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Journal

Environmental Technology, 42(9)

ISSN

0959-3330

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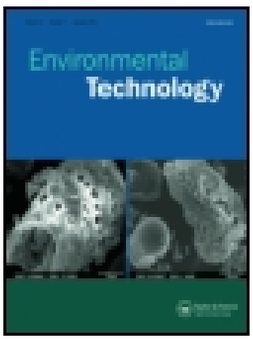
Publication Date

2021-04-16

DOI

10.1080/09593330.2019.1670267

Peer reviewed



Environmental Technology

ISSN: 0959-3330 (Print) 1479-487X (Online) Journal homepage: https://www.tandfonline.com/loi/tent20

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To cite this article: Xianlei Cai, Ling Yao, Qiyue Sheng, Luyao Jiang, Ting Wang, Randy A. Dahlgren & Huanhuan Deng (2019): Influence of a biofilm bioreactor on water quality and microbial communities in a hypereutrophic urban river, Environmental Technology, DOI: 10.1080/09593330.2019.1670267

To link to this article: https://doi.org/10.1080/09593330.2019.1670267

Accepted author version posted online: 20 Sep 2019.
Published online: 28 Sep 2019.

ENVIRONMENTAL TECHNOLOGY
https://doi.org/10.1080/09593330.2019.1670267

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Influence of a biofilm bioreactor on water quality and microbial communities in a hypereutrophic urban river

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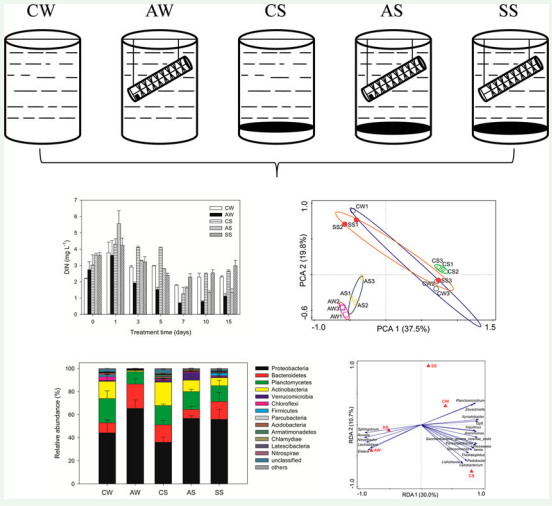
ABSTRACT

Biofilms play an important role in degradation, transformation and assimilation of anthropogenic pollutants in aquatic ecosystems. In this study, we assembled a tubular bioreactor containing a biofilm substrate and aeration device, which was introduced into mesocosms to explore the effects of bioreactor on physicochemical and microbial characteristics of a hypereutrophic urban river. The biofilm bioreactor greatly improved water quality, especially by decreasing dissolved inorganic nitrogen (DIN) concentrations, suggesting that biofilms were the major sites of nitrification and denitrification with an oxygen concentration gradient. The biofilm bioreactor increased the abundance of planktonic bacteria, whereas diversity of the planktonic microbial community decreased. Sequencing revealed that Proteobacteria, Bacteroidetes, Planctomycetes, and Actinobacteria were the four predominant phyla in the planktonic microbial community, and the presence of the biofilm bioreactor increased the relative abundance of Proteobacteria. Variations in microbial communities were most strongly affected by the presence of the biofilm bioreactor, as indicated by principal component analysis (PCA) and redundancy analysis (RDA). This study provides valuable insights into changes in ecological characteristics associated with self-purification processes in hypereutrophic urban rivers, and may be of important for the application of biofilm bioreactor in natural urban river.



ARTICLE HISTORY
Received 27 March 2019
Accepted 14 September 2019

KEYWORDS
Aeration; biofilms; bioreactor; water quality; microbial community

1. Introduction

Urban rivers are an important component of urban ecological systems [1–3], and play an especially important ecological role in densely distributed river networks, such as those found in southeast China. Due to rapid economic development and urbanization with lagging infrastructure development throughout the developing world, urban river systems have been exposed to increased nutrient and pollutant loading resulting in a serious decline in ecosystem health. Currently, up to 80% of urban rivers in China are reported to be highly polluted and degraded [4,5]. Serious organic, heavy metal and nutrient pollution in the water column and sediments, insufficient water flows, low oxygen

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reaeration-high oxygen demand, and slow self-purification processes are commonly encountered in urban rivers. Hence, urban river ecological restoration is a high priority in governance of urban environments in China and throughout the developing world.

As an ecosystem, river systems have a certain self-purification capacity that results from integrated physical, chemical and biological processes to degrade/transform pollutants. Microorganisms are the primary engine driving nutrient cycling and degradation/transformation processes [6–8], thereby playing a dominant role in the remediation of anthropogenic pollutants [9–11]. Therefore, bioremediation technologies based on microorganisms have been widely used in the remediation of polluted waters [12,13]. The surfaces of substrates immersed in aquatic environments are rapidly colonized by a wide variety of microorganisms that forms biofilms [11,14–16], which can attenuate anthropogenic pollutants [17–19]. Hence, providing instream artificial substrates (e.g. plastic materials) to increase microorganism densities and diversity, and to enhance nutrient metabolism is a low-cost, environmental friendly method for nutrient attenuation in hypereutrophic waterways.

In fact, there were several types of bioreactors using biofilms to clarify surface waters and wastewater, including algal turf scrubber [20], algal biofilm membrane photobioreactor [21], rotating algal biofilm reactor [22], tubular biofilm photobioreactor [23,24], and so on. Each biofilm bioreactor has advantages and limitations that must be considered in planning the application in bioremediation processes [25]. In hypereutrophic urban rivers, dissolved oxygen (DO) levels are generally hypoxic or anaerobic in both the water column and underlying sediments [26,27]. DO is an important parameter in aquatic environments because its presence or absence affects many geochemical and microbiological processes (i.e. redox processes) [28]. Consequently, artificial aeration is a commonly employed technique used to increase DO in hypereutrophic urban river systems [29]. However, the air bubbles produced by many aeration devices are rapidly lost in shallow urban rivers leading to low oxygen transfer and utilization efficiencies by biological processes. Meanwhile, considering the shipping and the small spaces available in hypereutrophic waterways, we designed a simple and space-saving tubular biofilm bioreactor with an aeration device and filaments to overcome DO deficiencies and maximize the biofilm remediation potential for various pollutants. Here, in addition to increasing DO, the air bubbles generated by the aeration can induce an upward water flow in the tubular biofilm bioreactor, which can not only produce higher oxygen transfer and utilization efficiencies by biological processes in the bioreactor at lower aeration rates, but also help to promote mixing with ambient water inside/outside of the bioreactor. In the present study, biofilm bioreactors were built with the following specific objectives: (1) to investigate the potential of biofilm bioreactor for enhanced bioremediation of hypereutrophic urban river waters; (2) to determine how the physicochemical and microbial characteristics of the water column were affected by the biofilm bioreactor; and (3) to increase understanding of self-purification processes in hypereutrophic urban river systems. Results of this study have several practical applications in designing and enhancing bioremediation strategies for restoring the ecological health of urban river systems.

2. Materials and methods

2.1. Study site

The sample site was located in the Shunao River (27° 55′47″ N, 120°42′19″ E), which is an urban tributary of the Wen-Rui Tang River, a typical coastal plain river system located in Wenzhou, eastern China. Due to rapid economic development and urbanization coupled with the lagging infrastructure development, Wen-Rui Tang River water quality has degraded dramatically since the 1990s, and is now in a state of serious eutrophication, characterized by high contents of total nitrogen, total phosphorus and ammonium [26,27,30]. The Shunao River is representative of impacts resulting from rapid urbanization over the past two decades.

Water and sediment samples used to construct mesocosms were collected in the Shunao River. For biofilm formation, polyethylene substrates (filaments: length 6 cm, diameter 0.06 cm, 100 filaments per replicate) were thoroughly washed with tap water, rinsed with distilled water, air dried, and then deployed for 20 d (20 Sept to 10 Oct, 2016) in the Shunao River. In situ water quality constituents were measured at the beginning and end of the culture period (mg L^{-1}): total nitrogen (TN) = 5.95–3.38; ammonium (NH_4^+–N) = 2.82–0.86; nitrate (NO_3^-–N) = 2.07–1.52; nitrite (NO_2^-–N) = 0.07–0.08; total phosphorus (TP) = 1.35–0.30; and orthophosphate (PO_4^{3-}–P) = 0.24–0.05.

2.2. Set-up of biofilm bioreactor and experimental design

The biofilm bioreactor consisted of a polyethylene tube (36 cm long × 6 cm diameter) with biofilms attached on filaments and an aeration device (Figure 1). The laboratory experiment was conducted in 15 high-density

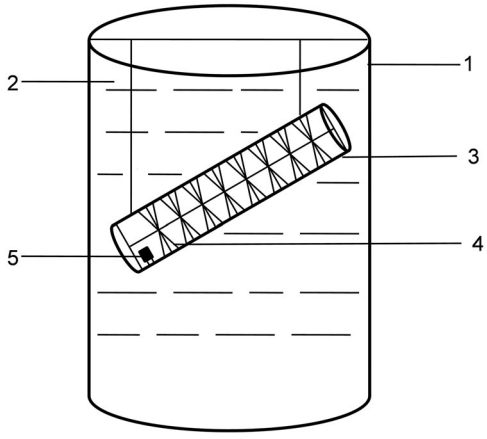

Figure 1. Schematic drawing of the experimental system: (1) bucket, (2) river water, (3) biofilm bioreactor, (4) biofilm attached on filaments, (5) aeration device.

polyethylene containers (top/ bottom diameters = 43/ 33 cm and depth = 50 cm) and began on 10 October 2016. The experiment was a completely randomized design with five treatments and three replicates per treatment: (1) river water (marked as CW); (2) river water with biofilm bioreactor (marked as AW); (3) river water with sediment (marked as CS); (4) river water with sediment and biofilm bioreactor (marked as AS); (5) river water with sediment and biofilm bioreactor (no aeration) (marked as SS). The initial sediment thickness was 4 cm. Aeration time was 6 h per day, from 9:00 to 15:00, and aeration rate was 2 L per minute.

Water quality measurements and sampling were conducted between 8:00 and 9:00 on 0, 1, 3, 5, 7, 10 and 15 days after treatment initiation. First, water temperature (T), pH, specific conductivity (EC) and dissolved oxygen (DO) were recorded using a freshly calibrated, multi-parameter probe (YSI 650MDS, YSI, Yellow Springs, Ohio). Then, water samples were collected from the mesocosm surface (top 20 cm) using a 500 mL Schindler sampler. Samples were analysed for ammonium (NH_4^+–*N*), nitrate (NO_3^-–*N*), nitrite (NO_2^-–*N*), total phosphorus (TP), total dissolved phosphorus (TDP), orthophosphate (PO_4^{3-}–*P*) and bacterial abundance. At the end of experiment, an additional water sample was collected for microbial community analyses.

2.3. Analytical methods for nitrogen and phosphorus

For the determination of dissolved constituents (NH_4^+–*N*, NO_3^-–*N*, NO_2^-–*N*, TDP and PO_4^{3-}–*P*), an aliquot was filtered through a 0.45 µm membrane filter. Total P and TDP were determined following oxidation with basic potassium peroxydisulfate using the ammonium molybdate spectrophotometry method [31]. Nesslerization colorimetric, ultraviolet spectrometry, N-(1-naphthyl)-ethylenediamine colorimetric and ammonium molybdate spectrometry methods were used for the quantification of NH_4^+–*N*, NO_3^-–*N*, NO_2^-–*N* and PO_4^{3-}–*P*, respectively [31]. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+–*N*, NO_3^-–*N* and NO_2^-–*N* concentrations.

2.4. Enumeration of bacteria

After sampling, 10 mL subsamples for bacteria enumeration were immediately fixed with a final concentration of 2.0% formaldehyde and stored at 4°C. All samples were enumerated within a month of collection using epifluorescence microscopy after staining with 1 µg mL^{-1} (final concentration) DAPI (4′, 6-diamidino-2-phenylindole) [32]. After staining with DAPI for 10 min, samples were filtered onto black polycarbonate membrane filters (0.2 µm pore size, 25 mm diameter; Millipore) with a <10 mm Hg vacuum to distribute the cells uniformly. A minimum of 400 bacterial cells were enumerated in at least 20 randomly selected fields per sample using a Leica fluorescent microscope (DM4000B, Germany).

2.5. Molecular analysis of microbial communities

Subsamples of 150 mL were filtered onto 0.2 µm polycarbonate membrane filters (47 mm diameter, Millipore), transferred to 2 mL sterilized microcentrifuge tubes and stored at −20°C for subsequent molecular analysis. Molecular analysis of microbial communities was performed according to previous studies [33]. In brief, microbial DNA was extracted from frozen filters using the E.Z.N.A.® Water DNA Kit (Omega, USA) according to manufacturer's protocols. Amplicons of V3-V4 regions of the 16S rDNA gene were sequenced on the Illumina HiSeq platform at Shanghai Xiangyin Biotechnology Co., using the universal primer set SD-Bact-0341-b-S-17/SD-Bact-0785-a-A-21 (5′-CCTACGGGNGGCWGCAG-3′ / 5′-GACTACHVGGGTATCTAATCC-3′) [34]. Denoised sequences were aligned and clustered at 97% sequence identity into operational taxonomic units (OTUs), and these OTUs were assigned taxonomic identities using the Ribosomal Database Project (RDP) classifier [35]. Alpha-diversity measures (observed OTUs, Good's coverage, abundance-based coverage estimator (ACE), Chao1 richness estimator, Shannon index and Simpson index) were calculated based on OTU data. All sequence data from this study were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number SRP148474.

2.6. Statistical analysis

Univariate statistical analyses were performed using SPSS Ver. 22.0 (IBM-SPSS Statistics, Chicago, IL, USA). Data were analysed using ANOVA, and significant differences among treatment means ($p < 0.05$) were determined by the Duncan test. Multivariate analysis of community data based on genus relative abundance was carried out using Canoco 5 software [36]. Principal component analysis (PCA) was used to investigate difference in community composition among treatments. Redundancy analysis (RDA) was used to investigate the influence of different treatments on microbial community composition. Additionally, Monte-Carlo permutation tests were conducted using 499 random permutations to determine the statistical significance of relationships between different treatments and their microbial communities.

3. Results

3.1. Influence of biofilm bioreactor on water quality parameters

During the experimental period, water temperature ranged from 23.2°C to 25.5°C and pH from 7.84 to 8.44. Mean values for water temperature (T), pH, specific conductivity (EC) and dissolved oxygen (DO) in the different treatments are shown in Table 1. DO and pH showed similar variations among treatments, following a trend of CW > AW > CS > AS > SS. Treatments AW and AS, containing the biofilm bioreactor, had a relatively lower values for water temperature and specific conductivity compared to their matched controls (CW and CS), indicating the air bubbles generated by the aeration induced water flow in tube and also promoted the adsorption and/or assimilation of the compounds and/or ions by biofilms.

Changes in different phosphorus and nitrogen parameters among treatments over time are presented in Figures 2 and 3. There were significant differences in nitrogen and phosphorus parameters between the experimental systems with and without sediments at the beginning of the study. There was a marked decline in TDP and PO_4^{3-} with time under AW and AS treatments relative to their matched controls (CW and CS). The concentrations of TP, TDP and PO_4^{3-} were highest in the SS treatment, and significantly increased after 3 days of incubation (Figure 2). These results suggested that there was a marked connection between DO and soluble phosphorus removal in biofilm bioreactor.

There were significant effects among treatments for DIN (Figure 3). With extension of incubation time, DIN concentration was significantly lower in the AW and AS treatments than in their matched controls (CW and CS) (Figure 3(A)). The three forms of DIN (NH_4^+-N, NO_2^--N and NO_3^--N) showed different trends. The NH_4^+-N concentration was higher in treatments with sediment compared to treatments without sediment at the beginning of the study; the temporal variation in NH_4^+-N

Table 1. Water temperature, pH, specific conductivity and dissolved oxygen observed throughout the study period.

Treatments	T (°C)	pH	DO (mg/L)	EC (mS·cm^{-1})
CW	24.3 ± 0.1	8.12 ± 0.04	6.66 ± 0.09	0.262 ± 0.0003
AW	24.1 ± 0.1	8.12 ± 0.03	4.78 ± 0.14	0.259 ± 0.0003
CS	24.3 ± 0.1	8.07 ± 0.03	3.41 ± 0.27	0.276 ± 0.0010
AS	24.2 ± 0.1	8.03 ± 0.03	3.36 ± 0.17	0.274 ± 0.0007
SS	24.3 ± 0.1	8.01 ± 0.02	1.08 ± 0.26	0.281 ± 0.0008

Values are mean ± standard error; T: water temperature; EC: specific conductivity; DO: dissolved oxygen; CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration).

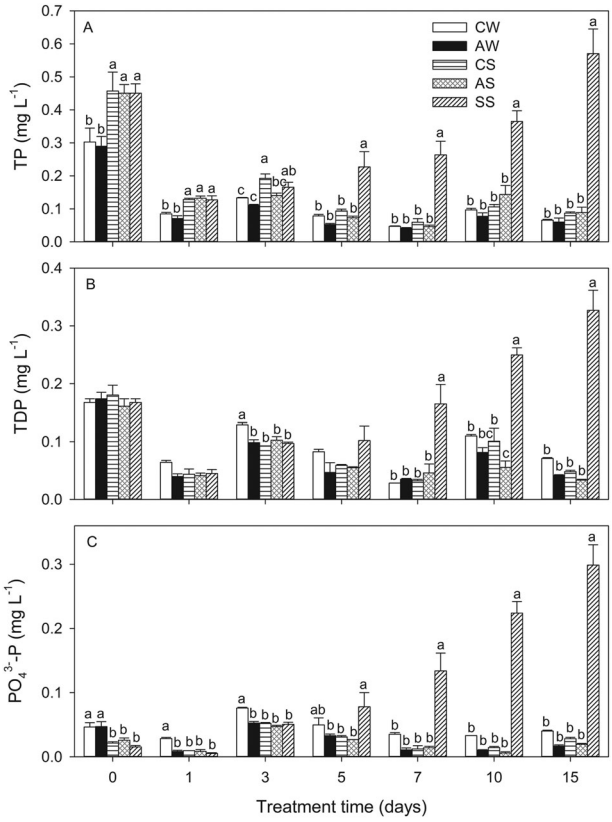

Figure 2. Temporal variation in three phosphorus parameters under different treatments. (A) total phosphorus (TP); (B) total dissolved phosphorus (TDP), (C) orthophosphate ($PO_4^{3-}-P$). CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration). Different letters indicate significant difference at $p < 0.05$ (determined by mean separation with Duncan test).

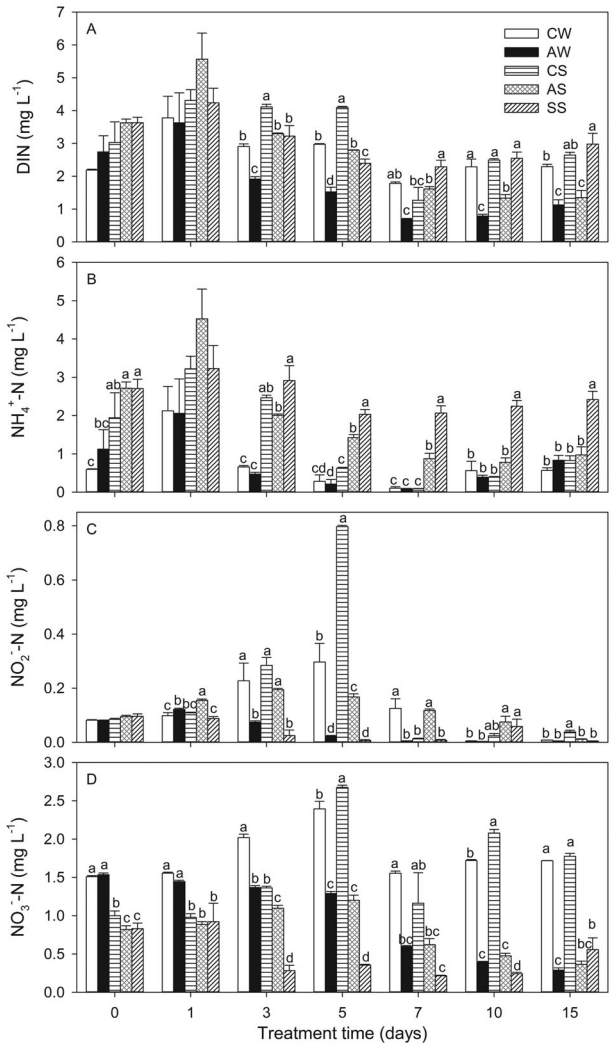

Figure 3. Temporal variation in four nitrogen parameters under different treatments. (A) dissolved inorganic nitrogen (DIN); (B) ammonium (NH_4^+-N); (C) nitrite (NO_2^--N); (D) nitrate (NO_3^--N). CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration). Different letters indicate significant difference at $p < 0.05$ (determined by mean separation with Duncan test).

concentration showed a decline after 1 d of treatment and then levelled off within a minimum range. The NO_2^--N concentration increased and then declined with time, especially in treatments CW and CS. These results showed that nitrification was the important mechanism for ammonia removal and was divided into two stages (ammonia oxidation to nitrite and the subsequent nitrite oxidation to nitrate) in our experiment. Obviously, the two stages of nitrification were coupled rapidly via the biofilm bioreactor, and therefore no high accumulation of NO_2^--N concentration was observed in the treatments with biofilm. Temporal

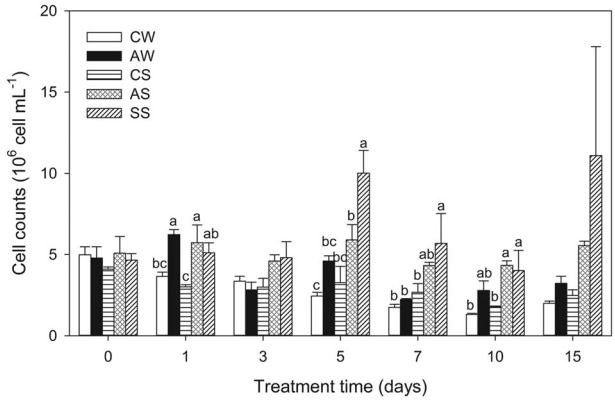

Figure 4. Temporal variation in cell counts under different treatments. CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration). Different letters indicate significant difference at $p < 0.05$ (determined by mean separation with Duncan test).

variation of NO_3^--N concentration was different among treatments showing a mark decline in NO_3^--N concentration with time under AW, AS and SS treatments relative to CW and CS. The multiple comparison test (Duncan test) showed significantly higher NO_3^--N concentrations for the CW and CS treatments than for the other three treatments after 7 days of incubation, indicating that biofilm was the major site of denitrification reaction which was no limitation due to aeration in our experiment.

3.2. Biofilm bioreactor influence on planktonic microbial abundance and community

Changes of bacterial abundance among different treatments with time showed a fluctuating decrease in bacterial abundance with time for the CW and CS treatments relative to other treatments (Figure 4). Although the abundance of bacteria in all treatments fluctuated throughout the 15-d incubation, the AW, AS and SS treatments, especially the SS treatment, containing the sediment and biofilm bioreactor had a relatively higher abundance of bacteria compared to their matched controls (CW and CS).

Species richness and diversity indices of microbial communities based on 16S rDNA Illumina reads are shown in Table 2. The mean Good's coverage was greater than 99%, indicating that the sequencing effort was sufficient to capture the relative complete diversity of these communities. Different treatments had significant effects on species richness and diversity indices of bacterial communities as evidenced by OTUs, Chao1, Shannon and Simpson indices (Table 2). In general, the

Table 2. Estimates of richness and diversity for operational taxonomic units (OTUs) with 97% similarity for each treatment.

Treatments	Reads	Coverage (%)	OTUs	ACE	Chao1	Shannon	Simpson
CW	62309 ± 4200	99.3 ± 0.03	1313 ± 56 a	1829 ± 98	1745 ± 93 a	4.614 ± 0.032 a	0.0326 ± 0.0022 b
AW	57734 ± 2290	99.4 ± 0.03	960 ± 23 b	1605 ± 118	1393 ± 34 c	3.454 ± 0.143 c	0.0872 ± 0.0213 a
CS	61770 ± 3955	99.4 ± 0.08	1350 ± 62 a	1727 ± 42	1663 ± 41 ab	4.736 ± 0.115 a	0.0300 ± 0.0056 b
AS	57174 ± 2483	99.3 ± 0.07	1041 ± 85 b	1811 ± 39	1499 ± 83 bc	3.855 ± 0.137 bc	0.0597 ± 0.0069 ab
SS	56031 ± 2311	99.3 ± 0.07	1096 ± 40 b	1780 ± 172	1511 ± 87 abc	4.185 ± 0.192 b	0.0459 ± 0.0069 b
F value	0.809	0.484	9.021	0.719	3.788	15.618	4.694
P value	0.547	0.748	0.002**:	0.598	0.040*	<0.001***	0.022*

*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$; Different letters indicate significant difference at $p < 0.05$ (determined by mean separation with Duncan test). ACE: abundance-based coverage estimator; CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration).

AW and AS treatments, containing the biofilm bioreactors had relatively lower species richness and diversity compared to their matched controls (CW and CS).

The four predominant phyla were Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria (Figure 5). Although these predominant phyla appeared in all treatments, there were marked variations in relative abundance of predominant phyla in different treatments. For example, the relative abundance of Proteobacteria was higher in the AW, AS and SS treatments than their match controls (CW and CS), whereas the relative abundance of Actinobacteria was lower for the AW, AS and SS treatments than their match controls (CW and CS).

To compare differences in planktonic microbial composition among treatments, principal component analysis (PCA) was used. The first two PCA axes explained more than 57% of variation in microbial community composition. Differences in microbial composition from different replicates were smaller in CS, AS and AW than in CW and SS. PCA analysis revealed that a clear distinction among treatments containing the biofilm bioreactor (AW and AS) compared to the other treatments (Figure 6). Redundancy analysis (RDA) indicated that the changes in microbial communities were related to the different treatments (Figure 7). Different treatments contributed significantly to the variance in microbial communities (Monte Carlo test $p = 0.006$), explaining 40.7% of the observed variation. Additionally, a few microbial genera stood out in the RDA analysis; *Sphingobium*, *Runella*, *Nitratifractor*, *Lacihabitans*, and *Elstera* were more closely associated with treatments AS and AW.

4. Discussion

Due to the worldwide increase in water pollution and degradation of aquatic ecosystem health, as well as increased public awareness, environmentally friendly measures based on microbial bioremediation have become important methods for removing pollutants from aquatic systems [13]. In the present study, we assembled a biofilm bioreactor in a tubular structure containing biofilm and aeration components. As expected, the aeration device generated a water flow in the tubular structure, resulting in both oxygen inputs and a circulating flow to mix inside/outside waters within the mesocosm. The relatively lower values for water temperature of the AW and AS treatments (both containing biofilm bioreactors) compared to their matched controls

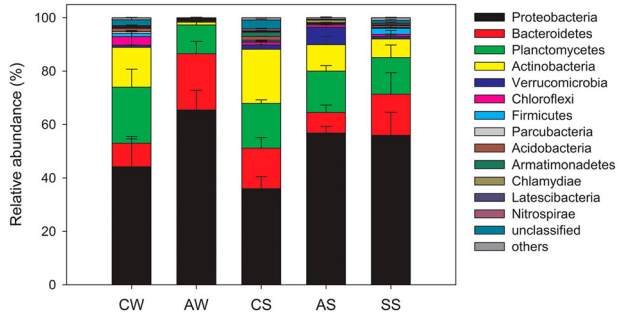

Figure 5. Changes of the dominant phyla of bacteria at different treatments. The sum relative abundance of dominant phyla exceeded 99%. CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration).

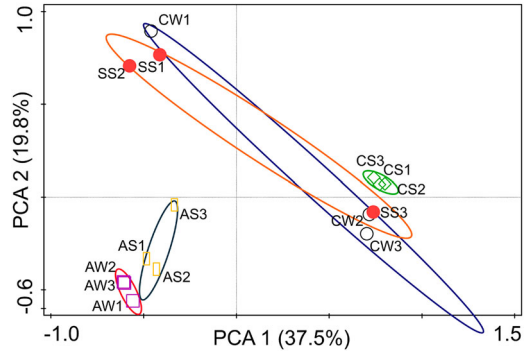

Figure 6. Principal component analysis (PCA) showing bacterial assemblages of each treatment. CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration).

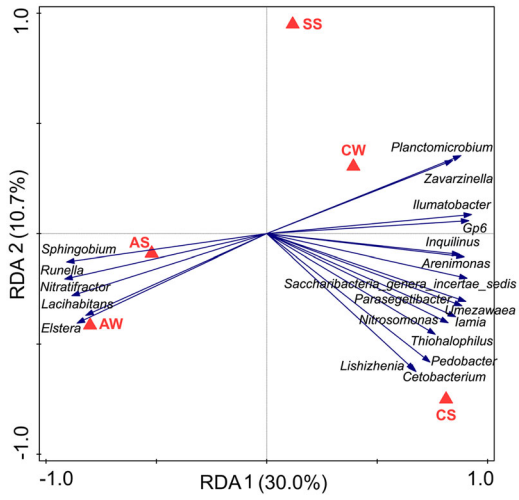

Figure 7. Redundancy analysis (RDA) showing the effects of treatment on bacterial community. The value % on the axes indicates the percentage of taxonomic data variation which they explain. For graph the genera that explain most variance are presented. CW: river water; AW: river water with biofilm bioreactor; CS: river water with sediment; AS: river water with sediment and biofilm bioreactor; SS: river water with sediment and biofilm bioreactor (no aeration).

(CW and CS) can be attributed to the different hydrodynamic mixing conditions (Table 1).

In general, biofilms have the potential for assimilating, transforming and degrading various pollutants including organic matter and inorganic nutrients [37–39]. When comparing the AW and AS treatments (containing biofilm bioreactors) with their matched controls (CW and CS), it was obvious that the biofilm bioreactor induced declines in EC, TDP and DIN (Table 1 and Figures 2(B) and 3(A)). However, the nutrient removal effects of the SS treatment (containing the biofilm bioreactor, without aeration) were limited or inferior to the other treatments (Table 1 and Figures 2 and 3). For phosphorus, PO_4^{3-}–*P* concentrations were strongly correlated with TDP (Figure 2(B,C)), suggesting that PO_4^{3-}–*P* was the main form of TDP responsible for changes in phosphorus concentrations, especially in the SS treatment. Although biofilms are purported to have a high affinity for inorganic phosphorus [40,41], our results showed a significant increase in PO_4^{3-}–*P* concentrations in treatments without aeration (SS) relative to the treatments with aeration (AS) demonstrating a strong linkage to DO levels (Table 1). For nitrogen, there were significant differences in NO_2^-–*N* and NO_3^-–*N* removal between treatments with and without biofilm bioreactors, and also a significant difference in NH_4^+–*N* removal between treatments with (AS) and without aeration (SS). This suggests that biofilms experiencing an oxygen concentration gradient (either spatially within biofilm communities or temporally with aeration events) were favourable for coupled nitrification-denitrification reactions.

Planktonic microorganisms are an important component of the aquatic ecosystem, and are highly sensitive to environmental changes, making them useful as bioindicator of aquatic ecosystem health and function [42]. In order to better understanding the effects of biofilm bioreactor on hypereutrophic aquatic ecosystem, the planktonic microbial abundance and community structure were investigated. Based on high-throughput sequencing and cell count data, the variations in microbial communities were most strongly affected by the presence of the biofilm bioreactor (Figures 4–7 and Table 2), indicating that the biofilm bioreactor played an important role in determining planktonic microbial communities. There are two explanations that may account for the differences in planktonic microbial communities among treatments associated with biofilm bioreactors. First, the biofilm bioreactor may exert a direct influence on the abundance and composition of planktonic microbial communities. For example, significant differences were observed in bacterial abundance between treatments with (AW, AS and SS) and without biofilms (CW and CS) (Figure 4). Biofilms have been defined as aggregates of microorganisms that have high cell densities, ranging from 10^8 to 10^{11} cells g^{-1} wet weight [43]. Due to presence of biofilms, the attached bacteria may dislodge from the biofilms and become suspended in the water. This may be an important reason for the relatively high bacterial abundance of treatments containing biofilm bioreactor (AW, AS and SS) as compared with their matched controls (CW and CS) (Figure 4). Additionally, a number of factors, such as dissolved organic carbon, DO, pH, nutrient concentrations and forms (e.g. NH_4^+–*N* vs NO_3^-–*N*), have been found to alter microbial communities in natural waters [44–48]. In the present study, biofilm bioreactors influence water quality characteristics (especially nutrients and DO), which may also affect planktonic bacterial communities. Our results may be of importance for the application of biofilm bioreactor in natural urban river, and may provide value insight into bioremediation of hypereutrophic urban river having insufficient water flows and low oxygen reaeration-high oxygen demand.

In conclusion, the biofilm bioreactor utilized in this study improved water quality of a hypereutrophic urban river, especially with regard to decreasing DIN concentrations. The biofilm bioreactor increased the abundance of planktonic bacteria, whereas the diversity of the planktonic microbial community decreased. Sequencing further revealed that Proteobacteria, Bacteroidetes, Planctomycetes, and Actinobacteria were the four

predominant phyla in the planktonic microbial community, and the presence of the biofilm bioreactor increased the relative abundance of Proteobacteria. Variations in microbial communities were affected most strongly by the biofilm bioreactor, as determined by principal component analysis (PCA) and redundancy analysis (RDA). This study demonstrated that a biofilm bioreactor utilizing an aeration device can generate a water flow in the tubular structure, which enhances the capacity of biofilms for bioremediation in hypoxic, hypereutrophic urban rivers. Mixing by the aeration device increases the chances of water pollutants coming into contact with the biofilms and creates a DO gradient, which favours coupled nitrification-denitrification reactions. These design features influence the abundance and diversity of planktonic microbial communities, and enhance the nutrient self-purification capacity of hypereutrophic urban rivers.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the National Natural Science Foundation of China (41601528); Zhejiang Provincial Natural Science Foundation of China (LQ16C030005); Wenzhou Science and Technology Project (S20180007) and Scientific Research Project of Wenzhou Medical University (QTJ13014).

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