# Development of downflow hanging sponge (DHS) reactor as post treatment of existing combined anaerobic tank treating natural rubber processing wastewater

Takahiro Watari, Trung Cuong Mai, Daisuke Tanikawa, Yuga Hirakata, Masashi Hatamoto, Kazuaki Syutsubo, Masao Fukuda, Ngoc Bich Nguyen and Takashi Yamaguchi

## ABSTRACT

Conventional aerated tank technology is widely applied for post treatment of natural rubber processing wastewater in Southeast Asia; however, a long hydraulic retention time (HRT) is required and the effluent standards are exceeded. In this study, a downflow hanging sponge (DHS) reactor was installed as post treatment of anaerobic tank effluent in a natural rubber factory in South Vietnam and the process performance was evaluated. The DHS reactor demonstrated removal efficiencies of 64.2 ± 7.5% and 55.3 ± 19.2% for total chemical oxygen demand (COD) and total nitrogen, respectively, with an organic loading rate of 0.97 ± 0.03 kg-COD $m^{-3}$ $day^{-1}$ and a nitrogen loading rate of 0.57 ± 0.21 kg-N $m^{-3}$ $day^{-1}$. 16S rRNA gene sequencing analysis of the sludge retained in the DHS also corresponded to the result of reactor performance, and both nitrifying and denitrifying bacteria were detected in the sponge carrier. In addition, anammox bacteria was found in the retained sludge. The DHS reactor reduced the HRT of 30 days to 4.8 h compared with the existing algal tank. This result indicates that the DHS reactor could be an appropriate post treatment for the existing anaerobic tank for natural rubber processing wastewater treatment.

**Key words** | DHS, natural rubber processing wastewater, post treatment

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## INTRODUCTION

The natural rubber production process discharges large amounts of wastewater containing ammonia, organic compounds and so on. Therefore, discharging natural rubber processing wastewater without an appropriate treatment can lead to environmental problems such as deterioration of water quality and eutrophication. The anaerobic tank had been widely applied for treatment of natural rubber processing wastewater in Southeast Asia because it has low operational and construction costs (Mohammadi *et al.* 2010; Nguyen & Luong 2012). The anaerobic tank process efficiently removes high concentrations of organic contaminants and is easy to operate and maintain (Mohammadi *et al.* 2010). However, the effluent from the anaerobic tank still contains organic matter and ammonia. Thus, anaerobic treatment is usually applied together with aerobic post treatment to achieve effluent standards. For post treatment of anaerobic tank effluent from treating natural rubber processing wastewater, several kinds of aerobic treatment systems have been applied (Mohammadi *et al.* 2010; Nguyen & Luong 2012). One of the most promising post-treatment systems is the conventional aerated tank, because an aerated tank has the ability to provide high effluent quality with superior organic and nitrogen removal efficiency. However, the process requires high electricity input for oxygen supplementation, and produces large amounts of excess sludge. The algal tank has also been applied to treat effluent from anaerobic tank treatment of natural rubber processing wastewater (Bich *et al.* 1999); this system efficiently removes organics and nitrogen, but it requires a long hydraulic retention time (HRT) and large treatment area, the same as a conventional aerated tank.

The downflow hanging sponge (DHS) reactor is a trickling filter system equipped with sponge as media, developed as a low cost aerobic treatment system (Tawfik *et al.* 2006; Tandukar *et al.* 2007). To date, six different types of sponge carriers have been proposed and their process performance was demonstrated in DHS reactors treating sewage (Tandukar *et al.* 2007; Onodera *et al.* 2014, 2016; Okubo *et al.* 2016). The highlight of the DHS reactor is that it can be operated without aeration or with low aeration requirements, as oxygen is naturally dissolved in wastewater. In addition, the sponge media supports a large amount of biomass as well as high microbial diversity in the surface and inner section of the sponge media. The high microbial diversity in this ecosystem, with an extremely long food chain, reduces the production of excess sludge (Araki *et al.* 1999; Uemura *et al.* 2010; Onodera *et al.* 2014; Kubota *et al.* 2014). Tandukar *et al.* (2007) reported that the volume of excess sludge production from the combination of an upflow anaerobic sludge blanket (UASB) – DHS system was 15 times smaller than a conventional activated sludge process. The DHS reactor has been applied for treatment of several kinds of industrial wastewaters, especially post treatment of high strength industrial wastewater treated in a UASB reactor (El-Kamah *et al.* 2011; Tanikawa *et al.* 2016; Watari *et al.* 2016). Our previous study reported effective organic removal through post treatment of UASB-treated natural rubber processing wastewater in North Vietnam (Watari *et al.* 2016). The study found that the post-treatment DHS reactor could accommodate approximately 0.7 kg-COD $m^{-3}$ $day^{-1}$ of organic loading rate (OLR) to achieve the Vietnamese effluent standard (Watari *et al.* 2016). Tanikawa *et al.* (2016) also reported that the post-treatment DHS reactor, for natural rubber processing wastewater treatment in Thailand, effectively oxidized the remaining organic matter and sulfide.

In this study, we installed a mini scale DHS reactor in a natural rubber processing factory in South Vietnam and investigated the process performance of the reactor. The microbial community structure of the DHS retained sludge was analyzed based on 16S rRNA gene sequencing.

## MATERIALS AND METHODS

### Experimental setup and operational conditions

A mini scale DHS reactor was installed at the natural rubber factory of the Rubber Research Institute of Vietnam, Binh Duong Province, Vietnam. A schematic diagram of the existing treatment system is shown in Figure 1. The factory produced 1,000 t $year^{-1}$ of ribbed smoked sheet (RSS) and discharged 10 $m^3$ wastewater per t-RSS produced. The RSS wastewater was treated by an anaerobic baffled tank (ABT), an algal tank and a polishing tank. The concrete ABT comprised 60 compartments separated by a baffled wall. The volume and depth of the ABT were 380 $m^3$ and 1.4 m, respectively. The volume and depth of the algal tank were 880 $m^3$ and 1 m, respectively. The HRTs of the ABT and the algal tank were 12 and 30 days, respectively. The dominant species in the algal tank is *Chlorella*. Figure 2 shows a schematic diagram of the mini scale post-treatment DHS reactor. Approximately 30 L of ABT effluent was collected every day and stored in the substrate tank for DHS influent. The

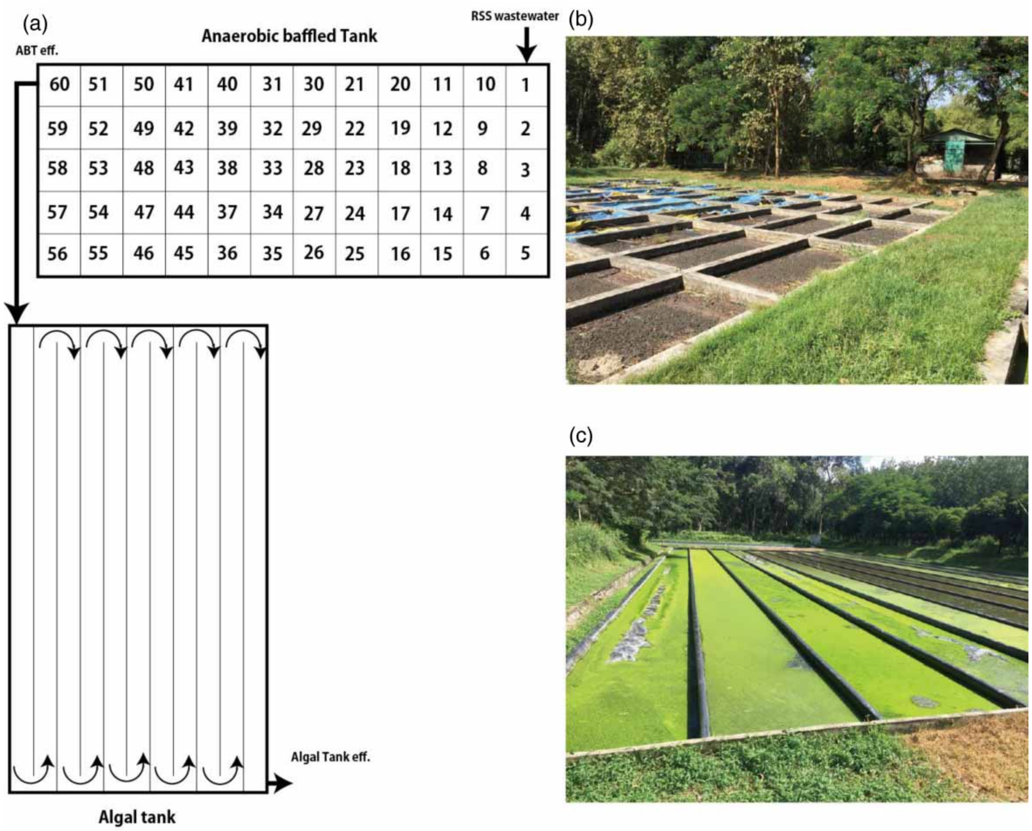


**Figure 1** | (a) Schematic diagram of treatment system in the natural rubber factory, (b) photograph of ABT, (c) photograph of algal tank.

stored ABR effluent was fed to the top of the DHS reactor by a pump (Master-flex model 7524–50). The DHS reactor was constructed from polyvinyl chloride pipe with a height of 150 cm. The DHS reactor was filled with 33 mm × 33 mm × 33 mm size sponge pieces, made from polyurethane sponges obtained from another DHS reactor previously operated for treating natural rubber processing wastewater. The reactor volume and sponge volume of the DHS reactor were 5.8 L and 3.9 L, respectively. The DHS reactor was supplied with air using an air pump for artificial ventilation. The DHS reactor was operated under ambient temperature ranging from 26.1 °C to 32.0 °C. The initial operational conditions of the DHS reactor are shown in Table 1.

## Analytical methods

Samples of ABT influent, ABT effluent, DHS effluent and algal tank effluent were collected for routine analysis. Temperature, pH and dissolved oxygen (DO) were measured on site (DOP-5F, Kasahara). The process performance of the ABT, DHS reactor and algal tank were evaluated by analysis of the total COD, soluble COD, total biochemical oxygen demand (BOD), soluble BOD, total suspended solids (TSS), total nitrogen (TN), ammonia, nitrate and nitrite. Total COD, soluble COD and TN were analyzed using a HACH DR-2800 water quality analyzer. The soluble COD and soluble BOD were determined after filtering through a 0.45 μm glass-fiber filter (GB-140, ADVANTEC). Ammonia, nitrite and nitrate concentrations were measured using an ion-exchange chromatograph (LC-10A, Shimadzu). Total BOD, soluble BOD, TSS, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analyzed as described in APHA (1998). Nitrification rate was calculated based on the reduction of ammonia.

## 16S rRNA gene sequencing

Sludge samples were collected from the upper, middle and bottom parts of the DHS reactor (20, 50 and 110 cm from the top) on day 35 and day 110. The retained sludge was extracted from the sponge media, gently washed with phosphate buffered saline (PBS) and stored at −20 °C until DNA was extracted. DNA extraction was performed using a FastDNA Spin Kit for Soil (MP Biomedicals). Polymerase

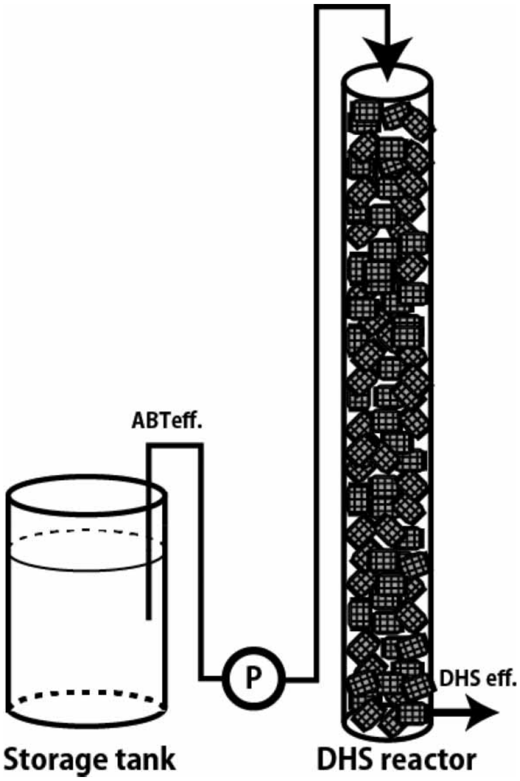


**Figure 2** | Schematic diagram of the mini scale post-treatment DHS reactor.

**Table 1** | Initial operational conditions of the DHS reactor from Phase 1 to Phase 3

| | Day | Flow rate L $day^{-1}$ | HRT h | OLR kg-COD $m^{-1}$ $day^{-1}$ | NLR kg-N $m^{-1}$ $day^{-1}$ |
|---|---|---|---|---|---|
| Phase 1 | 1–64 | 24 | 4.8 | 0.97 ± 0.03 | 0.57 ± 0.21 |
| Phase 2 | 65–109 | 32 | 3.0 | 2.4 ± 0.77 | 1.3 ± 0.44 |
| Phase 3 | 110–148 | 32 | 3.0 | 3.2 ± 0.21 | 1.5 ± 0.35 |

chain reaction (PCR) amplification of 16S rRNA genes was performed with the universal forward primer Univ515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and the universal reverse primer Univ806R (5′ -GGA CTA CHV GGG TWT CTA AT-3′) for whole bacteria and archaea (Caporaso *et al.* 2012). Purification of PCR products was conducted using a QIAquick PCR purification kit (OIAGEN). Massive parallel 16S rRNA gene sequencing was carried out using a Miseq reagent kit v.2 with the Miseq system (Illumina). Sequence data analysis was conducted using the QIIME software package v.1.7.0 (Caporaso *et al.* 2010). Operational taxonomic units (OTUs) were classified at 97% sequence identity. Taxonomic classification was determined using the Greengenes database v.13_5. The related strains of the representative sequences were identified using a web-based BLAST search in the NCBI database.

## RESULTS AND DISCUSSION

### Process performance of the DHS reactor and algal tank

The RSS wastewater was first treated by the ABT and was then continuously fed to the DHS reactor and the algal tank. Table 2 shows the process performance of the ABT during the entire experimental period. The ABT showed 92.0 ± 2.8% total COD removal efficiency and 92.7 ± 2.4% soluble COD removal efficiency with average OLR in the ABT of 0.30 ± 0.06 kg-COD $m^{-3}$ $day^{-1}$; these values are similar to those reported in previous studies (Mohammadi *et al.* 2010; Nguyen & Luong 2012). However, the effluent quality of the ABT did not achieve the Vietnamese effluent standards. In addition, ammonia was increased in the ABT effluent, thus it required post treatment for discharge to the aquatic environment.

The time course of TSS, total COD and soluble COD during the entire experimental period is shown in Figure 3. During Phase 1, the DHS reactor was operated at an HRT of 4.8 h, corresponding to an average OLR of 0.97 ± 0.03 kg-COD $m^{-3}$ $day^{-1}$. The DHS reactor achieved soluble COD and soluble BOD removal efficiencies of over 60% within 2 weeks of the reactor startup. The quick startup of the DHS reactor could be because the sponge carrier was collected from another DHS reactor that had previously been operated for 1 year. During Phase 1, the average total COD and soluble COD removal efficiencies of the DHS reactor were 64.2 ± 7.5% and 79.4 ± 1.5%, respectively. Similarly, the total BOD and soluble BOD removal efficiency of the DHS reactor were 67.0 ± 6.0% and 69.4 ± 27.3%, respectively. This result indicated that organics were removed with

**Table 2** | Process performance of the ABT during the entire experimental period

| Parameter | Unit | RSS wastewater | ABT eff. |
|---|---|---|---|
| pH | | 5.5 ± 0.2 | 6.9 ± 0.6 |
| Total COD | mg-COD $L^{-1}$ | 3,700 ± 640 | 280 ± 100 |
| Soluble COD | mg-COD $L^{-1}$ | 3,370 ± 690 | 250 ± 95 |
| Total BOD | mg $L^{-1}$ | 3,450 ± 690 | 202 ± 98 |
| Soluble BOD | mg $L^{-1}$ | 2,800 ± 640 | 150 ± 71 |
| TSS | mg $L^{-1}$ | 200 ± 58 | 72 ± 33 |
| TN | mg-N $L^{-1}$ | 220 ± 83 | 156 ± 54 |
| Ammonia | mg-N $L^{-1}$ | 108 ± 15 | 142 ± 55 |

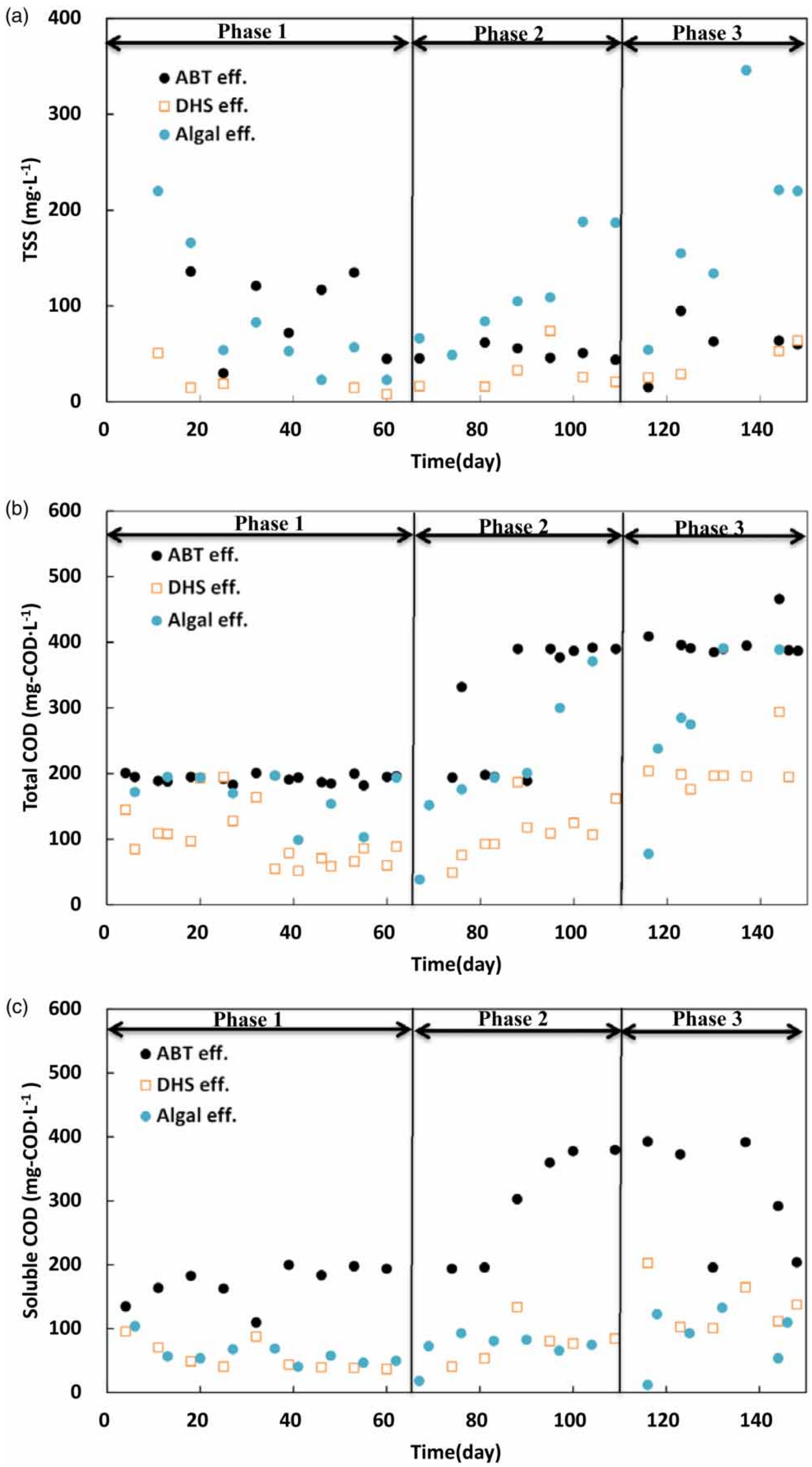


**Figure 3** | Time course of (a) TSS, (b) total COD and (c) soluble COD concentration of anaerobic baffled tank effluent (ABT eff.), DHS effluent (DHS eff.) and algal tank effluent (Algal eff.).

high efficiency in the DHS reactor. The DHS reactor also showed 75.1 ± 21.9% TSS removal efficiency. The concentrations of TN, nitrate and nitrite from Phase 1 to Phase 3 are shown in Table 3. Approximately 60% of ammonia was converted to nitrate and nitrite during Phase 1. There was only 1.8 ± 1.0 mg $L^{-1}$ of DO in the DHS effluent, which is relatively low compared with DO in the study by Araki et al. (1999), who showed a good nitrification rate. Therefore, the nitrite concentration of 19 ± 18 mg-N $L^{-1}$ remained in the DHS effluent. The nitrification rate in the present study (based on ammonia oxidation of 0.34 ± 0.13 kg-N $m^{-3}$ $day^{-1}$) was greater than for the same sponge type DHS reactor treating sewage effluent (Tawfik et al. 2006). Therefore, the DHS reactor is efficient for nitrification of this wastewater. The DHS reactor showed 55.3 ± 19.2% TN removal efficiency during Phase 1. This level of TN removal in the DHS reactor indicated that denitrification was continuously occurring in the DHS reactor, most likely deep inside the sponge carrier. The DHS effluent, containing 102 ± 46 mg-COD $L^{-1}$ total COD, 35 ± 13 mg $L^{-1}$ total BOD, 19 ± 16 mg $L^{-1}$ TSS, 57 ± 26 mg-N $L^{-1}$ TN and 20 ± 20 mg-N $L^{-1}$ ammonia, achieved the national technical regulation on effluents from natural rubber processing industry B (QCVN01:2008/BTNMT; Table 4). This result shows that the DHS reactor could be applied for the post treatment of an existing ABT treating natural rubber processing wastewater.

In Phase 2, the HRT of the DHS reactor was reduced to 3.0 h to increase the OLR. The water quality of the ABT effluent deteriorated because of high RSS production in the factory. The OLR of the DHS reactor increased to 2.4 ± 0.77 kg-COD $m^{-3}$ $day^{-1}$. Similarly to Phase 1, the DHS reactor showed organic removal efficiencies of 60.6 ± 13.2% of total COD and 78.1 ± 14.0% of total BOD. During Phase 2, the DHS reactor effluent contained 110 ± 40 mg-COD $L^{-1}$ total COD and 44 ± 14 mg $L^{-1}$ total BOD, respectively. Thus, the DHS reactor in this study had the potential to treat wastewater with an OLR of 2.5 kg-COD $m^{-3}$ $day^{-1}$. The ammonia concentrations of the DHS influent and effluent were 176 ± 16 mg-N $L^{-1}$ and 40 ± 25 mg-N $L^{-1}$, respectively. The DHS reactor showed a high nitrification rate of 0.68 ± 0.12 kg-N $m^{-3}$ $day^{-1}$. During Phase 2, the TN removal efficiency of the DHS reactor was 52.7 ± 25.2%. During this phase, the TN and ammonia of the DHS effluent exceeded the effluent standards because the influent concentrations of TN and ammonia were increased and there was short HRT

**Table 3** | Summary of nitrogen concentrations from Phase 1 to Phase 3

| | | TN mg-N $L^{-1}$ | Ammonia mg-N $L^{-1}$ | Nitrate mg-N $L^{-1}$ | Nitrite mg-N $L^{-1}$ |
|---|---|---|---|---|---|
| Phase 1 | ABT eff. | 115 ± 43 | 91 ± 34 | 0.3 ± 0.5 | N.D |
| | DHS eff. | 57 ± 26 | 20 ± 20 | 34 ± 40 | 19 ± 18 |
| | Algal eff. | 73 ± 25 | 46 ± 19 | 0.8 ± 0.9 | 0.0 ± 0.1 |
| Phase 2 | ABT eff. | 178 ± 58 | 176 ± 16 | 0.5 ± 0.9 | N.D |
| | DHS eff. | 80 ± 36 | 40 ± 25 | 32 ± 32 | 17 ± 18 |
| | Algal eff. | 97 ± 27 | 100 ± 12 | 1.2 ± 1.8 | N.D |
| Phase 3 | ABT eff. | 183 ± 44 | 182 ± 53 | 1.9 ± 1.24 | N.D |
| | DHS eff. | 122 ± 43 | 91 ± 25 | 14 ± 20 | N.D |
| | Algal eff. | 131 ± 120 | 107 ± 14 | 1.6 ± 0.6 | N.D |

ND, Not detected.

**Table 4** | Comparison of different treatment systems for natural rubber processing wastewater treatment

| | | ABT -DHS | | ABT -Algal Tank | | Decantation-UASB-aeration tank – settling and filter | | Decantation-flotation-UASB-aeration tank – settling and filter | | BR-UASB-DHS | | Oxidation ditch | | Vietnamese discharge effluent standard B (QCVN01: 2008/BTNMT) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Unit** | **Inf.** | **Eff.** | **Inf.** | **Eff.** | **Inf.** | **Eff.** | **Inf.** | **Eff.** | **Inf.** | **Eff.** | **Inf.** | **Eff.** | |
| pH | | 5.5 | 8.1 | 5.5 | 8.1 | 9.2 | 6.83 | 8.09 | 7.88 | 5.3 | 7.6 | 6.2 | 78 | 6–9 |
| Total COD | mg-COD $L^{-1}$ | 3,700 | 102 | 3,700 | 222 | 18,885 | 123 | 13,981 | 127 | 8,430 | 120 | 4,120 | 71 | 150 |
| Total BOD | mg $L^{-1}$ | 3,450 | 35 | 3,450 | 92 | 10,780 | 57 | 7,590 | 61 | – | – | 2,678 | 28 | 50 |
| TSS | mg $L^{-1}$ | 200 | 27 | 200 | 126 | 900 | 70 | 468 | 39 | 1,470 | 36 | 4,637 | 1,246 | 100 |
| TN | mg-N $L^{-1}$ | 220 | 57 | 220 | 97 | 611 | 35.3 | 972 | 129 | 420 | 220 | 531 | 26 | 60 |
| Ammonia | mg-N $L^{-1}$ | 108 | 20 | 108 | 77 | 342 | 30.8 | 686 | 30.3 | 200 | 100 | 354 | 12 | 40 |
| | | This study | | This study | | Nguyen & Luong (2012) | | Nguyen & Luong (2012) | | Watari et al. (2016) | | Ibrahim (1980) | | |

–, Not analyzed.

operation. Thus, the DHS reactor required further modification, such as an increase in sponge volume, to improve nitrogen removal efficiency.

During Phase 3, the COD, BOD and TN of the ABT effluent were 400 ± 30 mg-COD $L^{-1}$, 330 ± 20 mg $L^{-1}$ and 180 ± 40 mg-N $L^{-1}$, respectively. Consequently, the OLR of the DHS reactor was increased to 3.2 ± 0.21 kg-COD $m^{-3}$ $day^{-1}$. The total COD and TN removal efficiencies of the DHS reactor were decreased to 48.8 ± 5.2% and 38.4 ± 11.4%. These results show that the optimal operational condition of the post-treatment DHS reactor might be an OLR of 1.0 kg-COD $m^{-3}$ $day^{-1}$ to achieve the Vietnamese effluent standard.

The algal tank is one of the most promising post-treatment systems for treating natural rubber processing wastewater (Bich *et al.* 1999). The process performance of the algal tank applied as post treatment of ABT was evaluated in this study. The algal tank showed 18.1 ± 21.4% total COD removal efficiency and 49.4 ± 28.1% total BOD removal efficiency during the entire experimental period. The TSS of the algal tank effluent was often increased because algae with low settleability were contained in the effluent. The algal tank has advantages for nutrient removal as algae desorb ammonia and phosphorus (Bich *et al.* 1999). The algal tank showed 43.3 ± 15.2% TN removal and 51.5 ± 11.8% ammonia removal efficiencies during the entire experimental period.

## Retained sludge in the DHS reactor

The MLSS and MLVSS of the DHS sponge media retained sludge on day 35 and day 110 are shown in Figure 4. The DHS sponge retained sludge concentrations of the top, middle and bottom part on day 35 and day 110 were evaluated. The sludge concentration and MLVSS/MLSS ratio were significantly increased on day 110. The highest sludge concentration was found in the middle part of the DHS reactor, because of the large amount of excess sludge observed on the surface of the sponge carrier. The DHS reactor had an average sludge concentration of 25.2 ± 18.5 g-VSS L-$sponge^{-1}$ on day 110, which is approximately the same as that reported for a DHS reactor treating sewage as post-treatment for a UASB reactor, reactive dye wastewater and onion dehydration wastewater (Tawfik *et al.* 2006; El-Kamah *et al.* 2011; Tawfik *et al.* 2013). Therefore, this high sludge concentration might be an indicator of the capability of the DHS reactor to treat natural rubber processing wastewater.

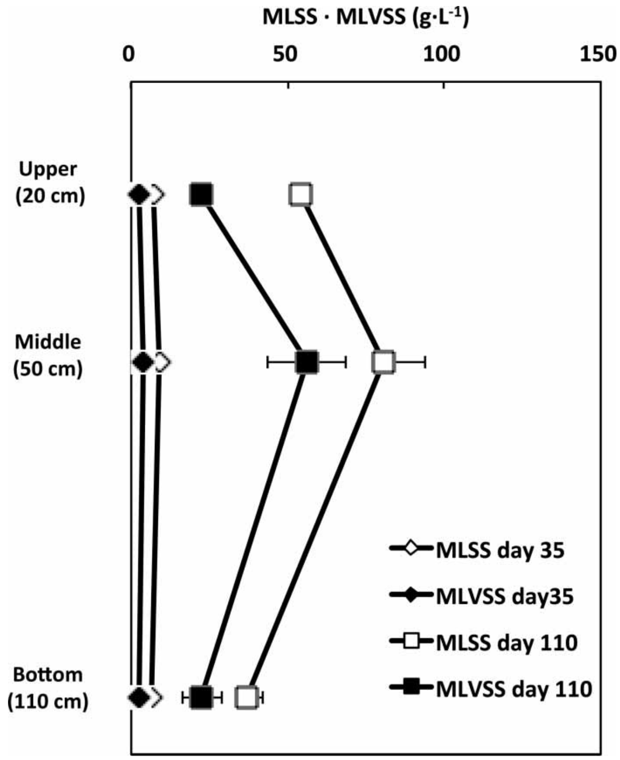


**Figure 4** | MLSS and MLVSS concentrations of DHS retained sludge on day 35 and day 110.

## Performance comparison of the ABT-DHS system and the current treatment system

The combination of the ABT (HRT = 12 days) and the DHS (HRT = 4.8 h) showed 96.4% total COD, 98.5% total BOD and 90.0% TSS removal efficiency in Phase 1. The effluent from this proposed system achieved the Vietnamese effluent standards. The conventional treatment system, consisting of the ABT and the algal tank (HRT = 42 days), showed 93.8% total COD, 97.0% total BOD and 31.4% TSS removal efficiencies, indicating that the effluent of the ABT-algal tank system exceeded the effluent standards (Table 4). Thus, the ABT-DHS system was considerably more efficient than the existing ABT-algal tank system. The HRT of the post-treatment DHS reactor was 0.6% of the algal tank.

The performance of other treatment systems for natural rubber processing wastewater is summarized in Table 4. Most factories applied an aeration tank and a settling tank for post treatment of anaerobic treatment systems such as UASB (Nguyen & Luong 2012). These existing treatment systems showed more than 99% total COD and total BOD removal efficiencies, however TN and ammonia removal was not sufficient to achieve the effluent standard. The oxidation ditch process has also been applied for treatment of natural rubber processing wastewater containing nitrogen pollutants. Ibrahim (1980) reported that a laboratory scale oxidation ditch process showed >90% nitrogen removal efficiency with natural rubber processing wastewater. However, the effluent of the full scale oxidation ditch process still contained high TN and ammonia concentrations. To remove TN and ammonia, external aeration has to be supplied to maintain DO levels of around 3.0 mg $L^{-1}$ (Nguyen & Luong 2012). The DHS can operate without or with low levels of external aeration because of its high oxygen transfer capacity (Tawfik et al. 2006; Tandukar et al. 2007; Onodera et al. 2014). Moreover, Tanikawa et al. (2016) reported the post-treatment DHS reactor can reduce 97% of power consumption and 98% of excess sludge production. Therefore, the DHS reactor can achieve advanced post treatment of natural rubber processing wastewater. Moreover, previous research demonstrated that the full-scale DHS reactor performed high organic mater and ammonia removal in sewage treatment (Okubo et al. 2016; Onodera et al. 2016). Thus, this mini scale experiment would be able to assure the success of a post-treatment DHS reactor at the full-scale system.

## Microbial community analysis

The microbial community structure of the DHS retained sludge was investigated by using 16S rRNA gene-based massively parallel sequencing analysis. Approximately 17,000–24,000 sequencing reads per sample were analyzed and 1,100–1,700 OTUs per sample were found at 97% identity (Table 5). Phylogenetic analysis showed that the principal microbial groups in the DHS retained sludge were the phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Chloroflexi* (Table 5). These microbial groups were also found in DHS reactors treating sewage and artificial cake-plant wastewater (Uemura et al. 2010; Kubota et al. 2014; Mac Conell et al. 2015). The phylum *Proteobacteria* was dominant in the DHS reactor, which is important in relation to the nitrification process observed (Kubota et al. 2014; Mac Conell et al. 2015).

The total sequence reads of nitrifying bacteria in the DHS reactor on day 35 and day 110 were shown in Figure 5. The massively parallel 16S rRNA gene sequencing of DHS retained sludge showed that the abundance of nitrifying bacteria was low. This low abundance of nitrifying bacteria has been reported in other microbial community analysis of DHS reactors (Kubota et al. 2014; Mac Conell et al. 2015). Ammonia-oxidizing bacteria such as *Nitrosomonas* spp., which are frequently found in sewage treatment plants (Limpiyakorn et al. 2006; Siripong & Rittmann 2007), were identified in 0.1% of total sequencing reads in the top and bottom of the reactor on day 35. Nitrite-oxidizing bacteria *Nitrospira* spp. was detected in the middle and bottom of the reactor on day 35. After operation under high OLR and nitrogen loading rate (NLR), the total sequence reads of *Nitrosomonas* spp. increased to 0.3–0.8%, and *Nitrosomonas* was predominant in the upper section on day 110. This shows that *Nitrosomonas* adapted to the high OLR and NLR conditions. A previous study reported that ammonia-oxidizing bacteria were

**Table 5** | Diversity indices and microbial community structure of the DHS reactor at phylum level

| | Day 35 | | | Day 110 | | |
|---|---|---|---|---|---|---|
| | Bottom | Middle | Upper | Bottom | Middle | Upper |
| No. of total sequence reads | 23,633 | 23,649 | 17,293 | 20,799 | 17,950 | 21,909 |
| No. of OTU | 1,542 | 1,679 | 1,453 | 1,613 | 1,180 | 1,355 |
| Phylogenetic affiliation | % of total sequence reads | | | | | |
| *Proteobacteria* | 17.9 | 23.3 | 25.4 | 54.3 | 56.3 | 50.9 |
| *Firmicutes* | 26.8 | 32.0 | 34.7 | 9.8 | 5.4 | 9.8 |
| *Bacteroidetes* | 11.8 | 3.8 | 4.3 | 17.9 | 12.8 | 14.4 |
| *Actinobacteria* | 11.5 | 20.1 | 20.2 | 4.1 | 3.7 | 4.0 |
| *Chloroflexi* | 14.6 | 11.7 | 8.7 | 4.5 | 13.2 | 9.0 |
| *Chlorobi* | 1.1 | 0.5 | 0.4 | 3.6 | 3.1 | 4.6 |
| *Planctomycetes* | 3.6 | 1.9 | 1.4 | 0.5 | 0.4 | 0.4 |
| *Acidobacteria* | 3.2 | 1.1 | 0.8 | 0.7 | 0.7 | 0.8 |
| Others | 9.6 | 5.6 | 4.2 | 4.6 | 4.4 | 6.3 |

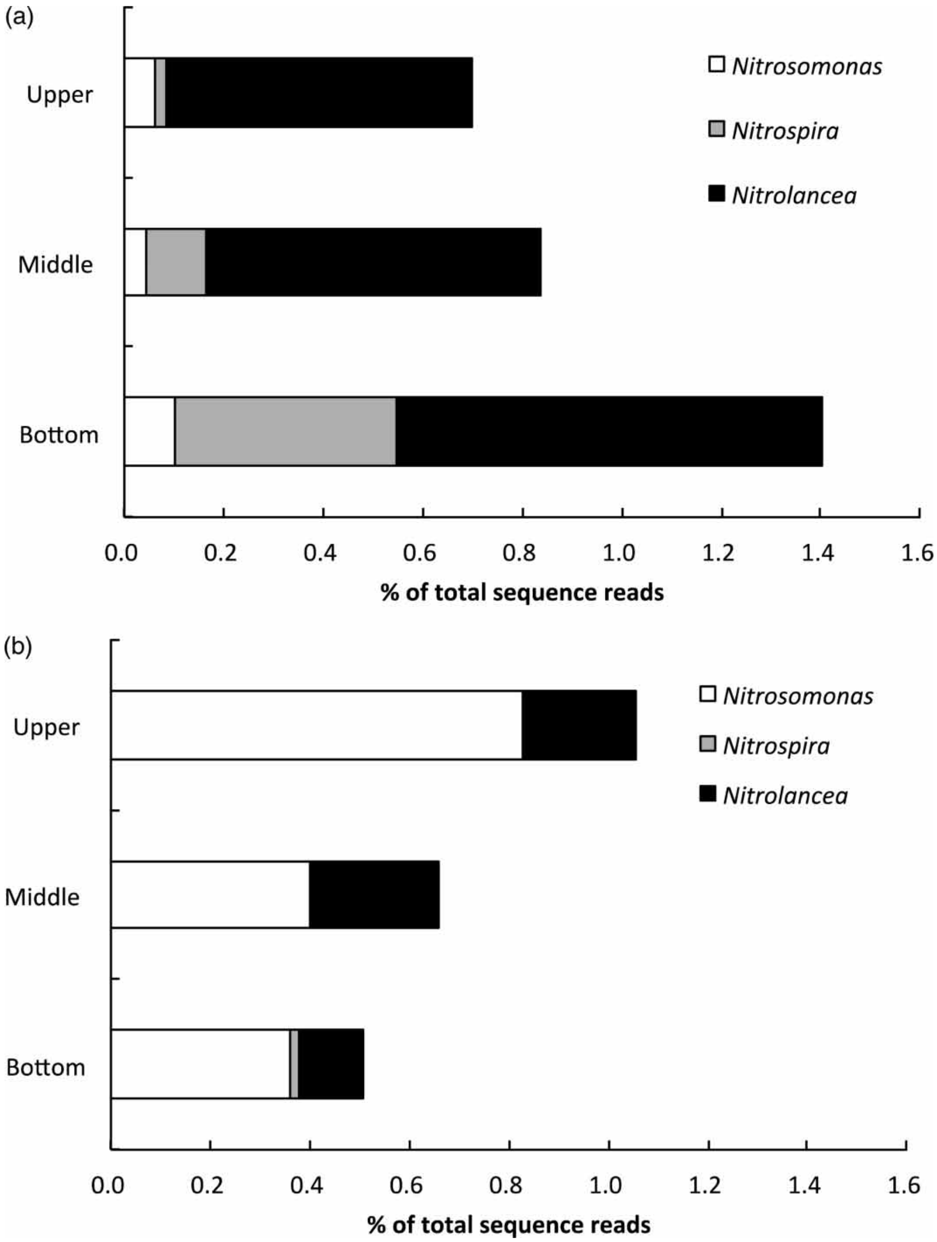


**Figure 5** | Total sequence reads of nitrifying bacteria in the DHS reactor on day 35 (a) and day 110 (b).

predominant in the upper compartment of a trickling filter when OLR ranged from 0.44 to 0.55 kg-COD $m^{-3}$ $day^{-1}$ (Mac Conell *et al.* 2013). Nitrite-oxidizing bacteria *Nitrospira* spp. was not found on day 110. However, nitrite was oxidized in the DHS reactor, thus other microorganisms might be responsible for oxidation of nitrite to nitrate. Mac Conell *et al.* (2015) reported that the nitrite-oxidizing bacteria *Nitrolancetus hollandicus* of phylum *Chloroflexi* was found in a DHS reactor treating pretreated municipal wastewater. Some sequences detected in this study were also closely related to *Nitrolancetus hollandicus*. Thus, operation of the DHS under high OLR could be related to the disappearance of *Nitrospira* from the DHS retained sludge; other microorganisms such as *Nitrolancetus hollandicus* might be responsible for the oxidation of nitrite to nitrate in this study. *Candidatus* Brocadia, a known anaerobic ammonia-oxidizing bacteria, was detected at a rate of 0.0–0.2% in the DHS reactor. This suggests that aerobic and anaerobic ammonia oxidation could be occurring in the DHS reactor.

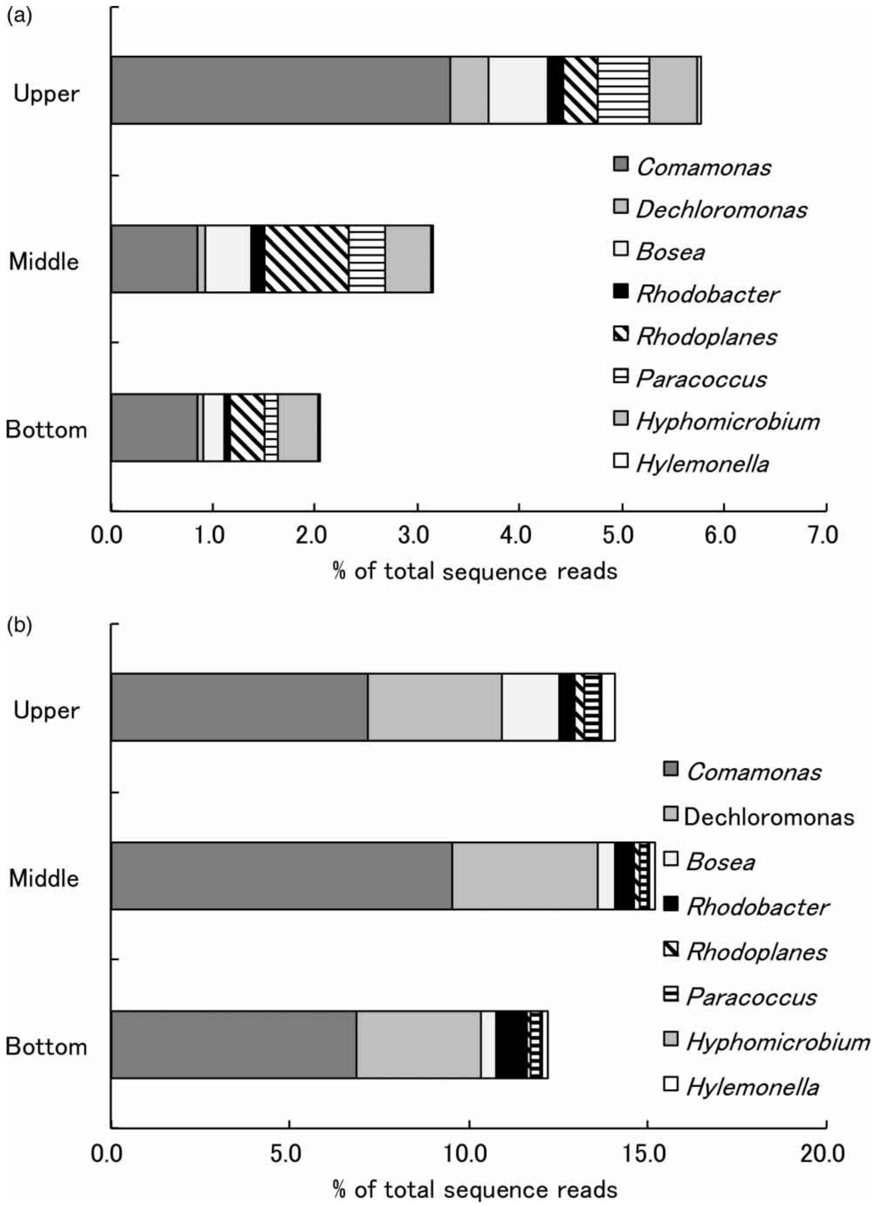


**Figure 6** | The main genera identified with known denitrification capabilities in the DHS reactor on day 35 (a) and day 110 (b).

The total sequence reads of denitrifying bacteria in the DHS retained sludge is shown in Figure 6. The total sequence reads of denitrifying bacteria was significantly increased on day 110 compared with that on day 35. *Comamonas* spp., a known denitrifying bacteria, was the most dominant in the DHS reactor on day 110. A previous study reported that *Comamonas* were observed in an acetate denitrifying system (Lu *et al.*, 2014). *Dechloromonas* spp. were found in high abundance at the top of the reactor on day 110. Kubota *et al.* (2014) also reported that *Dechloromonas* was most abundant in the top of a DHS reactor treating sewage. *Dechloromonas* is capable of utilizing volatile fatty acids (VFAs) and other intermediate compounds as carbon sources (Horn *et al.* 2005). Thus, *Comamonas* and *Dechloromonas* might be dominant because the natural rubber processing wastewater contains large amounts of VFA (Watari *et al.* 2016) and the remaining VFA might be supplied to the DHS reactor.

The results of the microbial analysis showed that nitrifying bacteria, denitrifying bacteria and anammox bacteria coexisted in the DHS sponge media, and different pathways were involved in nitrogen removal in the DHS reactor.

## CONCLUSIONS

The post-treatment DHS reactor performed efficient organic and nitrogen removal for treating natural rubber processing wastewater. The total COD and TN of the DHS effluent were 102 ± 46 mg COD $L^{-1}$ and 57 ± 26 mg N $L^{-1}$, respectively, operated with an OLR of 0.97 kg COD $m^{-3}$ $day^{-1}$. The DHS effluent quality was greater than the existing algal tank-treated effluent with a substantially lower HRT. 16S rRNA gene-based sequence analysis showed that nitrifying bacteria, denitrifying bacteria and anammox bacteria were found in the DHS reactor. This result indicated that the DHS reactor has various pathways for nitrogen removal in the treatment of natural rubber processing wastewater. It was demonstrated that the DHS reactor could be suitable for post treatment of natural rubber processing wastewater.

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