

## Research Article

# Degradation of High-Concentration Nitrate Nitrogen in Groundwater: A Laboratory Study

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To investigate effective and reasonable methods for the remediation of nitrate nitrogen pollution in groundwater, two groups of laboratory denitrification experiments were conducted: one on the effect of native denitrifying microbes in groundwater and another on the effect of artificially added denitrifying microbes. The water used in the experiment was typical groundwater with a high concentration of nitrate nitrogen. The temperature was controlled at 15°C. Both groups of experiments established four types of culture environments: anaerobic, anaerobic with an added carbon source (glucose), aerobic, and aerobic with an added carbon source (glucose). The results indicated that native denitrifying microbes in the groundwater have almost no ability to remove high concentrations of nitrate nitrogen. However, artificially added denitrifying microbes can effectively promote denitrification. Artificially added denitrifying microbes had the highest activity in an anaerobic environment in which a carbon source had been added, and the rate removal of a high concentration of nitrate nitrogen in groundwater was the highest and reached as high as 89.52%.

## 1. Introduction

Groundwater is an important part of water resources, with a stable quantity of high-quality water. It is one of the important water sources for agricultural irrigation, industry, mining, and urban life. However, with the development of society and economy and the influence of human activities, increasing amounts of environmental pollution problems have gradually been exposed, particularly the pollution of groundwater, whose scope has expanded from point to surface and from shallow to deep. In addition, the number of types of pollutants has been increasing. The degree of pollution is becoming increasingly serious [1–4]. Owing to the discharge and leakage of domestic sewage and substandard industrial wastewater, an unreasonable recharge of sewage, the leaching of fecal and solid waste, the application of a large number of chemical fertilizers and pesticides in rural areas, dry and wet deposition of nitrogen oxides in the

atmosphere, and excessive exploitation of groundwater, the concentration of nitrate nitrogen in groundwater has been increasing, thus, resulting in serious pollution to the environment [5–8]. Groundwater with a high concentration of nitrate nitrogen poses a major threat to human beings and other organisms, since the excessive intake of nitrate results in its reduction to toxic nitrite in the human body [9–11], which can be further transformed into nitrosamines. Nitrosamine is a “three-cause” substance, which easily causes digestive system diseases, methemoglobinemia (blue baby disease), and other problems [12–15].

The problem of nitrate pollution of groundwater has aroused widespread concern in academic circles. The effective remediation of nitrate pollution of groundwater has become the focus of widespread concern. The conventional processes (flocculation sedimentation-filtration-chlorine disinfection) applied for water potability do not eliminate nitrate from the groundwater [16], and special treatment

processes are required to lower the concentration of nitrate to acceptable levels. Currently, the main remediation methods of nitrate pollution in groundwater are physical, chemical, and biological methods. Although there are many methods, each has its own limitations in practical application [17–20]. In terms of eliminating nitrate pollution in groundwater and reducing the cost of denitrification, the biological method is the most effective method that is currently used [21, 22]. This method is characterized by high efficiency and low consumption, so it has been extensively studied. Bioremediation methods primarily rely on the denitrification of microorganisms to remove pollutants from groundwater [23, 24]. Most of the denitrifying microbes involved in the denitrification process are heterotrophic facultative anaerobes, which cannot synthesize organic matter themselves and need to consume additional carbon sources to produce energy and conduct cell synthesis and deoxidation [25]. Currently, the research on bioremediation methods is primarily focused on the selection of denitrifying microbes and carbon sources.

Trudell et al. [26] conducted a large number of experiments that proved that solid organic carbon can also be used as a substrate for denitrification. Schipper et al. [27], Robinson-Lora et al. [28], and Moorman et al. [29] selected solid carbon sources for denitrification experiments and achieved good denitrification results. However, solid carbon sources are mostly natural materials, such as sawdust, rice straw, and bark, and their carbon release is uncontrollable, which easily causes secondary pollution, aquifer blockage, and other problems [30, 31]. Compared with solid carbon sources, liquid carbon sources have a fast dissolution rate and highly efficient reactions. Studies have shown [32–39] that common carbon sources, such as methanol, ethanol, acetic acid, sucrose, and glucose can promote denitrification. In addition, different carbon sources have varying effects on denitrification. There are also many studies on denitrifying microbes [40–43]. Wang et al. [44] successfully isolated denitrifying microbes from groundwater in intensive vegetable growing areas. Zhang et al. [45] isolated denitrifying microbes from soil and verified their ability to perform denitrification. Using a shaking flask experiment, Wang [46] revealed that the groundwater polluted by nitrate contains a large number of indigenous microorganisms, which can degrade the nitrate in the presence of an additional carbon source. Li et al. [47] showed that the sludge of the anaerobic section of a sewage treatment plant contains denitrifying microbes, which are highly active after enrichment and culture. Zhang et al. [37] and Zhang et al. [38, 39] proved that the combination of optimized artificial denitrification agents and common carbon sources such as ethanol can clearly remove nitrate pollution from groundwater. Previous studies have made substantial progress in verifying that both liquid and solid carbon sources can promote denitrification. There is denitrification by indigenous microorganisms in groundwater, which can be enhanced by adding carbon sources to degrade nitrate pollution. Biological bacteria and fungi are beneficial for removing nitrate nitrogen in groundwater. However, research mostly adopts groundwater and synthetic water with low concentrations of nitrate

nitrogen. There are few research results on the comparison of effects of native denitrifying microbes in groundwater with those that have been artificially added on the remediation of groundwater with a high concentration of nitrate nitrogen. In view of these considerations, this study selected glucose as the carbon source and attempted to investigate the effect of the denitrification of native denitrifying microbes in groundwater and artificially added denitrifying microbes under different combinations of oxygen and carbon sources.

## 2. Materials and Methods

**2.1. Sampling.** Studies have shown that, owing to the long-term application of nitrogen fertilizer in vegetable growing areas, the content of nitrate nitrogen in groundwater is very high. The water used in the experiments was taken from the groundwater in a vegetable growing area (36.71565°N, 120.31963°E), and the sampling depth was 5 m. After sampling on the spot, the water was placed into a polyethylene bottle that was washed twice with water samples and sealed with sealing film. The samples were transported to the laboratory in an incubator at 4°C with a built-in ice bag and processed within 24 hours.

**2.2. Enrichment and Cultivation of Sludge.** To investigate the effect of artificial addition of denitrifying microbes on denitrification, the sludge containing denitrifying microbes was collected from the aeration tank of a sewage treatment plant (35.94553°N, 120.21454°E), placed in a polyethylene bottle, transported to the laboratory at 25°C, and treated and cultured within 24 hours.

The nutrient solution was prepared with distilled water, and the main components were KNO<sub>3</sub> (0.37 g/L), C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> (0.1562 g/L), CH<sub>3</sub>OH (320 uL/L), and KH<sub>2</sub>PO<sub>4</sub> (0.044 g/L).

The collected sludge was statically placed, and the supernatant was removed. The sedimentation of the lower layer of the sludge was packed into six 1 L conical bottles, which each contained 700 mL. A total of 350 mL of the nutrient solution was added to each conical bottle and sparged with high-purity nitrogen for 5 minutes. They were then sealed with film designed to maintain freshness and film to seal to maintain the anaerobic environment and cultured at 25°C for 14 days. During the period of cultivation, the nutrient solution was changed every 48 hours. The supernatant in the conical bottle was removed, and then, 350 mL of the newly configured nutrient solution was added and sparged with high-purity nitrogen for 5 minutes. The bottles were sealed with both types of film and cultured at 25°C. A volume of 350 mL of supernatant was discarded from the enriched sludge in each conical bottle, and all the excess sludge in the conical bottle was poured into a 5 L beaker and shaken well for inoculation. The settling ratio (SV%) of the mixed sludge was 90%. The concentration of the mixed sludge was 9838 mg/L. The C/N ratio of the mixed sludge was 16.3.

**2.3. Experimental Design.** In this study, two groups of experiments were designed to investigate the efficiency of denitrification of native denitrifying microbes in

groundwater and artificially added denitrifying microbes. Four conditions were established in each group, which were anaerobic, anaerobic with an added carbon source, aerobic, and aerobic with an added carbon source conditions. The anaerobic conditions were introduced by sparging with high-purity nitrogen for 3 minutes and sealing the bottle cap with sealing film.

Analytically pure glucose was used as the sole carbon source for microbial growth, and its concentration was controlled as 1 g/L. Each time, 100 mL of water samples was taken and placed in 150 mL volume serum bottles. The 15 mL of sludge that was cultured and enriched was inoculated to water. The serum bottle was placed in a BOD constant temperature incubator (American Hash Company, 205-2). The temperature was controlled at 15°C. The water samples were collected, and the indices were determined every 24 hours. The control consisted of three parallel samples. The specific experimental design is shown in Figure 1.

**2.4. Test Index and Determination Method.** The experimental indices include the pH, Oxidation-Reduction Potential (ORP), nitrate nitrogen ( $\text{NO}_3^-$ -N), nitrite nitrogen ( $\text{NO}_2^-$ -N), and ammonia nitrogen ( $\text{NH}_4^+$ -N).

The supernatant of cultured water samples was tested, in which the pH and ORP were determined directly. The water samples were then filtered through a 0.45  $\mu\text{m}$  filter membrane to determine the amounts of nitrate nitrogen ( $\text{NO}_3^-$ -N), nitrite nitrogen ( $\text{NO}_2^-$ -N), and ammonia nitrogen ( $\text{NH}_4^+$ -N). All of the detection methods were conducted in accordance with the national standard method [48], and the main analytical methods are shown in Table 1.

**2.5. Data Processing and Drawing Methods.** The mean and standard deviation of three parallel samples were calculated under each culture condition. Origin software (OriginLab, Northampton, MA, USA) was used to draw the figures.

$$\text{the removal rate of nitrate nitrogen} = \frac{\text{reduced nitrate nitrogen}}{\text{initial nitrate nitrogen}} \cdot 100\% \quad (1)$$

### 3. Results and Discussion

The results of two groups of experiments were compared to determine the effects of native denitrifying microbes in groundwater and denitrifying microbes that were artificially added on denitrification in groundwater with a high concentration of nitrate nitrogen.

**3.1. Removal of Nitrate Nitrogen.** The changes in concentration of nitrate nitrogen in water samples under the action of native denitrifying microbes are shown in Figure 2(a). The trend of change of concentration of nitrate nitrogen in the water samples under the four culture conditions was basically the same and unstable. During the culture period, there was both an increase and a decrease in nitrate nitrogen. The

overall rate of removal increased during the first 5 days of the experiment. It reached its maximum value of 10% on day 4 under the condition of aerobic with an added carbon source. This indicated that denitrification occurred, and the degree of denitrification was the highest on day 4. The nitrate nitrogen only increased on day 3, possibly owing to ammoxidation [49]. The increase was the largest under the condition of anaerobic with an added carbon source, which changed from an initial concentration of 281.81 mg/L to 309.77 mg/L. The overall rate of removal decreased from days 6 to 12 of the experiment as the nitrate nitrogen accumulated. The rate of removal under the anaerobic condition decreased to 0.78% on day 12. In addition, from days 6 to 8, the nitrate nitrogen increased continuously under the condition of anaerobic with an added carbon source. The change in concentration was slightly different from those under the other three conditions. The concentration of nitrate nitrogen under the other three conditions increased at first and then decreased.

As seen in Figure 2(a), the rate of removal of nitrate nitrogen in the water samples was not obvious under the four conditions. The denitrification efficiency cannot be effectively promoted by adding a carbon source and creating an anaerobic condition. The final concentration of nitrate nitrogen was close to that of the initial concentration, and there was basically no removal of nitrate nitrogen. This shows that the native denitrifying microbes in water samples cannot efficiently promote denitrification when glucose is added as a carbon source and whether oxygen is provided or not, so they were unable to reduce the high concentration of nitrate nitrogen in water samples.

In contrast, as shown in Figure 2(b), the denitrification efficiency of artificially added denitrifying microbes in the four culture conditions showed obvious differences with the passage of culture time. The concentration of nitrate nitrogen in the water samples decreased to varying degrees. The efficiency of denitrification was the most effective under the condition of anaerobic with an added carbon source. The rate of removal increased gradually with the duration of the experiment. On day 7, the concentration of nitrate nitrogen decreased from an initial concentration of 266.2 mg/L to 27.9 mg/L, and the rate of removal reached 89.52%. The denitrification effect under the condition of aerobic with an added carbon source was less than that of anaerobic condition in which a carbon source was added. The concentration of nitrate nitrogen decreased gradually with the experiment, and the rate of removal gradually increased. On day 7, the concentration of nitrate nitrogen decreased from the initial concentration of 266.2 mg/L to 103.8 mg/L, and the rate of removal reached 55.16%. Denitrification was not performed effectively under anaerobic or aerobic conditions, and the concentration of nitrate remained basically the same. During the experiment, the concentration of nitrate nitrogen increased and decreased but decreased as a whole. On day 7 under the anaerobic condition, the concentration of nitrate nitrogen decreased from an initial concentration of 266.2 mg/L to 220.3 mg/L, and the rate of removal was 17.24%. The concentration of nitrate nitrogen increased slightly on days 2 and 5 of the experiment, which may be

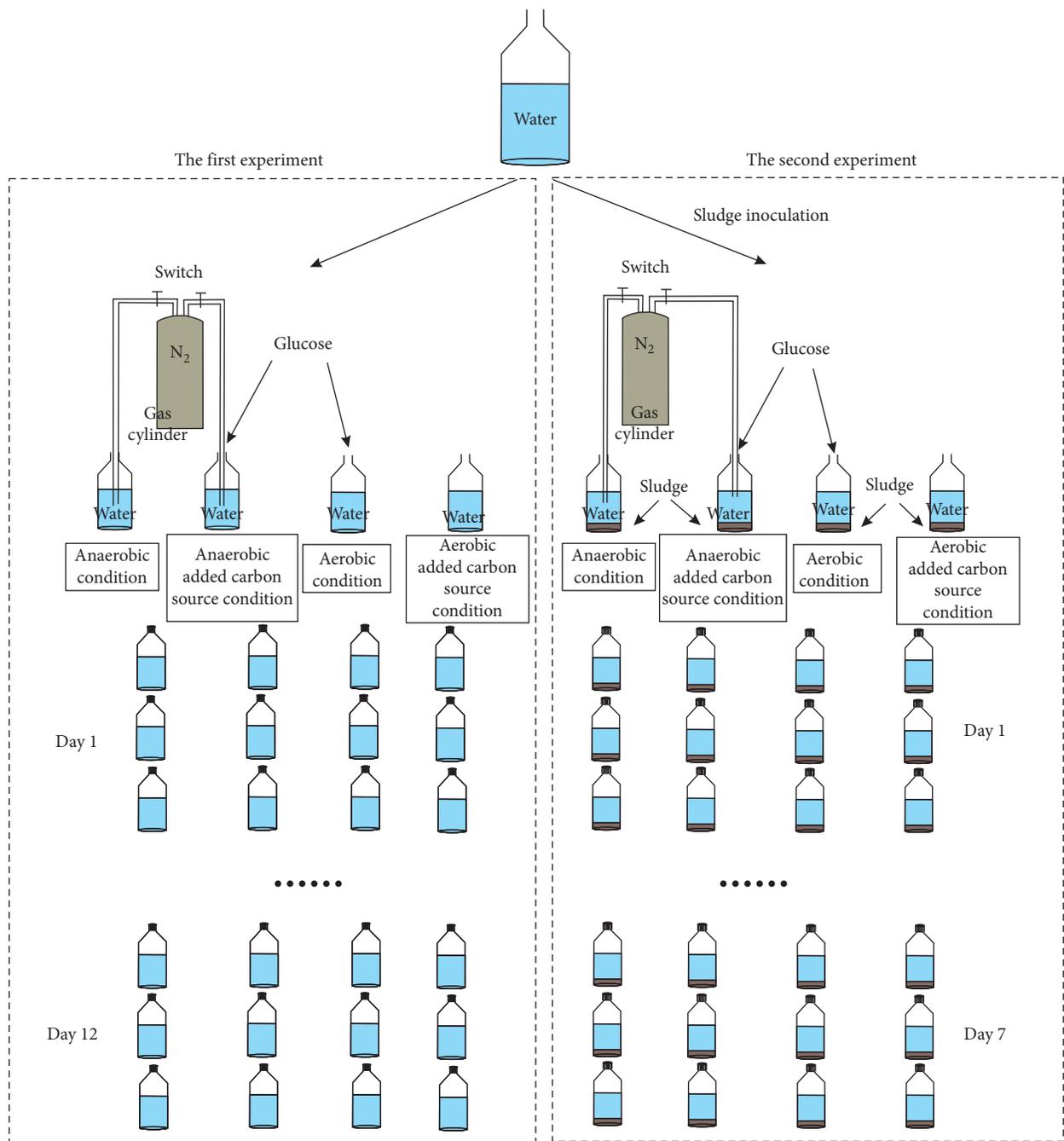


FIGURE 1: Diagram of the experimental design.

TABLE 1: Detection method of test index.

Serial number	Index	Detection method
1	pH	Hash electrode method
2	ORP	Hash electrode method
3	NO <sub>3</sub> <sup>-</sup> -N	Ultraviolet spectrophotometry
4	NO <sub>2</sub> <sup>-</sup> -N	N-(1-naphthyl)-ethylenediamine spectrophotometry
5	NH <sub>4</sub> <sup>+</sup> -N	Nessler's reagent spectrophotometry

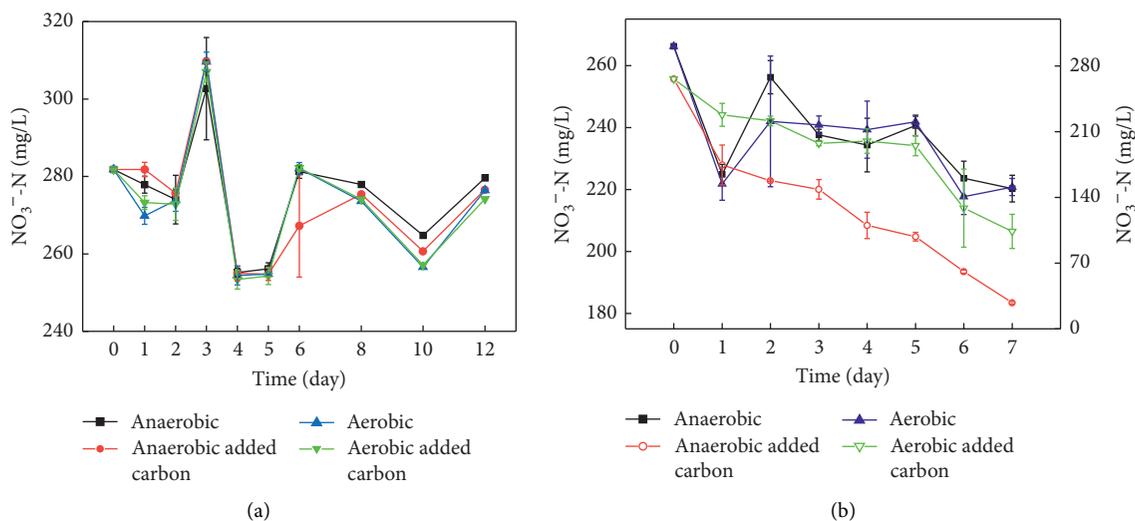


FIGURE 2: Changes in nitrate nitrogen. The solid point is based on the left longitudinal axis, and the hollow point is based on the right longitudinal axis.

owing to ammonification [49]. Under aerobic conditions, the concentration of nitrate nitrogen decreased from an initial concentration of 266.2 mg/L to 217.67 mg/L, and on day 6, the rate of removal was 18.23%. The concentration of nitrate nitrogen increased slightly on days 2, 5, and 7 of the experiment, which may be owing to ammonification [49].

Comparing the experimental results of the two groups, there was a significant difference in the rate of removal of nitrate nitrogen under the influence of the two types of microbes. The rate of removal of nitrate nitrogen in the water samples in which artificially added denitrifying microbes had been added was significantly better than that under the action of native denitrifying microbes. In addition, the maximum rate of removal of nitrate nitrogen under the action of native denitrifying microbe was only 10%. The rate of removal is not obvious, which shows that the native denitrifying microbes in groundwater are not capable of removing high concentrations of nitrate nitrogen in water samples. In addition, large amounts of denitrifying microbes are needed to promote the denitrification effect, so as to achieve the purpose of reducing high concentrations of nitrate nitrogen in groundwater.

In addition, when the changes in concentration of nitrate nitrogen of artificially added denitrifying microbe water samples under four culture conditions were compared, it can be seen that artificially added denitrifying microbes produced the strongest activity under anaerobic condition in which glucose was provided as a carbon source. It was highly effective at denitrifying a high concentration of nitrate nitrogen in groundwater with a rate of removal rate as high as 89.25%. The experimental results showed that for the groundwater with a high concentration of nitrate nitrogen, the introduction of denitrifying microbes and the provision of an anaerobic environment while using glucose as a carbon source could significantly reduce the concentration of nitrate nitrogen in the groundwater. However, the lowest concentration of nitrate nitrogen in the water samples was 27.9 mg/L, which still does not meet the quality standard of

class III groundwater ( $\leq 20$  mg/L). The experimental culture time was too short and did not provide adequate time for the complete denitrification of the nitrate nitrogen.

**3.2. Accumulation of Nitrite Nitrogen.** It can be seen in Figure 3(a) that under the action of native denitrifying microbes, the accumulation of nitrite nitrogen in the water samples under the four culture conditions was not obvious. The concentration of nitrite nitrogen increased at first and then decreased during the experiment. The change in concentration of nitrite nitrogen under the condition of anaerobic with an added carbon source was more obvious than that under the other three conditions. The concentration was the highest on day 12 of the experiment, which increased from an initial concentration of 0.004 mg/L to 0.6 mg/L. The concentration of nitrite nitrogen under the condition of aerobic with an added carbon source changed more gradually than that under the condition of anaerobic with an added carbon source. Additionally, the concentration was highest on day 8, representing an increase from an initial concentration of 0.004 mg/L to 0.123 mg/L. With the progression of the experiment, the concentration of nitrite nitrogen decreased again, and the concentration decreased to 0.057 mg/L on day 12 of the experiment. The change in concentration of nitrite nitrogen under anaerobic and aerobic conditions tended to be consistent, and it was very small. After 12 days of culture, the concentration of nitrite nitrogen under the anaerobic condition changed from 0.004 mg/L to 0.008 mg/L. Under the aerobic condition, the concentration of nitrite nitrogen changed from 0.004 mg/L to 0.009 mg/L.

Thus, it can be seen that under the action of native denitrifying microbes, the accumulation of nitrite nitrogen in water samples under the four culture conditions was not obvious. The concentration of nitrate nitrogen was basically unchanged, which provides additional evidence that the degree of denitrification was not high enough. The native

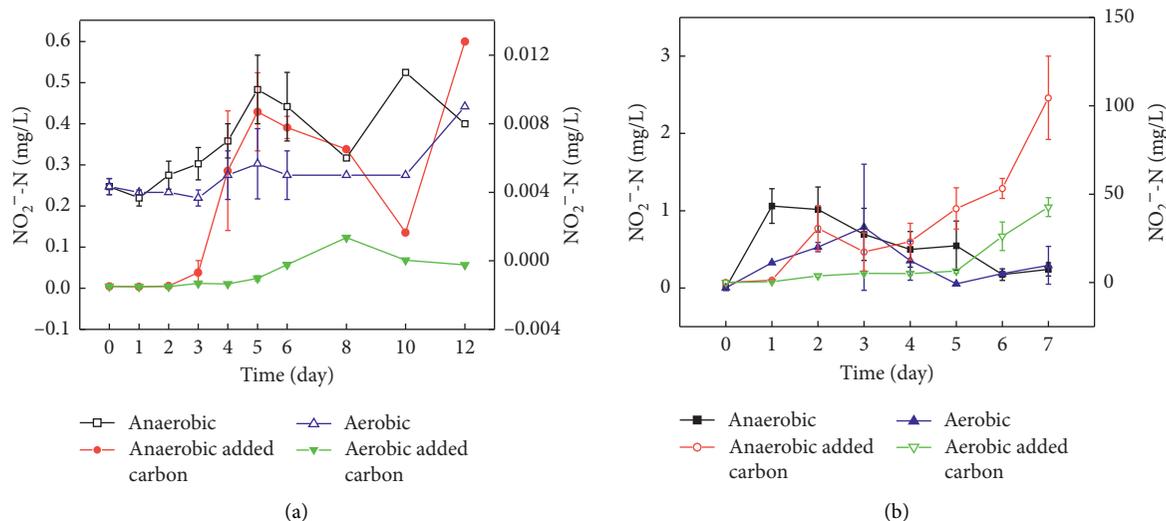


FIGURE 3: Changes in nitrite nitrogen. The solid point is based on the left longitudinal axis, and the hollow point is based on the right longitudinal axis.

denitrifying microbes in water samples failed to effectively promote denitrification under the combined conditions of whether or not glucose was added as a carbon source, and oxygen was provided.

As shown in Figure 3(b), the concentration of nitrite nitrogen in the water samples increased as a whole under anaerobic and aerobic conditions with an added carbon source, and nitrite nitrogen accumulated to greater amounts. The concentration of nitrite nitrogen in the anaerobic condition with an added carbon source increased the most significantly compared with the other three conditions, and the concentration increased from 0.003 mg/L to 104.533 mg/L on day 7 of the experiment. Under the condition of aerobic with an added carbon source, the concentration of nitrite nitrogen increased significantly, from the concentration of 0.003 mg/L to 42.667 mg/L on day 7 of the experiment. The overall change in the concentrations of nitrite nitrogen under anaerobic and aerobic conditions was almost the same, showing a trend of increasing at first and then decreasing. Compared with the original water sample, there was an accumulation of nitrite nitrogen, but it was very low. Under the anaerobic condition, the concentration of nitrite nitrogen was the highest on the first day of the experiment and increased from 0.003 mg/L to 1.061 mg/L. The concentration of nitrite nitrogen decreased again to 0.24 mg/L on day 7 of the experiment. Under the aerobic condition, the concentration of nitrite nitrogen was the highest on day 3 of the experiment, which resulted in an increase from 0.003 mg/L to 0.787 mg/L. The concentration of nitrite nitrogen decreased again to 0.293 mg/L on day 7 of the experiment.

Under the action of artificially added denitrifying microbes, nitrite nitrogen in the water accumulated to varying degrees under the four culture conditions but only slightly under anaerobic and aerobic conditions. The accumulation under the conditions of anaerobic with an added carbon source and aerobic with an added carbon source was more

obvious, particularly under the condition of anaerobic with an added carbon source. Simultaneously, the nitrate nitrogen declined the most quickly under the condition of anaerobic with an added carbon source, which showed that the denitrification effect was the strongest in the water sample under the condition of anaerobic with an added carbon source, and the artificially added denitrifying microbes played a role and effectively promoted denitrification.

Nitrite nitrogen is the intermediate product of the denitrification process, and this compound accumulated during both experiments. In addition, the faster the concentration of nitrate decreased, the more nitrite nitrogen accumulated, which is the incomplete embodiment of denitrification. The reason for this phenomenon could be that the culture time was not long enough; the reaction container was a serum bottle; the volume was fixed, and denitrification was not complete. During the process of denitrification, the rate of action of nitrate reductase is faster than that of nitrite reductase. Under the conditions suitable for denitrification, a large amount of nitrate can be rapidly reduced to nitrite, resulting in a large amount of accumulation. Only when denitrification is complete will the accumulation of nitrite gradually decrease. In addition, the culture temperature of this experiment was set at 15°C. Zhang Lin et al. [50] found that the temperature had a substantial effect on the accumulation of nitrite nitrogen during the process of denitrification, decreasing the activity of the enzyme and inhibiting the transformation of nitrite nitrogen to ammonia nitrogen at 15°C, thus, resulting in the accumulation of nitrite nitrogen. The factors that affect the rate of growth of microorganisms, such as C/N and the type of carbon source, also led to the accumulation of nitrite nitrogen [2, 51–53]. At the beginning of the experiments, the concentration of carbon source was 1 g/L, and the C/N was not well controlled. The lack of a carbon source in the latter stage of the reaction may also lead to the accumulation of nitrite nitrogen. In future experiments, we can choose a larger

reaction container to prolong the continuous culture time and change the concentration of carbon source and the type of carbon source to explore the nutrient carbon source that can enhance the effect of microbial denitrification.

**3.3. Accumulation of Ammonia Nitrogen.** As shown in Figure 4(a), the concentration of ammonia nitrogen in water increased at first and then decreased under the four culture conditions. Additionally, ammonia nitrogen did not obviously accumulate during the experiment. Under the anaerobic condition, the concentration of ammonia nitrogen reached its maximum on day 3 and increased from 0.037 mg/L to 0.39 mg/L. As the experiment continued, the concentration of ammonia nitrogen decreased to 0.163 mg/L on day 5 and tended to be stable. The concentration of ammonia nitrogen was 0.14 mg/L on day 12 of the experiment. Under the condition of anaerobic with an added carbon source, the change of ammonia nitrogen concentration was small, and the trend was relatively moderate. The concentration of ammonia nitrogen reached its maximum on day 2 of the experiment, increasing from 0.037 mg/L to 0.157 mg/L and gradually stabilizing with the experiment. The concentration was 0.07 mg/L on day 12 of the experiment. The concentration of ammonia nitrogen under the anaerobic condition and the condition of anaerobic with an added carbon source barely changed and was less than 0.5 mg/L, which meets the Chinese drinking water standard. Under the aerobic condition, the concentration of ammonia nitrogen increased from 0.037 mg/L to 0.27 mg/L on the first day, then decreased to 0.123 mg/L on day 3, and stabilized from days 3 to 6. With the progress of the experiment, the concentration increased rapidly from days 6 to 8, reached a maximum value of 0.56 mg/L on day 8, decreased gradually, and then decreased to 0.03 mg/L on day 12 of the experiment. Compared with the original water sample, there was no accumulation of ammonia nitrogen. Under the condition of aerobic with an added carbon source, ammonia nitrogen accumulated the most on day 5, increased from the initial concentration of 0.037 mg/L to 0.27 mg/L, decreased to 0 on day 6, and then almost no longer accumulated.

The formation of ammonium suggested that dissimilatory nitrate reduction to ammonia (DNRA) was occurring. DNRA is a side reaction of denitrification and competes with denitrification [54–56]. The balance of denitrification and DNRA depends on temperature, oxygen, nitrate availability, and organic carbon [54, 57]. Some data showed that using glucose as an organic carbon source can easily stimulate the formation of ammonia nitrogen [58]. Ammonia nitrogen is unstable and easily oxidized to nitrate. Under the aerobic condition, the concentration of ammonia nitrogen increased significantly only on the first day and from days 6 to 8. Under the aerobic condition with an added carbon source, the concentration of ammonia nitrogen only increased from days 3 to 5, which could be owing to the decrease in nitrate concentration and the occurrence of DNRA reaction. In addition, the concentration of ammonia nitrogen was less than 0.2 mg/L during the whole experiment. Anaerobic condition with an added carbon source is more conducive to denitrification, less nitrate is dissimilated

to ammonium, and the concentration of ammonia nitrogen was always less than 0.2 mg/L. Under the anaerobic condition, ammonia nitrogen is not easily oxidized, and the concentration of ammonia nitrogen increased many times over the course of the experiment.

As seen in Figure 4(b), the change of ammonia nitrogen under the conditions of anaerobic with an added carbon source and aerobic with an added carbon source was basically the same, and the trend line is relatively smooth, showing a trend of increasing at first and then decreasing. Under the anaerobic condition, the concentration of ammonia nitrogen reached its maximum on day 2 and increased from 0.08 mg/L to 0.54 mg/L. With the progression of the experiment, the concentration decreased gradually to 0.3 mg/L on day 7. Under the condition of aerobic with an added carbon source, the concentration of ammonia nitrogen reached its maximum on day 3 and increased from 0.08 mg/L to 2.4 mg/L. The concentration decreased gradually during the course of the experiment, dropping to 0.617 mg/L on day 7 of the experiment. Under the condition of anaerobic with an added carbon source, the concentration of ammonia nitrogen increased as a whole. It consistently accumulated during the first 3 days of the experiment from an initial concentration of 0.08 mg/L to the maximum concentration of 3.67 mg/L and decreased to 0.67 mg/L on day 4. It then increased gradually with the progress of the experiment, reaching 3.15 mg/L on day 7 of the experiment. Under the aerobic condition, the concentration of ammonia nitrogen increased at first and then decreased. The trend line fluctuated greatly. The accumulation of ammonia nitrogen was the highest on day 2 of the experiment, representing an increase from 0.08 mg/L to 0.92 mg/L. With the progress of the experiment, it decreased to 0 on day 6 and remained stable until the end of the experiment.

Denitrification and DNRA are two processes that exist simultaneously and compete with each other in both aerobic and anaerobic environments. Under the aerobic condition and the condition of aerobic with an added carbon source, ammonia nitrogen obviously accumulated only on day 2 owing to DNRA, but with the progress of the experiment, the ammonia nitrogen was easily oxidized; the concentration decreased gradually, and the final concentration was low. Additionally, the concentration of ammonia nitrogen gradually decreased to 0 under aerobic conditions. On the whole, the accumulation of ammonia nitrogen in the water sample under the condition of anaerobic with an added carbon source was more obvious than that under the other three conditions. Ammonia nitrogen is not easily oxidized, and the DNRA process produces ammonia nitrogen, while glucose as a carbon source readily stimulates the formation of ammonia nitrogen [58]. However, not much of this compound accumulates. Ammonia oxidation [49] may have only occurred on day 4, and the concentration of ammonia nitrogen decreased. Under anaerobic conditions, ammonia nitrogen is not easily oxidized. However, owing to the addition of denitrifying bacteria, the amount of denitrification is relatively strong. DNRA only occurs at the beginning of the experiment, and a small amount of ammonia nitrogen accumulates.

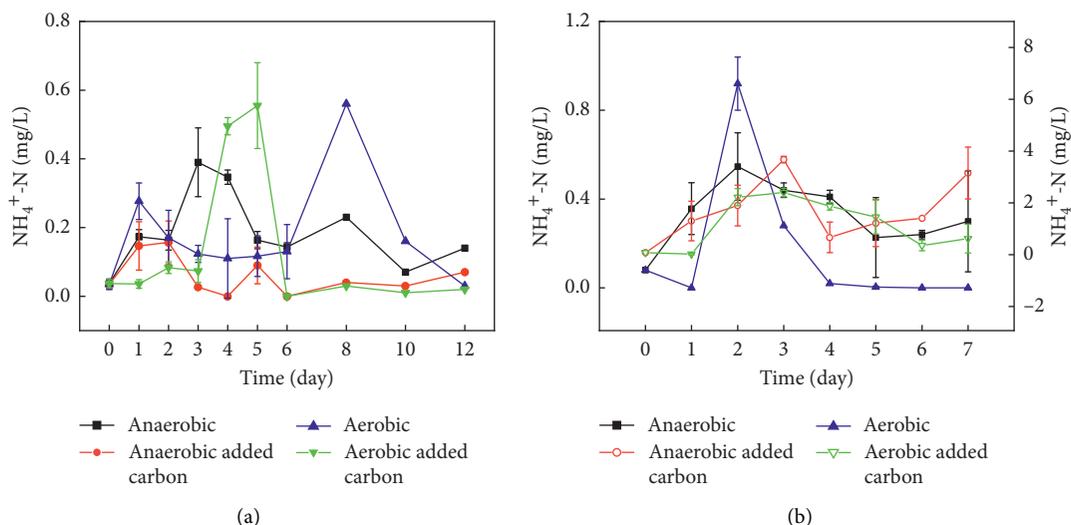


FIGURE 4: Changes in ammonia nitrogen. The solid point is based on the left longitudinal axis, and the hollow point is based on the right longitudinal axis.

At the beginning of the experiment, ammonia nitrogen accumulated to varying degrees under the four culture conditions, which may be owing to the sufficient dissolved organic matter or added carbon source in the water sample at the initial stage, and the dissimilation of nitrate nitrogen to ammonia nitrogen under the action of artificially added denitrifying microbes and native denitrifying microbes in water. In the latter stage, the denitrification effect gradually became strong, the effect of DNRA was weakened, and the difference of the four culture conditions led to a difference in the final accumulation of ammonia nitrogen.

In the two groups of experiments, the accumulation of ammonia nitrogen was not obvious as a whole, and there was only a certain accumulation of ammonia nitrogen in the water when denitrifying microbes were added artificially under the condition of anaerobic with an added carbon source. It is hypothesized that the reason for this phenomenon could be that the DNRA process and the existence of microorganisms and anaerobic conditions with an added carbon source promote the activity of microorganisms and increase the rate of release of ammonia nitrogen.

**3.4. Changes in pH.** The changes in pH in water under four culture conditions are shown in Figure 5(a). The change of pH under anaerobic and aerobic conditions was basically the same, and the whole trend increased at first and then decreased. On the first day, the water samples changed from weakly acidic to weakly alkaline and maintained this alkaline environment throughout the experiment. Combined with the change in nitrate nitrogen, denitrification occurred at the beginning of the experiment, the production of alkaline substances increased the pH, and the water sample was weakly alkaline [59]. There was a sudden increase in pH from day 6 to 8 of the experiment. The pH increased to 8.24 under anaerobic conditions and to 7.85 under aerobic conditions. Combined with the change in nitrate nitrogen,

this may be owing to the enhancement of denitrification and the production of alkaline substances [59]. The pH value increased. With the progress of the experiment, the pH gradually decreased. On day 12 of the experiment, the pH decreased to 7.36 under anaerobic conditions and to 7.11 under aerobic conditions. The natural denitrifying microbes in the water samples can produce organic acids [59] by biochemical reactions with the help of original organic matter, resulting in a gradual decrease in the pH. The change in pH was basically the same under the conditions of anaerobic with an added carbon source and aerobic with an added carbon source. On the first day of the experiment, the water samples changed from weakly acidic to weakly alkaline, but the trend line was relatively smooth. Additionally, the water sample was weakly alkaline all of the time. On day 12 of the experiment, the pH was 7.05 under the condition of anaerobic with an added carbon source and 7.11 under the condition of aerobic with an added carbon source. Combined with the change in nitrate nitrogen, denitrification occurred at the beginning of the experiment, and the production of alkaline substances increased the pH. The water sample was weakly alkaline [59].

As shown in Figure 5(b), the change in pH under anaerobic and aerobic conditions was basically the same. The trend line was relatively flat, and the value changed from 6.68 to 7.5. The water sample was weakly alkaline. Under the condition of anaerobic with an added carbon source, the change in pH was relatively smooth. The value always fluctuated around 6.68, but it was always less than 7, and the water sample was weakly acidic. Under the condition of aerobic with an added carbon source, the pH fluctuated greatly, and the value changed from 6.68 to 8.2. The sample fluctuated from 5 to 8.5. Owing to the alkaline substance produced by denitrification [59], the water sample changed from weakly acidic to weakly alkaline on the first day. It became acidic from day 2 to 5, which may be owing to the strong biochemical effect of microbes using external a carbon source

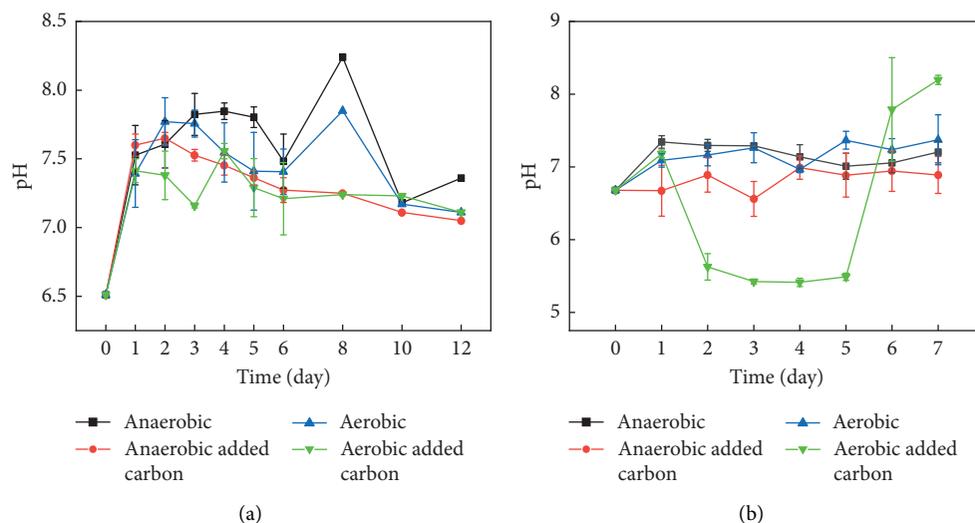


FIGURE 5: Changes in pH.

and the production of more organic acids [59]. In addition, the pH was between 5 and 6. It became weakly alkaline from day 6 to 7. Combined with the change in nitrate nitrogen, the amount of denitrification was stronger during this period, resulting in an increase in the pH.

Denitrification itself can increase the pH by releasing  $\text{CO}_2$  and hydroxide ( $\text{OH}^-$ ). Normally, these combine to yield  $\text{HCO}_3^-$ , but if the production of  $\text{OH}^-$  exceeds that of  $\text{CO}_2$ , the pH can increase [60]. The dynamic balance of alkaline substances produced by denitrification and organic acids produced by biochemical reactions will affect the change in the pH of the system.

Under the action of artificially added denitrifying microbes, combined with the changes in nitrate nitrogen, the changes in pH in water samples as a whole were visible. Under anaerobic and aerobic conditions, the water sample was maintained in a weakly alkaline environment, which could be that more alkaline substances were produced by denitrification than organic acids produced by biochemical action [59]. And it was in a relatively stable state, so that the pH value of the water sample was more stable. Under the condition of anaerobic with an added carbon source, the water sample was maintained in the weakly acidic environment. The reaction was relatively strong, and more organic acids were produced following the addition of glucose as the carbon source. Additionally, denitrification was very strong, and the two effects were in a state of equilibrium to ensure that the water sample maintained a weakly acidic environment. The substantial change of pH value under the condition of aerobic with an added carbon source may be owing to the instability of denitrification and biochemical reactions under these culture conditions.

Too high or too low a pH value will affect the denitrification effect. Although denitrification still occurs when the pH value exceeds the optimal range, it may lead to the accumulation of intermediate toxic products (such as nitrite) [50]. When the changes of pH in the two groups of experiments were compared and connected with the changes

in nitrate nitrogen and nitrite nitrogen concentration under different conditions, a pH value between 6.5 and 7 was found to be suitable for the growth and reproduction of denitrifying microbes. It aided in denitrification.

**3.5. Changes in the ORP.** As shown in Figure 6(a), the change of ORP under anaerobic and aerobic conditions was basically the same and showed a downward trend as a whole. The ORP fluctuated greatly from days 4 to 10 of the experiment. On day 12 of the experiment, the ORP decreased from 211.2 mV to 132 mV under anaerobic conditions. In addition, the ORP decreased from 211.2 mV to 146.1 mV under aerobic conditions. Under the conditions of anaerobic with an added carbon source and aerobic with an added carbon source, the change in ORP was basically the same. It showed a downward trend as a whole, and the trend line in the later stage of the experiment was relatively flat. On day 12 of the experiment, the ORP decreased from 211.2 mV to 149.4 mV under the condition of anaerobic with an added carbon source, and the ORP decreased from 211.2 mV to 154.4 mV under the condition of aerobic with an added carbon source.

The ORP can characterize the relative degree of oxidation or reduction. When the ORP is positive, the solution can oxidize to some extent. When the ORP is negative, the solution shows some degree of reduction. Its numerical change reflects the relative change of oxidation and reduction. The ORP decreased on the first day of the experiment. Combined with the change in nitrate nitrogen, it showed that denitrification occurred, the ability to oxidize decreased, and reduction occurred in the system. With the progress of the experiment, the ORP fluctuated and gradually stabilized, indicating that denitrification and ammonia oxidation occurred in the system. Denitrification is a reduction and will reduce the ability to oxidize and ORP. Ammonia oxidation consists of oxidation, which will increase the ability to oxidize and ORP. The change in ORP reflected the relative degree of denitrification and

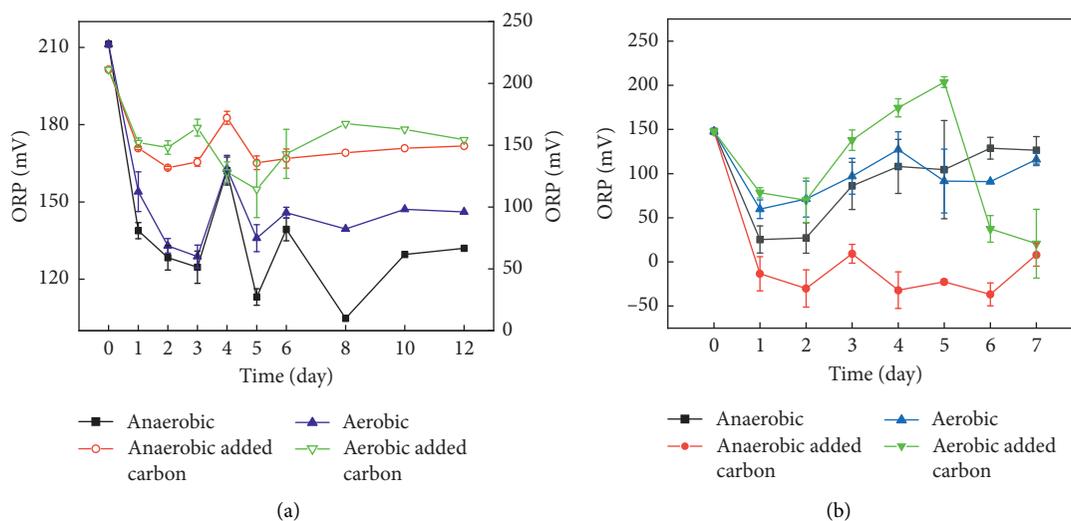


FIGURE 6: Changes in ORP. The solid point is based on the left longitudinal axis, and the hollow point is based on the right longitudinal axis.

ammoxidation. Thus, it can be seen that there was some ability to oxidize in the water under the four culture conditions. Denitrification occurred in the water, but it was not strong enough to make the water sample reductive.

As shown in Figure 6(b), the changes of ORP under anaerobic and aerobic conditions were basically the same, showing a trend of decreasing at first and then increasing. Under anaerobic conditions, the ORP decreased from 147.8 mV to 27.1 mV on the first day, and the tendency to oxidize in the water sample decreased. It then gradually increased with the progress of the experiment. The ORP increased to 126.33 mV on day 7. Under aerobic conditions, the ORP decreased from 147.8 mV to 59.7 mV on the first day, and the tendency of the water sample to oxidize decreased. It then gradually increased with the progress of the experiment. The ORP increased to 116.03 mV on day 7. Combined with the changes of nitrate nitrogen under these two conditions, denitrification was strong at the beginning of the experiment owing to the artificial addition of denitrifying microbes. The ORP decreased rapidly on the first day, and the ability of the water sample to oxidize decreased. With the progress of the experiment, ammoxidation occurred in the system to enhance the ability of the water to oxidize, and the ORP gradually increased. Simultaneously, there was no carbon source added. Because of the lack of organic carbon in water, the weakening of denitrification may also lead to an increase in ORP.

Under the condition of aerobic with an added carbon source, the ORP decreased during the first 5 days and then increased. At the beginning of the experiment, owing to the artificial addition of the denitrifying microbes and the addition of a carbon source, the process of denitrification was strong. Additionally, the ORP decreased rapidly on days 1 and 2, and the ability of the water sample to oxidize weakened. In the later stage, the aerobic environment enhanced the occurrence of ammoxidation, the ORP increased, and the ability to oxidize increased. The ORP then quickly decreased to 20.6 mV from days 6 to 7. Combined with the changes in nitrate nitrogen,

denitrification was enhanced, and the ability of water samples to oxidize decreased during this period. Under the condition of anaerobic with an added carbon source, the changes in the ORP showed a downward trend as a whole, which decreased rapidly to a negative value on the first day. The trend line changed smoothly from days 2 to 7. Most of the ORP had a negative value. In the reaction system, denitrification was the strongest, ammoxidation was relatively weak, and the water samples showed a tendency to be reduced.

The ORP value reflected the redox property to some extent. A comparison of the data of two groups of experiments indicated that the water sample with artificially added denitrifying microbes showed some degree of reducibility under the condition of anaerobic with an added carbon source, which was beneficial to denitrification.

The experimental results showed that with the effect of the artificial addition of denitrifying microbes, the denitrification effect was the most effective under the condition of anaerobic with an added carbon source, which could effectively reduce the content of nitrate nitrogen. During the course of the experiment, the amount of nitrate nitrogen continuously decreased, and the rate of removal was the highest. Both nitrite nitrogen and ammonia nitrogen accumulated. Ammonia nitrogen accumulated to a lower degree, and the accumulation of nitrite was obvious. Sludge as denitrifying microbe can effectively promote denitrification.

Future areas of research will involve how to reduce the accumulation of nitrite nitrogen at the same time as removing the nitrate nitrogen and completely promoting denitrification and how to better apply bioremediation to the actual remediation of groundwater pollution.

#### 4. Conclusions

Through two groups of laboratory experiments, this paper explored the feasibility and effectiveness of native denitrifying and artificially added denitrifying microbe sat

removing high concentrations of nitrate nitrogen in groundwater under different combinations of oxygen and carbon source.

Under the setting of four culture conditions, the concentration of nitrate nitrogen in the water sample that was subjected to native denitrifying microbes basically changed the same amount, and the change was unstable. There were processes of both reduction and an increase in nitrate nitrogen. In addition, the highest rate of removal of nitrate nitrogen was only 10%. This percentage is inadequate for remediation effects. The native denitrifying microbes in the water lacked the ability to remove the high concentration of nitrate nitrogen in the water sample.

Under the same four culture conditions, the concentration of nitrate nitrogen in water samples to which denitrifying microbes were artificially added changed significantly, and they decreased significantly with the increase in culture time. The change in concentration of nitrate nitrogen and the changes in nitrite nitrogen, ammonia nitrogen, pH, and ORP can reflect the ability of denitrifying microbes to remove nitrate nitrogen in the water under the four culture conditions. The removal was most effective under the condition of anaerobic with an added carbon source, and the rate of removal reached as high as 89.52%. In addition, the rate of removal under the condition of aerobic with an added carbon source was more effective, and the rate of removal was 55.16%. The rate of removal under anaerobic and aerobic conditions was not very high, and both the removal rates were less than 20%. Therefore, the artificially added denitrifying microbes have higher activity and a more significant effect under anaerobic conditions with the provision of glucose as a carbon source, which can effectively promote denitrification in water and reduce the concentration of nitrate nitrogen in the water.

Compared with the changes in five indices in water treated by microbes from different sources under the same culture condition, artificially added denitrifying microbes functioned significantly better than the native denitrifying microbes in water samples, which could better promote denitrification.

## Data Availability

The raw data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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