

Study on the effect of Anammox/Fe⁰ coupling treatment of nitrate wastewater under neutral conditions

Bing Wang¹; Bing Sun^{1*}; Yunlong Liu¹; Lin Yang¹

¹ School of Municipal and Environmental Engineering, Shenyang Jianzhu University, Shenyang Liaoning 110168, P.R.China;

Abstract. Batch experiments were conducted to explore the effect of Anammox bacteria on the nitrate reduction efficiency of Fe⁰ under neutral conditions and to analyze the ways of its enhancement. The effects of Fe²⁺ concentration on the activity of Anammox bacteria in the system were investigated by detecting the nitrate content in the system under neutral conditions. The upflow reactor was set to verify the possibility of stable operation of Fe⁰/Anammox system in continuous flow. The results showed that in neutral environment, Anammox bacteria can significantly promote the reduction of nitrate by Fe⁰, and the denitration rate can reach more than 75% under the dual biological and chemical effects. In the continuous flow system of nitrate reduction by Fe⁰/Anammox bacteria, the stable treatment period was about 7 days. Through regular replacement of iron powder, the denitration rate can be maintained at more than 75% for a long time, and the effluent NH₄⁺, NO₂⁻ concentration is low. The treatment of nitrate wastewater by zero-valent iron as a reducing agent has been widely paid attention, but the treatment effect is seriously affected by pH. Anammox bacteria were added to coupling with Fe⁰ can not only promote each other's reactivity to adapt to more non-ideal environment, but also reduce secondary pollution, which provides a new idea for the practical application of nitrate wastewater treatment.

1 Introduction

With the rapid development of industry and agriculture, nitrate wastewater has become a serious environmental pollution problem^[1]. Excessive nitrate content in water will not only cause ecological damage such as eutrophication, but also produce nitrite intermediate products which will threaten human health. China is the largest producer and consumer of chemical fertilizer in the world, and the use of chemical fertilizer leads to serious groundwater pollution^[2]. Therefore, the efficient treatment of nitrate wastewater has become an important subject in the field of environment.

Although physical and chemical methods such as ion exchange and reverse osmosis are effective, they are not ideal methods due to the need for frequent regeneration of media. Iron powder is not only highly restorative but also cheap and readily available. Using iron as reducing agent to remove nitrate from water is a promising wastewater treatment technology. Zawaideh et al.^[3] found that iron powder can effectively and rapidly reduce nitrate under acidic conditions. However, when pH is between 6 and 8, nitrate reduction rate decreases rapidly and is lower than 40%^[4]. This is because iron powder in neutral environment is easy to passivation, which affects its reaction ability. Therefore, in order to maintain a high

denitration rate in the system, it is necessary to continuously add acid to maintain pH, which not only increases the cost but also is not conducive to equipment maintenance. Besides, the low pH leads to the reduction of nitrate into a large amount of ammonia nitrogen, causing serious secondary pollution. Therefore, how to make iron powder still have the ability to efficiently remove nitrate in neutral environment has more practical significance^[5].

Studies have shown that Fe²⁺/Fe⁰ has the potential to reduce NO₃⁻ under the action of Anammox bacteria^[6]. In 2013, Oshiki^[7] found that Fe²⁺ can reduce nitrate to nitrite and ammonia in the Anammox system. Xing et al.^[8] found that the addition of anaerobic ammonium oxidation sludge can enhance the reduction of nitrate by Fe⁰. Therefore, coupling Fe⁰ with Anammox bacteria in neutral environment can not only improve the denitration rate through biological/chemical dual effects, but also achieve the effect of removing ammonia-nitrogen byproducts, which is an important research direction in the field of nitrate wastewater.

In this paper, batch experiments were set to investigate the concentration changes of NO₃⁻, NH₄⁺ and NO₂⁻ in the reaction process, to explore the influence of Anammox bacteria on the reduction of nitrate by Fe⁰ under neutral temperature conditions. The possibility of long-term stable operation of Fe⁰/Anammox was explored by setting continuous flow experiment, which

*Corresponding author: 415494033@qq.com

provided experimental data for the practical application of nitrate wastewater treatment.

2 Materials and methods

2.1 Experimental equipment

2.1.1 Batch experiments

A 110mL anaerobic serum flask was used as the experimental device, and the constant volume of the culture medium was 100mL. Before the experiment, the serum bottle was aerated with 99% pure nitrogen for 30 minutes to remove the dissolved oxygen, and the bottle mouth was quickly sealed with rubber stopper and aluminum cap. The bottle was placed in a thermostatic oscillator that was protected from light, and the rotating speed was maintained at 100rpm. During the reaction, continuous sampling and measurement were conducted.

2.1.2 Continuous flow experiment

An upflow reactor with an effective volume of 0.8L was used in the experiment, and 80mL Anammox sludge (MLVSS=3000 mg/L) was added. The inlet water was deoxygenated with nitrogen of 99.5% purity. The internal outlet pipe of the device was connected with non-woven fiber membrane to control the microorganism not to be discharged with the outlet water. The outer glass tube of the device was wrapped with opaque cloth to prevent the effect of light on the activity of iron bacteria. The reactor was controlled at 32 ° C by heating the water tank.

2.2 Inoculation sludge and experimental water quality

The inoculated sludge used in this paper was Anammox sludge, which was taken from the laboratory Anammox reactor which had been in good operation for a long time. The sludge had good properties and was brick red. The simulated wastewater used in the experiment was manually configured, and its main components were: NaNO₃, KHCO₃, KH₂PO₄, trace elements (including CaCl₂·2H₂O, NaCl, MgSO₄·7H₂O, KCl). The Fe⁰ used in this experiment was iron powder with particle size of 150um, which is activated before use. First, 0.5mol/L HCl is used to clean and remove the surface oxides, and then deoxidized primary water is used to rinse repeatedly until the pH of the leach solution is neutral. The Fe⁰ used in this experiment is all iron powder with particle size of 150um. Before the experiment, the iron powder was activated. First, the iron powder was cleaned with 0.5mol/L HCl to remove its surface oxides. Then, the iron powder was repeatedly washed with deoxidized primary water until the pH of the leach solution was neutral. To avoid air oxidation, the activated iron powder should be used immediately. Factors such as temperature and pH vary slightly according to the purpose of the experiment, and the specific conditions will be described

in detail in the corresponding sections.

2.3 Analysis method

In the process of reaction, pH value was determined by pH meter. NH₄⁺-N was determined by NaCl reagent spectrophotometry, NO₂⁻-N was determined by N¹-naphthalene - ethylenediamine spectrophotometry, NO₃⁻ was determined by ultraviolet spectrophotometry,.

3 Results and analysis

3.1 Nitrate denitration under neutral conditions

In order to explore the effect of adding Anammox bacteria on the reduction of nitrate wastewater by iron powder in neutral environment, we designed a group of control experiments. The pH values were set as 2,6,7,8, and the nitrate removal efficiency of the two groups of different substrates in the simulated wastewater was observed(R1:Fe⁰,R2:Fe⁰+Anammox). In the initial conditions, influent NO₃⁻ concentration was set as 50 mg/L, iron powder concentration was 5 g/L, and the temperature was 35°C. As can be seen from Figure 1, R1 was significantly affected by pH, and the denitration rate was the best under strong acid condition, while R2 showed no significant difference in denitration rate under different pH conditions. When pH =2, the reduction rate of nitrate in both groups could reach more than 80%, and R2 could hardly detect nitrate after 60h. However, in neutral environment, the denitration effect of R1 was not obvious, and with the increase of pH, the denitration rate decreased. When pH was 8, the denitrification rate was only 10%. This is because the surface of Fe⁰ will corrode and produce corrosion products FeOOH and Fe₃O₄[9]. Under acidic conditions, a larger proportion of Fe₃O₄ in the product, the loose surface structure of the product has a greater adsorption effect on Fe²⁺, and the contact area of iron powder is larger, which will promote the reduction of more NO₃⁻ by Fe⁰. Besides, the reduction of nitrate by iron powder is an acid-driven process. When the concentration of H⁺ in the system is high, the generated H₂ receives more electrons, and a large number of H atoms on the surface of Fe⁰ will effectively reduce NO₃⁻. In neutral environment, Fe⁰ is difficult to corrode but easy to passivate, and it is difficult to generate highly reductive H atoms. Therefore, the reduction rate of NO₃⁻ is greatly reduced. As shown in Figure 2, although the denitration rate in R2 was inversely proportional to pH, it can all reach more than 88%. The results indicated that Anammox bacteria could improve the nitrate loss in the neutral environment. There are two reasons for this phenomenon: □NH₄⁺ and NO₂⁻ as products of the reduction of NO₃⁻ by Fe⁰, the anaerobic ammonium oxidation reaction occurred under the action of Anammox bacteria. By consuming the products, the chemical equilibrium was changed so that the reaction moved forward, resulting in the increase of NO₃⁻ loss. □ In addition to the anaerobic ammonium oxidation

reaction, Anammox bacteria can directly participate in the reduction of NO_3^- . Some scholars found that Anammox bacteria can use $\text{Fe}^{2+}/\text{Fe}^0$ as electron donor to reduce NO_3^- . In order to explore whether Anammox bacteria can improve the denitration rate through chemical reaction or biochemical dual action, we designed a verification experiment. When $\text{pH} = 7$, Fe^0 and zeolite were added, and after 60mins, the nitrate removal rate was 20%. At this time, well-run Anammox sludge was added, and the nitrate removal rate was 80% after 36h. As shown in Figure 3, in the first stage, zeolite continuously absorbed NH_4^+ generated by Fe^0 and NO_3^- , but the denitration rate did not significantly improve. The experiment showed that the promotion of reaction forward through consumption of products is not the key reason to increase the denitration rate. When 45mins, NO_3^- was no longer removed, indicating that the chemical reaction between Fe^0 and NO_3^- had stopped. In the second stage, the concentration of NO_3^- decreased by 29 mg/L after the addition of Anammox bacteria, indicating that Anammox bacteria directly participated in the reduction of NO_3^- through biological action.

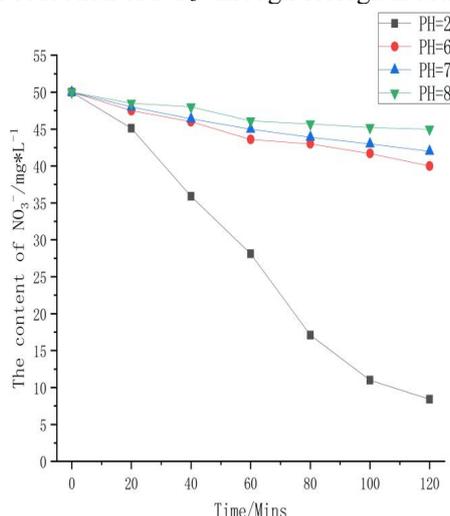


Figure 1. The effect of neutral conditions on nitrate reduction without the participation of Anammox bacteria

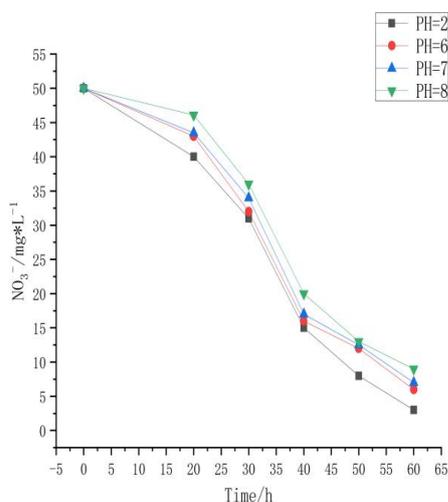


Figure 2. The effect of neutral conditions on nitrate reduction with the participation of Anammox bacteria

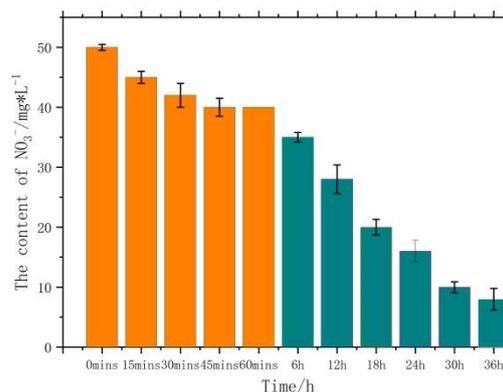


Figure 3. The effect of Anammox bacteria on nitrate reduction rate under $\text{Fe}^0/\text{zeolite}$ conditions

3.2 $\text{Fe}^0/\text{Anammox}$ continuous flow operation experiment

Batch experiments had verified that $\text{Fe}^0/\text{Anammox}$ can promote the removal of nitrate in a short period of time, but whether the continuous and stable operation of the reaction can be ensured remains to be studied. Therefore, we carried out continuous flow experiments for further investigation.

The changes in the concentration of each element in the reactor are shown in Figure 4. At the initial stage of reaction, the effluent NO_3^- concentration was high and the removal rate was only 45%. This stage was the adaptation period of Anammox bacteria. From the 5th day to the 12th day, the effluent NO_3^- concentration was relatively stable, and the denitrification rate was above 70%. From the 13th day, the concentration of NO_3^- in the effluent water began to rise, and the nitrate removal rate was only 15% on the 15th day. It is worth noting that the concentration of Fe^{2+} that can be detected in the liquid was proportional to the rate of denitration, and as the rate of denitration decreased, the concentration of Fe^{2+} in the system also decreased. For example, the total iron concentration was 192.7mg/L on day 10 and decreased to 132.1mg/L on day 15. This indicated that Fe^0 was gradually passivated and its nitrate reducing ability was gradually reduced as the reaction proceeded. When the iron powder in the reactor was replaced on the 22nd day, the concentration of NO_3^- decreased accordingly, and the effluent was about 13mg/L. On the 30th day, the concentration of NO_3^- rose again. The results showed that the stable period of efficient Fe^0 treatment was about 7 days, and the reactor began to collapse after this time range. Iron powder was replaced every 7 days from the 35th day. As shown in Fig. 4, the nitrate removal rate in the reactor from the 35th day to the 60th day was stable at around 75%, and the effluent NO_3^- concentration was even lower than 6mg/L on the 60th day. In summary, the stable period of $\text{Fe}^0/\text{Anammox}$ bacteria in treating nitrate in continuous flow was short, and the stable operation of the system can be maintained by replacing iron powder regularly.

The accumulation of NH_4^+ and NO_2^- can be detected in the whole process, and the changing trend of ammonia nitrogen concentration in the effluent was consistent

with the changing trend of nitrate removal rate. On day 24-30, the average effluent concentration of ammonia nitrogen was 11.9mg/L in the stable phase of nitrate removal. On day 33, the nitrate removal rate was reduced, and the effluent concentration of ammonia nitrogen was reduced to 8mg/L. On day 35, it was further reduced to 5mg/L. On the 38th day, with the replacement of iron powder, the treatment effect of the system on nitrate was enhanced, and the concentration of ammonia nitrogen increased again. Then, the effluent ammonia nitrogen stabilized at about 12.1mg/L on the 38-60 days. The concentration of nitrite in the whole process was (2.8 ± 0.3) mg/L, which was consistent with the nitrogen transformation pathway of " $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^{+}$ " proposed by Li Tielong et al. [10] for the reduction of NO_3^- by Fe^0 . When Fe^0 was used alone, nitrate will be reduced to a large amount of ammonia nitrogen and nitrite nitrogen, resulting in secondary pollution. Besides, the treatment effect of free ammonia (FA) and nitrite itself inhibiting Anammox in neutral environments is widely recognized [10]. In order to maintain the stable operation of the Anammox system, the optimal FA and nitrite concentrations are controlled below 20-25mg/L and 100mg/L, respectively. In this experiment, the effluent FA and nitrite concentrations were far below their respective inhibition thresholds, indicating that the denitrification products did not affect the anaerobic ammonium oxidation reaction activity in the system.

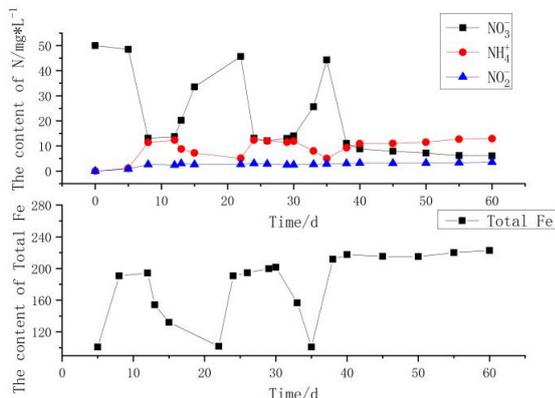


Figure 4. Changes in the concentration of each element in the continuous flow experiment

4 conclusion

(a) Under neutral conditions, the efficiency of nitrate reduction by Anammox bacteria / Fe^0 coupling was significantly higher than that of the control group with Fe^0 only. The results showed that the enhancement of Anammox bacteria was not related to the change of chemical equilibrium by the consumption of products. The increased nitrogen loss in the experiment was due to the biological reduction of nitrate by microorganisms using Fe^{2+} dissolved by Fe^0 .

(b) The nitrate reduction by Fe^0 /Anammox bacteria can operate in a continuous flow, but Fe^0 is easy to be passivated, which leads to a short stabilization period in the reactor. Fe^0 reduces nitrate to produce

by-products NO_2^- and NH_4^+ , which were further reduced to nitrogen under the action of Anammox. Therefore, the concentration of nitrogen by-products in the Fe^0 /Anammox reactor was low and the secondary pollution was less.

Fund projects

1. Study on three-phase synergistic phosphorus recovery mechanism in anaerobic treatment of phosphorus rich wastewater (2019-ZD-0298), Project of Science and Technology Department of Liaoning Province, 2019,

2. Food waste induces surplus sludge to enhance the fermentation performance of L-lactic acid bacteria (1nqn202011), Project of Education Department of Liaoning Province, 2020

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