

Article

Mixotrophic Denitrification of Glucose Polymer-Based Pyrite Tailings for Enhanced Nitrogen and Phosphorus Removal of Municipal Tailwater

Jie Mao ^{1,2,3}, Haojie Che ^{2,3}, Xinyu Wang ⁴, Mengqi He ^{2,3}, Chun Cheng ^{2,3}, Meng Wang ³, Bai Sun ^{1,2,3} and Shuguang Zhu ^{1,3,5,*}

- ¹ Engineering Research Center of Ministry of Education, Anhui Jianzhu University, Hefei 230601, China; maojie@ahjzu.edu.cn (J.M.); bsun@mail.ustc.edu.cn (B.S.)
- ² Key Laboratory of Water Pollution Control and Wastewater Recycling of Anhui Province, Hefei 230601, China; 18326721311@163.com (H.C.); hhmkk1220@163.com (M.H.); hayamimio@outlook.com (C.C.)
- ³ School of Environmental and Energy Engineering, Anhui Jianzhu University, Hefei 230601, China; m18455940817@163.com
- ⁴ Nanjing University Environmental Planning and Design Research Institute Group Co., Nanjing 211500, China; wxy@njuae.cn
- ⁵ Energy Saving Research Institute, Anhui Jianzhu University, Hefei 230601, China
- * Correspondence: zhushuguang@ahjzu.edu.cn; Tel.: +86-181-1965-8115

Abstract: In order to improve the removal ability of nitrogen and phosphorus pollutants from sewage with low C/N ratio, this study prepared the glucose polymer-based pyrite tailings with core-shell structure through glucose polymerizing on the surface of pyrite tailings particles and constructed a heterotrophic-sulfur autotrophic mixed-denitrification system. The experimental results show that compared with ordinary pyrite tailings, pyrite tailings modified by glucose polymer can improve the water quality of pH, enhance the ability to remove NO_3^- in water, and prolong the ability of mineral to continuously treat sewage, which also has a good removal effect on PO_4^{3-} in water. The results of this study are of great significance to solve the excessive nitrogen and phosphorus in the secondary effluent and alleviate the eutrophication of the natural water.

Keywords: pyrite tailings; autotrophic denitrification; heterotrophic denitrification



Citation: Mao, J.; Che, H.; Wang, X.; He, M.; Cheng, C.; Wang, M.; Sun, B.; Zhu, S. Mixotrophic Denitrification of Glucose Polymer-Based Pyrite Tailings for Enhanced Nitrogen and Phosphorus Removal of Municipal Tailwater. *Water* **2022**, *14*, 1868. <https://doi.org/10.3390/w14121868>

Academic Editor: Stefano Papirio

Received: 11 April 2022

Accepted: 6 June 2022

Published: 10 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

With the rapid development of society and the accelerating process of industrialization, the domestic sewage and industrial wastewater rich in N and P are produced in large quantities [1,2]. However, at present, the conventional treatment process of China sewage plants is not enough to effectively remove nitrogen and phosphorus in sewage. The concentration of nitrogen and phosphorus in the effluent of some sewage plants is higher than that of natural waters, which causes and aggravates the water eutrophication [3,4]. The survey shows that 82% of China's natural waters is polluted to varying degrees, among which eutrophic lakes account for three-fourths of the total natural water [5]. Eutrophication of water sources such as rivers and lakes seriously affects the safety of water supply. Therefore, it is very necessary to adopt technically feasible and economically favorable methods to reduce the nitrogen and phosphorus content of the effluent water.

At present, the main methods of sewage nitrogen removal include physical, chemical, and biological methods [6,7] because the high-efficiency and low-cost biological method has attracted wide attention. According to the type of electron donor, biological denitrification can be divided into heterotrophic and autotrophic types [8,9]. In the heterotrophic denitrification process, the carbon source is an indispensable material and directly affects the denitrification rate and effect [10,11]. However, domestic sewage is generally characterized by insufficient carbon source and low C/N ratio of influent water [12,13]. In order to ensure

nitrogen removal successfully and efficiently, heterotrophic denitrification needs to add a large number of additional carbon sources as electronic donors [14–16], which increases additional operating costs and can easily cause secondary pollution [17]. Compared with heterotrophic denitrification, autotrophic denitrification can rely on H_2 , Fe, S, and other substances as electron donors without adding organic carbon sources, which is an efficient, safe, and economic advanced nitrogen-removal technology [18–20]. Sulfur-based autotrophic denitrification, as a typical autotrophic denitrification process, has been widely used in wastewater treatment due to its good stability, non-toxicity, and economic benefits [21]. As a sulfur-containing mineral, pyrite can be used as a sulfur source to promote sulfur autotrophic denitrification [22]. In addition, the iron contained in pyrite can also promote ferrous autotrophic nitrite [23,24]. However, the direct use of pyrite is also economically costly, which limits the large-scale extension of the process. As byproducts after pyrite mining, the tailings themselves contain the same active ingredients as pyrite, which can also provide an electronic source for autotrophic denitrification [25]. Therefore, if pyrite tailings are used instead of pyrite as the electronic donor for biological nitrogen removal, it is of great practical significance to reduce the cost of wastewater nitrogen removal and can also solve the problem of solid waste pollution derived from tailings [26]. However, fewer residual sulfur and iron active components in pyrite tailings result in low denitrification efficiency. Therefore, determining how to improve the autotrophic denitrification efficiency of pyrite tailings is the key to realizing its practical application.

Klimenko et al. [27] and Goncharuk et al. [28] utilized activated carbon as the microbial carrier to improve the water quality of tap water. However, the method has disadvantages of a long experimental cycle and high cost. Additionally, at present, there are no relevant studies on reported the coating of carbohydrate polymers on to the surface of pyrite tailings. Therefore, in this study, a core-shell structure of glucose polymer-based pyrite tailings was prepared by the polymerization reaction of glucose on the surface of pyrite tailings particles under hydrothermal conditions to construct a heterotrophic-sulfur autotrophic mixed-denitrification system (Figure 1). The main features of this study are: (1) The glucose-polymer layer coated in the surface of tailings particles can continuously provide carbon source for the heterotrophic denitrification process and play the role of slow release. It can not only overcome the leakage of conventional carbon sources to water in sewage-treatment process but also avoid the problem of uneven dispersion in water in conventional solid carbon sources. (2) With the progress of heterotrophic denitrification and the consumption of carbon sources, the exposed pyrite tailings can participate in the process of autotrophic denitrification, while the free iron ions can be used to remove phosphorus. (3) Through the heterotrophy-sulfur autotrophy-programmed process constructed in this study, the slow growth rate and slow denitrification rate of sulfur autotrophic denitrification bacteria can be compensated by the fast heterotrophic denitrification reaction, which is helpful to improve the denitrification efficiency in the whole reaction stage.

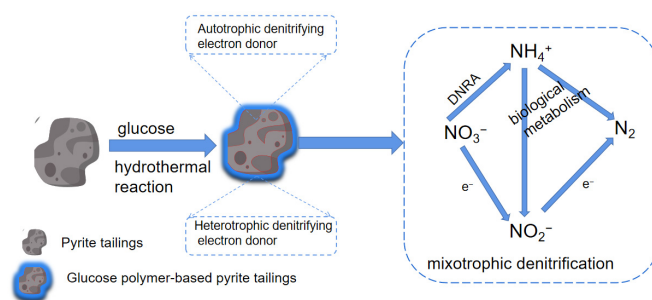


Figure 1. Heterotrophic-sulfur autotrophic mixed denitrification system.

2. Materials and Methods

2.1. Materials

The pyrite tailings used in the experiment were taken from Longqiao mine in Tongling, Anhui Province, and the activated sludge was taken from the secondary sedimentation tank of sewage treatment plant in Hefei Economic Development Zone. Glucose, NaCl, potassium nitrate, ammonium chloride, potassium dihydrogen phosphate, sodium bicarbonate, and anhydrous ethanol were all analytically pure. The glass instruments used were soaked in 10% nitric acid solution overnight. All aqueous solutions were prepared with de-ionized water.

2.2. Preparation of Materials

2.2.1. Pyrite Tailings Treatment

The pyrite tailings used were crushed by a wall-breaking machine. The mineral particles with particle size of 0.10–0.15 mm were obtained by screening (100–150 mesh) after grinding. The obtained mineral particles were washed with deionized water for several times to clarify the supernatant. Then, ultrasonic vibration was applied for 1 h; cleaning was performed with deionized water again to clarify the supernatant; and the cleaned ore particles were placed in an oven and dried at 70 °C for 6 h.

2.2.2. Preparation of Glucose Polymer-Based Pyrite Tailings

Next, 10 g pyrite tailings particles was added into 500 mL of glucose solution; the mass ratio of pyrite tailings to glucose was 1:1. The obtained mixture solution was stirred for 20 min; then, it was transferred into a 100 mL Teflon-lined stainless autoclave, sealed, and maintained at 180 °C for 3 h. After being cooled down to room temperature, the sample was taken out from the reactor and then washed several times with deionized water and ethanol. Finally, the extracted sample was dried at 80 °C for 12 h and crushed into powder and placed in sealed bags for later use.

2.2.3. Simulation of Sewage Quality

The secondary effluent of the laboratory-simulated sewage-treatment plant was prepared with deionized water. The simulated sewage quality was configured according to the actual secondary effluent concentration of the sewage treatment plant. In the experimental sewage, the concentration of nitrate nitrogen prepared by potassium nitrate (KNO_3) was 15 mg/L; the concentration of ammonia nitrogen prepared by ammonium chloride (NH_4Cl) was 10 mg/L; and the concentration of total phosphorus prepared by potassium dihydrogen phosphate (KH_2PO_4) was 3 mg/L. Glucose was used to simulate the residual organic matter in sewage, and the concentration of residual organic matter was set at about 60 mg/L by chemical oxygen demand meter. Sodium bicarbonate (NaHCO_3) was used as autotrophic denitrification carbon source, and its concentration is 90 mg/L.

2.3. Method

The activated sludge used in the experiment was taken from the second sedimentation tank of the sewage-treatment plant in Hefei Economic Development District. The activated sludge of the secondary sedimentation tank was placed for a period of time. After it settled, we poured out the supernatant, stored the remaining sludge under the condition of avoiding light and freezing, added the nutrients necessary for the sludge regularly, and changed the culture solution once a week. Before the sludge was inoculated into the experimental conical flask, it needed to be pretreated to remove the interfering ions in the sludge. First, we took a certain amount of activated sludge from the sludge storage bucket and placed it in the beaker. Then, after standing for 30 min, we poured out the supernatant, added 0.7% NaCl solution prepared in advance, let it stand for 30 min again to make the sludge settle again, and poured out the supernatant. Finally, we repeated the cleaning test for 3 times. We allowed it to stand all night for the last time and, finally, poured out the sludge supernatant again for inoculation.

Then, 800 mL of synthetic sewage was added to a 1000 mL conical flask; then, we took 30 g the mineral particles coated with glucose and 5 mL of clean sludge, put them into the 1000 mL conical flask, fastened with a rubber plug, and placed it in a constant-temperature shaking table. Under the condition of continuous operation at 150 R/min and 30°C, it continued to run for 132 h, and samples were taken every 12 h to measure the concentration of PH, total nitrogen, total phosphorus, and sulfate in the effluent. Two parallel experiments were thus set up.

2.4. Characterization

2.4.1. X-ray Diffraction Spectrometry (XRD)

Panalytical X-ray diffractometer of Panaco Company was used for X-ray diffraction analysis. The scanning speed was 10 °/min, and the scanning diffraction angle was (2θ) from 10~80°; the working voltage was 40 kV, and the current was 40 mA.

2.4.2. FTIR Analysis Method

The ftir-7600 infrared spectrometer of Lambda Company in Australia was used for Fourier-transform infrared spectroscopy. The scanning range was 500~4000 cm⁻¹, the instrument resolution was 4 cm⁻¹, the scanning times were 64, the test temperature was 25 °C, and the potassium bromide tablet pressing method was used for sample preparation.

2.4.3. Zeta Analysis Method

The MS2000 zeta potential analyzer of Malvern Company in the UK was used for zeta potential analysis. The dried mineral particles were mixed with deionized water, vibrated to uniform with a vibrator, and the surface potential of the mineral before and after coating was measured.

2.5. Water Quality Analysis Methods

All water samples were filtered by a 0.45 µm membrane, and pH was measured with a pH meter. Water samples were analyzed according to “Water and Wastewater Monitoring and Analysis Methods Fourth Edition” (2002). Denitrification and phosphorus removal reaction system with Pyrite tailings as the substrate was denoted by PW. Denitrification and phosphorus removal reaction system with glucose polymer-based pyrite tailings as the substrate was denoted by GPW. Specific indicators and analysis methods are shown in the Table 1.

Table 1. Water-quality analysis methods.

Analysis Index	Analytical Method	Analytical Instrument	Instrument Model
NO ₃ ⁻ -N	Ultraviolet spectrophotometry	Ultraviolet/visible spectrophotometer	UV-5100
NO ₂ ⁻ -N	N-1-naphthalene-ethylenediamine spectrophotometric method		
NH ₄ ⁺ -N	Sodium reagent spectrophotometry		
PO ₄ ³⁻ -P	Molybdenum antimony anti spectrophotometric method	ion chromatograph	ICS900DionexIonPac
SO ₄ ²⁻	Ion chromatography		

3. Results and Discussion

3.1. Characterization of Pyrite Tailings and Glucose Polymer-Based Pyrite Tailings

Figure 2 shows XRD and FTIR before and after pyrite tailings modification. According to XRD spectra, pyrite tailings has an obvious crystal structure, which matches FeS₂ characteristic at peaks of 28.6°, 33.1°, 37.2°, 40.9°, and 47.6°, indicating iron sulfide in pyrite tailings. However, the XRD spectrum of glucose polymer-based pyrite tailings shows that the position and intensity of the main peak at 2θ of 28~56° are basically unchanged,

demonstrating that the hydrothermal reaction process does not affect the crystal structure of the pyrite tailings. In addition, glucose polymer-based pyrite tailings have a characteristic peak at 1720 cm^{-1} , providing the first indication for the formation of a glucose polymer. Meanwhile, according to the data of Zeta potential, the surface charge changed from 4.34 mV to -33.9 mV , further verifying that the glucose polymer successfully coated the pyrite tailings surface.

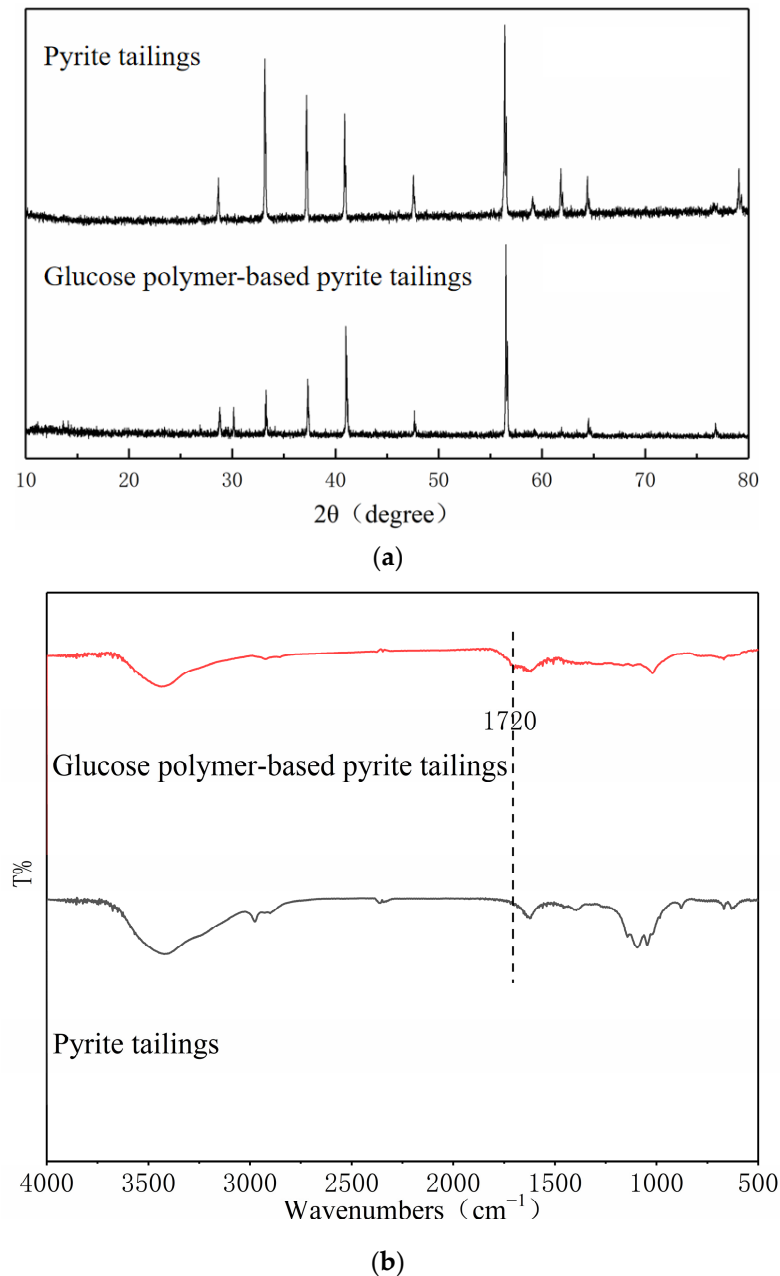


Figure 2. XRD pattern of pyrite tailings before and after coating (a). FTIR spectra of pyrite tailings before and after coating (b).

3.2. Analysis of Effluent Quality

3.2.1. Change of Effluent pH

Figure 3 shows the change trend of pH of denitrification system solution with time before and after glucose coating of pyrite tailings.

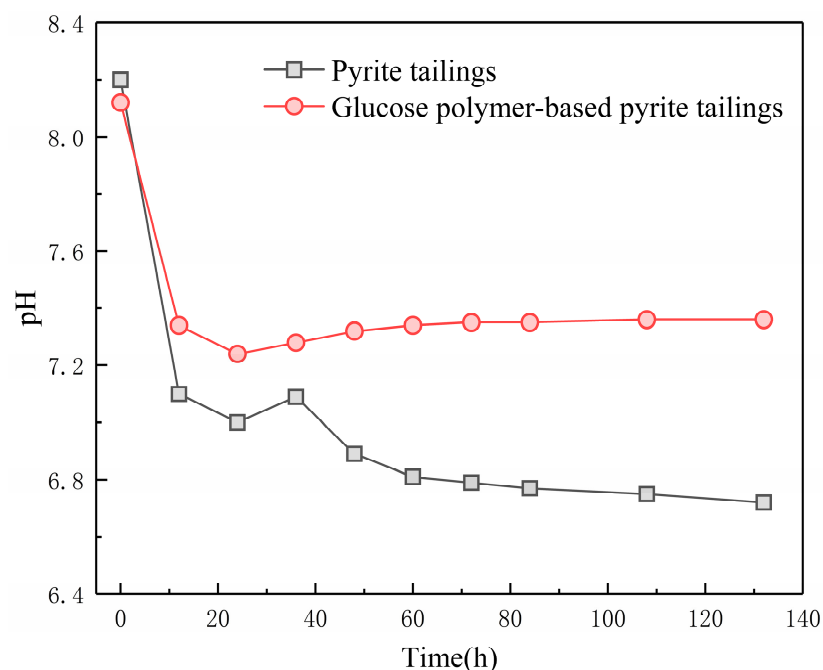
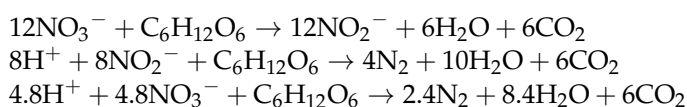


Figure 3. Change diagram of pH in denitrification system before and after pyrite tailings coating.

As can be seen from the Figure 3, the initial pH of PW solution is 8.20, which rapidly decreases to 7.1 at 12 h. This may be due to the oxidation of surface mineral during tailings cleaning, resulting in acid produced after putting tailings into water. Then, the pH of the solution increases to 7.09, which may be attributed to the alkaline substances derived from heterotrophic denitrification reaction caused by the trace carbon source in the system. In the subsequent stage, the pH of the solution decreases, mainly due to the release of SO_4^{2-} by the sulfur autotrophic denitrification process, resulting in an increase in the acidity of the aqueous solution. The initial pH of GPW solution is 8.12, which rapidly decreases to 7.34 at 12 h, probably because during the material preparation process, the glucose polymer on the surface of the material is oxidized, releasing the proton after encountering water and reducing the solution pH. Then, at 12–24 h, the solution pH slowly decreases to 7.24, probably due to the partial hydrolysis of the pyrite surface-coated glucose polymer, resulting in a decrease in the solution pH value. After 24 h, the pH of the solution continues rising, mainly originating from the alkaline material produced by the heterotrophic denitrification process. The experiments show that glucose coating can significantly improve the water quality of pH, keeping pH in the range suitable for microbial growth.

3.2.2. Change of Nitrogen in Effluent

When glucose is used as the carbon source for heterotrophic denitrification, the following reactions occur, which can result in changes in the concentrations of NO_3^- -N and NO_2^- -N in solution of PW and GPW.



The trend of NO_3^- -N changes in the denitrification system solution of PW and GPW is presented in Figure 4, respectively. It can be seen that the initial concentration of NO_3^- -N in the experimental group of PW is 24.87 mg/L, and the concentration of NO_3^- -N in the solution rises to 26.1 mg/L in the first 12 h. At this stage, microorganisms are in the growth and adaptation period and may release some nitrogenous substances, which may increase the concentration of NO_3^- -N in the solution, and then rapidly decrease to 5.7 mg/L in

12–84 h. At this stage, heterotrophic denitrification mainly occurs in the solution, and the speed of the reaction is relatively faster. Then, the concentration slowly declines to 5.4 mg/L in 84–132 h. Autotrophic denitrification mainly occurs at this stage, and the speed of reaction slows down; the initial concentration of NO_3^- -N in GPW was 25.73 mg/L, and in the first 12 h, the concentration of NO_3^- -N increased to 26.73 mg/L. At this stage, the microorganism was in the growth and adaptation period and might release some nitrogenous substances, which may raise the concentration of NO_3^- -N. After that, the concentration rapidly decreases to 3.69 mg/L in 12–84 h and continuously drops to 1.32 mg/L in 84–132 h. This stage mainly occurs during the heterotrophic denitrification process, and the speed of the reaction is fairly fast. Therefore, the GPW can enhance the ability of removing NO_3^- in water and prolong the ability of continuous treatment of sewage by mineral.

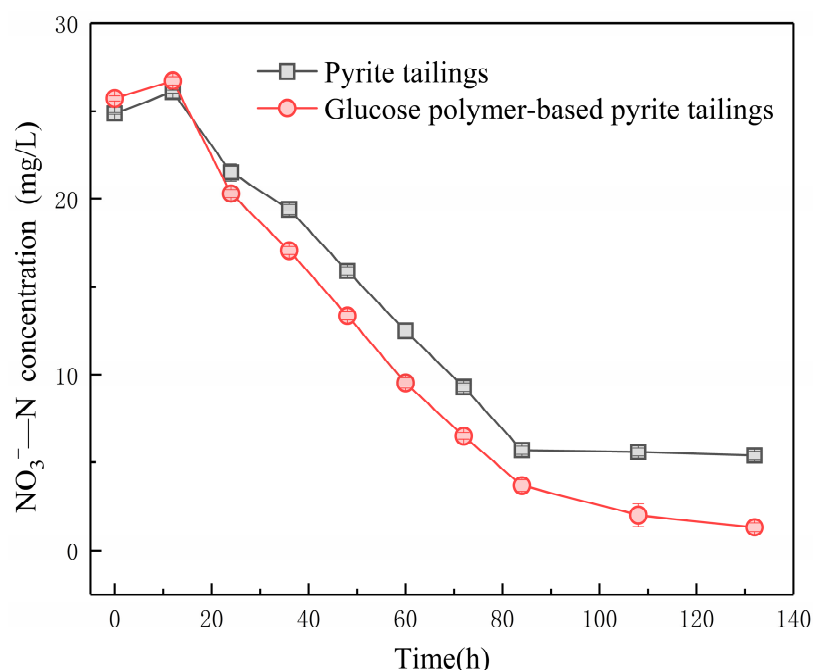


Figure 4. Variation diagram of NO_3^- -N concentration in denitrification system before and after pyrite tailings coating.

Figure 5 displays the trend of NO_2^- -N changes in denitrification system solution of PW and GPW. As the Figure 5 shows, before coating, the concentration of NO_2^- -N accumulated to 0.31 mg/L in the first 12 h and then decreased to 0.21 mg/L in 24 h. At 72 h, it piled up to the highest level, reaching 1.50 mg/L, and subsequently dropped to 0.11 mg/L in 132 h. The first 12 h comprise the stage of the microorganism adapting to the environment, and thus, the concentration of NO_2^- accumulated less. In 12–84 h, heterotrophic denitrification mainly occurred, and the reaction rate of NO_2^- was lower than that of degradation, so NO_2^- accumulated more at this stage. After 84 h, due to the reduction of the concentration of NO_3^- -N, the ability to inhibit the NO_2^- was reduced, and this stage mainly occurred during autotrophic denitrification. The concentration of NO_2^- -N in the GPW reached 0.30 mg/L in the first 12 h and up to 2.22 mg/L at 72 h, then decreased to 0.40 mg/L at 132 h. In the first 12 h, the microorganism adapted to the environment, and thus, the accumulation of NO_2^- was less. In the 12–84 h, the heterotrophic denitrification process mainly occurred, and the speed of reaction was faster, so NO_2^- accumulated more in this stage. After 84 h, on account of the decrease of the concentration of NO_3^- , the inhibition ability to degrade NO_2^- decreased, so the speed of the reaction was lower than the degradation. Therefore, the GPW is able to have a higher NO_2^- accumulation.

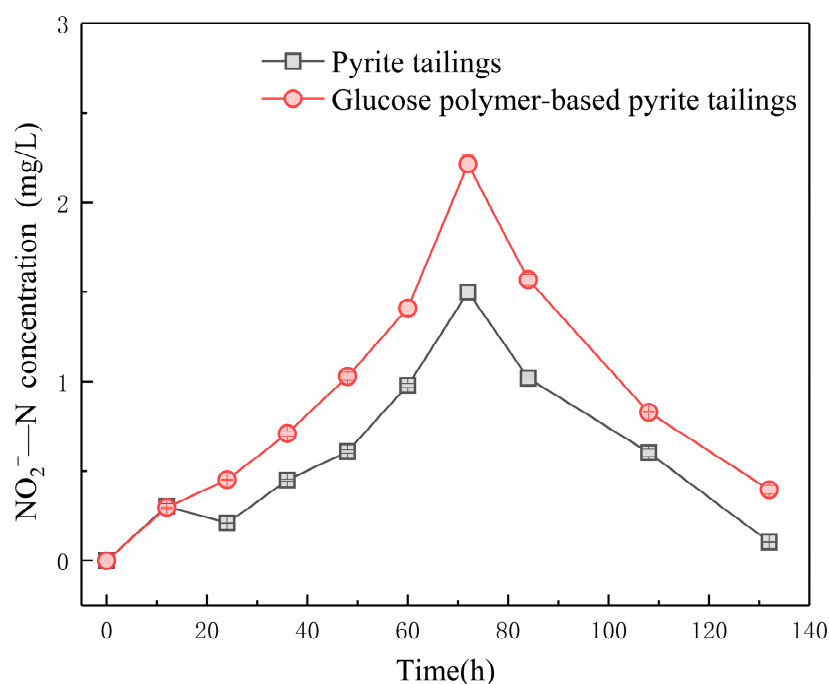


Figure 5. Variation diagram of NO_2^- -N concentration in denitrification system before and after pyrite tailings coating.

As can be seen from the Figure 6, the initial concentration of NH_4^+ -N in the experimental group of PW was 4.50 mg/L and accumulated to 12.60 mg/L in the first 24 h. After that, it decreased to 9.80 mg/L in 24–72 h, increased to 10.50 mg/L in 84 h, and then decreased to 9.50 mg/L in 132 h. This is due to the fact that in the first 24 h, the microorganisms in the solution are at the stage of growth and adaptation, and the solution is in the stage of heterotrophic denitrification and degradation of NH_4^+ during 24–72 h. Heterotrophic denitrification will consume part of NH_4^+ , while the growth of microorganisms will also consume part of NH_4^+ ; thus, the NH_4^+ -N in the solution is in a downward trend at this stage. At 84–132 h, the solution is in the stage of autotrophic denitrification, and autotrophic denitrification will also consume some NH_4^+ . At the same time, the DNRA (dissimilatory nitrate reduction to ammonium) process will take place in the solution, which will transform NO_3^- into NH_4^+ . The initial concentration of NH_4^+ -N in the GPW was 4.90 mg/L, accumulated to 13.20 mg/L in the first 24 h, and then decreased to 8.92 mg/L in 132 h. This is owing to the coated mineral, which was basically in the heterotrophic denitrification stage in 0–132 h, and the heterotrophic denitrification would consume a part of NH_4^+ , and the growth of microorganisms would also consume a part of NH_4^+ at the same time, so the concentration of NH_4^+ -N in the solution was reduced. Therefore, the GPW is capable of reducing the concentration of NH_4^+ -N in water.

3.2.3. Change of Positive Phosphorus (PO_4^{3-} -P) in Effluent

According to Figure 7, the initial concentration of PO_4^{3-} -P in PW is 3.05 mg/L, then decreases below the detection limit at 24 h, and the concentration of PO_4^{3-} -P always remains below the detection limit. The initial concentration of PO_4^{3-} -P in GPW is 3.2 mg/L, then decreases below the detection limit at 24 h, and the concentration of PO_4^{3-} -P also always remains below the subsequent reaction. Obviously, PW and GPW show the same ability to degrade PO_4^{3-} -P, indicating that the glucose-coated system has no significant effect on the removal ability of PO_4^{3-} -P.

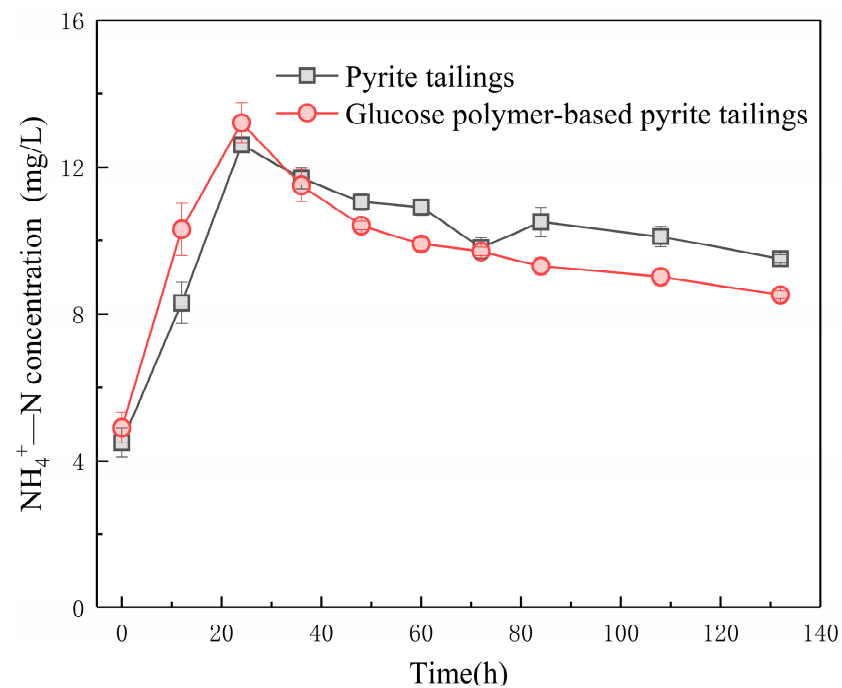


Figure 6. Variation of $\text{NH}_4^+\text{-N}$ concentration in denitrification system before and after pyrite tailings coating.

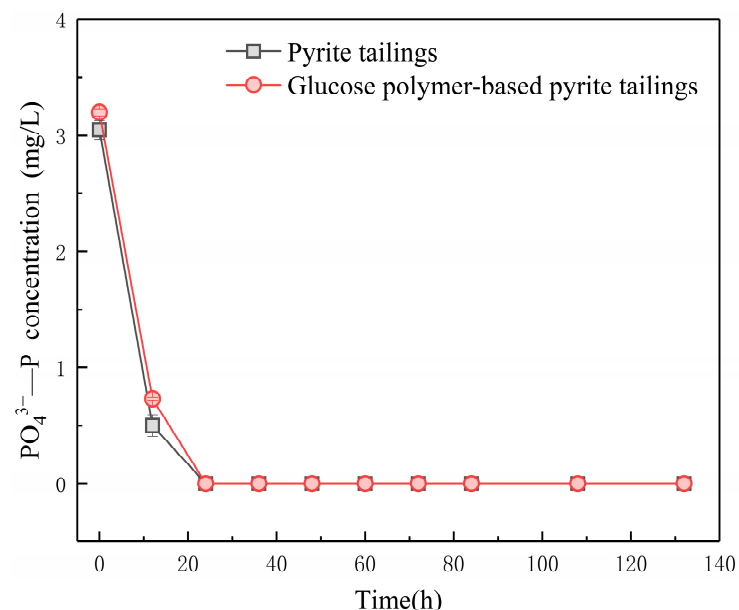
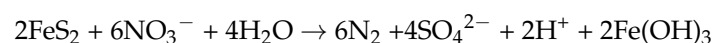


Figure 7. Variation diagram of $\text{PO}_4^{3-}\text{-P}$ concentration in denitrification system before and after pyrite tailings coating.

3.2.4. Change of Effluent Sulfate

The stoichiometry of the autotrophic denitrification reaction with iron tailings as a sulfur source is as follows:



As autotrophic denitrification proceeds, the concentration of SO_4^{2-} increases. As shown in the Figure 8, the initial concentration of SO_4^{2-} in PW is 0 mg/L, which rapidly increases to 67.3 mg/L at 12 h, SO_4^{2-} to 137.6 mg/L at 12–84 h, and then SO_4^{2-} to 163.4 mg/L at 84–132 h, accumulating a total of 96.1 mg/L of SO_4^{2-} between 12–132 h. The

initial concentration of SO_4^{2-} in GPW is 0 mg/L, which is rapidly increased to 47.2 mg/L at 12 h and increases at 125.9 mg/L for SO_4^{2-} at 12–132 h, accumulating a total of 78.7 mg/L of SO_4^{2-} at 12–132 h. It follows that the coated glucose polymer can effectively reduce the accumulation of SO_4^{2-} in water.

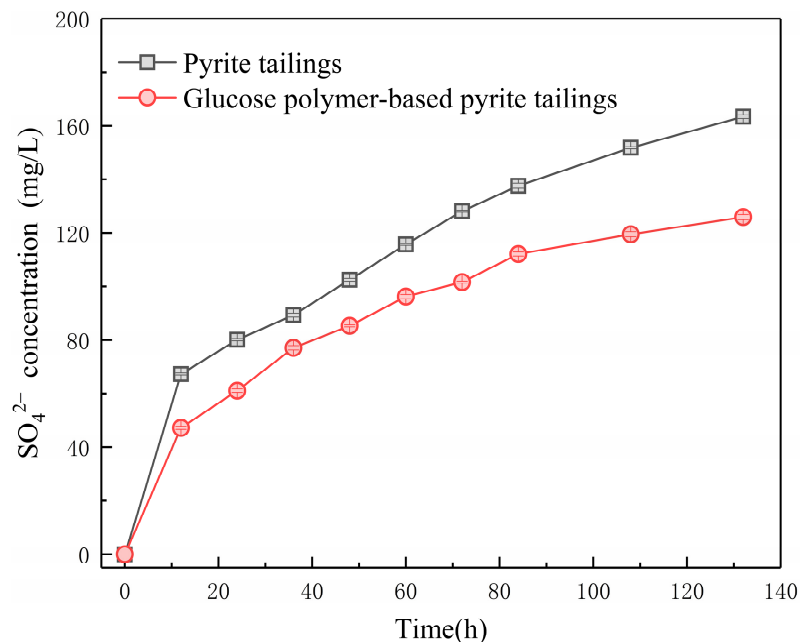


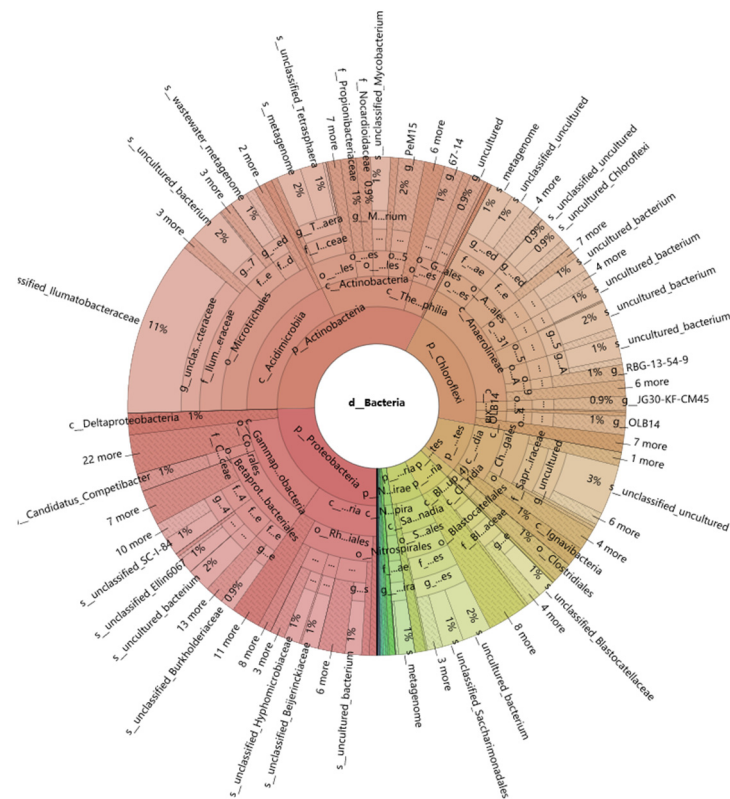
Figure 8. Variation of SO_4^{2-} concentration in denitrification system before and after pyrite tailings coating.

3.3. Study on Nitrogen- and Phosphorus-Removal Mechanism

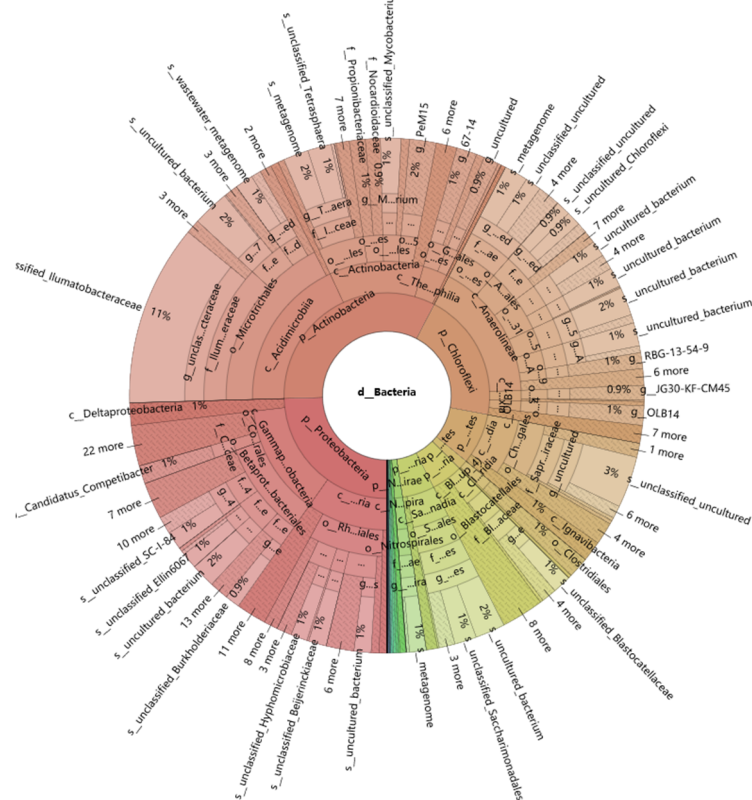
Microbial Community Analysis

In order to deeply understand the process and mechanism of nitrogen and phosphorus removal in pyrite heterotrophic autotrophic denitrification system, the microorganisms in sludge before and after the reaction were sequenced, the microbial community structure was analyzed, and the changes of microbial population and quantity in sludge before and after the reaction were understood.

Figure 9a shows the community structure and relative abundance of microorganisms in the sludge of the secondary sedimentation tank of the sewage plant. It can be seen from the Figure 9a that the dominant bacterial groups in the non-domestic sludge are mainly Actinobacteria, Proteobacteria, and Chloroflexi, and the corresponding abundances are 33%, 25%, and 20%. Several colonies in proteobacteria flora, such as Alphaproteobacteria, betaproteobacteria, and epsilon Proteobacteria, can carry out denitrification reaction. Among them, the microorganisms in betaproteobacteria are mainly heterotrophic denitrifying microorganisms, and the microorganisms in epsilon Proteobacteria are mainly autotrophic denitrifying microorganisms



(a)



(b)

Figure 9. Community structure and relative abundance of microorganisms before domestication (a). Community structure and relative abundance of Proteobacteria microorganisms before domestication (b).

The communities and relative abundances of Proteobacteria microorganisms are shown in Figure 10b. Among them, gammaproteobacteria, Alphaproteobacteria, and deltaproteobacteria are the dominant flora, and the corresponding relative abundances are 54%, 40%, and 6%, respectively. Thiobacillus is a typical chemoautotrophic denitrifying bacterium detected in gammaproteobacteria, which can use pyrite as electron donor for denitrification under anaerobic conditions.

Figure 10a shows the community structure and relative abundance of microorganisms in sludge after experimental reaction. It can be seen from Figure 10a that the main dominant flora in the domesticated microorganisms is Proteobacteria, with a relative abundance of 65%, followed by Bacteroidetes, Firmicutes, and Actinobacteria, with a corresponding relative abundance of 11%, 9%, and 6%, respectively.

Figure 10b shows the community and relative abundance of Proteobacteria microorganisms after the experimental reaction. It can be seen from the Figure 10b that Alphaproteobacteria and gammaproteobacteria are the dominant flora in proteobacteria, with relative abundances of 71% and 17%, respectively.

From the changes of microbial population before and after the experimental reaction, it can be seen that the microorganisms in the sludge after the experiment are mainly Proteobacteria, which increased to 65% compared with 25% before the experiment. The proportion of Actinobacteria in the biological community decreased significantly, indicating that denitrifying bacteria are mainly Proteobacteria and became a dominant population in the community after domestication.



Figure 10. Cont.

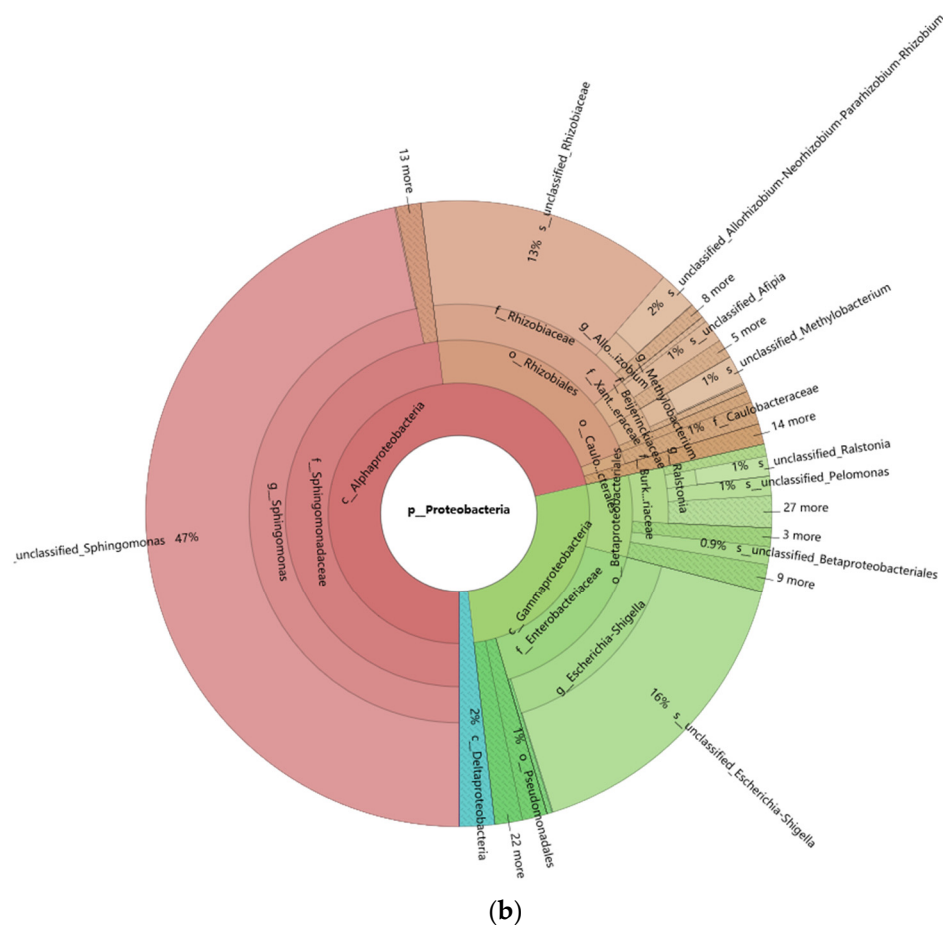


Figure 10. Community structure and relative abundance of microorganisms after domestication (a). Community and relative abundance of Proteobacteria microorganisms after domestication (b).

After the experimental reaction, the relative abundance of phosphorus accumulating bacteria in the microbial population was very low, and the degradation effect of PO_4^{3-} in water was low. It is considered that the effect of microbial removal of PO_4^{3-} in water is poor. Dechloromonas microorganisms were detected in the detection. Dechloromonas can oxidize Fe^{2+} ions in water into Fe^{3+} ions. Fe^{3+} ions in water can combine with PO_4^{3-} to form complex precipitation or hydrolyze to form $\text{Fe}(\text{OH})_3$ precipitation to remove PO_4^{3-} flocculation precipitation.

4. Conclusions

Successful polymerization coating of glucose on the pyrite tailings surface was demonstrated by XRD, FTIR, and zeta potential characterization of the pyrite tailings before and after the modification. Through the study of the effluent quality, it was found that the pH of the modified iron tailings was significantly higher than that before the reaction period, and the removal capacity of the pyrite tailings was improved, and the NO_2^- accumulation amount in the effluent sample at the 132 h monitoring point was not high. In the water sample of the 24 h monitoring point, PO_4^{3-} was completely removed from the two experimental groups of pyrite tailings before and after the modification, and the PO_4^{3-} concentration in water was kept below the detection limit in the subsequent reaction process, indicating that the coating of glucose polymer will not affect the ability of pyrite tailings to remove PO_4^{3-} in water. Moreover, the SO_4^{2-} produced during the modified pyrite tailings reaction is also significantly reduced, indicating that the glucose polymer-coated pyrite tailings can reduce the SO_4^{2-} production.

Author Contributions: Conceptualization, J.M. and S.Z.; formal analysis, J.M., H.C., B.S. and S.Z.; funding acquisition, J.M. and B.S.; investigation, M.H., C.C., M.W. and S.Z.; methodology, H.C., X.W. and S.Z.; project administration, S.Z.; validation, S.Z.; visualization, J.M.; writing—original draft preparation, H.C., X.W., M.H. and M.W.; writing—review and editing, M.H., C.C. and B.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Project of National Key Research and Development Program (2019YFC0408505); National Natural Science Foundation of China (52103104); The Scientific Research Start-up Foundation for Introduction of Talent, Anhui Jianzhu University (2020QDZ30); The Natural Science Research Project of the Higher Education Institutions of Anhui Province (KJ2021A0616); Fujian Provincial Key Laboratory of Fire Retardant Materials, College of Materials, Xiamen University (FH202103); and The 2021 National Innovation Training Program for College Students (202110878024).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rakesh, S.-A.; Santosh, K.-S.; Shweta, S.; Megha, S. Wastewater Treatment by Effluent Treatment Plants. *SSRG Int. J. Civ. Eng.* **2016**, *3*, 29–35.
2. Sun, S.; Lin, H.; Lin, J.; Quan, Z.; Zhang, P.; Ma, R. Underground sewage treatment plant: A summary and discussion on the current status and development prospects. *Water Sci. Technol.* **2019**, *80*, 1601–1611. [\[CrossRef\]](#)
3. Preisner, M.; Neverova-Dziopak, E.; Kowalewski, Z. Analysis of eutrophication potential of municipal wastewater. *Water Sci. Technol.* **2020**, *81*, 1994–2003. [\[CrossRef\]](#)
4. Nicholas, D.-I.; Michael, A.-M.; Lawrence, B.-C.; Douglas, W.-G.; Peter, B.-Z. Nutrient dynamics in a eutrophic blackwater urban lake. *Lake Reserv. Manag.* **2022**, *38*, 28–46.
5. Cheng, X.; Li, S. An analysis on the evolvement processes of lake eutrophication and their characteristics of the typical lakes in the middle and lower reaches of Yangtze River. *Sci. Bull.* **2006**, *51*, 1603–1613. [\[CrossRef\]](#)
6. Lee, S.; Maken, S.; Jang, J.; Park, K.; Park, J. Development of physicochemical nitrogen removal process for high strength industrial wastewater. *Water Res.* **2006**, *40*, 975–980. [\[CrossRef\]](#)
7. Benekos, A.K.; Tsigara, M.; Zacharakis, S.; Triantaphyllidou, I.-E.; Tekerlekopoulou, A.G.; Katsaounis, A.; Vayenas, D.V. Combined electrocoagulation and electrochemical oxidation treatment for groundwater denitrification. *J. Environ. Manag.* **2021**, *285*, 112068. [\[CrossRef\]](#)
8. Song, T.; Zhang, X.; Li, J.; Wu, X.; Feng, H.; Dong, W. A review of research progress of heterotrophic nitrification and aerobic denitrification microorganisms (HNADMs). *Sci. Total Environ.* **2021**, *801*, 14939. [\[CrossRef\]](#)
9. Zhang, Q.; Xu, X.; Zhou, X.; Chen, C. Recent Advances in Autotrophic Biological Nitrogen Removal for Low Carbon Wastewater: A Review. *Water* **2022**, *14*, 1101. [\[CrossRef\]](#)
10. Chang, Y.; Liu, J.; Tang, Q.; Sun, L.; Cui, J.; Liu, X.; Yao, D.; Han, S. Reed Biochar Addition to Composite Filler Enhances Nitrogen Removal from BDBR Systems in Eutrophic Rivers Channel. *Water* **2021**, *13*, 2501. [\[CrossRef\]](#)
11. Chen, D.; Chen, P.; Zheng, X.; Cheng, W.; Wang, Q.; Wei, X. Enhanced Denitrification of Integrated Sewage Treatment System by Supplementing Denitrifying Carbon Source. *Int. J. Environ. Res. Public Health* **2021**, *18*, 9569. [\[CrossRef\]](#)
12. Didem, G. Effects of Different Carbon Sources on Denitrification Efficiency Associated with Culture Adaptation and C/N Ratio. *CLEAN Soil Air Water* **2009**, *37*, 565–573.
13. Neda, M.; Eisa, E.; Seyed, A.; Julie, E. Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc systems with different carbon sources and input C:N ratios. *Aquaculture* **2019**, *512*, 734235.
14. Khanichadecha, W.; Nakaruk, A.; Ratananikom, K.; Eamrat, R.; Kazama, F. Heterotrophic nitrification and aerobic denitrification using pure-culture bacteria for wastewater treatment. *J. Water Reuse Desalination* **2019**, *9*, 10–17. [\[CrossRef\]](#)
15. Wang, H.; Chen, N.; Feng, C.; Deng, Y. Insights into heterotrophic denitrification diversity in wastewater treatment systems: Progress and future prospects based on different carbon sources. *Sci. Total Environ.* **2021**, *780*, 146521. [\[CrossRef\]](#)
16. Fu, X.; Hou, R.; Yang, P.; Qian, S.; Feng, Z.; Chen, Z.; Wang, F.; Yuan, R.; Chen, H.; Zhou, B. Application of external carbon source in heterotrophic denitrification of domestic sewage: A review. *Sci. Total Environ.* **2022**, *817*, 153061. [\[CrossRef\]](#)
17. Wang, S.; Cheng, H.; Zhang, H.; Su, S.; Sun, Y.; Wang, H.; Han, J.; Wang, A.; Guadie, A. Sulfur autotrophic denitrification filter and heterotrophic denitrification filter: Comparison on denitrification performance, hydrodynamic characteristics and operating cost. *Environ. Res.* **2021**, *197*, 111029. [\[CrossRef\]](#)
18. Li, Y.; Liu, L.; Wang, H. Mixotrophic denitrification for enhancing nitrogen removal of municipal tailwater: Contribution of heterotrophic/sulfur autotrophic denitrification and bacterial community. *Sci. Total Environ.* **2022**, *814*, 151940. [\[CrossRef\]](#)
19. Lorna, G.; Juan, P.; Maria, A.; Andrea, B.; Cesar, H.; Silvio, M.; Rafael, B. Autotrophic and heterotrophic denitrification for simultaneous removal of nitrogen, sulfur and organic matter. *J. Environ. Sci. Health Part A* **2016**, *51*, 1159875.

20. Francesco, D.; Francesco, P.; Lens, P.N.L.; Giovanni, E. Electron donors for autotrophic denitrification. *Chem. Eng. J.* **2019**, *362*, 922–937.
21. Kong, Z.; Li, L.; Feng, C.; Dong, S.; Chen, N. Comparative investigation on integrated vertical-flow biofilters applying sulfur-based and pyrite-based autotrophic denitrification for domestic wastewater treatment. *Bioresour. Technol.* **2016**, *211*, 125–135. [[CrossRef](#)]
22. Wang, J.; Xu, L.; Huang, B.; Li, J.; Jin, R. Multiple electron acceptor-mediated sulfur autotrophic denitrification: Nitrogen source competition, long-term performance and microbial community evolution. *Bioresour. Technol.* **2021**, *329*, 124918. [[CrossRef](#)]
23. Deng, S.; Peng, S.; Huu, H.; Sam, J.; Hu, Z.; Yao, H.; Li, D. Characterization of nitrous oxide and nitrite accumulation during iron (Fe(0))- and ferrous iron (Fe(II))-driven autotrophic denitrification: Mechanisms, environmental impact factors and molecular microbial characterization. *Chem. Eng. J.* **2022**, *438*, 135627. [[CrossRef](#)]
24. Mai, Y.; Liang, Y.; Cheng, M.; He, Z.; Yu, G. Coupling oxidation of acid volatile sulfide, ferrous iron, and ammonia nitrogen from black-odorous sediment via autotrophic denitrification-anammox by nitrate addition. *Sci. Total Environ.* **2021**, *790*, 147972. [[CrossRef](#)]
25. Chen, Y.; Shao, Z.; Kong, Z.; Gu, L.; Fang, J.; Chai, H. Study of pyrite based autotrophic denitrification system for low-carbon source stormwater treatment. *J. Water Process Eng.* **2020**, *37*, 101414. [[CrossRef](#)]
26. Oznur, K.; Claudio, C.; Krishna, R.-R. Mine tailing disposal sites: Contamination problems, remedial options and phytocaps for sustainable remediation. *Rev. Environ. Sci. Bio Technol.* **2018**, *17*, 205–228.
27. Klimenko, N.-A.; Savchina, L.-A.; Kozyatnik, I.-P.; Goncharuk, V.-V.; Samsoni-Todorov, A.-O. The Effect of Preliminary Ozonization on the Bioregeneration of Activated Carbon during Its Long-Term Service. *Phys. Chem. Water Treat. Processes* **2009**, *31*, 220–226. [[CrossRef](#)]
28. Goncharuk, V.-V.; Kozyatnik, I.-P.; Klimenko, N.-A.; Savchina, L.-A. Natural Bioregeneration of Activated Carbons in Filters for Add-on Treatment of Drinking Water during Their Continuous Operation. *J. Water Chem. Technol.* **2007**, *29*, 300–306. [[CrossRef](#)]