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Removal of nitrogen from livestock wastewater by iron cycling under Feammox and NO_3^--dependent Fe(II) oxidation coupling reaction

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ABSTRACT

In this paper, three groups of controlled variable experiments were compared for 64 d, which was based on the coupling reaction of Feammox (NH_4^+ oxidation with Fe(III) reduction) and NDFO (NO_3^--dependent Fe(II) oxidation), to explore the nitrogen removal effect of Feammox and NDFO coupling technology on livestock wastewater and iron recycling mechanism under anaerobic conditions. Through the analysis of transformation in the contents of various nitrogen and iron in the experimental operation system, it was found that the simultaneous nitrogen removal occurred with the presence of interconversion between Fe(II) and Fe(III). The Fe(III) added in the C2 group advanced the progress of Feammox and realized the iron cycle to remove nitrogen under the combination of Feammox and NDFO. However, after 50 d of operation, due to the insufficient NO_3^- content, the nitrogen removal was weakened, and the final ammonia nitrogen removal rate was 65.1%, and the total nitrogen removal rate was 71.2%. Comparatively, the C3 group added NO_3^- regularly based on the C2 group which promoted the NDFO reaction and further realized the circle of Feammox and NDFO. The final NH_4^+ removal rate reached 88.9%, and the total nitrogen removal rate reached 84.6%, which was significantly better than the C2 group and the blank group. The analysis of the microbial community by 16S rRNA high-throughput sequencing technology concluded that the main functional microorganism under the system was Ignavibacteria. This study provides a new theoretical basis for the exploration of Fe/N cycling mechanism under Feammox-NDFO coupling technology and nitrogen treatment in livestock wastewater in the future.

Keywords: Feammox; NO_3^--dependent Fe(II) oxidation; Iron cycle; Microbial analysis

1. Introduction

Livestock wastewater has the characteristics of high pollutant content such as NH_4^+ and chemical oxygen demand (COD) and large discharge volume [1]. If the wastewater is not properly treated and discharged, it will easily cause eutrophication and damage to the ecological environment [2]. In order to protect the water environment, it is necessary to remove nitrogen from livestock wastewater. Denitrification and anammox are used as the traditional nitrogen removal methods. Denitrification refers to the conversion of NO_3^-–N generated by the nitrification reaction into N_2 by denitrifying bacteria under anaerobic conditions. Although the denitrification effect is better, it's easily affected by carbon sources shortage [3]. Anammox is also a research hotspot, which can convert ammonia nitrogen into N_2 using NO_2^- as an electron acceptor under anaerobic conditions ($NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$). And anammox has been widely used in industrial wastewater, aquaculture wastewater, and landfill leachate [4–6]. But there still exists limitations such as difficult control of biomass, long growth cycle, and environmental sensitivity [7,8].

In addition, Feammox has received extensive attention in recent years. According to some research, Feammox has

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been confirmed to exist in rice fields [9], wetlands [10], riparian zones [11], and other places. Feammox can convert NH_4^+ into NO_3^-, NO_2^-, N_2, as well as reducing Fe(III) to Fe(II) [Eqs. (1)–(3)] [12]. For example, Yang et al. [13] induced the continuous occurrence of Feammox by supplementing iron compounds in the anaerobic digester, and found that NO_2^- and NO_3^- were generated in the Fe(III)-added reactors, After 40 d, 20.1% of total nitrogen was removed in the reactor where $Fe(OH)_3$ was added. Li et al. [14] found that the simultaneous conversion of Fe(III) and NH_4^+ were achieved by inoculating anammox sludge and starting the Feammox reaction. After 160 d, the ammonia conversion rate in the reactor was higher than 80%, and a large amount of NO_3^- and a small amount of NO_2^- were produced at the same time. The total nitrogen removal rate is as high as 71.8%. Therefore, Feammox is expected to become a new type of wastewater nitrogen removal method, but the iron sludge formed is retained in the reactor and cannot operate for a long time, so it still needs further research.

$$3\mathrm{Fe}\left(\mathrm{OH}\right)_3 + 5\mathrm{H}^+ + \mathrm{NH}_4^+ \rightarrow 3\mathrm{Fe}\left(\mathrm{II}\right) + 9\mathrm{H}_2\mathrm{O} + 0.5\mathrm{N}_2 \tag{1}$$

$$6\mathrm{Fe}\left(\mathrm{OH}\right)_3 + 10\mathrm{H}^+ + \mathrm{NH}_4^+ \rightarrow 6\mathrm{Fe}\left(\mathrm{II}\right) + 16\mathrm{H}_2\mathrm{O} + \mathrm{NO}_2^- \tag{2}$$

$$8\mathrm{Fe}\left(\mathrm{OH}\right)_3 + 14\mathrm{H}^+ + \mathrm{NH}_4^+ \rightarrow 8\mathrm{Fe}\left(\mathrm{II}\right) + 21\mathrm{H}_2\mathrm{O} + \mathrm{NO}_3^- \tag{3}$$

NO_3^--dependent Fe(II) oxidation (NDFO) is also a new approach to remove nitrogen, which can oxidize Fe(II) to Fe(III) by using NO_2^- and NO_3^- produced by Feammox reaction as electron acceptors simultaneously generate N_2 to achieve the effect of nitrogen removal [Eqs. (4) and (5)]. Li et al. [15] used Fe(III) as a catalyst to explore the feasibility of using Feammox coupled with NDFO to induce the conversion of nitrogen. After 61 d of operation, 67.6% of ammonia nitrogen and 58.8% of NO_3^-N were converted simultaneously. At the same time, there was the mutual transformation of Fe(III) and Fe(II). Yang et al. [16] investigated Feammox's nitrogen removal performance and iron changed by adding anthraquinone-2,6-disulfonate (AQDS) in an anaerobic wastewater reactor. The results showed that the nitrogen removal efficiency of the AQDS-Fe_2O_3 group reached 82.6%, and found that Fe(III) could be regenerated from NDFO to participate in the next round of Feammox. Yang et al. [12] studied the effect of coupling Feammox and NDFO to treat urban wastewater digestion liquid using NO_3^- as an electron acceptor in the absence of anammox bacteria, which realized the cycle conversion between Fe(III) and Fe(II), the total nitrogen removal rate of the nitrate addition group was 90.1%.

$$6\mathrm{Fe}\left(\mathrm{II}\right) + 2\mathrm{NO}_2^- + 8\mathrm{H}^+ \rightarrow 6\mathrm{Fe}\left(\mathrm{III}\right) + \mathrm{N}_2 + 4\mathrm{H}_2\mathrm{O} \tag{4}$$

$$10\mathrm{Fe}\left(\mathrm{II}\right) + 2\mathrm{NO}_3^- + 12\mathrm{H}^+ \rightarrow 10\mathrm{Fe}\left(\mathrm{III}\right) + \mathrm{N}_2 + 6\mathrm{H}_2\mathrm{O} \tag{5}$$

According to previous related studies, Fe(III) can promote the Feammox process to produce Fe(II) and carry out NDFO reaction with NO_2^- and NO_3^-. It is reasonable to speculate that NO_3^- can enhance NDFO reaction to regenerate Fe(III) which will undergo the next round of Feammox, forming a cyclic conversion between Fe(III) and Fe(II), and continuously removing nitrogen. At present, few people have studied Feammox and NDFO coupling under the action of Fe(III) and nitrate to achieve iron cycle nitrogen removal technology, and rarely use this technology to treat livestock wastewater. The high ammonia nitrogen of livestock wastewater has always been a difficulty in wastewater treatment. In order to further explore the feasibility of Feammox and NDFO coupling nitrogen removal technology to treat livestock wastewater, this experiment took certain livestock wastewater in Shenyang as the research object, explored the effect of Feammox-NDFO to remove ammonia nitrogen and NO_3^- in livestock wastewater under the action of Fe(III) and nitrate. At the same time, the Fe/N conversion mechanism under the combined action of Feammox and NDFO was analyzed, and the microbial abundance and functional microorganisms in the system were explored.

2. Methods and materials

2.1. Materials and setup

The water samples used in this experiment were taken from a livestock farm, filtered through a sieve and diluted 5 times, then stored at 4°C for later use. The inoculated sludge (with a small amount of iron) came from the second sedimentation tank of a sewage treatment plant.

In this experiment, three 500 mL glass bottles were used as anaerobic digestion reactors, and 400 mL of a mixture of livestock wastewater and inoculated sludge were added to the three reactors (C1, C2, C3), and the ratio of livestock wastewater to inoculated sludge is 3:1. The initial content of pollutants measured in each reactor is shown in Table 1. After that, using the C1 reactor as the blank group, the Fe(III) concentration in the C2 and C3 reactors was increased to 50 mg/L. From the 10th day, 1 mL $NaNO_3$ solution with a concentration of 9.6 g/L was added every 10 d in the C3 reactor (10, 20, 30, 40, 50 d), the other two groups were replaced with the same amount of deionized water. In each reactor, the pH is controlled at 5.5–6.5, and the acid-base adjusting solution is 0.5 mol/L HCL and 0.5 mol/L NaOH. Before the reaction, purge the solution with pure nitrogen or helium for 30 min to remove oxygen in the inner and headspace of the glass bottles, and then seal it with a lid, and then drill two holes in the lid to connect the biogas sampling bag and the liquid sampling pipe. Each reactor was wrapped with a light-shielding cloth. Finally, put the glass bottle on a thermostatic magnetic stirrer with a set temperature of 32°C ± 2°C and a rotating speed of 120 rpm. The entire experiment was carried out under anaerobic conditions, and the changes of nitrogen and iron in the reactor were observed within 64 d. The experiment was repeated three times.

Table 1
Initial content of pollutants in each reactor

Project	Content (mg/L)	Project	Content (mg/L)
NH_4^+–N	125.6 ± 1.23	Fe(II)	85.2 ± 4.21
Total nitrogen	350.6 ± 5.89	Fe(III)	12.7 ± 1.15
NO_3^-–N	19.7 ± 0.14	Protein	367.2 ± 3.02
COD	3,750.3 ± 10.35		

2.2. Analysis method

Take 1 mL water sample to dilute for each measurement. Determine the COD content in water samples according to the method described by Morita et al. [17]. The protein was determined according to Yang et al. [18]. NH_4^+–N concentration was analyzed using Nessler's reagent spectrophotometry (DR2800, HACH, USA). NO_2^-–N and NO_3^-–N were detected by the method used by Zhu et al. [19]. Total nitrogen was determined by the method of Yang et al. [20], and the pH value was directly measured with a pH meter. Volatile fatty acids (VFA, including acetic acid, propionic acid, and butyric acid) were analyzed by gas chromatography (Shimadzu, GC2010). Dissolved oxygen was determined by a DO meter (Modle 55/12, YSI, USA). The o-phenanthroline spectrophotometric method has used the concentration of Fe(II) and Fe(III), and the analysis of microbial community analysis adopts 16S rRNA high-throughput sequencing technology.

Use Origin 9.0 (Inc., OriginLab, Northampton, MA) software to draw graphics, and use one-way analysis of variance (One-Way ANVON), the average comparison is analyzed by LSD method, and the most suitable change equation uses regression analysis and correlation analysis was performed for fitting, $P < 0.05$.

3. Results and discussion

3.1. Conversion of nitrogen

According to the results in Fig.1, NH_4^+–N in the three reactors accumulated during the initial 16 d due to the ammonium nitrogen generation from protein decomposition [21]. Among them, the accumulation of NH_4^+–N in the C1 group was the least, only 214.5 mg/L. The accumulated of NH_4^+–N in the C2 group was 283.2 mg/L, which was significantly higher than that of C1, because the addition of Fe(III) enhanced the occurrence of Fe(III) dissimilatory reduction and promoted the decomposition of protein [22]. Comparatively, the C3 group had the highest accumulation of NH_4^+–N, reached 353.7 mg/L. It was reasonable to speculate that the addition of nitrate raised the degradation of sludge and further promoted the decomposition of protein to produces more NH_4^+–N [12]. The above conjecture can also be confirmed from Fig. 2. The initial protein content was 367.2 mg/L. After 64 d of experiment, the protein content of C1, C2, and C3 group were 273.20, 170.01, 114.57 mg/L, and the protein removal rate was 25.6%, 53.7%, 68.8%, respectively. It supports that the addition of Fe(III) could accelerate the decomposition of protein into NH_4^+–N, and nitrate further advanced the decomposition of protein.

After 16 d, the NH_4^+–N content of each reactor began to decrease. Among the three groups, the C2 group had a better NH_4^+–N removal effect on the 16th–45th day, because sufficient Fe(III) accelerated the Feammox reaction and made a large amount of NH_4^+–N reacted with Fe(III) to generate NO_3^-, NO_2^- and N_2. Feammox process may be accompanied by anammox reaction [23]. However, the decline of NH_4^+–N slowed down after 45 d, it may be because the Feammox reaction no longer dominated and most of the reaction products generate N_2 released, and only a small part generated nitrate [24]. The consumption of nitrate was much greater than the production, which would result in NDFO to weaken or stagnate after 45 d and could not continue to produce Fe(III). The gradual decrease of Fe(III) content inhibited the progress of Feammox reaction, and the circulation of NDFO and Feammox was blocked.

Remarkably, the C3 group had the highest accumulation of NH_4^+–N, but the removal rate of ammonia nitrogen was also the fastest. The NH_4^+–N content of C3 decreased from 353.7 to 121.7 mg/L by the 39th day of the reaction. During the same period, the NH_4^+–N content of C1 and C2 were 146.7 and 152.6 mg/L. By the 64th day, the NH_4^+–N content of C3 was 39.3 mg/L, and the removal rate was 88.9%, which was the highest among the three groups. The NH_4^+–N removal rates of the other two groups were 37.1% and 65.1%. This may be due to the addition of nitrate

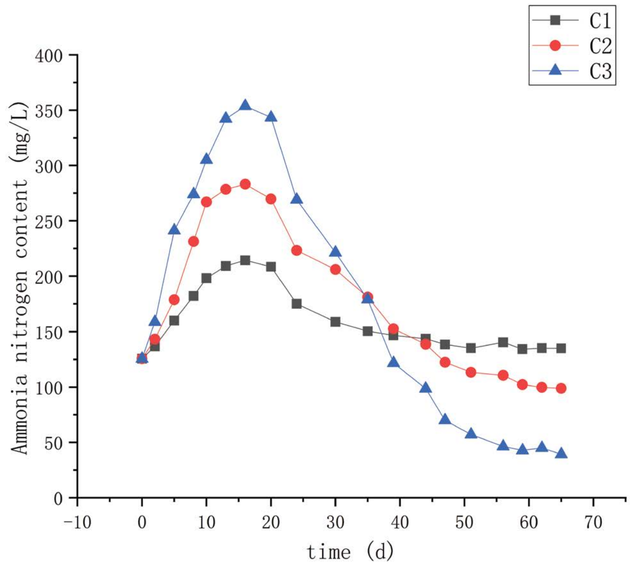

Fig. 1. Change of ammonia nitrogen content in C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) within 64 d.

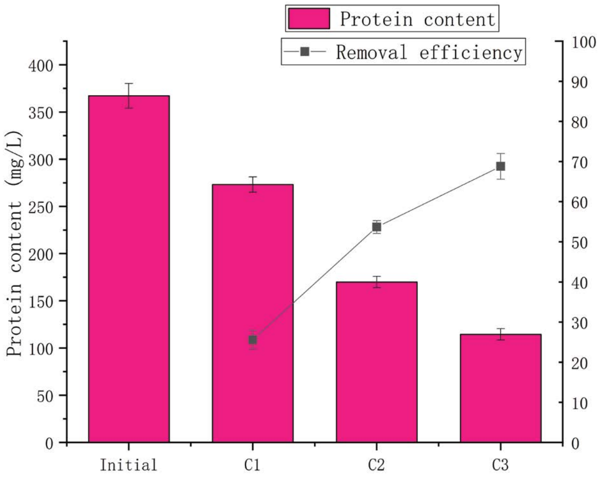

Fig. 2. Protein content and removal rate of C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) after 64 d.

on the basis in C3 reactor, which repeatedly increased the nitrate content, promoted the occurrence of NDFO, and continuously produced a large amount of Fe(III). The newly generated Fe(III) participated in the removal of ammonia nitrogen by Feammox, forming a virtuous cycle of NDFO and Feammox. The NH_4^+–N content of the C3 group tended to balance on the 55th day, because the nitrate added on the 50th day was consumed a lot and the NH_4^+–N content was also low. The lack of reactants of NDFO and Feammox resulted in almost stagnation of the reaction. In addition, it should be noted that although Fe(III) and nitrate were not added in the C1 group, there was still 37.1% NH_4^+–N loss. Considering C1 also contains a small amount of nitrate and Fe(III), it was reasonably assumed that C1 may also have a lower degree of NDFO and Feammox in addition to anammox. This part of the guess will be further confirmed in the subsequent analysis.

As shown in Fig. 3, the NO_3^- of the C1 group continues to decrease, which confirms the previous guess. The C2 and C3 groups accumulated NO_3^-, while the NO_3^- accumulation of C2 reached the maximum value of 27.4 mg/L. It showed that the addition of Fe(III) accelerated the Feammox reaction to oxidize NH_4^+ and produced NO_3^-. Subsequently, the occurrence of NDFO led to rapid consumption of NO_3^-. And the alternating dominant phenomenon of Feammox and NDFO makes the NO_3^- content of C2 decreased along with two little peaks on day 26 and day 38, and it tends to be flat by the 45th day. After 50 d of operation, the NO_3^- content was only 2.1 mg/L, this also certificated the phenomenon that the NDFO reaction was inhibited due to the insufficient nitrate of the C2 group, which resulted in a low ammonia nitrogen removal rate in the later stage. The C3 group had the same phenomenon of NO_3^- accumulation as C2 in the first 10 d. After NO_3^- was added on the 10th day, its content increased sharply. As the increase of NO_3^- promoted the NDFO reaction, NO_3^- reacted with Fe(II) and oxidized to produce Fe(III), which led to rapid consumption after the increase in NO_3^- content. Similarly, after the 20th, 30th, 40th, and 50th d, the NO_3^- content appeared to be sharply increased and rapidly decreased. Finally, NO_3^- tended to the lowest level of 1.7 mg/L after the 55th day, this also explains why the NH_4^+–N removal rate of the C3 group had been at a high level during the first 55 d.

Fig. 4 demonstrates the changes in NO_2^- content of the three groups within 64 d. The NO_2^- content of the C1 group was almost undetectable except for a small amount detected within the first 15 d, but this does not mean that there is no production of NO_2^- during the experiment. According to the previous analysis [13], it was likely that the NO_2^- produced was rapidly denitrified when exposed to sufficient organic matter. The NO_2^- content in the first 15 d of the C2 group was extremely low and the reason was the same as that of C1. This can also be reflected in Fig.5. The VFA content of the three groups was rapidly consumed in the first 15 d, and it was almost undetectable after 20 d. A small amount of NO_2^- accumulated in the C2 group after 15 d, because the organic matter was almost exhausted, and the Feammox reaction between Fe(III) and ammonia nitrogen occupied the main position to produce NO_2^-. In the C2 group, almost no NO_2^- could be detected after 32 d. In group C3, NO_2^- reached the peak soon after each nitrate addition, and then decreased rapidly, which indicated that NO_2^- produced by NO_3^- reduction accelerated the NDFO reaction, and NO_2^- reacted with Fe(II) to form Fe(III). No NO_2^- was detected in C3 group after 60 d.

3.2. Iron changes

Figs. 6 and 7 show the mutual transformation of Fe(III) and Fe(II) in the three groups within 64 d. The consequences showed that the Fe(III) and Fe(II) in the C1 group had slight fluctuations, which indicated that there was a slight mutual transformation between Fe(III) and Fe(II) and further proved the previous hypothesis: the combined

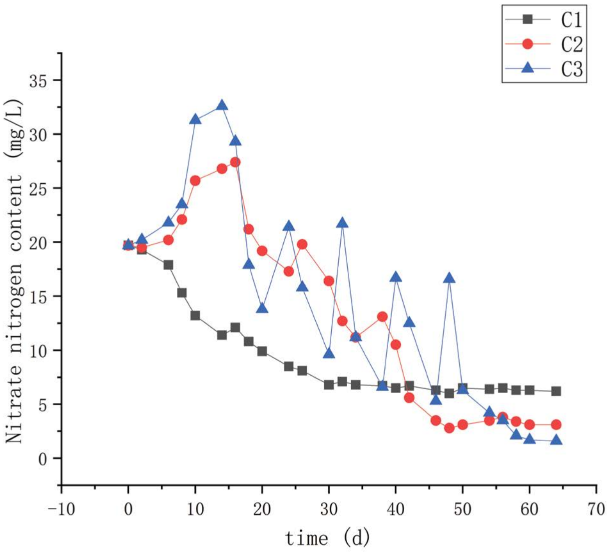

Fig. 3. NO_3^- content change in C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) within 64 d.

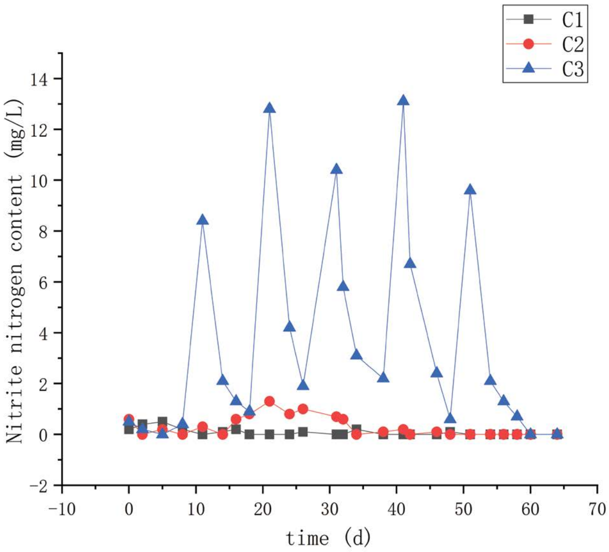

Fig. 4. NO_2^- content change in C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) within 64 d.

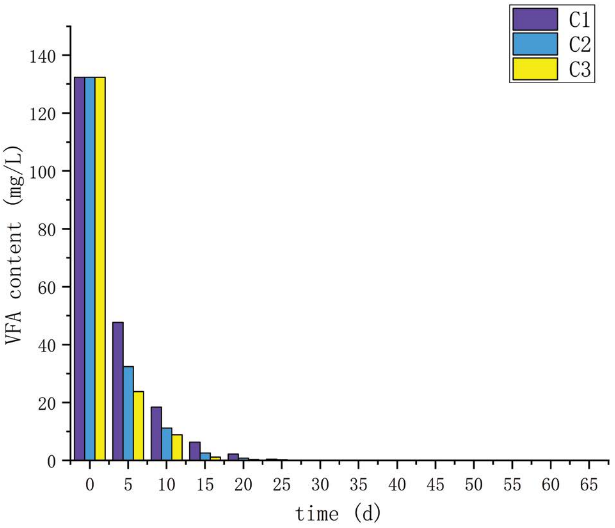

Fig. 5. VFA content changes in C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) within 64 d.

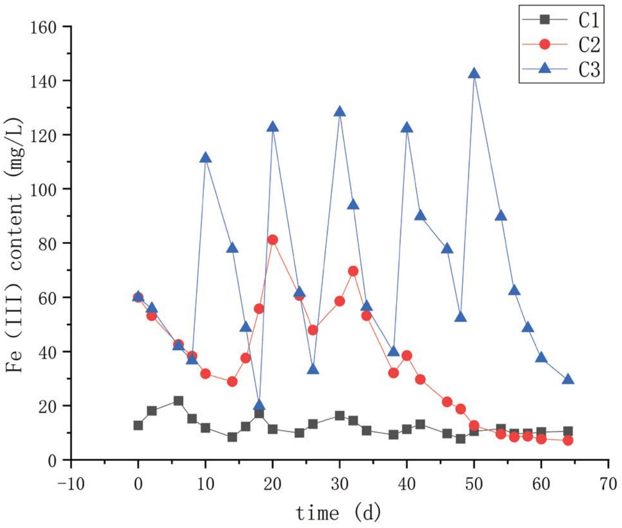

Fig. 6. Fe(III) changes within 64 d of C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$).

reaction of Feammox and NDFO also occurred to a lesser degree in C1. Fe(III) decreased and Fe(II) increased in C2 group for the first 15 d. This may be related to the high content of organic matter in the initial stage of the reaction, which limited the oxidation of Fe(II), and Fe(III) reacted organic matter as an electron donor to produce Fe(II). Thermodynamically, organic matter was preferentially used as the electron donor for Fe(III) reduction than ammonium [25,26]. After 15 d, the organic matter was almost exhausted, and a large amount of Fe(II) participated in the NDFO reaction and oxidized to produce Fe(III). Therefore, the Fe(III) content rebounded and further facilitated the next round of Feammox. Due to this alternate dominant phenomenon of Feammox and NDFO, Fe(III) and Fe(II) fluctuated twice in C2 group. Subsequently, with the massive consumption of nitrate, NDFO could not continue to produce a large amount of Fe(III), Fe(III) was gradually consumed in the Feammox reaction, and Fe(II) continued to increase. By the 64th day, Fe(III) was only 7.2 mg/L, and Fe(II) increased to 159.6 mg/L.

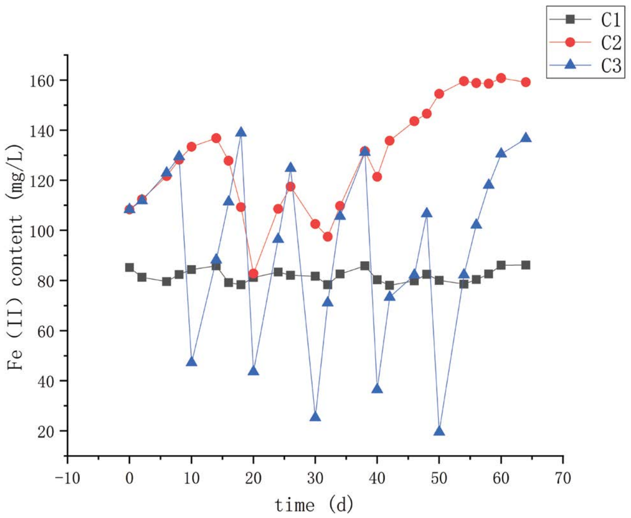

Fig. 7. Fe(II) changes within 64 d of C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$).

The fluctuation of Fe(III) and Fe(II) in the C3 group was periodic with the time interval between two nitrate additions. In the early stage, Fe(III) continued to decrease and Fe(II) continued to increase with sufficient organic content. After adding nitrate on the 10th day, the Fe(III) content increased sharply and Fe(II) decreased rapidly during the same period, which revealed that the addition of nitrate extremely stimulated the dominant position of NDFO. Then the effect of NDFO weakened with the consumption of nitrate content and Feammox occupied the dominant position, Fe(III) began to decreased and Fe(II) increased again. When the next nitrate addition time reached (day 20), there existed a new round of iron conversion cycle. The results showed that the regular addition of nitrate could promote the mutual transformation of Fe(III) and Fe(II), advance the virtuous cycle of Feammox and NDFO, make them continuously carry nitrogen, and maintain good nitrogen removal effect. After the last addition of nitrate, the final Fe(III) decreased to 29.4 mg/L, and Fe(II) accumulated to 136.7 mg/L with the consumption of nitrate.

3.3. Microbial community analysis

In order to investigate functional microorganisms under the synergistic nitrogen removal system of Feammox and NDFO, 16S rRNA high-throughput sequencing technology was applied to initial sludge and sludge that reacted to 30 and 64 d in a C3 group (Fig.8). The composition of microbial species in the early, middle and late stages of the C3 group was explored, and the variation of microbial community type and abundance was analyzed under the combined operation of Feammox and NDFO. From Fig. 8, the sequencing results illustrated that the dominant microorganisms in the inoculated sludge were mainly Anaerolineae,

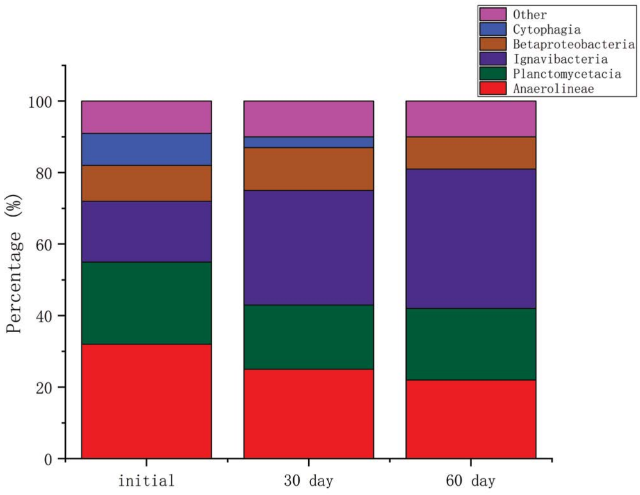

Fig. 8. Comparison of microbial abundance in different stages of C3(Fe(III)+$NaNO_3$) reactor.

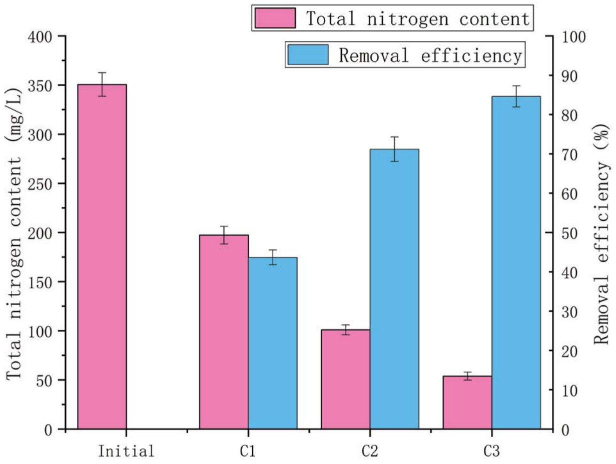

Fig. 9. The total nitrogen content and removal rate of C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) after 64 d.

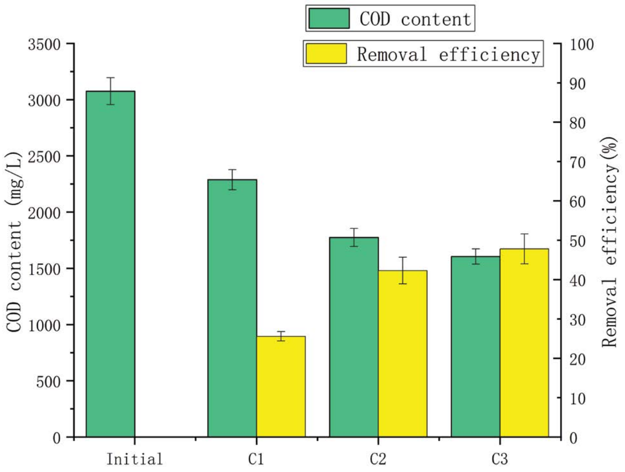

Fig. 10. COD content and removal rate of C1(control), C2(Fe(III)) and C3(Fe(III)+$NaNO_3$) after 64 d.

Planctomycetacia, Ignavibacteria, Betaproteobacteria, and Cytophagia. Among them, the abundance of Anaerolineae was very high in different stages, and there were no such species in the previous reports of anammox functional related microorganisms [26]. The living conditions of these bacteria are similar to those of anammox bacteria, but the effect of nitrogen removal remains to be investigated. Planctomycetacia was the main genus of bacteria involved in the anammox reaction, which accounted for a relatively large proportion of the sequencing results. The environment in the reactor changed with the progress of the reaction, and the advantage and proportion of Planctomycetacia decreased, but still existed, which indicates that there was certain anammox function in the system. Ignavibacteria was the most significant change in microbial abundance, as the reaction progresses, the abundance of bacteria increased from 17% to 39%, becoming the most dominant bacteria. It can be basically concluded that Ignavibacteria is the main functional microorganism involved in the collaborative nitrogen removal system of Feammox and NDFO. The abundance of Betaproteobacteria that reacted for 64 d was not much different from the initial sludge. As the reaction progresses, the abundance of Cytophagia continued to decrease, and eventually it was almost completely eliminated.

3.4. Treatment effect and discussion

After 64 d of reaction in the three groups, the total nitrogen and COD content in each group were measured, as shown in Figs. 9 and 10. It was found that the total nitrogen content in C1, C2, and C3 groups dropped from the initial 350.6 to 197.39, 100.9, and 53.9 mg/L, with removal rates of 43.7%, 71.2%, and 84.6%, respectively. Although C2 group can increase the nitrogen removal rate by adding Fe(III) to realize the cycle of Feammox and NDFO, the iron cycle of NDFO will be stopped and interrupted due to the lack of nitrate in the later stage, and excessive addition of Fe (III) will generate $Fe(OH)_3$, which will affect the normal operation of the reactor and increase the difficulty of cleaning [15]. Therefore, it is unrealistic to continuously add Fe(III) to boost the circulation of Feammox and NDFO. However, When Fe(III) was in a certain amount, the occurrence of NDFO was continuously promoted by adding nitrate to C3 reactor. The NDFO continuously produced new Fe(III) and then applied it to the next round of Feammox, realizing a virtuous cycle of Feammox and NDFO without too many by-products. Therefore, this is a desirable new nitrogen removal technology.

After 64 d of the experiment, the COD content in the three groups decreased from 3,075.3 to 2,288.1, 1,774.45, and 1,605.3 mg/L, with removal rates of 25.6%, 42.3%, and 47.8%, respectively. The COD removal rate of these three groups of reactors is relatively low, and the reason for which remains to be explored. The reason for the low COD removal rate in the experiment, how to solve this problem and microbial analysis are the contents of our future research.

4. Conclusion

- In this experiment, the Fe(III)-added C2 group realized the circulation of Feammox and NDFO, but the cycle was weakened or even stopped due to insufficient nitrate after 45 d. The final ammonia nitrogen removal rate was 65.1%, and the total nitrogen removal rate was 71.2%.
- After adding nitrate regularly in the C3 reactor based on the C2 reactor, it can form a virtuous cycle of Feammox and NDFO without producing too many by-products. The final ammonia nitrogen removal rate reached 88.9%, and the total nitrogen removal rate reached 84.6%, the effect was the best among the three groups, which certificated that adding nitrate was more suitable to accelerate the nitrogen removal technology of Feammox and NDFO. It provided a new experimental reference for the future livestock wastewater treatment technology.
- The results of 16S rRNA high-throughput sequencing technology on the initial sludge and the sludge on the 30th and 64th days of the C3 group showed that Ignavibacteria were the main functional microorganism of the Feammox and NDFO collaborative nitrogen removal system, and the system also accompanied anammox function.

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