Reed Biochar Addition to Composite Filler Enhances Nitrogen Removal from BDBR Systems in Eutrophic Rivers Channel

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Abstract: With the rapid development of urbanization in China, the eutrophication or black stink of urban rivers has become a critical environmental problem. As a research hotspot in wastewater purification, biofilm technology has shortcomings, such as insufficient carbon sources for denitrification. This study used a Biofilm Denitrification Batch Reactor (BDBR) system constructed using reed biochar as the carbon source required in denitrification, significantly accelerating the biofilm formation. To determine the suitable amount of biochar for water purification from the urban eutrophic rivers by the BDBR system, 0%, 5%, 10%, and 15% reed biochar was added to the viscose fiber combined packing. The combined packing reactor involved in this study had a high removal efficiency of the eutrophication channel COD throughout the experiment. However, adding 5% and 10% biochar in the combined filler effectively increased the number of nitrifying and denitrifying bacteria on the biofilm, improved the dominant bacteria diversity and microbial activity, and enhanced denitrification efficiency in the BDBR system. It provides new ideas and methods for developing and applying in situ denitrification technology for urban polluted rivers.

Keywords: reed biochar; BDBR system; eutrophic channel; nitrogen removal efficiency; microbial diversity

1. Introduction

Recently, the eutrophication or black stink of urban rivers has become a crucial environmental problem due to the rapid development of urbanization in China [1–3]. Due to immobilization and limited area of the surrounding buildings, the in situ treatment is often used to remediate urban polluted rivers [3–5]. The remediation technologies mainly include physical, chemical, and biological methods [6–8]. Of these methods, biological methods are mostly encouraged and recommended because they are more economical and environmental-friendly than physical or chemical methods for urban rivers remediation [6,9]. However, biological methods based on purification of the aquatic plants and microbial degradation also have some drawbacks in practical applications. For example, the purification performance of aquatic plants is low in winter, and nutrients would re-enter the waterbody after the plants withered [10]; the added external microorganisms might not reproduce rapidly to become a dominant flora due to their poor adaptability to a new environment.
environment [11,12]; and biofilm reactors require a long time for biofilm formation and sufficient carbon supply for nitrogen removal by denitrification [13,14].

Even so, biofilm reactors are still one of the research hotspots in wastewater purification at the present time [15,16]. Recent studies on sequencing batch biofilm reactor (SBBR), which is based on the coupled effect of the sequencing batch reactors (SBRs) and biomass in the form of a biofilm, indicated the vast potential for practical application [17]. The main advantages of the coupled effect for the SBBR includes: (1) It can effectively improve the retention capacity of microorganisms and enhance the stability and flexibility of bioprocesses of wastewater treatment in the event of varying loads of pollutants [18,19]; (2) it has strong ability of simultaneous nitrification and denitrification, resulting from anoxic anaerobic micropores of the inner layers of the biofilm [20]; (3) it is tolerant to changes in pH value and temperature compared with external microorganism addition, [17,21]; and (4) it is characterized by a less space requirement and better biomass sedimentation properties. Therefore, solving the drawbacks of the insufficient carbon supply and long biofilm formation time has become the key point for optimizing SBBR. In addition, it has been a restriction factor for the stable application of the SBBR for in situ remediation of eutrophic river channels.

Obviously, the addition of external organic carbon is a good way to supplement the lack of carbon source in the SBBR system. However, the selection of organic carbon sources should be driven by their availability, price, holistic approach to environmental protection involving waste materials as an external carbon source, and the possibility of their storage and feeding into a bioreactor [22]. It has been discovered that adding a biodegradable carbon source (such as cotton, crop straw, crop husk, and so on) directly to the SBBR may bring clogging or even damage to the reactors, which will affect the stable operation of the SBBR system and lead to secondary pollution [23,24]. Moreover, many studies indicated that small molecule carbon sources (such as methanol, ethanol, and CO₂) have the advantage of being easily utilized by microorganisms [25,26], but the promotion of denitrification will not be effectively achieved due to the fluidity and instability of river water. Biochar, which is usually prepared from biomass of agricultural and forestry waste by high-temperature anaerobic pyrolysis, is a carbon-rich substance providing carbon source. It has been widely used in carbon sequestration, heavy metals and organic matter adsorption, soil amelioration, and water purification [27-29], owing to its strong adsorption properties, large specific surface area and suitable habitats for environmental microorganisms [30,31]. Reed, which is commonly grown in wetland systems of China, is an ideal organic carbon resource for biochar preparation because of fast growth, large biomass amount, convenient access, and low cost [32-34]. However, the practice of adding reed biochar into a composite filler, which is then used in the SBBR for constructing a reliable Biofilm Denitrification Batch Reactor (BDBR) system, is very limited yet for in situ nitrogen removal of eutrophic urban river channel.

In this study, we aims to provide a reference idea that kills three birds with one stone: (1) Using the expanding reed in China as a biological resource to produce biochar can achieve the purpose of reed resource recycling utilization; (2) reed biochar composite filler was not only used as a carrier of microbial membranes, but also used as a slow-release carbon source for the denitrification process, and the sufficient and continuous carbon source supply could ensure rapid startup and stable operation of the SBBR system; (3) the SBBR was finally applied to enhance nitrogen removal efficiency and repair eutrophic river channel by the in situ denitrification system process via the BDBR system. Thus, the startup time, nitrogen removal efficiency, and bacterial community structure were analyzed for BDBR under different percentages of reed biochar in the composite filler, in order to provide a novel BDBR system for the bioremediation of eutrophic urban river.
2. Materials and Methods

2.1. Preparation of Reed Biochar Composite Filler

The reed sample was collected from the wetland in the Nanjing Botanical Garden, Mem. Sun Yat-Sen, Jiangsu Province. The reed aerial stems were cleaned, air-dried, cut into 1 cm × 5 cm pieces, and finally baked at 65 °C for three days. Briefly, the dried reed raw material was placed in an anaerobic muffle furnace (O-KTF1200, JIU GONG, Shanghai, China). Considering the fact that the physical and chemical characteristics of biochar are directly affected by pyrolysis temperature, we first determined the optimal pyrolysis temperature for reed biochar preparation through preliminary experiments. Moreover, the pre-experiment results showed that although the specific surface area and total pore volume of reed biochar increased with the increase in pyrolysis temperature, the productivity of biochar decreased from 44.3% to 31.2% as the temperature rose from 300 °C to 700 °C (Supplementary Tables S1 and S2). Especially, when the temperature exceeded 500 °C, the product amount of biochar prepared by reed was significantly reduced with the same weight of reeds, and the average pore size did not change significantly (Supplementary Tables S1 and S2). In addition, the total acidic oxygen-containing functional groups on the surface of reed biochar decreased with the increase in pyrolysis temperature, indicating that the higher pyrolysis temperature of the reed biochar preparation led to more decomposition and loss of oxygen-containing functional groups on the surface of the reed biochar (Supplementary Table S2 and Figure S1b). Therefore, combined with the analysis of the relationship between physical and chemical indexes and pyrolysis temperature (Supplementary Figure S1), the pyrolysis temperature of 500 °C was selected to prepare reed biochar for the present study.

The air in the muffle furnace was successfully expelled by continuously adding nitrogen for 30 min, and then the furnace temperature was increased from room temperature to 500 °C at a rate of 10 °C/min and maintained at 500 °C for 2 h. After pyrolysis, the material was naturally cooled to room temperature, pulverized, passed through a 120-mesh sieve, and ultimately stored in a desiccator until further use. The biochar composite filler was produced by Shandong Hailong Textile Co., Ltd., Weifang, China. First, the pretreated 200 nm diameter reed biochar, with a mass percentage of 0%, 5%, 10%, or 15%, were mixed with the viscous solution. Then, the mixture was stirred in a water bath for 1.5 h, subjected to vacuum degassing treatment, and then spun in a micro-spinneret (0.08 mm, 200 holes) to obtain a biochar viscose fiber. After being dried, the biochar viscose fiber was incorporated into a circular fiber net with a 5 cm diameter. The resulting composite fillers had a biochar content of 0%, 5%, 10%, and 15%.

2.2. Construction of Novel Sequencing Batch Biofilm Reactor (SBBR)

A sequencing batch biofilm reactor (SBBR) with biochar composite filler, which accounts for 50% of the volume of SBBR, was used to construct the BDBR system. The reactor was made of plexiglass with an effective volume of 10 L, a height of 45 cm, and an inner diameter of 20 cm (Figure 1). Each reactor was inoculated by directly injecting 700 g wet weight of activated sludge containing functional microorganisms. The activated sludge was collected by obtaining 0–30 cm silt at the bottom of the Shiligou River in Nanjing, Jiangsu Province, China, using stainless steel sludge sampler (Model: Bz-3798, Nanjing, China). The average total nitrogen (TN), total phosphorus (TP), and organic matter (SOM) in the activated sludge sediment were 3.05 mg g⁻¹, 0.72 mg g⁻¹, and 0.45 mg g⁻¹, respectively. The influent water of the experimental reactor was from the Shiligou River with chemical oxygen demand (COD), ammonium nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N), TN, and TP of 31.00 mg L⁻¹, 7.75 mg L⁻¹, 1.01 mg L⁻¹, 10.24 mg L⁻¹, and 0.78 mg L⁻¹, respectively. After the sediment and the influent water were thoroughly mixed and aerated, the biofilm was constructed intermittently by influent water from the reactor. The temperature inside the biofilm reactor was maintained at 25 °C by a heating rod and a temperature controller.
The constructed SBBRs with 0%, 5%, 10%, and 15% biochar filler were sequentially named BC0, BC5, BC10, and BC15. The operating parameters for all SBBRs were 0.25 h of influent water for 7.5 h of aerated, 4 h for anoxia, and 0.25 h for sedimentation drainage, for a total of 12 h per cycle. Meanwhile, equal weights and volumes of activated sludge and eutrophic water were placed into an identical plexiglass tank reactor without composite fillers and with aeration treatment as the control. Three replicates per experimental and control group were performed.

2.3. Sampling and Water Quality Analysis

The inlet and outlet water COD, TN, NH$_4^+$-N, and NO$_3^-$-N indexes were tested every two operating cycles (i.e., 24 h) for each SBBR. On the 8th day, the composite fillers in BC0, BC5, BC10, and BC15 groups were removed to analyze the numbers and biomass of nitrifying and denitrifying bacteria, and the microbial community structure. The COD of water was measured by the potassium dichromate method, and the NH$_4^+$-N concentration was determined by Nessler’s reagent method. The NO$_3^-$-N and TN contents were tested by ultraviolet spectrophotometry [35,36].

2.4. Biofilm Biomass and Microbial Activity

Biofilm biomass was tested by the lipid phosphorus method [37] to indicate the number of active microorganisms using lipid phosphorus content (nmol P) per gram (g) of dry fat (DW). Namely, the phosphorus (P) content of 1 nmol was approximately equal to $10^8$ coli-sized microorganisms, and thus the unit of biomass is expressed by nmol P/g DW [37]. To determine lipid and phosphorus content, the phosphorus (lipid, Lipid-P) in the phospholipids of the microbial cell membranes were sequentially extracted by chloroform, methanol, and water. The extract obtained was tested by ammonium molybdate spectrophotometry for total phosphorus quantification. The amount of nitrification and denitrifying bacteria on biofilms was determined by the MPN-Griess method [38].

Dehydrogenase is an important substance for measuring the activity of microorganisms. In the experiment, the artificial hydrogen acceptor, 2,3,5-triphenyltetrazolium chloride (TTC), was used as the hydrogen acceptor. After reduction, TTC was reduced to red triphenylformamidine (TF); the TF produced was measured by spectrophotometry and expressed in µg [39]. Therefore, the amount of TF produced indicates the magnitude of TTC-dehydrogenase activity, and the ratio of TTC-dehydrogenase activity to membrane biomass (nmol) indicates the microbial activity of different filler biofilms in µg · nmol$^{-1}$. 

![Figure 1. Schematic diagram of SBBR constructed with reed biochar composite filler.](image-url)
2.5. DNA Extraction and Biofilm Microbial Diversity

According to the manufacturer’s instructions, the total genomic DNA was extracted from biofilm samples using a PowerWater® Sterivex™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The extracted genomic DNA was detected using 1.2% agarose gel electrophoresis and stored at \(-20^\circ C\) until further processing.

Microbial diversity in biofilms was analyzed by the PCR-DGGE method. First, the hypervariable region of the 16S rDNA was amplified using the universal primers 5′-CGCCCGCCGCGCCGCGCCGCGGCGGCCGGCCGGGACGGGCGGGGCCCCATACGGAGGCAGCAG-3′ and 5′-ATTACCGCGGCTGCTGG-3′. The PCR products were purified and recovered (OMEGA DNA Gel Extraction Kit), examined using a Gel-Doc2000 gel imaging system (Bio-Rad Laboratories Inc., Hercules, USA), and isolated by DGGE (The DcodeTM Universal Mutation Detection System, Bio-Rad Laboratories Inc., Hercules, USA, denaturation gradient was 35–55%). Finally, the electrophoresis band was observed and photographed in the BIO-RAD Versa Doc IMAGING SYSTEM to obtain a DGGE fingerprint of the sample.

2.6. Statistical Analysis

The data were processed and analyzed using Excel (Microsoft Office 2010), and presented as mean ± standard error (SE) of the three replicates.

\[
W_i(\%) = \frac{(C_0 - C_i)}{C_0} \times 100\% ,
\]

where \(W_i(\%)\) is the removal rate, \(C_0\) is influent concentration, and \(C_i\) is the water concentration \([40]\).

The number and density of each sample strip in the PCR-DGGE fingerprint was digitized using “Quantity one” software.

3. Results

3.1. Removal of COD and TN for Eutrophic River Channel by SBBR Constructed with Reed Biochar Composite Filler

Four SBBR reactor groups (BC0, BC5, BC10, and BC15) were constructed with reed biochar-composite filler. Although there were no significant differences in the COD removal rates from the eutrophication channels among the four treatment reactors, their performance can be divided into three stages throughout the experiment (Figure 2). The concentration of COD in eutrophic water dropped rapidly during the first 6 days (stage 1) for all reactors during the experiment. After stage 1, the COD concentration of effluent water in BC0, BC5, BC10, and BC15 reactors decreased to 4.34, 3.98, 4.72, and 4.47 mg \(\text{L}^{-1}\), respectively, with corresponding COD removal rates of 88%, 84%, 87%, and 88%, respectively. Then, the COD concentration in the effluent water dropped slowly from the 6th to the 12th day of the experiment (stage 2) from each treatment reactor. After stage 2, the removal rate of COD reached about 90% and remained stable for the remaining reaction (stage 3). However, the control group without combined filler had an average removal rate of 46% for COD throughout the entire experimental period (26 days), much lower than the four treatment reactors with combined fillers. This indicates that reactors packed with combined fillers contribute to COD removal in eutrophic river channels.

As shown in Figure 3, among the four treatment groups, the TN concentration of the effluent water from BC5 and BC10 reactors decreased the fastest. When the BC5 and BC10 reactors operated until the 6th day, the TN concentration of effluent water decreased to 4.34, 3.98, 4.72, and 4.47 mg \(\text{L}^{-1}\), respectively, with corresponding COD removal rates of 88%, 84%, 87%, and 88%, respectively. Then, the COD concentration in the effluent water dropped slowly from the 6th to the 12th day of the experiment (stage 2) from each treatment reactor. After stage 2, the removal rate of COD reached about 90% and remained stable for the remaining reaction (stage 3). However, the control group without combined filler had an average removal rate of 46% for COD throughout the entire experimental period (26 days), much lower than the four treatment reactors with combined fillers. This indicates that reactors packed with combined fillers contribute to COD removal in eutrophic river channels.
and 78.5%, respectively. Moreover, throughout the experiment, the average TN removal rates from effluent water for BC0 and BC15 reactors were 77.0% and 75.7%, respectively, which were significantly lower than the 89.3% and 89.8% of the BC5 and BC10 reactors. However, the blank control reactor’s average TN removal rate from effluent water was only 23.5%, which was much lower than any of the combined packing treatment reactors.

Figure 2. COD concentration change in effluent water of SBBR constructed with reed biochar composite filler. The SBBR of BC0, BC5, BC10, and BC15 represents 0%, 5%, 10%, and 15% mass percentage of reed biochar contained in the reed biochar composite filler. A plexiglass tank without composite fillers and aeration treatment was used as the control. Different letters indicate significant differences between different samples ($p < 0.05$).

Figure 3. The change of TN concentration in effluent water from four reactor groups of SBBR constructed with reed biochar composite filler. Different letters indicate significant differences between different samples ($p < 0.05$). The BC0, BC5, BC10, and BC15 of SBBR have the same meaning as Figure 2.
3.2. Removal of NH$_4^+$-N and NO$_3^-$-N from Eutrophic Rivers by SBBR Constructed with Reed Biochar Composite Filler

As presented in Figure 4, throughout the experiment, the NH$_4^+$-N concentration in effluent water of the biochar-containing groups BC5, BC10, and BC15 decreased faster than that in the BC0 group without biochar. The NH$_4^+$-N removal rates in the effluent water were more than 90% when the biochar-containing reactors operated until the 6th day, and the average NH$_4^+$-N removal rates from the effluent water after 10 days were 94.5%, 95.6%, and 92.7%, respectively. After 10 days of operation, the average NH$_4^+$-N concentration in the effluent water of the BC5 and BC10 reactors were 0.42 and 0.32 mg·L$^{-1}$, respectively, meeting the requirements of Class II Water specified in China’s Surface Water Environmental Quality Standard (GB3838-2002). During the experiment, the removal rate of NH$_4^+$-N in effluent water by the BC0 reactor without biochar remained at about 74%, while the average NH$_4^+$-N removal rate in the control reactor was 39.9%.

![Figure 4](image)

**Figure 4.** The NH$_4^+$-N and NO$_3^-$-N concentration change in effluent water from four reactor groups of SBBR constructed with reed biochar composite filler. Different letters indicate significant differences between different samples ($p < 0.05$). The BC0, BC5, BC10, and BC15 of SBBR have the same meaning as Figure 2.
As shown in Figure 4, the effluent concentration of NO\textsubscript{3}−-N from the biochar-containing groups of BC5, BC10, and BC15 decreased sharply during the first 6 days of operation. However, the NO\textsubscript{3}−-N concentrations in the effluent water were significantly higher than the influent concentration of the system (1.01 mg·L\textsuperscript{-1}) in the first 10 days of system operation, which may be because NO\textsubscript{3}−-N nitrification oxidized the organic nitrogen in the overlying water and sediment, and NO\textsubscript{3}−-N was not consumed by the denitrifying bacteria in the system in time, resulting in NO\textsubscript{3}− accumulation in the system [41,42]. When the experiment was carried out for 10 days, the NO\textsubscript{3}−-N effluent concentrations from the BC0 and BC15 reactors were 0.98 and 0.92 mg·L\textsuperscript{-1}, respectively, while those of the BC5 and BC10 reactors remained at 0.58 and 0.53 mg·L\textsuperscript{-1}, respectively. Among the four treatment groups, the NO\textsubscript{3}−-N concentration of BC5 and BC10 reactors’ effluents was significantly lower than the influent concentration (1.01 mg·L\textsuperscript{-1}) with the corresponding average removal rates of 37.9% and 44.3%, respectively. The average removal rate of NO\textsubscript{3}−-N in the control reactor without combined filler was 27.7% during the whole experiment.

3.3. Biofilm Biomass and Microbial Activity in SBBR Constructed with Reed Biochar Composite Filler

As shown in Table 1, biofilm biomass in the BC5, BC10, and BC15 reactors containing biochar was significantly higher than that in the BC0 group without biochar. The biofilm biomass of the BC5 reactor was 13.91 × 10\textsuperscript{6} nmol P·g\textsuperscript{-1}·DW\textsuperscript{-1}, which was equivalent to 13.91 × 10\textsuperscript{14} microorganisms growing on the surface of the combined filler. Moreover, as the mass percentage of reed biochar in the combined filler increased from 5% to 15%, the corresponding reactor biofilm biomass increased from 8.35 × 10\textsuperscript{6} nmol P·g\textsuperscript{-1}·DW\textsuperscript{-1} to 22.08 × 10\textsuperscript{6} nmol P·g\textsuperscript{-1}·DW\textsuperscript{-1}. These show that biofilm biomass on the reactor’s filling differed significantly depending on biochar content (Table 1). The results demonstrate that the addition of reed biochar helps to increase the biofilm biomass of the composite filler, and the biofilm biomass increased as the mass percentage of reed biochar weight increased. Furthermore, the number of nitrification and denitrifying bacteria on the biofilm of the BC5 and BC10 reactors was significantly higher than that of the BC0 reactor without biochar fillers (Table 1). However, the number of nitrification and denitrifying bacteria on the biofilm of the BC15 reactor was less than that of the BC5 and BC10 reactors. In addition, comparing the four treatment groups, the dehydrogenase activity of the biofilm of the BC0 reactor was the lowest, being 9.3 µg·nmol\textsuperscript{-1}, while the biofilm of the BC10 reactor had the highest dehydrogenase activity of 54.2 µg·nmol\textsuperscript{-1}.

### Table 1. Biofilm biomass and microbial activity in SBBR constructed with reed biochar composite filler.

|                      | BC: 0%       | BC: 5%       | BC: 10%      | BC: 15%      |
|----------------------|--------------|--------------|--------------|--------------|
| **Biomass**  
(10\textsuperscript{6} P·g\textsuperscript{-1}·DW\textsuperscript{-1}) | 8.35 d\textsuperscript{1} | 13.91 c      | 16.90 b      | 22.08 a      |
| Nitrifying bacteria  
(10\textsuperscript{4} MPN·g\textsuperscript{-1}·DW\textsuperscript{-1}) | 0.29 d       | 3.21 b       | 7.13 a       | 1.94 bc      |
| Denitrifying bacteria  
(10\textsuperscript{5} MPN·g\textsuperscript{-1}·DW\textsuperscript{-1}) | 0.20 d       | 4.56 b       | 8.89 a       | 1.75 c       |
| TTC-dehydrogenase activity  
(µg·nmol\textsuperscript{-1}) | 9.60 d       | 26.63 bc     | 55.56 a      | 20.81 b      |

\textsuperscript{1} Different letters indicate significant differences between different samples (p < 0.05).

3.4. Diversity of Dominant Bacteria on Biofilm in SBBR Constructed with Reed Biochar Composite Filler

In this study, the semi-quantitative method PCR-DGGE analyzed the microbial diversity on the biofilm of each reactor. Each strip separated in the general map represents a microbial species; the intensity of the strip represents the abundance of the corresponding species, and the brighter the intensity, the higher the abundance [43]. In the DGGE map in Figure 5, the separated bands on the 1st to the 4th electrophoresis lanes represent the
biofilm microbial diversity of the BC0, BC5, BC10, and BC15 reactors on the 8th day of operation. The strips intensity diagram in Figure 5A shows that a total of 64 clear and bright bands are resolved in lanes 1 to 4 of the electrophoresis. Digital analysis by Quantity one software shows that many bands appeared in the same horizontal position in lanes 1 to 4, such as strips 4, 29, and 33. However, the intensity of these bands varied among the four SBBRs, indicating the differential abundance of the same microbial species among treatment groups. At the same time, some ectopic strips have appeared in the four processing groups, such as strips 1, 2, 3, 5, and 12, indicating a change in the biofilm microbial species in the four treatment groups. As presented in Figure 5B, the number of dominant bands in the BC5 and BC10 biofilm samples was 41 and 43, respectively. However, the number of dominant bands in the BC15 biofilm samples was reduced to 29. Altogether, these results indicate that the dominant bacteria diversity on the BC5 and BC10 reactors’ biofilm was higher than that of the BC15 reactor.

![Similarity analysis based on No. 1](image)

**Figure 5.** The microbial diversity on the biofilm for each reactor. (A) The relative luminance of the PCR-DGGE map; (B) Sketch map of band distribution. The separated bands on the 1st to the 4th electrophoresis lanes represent the biofilm microbial diversity of the BC0, BC5, BC10, and BC15 reactors on the 8th day of operation.
4. Discussion

For urban eutrophic rivers, anthropogenic activity inputs many aerobic organic materials, nitrogen, and phosphorus compounds, leaving the river water in a chronic state of anoxia and prone to odor [10,44]. This poses ecological and sanitary problems [45]. This study constructed a BDBR system based on a composite filler containing reed biochar to achieve in situ high-efficiency denitrification of eutrophic rivers. The COD, TN, nitrate-nitrogen, and ammonia removal from eutrophic river water by the BDBR system can be divided into 3 stages: the first phase is a 6-day system construction and rapid startup phase; the second phase is the transition period from day 6 to day 10; and the third phase is a stable operation period after 10 days (Figures 2–4). The time needed for complete denitrification in the SBBR type system is approximately 43 days in the presence of organic carbon in waste beer and approximately 48 days where citric acid serves as the organic substrate [17,46]. These results indicated that the exogenous addition of organic carbon can shorten the denitrification time of the SBBR system. In our study, we found that 26 days are enough for biofilm formation in a BDBR system under the operating parameters, significantly accelerating the biofilm formation process and achieving the rapid startup and stable operation of the BDBR system by adding the reed biochar into the combined filler. This result is consistent with reports that a 30-day operation period for a reactor with biofilm is sufficient to achieve a stable work after technological conditions modification [47,48].

As shown in Figure 2, after stage 1, the COD removal rate in the eutrophication channel of the 0%, 5%, 10%, and 15% (group BC0, BC5, BC10, and BC15) combined filler reactor was above 84%. When the system reached stage 3, the COD removal rates of the combined packed reactors remained around 90%. On the one hand, the COD in the reaction system was removed effectively by aeration and resuspension of the sediment. On the other hand, the high COD removal rate in a short time also shows that the fixed biofilm effectively maintained and enriched the microorganisms’ function, benefitting the startup, steady operation, and the COD degradation in the BDBR system [49]. The biofilm biomass results for BDBR with composite filler also confirmed that although the biochar content in the filler varied, the biofilms of all treatment groups had a large number of microorganisms between $8.35 \times 10^6$ and $22.08 \times 10^6$ nmol P·g$^{-1}$·DW$^{-1}$ (Table 1). In addition, the average COD removal rate (46%) by the reactor without the combined packing, which was used as control, was much lower than that of the reactor containing the combined packing during the experiment. However, there was no significant difference between the COD removal rates of the BDBR system without reed biochar (BC0) and with reed biochar (the BC5, BC10, and BC15) after stage 1. These results not only confirmed the potential of COD degradation in eutrophic river based on the coupling effect of sequencing batch reactor (SBR) and biomass in the form of SBBR biofilm [17], but also showed that this potential was not related to the addition of biochar.

Nevertheless, various substances, such as methanol, ethanol glucose, sliru odium acetate, and glycerol, are added to wastewaters to provide organic carbon to the denitrification organisms [19,25,50,51]. Additionally, anoxic microzones are provided in the inner layers of the biofilm by the SBBR system, under aeration conditions in the BDBR system, promoting simultaneous nitrification and denitrification [20]. In the present study, the BDBR system constructed with the composite filler with 5% and 10% biochar content (group BC5 and BC10) had significantly higher TN removal rates (89.3% and 89.8%) than the combined filler reactor without biochar (group BC0) and that with 15% biochar content (group BC15) (Figure 4). This could be explained by the fact that the amount of nitrifying and denitrifying bacteria on the biofilm of the BC5 and BC10 reactors was significantly higher than in the BC0 and BC15 reactors (Table 1). Therefore, the denitrification efficiency in BC5 and BC10 reactors was relatively high, which is why the effluent nitrate concentration of the two reactors after entering the stable operation period was significantly lower than the system influent concentration (1.01 mg·L$^{-1}$). In addition, as shown in Figure 5, in the various operational stages of the BDBR system, the $\text{NH}_4^+$-N removal rate from the eutrophic river water in reed and biochar-combined reactors (groups BC5, BC10, and BC15)
was higher than that of the filler reactor without biochar (group BC0). When the BDBR system entered a stable operation period, the ammonia nitrogen average removal rates in BC5 and BC10 reactors were as high as 94.6% and 95.6%, respectively, which were higher than the removal rate of the reactor with the highest biochar content (group BC15). At the same time, the dominant bacteria diversity and microbial activity on the biofilms of the BC5 and BC10 reactors were also higher than those of the BC15 group (Figure 5 and Table 1). This further illustrates that although the biofilm biomass increased with the reed biochar mass percentage, 5% and 10% biochar in the combined filler can effectively increase the number of nitrifying bacteria in the BDBR system and improve the dominant bacteria diversity and microbial activity.

Although almost all wastewater from human activities is treated in China, river pollution is still a concern due to inadequate or insufficient wastewater treatment coverage in small communities [44,52]. Usually, biochar has porosity and a large specific surface area. Reed, commonly grown in wetland systems, is a plant resource suitable for preparing biochar because of convenient access and low cost in China [32]. In denitrification systems containing reed biochar as the carrier, the composite filler first adsorbs COD, nitrogen, phosphorus, and other nutrients from the river water, forming a higher concentration of nutrients around the filler. When the microorganisms in the river sediment are activated and resuspended by aeration, they accumulate on the nutrient-rich composite packing surface to grow and multiply rapidly. On the other hand, the biofilms’ formation mainly occurs on the surface of the filler by the extracellular polymer (EPS) secreted by the microorganisms [50]. Microbial community composition, microbial activity, and denitrification efficiency depend principally on the microorganisms’ capability to produce EPS [53]. EPS immobilize microorganisms in biofilm and provide an on–off mechanism for modifying the concentration of molecules in the biofilm matrix [47]. The active group on the surface of reed biochar is beneficial to the combination of EPS and filler. Therefore, the higher the biochar content in the composite filler, the stronger the adsorption performance of the filler on microorganisms. Thus, as the biochar content increases from 5% to 15%, the biomass on the biofilm of the corresponding composite filler increases. However, as the system runs longer, more microorganisms and sediment are adsorbed on the filler’s surface, increasing the biofilm’s thickness. Excessive biofilm thickness would affect the mass transfer of DO and nutrients in the film and biofilm activity. Therefore, the number of nitrifying and denitrifying bacteria, dehydrogenase activity, and diversity of dominant bacteria on the 15% biochar-combined filler biofilm are lower than that on the 5% and 10% biochar-combined filler biofilm. Hence, nitrogen removal efficiency of BC15 with a biochar content of 15% was lower than that of the BC5 and the BC10 with a biochar content of 5% and 10%, respectively, in combined filler biofilms for the BDBR system.

5. Conclusions

In the present study, we found that 26 days were enough for biofilm formation in a BDBR system under the operating parameters, significantly accelerating the biofilm formation process and achieving the rapid startup and stable operation of the BDBR system by adding the reed biochar into the combined filler. Meanwhile, the results discovered that although the biofilm biomass increased with the biochar content in the combined packing, adding 5% and 10% biochar, not 15% in the combined filler, can effectively increase nitrifying and denitrifying bacteria in the BDBR system and improve the dominant bacteria diversity and microbial activity. Hence, the removal efficiencies of nitrogen are significantly higher in the BDBR with 5% and 10% reed biochar than the BDBR with 0% and 15% biochar. These results indicated that reed biochar composite filler prepared in this study can be used as a slow-release carbon source for the denitrification process in the BDBR system under conditions of the in situ bioremediation of eutrophic urban river. Moreover, using the expanding reed in China as a biological resource to produce biochar-composite filler for the BDBR system can not only enhance nitrogen removal efficiency and repair eutrophic river channel by the in situ denitrification system process via the BDBR system, but can also
achieve the purpose of reed resource recycling utilization. Therefore, our study provides a new idea for the development and application of in situ denitrification technology for urban polluted rivers by utilization of the BDBR system with a suitable amount of biochar.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/w13182501/s1, Figure S1: Relationship of the pyrolysis temperature and the physical and chemical properties; Table S1: Chemical properties of the reed biochar under different pyrolysis temperature; Table S2: Physical properties of the reed biochar under different pyrolysis temperature.

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