

Article

Chemical and Biological Cascade Responses Triggered by Nitrate-Driven In Situ Iron Corrosion: Mechanism of Simultaneous Nitrogen and Phosphorus Removal from Piggery Tailwater in Iron–Carbon Biofilter

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Abstract

A synergistic system integrating iron corrosion, chemical phosphorus removal, and biological nitrogen removal was established for advanced piggery tailwater treatment. Increasing NO_3^- -N concentration from 15 to 60 mg/L enhanced Fe^{2+} dissolution in iron–carbon biofilter (ICBF) by 61.7%. The in situ released iron ions improved the phosphate removal efficiency to 96.08% via chemical precipitation. Iron corrosion provided additional electron donors, enriching an integrated autotrophic–heterotrophic denitrifying community (*Dechloromonas*, *Thermomonas*, and *Limnobacter*). This elevated the total nitrogen removal efficiency to 79.07%, representing an 81.62% improvement over conventional biofilter BF-C. After sodium humate addition, microbial activity influenced secondary mineral formation, which may be associated with partial mitigation of iron passivation in the ICBF. This study clarified the nitrate-driven in situ iron corrosion and its associated chemical-biological cascade responses, providing a novel strategy and theoretical basis for achieving simultaneous nitrogen and phosphorus removal in low C/N wastewater.

Keywords: in situ nitrate-driven; iron–carbon biofilter; piggery tailwater; low C/N ratio; simultaneous nitrogen and phosphorus removal



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1. Introduction

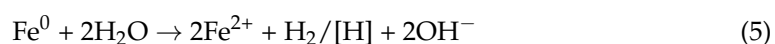
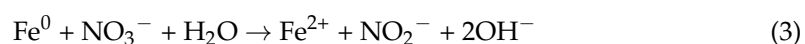
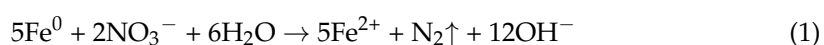
Advanced nitrogen and phosphorus removal from piggery tailwater (PTW) remains a major challenge in livestock and poultry wastewater management. As the world's largest producer and consumer of pork [1], China generates over 160 million tons of swine wastewater annually [2]. After conventional anaerobic digestion, aerobic, and anoxic treatment processes [3], the effluent (PTW) typically exhibits high nitrogen and phosphorus concentrations and a low carbon-to-nitrogen ratio (C/N) [4], with nitrate nitrogen (NO_3^- -N) and orthophosphate (PO_4^{3-} -P) as the dominant residual forms. Additionally, residual refractory humic-like substances [5,6] further increase the difficulty of treatment. With increasingly stringent environmental regulations worldwide, livestock wastewater management has become more rigorous. For example, the U.S. Clean Water Act classifies concentrated animal feeding operations as point sources and implements dynamic quota

management, while the EU sets strict phosphorus discharge limits under Best Available Techniques conclusions [7]. In China, the Guangdong provincial discharge standard of pollutants for livestock and poultry breeding (DB44/613-2024) [8] has tightened the total phosphorus (TP) discharge limit from 8 mg/L to 3 mg/L and introduced a new total nitrogen (TN) control limit of 40 mg/L. Therefore, developing efficient advanced treatment technologies for PTW has significant practical implications for meeting environmental protection requirements, promoting the green and sustainable development of the livestock and poultry farming industry, and protecting the aquatic environment.

However, conventional treatment of PTW is constrained by its reliance on external inputs. Conventional heterotrophic denitrification and enhanced biological phosphorus removal heavily depend on readily degradable carbon sources [9], often requiring external carbon addition to sustain performance, which results in increased operational costs and secondary pollution risks [10]. On the other hand, soluble iron salts are often used for phosphate precipitation or as autotrophic denitrification electron donors, yet continuous and excessive dosing is usually required. Chemical phosphorus precipitation often requires Fe/P molar ratios of 1.0–3.9 [11]. Moreover, Fe(II)-driven reduction of 1 kg of NO_3^- -N requires 19.9 kg of Fe(II), while practical Fe(II) dosages typically range from 400 to 1500 mg/L and may reach up to 6000 mg/L in certain cases [12]. Such soluble iron dosing results in high turbidity and color in the effluent and large volumes of sludge production.

Against this background, Fe(0)-based materials have attracted increasing attention for low C/N wastewater treatment. Fe(0)-based materials generate Fe(II) in situ, which subsequently oxidizes and hydrolyzes into Fe(III) and iron (hydr)oxides. All of these components can immobilize phosphate through adsorption, flocculation, and precipitation [13]. Meanwhile, iron corrosion processes can also facilitate nitrate chemical reduction and autotrophic denitrification [10].

In PTW, nitrate may act not only as a residual pollutant but also as an oxidant that promotes Fe(0) corrosion (Equations (1)–(4)). In nitrate-free systems, iron corrosion is mainly coupled with water reduction (Equation (5)), which proceeds slowly under near-neutral conditions [13]. In contrast, nitrate reduction is thermodynamically more favorable than water reduction, thereby promoting iron dissolution. He et al. [14] reported that elevated nitrate concentrations enhanced iron corrosion, leading to increased release of Fe(II). Previous batch experiments also showed that increasing nitrate concentration intensified iron dissolution, improving phosphate removal [14,15]. Simultaneously, Fe(II) can serve as an inorganic electron donor for autotrophic denitrification [10].



However, regular Fe(0) materials generally require acidic conditions (pH 2–4) for efficient NO_x reduction [16], which is incompatible with denitrifying bacteria. Iron–carbon (FeC) composites can retain reactivity even at neutral pH by forming numerous micro-galvanic cells that enhance electron transfer [16]. In addition, FeC micro-electrolysis can degrade humic substances in PTW [17], potentially improving wastewater biodegradability and supporting heterotrophic denitrification under low C/N conditions. Nevertheless, the deposition of corrosion products and phosphate precipitates can passivate the material, diminishing its reactivity [10]. Microorganisms may mitigate this limitation by sustaining

iron corrosion and mediating the Fe(II)/Fe(III) cycle. Kim et al. found that soluble iron concentrations in a ZVI–microbe system were 1.7-fold higher than those in an abiotic ZVI system [18]. In particular, dissimilatory iron-reducing bacteria (DIRB) can reduce insoluble Fe(III) minerals to Fe(II), driving a Fe(II)/Fe(III) cycle that regenerates reactive iron [19,20]. Therefore, a bio-coupled FeC system may sustain nitrate-driven iron corrosion while enabling phosphate immobilization and multiple nitrogen removal pathways in PTW.

Previous FeC systems have mostly been applied to relatively low-strength wastewater, with phosphorus concentrations often much lower than those in PTW [21–23]. Although batch studies have shown that nitrate, as a coexisting anion, can enhance phosphate removal by iron-based materials [14,15], nitrate has rarely been considered as an in situ driver of Fe(0) corrosion for the simultaneous removal of nitrogen and phosphorus from PTW. Therefore, based on the actual water quality characteristics of PTW, this study constructed an iron–carbon biofilter utilizing in situ nitrate-driven iron corrosion. FeC filters and conventional biofilters were employed as controls. This study aimed to: (1) elucidate the “in situ driving” role of nitrate in iron corrosion; (2) reveal the cascade responses of chemical phosphorus removal and biological multi-pathway denitrification triggered by iron corrosion; (3) clarify the mechanism of in situ iron corrosion–chemical–biological cascade coupling nitrogen and phosphorus removal. The study is expected to provide new insights for the advanced treatment of piggery tailwater and to offer a scientific basis for its practical engineering application and optimization.

2. Materials and Methods

The experimental design was established to evaluate nitrate-driven iron corrosion and the associated nitrogen and phosphorus removal processes. Six column reactors were used to compare abiotic FeC filters, FeC biofilters, and conventional biofilters under different influent nitrate conditions. The following sections describe the reactor setup, filler materials, synthetic wastewater, analytical methods, microbial analysis, and data treatment.

2.1. Reactor Set-Up and Operation

Six column reactors (600 mm in height; 75 mm in inner diameter) were constructed and operated in up-flow mode (Figure 1). The reactors were divided as follows: (1) FeC filter group (ICF-a, ICF-A): FeC filler without sludge inoculation; (2) FeC biofilter group (ICBF-b, ICBF-B): FeC filler with acclimated anaerobic sludge; (3) conventional biofilter group (BF-c, BF-C): quartz sand with acclimated anaerobic sludge. Biofilm was expected to develop mainly on the surface of fillers. Lowercase and uppercase suffixes in each reactor name denote operation under low and high nitrate concentrations, respectively. Each treatment was represented by one reactor, and no parallel reactors were set up. The lower 10 cm of each reactor was packed with quartz sand as the support layer, and the upper 30 cm was packed with either FeC filler or quartz sand, with a total packing volume of 1.77 L. The pore volumes of ICF/ICBF and BF were 0.84 L and 0.71 L. Given these different porosities of fillers (FeC filler around 50%, quartz sand around 40%), we regulated the outlet height of each reactor individually to ensure an identical effective liquid volume of 1.0 L. The 12 h HRT was selected as the baseline condition by referring to reported HRT ranges for iron-based or biofilter systems treating low-C/N wastewater [24–27], while the shorter HRTs were applied to evaluate system performance under increased hydraulic loading. By regulating the influent flow rate via calibrated peristaltic pumps, three hydraulic retention times (12 h, 10 h and 8 h) were realized. The matching influent flow rates were 2.0 L/d, 2.4 L/d and 3.0 L/d, respectively.

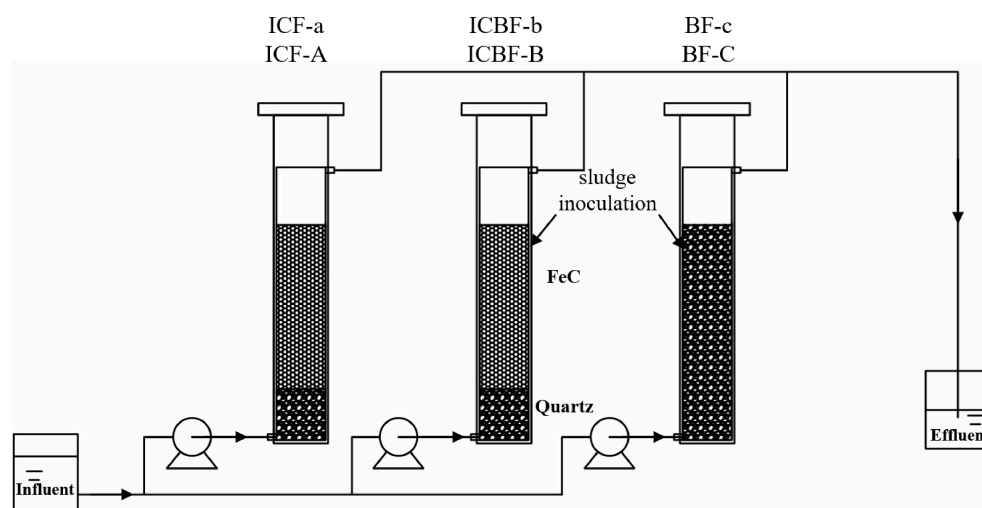


Figure 1. Schematic diagram of reactors.

All reactors were wrapped with aluminum foil to eliminate light interference and operated in up-flow mode at 25–30 °C. The experiment consisted of four phases. In phase I (day 1–day 50), sodium acetate (NaAc) was used as the carbon source. In phase II (day 50–day 90), III (day 90–day 110), and IV (day 110–day 130), sodium humate (NaHA) was introduced as recalcitrant organic matter to simulate refractory compounds in PTW [5]. The influent contained NaAc and NaHA at a COD-equivalent ratio of 1:1. All reactors ran through the first two phases with a constant HRT of 12 h. For ICF-A, ICBF-B and BF-C, operations of Phase III and Phase IV were conducted to explore the influence of varying influent loading rates. Correspondingly, the HRT was sequentially reduced to 10 h in Phase III and 8 h in Phase IV.

2.2. Materials, Sludge, and Synthetic Wastewater

In this study, commercial spherical FeC fillers (5–8 mm; Tan Nuo Company, Pingdingshan, China) and quartz sands (4–8 mm; Sheng Feng Company, Gongyi, China) were selected. The detailed physicochemical properties of the FeC filler are provided in Table S1. FeC fillers were pretreated with 1% H₂SO₄ for 20 min and rinsed with deionized water until the rinse water reached a neutral pH. Quartz sands were washed and dried. The above-mentioned acclimated anaerobic sludge was originally collected as seed sludge from the secondary sedimentation tank of the Lijiao Wastewater Treatment Plant in Guangzhou, Guangdong Province, China, and acclimated in the laboratory before use. Detailed acclimation procedures are summarized in Text S1. Before the experiments began, the sludge underwent three washes with deionized water. The inoculation sludge concentration was 3.5 g/L (MLVSS 2.4 g/L). N₂ was introduced at a flow rate of 25–50 mL/min to the reactor bottom during the initial stages to promote biofilm formation.

Synthetic wastewater was formulated to match the key water quality characteristics of a large-scale pig farm in Jiangmen, China (Table 1). NaNO₃ served as the nitrate source, and KH₂PO₄ as the phosphate source. Trace element solution was added at a ratio of 1 mL/L relative to the influent. The preparation method was as follows (g/L): EDTA 5.0, CuSO₄·5H₂O 0.03, KI 0.18, MnCl₂·4H₂O 0.12, NaMoO₄·2H₂O 0.06, ZnSO₄·7H₂O 0.12, CoCl₂ 0.15, H₃BO₃ 0.3. Reactors with lowercase suffixes (ICF-a, ICBF-b, BF-c) received Influent 1 (low nitrate strength—15.29 ± 0.76 mg/L NO₃[−]-N), while those with uppercase suffixes (ICF-A, ICBF-B, BF-C) received Influent 2 (high nitrate strength—61.53 ± 2.07 mg/L NO₃[−]-N). The low and high nitrate conditions were selected based on monitoring data from actual piggery tailwater (PTW) collected from a swine farm in Jiangmen, Guangdong.

Unless otherwise stated, the influent pH was maintained at 6.5–7.0. During days 25–50, the influent pH of FeC reactors was adjusted to 5.5–6.0 before feeding.

Table 1. Water quality characteristics (mg/L).

	COD	NO ₃ [−] –N	TN	TP
Influent 1	45.36 ± 0.38	15.29 ± 0.76	15.29 ± 0.76	20.23 ± 0.52
Influent 2	181.21 ± 0.76	61.53 ± 2.07	61.53 ± 2.07	20.45 ± 0.45
PTW range	45.00~230.00	15.00~77.49	15.00~81.72	19.11~22.98
PTW typical	180.00	60.00	60.00	20.00

Notes: PTW typical values are representative concentrations calculated from actual monitoring data. In the synthetic influents (Influent 1 and 2), TN was composed solely of NO₃[−]–N.

2.3. Analytical Methods

Water samples were taken every two days and were filtered through 0.45 µm membrane filters for further analysis. All water sample analyses were performed in triplicate, and the mean values were used for data analysis. pH and dissolved oxygen (DO) were measured immediately after sample collection using a pH meter (pHS-3C, Lei Magnetic, Shanghai, China) and a DO meter (YSI550A, YSI Inc., Yellow Springs, OH, USA). TP, TN, NH₄⁺–N, NO₃[−]–N, NO₂[−]–N, PO₄^{3−}–P, total iron (TFe), Fe²⁺ were analyzed according to Standard Methods [28]. TP and TN were determined after alkaline potassium persulfate digestion followed by UV spectrophotometry; NH₄⁺–N was measured using the Nessler's reagent spectrophotometry; NO₃[−]–N was determined by UV spectrophotometry; NO₂[−]–N was measured using the N-(1-naphthyl)-ethylenediamine spectrophotometry; PO₄^{3−}–P was determined by the molybdenum blue method; TFe and Fe²⁺ were measured using the 1,10-phenanthroline spectrophotometry. In this study, TN refers to aqueous-phase total nitrogen in filtered water samples. The measured TFe and Fe²⁺ concentrations referred to the residual aqueous-phase Fe in water samples, rather than total Fe released from the FeC fillers.

Raw FeC filler (unreacted), reacted FeC fillers, and suspended sludge samples were collected for physicochemical characterization. For reactors ICF-A and ICBF-B, reacted FeC filler samples were collected at the end of phase I (Day 50) and phase II (Day 90), while suspended sludge was taken only upon completion of phase II. High-resolution field-emission scanning electron microscopy (SEM, Tescan CLARA, Brno, Czech Republic) was used to observe the surface morphology of the FeC fillers. X-ray diffraction (XRD, Bruker D8 ADVANCE, Karlsruhe, Germany) was employed to analyze the crystal structures in both FeC fillers and suspended sludge. X-ray photoelectron spectroscopy (XPS, Thermo Escalab 250Xi, Waltham, MA, USA) was used to determine the surface elemental composition and chemical valence states of the suspended sludge. Three-dimensional excitation–emission matrix (3D-EEM) fluorescence spectroscopy was applied to characterize dissolved organic matter (DOM).

2.4. Microbial Community Analysis

Sludge samples were collected at the end of phases I and II and were denoted as B-I, C-I, B-II, and C-II, respectively. These samples were used to evaluate the dynamic changes in the microbial community across different systems and explore the mechanisms of microbial involvement in nitrogen and phosphorus removal. DNA extraction was performed using the E.Z.N.ATM Mag Bind Soil DNA Kit (Omega, M5635-02, Norcross, GA, USA). The V3-V4 region of the 16S rRNA gene was amplified by PCR with primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The amplified products were sent to Shenggong Biotech (Shanghai, China) for sequencing. Sequences

were analyzed by complementary base pairing and compared with reference sequences in the Silva 16S database for taxonomic classification.

2.5. Data Analysis

This study utilized Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) and SPSS 26.0 (IBM Corporation, Armonk, NY, USA) to calculate the arithmetic means and standard deviations. Data visualization was performed using Origin 2021 (OriginLab, Corporation, Northampton, MA, USA).

3. Results

The results are presented by first examining effluent soluble iron concentrations, followed by pollutant removal performance and the associated chemical-biological mechanisms.

3.1. Characteristics of Iron Corrosion and Soluble Fe Dynamics

To investigate the in situ driving effect of NO_3^- in PTW on iron corrosion in FeC filters, iron dissolution experiments were carried out under high and low NO_3^- concentrations. Figure 2 shows the variations in effluent Fe(II) and total Fe (TFe) concentrations. The measured iron concentrations represented the residual aqueous fraction after Fe release, precipitation, adsorption, and retention within the filter bed, rather than the total Fe released from the FeC fillers.

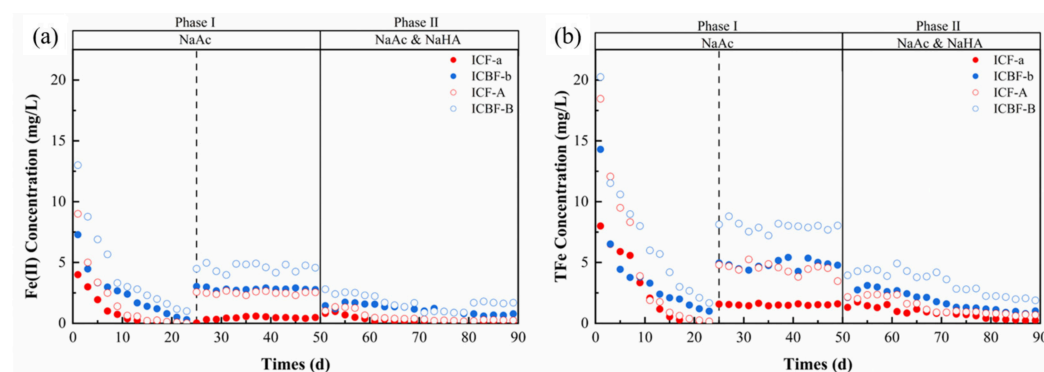


Figure 2. Variations in effluent soluble iron concentrations in different systems: (a) Fe(II) concentration; (b) total iron (TFe) concentration.

Influent nitrate concentration showed a pronounced effect on the effluent soluble Fe concentrations in the FeC systems. Higher Fe^{2+} and total iron (TFe) concentrations were observed in ICF-A and ICBF-B under high influent nitrate loading. However, during iron corrosion, H_2O was reduced to OH^- , which led to a rapid rise in system pH (Figure S1). Because pH critically influences both iron solubility and iron precipitates [29], the concentration of soluble iron ions decreases substantially under strongly alkaline conditions (above 9.5).

Influent pH adjustment was applied during days 25–50 to mitigate excessive alkalinity in the FeC systems. And no further pH adjustment was implemented after day 50. Because pH can influence Fe solubility, iron precipitation, and microbial activity, the results obtained during this period were interpreted within the context of pH-regulated operation. During the pH-regulated operation period, the concentrations of Fe^{2+} and TFe in ICF-A (high nitrate) were 2.51 mg/L and 4.57 mg/L, which were significantly higher than the 0.42 mg/L and 1.53 mg/L released by ICF-a (low nitrate). This pattern suggested that, under the tested conditions, higher nitrate concentration was associated with enhanced in situ iron corrosion. Furthermore, microbial involvement further enhanced the iron corrosion process. The Fe^{2+} and TFe concentrations in ICBF-b were 2.83 and 4.84 mg/L, respectively, while

those in ICBF-B reached 4.58 and 7.97 mg/L, higher than those in their corresponding abiotic filters (ICF-a and ICF-A).

In phase II, the introduction of NaHA inhibited iron release, potentially via active site occupation and pore clogging by HA [30]. Despite this, the high-nitrate groups (ICF-A, ICBF-B) still showed higher Fe^{2+} and TFe concentrations than the corresponding low-nitrate groups (ICF-a and ICBF-b). The biotic systems demonstrated higher soluble Fe concentrations than their abiotic counterparts. These observations suggested that nitrate and microbial activity contributed to iron corrosion under the tested conditions.

3.2. Characteristics of Pollutant Removal Under Chemical Biological Cascade Responses

The cascade responses triggered by nitrate-driven iron corrosion involved chemical phosphorus immobilization and multi-pathway biological nitrogen removal. The performance of each system was detailed in the following subsections.

3.2.1. Chemical Phosphorus Removal

Figure 3a,b illustrates the dynamic variations in influent and effluent $\text{PO}_4^{3-}\text{-P}$ concentrations and the corresponding removal efficiency of different systems. All reactors were operated under anoxic conditions (with DO consistently below 0.8 mg/L), which are unfavorable for the growth of phosphate-accumulating organisms. Thus, the negligible phosphate removal (<10%) in BF-c and BF-C was attributable primarily to limited microbial assimilation. In contrast, phosphate removal was significantly improved in FeC systems, indicating the dominant role of Fe-mediated phosphate immobilization.

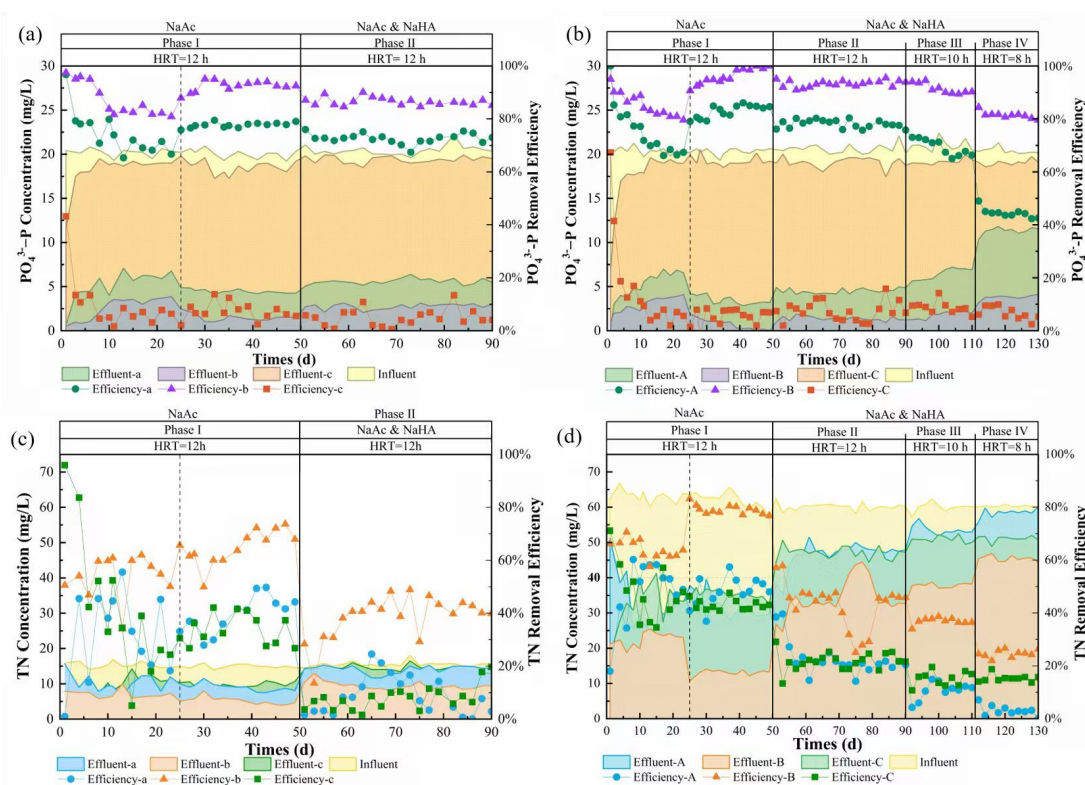


Figure 3. Variations in $\text{PO}_4^{3-}\text{-P}$ (a,b) and TN (c,d) concentration in low (a,c) and high (b,d) nitrate groups. Abbreviated reactor labels are used in the figure legend: a = ICF-a, A = ICF-A, b = ICBF-b, B = ICBF-B, c = BF-c and C = BF-C.

During the initial operation period, the system pH increased sharply (Figure S1), which reduced the availability of dissolved reactive iron species for phosphate precipitation. Excessive OH^- could also compete with phosphate ions for surface binding sites and reduce

the positive surface charge of iron (hydr)oxides, attenuating electrostatic adsorption of phosphorus [31]. Consequently, a decline in phosphate removal performance was observed in the FeC systems. During the pH-regulated period (day 25 to day 50), the average effluent $\text{PO}_4^{3-}\text{-P}$ concentration in ICF-A (high nitrate) was 3.48 mg/L, which was lower than 4.47 mg/L in ICF-a (low nitrate). Furthermore, the involvement of microorganisms further improved the phosphorus removal, as the effluent $\text{PO}_4^{3-}\text{-P}$ concentrations in ICBF-b and ICBF-B were 1.53 mg/L and 0.92 mg/L, respectively, lower than those in ICF-a and ICF-A.

In phase II, part of NaAc was replaced by NaHA to simulate the complex organic components of actual wastewater. HA may inhibit phosphorus removal through competitive adsorption, charge repulsion, and steric hindrance [32]. The average phosphate removal efficiency of all FeC systems decreased slightly compared to phase I (Figure 3a,b). Nevertheless, the high-nitrate systems maintained lower effluent $\text{PO}_4^{3-}\text{-P}$ concentrations than the corresponding low-nitrate systems. The effluent $\text{PO}_4^{3-}\text{-P}$ concentrations of ICF-A and ICBF-B were 4.49 and 1.38 mg/L, respectively, lower than those of ICF-a (5.59 mg/L) and ICBF-b (2.73 mg/L). ICBF-B achieved an average $\text{PO}_4^{3-}\text{-P}$ removal efficiency of 93.28% and the highest removal loading rate of 37.82 g P/(m³·d), indicating that iron corrosion promoted by high nitrate conditions and microbial participation strengthened phosphorus removal performance even in the presence of refractory organic matter.

3.2.2. Biological Multi-Pathway Denitrification

During the stable operation phase, differences in nitrogen removal efficiency and conversion pathways were observed among the systems. Under the multi-pathway synergistic denitrification associated with in situ nitrate-driven iron corrosion, ICBF consistently showed optimal nitrogen removal performance.

In phase I (NaAc as carbon source), the average effluent TN concentrations in ICBF-b and ICBF-B were 5.23 mg/L and 12.97 mg/L, respectively, lower than those of ICF-a/ICF-A (9.05 and 32.29 mg/L) and BF-c/BF-C (9.88 and 35.25 mg/L) (Figure 3c,d). Nitrogen transformation in each reactor was further evaluated by comparing the temporal profiles of effluent $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NH}_4^+\text{-N}$ (Figure S2). In conventional biofilters, nitrogen removal mainly depended on heterotrophic denitrification. Under low C/N conditions, BF-c and BF-C showed considerable residual $\text{NO}_3^-\text{-N}$ (8.21 ± 0.92 and 16.06 ± 1.16 mg/L) and $\text{NO}_2^-\text{-N}$ accumulation (1.75 ± 0.41 and 20.48 ± 2.72 mg/L), indicating heterotrophic denitrification was limited under low C/N conditions. In contrast, FeC systems changed the distribution of nitrogen species. In ICF, $\text{NO}_2^-\text{-N}$ accumulation was lower than that in BF, while $\text{NH}_4^+\text{-N}$ was detected, suggesting that iron-mediated chemical reduction partly converted nitrate/nitrite to ammonium. ICBF-b and ICBF-B achieved complete nitrate conversion and the lowest accumulation of $\text{NO}_2^-\text{-N}$ (<1 mg/L), while $\text{NH}_4^+\text{-N}$ accumulation further increased (4.27 mg/L and 12.43 mg/L). Consistent with the enhanced Fe release observed in ICBF, this result further suggested that microbial activity might have promoted FeC corrosion and the coupled reduction of nitrate/nitrite to ammonium. The higher TN removal efficiency of ICBF-B (79.07%) was associated with the coupling of chemical reduction and biological denitrification. ICBF-B achieved a volumetric TN removal loading rate of 97.12 g N/(m³·d), far exceeding the 59.58 g N/(m³·d) of conventional biofilter BF-C.

In phase II, the addition of NaHA inhibited nitrogen removal in all systems (adding NaHA). The TN removal efficiency of BF-C decreased from 43.45% in phase I to 21.78%. The effluent $\text{NO}_3^-\text{-N}$ concentration of BF-C increased to 43.07 ± 1.77 mg/L, while $\text{NO}_2^-\text{-N}$ decreased to 3.95 ± 0.71 mg/L, indicating that conventional heterotrophic denitrification was strongly constrained under the refractory organic matter condition. NaHA also inhibited nitrate chemical reduction with the effluent TN concentration of ICF-A increasing to 46.47 mg/L. In contrast, ICBF-B still achieved a better TN removal performance, with an ef-

fluent TN concentration of 32.25 mg/L. Iron–carbon micro-electrolysis might transform part of HA into lower-molecular-weight products with potentially higher bioavailability [33,34]. Together with the inorganic electron donors generated in situ, ICBF supported multiple possible nitrate transformation pathways under carbon-limited conditions. Therefore, the ICBF showed greater adaptability to refractory organic matter.

Furthermore, NaHA addition led to a notable increase in iron sludge production. Excessive accumulation of these precipitates within the filler pores and on the biofilm surface might impede mass transfer and microbial activity [35] and micro-electrolysis processes, causing an increase in effluent TN concentration from day 73 to day 79. Therefore, a one-time hydraulic flushing was conducted by cutting off the influent and applying upflow tap water cleaning, and the reactor performance was restored subsequently. To ensure the long-term operational stability of this synergistic system, regular backwashing is required to remove accumulated iron precipitates [36], restore the active surface of FeC fillers, and maintain an optimal biofilm thickness [37].

3.2.3. Treatment Capacity Under Increased Volumetric Loading Rates

To comprehensively evaluate the treatment capacity, the influent volumetric loading rate was progressively increased by reducing the HRT from 12 h to 10 h and finally to 8 h in the later operational phases. Accordingly, the influent $\text{PO}_4^{3-}\text{-P}$ and TN loading rates increased gradually from 40.60 to 61.35 g P/(m³·d) and from 123.86 to 184.59 g N/(m³·d), respectively. The comparative performance under these varying loads is summarized in Table S2.

For phosphorus removal, ICBF-B demonstrated sustained efficacy throughout the long-term operation, consistently maintaining a $\text{PO}_4^{3-}\text{-P}$ removal efficiency above 80%. It achieved the highest removal loading rate of 50.13 g P/(m³·d) at HRT 8 h. In contrast, the performance of ICF-A degraded over time, with its removal efficiency dropping to 44.93% under the same 8 h HRT condition. For nitrogen removal, although all systems experienced a decline in TN removal loading rate due to the limitation of contact time, ICBF-B consistently outperformed the other systems across all loading conditions.

In summary, ICBF-B was the only process that met the Class I discharge limits specified in DB44/613-2024 (Figures 3 and S2). At HRT of 10 h, the effluent concentrations of $\text{PO}_4^{3-}\text{-P}$, TN, and $\text{NH}_4^+\text{-N}$ were 2.04 mg/L, 38.04 mg/L, and 8.31 mg/L, respectively, all of which met the standard limits. As the HRT was further reduced to 8 h, the effluent quality failed to meet the discharge standard, indicating that 10 h was the boundary hydraulic retention time for ICBF-B. The results supported the feasibility of the “in situ nitrate-driven chemical–biological cascade response” system for advanced treatment of PTW.

3.3. Analysis of the Synergistic Mechanism in ICBF

To further interpret the performance differences among the reactors, mineral transformation, DOM variation, and microbial community structure were analyzed.

3.3.1. Iron Corrosion and Mineral Transformation

The morphology of the FeC filler before and after reaction was examined by SEM. The surface of the raw FeC filler was clean and smooth (Figure S3), with XRD identifying zero-valent iron (Fe^0) as the dominant phase alongside minor initial oxides.

After phase I operation, the FeC systems exhibited varying degrees of corrosion and the deposition of secondary minerals (Figure 4a,b). Correspondingly, XRD (Figure 4e) showed a decrease in Fe^0 peak intensity and the formation of typical iron corrosion products. The Fe^{2+} generated by corrosion underwent further oxidation and hydrolysis, forming a series of iron (hydr)oxides, identified as lepidocrocite, goethite and magnetite [33,38], which could pro-

vide more reactive sites for phosphate adsorption [39]. ICBF-B displayed more pronounced corrosion than the abiotic system ICF-A, attributed to microbial involvement [40].

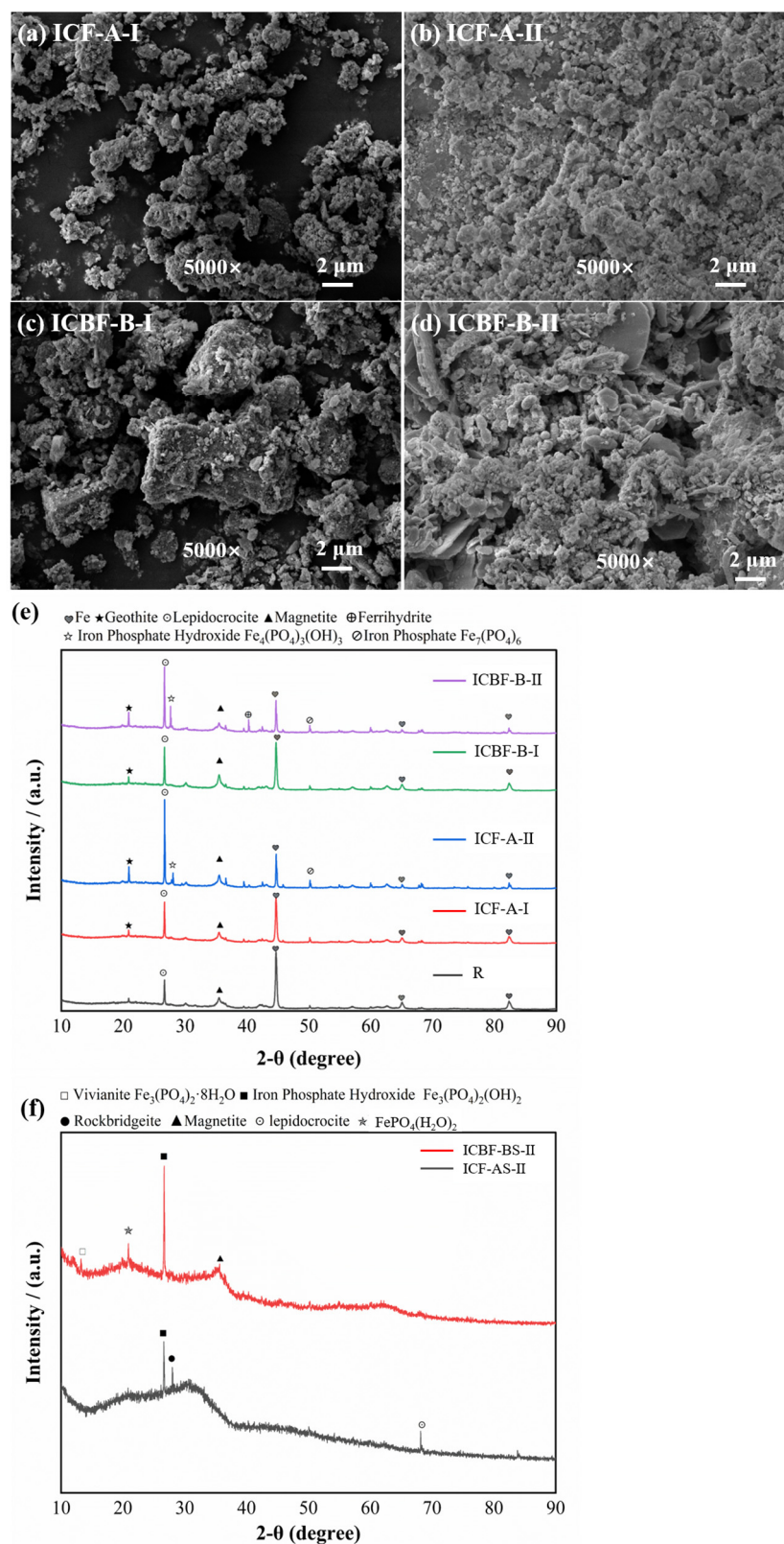


Figure 4. (a–d) SEM images of FeC fillers after treatment; (e,f) XRD patterns of FeC fillers and suspended sludge.

After phase II, the mineral deposits appeared relatively dense and compact in ICF-A. In contrast, ICBF-B exhibited a loose and porous attached layer, which might favor the exposure of reactive iron surfaces and ensure the continuous progress of corrosion. The evident colonization by rod-shaped bacteria (Figure 4b) indicated microbial participation in iron corrosion and secondary mineral formation. Further declines in Fe^0 diffraction intensity signaled sustained corrosion, accompanied by intensified accumulation of iron (hydr)oxides. However, the peak intensity of lepidocrocite in ICBF-B was notably lower than that in ICF-A. With a large surface area, lepidocrocite was more available to microbial Fe(III) reduction [41]. This decrease in peak intensity was consistent with the possible occurrence of DIR in the biotic system. In addition, ferrihydrite detected in ICBF-B was reported to be associated with iron-oxidizing bacteria [42]. With high specific surface area and reactivity, ferrihydrite could adsorb organic molecules [43] and might co-precipitate with humic acid to form a new negatively charged surface, providing additional active sites [44].

Notably, the addition of NaHA could inhibit crystallization and phase transformation processes. The complexes formed between HA and $\text{Fe}^{2+}/\text{Fe}^{3+}$ promoted the detachment of iron oxides from the iron surface [45], which explains the increase in iron sludge production observed in this stage. Phosphate removal may occur on the surface of the iron-based material or byproducts flaking off into the water [46]. XRD analysis (Figure 4e,f) confirmed that a series of iron–phosphorus compounds (FePs) were formed on both filler and sludge, demonstrating the presence of chemical precipitation for phosphate removal in FeC systems. The peak intensities of these FePs in ICBF-B were higher than those in ICF-A, consistent with the superior phosphorus removal performance of ICBF-B. In particular, vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) was detected in ICBF-B, a typical iron-phosphate mineral generated via iron reduction [47].

Further XPS analysis was performed on the suspended sludge within the reactors. As shown in the Fe 2p spectra (Figure S4c,f), the $\text{Fe(II)}/\text{Fe(III)}$ ratio in ICBF-B (44.63%:55.37%) was higher than that in ICF-A (39.12%:60.88%). This difference may be related to microbial dissimilatory iron reduction (DIR). Previous studies have reported that humic acid could serve as an electron shuttle to promote DIR [17,48,49]. However, because electrochemical measurements and electron-flux analyses were not conducted in this study, the possible redox-mediating role of HA was discussed only as a literature-supported hypothesis rather than direct experimental evidence. Microorganisms not only altered the valence state of iron but also influenced the pathways of mineral formation. In ICBF-B, the proportion of the Fe-OH component representing highly reactive, amorphous iron (hydr)oxides (11.69%) was nearly twice that of ICF-A (5.96%). The proportion of Fe-O components, which represent a stable crystalline iron oxide phase, decreased from 39.48% in ICF-A to 28.35% in ICBF-B. These findings suggested that microbial activity may have influenced Fe mineral transformation and helped maintain reactive Fe availability, possibly through the formation of poorly crystalline amorphous iron (hydr)oxides [50], which possess a higher specific surface area and enhanced reactivity. This might partially alleviate FeC surface passivation.

Additionally, the C 1s spectra (Figure S4a,d) revealed that compared with ICF-A, the C=O bond proportion in ICBF-B decreased from 14.19% to 13.45%, while the proportion of C-O-C bonds increased from 23.24% to 26.05%. Corresponding changes were observed in the O 1s spectra (Figure S4b,e), where the proportion of organic C=O decreased, while organic C-O increased from 9.84% in ICF-A to 16.07% in ICBF-B. These observations suggested that carbonyl/carboxyl groups (C=O) were more extensively converted into smaller molecular products containing C-O bonds.

In summary, in ICF-A, the filler surface tended to form a dense mineral layer. By contrast, in ICBF-B, nitrate and microorganisms synergistically enhanced iron corrosion. Furthermore, the combined effect of microorganisms and humic acid drove the trans-

formation of iron minerals into highly active, bioavailable forms (e.g., ferrihydrite and lepidocrocite) as well as amorphous phases, thereby preventing the formation of a dense passivation layer.

3.3.2. Analysis of DOM Transformation Based on 3D-EEM

The changes in DOM before and after wastewater treatment were analyzed by 3D-EEM. Figure S5 showed a distinct fluorescence peak at Ex/Em = 270/415 nm corresponding to humic acid-like substances [51]. In ICBF-B, iron-carbon micro-electrolysis disrupted the long-chain structure of HA [34]. Humic acid may serve as an electron acceptor and be reduced by certain bacteria [52,53]. Concurrently, the iron-based materials enhanced microbial activity and promoted the utilization of aromatic proteins [54]. The fluorescence intensity was remarkably lowered in the effluent of ICBF-B, indicating stronger degradation of HA. This transformation of refractory organic matter may generate lower-molecular-weight products with improved bioavailability, thereby providing possible support for heterotrophic microorganisms under carbon-limited conditions and contributing to the TN removal performance in ICBF-B.

3.3.3. Analysis of Microbial Community Structure and Function

As shown in Table S3, the coverage indices of all samples were above 0.999, which confirmed the reliability and representativeness of the findings. The Chao and Ace indices were used to evaluate species richness, with higher values indicating a greater total number of species. The Shannon index was employed to assess community diversity. Chao and Ace values in ICBF-B were consistently higher than those of BF-C, which aligned with previous reports that ferrous ions released from iron-based materials promote biomass augmentation [40,55]. The Shannon index in ICBF-B was lower than that in BF-C. This suggested that FeC filler may have shaped the microbial community and favored the enrichment of several functionally specialized populations.

In phase II, the addition of refractory substances, NaHA, exerted a significant selective pressure on the microbial communities. Species richness (Chao and Ace indices) plummeted by approximately 46% in ICBF-B and 47% in BF-C. A large number of microorganisms were eliminated, which might lead to a decrease in TN removal efficiency. However, the Shannon indices did not decline proportionally, suggesting that despite the reduction in total species number, community evenness improved. This pattern implied that the microbial community likely underwent structural reorganization under the altered organic composition.

At the phylum level in phase I (Figure 5a), ICBF-B was dominated by *Pseudomonadota* (37.41%), *Chloroflexota* (31.79%), and *Bacillota* (8.91%). In contrast, the relative abundances of these phyla were lower in the conventional biological filter BF-C, indicating that the FeC-associated environment shaped the microbial community structure. The *Pseudomonadota* phylum contains various denitrifying bacterial genera [34], and its enrichment supported the superior TN removal in ICBF-B. *Chloroflexota* contains facultative anaerobic members [56] capable of degrading organic compounds and readily enriched in iron-rich environments [57]. Their filamentous morphology was considered to contribute to the formation of stable biofilm, potentially facilitating the colonization of functional microorganisms and supporting efficient nitrogen removal [58]. Additionally, *Bacteroidota* (5.44%) and *Actinomycetota* (2.74%) were also enriched. These Gram-positive bacterial groups generally exhibited stronger tolerance to metal-related environmental stresses [56,59].

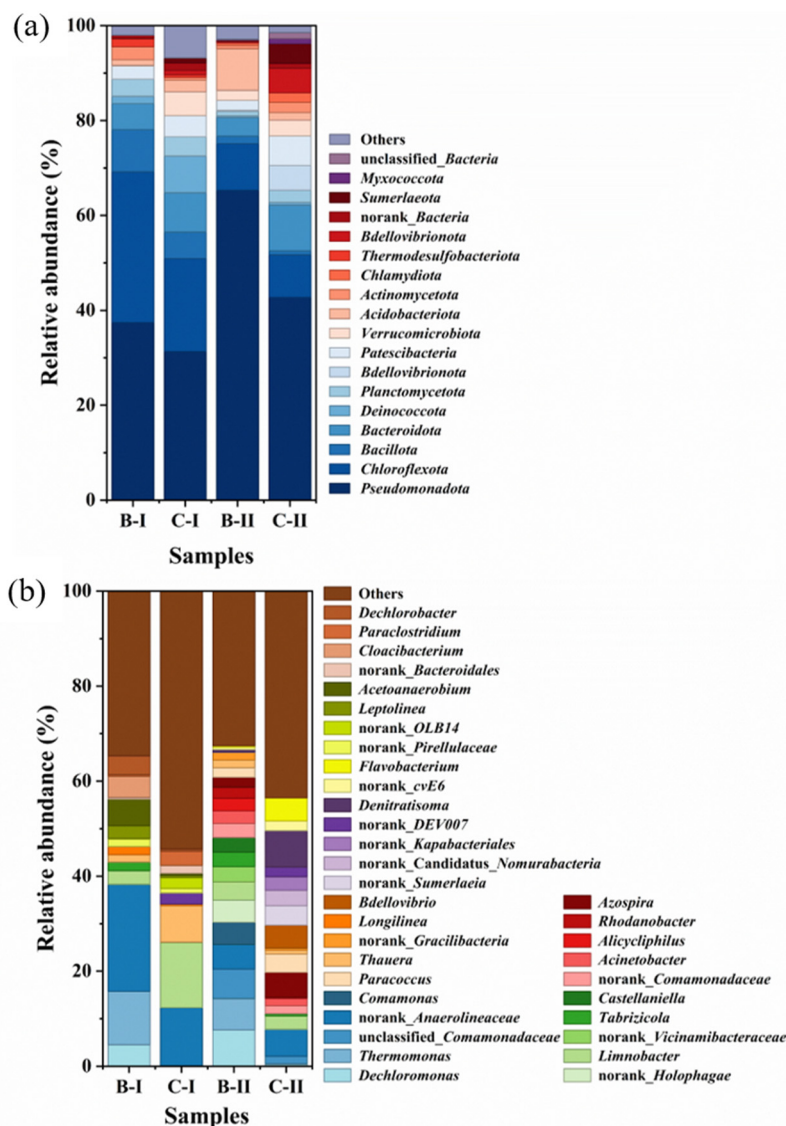


Figure 5. Microbial communities: (a) phylum level; (b) genus level. Abbreviated reactor labels are used in the figure legend: B-I, C-I, B-II, and C-II represent ICBF-B-I, CF-C-I, ICBF-B-II, and CF-C-II, respectively.

The microbial community structure was restructured in phase II. BF-C exhibited functional decline characterized by the enrichment of parasitic *Patescibacteria* (6.21%) [60] and predatory *Bdellovibrionota* (5.18%) [61]. In contrast, *Pseudomonadota* dominated in ICBF-B, reaching 65.3%. Under nutrient-limited conditions, the system selectively enriched microorganisms with high nutrient utilization efficiency [62]. The relative abundances of copiotrophic phyla such as *Bacillota* and *Bacteroidota* [63] decreased markedly. Conversely, the oligotrophic *Acidobacteriota* increased markedly from 1.14% to 8.73%, which has been reported to play an important role in carbon, nitrogen, and iron cycling environments [64,65].

At the genus level in phase I (Figure 5b), nitrogen removal in BF-C was primarily driven by conventional heterotrophic denitrifiers such as *Limnobacter* (13.72%) and *Thauera* (7.72%) [66]. Notably, the unique habitat created by the FeC filler in ICBF-B fostered the enrichment of microbial genera with diverse metabolic capabilities. *Norank_Anaerolineaceae* and *Anaerolineaceae*-related taxa have been reported to participate in organic matter decomposition, thereby potentially fueling denitrification [67–69]. *Acetoanaerobium* (5.62%) of the phylum *Bacillota* has been reported to adapt to iron-rich environments through fermentation [27]. Electroactive microorganisms, including *Leptolinea* (2.67%) [70] and *Cloacibacterium*

(4.45%) [71] were identified. Additionally, the autotrophic denitrifier *Thermomonas*, which has been reported as a nitrate-dependent Fe(II)-oxidizing bacterium (NDFO) in previous studies [72–75], exhibited a relative abundance of 11.26% autotrophic denitrification bacteria *Dechloromonas* (4.51%), which also possesses ferrous-oxidizing capacity [57,76]. Concurrently, heterotrophic denitrifiers such as *Dechlorobacter* (3.97%) [77], *Tabrizicola* (1.83%) [78], and norank_f_Pirellulaceae [79] were also enriched. These taxa suggested the possible coexistence of multiple nitrogen-transformation processes in ICBF-B, but their specific functions remain putative.

In phase II, the core heterotrophic genera in BF-C (e.g., *Limnobacter* (2.76%), *Thauera* (0.80%)) declined significantly, accompanied by significant increases in predatory bacteria such as *Bdellovibrio* (4.85%) [80], saprophytic bacteria *Flavobacterium* (4.86%). In ICBF-B, iron corrosion continuously supplied additional electron donors, while FeC micro-electrolysis might break down HA into bioavailable low-molecular-weight organic compounds [34], which might provide multi-electron donors. ICBF-B retained higher abundances of autotrophic denitrifier *Dechloromonas* (7.66%) and *Thermomonas* (6.57%), alongside heterotrophic denitrifier *Limnobacter* (3.85%) and *Thauera* (1.65%). While certain copiotrophs decreased, genera capable of utilizing aromatic and complex organic compounds, such as *Comamonas* (4.68%) [81] and norank_Holophagae, were enriched. *Comamonas* [82] and *Acinetobacter* (2.69%) [83] were previously identified as DIRB [82]. *Alicyclophilus* (2.62%), previously reported as Feammox bacteria [84], was also observed in ICBF-B. These microbes have been implicated in Fe(III)/Fe(II) cycling and may help mitigate iron passivation. In summary, ICBF-B enriched taxa are putatively associated with nitrate transformation, organic matter degradation, and iron cycling. It exhibited stronger adaptability to adverse water quality conditions and greater application potential [85].

3.3.4. Potential Mechanism of Simultaneous Nitrogen and Phosphorus Removal

In this study, nitrate, as a target pollutant in PTW, played a crucial in situ driving role (Figure 6). As an oxidizing agent, nitrate promoted iron corrosion and further triggered a chemical-biological cascade response. On the one hand, corrosion-generated reactive iron ions and secondary minerals efficiently removed phosphates from the wastewater through precipitation, adsorption, and flocculation. On the other hand, the inorganic electron donors generated during iron corrosion, together with available organic substrates, supported multiple nitrogen removal pathways including autotrophic denitrification, heterotrophic denitrification and chemical reduction. Meanwhile, iron–carbon micro-electrolysis could produce strongly reducing substances, which could break the long-chain structures of refractory organic substances. HA was partially degraded, which might provide available carbon sources for heterotrophic denitrification. In addition, HA appeared to indirectly influence secondary mineral transformation, possibly through inhibition of secondary mineral crystallization, and redox-mediating effects reported in the literature. Taxa potentially associated with iron reduction and iron oxidation may have contributed to Fe(II)/Fe(III) cycling within the system. The synergistic system tended to generate loose mineral layers, thus mitigating the development of a dense passivation layer. As a result, part of the iron minerals may have functioned as reactive redox and adsorption interfaces rather than only inert reaction end products, which could help maintain reactive Fe availability and partially alleviate filler passivation.

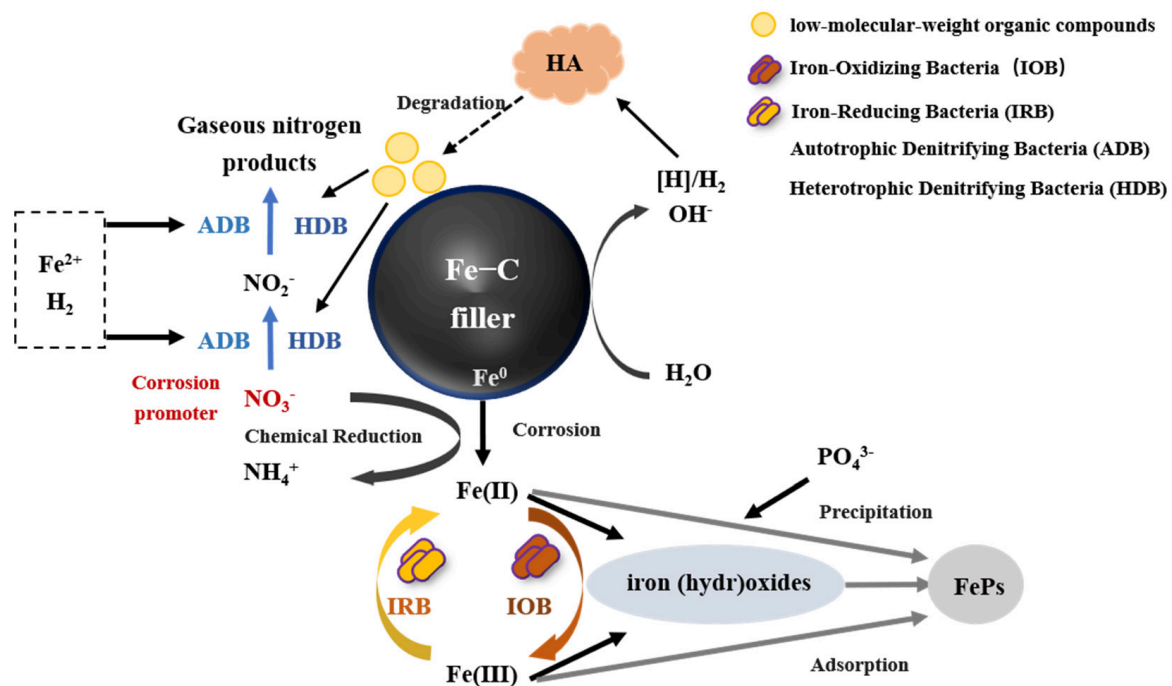


Figure 6. Mechanism of simultaneous nitrogen and phosphorus removal in ICBF.

3.4. Practical Implications and Scale-Up Considerations

This study suggested that the ICBF system has potential as a polishing unit for low-C/N wastewater. A brief comparison with representative nutrient-removal technologies was provided in Table S4. Conventional biological nitrogen removal generally requires an extra carbon source to maintain denitrification performance [86], while chemical precipitation for phosphorus control increases chemical consumption and sludge production [87]. Constructed wetlands have low energy demand but require larger land areas and are sensitive to seasonal and influent fluctuations [88,89]. Previous iron-based systems have shown enhanced nitrogen and phosphorus removal [85,90,91], supporting the role of iron-based materials in nutrient removal. Consistent with these studies, the ICBF system integrated nitrate transformation and phosphorus immobilization within a single unit, without external carbon addition, chemical coagulant dosing, or aeration. Thereby this system potentially curtails both chemical expenditure and energy consumption.

For engineering applications, further evaluation should focus on several aspects. First, long-term operational stability and maintenance should be assessed using real PTW, such as backwashing strategy, filler consumption, and replacement intervals. The complex matrix of real wastewater (e.g., suspended solids, antibiotics, and trace metals) may affect treatment performance. Consequently, long-term pilot-scale validation using real PTW is essential before full-scale application. Second, Fe/P fate and FeC corrosion-passivation behavior require further quantification to support the evaluation of operational cost and secondary waste generation. Future studies should combine FeC mass-loss analysis with electrochemical measurements to evaluate filler replacement and verify the corrosion-passivation mechanism. In practical operation, the replacement interval of FeC fillers could be correlated with pollutant removal deterioration and FeC mass loss. Third, potential gaseous emissions, such as NH₃ and N₂O, should be monitored, and corresponding mitigation strategies, such as pH optimization [92] and the C/N ratio regulation [93], should be evaluated. Fourth, future studies should incorporate functional gene analysis or metagenomics to elucidate the underlying microbial metabolic pathways. Overall, these scale-up consid-

erations inform the potential application of ICBF as an advanced treatment and provide guidance for its full-scale implementation.

4. Conclusions

This study investigated in situ nitrate-driven iron corrosion and the associated chemical-biological cascade response for simultaneous nitrogen and phosphorus removal in an ICBF treating PTW. The results demonstrated that the high-nitrate FeC systems released more Fe^{2+} than the corresponding low-nitrate systems, supporting the role of nitrate in promoting iron corrosion under the tested conditions. The released Fe species contributed to efficient phosphate immobilization, with the $\text{PO}_4^{3-}\text{-P}$ removal efficiency of ICBF-B reaching 96.08%. Meanwhile, iron corrosion provided additional inorganic electron donors that could support multiple nitrogen-transformation pathways. ICBF-B successfully enriched a composite functional community composed of autotrophic and heterotrophic denitrifying bacteria, consistent with its superior nitrogen removal performance. Moreover, the iron-carbon micro-electrolysis might facilitate the breakdown of refractory organic compounds into smaller molecules, thereby potentially supplementing available carbon for heterotrophic denitrification.

The influence of hydraulic loading was further evaluated by reducing the HRT from 12 h to 10 h and then to 8 h. Although effluent pollutant concentrations increased as the HRT decreased, ICBF-B consistently outperformed the other reactors. At 10 h HRT, the effluent $\text{PO}_4^{3-}\text{-P}$, TN, and $\text{NH}_4^+\text{-N}$ concentrations of ICBF-B were 2.04, 38.04, and 8.31 mg/L, respectively, meeting the Class I discharge limits specified in DB44/613-2024.

Overall, this study provides evidence that nitrate-associated iron corrosion can contribute to coupled phosphorus and nitrogen removal. Practically, this strategy can reduce reliance on chemical dosing and readily biodegradable organic substrates, providing a potentially feasible approach for advanced treatment of nitrate-rich, low-C/N PTW. Before full-scale application, further work is needed to quantify gas emissions, long-term FeC filler consumption, replacement requirements, and Fe/P fate, and to validate process performance under real PTW conditions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/w18141757/s1>, Text S1. Sludge acclimation procedure; Table S1. Physicochemical properties of the FeC filler; Table S2. Nutrient removal performance of different reactors; Table S3. Diversity indices; Table S4. Comparison of technologies for N and P removal; Figure S1. pH in each filter; Figure S2. Composition of nitrogen: (a) ICF-a, (b) ICBF-b, (c) BF-c, (d) ICF-A, (e) ICBF-B, (f) BF-C; Figure S3. SEM image of raw FeC filler; Figure S4. XPS C 1s, O 1s, Fe 2p spectra of suspended sludge in ICF-A (a–c) and ICBF-B (d–f); Figure S5. 3D-EEM fluorescence spectra of wastewater before and after treatment: (a) raw wastewater, (b) after treatment in ICF-A, (c) after treatment in ICBF-B, (d) after treatment in BF-C.

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