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Remediation of low C/N wastewater by iron–carbon micro-electrolysis coupled with biological denitrification: Performance, mechanisms, and application

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ABSTRACT

The ecological safety problems caused by nitrogen pollution in water have attracted much attention. An organic carbon source is the key factor that restricts nitrogen removal in low COD/total nitrogen (C/N) sewage. In this study, iron-carbon micro-electrolysis carrier was used for micro-electrolysis combined with biological denitrification (MEBD) of samples under low C/N conditions (C/N = 1.33). The nitrogen removal performance, the factors influencing nitrogen removal, and the underlying mechanisms of the removal system are studied using the response surface curve method and high-throughput sequencing. The results showed that when pH was 7, the dosage of denitrifying bacteria was 5%, and the dosage of iron-carbon was 10%, the optimal denitrification effect for low C/N wastewater was 97.86% and 97.72% for total nitrogen and COD removal in 20 days, respectively. The addition of an iron-carbon filler changed the community abundance and species diversity in MEBD system, and the structure of the dominant denitrifying bacteria changed. Moreover, hydrogen autotrophic denitrifying bacteria had been identified in the system. The synergistic effect of MEBD coupled denitrification system was due to the combined action of autotrophic denitrification, heterotrophic denitrification, micro–electrolysis, and electrochemical reduction. These findings provide a theoretical basis and technical support for nitrogen remediation of low C/N sewage.

1. Introduction

Nitrogen pollution primarily results from the use of nitrogen-containing fertilizers, animal waste, septic systems, industrial processes, atmospheric deposition from nitrogen oxide emissions, and irrigation and storm runoffs from farmlands [1]. Nitrogen contamination of surface and groundwater waters is an increasingly ubiquitous issue worldwide [2], posing potential risks to human health and aquatic ecosystems. Also, nitrogen removal in wastewater treatment, water resources conservation, and water supply remains a challenge.

Wastewater with a low carbon–to–nitrogen ratio (C/N), such as the tail water from a municipal wastewater treatment plant and nitrate–contaminated groundwater, lacks carbon sources to advance removal requirements. Biological nitrogen removal technologies have undergone extensive development in the area of water treatment due to their low cost and pollution–free products [3,4]. In the process of biological denitrification, C/N is a key indicator and organic carbon sources can affect the denitrification efficiency of heterotrophic denitrifying bacteria (DB) [5]. The current domestic sewage generally has the characteristics of insufficient carbon sources and low C/N [6]. Therefore, when the C/N

Abbreviations: C/N, carbon–to–nitrogen ratio; MEBD, micro-electrolysis combined with biological denitrification; DB, denitrifying bacteria; RSM, response surface methodology; HTS, high-throughput sequencing; COD, chemical oxygen demand; TN, total nitrogen; DO, dissolved oxygen; Fe-C, iron–carbon; BBD, Box–Behnken design.

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in wastewater is low, it is necessary to use an additional carbon source as an electron donor for denitrification, to increase the denitrification rate of the system and reduce the concentration of nitrite in the effluent, and then completely remove nitrate or nitrite [7,8]. This results in waste and the risk of organic residues and additives. Several autotrophic denitrification-based methods, such as autohydrogenotrophic denitrification, sulfur–based denitrification, and anaerobic Fe^{2+} oxidization denitrification, have been already developed for nitrogen removal from low C/N wastewater [9]. However, whether sulfur or Fe are used as the donor of electrons, autotrophic denitrification for the removal of total nitrogen (TN) is insufficient [3,4]. Therefore, it is important to seek an efficient nitrogen removal technology at the condition of low C/N.

Electrochemical methods have been and continue to be widely studied for their oxidation ability and high degradation efficiency in wastewater treatment [10]. The chemical oxygen demand (COD) and ammonia in wastewater can be efficiently degraded using anodic oxidation, but this requires high electricity consumption. Metallic iron (Fe^0) or nanoscale zero valent iron has been adopted as an electrode material and applied in NO_x reduction. The primary product ammonia in the treated wastewater still poses a risk for human beings and ecosystems. In this regard, micro–electrolysis combined biological denitrification (MEBD) has been developed for nitrate removal from low organic carbon water. DB are cultured on the cathode, and they can use electrons thereon for denitrification [11,12]. The operational processing efficiency of MEBD is affected by several factors including micro-electrolytic materials, DB density or abundance, pH and so on. Response surface methodology (RSM) is a robust mathematical and statistical tool for optimizing the experimental parameters and evaluating the interactions of important factors, which can be utilized to optimize the experimental conditions for the application of MEBD system.

Deng et al. [13] adopted Fe^0-carbon micro-electrolysis combined autotrophic denitrification to remove nitrate from low C/N wastewater, and the excellent performance and the microbial communities of the reaction system were verified by applying organic–free conditions and high-throughput sequencing. It was found that MEBD technology can reduce the addition of organic carbon source, which was suitable for the treatment of low C/N wastewater. In addition, the removal rate of NO_3^--N can reach 95% during 50 days of operation under given conditions (C/N = 1, T = 27 ± 1 °C and pH = 8.0 ± 0.3) [14]. Xu et al. [15] found that electrochemical combined with constructed wetlands could effectively improve the nitrogen removal efficiency through the combined effect of autotrophic and heterotrophic denitrification. The origin and roles of microorganisms in the coupling system still require further investigation. Although this bio-electrochemical coupling technology has theoretical potential for development, it has not been promoted and applied for the treatment of low C/N wastewater because the underlying mechanisms and optimal operating conditions remain unrevealed.

A native microorganism that utilizes denitrification was screened from the sediment of a river in Chengdu to investigate the feasibility and mechanisms of the proposed bio–electrochemical coupling technology for nitrogen removal. An iron–carbon (Fe-C) micro–electrolysis coupled biological denitrification (MEBD) system was then developed. After a series of single factor experiments, the operating conditions, including the Fe-C dosage, the DB dosage, and the initial pH, were optimized using RSM. The distribution of the microbial community structure during nitrogen removal was explored using high-throughput sequencing (HTS), and the synergistic denitrification mechanism of MEBD system was analyzed in combination with the difference in pollutant removal. This study further reveals the mechanism and optimal operating conditions of MEBD system for nitrogen removal and provides important data for the promotion and application of the proposed technique.

2. Materials and methods

2.1. Chemicals

Fe-C materials was purchased from the Shenzhen Lantaixing Environmental Protection Materials Co., Ltd. The filler is mainly composed of zero-valent iron (Fe^0), activated carbon (AC) and catalyst by high temperature fusion. Its appearance is spherical, and its main characteristics and components are shown in the Table S1. Other chemical reagents, including glucose, potassium sodium tartrate tetrahydrate (purity, 99%), hydrochloric acid (AR), sulfuric acid (purity 98%), sodium hydroxide (purity, 98%), ammonium iron (II) sulfate hexahydrate (purity, 99.5%), potassium dihydrogen phosphate (purity, 99.5%), and mercuric iodide, were purchased from the Chengdu Kelong Chemical Reagent Factory. Ultrapure water was obtained using an OKP ultrapure water system (Shanghai Lichen Bangxi Instrument Technology Company).

2.2. Screening and culturing of strains

The DB strains were screened from the sediments of a pond located near the Chengdu Institute. The medium was prepared using deionized water and adjusted to pH 7.0–7.2 using 1 mol/L HCl or NaOH. It was then sterilized at 121 °C in an autoclave for 30 min (BXM-30R, Shanghai Boni Industrial Co., Ltd. Medical Equipment Factory). Purification and enrichment of DB strains were performed at the Chengdu Institute of Biology, Chinese Academy of Sciences. After enrichment, primary screening, secondary screening, and domestication, the DB strains were stored in a 4 °C refrigerator for further use.

2.3. Batch experiments

2.3.1. Nitrogen removal performance of MEBD

The low C/N wastewater (C/N = 1.33) was simulated using tap water according to Zhao et al. [16]. KNO_3 and glucose were mainly adopted to simulate the TN and COD, respectively. The composition of other trace element were shown in Table S3.

DB, Fe-C, and MEBD system were adopted to remove nitrogen from the simulated wastewater. The nitrogen removal performance of the three systems was compared to verify the denitrification performance of MEBD. DB were cultured to the bacterial solution at OD_{600} = 0.869 in the logarithmic growth phase for experiment. The dosage of DB was set to 5% (v/v) in the group of DB and Fe-C, the filling ratio of the Fe-C was 0.1. MEBD system is a mixture of 5% DB and 10% Fe-C, which was added into 500 mL conical flask, and the volume and pH of simulated low C/N wastewater were controlled at 200 mL and 7.0, respectively. N_2 was used for 10 min to remove oxygen. The experimental groups were placed in a 30 °C incubator for the experiments. The samples were collected once every two days and filtered using a 0.45 μm filter membrane before analysis. The concentration of COD, NO_3^-, NO_2^- and TN were determined according to standard methods [17].

2.3.2. Influencing factors of nitrogen removal in the MEBD system

The effects of initial pH, Fe-C dosage (m/v), and DB dosage (v/v) on nitrogen removal of the MEBD system were investigated using single factor experiments. Based on the pH range in the polluted waters, three levels of pH (pH = 5, 7, 9) were designed to investigate their effect on nitrogen removal in the MEBD, as well as using Fe-C and DB dosages of 10% and 5%, respectively. Effects of Fe-C dosage (m/v) and DB dosage (v/v) on nitrogen removal in MEBD system experimental design is shown in the Table S2. The experimental wastewater volume of each experimental group was 200 mL, and the dosage of the trace elements was 2 mL/L (Table S3). After adding the required materials, N_2 was used for deoxygenation. The reactors were then sealed with rubber plugs and sealing membranes. Finally, the reactors were placed in a constant temperature incubator at 30 °C for the experiment. The samples were collected every two days during the experiment, and the obtained

samples were filtered through a 0.45-μm filter membrane. The concentrations of COD, NO_2^- and NO_3^- were measured using the methods mentioned above. Fe^{2+} concentrations were measured using the revised ferrozine assay [18].

Each experiment was repeated at least three times. The experimental data were analyzed using a single factor analysis of variance in SPSS 25 software and the significant difference was accepted when $p < 0.05$. Origin 2017 software was used for drawing.

2.3.3. Application of MEBD

To investigate the remediation performance of MEBD for actual wastewater, the low C/N wastewater and sediment used in the experiment were collected from the pond located near the research institute in Chengdu. The main information of the actual wastewater was concluded that the concentration of NO_3^--N and COD was 30 mg/L and 40 mg/L, and the pH was 7.60. The variation in the water quality and nitrogen removal mechanism during MEBD degradation were investigated. The experimental device is shown in Fig. S1. Four experimental groups, namely the control group, the systems of DB, Fe-C, and MEBD were established separately and then compared to investigate the denitrification performance of MEBD. All of the experiments were conducted outdoors at temperatures ranging from 25 to 32 °C. The experiments lasted 20 days, and samples were collected at 10 a.m. every day. The water samples were extracted using a disposable syringe (10 mL & 20 mL, the Jiangsu Zhiyu Medical Equipment Co., Ltd.), and filtered using a 0.45 μm filter. They were then brought back to the laboratory to determine the concentrations of nitrogen (TN, NO_3^--N, NO_2^--N) and COD, and the dissolved oxygen (DO) and pH were measured in the field.

2.3.4. Parameter optimization

The Box–Behnken design (BBD) model was used to investigate the influence of three factors and three levels using 17 tests on the NO_3^--N degradation efficiencies. The BBD design matrix is shown in Table S4, and the relevant experimental data were analyzed in detail using Design Expert 8.0.6 software. According to the single factor test results, the factors and levels were designed, and these included the pH, DB dosage, and Fe-C dosage (Table S5). The optimum nitrogen removal conditions of the MEBD were obtained using response surface fitting, and this provided the basis for the design parameters of the MEBD treatment of low C/N wastewater.

2.3.5. Microbial diversity analysis method

To further reveal the denitrification mechanism of MEBD system, water samples were taken for HTS at different time in the experimental process and the evolution of microbial community structure was analyzed. The species of DB in MEBD system sampled on day zero, ten and twenty were labeled as DB, D10 and D20, respectively. All samples were filtered by 0.45-μm membrane and stored in −20 °C refrigerator, then sent to Bioengineering (Shanghai) Co., Ltd. for HTS. The primers set 515F (GTGCCAGCMGCCGCGGTAA) and 909R (CCCCGYCAATTCMTTTRAGT) were used for the amplification of V4–5 sequences [19].

3. Results and discussion

3.1. Performance of MEBD for low C/N water treatment

As shown in Fig. 1, the removal rate of NO_3^--N by MEBD system was approximately 60% after eight days of operation. This was significantly better than the removal rate of DB and Fe-C system, and NO_2^--N accumulation was not found in MEBD system. In DB system, due to the lack of a carbon source in the simulated wastewater, the removal of NO_3^--N in the system was poor, and there was a certain accumulation of NO_2^--N in the system. In Fe-C system, after the beginning of the reaction, NO_2^--N as the intermediate product of the micro-electrolysis reaction, accumulated in the system. Due to the Fe-C micro-electrolysis reaction, the system was in a strong reductive environment, NO_2^--N was continuously reduced to NH_4^+ or other products with lower valence [11,12]. Fe-C system had a faster removal rate of NO_3^--N during the first two days, and this was due to the larger pore size of the filler and the strong adsorption of NO_3^--N [20]. After four days, the removal rate of NO_3^--N in the system began to decrease due to desorption from the iron-carbon filler. Whereafter, Fe-C system maintained a fixed pollutant removal effect due to micro-electrolysis.

In addition, the removal rate of COD by MEBD system reached 82%, and this might have been due to the coupling effect of micro–electrolysis and biological denitrification. Heterotrophic microorganisms can use organic carbon sources for traditional denitrification, and a large number of electrons would be generated during the micro-electrolysis process that could be used by autotrophic DB [21], thereby reducing the COD concentration. There was no significant difference in the COD degradation efficiency between the DB and Fe-C systems. The COD removal rates of the two systems were 66% and 60%, respectively, which were lower than the removal rates of the MEBD system.

3.2. Effects of the key parameters of MEBD

3.2.1. Effect of pH

When the pH were 5, 7, and 9, the removal rates of NO_3^--N by MEBD system were 79.2, 57.9, and 39%, respectively (Fig. 2). The denitrification efficiency of MEBD system under acidic conditions was significantly higher than under neutral and alkaline conditions. In MEBD system, when the pH was 5, NO_3^--N was rapidly converted to NO_2^--N, resulting in the accumulation of NO_2^--N in the system. Under neutral conditions, heterotrophic denitrifying bacteria proliferated in large numbers, and this was coupled with micro–electrolysis and denitrification. Most of NO_3^--N was reduced to N_2, thereby achieving complete

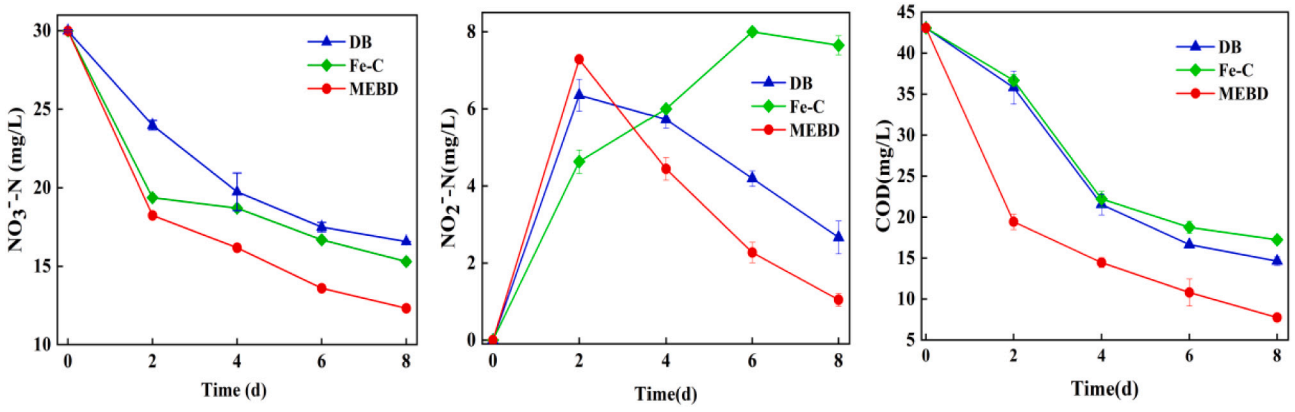

Fig. 1. Changes in the NO_3^--N, NO_2^--N, and COD concentrations over time in each system.

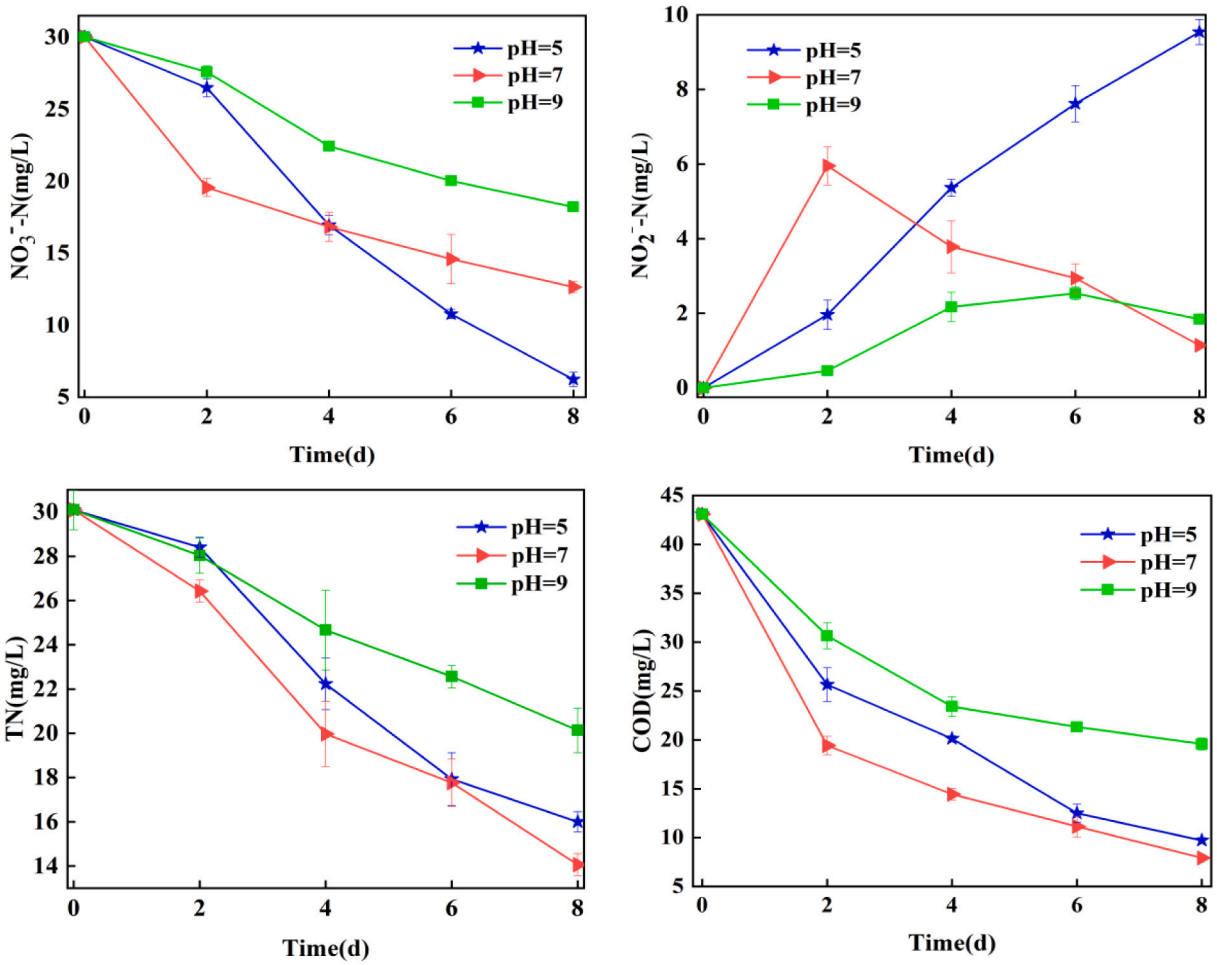

Fig. 2. Variation in the NO_3^--N, NO_2^--N, TN and COD concentration with time in MEBD system under different pH conditions.

nitrogen removal. In addition, the MEBD system had the highest removal rate of TN. Although the DB had higher activity and faster denitrification rate under alkaline condition (pH = 8) [14], the reduction rate of nitrate nitrogen decreased due to the decrease of Fe^{2+} production rate in micro-electrolysis reaction [22]. Furthermore, the presence of activated carbon might reduce the pH of the water [23]. According to the above, it seemed reasonably that the optimal pH condition in the MEBD system was 7.

With an increase in the pH, the COD removal showed a trend of first increasing and then decreasing (Fig. 2). Under neutral conditions, the removal rate of COD by MEBD was 81.6%. Under acidic conditions, the removal rate of COD was 77.4%, and the removal rate of COD was only 54.6% under alkaline conditions. This study found that MEBD system had a better removal effect on NO_3^--N under acidic conditions, and under acidic and neutral conditions, the [H] (reducing hydrogen) generated by micro-electrolysis reaction could react with the organic matter in the wastewater, which reduced the COD concentration in the wastewater. Under an alkaline environment, due to the difference between potential of the anode and the cathode, the micro–electrolysis effect was weakened, and the reduction substances were reduced, resulting in poor COD removal effect. Under neutral conditions, it was not only conducive to the proliferation of DB, but also had weak inhibitory effect on micro-electrolysis reaction, and the removal effect of NO_3^--N and COD was better.

3.2.2. *Effect of the Fe-C dosage*

Generally, the TN and NO_3^--N concentration in the MEBD system decreased with the increasing of Fe-C dosage (Fig. 3). After eight days, the NO_3^--N concentration was similar (18.4–19 mg/L) for all the treatments with an increase in the Fe-C dosage of 15%–20%, and the removal rates were 24.4% and 20.3%. However, the NO_3^--N concentration was only 14.1 mg/L at an Fe-C dosage of 10%, and the removal rate was 50.2%. This result indicated that the NO_3^--N removal rate did not increase with the increasing of Fe-C dosage. On the contrary, when the dosage exceeded 10%, the NO_3^--N removal rate decreased. According to previous studies, when the Fe-C matrix is small, the number of micro-primary batteries formed by micro-electrolysis decreases, and the amount of electron donors would be insufficient, which is not conducive to denitrification [24]. When the Fe-C matrix is too large, micro–electrolysis would inhibit denitrification, and this is not conducive for the removal of NO_3^--N. In addition, the concentration of NO_2^--N in each experimental group increased first and then decreased. When the dosage of Fe-C exceeded 15%, the accumulation of NO_2^--N was serious. Therefore, MEBD system had a better denitrification effect when the dosage of Fe-C was 10%.

With an increase in Fe-C dosage, the removal rate of COD in MEBD system was first high and then low. When Fe-C dosage was 5%, 10%, 15%, and 20%, the removal rates of COD were 60.3%, 73.2%, 42.8%, and 37.5%, respectively. These findings demonstrated that the Fe-C matrix content had a great influence on the removal of organic matter by MEBD system. The micro-electrolysis reaction generated reduction state [H] reacted with organic matter in the wastewater to degrade macromolecular organic matter and promote the removal of COD. The higher the iron-carbon content in Fe-C system, the more conducive to the removal of COD. On the other hand, an excessive Fe-C matrix would inhibit microbial denitrification, resulting in a decrease of the COD

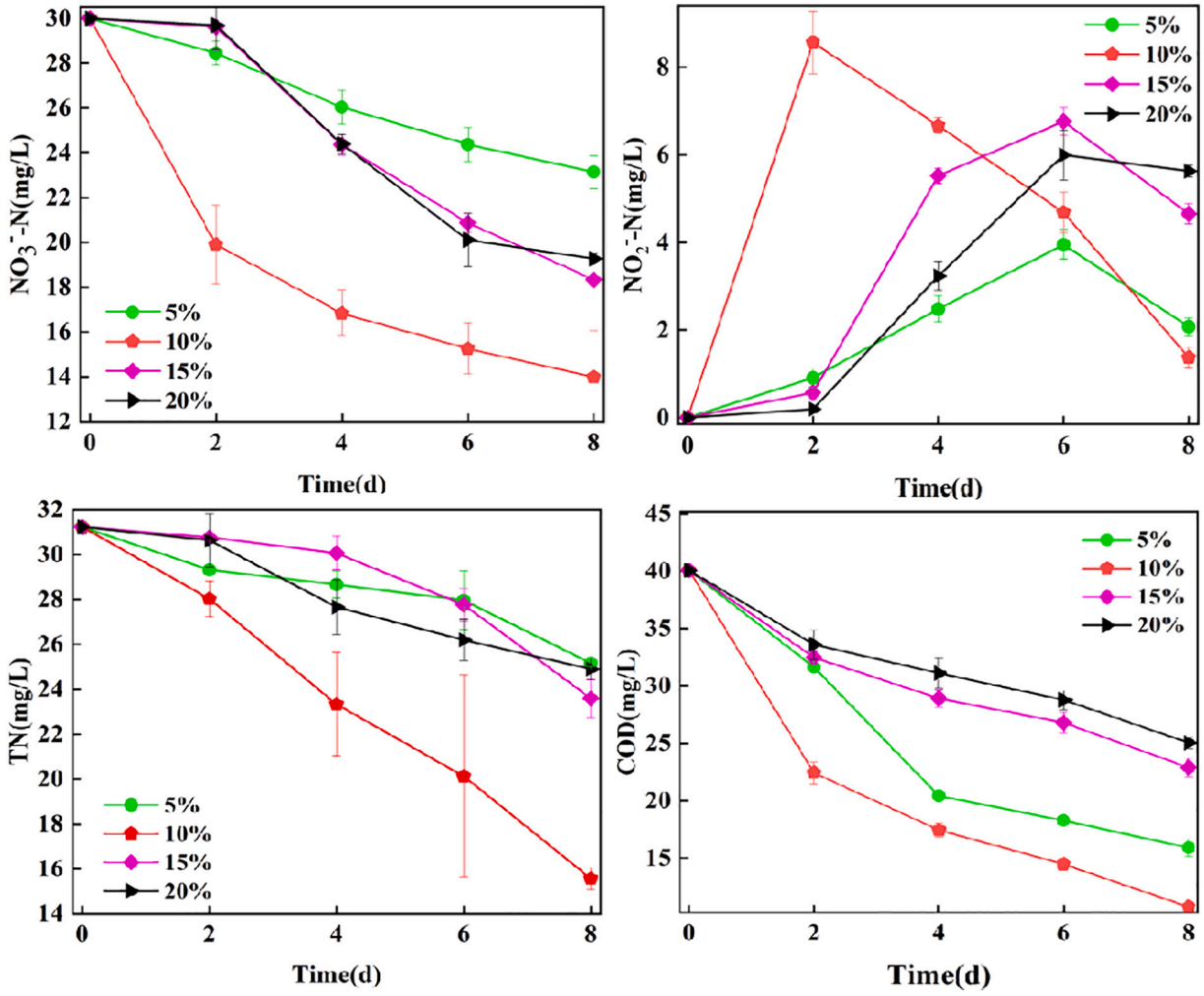

Fig. 3. Changes in the NO_3^--N, NO_2^--N, TN, and COD concentrations over time under different Fe-C dosages.

removal rate.

3.2.3. *Effect of the DB dosage*

The removal rates of TN and NO_3^--N in MEBD system showed a continuous upward trend with an increase in the DB dosage (Fig. 4). The greater the number of microorganisms, the faster the denitrification rate, and the higher the removal rate. When the dosage of DB was 5%, the TN removal rate was 55.7%, while when the dosage of DB was 7%, the TN removal rate was only 57.6%. This result indicated that the removal rate would not increase significantly when the dosage exceeded 5%. This might have been due to the increased number of microorganisms resulting in the intraspecific competition, and the nutrients in the system were insufficient to maintain an efficient denitrification rate. Generally, NO_2^--N showed a trend of first increasing and then decreasing. When the dosage was 1%, the cumulative concentration of NO_2^--N was high. The removal curves of NO_3^--N and TN were similar, and the removal rate of NO_3^--N also did not significantly increase when the dosage of DB exceeded 5%.

Overall, the removal rate of COD increased with an increase in DB dosage. When the DB dosage was 1%, 3%, 5%, and 7%, the removal rates of COD by MEBD were 57.1%, 73.1%, 81.6%, and 82.1%, respectively. As shown in Fig. 4, it was difficult to further improve the removal effect of COD when the dosage was higher than 5%. The reduction state [H] generated by the micro-electrolysis reaction can react with organic matter in sewage to improve biodegradability. Fe^{2+} generated after the corrosion of the zero–valent iron can promote autotrophic denitrification in the MEBD system so that COD has been removed. Therefore, the application of 5% DB dosage not only saved cost, but it also produced a good denitrification performance.

3.3. *Parameter optimization*

The response surface diagram of the effect of the composite factors on NO_3^--N removal was shown in Fig. S2. It can be seen that the response curves of the three graphs have obvious peaks, and this means that there is a maximum response (the optimal initial pH, DB dosage, and Fe-C dosage) [25]. In addition, the contour maps corresponding to the three factors all had a certain degree of ellipticity, and the interaction between the Fe-C dosage and pH had the highest degree of ellipticity. This indicated that the interaction between the Fe-C dosage and pH has the greatest impact on the NO_3^--N removal rate. In addition, the optimal conditions of the RSM fitting were shown in Table S6. The results showed that the optimized operation conditions of MEBD system were pH = 7.02, DB dosage = 5.02%, and Fe-C dosage = 10.14%. The results were consistent with the single factor experiments.

3.4. *Application of MEBD and analysis of the denitrification mechanism*

3.4.1. *Application of MEBD*

MEBD was applied to denitrification in actual wastewater. The initial pH of the experiment was about 7.60, and the addition of DB and Fe-C in the system was shown in Fig. S1. The changes in pH and dissolved

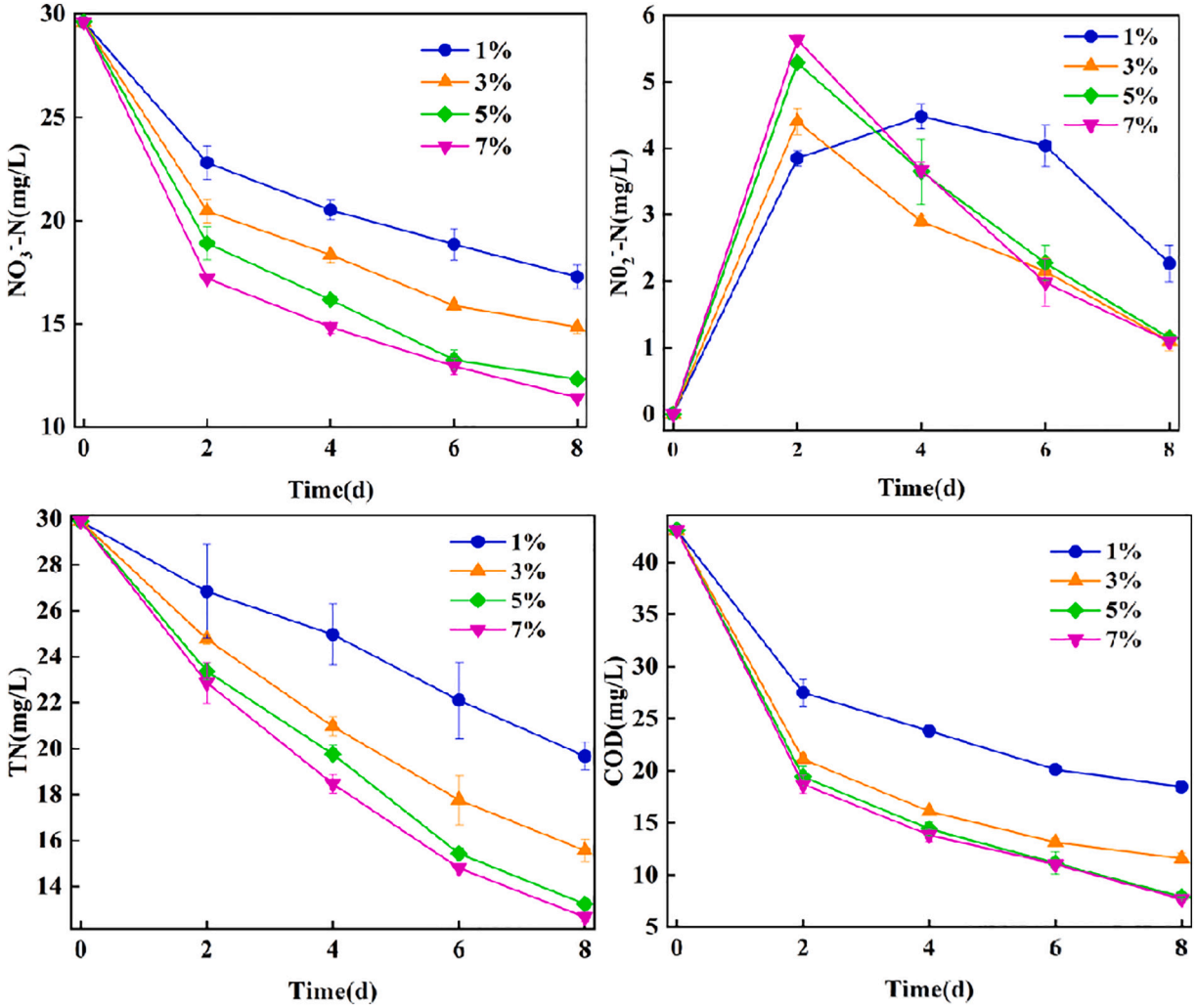

Fig. 4. Changes in the NO_3^--N, NO_2^--N, TN, and COD concentrations with time under different DB dosage conditions.

oxygen (DO) in each experimental group within 20 days are shown in Fig. 5(a, b). In the control group, the pH varied from 7.60 to 7.82, while in DB system, the pH fluctuated from 7.60 to 7.96. The pH of Fe-C system increased from 7.62 to 8.31 and then decreased from 8.31 to 8.03. The pH of MEBD system increased first and then tended to stabilize. At the end of the experiment, the two systems were weakly alkaline. The pH of Fe-C group was the highest, and the Fe-C micro-electrolysis produced reduced hydrogen [H] [26–28]. With the continuous consumption of H^+, the pH in wastewater gradually increased. During the experiment, the pH of MEBD system maintained a continuous upward trend. The microorganisms in the coupling system used organic substances for denitrification. The pH of MEBD system increased with the decreasing of NO_3^--N concentration. In addition, the electrons released by micro-electrolysis could be used by autotrophic DB, and the reduced hydrogen [H] could be continuously consumed, and this would also lead to an increased pH of the wastewater [29,30].

Due to atmospheric reoxygenation, the DO of each experimental system increased in the early stage of the experiment. In the reaction, O_2 was consumed, and dissolved oxygen began to decline. The trend of DO concentrations in the control group and in the DB group was similar, ranging from 0.10 to 1.22 mg/L. The DO in Fe-C and MEBD system increased first and then decreased and then increased. At the end of the experiment, the DO concentration of Fe-C system was still high. At this time, a micro-electrolysis reaction under aerobic conditions was being conducted in the system, and a large amount of OH^- was generated, resulting in pH greater than 8. According to the trend of DO concentration in MEBD system, the course of nitrogen removal process could be explained. In the early part of the process, DO concentration was low and the MEBD system was in a strong reductive environment. Hence, NO_3^--N was rapidly removed. In the middle of the experiment, the increasing of DO concentration was not conducive to denitrification, and the removal rate of NO_3^--N slowed down. During the later stage of the experiment, the DO concentration decreased again. At this time, there might have been autotrophic DB in MEBD system that further ensured the denitrification process.

At the end of the experiment, the NO_3^--N removal rates of control group, DB system, Fe-C system, and MEBD system were 38.6%, 56.0%, 51.3%, and 99.9%, respectively (Fig. 5(c)). Obviously, the removal of NO_3^--N in MEBD system was significantly different from other three experimental systems. Micro-electrolysis was coupled with DB. Fe^{2+} and [H] produced by Fe-C micro-electrolysis can provide electrons for autotrophic DB [31]. Micro–electrolysis accelerated the electron transfer and promoted autotrophic denitrification [32]. Additionally, iron can promote the growth and reproduction of microorganisms. The concentration of NO_3^--N in the control group decreased with time, and this might have been due to the presence of some denitrifying microorganisms in the sediment. The removal rate of NO_3^--N in DB group was low. This was because the added DB could not adapt to the new environment, resulting in a change in the microbial community structure in the system. The Fe-C group had a certain effect on the removal of NO_3^--N, which might be due to the reduction of nitrate by micro-electrolysis.

The cumulative curves of NO_2^--N in control group, DB system, and

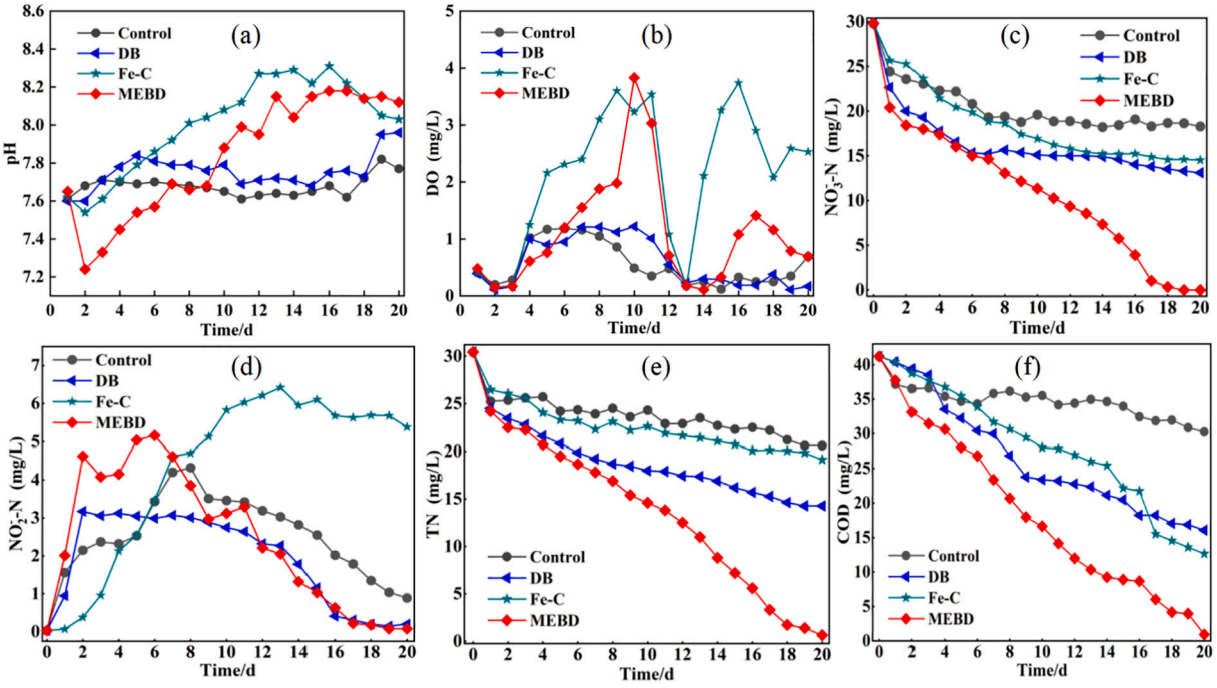

Fig. 5. Variation in the pH, DO, NO_3^--N, NO_2^--N, TN and COD concentrations with time in the different systems (DB, Fe-C and MEBD).

MEBD system were similar (Fig. 5(d)). NO_2^--N in each group showed a trend of going up first and then going down. During denitrification, NO_3^--N was first reduced to NO_2^--N. Therefore, in the early stage of the experiment (0–7 d), the accumulation of NO_2^--N was greater in the system of DB and MEBD. With denitrification, NO_2^--N was gradually degraded. At the end of the experiment, there was no NO_2^--N accumulation in the system of DB and MEBD. The accumulation of the NO_2^--N concentration in Fe-C system showed a continuous upward trend, and the accumulation of NO_2^--N in this group rose sharply, and it was finally stabilized at approximately 5.5 mg/L. The continuous accumulation of NO_2^--N indicated that micro–electrolysis reduced NO_3^--N to NO_2^--N. Previous studies have shown that the micro–electrolysis reaction is an acid-promotion reaction, and the reduction products are primarily NH_4^+-N [33,34]. Combined with the removal of pH and NO_3^--N, it was inferred that NO_3^--N was reduced to NO_2^--N in Fe-C system.

The removal rates of TN in MEBD system were significantly different from those in the other three experimental groups (Fig. 5(e)). The removal rates of TN in control group, DB system, Fe-C system, and MEBD system were 32.3%, 53.1%, 37.2%, and 97.9%, respectively. The C/N in the wastewater was 1.33, and the organic carbon source was insufficient, while the TN removal rate was high in MEBD system. It can be inferred that there was an autotrophic denitrification process during the MEBD denitrification process. In the early stage of the experiment, the sewage contained a certain amount of an organic carbon source, and DB could use electronic denitrification provided by organic matter. In the middle and late stages of the experiment, the supply of organic matter was insufficient, and MEBD system still maintained a high denitrification rate, indicating that Fe^{2+} and [H] generated by micro-electrolysis provided electrons for autotrophic denitrification microorganisms so as to ensure the denitrification process continued until complete denitrification was achieved.

Obviously, the COD of each system except of the control group showed a rapid downward trend (Fig. 5(f)). There was no significant difference in the removal efficiency of COD between DB and Fe-C system, and the removal rates of COD were 61.0 and 69.2%, respectively. Under the coupling effect of micro–electrolysis and denitrification, the COD in MEBD system still displayed a downward trend, and the removal rate of COD was 97.7% at the end of the experiment. Denitrifying microorganisms in DB system could use organic nitrogen to remove COD from the wastewater. In the mid and late stages of the experiment, the organic carbon source was insufficient, and this amount could not maintain the growth and metabolism of DB in the system. Hence, the utilization rate of COD decreased. There was a micro–electrolysis reaction in Fe-C system that could convert macromolecular organic matter into small molecular compounds [35]. Fe-C also had an adsorption effect, which could remove small molecular compounds through adsorption, thereby reducing the COD concentration in wastewater [36].

The changes of Fe^{2+} concentration and pH in MEBD system were shown in Fig. S3. The concentration of Fe^{2+} in MEBD system increased first and then decreased. The concentration of Fe^{2+} reached the maximum after 9 d of 43.21 mg/L. Since then, the Fe^{2+} concentration in the system gradually decreased to 3.14 mg/L. The change of Fe^{2+} concentration in the wastewater indicated that even under neutral conditions, the Fe-C micro–electrolysis reaction still occurred in MEBD system, and under the action of micro–electrolysis, the corrosion rate of Fe^0 accelerated. It is noteworthy that Fe-C micro–electrolysis produced hydrogen and ferrous in the meantime [37]. This resulted in an increasing of Fe^{2+} concentration in the system. As the reaction proceeded, the pH of MEBD system continued to rise. Finally, the system was weakly alkaline, and the pH was 8.12. It was worthy to note that after 10 days, the concentration of Fe^{2+} in MEBD system decreased rapidly. This might have been due to precipitation, and the pH of the solution was weakly alkaline. Therefore, it was speculated that the condition of Fe^{2+} precipitation in MEBD system was pH = 7.6.

3.4.2. *Analysis of the microbiological community*

The richness and diversity of the bacterial communities in water samples collected at different times are described in Table S8. Alpha diversity index indicated that the highest numbers of diverse bacterial communities were found in the water samples, indicating the importance of the Fe-C for species diversity. The addition of Fe-C filler have changed the community abundance and species diversity in MEBD system, and Fe-C micro-electrolysis affected the structure of the original DB. The rank-abundance curve was used to intuitively characterize species diversity (Fig. S4). It can be seen that the curve width of each sample was not very different, but the curve of the DB sample was the

flattest. This indicated that the species abundance of the three samples were close, and the species evenness of the DB sample was the highest.

Fig. 6 showed the relative abundance of the different phyla and classes in the three samples (DB, D10, and D20). Over 80% of the reads belonged to five representative phyla in DB: *Firmicutes* (96.04%), *Actinobacteria* (1.28%), *Proteobacteria* (1.09%), *Bacteroidetes* (0.74%), and *Chlamydiae* (0.67%). *Firmicutes* are Gram-negative bacteria which can degrade COD and are closely related to denitrification [32,38,39]. Xie et al. [40] found that *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Firmicutes*, and *Nitrospirae* were the dominant phyla in the sludge of sewage treatment plant. This was similar to the microbial community structure in the pond, and the strains screened from the actual environment might have stronger environmental adaptability, better tolerance to the actual water body and shorten the denitrification cycle. *Firmicutes* had been identified as a kind of DB according to the study of Wang et al. [41]. The abundances of D10 and D20 were similar. The phylum *Proteobacteria* increased higher in D10 and D20, accounting for 37.26% and 40.23% of all sequences, respectively, followed by *Bacteroides* (34.40%, 20.39%), *Chlamydia* (17.67%, 23.98%), *Planctomycetes* (2.35%, 1.90%), *Verrucomicrobia* (1.18%, 5.74%), and *Actinobacteria* (1.05%, 3.89%). Previous studies showed that *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Actinobacteria* contained a large number of DB [42,43]. This study showed that *Chlamydiae* and *Verrucomicrobia* might also contain denitrifying functional bacteria.

The community structure distribution of microorganisms in the samples at the class level was shown in Fig. 6(b). The relative abundance of *Clostridia* in DB samples was the highest, accounting for 82.94%. It was found that *Clostridia* was more likely to survive in various anaerobic environments, some of which were found to have good denitrification in biochemical systems [44]. The main microbial population structures of D10 and D20 samples at the class level were roughly the same, but the distribution degrees were slightly different. Among them, the relative abundance of *Betaproteobacteria* β was the highest, accounting for 22.70% and 29.50%, respectively. *Chlamydiia* was also relatively abundant, accounting for 17.67% and 23.98%, respectively.

The microbial community structures of the three operative stages at the genus level were shown in Fig. 7(a). The biological distribution of sample DB was quite different from that of D10 and D20. The species structure of DB was simple, while that of D10 and D20 was complex. There were three primary species in the DB samples, *Clostridium-sensitiveu-stricto* (82.72%), *Bacillus* (13.01%), and *Acidovorax* (0.16%) and these bacteria had been idenditied as common DB according to Chakraborty et al. [45].

Moreover, some unknown genera appeared in D10 and D20, and the diversity of the microbial community structure in the MEBD system could be further studied. Specifically, *Runella* (15.70%) was the dominant genus in D10, and in addition to *Runella* were *Flavobacterium*, *Dyadobacter*, *Gemmobacter*, and *Acidovorax*, with relative abundances of 7.66%, 7.34%, 5.32% and 2.59%, respectively. *Flavobacterium*, *Gemmobacter* and *Acidovorax* all have denitrification functions. In addition, the relative abundance of *Unclassified-Comamonadaceae* in D10 was 10.28%, and according to the GenBank database, the sequence shared 98.5% similarity with one species of *Comamonas* [46]. It had been found that *Comamonas* has a denitrification function, and this microorganism could use polymers as carbon sources for denitrification [47]. The genus *Unclassified-Parachlamydiaceae* had the highest relative abundance in the D20 samples at 23.90%. Combined with the performance of MEBD system, it can be concluded that *Unclassified-Parachlamydiaceae* had the function of nitrogen removal. The dominant genera in the D20 samples also included *Acidovorax*, *Sediminibacterium*, *Opitutus*, *Pedobacter*, *Novosphingobium*, and *Gemmobacter*, accounting for 8.89%, 7.78%, 5.69%, 3.5%, 2.98%, and 2.34%, respectively. D20 also contained a small amount of *Hydrogenophaga* [48,49], which is a kind of hydrogen autotrophic DB which can use hydrogen as an electron donor.

3.4.3. Mechanism and denitrification pathways

The results of the micro-electrolysis treatment of low C/N wastewater, the MEBD denitrification single factor experiment, and the evolution of the microbial community structure during the process of MEBD remediation of low C/N wastewater indicate a possible mechanism of the effect of MEBD on nitrate reduction and COD degradation, as shown in Fig. 8. This mechanism primarily includes four aspects: autotrophic denitrification, heterotrophic denitrification, micro electrolysis, and electrochemical reduction.

Unlike a single Fe-C micro-electrolysis system, there was nearly no accumulation of NO_2^--N during the denitrification process of MEBD system, and this indicated that there was a coupling effect between micro-electrolysis and biological denitrification. When Fe (anode) and AC (cathode) were mixed and contacted in the wastewater, a large number of microcells spontaneously formed in MEBD system. The electrons produced in the anode transferred to the cathode. The adsorbed H^+ or H_2O molecules on the AC surface accepted the electrons, and they were converted into adsorbed [H] and then rapidly reduced the neighboring adsorbed NO_3^--N to N_2 and NH_4^+-N. The Fe^{2+} generated at the anode and the [H] and O· generated at the cathode have strong chemical activities and can effectively break the carbon chains in organic contaminants and mineralize the contaminants. Fe^{2+} then promotes microbial reproduction, and the added AC can adsorb these small–molecule organic compounds [50]. Autotrophic DB used [H] to remove nitrogen, while heterotrophic DB used the organic carbon source to remove nitrogen. Under the synergistic effect of these, nitrate was finally reduced to N_2 and released into the environment through a series

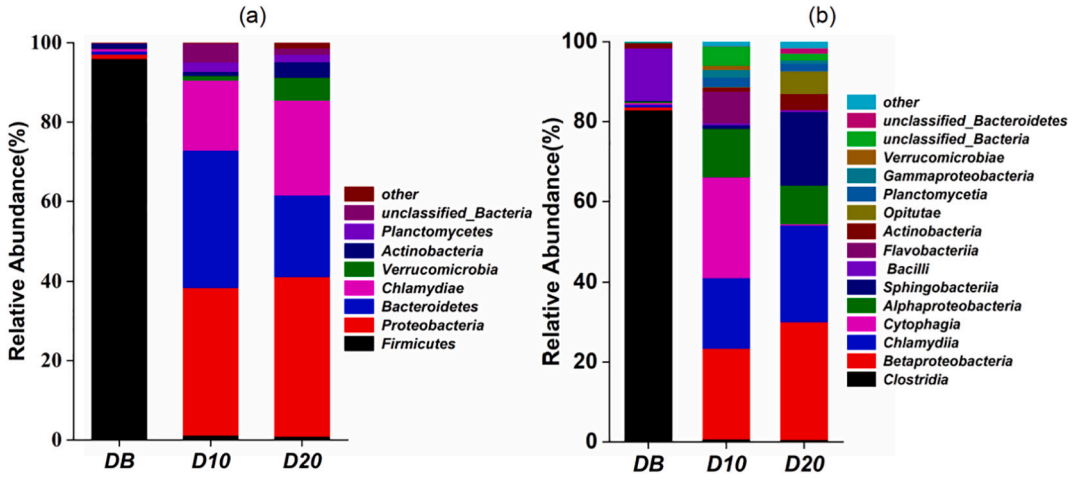

Fig. 6. Microbial community structure at the level of phylum (a) and class (b).

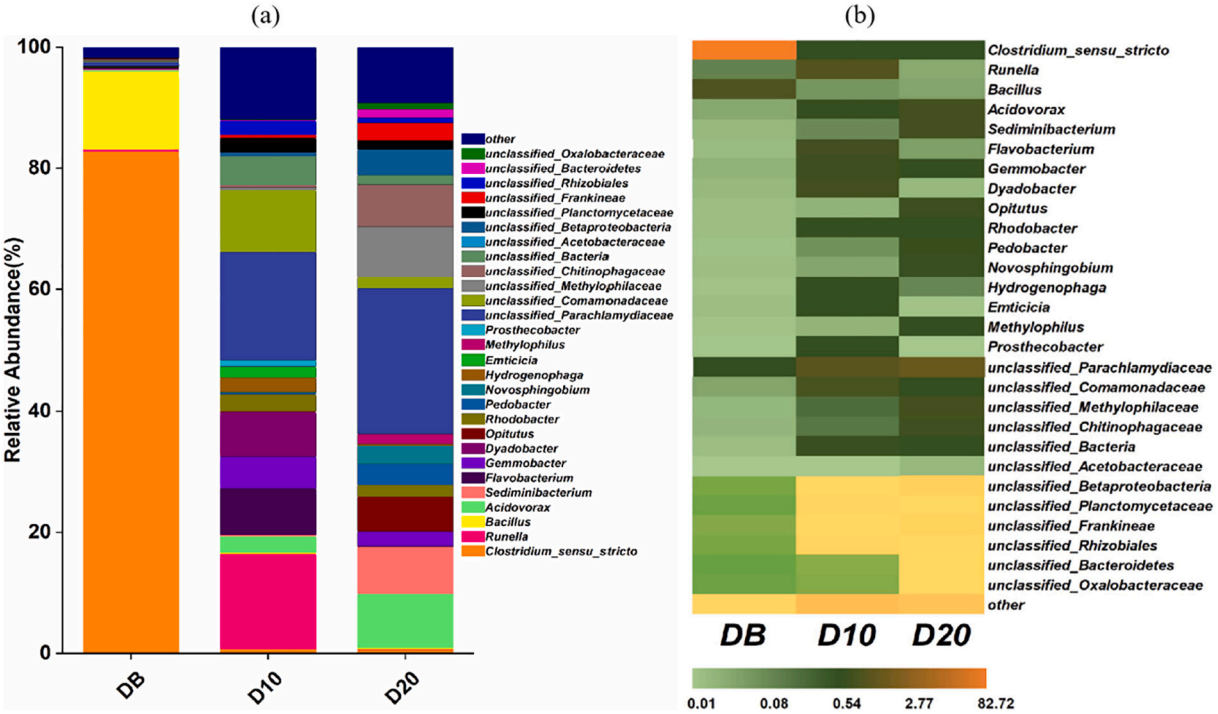

Fig. 7. Distribution of microorganisms at the genus level in MEBD system (a) and the heat map (b).

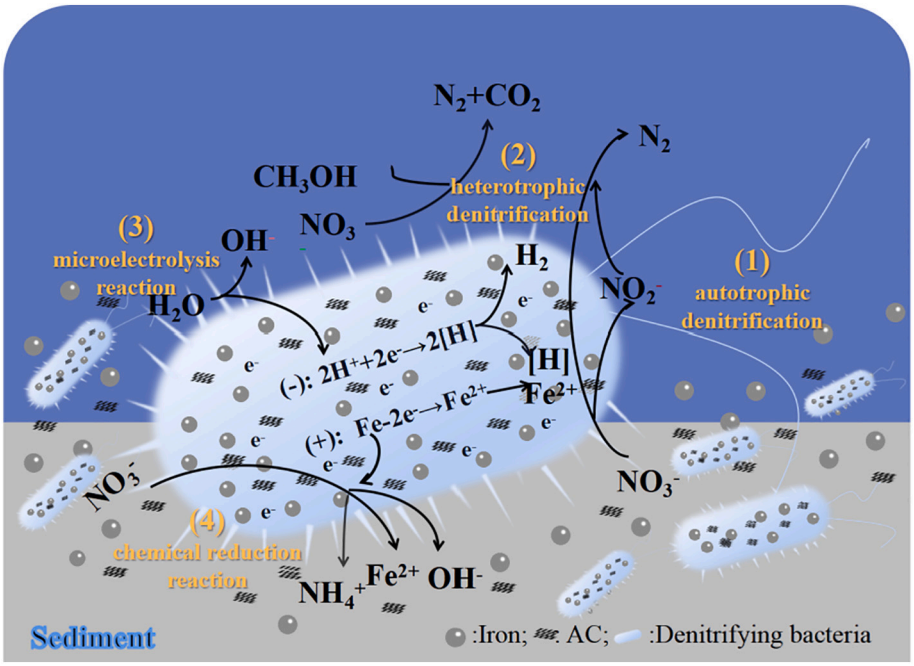

Fig. 8. Mechanisms of nitrogen removal in the MEBD coupling system.

of reductions (NO_3^- → NO_2^- → NO→N_2O → N_2) [51]. It was found that Fe^{2+} was nearly completely precipitated during the MEBD denitrification, and this might have been caused by the co-precipitation of Fe^{2+} oxidized to Fe^{3+} and hydroxylgel (FeOOH) generated by microelectrolysis. Based on the analysis above, it was speculated that the reduction of nitrate by MEBD was as follows [52,53]:

$$10\,Fe^0 + 6\,NO_3^- + 3\,H_2O \rightarrow 5\,Fe_2O_3 + 6\,OH^- + 3\,N_2 \quad (1)$$

$$10\,Fe^{2+} + 2\,NO_3^- + 14\,H_2O \rightarrow 10\,FeOOH + 18H^+ + N_2 \quad (2)$$

Bio-denitrification was the primary process involved to completely remove NO_3^--N. Micro-electrolysis could convert some non-biodegradable organic pollutants such as antibiotics into small molecular organic matter [27,28], which can provide additional carbon sources for heterotrophic DB and promote the efficient nitrogen removal by the MEBD. In addition, Fe^0 and Fe^{2+} formed by electrode corrosion reduced a portion of NO_3^--N to NH_4^+-N [54] and then the oxidation reaction occurred on the anode to generate NO_3^-/NO_2^-. Meanwhile, the NO_3^-/NO_2^- as the over-oxidation by-product of NH_4^+ could be recirculated into the cathode and continuously reduced to N_2 [22].

The traditional water treatment process consumes large energy, needs large organic carbon source and releases large amount of CO_2 [55,56]. In MEBD system, the [H] generated by micro-electrolysis was utilized by *Hydrogenophaga* and then to complete the autotrophic denitrification. Moreover, due to the existence of autotrophic DB, the addition of organic carbon source could be reduced in water treatment process, which was suitable for nitrogen removal in oligotrophic lakes and rivers. Additionally, the large pore size of Fe-C could provide habitats for microorganisms, enhance the shock resistance of microorganisms, reduce the loss of strains in the treatment process, and affect the distribution of microbial communities, which was conducive to improving the removal of NO_3^- -N and COD in the actual wastewater.

4. Conclusions

The denitrification performance, factors affecting the removal process, and mechanisms of MEBD system for low C/N wastewater remediation were investigated. Efficient denitrification was achieved under the conditions of Fe-C dosage of 10%, DB dosage of 5%, and pH = 7, obtaining TN and COD removal efficiencies of 97.9% and 97.8%, respectively, and nearly no NO_2^--N accumulation. The nitrogen removal processes were primarily autotrophic denitrification, heterotrophic denitrification, micro–electrolysis, and chemical reduction. The dominant microorganisms in MEBD system belonged to the phyla *Proteobacteria*, *Bacteroidetes*, *Chlamydiae*, and *Planctomycetes*. The relative abundance of microorganisms related to organic matter degradation and nitrate reduction in the MEBD system increased significantly, and the emergence of autotrophic DB indicated that there was denitrification in MEBD system. Hydrogen autotrophic DB in the system can utilize electrons generated by micro-electrolysis and then provide energy for growth and metabolism, whicn can avoid secondary pollution caused by adding organic carbon sources. The results confirmed the functional communities present in the system and the successful operation of the recommended MEBD process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material (https://doi.org/10.1016/j.jwpe.2022.102899) contains the wastewater formulation and trace element types, experimental device design, response surface design of MEBD system, parameter optimization results, and microbial diversity analysis in the unit.

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