The effect of heavy metals on sulfur autotrophic short-cut denitrification

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Abstract: A batch experiment was used to investigate the influence of common heavy metals Cr(Ⅵ), Ni(Ⅱ), Cu(Ⅱ)) on sulfur autotrophic short-cut denitrification. Experimental results show that low concentrations (1-3 mg/L) of heavy metals have little effect on sulfur autotrophic short-cut denitrification, and high concentrations (4-10 mg/L) of heavy metals have obvious effects on sulfur autotrophic short-cut denitrification. Inhibition. The doserape model is used to fit the dose-effect curve of heavy metals, and the IC⁵₀ is used to judge the toxicity. The order of the IC⁵₀ of each heavy metal is: Cr(Ⅵ)>Ni(Ⅱ)>Cu(Ⅱ).

The effects of two heavy metals in the binary system of Cu(Ⅱ) and Ni(Ⅱ) are antagonistic.

1. Introduction

Anammox technology has received widespread attention because of its high nitrogen removal efficiency and low energy consumption. However, the practical application of anammox technology is still far away. There are many factors that limit the practical application of anammox technology, such as the way to obtain stable nitrous nitrogen. Sulfur autotrophic short-cut denitrification, as a way to stably produce nitrous nitrogen, has gradually attracted the attention of scientific researchers in recent years[1].

At present, the research on sulfur autotrophic short-cut denitrification mainly focuses on the configuration of the reactor and the stability control of the process[2].Zhang et al.[3], produced short-cut denitrification by adding sulfur stone to the anammox system to improve the denitrification effect of anammox. The results showed that the coupled system can reduce sulfur consumption by nearly 55%. Deng et al.[4] used sulfide stress to achieve short-range denitrification, and combined it with the anaerobic ammonia oxidation process, the nitrogen removal efficiency was stable, and the nitrogen removal efficiency and nitrogen removal rate were maintained at 80% and 0.29 kgN/(m³ •d).

The actual water body often contains some toxic and harmful substances, and these toxic and harmful substances often have certain effects on organisms. Li et al.[5] studied the effects of four heavy metals on the denitrification process. Cd(II), Ni(II), Mn(II), and Pb(Ⅵ) all have obvious effects on the denitrification process. Nitrate removal efficiency (NRE) is reduced to 7.98-26.80%. Zheng et al. [6]studied the denitrification efficiency of activated sludge by nano-titanium dioxide in the environment. The experimental results showed that the nano-titanium dioxide in the environment has no obvious effect on the short-term exposure of activated sludge, while the 50 mg/L nano-titanium dioxide has no obvious effect on the short-term exposure of activated sludge. Titanium dioxide will greatly reduce the denitrification effect of sludge under long-term exposure conditions. The current research on toxic and harmful substances focuses on the denitrification and phosphorus removal of activated sludge, but there are relatively few studies on the process of sulfur autotrophic short-cut denitrification by toxic and harmful substances. Sulfur autotrophic short-cut denitrification can be used in industrial wastewater as
part of the deep denitrification process of industrial wastewater, but industrial wastewater often contains a large amount of heavy metal substances. Therefore, studying the influence of heavy metals on the sulfur autotrophic short-cut denitrification process is of great significance for the application of sulfur autotrophic short-cut denitrification to actual wastewater.

2. Materials and Methods
Sequencing batch experiment: This experiment adopts the method of sequencing batch experiment to comprehensively evaluate the influence of heavy metals on sulfur autotrophic short-cut denitrification. Sequencing batch experiments were carried out in serum bottles, and the same volume of sulfur autotrophic short-cut denitrification sludge was taken before the experiment. Add the medium shown in the table and the required heavy metals. Blow in high-purity nitrogen for 10 minutes to ensure that the serum bottle maintains an oxygen-free environment. The serum bottle was cultured in a constant temperature shaker in a water bath at a temperature of 35°C and a rotation speed of 150 r/min. The pre-reaction was performed every 2 hours after 1 hour. Use a sterile syringe to draw a water sample through a 0.45μm filter membrane and measure the relevant indicators, and calculate the corresponding sludge activity. Two sets of parallel tests are set for each concentration.

Sludge and culture medium: sulfur autotrophic short-cut denitrification sludge is taken from the sludge in the sulfur autotrophic short-cut denitrification reactor that operates stably. The water quality of the culture medium adopts the influent water quality of Zhang[7], as shown in Table 1.

| Compounds  | Concentration (g/L) | Proportion (mL/L) |
|------------|---------------------|------------------|
| NaNO₃      | 0.61                |                  |
| KH₂PO₄     | 0.013               |                  |
| NaCO₃      | 1.0                 |                  |
| CaCl₂      | 0.01                |                  |
| MgSO₄      | 0.001               |                  |
| Heavy metal| Add on demand       |                  |

Statistical Analysis: Carry out linear regression analysis on the concentration of nitrous nitrogen in the substrate to obtain the sludge activity of sulfur autotrophic short-cut denitrification. Set the sludge activity of the blank group to 1, and the sludge activity after adding heavy metals is the relative activity compared with the blank group. The dose rape model is used to fit the relative short-range denitrification activity of the sludge, and the relative short-range activity is obtained. The concentration of heavy metals at 50% denitrification is the IC₅₀. The equation of the fitted model is shown in Equation 1.

\[ y = A + \frac{B-A}{1+10^{(\log C-x)/p}} \]  

3. Results & Discussion

3.1. The effect of Cr (VI) on sulfur autotrophic short-cut denitrification
Taking the activity of sulfur autotrophic short-cut denitrification sludge as the criterion, the effect of Cr(VI) on sulfur autotrophic short-cut denitrification sludge was explored, and the results are shown in Figure 1. It can be seen from Figure 1(a) that with the increase of Cr(VI) concentration, the sludge activity of sulfur autotrophic short-cut denitrification continues to decrease. After adding Cr(VI) greater than 5 mg/L, the activity of short-cut denitrification sludge no longer changes significantly, and the activity of short-cut denitrification sludge is reduced to 1.3 mg-N·L⁻¹·g vss⁻¹. Compared with the sludge activity of short-cut denitrification without Cr(VI), the sludge activity of 5 mg/L Cr(VI) was reduced by 83.9%. This indicates that Cr(VI) has a relatively obvious inhibitory effect on sulfur autotrophic short-cut denitrification sludge.
Taking the short-cut denitrification sludge without Cr(VI) as the control group, the activity of the short-cut denitrifying sludge with different concentrations of Cr(VI) and the activity of the short-cut denitrifying sludge without Cr(VI) added were divided. Get relative activity. The Doserape model was used to fit the relative activity, and the dose-effect curve of short-cut denitrification sludge Cr(VI) was obtained. It is calculated that the concentration value of Cr(VI) at which the relative activity of short-range denitrification is 50% on the curve is the IC$_{50}$.

As shown in Figure 1(b), the relative activity of the sludge under different concentrations of Cr(VI) stress was fitted, and the fitting equation is shown in Table 2.

The IC$_{50}$ of Cr(VI) for short-cut denitrification 2.214 mg/L, compared with the IC$_{50}$ of Cr(VI) for other types of activated sludge in the literature, the IC$_{50}$ of sulfur autotrophic short-cut denitrification sludge is lower.

It shows that the sulfur autotrophic short-cut denitrification sludge is more sensitive to Cr(VI) compared with other sludges.

### Table 2  dose-effect curve

| Heavy metal | Dose-effect curve equation | $R^2$ | IC$_{50}$ |
|-------------|---------------------------|-------|----------|
| Cr(VI)      | $y=0.13233+\left(1.14284-0.13233\right)/\left(1+10^{0.4636\left(1.69024-X\right)}\right)$ | 0.903 | 2.214 |

3.2. The effect of Cu (II) on sulfur autotrophic short-cut denitrification

The effect of Cu(II) on sulfur autotrophic short-cut denitrification sludge was investigated, and the experimental results are shown in Figure 2. It can be seen from Figure 2(a) that when Cu(II) is not added, the activity of short-cut denitrification sludge is 9.26 mg-N·L$^{-1}$·g vss$^{-1}$, which is the same as the experimental result of 9.42 mg-N·L$^{-1}$·g vss$^{-1}$ approximation. When adding 1-2 mg/L of Cu(II), the activity of short-cut denitrification sludge does not change much, maintaining around 7 mg-N·L$^{-1}$·g vss$^{-1}$. When the addition of Cu(II) increases to After 3 mg/L, the activity of short-cut denitrification sludge dropped sharply to 3 mg-N·L$^{-1}$·g vss$^{-1}$. After the concentration of added Cu(II) is greater than 5 mg/L, as the concentration of added Cu(II) increases, the activity of short-cut denitrification sludge does not change much, maintaining at 1.3 mg-N·L$^{-1}$·g vss$^{-1}$. 

![short-cut denitrification active](a) 
![Dose response curve](b) 

Figure 1 The effect of Cr(VI) on short-range denitrification activity and the dose-response curve
The relative activity of the sludge under different concentrations of Cu(II) stress was fitted. The fitting equation is shown in Table 3. The IC$_{50}$ of Cu(II) for short-range denitrification = 2.834 mg/L, and 1 Cr(VI) Compared with the IC$_{50}$ of Cu(II), the IC$_{50}$ of Cu(II) is higher, which shows that the acute toxicity of Cr(VI) is stronger than that of Cu(II).

| Heavy metal | Dose-effect equation | $R^2$ | IC$_{50}$ |
|-------------|----------------------|-------|-----------|
| Cu(II)      | $y = 0.077 + \left(1.08632 - 0.077\right) / \left(1 + 10^{(2.18006 - X) * 0.4720}\right)$ | 0.921 | 2.834 |

3.3. The effect of NI (II) on sulfur autotrophic short-cut denitrification

As shown in Figure 3(a), in the blank group without NI (II), the short-range denitrification activity was 9.04 mg-N L$^{-1}$ g vss$^{-1}$, slightly lower than that in 1 and 2. The activity of the blank group. With the increase of the concentration of the external NI(II), the activity of the short-cut denitrification sludge is also continuously degraded, especially when the concentration of the external NI(II) increases to 2 mg/L, the activity of the short-cut denitrification sludge decreases to 4.2 mg-N L$^{-1}$ g vss$^{-1}$, the inhibition rate was 52%. When the concentration of the external NI (II) increases to 5 mg/L, the activity of the short-cut denitrification sludge does not change significantly, and it is maintained at around 1.5 mg-N L$^{-1}$ g vss$^{-1}$, which is different from the addition of Cu. The experimental results of (II) and Cr(VI) are similar.
Figure 3(b) is the dose-effect curve fitted by Ni(II). The equation of the curve is shown in Table 4. The IC$_{50}$ of Ni(II) for short-range denitrification is 2.092 mg/L, which is lower than Cr(VI) and Cu. (II) IC$_{50}$, which shows that Ni(II) is more toxic to short-cut denitrification sludge than Cr(VI) and Cu(II).

3.4. Effect of Cu(II) and Ni(II) binary mixed system on sulfur autotrophic short-cut denitrification

Two typical heavy metals are selected for binary mixing experiment, Cu(II) and Ni(II) are mixed with equal effect, and the mixing experiment is carried out with the equal effect concentration method, and the half-inhibition concentration ratio of heavy metal is selected for this special ray conduct experiment. The experiment was carried out using the same experimental method as in 1, and the experimental results are shown in Figure 4. It can be seen from the figure that the sludge activity of the blank group without heavy metal addition is 8.532 mg-N·L$^{-1}$·g vss$^{-1}$, which has little change compared with the sludge activity detected in the previous two chapters, with the increase of heavy metal concentration, The activity of short-cut denitrification sludge is also continuously decreasing. When the concentration of the binary mixture is 5 mg/L, the activity of short-cut denitrification sludge is reduced to 3.191 mg-N·L$^{-1}$·g vss$^{-1}$, The inhibition rate was 62.6%, and the inhibition effect was more significant.

The S-shaped curve is fitted to the binary mixed system of Cu (II) and Ni (II). The relevant equation parameters of the fitted curve are shown in Table 4. It can be seen from the table that the IC$_{50}$ of the binary mixture system of Cu(II) and Ni(II) for sulfur autotrophic short-cut denitrification sludge is 4.196 mg/L, which is higher than the IC$_{50}$ when the two act alone. It is speculated that the two heavy metals in the Cu(II) and Ni(II) binary systems have antagonistic effects. The binary mixed system is less toxic to the sulfur autotrophic short-cut denitrification sludge, which makes the IC50 of the binary mixed system increase.

4. Conclusions

1. Cu(II), Ni(II) and Cr(VI) have little inhibitory effect on sulfur autotrophic short-cut denitrification when the concentration of heavy metals is low, and has a greater inhibitory effect when the concentration is greater than 4 mg/L.

2. The order of the toxicity of three heavy metals on sulfur autotrophic short-term denitrification is: Cr(VI)>Ni(II)>Cu(II)
3. The Cu(II) and Ni(II) binary system is antagonistic to the effect of autotrophic short-term denitrification.

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