, R., Harada, H., Syutsubo, K., Okubo, T., Uemura, S., Araki, N., Yamada, M., Yamauchi, M., Yamaguchi, T. 2013a. Characterization of the retained sludge in a down-flow hanging sponge (DHS) reactor with emphasis on its low excess sludge production. *Bioresour. Technol.*, **136**, 169-175.

Onodera, T., Sase, S., Choeisai, P., Yoochatchaval, W., Sumino, H., Yamaguchi, T., Ebie, Y., Xu, K., Tomioka, N., Mizuochi, M., Syutsubo, K. 2013c. Development of a treatment system for molasses wastewater: the effects of cation inhibition on the anaerobic degradation process. *Bioresour. Technol.*, **131**, 295-302.

Onodera, T., Tandukar, M., Sugiyana, D., Uemura, S., Ohashi, A., Harada, H. 2014. Development of a sixth-generation down-flow hanging sponge (DHS) reactor using rigid sponge media for post-treatment of UASB treating municipal sewage. *Bioresour. Technol.*, **152**, 93-100.

Pattananuwat, N., Aoki, M., Hatamoto, M., Nakamura, A., Yamazaki, S., Syutsubo, K., Araki, N., Takahashi, M., Harada, H., Yamaguchi, T. 2013. Performance and Microbial Community Analysis of a full-scale Hybrid Anaerobic-Aerobic Membrane System for Treating Molasses-Based Bioethanol Wastewater. *Int. J. Environ. Res.*, **7** (4), 979-988.

Seghezzo, L., Zeeman, G., van Lier, J.B., Hamelers, H.V.M., Lettinga, G. 1998. A review: The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresour. Technol.*, **65** (3), 175-190.

Sumino, H., Takahashi, M., Yamaguchi, T., Abe, K., Araki, N., Yamazaki, S., Shimozaki, S., Nagano, A., Nishio, N. 2007. Feasibility study of a pilot-scale sewage treatment system combining an up-flow anaerobic sludge blanket (UASB) and an aerated fixed bed (AFB) reactor at ambient temperature. *Bioresour. Technol.*, **98** (1), 177-182.

Takahashi, M., Ohya, A., Kawakami, S., Yoneyama, Y., Onodera, T., Syutsubo, K., Yamazaki, S., Araki, N., Ohashi, A., Harada, H., Yamaguchi, T. 2011a. Evaluation of Treatment Characteristics and Sludge Properties in a UASB Reactor Treating Municipal Sewage at Ambient Temperature. *Int. J. Environ. Res.*, **5** (4), 821-826.

Takahashi, M., Yamaguchi, T., Kuramoto, Y., Nagano, A., Shimozaki, S., Sumino, H., Araki, N., Yamazaki, S., Kawakami, S., Harada, H. 2011b. Performance of a pilot-scale sewage treatment: an up-flow anaerobic sludge blanket (UASB) and a down-flow hanging sponge (DHS) reactors combined system by sulfur-redox reaction process under low-temperature conditions. *Bioresour. Technol.*, **102** (2), 753-7.

Tanaka, H., Takahashi, M., Yoneyama, Y., Syutsubo, K., Kato, K., Nagano, A., Yamaguchi, T., Harada, H. 2012. Energy saving system with high effluent quality for municipal sewage treatment by UASB-DHS. *Water Sci. Technol.*, **66** (6), 1186-94.

Tawfik, A., Ohashi, A., Harada, H. 2006. Sewage treatment in a combined up-flow anaerobic sludge

blanket (UASB)-down-flow hanging sponge (DHS) system. *Biochem. Eng. J.*, 29 (3), 210-219.

van Lier, J.B., Mahmoud, N., Zeeman, G. 2008. Anaerobic wastewater treatment. in: *Biological Wastewater Treatment: Principles, Modeling and Design.* , (Eds.) M. Henze, M.C.M.v. Loosdrecht, G.A. Ekama, D. Brdjanovic, IWA, pp. 415-455.

Figure legends:

Fig. 1 Time course of sewage temperature and total COD (A), total BOD (B), and SS concentrations (C).

■ particulate COD ■ soluble COD ▨ sulfate reduction
 ■ methane gas □ dissolved methane ■ excess sludge

Fig. 2 COD balance of UASB (A), DHS (B), and A₂SBR (C).

Fig. 3 Vertical profiles of water quality in the UASB reactor. (A) Operation date 826, and influent sewage temperature of 26.8°C. (B) Operation date 980, and influent sewage temperature of 10.0°C.

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7 Table 1 Treatment performance of the UASB–DHS–A₂SBR system.

	Summer* (days 729–928)				Winter* (days 700–728, 929–956)				Total period			
	Sewage	UASB eff.	DHS eff.	A ₂ SBR eff.	Sewage	UASB eff.	DHS eff.	A ₂ SBR eff.	Sewage	UASB eff.	DHS eff.	A ₂ SBR eff.
Total CODCr (mg/L)	336 (96) [#]	177 (78)	48 (43)	45 (41)	295 (104)	180 (60)	65 (45)	44 (63)	321 (101)	178 (73)	53 (44)	45 (44)
Soluble CODCr (mg/L)	161 (56)	88 (34)	27 (14)	27 (16)	128 (55)	110 (39)	38 (30)	20 (12)	150 (58)	95 (37)	31 (21)	25 (16)
Total BOD (mg/L)	227 (115)	88 (48)	8 (8)	8 (10)	185 (107)	99 (39)	15 (11)	9 (7)	214 (113)	91 (46)	10 (9)	8 (9)
Soluble BOD (mg/L)	106 (45)	51 (33)	4 (4)	4 (5)	83 (53)	64 (30)	9 (10)	4 (2)	99 (48)	55 (32)	5 (7)	4 (5)
SS (mg/L)	97 (57)	40 (30)	20 (25)	18 (28)	80 (32)	35 (19)	23 (15)	23 (53)	92 (51)	38 (27)	21 (22)	19 (33)
VSS (mg/L)	84 (42)	33 (21)	13 (14)	14 (23)	72 (32)	30 (17)	19 (14)	20 (45)	80 (40)	32 (20)	15 (14)	16 (28)
TKN (mg-N/L)	35 (16)	34 (14)	8 (11)	7 (11)	26 (11)	25 (10)	13 (12)	7 (6)	33 (16)	31 (14)	10 (11)	8 (10)
NH ₄ ⁺ (mg-N/L)	28 (24)	26 (7)	8 (11)	7 (15)	21 (7)	21 (8)	11 (7)	8 (9)	26 (20)	24 (7)	9 (9)	8 (14)
NO ₃ ⁻ (mg-N/L)	-	-	16 (8)	2 (5)	-	-	6 (5)	3 (3)	-	-	13 (8)	3 (4)
TN (mg-N/L)	36 (16)	33 (12)	23 (8)	8 (6)	28 (12)	25 (10)	18 (9)	10 (4)	34 (15)	30 (12)	21 (8)	9 (7)
TP (mg-P/L)	3.9 (0.8)	4.0 (0.7)	4.1 (0.8)	0.7 (0.3)	3.2 (1.0)	2.9 (0.6)	3.0 (1.0)	0.9 (0.5)	3.7 (1.0)	3.7 (1.0)	3.8 (1.0)	0.8 (0.6)
Sulfide (mg-S/L)	2 (2)	33 (13)	0 (1)	0 (1)	1 (2)	18 (11)	1 (1)	0 (0)	2 (2)	28 (14)	0 (1)	0 (1)
Sulfate (mg-S/L)	45 (35)	12 (23)	44 (27)	54 (18)	70 (36)	52 (33)	68 (33)	63 (25)	53 (37)	25 (32)	51 (31)	54 (21)

8 *: The period with an influent sewage temperature of ≥ 17 °C was considered summer and the period with an influent sewage temperature < 17 °C was considered
9 winter.

10 [#]: Figures in parentheses are standard deviations.

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