Purification of Eutrophic River Water Using a Biological Aerated Filter with Functional Filler

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Abstract

Biological aerated filter was used to treat eutrophic river water at different filler mass ratio and temperature on the removal of contaminants. The filter contained a functional composite filler which was made of activated iron and ceramsite. The results showed that the reactor was stable after 30 days of start-up at room temperature and that the proportion of filler mass exerted little effect on COD removal. At different temperatures, the denitrification and phosphorus removal capacity of reactor #1 was better than that of reactor #2, in which the composite filler with an activated iron and ceramsite mass ratio was 1:1 (reactor #1) and 1:3 (reactor #2). At 10°C, both reactor #1 and #2 had removed COD, TN and TP effectively. The relative abundance of species and the diversity of microorganisms in reactor #1 were higher than those in reactor #2. According to the analysis at the phylum and the genus level, bacteria with nitrification and denitrification functions in #1 and #2 were dominant and the proportion of activated iron affected the distribution of microbial population.

Keywords: functional filler, eutrophic river water, low temperature, nitrogen removal, phosphorous removal

Introduction

As improving the quality of surface water such as lakes, rivers and their tributaries is of great significance for improving the ecological environment and living environment. Improving the water ecological environment has become a hot spot in China. Excessive discharge of nitrogen and phosphorus in the eutrophic surface water is the key factor [1] restricting the improvement of water ecological environment. The conventional treatment for eutrophic water bodies includes physical method, chemical method and biological method [2]. The economic cost of physical method and chemical method is high, and their application is limited. Biological methods generally use activated sludge and biofilm. Biological Aerated Filter (BAF) belongs to biofilm process, which has the advantages of small area, high treatment efficiency, strong resistance to impact load, no sludge bulking, simultaneous removal of suspended...
solids (SS) and organic matter, low operation cost of capital construction and convenient maintenance and management in the later stage [3-10]. In addition, the highly integrated BAF system is easy to realize decentralized sewage treatment and is suitable for the treatment of eutrophic water bodies [11]. The BAF has a good function of biological nitrogen removal with less sludge discharge and weak phosphorus removal [12]. How to develop some functional fillers to enhance the ability of phosphorus removal in BAF, to achieve the simultaneous nitrogen and phosphorus removal, has become a research hotspot in the purification of eutrophic surface water.

Filler is an important factor that affects the operation and treatment effect of BAF [13], as well as largely determining the capital construction investment and operation costs [14]. Therefore, as a highly efficient biological carrier, filler is the research focus of biofilm treatment technology, and its surface structure, physical and chemical properties play an important role in the attachment, growth and reproduction of biofilm [15-17]. Smith et al. [18] showed that small particle size fillers can provide larger specific surface area for biofilm growth. Hall-Stoodley et al. [19] reported that the growth and shedding of biofilms were closely related to surface characteristics of the carrier incorporated in biofilm systems. The carrier should have the characteristics of high mechanical strength, abrasion resistance, good biocompatibility, resistance to microbial decomposition, wide source availability, low cost [20]. Bao et al. [21] converted coal tar pitch into a low-cost carbon foam and used it as a BAF filler in wastewater treatment. Studies have found that carbon foam can provide a larger bacterial growth area than ceramic particles, as carbon foam filler has a coral-like pore structure, securely trapping the biofilm within pores and achieving a stable removal effect.

Sponge iron contains a large amount of zero-valent iron, which has excellent physical and chemical properties such as a large specific surface area, high specific surface energy, strong electrochemical enrichment, reducibility, physical adsorption, flocculation and precipitation. It has certain application and research in the treatment of boiler water deoxidization, phosphorus-containing wastewater, organic printing and dyeing wastewater, coking wastewater and nitrate wastewater [22, 23]. Li et al. [24] found that when sponge iron was used as a water treatment material, problems such as pulverization, purification and hardening occur, resulting in a decrease in permeability in the sponge iron filler layer and an increase in inlet and outlet water pressure, thus increasing the operational costs. Wang et al. [25] found that traditional BAF usually uses single ceramsite as the filter material, with a relatively single surface structure, the surface structure of the ceramsite is relatively simple, and the biological adhesion and richness are limited, resulting in unstable purification effect. However, there is a lack of relevant research on biological aerated filter systems which use the mixture of activated iron and ceramsite to treat the eutrophic river water, and achieve the effect of simultaneous nitrogen and phosphorus removal at different temperatures.

In this study, self-made functional activated iron A and ceramsite B were mixed according to different mass ratios as BAF filler. The effects of different mass ratios of composite fillers on the removal of COD, ammonia nitrogen, total nitrogen and total phosphorus were studied, and the correlation between microbial community diversity and the nitrogen removal performance of the system was analyzed. These results provide a valuable technical reference for the treatment of polluted river water under low temperatures in winter.

### Materials and Methods

#### Materials

The fillers used in the experiment were self-made functional activated iron A and ceramsite B. Material A is a kind of self-made porous material of activated iron (pending patent application), with a specific surface area of 80 m²/g, high porosity, high surface energy and strong reducibility. The test influent was taken from a river located near university. The river ran through villages and schools, serving as the receiving water body for the school sewage treatment plant. The quality of the river water during the test period is shown in Table 1.

#### Reactor and Operation

The test device of BAF was shown in Fig. 1a) and b). The biological aerated filter was made of plexiglass, the length, width and height of the BAF is 40 cm × 12 cm × 60 cm, and the effective volume is 26.40 L. The inlet water was a continuous flow, which was pumped into the reactor by peristaltic pump according to the designed flow rate, the water flows vertically through the filling layer, and the aeration

<table>
<thead>
<tr>
<th>Content</th>
<th>COD</th>
<th>NH$_4^+$-N</th>
<th>TN</th>
<th>TP</th>
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<tr>
<td>Average (mg L$^{-1}$)</td>
<td>106.90</td>
<td>19.28</td>
<td>24.56</td>
<td>2.00</td>
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<tr>
<td>Range (mg L$^{-1}$)</td>
<td>39.20–257.60</td>
<td>8.28–43.50</td>
<td>11.58–48.09</td>
<td>0.60–6.72</td>
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</table>
device is uniformly arranged at the bottom. The dissolved oxygen concentration was 6.69-8.40 mg/L and the HRT was 48 h. The filter in the reactor before and after operation was shown in Fig. 1c). In the design phase of the experimental scheme, the SS in the river water was detected, and the SS values were all less than 60 mg/L. Therefore, SS was not detected during the experiment, and no obvious blockage was found during the experiment.

The experiment was divided into two groups, (#1 and #2), and the total mass of filter material used in each group was 18.96 kg. Material A and B were mixed uniformly to generate mass ratios of 1:1 and 1:3, then packed into the reactors accordingly with the filling rate was 54.55%. The experimental period was divided into three stages: the first stage was to start self-cultivation for one month; the second stage ran steadily for one month; the third stage ran at 27ºC, 20ºC and 10ºC respectively. The COD, NH\textsubscript{4}+-N, TN, and TP removal capacity of BAF #1 and #2 were investigated at different temperatures.

Adsorption Experiments

Adsorption test of phosphorus by fillers. Filler A and B were washed with deionized water and dried at 120ºC. 0.5g of fillers were weighed in 50 mL centrifuge tubes, respectively, and KH\textsubscript{2}PO\textsubscript{4} solutions containing 5, 10, 20, 40, 80 and 100 mg/L of 20 mL with P concentrations of 5, 10, 20, 40, 80 and 100 mg/L were added successively. After shaking for 24 hours at 25ºC and 170 r/min, the content of P in the supernatant was determined by centrifugation 5 min (3000 r/min), and the equilibrium adsorption capacity of the packing was calculated as the isothermal phosphorus adsorption curve of the packing.

The isothermal adsorption data were fitted by Langmuir and Freundlich isothermal adsorption equations.

Langmuir isothermal adsorption equation:

\[
q_e = \frac{q_m K_e c_e}{1 + K_e c_e}
\]

Freundlich isothermal adsorption equation:

\[
q_e = K_F c_e^n
\]

In the equation, \(q_e\) is the adsorption amount at adsorption equilibrium, mg/g, \(q_m\) is the maximum adsorption capacity, mg/g, \(c_e\) is the solution concentration at adsorption equilibrium, mg/L, \(K_e\) is Langmuir adsorption equilibrium constant, \(K_F\) is Freundlich adsorption equilibrium constant, and \(1/n\) is the adsorption difficulty degree of the filler, which is related to temperature and adsorption heat.

Analytical Methods

The influent and effluent samples were collected at specified times during the operation of the reactor. The concentrations of COD, NH\textsubscript{4}+-N, NO\textsubscript{2}--N, NO\textsubscript{3}--N, TN, and TP were analyzed according to standard methods [26].

The surface morphology and structural characteristics of fillers A and B were characterized and analyzed by scanning electron microscope (SEM) and X-ray diffraction (XRD).

Detection of microbial community diversity on the filler surface was performed after the system became stable, with the biofilm at 5 cm below the surface of the inlet filler taken for high-throughput sequencing. The 16S rDNA gene is the most commonly used molecular marker technology in phylogenetic taxonomy research.
Highly variable region sequences were sequenced using the high-throughput sequencing platform Hiseq or Miseq. Sequence analysis and species annotation were carried out using the bioinformatics analysis method to analyze the community composition of samples and the differences between samples were further compared by Alpha diversity analysis.

Results

Filter Appearance and Structural Characteristics

The fillers used in this test was shown in Fig. 2a) and b). It can be seen from Fig. 2c) that the filler A has a prominent three-dimensional structure, which is conducive to the attachment, growth and reproduction of microorganisms. As shown by Fig. 2e), the phase of filler A is mainly pure Fe, and FeO is also present. It can be seen from Fig. 2d) that the surface of the ceramsite is relatively smooth, the three-dimensional structure is not prominent, and the pores is almost invisible. As shown in Fig. 2f), the phase of ceramsite mainly consists of Quartz, while Hematite and Dolomite also exist.

Reactor Performance during the Start-Up Stage and Steady-State Stage

The water quality was continuously monitored from the start-up stage, with the operating temperatures of 15-25°C (average 19.8°C). As shown in Fig. 3a), the influent COD concentration ranged from 39.20 mg/L to 257.60 mg/L and fluctuated greatly, while the effluent COD concentration remained stable at a low level. And the COD removal rate was little affected by temperature during operation. In the start-up stage, effluent COD concentrations of reactor #1 and #2 were 19.60-66.00 mg/L (average 43.00 mg/L) and 22.40-61.60 mg/L (average 39.60 mg/L), and the average removal rates were 49.52% and 53.44%. The effluent COD concentrations during the steady-state stage of operation were 8.00-57.60 mg/L (average 33.51 mg/L) and 10.40-52.56 mg/L (average 31.43 mg/L) of #1 and #2, and the average removal rate of 56.45% and 54.98%.

As shown in Fig. 3b), the influent TN concentration was 11.58-27.42 mg/L (average 17.71 mg/L) and fluctuates greatly. During the start-up stage, the effluent TN concentrations of #1 and #2 were 3.15-5.76 mg/L (average 4.79 mg/L) and 2.29-4.87 mg/L (average 3.47 mg/L). At the end of the steady-state stage (after 45 days), the effluent TN concentrations were 1.40-3.21 mg/L (average 2.64 mg/L) and 0.98-3.33 mg/L (average 2.23 mg/L). The nitrogen removal effect was stable and there were no significant differences between treatments #1 and #2. Fig. 3c) shows the ammonia nitrogen removal effects of #1 and #2. At the later stage of the steady-state operation (after 45 days), the effluent NH₄⁺-N concentrations of #1 and #2 were 0.02-0.55 mg/L (average 0.33 mg/L) and 0.06-0.77 mg/L (average 0.40 mg/L), and the average removal rates were 97.70% and 97.17%. Overall, the ammonia nitrogen removal performance of the two reactors is stable and the removal rate is similar.

During the test period, the influent TP concentration was 0.60-3.68 mg/L (average 1.63 mg/L). It can be seen from Fig. 3 (D), the effluent TP concentrations of the two reactors were similar during the start-up stage and the steady-state stage, both of which exhibited fluctuation within 0.04-0.35 mg/L. The change of influent TP concentration and temperature induced no obvious effect on effluent TP concentrations.

Fig. 2. Filler appearance and structural characteristics: a) Filler A, b) Filler B, c) SEM image of Filler A (10 μm), d) SEM image of Filler B (10 μm), e) XRD patterns of Filler A, f) XRD patterns of Filler B.
The Influence of Temperature on Pollutant Removal

To investigate the effect of temperature on the removal of contaminants in the system, #1 and #2 treatment reactors were controlled at temperatures of 27°C, 20°C and 10°C, accordingly. The influent and effluent were monitored during the operational period and the test results are shown in Figs 4, 5 and 6.

Fig. 4 shows the change in influent and effluent quality at different temperatures, with influent COD concentrations between 41.00-235.94 mg/L. At 27°C, 20°C and 10°C, the average COD concentrations in inffluents were 184.54 mg/L, 104.50 mg/L and 77.78 mg/L, respectively. The average COD concentrations in effluent of system #1 were 35.27 mg/L, 30.63 mg/L and 31.36 mg/L, while the average removal rates were 78.58%, 67.07% and 53.24%. The average COD concentrations in effluent of system #2 were 30.84 mg/L, 36.45 mg/L and 28.36 mg/L, at 27°C, 20°C and 10°C, with average removal rates of 80.82%, 60.55%, and 58.83%. It can be seen from Fig. 4 that influent COD concentrations fluctuated greatly, although the effluent COD concentration remained stable below 40 mg/L and the system showed a strong capacity to resist the COD impact load. When the reactor temperature decreased from 20°C to 10°C, the effluent COD concentration did not increase. At different temperatures, the effluent COD concentrations of the two reactors remained close, exhibiting no significant differences.

Fig. 5 shows the effect of temperature on the nitrogen removal performance. Influent NH₄⁺-N and TN concentrations were 10.16-43.50 mg/L and 18.08-48.09 mg/L during the operational period. As shown in Fig. 5a), at 27°C, 20°C and 10°C, the average NO₃⁻-N concentration in the effluent of treatment #1
gradually increased with the decrease in temperature (0.33 mg/L, 2.04 mg/L and 4.87 mg/L, respectively), with an average NO$_3$-N concentration in effluent was 0.67 mg/L, 0.49 mg/L and 0.41 mg/L, respectively. Fig. 5c) shows that at 27ºC, 20ºC and 10ºC, the average NO$_3$-N concentration in effluent of system #2 was 5.25 mg/L, 5.39 mg/L and 6.75 mg/L, with average NO$_2$-N concentrations in effluent of 0.05 mg/L, 0.01 mg/L and 0.22 mg/L, respectively. The effluent NO$_3$-N concentration in system #1 was significantly lower than in system #2, although the effluent NO$_2$-N concentration in #1 was higher than in #2. In addition, as shown in Fig. 5b), the average effluent TN concentration in #1 gradually increased with decreasing temperature (1.31 mg/L at 27ºC, 2.95 mg/L at 20ºC and 7.54 mg/L at 10ºC), with the removal rates of 96.63%, 87.32% and 68.73%, respectively. Fig. 5d) shows that at 27ºC, 20ºC and 10ºC, the average effluent TN concentrations in #2 were 6.13 mg/L, 5.72 mg/L and 8.85 mg/L with removal rates of 83.15%, 76.09% and 63.06%, respectively. Based on these results, it can be seen that the denitrification effect of #1 was significantly better than that of #2, with the denitrification efficiency decreasing with the decrease in temperature. From Fig. 5b) and d), it can be seen that the two reactors exhibited very good removal effects for ammonia nitrogen at different temperatures.

At 10ºC, the effluent NH$_4^+$-N concentration was consistently stable below 1 mg/L in both reactors #1 and #2, with the removal rate remaining above 94%, resulting in average effluent TN concentrations of 7.54 mg/L and 8.85 mg/L, respectively.

As shown in Fig. 6, when the temperature were 27ºC, 20ºC and 10ºC, the average effluent TP concentrations in system #1 were 0.10 mg/L, 0.09 mg/L and 0.11 mg/L, while the average effluent TP concentrations...
in system #2 were 0.15 mg/L, 0.13 mg/L and 0.16 mg/L, respectively. The removal effect of TP of #1 was obviously better than that of #2, and the removal of phosphorus is less affected by temperature in the range of experimental temperature.

Discussion

The Effect of Functional Composite Filler on Pollutant Removal

It can be seen from the above test results that the BAF with functional composite fillers can effectively remove COD, nitrogen and phosphorus in sewage. In the range of the mass ratio of filler components given in the test, the filler ratio of different components has no obvious effect on the removal of pollutants.

The effluent COD concentrations in #1 and #2 fluctuated uniformly and were relatively stable during the start-up stage and the steady-state stage. The reason was that the composite packing contained many pores and a large specific surface area, which is beneficial to the formation of biofilms. A large number of biofilms are grown on the surface of combined fillers, which can trap, adsorb and biodegrade both microorganisms and suspended matter in the influent. A large number of biofilms are grown on the surface of combined fillers, which can trap, adsorb and biodegrade both microorganisms and suspended matter in the influent. BAF has a good effect on the removal of TN in polluted river water, but because biofilm plays a major role in biological removal, biological phosphorus removal is limited, so it cannot achieve the purpose of nitrogen and phosphorus removal at the same time. In this experiment, the biological filter system was combined with self-made activated iron A and ceramsite B to explore the contribution of fillers in the removal of total phosphorus from polluted river water.

Studies [25] have shown that using the ceramsite biological aerated filter, the NH$_4^+$-N removal rate remains stable at above 85%, with average TN removal rates of 25%-60%. The results of this experiment show that NH$_4^+$-N and TN can be removed continuously and consistently after system start-up and operation for 45 days, with removal rates reaching 97% and 87%, respectively. The reason for this was that the composite filler used in the experiment was conducive to the formation of biofilms, allowing the growth and reproduction of nitrifying bacteria. The macro aerobic environment was beneficial to nitrification reactions by organisms and the nitrification of ammonia nitrogen. The formation of a local anoxic environment at the micro level was conducive to the occurrence of denitrification reactions. Functional activated iron can not only be used as the carrier for immobilized microorganism, but also has certain chemical denitrification effects because its main component is zero-valent iron.

Studies [28, 29] have shown that zero-valent iron can produce Fe$^{2+}$ through electrochemical corrosion and biological corrosion, with ferrous ion having strong reducibility, which can react with nitrate in sewage for oxidation-reduction, removing nitrogen elements from sewage. During the stable operation of #1 and #2, the concentration of ammonia and nitrogen in the effluent was always stable below 1mg/L, and the removal rate was more than 95%. Some studies [30] have shown that when the sewage BOD$_5$/TKN>(4~6), the denitrification carbon source can be considered sufficient. The average C/N ratio in the influent in the present study was 6.00, showing that the carbon source was sufficient and NO$_3^-$ accumulation was not found. However, from the effluent after 45 days, it could be shown that the denitrification effect of #1 and #2 was similar, which may be due to that the dosage of activated iron has far exceeded the threshold value and the change in activated iron could not induce an obvious change in denitrification effect. From the view of denitrification, the following experiments can be performed to further establish the optimal ratio of activated iron.

BAF has a good effect on the removal of TN in polluted river water, but because biofilm plays a major role in biological removal, biological phosphorus removal is limited, so it cannot achieve the purpose of nitrogen and phosphorus removal at the same time. In this experiment, the biological filter system was combined with self-made activated iron A and ceramsite B to explore the contribution of fillers in the removal of total phosphorus from polluted river water.

After 45 days of stable operation of the biofilter system, the phosphorus removal efficiency of system #1 was slightly higher than that of system #2, the effluent TP concentration was lower than 0.20 mg/L and the average removal rate was above 93%. XIE et al. [31] used the self-made fly-ash ceramsite biological filter to treat urban sewage, reporting an average TP removal rate of 44.16% and an effluent TP concentration of less than 3 mg/L, showing that the phosphorus removal effect was worse than that of the functional composite biofilter used in this experiment. It can be inferred that the main phosphorus removal effect was due to activated iron in the mixed filler. An isothermal adsorption test of self-made activated iron and ceramsite was carried out (Fig. 7) and the results show that the TP adsorption

![Fig. 7. Adsorption isotherm of TP by the filler at 25°C.](image-url)
amount by filler A increased with the increase in initial concentration. When the initial concentration was 80 mg/L, the saturated adsorption amount of Filler A to TP reached 2.30 mg/g and the saturated adsorption amount of Filler B to TP was much lower at below 0.21 mg/g. Zhang et al. [32] studied sponge iron with similar properties and also showed that the internal porous three-dimensional structure can increase the specific surface area of the filler and provide more adsorption sites for phosphate. Zhou et al. [33] found that zero-valent iron can be corroded in water to produce Fe^{0}, which can chemically precipitate with phosphate and co-precipitate iron hydroxide with phosphate. Because there was no sludge discharge in this biofilter system, functional activated iron played a major role in phosphorus removal, which occurred mainly by physicochemical processes.

The Langmuir isotherm model is a chemical adsorption, a monolayer adsorption model. When the surface of the adsorbent is covered with a layer of adsorbate, adsorption will no longer occur [34]. The Freundlich adsorption equation is mainly used to judge the properties of the adsorption process, that is, physical and chemical adsorption [35]. The Langmuir and Freundlich fitting isotherm adsorption equations and correlation coefficients of filler to P were shown in Table 2.

It can be seen from Table 2 that the correlation coefficient R² in the fitting equation was greater than 0.95, indicating that both the Freundlich and the Langmuir isotherm adsorption equations can describe the isotherm adsorption process of filler A and B on phosphorus, and the adsorption effect of these two fillers on phosphorus included physical and chemical adsorption. By comparing the correlation coefficient R² in the adsorption isotherm equations of fillers A and B, it was found that R² in the Langmuir equation was greater than R² in the Freundlich equation. The adsorption effect of filler on phosphorus was mainly chemical adsorption. In addition, the sludge was not discharged during the operation of the biological filter system, so it can be inferred that functional activated iron A plays a major role in the chemical removal of phosphorus. Therefore, the BAF with activated iron had a continuous, high efficiency and stable phosphorus removal function, which is coupled with the biological denitrification in the system to removal of nitrogen and phosphorus pollutants in eutrophic river water at the same time. It was of great significance for the restoration of river ecological function and the sustainable development of water resources.

The Influence of Temperature on Pollutant Removal

The results show that the COD removal efficiency of #1 and #2 were highly similar, with both showing a downward trend with the decrease in temperature, which is similar to the results of Gómez-Silván et al. [36]. Analysis shows that the enzyme activity decreased with the temperature decreasing, the growth and reproduction of bacteria were limited and the oxidation and degradation ability of organic matter was weakened.

According to Fig. 5b) and d), in the temperature range of 10–27ºC, the effluent NH₄⁺-N and TN concentration of the reactors #1 and #2 were consistently below 1.40 mg/L and 10 mg/L, respectively. At 10ºC, the effluent NH₄⁺-N removal rates of #1 and #2 were 94.11% and 96.10%, respectively. The TN removal rates were 68.73% and 63.06%, with the nitrogen removal performance was much better than that studied by Dou et al. [37] When the temperature was 18ºC (low temperature), the removal rate of NH₄⁺-N by BAF was less than 60%. The decrease in nitrification capacity of BAF in low temperature environments was obvious, because most nitrifying functional bacteria are mesophilic microorganisms [38]. However, the NH₄⁺-N removal rate did not decrease significantly with the reduction in temperature in the present study. The system maintained a good nitrification effect at low temperatures of 10ºC, with no significant NO₃⁻-N accumulation. The effluent NO₃⁻-N concentration of the reactors #1 and #2 were consistently below 10 mg/L and the system was able to operate stably. The effluent NO₃⁻-N concentration of system #1 was always lower than that of #2 and the denitrification effect of #1 was better than that of #2. Therefore, these results show that low temperatures had no obvious effect on the nitrification process but did exert an obvious effect on the denitrification process, and the effect on system #1 was greater than that on system #2. The reason for this is that material A contains a large amount of zero-valent iron, which has strongly reductive properties. Liu et al. [39] showed that zero-valent iron mostly removes oxygen from the environment through chemical reduction, rapidly creating an anaerobic environment that is beneficial to biological nitrogen removal and therefore, increasing the proportion of material A in the
system can effectively improve the nitrogen removal capacity of the system. The test results also showed that the phosphorus removal rate did not change significantly at different temperatures, which further proved that the removal of phosphorus in the system occurred mainly via physical and chemical processes with activated iron. This provides new techniques for low temperature nitrogen and phosphorus removal. The activated iron and ceramsite composite packing BAF can be used for low-temperature polluted water denitrification and dephosphorization treatment. Increasing the ratio of activated iron can increase nitrogen and phosphorus removal efficiency and stability. It has important popularizing value in the treatment of polluted eutrophicated river water in northern China or seasonal low temperature areas.

Microbiological Analysis

In order to verify the effect of the reactor on the removal of contaminants, the biofilm on the surface of the packing was scraped for high-throughput sequencing after the reactor operated stably at 20ºC for 35 days. The population diversity index is shown in Table 3. The Sobs, ACE and Chao indices are used ecologically to indicate the richness of microbial communities, with larger values showing higher community richness [40]. The Shannon index and the Simpson index are ecologically used to characterize the diversity of

![Fig. 8. Relative abundance distribution: a) Relative abundance distribution at Phylum level, b) Relative abundance distribution at Genus level.](image-url)
Ten known phylum were detected by sequencing and Fig. 8a) shows the species with relative abundances of no less than 1%. At the Phylum level, Proteobacteria was dominant in the two groups of reactors, accounting for 43.88% and 45.38%, respectively. In addition, Chloroflexi (15.33%), Actinobacteria (9.56%), Firmicutes (6.23%), Bacteroidetes (4.24%), Acidobacteria (3.81%) and Nitrospirae (2.96%) were present in #1, while reactor #2 included Bacteroidetes (27.98%), Acidobacteria (11.27%), Nitrospirae (6.42%) and Chloroflexi (2.33%). Brock et al. [42] reported that most of the microorganisms with nitrogen and phosphorus removal functions belong to Proteobacteria and some to Bacteroidetes. Xu et al. [43] found that some of the bacteria in Firmicutes and Nitrospirae exhibited better carbon and nitrogen removal ability. Combined with the findings of the present study, it can be inferred that systems #1 and #2 exhibit denitrification functions.

At the genus level, there were 34 known genera with an abundance greater than 1% and the relative abundance of the genera level microbial community is shown in Fig. 8b). The dominant bacteria in #1 included norank_f_Anaerolineaceae (5.23%), Rhodobacter (5.16%), Nitrospirae (4.24%), Hydrogenophaga (2.96%) and Acidovorax (2.41%). The dominant bacteria in #2 included Flavobacterium (8.85%), Nitrospirae (6.42%), Hydrogenophaga (5.49%), Acidovorax (5.36%) and Rhodobacter (2.17%), most of which have the function of nitrification and denitrification. Rhodobacter has a nitrogen fixation function and belongs to aerobic denitrifying bacteria [44]. Xiao et al. [45] reported that Nitrosomonas and Nitrospirae play an important role in the conversion of NH$_4^+$-N into NO$_2^-$-N and NO$_3^-$-N. Hydrogenophaga and Acidovorax are heterotrophic nitrifying and aerobic denitrifying bacteria and Li et al. [46] reported that they were abundant in aerobic pools with sufficient organic carbon, making important contributions to simultaneous nitrification and denitrification. At the genus level, it was proven that systems #1 and #2 had the function of denitrification, although the population structure of microorganisms in both systems #1 and #2 were very different, which may be due to the different proportion of activated iron and ceramsite in the filler, resulting in micro electrochemical environments and ultimately affecting the growth and reproduction of microorganisms and population distribution.

### Conclusions

In BAF reactors #1 and #2, containing activated iron and ceramsite mass ratios of 1:1 and 1:3, after the 45th day of stable operation the average COD removal rates were 63.22% and 58.27%, the TN removal rates were 87.51% and 89.45%, the NH$_4^+$-N removal rates were 97.70% and 97.17%, and the TP removal rates were 96.28% and 94.84%, respectively. It was shown from the adsorption test results that the saturated adsorption capacity of material A and B for phosphate was 2.30 mg/g and 0.21 mg/g, respectively, and the removal of phosphorus mainly depending on the chemical adsorption of material A.

The COD and TP removal rate in the two reactors showed a decreasing trend with a reduction in temperature, although the impact was not significant. The effluent COD and TP concentrations were maintained at 28.36-36.45 mg/L and 0.09-0.16 mg/L, respectively. With a reduction in temperature from 27°C to 20°C, to 10°C, the TN removal rate was significantly reduced, with system #1 rates of 96.63%, 87.32% and 68.73%, and system #2 rates of 83.15%, 76.09 % and 63.06%, respectively. The NH$_4^+$-N removal rate was relatively stable, maintaining above 94.00%. NO$_3^-$-N accumulated in the reactor and the denitrification effect of #1 was better than that of #2 at different temperatures, especially as the system still maintained good nitrogen removal capacity at a low temperature of 10°C.

The relative abundance and microbial diversity in system #1 were greater than that in system #2. Analysis at the phylum and the genus level, showed that bacteria with nitrification and denitrification functions were dominant in system #1 and #2. The population distribution at the phylum level

### Table 3. Analysis of microbial population abundance and diversity.

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</table>
exhibited no significantly differences, although the difference in population distribution at the genus level was large. The proportion of activated iron significantly affected the distribution of microbial populations.

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Conflict of Interest

The authors declare no conflict of interest.

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