The screening of high-efficiency ammonia-nitrogen degrading bacteria and their influencing factors were studied

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Abstract. By enriching the culture medium, three dominant bacteria(X₁, X₂ and X₃) were selected from the farmland sludge. The degradation effect of X₁, X₂, X₃ and mixed bacterium(X₁₂₃) under different initial ammonia nitrogen(NH₃-N) concentration, pH and C/N were studied under laboratory, the effect of X₁₂₃ was compared with purchased bacteria(S₁, S₂ and S₃) and strains in published literature. The results showed that X₁₂₃ had the best effect when the initial NH₃-N concentration was 20 mg L⁻¹, pH was 7 and C/N was 10, the degradation rate was 96.4% after 2 days of culture. Comparing X₁₂₃ with S₁, S₂ and S₃, it was found that X₁₂₃ had the best effect. Comparing X₁₂₃ with the strains in published literature, it can be seen that the difference for the degradation of NH₃-N was very small, and the growth conditions of the strain were almost same. It was shown that X₁₂₃ can effectively degrade NH₃-N from wastewater, which has potential of application for treatment of nitrogen polluted wastewater.

1. Introduction

Nitrogen pollution is one of the important components of water pollution. A large number of nitrogen-containing compounds was discharged from industrial and agricultural production, causing the problem of nitrogen pollution in the water environment. According to The Bulletin on China’s ecological environment in 2019, among the 1,610 water quality sections monitored by rivers across the country, 3.0% were inferior to Grade V [1]. The main pollution indicators were COD, MnO₄⁻, and NH₃-N. Among the 107 important lakes (reservoirs) monitored, 9.4% were in poor nutrition state, 62.6% in medium nutrition state and 28% in eutrophic state. Excessive nitrogen concentration in the water not only destroyed the soil planting ability, but also caused the eutrophication of water, which will cause algae to proliferate, reduce dissolved oxygen, reduce transparency, increase turbidity and chromaticity, and seriously threaten the survival of aquatic animals [2-9]. NH₃-N is the main component of nitrogen pollution, and also the main pollution factor in water pollution.

The methods for treating nitrogen-polluted wastewater are divided into physical and chemical denitrification methods and biological denitrification methods. The physical and chemical methods [10-11] mainly include ion exchange method, breakpoint chlorination method, electrochemical method, etc. However, the physical and chemical methods not only need a large amount of capital and floor space, but also cause secondary pollution, which is difficult to achieve the effect of removing NH₃-N. Notwithstanding the biological denitrification method [12-18] uses the assimilation or dissimilation by
the microorganisms to convert nitrogen pollution into gaseous nitrogen and discharge it into the atmosphere to achieve the purpose of nitrogen removal. Compared with physical-chemical denitrification, biological denitrification is easy to operate, relatively stable in effect, and does not cause secondary pollution. Environmental microorganisms are the main workers of biological nitrogen removal, and the activity of environmental microorganisms is also the main factor affecting the efficiency of biological nitrogen removal. Therefore, it is very important to seek efficient and stable environmental microorganisms for biological nitrogen removal. The growth characteristics of environmental microorganisms in different regions are different, so screening local dominant strains is more adaptive and can achieve better degradation effects. This experiment will screen for native dominant bacteria and study the effects of environmental impact factors on their degradation performance of NH₃-N, and the dependence of degradation efficiency on initial NH₃-N concentration, pH and C/N were examined, in order to find the best survival conditions for dominant bacteria, to lay a foundation for the engineering application of the bacterium and theoretical study of NH₃-N degradation pathways.

2. Materials and methods

2.1. Experimental materials

2.1.1. Sample source. The bacterium selected in this experiment were collected from a rice farmland sludge in Lishui Town, Foshan, Guangdong, China.

2.1.2. Culture medium [19]. The enrichment medium contained 5000 mg·L⁻¹ of CaH₂O₆, 500 mg·L⁻¹ of (NH₄)₂SO₄, 1000 mg·L⁻¹ of K₂HPO₄, 300 mg·L⁻¹ of MgSO₄·7H₂O, 300 mg·L⁻¹ of NaCl, 30 mg·L⁻¹ of CaCl₂, 30 mg·L⁻¹ of FeSO₄·7H₂O, pH 7. The separation medium was made on the basis of the enrichment medium to which agar powder was added (1.5-2%, mass fraction). The activation medium contained 10 g·L⁻¹ of NaCl, 5 g·L⁻¹ of Yeast extract fermentation, 10 g·L⁻¹ of Casein tryptone, pH 7. The screening medium contained 5000 mg·L⁻¹ of CaH₂O₆, 470 mg·L⁻¹ of (NH₄)₂SO₄(100 mg·L⁻¹ NH₃-N), 1000 mg·L⁻¹ of K₂HPO₄, 300 mg·L⁻¹ of MgSO₄·7H₂O, 300 mg·L⁻¹ of NaCl, 30 mg·L⁻¹ of CaCl₂, 30 mg·L⁻¹ of FeSO₄·7H₂O, pH=7.

2.2. Experimental methods

2.2.1. Enrichment of bacteria. In this experiment, 1g of sludge was put into 100 mL enrichment medium and cultured in a shaker at 30 °C and 140 r·min⁻¹ for 3 days, and the culture was observed.

2.2.2. Bacteria coating separation. We coated the culture solution in the enrichment medium into the separation medium, 3 replicates for each dilution concentration, and then placed the coated culture medium in 30 °C incubator for 3 days. Finally, we selected different bacteria for streaking.

2.2.3. Bacteria purification. We isolated different bacteria from the appropriate diluted medium, as the initial bacterium, marked it, and then streaked each bacterium in the new separation medium, cultivated it for 3 days in incubator at 30 °C. After 3 days, we streaked the bacterium into a new separation medium, cultured in 30 °C incubator for 3 days, repeated the operation more than two times to improve the purity of the strains and ensure that there was only single bacterium in each medium, then transferred bacteria to slant medium for storage.

2.2.4. Screening of dominant bacterium. We moved the screened bacteria to the activation medium, cultured it for 2 days in a shaker at 30 °C and 140 r·min⁻¹, then took 10 mL of the culture liquid and centrifuged it for 5 min (3000 r·min⁻¹), and then removed the supernatant. The obtained pellet was washed 2-3 times with physiological saline and centrifuged, and the centrifuged product was inoculated 3% to 100 mL of screening medium, each strain was repeated three times, and cultured for
2 days. We measured the average concentration of NH$_3$-N in the medium every day, and calculated the degradation rate of NH$_3$-N.

2.2.5. Analysis of the effects of dominant bacteria and purchased strain on degradation of NH$_3$-N. Three kinds of NH$_3$-N degrading bacteria were purchased from the market, namely autotrophic nitrifier solution $S_1$, nitrifier powder $S_2$ and heterotrophic nitrifier solution $S_3$. The dominant bacterium and the purchased strain were cultured in a shaker at 30 °C and 140 r·min$^{-1}$ for 2 days, then we took 10 mL of the culture liquid and centrifuged it for 5 min (3000 r·min$^{-1}$), and then removed the supernatant. The obtained pellet was washed 2-3 times with physiological saline and centrifuge, and the centrifuged product was inoculated 3% into 20 mg·L$^{-1}$ and 106 mg·L$^{-1}$ NH$_3$-N wastewater, each strain was repeated three times, and cultured for 2 days. We measured the average concentration of NH$_3$-N in the medium every day, and calculated the degradation rate of NH$_3$-N.

2.2.6. The influence of different environmental factors on the degradation of NH$_3$-N by dominant bacteria. There are many factors that affect the performance of bacteria to degrade NH$_3$-N, such as initial concentration of NH$_3$-N, pH, C/N and so on. If the concentration is too low, the lack of nutrients causes growth inhibition. If concentration was too high, the environment deteriorates and the growth is hindered [20-21]; pH is known to affect both the growth of bacteria and the effectiveness of denitrification [22]; C/N is also one of the main factors, because when it is too low, there is insufficient carbon source. The growth and development of the bacteria is inhibited, making the bacteria unable to carry out reaction normally. On the other hand, too high C/N would cause a waste of resources [23]. Therefore, finding suitable initial concentration of NH$_3$-N, pH and C/N for bacteria growth is of great significance to the denitrification performance of strain.

2.2.6.1. The initial concentration of NH$_3$-N. 3% of dominant bacteria was inoculated into 20, 50, 100, 150, 200, 300 mg·L$^{-1}$ NH$_3$-N wastewater respectively, each strain was repeated three times, cultured them in a shaker with a 30 °C and 140 r·min$^{-1}$ for 2 days, measured the average concentration of NH$_3$-N, and calculated the degradation rate of NH$_3$-N.

2.2.6.2. The Initial pH. We adjusted the pH of the wastewater ($C_{NH_3-N}$=20 mg·L$^{-1}$) with 0.1 mol·L$^{-1}$ HCl and NaOH to pH of 3, 5, 7, 9 and 11 respectively. 3% of dominant bacteria was inoculated in the wastewater, each strain was repeated three times, and cultured for 2 days. We measured the average concentration of NH$_3$-N, and calculated the degradation rate.

2.2.6.3. The Initial C/N. We changed the concentration of C$_6$H$_5$O$_6$ in the wastewater ($C_{NH_3-N}$=20 mg·L$^{-1}$) to make the C/N of 2, 5, 10, 15 and 20 respectively. 3% of dominant bacteria was inoculated in the wastewater, each strain was repeated three times, and cultured for 2 days. We measured the average concentration of NH$_3$-N in the wastewater, and calculated the degradation rate.

2.2.7. Detection method. NH$_3$-N was determined by Nessler's reagent spectrophotometry.

3. Results and discussion

3.1. Bacteria screening results
In this experiment, a total of nine purified single bacterium were screened out and named X$_1$-X$_9$ respectively.

3.2. Screening of dominant bacterium
The X$_1$-X$_9$ were respectively inoculated into the screening medium. After cultivating for 2 days, the degradation rate of NH$_3$-N was shown in Figure 1. Among them, the degradation rates of X$_1$, X$_2$ and
X₁ were relatively high, 51.1%, 48.7% and 57.3% respectively, so X₁, X₂, and X₃ were selected for subsequent testing.

We inoculated X₁, X₂, X₃ and X₁23 into 20 mg-L⁻¹ and 106 mg-L⁻¹ NH₃-N wastewater respectively. The change in concentration of NH₃-N after 2 days was shown in Figure 2. As it can be seen from Figure 2, at the concentration of 20 mg-L⁻¹, the X₁23 was the most efficient in NH₃-N degradation, with a degradation rate of 96.4%. The effect of single bacterium was obviously lower than that of mixed bacterium, among which X₃ had the best effect, its degradation rate was 90.7%. At the concentration of 106 mg-L⁻¹, X₁23 also had the best effect, the degradation efficiency was only 69.7% because the concentration was too high. The effect of single bacterium was not ideal, of which X₃ effect was relatively good, degradation rate of 57.3%.

3.3. **Comparative analysis of degradation effects between X₁23 and purchased bacteria**

The degradation effect of X₁23 and S₁, S₂, S₃ in wastewater after 2 days of culture was shown in Figure 3. As it can be seen from Figure 3, when the initial NH₃-N concentration was 20 mg-L⁻¹, the degradation was more efficient. The most pronounced effect was observed for X₁23, displaying degradation rate 96.4%. The degradation effect was not ideal at the concentration of 106 mg-L⁻¹, where the degradation effect of S₂ was relatively low, was 38.2%.

3.4. **The influence of different environmental factors on the degradation of NH₃-N by X₁23**

3.4.1. **The initial NH₃-N concentration.** Figure 4 shows the degradation effect of X₁23 at different initial concentration. It can be seen that the higher the initial NH₃-N concentration, the worse the degradation effect, when the NH₃-N concentration was 300 mg-L⁻¹, the degradation rate was only 48.1%. Too low initial NH₃-N concentration also affected degradation effect. When the NH₃-N
concentration was 20 mg·L⁻¹, the degradation rate was 96.4%, and when the NH₃-N concentration was 5 mg·L⁻¹, the degradation rate was 80.5%.

Figure 4. The degradation effect of X₁₂₃ at different initial concentration.

3.4.2. The Initial pH. Figure 5 shows the degradation effect of X₁₂₃ at different pH. It can be seen from the figure that when pH was 3, the ability of X₁₂₃ to degrade NH₃-N was inhibited, and the degradation rate was extremely low. As the pH tends to be neutral, the degradation effect was getting better and better, and the degradation effect was best when pH was 7, the degradation rate was 96.4% after incubating for 2 days. When pH > 7, the ability of X₁₂₃ to degrade NH₃-N was inhibited and the degradation rate begins to decline. When pH was 11, the degradation rate was extremely low. Therefore, the optimal pH of X₁₂₃ was 7.

Figure 5. The degradation effect of NH₃-N by X₁₂₃ at different pH.

3.4.3. The Initial C/N. Figure 6 shows the degradation effect of X₁₂₃ at different C/N. It can be seen that the degradation rate in the range of 2 - 20 showed a tendency to increase first and then stabilize. When C/N = 2, the degradation rate was low, because the carbon source was limiting at this time, which seriously affected the growth of the strains. With the continuous increase of C/N, the degradation rate was getting higher and higher. When C/N = 10, the degradation rate reached its maximum. After 2 days, the degradation rate was 96.4%. For higher C/N the degradation rate was basically unchanged when compared to C/N=10. Therefore, the best C/N of X₁₂₃ was 10.
3.5. Comparative analysis of the effect by dominant bacteria and nitrifier in literature on the degradation of NH$_3$-N

Yang Lei [24] isolated three strains that heterotrophic nitrifiers with high-efficiency denitrification ability from the Sequencing Batch Reactor (SBR) and named them YB, YH and YL respectively. Among them, YH had the best ability to degrade ammonia nitrogen. When the initial NH$_3$-N concentration was 200 mg·L$^{-1}$, the degradation effect of NH$_3$-N was the highest, which was 99.83%, which shows that it had strong resistance to high ammonia. Its optimal pH was 7 and C/N was 10. Si Wengong [25] isolated a heterotrophic nitrifier from activated sludge of sewage treatment plant, soil of chemical fertilizer plant and farmland soil. The degradation rate of NH$_3$-N was highest when pH was 5-9 and C/N was 12, which was 98.3%-98.9%. Wang Jie [26] isolated a heterotrophic nitrifier from the outlet of the pig farm's biogas digester. The degradation rate of NH$_3$-N was the highest when pH was 8-9 and C/N was 8, which was 90.8%. The dominant bacteria X$_{123}$ in this article had the best ammonia degradation effect when pH was 7 and C/N was 10, which was 96.4%. It can be seen that the difference between X$_{123}$ and the strains that was screened in the literature for the degradation of NH$_3$-N was very small, and the growth conditions of the strain were almost same.

4. Conclusions and prospects

Among the nine bacteria we isolated from the sludge and screened, X$_1$, X$_2$ and X$_3$ had a better effect on the degradation of NH$_3$-N. The X$_{123}$ degradation effect was the best, after culturing in NH$_3$-N of 20 mg·L$^{-1}$ and 106 mg·L$^{-1}$ for 2 days, the degradation rate was 96.4% and 69.7%, respectively. The degradation effect of X$_{123}$ under different initial concentration, pH and C/N was studied. It was found that X$_{123}$ had the best degradation effect when the initial concentration was 20 mg·L$^{-1}$, pH = 7 and C/N = 10, and the degradation rate was 96.4% after 2 days of cultivation. By comparing the NH$_3$-N degradation effect of S$_1$, S$_2$, S$_3$ and X$_{123}$, it was found that X$_{123}$ had the best NH$_3$-N degradation effect. And comparing it with the nitrifier that was screened in the literature, it was found that the difference between X$_{123}$ and the strains that was screened in the literature for the degradation of NH$_3$-N was very small, and the growth conditions of the strain were almost same. To sum up, the optimal conditions for X$_{123}$ are more suitable for wastewater with low NH$_3$-N concentration and neutral pH. Because the experiments were all carried out in the laboratory, the application in natural water may be different. At present, we are also doing some pilot experiments to make contribution to the treatment of NH$_3$-N in natural water in the future.
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