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A novel pilot-scale tubular bioreactor-enhanced floating treatment wetland for efficient in situ nitrogen removal from urban landscape water: Long-term performance and microbial mechanisms

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Abstract

In order to strengthen in situ nitrogen removal of urban landscape water, a novel pilot-scale tubular bioreactor-enhanced floating treatment wetland (TB-EFTW) was constructed, and the long-term performance and responsible microbial mechanisms were investigated in this study. The results showed that the system could remove 81.5% nitrogen from the landscape water after 240 days' operation. Moreover, the contribution rate of plant absorption to nitrogen was low (8.3%), which indicated that microbial biotransformation rather than plant absorption played a more key role in nitrogen removal in TB-EFTW system. The declining dissolved oxygen (DO) concentration along the axial direction of tubular bioreactor (TB) resulted in the sequential bacterial community of nitrifying, aerobic denitrifying, and anoxic denitrifying bacteria in the front, middle, and final part of TB. High-throughput sequencing results demonstrated that the internal environment of the system realized the coexistence of nitrifying, aerobic denitrifying and anoxic denitrifying process. The reason was mainly because that oxic-anoxic (O-A) areas were formed in sequence along the axial direction of tubular bioreactor. Overall, a unique advantage in nitrogen removal was achieved in TB-EFTW, which could provide important references for in situ treatment of urban landscape water.

Practitioner points

- TB-EFTW strengthened nitrogen removal for in situ urban landscape water treatment.
- Microbial conversion played a key role in nitrogen removal of the TB-EFTW system.
- The unique distribution of oxic-anoxic (O-A) areas was formed in sequence along the TB.
- Nitrification, aerobic, and anoxic denitrification were synergistically involved in the TB.

Key words

agriculture; biological treatment; nitrogen removal; surface water

Introduction

The urban landscape water usually lacks necessary hydraulic circulating, and the water speed tends to be zero, which easily lead to nitrogen accumulation by runoff pollution, domestic sewage discharge, and precipitation (Hsieh & Davis, 2005). The excessive nitrogen in landscape water may cause eutrophication, algal blooms, and even seriously threatens ecological safety and human health (Heisler et al., 2008). Hence, it is urgent to strengthen the efficiency of nitrogen removal from urban landscape water.

The conventional purification methods of urban landscape water include chemical, biological, and ecological methods. Although chemical methods own quick

effects, secondary pollution has restricted their rational utilization. Biological and ecological restoration technologies, such as aquatic plants and constructed wetland (CW), have been applied in landscape water restoration due to its low investment and easy implementation (Bustamante, Mier, Estrada, & Domiguez, 2011; Li, Song, Li, Lu, & Nishimura, 2010). Recent years, as an innovative type of CWs, floating treatment wetlands (FTW) has shown obvious advantages of no additional land occupancy and in situ purification adaptability in the treatment of urban landscape water (Borne, Fassman-Beck, & Tanner, 2014; Headley & Tanner, 2012). Conventional floating treatment wetland (CFTW) is mainly composed of floating bodies and plants with admirable pollution resistance and absorption capacity, such as *Ipomoea aquatic, Acorus calamus,* and *Hydrocotyle vulgaris* (Chang, Cui, Huang, & He, 2017). Unfortunately, the traditional FTW still has some limitations in practical application, including limited biomass, insufficient hydraulic circulating, and lack of carbon source (Gao et al., 2018; He, Wang, Li, Li, & Zhou, 2018).

Enhanced floating treatment wetlands (EFTWs) have been developed as an innovative ecological restoration technology based on FTWs. The biofilm carriers can provide additional surface area for the attachment of microorganisms (Wu, Hu, Li, Peng, & Zhao, 2016; Zhang et al., 2016). For intensifying the biomass, the EFTWs are integrated with biofilm carriers such as plastic filling materials, elastic packing, and hemp fiber (Cao & Zhang, 2014; Zhao et al., 2012). What's more, aeration facilities are also added to EFTWs, so as to enhance the oxygen dissolution and hydraulic circulating (Chang et al., 2017). Nevertheless, the denitrification performance of EFTWs is still unsatisfactory in the treatment of landscape water. For instance, the EFTWs containing *Iris pseudacorus* L. and *Canna indica* L. along with fiber filters were used to treat the eutrophic water from an urban lake, and the highest TN removal efficiency was lower than 50% during the 112 days' operation (Wu et al. (2016).

One important reason for the inefficient TN removal is the lack of biodegradable organic carbon in EFTW system (Gao et al., 2018). It has therefore become quite necessary to add external carbon sources during the nitrogen removal process. The frequently used carbon sources in traditional denitrification processes such as methanol, ethanol, glucose, and sodium acetate are usually highly costly, and sometimes may cause secondary pollution (Xu, Dai, & Chai, 2018). Agricultural waste carbon sources, such as bagasse, corncob, wheat straw, and wood chips, have been widely used in CWs due to their slow-release characteristic and low cost. However, the application of agricultural waste carbon sources in EFTWs is rarely reported. As a result, agricultural waste carbon sources, such as bagasse, corncob, wheat straw, and wood chips, have already attracted researchers' attention due to their slow-release characteristic and low cost in EFTWs (Cao & Zhang, 2014; Zhao et al., 2012).

Another reason for the low TN removal rate is the deficiency of the anoxic environment, which is necessary for traditional anoxic denitrification. On the contrary, the aerobic environment is necessary for microbial nitrification process (Kulikowska, Jozwiak, Kowal, & Ciesielski, 2010). One possible solution is the integration of plug flow reactor (PFR) in EFTWs, because variable reactant concentration profiles along the axial direction, and the subdivision control of dissolved oxygen (DO) can be more easily implemented in the PFR (Yun & Kim, 2003).

In this study, a novel pilot-scale tubular bioreactor-enhanced floating treatment wetland (TB-EFTW) was constructed to strengthen the nitrogen removal of urban landscape water. The system was mainly consisted of tubular bioreactors (TBs), plants, and an injector aerator. The TB was a novel PFR filled with palm fiber as biofilm carrier and bagasse as a carbon source. The landscape water was pumped and delivered into TBs by the injector aerator. The pollutants in water would be removed by the absorption of plant roots and the biotransformation of microorganisms when the water flows through TBs in a continuous circulation model. As a result, landscape water was purified by the continuous circulation in TB-EFTW. The aims of this study were (a) to evaluate the nitrogen removal efficiency in a pilot-scale TB-EFTW system; (b) to reveal the nitrogen removal mechanism, which mainly included the physicochemical factors affecting nitrogen removal along the TB, as well as the contribution of plant absorption; and (c) to understand the microbial community diversity in the system through high-throughput sequencing analysis.

Materials and Methods

Experimental setup

The pilot-scale TB-EFTW system was established in an outdoor landscape pool located in East China Normal University, Shanghai, China. The effective volume of the pool was 50 m^3. The structure diagram of the TB-EFTW system is shown in Figure 1, and the on-site photograph is shown in Figure S1. The system consisted of fifteen multiplied TBs, a floating bed, plants, an injector aerator, and a collection tank (0.5 × 0.5 × 1 m).

The TBs were made of polythene pipes (12 m in length, 10 cm in diameter, and 0.3 mm in thickness) with internal fillers. The internal fillers accounted for 33% of the empty polythene pipes and were consisted of palm fiber and bagasse with the volume ratio of 3:1. The hemp fiber was considered as biofilm carrier while bagasse as carbon source (Tangsir et al., 2017). The basic properties of the fillers in TBs are shown in Table 1. The fifteen TBs were coiled into circles (2.5 ± 0.5 m in diameter) and fixed on the floating bed, which was made of moso bamboo. Planting holes (1 cm in diameter) were made on the top of TBs, and the distance between each hole was 50 cm along the TB. One to two plants were planted in the hole. The test plants were chosen as *Ipomoea aquatic* with the initial average height and weight of 8.6 ± 2.3 cm and 7.9 ± 1.1 g, respectively.

The landscape water was supplied to the fifteen TBs by the injector aerator with the power consumption of 1.8 (kWh)/day and then purified when flowing through the fillers and plant roots in TBs. The influent flow of each TB was 7.9 L/hr, the water flow velocity was 1.5 m/hr, and the hydraulic retention time was 8.0 hr. The determination of above parameters was based on the results of our preliminary experiment, where the fillers in TBs would not be rushed out

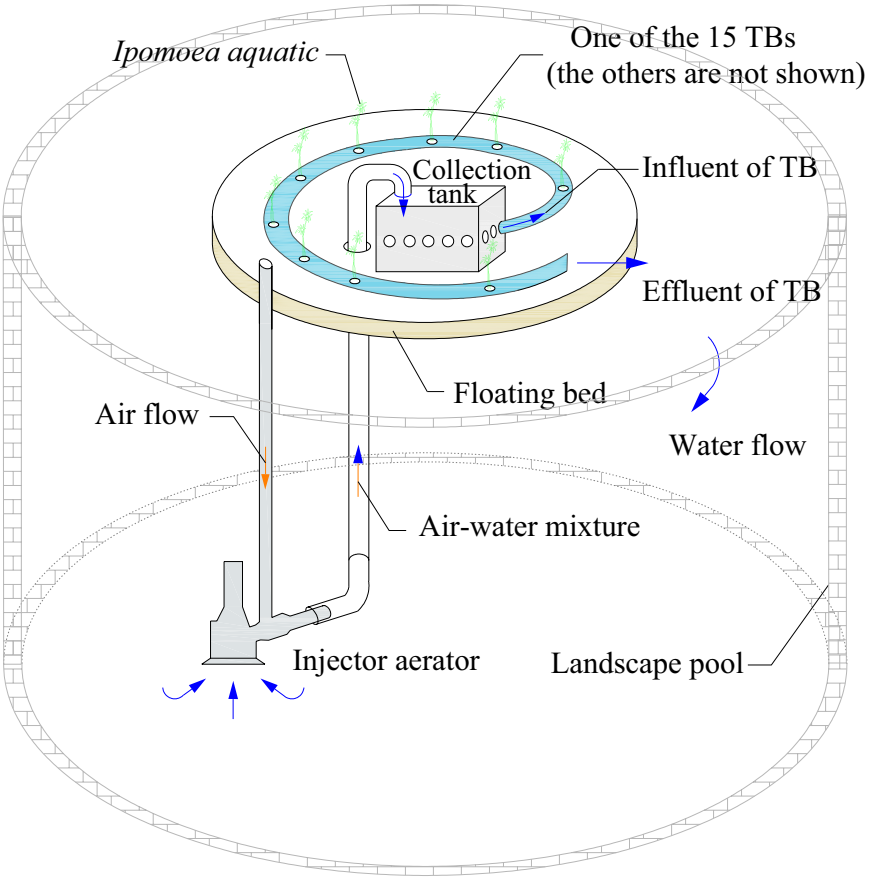

Figure 1. The schematic diagram of the pilot-scale TB-EFTW.

or got blocked, and the high purification efficiency of the system could be guaranteed. Moreover, to maintain the system performance once the system encountered severe weather disturbing, an open type exhaust canopy was set on top of the landscape pool, which could protect the pilot-scale system from rainfall. Besides, the top of the landscape pool wall was about 0.7 m higher than ground level, which ensured little affect from runoff.

Landscape water and operation procedure

The landscape water investigated in this study was taken from an urban lake, which was located in a botanical garden of Shanghai, and the rainwater runoff had caused excess nitrogen accumulation. The water quality of the lake was worse than Grade V of Chinese National Surface Water Environmental Quality Standards (GB3838-2002, 2002). The quality of the test water and the water quality standards are shown in Table 2.

During the 90 days' TB-EFTW construction period, the test water in the pool showed a low nitrogen removal efficiency (<5%), which indicated that the landscape water was hardly purified without EFTWs. After the construction period, the pilot-scale system was operated with a domestication period of plants and microorganisms for one month at first. In April 2016, the water in the pool was renewed by the landscape water, and then, the operation process was carried out for 240 days. During the operation, the landscape pool was refilled with tap water every month to compensate for evaporation.

Sampling and analyses

Water samples were collected in triplicate from the TB-EFTW system monthly for measuring the physicochemical parameters. The concentration of DO and water temperature (WT) of the system were determined in situ by a dissolved oxygen meter (HQ30d 53LEDTM, HACH, USA). The concentrations of TN,

Table 1. Basic properties of fillers in the tubular bioreactors

FILLERS MATERIAL	PORINESS (%)	MOISTURE CONTENT (%)	DENSITY (G/CM^3)	DISSOLUTION RATE (MG COD_{CR} [L/DAY])
Bagasse	31 ± 1.20	2.53 ± 0.21	0.43 ± 0.02	23.94 ± 0.35
Hemp fiber	79 ± 2.37	1.38 ± 0.13	0.73 ± 0.03	2.49 ± 0.11

Table 2. Water quality of the test water and water quality standards

WATER QUALITY PARAMETERS	COD_{CR} (MG/L)	TN (MG/L)	NH_4^+-N (MG/L)	NO_3^--N (MG/L)
Water quality of raw water	22.56 ± 2.10	2.32 ± 0.3	1.52 ± 0.26	0.21 ± 0.05
Water quality standards (Grade V)	40	2.0	2.0	–
Water quality standards (Grade II)	20	0.5	0.5	–

ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and chemical oxygen demand (COD_{Cr}) were obtained in the laboratory following the standard methods (APHA, 2005).

Plant samples were collected from the front (1.0 m from the TB inlet), middle (6.0 m from the TB inlet), and final (1.0 m from the TB outlet) parts of the 15 TBs both in the initial and final periods of the operation. Height and weight of these plant samples were obtained through measurement and gravimetric method, respectively. The total nitrogen concentration in plants was also tested using alkaline diffusion method (Chen, Wen, Zhou, & Vymazal, 2014). Furthermore, the surface morphology of the fillers in TBs before and after the operation was detected by Quanta 2000 Scanning Electron Microscope (SEM; Yang, Jiang, Song, Gu, & Xia, 2015).

Statistical analyses

Removal rate of nitrogen (RR, %) is calculated following Equation (1); growth rate of COD_{Cr} is calculated following Equation (2); TN mass removal in TB-EFTW system (MR, mg) is obtained through Equation (3); TN mass removal via plants absorption (MR_{plant}, mg) and its contribution rate in TN removal (CR_{MR}, %) are afforded by Equation (4) and Equation (5), respectively:

$$RR = (C_i - C_m)/C_i \times 100\% \quad (1)$$

$$GR = (C_m - C_i)/C_i \times 100\% \quad (2)$$

$$MR = C_i \times Q_i - C_f \times Q_f \quad (3)$$

$$MR_{plant} = P_f \times B_f - P_i \times B_i \quad (4)$$

$$CR_{MR} = MR_{plant}/MR \times 100\% \quad (5)$$

where C_i (mg/L) is the initial concentration of nitrogen at the beginning of experiment, C_m (mg/L) is the concentration of nitrogen measured at a certain month during the experimental operation, and C_f (mg/L) is the final concentration at the end of experiment; P_i (mg/g) and P_f (mg/g) are initial and final nitrogen mass in plant organs and tissues, respectively; B_i (g) and B_f (g) are the initial and final dry weight of plants, respectively; Q_i (L) and Q_f (L) are the initial and final water quantity in the tanks, respectively.

Microbial community analysis

In order to evaluate the diversity of microbial community structure of the pilot-scale TB-EFTW system, three biofilm samples were collected from the TB at day 240 of the operation. The biofilm samples taken from the front (1.0 m from the TB inlet), middle (6.0 m from the TB inlet), and final (1.0 m from the TB outlet) parts of TB were named P1, P2, and P3, respectively.

Sample pretreatment methods of biofilm were as following: 10 g TB filler samples and 5 ml sterile water were mixed in 25-ml sterile conical flask and then oscillated on oscillator for 30 min at a speed of 200 rpm. The suspension was poured into centrifugal tubes for centrifugation by 2000 rpm, and the solid part was then frozen at −20°C for DNA extracting.

Power Soil DNA isolation kit was used to extract DNA from samples, and 5 μl DNA of the extracted bacteria was detected by agarose gel electrophoresis of 1%. The V3–V4 hypervariable region fragments of 16S rRNA gene were amplified by polymerase chain reaction (PCR) with universal primers 515F (50-GTGCCAGCMGCCGCGGGTAA-30) and 919R (50-CCCCGYCAATTCMTRAGT-30). PCR templates and programs accorded to the study of Huang, Dong, Wang, and Jiang (2017). Then high-throughput sequencing analysis was carried out on Illumina MiSeq platform by Shenggong Biotechnology Company, Shanghai, China.

The original image data files were transformed into original sequencing sequence by CASAVA base recognition analysis, and the results were stored in FASTQ file format. Mothur was used to correct the original sequence and get the optimized sequence by removing the chimeras in these sequences. Sequences were divided into operational taxonomic units (OTUs) at 97% similarity level. RDP Classifier Bayesian algorithm was used to classify and analyze the OTU representative sequences at 97% similarity level, and the calculated Coverage index, Chao1 index, and Shannon diversity index. Besides, community composition of each sample was calculated at phylum and genus levels.

Results and Discussion

Performance of nitrogen removal by TB-EFTW

As shown in Figure 2a, in the first 90 days' operation, the NH_4^+-N concentration of the landscape water sharply decreased from 1.52 to 0.45 mg/L, which met Grade II (NH_4^+-N ≤ 0.5 mg/L) of Chinese National Surface Water Environmental Quality Standards (GB3838-2002, 2002). Compared with plant absorption, the nitrification of microorganisms contributes much more for NH_4^+-N removal in EFTWs (Cao & Zhang, 2014). Hence, the sound NH_4^+-N removal in the initial operation period might be mainly attributed to the efficient nitrification in TB-EFTW system. After 90 days' operation, the decrease in NH_4^+-N became slow, which was mainly because the NH_4^+-N

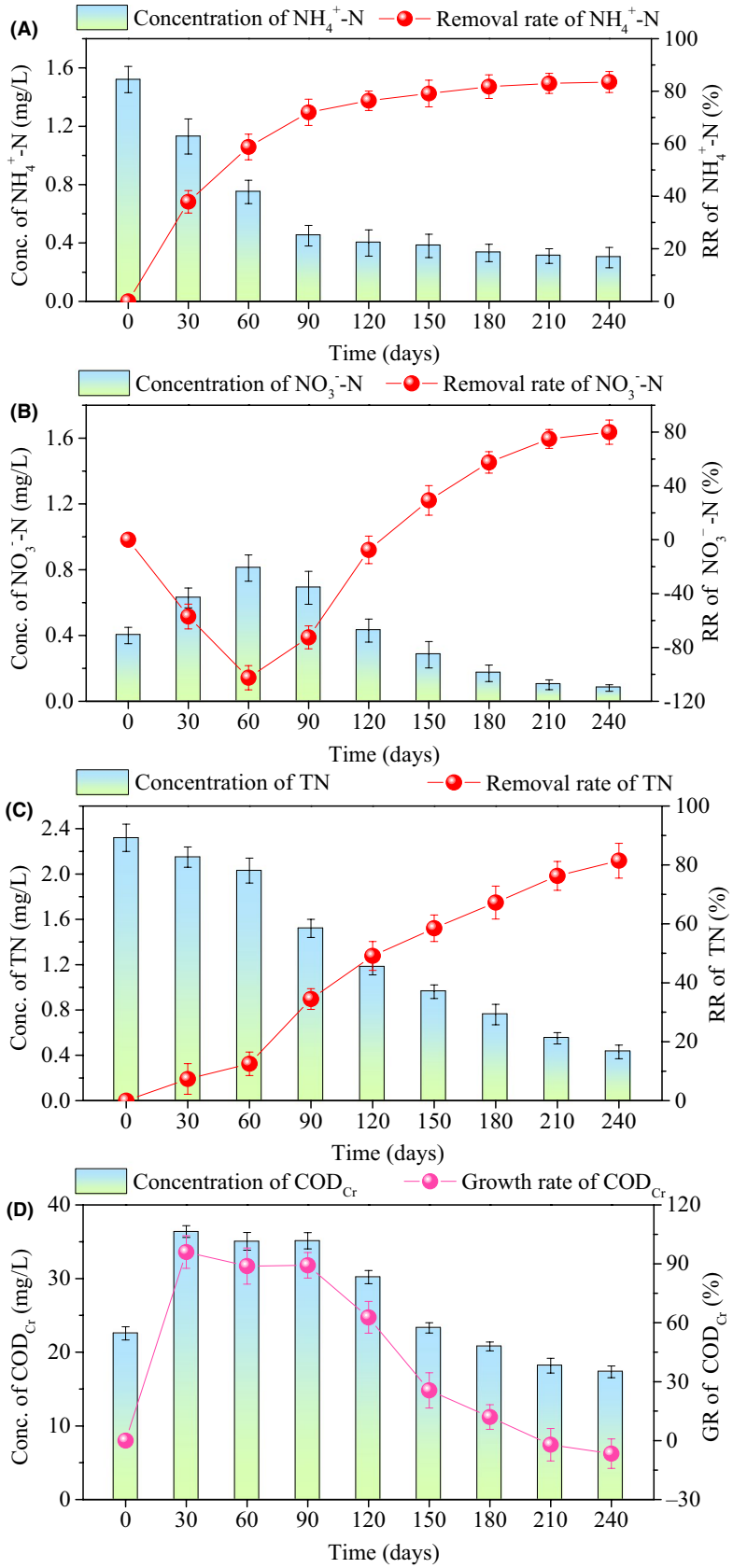

Figure 2. Variation of (a) NH_4^+-N, (b) $N0_3^-$-N, (c) TN, and (d) COD_{Cr} in the landscape water during the operation of TB-EFTW.

concentration had reached a low level. At the end of the operation, the removal rate of NH_4^+-N in TB-EFTW reached 80.3%. The efficient ammonia removal performance achieved in TB-EFTW system might be attributed to the synthesis of aeration by the injector aerator and biofilm enhancement of TBs, which could lead to high DO in the influent of TBs and abundant enrichment of nitrifying bacteria.

Figure 2b shows the variation of NO_3^--N concentration and removal efficiency in the landscape water. The NO_3^--N concentration increased from 0.40 to 0.81 mg/L in the first 60 days. The reason was presumably due to the effect of nitrification process at the beginning of the operation, which could transform NH_4^+-N to NO_3^--N, and lead to the increase of NO_3^--N in water (Lazarova, Nogueira, Manem, & Melo, 1998). After 60 days' operation, the concentration of NO_3^--N began to decrease rapidly. There was a dynamic balance between nitrification and denitrification processes in TB-EFTW system. When the denitrification rate was higher than the nitrification rate, the NO_3^--N concentration of the system would decrease. During the period of day 60–240, the NO_3^--N concentration of landscape water gradually decreased to 0.08 mg/L.

Besides, the NO_2^--N concentration of this system was also monitored. The accumulation of NO_2^--N in a bioreactor usually suggested the incompleteness of nitrification or denitrification process (Chen et al., 2017). The result shows a low concentration of NO_2^--N in TB-EFTW system during the whole operation, which reflected that the nitrification and denitrification processes were complete.

As shown in Figure 2c, during the 240 days' operation, the TN concentration of the landscape water gradually decreased from 2.32 to 0.43 mg/L, which met Grade II (TN ≤ 0.5 mg/L) in Chinese GB3838-2002 (2002). The final TN removal efficiency of TB-EFTW system was 81.5%. In our current study, the final removal efficiency of TN and NO_3^--N reached up to 81.5% and 80.0%, showing an extraordinary nitrogen removal performance of TB-EFTW system.

It is considered that TN removal of EFTWs is synergistic involvement of multiple pathways including plant absorption, nitrification, denitrification, and microbial assimilation (Li et al., 2018; Wu et al., 2016). Among them, microbial denitrification is regarded as the limiting step to realize TN removal from landscape water, due to lack of bioavailable carbon source in EFTW system (He et al., 2018). As for TB-EFTW system, besides the endogenous carbon sources from rhizosphere secretion, plant litter, and biofilm detachment, it was also supported by supplemental carbon sources from bagasse. This might be the reason that the TB-EFTW system offered a distinct advantage over nitrogen removal.

As can be seen from Figure 2d, in the first 30 days' operation, the concentration of COD_{Cr} in landscape water increased from 22.56 to 36.38 mg/L, which was possibly due to the carbon release from the bagasse filled in TBs. In the following 60 days' operation, the COD_{Cr} concentration remained relatively constant. After the 120 days' operation, the COD_{Cr} concentration gradually decreased and finally stabilized at 17.33 mg/L, which was because the removal rate of organics by TBs and the released rate of organics from bagasse reached dynamic equilibrium. This is consistent with Yang et al. (2015), who reported that the addition of agricultural wastes in a bioreactor quickly increased COD_{Cr} concentration in the first 10 days, and then gradually decreased until maintaining at a stable level. Nonetheless, the COD_{Cr} concentration in the landscape water was lower than Grade V (COD_{Cr} ≤ 40 mg/L) in Chinese GB3838-2002 (2002) during the whole operation, which indicated that over-proof organic pollutant concentration was not found in TB-EFTW system.

Physicochemical factors affecting nitrogen removal along the TB

In biological treatment process, the ratio of COD_{Cr} and TN (C/N) and DO concentration were essential for denitrification of microorganisms to remove NO_3^--N. As shown in Figure 3a, the C/N and DO concentration along one of the TBs were explored on days 30, 120, and 210 of operation. The result shows that the C/N increased continuously along TB-EFTW, which was mainly due to the carbon release from the bagasse, while the DO concentration kept declining along TB-EFTW as the oxygen consumption from the metabolism of aerobic microorganisms on the internal fillers. As a result, the unique DO distribution (oxic-anoxic (O-A) areas) was formed in sequence along the axial direction TB.

Generally speaking, the suitable DO concentration for nitrification is required to exceed 2.0 mg/L (Lazarova et al., 1998). Hence, microbial nitrification process might exist in the initial part of TB with higher DO concentration (>5.0 mg/L). Besides, the suitable C/N and DO concentration for aerobic denitrification were above 10 and ranged 1.0–4.0 mg/L, respectively, which met the requirements for anoxic denitrification (C/N: >6, and DO: <0.5 mg/L) (Ahmad, Xu, Chen, Liu, & Liu, 2008; Ji et al., 2015). Therefore, the process of aerobic denitrification and anoxic denitrification might come up in the middle part and final part of TB-EFTW, respectively.

The changes in NH_4^+-N, NO_3^--N, and TN concentrations are shown in Figure 3b. The result showed that the NH_4^+-N concentration rapidly decreased in the initial part of TB, which was mainly because that higher DO in the influent promoted the nitrification, while the NO_3^--N concentration in the initial TB increased as the nitrification process could convert NH_4^+-N into NO_3^--N (Lazarova et al., 1998). In the latter part of TB, the decrease in NH_4^+-N tended to be stopped, and the concentration of NO_3^--N started to fall off. The main reason for the phenomenon was that the decreased DO (<2 mg/L) had inhabited nitrification process. In the middle part of TB, the growing C/N (>10) and suitable DO (0.5–2.0 mg/L) might promote heterotrophic aerobic denitrification. And in the final part of TB, the NO_3^--N and TN kept declining, which was probably due to the effect of heterotrophic anoxic denitrification.

In addition, the growth of the plants and the nitrogen removed by plants absorption were also detected during the operation. The final average height and weight of plants (45.3 ± 6.8 cm and 57.6 ± 2.3 g) were much higher than the

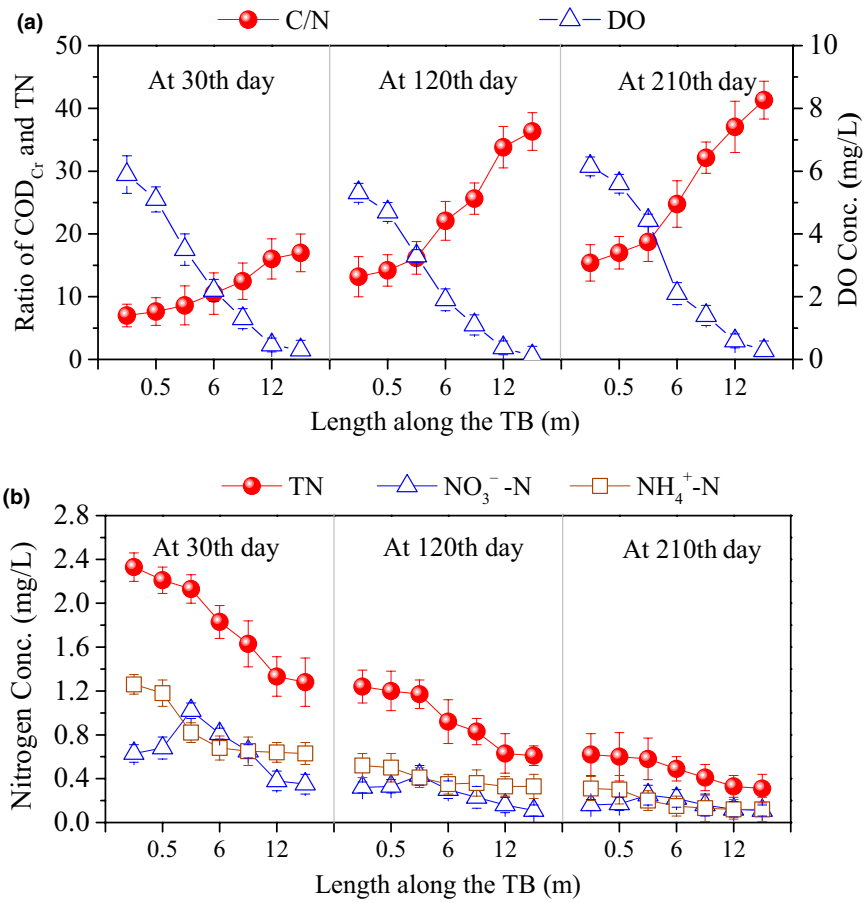

Figure 3. Variation of DO and C/N ratio (a), as well as different forms of nitrogen (b) along the TB during the operation of TB-EFTW.

initial (8.6 ± 2.3 cm and 7.9 ± 1.1 g), which reflected that the TB-EFTW system was beneficial for the growth of plants. The total plant biomass of TB-EFTW system was 14.9 kg at the end of the experiment, and the plant density was 2.1 kg Plant/m^2. It is reported that *Ipomoea aquatic* was also chosen as the EFTW plant in a eutrophic pond to realize water purification (Chang et al., 2017). In that study, the plant density reached as high as 7.0–7.3 kg Plant/m^2 in the harvest, while the TN removal efficiency of their study was stable at 36.6%, which was 44.9% lower than TB-EFTW system.

The TN removal rate in TB-EFTW system was 213.5 mg/day, while the TN removal rate via plants absorption was 17.7 mg/day. Moreover, the contribution rate of plant absorption in nitrogen removal was only 8.3%, providing a solid evidence that plant absorption might not be the main factor in the nitrogen removal (Li, He, Ji, Zhi, & Sheng, 2015; Zhang, Sun, Xie, Wu, & Cheng, 2018). In biological treatment process, NO_3^--N was the stable form of nitrogen before it was removed by biotransformation (mainly by biological denitrification) from water. Therefore, it could be inferred that the super nitrogen removal efficiency in TB-EFTW system was mainly depended on nitrification and denitrification performance.

The photographs of hemp fiber and bagasse before and after the operation are shown in Figure S2, and the corresponding SEM images are shown in Figure 4. Obviously, the hemp fiber had been attached with a large number of microorganisms, while the bagasse showed obvious signs of decomposition and consumption after a long time of operation, which indicated that active microbial metabolisms had been proceeding during the treatment process. In order to understand the mechanisms of this process, it is necessary to analyze the microbial community structure on the filler.

Microbial mechanism of nitrogen removal by TB-EFTW

In order to elucidate the pollutant degradation mechanism by microorganism in TBs, high-throughput sequencing was carried out for the biofilm samples collected from the front, middle, and final parts of the TB at the end of the operation. After eliminating the invalid sequence and chimeric sequence, three samples obtained a total of 215,194 high-quality sequences, and a total of 20,103 OTUs were obtained when sequences were divided by 97% similarity. Coverage of sample libraries was 92.8%–95.0%, which indicated that this sequencing could represent the real situation of microbial community.

Microbial community richness and diversity. Microbial community richness and biodiversity were demonstrated in

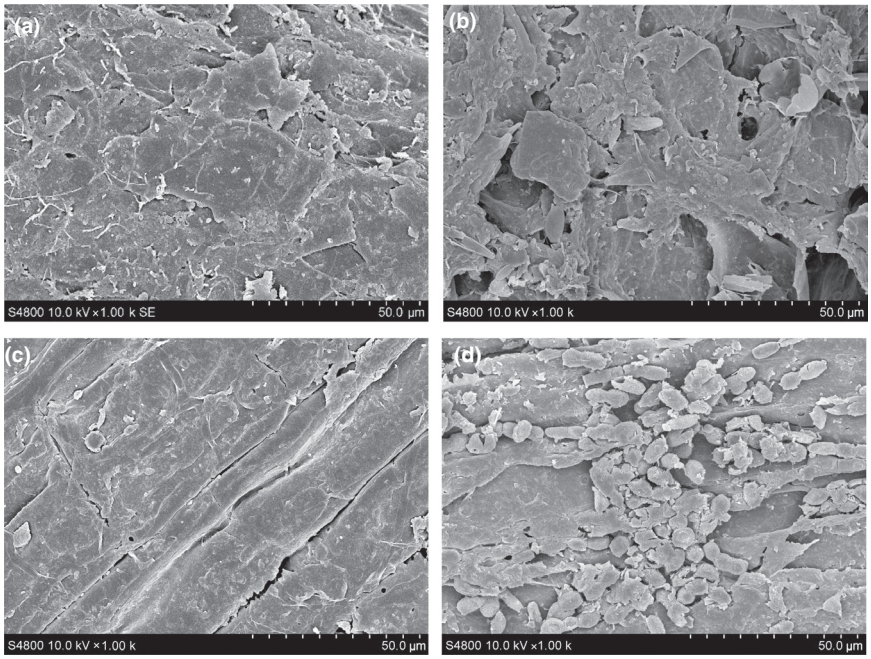

Figure 4. Scanning electron microscope images of hemp fiber (a: before operation, b: after operation) and bagasse (a: before operation, b: after operation) filled in TBs.

Table 3. The Chao 1 index is used to describe the bacterial community richness. Meanwhile, microbial community biodiversity could be reflected by Shannon index. It is well known that higher Chao 1 index indicated more microbial richness, and higher Shannon index represented more diversity (dC Rubin et al., 2017).

The result shows that the highest Chao 1 index was achieved in P1 (28,044), followed by P2 (24,306) and P3 (19,408). Furthermore, the Shannon index of the three decreased from P1 (6.49) > P2 (6.29) > P3 (6.28), whose change rules were similar to Chao1. The reason for the decreased microbial diversity along the TB was mainly due to the carbon source released from bagasse promoted the growth of heterotrophic bacteria in the TB-EFTW system after long-term operation, and then declined the proportion of other minor bacteria. Therefore, the increasing C/N along TB was conducive to the enrichment and growth of specific heterotrophic bacteria.

Microbial composition and nitrogen removal related bacteria. Microbial community composition was analyzed at phylum level (Figure 5a), and the biofilm samples were dominated by three phyla, namely Proteobacteria, Bacteroidetes, and Acidobacteria. The proportion of Proteobacteria was the highest in all samples, accounting for 60.92%, 68.70%, and 56.41% for P1, P2, and P3, respectively. Besides, the proportions of Bacteroidetes ranged from 7.27% to 15.31% in three samples, while that of Planctomycetes fluctuated between 3.86% and 7.45%.

Typical nitrogen removal microorganisms are dominant in Proteobacteria, Bacteroidetes, and Planctomycetes, and they are detected in different sewage treatment processes (Tian et al., 2015). Proteobacteria have been reported by many studies to contain common denitrifiers and play a major role in N-cycle for the removal of nitrogen (Shu, He, Yue, & Wang, 2016; Tan et al., 2019). Bacteroidetes has been shown to be associated with the degradation of cellulose-rich organic wastes such as plant residues and agricultural wastes (Dennehy et al., 2017), and sludge fermentation system (Huang, Dong, Wang, & Feng, 2018). Planctomycetes is critical strain in nitrogen cycle, and it can use NO_2^--N and NH_4^+-N to generate nitrogen to obtain energy in anoxic environment (Wilsenach et al., 2014). During the operation process, enriched Proteobacteria explained why TB-EFTW owned wonderful nitrogen removal efficiency.

Figure 5b shows the microbial community composition at genus level. The result shows that the dominant genera of three biofilm samples were quite different from each other, which implied the alien habitats in different parts of TB. The dominant genera of P1 were *Arenimonas*, *Chryseobacterium,*

Table 3. Microbial sequences, richness, and diversity of the three biofilm samples

SAMPLES	SEQUENCES	OTUS	COVERAGE (%)	CHAO 1	SHANNON
P1	78,031	7,137	93.2	28,044	6.49
P2	63,103	6,987	92.8	24,306	6.29
P3	74,060	5,979	95.0	19,408	6.28

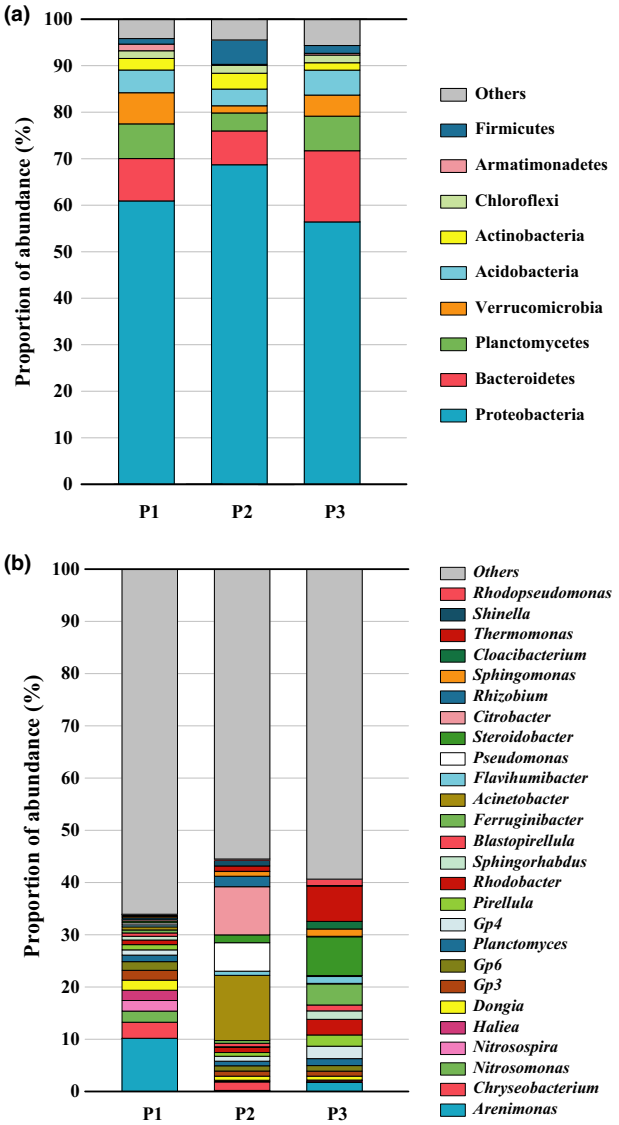

Figure 5. Relative abundances of (a) phylum and (b) genus level of the three biofilm samples. Phyla or genera making up less than 1.0% of total composition were defined as "others".

Nitrosomonas, and *Nitrosospira*. *Arenimonas* is reported as an aerobic bacterium which can degrade various sugars and amino acids (Young et al., 2007), while *Chryseobacterium* can utilize O_2 to degrade macromolecular organic compounds like fatty acids and cellulose (McBride et al., 2009). *Nitrosomonas* and *Nitrosospira* are both nitrification-related bacteria, which can transform ammonia into oxidized nitrogen under aerobic environment (Lazarova et al., 1998). Therefore, the dominant bacteria in the front of TB were all aerobic bacteria, which might be caused by the high DO concentration (1.9–3.3 mg/L) of the initial water flow

The dominant microorganisms of P2 were *Acinetobacter*, *Citrobacter*, *Pseudomonas,* and *Rhizobium*. Coincidentally, all of them have been reported to own the function of aerobic denitrification, which can remove oxidized nitrogen from water under aerobic conditions (Chen & Strous 2013; Ji et al., 2015; Ren, Yang, & Liang, 2014). Moreover, *Steroidobacter*, *Thermomonas*, *Ferruginibacter,* and *Rhodobacter* were found as the dominant bacteria genera in P3. *Steroidobacter*, *Thermomonas,* and *Rhodobacter* were typical anoxic denitrifying bacteria (Fahrbach et al., 2008; Idi, Ibrahim, Mohamad, & Majid, 2015). *Ferruginibacter* normally functioned in the anoxic/oxic/membrane reactor (AO/MR), and the extracellular polymers (EPS) could release carbon sources to promote the denitrification (Han, Zhou, Mei, Ma, & Xie, 2018). The proper conditions with abundant carbon source and low oxygen in the final part of TB could promote microbial anoxic denitrification.

To reveal the core role of the nitrogen removal bacterial communities in TB-EFTW system, the proportions of nitrogen removal related genus of the three biofilm samples were investigated. The principal nitrogen removal process among the three samples mainly included autotrophic aerobic nitrification, heterotrophic aerobic denitrification, and heterotrophic anoxic denitrification as shown in Table 4. By the way, the proportions of both autotrophic denitrifying and anaerobic ammonium oxidation-related genera were <0.5%, which demonstrated that the main denitrification process was heterotrophic metabolism in TB-EFTW system.

It was clear from the results that the efficient nitrification was dependent on the existence of *Nitrosomonas*, *Nitrosospira*, *Nitrobacter,* and *Nitrospira*, which occupied a dominant position in P1 (4.79%). The main reason for the higher abundance of nitrifying bacteria in P1 was the sufficient DO (>3.3 mg/L) in the front part of the TB. The growth and metabolism of nitrifying bacteria will be inhibited when DO in water is <2.0 mg/L (Lazarova et al., 1998). This explained why the abundance of nitrifying bacteria decreased in the middle (0.30%) and end (0.17%) of the TB. Therefore, the nitrification in TB-EFTW system strongly depended on the nitrifying bacteria in the front part of TBs.

Besides, the highest abundance of heterotrophic aerobic denitrification-related genera was obtained in P2 (30.57%), while the most anoxic denitrification-related genera were found in P3 (19.52%). In other words, the denitrifying bacteria in the middle of TB were mainly aerobic denitrifying bacteria, while that in the end of TB were mainly anoxic denitrifying bacteria. In the operation process, a large amount of biofilm was enriched on the fillers, while aerobic and anoxic environments were formed on fillers, which leads to the existence of complex denitrifying bacteria in TB-EFTW system. In particular, the long-term aeration and the presence of large amounts of NO_3^--N along with organic matter provided a suitable condition for aerobic denitrification bacteria. The high abundance of aerobic denitrifiers (30.57%) existing in the middle of TB was much higher than that of typical floating treatment wetland with the 0.31% aerobic denitrifiers (Gao et al., 2017).

On one hand, DO concentration in front of TB was comparatively high (above 3.3 mg/L) and then dropped gradually (down to 0.3 mg/L) along TB due to the oxygen consumption of aerobic microorganisms, thus leading to the distribution of "oxic-anoxic (O-A) area in sequence" along TB. On the other

Table 4. Relative abundance of nitrogen removal related genus in the biofilm samples

TYPES OF NITROGEN REMOVAL	RELATED GENUS	P1	P2	P3
Autotrophic aerobic nitrification	*Nitrosomonas*	2.13	0.00	0.00
	Nitrosospira	2.03	0.15	0.04
	Nitrobacter	0.37	0.03	0.04
	Nitrospira	0.26	0.12	0.09
	Sum	4.79	0.30	0.17
Heterotrophic aerobic denitrification	*Acinetobacter*	0.58	12.47	0.12
	Citrobacter	0.33	9.22	0.03
	Pseudomonas	0.40	5.45	0.20
	Rhizobium	0.30	2.00	0.06
	Bacillus	0.04	0.91	0.34
	Achromobacter	0.06	0.33	0.01
	Enterobacter	0.04	0.13	0.00
	Paracoccus	0.05	0.06	0.01
	Sum	1.80	30.57	0.77
Heterotrophic anoxic denitrification	*Rhodobacter*	0.90	0.97	2.99
	Steroidobacter	0.33	1.50	7.43
	Thermomonas	0.11	1.02	6.75
	Flavobacterium	0.31	0.07	0.99
	Hyphomicrobium	0.25	0.76	0.77
	Azospira	0.02	0.13	0.54
	Hydrogenophaga	0.09	0.06	0.05
	Sum	2.01	4.51	19.52

hand, C/N rose constantly (from 6.2 to 40.3) along TB as the release of carbon source and the decline of nitrogen concentration. As a result, the unique O-A area distribution with the abundant C/N forming along TB might promote the growth of both aerobic and anaerobic denitrifying bacteria.

Overall, TB-EFTW system stimulated the growth of microorganism distribution featuring "nitrification, aerobic denitrification, and anoxic denitrification" inside TBs. This coexistence of "nitrification-, aerobic denitrification-, and anoxic denitrification-" related bacteria in this system demonstrated a unique advantage in nitrogen removal and could provide an important reference value for the treatment of urban landscape water.

Conclusions

During 240 days' operation, the TN concentration of the landscape water decreased from 2.32 to 0.43 mg/L, and the final TN removal rate reached 81.5%, which demonstrated the efficient nitrogen removal performance of the pilot-scale TB-EFTW system. The unique distribution of oxic-anoxic (O-A) areas was formed in sequence along the axial direction TB. The low contribution rate (8.3%) of plant absorption to nitrogen removal suggested the key role of microbial conversion in the system. Nitrifiers, aerobic denitrifiers, and anoxic denitrifiers were enriched in the front, middle, and end of the TB, with the abundance of 4.79%, 30.57%, and 19.52%, respectively. The ordinal distribution of "nitrifying, aerobic denitrifying, and anoxic denitrifying: along the TB suggested a special superiority in nitrogen removal for in situ treatment of landscape water.

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Conflict of Interest

The authors do not have any possible conflict of interest.

References

Ahmad, N. U. D., Xu, H. Y., Chen, L. P., Liu, Z. P., & Liu, S. J. (2008). Enhanced biological nutrient removal by the alliance of a heterotrophic nitrifying strain with a nitrogen removing ecosystem. *Journal of Environmental Sciences*, *20*(2), 216–223. https://doi.org/10.1016/S1001-0742(08)60034-0

APHA (2005). *Standard methods for the examination of water and wastewater*, 21st ed. Washington, DC: APHA, AWWA and WPCF.

Borne, K. E., Fassman-Beck, E. A., & Tanner, C. C. (2014). Floating treatment wetland influences on the fate of metals in road runoff retention ponds. *Water Research*, *48*, 430–442. https://doi.org/10.1016/j.watres.2013.09.056

Bustamante, M. A. O., Mier, M. V., Estrada, J. A. E., & Domiguez, C. D. (2011). Nitrogen and potassium variation on contaminant removal for a vertical subsurface flow lab scale constructed wetland. *Bioresource Technology*, *102*(17), 7745–7754. https://doi.org/10.1016/j.biortech.2011.06.005

Cao, W. P., & Zhang, Y. Q. (2014). Removal of nitrogen (N) from hypereutrophic waters by ecological floating beds (EFBs) with various substrates. *Ecological Engineering*, *62*, 148–152. https://doi.org/10.1016/j.ecoleng.2013.10.018

Chang, Y. Y., Cui, H., Huang, M. S., & He, Y. (2017). Artificial floating islands for water quality improvement. *Environmental Reviews*, *25*(3), 350–357. https://doi.org/10.1139/er-2016-0038

Chen, D. Y., Chen, X. C., Huang, X. B., He, S. B., Huang, J. C., & Zhou, W. L. (2017). Controlling denitrification accompanied with nitrite accumulation at the sediment-water interface. *Ecological Engineering*, *100*, 194–198. https://doi.org/10.1016/j.ecoleng2016.12.019

Chen, J. W., & Strous, M. (2013). Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution. *Biochimica Et Biophysica Acta-Bioenergetics*, *1827*(2), 136–144. https://doi.org/10.1016/j.bbabio.2012.10.002

Chen, Y., Wen, Y., Zhou, Q., & Vymazal, J. (2014). Effects of plant biomass on denitrifying genes in subsurface-flow constructed wetlands. *Bioresource Technology*, *157*, 341–345. https://doi.org/10.1016/j.biortech.2014.01.137

dC Rubin, S. S., Marín, I., Gómez, M. J., Morales, E. A., Zekker, I., San Martín-Uriz, P., … Amils, R. (2017). Prokaryotic diversity and community composition in the Salar de Uyuni, a large scale, chaotropic salt flat. *Environmental Microbiology*, *19*(9), 3745–3754. https://doi.org/10.1111/1462-2920.13876

Dennehy, C., Lawlor, P. G., Gardiner, G. E., Jiang, Y., Cormican, P., McCabe, M. S., & Zhan, X. M. (2017). Process stability and microbial community composition in pig manure and food waste anaerobic co-digesters operated at low HRTs. *Frontiers of Environmental Science & Engineering*, *11*(3). https://doi.org/10.1007/s11783-017-0923-9

Fahrbach, M., Kuever, J., Remesch, M., Huber, B. E., Kampfer, P., Dott, W., & Hollender, J. (2008). Steroidobacter denitrificans gen. nov., sp nov., a steroidal hormone-degrading gammaproteobacterium. *International Journal of Systematic and Evolutionary Microbiology*, *58*, 2215–2223. https://doi.org/10.1099/ijs.0.65342-0

Gao, L., Zhou, W., Huang, J., He, S., Yan, Y., Zhu, W., … Zhang, X. U. (2017). Nitrogen removal by the enhanced floating treatment wetlands from the secondary effluent. *Bioresource Technology*, *234*, 243–252. https://doi.org/10.1016/j.biortech.2017.03.036

Gao, L., Zhou, W. L., Wu, S. Q., He, S. B., Huang, J. C., & Zhang, X. (2018). Nitrogen removal by thiosulfate-driven denitrification and plant uptake in enhanced floating treatment wetland. *Science of the Total Environment*, *621*, 1550–1558. https://doi.org/10.1016/j.scitotenv.2017.10.073

GB3838-2002 (2002). *Chinese national environmental quality standards for surface water*. China: Ministry of Environmental Protection.

Han, X. M., Zhou, Z., Mei, X. J., Ma, Y., & Xie, Z. F. (2018). Influence of fermentation liquid from waste activated sludge on anoxic/oxic-membrane bioreactor performance: Nitrogen removal, membrane fouling and microbial community. *Bioresource Technology*, *250*, 699–707. https://doi.org/10.1016/j.biortech.2017.11.090

He, S., Wang, Y. M., Li, C. S., Li, Y. C., & Zhou, J. (2018). The nitrogen removal performance and microbial communities in a two-stage deep sequencing constructed wetland for advanced treatment of secondary effluent. *Bioresource Technology*, *248*, 82–88. https://doi.org/10.1016/j.biortech.2017.06.150

Headley, T. R., & Tanner, C. C. (2012). Constructed wetlands with floating emergent macrophytes: An innovative storm water treatment technology. *Critical Reviews in Environmental Science and Technology*, *42*(21), 2261–2310. https://doi.org/10.1080/10643389.2011.574108

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., … Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, *8*(1), 3–13. https://doi.org/10.1016/j.hal.2008.08.006

Hsieh, C. H., & Davis, A. P. (2005). Evaluation and optimization of bioretention media for treatment of urban storm water runoff. *Journal of Environmental Engineering*, *131*(11), 1521–1531.

Huang, X., Dong, W. Y., Wang, H. J., & Feng, Y. Y. (2018). Role of acid/alkali-treatment in primary sludge anaerobic fermentation: Insights into microbial community structure, functional shifts and metabolic output by high-throughput sequencing. *Bioresource Technology*, *249*, 943–952. https://doi.org/10.1016/j.biortech.2017.10.104

Huang, X., Dong, W. Y., Wang, H. J., & Jiang, S. L. (2017). Biological nutrient removal and molecular biological characteristics in an anaerobic-multistage anaerobic/oxic (A-MAO) process to treat municipal wastewater. *Bioresource Technology*, *241*, 969–978. https://doi.org/10.1016/j.biortech.2017.05.161

Idi, A., Ibrahim, Z., Mohamad, S. E., & Majid, Z. A. (2015). Biokinetics of nitrogen removal at high concentrations by *Rhodobacter sphaeroides* ADZ101. *International Biodeterioration & Biodegradation*, *105*, 245–251. https://doi.org/10.1016/j.ibiod.2015.09.014

Ji, B., Yang, K., Zhu, L., Jiang, Y., Wang, H. Y., Zhou, J., & Zhang, H. N. (2015). Aerobic denitrification: A review of important advances of the last 30 years. *Biotechnology and Bioprocess Engineering*, *20*(4), 643–651. https://doi.org/10.1007/s12257-015-0009-0

Kulikowska, D., Jozwiak, T., Kowal, P., & Ciesielski, S. (2010). Municipal landfill leachate nitrification in RBC biofilm – Process efficiency and molecular analysis of microbial structure. *Bioresource Technology*, *101*(10), 3400–3405. https://doi.org/10.1016/j.biortech.2009.12.050

Lazarova, V., Nogueira, R., Manem, J., & Melo, L. (1998). Influence of dissolved oxygen on nitrification kinetics in a circulating bed reactor. *Water Science and Technology*, *37*(4–5), 189–193. https://doi.org/10.1016/S0273-1223(98)00103-6

Li, D., Zheng, B. H., Liu, Y., Chu, Z. S., He, Y., & Huang, M. S. (2018). Use of multiple water surface flow constructed wetlands for non-point source water pollution control. *Applied Microbiology and Biotechnology*, *102*(13), 5355–5368. https://doi.org/10.1007/s00253-018-9011-8

Li, L. Z., He, C. G., Ji, G. D., Zhi, W., & Sheng, L. X. (2015). Nitrogen removal pathways in a tidal flow constructed wetland under flooded time constraints. *Ecological Engineering*, *81*, 266–271. https://doi.org/10.1016/j.ecoleng.2015.04.073

Li, X. N., Song, H. L., Li, W., Lu, X. W., & Nishimura, O. (2010). An integrated ecological floating-bed employing plant, freshwater clam and biofilm carrier for purification of eutrophic water. *Ecological Engineering*, *36*(4), 382–390. https://doi.org/10.1016/j.ecoleng.2009.11.004

McBride, M. J., Xie, G., Martens, E. C., Lapidus, A., Henrissat, B., Rhodes, R. G., … Stein, J. L. (2009). Novel Features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. *Applied and Environmental Microbiology*, *75*(21), 6864–6875. https://doi.org/10.1128/Aem.01495-09

Ren, Y. X., Yang, L., & Liang, X. (2014). The characteristics of a novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Acinetobacter junii* YB. *Bioresource Technology*, *171*, 1–9. https://doi.org/10.1016/j.biortech.2014.08.058

Shu, D. T., He, Y. L., Yue, H., & Wang, Q. Y. (2016). Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems. *Chemical Engineering Journal*, *290*, 21–30. https://doi.org/10.1016/j.cej.2016.01.024

Tan, X. U., Yang, Y., Liu, Y., Li, X., Fan, X., Zhou, Z., … Yin, W. (2019). Enhanced simultaneous organics and nutrients removal in tidal flow constructed wetland using activated alumina as substrate treating domestic wastewater. *Bioresource Technology*, *280*, 441–446. https://doi.org/10.1016/j.biortech.2019.02.036

Tangsir, S., Moazed, H., Naseri, A. A., Garmdareh, S. E. H., Broumand-Nasab, S., & Bhatnagar, A. (2017). Investigation on the performance of sugarcane bagasse as a new carbon source in two hydraulic dimensions of denitrification beds. *Journal of Cleaner Production*, *140*, 1176–1181. https://doi.org/10.1016/j.jclepro.2016.10.044

Tian, M., Zhao, F., Shen, X., Chu, K., Wang, J., Chen, S., … Liu, H. (2015). The first metagenome of activated sludge from full-scale anaerobic/anoxic/oxic (A2O) nitrogen and phosphorus removal reactor using Illumina sequencing. *Journal of Environmental Sciences*, *35*, 181–190. https://doi.org/10.1016/j.jes.2014.12.027

Wilsenach, J., Burke, L., Radebe, V., Mashego, M., Stone, W., Mouton, M., & Botha, A. (2014). Anaerobic ammonium oxidation in the old trickling filters at daspoort wastewater treatment works. *Water SA*, *40*(1), 81–88. https://doi.org/10.4314/wsa.v40i1.10

Wu, Q., Hu, Y., Li, S. Q., Peng, S., & Zhao, H. B. (2016). Microbial mechanisms of using enhanced ecological floating beds for eutrophic water improvement. *Bioresource Technology*, *211*, 451–456. https://doi.org/10.1016/j.biortech.2016.03.113

Xu, Z. S., Dai, X. H., & Chai, X. L. (2018). Effect of different carbon sources on denitrification performance, microbial community structure and denitrification genes. *Science of the Total Environment*, *634*, 195–204. https://doi.org/10.1016/j.scitotenv.2018.03.348

Yang, X. L., Jiang, Q., Song, H. L., Gu, T. T., & Xia, M. Q. (2015). Selection and application of agricultural wastes as solid carbon sources and biofilm carriers in MBR. *Journal of Hazardous Materials*, *283*, 186–192. https://doi.org/10.1016/j.jhazmat.2014.09.036

Young, C.-C., Kampfer, P., Ho, M.-J., Busse, H.-J., Huber, B. E., Arun, A. B., … Rekha, P. D. (2007). Arenimonas malthae sp nov., a gammaproteobacterium isolated from an oil-contaminated site. *International Journal of Systematic and Evolutionary Microbiology*, *57*, 2790–2793. https://doi.org/10.1099/ijs.0.64975-0

Yun, H. J., & Kim, D. J. (2003). Nitrite accumulation characteristics of high strength ammonia wastewater in an autotrophic nitrifying biofilm reactor. *Journal of Chemical Technology and Biotechnology*, *78*(4), 377–383. https://doi.org/10.1002/jctb.751

Zhang, L. L., Sun, Z. Z., Xie, J., Wu, J., & Cheng, S. P. (2018). Nutrient removal, biomass accumulation and nitrogen-transformation functional gene response to different nitrogen forms in enhanced floating treatment wetlands. *Ecological Engineering*, *112*, 21–25. https://doi.org/10.1016/j.ecoleng.2017.12.021

Zhang, L. L., Zhao, J., Cui, N. X., Dai, Y. R., Kong, L. W., Wu, J., & Cheng, S. P. (2016). Enhancing the water purification efficiency of a floating treatment wetland using a biofilm carrier. *Environmental Science and Pollution Research*, *23*(8), 7437–7443. https://doi.org/10.1007/s11356-015-5873-9

Zhao, F. L., Xi, S., Yang, X. E., Yang, W. D., Li, J. J., Gu, B. H., & He, Z. L. (2012). Purifying eutrophic river waters with integrated floating island systems. *Ecological Engineering*, *40*, 53–60. https://doi.org/10.1016/j.ecoleng.2011.12.012