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Enhancement and mechanisms of iron-assisted anammox process

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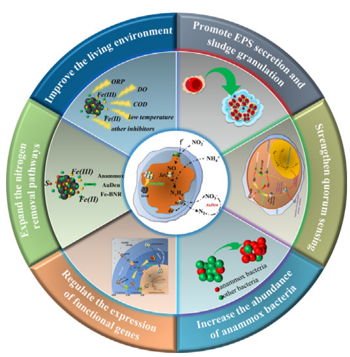
HIGHLIGHTS

- Recent advances in the effects of iron on anammox process were reviewed.
- Fe (II) showed better strengthening performance but remains limitation.
- Possible mechanisms of iron-fortified anammox process were discussed.
- Future research prospects were envisaged.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic ammonium oxidation (anammox) is a sustainable biological nitrogen removal technology that has limited large-scale applications owing to the low cell yield and high sensitivity of anammox bacteria (AnAOB). Fortunately, iron-assisted anammox, being a highly practical method could be an effective solution. This review focused on the iron-assisted anammox process, especially on its performance and mechanisms. In this review, the effects of iron in three different forms (ionic iron, zero-valent iron and iron-containing minerals) on the performance of the anammox process were systematically reviewed and summarized, and the strengthening effects of Fe (II) seem to be more prominent. Moreover, the detailed mechanisms of iron-assisted anammox in previous researches were discussed from macro to micro perspectives. Additionally, applicable iron-assisted methods and unified strengthening mechanisms for

Abbreviations: Anammox, anaerobic ammonium oxidation; AnAOB, anammox bacteria; N-cycling, nitrogen-cycling; NRR, nitrogen removal rate; NRE, nitrogen removal efficiency; TNRE, total nitrogen removal efficiency; ZVI, zero-valent iron; SBR, sequencing batch reactor; SBBR, sequencing batch biofilm reactors; UASB, up-flow anaerobic sludge blanket; IC50, half-maximum inhibitory concentration; mZVI, micro zero-valent iron; nZVI, nano zero-valent iron; DNA, deoxyribonucleic acid; FeS, mackinawite; $Fe_{1-x}S$, x = 0–0.125, pyrrhotite; FeS_2, pyrite; AuDen, autotrophic denitrification; γ-Fe_2O_3, ferric oxide; Fe_3O_4, magnetite; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; N_2, nitrogen gas; NPs, nanoparticles; EPS, extracellular polymer substance; LB-EPS, loosely bound EPS; TB-EPS, tightly bound EPS; DO, dissolved oxygen; ORP, oxidation-reduction potential; PN, proteins; PS, polysaccharides; QS, quorum sensing; AHLs, acyl homoserine lactones; AIP, autoinducing peptides; AI-2, autoinducer 2; DSF, diffusible signal factor; DGC, *c*-di-GMP synthesis protein; PDE, *c*-di-GMP degradation protein; *nir*, nitrite reductase; *hzs*, hydrazine synthase; *hdh*, hydrazine dehydrogenase; *dha*, dehydrogenase activity; Feammox, anammox-bound ferric reduction; NDFO, nitrate-dependent anaerobic ferrous oxidation; DNRA, dissimilatory nitrate reduction to ammonium; NDZO, nitrate-dependent anaerobic ZVI oxidation; FeOB, iron-oxidizing bacteria; FeRB, iron-reducing bacteria; CO_2, carbon dioxide; Fe-BNR, iron-driven autotrophic biological nitrogen removal technology; AAFEB, anammox attached film expanded bed; EGSB, expanded granular sludge bed; SAA, specific anammox activity; SS, suspended solid; VSS, volatile suspended solid; ASBR, Anammox-anaerobic sequencing batch reactor; NLR, nitrogen loading rate.

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improving the stability of nitrogen removal and shortening the start-up time of the system in anammox processes were suggested to explore in future studies. This review was intended to provide helpful information for scientific research and engineering applications of iron-assisted anammox.

Contents

1. Introduction

Human activities significantly impact the aquatic environment. Large amounts of nitrogen-containing wastewater from domestic and industrial sources are discharged annually. Notably, a large amount of nitrogen remains owing to many wastewaters lack sufficient biodegradable carbon sources, which dramatically disrupts N-cycling and causes deterioration in aquatic ecosystems (Canfield et al., 2010; de Lille et al., 2015; Zhang et al., 2016; Zou et al., 2020). Various biological nitrogen removal techniques have been investigated and developed to meet the increasingly stringent nitrogen discharge standards. Mainly including the complete, the shortcut and the simultaneous nitrification-denitrification process. Nevertheless, the disadvantages of traditional biological nitrogen removal technology, such as complicated processes, high energy consumption and cost, secondary pollution and insufficient efficiency, are becoming increasingly prominent (Du and Peng, 2022; Zhang et al., 2022d). Therefore, a technical revolution in the biological nitrogen removal process is urgently required. Fortunately, nitrogen removal processes based on anaerobic ammonia oxidation (anammox) had become the development trend. This eco-friendly process can create a shortcut in N-cycling by oxidizing ammonium directly to dinitrogen with nitrite as the electron acceptor as illustrated by Eq. (1). In theory, 100 % of the external organic carbon source, 80–90 % of the sludge production, 64 % of the aeration and 90 % of the operating costs could be reduced in the anammox process (Bi et al., 2014b). Hence, it had been regarded by the academic community as one of the most promising biotechnologies involving the nitrogen removal process with the highest sustainable development potential (Kartal et al., 2010; Ma et al., 2017). With theoretical progress and technological breakthroughs, anammox-based nitrogen removal processes had been widely employed to treat ammonium-rich wastewater (Cao et al., 2017). Up to now, >100 anammox wastewater treatment facilities had been built worldwide, but most were sidestream treatment installations (Ali and Okabe, 2015; Lackner et al., 2014).

$$\begin{aligned}&1NH_4{}^{+} + 1.32NO_2{}^{-} + 0.066HCO_3{}^{-} + 0.13H^{+} \rightarrow 1.02N_2 + 0.26NO_3{}^{-}\\&+0.066CH_2O_{0.5}N_{0.15} + 2.03H_2\end{aligned} \tag{1}$$

Despite the great superiorities of anammox in theory, it remains challenges in the practical scale-up of this biotechnology. More importantly, the anaerobic ammonium oxidizing bacteria (AnAOB) has a considerably long cell multiplication time (up to 19 days) and low cell yield, resulting in the long start-up time of anammox processes. In addition, AnAOB is sensitive to environmental conditions, which makes the nitrogen removal performance of anammox vulnerable to environmental factors and leads to a low total nitrogen removal efficiency (TNRE). These make anammox processes difficult to be used for the mainstream process of current wastewater treatment (Feng et al., 2022; Reino et al., 2018; Strous et al., 1998; van der Star et al., 2007; Wu et al., 2022). Currently, many studies were devoted to promoting the rapid start-up and enhancing the nitrogen removal performance of anammox processes (Tang et al., 2020; Wang et al., 2021b; Yang et al., 2019). Compared with cost-intensive methods such as applied electric field (Li et al., 2022; Yin et al., 2015), magnetic field (Liu et al., 2008) and ultrasonic wave (Zhang et al., 2022a; Zhang et al., 2022e), the exogenous additives could be more economical and convenient to solve the above problems by efficiently retaining microorganisms, shortening the cell multiplication time of AnAOB, and improving the activity of functional enzymes (Adams et al., 2020; Zhang et al., 2022d).

Iron is widely used as an exogenous additive, and commonly exists in nature. It is an essential element as well as the most abundant transition metal in biological cells (Liu and Horn, 2012; Wang et al., 2022). It acts as a coenzyme factor of metalloproteinases or some functional enzymes, together with participating in catalytic, redox and regulatory processes in the tricarboxylic acid cycle and electron transfer (Ferousi et al., 2017; Qiao et al., 2013). Consequently, an appropriate amount of iron could significantly enhance microbial community diversity and metabolic activity (Ferousi et al., 2017; Zhang et al., 2018b). In particular, iron plays a greater role in the metabolism of AnAOB. The anammoxosome, a unique organelle of AnAOB, contains abundant iron-containing substances (e.g., heme C, iron-sulfur cluster, iron-nickel protein and other iron-containing cofactors) that can be used for nitrogen metabolism, which are 1–2 orders of magnitude higher than that of *E. coli*. Also, the activities of three key enzymes involved in anammox reactions are regulated by iron (Ferousi et al., 2017; van Niftrik et al., 2008a; Wang et al., 2022; Wang et al., 2021a).

Iron has attracted extensive attention as an exogenous additive for optimizing the anammox process. Researchers believed that iron in different forms (i.e., ionic iron, zero-valent iron and iron-containing minerals) had great potential in accelerating the start-up of the anammox system and enhancing its operational performance and stability (Bi et al., 2014b;

Chen et al., 2014; Erdim et al., 2019; Liu and Ni, 2015; Wang et al., 2022; Zhang et al., 2022d; Zhang et al., 2019b). A more systematic review of iron-assisted anammox processes and their mechanisms would be beneficial to future scientific research, despite the efforts of Zhang et al. (2022d) and Ma et al. (2022). Therefore, this review aimed to clarify the iron-assisted anammox process to promote its application. Herein, the enhancement effects of iron on anammox operational performance were reviewed. Furthermore, the strengthening mechanisms of iron in anammox processes were revealed. Finally, the future development trends and research directions were put forward.

2. Iron enhances anammox performance

Previous studies have shown that iron in different forms is beneficial to promoting microbial activity and enhancing the anammox system. Generally, the strengthening effects of iron are mainly reflected in shortening the start-up time and improving the nitrogen removal rate (NRR) and efficiency (NRE) (Zhang et al., 2022d; Zhou et al., 2021). Relevant studies have mainly investigated the effects of iron in different forms on anammox processes in synthetic wastewater, as summarized in Tables 1 and 2. Overall, the effects of iron on anammox processes are concentration-dependent,

Table 1
Effects of Fe (II) and Fe (III) additives on the anammox process.

Mental ion	Concentration (mg/L)	Reactor type (effective volume)	Temperature (°C)	Influent or initial N concentration (mg/L)	Sludge concentration	Effect	Reference
Fe (II)	1–50	Biofilter (1 L)	25 ± 1	NH_4^+: 50; NO_2^-: 50	nitrifying sludge (4.1 g/L) and anammox sludge (5.7 g/L)	1–5 mg/L: TNRE was increased by 6.4 %, 10–50 mg/L: TNRE was reduced by 2 %–38.5 %.	(Zhang et al., 2018b)
	5–20	AAFEB (2.5 L)	34 ± 2	TN: 350	–	TNRE was increased by 0.09 %–12.06 %, the optimum iron dosage is 20 mg/L.	(Zhou et al., 2021)
	1.12–5.6	SBR (2.6 L)	32 ± 3	NH_4^+: 120; NO_2^-: 156	–	TNRE was increased by 1.90 %–5.58 %, the optimum iron dosage is 4.48 mg/L.	(Shu et al., 2016)
	1.68–10.08	Up-flow fixed-bed column reactor (0.3 L)	35 ± 1	NH_4^+: 100; NO_2^-: 130	3683 mg VSS/L	TNRE was increased by 9.25 %–19.32 %, the optimum iron dosage is 5.04 mg/L.	Liu and Ni, 2015; Qiao et al., 2013)
	1–120	SBR (7 L)	15	NH_4^+: 100; NO_2^-: 132	1244 mg SS/L	1–4 mg/L: NRE (NH_4^+) was increased by 10.33 %–100 %, NRR (NO_2^-) was increased by 3.5 %–89 %; 6–120 mg/L: NRE (NH_4^+) maintained 100 % but NRR (NO_2^-) declined.	(Feng et al., 2020)
	1.68–42	Serum bottles (115 mL)	35	NH_4^+: 50; NO_2^-: 66	–	1.68–6.72 mg/L: NRE (NH_4^+) was increased by 39.80 %–144.30 %, NRR (NO_2^-) was increased by 39.20 %–141.90 %; 42 mg/L: NRE (NH_4^+) was reduced by 39.50 %, NRR (NO_2^-) was reduced by 34.20 %.	(Ding et al., 2021b)
	3.36–5.04	Up-flow column reactor (0.5 L)	35 ± 1	NH_4^+: 50–170; NO_2^-: 50–170	2540 mg SS/L	the start-up time was shortened by 17.14 %–28.57 %, TNRE was increased by 15.43 %–34.28 %; the optimum iron dosage is 5.04 mg/L.	(Bi et al., 2014b)
	2–50	Up-flow anoxic reactor (1 L)	–	NH_4^+: 35–100; NO_2^-: 45–130	8670 mg SS/L, 5809 mg VSS/L	2–5 mg/L: TNRE was increased by 0.80 %–11.80 %, 8–50 mg/L: TNRE was reduced by 1.20 %–31.00 %.	(Mishra et al., 2021)
	3.36–10.08	Serum bottles (100 mL)	35 ± 1	NH_4^+: 50; NO_2^-: 50	1850 mg VSS/L	TNRE was increased by 7.70 %–11.30 %, the optimum iron dosage is 5.04 mg/L.	(Qiao et al., 2013)
	5–30	UASB (0.42 L)	35 ± 1	NH_4^+: 28.55–114.96; NO_2^-: 37.68–147.78	8–10 g VSS/L	5–15 mg/L: SAA was increased by 3.43 %–4.36 %, 30 mg/L: SAA was reduced by 10.63 %.	(Chen et al., 2021b)
	2.24–4.48	SBBR (1.5 L)	35 ± 1	NH_4^+: about 150; NO_2^-: about 180	–	TNRE was increased, the optimum iron dosage is 4.48 mg/L.	(Huang et al., 2014)
	1.5–4.5	Up-flow anaerobic coupled biofilm reactor (6.28 L)	30–32	NH_4^+: 40; NO_2^-: 53	–	NRE (NH_4^+) was increased by 9.31 %–15.66 %, the optimum iron dosage is 4.5 mg/L.	(Yan et al., 2022)
	2.24–11.2	SBR (1 L)	35	NH_4^+: 50; NO_2^-: 50	3000 mg VSS/L	2.24–4.48 mg/L: NRE (NH_4^+) and NRE (NO_2^-) were increased, 11.2 mg/L: NRE (NH_4^+) and NRE (NO_2^-) were reduced.	(Mak et al., 2019)
Fe (III)	5–30	UASB (0.42 L)	35 ± 1	NH_4^+: 114.96 ± 3.55; NO_2^-: 147.78 ± 4.38	8–10 g VSS/L	SAA was reduced by 18.41 %–22.14 %.	(Chen et al., 2021b)
	4–250	SBR (7 L)	15	NH_4^+: 80; NO_2^-: 105.6	–	NRE (NH_4^+) and NRE (NO_2^-) were increased.	(Li et al., 2020)
	2.24–7.84	EGSB (0.8 L)	35	–	20.0 g SS/L	TNRE was increased by 2.40 %–3.10 %.	(Wang et al., 2021a)
	3.68–6.61	UASB (1 L)	35 ± 1	NH_4^+: 130–280, NO_2^-: 150–280	11.1 g SS/L, 8.2 g VSS/L	TNRE was increased by 3.00 %–7.22 %.	(Chen et al., 2014)
	5–100	SBR (1 L)	33 ± 1	NH_4^+: 90–135; NO_2^-: 117–175	5200 mg SS/L	5–10 mg/L: TNRE was increased by 0.05 %–5.32 %, 50–100 mg/L: TNRE was reduced by 12.45 %–27.10 %.	(Zhang et al., 2021b)
	2.24–6.72	ASBR (2.6 L)	35 ± 2	NH_4^+: 100; NO_2^-: 120	3500 mg SS/L, 2000 mg VSS/L	TNRE was increased by 3.09 %–7.67 %, the optimum iron dosage is 4.48–5.6 mg/L.	(Wang et al., 2016)

Note. the optimum iron dosage means that the iron dosage with which anammox systems could have the best nitrogen removal performance in the current study; AAFEB, anammox attached film expanded bed; TN: total nitrogen; TNRE (%): total nitrogen removal efficiency; NRE (%): nitrogen removal efficiency; NRR (kg N/m^3/d): nitrogen removal rate; SBR: sequencing batch reactor; SBBR, sequencing batch biofilm reactors; SAA, specific anammox activity; SS, suspended solid; VSS, volatile suspended solid; UASB: up-flow anaerobic sludge blanket; ASBR, anammox-anaerobic sequencing batch reactor; NLR (kg N/m^3/d), nitrogen loading rate.

with low concentrations promoting and high concentrations inhibiting the anammox process (Zhou et al., 2021).

2.1. Ionic iron

Fe (II) was essential for anammox processes. It could promote a rapid start-up and an efficient operation of anammox processes within a certain concentration range. Moreover, the strengthening effect is enhanced with the increase of Fe (II) concentration. Also, a lack of Fe (II) supply would severely limit the nitrogen removal performance of AnAOB (Ribeiro et al., 2017; Zhang et al., 2018b). Bi et al. (2014b) studied in an up-flow column reactor and found that when the influent Fe (II) was 3.36 mg/L, the start-up time was 12 days faster than that of 1.68 mg/L, which was shortened to 58 days. In addition, the start-up speed was 20 days faster under the influent Fe (II) concentration which was three times that of the control group. Shu et al. (2016) explored the effects of Fe (II) at concentrations ranging from 1.12 to 5.6 mg/L on the anammox process. The results suggested that it could play a stable and enhanced role in the nitrogen removal performance within this concentration range. However, Fe (II) was a double-edged sword under the excessive influent Fe (II) concentration. Zhang et al. (2018b) employed 1–50 mg/L Fe (II) in the anammox process. The results indicated that the nitrogen removal performance of the system would be inhibited when the Fe (II) concentration exceeded 10 mg/L. Irreversible inhibition would occur when Fe (II) concentration exceeded 50 mg/L. A similar inhibition phenomenon was suggested by Mak et al. (2019). They found out that Fe (II) would decrease the nitrogen removal performance

Table 2
Effects of ZVI and iron-containing minerals additives on the anammox process.

Mental type	Particle size	Concentration (mg/L)	Reactor type (effective volume)	Temperature (°C)	Influent or initial N concentration (mg/L)	Sludge concentration	Effect	Reference
nZVI	50 nm	1–10 10–200	UASB (1 L)	35 ± 1	NH_4^+: 280; NO_2^-: 280	12.0 g VSS/L	The NRE slightly changed The NRE was reduced, but the worst performance at the nZVI = 20–50 mg/L, the NRE was maintained at 85.7 ± 1.4 % in the presence of 200 mg/L nZVI.	(Zhang et al., 2017)
ZVI	45 μm	1–16	UASB (2.5 L)	35	NH_4^+: 208.48 ± 20.36; NO_2^-: 213.36 ± 15.94	2 g SS/L	TNRE was increased by 2.26 %–7.66 %, the optimum iron dosage is 16 mg/L.	(Zhang et al., 2022b)
ZVI	–	10^5	UASB (2 L)	–	NH_4^+: 63–252; NO_2^-: 75.7–302.7	18,750 ± 200 mg SS/L, 11660 ± 260 mg VSS/L	Shorten the activity-recovery period during the start-up process (12 d, the control group is 16d) and improves the adaptability of AnAOB at low temperature.	(Ren et al., 2016)
ZVI	–	25 g mZVI (4.75 L) 25 g nZVI (4.75 L)	Up-flow anaerobic sludge blanket reactor (4.75 L)	32–35	NLR = 630 ± 20 mg N/L/d	2000 ± 20 mg SS/L	The start-up time was shortened by 16.7 %, the NRE (NO_2^-) is increased by 2.3 % The start-up time was shortened by 33.4 %, the NRE (NO_2^-) is increased by 5.4 %	(Ren et al., 2015)
nZVI	50 nm	50–100	UASB (1 L)	35 ± 1	NH_4^+: 280; NO_2^-: 280	–	50–75 mg/L: TNRE was increased by 0.03 %–1.44 %, 100 mg/L: TNRE was reduced by 6.22 %.	(Xu et al., 2018)
ZVI	150 μm	100 1000	Serum bottle (0.6 L)	32 ± 1	NH_4^+: 280; NO_2^-: 280	8000 ± 20 mg VSS/L	TNRE > 90 %, had no significant effect on shortening the start-up time, the anammox abundance increased by 44.5 % TNRE >90 %, it shortened the start-up time by nearly 10 %, and the anammox abundance increased by 54 %	(Guo et al., 2019)
nZVI	100 nm	5.6	Serum bottle (0.5 L)	35 ± 1	NH_4^+: 60–200; NO_2^-:60–200	–	The SAA is increased by 37.88 %, and the NRR is increased by 18.8 %	(Peng et al., 2022)
Fe_3O_4	100 μm 100 μm 20 μm 20 μm 200 nm 200 nm	100 500 100 500 100 500	Serum bottles (120 mL)	35 ± 1	NH_4^+: around 50; NO_2^-: around 50	11.5 g SS/L, 8.6 g VSS/L	SAA is reduced by 4.5 % SAA is increased by 6.7 % SAA is increased by 14.1 % SAA is increased by 16.3 % SAA is increased by 33.3 % SAA is increased by 28.9 %	(Chen et al., 2021a)
Fe_3O_4	20 nm	2–200	UASB (1 L)	35 ± 1	NH_4^+: 280; NO_2^-: 280	18.3 g VSS//L	The NRE and NRR had no significant differences.	(Xu et al., 2020)
Fe_3O_4	–	Using magnetite as a functional bio-carrier	UASB (4.7 L)	31	NH_4^+: 100; NO_2^-: 120	4250 mg SS/L	The endogenous denitrification phase ended on day 5, while the control group ended on day 14. Anammox system Successfully started up on day 53, and the TNRE is 6.5 % higher than that of control group.	(Lv et al., 2016)
γ-Fe_2O_3	20 nm	1–200	UASB (1 L)	35 ± 1	NH_4^+: around 280; NO_2^-: around 280	12.0 g VSS/L	SAA was increased by 2.70 %–40.75 %.	(Zhang et al., 2018d)
γ-Fe_2O_3	<50 nm	25–150	Serum bottle (75 mL)	35 ± 1	NH_4^+: 100; NO_2^-: 100	2500 mg VSS/L	The addition of 25–150 mg/L γ-Fe_2O_3 promoted the nitrogen removal, and the order from large to small was 100, 150, 50 and 25 mg/L	(Elreedy et al., 2021)
FeS	–	30 g (1.5 L)	Fluidized bed bioreactor (1.5 L)	30	NH_4^+: 25–100; NO_3^-: 25–100	3000 mg SS/L	The NRE (NO_3^-) = 100 %, the effluent NH_4^+ = 8.11 mg/L	(Ma et al., 2021)
FeS	–	3 g/L	UASB (5 L)	32 ± 1	NH_4^+: 50–100; NO_2^-: 60–120 NH_4^+: 150–200; NO_2^-: 180–245	5500 ± 50 mg SS/L	The TNRE was increased by 2.3 %. FeS effectively shortened the start-up time to 51 days. With the increase of NLR, effluent NH_4^+-N and NO_2^--N concentrations were significantly lower than that of control group, suggesting FeS could improve the stability of anammox reactor.	(Zou et al., 2020)

with the concentration of Fe (II) exceeded 4.5 mg/L, and proposed that the half-maximum inhibitory concentration (IC50) of Fe (II) on anammox was 11.2 mg/L.

Similar effects of Fe (III) on anammox processes were also observed. The addition of 3.68 mg/L Fe (III) increased the anammox activity by 5-fold compared with the control group (Chen et al., 2014). In another study, NRR increased by 1.9 times when 7.8 mg/L Fe (III) was added (Wang et al., 2021a). Wang et al. (2016) came to a similar conclusion that the specific growth rate of AnAOB was significantly enhanced when the influent concentration of Fe (III) was increased from 2.2 mg/L to 5.6 mg/L in a SBR reactor. The experiment by Zhang et al. (2021b) suggested that long-term exposure with low concentration Fe (III) (5 and 10 mg/L) exposure remarkably improved the NRE of the anammox process, while high-concentration Fe (III) (50 and 100 mg/L) decreased the NRE.

Regardless of the operating performance of the anammox system, the concentration of Fe (II) with a strengthening effect on the anammox process occurs between 1.68 and 5.04 mg/L, while that of Fe (III) could be higher (Table 1). The reason might be that Fe (II) is a growth substrate that stimulates the metabolism of AnAOB (Feng et al., 2022). Moreover, Fe (II) has natural reduction characteristics. A small amount of Fe (II) could reduce dissolved oxygen (DO), oxidation-reduction potential (ORP) and adjust pH conditions, which is very helpful to promote the biological activity of AnAOB (Bi et al., 2014b; van Niftrik et al., 2008b). In contrast, the utilization of Fe (III) by microorganisms was generally inferior to that of Fe (II) (Wan et al., 2021). However, under neutral environmental conditions, Fe (II) could be easily converted into Fe (III), and Fe (III) is easily hydrolyzed and precipitated (Wan et al., 2021; Wang et al., 2022). Therefore, researchers have explored zero-valent iron (ZVI) and iron-containing minerals which could stably and continuously release ionic iron for sustainable anammox enhancement.

2.2. Zero-valent iron

The use of zero-valent iron (ZVI) in environmental remediation and wastewater treatment has attracted increasing attention (Crane and Scott, 2012). ZVI is an effective bio-augmenter for enhancing the performance of anammox, on account of its high reducibility, ecological sustainability and stable release of Fe (II) (Gao et al., 2014; Guo et al., 2019).

Sponge iron in UASB reactors could effectively shorten the activity-recovery period during the start-up of the anammox system, which was 1/4 faster than that of the control group (Ren et al., 2016). Compared with micro zero-valent iron (mZVI), nano zero-valent iron (nZVI) had stronger reducing activity in the process of environmental remediation due to its size advantage. In the study of Ren et al. (2015), the start-up time of the anammox system was shortened from 126 days to 105 days and 84 days with the addition of mZVI and nZVI, which were shortened by 16.7 % and 33.4 %, respectively. Also, both ammonium and nitrite utilization rates increased apparently with continuous nZVI addition (Erdim et al., 2019). However, previous research has shown that nZVI had no obvious function in shortening the start-up time of the anammox system although it could improve the activity of AnAOB at the dosage of 100 mg/L (Guo et al., 2019). Moreover, Guo et al. (2019) suggested that ZVI might directly partake in the anammox reaction pathway and improve the TNRE.

Interestingly, the effects of nZVI on the anammox process showed the pattern of first inhibition and then promotion with increasing nZVI concentration (Gao et al., 2014; Guo et al., 2019). In long-term experiments, Zhang et al. (2017) found that low concentrations of nZVI (10 mg/L or less) had little effect on the performance of anammox. A moderate nZVI concentration (10–50 mg/L) would deteriorate the reaction system first and then gradually recover. However, a high nZVI concentration (50–200 mg/L) would directly enhance the nitrogen removal performance of the system. This phenomenon might be due to the gradual increase in the adaptive ability of AnAOB and the reaction between NO_3^- and nZVI or Fe (II) released from nZVI (Gao et al., 2014). Unfortunately, the effects of nZVI on the anammox system are not as clear as that of ionic iron.

The possible explanation for the deterioration of TNRE is that nZVI with improper exposure time and dosing level might affect the normal metabolism of microorganisms, damage microbial cell membranes and DNA together with inactivating enzymes (Crane and Scott, 2012; Diao and Yao, 2009; Li et al., 2019). Therefore, exploring its appropriate exposure methods is needed while applying nZVI for environmental remediation and wastewater treatment. It should be noted that the intermittent addition of low concentrations of nZVI has been proven to be an effective solution for enhancing the metabolism of AnAOB (Erdim et al., 2019; Li et al., 2019).

2.3. Iron-containing minerals

Iron-containing minerals that exist in nature could also enhance the anammox process and act as a Fe (II) release agent, mainly including iron oxides and iron sulfides.

Magnetic iron oxides nanoparticles were capable of superparamagnetism, high biocompatibility, chemical stability and promotion of co-trophic metabolism, which has received extensive attention in enhancing the biological nitrogen removal capability of anammox processes (Tang and Lo, 2013; Xu et al., 2020; Zhang et al., 2018d). They are more easily adsorbed on the surface of the anammox granular sludge, which could accelerate the release of Fe (II) or Fe (III), and finally achieve the target of intensifying the anammox process. The research by Xu et al. (2020) demonstrated that the addition of 2–200 mg/L of nano-magnetite had no inhibitory effect on anammox processes, and the metabolism of AnAOB increased significantly with the increase of nano-magnetite concentration. Zhang et al. (2018d) came to a similar conclusion when they studied the effects of maghemite nanoparticles on the anammox system. However, a different conclusion was put forward by Elreedy et al. (2021), who suggested that the promotion effect of γ-Fe_2O_3 on the anammox system was mainly abiotic. The introduction of magnetite could also promote anammox systems start up rapidly. Research manifested that using magnetite as a functional biological carrier in the UASB reactor could shorten the endogenous denitrification time by approximately 65 %, and successfully start up the anammox system on the 53rd day (Lv et al., 2016). In summary, the enhancement of anammox processes by ferromagnetic nanoparticles may be attributed to the combined effect of iron ion release and its internal magnetic biological effect (Li et al., 2018; Xu et al., 2020).

Iron sulfide could promote the growth of bacteria and reduce by-product nitrate, which has a remarkable potential in enhancing anammox process (Feng et al., 2022; Ma et al., 2021; Zou et al., 2020). Iron sulfide mainly exists in the form of mackinawite (FeS), pyrrhotite ($Fe_{1-x}S$, x = 0–0.125) and pyrite (FeS_2), which are the most abundant sulfide mineral (Hu et al., 2020). Dosing FeS to anammox systems would significantly increase the abundance of AnAOB and effectively shorten the start-up time to 51 days. Moreover, the addition of FeS effectively improved the nitrogen removal performance and resistance to the shock loading rate of the system (Zou et al., 2020). Ma et al. (2021) also analyzed the feasibility of FeS-driven autotrophic denitrification (AuDen) and anammox coupling system, in which 100 % of NO_3^--N removal efficiency could be achieved. Feng et al. (2022) demonstrated that FeS_2 could also produce similar effects on the anammox system as FeS.

Notably, iron-based nanoparticles (NPs) usually play a stronger role in anammox processes than other iron-based additives with large sizes (Ren et al., 2015). However, metal NPs generally exhibited negative effects on anammox processes (Li et al., 2019; Zhang et al., 2018a). The main reasons for this were that the metal NPs had low toxicity and persistent effects on anammox (Zhang et al., 2022c; Zhang et al., 2018a; Zhang et al., 2018c). Fortunately, unlike most metal NPs, the Fe (II) released from the iron-based NPs during the anammox process could effectively offset the negative effects of the NPs and significantly improve the nitrogen removal performance (Bi et al., 2014a; Yu et al., 2016; Zhang et al., 2022c).

To sum up, Fe (II) enhanced the anammox process better than other forms of iron (Tables 1 and 2, Fig. 1). Since AnAOB had higher bioavailability of soluble Fe (II), while others needed to be dissolved or reduced to Fe

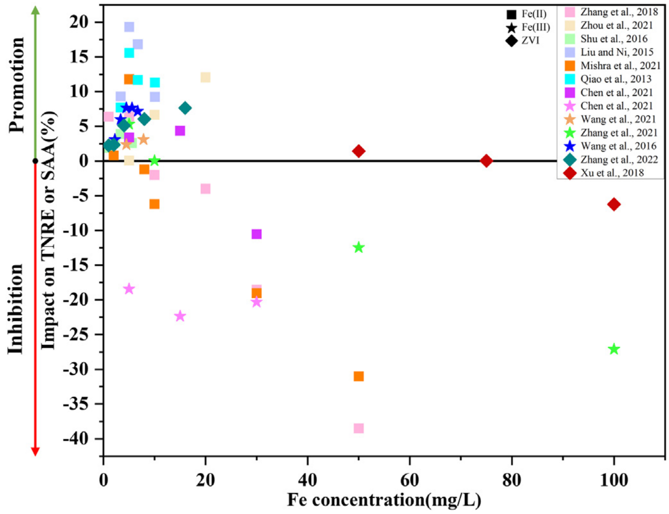

Fig. 1. Effects of different concentrations of iron on TNRE or SAA in anammox processes (Chen et al., 2021b; Liu and Ni, 2015; Mishra et al., 2021; Qiao et al., 2013; Shu et al., 2016; Wang et al., 2021a; Wang et al., 2016; Xu et al., 2018; Zhang et al., 2022b; Zhang et al., 2021b; Zhang et al., 2018b; Zhou et al., 2021).

(II) for efficient absorption and utilization by AnAOB (Kappler et al., 2021; Wang et al., 2022; Zhang et al., 2022d). Nevertheless, it is difficult for Fe (II) to exist stably for a long time and the continuous addition of Fe (II) leads to high investment. Hence, both the strengthening effects and the cost-effectiveness should be taken into consideration, and a future research direction is to search for an iron-based additive with low price and excellent performance.

3. Mechanisms of iron-assisted anammox

Iron plays an important role in strengthening anammox systems. Understanding its strengthening mechanisms is of great significance for its engineering applications. Herein, this review summarized the possible mechanisms of the iron-assisted anammox process from macro and micro perspectives (Fig. 2). Generally, Fe (II) whether dosed directly

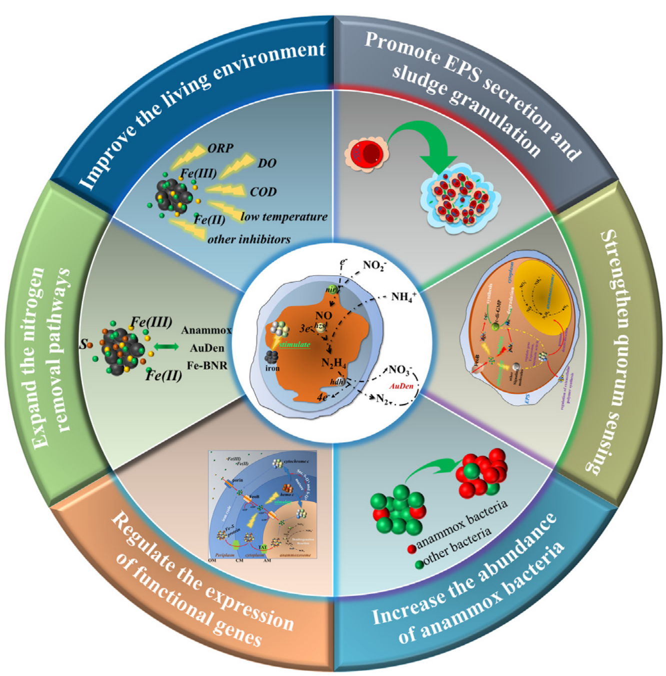

Fig. 2. Mechanisms of iron-assisted anammox processes.

(ferrous salt) or released by iron-containing substances (insoluble iron such as ZVI and iron-containing mineral) plays a major role in the strengthening process via various mechanisms. Moreover, the addition of ZVI and iron-containing minerals could promote sludge granulation and biofilm formation by acting as the nuclei or carriers. Also, ZVI could play an important role in improving the living environment of functional microorganisms. In addition, Fe (III) combined with Fe (II) and iron sulfides mainly make a difference in expanding nitrogen removal pathways.

3.1. Improvement of living environment for functional microorganism

The performance of the anammox system is strongly affected by the surrounding environment and various pollutants, such as temperature, pH, DO, organic matter, metal ions and emerging pollutants (e.g., NPs, microplastics and antibiotics) (Huang et al., 2022; Tomaszewski et al., 2017). Studies have revealed that an appropriate amount of iron could alleviate the inhibition of some factors on anammox (Chen et al., 2021a; Feng et al., 2022; Li et al., 2020; Yan et al., 2022).

The addition of ZVI could optimize the living environment of AnAOB, and resist some of the adverse conditions that trigger the decline of anammox activity (Guo et al., 2019; Yan et al., 2019). This method could enable the rapid enrichment of functional microorganisms in the system (Gao et al., 2014). ZVI and Fe (II) could be regarded as effective deoxidizers, which were beneficial to maintaining anaerobic conditions for the proliferation of anaerobic bacteria. However, the enhancement effect of Fe (II) for the activity recovery of AnAOB after oxygen exposure was inferior to that of ZVI (Ren et al., 2015; Yan et al., 2019). ZVI is extremely electronegative, with strong reduction ($E^{\circ} = -0.44$ V) and adsorption properties (Crane and Scott, 2012). Hence, the introduction of ZVI could release Fe (II) and reduce DO and ORP, together with regulating the pH of the anammox system (Guo et al., 2019; Li et al., 2020).

The addition of iron could stimulate the formation of granular sludge and facilitate the joint action of multiple nitrogen removal pathways, resulting in the enhanced nitrogen removal performance of anammox processes at low temperature. Chen et al. (2021a) studied the effects of magnetite with different particle sizes on the performance of anammox systems which was cultivated at low temperature (10–25 °C). The results showed that nano-magnetite greatly improved the adaptability of AnAOB under low temperature conditions. Furthermore, Ren et al. (2016) suggested that the performance of anammox under low-temperature (10–15 °C) shock condition could be enhanced with the use of novel ZVI to assemble an up-flow anaerobic sludge bed reactor.

An appropriate concentration of Fe (II) could also alleviate the inhibition of organic on anammox process. Yan et al. (2022) suggested that the addition of Fe (II) could accelerate the growth of AnAOB in the organic-inhibited anammox reactor. In their study, the performance of nitrogen removal was enhanced with the increase of influent Fe (II) concentration in the range of 0.15–4.5 mg/L, owing to the abundance of denitrifying bacteria and AnAOB increased collectively with Fe (II) addition. Moreover, iron-containing minerals could even alleviate the inhibition of heavy metals during the anammox process. Some researchers have pointed out that pyrite was a promising Cr (VI) reductant, reducing the inhibition of anammox by Cr (VI) (Feng et al., 2022).

3.2. Promoting the secretion of EPS and sludge granulation

Sludge aggregation to form granular sludge and biofilm is an effective way to retain AnAOB, which tends to self-aggregate. Moreover, AnAOB has a double-layer EPS structure, tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS) surrounding AnAOB. EPS plays an essential role in the aggregation of AnAOB. The AnAOB in the outer region of the sludge is entangled by weak interactions through LB-EPS. The TB-EPS are responsible for AnAOB adhesion and attachment in the inner sludge structure through strong interactions (Jia et al., 2017; Liu et al., 2010; Wang et al., 2020c). Moreover, the formation of EPS played important functions in the anammox system, such as structural stability, water retention, and a protective barrier for AnAOB (Izadi et al., 2021).

EPS content in the anammox system was positively correlated with bacterial activity, so the increase of EPS content might suggest an enhanced anammox activity (Duan et al., 2011; Zhang et al., 2021b). Moreover, studies proved that the changes in EPS content in the biofilm were consistent with iron concentration. Indicating that the increase in EPS could promote more Fe (II) adsorption, thereby improving the resistance of AnAOB to inhibitors (Zhang et al., 2020; Zhang et al., 2018b). However, a previous study by Tang et al. (2020) showed that the concentration of EPS decreased with the enlargement of granular sludge's diameter. This caused the decreased adsorption capacity of EPS to Fe (II), which facilitate more Fe (II) to be transported into the AnAOB to intensify the anammox process. Elreedy et al. (2021) obtained a similar conclusion in their study on the effects of Fe_2O_3 NPs on the anammox system. Significantly, the EPS content would increase with the rise of Fe_3O_4 NPs within a certain concentration range (Xu et al., 2020). However, excessive amounts of Fe (III) would destroy the constituents of EPS and makes AnAOB more vulnerable to an adverse environment. Also, excessive amounts of Fe (II) might cause sediment to wrap granular sludge and inhibit the NRE of the anammox system (Chen et al., 2021b).

The introduction of iron could stimulate AnAOB to secrete EPS, which could strengthen the anammox system. Fe (II) and ZVI could reduce the proteins (PN)/polysaccharides (PS) ratio to improve the hydrophobicity and settling properties of sludge, which was very beneficial to sludge granulation (Batstone and Keller, 2001; Ren et al., 2015; Tang et al., 2020). The interaction of iron-based particles and ions with EPS was proposed to favor sludge granulation. Owing to charge neutralization, Fe (II) or Fe (III) compressed the electric double layer to reduce zeta potential and promoted the granulation of anammox sludge (Tang et al., 2020). In addition, Fe (II) or Fe (III) is usually combined with hydroxyl ions (OH^-), which stimulated the formation of active flocculation groups to promote sludge sedimentation (Tang et al., 2020; Zhao et al., 2014). Also, ferrihydrite, magnetite, FeS, ZVI and other iron-based particles could promote granulation by acting as nuclei for bacterial attachment and improve the settling performance of biomass along with the reactor's stability and resistance to shock loads (Gao et al., 2014; Ren et al., 2015; Ren et al., 2018; Wang et al., 2022; Zou et al., 2020). The iron-promoted anammox granular sludge formation process is as the following (Fig. 3). Firstly, AnAOB aggregates into small particles with the help of EPS along with iron; Subsequently, anammox small particles further aggregate and adhere to each other to form large-diameter anammox granular sludge via bridging and netting interactions; Finally, granulation completed (Liang et al., 2022; Tang et al., 2020; Wang et al., 2020a).

3.3. Strengthening the quorum sensing in anammox systems

Quorum sensing (QS) acts as a bacterial communication mode that favors the regulation of microbial aggregation behavior and density, which was first found in *Vibrio fischeri* (Nealson and Hastings, 1979; Zhang et al., 2021a). Bacteria with QS could synthesize and release chemical signal molecules, such as acyl homoserine lactones (AHLs), autoinducing peptides (AIP), autoinducer 2 (AI-2) and diffusible signal factor (DSF), and their external concentration was significantly correlated with biomass concentration (Zhang et al., 2021a). The secondary messenger *c*-di-GMP is a major intracellular signal that regulates gene expression and enzymatic activity (Wang et al., 2021b). QS occurs when the bacterial density of the anammox system exceeds 10^{10}–10^{11} cells/L (Ali et al., 2018; Kartal et al., 2012). Meanwhile, AnAOB has an extremely strong aggregation ability, which implies the potential existence of QS (Jia et al., 2017; Shrout and Nerenberg, 2012; Wang et al., 2020c).

QS has been shown to intensify the anammox process. Zhao et al. (2018) used AHL-containing supernatants and successfully achieved a rapid start-up of the anammox system within 66 days, and the NRE of this reactor reached 98 %. QS could also promote EPS secretion, accelerating the aggregation of AnAOB to form granular sludge (Tang et al., 2015; Zhang et al., 2019a). Studies have suggested that the addition of iron could enhance the QS of anammox systems to promote the anammox process and sludge granulation (Fig. 4). Gao et al. (2014) successfully obtained anammox granular sludge with ZVI and Fe_3O_4 as the nuclei and pointed out that the release of Fe (II) and Fe (III) at appropriate concentrations promoted the granulation and nitrogen removal performance of AnAOB by maintaining a high redox potential and aggregating AHLs. In the anammox system, nZVI at 50 mg/L could effectively increase the abundance of *c*-di-GMP synthesis protein (DGC) and inhibit the synthesis of *c*-di-GMP degradation protein (PDE). The enrichment of *c*-di-GMP could reduce the motility of microorganisms in anammox systems and enhance the secretion of EPS by them, which

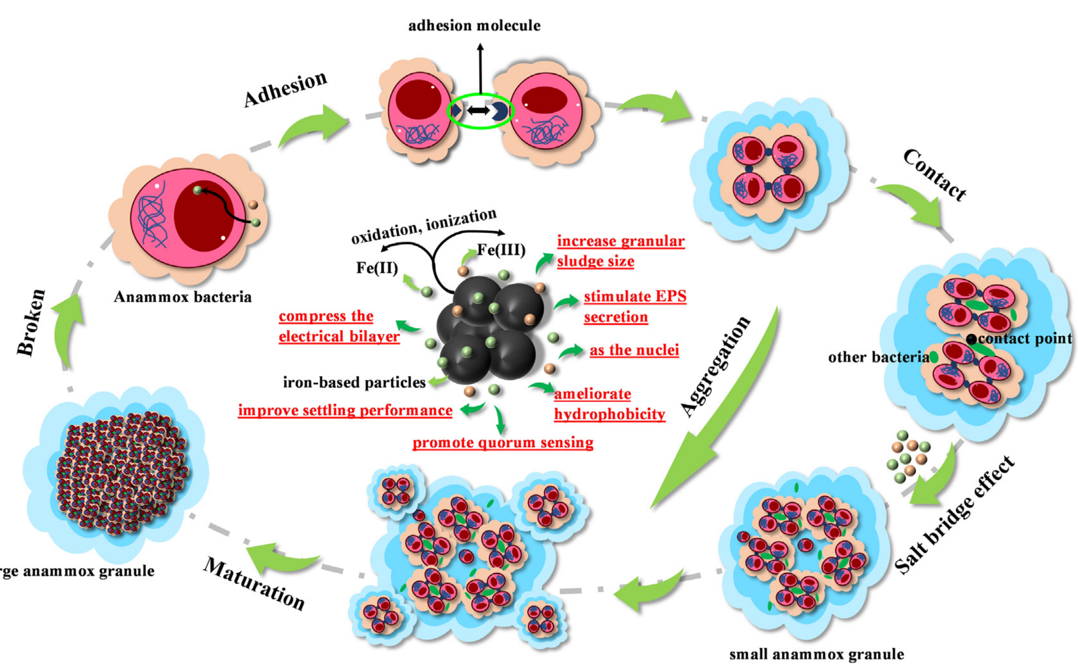

Fig. 3. Iron promotes the formation of anammox granular sludge (Adams et al., 2022; Gao et al., 2014; Tang et al., 2020; Wang et al., 2020a; Wang et al., 2020b; Wang et al., 2020c; Zhang et al., 2021a).

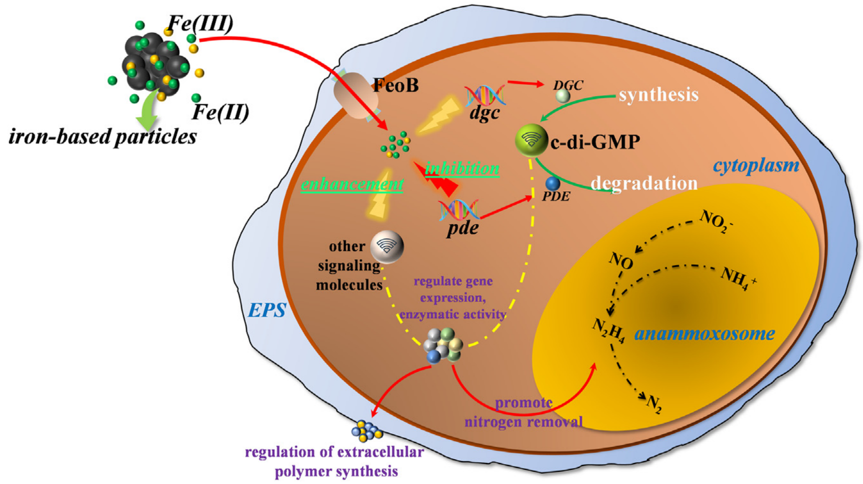

Fig. 4. Iron strengthens the anammox process by enhancing quorum sensing (Gao et al., 2014; Lau et al., 2016; Wang et al., 2021b; Zhang et al., 2021a).

was beneficial to sludge granulation (Wang et al., 2021b). Nevertheless, the research on QS in the iron-assisted anammox process is far from enough, and more comprehensive research is required.

3.4. Regulating the expression of key enzymes and functional genes

The metabolism of AnAOB relies on a specific set of heme proteins, and three functional genes play important roles in the nitrogen removal metabolism process: (1) nitrite reductase (*nir*), which catalyzes the conversion of nitrite to nitric oxide; (2) hydrazine synthase (*hzs*), which induces the conversion of nitric oxide to hydrazine; and (3) hydrazine dehydrogenase (*hdh*), which converts hydrazine to nitrogen gas (Gamon et al., 2022; Kartal et al., 2011; Li et al., 2011). Significantly, the addition of iron could positively regulate the expression of relevant functional genes and enzymes synthesis (Fig. 5).

At the Fe (II) concentration of 5.04 mg/L, the total iron content, heme c level and *hdh* activity in AnAOB were remarkably increased, which were 2.0, 2.1 and 2.35 times higher than those in the control group, respectively. Moreover, moderately increasing the Fe (II) concentration of influent could promote more heme c synthesis and enhance *hdh* activity (Bi et al., 2014b; Qiao et al., 2013). In anammox systems, the dehydrogenase activity (*dha*) response to Fe (II) was similar but faster than microbial activity, suggesting *dha* was an effective indicator for anammox performance (Tan et al., 2017; Zhang et al., 2018b). Nevertheless, some researchers pointed out that 0.09 mM and 0.12 mM Fe (II) could induce more gene expression, but had no enhancement effect on the relative expression level of anammox functional genes, which might be due to the inconsistent conditions of their experiments (Ding et al., 2021b).

The anammoxosome of AnAOB contains a large number of iron-rich substances. Almost all iron acts as a cofactor for heme c and Fe—S proteins, which play an important role in the anammox metabolism, and are effective indicators to characterize the nitrogen removal performance of the anammox system (Ferousi et al., 2017; Kartal et al., 2013). The functional proteins involved in the anammox process cannot lack heme c, as it is an extremely important cofactor, and >30 % of the total cellular protein of AnAOB is the cytochrome *c* protein (Kang et al., 2018; Schalk et al., 2000). A previous study showed that the amount of heme c enriched with the increase of Fe_3O_4 NPs addition and reached the maximum at 200 mg/L Fe_3O_4 NPs, which was 66.9 % higher than that of the control group. This might be related to the release of Fe (II) from Fe_3O_4 NPs (Xu et al., 2020). However, the metagenomic analysis showed that the metabolism of AnAOB could not be effectively enhanced by dosing additional Fe (III)-minerals when Fe (II) was sufficient. This might imply that the enhancement of performance in anammox systems is mainly due to the effects

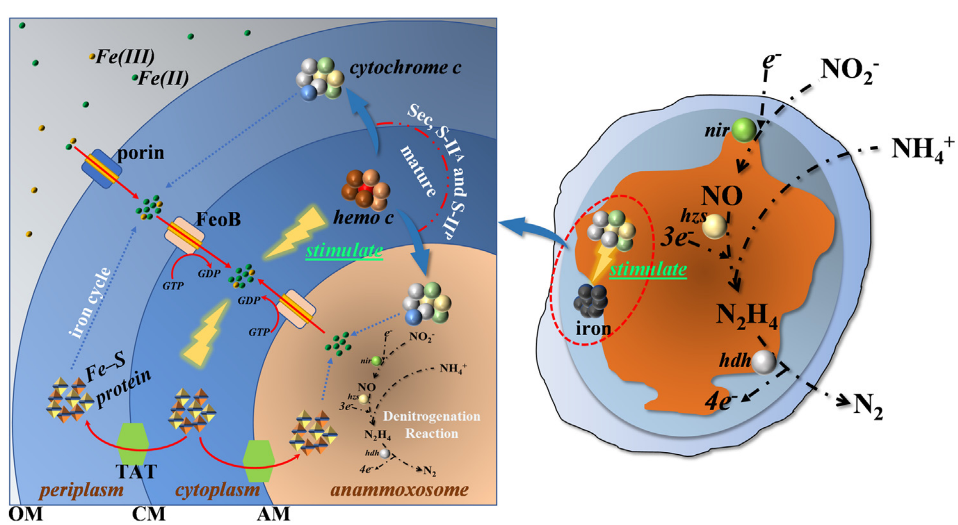

Fig. 5. Iron regulates the expression of functional genes and the synthesis of enzymes to enhance the anammox process. Sec-translocon (Sec) and cytochrome *c* maturation systems $S\text{-}II^A$ and $S\text{-}II^P$ are key factors for the maturation of cytochrome *c* in anammoxosome and periplasm, respectively. Fe—S proteins could be transferred to periplasm and anammoxosome through TAT pathway (Ferousi et al., 2017; Lau et al., 2016; Tang et al., 2020; Zhang et al., 2022c).

of Fe (II) (Wang et al., 2022). Adequate iron could support the stable production of heme c, but excessive iron addition exhibits inhibitory effects. The gene *fur* is a regulator of iron acquisition and iron balance in AnAOB. Downregulating the gene *fur* expression could prevent AnAOB from accumulating excessive iron and inhibiting the process of anammox (Fillat, 2014; Touati, 2000). In addition, the abundance regulation of gene *fur* enabled AnAOB always maintain high activity under different pH and ZVI dosages (Zhang et al., 2022b).

3.5. Increasing the anammox bacterial abundance

Up to now, six AnAOB generas have been proposed, including "*Candidatus Brocadia*", "*Candidatus Anammoxoglobus*", "*Candidatus Jettenia*", "*Candidatus Kuenenia*", "*Candidatus Scalindua*" and "*Candidatus Anammoximicrobium*" in the phylum of *Planctomycetes* (Gao et al., 2018; Kuenen, 2008; Shu et al., 2016).

Appropriate amounts of ionic iron could promote the proliferation of AnAOB (Bi et al., 2014b). 16S metagenomic analysis showed that the introduction of Fe (II) enhanced the relative abundance of *Candidatus Brocadia* in anammox systems, and the abundance of *Planctomycetota*, *Candidatus Jettenia* and *Candidatus Kuenenia* with anammox function were also increased (Sindhu et al., 2021; Yan et al., 2022; Zhang et al., 2019b). During the start-up phase of anammox systems, the dominant phylum of the system was transformed into *Planctomycetes*, indicating the enrichment of AnAOB (Zhang et al., 2018b). Fe (III) could promote the enrichment of *Candidatus Brocadia* in the anammox system and the promotion effect was positively correlated with the Fe (III) concentration (Wang et al., 2021a). Iron-based particles could also enrich the functional bacteria of the anammox system. In the presence of different concentrations of nZVI, *Candidatus Kuenenia* had always been the dominant genus (Zhang et al., 2017). With the increase of maghemite NPs concentration in the anammox system, the community structure shifted to *Candidatus Kuenenia* with a high abundance (Zhang et al., 2018d).

In general, iron is a necessary element in many microbial metabolic processes. As summarized above, iron would not have a significant effect on the microbial community structure in anammox systems to some extent. However, previous studies have proved that the introduction of iron could increase the relative abundance of denitrogenation bacteria in the anammox system (Shu et al., 2016; Zhang et al., 2022b). Hence, exploring the effects of iron on various microorganisms in anammox systems should be concerned to better understand the iron-assisted anammox process.

3.6. Expanding the nitrogen removal pathways

The excellent nitrogen removal process of the anammox system does not depend solely on AnAOB, which can be explained according to its reaction equation (Eq. (1)), the theoretical TNRE by anammox is only 88.79 %, and 11.21 % of the nitrate accumulated in the system as a by-product (Kartal et al., 2010; Kuenen, 2008). Therefore, it's also essential to focus on the influence of iron on other nitrogen removal pathways and their combination with anammox.

Iron could enhance the metabolism of AnAOB in anammox systems. Also, it serves as a potential energy source that can be used as an electron donor or electron acceptor to participate in nitrogen removal (Tan et al., 2022; Wan et al., 2021). When iron was added to anammox systems, several reactions could occur, such as NH_4^+-N oxidation by Fe (III) (anammox-bound ferric reduction, feammox; Eqs. (2)–(4)), NO_3^--N reduction by Fe (II) (nitrate-dependent anaerobic ferrous oxidation, NDFO; Eqs. (5)–(8)), dissimilatory nitrate reduction to ammonium (DNRA; Eqs. (7), (8) and (10)), NO_3^--N reduction by ZVI (NDZO; Eqs. (9)–(11)), denitrification, etc. Their roles in nitrogen removal are shown in Fig. 6 briefly, demonstrating that iron plays an important role in nitrogen removal and N-cycling (Li et al., 2021; Shu et al., 2016; Yang et al., 2020a; Yang et al., 2021). Additionally, it would be beneficial to combine anammox with other nitrogen transformation processes in order to enable more efficient use of the nitrogen and reduce the limitations of the current methods.

$$3Fe(OH)_3 + 5H^+ + NH_4{}^+ \rightarrow 3Fe(II) + 9H_2O + 0.5N_2 \tag{2}$$

$$6Fe(OH)_3 + 10H^+ + NH_4{}^+ \rightarrow 6Fe(II) + 16H_2O + NO_2^- \tag{3}$$

$$8Fe(OH)_3 + 14H^+ + NH_4{}^+ \rightarrow 8Fe(II) + 21H_2O + NO_3^- \tag{4}$$

$$2NO_3^- + 10Fe(II) + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+ \tag{5}$$

$$2NO_2^- + 6Fe(II) + 14H_2O \rightarrow 6Fe(OH)_3 + N_2 + 10H^+ \tag{6}$$

$$6Fe(II) + NO_2^- + 8H^+ \rightarrow 6Fe(III) + NH_4{}^+ + 2H_2O \tag{7}$$

$$8Fe(II) + NO_3^- + 10H^+ \rightarrow NH_4{}^+ + 8Fe(III) + 3H_2O \tag{8}$$

$$5Fe^0 + 2NO_3^- + 12H^+ \rightarrow 5Fe(II) + N_2 + 6H_2O \tag{9}$$

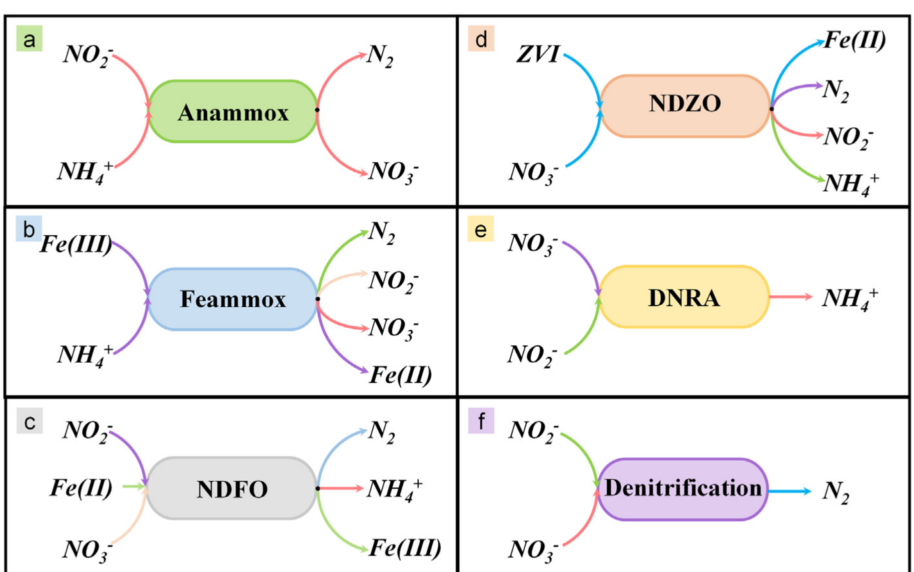

Fig. 6. The nitrogen removal pathways that usually occurred in iron-assisted anammox processes. Arrows of the same color in each pathway indicate that the reactant or product exists in every possible pathway, and similarly, arrows of different colors indicate that the reactant or product exists only in one possible pathway.

$$4Fe^0 + NO_3^- + 10H^+ \rightarrow 4Fe(II) + NH_4^+ + 3H_2O \quad (10)$$

$$Fe^0 + 2NO_3^- + 4H^+ \rightarrow Fe(II) + 2NO_2^- + 2H_2O \quad (11)$$

Feammox combined with anammox was considered as a potential nitrogen loss pathway in farmland and aquatic ecosystems, especially in paddy soil (Ding et al., 2022; Ding et al., 2021a; Qin et al., 2019). Meanwhile, anammox combined with DNRA process could achieve more efficient nitrogen removal, which was expected as an effective way to solve the incomplete nitrogen removal of anammox (Ahmad et al., 2021; Valiente et al., 2022). Among microorganisms, Fe-cycling is catalyzed by iron-oxidizing bacteria (FeOB) and iron-reducing bacteria (FeRB), which play key roles in global nitrogen and iron cycling (Melton et al., 2014). Up to now, only one species of functional feammox bacteria (*Acidimicrobiaceae* sp. *A6*) has been thoroughly probed, which could use Fe (III) to complete the feammox process with CO_2 as the carbon source (Huang and Jaffé, 2018). Certainly, the researchers have also found that some other iron-reducing bacteria could promote the feammox operation (Tan et al., 2022). Unfortunately, the reaction of feammox is slower than that of anammox. The reason is that most of Fe (III) in the natural state exists in the form of insoluble iron oxide, which limits the utilization of Fe (III) by microorganisms. Generally, Fe (III) utilization is promoted through direct contact, iron chelation and electron shuttle (Wan et al., 2021). A large number of microorganisms that could participate in the DNRA process had been identified, but the role of DNRA in N-cycling and the factors controlling DNRA need further research (Pandey et al., 2020; van den Berg et al., 2015).

Feammox combined with NDFO could form a nitrogen removal system similar to the anammox process. This process could complete the removal of ammonia and nitrate without the participation of AnAOB. Yang et al. (2021) even found that the NDFO process could achieve the reduction of NO_2^--N without the participation of bacteria. It indicated that the iron-driven autotrophic biological nitrogen removal technology (Fe-BNR) could be microbial mediated or abiotic mediated (Pang et al., 2022; Yang et al., 2020b; Yang et al., 2021). Although Fe-BNR shows great potential in wastewater treatment. Unluckily, its underlying microbiological processes have not been yet elucidated, and further research is needed to achieve breakthroughs (Yang et al., 2018a).

Since various iron-based nitrogen conversion pathways could be generated in iron-assisted anammox processes, it deserves further discussion about the condition for the coexistence of AnAOB and other related bacteria to give full play to the functions of anammox and iron-based nitrogen removal pathways. Similar to AnAOB, FeOB and FeRB were affected by various external conditions (pH, temperature, DO, organics, etc.), but fortunately, they were stronger than AnAOB. Therefore, it was allowed to consider more about the survival needs of AnAOB during the operation of iron-assisted anammox processes (Tan et al., 2022; Zhu et al., 2021). Previous studies have proven that iron-based nitrogen removal processes could be completed independently without the participation of AnAOB. However, continuous iron supplementation is required in this process, which is not feasible in practice (Yang et al., 2020b; Yang et al., 2021). Hence, maintaining the dominant position of AnAOB in the iron-assisted anammox process was needed (Kartal et al., 2010; Ma et al., 2017). In addition, precipitations of Fe (II) and Fe (III) would inhibit microbial metabolic activity during iron-assisted anammox, it is important to apply appropriate iron sources. Liang et al. (2022) suggested that the addition of polymeric ferric sulfate could alleviate the potential $Fe(OH)_3$'s inhibition. Meanwhile, the Fe—C carrier had also been proven to be an effective mitigation measure (Hu et al., 2022; Xie et al., 2020).

4. Conclusions and prospects

Anammox is ideal for treating ammonium-rich and low-carbon-containing wastewater. However, many inhibitors limit its large-scale application. Fortunately, in iron-assisted anammox systems, especially in the anammox system with stable and continuous Fe (II) supply, fast start-up, stable operation and high TNRE could be achieved and showed various strengthening mechanisms. Mainly incorporates improving the living environment of AnAOB along with increasing its abundance and activity; stimulating the secretion of EPS and granulation of sludge; strengthening the QS; regulating the expression of functional genes and the synthesis of enzymes together with expanding the nitrogen removal pathways. However, previous studies on the performances and mechanisms of iron-assisted anammox processes are not comprehensive enough. Future research should mainly focus on the following aspects:

(1) Under the conditions of different reactor types, reaction conditions, and microbial community structures, the results obtained by iron-assisted anammox are quite different. Several general iron dosing amounts, adding ways and iron occurrence forms to maintain the sustainable positive effects of iron for anammox systems should be determined in further research. As a cheap and easily available industrial by-product, intermittent addition of waste scrap iron releases Fe (II) continuously and stably, which has great potential to strengthen the anammox process.
(2) Iron enhanced nitrogen removal processes had been used to treat real wastewater. For example, Wang et al. (2018) used iron scraps to strengthen the treatment of digested effluent, and increased the TNRE from 1.83 % to 93.3 %. Yang et al. (2018b) found that 20.1 % of TN was removed by dosing $Fe(OH)_3$ during the anaerobic digestion of sludge. However, there is a great lack of research on iron-assisted anammox processes used in actual scenarios. Although iron could enhance the anammox process in synthetic wastewater, the pollutants in the real wastewater are diverse and complex. Therefore, the relationship between different forms of iron and other pollutants, as well as the enhancement performance of iron on anammox processes in real wastewater, need to be further studied and explained.
(3) Meanwhile, it is also necessary to further study the strengthening mechanisms of iron in anammox processes from the microscopic perspective. Although many possible mechanisms have been proposed at present, there is still a lack of a unified and clear mechanism on how iron in different forms affects the anammox process. In future research, more genomics, proteomics and transcriptomics should be used to fully explain the proposed possible mechanisms and to propose new mechanisms, to better understand the iron-anammox process.
(4) Anammox process produces by-product nitrate, which is a fatal blow to the TNRE of the nitrogen removal system. Therefore, the research should focus on the iron-based combined nitrogen removal measures and achieve the effective combination of anammox and AuDen to improve the TNRE of the system. Both iron and inorganic carbon additions to the anammox system could enhance the anammox process. Hence, iron combined with carbon such as Fe—C micro-electrolysis and iron-modified inorganic carbon could be promising.
(5) Iron-assisted nitrogen removal process has not been used yet in full-scale plants. The reason could be contributed to the effects of iron on excess sludge have not been fully evaluated yet. Therefore, it is also of great significance to clarify the effects of iron on excess sludge and the recovery of iron in anammox processes.

CRediT authorship contribution statement

Ben Dai: Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

Yifeng Yang: Funding acquisition; Writing – review & editing.

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Jiangming Wang: Formal analysis, Visualization.

Lin Yang: Formal analysis, Writing – review & editing.

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Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare no competing interests.

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