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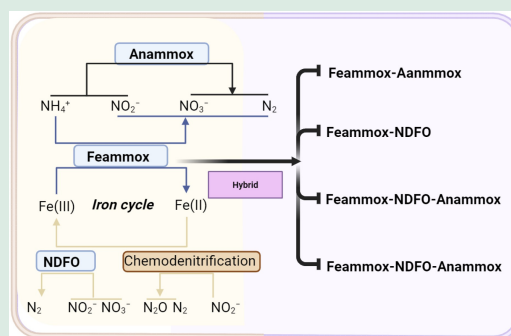
A critical review of iron redox-coupled nitrogen conversion for sustainable wastewater treatment

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HIGHLIGHTS

- This review evaluates Fe redox-coupled N conversion in wastewater treatment.
- It reveals competition and instability among coupled Fe-N transformation pathways.
- Engineering barriers including kinetics, encrustation, and N_2O risks are identified.
- Strategies for scalable and sustainable Fe-mediated N removal are proposed.



ABSTRACT: Sustainable nitrogen removal remains a central challenge in wastewater treatment, as conventional biological processes depend extensively on aeration and external carbon addition, resulting in substantial energy consumption, operational costs, and greenhouse gas emissions. Iron redox-coupled nitrogen conversion has emerged as a promising alternative, linking nitrogen transformation to Fe(II)/Fe(III) cycling to enable pathways for low-energy and low-carbon wastewater treatment. This review provides a critical evaluation of current knowledge on key iron-mediated nitrogen-conversion pathways, including Fe(III)-dependent ammonium oxidation (Feammox), nitrate-dependent ferrous oxidation, iron-enhanced anaerobic ammonium oxidation (anammox), and abiotic chemodenitrification, with emphasis on their interactions in engineered wastewater systems. Rather than considering these pathways as isolated processes, this review highlights how competition among pathways, kinetic mismatch, iron bioavailability, mineral precipitation, and abiotic side reactions fundamentally govern system performance and stability. Key operational regulators, such as iron concentration and speciation, dissolved oxygen, pH, temperature, and electron-transfer mediators, are critically examined from an engineering control perspective. Major translational barriers, including unresolved microbial mechanisms, slow reaction kinetics, uncertain pathway partitioning, mineral encrustation, and the risk of greenhouse gas emissions, are discussed. Finally, future research priorities are proposed to accelerate the development of robust and scalable iron-mediated nitrogen-removal technologies. This review offers an important engineering perspective for advancing sustainable wastewater treatment via iron redox-driven nitrogen conversion.

KEYWORDS: Iron redox cycling, Feammox, Nitrate-dependent ferrous oxidation, Anammox, Sustainable wastewater treatment, Nitrogen removal

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1 Introduction

Nitrogen removal is a fundamental requirement in wastewater treatment to prevent eutrophication, ecosystem degradation, and downstream greenhouse gas emissions (van Maanen et al., 1994; Zhou et al., 2024). Conventional biological nitrogen removal, based on nitrification and denitrification, has facilitated effective control of ammonium and total nitrogen for decades; however, these processes are intrinsically energy- and resource-intensive, requiring substantial aeration for nitrification and external organic carbon for denitrification, while contributing considerably to operational costs and greenhouse gas emissions (Lackner et al., 2014; Zhou et al., 2024). As wastewater treatment increasingly transitions toward energy-efficient and low-carbon infrastructure, the development of alternative nitrogen-removal strategies has become a major research and engineering priority. Over the past two decades, innovative biological nitrogen-removal technologies such as anaerobic ammonium oxidation (anammox), partial nitrification/anammox (PN/A), and partial denitrification/anammox have substantially improved the sustainability of nitrogen removal by reducing oxygen demand, sludge production, and external carbon requirements (Lackner et al., 2014; Wang et al., 2022; Shah, 2023; Zuo et al., 2026). Despite these advances, these systems remain constrained by operational instability, stringent process control requirements, and dependence on favorable wastewater characteristics, particularly under low-strength, variable, or decentralized treatment conditions (Deng et al., 2025; Chang et al., 2026). Accordingly, alternative redox pathways that may further enhance nitrogen removal efficiency while reducing energy and chemical inputs have garnered increasing attention.

Among emerging approaches, iron redox-coupled nitrogen conversion has attracted growing interest as a potentially transformative pathway for sustainable wastewater treatment. Iron is one of the most abundant redox-active elements in natural and engineered environments (Huang et al., 2026; Zhang et al., 2026), and can function as both electron donor and acceptor in nitrogen-transformation pathways (Kappler et al., 2021). Beyond its conventional roles in wastewater treatment as a micronutrient, coagulant, and phosphorus precipitant, iron can fundamentally reshape nitrogen conversion via multiple interconnected processes, such as Fe(III)-dependent ammonium oxidation (Feammox), nitrate-dependent ferrous oxidation (NDFO), iron-enhanced anaerobic ammonium oxidation (anammox),

and abiotic chemodenitrification (Sun et al., 2023; Yang et al., 2023; Liu et al., 2024). These pathways present opportunities for low-carbon nitrogen removal under anaerobic or carbon-limited conditions by partially replacing conventional oxygen- and carbon-dependent biological processes. However, the engineering feasibility of iron-mediated nitrogen conversion remains highly uncertain. Feammox and NDFO remain emerging pathways with unresolved microbial mechanisms, slow and variable kinetics, uncertain relative contributions, and strong dependence on iron speciation and environmental conditions (Kappler et al., 2021; Liu et al., 2024). Moreover, the coexistence of multiple biological and abiotic reactions introduces considerable complexity, including competition for nitrogen intermediates, mineral precipitation, microbial encrustation, redox imbalance, and unintended greenhouse gas emissions such as N₂O (Jones et al., 2015; Kappler et al., 2021; Chen et al., 2021). These interactions indicate that iron-mediated nitrogen conversion should not be viewed simply as a set of complementary reactions. Instead, it functions as a dynamic, potentially unstable redox network requiring critical evaluation from an engineering perspective.

Several recent reviews have summarized iron-mediated nitrogen conversion, mainly focusing on individual reaction mechanisms, microbial communities, or pollutant-removal performance (Shi et al., 2024; Yuan et al., 2025). However, a critical engineering synthesis remains lacking. In particular, limited attention has been paid to the interactions and competition among coupled iron-mediated pathways, the operational constraints governing process stability, and the translational barriers to practical implementation in engineered wastewater systems. Accordingly, this review critically evaluates the current understanding of iron redox-coupled nitrogen conversion for sustainable wastewater treatment. Specifically, it 1) examines the thermodynamic and mechanistic basis of major iron-mediated nitrogen transformation pathways; 2) analyzes the interactions, synergies, and competition among coupled biological and abiotic processes; 3) evaluates key operational regulators influencing process performance and controllability; and 4) identifies major translational barriers and research priorities for scalable implementation. By integrating mechanistic understanding with engineering considerations, this review aims to provide a critical framework for advancing iron-mediated nitrogen removal in practical wastewater treatment applications.

2 Iron redox pathways shaping nitrogen transformation

In engineered wastewater-treatment systems, iron redox cycling can fundamentally restructure nitrogen transformation by introducing additional biological and abiotic pathways beyond conventional nitrification–denitrification. Depending on iron speciation, microbial community structure, and environmental conditions, Fe(II)/Fe(III) can stimulate established biological routes, facilitate emerging iron-dependent nitrogen conversions, or drive abiotic nitrogen transformations (Kappler et al., 2021; Liu et al., 2024). These pathways differ markedly in terms of thermodynamic favorability, kinetics, controllability, and engineering maturity, making comparison evaluation essential for assessing their practical potential in sustainable wastewater treatment (Fig. 1).

2.1 Iron-enhanced anammox: strengthening anaerobic nitrogen removal

Anammox is an established autotrophic nitrogen-removal pathway that substantially reduces aeration demand, sludge production, and external carbon requirements compared to conventional biological nitrogen removal (Lackner et al., 2014). Iron can further improve anammox performance via multiple mechanisms, making this type of iron-mediated interaction one of the most practically relevant in wastewater treatment systems. As an essential micro-nutrient, iron participates in the synthesis of heme-containing proteins, cytochromes, and other enzymes involved in electron transport and nitrogen metabolism,

thereby supporting anammox microbial activity (Li et al., 2025). Additionally, conductive iron-bearing minerals such as magnetite (Fe_3O_4) may facilitate extracellular electron transfer (EET), promote microbial aggregation, and stabilize biomass retention, thereby enhancing reactor performance (Zhang et al., 2021; Jiang et al., 2023). Furthermore, iron supplementation has been linked to increased extracellular polymeric substance secretion, which may further strengthen granule structure and improve microbial resilience under operational stress (Li et al., 2025).

However, the beneficial effects of iron are strongly contingent on its concentration and chemical speciation. Moderate iron supplementation can stimulate anammox activity, whereas excessive dissolved iron can induce toxicity, promote mineral precipitation, or impose mass-transfer constraints that impair biological performance (Zhang et al., 2021; Jiang et al., 2023). From an engineering perspective, iron-enhanced anammox is the most mature iron-mediated nitrogen-conversion strategy presently available. However, its practical effectiveness depends on controlled iron management rather than uncontrolled supplementation.

2.2 Feammox: an emerging anaerobic ammonium-oxidation pathway

Feammox has emerged as a promising pathway for anaerobic ammonium removal, in which ammonium is oxidized using Fe(III) as the terminal electron acceptor. Although the corresponding stoichiometric pathways can generate N_2 , nitrite, or nitrate, available isotope-tracing and nitrogen mass-balance studies generally identify N_2 as the dominant terminal product, with nitrite and nitrate representing minor or condition-

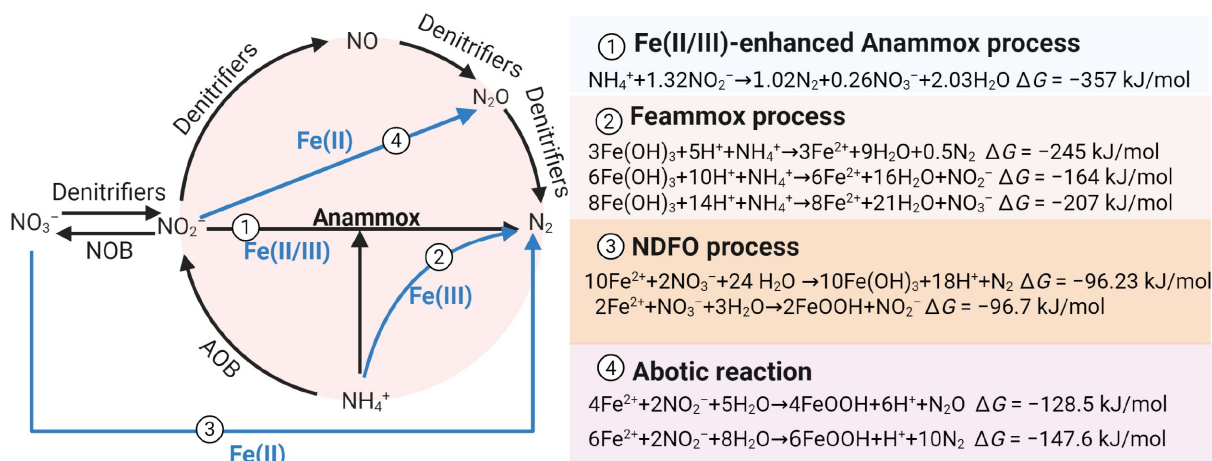


Fig. 1 Conceptual illustration of iron-mediated nitrogen-transformation pathways in wastewater treatment, showing synergistic and competing biological and abiotic reactions regulated by Fe(II)/Fe(III) redox cycling.

dependent fractions (Yang et al., 2012, 2023). The stoichiometric feasibility of nitrite- and nitrate-producing reactions should therefore not be interpreted as evidence that these intermediates are generated at rates sufficient to sustain downstream anammox or NDFO.

Unlike anammox, Feammox does not depend on nitrite as an obligate electron acceptor, making it theoretically attractive for carbon-limited anaerobic wastewater systems. Feammox is thermodynamically favorable, with ΔG values ranging from approximately -164 to -245 kJ/mol depending on the reaction pathway, although it remains less favorable than anammox (-357 kJ/mol). Nevertheless, thermodynamic feasibility does not solely determine engineering performance, as Feammox is strongly constrained by slow reaction kinetics, limited Fe(III) bioavailability, and inefficient EET (Kappler et al., 2021; Liu et al., 2024).

Mechanistically, Feammox remains insufficiently resolved. Proposed mechanisms include direct EET mediated by outer membrane *c*-type cytochromes, indirect transfer via soluble electron shuttles such as flavins and humic substances, and conductive microbial appendages that facilitate electron movement at the microbe–mineral interface (Kappler et al., 2021; Zhu et al., 2021; Liu et al., 2024). Direct EET through cell-associated cytochromes or conductive appendages may be particularly relevant when microorganisms are in close contact with iron minerals, whereas soluble electron shuttles can extend electron transfer beyond the cell–mineral interface. However, the relative contributions of these mechanisms remain unresolved, and soluble shuttles are generally more amenable to operational manipulation than biologically regulated direct EET pathways. Iron also serves as a cofactor in essential metalloenzymes and heme-containing proteins involved in microbial metabolism (Qiao et al., 2013; Baek et al., 2019). Nevertheless, direct mechanistic confirmation remains limited. To date, *Acidimicrobia-ceae bacterium* A6 is the only confirmed direct Feammox microorganism (Huang and Jaffé, 2018), although broader microbial consortia likely contribute indirectly via iron cycling, electron shuttling, or intermediate nitrogen transformation (Li et al., 2019; Yao et al., 2019; Ahmed et al., 2021).

2.3 NDFO: microbial and hybrid iron-driven nitrate reduction

NDFO describes nitrogen-conversion processes in which Fe(II) oxidation is coupled to nitrate or nitrite reduction, regenerating Fe(III) while removing oxidized

nitrogen species (Chen et al., 2021). Conceptually, NDFO is especially attractive because it can complement Feammox by enabling internal Fe(II)/Fe(III) redox cycling, thereby supporting integrated iron-mediated nitrogen conversion systems. However, NDFO remains mechanistically complex and, in many cases, debated. Reported NDFO activity has been attributed to direct enzymatic Fe(II) oxidation, indirect microbial denitrification coupled with abiotic iron oxidation, or hybrid biotic–abiotic interactions (Kim et al., 2016; Wang et al., 2020). Experimentally distinguishing these pathways remains challenging, particularly in complex engineered systems where multiple nitrogen and iron transformations occur simultaneously.

Thermodynamically, NDFO is less favorable than anammox or Feammox, with a Gibbs free energy change of approximately -96 kJ/mol, suggesting stronger dependence on microbial catalysis and environmental conditions (Kappler et al., 2021). A wide range of microorganisms, including autotrophic Fe(II) oxidizers, heterotrophic denitrifiers, and mixotrophic bacteria such as *Acidovorax* and *Pseudomonas stutzeri* (Melton et al., 2012; Klueglein et al., 2014; Jiang et al., 2023), have been implicated in NDFO-related activity. However, the relative contribution of true enzymatic NDFO remains unresolved in many reported systems.

2.4 Chemodenitrification: the abiotic competitor in iron-mediated nitrogen conversion

In addition to biologically mediated pathways, abiotic chemodenitrification is a major competing nitrogen transformation pathway in iron-rich systems. In this process, Fe(II) chemically reduces nitrogen oxides, particularly nitrite, generating gaseous products such as NO, N₂O, and N₂ (Jones et al., 2015; Kappler et al., 2021). Chemodenitrification is thermodynamically favorable, with ΔG ranging from approximately -128 to -148 kJ/mol depending on reaction conditions. More importantly, it is often kinetically rapid, especially in the presence of reactive Fe(II)-bearing minerals, allowing it to compete effectively with biological nitrogen-removal pathways. Unlike microbial processes, chemodenitrification bypasses biological regulation and rapidly consumes nitrogen intermediates, particularly nitrite. From an engineering perspective, chemodenitrification presents a double-edged effect. Although it may contribute to nitrogen removal, it reduces process controllability, complicates pathway attribution, and introduces the risk of unintended greenhouse gas emissions, especially N₂O.

3 Coupled iron-mediated nitrogen conversion: interactions, synergies, and instability

In engineered wastewater systems, iron-mediated nitrogen transformations rarely occur in isolation. Instead, multiple Fe(II)/Fe(III)-coupled biological and abiotic pathways typically coexist and interact (Fig. 2). These interactions may enhance nitrogen removal via complementary substrate exchange and internal iron redox cycling. However, they may also destabilize process performance owing to kinetic mismatches, mineral precipitation, and competition for shared redox-active substrates and intermediates, including NH_4^+ , NO_2^- , NO_3^- , Fe(II), and Fe(III). For example, NO_2^- is consumed by anammox, NDFO, and chemodenitrification, while Fe(II) can serve as an electron donor for NDFO and abiotic nitrite reduction or be oxidized by residual oxygen. The resulting competition affects nitrogen removal efficiency as well as iron recycling, pathway attribution, and the formation of NO and N_2O . Understanding these coupled interactions is thus essential for evaluating the practical feasibility of iron-mediated nitrogen-removal systems. Accordingly, the following subsections examine Fe–N coupling across increasing levels of pathway complexity, from binary interactions between Feammox and anammox or NDFO to integrated biological–abiotic networks, with

representative studies summarized in Table 1.

3.1 Feammox–anammox

The coupling of Feammox and anammox has attracted considerable attention as a potential strategy for autotrophic nitrogen removal, as they are metabolically complementary pathways. In this configuration, Feammox oxidizes ammonium using Fe(III) as the electron acceptor, generating nitrite and/or nitrate as intermediates, which subsequently act as electron acceptors for anammox to produce dinitrogen gas (Huang and Jaffé, 2018; Yang et al., 2023). Conceptually, this coupling is attractive because it facilitates anaerobic nitrogen removal without external organic carbon input while partially reducing dependence on conventional nitrification for nitrite generation. However, the practical feasibility of this coupling is fundamentally constrained by pronounced kinetic asymmetry between the two pathways. As discussed in Section 2.2, the intrinsically low Feammox rate creates a substantial kinetic mismatch with anammox (Kappler et al., 2021). By contrast, anammox is a well-established and comparatively efficient autotrophic nitrogen-removal pathway that rapidly consumes nitrite when sufficient substrate is available (Lackner et al., 2014). Thus, endogenous nitrite generation via Feammox is unlikely to sustain high-rate anammox activity in engineered systems. From a substrate mass-balance perspective, only the minor

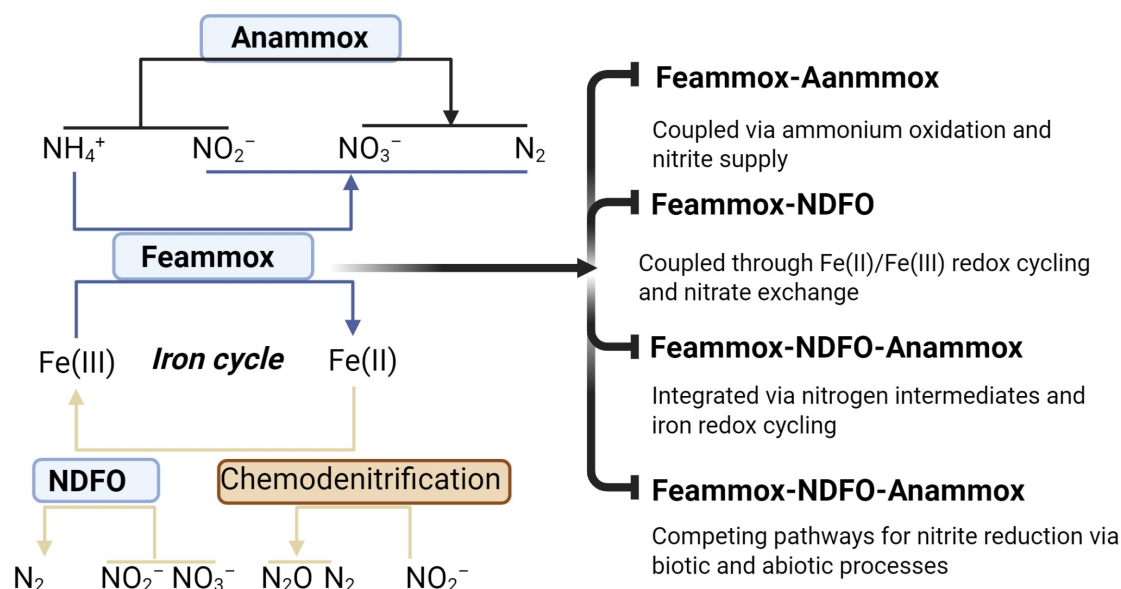


Fig. 2 Interaction framework of coupled iron-mediated nitrogen-conversion pathways in wastewater treatment, illustrating synergistic, competitive, and abiotic process interactions regulated by Fe(II)/Fe(III) redox cycling.

Table 1 Representative studies of coupled iron-mediated nitrogen-conversion systems and their engineering characteristics

Hybrid processes	Reactor type	Inoculum	Reactions	Temperature	pH	Initial nitrogen concentration	Removal performance	Reference
Feammox–anammox	UASB	Cultivated Feammox sample and activated sludge	Feammox, PN/A	30 °C	6.0–8.0	NH ₄ ⁺ -N: 261.8 mg/L	NH ₄ ⁺ -N: 66.05% phosphate: over 40%	Xu et al., 2024
	SBR	Anaerobic sludge and floc anammox	Feammox, anammox	22 °C	7.0	NH ₄ ⁺ -N: 250 mg/L	NH ₄ ⁺ -N: 50.8%	González et al., 2024
Feammox–NDFO		Anammox granular sludge	Feammox, anammox	32 ± 2 °C	7.0–7.5	NH ₄ ⁺ -N: 100.0 mg/L	TNR: 71.8%	Li et al., 2018a
	Up-flow anaerobic biological filter based on limonite	Anaerobic municipal sludge	Feammox, NDFO	20 ± 2 °C	7.0 ± 0.1	NH ₄ ⁺ -N: 30.0 mg/L TN: 33.5 mg/L	NH ₄ ⁺ -N: 98.9% COD: 96.9%	Ma et al., 2022
	SBR	Sludge from secondary sedimentation tank	Feammox, NDFO	25–30 °C	7.6 ± 0.2	NH ₄ ⁺ -N: 26.6–63.9 mg/L	NH ₄ ⁺ -N: 100% TNR: 92.45%	Hao et al., 2024
	Biofilm	Anaerobic digestion sludge	Feammox, NDFO	28 ± 1 °C	Change with reaction	NH ₄ ⁺ -N: 20 mg/L	NH ₄ ⁺ oxidation efficiency and rate of 42.3% and 0.032 kg-N/(m ³ ·d), respectively	Yao et al., 2020
	Moving bed biofilm reactor	LEVAPOR bio-carriers	Feammox, NDFO	NR	7.0	NH ₄ ⁺ -N: 30.1 mg/L NO ₃ ⁻ -N: 14.8 mg/L	NH ₄ ⁺ -N: 79% ± 4% NO ₃ ⁻ -N: 98% ± 4%	Sheng et al., 2024
	Bioelectrochemical systems	Anaerobic sludge	Feammox, NDFO	NR	6.8–7.2	NH ₄ ⁺ -N: 52.4–104.7 mg/L	NH ₄ ⁺ -N: 0.08062 kg-N/(m ³ ·d)	Zhu et al.,
Feammox–NDFO–anammox	UASB	Granular anammox sludge	Feammox, NDFO, anammox	35 ± 1 °C	7.0 ± 0.3	NH ₄ ⁺ -N: 151.01 ± 2.11 mg/L NO ₂ ⁻ -N: 200.94 ± 4.73 mg/L	TNR: 10.97 ± 0.36 kg-N/(m ³ ·d)	Jia et al., 2025
	Sponge iron Biofilter	Nitrifying activated sludge, partial nitrification–anammox granule sludge	Feammox, NDFO, nitrification, anammox	25 °C	7.5–8.0	NH ₄ ⁺ -N: 60.2 mg/L	TNR: 77.2%	Li et al., 2022, 2023
	SBR	Nitrification sludge, anammox granular sludge	Feammox, NDFO, anammox	NR	NR	NH ₄ ⁺ -N: 50 ± 10 mg/L	TNR: 83%, phosphate: 78.7%	Kao et al., 2022
	SBR	Marine anammox sludge	Feammox, NDFO, anammox	15 °C	7.5 ± 0.1	NH ₄ ⁺ -N: 80 mg/L NO ₂ ⁻ -N: 105.6 mg/L	NH ₄ ⁺ -N: 0.85 kg-N/(m ³ ·d) NO ₂ ⁻ -N: 0.90 kg-N/(m ³ ·d)	Feng et al., 2020
	SBR	Anammox sludge	Feammox, NDFO, anammox	30 ± 1 °C	7.0 ± 0.2	NH ₄ ⁺ -N: 40 mg/L	TNR: 91.6%	Wang et al., 2025b
Feammox–NDFO–chemodenitrification	UASB	Granular anammox sludge	Feammox, NDFO, chemodenitrification	23 ± 3 °C	7.0–7.3	NH ₄ ⁺ -N: 100 mg/L	TNR: ~90%	Cheng et al., 2025
	UASB	Anaerobic municipal sludge	Feammox, NDFO, chemodenitrification	30 °C	5.5	NH ₄ ⁺ -N: 70 mg/L NO ₂ ⁻ -N: 70 mg/L NO ₃ ⁻ -N: 42 mg/L	NH ₄ ⁺ -N: 42.7%–50.3%	Yang et al., 2023

Notes: NR: not reported.

fraction of ammonium converted to nitrite is available for subsequent anammox. Therefore, practical Feammox–anammox coupling may require an additional nitrite source. This kinetic limitation is supported by experimental observations. González et al. (2024) found that in a coupled Feammox–anammox reactor, anammox accounted for the dominant contribution to total nitrogen removal, whereas Feammox served only as a supplementary ammonium-oxidation pathway. Similar findings have been reported for other hybrid systems, where the coexistence of both pathways did not translate into balanced functional coupling (Huang and Jaffé, 2018). Beyond kinetic mismatch, pathway attribution remains challenging because Feammox-derived nitrogen intermediates may overlap with those generated via other biological or abiotic processes, particularly in complex mixed-culture systems. This uncertainty complicates direct evaluation of the actual contribution of Feammox to overall reactor performance.

3.2 Feammox–NDFO

Feammox and NDFO are conceptually linked via reciprocal iron redox transformations. This makes their coupling one of the most theoretically attractive configurations in iron-mediated nitrogen-conversion systems. In Feammox, ammonium is oxidized using Fe(III) as the electron acceptor, generating Fe(II) along with nitrite and/or nitrate. Conversely, NDFO oxidizes Fe(II) coupled with nitrate or nitrite reduction, regenerating Fe(III). This reciprocal relationship indicates the possibility of an internally sustained Fe(II)/Fe(III) redox cycle that can support integrated nitrogen removal with reduced external iron demand. Nevertheless, the limited endogenous production of nitrate and nitrite by Feammox may be insufficient to sustain NDFO. Consequently, an internally closed Feammox–NDFO cycle would probably require an additional source of oxidized nitrogen. Experimental studies have demonstrated the feasibility of such coupling under controlled conditions. Hao et al. (2024) established a coupled Feammox–NDFO sequencing batch reactor inoculated with anaerobic municipal sludge, achieving complete ammonium removal within 48 h. Likewise, other studies have reported simultaneous Fe(III) reduction and nitrate-dependent Fe(II) oxidation in engineered systems, supporting the coexistence of both pathways (Yao et al., 2020; Ma et al., 2022; Sheng et al., 2024).

Despite this conceptual appeal, the practical stability of Feammox–NDFO coupling remains highly uncertain. Effective integration requires close synchro-

nization between Fe(III) reduction in Feammox and Fe(II) oxidation in NDFO. However, these processes are governed by distinct microbial communities, diverse kinetic constraints, and variable substrate availability, which makes redox balance difficult to maintain. Disruption of this balance can cause Fe(II) or Fe(III) buildup, weakening iron recycling efficiency and reducing overall nitrogen-removal performance. Further, mineral precipitation introduces an additional operational challenge. During NDFO, regenerated Fe(III) typically forms insoluble mineral phases that can precipitate on microbial cell surfaces, within the periplasm, or even intracellularly, causing physical encrustation and metabolic inhibition (Picardal, 2012; Klueglein et al., 2014). Such mineral accumulation may obstruct substrate transport, disrupt enzymatic activity, and progressively destabilize reactor performance. Moreover, excessive precipitation reduces iron bioavailability, further weakening the intended redox-regeneration loop. Additionally, mechanistic ambiguity in NDFO creates further uncertainty. In many reported systems, nitrate reduction coupled with Fe(II) oxidation may arise from true enzymatic NDFO as well as indirect microbial denitrification or hybrid biotic–abiotic processes (Wang et al., 2020; Kappler et al., 2021). Thus, the extent to which Feammox–NDFO represents a controllable biological coupling rather than a loosely interacting mixed redox system remains unresolved.

3.3 Integration of Feammox–NDFO–anammox

The coexistence of Feammox, NDFO, and anammox is among the most complex iron-mediated nitrogen-conversion arrangements proposed for wastewater treatment. In principle, this hybrid system integrates ammonium oxidation, nitrate reduction, and Fe(II)/Fe(III) redox cycling, creating a theoretically attractive framework for enhanced autotrophic nitrogen removal via complementary substrate exchange and internal iron regeneration. Several studies have demonstrated the feasibility of such multipathway systems. Huang and Jaffé (2018) reported the simultaneous occurrence of Feammox, NDFO, and anammox in an anaerobic culture system, achieving ammonium- and nitrate-removal efficiencies of 67.6% and 58.8%, respectively. Gao et al. (2021) further integrated PN/A with Feammox and NDFO, reporting improved nitrogen-removal performance in engineered bioreactors. Other studies have similarly suggested functional coexistence of these pathways under controlled conditions (Feng et al., 2020; Jia et al., 2025; Wang et al., 2025).

Despite such theoretical promise, practical system behavior is likely governed less by ideal synergy than by hierarchical competition for key intermediates, particularly nitrite. Anammox is a well-established, kinetically efficient autotrophic nitrogen-removal pathway that rapidly consumes nitrite once enriched in engineered reactors (Lackner et al., 2014). In comparison, Feammox and NDFO are slower and strongly constrained by iron bioavailability, mineral accessibility, and complex electron-transfer requirements (Kappler et al., 2021; Liu et al., 2024). Consequently, nitrite generated within hybrid systems is expected to be preferentially consumed by anammox, limiting its availability for alternative iron-mediated pathways. Furthermore, pathway attribution remains particularly challenging in such complex systems, as overlapping biological and abiotic nitrogen transformations can occur simultaneously. Nitrite and nitrate may be generated or consumed via multiple routes, including Feammox, NDFO, anammox, denitrification, and chemodenitrification, making direct quantification of pathway contributions highly uncertain. Reported improvements in nitrogen-removal performance may therefore reflect collective system effects rather than well-controlled synergistic coupling. Importantly, simultaneous substrate removal, changes in Fe(II)/Fe(III), or enrichment of putative functional microorganisms do not independently confirm the concurrent activity of Feammox, NDFO, and anammox. More robust attribution requires integrating ^{15}N isotope tracing, abiotic controls, iron speciation, and nitrogen mass balances, as microbial community analyses alone reveal metabolic potential instead of direct pathway activity. Therefore, current evidence supports pathway coexistence more strongly than clearly quantified metabolic coupling.

3.4 Potential role of chemodenitrification

In iron-mediated wastewater-treatment systems, chemodenitrification plays a critical yet often underappreciated role by introducing abiotic nitrogen-conversion pathways that compete with biologically mediated processes. In the presence of reactive Fe(II) and oxidized nitrogen species, particularly nitrite, abiotic reduction occurs rapidly, producing gaseous nitrogen products such as NO , N_2O , and N_2 without direct microbial involvement (Jones et al., 2015; Kappler et al., 2021). The importance of chemodenitrification becomes particularly evident in complex hybrid systems involving Feammox and NDFO. Both pathways can create conditions that favor abiotic nitrogen conversion via Fe(II) accumulation and nitrite

production. Under such conditions, nitrite may be rapidly consumed through chemical reduction rather than biologically controlled pathways, thereby altering nitrogen-conversion routes and reducing process controllability. Experimental evidence suggests that chemodenitrification is difficult to avoid in iron-rich nitrogen conversion systems. Yang et al. (2023) observed substantial abiotic nitrogen transformation under Feammox-favorable conditions, despite reactor operation intended to promote biological ammonium oxidation. Similarly, Cheng et al. (2025) reported unavoidable chemodenitrification in coupled Feammox–NDFO systems under Fe(II)- and nitrite-rich conditions. These findings suggest that abiotic nitrogen conversion should not be considered a minor side reaction but rather an inherent component of many iron-mediated treatment systems. Beyond process controllability, chemodenitrification introduces important environmental concerns due to the formation of reactive nitrogen intermediates and greenhouse gases, particularly N_2O (Jones et al., 2015; Chen et al., 2021). As these emissions may partially offset the intended sustainability benefits of iron-mediated nitrogen removal, their contribution to total emissions requires explicit consideration in process evaluation and reactor design.

4 Operational regulators governing iron-mediated nitrogen conversion

The performance of iron-mediated nitrogen-conversion systems is governed by the intrinsic characteristics of individual biological and abiotic pathways as well as operational conditions that regulate pathway selection, reaction kinetics, and system stability. Unlike conventional biological nitrogen-removal processes, in which microbial competition is primarily controlled by oxygen, substrate availability, and sludge retention, iron-mediated systems introduce additional complexity via Fe(II)/Fe(III) redox dynamics, mineral precipitation, and abiotic nitrogen transformations. Operational parameters such as iron availability, dissolved oxygen (DO), pH, and temperature influence not only microbial activity but also iron speciation, electron-transfer efficiency, and the relative contributions of competing biological and abiotic pathways. Consequently, these factors do not simply affect treatment performance quantitatively; they can qualitatively reshape nitrogen-conversion routes within engineered systems. Effective operational control is thus essential for maintaining

process stability, improving nitrogen-removal efficiency, and minimizing unintended outcomes such as iron accumulation, microbial inhibition, or greenhouse gas emissions (Fig. 3).

4.1 Iron availability

Iron availability is the primary operational regulator of iron-mediated nitrogen conversion because Fe(II)/Fe(III) serves not only as a redox-active participant in nitrogen transformation but also as a determinant of microbial activity, electron-transfer efficiency, and abiotic reaction potential. Unlike conventional biological nitrogen-removal systems, in which iron typically functions as a micronutrient or chemical additive, iron-mediated nitrogen conversion depends fundamentally on maintaining an appropriate balance among iron availability, bioaccessibility, and redox stability. In Feammox systems, Fe(III) is often a limiting factor, with concentrations below ~25 mmol/L restricting activity, while increasing Fe(III) typically enhances ammonium oxidation by alleviating electron acceptor limitation (Hu et al., 2022; Xia et al., 2022). However, excessive Fe(III) may reduce bioavailability via precipitation rather than direct toxicity. A similar concentration-dependent behavior is observed in NDFO

and anammox processes. Moderate Fe(II) concentrations promote NDFO kinetics by enhancing electron-transfer capacity, whereas excessive Fe(II) can induce cellular stress and inhibit nitrogen removal (Liu et al., 2017). In anammox systems, iron exhibits a narrower tolerance window: low Fe(III) concentrations (~5–10 mg/L) stimulate activity, while higher levels (≥ 50 mg/L) become inhibitory, and prolonged exposure to elevated Fe(II) (~70–80 mg/L) can impair cellular function (Zhang et al., 2021; Jiang et al., 2023). These observations suggest that iron plays a dual role, functioning as an essential nutrient and a potential inhibitor, with process-specific sensitivity.

In addition to concentration, iron speciation strongly influences system performance by affecting bioavailability and redox reactivity. Diverse Fe(III) minerals (e.g., Fe(OH)₃, Fe₂O₃, and Fe₃O₄) have exhibited inconsistent promoting effects across studies, signifying strong dependence on reactor configurations, operational conditions, and microbial community structure (Yang et al., 2018; Zhu et al., 2022). In NDFO systems, both dissolved and solid-phase Fe(II) sources are applicable, while natural minerals and industrial by-products provide cost-effective alternatives with variable physicochemical properties, such as solubility, crystallinity, and surface area, which ultimately

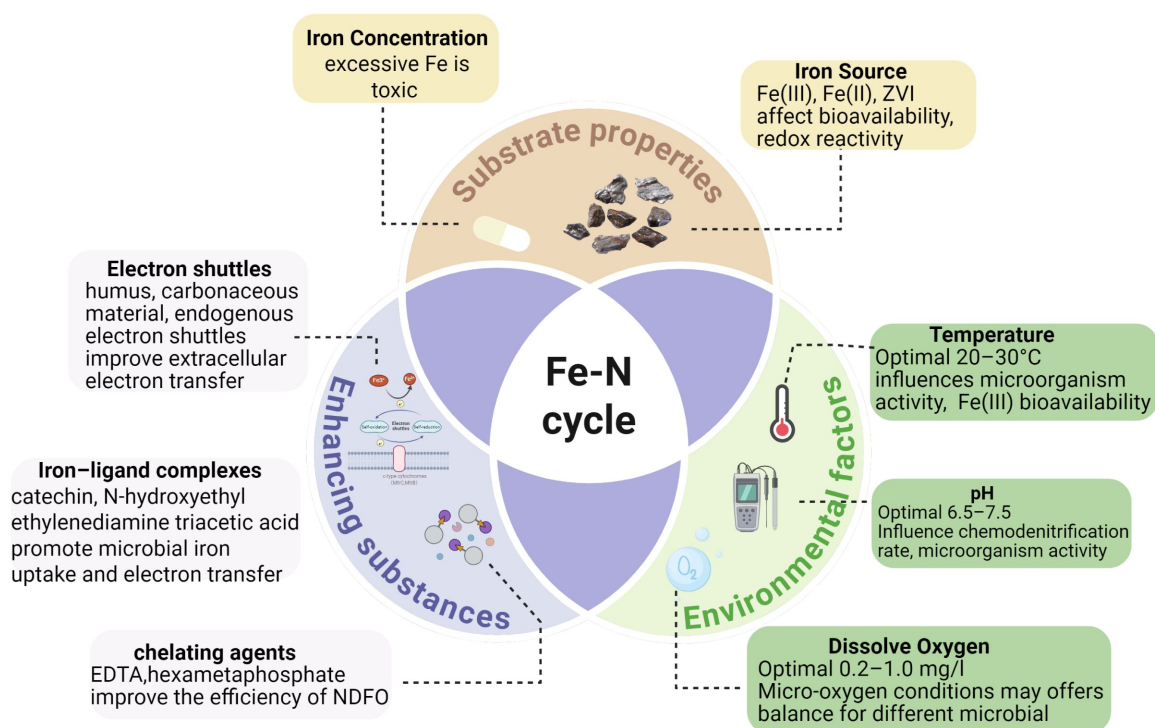


Fig. 3 Principal operational regulators governing iron-mediated nitrogen conversion in wastewater treatment, highlighting their influence on pathway selection, microbial activity, iron redox cycling, and abiotic reactions.

determine microbial accessibility and reaction efficiency.

Overall, in hybrid iron-mediated systems, the optimal iron regime is constrained by the divergent metabolic requirements of Feammox, NDFO, and anammox. A moderate concentration window appears most suitable, typically with Fe(II) maintained at approximately 10–50 mg/L and Fe(III) above the limiting threshold but without excessive accumulation. Within this range, Feammox and NDFO can be sustained with minimal inhibition of anammox. Conversely, excessive iron, particularly Fe(II) above ~70 mg/L, may induce toxicity, promote mineral precipitation, and disrupt system stability. Therefore, effective operation relies not on maximizing iron availability but on maintaining a balanced and dynamic Fe(II)/Fe(III) pool, underscoring the role of iron as a key regulator of coupled nitrogen-transformation processes. In practice, dissolved Fe(II) can be monitored using automated colorimetric methods, while DO and oxidation–reduction potential can serve as complementary indicators of changes in reactor redox conditions. These measurements can be integrated with feedback-controlled iron dosing to prevent excessive Fe(II) buildup and maintain a stable Fe(II)/Fe(III) balance.

4.2 Oxygen supply

DO is a critical operational parameter governing iron-mediated nitrogen conversion, as it simultaneously affects microbial metabolism, iron redox chemistry, and competition between biological and abiotic pathways. Compared to conventional biological nitrogen removal, oxygen control in iron-mediated systems is complex, as excessive oxygen can suppress anaerobic microbial processes while driving the rapid abiotic oxidation of Fe(II), which disrupts the iron redox cycle. Feammox-related functional microorganisms, typified by *Acidimicrobiaceae bacterium A6*, display obligately anaerobic metabolic preferences (Huang et al., 2016). Consistent with this observation, Yao et al. (2020) reported progressive declines in ammonium oxidation and nitrogen-removal rates with increasing DO in biofilm-based iron-mediated systems. Elevated oxygen concentrations may inhibit Feammox biologically by suppressing anaerobic microbial activity and chemically by oxidizing Fe(II) and altering the intended Fe(II)/Fe(III) redox balance. Likewise, NDFO is generally favored under low-oxygen or anoxic conditions, where Fe(II) remains sufficiently available to support microbial nitrate reduction (Yang et al., 2024). Under elevated DO, abiotic Fe(II) oxidation can rapidly consume bioavailable ferrous iron, resulting in

uncontrolled mineral precipitation and reduced redox efficiency. This creates a strong operational incentive to limit oxygen exposure when the objective is to preserve iron-mediated nitrogen conversion.

However, strict anaerobic conditions may not always represent the most practical or stable operating regime in engineered wastewater systems, especially when mixed microbial communities are involved. Under appropriately controlled conditions, micro-aeration may support partial nitrification and generate oxidized nitrogen intermediates, particularly nitrite for anammox and, where further oxidation occurs, nitrate for NDFO, thus partially compensating for their limited endogenous production by Feammox (Hu et al., 2022; Wang et al., 2025). Wang et al. (2025) reported enhanced ammonium oxidation under low DO conditions (0.2–1.0 mg/L) compared to strictly anaerobic operation in a mixed-culture Feammox system. Notably, deliberate micro-aeration can be employed to regulate the supply of nitrite and nitrate, whereas uncontrolled oxygen intrusion can disrupt anaerobic metabolism, accelerate abiotic Fe(II) oxidation, and promote mineral precipitation. Therefore, micro-aeration must be carefully controlled to balance the beneficial production of oxidized nitrogen substrates against the risks of Fe(II) depletion and inhibition of anaerobic nitrogen-converting microorganisms.

4.3 pH control

pH is a critical operational regulator in iron-mediated nitrogen conversion because it concurrently governs microbial activity, iron speciation, mineral stability, and abiotic reaction kinetics. In addition, pH in iron-mediated systems directly determines Fe(II)/Fe(III) solubility, redox reactivity, and the likelihood of unintended chemical nitrogen transformations. For Feammox, pH strongly influences microbial activity and ferric iron bioavailability. The only confirmed direct Feammox microorganism, *Acidimicrobiaceae bacterium A6*, remains active over a broad pH range (3.4–7.3), with optimal performance reported under mildly acidic conditions (pH 4.5–6.5) (Huang et al., 2016). Acidic conditions may also enhance Fe(III) dissolution and improve electron acceptor accessibility via proton-mediated mineral solubilization (Weber et al., 2006; Li et al., 2019). Nevertheless, Feammox-associated microbial communities are likely more diverse than currently confirmed isolates, with some reported systems showing activity closer to neutral pH (Li et al., 2019). Consequently, the optimal pH for Feammox may be strongly influenced by microbial

community composition and iron mineral characteristics, rather than conforming to a single universal range.

For NDFO, pH similarly influences microbial activity and iron chemistry, although the optimal operating window appears closer to neutral conditions. Zhang et al. (2015) reported improved nitrogen removal with increasing pH between 3.0 and 7.2, with performance exceeding 95% above pH 6. This trend possibly reflects enhanced microbial denitrification activity and more favorable thermodynamic conditions for Fe(II)-driven nitrate reduction (Wang et al., 2023b). However, excessively alkaline conditions may reduce Fe(II) solubility and bioavailability via rapid precipitation, limiting substrate accessibility for microbial oxidation (Kiskira et al., 2017; Yan et al., 2019). This finding underscores the need to balance thermodynamic favorability against iron accessibility.

Beyond biological pathways, pH exerts strong control over abiotic nitrogen transformations. Chemodenitrification is highly sensitive to pH; however, reported trends vary depending on whether reactions occur in homogeneous aqueous systems or on mineral surfaces (Tai and Dempsey, 2009; Chen et al., 2020; Dhakal et al., 2021). Furthermore, highly reactive iron materials such as zero-valent iron may induce substantial pH fluctuations via rapid redox reactions with water and nitrogen species, potentially disrupting microbial activity and altering pathway distribution (Huang et al., 2020). Accordingly, effective pH management must balance microbial performance, iron solubility, and suppression of undesirable abiotic reactions.

4.4 Temperature effects

Temperature is a vital operational factor influencing iron-mediated nitrogen conversion because it affects microbial activity, reaction kinetics, iron redox chemistry, and overall process stability. For Feammox, reported activity spans a broad temperature range, typically between 15 and 37 °C (Feng et al., 2020; Zhu et al., 2022), indicating a degree of environmental adaptability. However, performance can vary considerably with temperature owing to shifts in microbial activity, iron reduction kinetics, and community composition. Ding et al. (2020, 2022) reported pronounced seasonal variation in Feammox activity and associated microbial abundance, while Yao et al. (2019) observed that Feammox rates were markedly higher in summer than in winter in eutrophic lake environments. These findings suggest that Feammox performance may be highly sensitive to temperature fluctuations,

particularly where microbial enrichment remains unstable.

NDFO also exhibits clear temperature dependence. Wang et al. (2022) found that NDFO activity increased with temperature under moderate conditions, with inhibition observed above 40 °C. Similarly, Liu et al. (2016) reported optimal Fe(II)-driven nitrate bioremediation performance near 25 °C within the tested range of 15–25 °C. Temperature may affect NDFO through microbial metabolism as well as changes in Fe(II) oxidation kinetics, solubility, and mineral transformation behavior.

Abiotic nitrogen transformations are also temperature-sensitive. Chen et al. (2021) revealed that chemodenitrification rates declined substantially as the temperature decreased from 35 to 5 °C, highlighting the strong kinetic dependence of abiotic redox reactions. Thus, temperature can alter not only overall nitrogen-removal rates but also the relative contribution of biological versus abiotic pathways.

4.5 Other emerging control strategies

Beyond conventional operational parameters, increasing attention has been paid to intervention strategies designed to overcome the intrinsic limitations of iron-mediated nitrogen conversion, particularly restricted iron bioavailability, inefficient EET, and mineral encrustation. These emerging approaches aim to improve nitrogen removal performance as well as enhance process stability and controllability in engineered wastewater systems.

An important strategy is using chelating agents to increase iron solubility and bioavailability, specifically in NDFO systems where rapid iron precipitation limits effective accessibility to electron donors. Chelators such as EDTA and hexametaphosphate maintain dissolved ferrous iron, reduce mineral encrustation, and improve nitrogen-removal performance in continuous reactor systems (Zhou et al., 2016; Wang et al., 2020). By stabilizing iron in a more bioavailable form, these additives may enhance microbial Fe(II) utilization and prolong reactor performance. Nevertheless, the use of persistent chelants such as EDTA may introduce additional environmental concerns owing to their limited biodegradability, potential to mobilize metals, and persistence in treated effluent. These effects are difficult to reconcile with the sustainability objective, unless the chelant can be effectively retained or recovered. Future studies should therefore prioritize biodegradable or recoverable ligands, naturally derived chelators, siderophore-like iron carriers, and immobilized mediators that improve iron bioavailability

without increasing the persistent organic load of the effluent.

Another major approach focuses on improving EET, which is widely considered a limiting factor in Feammox and related iron-mediated pathways. Electron shuttles such as humic substances, 9,10-anthraquinone-2,6-disulfonate (AQDS), activated carbon, biochar, and sulfur-based materials have been examined as redox mediators to accelerate electron transfer between microorganisms and iron minerals (Sun et al., 2023; Wang et al., 2023). Zhou et al. (2016) and Cao et al. (2022) reported enhanced nitrogen removal following the addition of AQDS or conductive carbon materials, with solid conductive materials generally exhibiting better long-term retention than soluble electron shuttles susceptible to washout. In addition, research has explored iron-ligand complex engineering to improve microbial iron uptake and intracellular iron utilization. Liu et al. (2025) demonstrated that siderophore-like iron carrier systems, including catechin and N-hydroxyethyl ethylenediamine triacetic acid, improved iron-uptake efficiency in anammox consortia and stimulated the synthesis of iron-dependent enzymatic cofactors. These findings suggest that targeted manipulation of iron bioavailability may offer a more sophisticated approach to regulating iron-mediated nitrogen conversion.

5 Translational barriers and future opportunities

Iron-mediated nitrogen conversion has emerged as a

conceptually attractive strategy for sustainable wastewater treatment, providing opportunities to reduce aeration demand, external carbon dependence, and overall greenhouse gas emissions. Nevertheless, despite growing mechanistic interest and promising proof-of-concept demonstrations, practical implementation remains limited. Current systems are constrained not by a single bottleneck but by interconnected scientific and engineering barriers, such as unresolved microbial mechanisms, limited process efficiency, poor pathway controllability, and uncertain scalability (Fig. 4). Addressing these challenges requires a transition from pathway discovery to engineering-oriented system design, operational control, and translational validation.

5.1 Resolving microbial mechanisms and pathway attribution

Compared to well-characterized pathways such as anammox, the microbial basis of Feammox and NDFO remains poorly resolved. To date, 27 anammox species have been identified with well-defined growth kinetics and doubling times (11–20 d) (Hu et al., 2010; Zou et al., 2020). Feammox is currently linked to only one confirmed microorganism: *Acidimicrobiaceae bacterium A6* (Huang and Jaffé, 2018). Although *Shewanella*, *Pseudomonas*, *Exiguobacterium*, *Fervidicella*, and *Nitrososphaeraceae* have been reported as iron-reducing bacteria capable of reducing Fe(III) with ammonium oxidation (Li et al., 2019; Yao et al., 2019; Ahmed et al., 2021), their roles remain ambiguous. Whether these strains directly catalyze Feammox or NDFO, or function indirectly by regulating iron redox

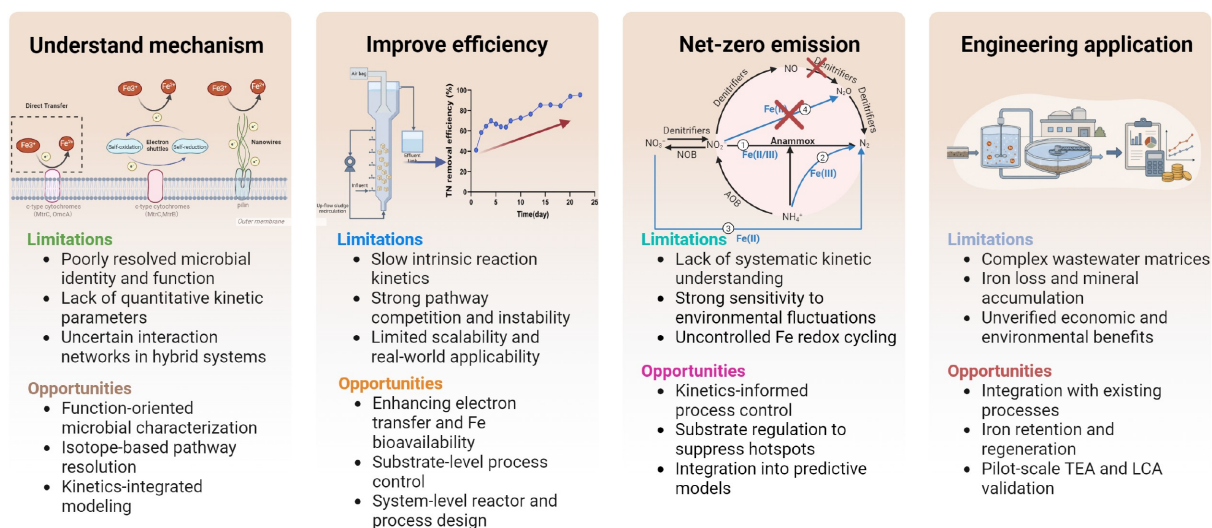


Fig. 4 Key translational barriers to practical implementation of iron-mediated nitrogen conversion and strategic opportunities for future development.

cycling, mediating electron shuttling, or transforming nitrogen intermediates, has not been conclusively established.

This uncertainty is further reflected at the kinetic level. Anammox bacteria are well characterized: their maximum specific growth rates range from 0.002 to 0.004 per hour, and their half-saturation constants for ammonium are as low as 0.05–0.3 mmol/L, indicating high substrate affinity that enables efficient ammonium uptake even at low concentrations (Ni et al., 2009; Liu and Ni, 2015). Conversely, the kinetic parameters of Feammox-associated microorganisms remain largely unresolved, with reliable maximum specific growth rate and substrate affinity constants yet to be systematically determined (Sima et al., 2023). Consequently, their competitiveness under substrate-limited conditions cannot be accurately assessed. This disparity indicates that ammonium concentration may serve as a key regulatory factor: low ammonium levels are likely to favor anammox dominance owing to its higher substrate affinity, whereas moderate levels may help alleviate competitive pressure and promote Feammox activity.

In hybrid systems, this microbial uncertainty translates into interaction-driven, dynamically regulated networks rather than discrete and predictable pathways. Feammox can provide a limited supplementary supply of nitrite or nitrate but is unlikely to sustain high-rate anammox or NDFO as the sole source of oxidized nitrogen. Thus, the overall system performance is determined by the balance between cooperation and competition among biological and chemical processes. However, the lack of quantitative kinetic parameters (e.g., growth rates and substrate affinities) for Feammox- and NDFO-associated microorganisms hinders prediction of their relative contributions and long-term stability.

Future research should transition from descriptive community analysis toward a functionally resolved, quantitatively constrained framework. In particular, efforts should focus on 1) isolating and enriching Feammox- and NDFO-associated microorganisms to verify their direct metabolic roles, 2) applying isotope tracing (e.g., ^{15}N and ^{57}Fe) and meta-omics approaches to distinguish direct enzymatic pathways from indirect processes such as iron redox cycling and electron shuttling, and 3) systematically quantifying key kinetic parameters, including maximum specific growth rates, substrate affinities, and electron-transfer rates under controlled conditions. Among these, kinetic parameters are especially critical, as they directly determine microbial competitiveness, pathway selection, and system response to environmental fluctuations. In the

absence of reliable kinetic constraints, predicting the relative contributions of Feammox, NDFO, and anammox, or developing operational strategies for selective enrichment remains challenging. In parallel, integrating these data into process-based or reactive transport models would enable prediction of pathway dominance and system stability under varying environmental conditions. Such combined experimental–modeling efforts are essential for establishing a predictive mechanistic framework that links microbial function, iron redox dynamics, and nitrogen transformation in complex systems.

5.2 Overcoming kinetic and operational limitations

Beyond mechanistic uncertainty, the practical implementation of iron-mediated nitrogen conversion is fundamentally constrained by limited reaction kinetics, unstable process performance, and narrow operational tolerance. Although the proof-of-concept feasibility of multiple iron-mediated pathways has been demonstrated, their performance remains considerably less mature and less predictable than established biological nitrogen-removal technologies.

As discussed in Section 2.2, Feammox exhibits substantially lower volumetric conversion rates than mature nitrogen-removal processes such as anammox. Reported Feammox rates of approximately 0.1–0.3 mmol $\text{NH}_4^+ / (\text{L} \cdot \text{d})$ correspond to only 1.4–4.2 mg N/(L·d) under laboratory conditions (Huang and Jaffé, 2018; Li et al., 2025). This kinetic limitation necessitates longer hydraulic retention times (HRTs), larger reactor volumes, and effective retention of slow-growing biomass and reactive iron solids. For instance, assuming Feammox is solely responsible for ammonium removal, treatment of wastewater containing 40 mg $\text{NH}_4^+ \text{-N/L}$ would theoretically require an HRT of approximately 9.5–28.6 d. Although biomass enrichment, biofilm formation, and improved iron accessibility may increase the effective volumetric rate, this simple calculation demonstrates why Feammox is currently more realistic as a supplementary pathway than as a standalone high-rate treatment process. Increasing Fe(III) bioavailability, particularly using poorly crystalline or amorphous iron minerals, stimulates Feammox activity (Pang et al., 2022; Park et al., 2026). Additionally, adding conductive materials or electron shuttles such as AQDS and biochar can alleviate electron-transfer limitations by facilitating EET and enhancing ammonium oxidation (Cao et al., 2022; Wang et al., 2023). Beyond these strategies, substrate regulation provides an additional lever for pathway control. Owing to the high substrate affinity of

anammox bacteria, low ammonium concentrations tend to favor anammox dominance, whereas moderate concentrations may reduce competitive pressure and promote Feammox activity. Similarly, controlling nitrite accumulation is essential for balancing its role as a key intermediate while preventing its rapid loss via competing processes such as chemodenitrification.

Most enhancement strategies have been validated only at laboratory scale using synthetic wastewater, and their applicability to real systems remains unverified. Real wastewater contains fluctuating substrates, complex microbial communities, and inhibitory compounds, all of which alter iron speciation, microbial activity, and pathway distribution. This discrepancy suggests that strategies effective under controlled conditions may not ensure stable performance in engineering systems. Future optimization must therefore consider both intrinsic kinetics and environmental robustness under dynamic conditions. Additionally, the lack of suitable reactor configurations and microbial management strategies further limits efficiency at larger scales. Although various reactor types, such as SBR and UASB, have been investigated (Yang et al., 2023; Hao et al., 2024), their comparative performance and scalability for iron-mediated nitrogen conversion remain poorly understood. Moreover, unlike anammox systems, which have well-established inoculation and operation protocols, Feammox lacks standardized inoculum sources and quantified growth kinetics, making enrichment and long-term stability difficult. Developing targeted enrichment strategies, optimizing reactor hydrodynamics, and integrating iron management with microbial selection are thus essential to translate laboratory findings into practical applications.

5.3 Managing abiotic reactions and greenhouse gas risks

N₂O emissions represent a serious environmental concern in iron-mediated nitrogen-conversion systems, as N₂O is a potent greenhouse gas with a global warming potential far exceeding that of CO₂. In hybrid systems, a substantial fraction of N₂O is attributed not to biological pathways but to chemodenitrification, where Fe(II) abiotically reduces nitrite to gaseous nitrogen products. Therefore, effective mitigation of N₂O emissions requires controlling chemodenitrification rather than solely optimizing biological nitrogen-removal pathways.

A key limitation in this regard is the absence of a systematic kinetic framework describing chemodenitrification under Fe–N-coupled conditions. Reported

reaction rate constants (k values) vary widely depending on environmental factors such as pH, Fe(II)/Fe(III) speciation, nitrite concentration, and the presence of ligands or mineral phases (Jones et al., 2015; Chen et al., 2020; Robinson et al., 2021). Among these variables, pH exerts a particularly strong influence on reaction kinetics by simultaneously affecting iron speciation, nitrite chemistry, and electron-transfer processes. pH variations alter the solubility and redox reactivity of Fe(II), as well as the speciation of nitrite, thereby modifying the availability of reactive intermediates involved in abiotic nitrogen transformation (Su et al., 2019; Chen et al., 2020; Hu et al., 2022). Additionally, pH-dependent changes in surface charge and mineral properties further affect interfacial electron transfer on Fe-bearing solids. These combined effects contribute to the wide variability in reported kinetic parameters and complicate chemodenitrification behavior prediction in hybrid systems. Moreover, Fe(III) facilitates electron transfer between Fe(II) and nitrite, effectively accelerating abiotic nitrogen transformations (Tai and Dempsey, 2009). This finding suggests that iron redox cycling is not merely a background process but actively regulates N₂O formation rates.

Environmental fluctuations further complicate emission control. For example, DO intrusion promotes abiotic oxidation of Fe(II), altering Fe speciation and indirectly influencing nitrite-reduction pathways (Park and Dempsey, 2005). Moreover, excessive accumulation of Fe(II) and nitrite creates favorable conditions for rapid chemodenitrification, diverting nitrogen from controlled biological pathways such as anammox and increasing N₂O production. These findings indicate that N₂O emissions are highly sensitive to transient imbalances in redox conditions and substrate availability.

To effectively reduce N₂O emissions, future research should focus on establishing a kinetically informed control strategy for chemodenitrification. First, systematically quantifying reaction kinetics under environmentally relevant conditions, with a focus on the coupled effects of Fe(II), Fe(III), nitrite, and pH, is essential. Second, regulating key intermediates, particularly nitrite and Fe(II), can help avoid threshold conditions that trigger rapid abiotic reactions. Maintaining moderate substrate levels and preventing nitrite accumulation are thus critical operational strategies. Third, controlling iron speciation and redox cycling by stabilizing Fe(II)/Fe(III) ratios or limiting unintended oxygen intrusion may reduce the acceleration of electron-transfer pathways for N₂O formation. Beyond experimental studies, integrating

chemodenitrification kinetics into process-based or reactive transport models would provide a predictive tool for assessing N₂O emission risks under dynamic conditions. Such models could help identify critical thresholds and guide operational strategies in engineered systems. Overall, N₂O emission mitigation requires a transition from empirical observations to a mechanistic understanding of abiotic nitrogen transformation, with particular emphasis on the kinetic control of chemodenitrification within Fe-mediated systems.

5.4 Engineering integration and sustainability assessment

The practical value of iron-mediated nitrogen conversion ultimately depends on its compatibility with existing treatment infrastructure and robustness under real wastewater conditions. Currently, these pathways are more realistically positioned as complementary modules than as replacements for established activated-sludge, PN/A, or conventional anammox processes. Potential applications include anaerobic or anoxic zones, iron-rich sidestreams, and polishing units for residual nitrogen removal. Integration with PN/A is particularly promising because controlled nitrification can compensate for the limited endogenous supply of nitrite and nitrate from Feammox (Gao et al., 2021; Wang et al., 2025). However, wastewater chemistry, specifically pH, complexing ligands, suspended solids, and competing ions, alters iron speciation, reduces bioavailability, and promotes precipitation, thereby disrupting Fe(II)/Fe(III) cycling and increasing sludge production (Klueglein et al., 2014; Kiskira et al., 2017; Wang et al., 2020).

Reactor configuration and iron management impose additional constraints. SBRs, UASB reactors, and biofilm systems have all been investigated for iron-mediated nitrogen conversion (Ma et al., 2022; Yang et al., 2023; Hao et al., 2024). From an engineering perspective, SBRs offer greater flexibility for iron dosing and redox control. Conversely, high-retention systems improve biomass and mineral retention but are more susceptible to clogging, carrier encrustation, and hydrodynamic disturbance. As direct comparisons under equivalent conditions remain limited, no universally preferred configuration has been identified. Continuous iron addition also increases chemical costs, sludge production, and solids-handling requirements. Practical implementation should accordingly prioritize internal Fe(II)/Fe(III) recycling and the recovery or reuse of reactive iron solids. Nevertheless, iron precipitation, cell encrustation, mineral ageing, and transformation into less reactive phases may limit

complete internal recycling (Picardal, 2012; Klueglein et al., 2014; Kappler et al., 2021).

The sustainability of iron-mediated nitrogen conversion cannot be inferred solely from its potential to reduce aeration and external carbon demand. Established PN/A processes can reduce oxygen demand by ~60% and largely eliminate external organic carbon input compared to conventional nitrification–denitrification (Lackner et al., 2014). Iron-mediated pathways may provide similar conceptual benefits under oxygen-limited conditions; however, these must be weighed against iron production and transport, chemical replenishment, mineral sludge management, and possible NO and N₂O emissions (Jones et al., 2015; Robinson et al., 2021). Without consistent full-scale data on energy use, iron consumption, sludge production, emissions, and treatment costs, conclusive sustainability claims remain premature. Future research should prioritize long-term pilot-scale validation with real wastewater, comparative reactor studies under equivalent loading conditions, complete iron mass balances, and integrated techno-economic and life cycle assessments. These efforts are essential for determining whether reductions in aeration and external carbon input translate into net environmental and economic benefits.

In summary, iron-mediated nitrogen conversion signifies a scientifically compelling and potentially transformative direction for sustainable wastewater treatment, providing opportunities to reduce dependence on aeration and external carbon while expanding the toolbox of low-energy nitrogen-removal strategies. However, current progress remains predominantly confined to mechanistic studies and laboratory-scale proof-of-concept demonstrations, with considerable gaps between conceptual feasibility and practical engineering implementation. Achieving real-world translation requires a decisive transition from pathway discovery to system engineering, including the development of robust reactor configurations, stable iron redox management strategies, reliable control of competing biological and abiotic pathways, and validation under long-term continuous operation with real wastewater matrices. Future assessments must also extend beyond nitrogen-removal performance to encompass techno-economic feasibility, operational complexity, sludge management, and comprehensive environmental impacts, especially greenhouse gas emissions. Ultimately, the practical value of iron-mediated nitrogen conversion will depend on its evolution into a controllable, scalable, and genuinely sustainable wastewater-treatment technology rather than its mechanistic novelty.

CRedit Authorship Contribution Statement

Rui Guo: Conceptualization, writing-original draft; **Xiaotong Cen, Cong Li, Zhiqiang Zuo:** writing-review and editing; **Min Zheng:** Conceptualization, writing-review and editing, project administration

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

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