

# Characterization of sludge properties for sewage treatment in a practical-scale down-flow hanging sponge reactor: oxygen consumption and removal of organic matter, ammonium, and sulfur

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## ABSTRACT

The characteristics of sludge retained in a down-flow hanging sponge reactor were investigated to provide a better understanding of the sewage treatment process in the reactor. The organic removal and sulfur oxidation conditions were found to differ between the first layer and the following three layers. It was found that 63% and 59% of the organic matter was removed in the first layer, even though the hydraulic retention time was only 0.2 h. It is thought that the organic removal resulted from aerobic and anaerobic biodegradation on the sponge medium. The sulfate concentration increased 1.5–1.9-fold in the first layer, with almost no subsequent change in the second to fourth layers. It was shown that oxidation of sulfide in the influent was completed in the first layer. The result of the oxygen uptake rate test with an ammonium nitrogen substrate suggested that the ammonium oxidation rate was affected by the condition of dissolved oxygen (DO) or oxidation–reduction potential (ORP).

**Key words** | developing country, down-flow hanging sponge, retained sludge, sewage treatment

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## INTRODUCTION

Down-flow hanging sponge (DHS) reactors have been developed as a low-energy-consumption sewage treatment technology for developing countries (Agrawal *et al.* 1997; Machdar *et al.* 2000; Tandukar *et al.* 2006, 2007; Tawfik *et al.* 2006; Onodera *et al.* 2014a). The DHS method is based on a conventional trickling filter, in which a sponge medium is used to retain the sludge. The influent wastewater is supplied to the top of the DHS reactor and goes through to the bottom due to gravity. The microorganisms retained on the sponge medium remove organic matter and ammonium nitrogen from the wastewater. Furthermore, the DHS method does not require aeration because oxygen is naturally supplied from the air, lowering this method's energy consumption.

Our research group has previously fabricated a bench-scale DHS reactor at a sewage treatment plant in Japan, and conducted various sewage treatment experiments using several types of sponge media. The results showed that the performance of the DHS for organic removal is satisfactory (Agrawal *et al.* 1997; Machdar *et al.* 2000; Tandukar *et al.* 2006, 2007; Tawfik *et al.* 2006; Onodera *et al.* 2014a). Especially, G3 sponge carrier which is cylindrical in shape and packed randomly in the reactor has widely used in experiment for sewage treatment (Tawfik *et al.* 2008, 2011; Mahmoud *et al.* 2010, 2011; Machdar *et al.* 2014; Onodera *et al.* 2014b; Okubo *et al.* 2016). The results showed that DHS reactor has high performance in removal of organic matter, for example, chemical oxygen demand (COD) removal rates were 56–96%, and COD of DHS effluent were 11.4–121 mg L<sup>-1</sup>. Moreover, full-scale experiment was conducted in India (Okubo *et al.* 2016). The DHS reactor was run under hydraulic retention time (HRT) 1.33 h condition, consequently, averaged COD and biochemical oxygen demand (BOD) of DHS effluent were 40 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup>. The water quality satisfied effluent standard in India (BOD < 30 mg L<sup>-1</sup>), i.e. it was confirmed that DHS reactor is applicable technology to sewage treatment in India.

To understand the sewage treatment process in the DHS reactor, water quality profiles and microbial activity of the retained sludge in the DHS reactors have been investigated using several laboratory-scale DHS reactors (Machdar *et al.* 2000; Tandukar *et al.* 2006, 2007; Onodera *et al.* 2014a). There is, however, only one example in the literature of a practical-scale DHS reactor (Okubo *et al.* 2015, 2016). According to Okubo *et al.* (2016), the average chemical oxygen demand (COD<sub>Cr</sub>) of the influent to the DHS reactor (i.e. the UASB effluent) was 168 mg L<sup>-1</sup>, lower than that of other sewage treatment plants (average: 419 mg L<sup>-1</sup>) in India (Sato *et al.* 2006). Thus, the organic matter concentration of the influent to the DHS reactor, which is primarily treated by UASB reactors, is often high in developing countries. No practical-scale reactor has ever been operated with influents that have such high COD<sub>Cr</sub> values, therefore, information on the treatment performance and degradation of organic matter in a DHS reactor under high organic concentration influent condition is required to adapt the design of DHS reactors for developing countries.

The aim of this study was to identify the mechanisms of the removal of organic matter, sulfur, and ammonium in a practical-scale DHS reactor under high-organic-concentration influent conditions. The oxygen consumption by the sludge retained in the sponge medium was evaluated, and the rates of oxygen consumption under different organic matter concentration conditions were compared with those from previous studies.

## MATERIALS AND METHODS

### DHS reactor

A practical scale DHS reactor was installed at a municipal sewage treatment plant in Agra, India. The raw sewage was treated by a screen, a grid channel, and an upflow

anaerobic sludge blanket (UASB) reactor. The HRT of the UASB reactor was set at 9.75 h. The effluent from the UASB reactor was sent to the DHS reactor.

A schematic diagram of the DHS reactor is shown in Figure 1. The DHS reactor consisted of a concrete cylinder, 16 m in diameter and 2.7 m in height. To supply oxygen to the inside of the reactor, ventilation windows were incorporated into the reactor wall. The sponge medium used to retain the sludge was made from hexagonal polyurethane cylinders, 25 mm wide and 40 mm long, that were supported by a polyethylene plastic net to prevent compaction. The center of the sponge cylinders was hollow to facilitate the entry of oxygen. A total of 4.7 million sponge cylinders with a total volume of 187 m<sup>3</sup> (excluding the center hole) were installed randomly in four layers, separated vertically, in the reactor. The sponge layer height was 200 mm for the first layer, and 600 mm for the subsequent layers. For this study, the influent volume supplied to the DHS reactor was set to 3,000 m<sup>3</sup> day<sup>-1</sup>.

## Sampling

Sponge sampling and profile analyses were conducted twice, in different seasons. Phase 1 was conducted 475–491 days after the reactor was initiated and phase 2 was conducted 551–565 days. Water samples were collected from the first to the fourth layers using a total of 12 sampling ports located every 2 m from the reactor center in a horizontal direction of the DHS reactor. DHS reactor influent samples, i.e. UASB reactor effluent samples, were taken at the same time. Sponge medium samples were randomly

collected from the 12 ports in the same manner as the water samples to measure the retained biomass concentration and the oxygen uptake rate (OUR) of the retained biomass.

## Analytical methods

Dissolved oxygen (DO) was measured using the Winkler method (APHA/AWWA/WEF 2005), while COD<sub>Cr</sub>, NH<sub>3</sub>-N, and SO<sub>4</sub><sup>2-</sup> were determined using the potassium dichromate method, Nessler method, and SulfaVer 4 method, respectively, using a DR5000 water quality analyzer (HACH). Glass fiber filter paper (pore diameter: 0.45 mm, ADVANTEC-GB140) was used to obtain filtered samples.

The three sponge samples collected were squeezed and washed with pure water until all of the retained biomass was extracted. The extracted sludge was then washed twice with pure water. The quantity of the retained biomass was analyzed following *Standard Methods* (APHA/AWWA/WEF 2005), and calculated as grams of suspended solids per liter of sponge (g-SS L<sup>-1</sup>-sponge), and grams of volatile suspended solids per liter of sponge (g-VSS L<sup>-1</sup>-sponge).

The results were averaged while in a horizontal direction (three points) on the DHS reactor.

## OUR test

The three sponge samples collected were squeezed and washed twice with a 10 mM phosphate buffer solution (Na<sub>2</sub>HPO<sub>4</sub> 12H<sub>2</sub>O; 1.432 g L<sup>-1</sup>, NaH<sub>2</sub>PO<sub>4</sub> 2H<sub>2</sub>O; 0.936 g L<sup>-1</sup>). The washed sludge sample (final concentration: 3–5 g-VSS L<sup>-1</sup>), nutrients, and substrate were placed in a cell, and a DO sensor was then inserted immediately into the cell to initiate measurement. The nutrient reagents used were a phosphate buffer solution, magnesium sulfate solution, calcium chloride solution, and ferric chloride solution, all of which are used for BOD analysis (APHA/AWWA/WEF 2005). The initial substrate concentrations were adjusted to 80 mg-COD<sub>Cr</sub> L<sup>-1</sup> for glucose and filtered sewage, and to 25 mg-N L<sup>-1</sup> for NH<sub>4</sub>Cl. To confirm endogenous oxygen uptake rate (EOUR), tests without any substrate were also conducted. To suppress any nitrification, the nitrification inhibitor allylthiourea (ATU) was added at a concentration of 2.0 mg L<sup>-1</sup> to the test slurries in all of the test systems, other than that for NH<sub>4</sub><sup>+</sup>-N. A YSI-5300A biological oxygen monitor (YSI Corp.) was used for DO measurement. A YSI-5301B water bath and stirrer assembly (YSI Corp.) was used throughout this work, and all tests were conducted at 20 °C. The tests were conducted twice, and the results were represented as averaged value.

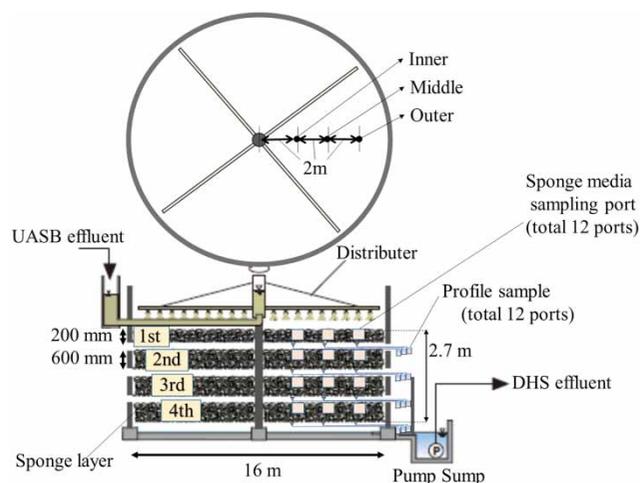
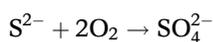


Figure 1 | Schematic of the DHS reactor.

## Calculation of oxygen consumption rate

The oxygen consumption of the DHS reactor has been evaluated as per sponge carrier volume (Hatamoto *et al.* 2010) and per weight of retained sludge (Okubo *et al.* 2016). Therefore, this study evaluated the oxygen consumption of the DHS reactor with the both viewpoints. For the oxygen consumption of the DHS reactor, the amount of oxygen consumed by oxidation of organic matter (COD<sub>Cr</sub>), sulfur, and ammonium were calculated with the results of water quality profile. The amounts of oxygen consumption in each item were calculated with the decrease of COD<sub>Cr</sub> and ammonium nitrogen, and increase of sulfate ion in water quality profile. The amount of oxygen required for oxidations of sulfur and ammonium were calculated following chemical reaction formula.



## RESULTS

### Profile analysis

The average water quality for the 2 weeks prior to the analysis of the DHS reactor is shown in Table 1. The temperatures of the DHS influent on the profile sampling dates were 26 °C (489 days (phase 1)) and 17 °C (565 days (phase 2)). The results of the profile analyses at 489 days (phase 1) and 565 days (phase 2) are shown in Table 2. The BOD/COD<sub>Cr</sub> ratio of the filtered sewage were 0.40 (±0.07) and 0.34 (±0.10) for phases 1 and 2, respectively. The values of the filtered DHS effluent were lowered to 0.23 (±0.08) and 0.15 (±0.06) for phases 1 and 2, respectively.

The DO at phase 1 is low in the first to the third layers (Table 2), especially at the middle port, with values lower than 1 mg L<sup>-1</sup>. However, the DO at phase 2 increases to 4.24 (±0.65) mg L<sup>-1</sup> in the first layer. These values show that DO differs for different seasons.

The COD<sub>Cr</sub> decreases by almost half in the first layer (Table 2), with reduction rates at 63% (phase 1) and 59% (phase 2). The COD<sub>Cr</sub> decrease proceeds gradually in the

**Table 1** | Summary of averaged water quality data gathered in the 2 weeks prior to profile analysis

Period	DHS influent temperature °C	DHS influent load kg-COD <sub>Cr</sub> m <sup>-3</sup> day <sup>-1</sup>	COD <sub>Cr</sub> influent mg L <sup>-1</sup>	COD <sub>Cr</sub> effluent mg L <sup>-1</sup>	BOD influent mg L <sup>-1</sup>	BOD effluent mg L <sup>-1</sup>	NH <sub>3</sub> -N influent mg L <sup>-1</sup>	NH <sub>3</sub> -N effluent mg L <sup>-1</sup>
Phase 1 (475–489 days)	27 (1)	4.69 (0.45)	338 (31)	106 (15)	101 (10)	24 (10)	40.0 (–)	29.5 (–)
Phase 2 (551–565 days)	19 (1)	3.54 (1.45)	385 (92)	136 (22)	121 (17)	27 (6)	45.4 (1.2)	32.9 (2.7)

The numbers in parentheses are standard deviations.

**Table 2** | Profiles for DO, COD<sub>Cr</sub>, and NH<sub>3</sub>-N along the main axis of the DHS reactor at phase 1 (489 days) and phase 2 (565 days)

Period	Phase 1				Phase 2			
	DO mg L <sup>-1</sup>	Unfiltered COD <sub>Cr</sub> mg L <sup>-1</sup>	NH <sub>3</sub> -N mg L <sup>-1</sup>	SO <sub>4</sub> <sup>2-</sup> mg L <sup>-1</sup>	DO mg L <sup>-1</sup>	Unfiltered COD <sub>Cr</sub> mg L <sup>-1</sup>	NH <sub>3</sub> -N mg L <sup>-1</sup>	SO <sub>4</sub> <sup>2-</sup> mg L <sup>-1</sup>
Influent	0.00 (–)	374 (–)	40.1 (–)	175 (–)	0.00 (–)	331 (–)	46.3 (–)	194 (–)
1 <sup>st</sup>	1.03 (1.78)	140 (29)	37.8 (4.8)	339 (29)	4.24 (0.65)	135 (25)	44.0 (1.2)	300 (30)
2 <sup>nd</sup>	0.33 (0.58)	140 (21)	37.3 (3.2)	332 (27)	4.47 (1.48)	90 (19)	37.6 (4.2)	285 (6)
3 <sup>rd</sup>	0.86 (1.33)	112 (19)	31.5 (3.2)	345 (31)	5.96 (2.30)	84 (6)	32.6 (1.8)	293 (7)
4 <sup>th</sup>	3.12 (1.36)	103 (22)	28.6 (3.1)	334 (26)	6.06 (3.13)	87 (20)	27.8 (6.1)	272 (13)

The values were averaged while in a horizontal direction on the DHS reactor. The numbers in parentheses are standard deviations.

next layer, and the combined decrease ratio for the second to fourth layers are 26% (phase 1) and 36% (phase 2). The  $\text{COD}_{\text{Cr}}$  decrease rates, based on the dry weight of the sludge, are shown in Figure 2(a). The results at the first layer are  $0.76 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  (phase 1) and  $0.54 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  (phase 2), respectively.

Very little  $\text{NH}_3\text{-N}$  removal is observed in the first and the second layers, and the concentration gradually falls after the third layer in phase 1 (Table 2). In contrast, at phase 2, the  $\text{NH}_3\text{-N}$  concentration falls gradually from the first to the bottom layers, with concentrations in the range  $2\text{--}6 \text{ mg-N L}^{-1}$  in each layer. The  $\text{NH}_3\text{-N}$  decrease rates based on the dry weight of the sludge are shown in Figure 2(b). The results are  $0.00373\text{--}0.00756 \text{ g-NH}_3\text{-N g}^{-1}\text{-VSS day}^{-1}$  in phase 1 (apart from the second layer) and

$0.00570\text{--}0.00728 \text{ g-NH}_3\text{-N g}^{-1}\text{-VSS day}^{-1}$  in phase 2, and the values are of the same range between phases 1 and 2.

The  $\text{SO}_4^{2-}$  concentration increases 1.9-fold (phase 1) and 1.5-fold (phase 2) in the first layer (Table 2), and the subsequent  $\text{SO}_4^{2-}$  concentrations are similar in the second to fourth layers.

### Oxygen consumption of the reactor

The oxygen consumption rates in each layer, calculated based on  $\text{COD}_{\text{Cr}}$ , ammonium, and sulfur oxidation, are shown in Figure 3. High oxygen consumption occurs in the first layer for both phases. The oxygen consumption rates derived from the  $\text{COD}_{\text{Cr}}$  decrease are  $27.6 \text{ kg-O}_2 \text{ m}^{-3} \text{ sponge day}^{-1}$  (phase 1) and  $21.9 \text{ kg-O}_2 \text{ m}^{-3} \text{ sponge day}^{-1}$

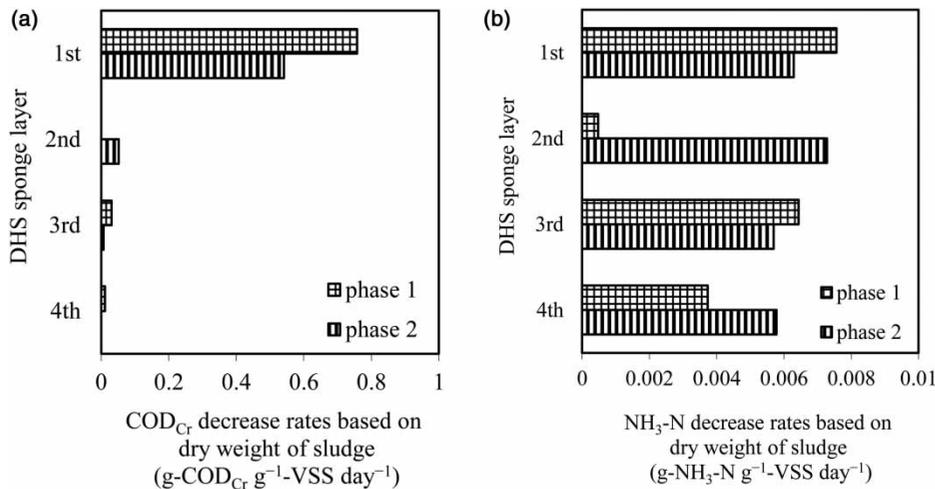


Figure 2 |  $\text{COD}_{\text{Cr}}$  (a) and  $\text{NH}_3\text{-N}$  (b) decrease rates based on dry weight of sludge at each layer.

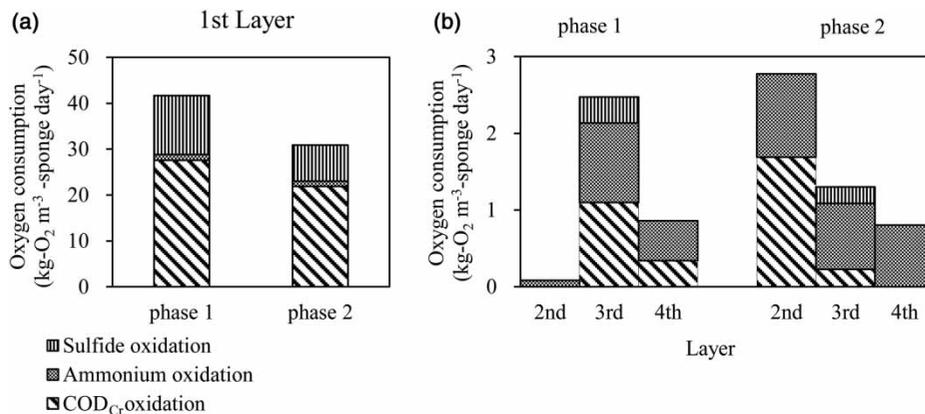
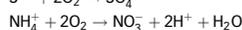
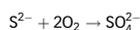


Figure 3 | Oxygen consumption rates at phase 1 (489 days) and phase 2 (565 days) in the DHS reactor in the first layer (a) and the second to fourth layers (b). The oxygen consumption rates are calculated from ammonia and  $\text{COD}_{\text{Cr}}$  reduction and sulfate production rates. Ammonia oxidation and sulfur oxidation are calculated by the following formulae:



(phase 2), and oxygen consumption derived from the oxidation of sulfur to sulfate are 12.9 and 7.87 kg-O<sub>2</sub> m<sup>-3</sup>-sponge day<sup>-1</sup> in phase 1 and phase 2, respectively. In the second to the fourth layers, the total oxygen consumption rates are in the range of 0–2.78 kg-O<sub>2</sub> m<sup>-3</sup>-sponge day<sup>-1</sup>.

### Retained biomass and OUR

The sludge concentrations in the DHS sponge medium are 36 (±9), 38 (±11), 35 (±6), and 30 (±5) g-VSS L<sup>-1</sup>-sponge (phase 1), and 40 (±8), 33 (±13), 33 (±1), 30 (±4) g-VSS L<sup>-1</sup>-sponge (phase 2) from the first layer to the fourth layers, respectively. The sludge concentration decreases along the downward vertical direction of the DHS reactor.

The results of the OUR tests are shown in Figure 4. The OUR values with filtered sewage as the substrate are 5.78–14.4 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> (phase 1), and 14.6–27.6 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> (phase 2). The OUR excluding the EOUR for the filtered sewage substrate is 1.69–9.65 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> in phase 1 and 7.42–15.9 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> in phase 2. The results for the OUR in phase 2 are higher than those in phase 1, especially in the lower layers. The OUR results for the ammonium nitrogen substrate in the third and the fourth layers are 6.92 and 5.24 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> (phase 1), and 17.1 and 12.6 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> (phase 2), respectively. For the third and fourth layers, the OUR excluding the EOUR for the ammonium nitrogen substrate is 2.45 and 2.35 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> in phase 1, and 2.78 and 4.26 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> in phase 2), respectively.

## DISCUSSION

### Oxygen consumption by oxidation of organic matter

The COD<sub>Cr</sub> decreases by almost half in the first layer. It is thought that this reduction is caused not only by aerobic biodegradation, but also absorption onto the sponge media followed by anaerobic biodegradation because the amount of oxygen consumed is very high (Figures 2 and 3, and Table 2). It is known that the oxygen consumption of the DHS reactor is 2.5 kg-O<sub>2</sub> m<sup>-3</sup>-sponge day<sup>-1</sup> (Hatamoto *et al.* 2010). However, the oxygen consumption calculated from COD<sub>Cr</sub> profile at the first layer are much higher previous report (27.6 kg-O<sub>2</sub> m<sup>-3</sup>-sponge day<sup>-1</sup> (phase 1) and 21.9 kg-O<sub>2</sub> m<sup>-3</sup>-sponge day<sup>-1</sup> (phase 2)). Therefore, it is suggested that the organic removal is caused by not only aerobic biodegradation. The thickness of the first sponge layer is 200 mm, which is only 10% of the total sponge thickness, and the HRT is only 0.2 h. Nevertheless, a large proportion of the COD<sub>Cr</sub> is eliminated in the first layer (Figures 2 and 3, and Table 2). This suggests that a large amount of organic matter is trapped on the sponge medium in the first layer of the DHS reactor; subsequently, the adsorbed organic matter is degraded by microorganisms retained on the sponge medium. Between 486 days (phase 1) to 553 days (phase 2), 6.0 tons of VSS is removed by the DHS reactor (average influent quantity: 2,210 m<sup>3</sup> day<sup>-1</sup>, average VSS removal: 41 mg L<sup>-1</sup>). This value is almost the same as the amounts of retained sludge in the DHS reactor, 6.2 tons (486 days (phase 1)) and 6.5 tons (553 days (phase 2)). In phases 1 and 2, the sludge concentrations in the first layer are similar, namely 27–45 and 35–49 g-VSS L<sup>-1</sup>-sponge, respectively. The same trend is observed for the

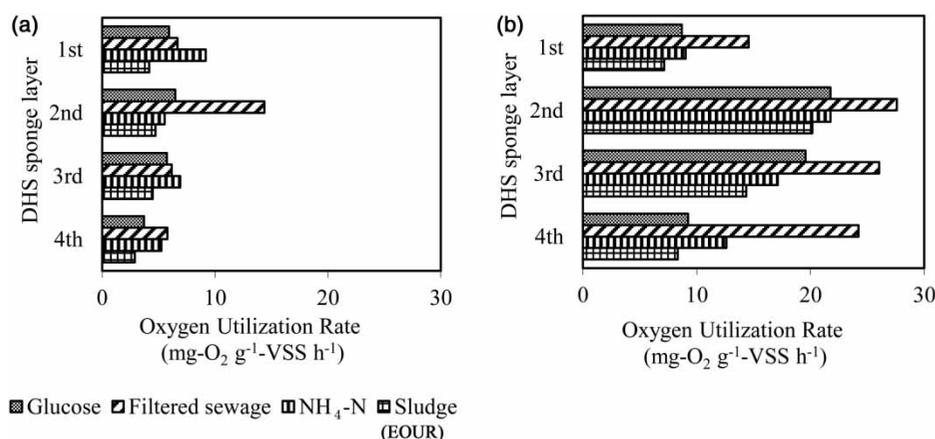


Figure 4 | OURs at phase 1 (486–491 days) (a) and phase 2 (555–563 days) (b).

second to fourth layers of the DHS reactor; therefore, the sludge concentrations do not change much during this period. These results indicate that a significant degree of  $\text{COD}_{\text{Cr}}$  decrease is derived from not only the capture of organic matter on the sponge medium, but also aerobic and anaerobic biodegradation.

The  $\text{COD}_{\text{Cr}}$  decrease rates, based on the dry weight of the sludge in the first layer, are  $0.76 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  (phase 1) and  $0.54 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  (phase 2). These values are higher than those reported in previous studies, namely,  $0.20\text{--}0.48 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  using the activated sludge method (von Sperling *et al.* 2001),  $0.14 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  for an anaerobic/oxic (A/O) plug flow reactor,  $0.17 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  using the oxidation ditch method, and  $0.20 \text{ g-COD}_{\text{Cr}} \text{ g}^{-1}\text{-VSS day}^{-1}$  for a sequential batch reactor (Li *et al.* 2014).

The OUR, using filtered sewage as the substrate, is  $5.78\text{--}14.4 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in phase 1 and  $14.6\text{--}27.6 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in phase 2 (Figure 4). These levels are similar to those reported in a previous study ( $6.87\text{--}27.4 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$ ) using a practical scale DHS reactor in India, conducted using a low- $\text{COD}_{\text{Cr}}$  influent (Okubo *et al.* 2016). Here, the OUR excluding the EOUR ranges from  $7.42\text{--}15.9 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in phase 2, similar to the range reported from a previous study ( $9\text{--}12 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$ ; Okubo *et al.* 2016). Thus, the microbial activity in terms of OUR is in the same range under low ( $168 \text{ mg L}^{-1}$ ; Okubo *et al.* 2016) and high ( $385 \text{ mg L}^{-1}$ ; phase 2) influent  $\text{COD}_{\text{Cr}}$  conditions. In contrast, the results for OUR in this study are at the same level or lower than those obtained with activated sludge in the previous study. The OUR values in the previous study are ca.  $5 \text{ mg-O}_2 \text{ g}^{-1}\text{-MLSS h}^{-1}$  (Chiemchaisri *et al.* 1994), and the OUR excluding the EOUR is  $4.5 \text{ mg-O}_2 \text{ g}^{-1}\text{-MLVSS h}^{-1}$  (ML denotes mixed liquor) in the glucose substrate (Witzig *et al.* 2002) and  $12\text{--}31 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in the acetate substrate (Kristensen *et al.* 1992). The oxygen consumption rates associated with  $\text{COD}_{\text{Cr}}$  reduction, calculated from the profile analysis of the reactor in phase 1, are  $17.9 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in the first layer and  $0\text{--}0.66 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in the second to fourth layers. The calculated value is higher than the OUR test data for the first layer, which suggests that the  $\text{COD}_{\text{Cr}}$  reduction in the first layer is due not only to aerobic biodegradation, but also anaerobic biodegradation. In the second to fourth layers, the results of the OUR tests are higher than the calculated oxygen consumption rate from the profile analysis of the reactor, and thus, it is assumed that the sewage used as the substrate in the OUR test included various types of easily decomposable organic matter. However, in the case of the actual reactor, it

is assumed that such easily decomposable organic matter is removed in the upper layer. As a result, it is thought that the difference is appeared between the results of the OUR tests and the calculated oxygen consumption rate from the profile analysis of the reactor.

In the second to fourth layers, the OUR excluding the EOUR for the filtered sewage substrate is higher in phase 2 than in phase 1. It is thought that the difference of the results between phase 2 and phase 1 is caused by DO condition of the influent. The influent DO values for the second to fourth layer at the middle port range from  $3.93\text{--}6.72 \text{ mg L}^{-1}$  in phase 2. In contrast, the influent DO is only  $0\text{--}1.00 \text{ mg L}^{-1}$  in phase 1. Samiotis *et al.* (2015) reported that the  $\text{COD}_{\text{Cr}}$  reduction ratio decreases when DO is less than  $0.8 \text{ mg L}^{-1}$ , and thus, it is assumed that it is assumed that the difference in OUR results is due to the effect of DO conditions.

### Oxygen consumption by oxidation of sulfur

The oxygen consumption resulting from sulfur oxidation is also very high in the first layer (Figure 3). Biological and chemical sulfide oxidation occurs in wastewater, and previous studies have reported that biological sulfide oxidation accounts for 3–56% of the total oxidation rate (Wilmot *et al.* 1988; Kotronarou & Hoffmann 1991; Nielsen *et al.* 2003; Preisler *et al.* 2007). Preisler *et al.* (2007) reported that the ratio of chemical to biological oxidation is 97:3 when sulfide is oxidized. Therefore, it may be concluded that sulfide oxidation occurs not only by biological reactions, but also by chemical reactions. The sulfate concentrations in the effluent are almost the same in the first to fourth layers (Table 2); thus, almost all the sulfur in the influent is oxidized in the first layer.

### Oxygen consumption by oxidation of ammonium

The  $\text{NH}_3\text{-N}$  decrease rates based on the dry weight of sludge remain the same throughout the first to the fourth layers in phase 2. Previous studies using a DHS reactor report  $\text{COD}_{\text{Cr}}$  loads of  $2.03\text{--}2.66 \text{ kg-COD}_{\text{Cr}} \text{ m}^{-3}\text{-sponge day}^{-1}$  accompanied by high removal ratios of the ammonium nitrogen (61–83%) (Machdar *et al.* 1997; Tandukar *et al.* 2007; Onodera *et al.* 2014a). On the other hand, the  $\text{COD}_{\text{Cr}}$  load in this study is  $3.13 \text{ kg-COD}_{\text{Cr}} \text{ m}^{-3}\text{-sponge day}^{-1}$  (phase 2), even in the fourth layer. The  $\text{NH}_3\text{-N}$  removal rate remains the same from the first to the fourth layer under a high organic matter load. The OUR excluding the EOUR for the ammonium substrate in the third and fourth layers is 2.45 and  $2.35 \text{ mg-O}_2 \text{ g}^{-1}\text{-VSS h}^{-1}$  in phase 1, and 2.78 and

4.26 mg-O<sub>2</sub> g<sup>-1</sup>-VSS h<sup>-1</sup> in phase 2, respectively. In the third layer, the results are almost the same for phases 1 and 2. The results of NH<sub>3</sub>-N decrease rates, based on the dry weight of the sludge, are also similar. Zhu & Chen (2002) reported that the ammonium oxidation rate does not depend on the temperature when it ranges from 14 and 27 °C. The temperatures employed in this study were 26 °C for phase 1 (486 days) and 17 °C for phase 2 (565 days), which are in the same range as those of the previous study. In the fourth layer, the OUR excluding the EOOR for the ammonium substrate in phase 2 is ca. twice that of phase 1. Furthermore, for the ammonium substrate in phase 2, the OUR excluding the EOOR in the fourth layer is higher than that in the third layer. It is thought that these differences are due to the effect of influent DO and the oxidation-reduction potential (ORP) of the sponge medium. Garrido *et al.* (1996) reported that ammonium oxidation is inhibited when DO is lower than 1 mg L<sup>-1</sup>. The DO values of the influent in the fourth layer at the middle port are 0.20 mg L<sup>-1</sup> in phase 1, and 6.72 mg L<sup>-1</sup> in phase 2. It is assumed that the differences in the OUR results for phase 1 and 2 are caused by the effect of DO, because influent DO is low in phase 1, and the retained sludge is affected by low DO conditions as the sponge medium was collected from the surface of the sponge layer. Furthermore, when the sponge medium samples were collected on day 553 (phase 2), the ORP values for retained liquid on the sponge medium samples of third and the fourth layers are -325 and -155 mV, respectively. The sponge medium in the third layer exists under virtually anaerobic conditions in phase 2; in contrast, the ORP in the fourth layer is higher than that in the third layer. Thus, it is believed that the OUR in the fourth layer is higher than that in the third layer because ammonium oxidation proceeds more easily in the fourth layer than in the third layer. Taken together, the above results and previous reports, DO and ORP have most influence for oxidation of ammonium in the DHS.

## CONCLUSION

The practical-scale DHS reactor receiving high-organic and sulfur concentration of influent removed about 60% of organic matter in the first layer, and thus, the organic removal was attributed to aerobic and anaerobic biodegradation on the sponge medium. The sulfur was almost oxidized to sulfate at the first layer with chemical and biological reaction. For the second to fourth layer, the organic matter removal was gradual, but it was shown that

the retained sludge at the second to fourth layer had potential of high rate of organic matter oxidation since the results of OUR test. The microbial activity in terms of OUR was found to be similar under low (168 mg L<sup>-1</sup>; previous study) and high (385 mg L<sup>-1</sup>; phase 2) influent COD<sub>Cr</sub> conditions. The NH<sub>3</sub>-N decrease rates based on the dry weight of sludge remain the same throughout the first to the fourth layers under high organic matter concentration.

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