Doctoral Thesis

# Development of a Novel Treatment System for Natural Rubber Processing Wastewater in Vietnam

# ベトナムの天然ゴム製造工程廃水を対象とした処理システムの開発

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Chapter 1

# General Outline and Objective

## 1.1 Outline

Natural rubber processing industry is one of the most economically important industries in South east Asian countries. Approximately 80% of natural rubber have been produced from Thailand, Indonesia, Malaysia and Vietnam. Natural rubber has a large stretch ratio, high resilience and is extremely waterproof compared with synthetic rubber. Thus, it is widely used for aircraft, automobile tires and so on. However, the production process of natural rubber such as coagulation, centrifugation, lamination, washing, and drying emits large amount of polluted wastewater (e.g. 25 $m^3$ of wastewater is generated per ton of ribbed smoke sheet rubber production in Vietnam) (Nguyen and Luong, 2012). This wastewater contains high concentration of organic compounds and ammonia. In addition, large amount of residual natural rubber particulars is remained in the wastewater. Unfortunately, some environmental pollution has been reported due not sufficient wastewater treatment of this wastewater in Southeast Asian countries.

A traditional anaerobic and aerobic lagoon has been widely applied to treat natural rubber processing wastewater in southeast Asian countries (Mohammadi et al., 2010; Nguyen and Luong, 2012). This conventional process can remove more than 95% of biochemical oxygen demand with easy maintenance. However, the system requires large lagoon area, long hydraulic retention time and further post treatment system for achievement of the local discharge standard. Moreover, our previous research disclosed that the anaerobic lagoon emits not only methane, but also nitrous oxide that had high global warming potential (Tanikawa et al., 2016). Therefore, development of an appropriate treatment system for natural rubber processing wastewater is necessary to solve thesis problems.

An upflow anaerobic sludge blanket (UASB) reactor is one of the most processing system, given its high organic loading capacity, low operational costs, and energy recovery in the form of methane for the treatment of different types of industrial wastewater (van Lier et al., 2015). A down-flow hanging sponge (DHS) reactor is one of the most effective aerobic treatment systems used as post treatment of UASB reactor in different types for industrial wastewater.

This thesis summarizes the findings of the research on laboratory scale and pilot scale continues flow experiment of UASB-DHS system for treating natural rubber processing wastewater, mini scale DHS reactor for post treatment of existing anaerobic baffled reactor treating this wastewater, and development of single stage partial nitritation - Anammox process in sponge-based trickling filter reactor. Furthermore, microbial community structures of these bioreactors were revealed by molecular biology approach.

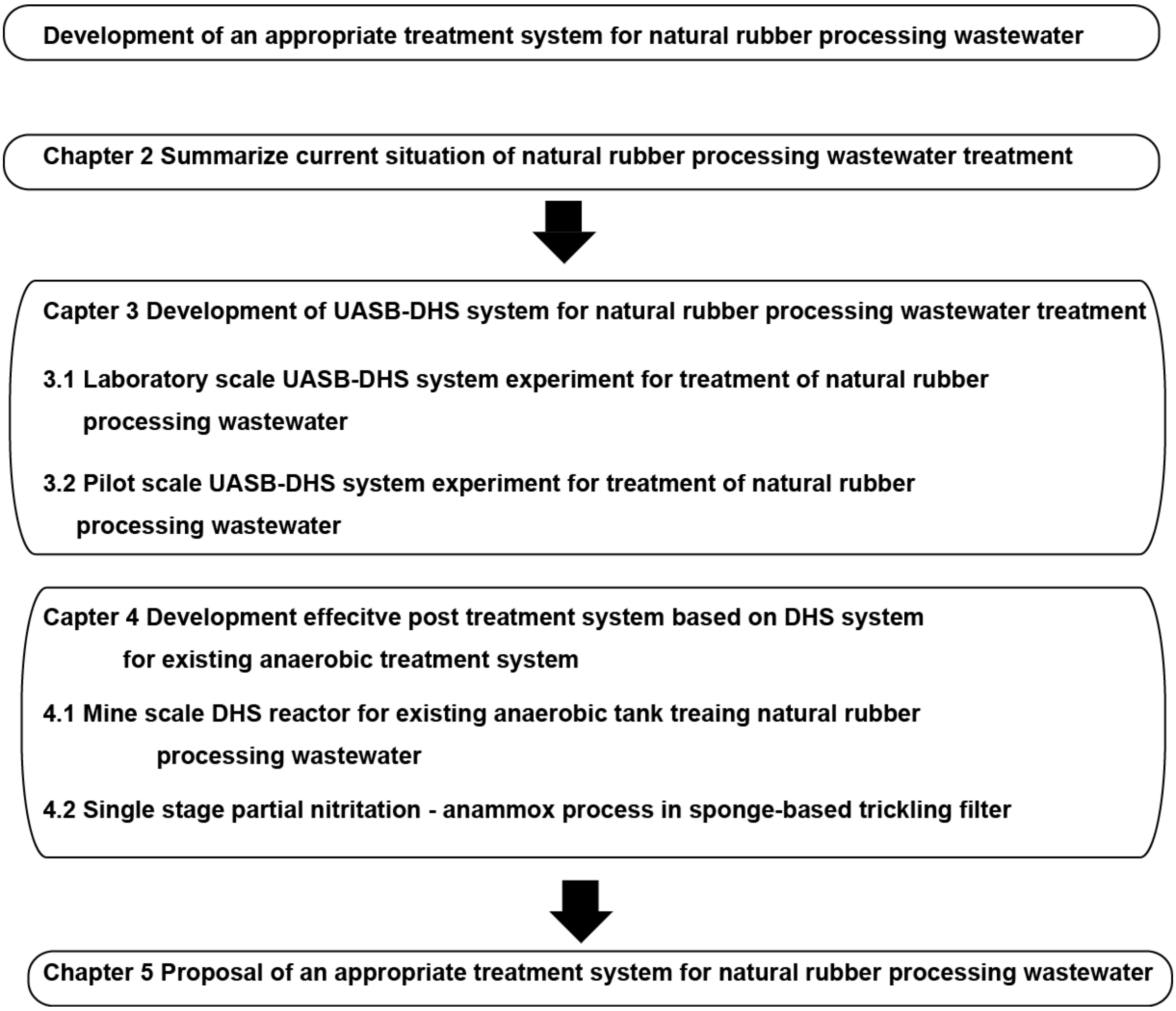


Figure 1.1 Outline of doctoral thesis

## 1.2 Objective

A combination system of UASB reactor and DHS reactor has been widely applied for sewage and industrial wastewater treatment in recent 20 years. This system has several advantages for energy recovery, less excess sludge production and short hydraulic retention time. Research on the treatment of natural rubber processing wastewater was started with application of UASB-DHS system. The objective of this research can define as flows

· Development of UASB-DHS system for treatment of natural rubber processing wastewater in Vietnam
· Evaluate process performance of UASB-DHS system and calculate greenhouse gases reduction when applied UASB-DHS system
· Investigate key microorganisms that degrade natural rubber processing wastewater

**Reference**

Nguyen Nhu Hien and Luong Thanh Thao (2012) Situation of wastewater treatment of natural rubber latex processing in the Southeastern region, Vietnam

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Tanikawa, D., Syutsubo, K., Watari, T., Miyaoka, Y., Hatamoto, M., Iijima, S., ... & Yamaguchi, T. (2016). Greenhouse gas emissions from open-type anaerobic wastewater treatment system in natural rubber processing factory. *Journal of Cleaner Production*, *119*, 32-37.

van Lier, J. B., Van der Zee, F. P., Frijters, C. T. M. J., & Ersahin, M. E. (2015). Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Reviews in Environmental Science and Bio/Technology*, *14*(4), 681-702.

Chapter 2

# Literature Review

## 2.1 Natural Rubber Industry
### 2.1.1 Natural rubber

Natural rubber consists of polymers of the organic compound isoprene, with minor impurities of other organic compounds and water. Rubber is harvested mainly in form of the latex from the rubber tree (*Hevea brasillensis*) or others (Figure 2.1). Figure 2.2 show the production process of rubber products in the natural rubber processing factory. The latex is a stickily, milky colloid drawn off by making incision in the bark and collecting the fluid in vessels in a process called “tapping”. Raw natural rubber latex is collected from a rubber tree and added ammonia in order to keep at low pH condition. After arriving at the factory, natural rubber latex is diluted by tap water, then added acid such as acetate or formic acids to coagulate natural rubber block. The coagulated rubber natural rubber is pressed to make a rubber sheet and smoked in the furnace. Finally, the rubber sheet is washed by tap water and dry in the sun.

The products of natural rubber latex manufactured 3 kinds of raw rubber sheets named technically specified rubber (TSR), concentrated latex (CL) and ribbed smoked sheet (RSS) in the local factory. The TSR is graded by a quality inspection after the forming. The TSR also calls “blocked rubber” or “crumb rubber” due to the morphology of TSR. The TSR has been the most used in US and Europe countries. The RSS is a smoked rubber sheet (Figure 2.1) and largely used in industries.

Natural rubber is one of the most valuable agricultural products in Southeast Asian countries. More than 60% of raw natural rubber latex was produced in Thailand, Indonesia and Malaysia (Mohammidi et al., 2010). According to data of Food and Agriculture Organization of the United Nations, the total production of natural rubber in 2014 was 13,244,825 ton in the world (Figure 2.3). Thailand and Indonesia produced almost half amount of natural rubber on 2014. Vietnam was located 3$^{rd}$ produced country and the produced amount was 961,104 ton. In Vietnam, the natural rubber has been grown the mostly in the South-East region, Binh Phuoc, Binh Duong, Tay Ninh, Dong Nai province (Nguyen and Luong, 2012).

Figure 2.1 Natural rubber plantation and natural rubber sheet (RSS).

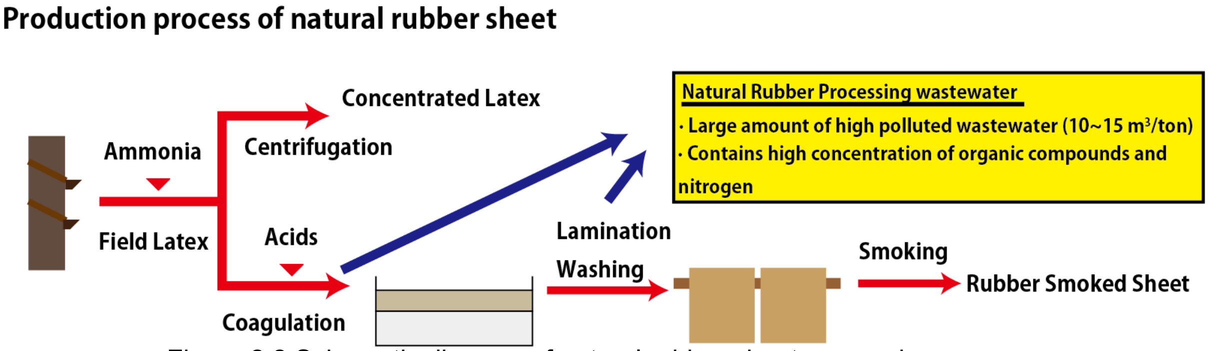


Figure 2.2 Schematic diagram of natural rubber sheet processing process

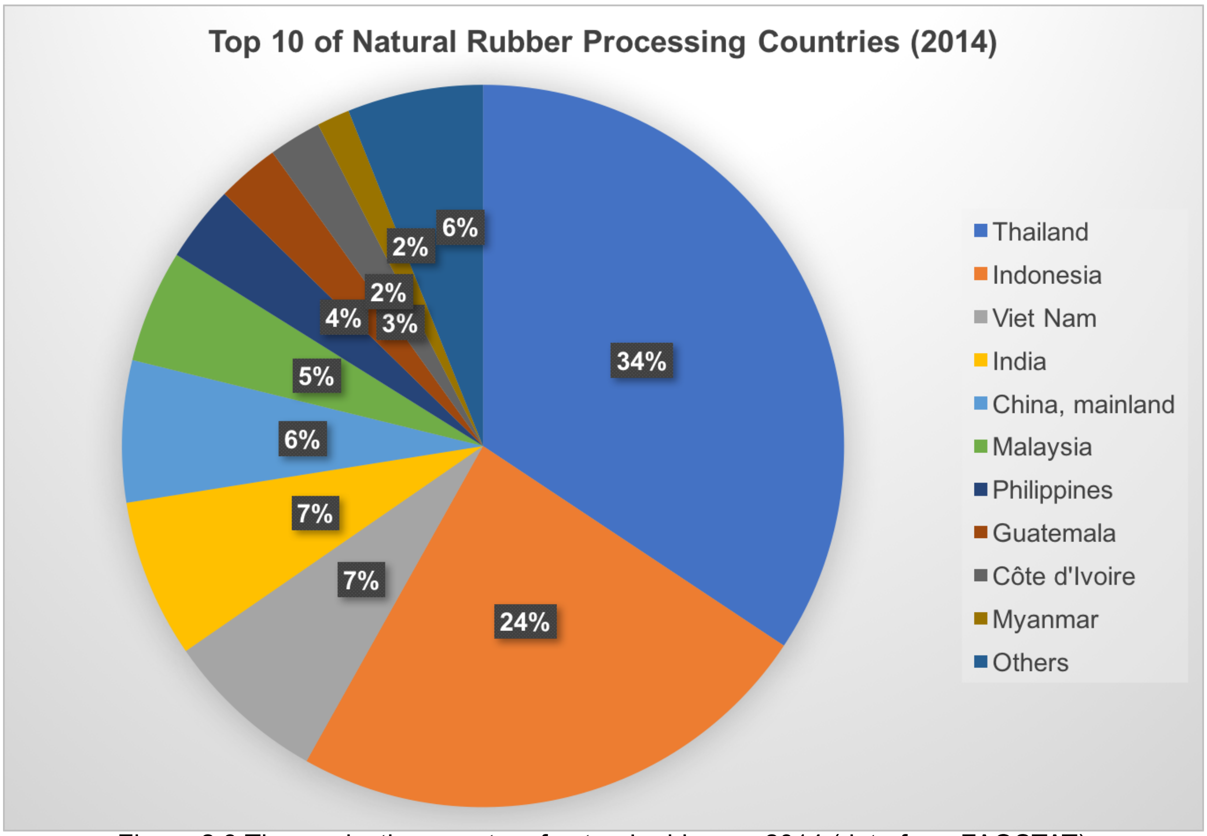


Figure 2.3 The production country of natural rubber on 2014 (data from FAOSTAT)

### 2.1.2 Natural rubber processing process and wastewater

The natural rubber processing industry generates large amounts of wastewater from several processes such as coagulation, centrifugation, lamination, washing, and drying (Figure 2.2). Nguyen and Luong (2012) reported that 25 – 35 $m^3$ and 18 $m^3$ of wastewater generated by 1 ton of technically specified rubber and ribbed smoked sheet, and concentrated latex production, respectively in Vietnam. Also, Mohammaid et al. (2010) reported that 20 tons of rubber and 410,000 L of effluent per day is produced by local rubber factory in Malaysia. This wastewater contains high concentrations of organic compounds, nitrogen, and other contaminants. The characteristics of natural rubber processing is largely different and its depend on how much add ammonia at transportation process and which kinds of acids used in the coagulation process. Thailand where the most natural rubber produced country and Malaysia usually use sulfuric acids for coagulation due to its strong oxidizing potential. Therefore, natural rubber processing wastewater where used sulfuric acid at the coagulation process does not contain large amount of residual rubber particles. However, it still has a large impact for aquatic environment. In fact, some environmental pollution has been reported due to daily discharge of 80 million liter of untreated rubber effluent to near streams in Malaysia (Mohammaid et al. 2010). Acetic acid and/or formic acid have been typically used for the coagulation process in Vietnam because they have a lower impact on the environment compared to sulfuric acid. But, the natural rubber processing wastewater from the Vietnamese factories contains large quantity of residual natural rubber.

Mohammadi et al. (2010) and Nguyen and Luong (2012) summarized current status of natural rubber processing wastewater treatment in Malaysia and Vietnam, respectively. Table 2.1 summarized the process performance of current treatment system treating natural rubber processing wastewater. An anaerobic lagoon has been widely used for treatment of this wastewater due to its easy operational maintenance and low operational cost. Recently, next generation treatment process such as an up-flow anaerobic sludge blanket (UASB) reactor was installed. A Ph.D study of Dr. Nguyen Trung Viet, group of Prof. Lettinga (Wageningen University, The Netherlands) demonstrated the possibility of application of the UASB reactor to natural rubber processing wastewater in 1999. Also, Tanikawa et al. (2016) reported the process performance of two-stage UASB system treating natural rubber processing wastewater in Thailand.

Table 2.1 Process performance of several systems for natural rubber processing wastewater

| | | | HRT | Influent concenration ($mg \cdot L^{-1}$) | | | | | | Effluent concentration ($mg \cdot L^{-1}$) | | | | | | Removal effciency (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| System | Country | Wastewater | days | pH | TCOD | TBOD | TSS | TN | Ammonia | pH | TCOD | TBOD | TSS | TN | Ammonia | TCOD | TBOD | TSS | TN | Reference |
| Decantation - UASB - aeration tank - settling and filiter | Vietnam | CL + SVR | - | 9.2 | 18,885 | 10,780 | 900 | 611 | 342 | 6.83 | 123 | 57 | 70 | 35.3 | 30.8 | 99 | 99 | 92 | 94 | Nguyen and Luong (2012) |
| Decantation - oxidation ditch - settling and filiter | Vietnam | CL | - | 9.1 | 26,914 | 8,750 | 740 | 766 | 361 | 8.39 | 567 | 50 | 74 | 160 | 137 | 98 | 99 | 90 | 79 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL | - | 8.55 | 19,029 | 7,830 | 2,220 | 813 | 302 | 8.23 | 466 | 70 | 300 | 40.6 | 34.5 | 98 | 99 | 86 | 95 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL + SVR | - | 8.23 | 14,466 | 9,200 | 850 | 450 | 350 | 7.39 | 107 | 92 | 60 | 65 | 47 | 99 | 99 | 93 | 86 | Nguyen and Luong (2012) |
| Decantation - flotation - oxidiation ditich - settling and filiter | Vietnam | CL + SVR | - | 9.42 | 26,436 | 13,820 | 1,690 | 651 | 285 | 8.14 | 120 | 85 | 60 | 74.9 | 33 | 99.5 | 99 | 96 | 88 | Nguyen and Luong (2012) |
| Decantation - flotation - UASB - aeration tank - settling and filiter | Vietnam | CL | - | 8.09 | 13,981 | 7,590 | 468 | 972 | 686 | 7.88 | 127 | 61 | 39 | 129 | 30.3 | 99 | 99 | 92 | 87 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL + SVR | - | 8.59 | 11,935 | 8,780 | 1,164 | 1,306 | 1,043 | 6.59 | 130 | 60 | 94 | 67 | 50 | 99 | 99 | 92 | 95 | Nguyen and Luong (2012) |
| Dissolved air flotation - anaerobic lagoon - anoxic lagoon - aerated tank | Vietnam | CL + SVR | - | 5.37 | 5,610 | - | 867 | 372 | 341 | 7.79 | 136 | - | 98 | 33 | 13 | 98 | - | 89 | 91 | Syutsubo et al. (2015) |
| Dissolved air flotation - lagoon - aeration tank - aerated tank | Vietnam | CL + SVR | - | 6.34 | 5,350 | - | 357 | 394 | 154 | 7.76 | 128 | - | 70 | 41 | 27 | 98 | - | 80 | 90 | Syutsubo et al. (2015) |
| UASB | Vietnam | RSS | 0.8 | 7.1 | 1,450 | - | 279 | - | - | 7.4 | 102 | - | 72 | - | - | 96 | - | 74 | - | Thanh et al. (2016) |
| UASB | Thailand | CL | 4 | 1.95 | 3,350 | 1,855 | 340 | 661 | 271 | - | - | - | - | - | - | 60 | - | - | - | Boonsawang et al., (2008) |
| UASB - UASB - DHS | Thailand | CL | 11.5 | 5.5 | 9,710 | 8,670 | 1,780 | 1,370 | - | - | - | - | - | - | - | 96 | - | - | - | Tanikawa et al. (2016) |
| Oxidation Ditch Process | Malaysia | CL | - | 7.16 | 2,675 | 1,871 | 3,645 | 231 | 17 | 7.1 | 56 | 22 | 1,313 | 36 | 0 | 98 | 99 | 64 | 84 | Ibrahim et al. (1980) |
| Stablilisation pond | Malysia | CL | - | - | - | - | - | - | - | - | - | - | - | - | - | 93 | 90 | - | - | Madhu et al. (2007) |

Note: ABR: anaerobic baffled reactor, CL: Concentrated latex, DHS: down flow hanging sponge, RSS: Ribbed smoked sheet, ST: settling tank, SVR: standard Vietnamese rubber, UASB: upflow anaerobic sludge

### 2.1.3 Natural rubber industrial effluent discharge standards

The industrial effluent discharge standards have been usually provided by government for the environmental protection. Natural rubber processing wastewater is one of the biggest sources of industrial wastewater pollution in Southeast Asian counties and usually set original and strong effluent standard for natural rubber processing factory. In Vietnam, ministry of natural resources and environment provides national technical regulation on the effluent of natural rubber processing industry (QCVN 01-MT : 2015/BTNMT). The water quality of Vietnamese effluent standards is shown in Table 2.2. Standard A is applied for effluent discharging to domestic water supply (used for daily activities, except drinking and cooking directly). Standard B is applied for other water supply not domestic water supply (eg. water transport, irrigation, aquaculture, cultivation ...). From the national technical regulation published 2015, there are two categories that new factory (start to operate after 31/March/2015) and previous factory (start to operate before 31/March/2015).

Table 2.2 National technical regulation on the effluent of natural rubber processing industry (QCVN 01-MT : 2015/BTNMT)

| Contents | | Unit | A | B |
|---|---|---|---|---|
| pH | | - | 6 - 9 | 6 - 9 |
| $BOD_5$ (20˚C) | | $mg{\cdot}L^{-1}$ | 30 | 50 |
| COD | New factory | | 75 | 200 |
| | Previous factory | $mg{\cdot}L^{-1}$ | 100 | 250 |
| TSS | | $mg{\cdot}L^{-1}$ | 50 | 100 |
| TN | New factory | | 40 | 60 |
| | Previous factory | $mg{\cdot}L^{-1}$ | 50 | 80 |
| Ammonia | New factory | | 100 | 40 |
| | Previous factory | $mg{\cdot}L^{-1}$ | 15 | 60 |

## 2.2 Anaerobic industrial wastewater treatment

### 2.2.1 Characteristics of anaerobic wastewater treatment and the degradation pathway of anaerobic digestion

Anaerobic digestion is the fermentation process that organic material is degraded and is produce biogas containing methane and carbon dioxide. This biodegradation occurred in many places where organic material is available and anaerobic or anoxic condition. Anaerobic treatment is more attractive treatment process compared with aerobic treatment. Anaerobic treatment can effectively remove biodegradable organic compounds leaving mineralized compounds such as $NH_4^+$, $PO_4^{3-}$, $S_2^-$ in the solution. The bioreactor of anaerobic treatment is very simple system and can be applied any scale and at almost any place. Most great benefit of anaerobic treatment is useful energy in the form of methane can be recovered by anaerobic digestion. In general, 40 ~ 45 $m^3$ of biogas can recovery from 100 kg-COD of influent (van Lier et al., 2008). In addition, anaerobic wastewater treatment can reduce excess sludge production. van Lier et al. (2008) summarized the reasons why the selection for anaerobic wastewater treatment was made, the following striking advantages of anaerobic wastewater treatment over conventional aerobic treatment systems.

· reduction of excess sludge production up to 90%
· up to 90% reduction in space requirement 20-35 kg COD per $m^3$ of reactor per day, requiring smaller reactor volumes
· high applicable COD loading rates reaching 20-35 kg COD per $m^3$ of reactor per day, requiring smaller reactor volumes
· no use of fossil fuels for treatment, saving about 1 kWh·kgCOD$^{-1}$ removed, depending on aeration efficiency.
· production of about 15.5 MJ $CH_4$ energy·kg-COD$^{-1}$ removed, giving 1.4 kWh electricity (assuming 40% electric conversation efficiency)
· rapid start up (< 1 week) using anaerobic granular sludge as seed material.
· no or very little use of chemicals
· plain technology with high treatment efficiencies
· anaerobic sludge can be stored unfed, reactors can be operated during agricultural campaigns only.
· excess sludge has a market value (sold as granular sludge)
· high rate system facilitates water recycling in factories (towards closed loops)

Biological organic compounds degradation under anaerobic condition is a multistep process of series and parallel reactions. This process of anaerobic degradation proceeds in three stages (Figure 2.4).

1st step: Hydrolyze complex organic compounds to dissolved and low molecular weight organic compounds.

2nd step: Ferment low molecular weight organic compounds, and produce volatile fatty acids and alcohols.

3rd step: Methane gas produce from acetate or hydrogen and carbon dioxide

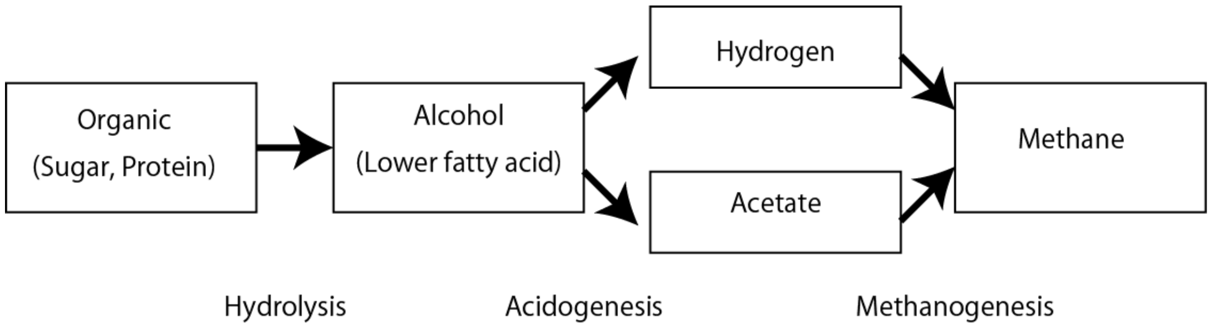


Figure 2.4 Anaerobic digestion scheme of organic compounds

In general, 1st step of anaerobic digestion (acidification) is slower than 2nd step (methane fermentation). If wastewater containing unbiodegradable compounds such as cellos, acidification would be rate-limiting. On the other hand, wastewater containing an easily biodegradable organic compound, volatile fatty acids (VFA) are rapidly produced and accumulated in the reactor. This produced VFAs inhibits methanogen. Therefore, consideration of methane production rate and organic loading rate is important for perform stable and high process performance in anaerobic treatment. The important factors for anaerobic treatment is shown in below.

Optimal temperature for anaerobic treatment is reported at 30˚C ~ 35˚C (mesophilic) and 50˚C ~ 60˚C (thermophilic). A thermophilic anaerobic treatment is 25~50% faster than mesophilic anaerobic wastewater treatment.

Optimal pH for acidogen and methanogen are ranged 5.0 ~ 6.0 and 6.8 ~ 7.2, respectively. Methanogen is more sensitive for pH (less than 6 or higher than 8.0) and activities of methanogen are significantly decreased. In anaerobic treatment, VFAs are produced as intermediate and lead pH deterioration. Therefore, supplement of alkalinity is required.

Nutrients such as phosphors and nitrogen require for growth anaerobic bacteria. The ratio of COD : N : P at the high organic loading rate (0.8 ~ 1.2 kg-COD·kg-VSS$^{-1}$·day$^{-1}$) and low organic loading rate (0.5 kg-COD·kg-VSS$^{-1}$·day$^{-1}$) are 350 : 7 : 1 and 1000 : 7 : 1, respectively (Michal and Geradi; 2003). In addition, optimal N/P and C/N ratio are 7 and at least 25.

The VFAs and ammonia are mention of inhibitors for anaerobic digestion. VFAs that acetate, propionate and lactic acid are intermediate of anaerobic digestion. The inhibition of methanogen could be occurred around 2,500 mg-COD·$L^{-1}$ of acetate accumulated in the reactor at pH 7.5. On the hand, ammonia inhibition happens 3,500 mg-N·$L^{-1}$ at mesophilic condition and 2,000 mg-N·$L^{-1}$ at thermophilic condition.

### 2.2.2 Anaerobic industrial wastewater treatment technology

During this 40 years, anaerobic wastewater treatment technology evolved from localizes lab-scale experiment to worldwide successful implementation in the various industries (van Lier et al., 2015). Nowadays, more than 1,600 of real scale anaerobic wastewater treatment plant have been operated all over the world (Macarie, 2000; Chan et al., 2009; van Lier et al., 2015). Previous studies have demonstrated the process performance of anaerobic treatment for treating many types of medium- and high-strength industrial wastewater (Chan et al., 2009). Especially, some agro-food industry such as sugar, potato, starch, yeast, pectin, citric acid, cannery confectionary, fruit, vegetables, dairy and bakery were highly applied due to its high biodegradability (Table 2.3).

One of the most advantage of anaerobic wastewater treatment in industrial wastewater is anaerobic treatment can operate with high organic loading rate. An UASB reactor is most successful technology for high OLR wastewater treatment and most widely implemented for the anaerobic industrial wastewater, having about 90% of the market share of all installed system (van Lier, 2008). In addition, anaerobic wastewater treatment can treat chemical wastewaters containing toxic compounds or wastewaters with a complex composition.

Table 2.3 Application of anaerobic technology to industrial wastewater (van Lier et al., 2015)

| Industrial sector | Type of wastewater | Installed reactors (% of total) |
|---|---|---|
| Agro-food industry | Sugar, potato, strach, yeast, pectin, citric acid, cannerym confectionary, fruit, vegetables, dairy, bakery | 36 |
| Beverage | Beer, malting, soft drinks, wine, fruit juices, coffee | 29 |
| Alcohol distillery | Can juice, cane molasses, beet molasses, grape wine, grain fruit | 10 |
| Pulp and paper industry | Recycle paper, mechanical pulp, NSSC, sulphite pulp, straw, bagasse | 11 |
| Miscellaneous | Chemical, pharmaceutical, sludge liqor, landfill leachate, acid mine water, minicipal sewage | 14 |

### 2.2.3 Anaerobic biological pond

Anaerobic biological pond has been widely applied for treatment of industrial wastewater from palm oil processing factory and natural rubber processing factory in Southeast asian countries (Figure 2.5). More than 500 of anaerobic biological pond have install in Malaysia for palm oil and natural rubber processing factories (Chaiprapat and Sdoodee, 2007; Madhu et al., 1994; Mohammadi et al., 2010; Thongnuekhang and Puetpaiboon, 2004). This treatment process is degraded organic compounds by anaerobic microorganism and settled suspended solid by long retention time. The chemical oxygen demand (COD) removal efficiency of this process is approximately 65 ~ 90% with easy operational control (Madhu et al., 1994). Our site survey in Southeast Vietnam reported the advanced open type anaerobic baffled lagoon in Rubber Research Institute of Vietnam performed 94.4% of total COD removal with hydraulic retention time (HRT) of 12 days (Tanikawa et al., 2016). However, these systems require a large lagoon area, high operating costs (especially for surface aeration), and long hydraulic retention times (e.g. over 1 month). In addition, improvements to the effluent water quality of existing treatment systems are required to comply with discharge standards. Moreover, our previous study reported that the anaerobic biological pond treating natural rubber processing wastewater in Vietnam emits not only methane but also nitrous oxide that has a higher global warming potential (Tanikawa et al., 2016).


Figure 2.5 anaerobic biological pond in (a) Rubber Research Institute of Vietnam (b) Mia Thao Rubber sheet processing factory.

### 2.2.4 Upflow anaerobic sludge blanket (UASB)

As mentioned in 2.2.1, an UASB reactor has been widely applied to high- or middle-strength industrial wastewater treatment. The UASB reactor was introduced by Prof. Letting on 1976 in Netherlands. The formation of well settleable sludge aggregates and on the application of a reverse funnel-shaped internal gas-liquid-solids separation (GLSS) devise is key technology for successful UASB reactor (Figure 2.6). The characteristics of UASB is shown as below

1) The influent is fed from bottom reactor in order to make upflow.
2) If the UASB reactor satisfactory operated, granulation could occur and formatted high settleability sludge in the reactor.
3) The UASB reactor has high contacting efficiency due to high biogas production
4) The washed out sludge has effectively collected by GLSS.
5) The excess sludge from the UASB reactor can reduce 90% compared with activated sludge process.

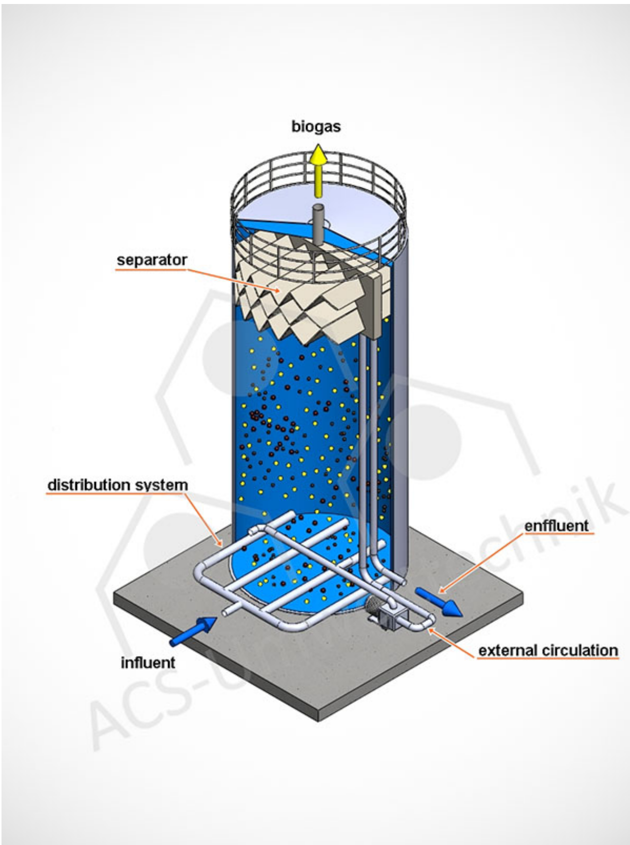


Figure 2.6 Schematic diagram of UASB reactor (http://www.acs-environment.com/en/reactor-types/uasb-reactor)]

The first application of UASB reactor to treatment of natural rubber processing wastewater in Vietnam was demonstrated by Nguyen (1999) as his Ph.D research in Wageningen University (Table 2.4). The results showed that the UASB reactor performed around 79.8% - 87.9% of total COD removal efficiency at organic loading rate of 28.5 kg-COD·$m^{-3}$·$day^{-1}$. However, the remaining natural rubber particulars affected the anaerobic biodegradation such as accumulation rubber particulars in the UASB column. Therefore, effective post treatment for removing residual natural rubber particulars is required for application of UASB reactor in Vietnam local natural rubber processing factory. Nguyen et al. (2016) reported that enhancement of granulation by using aluminum chloride and the UASB reactor performed total COD removal efficiency rose to 96.5 ± 2.6%, with a methane recovery rate of 84.9±13.4% in natural rubber processing wastewater in Vietnam.

The UASB technology in natural rubber processing wastewater treatment is actively researched in Thailand because of wastewater did not contain much amount of suspend solids due to using sulfuric acid in coagulation process. Jawjit and Liengcharernsit (2008) investigated the treatment performance of the two-stage UASB reactor applied to concentrated latex processing wastewater. As result, the UASB reactor performed high process performance when pH controlled at 7 and operated at the mesophilic conditions (35˚C) at laboratory scale level. Tanikawa et al. (2016) demonstrated the pilot scale two stage UASB reactor (volume of 997 L and 597 L. respectively) in the Von Bundit natural rubber processing factory in Stat Thani, Thailand. The system achieved a COD removal efficiency of 95.7% ± 1.3% at an OLR of 0.8 kgCOD·$m^{-3}$·$d^{-1}$. Bacterial activity measurement of retained sludge from the UASB showed that s

ulfate-reducing bacteria (SRB), especially hydrogen-utilizing SRB, possessed high activity compared with methane- producing bacteria (MPB).

Table 2.4 Previous studies of treating natural rubber processing by UASB reactor

| Reactor type | | Volume L | Seed sludge | Organic removal rate (kg-COD·$m^{-3}$·$day^{-1}$) | COD removal % | Reference |
|---|---|---|---|---|---|---|
| Single | Vietnam | 8.55 | Digested pig manure sludge | 28.5 | 79.8-87.9% | Nguyen (1999) |
| Single | Vietnam | 17 | Anaerobic digester trating casava wastewater | 2.65 | 96.5 ± 2.6 | Thanh et al., (2015) |
| Two stage | Thailand | 24.8 | Concentrated latex mill | 1.41 | 82 | Jawjit and Liengcharernest (2010) |
| Two stage | Thailand | 997 + 597 | Anaerobic pond in the rubber factory | 0.8 | 96.57 ± 1.3 | Tanikawa et al., (2016) |

### 2.2.5 Anaerobic baffled reactor

An aerobic baffled reactor (ABR) has been designed since the early 1980s and has several advantages over well established system such as UASB and anaerobic filter (Barber and Stuckey; 1998). These advantages are better resilience to hydraulic and organic shock loadings, longer biomass retention times, lower sludge yields, and the ability to partially separate the various phases of anaerobic catabolism. The most significant advantages of the ABR is the typical reactor configuration that can separate acetogen and methanogen longitudinally down the reactor. This two phases operation can enhance acetogen and methanogen activity by a factor of up to four as acetogen accumulate within the first stage, and different microbial group can develop under more favorable conditions. Therefore, the ABR has been applied to treatment of various industrial wastewaters. Barber and Stuckey, (1999) summarized advantages associated with the ABR shown in below:

Constriction

1) Simple design; 2) No moving part; 3) No mechanical mixing; 4) Inexpensive constriction; 5) High void volume; 6) Reduced clogging; 7) Reduced sludge bed expansion;

Biomass

1) No requirement for biomass with unusual settling properties; 2) Low sludge generation; 3) High solids retention time; 4) Retention of biomass with fixed media or solid-settling chamber; 5) No special gas or sludge separation required

Operation

1) Low HRT; 2) Intermittent operation possible; 3) Extremely stable to hydraulic shock loads; 4) Protection from toxic materials in influent; 5) Long operation times with out sludge wasting; 6) High stability to organic shock

Figure shows that various reactor configuration of ABR. The first report of ABR was equipped several partitions in the reactor to keep high concentration of methanogens. This study reported the methane recovery rate was increased to 30 ~ 55% in organic loading rate of 1.6 kg-COD·m$^{-3}$·day$^{-1}$ (Fannin et al., 1981). Figure 2.7 (A) is basic design of ABR that vertically separated by the wall. Figure 2.7 (B) installed a chamber for settling and a gas sampling line in each compartment for improvement of retention time of waste solid. In Figure 2.7 (C), the diameter of downflow compartment made narrow, the increased sludge retention time in the up-flow compartment.

Akunna and Clark (2000) reported the performance of a granular-bed anaerobic baffled reactor (GRABBA) applied in the treatment of a whisky distillery wastewater. The GRABBA used granular sludge for inoculation and its can compatible with the both advantage of UASB reactor and ABR.

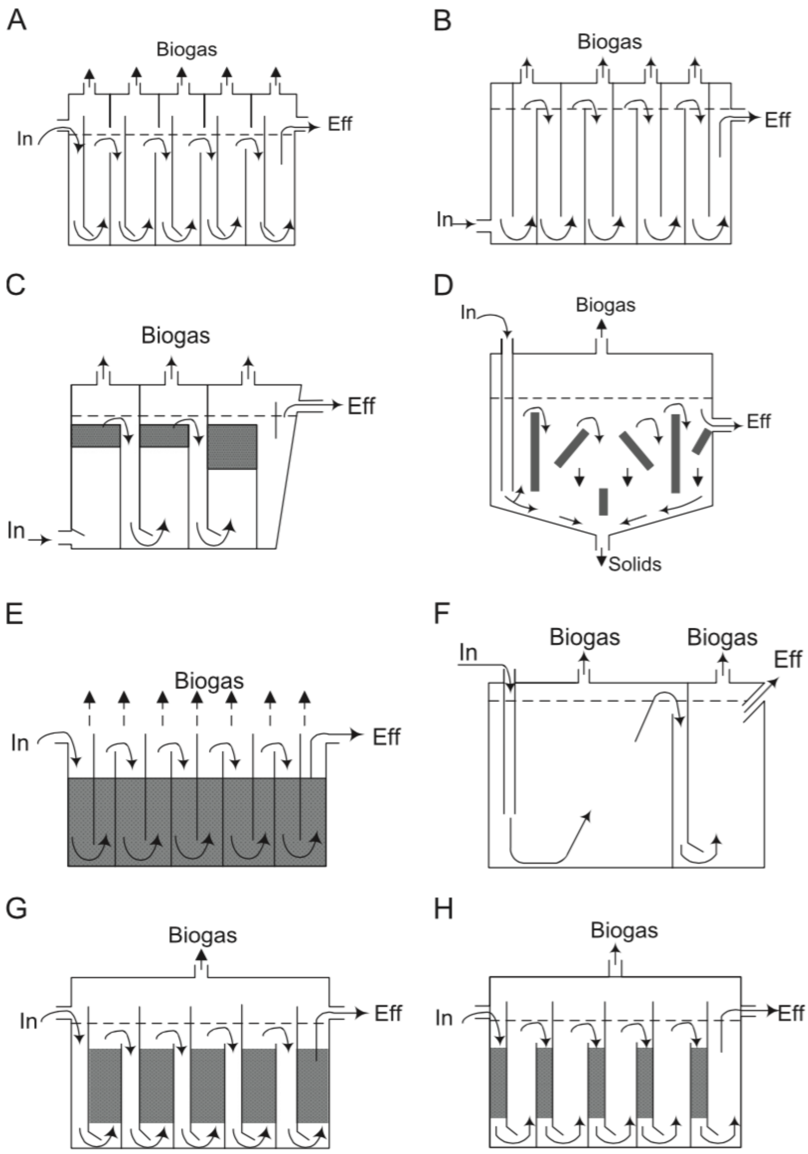


Figure 2.7 various reactor configuration of ABR (Barber and Stuckey, 1999)

## 2.3 Aerobic industrial wastewater treatment
### 2.3.1 Characteristics of aerobic wastewater treatment and the degradation

An aerobic treatment is the removal process that oxidize organic compounds, ammonia, smell and iron by several aerobic bacteria under the oxygen available condition (Figure 2.8). The bacteria or floc absorbed organic compounds and degrade to water and carbon dioxide to get energy for own breeding. The oxidation of organic compounds and compose of bacteria cell can show in reaction equation follow as;

Oxidation of organic compounds

$ClHnOm + O_2 \rightarrow CO_2 + H_2O + Energy$

Oxidation of glucose
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 3.7\ kcal \cdot g^{-1}$

Composition of bacteria cell
$ClHnOm + N + P + O + E \rightarrow CO_2 + H_2O +$ Bacteria cell
$8(CH_2O) + 3O_2 + NH_3 \rightarrow C_5H_7NO_2$ (as bacteria cell) $+ 3CO_2 + 6H_2O$

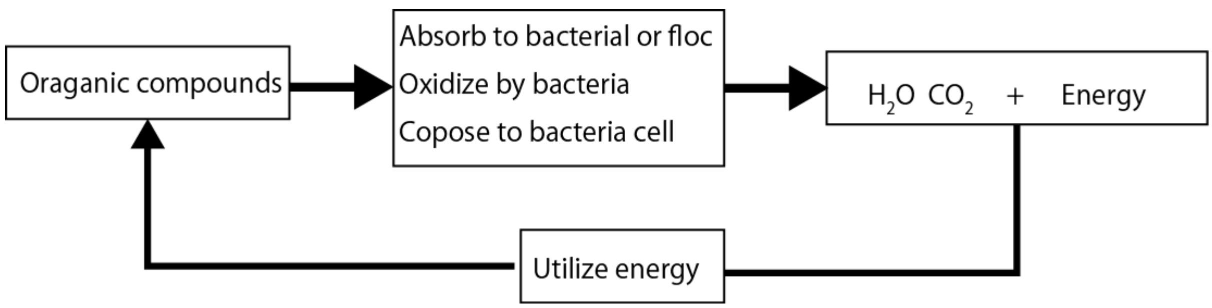


Figure 2.8 Aerobic biological degradation pathway

### 2.3.2 Activated sludge process

An activated sludge process that is one of major treatment system in aerobic treatment consist by aeration tank and settling tank. The process of conventional activated sludge was shown in Figure 2.9. The aeration tank equips diffusing pipe and supplied oxygen to the tank in order to keep aerobic condition. The aeration tank retained the floc that containing 2,000 ~ 5,000 mg·L$^{-1}$ of bacteria. The main bacterial group in the aeration tank are phyla *Psudomonas*, *Bacillus*, *Microbacterium*, *Acinetobacter* and *Nocardia*. Also, Protozoa and metazoa are growth in the aeration tank and this high microbial diversity in this ecosystem with an extremely long food chain. The settling tank is installed for separation of effluent and the floc. The activated sludge process can widely apply to low and middle strength industrial wastewater.

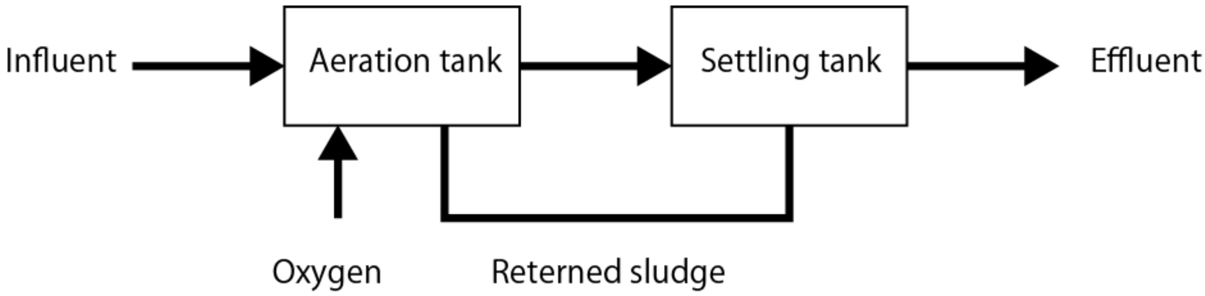


Figure 2.9 Basic water flow in conventional activated sludge

### 2.3.3 Downflow hanging sponge reactor

A down-hanging sponge (DHS) reactor is a trickling filter used sponge as media (Figure 2.10). The group of Prof. Harada and collaborators developed in 1997 for the first time a sponge-based bioreactor, as a novel cost effective post-treatment methods for anaerobically pre-treated sewage (Machdar et al., 1997). Nowadays, many research papers for performance of DHS reactor treating sewage were documented (Machdar et al., 1997, 2000; Tandukar et al., 2006, 2007; Tawfik et al., 2006; Onodera et al., 2014; Okubo et al., 2015, 2016). To days, sixth types of sponge carries were proposed and demonstrated process performance (Tawfik et al., 2006; Onodera et al., 2014). The most promising post- treatment systems is a 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. 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 same as conventional aerated tank. The 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, sixth type of sponge carriers were proposed and demonstrated process performance of DHS reactor treating sewage (Tandukar et al. 2007; Onodera et al. 2014, 2016; Harada, 2014; Okubo et al. 2016). Figure 2.11 shows the summary of sixth sponge carrier developed for DHS reactor. Nowadays, G3 type sponge has been widely used due to its high process performance. 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 combination of UASB – DHS system was 15 times smaller than conventional activated sludge process. The DHS reactor has been applied for treatment of several kinds of industrial wastewaters especially post treatment of UASB reactor treated high strength industrial wastewater (El-Kamah et al. 2011; Tanikawa et al. 2016; Watari et al. 2015). There are several papers reported treatment of molasses wastewater using UASB-DHS system (Onodera et al., 2013; Choeisai et al., 2014, Kuroda et al., 2015). Besides that, the DHS reactor has been applied treatment of reactive dye wastewater (Tawfik et al., 2013), freshwater aquarium (Furukawa et al., 2016; Adlin et al., 2017) and ethylene glycol containing industrial wastewater (Watari et al., 2015).

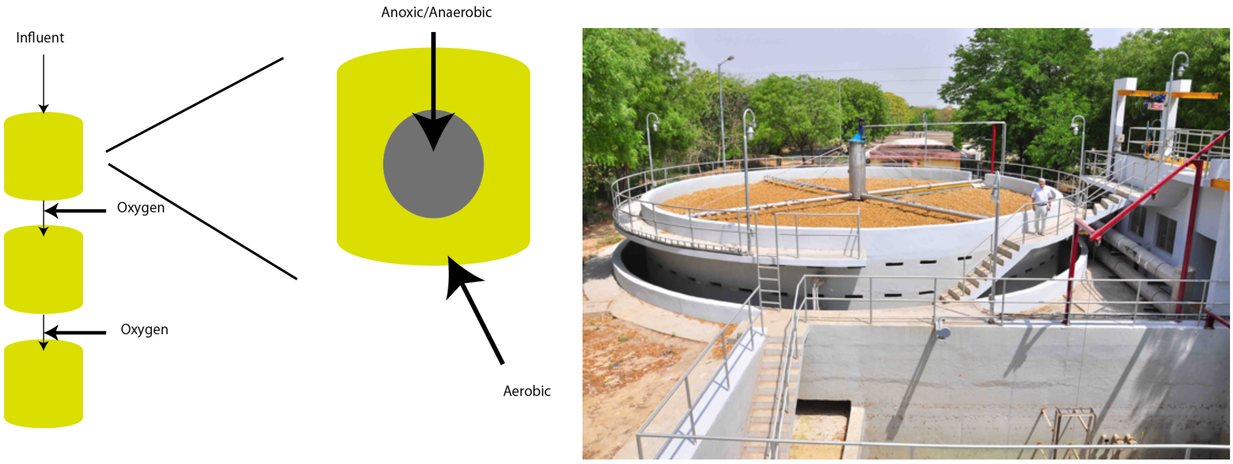

Figure 2.10 Principle of downflow hanging sponge reactor and full scale DHS in India.

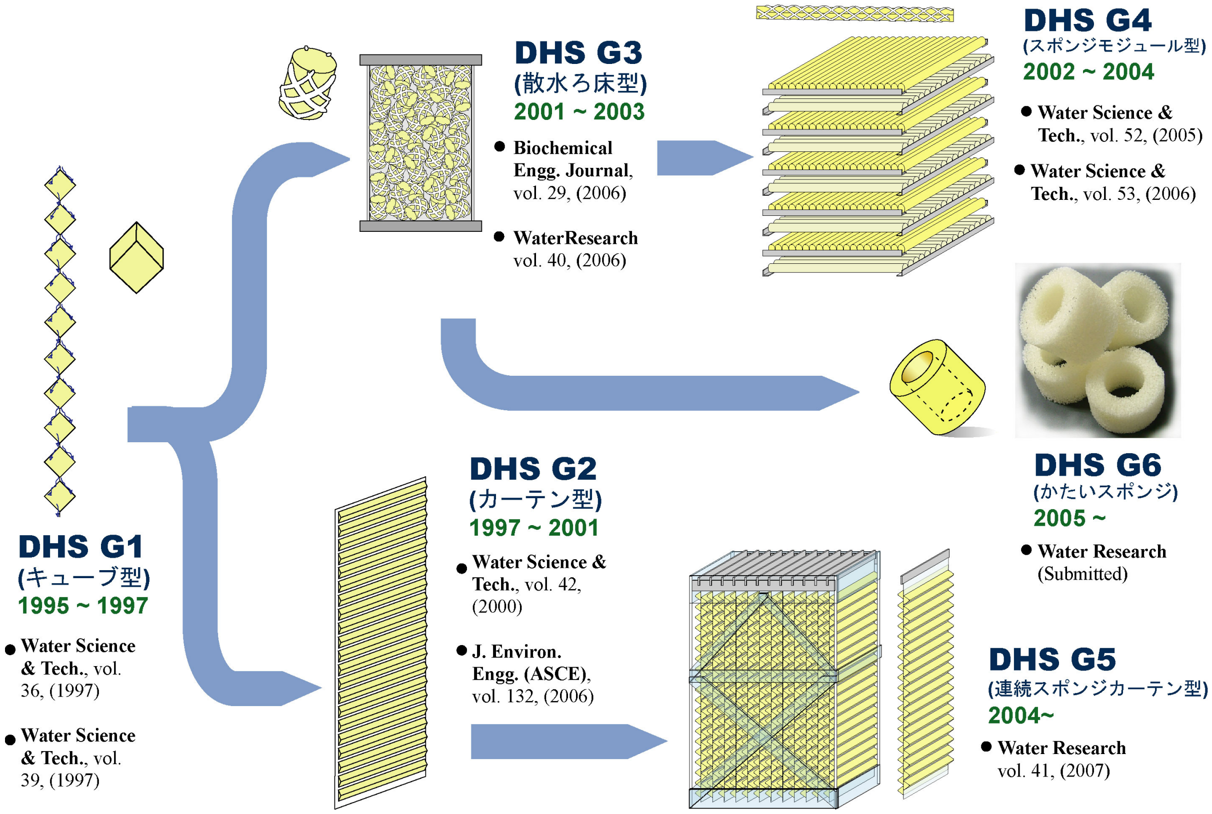


Figure 2.11 Development history from DHS G1 to DHS G6 (Harada, 2014)

## 2.4 Nitrogen removal
### 2.4.1 Nitrification

Nitrification is the process that oxidation of ammonia to nitrate. This process is consisted by two process nitritation ($NH_4^+ \rightarrow NO_2^-$) and nitratation ($NO_2^- \rightarrow NO_3^-$). These processes assumed by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) that utilized ammonia and nitrite as electron donor and oxygen as electron acceptor. The equations of *Nitrosomonas* (AOB) and *Nitrobacter* (NOB) are written follow as

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^-$$
$$\rightarrow C_5H_7O_2N + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3 \quad (\textit{Nitrosomonas}, \text{AOB})$$

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + 195\ O_2 + HCO_3^-$$
$$\rightarrow C_5H_7O_2N + 400\ NO_3^- + 3\ H_2O \qquad (\textit{Nitrobacter}, \text{NOB})$$

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^-$$

$\rightarrow$ 0.21 $C_5H_7O_2N$ + 0.98 $NO_3^-$ + 1.041 $H_2O$ + 1.88 $H_2CO_3$ (whole)

According to this equation, the growth yield and oxygen consumption of *Nitrosomonas* and *Nitrobacter* are 0.15 mg-$O_2$ cell·mg-$NH_4^+$-$N^{-1}$ and 0.2 mg-$O_2$ cell·mg-$NO_2^-$-$N^{-1}$, and 3.15 mg-$O_2$· mg-$NH_4^+$-$N^{-1}$ and 1.11 mg-$O_2$· g-$NO_2^-$-$N^{-1}$, respectively. In addition, 7.07 mg (as $CaCO_3$) are required to oxidize 1 mg $NH_4^+$-N of ammonia. Thus, pH deterioration could be occurred in nitrification.

### 2.4.2 Denitrification

Denitrification is occurred by heterotrophic microorganisms under the anaerobic condition. These microorganisms utilized nitrate or nitrite as electron acceptor and organic carbon as electron donor to deoxidize nitrogen gas. Several kinds of denitrifying bacteria have been reported and its classified to $\alpha$ -*Proteobactreia* or $\beta$ -*Protobacteria*. Nowadays, methanol, acetate, glucose and ethanol have been widely used for electron donor. The equation that nitrate deoxidized to nitrogen gas by using methanol as electron donor is shown as below,

$NO_3^-$ + 1.08 $CH_3OH$ + 0.24 $H_2CO_3$
$\rightarrow$ 0.056 $C_5H_7O_2N$ + 0.47 $N_2$ + 1.68 $H_2O$ + $HCO_3^-$

From this equation, 2.47 mg $CH_3OH$ required to deoxidize 1 mg $NO_3^-$N.

### 2.4.3 Anammox

An anaerobic ammonia oxidation (Anammox) process was firstly found in 1995 from the denitrifying fixed fluidized bed reactor treating methane fermented effluent (Mulder et al., 1995). The Anammox reaction is occurred by bacteria belonging to phylum, *Plamctomycete* but until now Anammox bacteria has not been isolated. The Anammox bacteria can utilize ammonia under anaerobic condition without organic, thus Anammox process is pay attention to cost effective novel nitrogen removal process during this 20 years. This Anammox process can show by following equation

$NH_4^+$ + $NO_2^-$ $\rightarrow$ $N_2$ + $H_2O$

$CO_2$ + 2 $NO_2^-$ + $H_2O$ $\rightarrow$ $CH_2O$ + 2$NO_3^-$

Anammox process requires the pretreatment of oxidize approximately 50% of ammonia to nitrite (partial nitritation). Partial nitritation is one of the difficult issue for application of Anammox process in the practical scale.

Recently, Hien et al. (2017) report that application of oxygen limited autotrophic nitritation / denitrification (OLAND) process to latex wastewater treatment by using a reactor with rotating bio-carrier and sequencing batch regime and its performed high TN and COD removal efficiencies of 94% and 61%, respectively.

### 2.4.4 Oxygen transfer in trickling filter

The basic knowledge for oxygen transfer was summarized by Poonam et at. (2014). Oxygen transfer from air to liquid was proposed by Lewis and Whiteman on 1924. The basic equation of oxygen transfer rate is

$N = K_LA\ (C_S - C_L)$ ·································(1)

$N$ = Mass oxygen transferred per unit time (g-$O_2$/day)
$K_LA$ = The liquid film coefficient (g-$O_2$/day $m^2$)
$A$ = Interfacial area in transfer ($m^2$)
$C_S$ = Saturation of concentration of the liquid (mg/L)
$C_L$ = Oxygen concentration in bulk of the liquid (mg/L)

(1) is usually rewritten in consternation units by dividing by volume of the system

$N/V = K_L\ (A/V)\ (C_S - C_L) = KL\ a\ (C_S - C_L)$ ·························(2)

$a = A/V$ = interfacial area per volume ($m^2/m^3$)
$K_La$ = the overall coefficient of oxygen transfer (g-$O_2$/day $m^3$)

In aeration tank contained activated sludge, oxygen utilization rate at microorganism is shown by following equation

$dC_L/dt = K_La\ (C_{sw} - C_L) - r$

r: The rate of oxygen utilization of microorganisms
$C_{SW}$: saturation concentration of oxygen in the wastewater
$C_L$: The operating concentration of dissolved oxygen

The value of $dC_L/dt$ are obtained by plotting $C_L$ (measured by DO test) vs time and determining slope at the selected time intervals

The oxygen transferred is estimated under the standard conditions (SC), corresponding to a temperature of 20 ˚C and standard atom temperature.

Oxygen capacity (OC) (mg-$O_2$/hour) (unit volume) = dCL/dt = ($K_La$)20c (Cs-Co) = ($K_La$) 20 c CS

The fraction oxygen transferred to the water due to pass one meter cubic of expressed as oxygenation efficiency (E) of the diffuser system, which can be written as

E = OC. H/I

E: oxygen efficiency
OC: Oxygenation capacity
H: the liquid depth in the tank in meter
I: the aeration intensity or volumetric air flux per unit area of the tank

Trickling filter is one of the most promising systems, given its low operational cost, simple process and easy maintenance, the size of treatment facility. The concept for the reactor is based on a conventional trickling filter. Air dissolves into the wastewater as it flows down the DHS reactor, so there is no need for external aeration. Previous studies reported DHS reactor performed high process performance as well as conventional activated sludge process without external aeration. However, this oxygen transfer from atmosphere to sponge carrier has not been studied. Uemura et al. (2016) firstly examined (1) water distribution in the sponge media and (2) oxygen mass transfer from the atmosphere to water flowing down the sponge media. The sponge media used in this study was polyether-based urethane sponge (cell sizeR" 500μm, porosity=98%) called "G-3". The ratio of actual hydraulic retention time (HRT) to theoretical HRT was determined by measuring the actual HRT of the sponge media using a tracer, because the ratio was assumed to be a good index to measure the water distribution in the sponge media. The actual HRT/ theoretical HRT ratio was 67% at the G3 type sponge carrier (Figure 2.12). In addition, they used the oxygen transfer coefficient ($K_La$) to evaluate the ability of the sponge medial to supply oxygen to the microorganisms. As a result, the $K_La$ of G3 type sponge was 1.67 $min^{-1}$ and increased together with increase flow rate (Figure 2.13). The DHS reactor has high $K_La$ same as aerated reactor without external aeration.

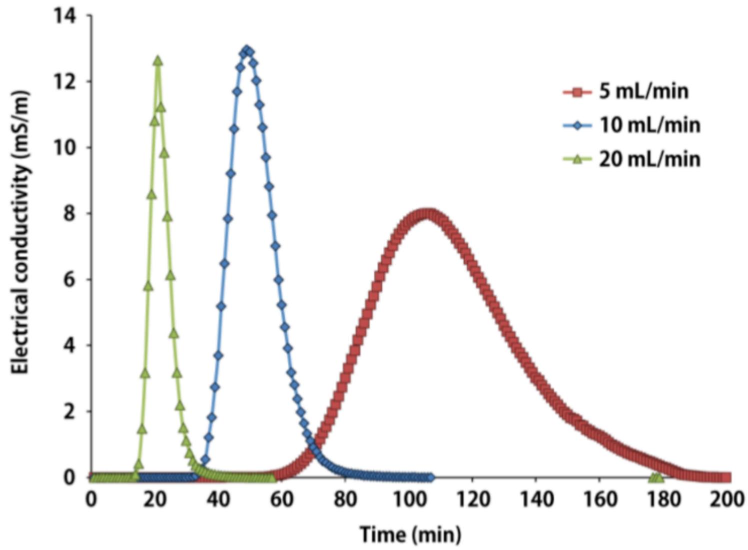


Fig. 2.12 Relationship between electrical conductivity and elapsed time for sponge media. (Uemura et al., 2015)

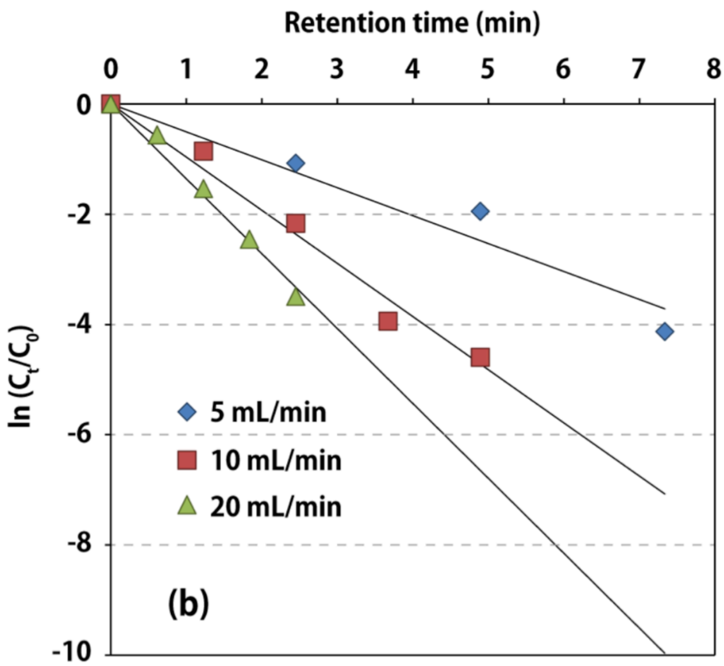


Figure 2.13 Calculation of $K_La$ for G3 sponge media (Uemura et al., 2015)

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Chapter 3

# Development of UASB-DHS system for Treating Natural Rubber Processing Wastewater

**3.1 Laboratory scale UASB-DHS system experiment for treatment of natural rubber processing wastewater**
**3.1.1 Introduction**
**3.1.2 Materials and Methods**
- **3.1.2.1 Raw wastewater**
- **3.1.2.2 System description and operational conditions**
- **3.1.2.3 Analytical methods**
- **3.1.2.4 Microbial community analysis**

**3.1.3 Results and Discussion**
- **3.1.3.1 Process Performance**
- **3.1.3.2 Microbial community analysis**

**3.1.4 Conclusion**

**Reference**

**3.2 Pilot scale UASB-DHS system experiment for treatment of natural rubber processing wastewater**
**3.2.1 Introduction**
**3.2.2 Materials and Methods**
- **3.2.2.1 Experimental setup and operational conditions**
- **3.2.2.2 Analytical methods**
- **3.2.2.3 16rRNA gene sequence**

**3.2.3 Results and Discussion**
- **3.2.3.1 Organic removal**
- **3.2.3.2 Nitrogen removal and greenhouse gas emissions**
- **3.2.3.3 Microbial community analysis of the UASB retained sludge**
- **3.2.3.4 Performance comparison of the ABR-UASB-ST-DHS system and other treatment systems for treating natural rubber processing wastewater**

**3.2.4 Conclusion**

**Reference**

### 3.1 Laboratory scale UASB-DHS system experiment for treatment of natural rubber processing wastewater

### 3.1.1 Introduction

Natural rubber is one of the most valuable agricultural products in Southeast Asian countries; however, the natural rubber processing industry generates large amounts of wastewater from several processes such as coagulation, centrifugation, lamination, washing, and drying. This wastewater contains high concentrations of organic compounds, nitrogen, and other contaminants. Natural rubber processing factories in Southeast Asian countries commonly utilize a combination of anaerobic–aerobic lagoon systems for treating natural rubber processing wastewater (Madhu et al., 1994; Saritpongteeraka and Chaiprapat, 2008; Mohammidi et al., 2010). Existing treatment systems have a high chemical oxygen demand (COD) removal efficiency of 65– 90% (Madhu et al., 1994). However, these systems require a large lagoon area, high operating costs (especially for surface aeration), and long hydraulic retention times (HRTs). In addition, improvements to the effluent water quality of existing treatment systems are required to comply with discharge standards (Nguyen and Luong, 2012).

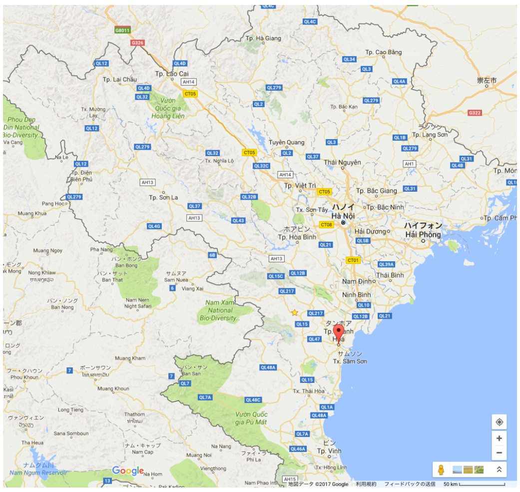
The upflow anaerobic sludge blanket (UASB) reactor is one of the most promising systems, given its high organic loading rate (OLR) capacity, low operational costs, and energy recovery in the form of methane for the treatment of different types of industrial wastewater (Lettinga, 1991). Previous studies have reported on the application of the UASB system to treat natural rubber processing wastewater (Mohammidi et al., 2010; Nguyen and Luong, 2012, Jawjit and Liengchaeranist, 2010). It was determined that natural rubber particles in the wastewater had a negative effect on the anaerobic biological process; therefore, the development of a pretreatment system to remove natural rubber particles is essential. The effluent from a UASB reactor treating high-strength industrial wastewater still contains high concentrations of organic compounds and nutrients. Thus, an aerobic treatment system has been typically applied as a post-treatment to remove residual organic matter and achieve effluent standards (Chan et al., 2009). The down-flow hanging sponge (DHS) is one of the most effective aerobic treatment systems used as a post-treatment for UASB reactors to process different types of industrial wastewater (Onodera et al., 2013; El-Kamah et al., 2011; Watari et al., 2015).

In this study, a laboratory-scale experiment was conducted to develop an appropriate wastewater treatment system for natural rubber processing wastewater consisting of a baffled reactor (BR), a UASB reactor, and a DHS reactor (a BR–UASB–DHS system). In addition, 16S rRNA gene sequencing of microorganisms in the retained sludge in the UASB reactor was undertaken to identify the microbial communities present.

### 3.1.2 Materials and Methods
#### 3.1.2.1 Raw wastewater

Raw wastewater was collected from the coagulation process in a natural rubber factory producing Standard Vietnamese Rubber in Thanh Hóa Province, Vietnam (Figure 3.1). The wastewater was collected from 2 month one time and kept in the refrigerator until used. The characteristics of the raw wastewater were as follows: 23,200 ± 6,000 mg-COD·L$^{-1}$ total COD; 19,700 ± 3,100 mg-COD·L$^{-1}$ soluble COD; 12,000 ± 3,100 mg·L$^{-1}$ biochemical oxygen demand (BOD); 1,450 ± 580 mg·L$^{-1}$ total suspended solids (TSS); 1,300 ± 350 mg·L$^{-1}$ volatile suspended solids (VSS); 4,000 ± 1,100 mg-COD·L$^{-1}$ acetate; 4,500 ± 2,600 mg-COD·L$^{-1}$ propionate; and 1,070 ± 100 mg-COD·L$^{-1}$ other volatile fatty acids (VFAs). The concentrations of raw natural rubber wastewater was fluctuated by the operational condition of natural rubber processing factory (eg: production amount, used chemical amount).

(A)



(B)


(C)

Figure 3.1 (A) Location of Thanh Hoa province, Vietnam, (B) Thanh Hoa Rubber Factory, (C) Coagulation process in natural rubber sheet producing process

### 3.1.2.2 System description and operational conditions

Figure 3.2 shows a schematic diagram of the laboratory- scale BR–UASB–DHS treatment system. The laboratory- scale treatment system was operated in Hanoi University of Science and Technology, Vietnam. The BR was constructed from polyvinyl chloride pipes with a working volume of 43.7 L. The UASB reactor had a working volume of 9 L, and was equipped with a gas–solid separator, a mixer, and five sampling ports 1–5 (5.5, 20, 35, 50, and 65 cm from the base of the reactor, respectively). The UASB reactor was inoculated with sludge obtained from an anaerobic digester treating livestock manure in Bac Ninh Province, Vietnam. The temperature of the UASB reactor was maintained at 35°C by the circulation of heated water, originating in a water bath and moving through the water jacket of the UASB column. The DHS reactor was equipped with a distributor and was 80 cm in height. The reactor and sponge volumes were 40 and 8.4 L, respectively. The sponge carrier consisted of a cubic sponge (33 mm × 33 mm × 33 mm) packed inside a plastic net- ring 33 mm in diameter and 33 mm long. The DHS reactor was operated at room temperature. The raw wastewater was diluted to the appropriate total COD concentration using tap water and was used as the influent of the system. A summary of the initial operational conditions for the two operating phases is provided in Table 3.1. During phase 2, the influent total COD concentration was adjusted to 8,500 mg-COD·L$^{-1}$ to attain the same conditions as real natural rubber processing wastewater.

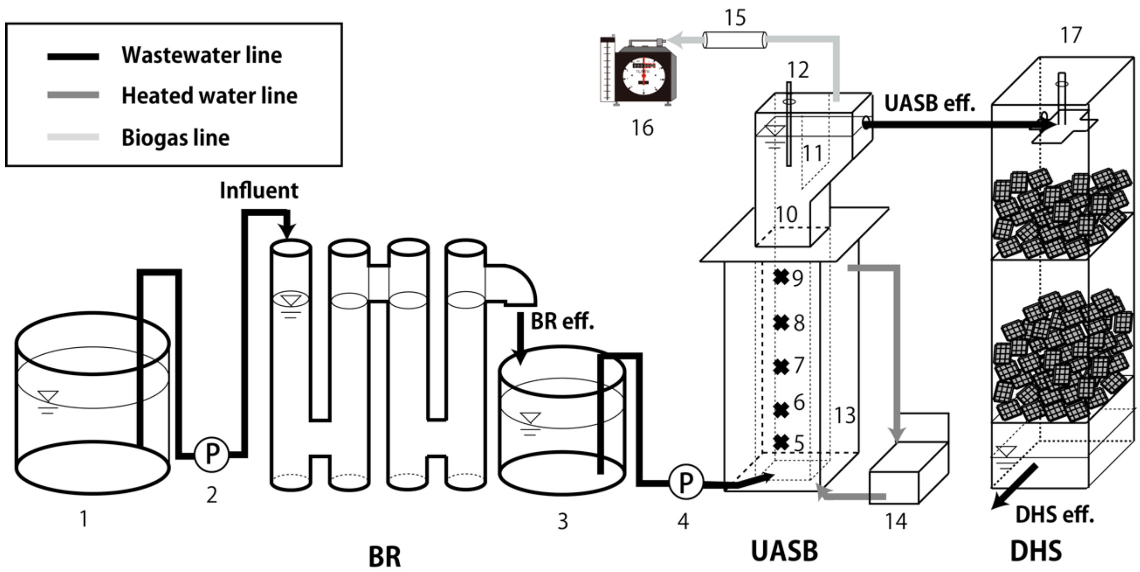



Figure 3.2 Schematic diagram of the baffled reactor (BR), upflow anaerobic sludge blanket (UASB), and downflow hanging sponge (DHS) combined system. (1) Substrate reservoir, (2) pump, (3) pretreatment tank, (4) pump, (5–8) sampling ports, (9) UASB column, (10) Gas solid separator, (11) mixer, (12) desulfurizer, (13) gas meter, and (14) distributor.

Table 3.1 Summary of the initial operational conditions for the two operating phases

| | Day | Influent COD | OLR | |
|---|---|---|---|---|
| | | | UASB | DHS |
| | | mg-COD·L$^{-1}$ | kg-COD·m$^{-3}$·day$^{-1}$ | kg-COD·m$^{-3}$·day$^{-1}$ |
| Phase 1 | 1–44 | 4,000 | 6.4 | 0.7 |
| Phase 2 | 45–125 | 8,500 | 12.0 | 1.3 |

### 3.1.2.3 Analytical methods

Samples of the influent and the BR, UASB, and DHS effluents were collected for routine analysis. Temperature was measured onsite. pH of each samples were determined by using portable pH meter (AS-212, Horiba). Total COD, soluble COD, and total nitrogen (TN) were determined using a HACH water quality analyser (DR-2800, HACH). The soluble COD was determined after filtering using a 0.45-μm glass-fibre filter (GB-140, ADVANTEC). The solid COD was calculated as the difference between total COD and soluble COD. VFA concentrations were determined using a gas chromatograph equipped with a flame ionization detector (GC-2014, Shimadzu). Ammonia, nitrate, and nitrite levels were measured using an ion chromatograph (LC- 20AD, Shimadzu). TSS and VSS were analysed using standard methods (APHA, 2015). Biogas production was measured using a wet gas meter (WS-1A, Shinagawa) after desulphurization. Biogas composition was analysed using a gas chromatograph equipped with a thermal conductivity detector (GC-8A, Shimazdu). The methane recovery rate was calculated from the methane gas production and the removed total COD of the UASB reactor (the difference between BR effluent and UASB effluent). The COD mass balance was calculated from the average concentrations of acetate, propionate, other soluble COD and solid COD in the influent, BR effluent, and UASB effluent as well as the methane recovery rate during phase 2.

### 3.1.2.4 Microbial community analysis

The retained sludge from the UASB reactor on day 100 was collected from the sampling ports, gently washed with phosphate-buffered saline to remove retained substrate that would inhibit polymerase chain reaction (PCR) amplification, and stored at −20°C. DNA was extracted using a Fast DNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. PCR amplification was performed using universal primers for whole bacteria and archaea for 515F (5′-GTG CCA GCM GCC GCG GTA A-3′ ) and 806R (5′ -GGA CTA CHV GGG TWT CTA AT-3′) at an annealing temperature of 50°C (Turner et al., 1999; Walters et al., 2011) PCR products were purified using a MinElute PCR purification kit (QIAGEN). Massive parallel 16S rRNA gene sequencing was carried out using Miseq reagent kit v.2 with the Miseq system (Illumina). Sequence data analysis was conducted using QIIME software package v.1.7.0. (Caporaso et al., 2010). Operational taxonomic units (OTU) were picked at 97% sequence identity, with chimeric sequences removed using ChimeraSlayer (Haas et al., 2011). Taxonomic classification was determined using the Greengenes database v.13_5 (available at http://qiime.wordpress.com/2013/05/20/greengenes-13_5/). The relative stains of the representative sequences were identified using a web-based BLAST search in the national center for biotechnology information database.

Fluorescent *in situ* hybridization (FISH) was conducted based on the method described by Sekiguchi et al. (1999). The 16S rRNA-targeted oligonucleotide probes used in this study were WWE1–1181 (5′-CTTCCTCTGCGTTGTTAC -3′) for phylum WWE1 and EUB338 (5′-GCTGCCTCCC GTAGGAGT-3′) for bacteria (Chouari et al., 2005; Amann et al., 1990). For double staining of the enrichment cultures, Cy3-labelled WWE1-1181 and Alexa 488-labelled EUB338 probes were simultaneously used. Formamide was added at a final concentration of 10% to each probe to ensure optimal hybridization stringency. An epifluorescence microscope BX-53 (Olympus) was used for observation.

### 3.1.3 Results and Discussions

#### 3.1.3.1 Process Performance

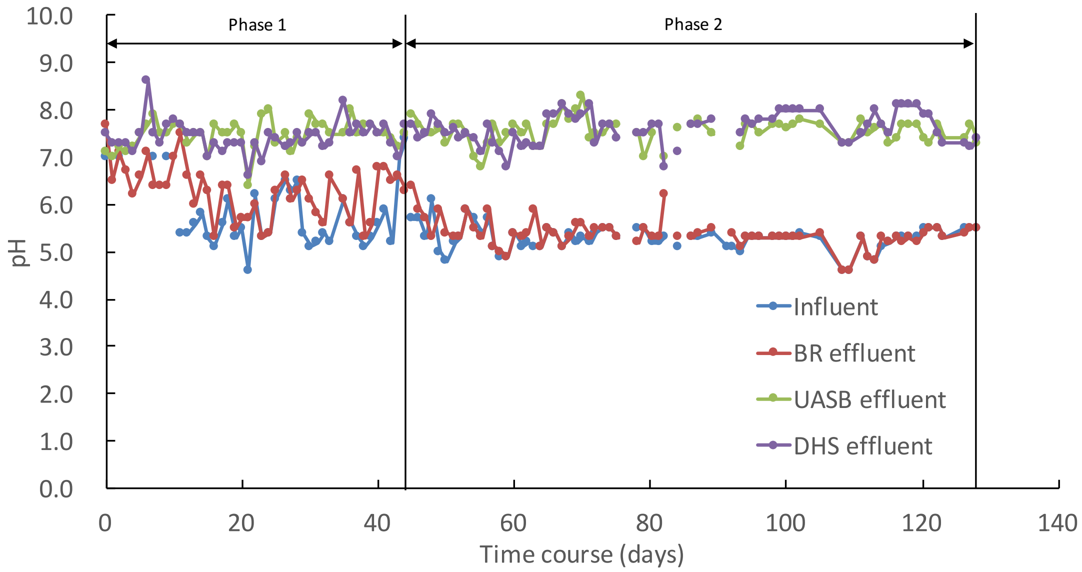
The system was started up with an influent total COD of 2,000 mg-COD/L. The OLRs of the UASB and DHS reactors were increased stepwise by increasing the influent total COD concentration. The process performance of the treatment system for phases 1 and 2 is summarized in Figure 3.3 and Table 3.2. The system showed good performance in the start-up period of phase 1 (days 1–45), and was operated for a total of 126 days. The influent of pH was 5.8 ± 0.7 and 5.3 ± 0.3, respectively and the proposed BR-UASB-DHS system performed without pH adjustment. Overall, high total COD removal of 98.6 ± 1.2% and TSS removal of 98 ± 1.4% were achieved with an HRT of 42.2 h during phase 2. Figure 3.5 shows the COD mass balance of the influent reactor, BR, and UASB reactor during phase 2.

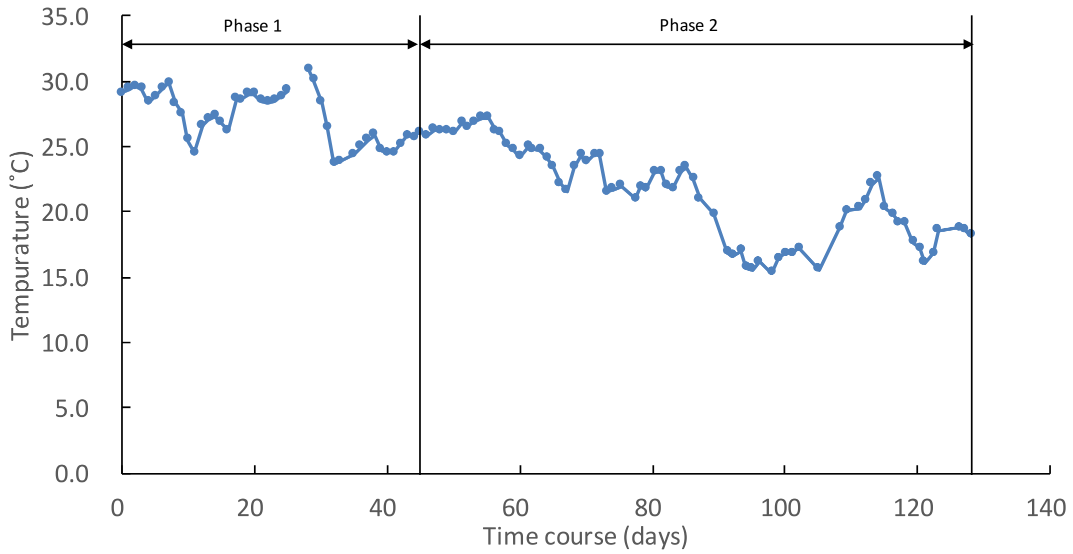
The BR acted as a pretreatment system for the removal of insoluble organic matter such as residual natural rubber particles. Coagulated residual natural rubber particles in the influent wastewater were observed at the surface of the BR. The BR steadily removed 42.3 ± 34.5% of TSS and 72.4 ± 38.2% of VSS during phase 2. Similarly, solid COD was removed and the concentrations of acetate and propionate increased (Figure 3.4). During the entirety of the experiment, accumulation of sludges was also observed in the BR. Therefore, accumulated sludges could be performed acidification of wastewater. The accumulated natural rubber particles were never removed from the BR. These results suggested that the BR acted as both a trapping tank for the residual rubber particles and an acidification tank.

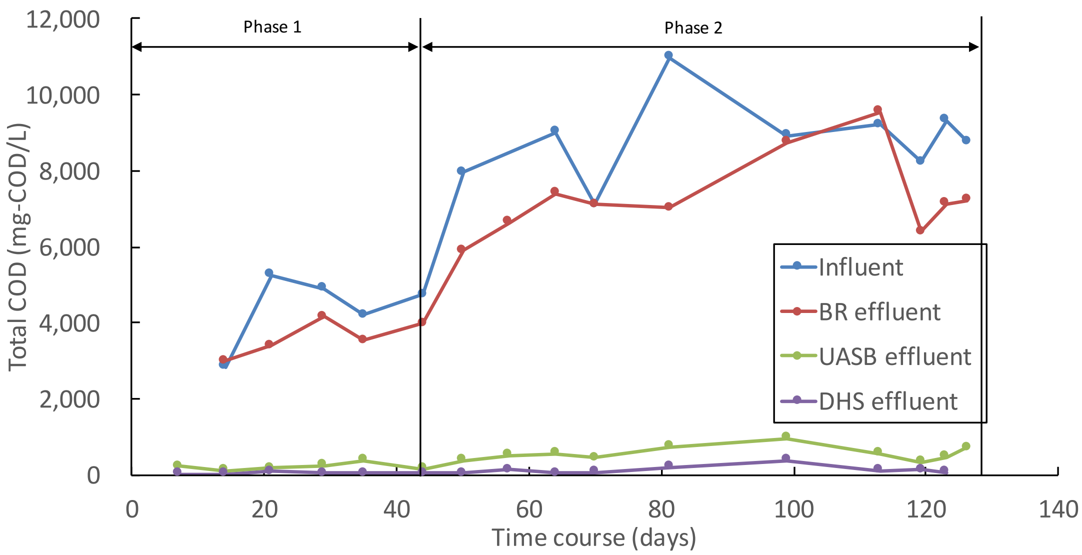
The UASB reactor removed most of the organic matter in the influent wastewater and recovered methane in the system. During phase 2, the total COD concentrations of the UASB influent and effluent were 7,010 ± 1,430 mg-COD·$L^{-1}$ and 530 ± 220 mg- COD·$L^{-1}$, respectively. The UASB reactor also performed at a high total COD removal efficiency of 92.7 ± 2.3% with an OLR of 12.2 ± 6.2 kg-COD·$m^{-1}$·$day^{-1}$. The amount of methane gas produced was 20.1 ± 4.5 L·$day^{-1}$ and 30.3 ± 9.9 L·$day^{-1}$ for phases 1 and 2, respectively. The composition of the biogas after the removal of hydrogen sulphide was 72.4 ± 15.6% methane, 27.4 ± 15.7% carbon dioxide, and 0.2 ± 0.5% nitrogen during phase 2. The methane recovery rate, calculated from the removed total COD, was 93.3 ± 19.3% for phase 2. The COD removal efficiency of the reactor was higher than previously reported values for other anaerobic reactors treating industrial wastewater (e.g. Saripongteeraka and Chaiprapat, 2008). High-level COD removal efficiency and methane recovery rates are thought to result from the efficient solid organic removal and acidification of the wastewater by the BR. A previous study reported that high concentrations of solid organic matter, such as natural rubber particles, had a negative effect on the performance of a UASB reactor treating natural rubber processing wastewater (Nguyen and Long, 2012). Therefore, the BR could be used as a pretreatment system for the UASB reactor.

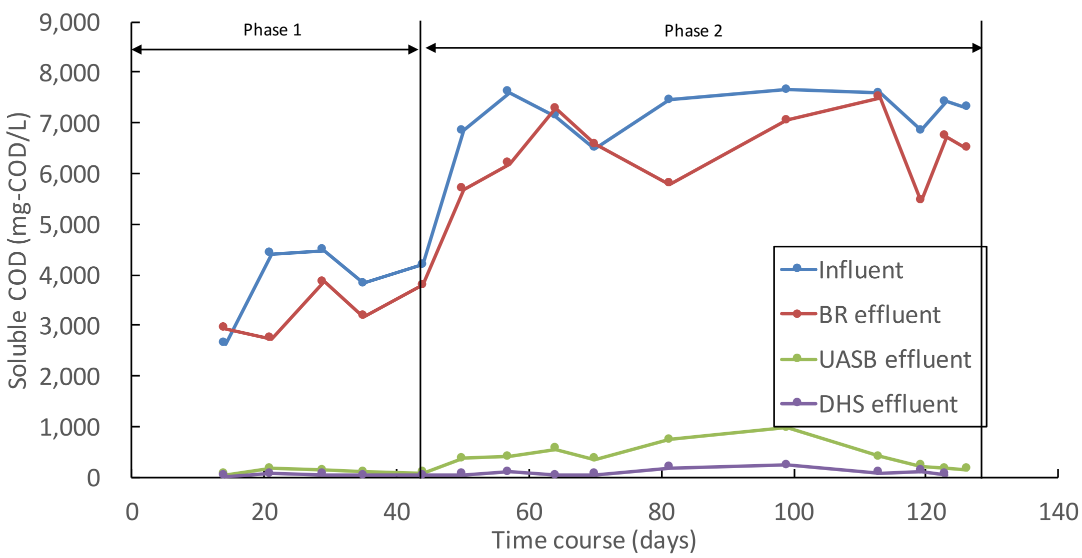
The DHS reactor removed residual organic matter in the UASB effluent and decreased TN without aeration. The DHS reactor performed at 77.8 ± 10.7% total COD removal efficiency and 86 ± 8.5% TSS removal efficiency during phase 2. In addition, approximately 20% of the ammonia was converted to nitrate and nitrite. Such low nitrification maybe high COD concentration in the DHS influent and oxygen was mainly consumed for oxidizing organic compounds. In addition, the temperature of DHS was not controlled and 21.6 ± 3.6˚C during phase 2 (Figure 3.3). The activity of nitrifying bacteria strongly affects to the temperature. The DHS reactor performed at 35.9 ± 20.5% TN removal efficiency. A previous study reported that the inner section of the sponge media of the DHS reactor is anaerobic, so denitrification is likely to have occurred to some degree (Machdar et al., 2000). Sponge replacement and excess sludge removal were not performed during the experimental period. The high-level COD removal efficiency suggests that the DHS reactor can be an effective post-treatment system for organic removal but further modifications, such as an increase in sponge volume, would be necessary for ammonia removal.

Existing systems for the treatment of natural rubber processing wastewater, such as the combined anaerobic–aerobic treatment system, require HRTs of longer than 30 days and have high operational costs for the aeration of the aerobic pond (Nguyen and Luong, 2012). The BR–UASB–DHS system can decrease the HRT; consequently, the land requirements of the system are smaller than those of currently used treatment systems. However, the total COD concentration of the DHS effluent was 120 ± 100 mg-COD·L$^{-1}$, which exceeded the 50 mg- COD·L$^{-1}$ discharge industrial standard for the natural rubber processing industry in Vietnam. During phase 1, the COD concentration of the DHS effluent satisfied the industrial effluent standard for influent total COD, which is 220 ± 120 mg-COD·L$^{-1}$ or an OLR of 0.67 ± 0.47 kg-COD·m$^{-1}$·day$^{-1}$. This result shows that the DHS reactor could process approximately 0.7 kg-COD·m$^{-1}$·day$^{-1}$ to achieve industrial effluent standards. In addition, ammonia remained in the DHS effluent and nitrogen removal was not adequate. An increase in the HRT of the DHS and the addition of a denitrification tank are required for improvement of nitrogen removal. Hence, the optimal operational conditions should be assessed and demonstrated in future studies.




Phase 1
Phase 2
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Tempurature (°C)
0
20
40
60
80
100
120
140
Time course (days)


Phase 1
Phase 2
12,000
10,000
8,000
6,000
4,000
2,000
0
Total COD (mg-COD/L)
Influent
BR effluent
UASB effluent
DHS effluent
0
20
40
60
80
100
120
140
Time course (days)


Phase 1
Phase 2
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Soluble COD (mg-COD/L)
Influent
BR effluent
UASB effluent
DHS effluent
0
20
40
60
80
100
120
140
Time course (days)

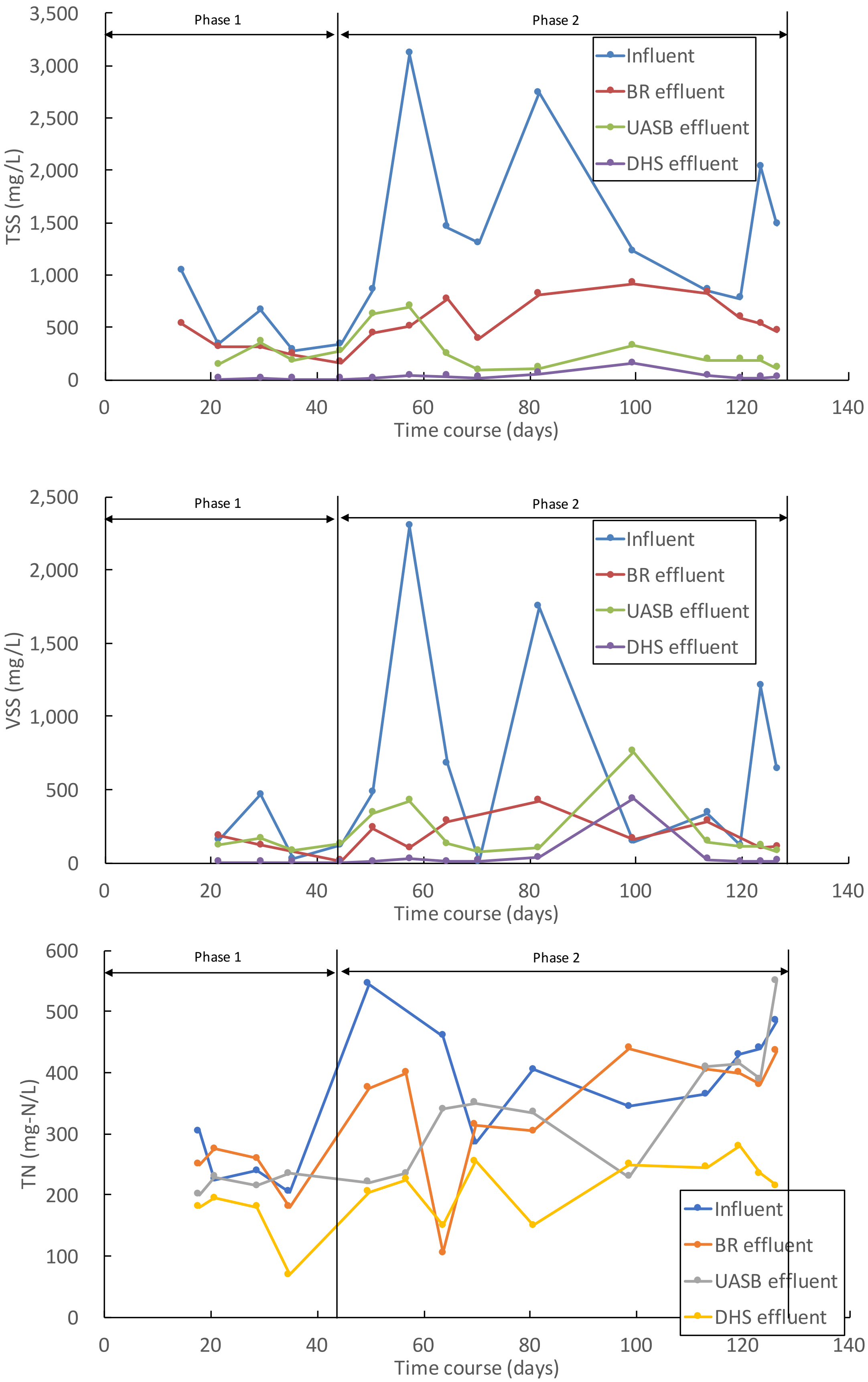


Figure 3.3 Time course of pH, temperature, Total COD, soluble COD, TSS, VSS and TN,

Table 3.2 Summary of process performance of the treatment system.

| | | pH | Total-COD mg-COD/L | Solube-COD mg-COD/L | Acetate mg-COD/L | Propionate mg-COD/L | TSS mg/L | VSS mg/L | TN mg-N/L | Ammonia mg-N/L | Nitrate mg-N/L | Nitrite mg-N/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phase 1 (day 1 to 44) | Influent | 5.8±0.7 | 4,300±1,070 | 3,840±850 | 1,108±482 | 1,240±620 | 580±350 | 220±230 | 240±43 | 160±61 | N.D. | N.D. |
| | BR eff. | 6.3±0.6 | 3,520±480 | 3,180±490 | 1,121±85 | 1,480±193 | 350±130 | 150±50 | 240±42 | 190±77 | N.D. | N.D. |
| | UASB eff. | 7.5±0.3 | 220±120 | 110±53 | 18±25 | 26±3 | 220±110 | 120±40 | 220±16 | 160±41 | N.D. | N.D. |
| | DHS eff. | 7.4±0.3 | 50±25 | 50±22 | N.D. | 1,2±1.7 | 4.7±1.2 | 4.4±0.7 | 160±58 | 160±41 | 47±9.6 | N.D. |
| Phase 2 (day 45 to 126) | Influent | 5.3±0.3 | 8,430±1,640 | 6,960±990 | 2,070±395 | 2,300±370 | 1,470±850 | 577±470 | 420±78 | 200±58 | N.D. | N.D. |
| | BR eff. | 5.4±0.3 | 7,010±1,430 | 6,240±1,040 | 2,200±360 | 2,280±320 | 580±230 | 170±100 | 360±99 | 190±77 | N.D. | N.D. |
| | UASB eff. | 7.6±0.3 | 530±220 | 400±270 | 62±69 | 43±29 | 270±200 | 110±26 | 350±100 | 160±41 | N.D. | N.D. |
| | DHS eff. | 7.6±0.3 | 120±100 | 100±68 | 9.7±9.9 | 7.3±9.3 | 36±41 | 13±7.2 | 220±43 | 100±96 | 28±25 | 9.4±11 |

N.D : Not detected

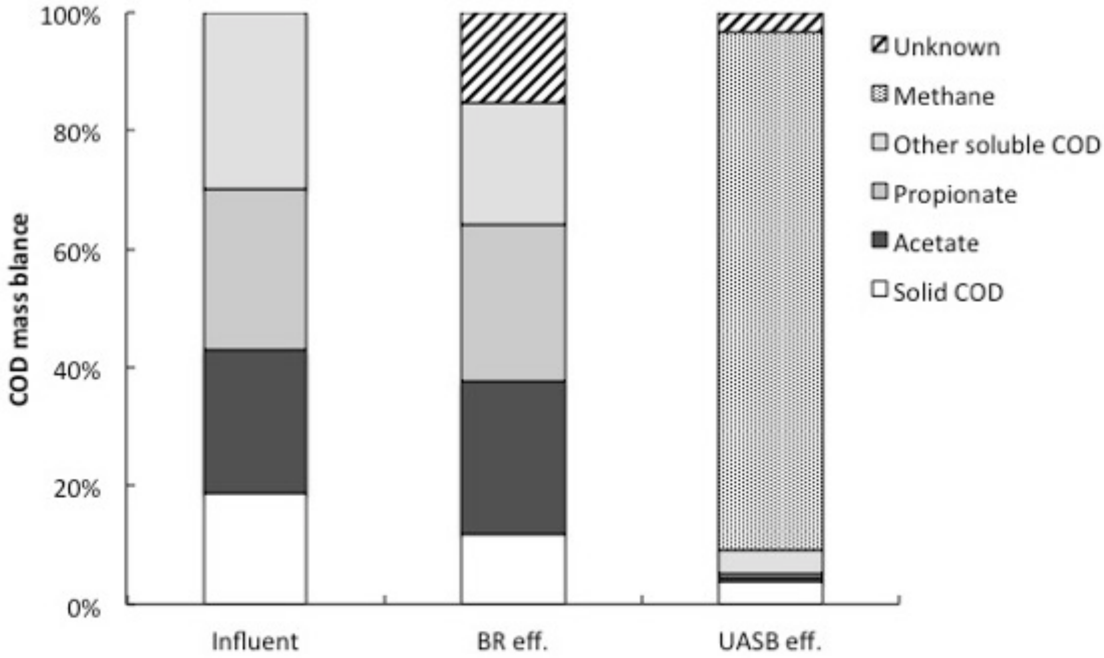



Figure 3.4 COD mass balance of the influent, BR effluent, and UASB effluent during phase 2

### 3.1.3.2 Microbial community analysis

The microbial community structures of the seed and retained sludges obtained from sampling ports 1–5 of the UASB reactor on day 100 were analysed based on the 16S rRNA gene sequence. A total of 154,826 sequencing reads were determined, and the median sequence length of the 16S rRNA genes sequencing was 251 bp. Approximately 20,000–30,000 sequencing reads were analysed per sample, and 1700–2000 OTUs per sample were detected at 97% identity.

Figure 3.5 shows the microbial community compositions of the seed sludge and the UASB reactor at the phylum level. In the seed sludge, phylum *Proteobacteria* predominated. Among phylum *Proteobacteria*, the denitrifying bacteria *Commamonas* spp. and *Dechloromonas* spp. were detected at 1.6% and 3.8% of total sequence reads, respectively. The principal microbial groups in the UASB retained sludges were the phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, WWE1, and *Euryarchaeota*. These microbial groups have been frequently and commonly encountered in methanogenic granular sludges (Watari et al., 2015; Sekiguchi, 2006). The most abundant bacterial phylum was *Bacteroidetes,* with 5.3–8.2% of total sequence reads in the UASB retained sludges. These bacteria were also detected in the seed sludge, with 18.5% of total sequence reads. The most closely related species of the most predominant phylotype was *Anaeroflexus maritimus*, with 92% 16S rRNA gene sequence similarity. Chin et al. reported that *A. maritimus* was isolated from paddy field soil and oxidized organic matter to acetate and propionate (Chin et al., 1999). Moreover, the influent of the UASB reactor contained large amounts of VFAs. Hence, fatty-acid-oxidizing bacteria, *Syntrophomonas* spp. and *Syntrophus* sp., were largely detected in the bottom and middle of the UASB reactor. Uncultured *Cloacamonaceae* species, belonging to the uncultured phylum WWE1, were also found in abundance in the UASB reactor, particularly in the upper part of the UASB reactor (Figure 3.5). WWE1 is an uncultured bacterial phylum that is frequently detected in mesophilic anaerobic digesters (Narihiro et al., 2009). Figure 3.6 (a) and (b) shows the detection of WWE1 bacteria in the UASB reactor retained sludge obtained from port 5. The FISH observations demonstrated that the cells targeted by a WWE1-specific probe had small cocci or short rods, a morphology consistent with previous reports (Chouari et al., 2005; Limam et al., 2014). In all the cells, members of the WWE1 showed a positive hybridization signal with the EUB338 mix. The uncultured bacteria belonging to WWE1 dominated the UASB reactor. It was thought that the bacteria may have a role in the degradation of organic compounds in wastewater. The genomic analysis of '*Candidatus* Cloacamonas acidaminovorans', a member of WWE1, showed it to utilize several sugars and amino acids (Pelletier et al., 2008). It was also suggested that '*Ca* Cloacamonas acidaminovorans' might be a syntrophic propionate-oxidizing bacterium (Limam et al., 2014). Recent reports have demonstrated that members of the WWE1 are involved in cellulose hydrolysis and the utilization of fermentation products (Pellertier et al., 2008). Several metabolic functions of WWE1 members have been suggested, but further research is needed to elucidate the function of WWE1 bacteria in the UASB reactor for the treatment of natural rubber processing wastewater.

For archaea, only 1.7% of total sequence reads of phylum *Euryarchaeota* was detected in the seed sludge. After 100 days operation, phylum *Euryarchaeota* increased to 9.4–13.7% in the UASB retained sludge. In the UASB retained sludge, the acetate-utilizing methanogen *Methanosaeta* sp. was predominantly detected, with abundances of 2.3–3.8%. *Methanosaeta* sp. is frequently found in methanogenic sludge treating industrial wastewater and has a role in the final step in the anaerobic bioconversion of organic compounds to methane (Narihiro et al., 2009a; Narihiro et al., 2009b). Narihiro et al. (2009a) reported that the population of *Methanosaetace* phylotypes was always high in reactors with a good propionate removal capability. On the other hand, the hydrogenotrophic methanogen *Methanolinea* sp. was also detected at abundances of 1.2–3.2% in the sludge. *Methanolinea* sp. is commonly present in high-salinity conditions (Kuroda et al., 2014). The natural rubber processing wastewater contained a high concentration of sodium (e.g. the sodium concentration of influent during phase 2 was 671 ± 105 mg/L). Thus, *Methanolinea* sp. could predominate in the UASB reactor. These results suggest that the archaean community of the UASB reactor may be capable of treating natural rubber processing wastewater.

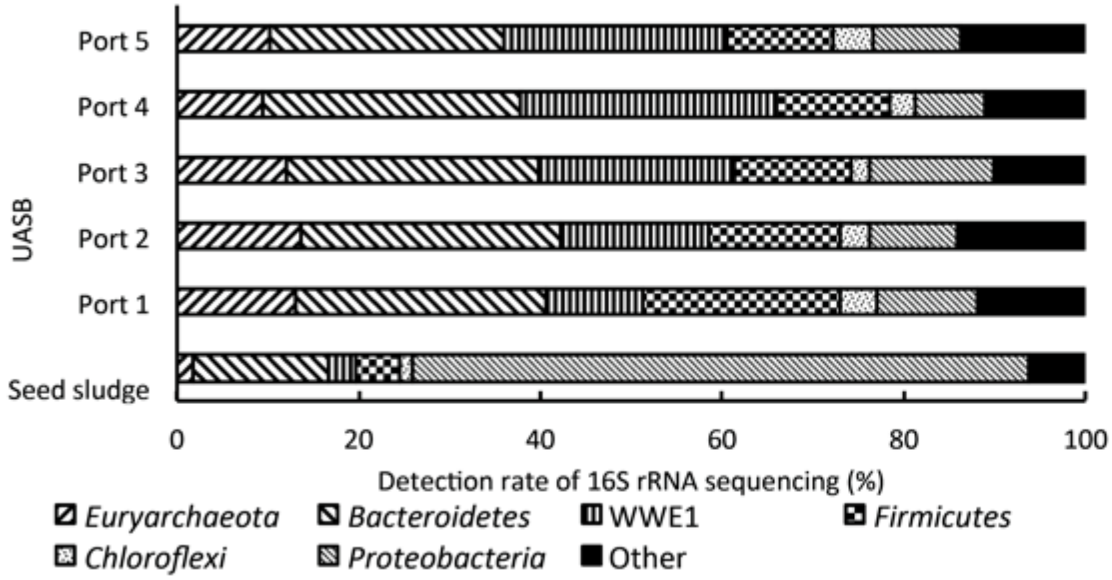



Figure 3.5 Phylum-level microbial community compositions of the seed sludge and the UASB reactor.

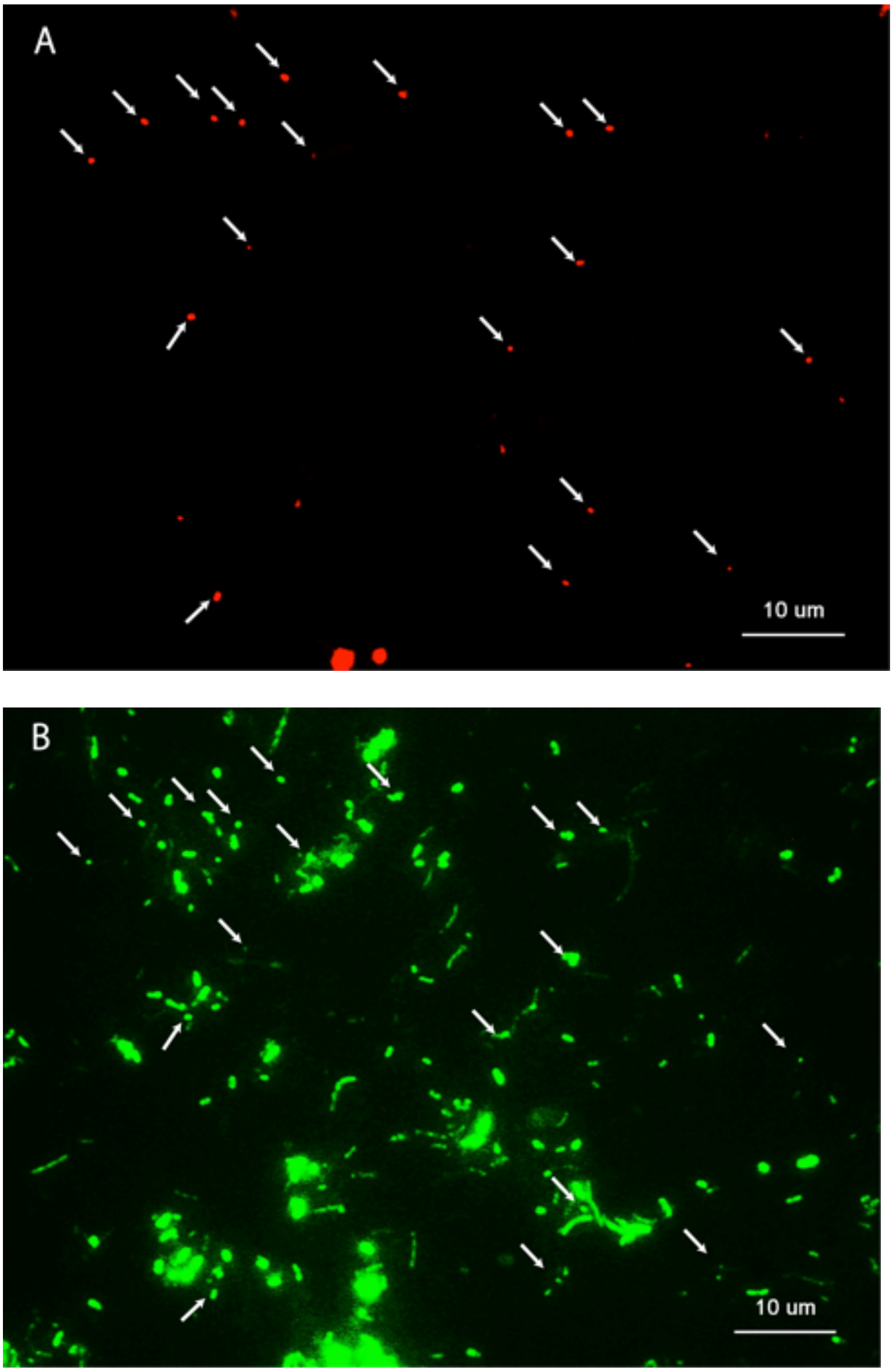



Figure 3.6 *In situ* detection of WWE1 bacterial cells in the retained sludge in the UASB reactor. Hybridization with Cy3-labeled WWE1-1181 probe (A) and Alexa 488-labeled EUB338 probe (B).

### 3.1.4 Conclusion

A laboratory-scale experiment demonstrated that the pro- posed BR–UASB–DHS system can efficiently treat natural rubber processing wastewater. The BR showed efficient solid organic removal efficiency and acidification of wastewater. The UASB reactor performed high-level total COD removal at 92.7 ± 2.3% with an OLR of 12.2 ± 6.2 kg-COD/m·day and 93.3 ± 19.3% methane recovery. The DHS reactor was an effective post- treatment system for the removal of residual organic matter. Microbial community analysis showed that uncultured bacteria belonging to phylum *Bacteroidetes* and candidate division WWE1 were the dominant bacteria in the UASB reactor. This finding suggested that these bacteria may have a role in organic matter degradation. These results demonstrate the great potential of the BR–UASB– DHS system for natural rubber processing wastewater treatment.

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### 3.2 Pilot scale UASB-DHS system experiment for treatment of natural rubber processing wastewater

#### 3.2.1 Introduction

Natural rubber is an important material because of its high strength compared to synthetic rubber. The production process of natural rubber from rubber latex discharges a large amount of wastewater containing high concentrations of organic compounds and nitrogen (e.g. 25 $m^3$ of wastewater is generated per ton of ribbed smoke sheet rubber production) (Nguyen and Luong, 2012). The combination of anaerobic and aerobic ponds is widely applied to treat this type of industrial wastewater due to its low construction and operational costs (Mohammadi et al., 2010; Nguyen and Luong, 2012). This conventional treatment system can remove more than 95% of biochemical oxygen demand (BOD) (Mohammadi et al., 2010). However, the system requires a large construction area, long hydraulic retention times (HRTs), and improvements in the effluent water quality to comply with discharge standards. In addition, the anaerobic pond emits not only methane but also nitrous oxide that has a higher global warming potential (Tanikawa et al., 2016a).

An upflow anaerobic sludge blanket (UASB) reactor is one of the most promising systems that can perform at high organic loading rates (OLR) with low operational costs and high energy recovery potential (in the form of methane) (van Lier et al., 2015). Several previous studies have reported the treatment of natural rubber processing wastewater by UASB reactors (Boonsawang et al., 2008; Jawjit and Liengcharernsit, 2010; Mohammadi et al., 2010; Nguyen and Luong, 2012, Watari et al., 2016a, Tanikawa et al., 2016b, Thanh et al., 2016). Most of the studies were conducted concentrated latex wastewater discharged from recovered skim latex process by using sulfuric acid in Thailand and Malaysia (Boonsawang et al., 2008; Jawjit and Liengcharernsit, 2010, Tankaiwa et al., 2016b). The natural rubber processing wastewater in these countries did not contain a large number of residual rubber particles, but it still had a large impact on aquatic environments. Acetic acid and/or formic acid have been typically used for the coagulation process in Vietnam because they have a lower impact on the environment compared to sulfuric acid. However, the natural rubber processing wastewater from the Vietnamese factories contains a large amount of residual rubber particles and these particles have a negative effect on the UASB reactor due to the accumulation of floating particles (Nguyen and Loung, 2012; Watari et al., 2016a). Our previous study reported that the UASB reactor achieved high chemical oxygen demand (COD) removal and energy recovery in the form of methane when combined with a pre-treatment anaerobic baffled reactor (ABR) (Watari et al., 2016a).

Anaerobic treatment systems require post-treatment because their effluents contain organic compounds and nitrogen (Chan et al., 2009). Conventional activated sludge process has been commonly applied as a post-treatment option for anaerobic treatment systems for natural rubber processing wastewater (Nguyen and Luong, 2012; Watari et al., 2016b). Although this existing post-treatment system has shown high residual organics removal, total nitrogen (TN) and ammonia removal is not sufficient for the required effluent standards. Moreover, the cost of electricity and disposal of excess sludge are great concerns for the industries.

A down-flow hanging sponge (DHS) reactor is a trickling filter using sponge media and has been developed as a low-cost treatment system especially for sewage treatment in developing countries (Tawfik et al., 2006; Tandukar et al., 2007; Okubo et al., 2016). The DHS reactor has been applied to the post-treatment of anaerobic treatment systems for natural rubber processing wastewater (Watari et al., 2016a, 2016b; Tanikawa et al., 2016b). The highlight of the DHS reactor is the retention of a large amount of sludge inside the sponges, contributing to high organic removal rates (Tandukar et al., 2007).

Previous studies reported that 16S rRNA gene-based microbial community structure of anaerobic reactors treated natural rubber processing wastewater by using cloning and massively parallel sequencing (Watari et al., 2016a; Tanikawa et al., 2016a, 2016b). The dominant archaea in the open-type anaerobic system that operated with low OLR was hydrogen-utilizing archaeal species of *Methanospirillum* (Tanikawa et al., 2016a). On the other hand, acetate-utilizing archaea *Methanosaeta spp*. dominated the UASB reactor that operated with high OLR (Watari et al., 2016a).

Recently, our research team reported that the laboratory scale UASB-DHS system is an energy efficient treatment system for natural rubber processing wastewater taken from north of Vietnam (Watari et al., 2016a). However, the process performance of the pilot scale treatment system was largely different from the laboratory scale treatment system. Moreover, our previous survey reported the existing open-type anaerobic treatment system that treats natural rubber processing wastewater in South Vietnam and emits substantial amounts of greenhouse gases (GHGs) (Tanikawa et al., 2016a). Therefore, the development of an effective closed treatment system for natural rubber processing wastewater is necessary.

In this study, we installed a pilot scale UASB-DHS system combined with an ABR and a settling tank (ST) in a natural rubber processing factory in South Vietnam. We investigated the process performance of the proposed system for 267 days. In addition, GHG emissions from the proposed system were evaluated. We also identified the microbial community structure of the UASB reactor in order to understand its relationship with the process performance.

### 3.2.2 Materials and Methods
#### 3.2.2.1 Experimental setup and operational conditions

Figure 3.7 shows the schematic diagram of the pilot scale natural rubber processing wastewater treatment system installed at the Rubber Research Institute of Vietnam, Binh Duong, Vietnam. The factory produced 1,000 t·year$^{-1}$ of ribbed smoked sheet (RSS) and discharged at 10 m$^3$·t$^{-1}$ –RSS of production. The system consisted of an ABR (76.5 m$^3$), a substrate reservoir (5 m$^3$), a UASB reactor (3 m$^3$), an ST (1 m$^3$), and a DHS reactor (2 m$^3$) with an effluent recirculation. The full-scale ABR comprised 60 compartments separated by baffled walls. Some of the wastewater was collected from the 17$^{th}$ compartment of the ABR as the UASB influent (ABR effluent) by using the submersible pump at 4 times a day. The tanks of the substrate reservoir, UASB reactor, ST, and the DHS reactor were bought ready-made. The UASB reactor and ST equipped with a distributor were built using polyvinyl chloride (PVC) pipes. The UASB reactor was inoculated with 900 L of dewatered aerobic sludge obtained from another natural rubber processing factory in Thai Ninh Province, Vietnam since anaerobic sludge was operated 186 days before starting this experiment, thus, was not available in the study location. The media in the DHS reactor were sponge cubes of polyurethane forms (33 mm × 33 mm × 33 mm) inserted in a net ring packed randomly. The sponge volume of the DHS reactor was 0.84 m$^3$. The HRT of the DHS reactor was calculated based on the sponge volume. The DHS reactor was equipped with a distributor and supplied air using an air pump for artificial ventilation. The initial operational condition was listed in Table 3.3.

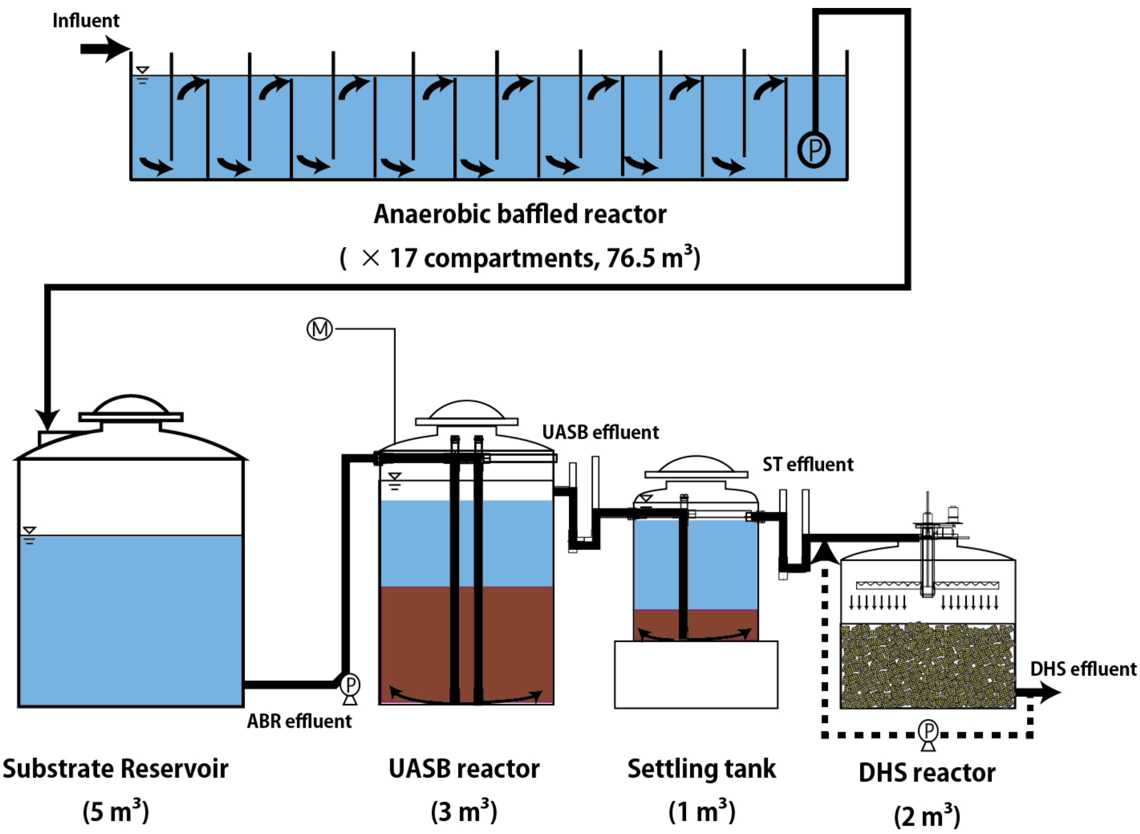


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Figure 3.7 Schematic and photo of the pilot scale ABR-UASB-ST-DHS system

Table 3.3 Initial operational conditions through phases 1 to 4.

| Phase | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Day | | 1 – 120 | 121 – 154 | 155 – 229 | 230 – 267 |
| Flow rate | $m^3 \cdot day^{-1}$ | 4.0 ± 1.0 | 1.3 ± 0.4 | 1.8 ± 0.4 | 2.8 ± 0.5 |
| HRT of UASB | hours | 19.3 ± 6.4 | 63.4 ± 23.5 | 42.0 ± 10.1 | 26.6 ± 5.3 |
| OLR of UASB | kg–COD$\cdot m^{-3} \cdot day^{-1}$ | 3.1 ± 1.3 | 0.7 ± 0.2 | 1.7 ± 0.6 | 3.1 ± 0.7 |
| DHS effluent recirculation ratio | | 0 | 1 | 4 | 4 |

During phase 1, the UASB reactor was started up with an approximate OLR of 3.0 kg-COD$\cdot m^{-3} \cdot day^{-1}$. However, a large amount of sludge was washed out from the UASB reactor and OLR of the UASB reactor was decreased by reducing HRT during phase 2 and phase 3. During phase 4, OLR of the UASB reactor was increased to 3.0 kg-COD$\cdot m^{-3} \cdot day^{-1}$ again. During phases 2 to 4, the DHS effluent was recirculated to the DHS influent in order to enhance nitrification and denitrification of the wastewater.

### 3.2.2.2 Analytical methods

Water samples were obtained from the influent, the ABR effluent, the UASB effluent, the ST effluent, and the DHS effluent for routine analysis. Temperature, pH, dissolved oxygen (DO), and oxidation–reduction potential (ORP) were measured on-site by using portable pH/DO/ORP meter (DOP-5F, Kasahara). BOD and total suspended solids (TSS) were analyzed using standard methods (APHA, 2005). TSS was measured using a 0.45 μm glass fiber filter (GB-140, Advantec). The total COD, soluble COD, and total nitrogen (TN) were determined by using a water quality analyzer (DR-2800, HACH). Ammonia, nitrate, and nitrite concentrations were measured using a high-performance liquid chromatography (LC-20AD, Shimadzu). Biogas production from the UASB reactor was measured using a wet gas meter (WS-1A, Shinagawa). Biogas composition was analyzed using a gas chromatograph equipped with a thermal conductivity detector (GC-8A, Shimadzu). Biogas from the ABR was collected using a collection chamber made from PVC pipes (Tanikawa et al., 2016a). Concentration of nitrous oxide was analyzed using gas chromatograph equipped with an electron capture detector (GC-2014, Shimadzu).

#### 3.2.2.3 16rRNA gene sequence

Sludge samples were collected from the bottom of the UASB reactor on day 1, day 41, day 155, day 228, and day 267. The collected sludge samples were gently washed with phosphate-buffered saline and stored at -20 ˚C until DNA was extracted using a FastDNA Spin Kit for Soil (MP Biomedicals). Polymerase chain reaction (PCR) amplification of 16S rRNA genes was performed with the universal forward primer Univ515F (5'-GTGCCAGCMGCCGCGGTAA-3') and the universal reverse primer Univ806R (5'-GGACTACHVGGGTWTCTAAT-3') at an annealing temperature of 50 ˚C (Caporaso et al., 2012). Purification of PCR products was conducted using a QIAquick PCR purification kit (QIAGEN). Massive parallel 16S rRNA gene sequencing was performed using MiSeq reagent kit v.2 with the Miseq system (Illumina). Sequence data analysis was conducted using QIIME software package v.1.7.0. (Caporaso et al., 2010). Operational taxonomic units (OTU) were picked at 97% sequence identity with chimeric sequences removed using ChimeraSlayer. The taxonomic classification was determined using the Greengenes database v.13_5. The relative species of the representative sequences were identified using a web-based BLAST search in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

### 3.2.3 Results and Discussion

#### 3.2.3.1 Organic removal

Table 3.4 lists the process performance of the treatment system during phases 1-4. The temperature was 29.6 ± 1.1˚C and the total COD and BOD removal efficiencies were 93.8 ± 3.7% and 93.3 ± 7.4%, respectively, during the entire experimental period (Figure 3.9).

The ABR was installed to remove residual natural rubber particles from the influent. In this study, a part of the existing full-scale ABR was diverted as the pre-treatment of the pilot scale UASB reactor. Previous studies have reported that remaining rubber particles adversely affected the anaerobic biological treatment (Nguyen and Luong, 2012; Watari et al., 2016a). The RSS wastewater contained a total COD of 3,470 ± 760 $mg \cdot L^{-1}$, soluble COD of 3,210 ± 850 $mg \cdot L^{-1}$, total BOD of 2,650 ± 950 $mg \cdot L^{-1}$, and soluble BOD of 2,550 ± 860

$mg \cdot L^{-1}$. The concentrations of organics in RSS wastewater gradually increased with increasing natural rubber sheet production. The ABR achieved a 31.6 ± 15.6% of total COD and 40.5 ± 16.0% of soluble COD removal efficiency during the entire experiment. Similarly, total BOD and soluble BOD removal efficiencies of the ABR were 45.1 ± 14.5% and 50.7 ± 14.3%, respectively. The total COD and total BOD concentration of the ABR effluent were 2,540 ± 710 $mg \cdot L^{-1}$ and 1,600 ± 620 $mg \cdot L^{-1}$, respectively. In addition, TSS in the ABR influent and effluent were 200 ± 58 $mg \cdot L^{-1}$ and 166 ± 65 $mg \cdot L^{-1}$, resulting in a TSS removal efficiency of 18.7 ± 41.1%. These results indicate that ABR roughly removed organic compounds in the RSS wastewater.

The UASB reactor achieved most of the organic removal and methane recovery in the system. During phase 1, the UASB reactor had a low total COD removal efficiency of 18.6 ± 17.0% likely due to the large amount of washed out sludge caused by the low settleability of seed sludge and high biogas production of 370 ± 250 $L \cdot day^{-1}$. However, soluble COD removal efficiency increased from 23.4 ± 15.4% to 63.3 ± 21.4% in phase 2. During phase 3, the UASB reactor demonstrated total COD and BOD removal efficiencies of 55.5 ± 16.1% and 77.8 ± 10.3% with OLR of 1.7 ± 0.6 kg-$COD \cdot m^{-3} \cdot day^{-1}$. The efficiencies were lower than our previous laboratory scale experiments and other anaerobic treatment systems treating natural rubber processing wastewater (Mohammadi et al., 2010; Nguyen and Luong, 2012; Watari et al., 2016a). On the other hand, the UASB reactor achieved high soluble COD and BOD removal efficiencies of 70.2 ± 19.6% and 76.3 ± 7.5% during phase 3. During phase 4, OLR of the UASB reactor increased to 3.0 kg-$COD \cdot m^{-3} \cdot day^{-1}$. The total COD and soluble COD removal efficiencies of UASB decreased to 33.9 ± 19.4% and 57.4 ± 12.2% in phase 4. In addition, TSS of the UASB effluent significantly increased to 245 ± 70 $mg \cdot L^{-1}$. In summary, high OLR operation of the UASB reactor led to the retained sludge wash out and the performance of the UASB reactor deteriorated. In addition, the accumulation of natural rubber particular was frequently occurred in the supply pipe (Figure 3.8) and required further modification for remove natural rubber particulars.

The composition of the biogas was 6.8 ± 10.1% nitrogen, 68.7 ± 10.9% methane, and 24.5 ± 5.5% carbon dioxide during phase 1, and 1.3 ± 0.7% nitrogen, 77.4 ± 1.9% methane, and 21.3 ± 1.7% carbon dioxide during phase 3. The average methane gas production of the UASB reactor in phases 1–4 was 250 ± 160 $L \cdot day^{-1}$, 202 ± 117 $L \cdot day^{-1}$, 323 ± 198 $L \cdot day^{-1}$, and 357 ± 234 $L \cdot day^{-1}$, respectively. Moreover, methane recovery ratio based on removed total COD were 32.7 ± 86.4%, 41.5 ± 29.3%, and 64.3 ± 71.6% for phase 1, phase 3, and phase 4, respectively (Table 3.5). During phase 2, a large amount of sludge wash out was observed in the UASB reactor. Therefore, methane recovery ratio could not be calculated in this phase. Such low methane recovery rates in the other phases can be attributed to the large number of natural rubber particles accumulated in the UASB reactor. Thus, further modifications to the system are required to remove residual natural rubber particles by adding processes, such as chemical precipitation.

A settling tank was installed for trapping washed out sludge and residual rubber particles from the UASB reactor. During phases 2 and 3, the ST efficiently removed total COD (76.0 ± 7.7% and 47.2 ± 18.1%, respectively). In addition, TSS removal efficiencies were 95.7 ± 1.8% and 60.4 ± 14.9% in phases 2 and 3, respectively. Therefore, the ST could be protected from the unexpected sludge washed out from the UASB reactor. The residual natural rubber particles floated to the surface of ST were removed once per month (data not shown). Thus, the UASB reactor should be equipped with an 'excess sludge and rubber particle removal system' in addition to a ST for treating natural rubber processing wastewater that contains a large amount of residual natural rubber particles.

The DHS reactor can serve as an effective post-treatment system for residual organic particles and TSS removal. In this study, the DHS reactor removed 83.5 ± 10.0% of total COD, 82.6 ± 11.2% of total BOD, and 73.5 ± 20.0% of TSS during the entire experiment. These organic removal efficiencies were higher than the post-treatment DHS reactor treating the ABR effluent (Watari et al., 2016). Dissolved oxygen level of the DHS effluent was only 0.5 ± 0.3 mg·L$^{-1}$ in phase 1. After the effluent was recirculated to DHS, DO increased to 0.9 ± 0.5 mg·L$^{-1}$ in the DHS effluent. BOD of the DHS effluent also increased to 30 ± 16 mg·L$^{-1}$ in phase 2. Okubo et al. (2016) reported that the effluent recirculation improved the DO levels in the system, however, DO was consumed very quickly to degrade high concentration organics in the upper part of the reactor. During phase 3, the organics concentration in the DHS effluent was 140 ± 64 mg·L$^{-1}$ for total COD and 31 ± 12 mg·L$^{-1}$ for total BOD, indicating that most of the biodegradable organic compounds were removed from the system. Thus, the DHS reactor can be used as an effective post-treatment process for treating this wastewater.

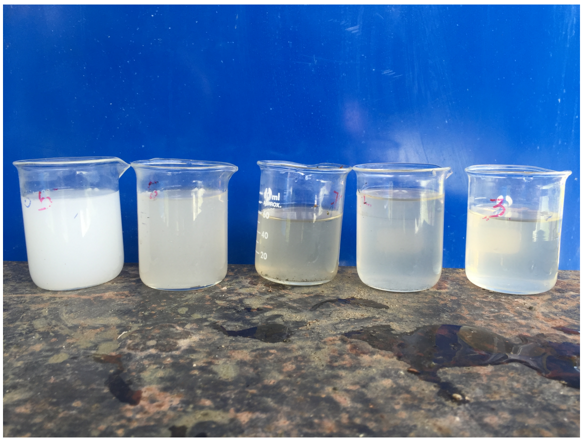

Figure 3.8 Accumulation of rubber particular in feed pipe and photo wastewaters

Table 3.4 Summary of the process parameters of the system during entire experimental period.

| Phase | Parameter | Unit | Influent | ABR eff. | UASB eff. | ST eff. | DHS eff. |
|---|---|---|---|---|---|---|---|
| Phase 1 | pH | | 5.0±0.3 | 5.6±0.3 | 5.9±0.4 | 6.0±0.4 | 6.4±0.4 |
| | TSS | $mg·L^{-1}$ | 220±67 | 180±45 | 350±175 | 380±180 | 100±70 |
| | Total COD | $mg·L^{-1}$ | 3,150±830 | 2,320±670 | 2,250±540 | 2,250±700 | 180±110 |
| | Soluble COD | $mg·L^{-1}$ | 2,760±940 | 1,660±570 | 1,340±580 | 1,060±610 | 154±60 |
| | Total BOD | $mg·L^{-1}$ | 2,160±1,100 | 1,370±620 | 1,290±580 | 1,220±690 | 130±80 |
| | Soluble BOD | $mg·L^{-1}$ | 2,020±1,070 | 1,200±460 | 980±440 | 830±480 | 100±52 |
| Phase 2 | pH | | 5.6±0.2 | 6.3±0.2 | 6.7±0.1 | 6.9±0.2 | 7.5±0.2 |
| | TSS | $mg·L^{-1}$ | 200±35 | 180±86 | 690±240 | 61±33 | 15±12 |
| | Total COD | $mg·L^{-1}$ | 2,870±650 | 2,000±340 | 2,550±1,050 | 640±360 | 120±110 |
| | Soluble COD | $mg·L^{-1}$ | 2,560±660 | 1,450±200 | 530±300 | 430±280 | 69±73 |
| | Total BOD | $mg·L^{-1}$ | 2,240±610 | 1,240±45 | 660±250 | 310±200 | 30±16 |
| | Soluble BOD | $mg·L^{-1}$ | 2,150±480 | 1,080±160 | 300±130 | 250±140 | 11±2 |
| Phase 3 | pH | | 5.5±0,3 | 6.1±0,5 | 6.7±0.4 | 7.0±0.2 | 7.7±0.3 |
| | TSS | $mg·L^{-1}$ | 170±17 | 150±74 | 98±62 | 83±77 | 46±32 |
| | Total COD | $mg·L^{-1}$ | 3,940±310 | 2,750±600 | 1,270±600 | 730±410 | 140±64 |
| | Soluble COD | $mg·L^{-1}$ | 3,500±310 | 1,890±510 | 600±470 | 400±390 | 36±12 |
| | Total BOD | $mg·L^{-1}$ | 3,320±410 | 1,830±170 | 760±980 | 300±320 | 31±12 |
| | Soluble BOD | $mg·L^{-1}$ | 3,120±560 | 1,550±210 | 440±270 | 270±290 | 10±4.6 |
| Phase 4 | pH | | 5.5±0.2 | 5.9±0.3 | 6.5±0.3 | 7.1±0.3 | 7.5±0.3 |
| | TSS | $mg·L^{-1}$ | 240±83 | 150±79 | 245±70 | 35±22 | 35±22 |
| | Total COD | $mg·L^{-1}$ | 3,860±630 | 3,020±710 | 1,990±790 | 940±670 | 155±50 |
| | Soluble COD | $mg·L^{-1}$ | 3,690±830 | 2,540±640 | 1,050±220 | 430±220 | 130±40 |
| | Total BOD | $mg·L^{-1}$ | 3,560±160 | 2,030±1,360 | 630±160 | 230±40 | 45±1.4 |
| | Soluble BOD | $mg·L^{-1}$ | 2,970±260 | 1,470±590 | 410±175 | 170±15 | 27±2.8 |

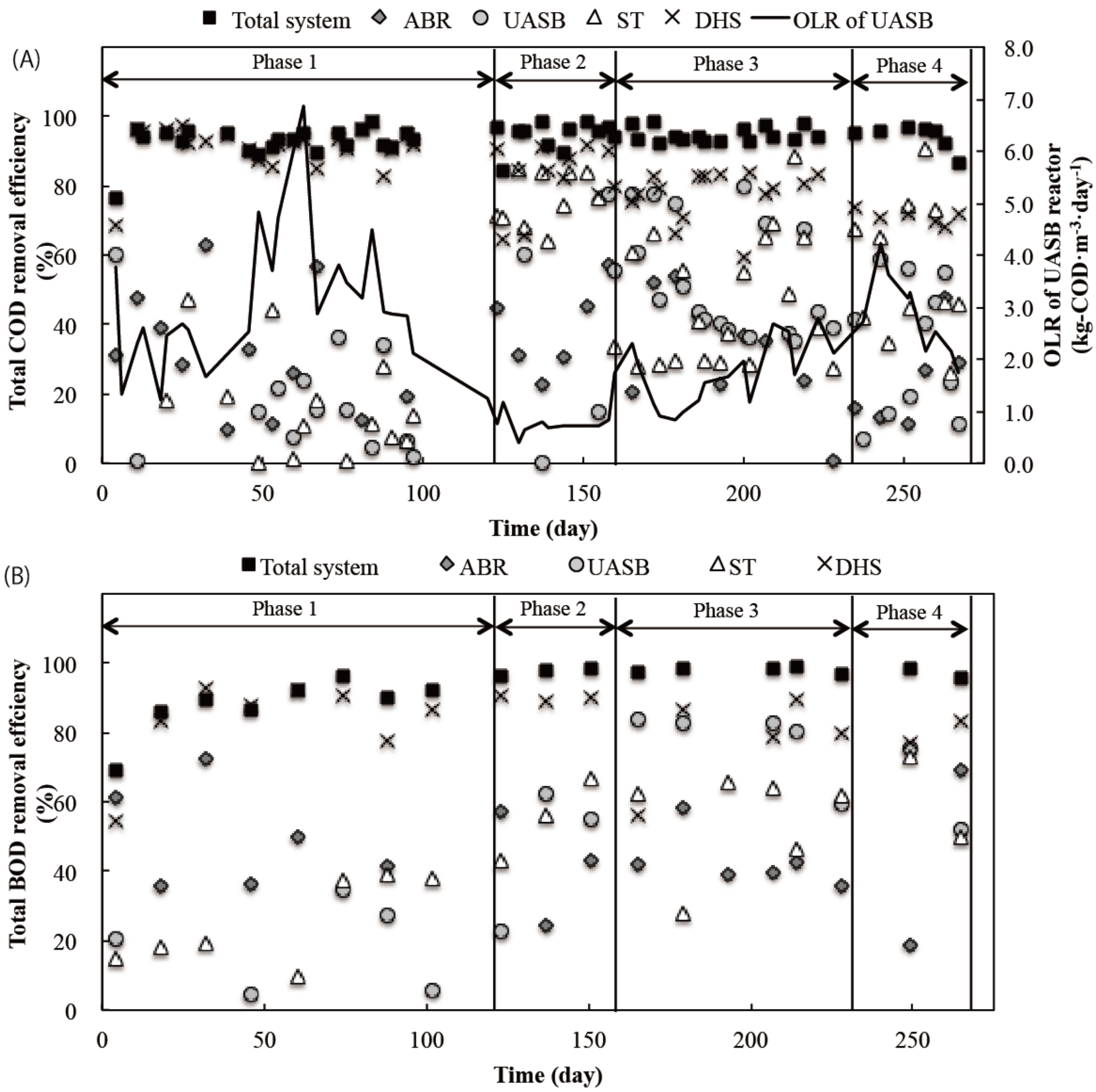


Figure 3.9 Time course of (A) Total COD removal efficiency and organic loading rate of UASB reactor, (B) Total BOD removal efficiency.

Table 3.5 Biogas production and compositions of the UASB reactor

| | Unit | Phase 1 | Phase 2 | Phase 3 | Phase 4 |
|---|---|---|---|---|---|
| Total biogas production | $L \cdot day^{-1}$ | 369 ± 252 | 250 ± 138 | 416 ± 249 | 454 ± 338 |
| Methane gas production | $L \cdot day^{-1}$ | 250 ± 160 | 202 ± 117 | 323 ± 198 | 357 ± 234 |
| Methane recovery rate | % | 32.7 ± 86.4 | – | 41.5 ± 29.3 | 64.3 ± 71.6 |
| Compostion of produced biogas | | | | | |
| Nitrogen | % | 6.8 ± 10.1 | 2.5 ± 1.9 | 1.3 ± 0.7 | 1.2 ± 0.8 |
| Methane | % | 68.7 ± 10.9 | 81.3 ± 4.0 | 77.4 ± 1.9 | 71.2 ± 12.2 |
| Carbon dioxide | % | 24.5 ± 5.5 | 16.2 ± 3.5 | 21.3 ± 1.7 | 27.6 ± 12.3 |

–: Not available

### 3.2.3.2 Nitrogen removal and greenhouse gas emissions

Table 3.6 lists the concentrations of TN, ammonia, nitrate, and nitrite in the treatment system. The TN in the ABR influent and effluent were 184 ± 93 mg-N·$L^{-1}$ and 155 ± 72 mg-N·$L^{-1}$, respectively. On the other hand, ammonia concentrations of the ABR influent and effluent were 122 ± 49 mg-N·$L^{-1}$ and 151 ± 70 mg-N·$L^{-1}$, indicating that ammonia could be produced from organic nitrogen by anaerobic digestion. In addition, small amounts of nitrate detected in the ABR effluent suggested the occurrence of nitrification in ABR. According to Tanikawa et al. (2016a), ammonia was oxidized to nitrate at the surface of ABR and nitrous oxide was emitted to the atmosphere. In fact, 213 ppm of nitrous oxide was detected in the biogas collected from ABR on day 190 of this study.

Nitrate reduction in the UASB reactor indicated the possibility of denitrification of wastewater in the UASB reactor. The concentrations of nitrous oxide in the biogas produced in UASB were 213 ppm, 72 ppm and 614 ppm on day 42, day 190 and day 264, respectively. The nitrous oxide production ratio from 1 $m^3$ of treated RSS wastewater was 4.737 × $10^{-6}$ $m^3$·$m^{-3}$-w.w during phase 3. The maximum nitrous oxide concentration of 614 ppm was observed on day 264. The production rate equivalent to carbon dioxide for 1 $m^3$ of treated RSS wastewater for nitrous oxide in this UASB reactor was calculated as 2.77 × $10^{-5}$ t-$CO_{2\,eq}$·$m^{-3}$-w.w during phase 3.

The DHS reactor was installed for the nitrification of wastewater as well as residual organics removal in this system. During phase 1, the DHS reactor demonstrated low TN and ammonia removal efficiencies of 38.8 ± 16.0% and 19.3 ± 5.8% (Figure 3.10). A small amount of nitrate production was also observed in the DHS reactor. However, TN and ammonia reduction suggested that nitrification occurred in the reactor and nitrification products were immediately utilized by denitrifying bacteria in the DHS reactor. Araki et al. (1999) and Machdar et al. (2000) reported that the inner section of the DHS sponge carrier is anaerobic. Therefore, denitrification would be occurred to some degree in the DHS reactor. In addition, the low BOD concentration in the DHS effluent suggested that autotrophic bacteria could be in abundance in that reactor. Several studies reported the coexistence of anaerobic ammonia-oxidizing bacteria and heterotrophs in the DHS reactor (Almeida et al. 2013; Mac Conell et al., 2015; Watari et al., 2017). During phase 2 through phase 4, the DHS effluent was recirculated back as DHS influent in order to enhance TN removal efficiency. The TN removal efficiency of the DHS reactor was increased to 52.9 ± 5.1% during phase 2. Ikeda et al. (2013) demonstrated that advantage of effluent recirculation up to 2.0 in the DHS reactor treating industrial wastewater containing a high concentration of organic and ammonia for enhancing denitrification. The nitrification ratio (based on ammonia oxidization) of the DHS reactor also increased to 0.42 ± 0.03 kg-N·$m^{-3}$·$day^{-1}$ during phase 4. This nitrification rate was greater than the same sponge-type DHS reactor treating sewage and natural rubber processing wastewater in

other studies (Tawfik et at., 2006, Watari et al., 2017). During phase 4, the TN removal efficiency of the DHS reactor was decreased to 35.9 ± 10.7% due to high nitrogen loading rate operation of 0.68 ± 0.22 kg-N·m$^{-3}$·day$^{-1}$. Nitrous oxide emissions from the DHS reactor were evaluated by a small closed cylinder with a sponge carrier that retained sludge. The biogas from the DHS reactor contained 99.2 % of nitrogen, 0.8% of carbon dioxide and 85.4 ppm of nitrous oxide on 190. The amount of the biogas from the DHS reactor was under the detection limit. Kampschreur et al. (2009) reported that low DO and low COD/N ratio were the most important operational parameters leading to nitrous oxide emissions. Thus, the DHS reactor could emit nitrous oxide from nitrification and/or denitrification processes. Kampschreur et al. (2008) also reported that 0.6% of the nitrogen load was emitted as nitrous oxide in full-scale nitrifying and denitrifying sewage treatment plants. According to this nitrous oxide emission ratio (0.6% of the nitrogen load), nitrous oxide emissions from the DHS reactor were calculated as 0.00026 t-$CO_{2\ eq}$·m$^{-3}$ - w.w. during phase 3 (Kampschreur et al. 2008). Therefore, nitrous oxide emissions from the DHS reactor are an important parameter to consider when designing full-scale treatment systems.
In total, the TN removal efficiency was 33.6 ± 17.7%, 51.3 ± 34.0%, 68.3 ± 15.1%, and 57.9 ± 7.0% in phases 1 to 4, respectively. However, ammonia and TN remained in the final effluent. Therefore, a system modification such as the addition of a denitrification tank or autotrophic nitrogen removal process is required. The emission ratios for 1 m$^3$ of RSS wastewater treatment for ABR, UASB, and DHS were calculated as 0.0129 t-$CO_{2eq}$·m$^{-3}$, 0.0045 t-$CO_{2eq}$·m$^{-3}$ and 0.00026 t-$CO_{2eq}$·m$^{-3}$, respectively. The UASB reactor can recover biogas as energy, thus GHGs emission ratio from the proposed system can be reduced to 0.013 t-$CO_{2eq}$·m$^{-3}$, corresponding to a 92% reduction of GHGs emissions

Table 3.6 Nitrogen concentrations (mg-N·L$^{-1}$) in the proposed system

| Phase | Parameter | Unit | Influent | ABT eff. | UASB eff. | ST eff. | DHS eff. |
|---|---|---|---|---|---|---|---|
| Phase 1 (R=0) | TN | mg-N·L$^{-1}$ | 150 ± 80 | 127±65 | 125±65 | 152±49 | 123±46 |
| | Ammonia | mg-N·L$^{-1}$ | 118±16 | 88 ± 30 | 113 ± 41 | 88 ± 17 | 77 ± 29 |
| | Nitrate | mg-N·L$^{-1}$ | 1.8 ± 2.8 | 1.6 ± 2.0 | 1.0 ± 1.8 | 0.1±0.2 | 2.1±2.1 |
| | Nitrite | mg-N·L$^{-1}$ | N.D | N.D | N.D. | N.D | N.D |
| Phase 2 (R=1) | TN | mg-N·L$^{-1}$ | 143±19 | 120±22 | 126±61 | 84 ± 38 | 53 ± 39 |
| | Ammonia | mg-N·L$^{-1}$ | 64 ± 36 | 69±58 | 99±48 | 60 ± 16 | 29±33 |
| | Nitrate | mg-N·L$^{-1}$ | N.D | N.D. | N.D. | N.D. | N.D. |
| | Nitrite | mg-N·L$^{-1}$ | N.D | N.D. | N.D. | N.D. | N.D. |
| Phase 3 (R=4) | TN | mg-N·L$^{-1}$ | 202±54 | 156±50 | 175±54 | 165±63 | 58±24 |
| | Ammonia | mg-N·L$^{-1}$ | 109±17 | 176±31 | 172 ± 29 | 153 ± 21 | 49±22 |
| | Nitrate | mg-N·L$^{-1}$ | N.D. | 4.0 ± 7.5 | 0.7 ± 0.6 | 0.9 ± 0.3 | 4.1±4.0 |
| | Nitrite | mg-N·L$^{-1}$ | N.D. | N.D. | N.D. | N.D. | N.D |
| Phase 4 (R=4) | TN | mg-N·L$^{-1}$ | 273±117 | 224±53 | 252±54 | 197±38 | 128±36 |
| | Ammonia | mg-N·L$^{-1}$ | 171 ± 52 | 232 ± 44 | 224 ± 4.3 | 227 ± 17 | 133 ± 4.8 |
| | Nitrate | mg-N·L$^{-1}$ | 1.5 ± 1.4 | 0.3 ± 0.4 | 0.5 ± 0.6 | 0.2 ± 0.5 | 0.2 ± 0.5 |
| | Nitrite | mg-N·L$^{-1}$ | N.D. | N.D. | N.D. | N.D | 0.1 ± 0.3 |

R: Recirculation ratio, N.D.: Not detected compared with the existing open-type anaerobic treatment systems (Tanikawa et al., 2016a). However, pre-treatment ABR emitted most of the GHGs emissions in this study. Therefore, the development of effective closed type pretreatment systems is needed for further reductions in GHGs emissions.

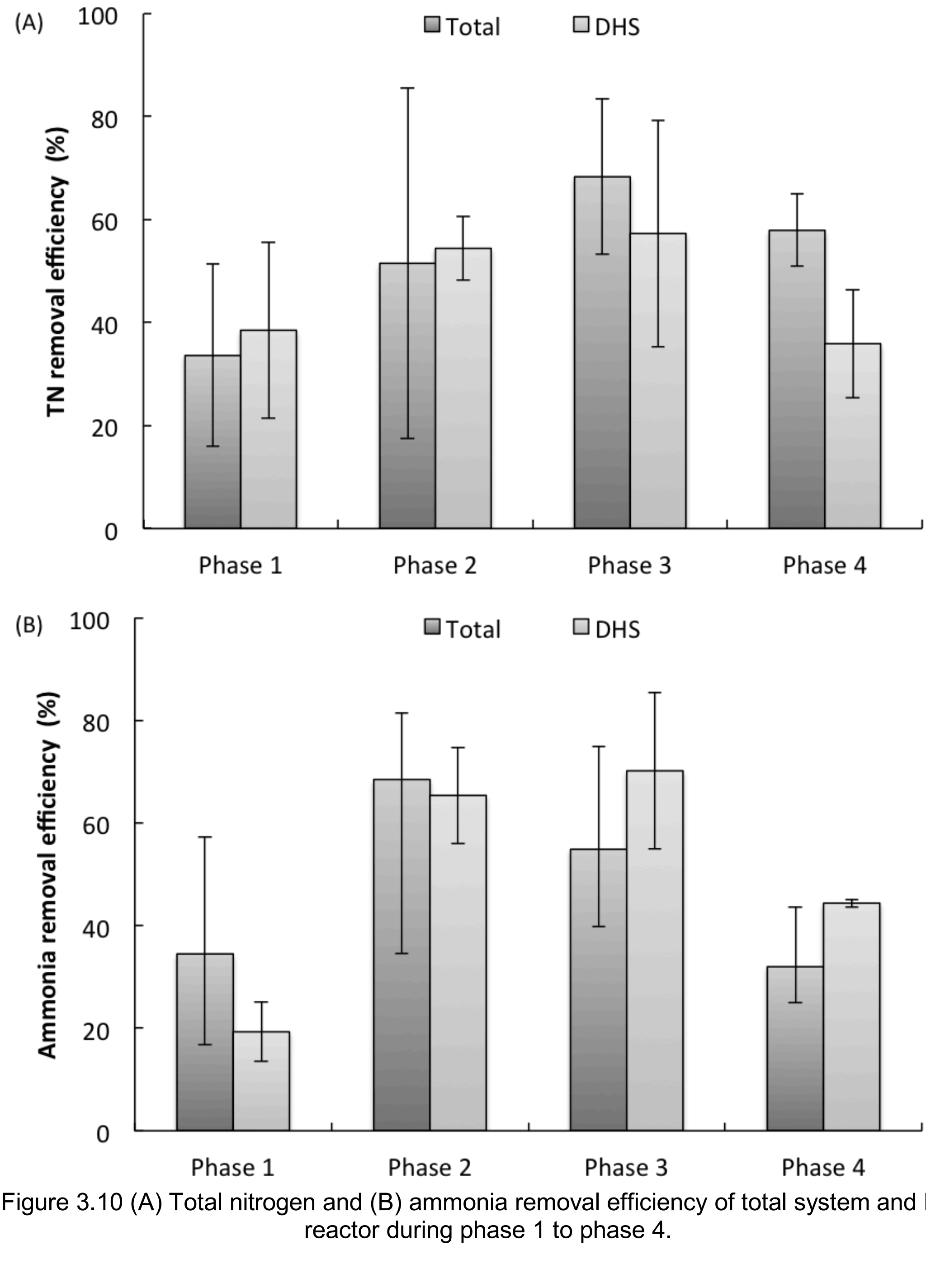


Figure 3.10 (A) Total nitrogen and (B) ammonia removal efficiency of total system and DHS reactor during phase 1 to phase 4.

### 3.2.3.3 Microbial community analysis of the UASB retained sludge

The microbial community structures of UASB that retained sludge on day 1, day 41, day 155, day 228, and day 267 were analyzed based on the 16S rRNA gene sequence (Table 3.7). A total of 140,288 sequence reads were analyzed and the median length of the 16S rRNA sequence was 251 bp. Approximately, 20,000–32,000 sequencing reads were determined per sample, and 1,500–2,000 OTUs per sample were detected at 97% sequence similarity.

Fig. 3.11 shows the microbial community structure of the UASB reactor on days 1, 41, 155, 228, and 267 at phylum level. The principal bacterial groups were from the phylum of *Chloroflexi*, *Proteobacteria*, *Bacteroidetes,* and *Firmicutes* in the UASB reactor. These microbial groups have been detected commonly and frequently in methanogenic granular sludges (Narihiro et al., 2009). The most abundant bacterial phylum was *Chroloflexi* with total sequence reads in the UASB reactor. Among phylum *Chroloflexi, Longlina* is the most dominated one. These organisms are frequently found in the mesophilic UASB reactors, and they degrade carbohydrates and cellular material (Yamada et al., 2005; Narihiro and Sekiguchi 2007). Thanh et al. (2016) reported that unclassified *Anaeroilinaceae* were found in a UASB reactor treating natural rubber processing wastewater suggesting that phylum *Chroloflexi* dominated the UASB reactor and could degrade organic material in the wastewater. Fatty acid oxidizing bacteria, *Syntrophomonas*, *Syntropus,* and *Syntrophobacter* were also found in this UASB reactor.

Hydrogen-utilizing archaea, *Methanobacterium,* was predominantly detected in the UASB reactor in this study (Fig. 3.12). *Methanobacterium* are typically detected in mesophilic or thermophilic anaerobic digesters (Narihiro et al., 2009) and in salty environments (Kuroda et al., 2014). Moreover, hydrogen-utilizing methanogen *Methanosopirillum* is one of the most frequently detected organisms in anaerobic wastewater treatment systems for natural rubber processing wastewater (Tanikawa et al., 2016a). Therefore, hydrogen-utilizing methanogens played a crucial role in the final step of the anaerobic digestion via $H_2$ in the UASB reactor in this study. On the other hand, acetate-utilizing methanogen, *Methanosaeta,* was predominant in the UASB reactor on day 155 when the UASB reactor demonstrated a high COD removal efficiency (e.g. total COD: 55.5 ± 16.1%, total BOD removal: 77.8 ± 10.3% during phase 3). Watari et al. (2016) also reported the abundance of *Methanosaeta* in the UASB reactor treating natural rubber processing wastewater. In addition, the proportion of *Methanosaeta* phylotypes was always high in the reactor well capable of removing propionate (Narihiro et al., 2009). Therefore, these archaea communities in UASB reactors are capable of treating natural rubber processing wastewater.

Table 3.7 Diversity indices of the upflow anaerobic sludge blanket reactor.

| | Day 1 | Day 41 | Day 155 | Day 228 | Day 267 |
|---|---|---|---|---|---|
| Total sequence reads | 21,128 | 28,542 | 30,543 | 28,022 | 32,053 |
| OTUs | 1,457 | 1,750 | 1,925 | 1,627 | 2,016 |
| Chao1 richness estimation | 2,707 | 3,537 | 4,107 | 3,309 | 3,989 |
| Shannon diversity index | 7.18 | 7.11 | 7.24 | 6.98 | 7.36 |
| Good's coverage (%) | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |

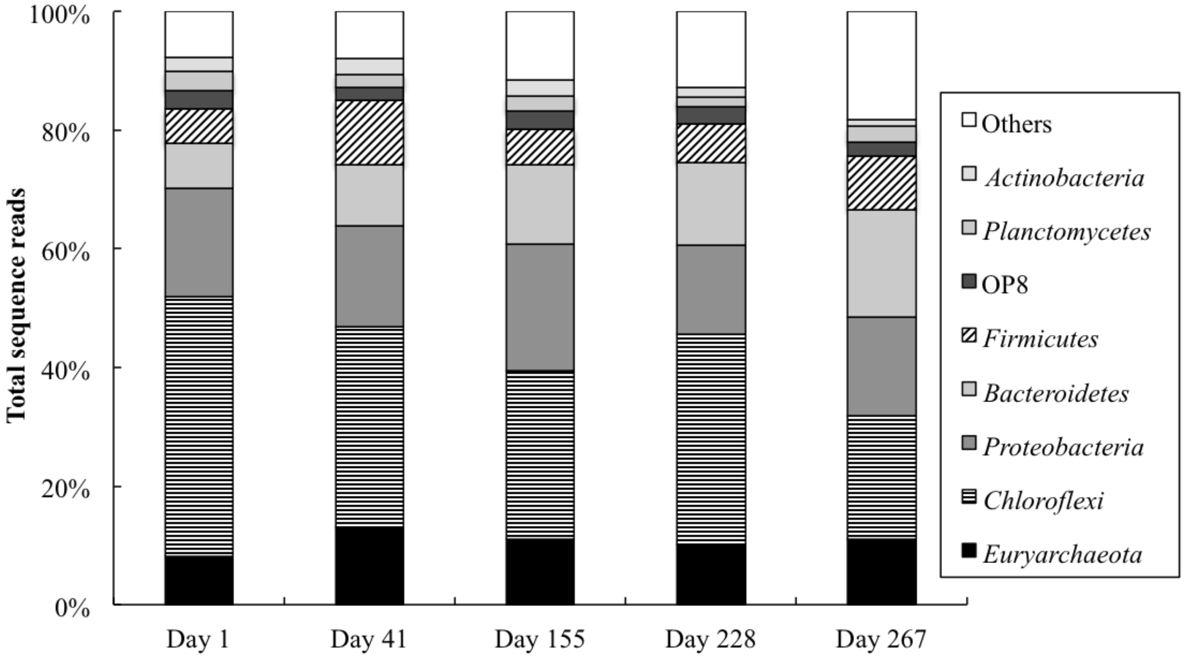


Fig. 3.11 Phylum-level microbial community compositions of the UASB reactor on days 1, 41, 155, 228, and day 267.

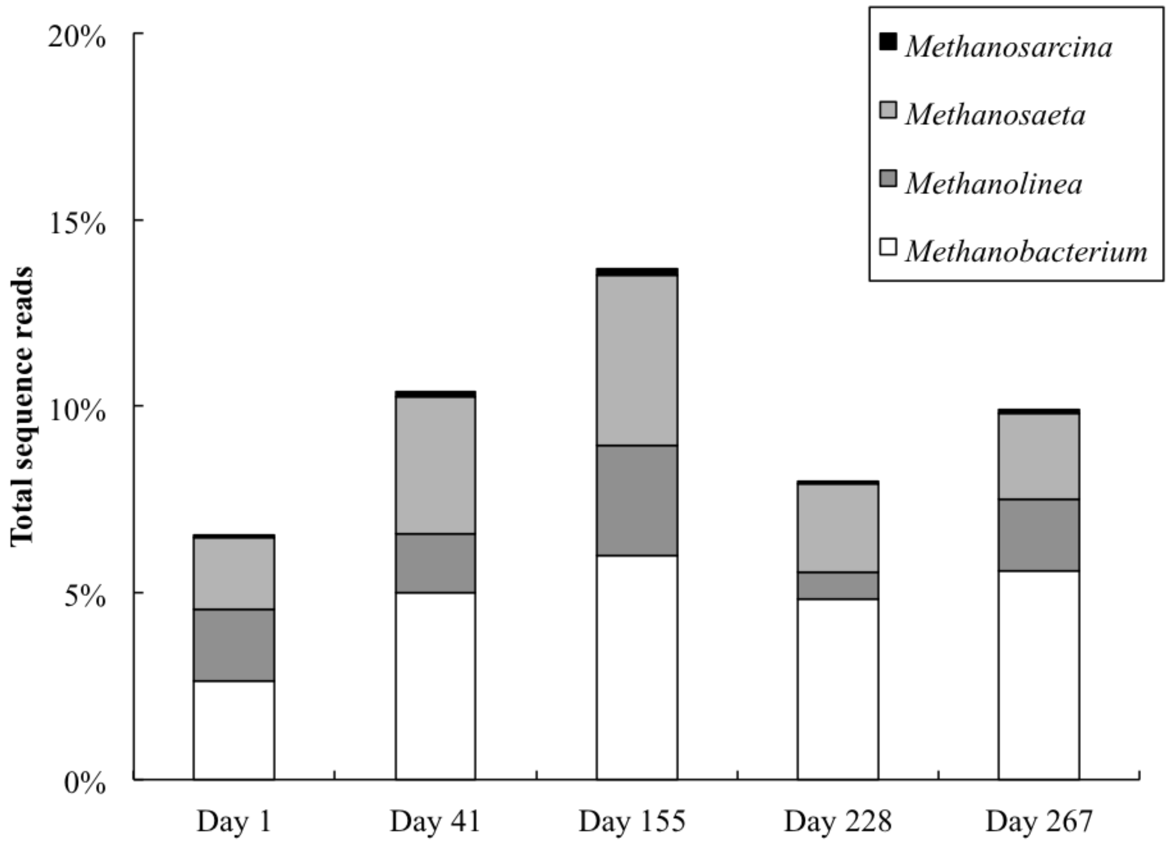


Fig. 3.12 Relative abundance of the predominant methanogen in UASB reactor on days 1, 41, 155, 228, and 267.

### 3.2.3.4 Performance comparison of the ABR-UASB-ST-DHS system and other treatment systems for treating natural rubber processing wastewater

Previous studies on the process performance of existing treatment systems for natural rubber processing wastewater are summarized in Table 3.8. The combined ABR (HRT=3.4 day)-UASB (HRT=1.8 day)-ST (HRT=0.6 day)-DHS (HRT=0.5 day) system removed 94.8 ± 2.1% of total COD, 98.0 ± 0.9% of total BOD, 71.8 ± 22.6% of TSS, and 68.3 ± 15.1% of TN during phase 3. A combination of anaerobic and aerobic lagoons has also been widely used in Thailand, Vietnam, and Malaysia because of its low operational cost and easy maintenance (Ibrahim et al., 1980; Nguyen and Luong, 2012; Syutsubo et al., 2015). Oxidation ditches were used for the treatment of the natural rubber processing wastewater due to their highly efficiency in removing nitrogen (Ibrahim, 1980, Nguyen and Luong, 2012). Syutsubo et al. (2015) reported that the process performance of full-scale dissolved air flotation–anaerobic lagoon–anoxic lagoon–aerated tank system achieved the removal efficiencies of 89% for TSS, 98% for total COD, 91% for TN in South Vietnam. Thus, the ABR-

UASB-ST-DHS system developed in this study demonstrated similar removal efficiencies to existing treatment systems. Moreover, our system could reduce approximately 80% of HRT similar to the existing systems (Nguyen and Luong, 2012; Syutsubo et al., 2015). The final effluent of our system met the required Vietnamese national technical regulation on effluent of the natural rubber processing industry-B except for the ammonia content (QCVN01: 2008/BTNMT, pH: 6-9, Total BOD: < 50 $mg \cdot L^{-1}$, Total COD: < 250 $mg \cdot L^{-1}$. TSS: < 100 $mg \cdot L^{-1}$, TN: < 60 $mg\text{-}N \cdot L^{-1}$, Ammonia: < 40 $mg\text{-}N \cdot L^{-1}$). Several current treatment systems exceed the effluent regulations in Vietnam (Nguyen and Luong, 2012). Thus, an effective nitrogen removal process is required in both the proposed and existing systems. However, the pilot scale ABR-UASB-ST-DHS system demonstrated high potential for the treatment of natural rubber processing wastewater in Vietnam. Furthermore, the full-scale UASB-DHS system that will be developed based on the results obtained in the pilot scale system in this study and in previous studies will likely achieve a high process performance and energy recovery potential in the form of methane.

Table 3.8 Process performance of the existing treatment system for treating natural rubber processing wastewater.

| | | | HRT | Influent concenration (mg·L$^{-1}$) | | | | | | Effluent concentration (mg·L$^{-1}$) | | | | | | Removal effciency (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| System | Country | Wastewater | days | pH | TCOD | TBOD | TSS | TN | Ammonia | pH | TCOD | TBOD | TSS | TN | Ammonia | TCOD | TBOD | TSS | TN | Reference |
| Decantation - UASB - aeration tank - settling and filiter | Vietnam | CL + SVR | - | 9.2 | 18,885 | 10,780 | 900 | 611 | 342 | 6.8 | 123 | 57 | 70 | 35.3 | 30.8 | 99 | 99 | 92 | 94 | Nguyen and Luong (2012) |
| Decantation - oxidation ditch - settling and filiter | Vietnam | CL | - | 9.1 | 26,914 | 8,750 | 740 | 766 | 361 | 8.4 | 567 | 50 | 74 | 160 | 137 | 98 | 99 | 90 | 79 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL | - | 8.55 | 19,029 | 7,830 | 2,220 | 813 | 302 | 8.2 | 466 | 70 | 300 | 40.6 | 34.5 | 98 | 99 | 86 | 95 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL + SVR | - | 8.23 | 14,466 | 9,200 | 850 | 450 | 350 | 7.4 | 107 | 92 | 60 | 65 | 47 | 99 | 99 | 93 | 86 | Nguyen and Luong (2012) |
| Decantation - flotation - oxidiation ditich - settling and filiter | Vietnam | CL + SVR | - | 9.42 | 26,436 | 13,820 | 1,690 | 651 | 285 | 8.1 | 120 | 85 | 60 | 74.9 | 33 | 99.5 | 99 | 96 | 88 | Nguyen and Luong (2012) |
| Decantation - flotation - UASB - aeration tank - settling and filiter | Vietnam | CL | - | 8.09 | 13,981 | 7,590 | 468 | 972 | 686 | 7.9 | 127 | 61 | 39 | 129 | 30.3 | 99 | 99 | 92 | 87 | Nguyen and Luong (2012) |
| Decantation -oxidation ditch - settling and filiter | Vietnam | CL + SVR | - | 8.59 | 11,935 | 8,780 | 1,164 | 1,306 | 1,043 | 6.6 | 130 | 60 | 94 | 67 | 50 | 99 | 99 | 92 | 95 | Nguyen and Luong (2012) |
| Dissolved air flotation - anaerobic lagoon - anoxic lagoon - aerated tank | Vietnam | CL + SVR | - | 5.37 | 5,610 | - | 867 | 372 | 341 | 7.8 | 136 | - | 98 | 33 | 13 | 98 | - | 89 | 91 | Syutsubo et al. (2015) |
| Dissolved air flotation - lagoon - aeration tank - aerated tank | Vietnam | CL + SVR | - | 6.34 | 5,350 | - | 357 | 394 | 154 | 7.8 | 128 | - | 70 | 41 | 27 | 98 | - | 80 | 90 | Syutsubo et al. (2015) |
| ABR - DHS | Vietnam | RSS | 42 | 5.5 | 3,700 | 3,450 | 200 | 220 | 108 | 8.1 | 102 | 35 | 27 | 57 | 20 | 97 | 99 | 87 | 74 | Watari et al. (2016b) |
| ABR - Algal Tank | Vietnam | RSS | 14.2 | 5.5 | 3,700 | 3,450 | 200 | 220 | 108 | 8.1 | 222 | 92 | 126 | 97 | 77 | 94 | 97 | 37 | 56 | Watari et al. (2016b) |
| ABR - UASB -DHS | Vietnam | RSS | 2.0 | 5.3 | 8,430 | - | 1,470 | 420 | 200 | 7.6 | 120 | - | 36 | 220 | 100 | 99 | - | 98 | 48 | Watari et al. (2016a) |
| UASB | Vietnam | RSS | 0.8 | 7.1 | 1,450 | - | 279 | - | - | 7.4 | 102 | - | 72 | - | - | 96 | - | 74 | - | Thanh et al. (2016) |
| UASB | Thailand | CL | 4 | 1.95 | 3,350 | 1,855 | 340 | 661 | 271 | - | - | - | - | - | - | 60 | - | - | - | Boonsawang et al., (2008) |
| UASB - UASB - DHS | Thailand | CL | 11.5 | 5.5 | 9,710 | 8,670 | 1,780 | 1,370 | - | - | - | - | - | - | - | 96 | - | - | - | Tanikawa et al. (2016) |
| Oxidation Ditch Process | Malaysia | CL | - | 7.16 | 2,675 | 1,871 | 3,645 | 231 | 17 | 7.1 | 56 | 22 | 1,313 | 36 | 0 | 98 | 99 | 64 | 84 | Ibrahim et al. (1980) |
| Stablilisation pond | Malysia | CL | - | - | - | - | - | - | - | - | - | - | - | - | - | 93 | 90 | - | - | Madhu et al. (2007) |
| ABR-UASB-ST-DHS | Vietnam | RSS | 6.3 | 5.5 | 3,940 | 3,320 | 170 | 200 | 110 | 7.7 | 140 | 36 | 46 | 53 | 53 | 95 | 98 | 72 | 67 | This study (during phase 3) |

Note: ABR: anaerobic baffled reactor, CL: Concentrated latex, DHS: down flow hanging sponge, RSS: Ribbed smoked sheet, ST: settling tank, SVR: standard Vietnamese rubber, UASB: upflow anaerobic sludge

### 3.2.4 Conclusion

· A pilot-scale UASB –DHS combined with an ABR and a ST was installed in a natural rubber processing factory in South Vietnam and its process performance was evaluated for 267 days. The UASB reactor achieved a total removal efficiency of 55.6 ± 16.6% for COD and 77.8 ± 10.3% for BOD with an organic loading rate of 1.7 ± 0.6 kg-COD·$m^{-3}$·$day^{-1}$. The final effluent of the proposed system had 140 ± 64 mg·$L^{-1}$ of total COD, 31 ± 12 mg·$L^{-1}$ of total BOD, and 58 ± 24 mg-N·$L^{-1}$ of TN. The post-treatment DHS reactor can perform removal efficiencies of 64.2 ± 7.5% and 55.3 ± 19.2% for total COD and TN, respectively. Also, the DHS reactor reduced that the HRT of 30 days to 4.8 hours compare with existing algae tank. 16S rRNA gene sequencing analysis of the DHS retained sludge also correspond to the result of reactor performance and both nitrifying and denitrifying bacteria were detected in the sponge carrier. In addition, Anammox bacteria was also found in the retained sludge.

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**Chapter 4**

# Development of an appropriate post-treatment system by using sponge-based trickling filter

**4.1 Development of downflow hanging sponge (DHS) reactor as post treatment of existing combined anaerobic tank treating natural rubber processing wastewater**
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**4.2.3 Results and Discussion**
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**4.2.3.3 Retained sludge in STF reactor**
**4.2.3.4 Microbial community structure of STF reactor**

**4.3 Conclusions**
**Reference**

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

### 4.1.1 Introduction

The natural rubber production process discharges large amounts of wastewater containing ammonia, organic compounds and so on. Therefore, discharge of natural rubber processing wastewater without an appropriate treatment can lead to environmental problems such as deterioration of water quality and eutrophication. An 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 of the anaerobic tank still contains organic matter and ammonia. Thus, the 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 a 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. 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 same as 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 al., 2006; Tandukar et al., 2007). To date, sixth type of sponge carriers were proposed and demonstrated process performance of DHS reactor 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 combination of upflow anaerobic sludge blanket (UASB) - DHS system was 15 times smaller than conventional activated sludge process. The DHS reactor has been applied for treatment of several kinds of industrial wastewaters especially post treatment of UASB reactor treated high strength industrial wastewater (El-Kamah et al., 2011; Watari et al., 2016; Tanikawa 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 the post-treatment DHS reactor for natural rubber processing wastewater treatment in Thailand effectively oxidized 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.

### 4.1.2 Materials and methods

#### 4.1.2.1 Experimental setup and operational conditions

A mini scale DHS reactor was installed at the natural rubber factory at the Rubber Research Institute of Vietnam (RRIV), 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 from 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 substrate tank for DHS influent. The 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 (PVC) pipe with a height of 150 cm. The DHS reactor was filled with 33 mm × 33 mm × 33 mm size sponge pieces made by 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 were shown in Table 1.

Table 4.1 Initial operational conditions of the DHS reactor from Phase 1 to Phase 3.

| | day | Flow rate $L{\cdot}day^{-1}$ | HRT h | OLR $kg\text{-}COD{\cdot}m^{-1}{\cdot}day^{-1}$ | NLR $kg\text{-}N{\cdot}m^{-1}{\cdot}day^{-1}$ |
|---|---|---|---|---|---|
| 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 |

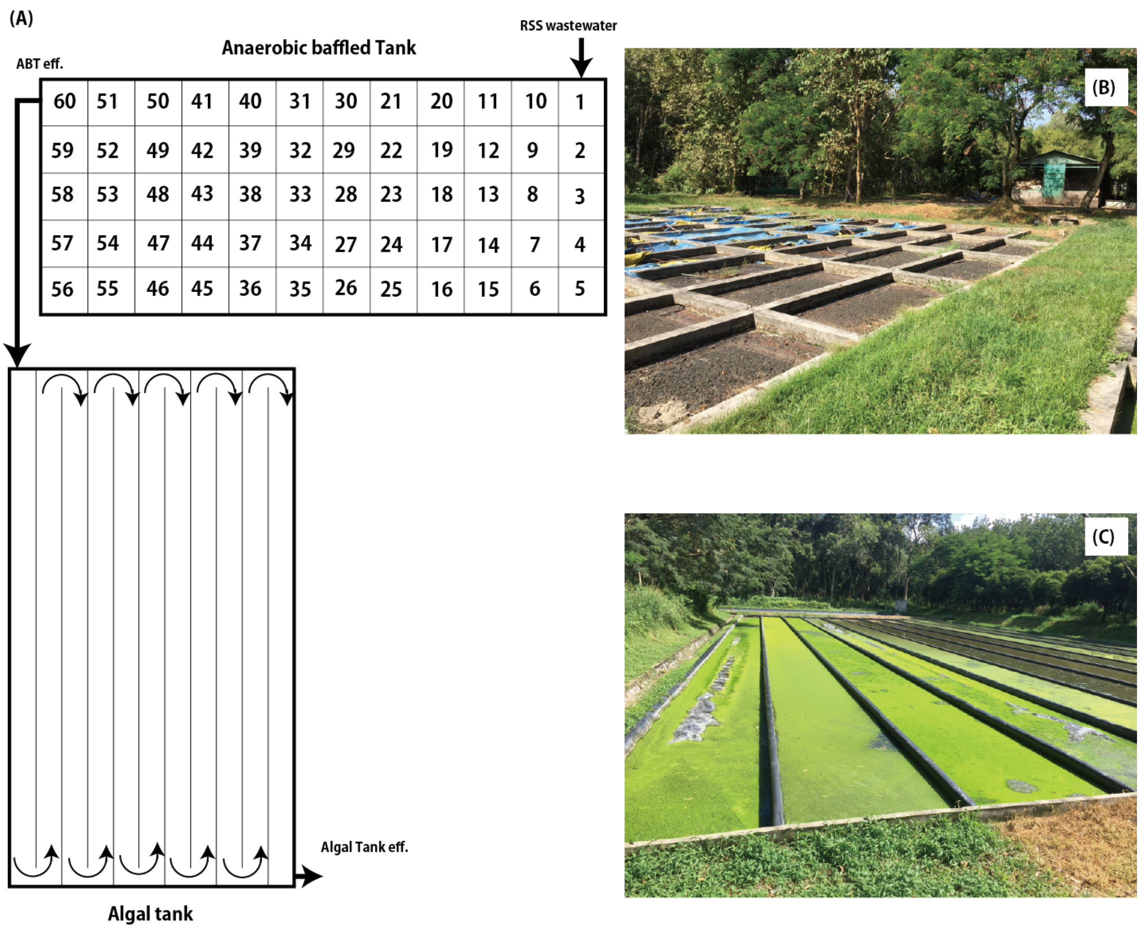


Figure 4.1 (A) Schematic diagram of treatment system in the natural rubber factory, (B) picture of anaerobic baffled tank, (C) picture of algal tank.

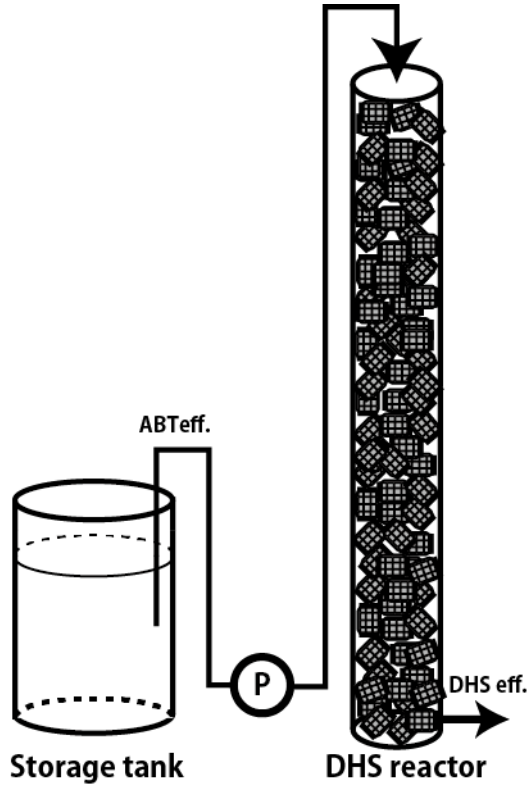


Figure 4.2 Schematic diagram of the mini scale post-treatment DHS reactor

Table 4.2 Process performance of the anaerobic baffled tank 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 |

### 4.1.2.2 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 chemical oxygen demand (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 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 reduction of ammonia.

### 4.1.2.3 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 PBS and stored at −20 °C until DNA was extracted. DNA extraction was performed using a FastDNA Spin Kit for Soil (MP Biomedicals). 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 achaea (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 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 (OTU) 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.

### 4.1.3 Results and Discussion

#### 4.1.3.1 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 organic loading rate (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 effluent standards in Vietnam. 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 was 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, 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 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 were 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 good nitrification rate. Therefore, nitrite concentration of 19 ± 18 mg-N·$L^{-1}$ was remained in the DHS effluent. 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 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 effluent of the DHS reactor 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, TN removal efficiency of the DHS reactor was 52.7 ± 25.2%. During this phase, TN and ammonia of the DHS effluent exceeded the effluent standards because the influent concentrations of TN and ammonia were increased and short HRT 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-$L^{-1}$, 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. 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.

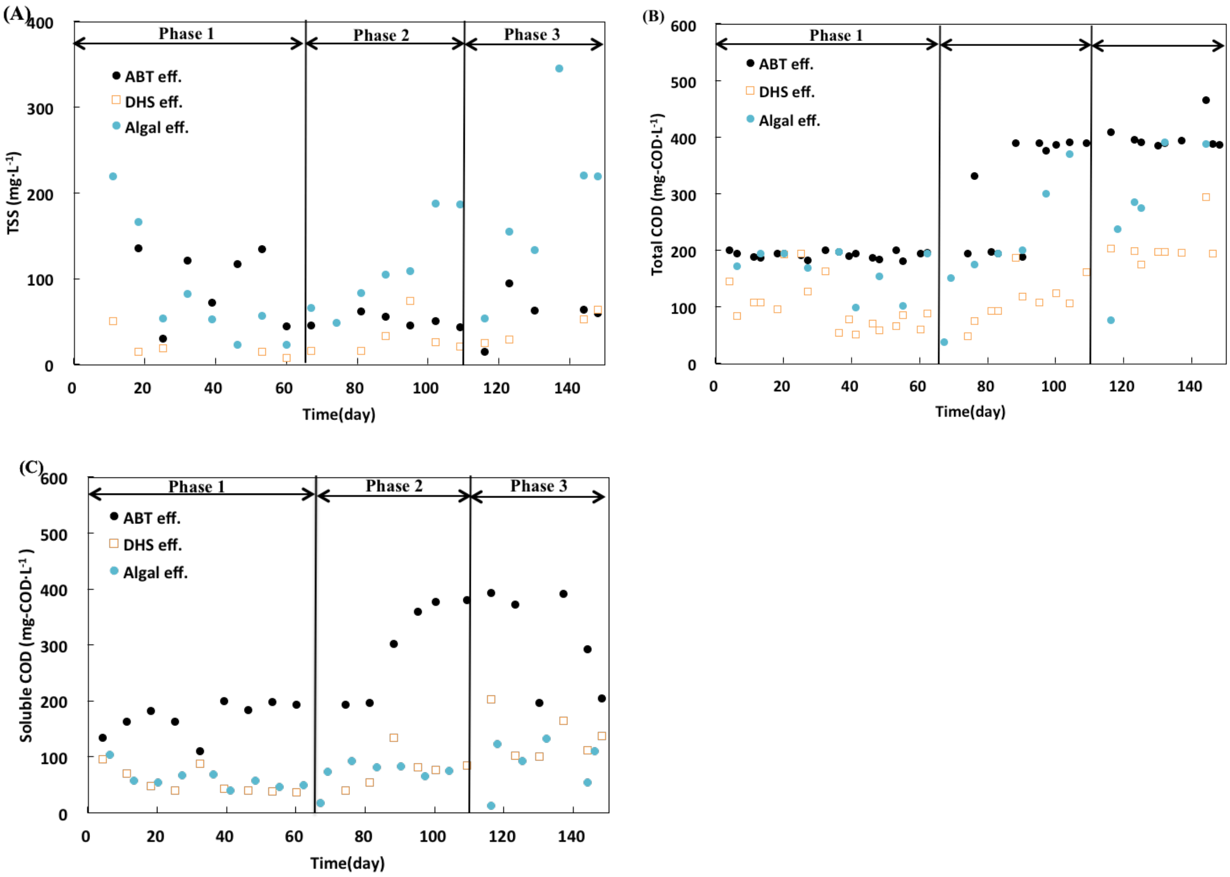


Figure 4.3 Time course of (A) total suspended solids (TSS), (B) Total chemical oxygen demand (COD) and (C) Soluble COD concentration of anaerobic baffled tank effluent (ABT eff.), downflow hanging sponge (DHS) effluent (DHS eff.) and algal tank effluent (Algal eff.)

Table 4.3 Summary of nitrogen concentrations from Phase 1 to Phase 3.

| | | TN $mg\text{-}N{\cdot}L^{-1}$ | Ammonia $mg\text{-}N{\cdot}L^{-1}$ | Nitrate $mg\text{-}N{\cdot}L^{-1}$ | Nitrite $mg\text{-}N{\cdot}L^{-1}$ |
|---|---|---|---|---|---|
| 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 |

N.D: Not detected

### 4.1.3.2 Retained sludge in the DHS reactor

The MLSS and MLVSS of the DHS sponge media retained sludge on day 35 and day 110 were 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 large amount of excess sludge was 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 of 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 for treating natural rubber processing wastewater.

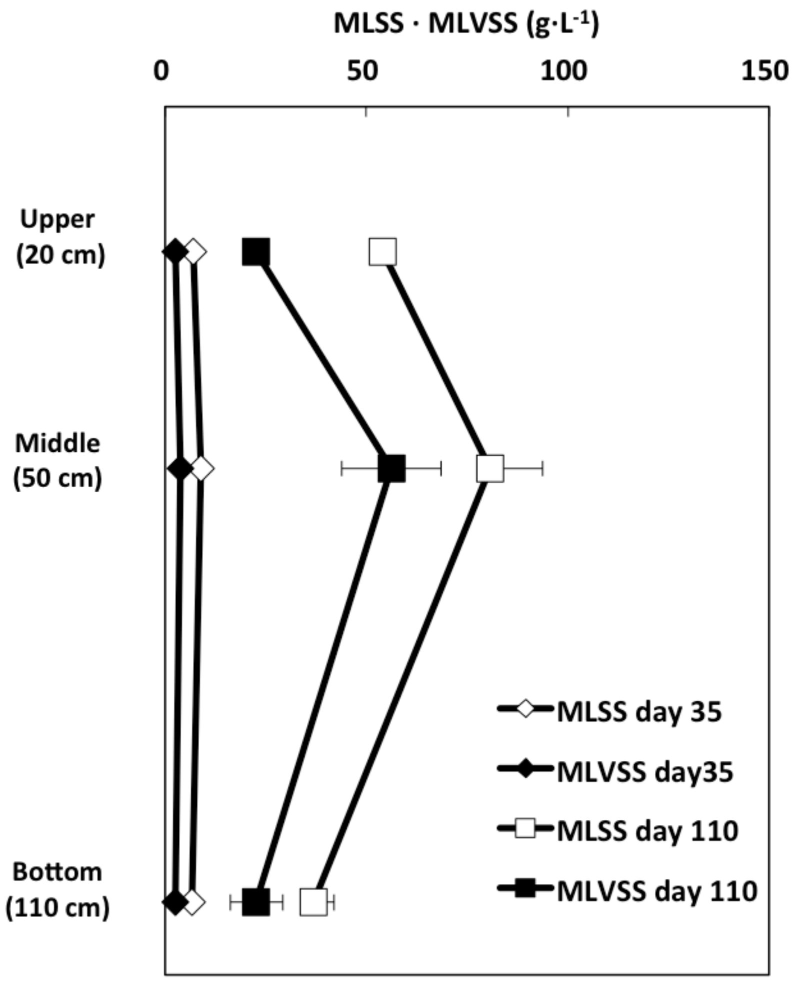


Figure 4.4 Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations of downflow hanging sponge (DHS) retained sludge on day 35 and day 110.

#### 4.1.3.3 Performance comparison of the ABT-DHS system and the current 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 $mg \cdot L^{-1}$. (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 full-scale DHS reactor performed high organic mater and ammonia removal in sewage treatment (Onodera et al., 2016; Okubo et al., 2016). Thus, this mini scale experiment would be able to assure the success of post-treatment DHS reactor at the full-scale system.

Table 4.4 Comparison of different treatment systems for natural rubber processing wastewater treatment.

| Parameter | Unit | Anaerobic baffled tank – DHS Inf. | Anaerobic baffled tank – DHS Eff. | Anaerobic baffled tank – Algal Tank Inf. | Anaerobic baffled tank – Algal Tank Eff. | Decantation–UASB–aeration tank – settling and filiter Inf. | Decantation–UASB–aeration tank – settling and filiter Eff. | Decantation–flotation–UASB–aeration tank – settling and filiter Inf. | Decantation–flotation–UASB–aeration tank – settling and filiter Eff. | BR–UASB–DHS Inf. | BR–UASB–DHS Eff. | Oxidation ditch Inf. | Oxidation ditch Eff. | Vietnamese discharge effluent standart B (QCVN01:2008/BTNMT) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 250 |
| 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 and Luong (2012) | | Nguyen and Luong (2012) | | Watari et al. (2016) | | Ibrahim (1980) | | Vietnamese discharge effluent standart B (QCVN01:2008/BTNMT) |

### 4.1.3.4 Evaluation of microbial community structure of DHS retained sludge

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). 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 4. 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 was 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., that are frequently found in sewage treatment plants (Siripong & Rittmann, 2007; Limpiyakorn et al., 2006), 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 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, known anaerobic ammonia-oxidizing bacteria, was detected with 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.

The total sequence reads of denitrifying bacteria in the DHS retained sludge was shown in Figure 5. The total sequence reads of denitrifying bacteria was significantly increased on day 110 compared with that on day 35. *Comamonas* spp., 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 (VFA) 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.

Table 4.5 Diversity indices and microbial community structure of the downflow hanging sponge (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 |
| Phylogenitic 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 |

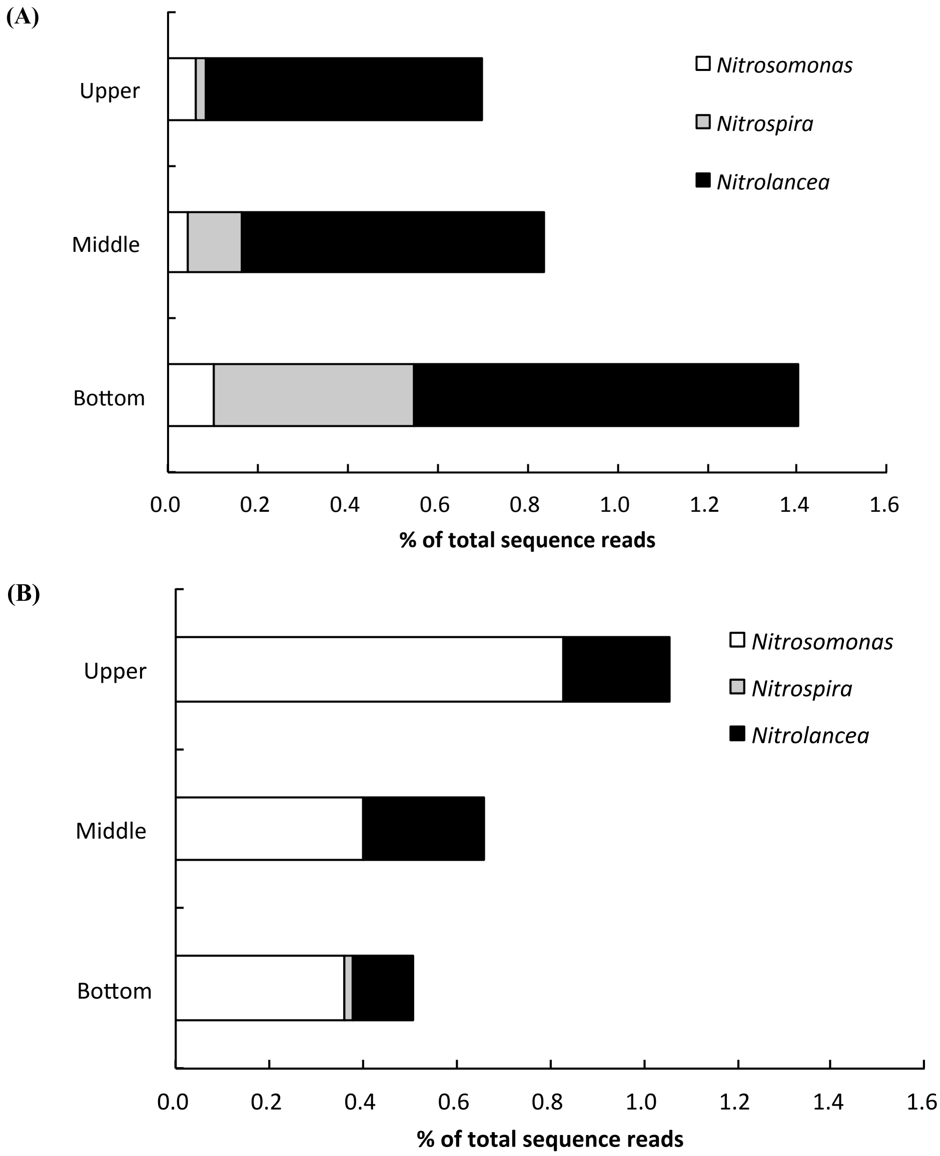


Figure 4.5 Total sequence reads of nitrifying bacteria in the downflow hanging sponge (DHS) reactor on day 35 (A) and day 110 (B).

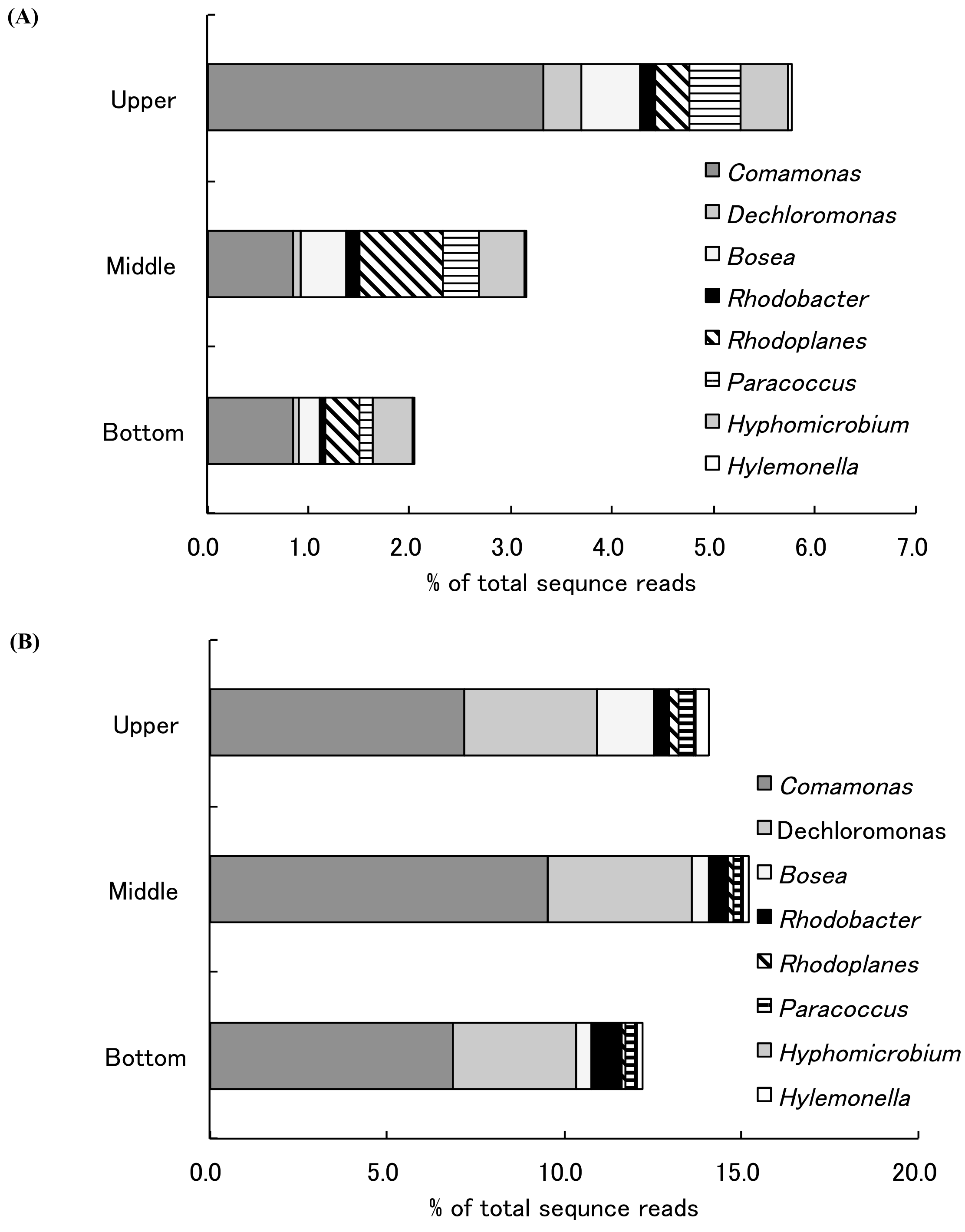


Figure 4.6 The main genera identified with known denitrification capabilities in the downflow hanging sponge (DHS) reactor on day 35 (A) and day 110 (B).

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### 4.2 Development of a single stage mainstream Anammox process using a sponge-bed trickling filter

#### 4.2.1 Introduction

An anaerobic treatment process has been widely used for sewage and industrial wastewater treatment due to its high organic removal efficiency and low operational costs (van Lier et al., 2015). However, the effluent of an anaerobic treatment process contains residual organic compounds and mineralised nutrients, i.e. ammonium and phosphorus. Thus, additional post-treatment processes are required to meet the local discharge standards.

In the search for cost-effective solutions to treat municipal sewage in a sponge-based trickling filter (STF) reactor is regarded one of the most promising systems for post-treating anaerobic effluents (Tandukar et al., 2007; Almeida et al., 2013). Machdar et al. (1997) firstly proposed that a sponge used for biomass retained carrier and it named downflow hanging sponge (DHS) reactor (Machdar et al., 1997). Nowadays, six types of sponge carriers were developed and evaluated its process performance in sewage treatment (Tandukar et al. 2007; Onodera et al. 2014, 2016; Okubo et al. 2016). The STF reactor is characterized by a high biomass retention capacity in the sponge carrier and extremely low excess sludge production without external aeration (Araki et al. 1999; Uemura et al. 2010; Onodera et al. 2014). In addition, the sponge media supports high microbial diversity on the surface and in the inner section of the sponge media (Araki et al., 1999; Kubota et al., 2014). Recent studies of microbial community structure in the sponge retained sludge showed that anaerobic ammonia oxidation (Anammox) bacteria was found and suggested both aerobic and anaerobic ammonia oxidation process occurred (Mac Conell et al., 2015; Watari et al., 2017)

A partial nitritation and Anammox process consists of two consecutive reactions: ammonium is partially oxidized to nitrite under the aerobic condition by ammonium oxidizing bacteria (AOB), and subsequently, the remaining ammonium reactors with nitrite to from nitrogen gas anaerobically by Anammox bacteria. This autotrophic nitrogen removal process can expect that 60% of reduction in oxygen demand for partial oxidation of ammonium to nitrite, almost 100% of elimination of carbon demand for denitrification and 80% reduction of excess sludge (Cao et al., 2017). In addition, Cao et al. (2017) reported more than 200 full-scale facilities have been operating successfully in the world. However, these partial nitritation – Anammox process such as sequence batch reactor requires complicated dissolved oxygen (DO) control and therefore elevated capital investment high operational costs and advanced technical expertise. The application of partial nitritation in the STF reactors was studied by Chuang et al. (2007), Uemura et al. (2011) and Guillén et al. (2015a). Chuang et al. (2007) attained partial nitritation in the DHS reactor by controlled oxygen conditions by using an air pump. Guillén et al. (2015a) studied partial nitritation under natural air circulation and showed the possibility of autotrophic nitrogen removal in STF reactors.

Also, Anammox bacteria was successfully cultivated in the closed STF reactor and the STF reactor performed around 75% of total nitrogen removal efficiency with a short HRT of 1 hour (Guillén et al., 2015b). The STF reactor had several advantages for application of partial nitritation – Anammox process such as a DO gradient from 7.5 mg-$O_2 \cdot L^{-1}$ in the external layers of sponge to around 0.2 mg-$O_2 \cdot L^{-1}$ in the inner layer (Machdar et., 2000), a large surface area can lead to an increased biomass retention capacity, thus being able to attain long solids retention time for favorable for slow growing organisms, potentially high microbial conversion as a consequence of the high biomass retention and high permeability which could be reflected in shorter hydraulic retention times (Guillén et al., 2015a).

The transfer of oxygen from air to a wastewater subject to biological aerobic treatment and play a crucial role in an oxygen-limited partial nitritation – Anammox process. Uemura et al. (2016) examined the overall volumetric oxygen transfer coefficient $K_La$ of the DHS reactor by supplying deoxygenated water from the top of device. The $K_La$ values of the DHS support media without external aeration were ranged 0.56 – 4.88 $1 \cdot min^{-1}$, surpassing those of other mechanically aerated process. This research aims to develop a single stage partial nitritation – Anammox process in STF reactor as a low cost post-treatment process using a synthetic substrate for simulating an ammonia rich effluent (100 mg of ammonia) from a upflow anaerobic sludge blanket reactor treating domestic sewage at 30˚C. In addition, oxygen mass transfer of the used STF reactor and microbial community structure of retained sludge were evaluated.

### 4.2.2 Materials and Methods
#### 4.2.2.1 Experimental appears

Figure 4.7 shows schematic diagram of STF reactor used in this study. The STF reactor made by transparent acrylic glass had height of 60.5 cm. The horizontally layered sponge carrier made by polyurethane sponge slabs BVB Sublime (BVB Substrates; De Lier, The Netherlands) was used in this study. The thickness of sponge carriers was 0.75 cm. The sponge volume of STF reactor was 991 $cm^3$ and hydraulic retention time (HRT) of STF reactor was calculated based on the sponge volume. The air put valves were equipped in each sponge carrier. The STF reactor was operated at 30˚C in a temperature controlled room. A water distributor was equipped in the top of STF reactor.

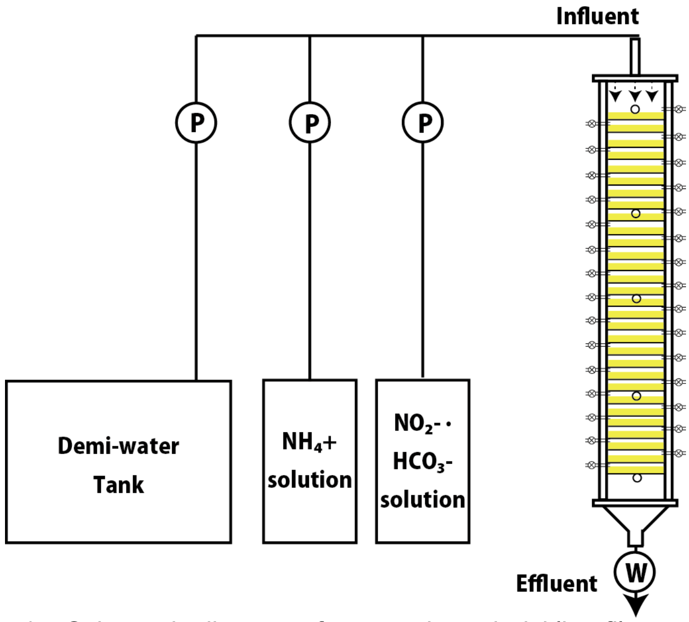


Figure 4.7 Schematic diagram of sponge-based trickling filter reactor.

#### 4.2.2.2 Synthetic wastewater

During phase 1, the mixture of ammonium and nitrite were supplied as substrate in order to cultivate anammox bacteria. The composition of these substrates per 1 liter of demineralized water was (i) ammonium feed: 2.9828 g $NH_4Cl$; 0.77 g $MgSO_4{\cdot}7H_2O$; 0.3906 g $KH_2PO_4$; 4.6875 g $CaCl_2{\cdot}2H_2O$; (ii) nitrite feed: 3.8504 g $NaNO_2$; 0.1786 g $FeSO_4{\cdot}7H_2O$; 19.531 g $KHCO_3$; 0.1786 g NaEDTA and 1.25 mL of trace element solution. The trace element solution contained per liter: 15 g Mg EDTA; 0.43 g $ZnSO_4$ $7H_2O$; 0.24 g $CoCl_2$ 6H2O; 0.99 g $MnCl_2$ $4H_2O$; 0.25 g $CuSO_4$ $5H_2O$; 0.22 g $Na_2MoO_4$ $2H_2O$; 0.19 g $NiCl_2$ 6H2O; 0.1076 g $Na_2SeO_4$; 0.014 g $H_3BO_3$; 0.05 g $NaWO_4$ $2H_2O$.

To achieve autotrophic nitrogen removal, ammonium was supplied as substrate. The composition of these substrates per 1 L of demineralized water was modified from van de Graaf et al. (1996) (i) in the ammonium-rich feed: 5.9656g $NH_4Cl$; 0.77 g $MgSO_4·7H_2O$; 0.3906 g $KH_2PO_4$; 4.6875 g $CaCl_2·2H_2O$; and, (ii) in the bicarbonate feed: 0.1786 g FeSO4·$7H_2O$; 19.531 g $KHCO_3$; 0.1786 g NaEDTA and 1.25 mL of trace element solution. During phase 3, the ammonia concentration was adjusted to 80 mg-N·$L^{-1}$.

#### 4.2.2.3 Operational conditions and inoculation

At the beginning of this study, 300 ml of anammox sludge from an existing lab-scale sequence batch reactor was inoculated to sponge-bed. The mixed liquor suspends solid (MLSS) of seed anammox sludge was 510 ± 50 mg·$L^{-1}$. The air put valves were closed to keep anaerobic condition in the STF reactor. After, nitrogen removal was observed 100 ml of activated sludge (MLSS: 6,400 ± 300 mg·$L^{-1}$, mixed liquors volatile suspended solid (MLVSS): 1,030 ± 60 mg·$L^{-1}$) was added in the sponge-bed in order to growth ammonia oxidizing bacteria. Summary of operational condition was shown in Table 4.6.

Table 4.6 Operational conditions for sponge-based trickling filter

| | | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|---|
| Day | | 1–25 | 26–97 | 98–181 |
| Flow rate | L·$day^{-1}$ | 9.6±0.5 | 10.6±2.4 | 7.1±2.2 |
| HRT | hours | 2.5±0.2 | 2.4±0.7 | 2,7±1.3 |
| Ammonia | mg–N·$L^{-1}$ | 52±3.6 | 133±27 | 79±19 |
| NLR | kg–N·$m^{-3}$·$day^{-1}$ | 0.48±0.03 | 1.47±0.27 | 0.55±0.20 |

#### 4.2.2.4 Analytical methods

The influent and effluent of STF reactors were collected for routine analysis. pH was measured with a pH meter (Model ProfiLine 3310. WTW, Germany). Dissolved oxygen (DO) concentrations of influent and effluent were measured by a portable DO meter (Oxi 3320, WTW). Nitrite-nitrogen ($NO_2^-$-N) and nitrate-nitrogen ($NO_3^-$-N) concentration was measured by using as ion-chromatography (ICS-1000, Thermo Scientific). Ammonia-nitrogen ($NH_4^+$-N) was analyzed by using a spectrometer (Lambda 365, Perkin Elmer). MLSS and MLVSS of retained sludge was measured by using a standard methods (APHA, )

#### 4.2.2.5 Evaluation of oxygen mass transfer in the STF reactor

The oxygen transfer coefficient ($K_La$) was measured to evaluate the ability of the sponge bed media to supply oxygen to the microorganisms. Deion water was stored in a 100 L tank and aerated with nitrogen gas to remove oxygen (70 mins). The de-oxygen water was supplied to the top of STF reactor and waiting 2 hours for stabilize water flow in the reactors. Dissolved oxygen (DO) concentration in sponge bed was measured by using a micro DO electrode (Unisense). The DO electrode was fix by an iron stand to measure the surface of sponge bed. $K_La$ is a coefficient indicating the ability of aeration tanks and devise to transfer oxygen from the gas phase to the liquid phase per time. This parameter has been frequently used to evaluate aeration tank in conventional activated sludge process. $K_La$ was calculated with following equation (JSWA, 2012, Uemura et al., 2015).

$$K_La = 1/t \times \ln (Cs \cdot (Cs-Ct)^{-1})$$

Cs: saturated dissolved oxygen concentration ($mg \cdot L^{-1}$)
Ct: dissolved oxygen concentration in the effluent at time t ($mg \cdot L^{-1}$)

The oxygen consumption rate of nitritation was calculated with following equation.

Oxygen consumption rate = 3.16 mg-$O_2$·mg-$NH_4$-N $\times$ 50% of influent ammonia concentration (mg-N·$L^{-1}$) $\times$ Influent flow rate (L·$day^{-1}$)

Oxygen capacity of STF reactor was calculated with following equation
Oxygen capacity = $K_LA$ (Cs - $C_0$)

#### 4.2.2.6 Massively parallel 16S rRNA gene sequence

The sludge sample was collected from 2nd, 15th and 25th sponge media. The retained sludge was extracted from the sponge media and gently washed with PBS and stored at -20 ˚C until DNA was extracted. DNA extraction was performed using a FastDNA Spin Kit for Soil (MP Biomedicals). 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 achaea (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 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.

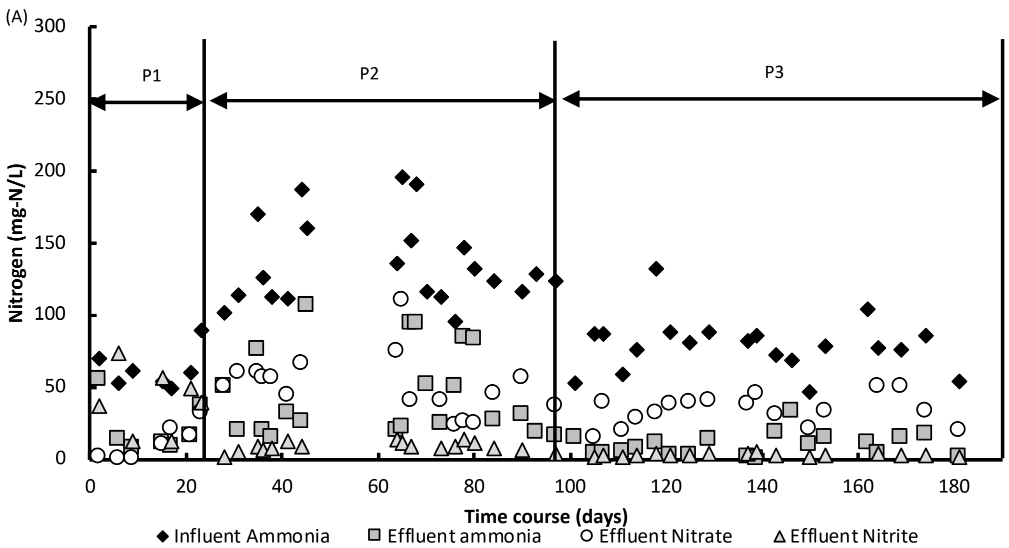
### 4.2.3 Results and Discussion

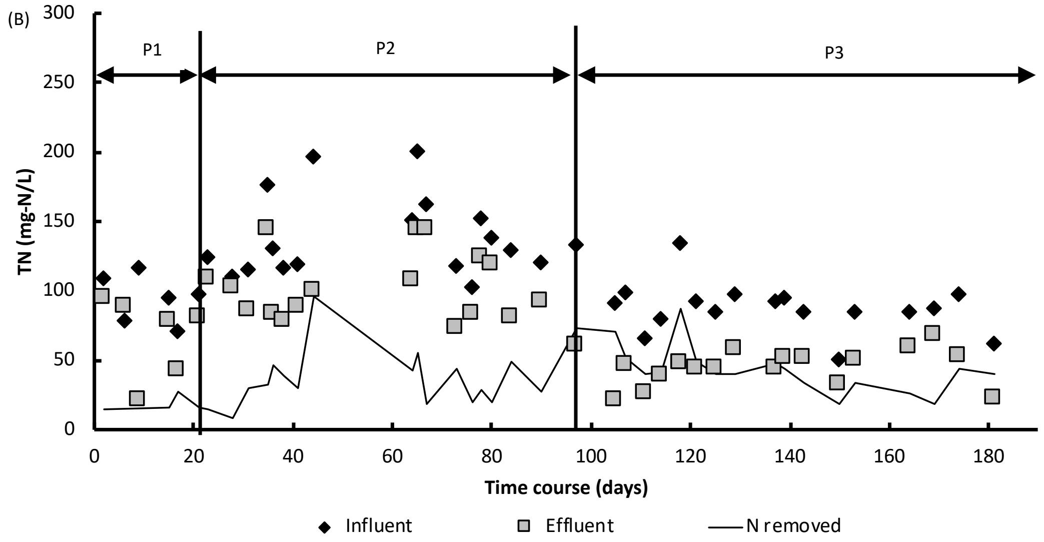
#### 4.2.3.1 Nitrogen removal performance

Figure 4.8 shows the process performance of the STF reactor during the entire experimental periods. During phase 1, ammonia and nitrite were fed to the reactor to grow Anammox bacteria in the sponge bed. After 6 days of operation, the ammonia removal efficiency reached to 70%, apparently related to a DO level in the influent of about 1.0 mg·$L^{-1}$. The fast growth of nitrifiers in the STF reactor under low DO concentrations was observed in previous studies in our lab (Guillén et al., 2015a). The average influent and effluent ammonia concentration were 62.3 ± 13.9 mg-N·$L^{-1}$ and 21.6 ± 17.6 mg-N·$L^{-1}$, respectively. The ammonia and TN removal efficiencies were 67.4 ± 22.4% and 18.0 ± 5.6%, respectively during this phase. The production of nitrite indicated that most of ammonia was oxidized to nitrite by ammonia oxidizing bacteria but Anammox bacteria did not function well.

In phase 2, ammonia was supplied to attain partial nitritation - Anammox process in the STF reactor. The DO concentration of effluent was around 0.3 ± 0.2 mg-$O_2$·$L^{-1}$. Chuang et al. (2007) reported partial nitritation in a closed DHS reactor operated with oxygen concentration in 0.42 mg-$O_2$·$L^{-1}$. The ammonia concentration of influent and effluent were 135 ± 28.4 mg-N·$L^{-1}$ and 40.2 ± 27.8 mg-N·$L^{-1}$, respectively. The STF reactor performance showed a high ammonia removal efficiency of 70.0 ± 19.1% and an ammonia removal rate of 0.97 ± 0.29 kg-N·$m^{-3}$·$day^{-1}$ at a nitrogen loading rate (NLR) of 1.41 ± 0.27 kg-N·$m^{-3}$·$day^{-1}$ even at oxygen limited condition. Tawifk et al. (2006) reported that the nitrification rate of DHS reactor installed post-treatment of UASB reactor treating sewage was 0.22 ± 0.07 kg-N·$m^{-3}$·$day^{-1}$. Chuang et al. (2007) demonstrated partial nitrification – Anammox process in the closed DHS reactor and the nitrification rate was 1.46 kg-N·$m^{-3}$·$day^{-1}$. Therefore, the STF reactor is efficient for ammonia oxidation. However, TN removal efficiency was as low as 28.1 ± 12.1%. Figure 4.9 shows pH, DO and nitrogen concentrations profiles in the STF reactor on day 92. The DO concentration of the surface of sponge carrier was also most kept under 1.5 mg-$O_2$·$L^{-1}$. Machdar et al. (2000) found DO in the 1 cm inner the sponge of DHS reactor was around 0.2 mg-$O_2$·$L^{-1}$. Thus, this oxygen concentration in the STF retained sponge could be favour condition for partial nitritation and Anammox process. The accumulation of nitrite observed in the top of reactor. In fact, pH was immediately decreased 8.5 to 7.5 in the upper part. This pH deterioration was also observed in our previous study (Guillén et al., 2015a) and showed high microbial activity in the top of reactor. Besides, effective nitrogen removal did not observe in the bottom of reactor. Previous research reported many factors for the inhibition of the Anammox process (Jin et al., 2012). In the bottom of STF reactor, pH was around 7.2 -7.4. Jaroszynski et al. (2012) noted that in the pH range 7-8, the decrease in Anammox activity was independent of pH and related only to the concentration of free ammonia. The free ammonia concentration of the bottom of STF reactor was calculated below 1.0 mg-N·L$^{-1}$ and this concentration was lower than the free ammonia inhibition concentration reported by previous study (Fernández et al., 2012). The ammonia, nitrite and nitrate concentrations in the bottom of reactor also was fell within the range of the inhibition of the Anammox process (Jin et al., 2012). However, the inhibition of Anammox bacteria was significantly observed in the downstream and further research in needed to application of STF reactor to single stage partial nitritation – Anammox process. Also, the fluctuations of ammonia and TN concentrations in the STF reactor were observed height due to short circuiting in the system. Therefore, the STF reactor requires further modification such as spilt influent feeding to improve nitrogen removal efficiency.

Since there was no improvement of TN removal efficiency, the NLR was decreased to 0.55 ± 0.20 kg-N·m$^{-3}$·day$^{-1}$at phase 3 by reducing influent ammonia concentration and flow rate. During this phase, the ammonia removal efficiency was as high as 89.8 ± 8.2% and the ammonia of effluent was 7.8 ± 6.1 mg-N·L$^{-1}$. This high ammonia removal efficiency could be the reduction of influent ammonia concentration led complete nitrification by enough oxygen in the STF reactor. This result shows the decrease of NLR leaded increase nitrite oxidizing bacteria activity and deterioration of TN removal efficiency of 42.7 ± 16.9%.





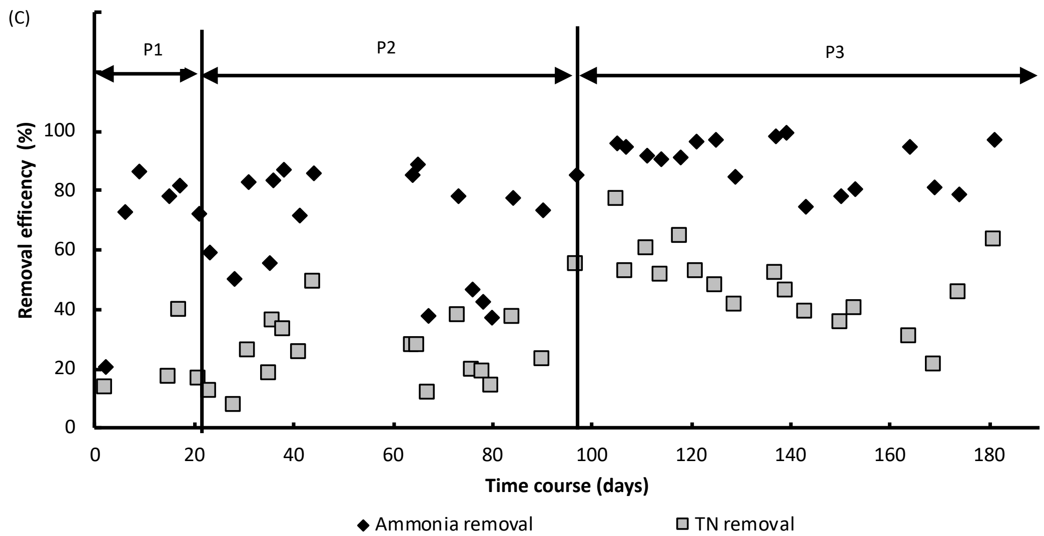




Figure 4.8 Time course of (A) ammonia, nitrate and nitrite, (B) TN and (C) ammonia and TN removal efficiency during the entire experimental periods.

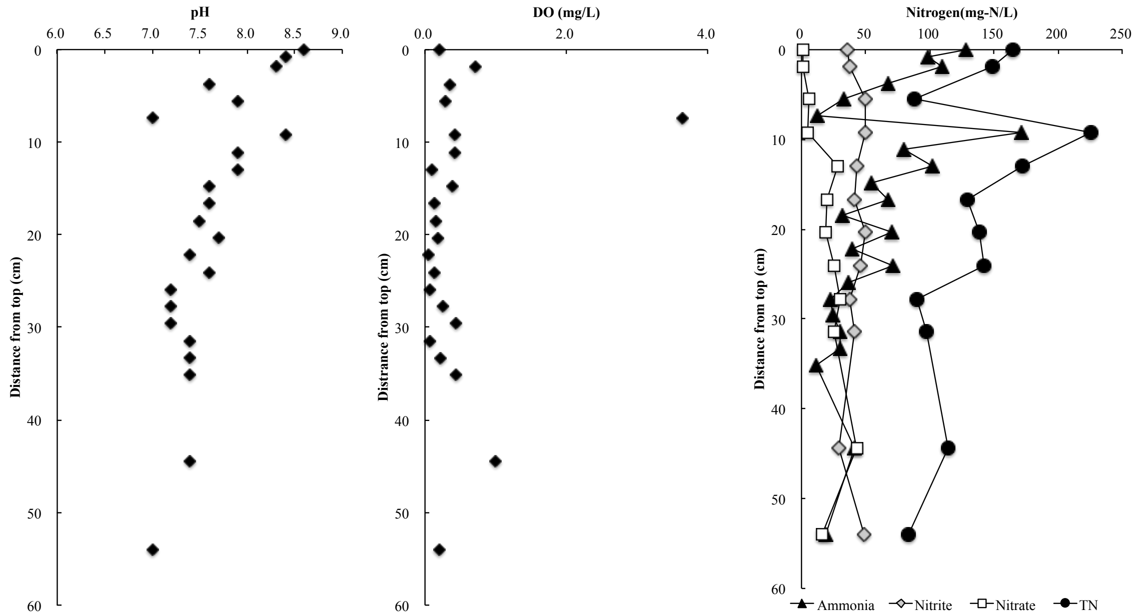



Figure 4.9 pH, DO and nitrogen profiles in STF reactor on day 92.

#### 4.2.3.2 Oxygen mass transfer in the STF reactor

Before starting the experiment, oxygen transfer coefficient of the STF reactor was measured to find an appropriate condition for partial nitritation – Anammox process. Figure 4.10 shows the DO profile with suppling dioxygen water in STF reactor at influent flow rate of 5 ml·min$^{-1}$, 10 ml·min$^{-1}$ and 20 ml·min$^{-1}$. The DO of water reached to saturation within 7 min at 20 ml·min$^{-1}$ and 10 ml·min$^{-1}$, respectively. On the other hand, the STF reactor operated at 5 ml·min$^{-1}$ took 20.5 min for reaching the DO saturation value. Uemura et al. (2016) also reported such quick oxygen acquisition in the DHS reactor. This result shows the STF reactor have a great potential for oxygen transfer.

The $K_La$ was calculated to evaluate the ability of the sponge bed media to supply oxygen to the microorganisms (Figure 4.10). The $K_La$ values at an influent flow rate of 20 ml·min$^{-1}$, 10 ml·min$^{-1}$ and 5 ml·min$^{-1}$ were 0.259 1·min$^{-1}$, 0.226 1·min$^{-1}$ and 0.074 1·min$^{-1}$, respectively. The assessed $K_La$ values in the lab-scale STF reactor were lower than most convective diffusion based down-flow hanging sponge reactors, but higher or similar to conventional activated sludge process (Uemura et al., 2016). In this study, oxygen consumption rate for nitritation of 50 mg-N·L$^{-1}$ of ammonia was 1,700 mg-$O_2$·L$^{-1}$. At 10.8 L·day$^{-1}$ flow rate, the reactor achieved 1,670 mg-$O_2$·day$^{-1}$. From this calculation, it is clear that appropriate oxygen was transferred in the reactor for nitritation.

The oxygen capacity and average oxygen consumption in phase

2 and phase 3 calculated based on ammonia and nitrite oxidation are shown in Figure 4.12. The oxygen capacity of STF reactor at phase 2 and phase 3 calculated based on flow rate and $K_La$ values were 1.68 kg-$O_2 \cdot m^{-3} \cdot day^{-1}$ and 0.81 kg-$O_2 \cdot m^{-3} \cdot day^{-1}$, respectively. The oxygen consumption at phase 2 and phase 3 of 3.05 ± 0.80 kg-$O_2 \cdot m^{-3} \cdot day^{-1}$ and 1.79 ± 0.47 kg-$O_2 \cdot m^{-3} \cdot day^{-1}$ were twice higher than the calculated oxygen capacity. Courtens et al., (2014) noted that physical data alone can thus provide misleading information on oxygen transfer mechanisms and rate, and pointed out the importance of biological activity in the total oxygen transfer. The enhancement factor was used for accounting the increase in oxygen transfer capacity resulting from biological activity (Garcia-Ochoa and Gomez, 2009). The enhancement factor of rotating biological conductor was up to 10. (Courtens et al., 2014) Therefore, some biological oxygen transfer could occur in the STF reactor. The total oxygen consumption rate of this reactor was almost same as previous studies (Hatamoto et al., 2010, 2011; Machdar et al., 1997; Tandukar et al., 2007). Using this value as a benchmark, the appropriate STF reactor for partial nitritation – Anammox process. However, further study needs to perform effective partial nitritation – Anammox process such as biological oxygen transfer.

The oxygen consumption of ammonia oxidation has the biggest variation during phase 2 and phase 3 (Figure 4.12). Hatamoto et al., (2011) also reported that ammonia oxidation showed the greatest variation due to sensibility of ammonia oxidation are low oxygen affinity of ammonia-oxidizing bacteria and lower free energy charge of ammonia oxidation compared with acetate, propionate, methane and sulfide oxidation.

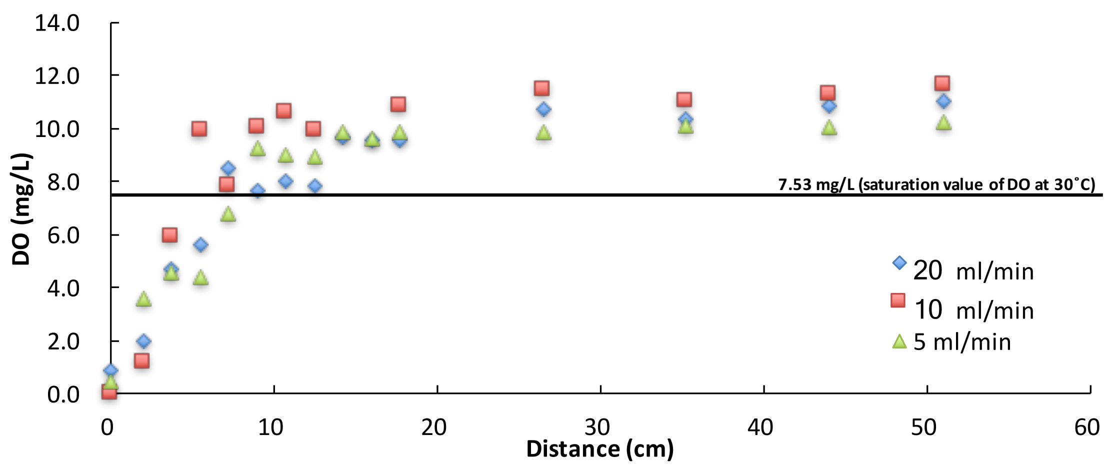



Figure 4.10 DO concentrations in sponge media at different flow rate.

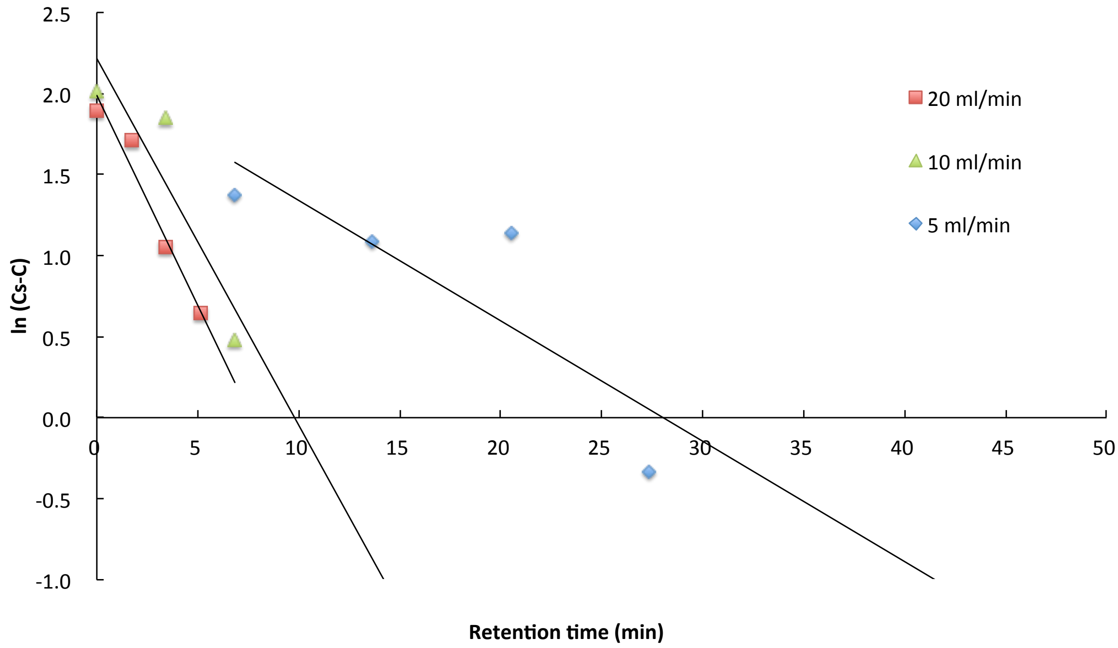



Figure 4.11 $K_La$ of the STF reactor at different flow rate.

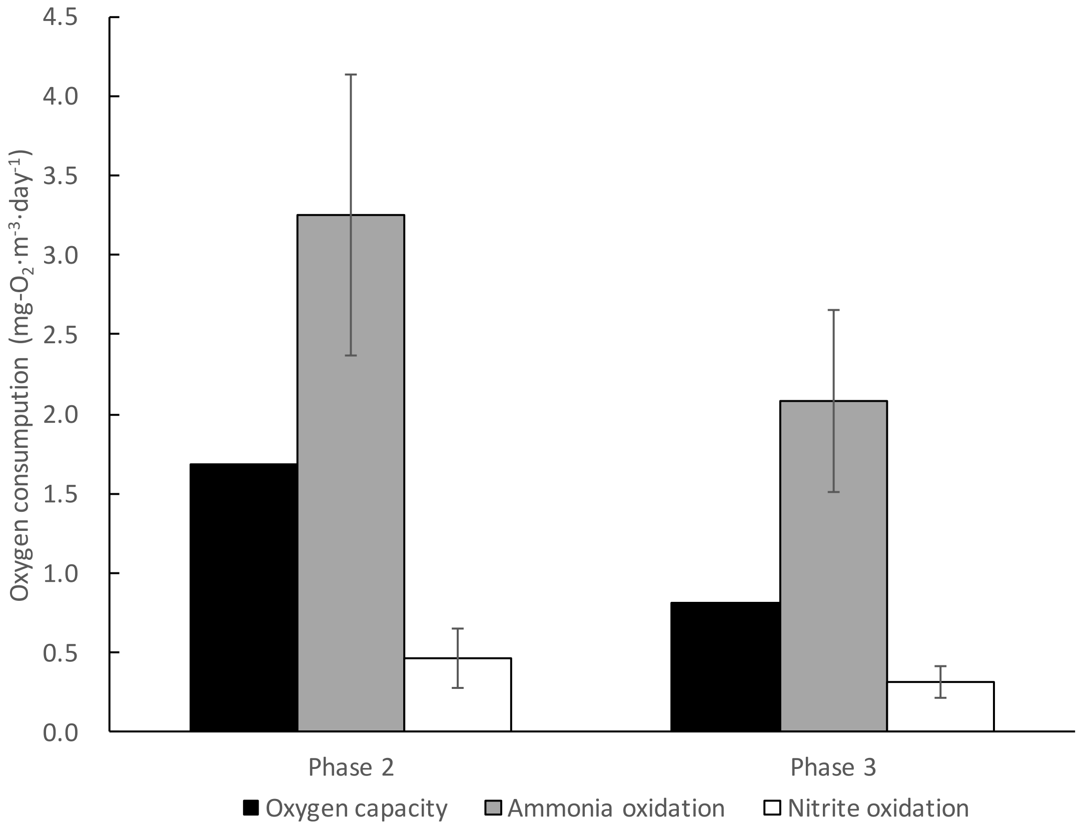



Figure 4.12 Oxygen capacity and oxygen consumption of ammonia oxidation and nitrite oxidation at phase 2 and phase 3. The error bars indicate the standard deviations.

#### 4.2.3.3 Retained sludge in STF reactor

The biomass development in the sponge carrier was visually inspected periodically. The higher biomass growth was observed in top of reactor. This biomass tendency was also observed in our previous study (Sanchez Guillen et al., 2015) and considered the direct availability of substrate at the upper sponge layer. During the entire experimental periods, the biomass washed-out was not observed from the sponge layers. At the end of experiment, the retained sludge concentrations in sponge bed was determined (Figure 4.13). The highest sludge concentration of 145.4 mg-MLSS·$L^{-1}$ and 82.2 mg-MLVSS·$L^{-1}$ was found in the top of reactor (sponge layer No.6). However, the concentrations of retained sludge were considerably lower than previous studies (Tawfik et al., 2006, 2013; Watari et al., 2016).

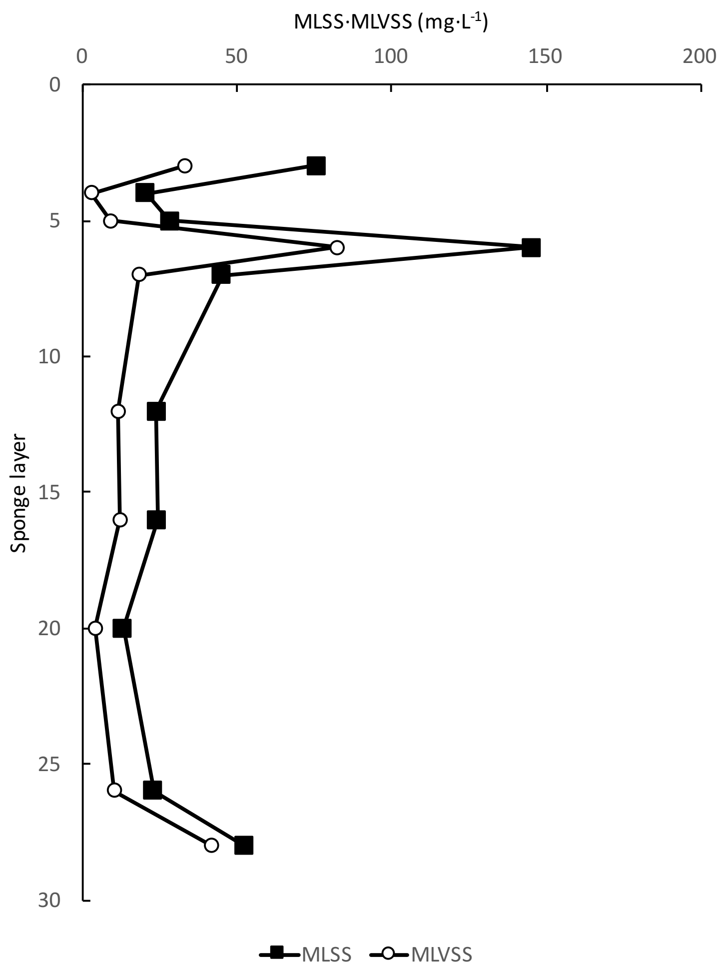



Figure 4.13 MLSS and MLSS concentration of the STD retrained sludge at the end of experiment

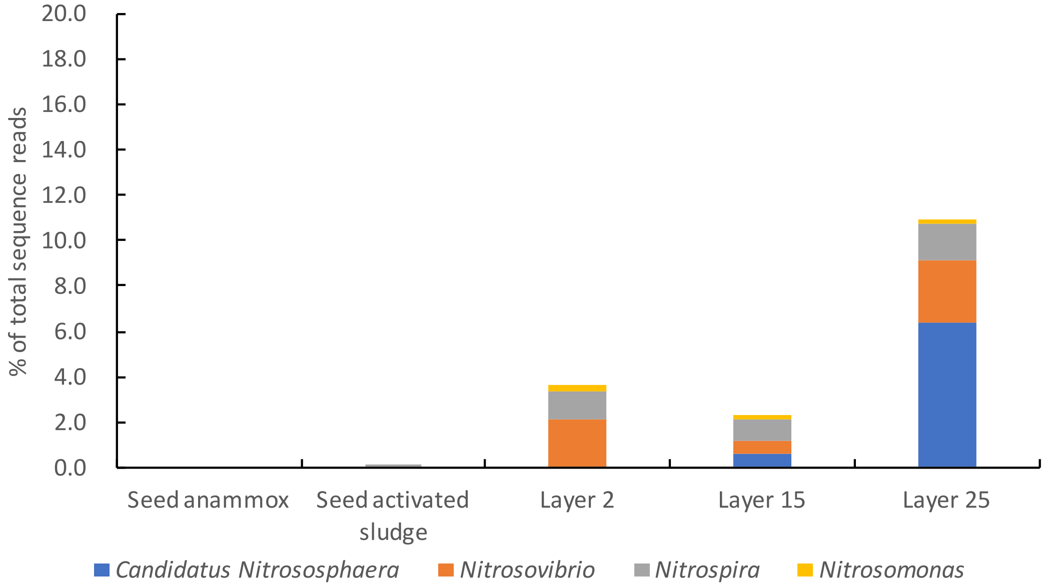
### 4.2.3.4 Microbial community structure of STF reactor

The microbial community structure of the seed sludges and the STF retained sludge were investigated by using 16S rRNA gene-based massively parallel sequencing analysis (Table 4.7, Figure 4.14). Ammonia oxidizing bacteria (AOB) *Nitrosovibrio* sp. and *Nitrosomonas* sp. were detected in the reactor. In addition, ammonia oxidizing archaea (AOA) *Candidatus* Nitrososphaera was highly found in the bottom of reactor. This difference of AOB and AOA dominance may be due to the concentration of ammonia (Erguder et al., 2009). Hatzenpichler et al., 2008) reported that *Candidatus* Nitrososphaera was partially inhibited at ammonia concentration of 43.1 mg·N·$L^{-1}$. Limpiyakorn et al. (2011) reported significant numbers of AOA amoA genes occurred in municipal WWTPs with ammonium levels in the influent and effluent of 5.6–11.0 mg-N·$L^{-1}$ and 0.2–3.0 mg-N·$L^{-1}$, respectively. Nitrite oxidizing bacteria *Nitrospira* sp. was detected at a relative amount of 1.0-1.5% of total sequence reads. With regard to the Anammox bacteria, *Candidatus* Brocadia was detected in the STF reactor, which is in agreement with observations of Huang et al. (2016), who also found the same species in a single stage partial nitritation – Anammox process. Moreover, several denitrifying bacteria *Rhodanobacter* sp. and *Dechloromonas* sp. were detected. Therefore, autotrophic and heterotrophic nitrogen removal probably occurred in the STF reactor.

Table 4.7 Microbial community structure of seed sludge and STF retained sludge.

| | Anammox seed sludge | Activate seed sludge | Upper | Middle | Bottom |
|---|---|---|---|---|---|
| | | % of total sequence reads | | | |
| *Proteobacteria* | 27.0 | 29.5 | 38.8 | 27.1 | 26.6 |
| *Acidobacteria* | 3.9 | 2.2 | 26.1 | 15.2 | 19.0 |
| *Firmicutes* | 0.5 | 14.3 | 4.4 | 18.4 | 9.6 |
| *Chlorobi* | 28.1 | 1.0 | 2.1 | 10.6 | 3.6 |
| *Bacteroidetes* | 4.8 | 13.8 | 8.1 | 7.3 | 10.5 |
| *Chloroflexi* | 16.3 | 7.0 | 5.9 | 3.4 | 4.8 |
| *Actinobacteria* | 7.1 | 24.9 | 1.2 | 1.3 | 1.1 |
| *Planctomycetes* | 6.4 | 1.4 | 5.5 | 7.5 | 7.2 |
| *Chlamydiae* | 0.0 | 0.1 | 1.3 | 2.9 | 5.9 |
| *Armatimonadetes* | 4.7 | 0.3 | 2.7 | 0.3 | 0.4 |
| Other | 1.1 | 5.6 | 4.0 | 5.9 | 11.5 |

(A)




(B)

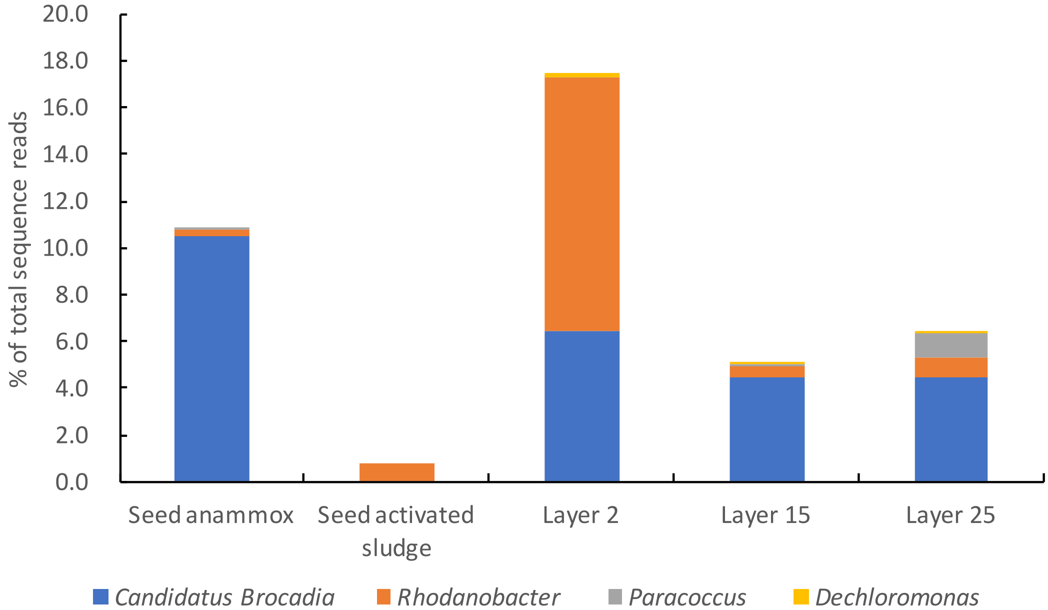



Figure 4.14 Total sequence reads of nitrifying bacteria and achaea (A) and Anammox and denitrifying bacteria in STF reactor and seed sludge

### 4.3 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.

A sponge-based trickling filter reactor was applied for single stage mainstream Anammox process. The oxygen capacity of STF reactor was almost equivalent for calculated oxygen consumption for partial nitritation. The STF reactor performance showed a high ammonia removal efficiency of 70.0 ± 19.1% and an ammonia removal rate of 0.97 ± 0.29 kg-N·$m^{-3}$·$day^{-1}$ at a nitrogen loading rate (NLR) of 1.41 ± 0.27 kg-N·$m^{-3}$·$day^{-1}$ even at oxygen limited condition. However, several limitations for nitrogen removal was observed in the downstream of STF reactor. Therefore, further modification and research are required for application of STF reactor to single stage nitritation – Anammox process. The microbial community structure analysis showed denitrifying bacteria was detected in the sponge retained sludge and could be occurred not only Annamox process, but also denitrification.

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**Chapter 5**

# Summary

**5.1 Summary of this thesis**
**5.2 Recommendations for improving the current treatment system**
**5.3 Design of an appropriate treatment system for a large natural rubber processing factory and calculation of electricity generation**

**Reference**

### 5.1 Summary of this thesis

In this thesis, an appropriate treatment system for natural rubber processing wastewater in Vietnam was developed by laboratory - and pilot- scale continues flow experiment. After the experiment, the several parameters such as effluent quality, hydraulic retention time (HRT) and greenhouse gas (GHGes) emission was compared with the existing system. In addition, further post-treatment system for natural rubber processing wastewater was developed by using sponge-based trickling filter. The experimental outcomes and conclusions in each chapter are as follows:

In Chapter 3, laboratory scale baffled reactor (BR) – upflow anaerobic sludge blanket (UASB) – downflow hanging sponge (DHS) system was installed in Hanoi University of Science and Technology and examined treatment of natural rubber processing wastewater obtained from Thanh Hoa province, Vietnam. The BR worked efficient solid removal such as residual natural rubber particulars as well as acidification of wastewater. The UASB reactor also performed at a high total COD removal efficiency of 92.7 ± 2.3% with an organic loading rate (OLR) of 12.2 ± 6.2 kg-COD·$m^{-1}$·$day^{-1}$. The methane recovery rate, calculated from the removed total COD, was 93.3 ± 19.3% in this phase. The application of UASB reactor to natural rubber processing wastewater was failed because of large amount of residual rubber accumulated in the reactor and wastewater supply line. However, combined system of pretreatment BR and UASB reactor had been successful for treatment of natural rubber processing wastewater in Vietnam. Microbial community analysis showed the acetate-utilizing methanogen *Methanosaeta* sp. was predominantly detected. The DHS reactor performed efficient organic and nitrogen removal without aeration. The pilot scale anaerobic baffled reactor - UASB - settling tank - DHS system was designed based on the laboratory scale experiment results and installed in the actual natural rubber factory in Binh Duong province. The 267 days continues flow experiment showed the high process performance of proposal system and the effluent quality was same as the existing treatment system with only 20% of HRT. The GHGes emission from the proposed system was calculated 0.015 t-CO2 eq·$m^{-3}$, corresponding to a 92% reduction of GHGes emissions with the existing treatment system. However, total nitrogen and ammonia concentrations of final effluent were exceeded the local discharge standards.

In Chapter 4, mini-scale DHS reactor was applied as post-treatment of existing anaerobic tank treating natural rubber processing wastewater and evaluated its process performance. The DHS reactor demonstrated removal efficiencies of 64.2 ± 7.5% and 55.3 ± 19.2% for total COD and TN, 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 reactor 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. Anaerobic ammonia oxidation to nitrogen gas using nitrite as electron acceptor (Anammox process) is considered cost-effective solution for nitrogen removal after an anaerobic pre-treatment process. In this study, we conducted a laboratory scale experiment to develop a single stage partial nitritation – Anammox process in a sponge-based trickling filter (STF) reactor inoculated with Anammox sludge in order to simulate treating domestic sewage (eg. Influent ammonia concentration of 100 mg-N/L). The $K_La$ of the STF reactor was higher than those observed for conventional activated sludge processes. The STF reactor performed 89.8 ± 8.2% and 42.7 ± 16.9% of ammonia and TN removal efficiency with a nitrogen loading rate of 0.55 ± 0.20 kg-N·m$^{-3}$·day$^{-1}$ calculated based on sponge volume. Microbial community of STF retained sludge shows both autotrophic and heterotrophic nitrogen removal occurred in the reactor.

These results demonstrated UASB technology could be an appropriate process for natural rubber processing wastewater in Vietnam that containing high residual rubber particulars by application with post-treatment baffled reactor. The STF reactor had a potential for improve effluent quality in this wastewater by nitrification-denitrification process and nitritation – Anammox process in a single reactor.

### 5.2 Proposal of optimal treatment system for natural rubber processing wastewater in Vietnam

Natural rubber processing industry has one of important industry in South-east Asian countries. From this thesis, it can be suggested that natural rubber processing wastewater treatment need to be improved for saving aquatic environment. In South-east Asian countries, the local effluent discharge is going to strict in near future.

Nowadays, anaerobic and/or aerobic lagoon system has been used for natural rubber processing wastewater but effluent quality of this system is still poor and require a large amount of space and energy consumption. In addition, our survey defined greenhouse gas such as methane and nitrous oxide emitted to atmosphere from anaerobic lagoon and should pay attention about this emission.

Finally, we can propose an optimal treatment system for natural rubber processing wastewater and some recommendations for improving the current treatment system. Figure 5.1 shows the block flow diagram of optimal treatment system for natural rubber processing wastewater. An UASB reactor is attractive treatment system for natural rubber processing wastewater in Vietnam, because acetate or formic acid use for coagulation of natural rubber particular and easy biodegradable. However, much amount of rubber particulars is remained in the wastewater compared with Thailand and Malaysia. Therefore, we should design effective post-treatment system such as baffled reactor. Also, advanced anaerobic baffled reactor that inoculated anaerobic granular sludge is recommended. The DHS reactor is effective for organic removal and nitrification as post-treatment of anaerobic reactor. However, further denitrification is required for achieving the local effluent discharge standard. Therefore, a denitrification tank equipped after the DHS reactor and perform denitrification of wastewater with carbon source supplement. Recently, some research reported that possibility of applying an autotrophic nitrogen removal such as Anammox to post-treatment of anaerobic treatment (Watari et al., 2017; Hien et al., 2017). Thus, the autotrophic nitrogen removal process will be applied as post-treatment. Finally, a settling tank works excess sludge removal.

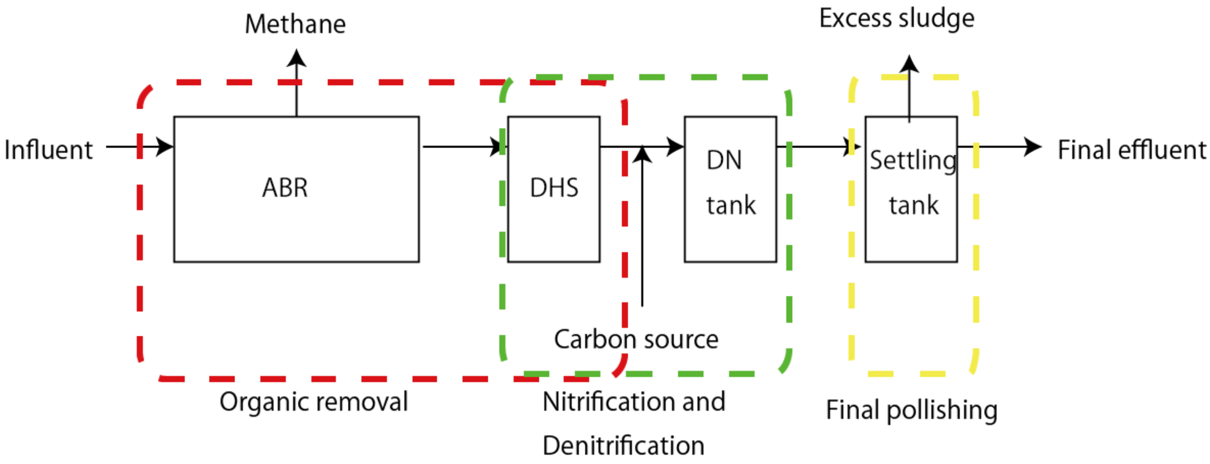


Figure 5.1 Schematic diagram of proposed system for natural rubber processing wastewater in Vietnam.

## 5.3 Design of an appropriate treatment system for a large natural rubber processing factory and calculation of electricity generation

As mentioned 5.2, combined system of ABR – DHS – Denitrification tank – Settling tank is recommended. In this section, the scale and cost of proposed system was calculated. A large scale natural rubber processing factory in South Vietnam is selected to calculation of the treatment system. The factory produced 200 ton of latex and 30 ton of cup lump per day. The factory discharged 1000 $m^3 \cdot day^{-1}$ of wastewater. The water quality of effluent is shown in Table

The design parameter of ABR reactor is

Flow rate: 1,000 $m^3$/day
Influent COD: 6,500 mg-COD/L
HRT: 4 days
Reactor volume: 4,000 $m^3$

No. of compartments: 10
Up-flow speed: 0.5 $m^3$/hour

Table Water quality of an actual factory discharged 1,000 m3/day of wastewater

| Sample | pH | T-COD (mg-COD/L) | S-COD (mg-COD/L) | SS (mg/L) | VSS (mg/L) | TN (mg/L) | VFA | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Actate (mg-COD/L) | Propionate (mg-COD/L) |
| Wastewater from washing process | 5.6 | 6,430 | 6,020 | 650 | 250 | 420 | 810 | 760 |
| Wastewater from coagulation process | 6.4 | 2,830 | 1,250 | 77 | 24 | | 27 | 9 |
| BT eff. | 5.9 | 3,970 | 3,040 | 510 | 42 | 220 | 800 | 284 |
| AT eff. | 5.9 | 3,390 | 6,560 | 420 | 91 | 300 | 830 | 220 |
| Facultative lagoon eff. | 7.0 | 730 | 480 | 100 | 6 | 60 | 125 | 34 |

The calculation of ABR is
$w \times l \times h = 400\ m^3$ (single compartment)
w: wide (m)
l: length (m)
h: height (m)

The upflow speed can be calculated by
83 $m^3$/hour (flow rate) / $H \times D = 0.5\ m^3$/hour

The bottom area is
$H \times D = 166\ m^2$

The size of bottom area can be calculated by
$H = D = \sqrt{116} = 13$ m

Height of ABR is
h = 400 / 166 = 2.4 m

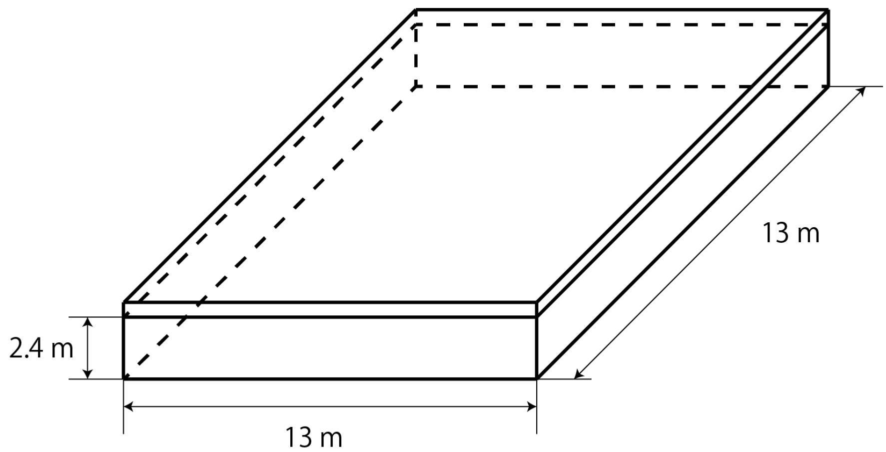


Figure design of ABR one compartment for 1,000 $m^3$/day treatment

The size of DHS was calculated flow as

Flow rate 1,000 $m^3$/day
HRT of DHS reactor: 4 hours
Sponge volume of DHS reactor: 250 $m^3$

According to the calculation for the treatment system for large scale natural rubber processing factory (1000 $m^3$/day), the volume of ABR and DHS reactor were 4,000 $m^3$ and 250 $m^3$, respectively. The proposed system required one time pumping in order to distribute wastewater to the DHS reactor. The spec of pump was selected by flowing parameter and estimated electricity consumption;

Flow rate: 2,000 $m^3$/day
Lifting height: 5 m

The centrifugal pump (GE-4M, Kawamoto pump) was selected for this calculation. The electricity consumption of this pump was estimated 7.5 kWh.

The ABR could recover energy in form as the methane. The energy recovery from ABR was calculated flow as;

Influent COD: 6,000 mg-COD/L
Influent flow rate: 1,000 $m^3$/day
Estimated methane recovery ratio (based on influent total COD): 60%

Estimated methane production (L-$CH_4$/day) =
6,000(mg-COD/L × 1,000 $m^3$/day × 60% / 2.857 (g-COD/L-$CH_4$)
= 1,260 $m^3$-$CH_4$/day

The manual for installation of biomass plant published by Ministry of Environment, Japan mentioned gas power generation unit is 1.8 kWh/ $m^3$-$CH_4$. The power generation from ABR can be calculated flow as;

The power generation from ABR (kWh) =
Methane gas production (1,260 $m^3$-$CH_4$/day) ×1.8 kWh/ $m^3$-$CH_4$
= 2,268 KWh/day

Compared with the electricity consumption and electricity generation, the ABR could be generated approximately 2,000 KWh/day. This generated electricity is enough for operating a factory.

To design the natural rubber processing wastewater treatment system in Vietnam should be considered and studied following things;

1) An UASB reactor is attractive treatment system for natural rubber processing wastewater in Vietnam, because acetate or formic acid use for coagulation of natural rubber particular and easy biodegradable. However, much amount of rubber particulars is remained in the wastewater compared with Thailand and Malaysia, and should design effective post-treatment system such as baffled reactor. Also, chemical addition should be considered.

2) In South-east Asian countries, it is not easy to prepare granular sludge. Thus, UASB reactor requires long start-up periods. Therefore, effective cultivation method of granular sludge should be studied.

3) As mentioned 1) and 2), combined the UASB reactor and the ABR reactor is proposed because of high TSS concentration of influent and difficulty of collecting granular sludge.

4) A sponge-based trickling filter reactor has great potential for post-treatment of anaerobic treatment. However, further denitrification of wastewater and coping with a large amount of washed-out sludge are required. Therefore, settling tank or screening equipment should installed before the DHS reactor. Also, a denitrification tank should be installed for improving nitrogen removal.

5) Both nitrous oxide and methane that emitted from the treatment of wastewater from a natural rubber processing factory must be considered as significant contributors to the GHGs. Therefore, closed treatment system process is recommended.

6) The microbial community structure of anaerobic system treating natural rubber processing wastewater was identified. Further research is required for identification of the relationship between process performance of the reactor and microbial community structure.

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# Achievement

## Journal Paper (Refereed)

1. Takahiro Watari, Daisuke Tanikawa, Kyohei Kuroda, Akinobu Nakamura, Masashi Hatamoto, Nanako Fujii, Fuminori Yoneyama, Osamu Wakisaka, Takashi Yamaguchi (2015) Development of UASB-DHS system for treating industrial wastewater containing ethylene glycol, Journal of Water Environment Technology, 13(2) p.131-140.
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## Oral Presentation in International Conference

1. Takahiro Watari, Daisuke Tanikawa, Kyohei Kuroda, Yuma Miyaoka, Akinobu Nakamura, Masashi Hatamoto, Nanako Fujii, Fuminori Yoneyama, Osamu Wakisaka, Takashi Yamaguchi, Development of UASB - DHS system for treatment of industrial wastewater containing ethylene glycol, 2nd International Symposium on Technology for Sustainability (ISTS), [p.225-226] Hongkong, China (2013/10)
2. Takahiro Watari, Nguyen Thi Thanh, Natsumi Tsuruoka, Dasuke Tanikawa, Kyohei Kuroda, Nguyen Lan Huong, Nguyen Minh Tan, Tan, Huynh Trung Hai, Masashi Hatamoto, Kazuaki Syutsubo, Masao Fukuda, Takashi Yamaguchi, Development of BR-UASB-DHS system for treating Natural Rubber Processing Wastewater, 9th IWA International Symposium on Waste Management Problems on Agro Industries - AGRO 2014 [p.66-73] Kouchi, Japan (2014/11)
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2. Takahiro Watari, Nguyen Xan Dan, Nguyen Thi Thanh, Daisuke Tanikawa, Nguyen Lan Huong, Nguyen Minh Tan, Takashi Yamaguchi, Study of restitution of wastewater from rubber processing factory and start-up for UASB-DHS system treatment of pretreated latex wastewater, 2nd ESCANBER workshop [P-10] Hanoi, Vietnam (2013/8)
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15. Takahiro Watari, Carlos Lopez Vazquez, Masashi Hatamoto, Takashi Yamaguchi, Jules B. van Lier (2018) Development of a single stage mainstream Anammox process using a sponge based trickling filter, LET2018, Nanjing, China (2018/5) 発表予定

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2. 谷川大輔，珠坪一晃，渡利高大，宮岡佑馬，飯島想，幡本将史，福田雅夫, Nguyen Ngoc Bich，山口隆司，天然ゴム製造工場における開放型嫌気性廃水処理システムからの温室効果ガス排出量の測定, ESCANBER シンポジウム [p.44] 長岡 (2014 年 3 月)
3. Daisuke Tanikawa, Kazuaki Syutsubo, Takahiro Watari, Masashi Hatamoto, So Iijima, Masao Fukuda, Nguyen Ngoc Bich, Takashi Yamaguchi, Evaluation of the green house gases emission from anaerobic lagoon treating wastewater of natural rubber factory, 日本水環境学会年会 [p.482] 大阪 (2013 年 3 月)
4. 谷川大輔，渡利高大，鶴岡夏海，幡本将史，福田雅夫，Nguyen Thi Thanh, Nguyen Lan Huong, Nguyen Minh Tan，珠坪一晃，山口隆司，ベトナムの天然ゴム製造工場に適した廃水処理システムの開発，日本水環境学会年会 [p.287] 仙台 (2014 年 3 月)
5. 渡利高大，谷川大輔，黒田恭平，藤井なな子，米山史紀，脇坂治，中村明靖，幡本将史，山口隆司，

UASB-DHS システムによる高濃度エチレングリコール含有廃水の処理特性評価，日本水環境学会年会 [p.502] 仙台 (2014 年 3 月)

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7. 渡利高大, 谷川大輔, 鶴岡夏海, Nguyen Thi Thanh, 黒田恭平, Nguyen Lan Huong, Nguyen Minh Tan, Huynh Trung Hai, 幡本将史, 福田雅夫, 珠坪一晃, 山口隆司, BR-UASB-DHS システムによるベトナムにおける天然ゴム製造工程廃水の処理特性評価，土木学会関東支部 [ⅤⅡ-49] 長岡 (2014 年 3 月)
8. 渡利高大, Nguyen Thi THANH, 鶴岡夏海, 谷川大輔, 黒田恭平, Nguyen Lan HUONG, Nguyen Minh TAN, Huynh Trung HAI, 幡本将史, 福田雅夫, 珠坪一晃, 山口隆司, ベトナムにおける天然ゴム製造工場廃水を対象とした処理システムの開発，日本水環境学会シンポジウム [p.164-165] 滋賀 (2014 年 9 月)
9. 渡利高大, Nguyen Thi THANH, 鶴岡夏海, 谷川大輔, 黒田恭平, Nguyen Lan HUONG, Nguyen Minh TAN, Huynh Trung HAI, 幡本将史, 福田雅夫, 珠坪一晃, 山口隆司 UASB-DHS システムを用いた天然ゴム製造工程廃水の処理手法開発，平成 26 年度土木学会全国大会 第 69 回年次学術講演会 [p.15-16] 大阪 (2014 年 9 月)
10. 渡利高大, Nguyen Thi THANH, 武笠巨尭, 谷川大輔, 黒田恭平, Nguyen Lan HUONG, Nguyen Minh TAN, Huynh Trung HAI, 幡本将史, 福田雅夫, 珠坪一晃, 山口隆司 前段 baffled reactor と UASB-DHS システムによる天然ゴム製造工程廃水の処理特性，第 49 回日本水環境学会年会 [p.51] 金沢 (2015 年 3 月)
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14. 中村嘉伸, 中村稔, 野本直樹, 段下剛, Alqadri Asri Putra, 平片悠河, 秦裕弥, ○渡利高大, 幡本将史, 山口隆司, 浄化槽の維持管理費用低減を目的とした除外装置の開発，第 30 回全国浄化槽技術研究集会 [4-3] 東京 (2016 年 8 月)
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16. 秦裕弥, 渡利高大, 松浦哲久, 星野貴宏, 星野善彦, 幡本将史, 山口隆司, DHS リアクターによる染色廃水の処理実験，第 34 回土木学会関東支部新潟会研究調査発表会 [7-209] 長岡 (2016 年 10 月)
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21. 秦裕弥，渡利高大，星野貴宏，星野善彦，牧慎也，幡本将史，山口隆司 (2018) 染色廃水処理を処理する無酸素·好気型 DHS システムの運転管理特性，第 52 回日本水環境学会年会，札幌 3-I-14-4, (2018 月 3 月)
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24. Tran Phuong Thao, Vu Thi Hoa, Nguyen Thi Thanh, Takahiro Watari, Masashi Hatamoto, Takashi Yamaguchi (2018) Upflow anaerobic reactor using bio-carrier for natural rubber processing wastewater treatment，第 52 回日本水環境学会年会，札幌, 3-D-11-1, (2018 月 3 月)

## Invited Presentation

1. Takahiro Watari, Global Research Strategy for Future Carrier, Japan-YWP 5th International Symposium in WET2016, Tokyo, Japan, (2016 年 8 月)

## Patent

1. 藤井なな子，米山史紀，脇坂治，山口隆司，幡本将史，渡利高大，グリコール類含有廃水の処理方法，グリコール類含有廃水処理装置及びこれらに用いられる汚泥，特開 2015-97994, (2015 年 5 月 28 日)

## Research Grants

1. Grant-in-Aid for JSPS Fellows, Establishment of energy recovery type treatment process for industrial wastewater treatment, 15JI12291, April/2015 – Feb/2017.
2. Grant-in-Aid for Research Activity start-up, Establishment of nitrogen removal process using sponge biomass carrier, 17H06703, October/2017-March/2019.

## Award

1. Best Presentation Award

Development of UASB-DHS system for Treatment of Natural Rubber Processing Wastewater, $69^{th}$ Japan Society of Civil Engineering Annual Meeting

2. Best Poster Award

Evaluation of process performance of BR-UASB-DHS system treating Natural Rubber Wastewater, IGCN2014