Isolation and characterization of heterotrophic nitrifying bacteria and the removal of pollutants in black and malodorous water bodies

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Abstract. Aiming at the problem of high concentration of COD and NH3-N in black and odorous water bodies, heterotrophic nitrifying bacteria which can remove ammonia nitrogen and organic pollutants were screened out in the laboratory simultaneously. A heterotrophic nitrifying functional group was constructed by using the strategy of bacterial source reorganization ecologically, and the removal effect of heterotrophic nitrifying functional bacteria on organic pollutants and ammonia nitrogen in water was studied. The results showed that the removal rate of ammonia nitrogen in the water was more than 52%, and the removal rate of COD was 57%~86%. The effect of heterotrophic nitrifying functional bacteria group 1 on the removal of COD and ammonia in water was better, and the removal rate was 76.5% and 54.8%, respectively. The optimum inoculation test showed that the optimum inoculation amount of the heterotrophic nitrification bacteria group 1 was 10 ppm.

Key words: black and malodorous water bodies; heterotrophic nitrifying bacteria; organic pollutants; ammonia nitrogen.

1. Introduction

With the rapid development of economy in our country, the sewage emissions increased dramatically, and some of the raw sewage was drained off into the water. The water self-purification ability was not desirable due to the small environment capacity of landscape water body. Thus it was easy to cause the increase of organic pollutants and nitrogen and phosphorus content in water body. Under the anoxic and anaerobic conditions, the pollutants were transformed into the foul-smelling such as ammonia nitrogen, hydrogen sulphide, volatile organic acids and the black material such as iron, manganese sulfide and etc [1-3]. The water body became black and emitted odorous gas, and the dissolved oxygen (DO) concentration reduced to approximately 0 mg/L, which caused the death of a large number of aquatic organisms. It had a severe effect on not only the industrial and agricultural production but also the domestic water quality of the surrounding cities and contributes to “water crisis” [4]. Therefore, it is of
great significance to control the black and smelly water body, improve the quality of urban water environment, and realize the sustainable development and utilization of urban water resources.

The heterotrophic nitrification bacteria were could remove the organic pollutants and ammonia nitrogen in the black and malodorous water bodies at the same time. Thus several bacteria strains were screened out in order to build the heterotrophic nitrifying functional group using self-adaption and ecological restructuring strategy. The effects of the heterotrophic nitrifying functional bacteria on removal of organic pollutants and ammonia nitrogen in water were investigated.

2. Materials and methods

2.1. Medium

Selective heterotrophic nitrifying bacteria liquid medium: CH$_3$COONa 0.5 g, NH$_4$Cl 0.1 g, CaCl$_2$ 0.05 g, K$_2$HPO$_4$ 0.1 g, MgCl$_2$ 0.05 g, H$_2$O 1 L, pH 7.5~8.0.

FM medium: CH$_3$COONa 0.2 g, NH$_4$Cl 0.04 g, CaCl$_2$ 0.02 g, K$_2$HPO$_4$ 0.04 g, MgCl$_2$ 0.02 g, H$_2$O 1 L, agar 20 g, pH 7.5~8.0.

XJ medium: CH$_3$COONa 0.1 g, NH$_4$Cl 0.02 g, CaCl$_2$ 0.01 g, K$_2$HPO$_4$ 0.02 g, MgCl$_2$ 0.01 g, H$_2$O 1 L, agar 20 g, pH 7.5~8.0.

2.2. Bacterial agent

The heterotrophic nitrifying bacteria used in this study were isolated from the sediment of black and smelly water bodies. A special culture method was employed for the enrichment culture of the heterotrophic nitrifying bacteria in our laboratory. After one month of domestication, the target bacteria became dominant. The heterotrophic nitrifying bacteria were isolated, and subjected to the combination experiments in order to obtain an optimal compound microbial community, which could be combined with chemicals and other microorganisms in source water to form a new micro-ecosystem with a stable metabolic growth and activity when degrading pollutants.

2.3. Source water

The main component of artificial wastewater in the experiment: CH$_3$COONa 0.5 g, NH$_4$Cl 0.1 g, CaCl$_2$ 0.05 g, K$_2$HPO$_4$ 0.1 g, MgCl$_2$ 0.05 g, H$_2$O 1 L.

2.4. Instruments

HZQ-F200 vertical air constant temperature oscillation incubator, HI839800 HANNA digestion instrument, DR 5000 UV visible spectrophotometer, SYQ-DSX-280B stainless steel portable sterilizer, DHP060 thermostatic incubator, CJ-1D super clean bench.

2.5. Analytical methods

Ammonia was determined by the Nesslerization method. COD$_{Cr}$ was determined by the COD rapid decomposition spectrophotometry method. NH$_4$-N and COD$_{Cr}$ was analyzed with Hach DR5000 ultraviolet spectrophotometer (Hach Company, Loveland, CO, USA).

Figure 1. Effect of various strains on the removal of COD and ammonia in water
3. Results and discussion

3.1. Screening of heterotrophic nitrifying bacteria
A total of 52 heterotrophic nitrifying bacteria were isolated and purified by the plate streaking. 5 strains with a better removal rate were selected. It was shown in Figure 1 that the effect of various strains on the removal of COD and ammonia in water under conditions of 25 °C temperature, 120 r/min shaking rotary speed, 8 days incubation time. The removal rates of ammonia nitrogen of strains in water were above 52%, and the corresponding COD removal rate range was between 57% and 86%.

3.2. Construction of heterotrophic nitrification bacteria group
In the practical engineering application, the single strain could not adapt to the environmental conditions well, and the mixed bacteria group had a much better adaptability to the environment. If the strains formed a protocooperation relationship, and produced synergies, mutually provide nutrients and other living conditions, the bacteria group could improve biodegradation efficiency as a whole. The complex scheme and removal effect of each functional bacteria group was shown in Table 1 and Figure 2.

| Number | 1         | 2         | 3         | 4         | 5         | 6         | 7         |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Group  | X12+X30+X31 | X31+X32  | X31+X37  | X12+X31  | X31+X32+X37 | X12+X31+X32 | X12+X31+X37 |
| COD/%  | 76.5      | 64.8      | 65.9      | 72.3      | 67.8      | 73.5      | 71.5      |
| Ammonia%/ | 54.8      | 45.3      | 50.2      | 49.6      | 49.3      | 51.3      | 50.6      |

Figure 2. Removal effect of heterotrophic nitrifying functional bacteria group 1 on organic pollutants and ammonia nitrogen under different inoculation conditions

It can be seen from Table 1 and Figure 2 that the removal rates of COD were between 64% and 77%, and the removal rates of ammonia nitrogen were between 49% and 55%. The results showed that the heterotrophic nitrification bacteria group 1 had the most desirable performance on the removal rate of COD and ammonia nitrogen with 76.5% and 54.8% respectively. The functional bacteria group 1 by the mixed bacteria system consisting of three strains had a wide ecological niche and a good adaptability to the environment.

3.3. Determination of the optimum amount of inoculation
The organic pollutants and ammonia nitrogen removal efficiencies of the heterotrophic nitrification bacteria group 1 under different dose conditions were given in Figure 3 (culture conditions as follows: 25 °C temperature, 120 r/min shaking rotary speed, 8 days incubation time).
Figure 3. Removal effect of heterotrophic nitrifying functional bacteria group 1 on organic pollutants and ammonia nitrogen under different inoculation conditions

As illustrated in Figure 3, if the inoculation amount was not optimal, it would affect the removal efficiency of organic pollutants and ammonia nitrogen of heterotrophic nitrification bacteria. Microorganisms would compete with each other for nutrients such as the carbon source, nitrogen source and etc, resulting in the lower degradation efficiency of pollutants. However, the low amount of microbial dosage could affect the removal efficiency of organic pollutants and ammonia nitrogen. Therefore, the appropriate inoculation amount should be determined to ensure the removal rate of pollutants. It could be seen from the results that when the inoculation amount was 10 ppm, the heterotrophic nitrification bacteria group had a desirable removal rate with 78.5% of COD and 55.6% of ammonia nitrogen in water. Therefore, the optimal dose was 10 ppm.

3.4. Discussion on mechanism of heterotrophic nitrification
Traditional nitrous acid bacteria and nitric acid bacteria belonged to chemoautotrophic bacteria with a long generation cycle and an extremely slow growth, and they were sensitive to environmental changes. Then the heterotrophic bacteria with a nitrification effect were paid more and more attention. In the late 19th and early 20th centuries, researchers found that a heterotrophic nitrification phenomenon occurred in the nitrification process of soil. Heterotrophic nitrifying bacteria could achieve the removal of organic matter and ammonia nitrogen simultaneously. Furthermore, there are a large number of heterotrophic nitrifying bacteria in the environment. And they had a rapid reproduction speed and a wide substrate range. Wan et al isolated a strain of nitrogen removal and phosphorus removal bacteria HW-15 with an effect of heterotrophic nitrification and aerobic denitrification in phosphorus-rich wastewater. The strain of ammonia nitrogen, nitrate nitrogen, nitrate nitrogen and phosphate removal rate could reach 99%, 88%, 59% and 73% in actual wastewater [5]. Sun et al identified a strain of heterotrophic nitrifying bacteria (S1) with a tolerance to metal, which has a good removal effect on nitrogen source pollutants in industrial wastewater containing heavy metals. Therefore, it is of certain significance to apply the heterotrophic nitrifying bacteria which could remove organic pollutants and ammonia nitrogen in water to the repair of black and malodorous water bodies [6].

4. Conclusion
The heterotrophic nitrifying bacteria which can remove ammonia nitrogen and organic pollutants were screened out in the laboratory simultaneously. These strains had a removal rate of ammonia nitrogen with above 50% and COD with 55%~89%.

The heterotrophic nitrifying bacteria group 1 was constructed by using the strategy of bacterial source reorganization ecologically. The removal rate of COD and ammonia nitrogen for the bacteria group 1 was 75.6% and 53.7% respectively, and the optimal inoculation dose was 10 ppm.

Heterotrophic nitrification bacteria could realize synchronous to remove organic matter and ammonia nitrogen. Heterotrophic nitrifying bacteria were abundant in the environment, and had a faster
propagation rate and a wider heterotrophic nitrification substrate range. Therefore, it was significant that applied them to the restoration of black and malodorous water bodies.

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