Bioresource Technology 280 (2019) 441–446

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Enhanced simultaneous organics and nutrients removal in tidal flow constructed wetland using activated alumina as substrate treating domestic wastewater

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ARTICLE INFO

Keywords:
Tidal flow constructed wetland
Domestic wastewater
Substrate
Activated alumina
Microbial community structure

ABSTRACT

A tidal flow constructed wetland (TFCW), a commonly applied system to clean wastewater, contains a substrate to assist pollutants removal, while the choice of substrate affects the formation of bacterial biofilms. Herein, activated alumina-TFCW (A-TFCW) with hydraulic load of $1.35\,m^3/(m^2{\cdot}d)$ parallel with shale ceramisite (S-TFCW) was investigated for treating domestic wastewater, aiming to enhance simultaneous long-term removal of organics, nitrogen and phosphorus. A-TFCW achieved significantly higher COD, NH_4^+-N, TN and TP removal efficiency than S-TFCW, with the removal efficiency of 85.9% COD, 85.4% NH_4^+-N, 72.8% TN and 96.4% TP respectively. Denitrifying bacteria dominated in both formed biofilms, with higher relative abundance of nitrifying bacteria and denitrifying bacteria in A-TFCW. These results demonstrated that AA substrate was more suitable to be applied in enhancing the removal performance in TFCW for the treatment of domestic wastewater.

1. Introduction

Constructed wetlands (CW), which simulate a natural wetland ecosystem and can be applied to wastewater treatment has the benefits of relatively low operation costs and simple maintenance (Vymazal, 2005). Combined with lower construction cost, easier management and larger treatment capacity, CW has been commonly applied in treatment of wastewater from cities and small towns alike (Wu et al., 2015). However, CW generally has a low treatment capacity and may suffer from regular clogging, which restricts its application in decentralized domestic wastewater treatment (Sears et al., 2012). CW usually can obtain high organics removal, although the nitrogen removal performance is poor because of the low oxygen supply capability (Ju et al., 2014). Tidal flow constructed wetland (TFCW) can greatly improve oxygen supply by a “tidal” operation strategy that repeats cycle of “saturated” and “unsaturated” periods. The oxygen transfer rate in TFCW can reach up to $450\,g/(m^2{\cdot}d)$ under operation with optimal duration of saturated and unsaturated phases (Wu et al., 2011).

The addition of substrates to CW play positive effect on pollutants removal and can prolong the operation life. Compared with a conventional gravel medium, unconventional substrates used for CW include organic wood-mulch, alum sludge, zeolite, volcanic rock and shale ceramisite. These can achieve substantial nitrogen and organics removal from wastewater under optimal operating conditions (Saeed and Sun, 2011a; Zhao et al., 2011; Liu et al., 2014; Drizo et al., 1997). These substrates not only possess higher porosity and larger specific surface area to provide optimal aerobic conditions, but also present reactive surfaces for microbial attachment that is essential for CW operation. In particular activated alumina (AA) has been extensively used as an adsorbent for ground water treatment in rural and urban applications because of its unique physical, chemical and textural properties (Changmai et al., 2017).

A remaining challenge of CW is sustainable removal of phosphorous. Most phosphorus is removal by substrate absorption and precipitation in CW (Wang et al., 2013). The phosphorus removal efficiency can be sufficient at the beginning of operation, but over time it significantly decreases, and eventually phosphorus can be release again. A pervious study has confirmed that AA has a strong affinity for phosphate, whereby almost all phosphorus could be adsorbed at low concentration. This characteristic is favorable to phosphorus removal in wastewater and micro-polluted water.

The choice of substrate not only plays a decisive role on pollutants removal, but also affects the formation of biofilm in CW, leading to various community that differ in their microbial diversity and structure.

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https://doi.org/10.1016/j.biortech.2019.02.036
Received 20 December 2018; Received in revised form 5 February 2019; Accepted 6 February 2019
Available online 07 February 2019
0960-8524/

The main bacterial genera in pyrite CW were *Anaeromyxobacter*, *Ramlibacter*, *Defluviicoccus*, *Azoarcus*, *Geobacter*, and the microbial communities were highly related to anaerobic and aerobic oxidation of pyrite (Ge et al., 2019). Substrates including gravel, steel slag and zeolite in three lab-scale vertical-flow CWs treating surface water produced different abundance of *Acidobacteria* and *Planctomycetes* that could enhance the nitrogen removal (Long et al., 2016). Compared to gravel, a substrate of sludge ceramisite used in intermittently aerated CW for treating decentralized domestic wastewater increased the fraction of nitrifying bacteria with 5–6%, achieving high removal of ammonium nitrogen (NH_4^+-N) and total nitrogen (TN) (Wu et al., 2016). There are fewer studies that used AA as substrate in CW, and bacterial community diversity and structure in AA-based CW remains unclear.

The objectives of this study were to investigate the removal of pollutants during long-term operation of TFCWs, especially for sustainable total phosphorous (TP) removal, using AA as substrate parallel with shale ceramisite (SC); additionally, to analyze the effect of different substrates on the microbial community in these TFCWs.

2. Materials and methods

2.1. Substrates

The two substrates, active alumina (AA) and shale ceramisite (SC), were purchased from An Bang Water Purification Material Co., Ltd (Zhengzhou, China). Before packed in a laboratory-scale TFCW, the substrates were washed and its main properties were investigated. The particle size of AA was 5–8 mm and that of SC was 10–15 mm. The main characteristics of fresh AA were as follows: micro-pore volume 393.1 mm^3/g, average micro-pore size 4.69 nm and BET specific surface area 334.07 m^2/g, and the corresponding characteristics of SC were 2.844 mm^3/g, 5.248 nm and 2.168 m^2/g, respectively.

2.2. Synthetic wastewater

Water treatment was conducted with synthetic wastewater, with a composition of 250 mg/L glucose, 188.4 mg/L NH_4Cl, 39.8 mg/L $K_2HPO_4{\cdot}3H_2O$, 10 mg/L $MgSO_4$, 10 mg/L $FeSO_4{\cdot}7H_2O$, and10 mg/L $CaCl_2$. During operation the synthetic wastewater was maintained at average influent concentration of 246.2 mg/L COD_{cr}, 49.3 mg/L NH_4^+-N, 53.9 mg/L TN and 5.41 mg/L TP, respectively. The temperature of the raw water during the entire experiment varied between 16 and 27 °C.

2.3. Experimental setup

The experiments were conducted in two lab-scale down-flow TFCWs, which were identically built, with A-TFCW using AA as substrate, and S-TFCW using SC, respectively. The system was made of cylindrical plexiglass, with a diameter of 20 cm and a height of 100 cm. The total depth of the TFCWs was 90 cm with working volume of 9.42 L in A-TFCW (initial porosity 37.5%) and 9.60 L in S- TFCW (initial porosity 38.2%). The bottom of the TFCWs was filled with pebble as the support layer with a height of 10 cm, followed by 80 cm of SC or AA as the main substrate layer, respectively. The water feed consisted of an 80 L tank and two magnetic pumps (MP-10R, shanghai, China) connecting to the two TFCWs in parallel.

Operation was controlled by programmable logic controller using the "tidal flow" mode, consisting of four stages (fill-contact-drain-rest). During fill, wastewater from the feed tank was loaded rapidly (2 min) onto the substrate bed from above, and after filling the duration of contact time was 4 h. After this all wastewater was drained out rapidly (2 min) and the bed was kept to rest for 2 h. The whole tidal cycle was 6 h, with a hydraulic load of 1.35 $m^3/(m^2{\cdot}d)$ for A-TFCW and 1.38 $m^3/(m^2{\cdot}d)$ for S-TFCW. The duration of the entire experiment with repeated cycles was 180 days. The substrates were inoculated with 2500 mg/L Mixed liquor suspended solids (MLSS) activated sludge taken from the secondary sedimentation tank of a municipal sewage plant (Beijng, China). Prior to the actual experiments, 80 L synthetic wastewater was mixed with 1 L activated sludge and this inoculum was fed to the TFCWs once a day for 10 days. After this, the experiment was started by feeding synthetic wastewater into the systems. Water samples were collected from the feeding tank and outlet of each system at 12 AM twice a week.

2.4. Microbial community analysis

The biofilms forming on the substrates were collected at the end of experiment and the bacterial community was analyzed by DNA-based molecular technique. Microbial DNA from the biofilm was extracted by E.Z.N.A. ® Soil DNA Kit (Omega Bio-tek, U.S.) following the manufacturer's protocols and stored at −20 °C before further analysis. The V3-V4 regions of the bacterial 16S rRNA gene of was amplified by primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) (Fu et al., 2018). Illumina MiSeq sequencing was conducted at Shanghai Sangon Biotech Co., Ltd (shanghai, China) to analyze the structure of microbial community. High-quality sequences were clustered into the operational taxonomic units (OTUs) at 97% similarity. Bacterial community richness was assessed by the ACE index and diversity by Shannon index, these were analyzed using the UPARSE pipeline (Edgar, 2013). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA514969).

2.5. Other analytic methods

Water samples collected from the feed tank and outlet were filtered using quantitative filter paper before chemical analysis to remove coarse material. Water samples were analyzed for content of NH_4^+-N, TN, chemical oxygen demand (COD_{cr}), and PO_4^{3-}-P according to standard methods (SEPA, 2002). Temperature and dissolved oxygen (DO) were measured by means of a portable Hach HQ30d multi-parameter analyzer (Hach Company, Loveland, Co., USA). The porous properties of the substrates were detected at room temperature by TriStar II 3020 (Micromeritics instrument (Shanghai) Ltd.). Before detecting pore size, substrates with a mass of 100 mg were evacuated at 200 °C under 0.1 Pa prior to the adsorption. The porosities of the substrate, including their microspore volume, pore distribution and specific surface area, were detected by multipoint Brunauer-Emmet-Teller N_2 adsorption–desorption isotherms. Surface morphologies of substrates were observed by scanning electron microscope (SEM) (FEI Nova nano450, Holland) operating at 5.0 kV.

3. Results and discussion

3.1. Effect of substrate types on organics removal

The variation of COD_{cr} in influent and effluent of the two TFCWs compared here is shown in Fig. 1. Two phases of the experiment could be recognized: a starting phase (Phase I) and a stable phase (Phase II). Duration of Phase I was 24 days for A-TFCW, which S-TFCW took twice that time to become stable, indicating that a stable biofilm had formed much faster in AA substrate than in SC substrate. During Phase I, the removal efficiency of COD_{cr} increased from 37.5% to 68.8% and 21.2% to 50.4% with effluent concentration changing from 180.4 mg/L to 82.1 mg/L and 227.4 mg/L to 132.9 mg/L in A-TFCW and S-TFCW, respectively. During Phase II, the average COD_{cr} removal efficiency in the A-TFCW and S-TFCW was 85.9% and 78.4%, respectively. The average COD_{cr} effluent concentration in A-TFCW was 35.1 mg/L, which was lower than that in S-TFCW (58.1 mg/L). These results suggested that COD_{cr} was removed more effectively in A-TFCW than S-TFCW.

The two main attributors to a reduction of organics are adsorption

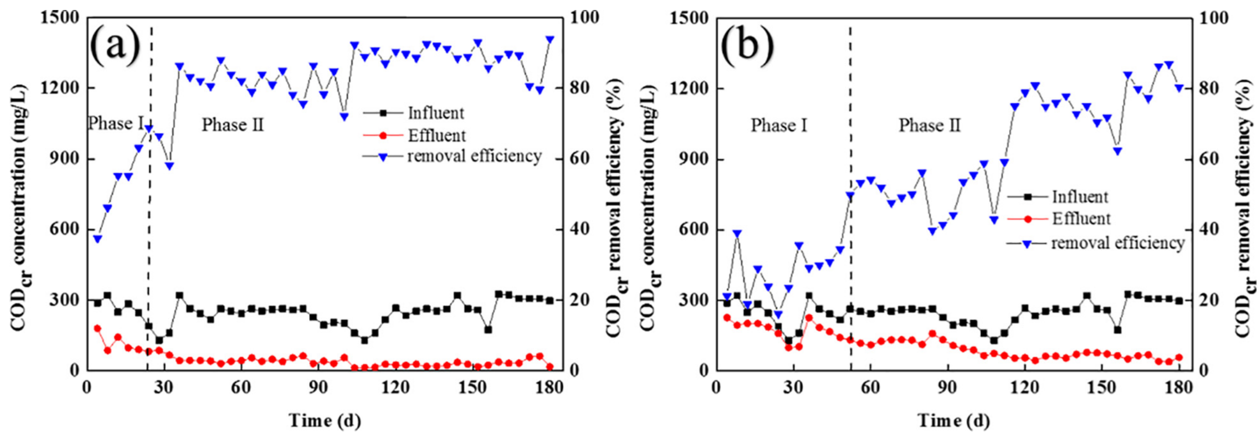

Fig. 1. CODcr removal in two TFCWs (a) A-TFCW; (b) S-TFCW. (The average influent concentration of CODcr was 246.2 mg/L).

by substrates and assimilation by microorganisms resulting in increased biomass. It was reported that a CW substrate with larger BET specific surface area and more micro-pore performed better in pollutants removal in CWs (Liu et al., 2014). The micro-pore volume, average micro-pore size and BET specific surface area of the AA and SC substrates after 180 days of operation were determined. The BET specific surface area of the used AA substrate reached up to 326.05 m^2/g, with a micro-pore volume and average micro-pore size of AA still 390.8 mm^3/g and 4.80 nm. In contrast, the BET specific surface area of the used SC had decreased to 0.466 mm^2/g, with a pore volume of 0.318 mm^3/g and a pore size of only 2.713 nm.

The microscopic surface structure of these substrates was investigated by SEM, prior to use and after 180d-operation. The surface of fresh AA was rough and porous, mainly consisting of micro-pores; cracks on the surface were visible to varying degrees, mainly with fine micro seams. The surface of fresh SC was more uniform and smooth, with few cracks and fewer micro-pores and seams than were observed with AA. Biofilm attached on these substrates was both compact and uniform. Large cracks were visible on the surface of used AA, while a densely grown biofilm was attached to SC that covered most of the pore on the surface. A porous substrate is beneficial as it allows the circulation of gas including oxygen, generating an aerobic environment throughout, which supports the growth of aerobic bacteria that depend on oxygen and utilize organics as they multiply. Thus, removal of organics will be more effective in a porous substrate that optimally supports their growth, propagation, and biochemical process.

3.2. Effect of substrate types on nitrogen removal

As illustrated in Fig. 2(panels (a) and (b)), the removal of NH_4^+-N become stable after 40 days, after which an average NH_4^+-N effluent concentration of 35.8 mg/L, and removal efficiency of 25.2% was reached in A-TFCW, while in S-TFCW these were 33.6 mg/L and 33.2%, respectively. During the phase I, the main mechanism for NH_4^+-N removal might be ascribed to the absorption of substrates, while during the phase II, bacterial nitrification dominated the NH_4^+-N removal. Fresh SC could achieve 20.3% removal of NH_4^+-N by adsorption, which was about twice the capacity of AA (11.5%), leading to a higher average NH_4^+-N removal efficiency in S-TFCW during the phase I. During phase II, A-TFCW achieved an average NH_4^+-N effluent concentration and a removal efficiency of 6.76 mg/L and 86.1%, compared to that of 17.1 mg/L and 64.9%, respectively, in S-TFCW. Thus, A-TFCW performed better for NH_4^+-N removal during phase II, which may be related to the higher porous characteristic of AA compared with SC, resulting in better microbial nitrification. DO concentration above 1.5 mg/L is essential for nitrification (Hu et al., 2014). A better oxygen environment (DO > 1.5 mg/L) is benefit to the growth of nitrifying bacteria, while a low DO during most time of a tidal cycle may restrict the nitrification process. In a typical tidal cycle with a contact time of 4 h, the average DO concentration in A-TFCW varied from 5.13 mg/L to 1.52 mg/L which was higher than that in S-TFCW (4.09–0.98 mg/L). Moreover, the duration of the period DO exceeded 1.5 mg/L was about 4 h in A-TFCW and porosity of the AA substrate in A-TFCW provided a higher DO of the outlet (1.5 ± 0.5 mg/L) than could be reached with S-TFCW (1.0 ± 0.4 mg/L). This illustrates that nitrification was promoted during most of the time of a tidal cycle in A-TFCW, benefitting the overall removal rate of ammonium nitrogen.

The removal performance of TN was also determined, and was found to be stable after 40 days (Fig. 2(c) and (d)). The TN concentration in A-TFCW effluent (14.3 mg/L on average) was much lower than that of S-TFCW (27.9 mg/L), corresponding to removal efficiencies of 72.8% and 47.2%, respectively. The higher TN removal performance of A-TFCW can be caused by three aspects: firstly, TN removal performance in A-TFCW could be favorable due to the higher porosity of this substrate and its larger micro-pores, causing a suitable oxygen supply and promoting simultaneous nitrification and denitrification. The porous properties of fresh AA were still partly retained after 180 d-operation, and this may have a positive effect on TN removal. Organic media with a higher porosity could enhancing denitrification in CWs (Patureau et al., 2000). Secondly, denitrifying microbes in the small-scale A-TFCW might not only included anaerobic denitrifiers, but also aerobic denitrifiers under DO concentration of 1.5 ± 0.5 mg/L. Presence of bacterial genera representing anaerobic denitrifiers and aerobic denitrifiers are discussed in the following Section 3.4. Generally, most denitrifiers are anaerobic bacteria capable of denitrification only when the oxygen concentration is below 0.5 mg/L, but aerobic bacteria that grow at DO higher than 1.7 mg/L within oxygen-enriched regions can be was capable of aerobic denitrification (Matos et al., 2017; Saeed and Sun, 2011b). Thirdly, from the SEM images of biofilms attached onto AA and SC, it was observed that the SC substrate was uniformly covered, increasing the potential of surface blockage of filter media, which would be consistent with the result of Robertson et al. (1986) who reported that the formation of substrate biofilm was an important reason for blockage of filter media, lowering TN removal.

3.3. Effect of substrate types on TP removal

The difference of TP removal in the two TFCWs during 180d-operation is shown in Fig. 3. A-TFCW resulted in more phosphorous removal that was also more durable compared to S-TFCW. Fresh AA could achieve 97.8% of TP removal efficiency by adsorption, which was higher than that of SC (38.1%), leading to higher removal ability for TP during the 180 d-operation in A-TFCW. The average TP concentration of A-TFCW effluent was 0.187 mg/L with a removal efficiency of 96.4%. The S-TFCW achieved the average effluent TP concentration and removal efficiency were 3.95 mg/L and 27.46%, respectively. Although

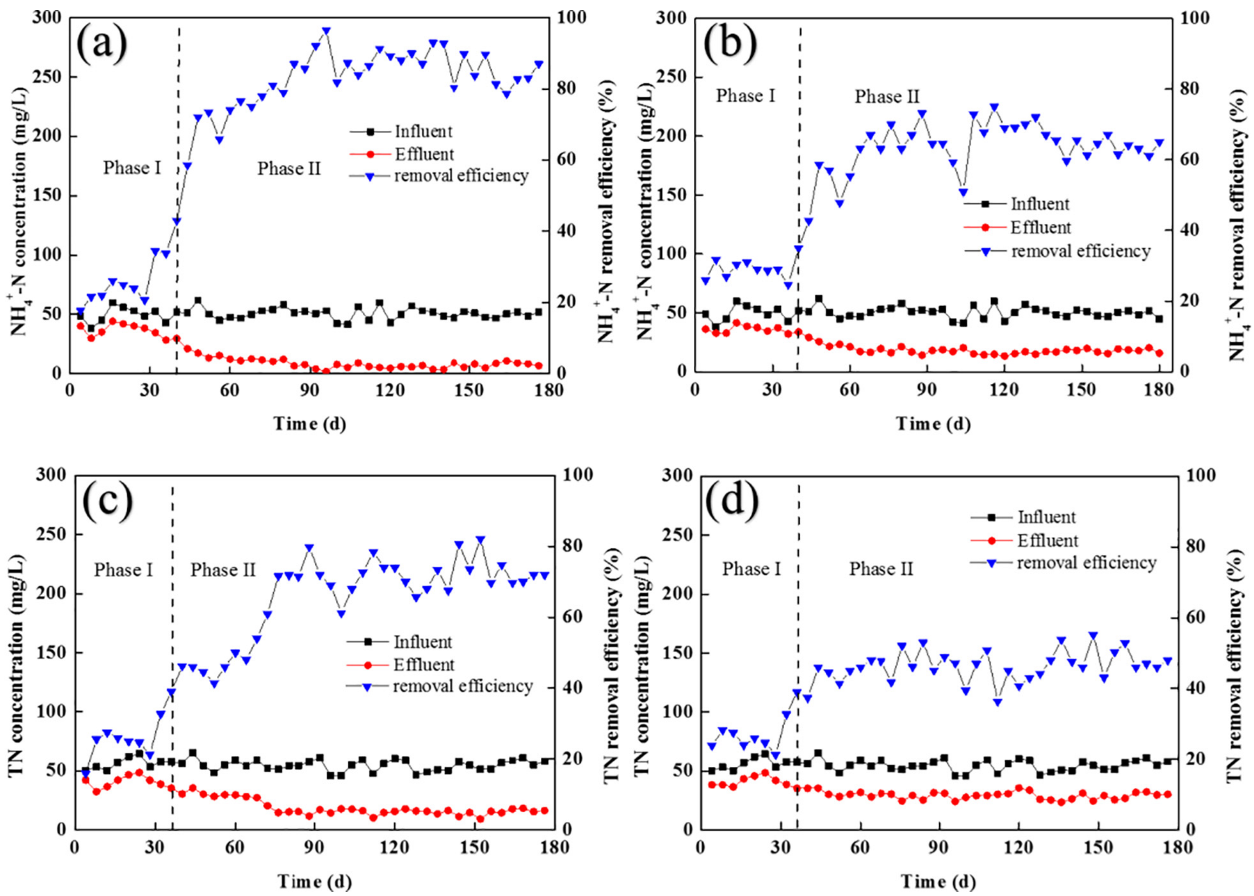

Fig. 2. Nitrogen removal in two TFCWs (a) NH4 + -N, A-TFCW; (b) NH4 + -N, S-TFCW; (c) TN, A-TFCW; (d) TN, S-TFCW (The average influent concentration of NH4 + -N and TN was 49.3 mg/L and 53.9 mg/L, respectively).

during the first 84 days, average TP effluent concentration and removal efficiency were 3.27 mg/L and 32.9%, respectively, over time the TP average removal efficiency in S-TFCW decreased to 22.5%.

There are two possible explanations for the higher TP removal performance of A-TFCW: either more active groups are available in AA compared to SC to bind phosphorus, or the effective reactive surface of AA is larger. The AA substrate is a mixture of mainly X-Al_2O_3 and α-AlOOH which contains high numbers of hydroxyl groups. These can react with phosphate. Moreover, the AA substrate has a surface morphology is porous and irregular so that more bonding sites are available. High adsorption capacity of a substrate is strongly affected available bonding sites (Kaewsarn and Yu, 2001). SC substrate is mainly composed of quartz (SiO_2) and crystals containing Fe_2O_3. The reaction products from SC substrate (such as $Fe(OH)_2$ and $Fe(OH)_3$) form flocs, which could fix the phosphorus (Jiang et al., 2014). The surface of SC substrate is smooth surface with fewer pores, which would present fewer bonding sites leading to less phosphorous removal. According to previous studies, the P adsorption capacity of AA substrate was about 50 times of that of SC substrate (Vohla et al., 2011), which supports the current results that TP removal in A-TFCW was better than that of S-TFCW.

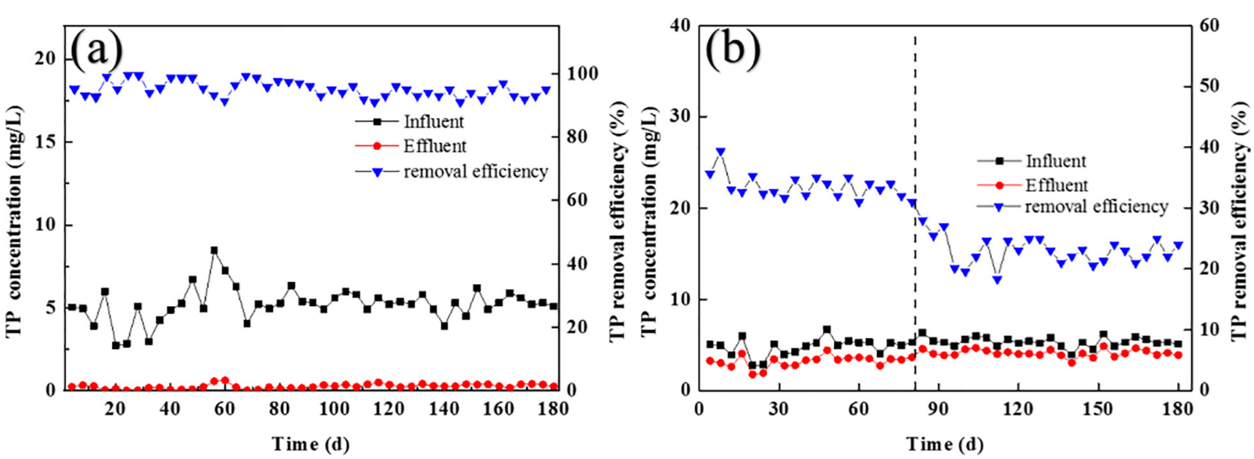

Fig. 3. TP removal in two TFCWs. (a) A-TFCW; (b) S-TFCW (The average influent concentration of TP was 5.41 mg/L).

Table 1
Comparison of microbial richness and diversity estimation from high-throughput sequencing.

Samples	OUTs	Shannon index	ACE index	Coverage
A-TFCW	3122	5.15	27160.4	0.95
S-TFCW	2869	5.06	18968.8	0.95

3.4. Effect of substrate types on bacterial diversity and microbial community structure

The presence of a better oxygenated environment was shown to be beneficial to the competition of microbe assimilation and contaminant decomposition, as well as the fluctuation of redox condition due to organics and ammonia (Chen et al., 2019). Consequently, the bacterial diversity and microbial community structure would be affected. High-throughput sequencing was conducted to analyze the bacterial community diversity and structure in the two TFCWs. Microbial samples were collected from the bottom of the TFCWs after 180 d-operation when the systems were stable. A total of 99,052 raw sequence reads of variable 16S region amplicons were obtained, and after normalization 81,202 could be used for comparison of bacterial community diversity at the same sequencing depth. As shown in Table 1, the OTUs diversity had been well captured, with an estimated coverage of 95%. The ACE index evaluating the richness of the microbial community was higher in A-TFCW than in S-TFCW. A higher Shannon index in A-TFCW indicated this also contained a more diverse microbial community (Zhang et al., 2015).

The phyla with relative abundance greater than 1% in the substrate samples are shown in Fig. 4. *Proteobacteria*, *Bacteroidetes* and *Acidobacteria* are generally the most prevalent phyla in CWs (Ansola et al., 2014). In A-TFCW, *Proteobacteria* was the most dominant phylum (75.87%), followed by *Bacteroidetes*, *Acidobacteria* and *Nitrospirae,* with proportion of 8.08%, 4.52% and 2.06%, respectively. *Proteobacteria* were also dominant in the S-TFCW (67.71%), which contained a larger relative fraction of *Bacteroidetes* (14.61%) and a considerable fraction of *Planctomycetes* (7.92%) but fewer *Acidobacteria* (2.36%)

As illustrated in Fig. 4, the proportion of *Proteobacteria* in A-TFCWs was 8.16% higher than that in S-TFCWs, which might be ascribed to the better removal of organic and nitrogen in A-TFCW. Members from *Proteobacteria* are important for the removal of conventional pollutants (Yan et al., 2017) *Planctomycetes* were only detected in S-TFCW, and anaerobic ammonium oxidation (anammox) bacteria were included in this phylum (Hassan et al., 2017). A limited oxygen supply existed in S-TFCW, creating an anaerobic oxygen environment for the growth of anammox bacteria.

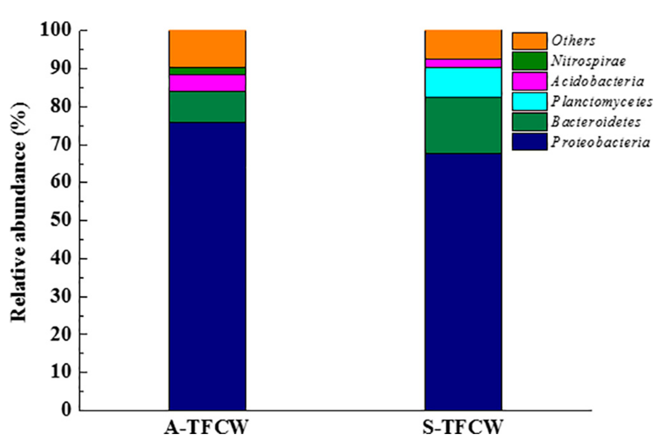

Fig. 4. Relative abundance of main phyla identified in AA and SC biofilm sample. Phyla with the relative abundance less than 1% of the total sequences were defined as "Others".

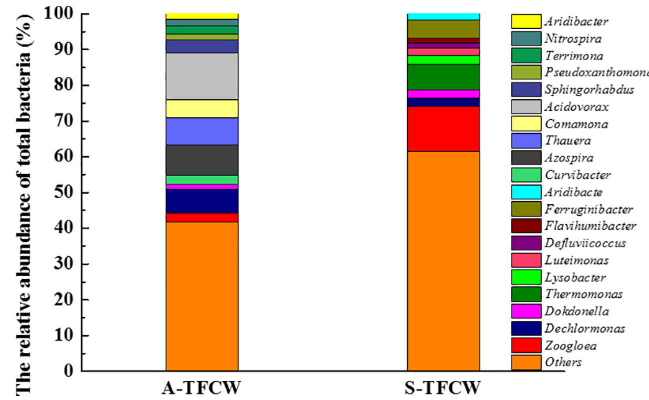

Fig. 5. Relative abundance of main genera identified in AA and SC biofilm sample. Genera with relative abundance less than 1% of the total sequences were defined as "Others".

The main genera present at relative abundances above 1% in the substrate samples are shown in Fig. 5. A total of 48 genera were identified, more genera were detected in A-TFCW, which was consistent with the higher Shannon index of that sample. In S-TFCW, In S-TFCW, *Zoogloea* species (*Beta-proteobacteria*) represented the highest proportion (12.47%), followed by *Thermomonas* (7.18%, *Gamma-proteobacteria*), *Ferruginibacter* (5.04%, *Bacteroidetes*), *Dechlormonas* (2.49%, *Beta-proteobacteria*) and *Lysobacter* (2.49%, *Gamma-proteobacteria*). *Zoogloea* and *Dechlormonas* were also observed in A-TFCW, with percentages of 2.70% and 6.53%, while other highly abundant genera were *Acidovorax* (13.23%), *Azospira* (8.44%), *Thauera* (7.64%), *Dechloromonas* (6.53%), *Comamonas* (4.90%) (all of these are *Beta-proteobacteria*) and *Nitrospira* (1.98%), which are members of a separate phylum.

In A-TFCW, seven genera including *Acidovorax*, *Comamonas*, *Thauera*, *Azospira*, *Dechloromonas*, *Zoogloea* and *Pseudomonas* are known to contain common dentrifiers, together these accounted for 45.19% of the mapped genera. (Wu et al., 2016; Tang et al., 2018; Yan et al., 2016; Zhong et al., 2015). In contrast, in S-TFCW, *Zoogloea*, *Thermomonas* and *Dechlormonas* were the main denitrifiers, making up 22.14%. The relative abundance of denirtifiers in A-TFCW was higher than S-TFCW, corresponding to the observed higher average TN removal efficiency (72.8% in A-TFCW *vs.* 47.2% in S-TFCW) (Fig. 2). However, a causative correlation should be drawn with care, as these genera were identified by DNA sequencing only, and metabolic activity or transcriptome analysis was not performed. A more detailed analysis in future studies would be needed to investigate the actual activity of these potential denitrifiers in A-TFCW and S-TFCW. In addition to denitrifiers, nitrifiers were also detected in both TFCWs, e.g., *Nitrospira* (Sun et al., 2016), which were present in higher relative abundance in A-TFCW (1.98%), resulting in a better nitrification performance in A-TFCW. Overall, these results indicated that compared with SC, AA substrate was more favorable as a supporter for the growth of denitrifiers and nitrifiers.

4. Conclusion

Higher removal efficiencies of 85.9% COD, 85.4% NH_4^+-N, 72.8% TN and 96.4% TP were obtained in A-TFCW for 180-d operation. Compared to that of 78.4% COD, 64.9% NH_4^+- N, 47.2% TN and 27.5% TP in S-TFCW. Denitrifying bacteria including *Acidovorax*, *Azospira*, *Thauera, Dechloromonas, Comamonas* and *Zoogloea* took up 45.19% in A-TFCW, which was higher than that in S-TFCW dominant by *Zoogloea, Thermomonas* and *Dechlormonas* accounting for 22.14%. Better pollutants removal in A-TFCW was ascribed to higher affinity and more porous structure of AA, as well as more abundant microbial community structure and diversity of biofilm attached by AA.

Acknowledgements

The authors acknowledge the financial support of the National Key R&D Program of China (2018YFC0406200 and 2018 YFC0704800), Science and Technology project of the Ministry of Housing and Urban-Rural Development of the People's Republic of China (K72018021), and China Construction Science and Technology Group Science and Technology Fund for Youth (Z2017Q05). We would like to give our sincere thanks to the peer-reviews for their suggestions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.02.036.

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