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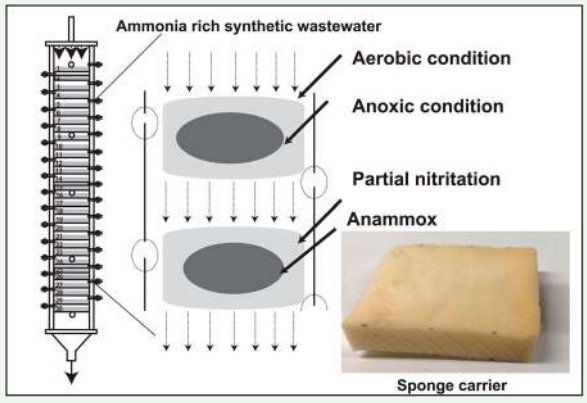
Development of a single-stage mainstream anammox process using a sponge-bed trickling filter

Takahiro Watari [a,b,c], Carlos Lopez Vazquez[b], Masashi Hatamoto[a], Takashi Yamaguchi[a] and Jules B. van Lier [b,d]

[a]Department of Civil and Environmental Systems Engineering, Nagaoka University of Technology, Nagaoka, Japan; [b]Environmental Engineering and Water Technology Department, UNESCO-IHE, Delft, The Netherlands; [c]Department of Chemical Engineering, Hanoi University of Science and Technology, Hanoi, Vietnam; [d]Faculty of Civil Engineering and Geosciences, Department of Water Management, Delft University of Technology, Delft, The Netherlands

ABSTRACT

Anaerobic ammonia oxidation to nitrogen gas using nitrite as the electron acceptor (anammox process) is considered a 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, simulating the treatment of anaerobically pretreated concentrated domestic sewage without mechanical oxygen control. The influent ammonia concentration was 100 mg-N·L^{-1}. The K_La of the STF reactor was higher than those observed for conventional activated sludge processes. The STF reactor performed at 89.8 ± 8.2% and 42.7 ± 16.9% ammonia and TN removal efficiency, respectively, with a nitrogen loading rate of 0.55 ± 0.20 kg-N·m^{-3}·day^{-1} calculated based on sponge volume. Microbial community analysis of the STF-retained sludge indicated that both autotrophic and heterotrophic nitrogen removal occurred in the reactor.



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Autotrophic nitrogen removal; sponge-based trickling filter; microbial community analysis

Highlights

- A single-stage mainstream anammox process is feasible in a sponge-bed reactor.
- The sponge-bed reactor has high oxygen transfer without external aeration.
- The STF reactor removed 89.8 ± 8.2% of ammonia and 42.7 ± 16.9% of TN.
- Nitrogen removal was due to the coexistence of nitrifiers, denitrifiers, and anammox bacteria.

1. Introduction

Anaerobic treatment processes have been used widely for sewage and industrial wastewater treatment due to their high organic removal efficiencies and low operational costs (e.g. [1]). However, the effluent of an anaerobic treatment process contains residual organic compounds and mineralised nutrients, i.e. ammonium and phosphates. Thus, additional post-treatment processes for the anaerobic treatment system are required to meet local discharge standards.

CONTACT Takahiro Watari watari@vos.nagaokaut.ac.jp; nut113359@gmail.com Department of Civil and Environmental Systems Engineering, Nagaoka University of Technology, 1603-1, Kamitomioka, Nagaoka, 940-2188, Niigata, Japan; Environmental Engineering and Water Technology Department, UNESCO-IHE, PO Box 3015, 2601 DA Delft, The Netherlands; Department of Chemical Engineering, Hanoi University of Science and Technology, No. 1 Dai C o Viet, Hanoi, Vietnam
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In the search for cost-effective solutions for treatment of municipal sewage, the sponge-based trickling filter (STF) reactor is regarded one of the most promising systems for post-treatment of anaerobic effluents [2–4]. Machdar et al. [5] first proposed using sponges as a carrier material for biomass retention in biological trickling filters. Currently, six types of sponge carriers have been developed and evaluated for their process performance in sewage treatment [2,6–9]. The STF reactor is characterised by a high biomass retention capacity in the sponge carrier and extremely low excess sludge production without external aeration [6,10,11]. In addition, the sponge medium supports high microbial diversity on its surface and in its inner section [10,12]. Recent studies on microbial community structure in the sponge-retained sludge have demonstrated the presence of anaerobic ammonia oxidation (anammox) bacteria, suggesting that both aerobic ammonia oxidation and the anammox process occur simultaneously [13,14].

A partial nitritation–anammox process consists of two consecutive reactions: ammonium is partially oxidised to nitrite under oxygen-limited aerobic conditions by ammonium oxidising organisms (AOO) and, subsequently, the remaining ammonium reacts with nitrite to form nitrogen gas anaerobically by anammox bacteria. Compared to the conventional nitrification–denitrification process, autotrophic nitrogen removal consumes 60% less oxygen, does not require any chemical oxygen demand (COD) for the denitrification step, and produces 80% less excess sludge [15]. In addition, Cao et al. [15] reported that more than 200 full-scale facilities have been operating successfully in the world. However, the existing partial nitritation–anammox processes require complex dissolved oxygen (DO) control, which increases capital investment and operation costs. The application of partial nitritation in STF reactors was studied by Chuang et al. [16], Uemura et al. [17], and Guillén et al. [18]. Chuang et al. [16] attained partial nitritation in the STF reactor by controlling oxygen conditions using an air pump. Guillén et al. [18] studied partial nitritation under natural air circulation and showed the great potential of autotrophic nitrogen removal in STF reactors with easy operational methods. Additionally, anammox bacteria were successfully cultivated in the closed STF reactor, which performed at about 75% total nitrogen (TN) removal efficiency, applying a short hydraulic retention time (HRT) of 1 h [19]. An STF reactor has several advantages for applying the partial nitritation–anammox process. Experimental results showed that DO transitioned from 7.5 mg-$O_2 \cdot L^{-1}$ in the surface layers of the sponge carrier to about 0.2 mg-$O_2 \cdot L^{-1}$ in the inner layer [20]. The large surface area can lead to an increased biomass retention capacity [8], and the difference in DO level in the sponge carrier as well as the large surface area can result in long solids retention times (SRT), favouring the accumulation of slow-growing organisms. The high biomass hold-up can potentially result in a high microbial conversion capacity at short HRTs [18].

The transfer of oxygen from air to wastewater is subject to biological aerobic treatment and plays a crucial role in an oxygen-limited partial nitritation–anammox process. Uemura et al. [21] examined the overall volumetric oxygen transfer coefficient K_La of the downflow hanging sponge (DHS) reactor by supplying deoxygenated water from the top of the device. The K_La values of the DHS support media without external aeration ranged from 0.56 to 4.88 1 min^{-1}, surpassing those of other mechanically aerated processes.

This research aimed to develop a single-stage partial nitritation–anammox process in an STF reactor as a low-cost post-treatment process using a synthetic substrate to simulate an ammonia-rich effluent (100 mg of ammonia) from an upflow anaerobic sludge blanket (UASB) reactor treating domestic sewage at 30°C. Our research group previously examined autotrophic nitrogen removal over nitrite in the STF reactor with activated sludge inoculation [18]. In the present research, pure anammox sludge was used for STF inoculation, and the in-growth of ammonium oxidisers without mechanical oxygen control. In addition, the oxygen mass transfer of the STF reactor and the microbial community structure of the retained sludge were evaluated.

2. Materials and methods

2.1. Experimental set-up

Figure 1 shows a schematic diagram of the STF reactor used in this study. The STF reactor was made of transparent acrylic glass with a height of 60.5 cm. The total volume of the STF reactor was 2.5 L. The horizontally layered sponge carriers were made of polyurethane sponge slabs (BVB Substrates, De Lier, The Netherlands). The sponge void ratio and sponge density were 98% and 28 kg·m^{-3}, respectively. The thickness of the sponge carriers was 0.75 cm. The sponge volume of the STF reactor was 991 cm^3, and the HRT was calculated based on the sponge volume. The STF reactor was operated at 30°C in a temperature-controlled room. Air circulation across the sponge medium was facilitated through lateral openings located above each sponge layer. Synthetic wastewater was fed from the top of the reactor with a water distributor.

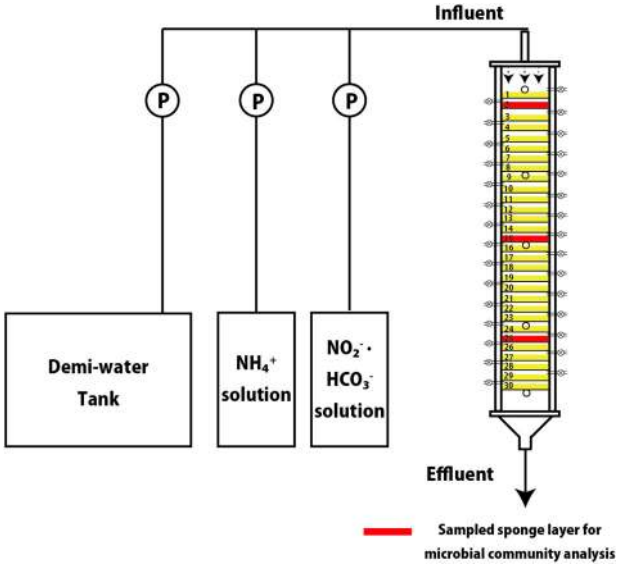

Figure 1. Schematic diagram of sponge-based trickling filter reactor.

2.2. Synthetic wastewater

During phase 1, ammonia and nitrite were fed to the reactor to stabilise and further cultivate the anammox bacteria in the sponge bed. The composition of the media was based on the work of Guillén et al. [18]. It contained the following per 1 L of demineralised water: (i) ammonium feed: 2.98 g NH_4Cl, 0.77 g $MgSO_4{\cdot}7H_2O$, 0.39 g KH_2PO_4, 4.69 g $CaCl_2{\cdot}2H_2O$; (ii) nitrite feed: 3.85 g $NaNO_2$, 0.18 g $FeSO_4{\cdot}7H_2O$, 19.5 g $KHCO_3$, 0.18 g 2-NaEDTA, and 1.25 mL of trace element solution. The trace element solution contained the following per litre: 15 g $C_{10}H_{12}K_2Mg\ N_2O_8{\cdot}\ 2\ H_2O$, 0.43 g $ZnSO_4\ \cdot\ 7H_2O$, 0.24 g $CoCl_2\ \cdot\ 6H_2O$, 0.99 g $MnCl_2\ \cdot\ 4H_2O$, 0.25 g $CuSO_4\ \cdot\ 5H_2O$, 0.22 g $Na_2MoO_4\ \cdot\ 2H_2O$, 0.19 g $NiCl_2\ \cdot\ 6H_2O$, 0.1076 g Na_2SeO_4, 0.014 g H_3BO_3, 0.05 g $NaWO_4\ \cdot\ 2H_2O$.

At phase 2, ammonium was supplied as the sole substrate to achieve autotrophic nitrogen removal. The composition of the substrates used per 1 L of demineralised water was the following: (i) in the ammonium-rich feed: 5.97 g NH_4Cl, 0.77 g $MgSO_4{\cdot}7H_2O$, 0.39 g KH_2PO_4, 4.69 g $CaCl_2{\cdot}2H_2O$; (ii) in the bicarbonate feed: 0.18 g $FeSO_4{\cdot}7H_2O$, 19.53 g $KHCO_3$, 0.18 g NaEDTA, and 1.25 mL of trace element solution. During phase 3, the ammonia concentration was adjusted to 80 mg-N·L^{-1} to simulate less concentrated sewage.

2.3. Operational conditions and inoculation

At the beginning of this study, 300 ml of anammox sludge obtained from a lab-scale anammox membrane bioreactor (MBR) was used for inoculation [22]. The mixed liquor suspended solids (MLSS) of the seed anammox sludge was 510 ± 50 mg·L^{-1}. After 21 days of operation, activated sludge from a full-scale sewage treatment plant (Harnaschpolder, Delft, The Netherlands) was added as a secondary inoculum (MLSS: 6,400 ± 300 mg·L^{-1}). A summary of the operating conditions is shown in Table 1. The nitrogen loading rate (NLR) was calculated based on the sponge volume and influent ammonia concentration.

Table 1. Operational conditions for sponge-based trickling filter.

Day		Phase 1 1–25	Phase 2 26–97	Phase 3 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

2.4. Analytical methods

The influent and effluent of the STF reactor were collected for routine analysis. pH was measured with a pH metre (Model ProfiLine 3310, WTW, Germany). DO concentrations of influent and effluent were measured by a portable DO metre (Oxi 3320, WTW). Nitrite-nitrogen (NO_2^-N) and nitrate-nitrogen (NO_3^-N) concentrations were measured by ion chromatography (ICS-1000, Thermo Scientific). Ammonia-nitrogen (NH_4^+-N) was analysed using a spectrometer (Lambda 365, Perkin Elmer). The MLSS and MLVSS of the retained sludge were measured using standard methods [23].

2.5. Evaluation of oxygen mass transfer in the STF reactor

The oxygen transfer coefficient (K_La) was measured to evaluate the oxygen supply to the microorganisms in the sponge bed media following the method of Uemura et al. [21]. Deionised water was stored in a 100 L tank, sparged with nitrogen gas to remove oxygen (70 min), and then supplied from the top of the STF reactor. The STF reactor was operated for 2 h in order to stabilise the water flow in the reactors before measuring DO concentrations. The DO concentration in the sponge bed was measured using a micro DO electrode (Unisense). The DO electrode was fixed using an iron stand to be able to measure the DO concentrations on the surface of the sponge bed. K_La is a coefficient indicating the ability of aeration tanks and other devices to transfer oxygen from the gas phase to the liquid phase per time unit. This parameter has been used frequently to evaluate aeration tanks in the conventional activated sludge process. K_La was calculated with the following

equation [21,24].

$$K_L a = 1/t \times \ln\left(Cs \cdot (Cs - Ct)^{-1}\right) \quad (1)$$

Cs: saturated DO concentration (mg·L^{-1}) *Ct*: DO concentration in the effluent at time t (mg·L^{-1})

The oxygenation capacity of STF reactor was calculated with the following equation.

$$\text{Oxygenation capacity} = K_L a \times (Cs - Ct) \quad (2)$$

Cs: saturated DO concentration (mg·L^{-1}) *Ct*: DO concentration in the effluent at time t (mg·L^{-1})

The oxygen consumption rate of ammonia and nitrite oxidation were calculated based on the theoretical oxygen demands of 3.43 mg-O_2·mg- NH_4^+-N^{-1} and 1.14 mg-O_2· mg-NO_2^- -N^{-1} from the NH_4^+-N and NO_2^- -N removal rate.

2.6. Massively parallel 16s rRNA gene sequence

The microbial community structures of the seed sludge and STF retained sludge on day 181 were analysed based on 16S rRNA gene sequencing. The sludge sample was collected from the 2nd (upper part), 15th (middle part), and 25th sponge media (bottom part) (Figure 1). The retained sludge was extracted from the sponge media, gently washed with phosphate buffered saline (PBS), and stored at −20°C until DNA was extracted. DNA extraction was performed using a FastDNA Spin Kit for Soil (MP Biomedicals). Polymerase chain reaction (PCR) amplification of 16S rRNA genes was performed with the universal forward primer Univ515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and the universal reverse primer Univ806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) for whole bacteria and archaea [25]. Purification of the PCR products was conducted using a QIAquick PCR Purification Kit (Qiagen). Massive parallel 16S rRNA gene sequencing was carried out using the MiSeq Reagent Kit v. 2 with the MiSeq System (Illumina). Sequence data analysis was conducted using the QIIME software package v. 1.7.0 [26]. Operational taxonomic units (OTUs) were classified at the 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.

3. Results and discussion

3.1. Nitrogen removal performance

Figure 2 shows the process performance of the STF reactor during the entire experimental period. During phase 1, ammonia and nitrite were fed to the reactor to stabilise and further grow the anammox bacteria in the sponge bed. After 6 days of operation, the ammonia removal efficiency reached 70% despite that the DO level in the influent was maintained at about 1.0 mg·L^{-1} (Table S-1). The rapid growth of nitrifiers in the STF reactor under low DO concentrations was also observed in previous studies in our lab [18]. The average influent and effluent ammonia concentrations 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 during this phase were 67.4 ± 22.4% and 18.0 ± 5.6%, respectively. The production of nitrite indicated that most of the ammonia was oxidised to nitrite by AOO, but the anammox bacteria were not yet active (Table S-1).

In phase 2, ammonia was supplied as the sole N source to attain a partial nitritation–anammox process in the STF reactor. The DO concentration of the effluent was about 0.3 ± 0.2 mg-O_2·L^{-1} in this operational phase. Chuang et al. [16] reported that effective partial nitritation was observed in a closed DHS reactor when the reactor was operated at 0.42 mg-O_2·L^{-1}. The ammonia concentrations in the influent and effluent in phase 2 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 an NLR of 1.41 ± 0.27 kg-N·m^{-3}·day^{-1} under oxygen limited conditions. These values are much higher than those observed by Tawfik et al. [27], who reported a nitrification rate of 0.22 ± 0.07 kg-N·m^{-3}·day^{-1} in a DHS reactor that was installed as post-treatment system of a UASB reactor treating municipal sewage. On the other hand, the results of Chuang et al. [16], who operated a closed DHS reactor with an ammonia-rich synthetic substrate, showed high ammonia removal rates of 1.46 kg-N·m^{-3}·day^{-1}. However, their TN removal efficiency was as low as 28.1 ± 12.1%. Table 2 shows pH, DO, and nitrogen concentration profiles in the STF reactor on day 92. The DO concentration on the sponge carrier surface was mostly below 0.5 mg-O_2·L^{-1}. Machdar et al. [20] found a DO concentration of 0.2 mg-O_2·L^{-1} at the 1 cm depth of the inner part of the sponges inside a DHS reactor. Thus, with regard to the prevailing oxygen concentrations, STF-retained sponges created favourable conditions for partial nitritation and the anammox process. The pH of the medium was immediately decreased from 8.5 to 7.5 in the upper part of the reactor, likely due to the ongoing ammonium oxidation. A similar pH drop in the top part of the reactor, which was concomitant with high microbial activity, was also observed in our

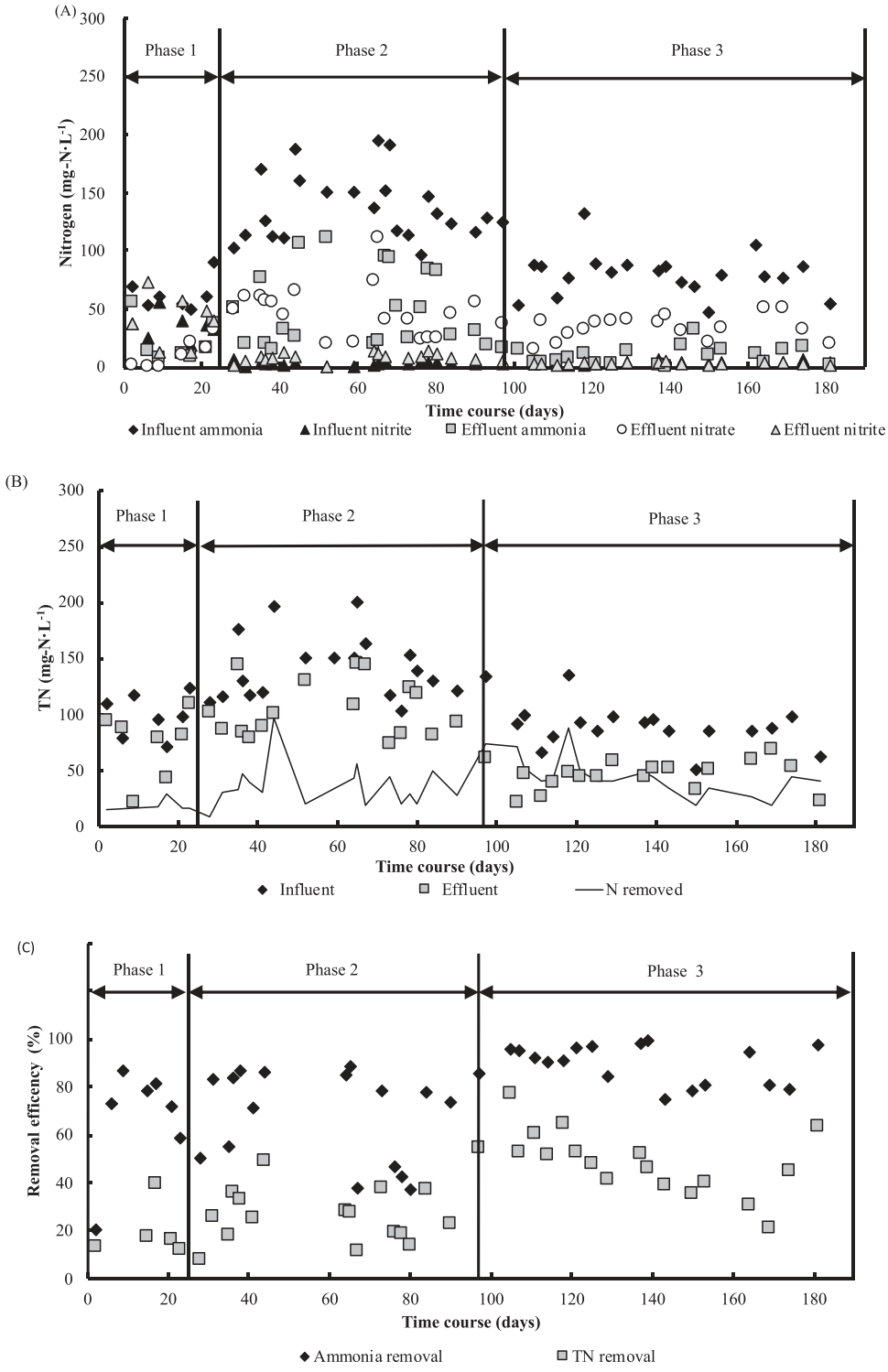

Figure 2. Time course of (A) ammonia, nitrate, and nitrite, (B) TN, and (C) ammonia and TN removal efficiency during the entire experimental periods.

previous study [18]. Although both nitrite and ammonium were present in the effluent, no effective nitrogen removal was observed at the bottom part of the reactor. Our previous research also found low nitrogen removal in the bottom part of the STF reactor [18]. At the bottom part of the STF reactor, the pH was about 7.2–7.4. Jaroszynski et al. [28] noted that in the pH range of 7–8, the decrease in anammox activity was independent of pH and related only to the concentration of free ammonia (NH_3). The free ammonia concentration at the bottom of the STF reactor was calculated to be below 1.0 mg-N·L^{-1}, and this concentration was lower

Table 2. pH, dissolved oxygen, ammonia, nitrite and nitrate concentration inside STF reactor at day 92.

Unit	Distance from top of reactor cm	pH	DO $mg{\cdot}L^{-1}$	Ammonia $mg{\cdot}N{\cdot}L^{-1}$	Nitrite $mg{\cdot}N{\cdot}L^{-1}$	Nitrate $mg{\cdot}N{\cdot}L^{-1}$
Influent	0.0	8.6	0.2	128.0	1.7	3.9
Layer 10	16.7	7.6	0.1	67.9	8.5	27.7
Layer 20	35.2	7.4	0.5	11.0	2.8	53.6
Effluent	54.0	7.0	0.2	19.0	5.0	51.1

than the inhibitory free ammonia concentration reported in a previous study [29]. Nonetheless, anammox bacteria did not grow and accumulate at the bottom part of the STF reactor in our present research and further research is required to optimise a partial nitritation–anammox process in a single-stage STF reactor.

In order to simulate less concentrated sewage, the NLR was decreased to 0.55 ± 0.20 kg-$N{\cdot}m^{-3}{\cdot}day^{-1}$ at phase 3 by reducing the influent ammonia concentration and flow rate. During this phase, the ammonia removal efficiency was as high as $89.8 \pm 8.2\%$ and the effluent ammonia concentration was 7.8 ± 6.1 mg-$N{\cdot}L^{-1}$. The decrease in both the influent ammonium concentration and NLR apparently were more compatible with the available nitrification capacity of the system. Nevertheless, the nitrogen removal efficiency only increased to a maximum of 63.5% during the final part of this phase. This nitrogen removal efficiency was similar to that in our previous study in which we inoculated the reactor with activated sludge [18].

3.2. Oxygen mass transfer in the STF reactor

Before starting the experiment, the oxygen transfer coefficient of the STF reactor was assessed to determine the proper conditions for the partial nitritation–anammox process. Figure 3 (A) shows the DO profile when supplying oxygen-free water to the STF reactor at an influent flow rate of 5, 10, and 20 $ml{\cdot}min^{-1}$. The DO of the water reached saturation within the 4th sponge layer (distance: 7.5 cm) in the entire experiment. Uemura et al. [21] also reported such quick oxygen acquisition in the DHS reactor. This result shows that the STF reactor has great potential for enhanced oxygen transfer.

The K_La was calculated to evaluate the ability to supply oxygen to the microorganisms in the used STF reactor (Figure 3 (B)). The K_La values at an influent flow rate of 20, 10, and 5 $ml{\cdot}min^{-1}$ were 0.259, 0.226, and 0.074 1 min^{-1}, respectively. The assessed K_La values in the lab-scale STF reactor were lower than those in most convective diffusion-based DHS reactors, but they were higher or similar to those of the conventional activated sludge process [21].

The oxygenation capacity of the STF reactor and average oxygen consumption rate in phase 2 and phase 3, calculated based on ammonia and nitrite oxidation, are shown in Figure 4. The oxygenation capacities of the STF reactor at phase 2 and phase 3, calculated based on the flow rate and the K_La values, were 1.68 and 0.81 kg-$O_2{\cdot}m^{-3}{\cdot}day^{-1}$, respectively. However, the oxygen consumption rates for ammonia and nitrite oxidation at phase 2 were estimated to be around 2.58 ± 0.64 kg-$O_2{\cdot}m^{-3}{\cdot}day^{-1}$ and 1.46 ± 0.40 kg-$O_2{\cdot}m^{-3}{\cdot}day^{-1}$, respectively, i.e. two times higher than the calculated oxygen transfer capacity of the STF reactor. (Figure 4). Courtens et al. [30] also noted that physical data alone can provide misleading information on oxygen transfer rate and pointed out the importance of biological activity in the total oxygen transfer. Garcia-Ochoa and Gomez [31] assessed an enhancement factor used for estimating the increase in oxygen transfer capacity resulting from biological activity. The enhancement factor of rotating biological contactors was as high as 10 [30]. Therefore, the difference in oxygen transfer capacity and oxygen consumption could be ascribed to the actual biochemical oxygen consumption in the STF reactor. The total oxygen consumption rate of this reactor was similar to that of previous studies [2,5,32,33]. The oxygen consumption for ammonia oxidation had the largest variation of 2.58 ± 0.64 kg-$O_2{\cdot}m^{-3}{\cdot}day^{-1}$ during phase 2 and phase 3 (Figure 4). Hatamoto et al. [33] also reported that ammonia oxidation showed the highest variation due to the low oxygen affinity of ammonia-oxidising organisms and the lower free energy change of the ammonia oxidation reaction compared with acetate, propionate, methane, and sulfide oxidation. By using the oxygenation capacity of the bioreactor and actual oxygen consumption rate as a benchmark, a proper STF reactor for the partial nitritation–anammox process can be designed.

3.3. Retained sludge in STF reactor

The biomass development in the sponge carriers was visually inspected periodically. Higher biomass growth and accumulation were observed in the top part of the reactor. A similar phenomenon was also observed in our previous study [18] and was considered to be related to the non-limited availability of substrate and the presence of inoculum at the upper sponge layers. During the entire experimental periods, there was no biomass wash-out from the sponge layers. At the end of the experiment, the retained sludge mass in the sponge bed was determined (Figure 5). The highest sludge concentrations of 145.4 mg-$MLSS{\cdot}L^{-1}$ and 82.2 mg-$MLVSS{\cdot}L^{-1}$ were found in the top part of the

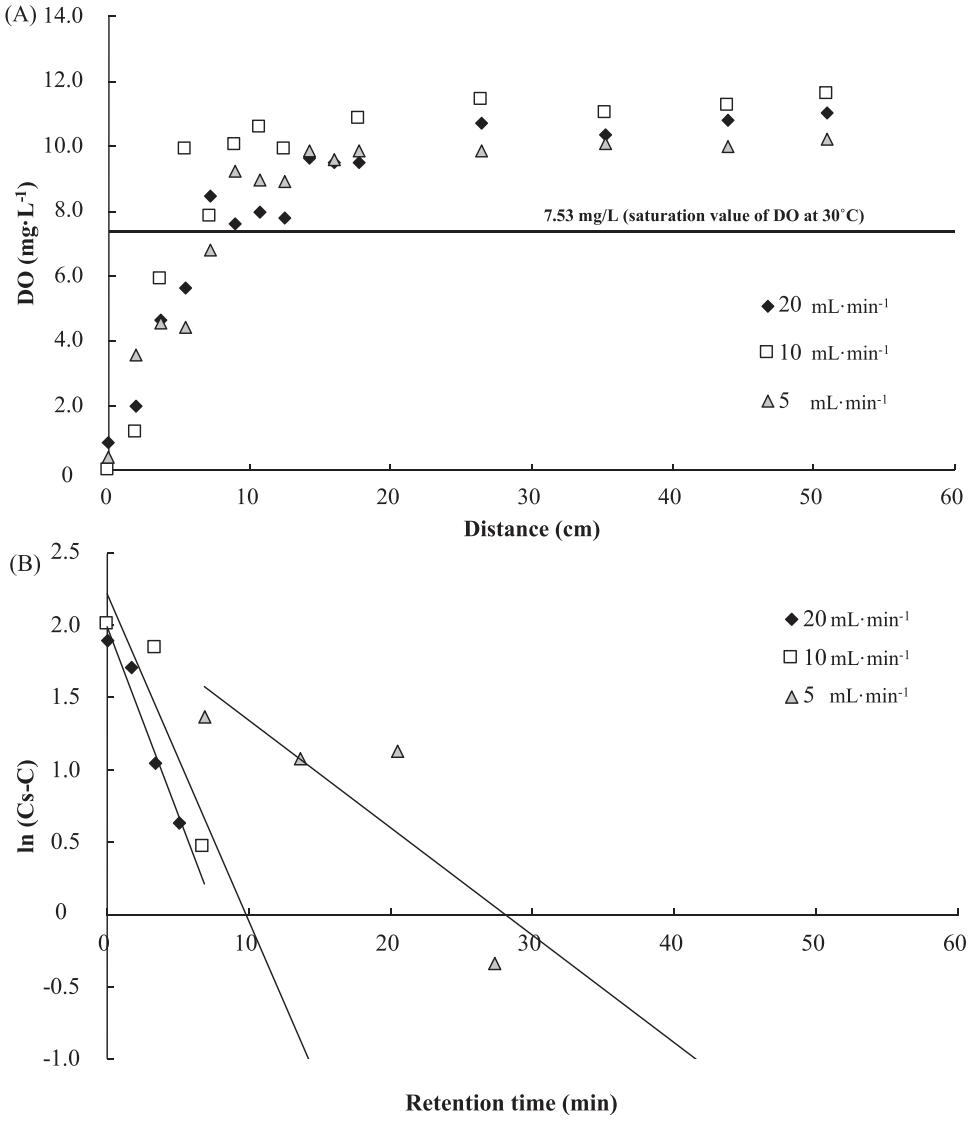

Figure 3. (A) DO concentrations and (B) K_La in sponge media at different flow rates.

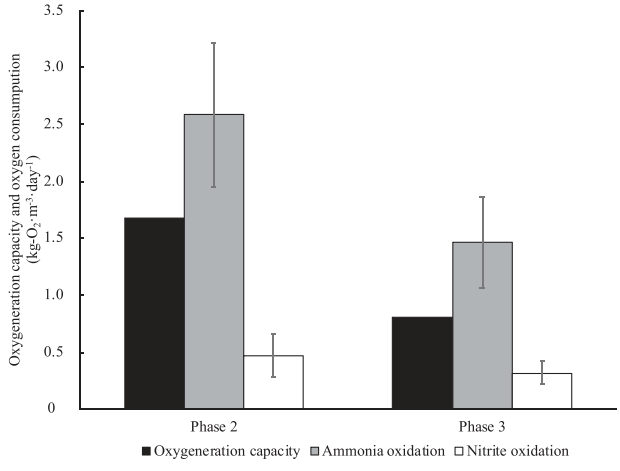

Figure 4. Oxygenation capacity of STF reactor and oxygen consumption of ammonia oxidation and nitrite oxidation at phase 2 and phase 3. The error bars indicate the standard deviation.

reactor (sponge layer No. 6). However, the concentrations of retained sludge were considerably lower than in previous studies [14,27,34]. This low biomass concentration was likely the main reason for the observed low TN removal efficiency in this STF reactor.

3.4. Microbial community structure of STF reactor

The microbial community structures of the seed sludges and the STF-retained sludge were investigated using 16S rRNA gene-based massively parallel sequencing analysis at the end of the experiment (Table 3). A total of 55,387 sequence reads were analysed based on the 16S rRNA gene sequence, and the median length of the 16S rRNA sequence was 251 bp. These gene sequences were then assigned to conduct downstream analyses and 794 (anammox seed sludge), 3,213 (activated seed

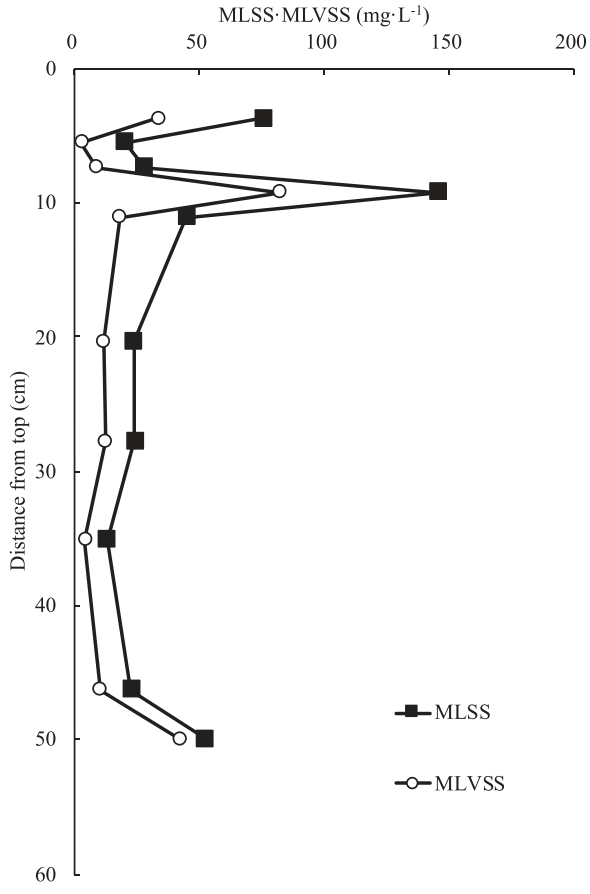

Figure 5. Retained sludge concentration in STF reactor at the end of the experiment.

sludge), 1,063 (5th layer), 1,370 (15th layer), and 766 (25th layer) OTUs were obtained at the sequence identity threshold. Species richness was also characterised by Chao values, and the Shannon value was used to represent the diversity, shown in Table 3. It was observed that the seed activated sludge had the highest richness and diversity. The Shannon indices in the STF reactor ranged from 6.17 to 6.83. These values were the same or lower than those of the conventional DHS reactor [35] and other sewage treatment plants [36].

The dominating phyla of the STF-retained sludge were *Proteobacteria*, *Acidobacteria*, and *Firmicutes*, which are frequently found in the DHS reactor treating sewage [12]. The phylum *Proteobacteria*, which is important in relation to the nitrification process, was most dominant in the retained sludge. The functional group of ammonia oxidising bacteria is represented by organisms affiliated with the Beta- and Gamma- *Proteobacteria* [37]. Beta-*Proteobacteria* was the most dominant in the STF reactor and Beta-Proteobacteria ammonia oxidising bacteria (AOB) *Nitrosospira* and *Nitrosomonas* belong to this bacterial group.

Figure 6 shows the dominant nitrifying organisms in the seed sludge and STF-retained sludge. The abundances of nitrifying bacteria in the anammox and activated seed sludge were low, but AOB were detected in all layers of the STF reactor at the end of the experiment. Within the AOB, *Nitrosomonas* accounted for 2.0–0.3% of the total reads and was most abundant in layer 2. Kubota et al. [12] and MacConell et al. [13] reported that within the microbial community structure of the DHS reactor treating sewage, AOB was highly detected in the lower part of the reactor. The difference in AOB abundance over the height of the DHS in their work was likely related to the concentration of organic matter in their influent. In contrast, our results show highest AOB abundance in the upper part of the DHS, which is related to the use of a synthetic substrate without any organic content. On the other hand, the ammonia oxidising archaea (AOA) *Candidatus* Nitrososphaera was highly found in the bottom part of the reactor. The observed difference between AOB and AOA dominance within the STF reactor may be due to the concentration of

Table 3. Microbial community structure of seed sludge and STF-retained sludge.

	Anammox sludge seed	Activated sludge seed	Layer 2	Layer 15	Layer 25
No. of total sequence reads	12,282	11,486	12,056	9,590	9,973
No. of OTUs	794	3,213	1,063	1,370	766
Chao 1 richness estimation	8,094	19,996	5,387	13,351	3,292
Shannon diversity index	4.51	9.01	6.42	6.83	6.17
Good's coverage (%)	0.94	0.78	0.94	0.88	0.95
		% 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

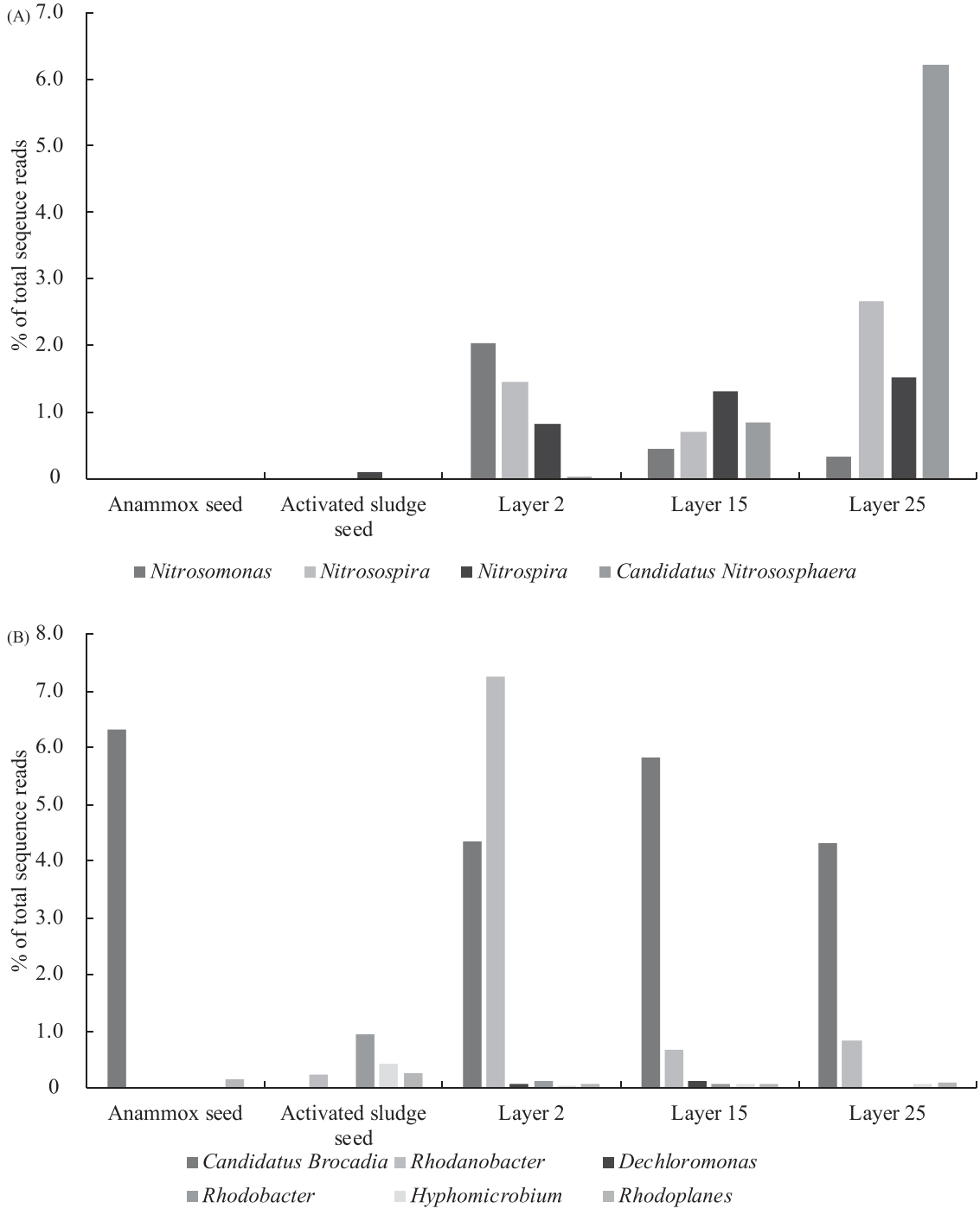

Figure 6. Total sequence reads of nitrifying bacteria and archaea (A) and anammox and denitrifying bacteria (B) in the STF reactor and seed sludge.

ammonia [38]. Hatzenpichler et al. [39] reported that *Candidatus* Nitrososphaera was partially inhibited at ammonia concentrations of 43.1 mg·N·L^{-1}. Limpiyakorn et al. [40] reported significant numbers of AOA amoA genes occurring in municipal WWTPs with low 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. Therefore, *Candidatus* Nitrososphaera likely became dominant in the bottom part of the reactor due to low prevailing ammonium concentrations (e.g. ammonia concentration at layer 20 was 11.0 mg-N·L^{-1} at day 92). The nitrite oxidising bacteria *Nitrospira* was detected at a relative amount of 1.0–1.5% of the total sequence reads. With regard to the anammox bacteria, *Candidatus* Brocadia was detected in the STF reactor and was also highly dominant in the seed anammox sludge, which was

Table 4. Performance comparison of STF reactors on single-stage partial nitritation–anammox process.

		Guillén et al. [18]	This study (phase2)	This study (Phase 3)
Seed sludge		Activated sludge	Anammox + Activated sludge	Anammox + Activated sludge
NLR	kg-N·m^{-3}·day^{-1}	1.68	1.47 ± 0.27	0.55 ± 0.20
HRT*	hours	1.71	2.4 ± 0.7	3.7 ± 1.3
Air circluation		No air supply to 7 sponge sheet	–	–
Influent NH_4^+-N	mg-N·L^{-1}	111.9 ± 5.5	135.0 ± 28.4	80.8 ± 18.2
Effluent NH_4^+-N	mg-N·L^{-1}	34.4 ± 3.6	40.2 ± 27.8	7.8 ± 6.1
Effluent NO_2^--N	mg-N·L^{-1}	0.3 ± 0.1	8.9 ± 2.9	2.9 ± 1.1
Effluent NO_3^--N	mg-N·L^{-1}	18.9 ± 3.4	51.4 ± 21.9	33.9 ± 10.5
NH_4^+-N removal	kg-N·m^{-3}·day^{-1}	1.17 (1.22)	0.97 ± 0.29	0.67 ± 0.17
NH_4^+-N removal effciency	%	69.3 (71.7)	70.9 ± 19.1	89.9 ± 8.2
N removal	mg-N·L^{-1}	58.4 (66)	40.7 ± 20.8	42.7 ± 16.9
N removal	kg-N·m^{-3}·day^{-1}	0.88 (0.99)	0.42 (0.75)	0.39 (1.11)
N removal	%	52.3 (60.4)	28.8 (54.4)	48.4 (77.0)

coming from the anammox-MBR reactor [22]. Nonetheless, our results are also in agreement with the observations of Huang et al. [41], who found the same species in a single-stage partial nitritation–anammox process. In addition, MacConell et al. [13] reported that *Candidatus* Brocadia was found in the downflow sponge-based trickling filter treating UASB effluent. On the other hand, several kinds of denitrifying bacteria were detected under the applied low-organic substrate loads. The main genera identified with known denitrification capabilities were *Rhodanobacter*, *Dechloromonas*, *Rhodobacter*, *Hyphomicrobium*, and *Rhodoplanes. Rhodanobacter* is described as acetate-utilizing denitrifying bacteria, isolated from a nitrate-rich environment and an autotrophic denitrification process [42,43]. The microbial community in hollow-fiber-membrane biofilm reactors treating high-strength nitrogen wastewater indicated that *Rhodanobacter* was dominant and utilised acetate and amino acids as electron donors [41]. These organic compounds could be provided by nitrifiers via the production of soluble microbial products [44,45]. *Dechloromonas* was found in a DHS reactor treating domestic sewage [12]. Therefore, both autotrophic and heterotrophic nitrogen removal likely occurred in the STF reactor.

3.5. Performance comparison with the previous study

The comparison of our previous study on the process performance of an STF reactor operated as a single-stage nitritation–anammox process in our lab and this study is summarised in Table 4. Guillén et al. [18] carried out a single-stage partial nitritation–anammox process in the STF reactor with activated sludge as inoculum. The ammonia removal efficiency was similar, but higher concentrations of nitrate were observed in the effluent compared with the results of Guillén et al. [18]. This result indicated that too much DO was likely available in the STF reactor and nitrite oxidising bacteria were growing in the sponge carrier. Therefore, further research for controlling the DO at a favourable level for partial nitritation is required. Recently, Roots et al. [46] found that ammonia oxidation was dominated by comammox in a main stream nitrification reactor operated at low DO concentration. Possibly, the high nitrate production and difference between oxygen capacity of the STF reactor and actual oxygen consumption rate might be attributed to the presence of commamox bacteria. Nevertheless, it is difficult to make a direct comparison of the efficiency of our previous study with this study because of the differently applied NLR and HRT. Roughly speaking, the nitrogen removal rates and efficiencies in both STF reactors were comparable, but the stability of performance was better in the previous study.

4. Conclusions

A single-stage mainstream anammox process in an STF reactor with anammox sludge as inoculum was demonstrated under the condition of natural air convection for oxygen supply. The STF reactor was characterised by a high ammonia oxidation rate and TN removal efficiency when applying NLR of 0.55 ± 0.20 kg-N·m^{-3}·day^{-1}. The STF reactor had good potential for oxygen transfer, which was explained by both high K_La values and biochemical conversion processes. Therefore, the STF reactor could be a promising technology for post–treatment of anaerobic effluents.

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ORCID

Takahiro Watari http://orcid.org/0000-0003-3781-3710
Jules B. van Lier http://orcid.org/0000-0003-2607-5425

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