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RESEARCH ARTICLE

Enhancement of iron-based nitrogen removal with an electric–magnetic field in an upflow microaerobic sludge reactor (UMSR)

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Abstract

Traditional denitrification often produces high operating costs and excessive sludge disposal expenses due to conventional carbon sources. A novel electric–magnetic field (MF) 48 mT with Fe^0 and C-Fe^0 powder in an upflow microaerobic sludge reactor (UMSR) improved nitrogen removal from wastewater without organic carbon resources and gave richness to the heterotrophic bacterial community. In the current study, the reactor was operated for 78 ± 2 days, divided into five stages (without Fe^0, with Fe^0, coupling with MF, without coupling with MF, and coupling with MF again), at a hydraulic retention time (HRT) of 2.5 h, with an influent loading of ammonium (NH_4^+-N) 50 ± 2 mg/L, at 25–27 °C, and less than 1.0 mg/L dissolved oxygen (DO). The results demonstrated nitrogen removal efficiency enhanced after coupling with MF on the levels of NO_3^--N by 76% with an effluent concentration of 8.7 mg/L, NH_4^+-N by 72% with an effluent concentration of 13.6 mg/L, and total nitrogen removal (TN) by 76%, respectively. After coupling the MF with the reactor, the microbial community data analysis showed the dominant abundance of ammonia-oxidizing bacteria, heterotrophic nitrifying bacteria, and denitrifying bacteria on the level of *Anaerolineaceae_uncultured* 2%, which is capable of denitrification that uses Fe^{2+} as an electron source, *Gemmatimonadaceae_uncultured* 4%, *Hydrogenophaga* 4% which is capable of catalyzing hydrogenotrophic denitrification and correlating to nitrate removal, denitrification and desulfurization bacteria *SBR1031_norank* 18%, anammox-bacteria *Saccharimonadales_norank* 2%, and (AOM) *Limnobacter* 3% in the sludge.

Keywords Magnetic field · Nitrogen removal · Nitrate removal · Nitrite removal · Microbial community richness

Introduction

In anoxic conditions, heterotrophic or/and autotrophic denitrification bacteria utilize organic carbon sources as electron donors to convert nitrite and nitrate into free nitrogen gas. A lack of organic material often slows denitrification; thus, a carbon source is added to the process to convert nitrate into nitrogen gas. However, external carbon sources in wastewater treatment plants (WWTPs) have increased the breadth of safety problems, high risk, and high operating management cost of heterotrophic denitrification associated with selecting optimal carbon sources. Instead of employing organic carbon, zero-valent iron (Fe^0) is used as the electron donor. Zero-valent iron (Fe^0) is a widely available, low-cost, non-toxic, easy-to-handle material (Fu et al. 2014). Many contaminants, including halogenated organics, nitrate, dyes, and phenol, have been successfully removed from groundwater and wastewater using Fe^0 (Jiang et al. 2008; Siddiqui et al. 2013; Fu et al. 2014). However, Fe^0 is used alone as an electron donor to remove nitrate from the environment by autotrophic and heterotrophic denitrification (Aslan and Türkman 2005; Hosseini et al. 2011; Zhao et al. 2012). As a result of the Fe^0 corrosion process, H_2, Fe^{2+}, and Fe^{3+} are released, and hydrogen can be used as the final electron donor for heterotrophic denitrification (Zhang 2002).

However, when Fe^0 is used to treat wastewater, oxidants such as dissolved O_2, H_2O, and NO_3^- consume the bulk of the Fe^0 (Noubactep et al. 2009); decreasing

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NO_3^- concentration by Fe^0 can represent the following Eq. (1) (Park et al. 2008):

$$4Fe^0 + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^- \quad (1)$$

The nitrite formation occurs by nitrate reduction to nitrite by the iron surface (Liu and Wang 2019), as shown in Eq. (2). It is an initial chemical step to reduce nitrate when electrons transfer from the Fe^0 surface to NH_4^+, as shown in Eq. (1), or reduced to nitrite by Fe^{2+}, as shown in Eq. (3), or an electron donor in anoxic, or by some bacteria as shown in Eq. (3).

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

$$2Fe^{2+} + NO_3^- \rightarrow NO_2^- + 2Fe^{3+} \quad (3)$$

The nitrate-dependent Fe^{2+} oxidized (NDFO) reaction has recently been found to oxidize NO_3^- to NH_4^+ by Fe^{2+} in an abiotic environment (Carlson et al. 2013) or chemically oxidized to N_2, as shown in Eqs. (4, 5) (S⊘ Rensen 1987), or by bacteria in the presence of hydrogen ion:

$$10Fe^{2+}_{(aq)} + 2NO_3^- + 24H_2O \rightarrow N_{2(g)} + 10Fe(OH)_{3(s)} + 10H^+_{(aq)} \quad (4)$$

$$10Fe^{2+} + 2NO_3^- + 12H^+ \rightarrow N_2 + 10Fe^{3+} + 6H_2O \quad (5)$$

Consequently, through the Feammox process, Fe^{3+} is utilized as an electron acceptor to oxidize NH_4^+. The NH_4^+ oxidation with Fe^{3+} reduction (Feammox) was defined as oxidizing NH_4^+ to produce N_2, NO_2^- or NO_3^- through reducing Fe^{3+} and reducing to Fe^{2+}, as shown in Eq. (6):

$$NH_4^+ + 2H_2O + 6Fe^{3+} \rightarrow NO_2^- + 6Fe^{2+} + 8H^+ \quad (6)$$

At the same time, Fe^{2+} is employed as an electron donor to decrease NO_3^-. Because Fe^{3+} has fewer electrons and is more stable than Fe^{2+}, coupling of the NDFO and Feammox reactions was studied (Xu et al. 2016b), with Fe cycling used as a catalyst to decrease the need for Fe ions to prevent sludge mineralization.

However, the conversion between nitrite and Fe^{2+} and decreasing pH will produce N_2O (Park et al. 2008), then N_2O can act as an electron acceptor by electrons donated by NO_3^- to produce N_2O and N_2 following Eqs. (7–9):

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + 6H_2O \quad (7)$$

$$NO_2^- + 2e^- + 2H^+ \rightarrow 0.5NO_2 + 1.5H_2O \quad (8)$$

$$0.5NO_2 + 2e^- + H^+ \rightarrow 0.5N_2 + 0.5H_2O \quad (9)$$

When Fe^0 is used, the aging of Fe^0 and its limited reactivity are critical issues for Fe^0-based technology (Xu et al. 2016b). Therefore, finding practical ways of significantly increasing Fe^0 reactivity is crucial. Many researchers employed various technologies to enhance Fe^0 reactivity, such as ultrasonic, acid washing, H_2-reducing pretreatment (Lai and Lo 2008), electrochemical shorthand (Chen et al. 2012), Fe^0-based bimetals (Lim et al. 2007), and nanosized Fe^0-nFe^0 (Huang et al. 2013). These technologies are always complicated, costly, and hazardous to the environment (Jiang et al. 2015). As a result, other technologies for increasing the reactivity of aging Fe^0, such as pre-magnetization or applying a weak permanent magnetic field (WMF) with Fe^0, were used to eliminate high concentrations of p-nitrophenol (PNP), SO_2 removal (Jiang et al. 2008; Siddiqui et al. 2013), accelerate chloroacetamide removal from drinking water, enhance phenol degradation, partial nitrification, CH_4 production, triethyl phosphate degradation, and antibiotic degradation (Wang et al. 2015; Wang et al. 2017; Chen et al. 2019; Huang et al. 2019; Pan et al. 2019, et al. 2019).

Additionally, several studies have found that the effects of permanent magnets or electromagnets can change water pH, oxidation–reduction potential (ORP) (Yin et al. 2011; Hassan and Rahman 2016), increase electron density, and promote electron transfer for redox reactions (Yap et al. 2021). The magnetic field mainly affects the material's properties, structure, photocatalysis, electrodynamics, synthesized reaction, isomerization reaction, nuclide enhancement reaction, increasing electron density in water, and metabolic reaction (Hassan and Rahman 2016). Coupling magnetic field (MF) with Fe^0 is chemical-free (Xu et al. 2016a); it contributes to the release of Fe^{2+} from the Fe corrosion process (Li et al. 2017b; Ren et al. 2018), which can significantly improve the pollutants' targeting, reduce Fe^0 doses, extend the operating pH range (Sun and Guan 2019; Wang et al. 2020b), and give energy to the donor electron (Salehani et al. 2010), and influence the anions' movement simultaneously with paramagnetic Fe^{2+} to keep electroneutrality (Sun and Guan 2019). The theoretically essential point of coupling electric–magnetic (MF) with Fe^0 in this study is to promote the increase of Fe^{2+}, accelerating the Fe^{3+} reduction to Fe^{2+} because the reduction rate of Fe^{3+} is much slower than the oxidation rate of Fe^{2+} because the concentration of Fe^{2+} would decrease rapidly during pollutants' depredation (Chu et al. 2021).

However, many researchers are working to achieve a low dose of Fe^{2+} with effective pollutant degradation and removal from wastewater (Taherdanak et al. 2016). Fe^{2+} will promote decreasing pH and increase the reaction rate of non-target substrates (H^+ and O_2) with Fe^0 and would undoubtedly increase the Fe^0 corrosion rate concomitantly with pollutants (Guan et al. 2015). Therefore, most bacteria species use Fe^{2+} as the electron donor for metabolism (Straub et al. 2004), promoting microbial abundance

diversity and increasing bacteria's metabolism by promoting Fe^{2+} (Li et al. 2017a). Anaerobic denitrifying Fe^{2+} oxidation bacteria grow by using Fe^{2+} as a source of energy and electron donor in marine environments or freshwater with a narrow pH range to 7 (Kappler et al. 2005; Hedrich et al. 2011). An essential point in this study is to enhance nitrogen removal efficiency in the upflow microaerobic sludge reactor (UMSR) by coupling the electric–magnetic field (MF) with Fe^0. By coupling the electric–magnetic field (MF) with Fe^0, there is a possibility of continuously releasing H_2, Fe^{2+}, and Fe^{3+} from the Fe^0 corrosion process to give activity to some heterotrophic nitrifying bacteria and denitrifying bacteria that depend on Fe^{2+} and Fe^{3+}, or H_2 for metabolism in the absence of the organic carbon source. Additionally, coupling the electric–magnetic field (MF) with Fe^0 may prevent Fe^{3+} precipitation on the bacterial cell surface by chemically reducing Fe^{3+} to Fe^{2+}. This research aimed to study a novel upflow microaerobic sludge reactor (UMSR) operated for 78 ± 2 days continuously, under five operating stages with and without coupling electric–magnetic field (MF) with Fe^0. At the same time, the NH_4^+-N, NO_3^--N, NO_2^--N, TN removal, and microbial community structure were evaluated in this research.

Methods and materials

As shown in Fig. 1, the UMSR has an inner volume of 4.9 L, and the HRT was 2.5 h; the influent was injected from the bottom of the reactor. A reflux aeration tank controlled dissolved oxygen (DO) in the reactor was held at less than 1.0 mg/L by a reflux aeration tank. An extra heating system maintained the temperature at 25–27 °C by a supplementary heating system. The electric–magnetic field of 40 mT was adjusted by 6 V–50 Hz alternate current (AC) and put deep into the sludge. The UMSR continuously operated for 78 ± 2 days, divided into five operating stages of 15 ± 1 days for each stage (without Fe^0, with Fe^0, coupling with MF, without coupling with MF, and coupling with MF again) until the Fe^0 corrosion was completed.

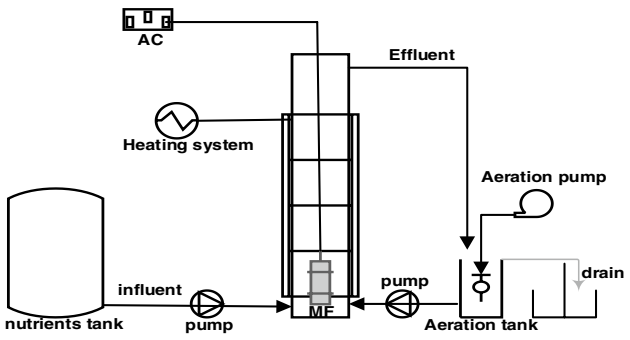

Fig. 1 Schematic diagram of USMR setup

Synthetic nutrients and sludge

The nutrients in this synthetic wastewater were composed of (mg/L): NH_4Cl (190), KH_2PO (25.2), $KHCO_3$ (124.8), $CaCl_2$ (300), and $MgSO_4$ (200). The inoculated sludge was taken from the Taiyuan wastewater treatment plant, with the mixed liquor suspended solids (MLSS) and the mixed liquor volatile suspended solids (MLVSS) being 2.42 mg/L and 1.936 mg/L, respectively. The ratio of Fe^0 powder and Fe–C powder was 3:1.

Analytical methods and equipment

Before experimenting with new conditions, the reactor was operated at its optimized condition for more than 360 days at 25–27 °C. An external heating wire and a temperature controller are used to regulate the inside temperature of the reactor (XH-W2140, China). The pH and temperature were measured using pH and temperature meters (Hanna Instruments CAL Check™ HI5221). The concentrations of NH_4^+-N, NO_3^--N, NO_2^--N, and TN in effluent were measured daily using standard methods (Association 1915).

Microbial community analysis

The sludge samples were taken from three continual operation conditions (before adding Fe^0, after adding Fe^0, and after coupling MF with Fe^0) labeled as (S1, s2, and s3) to identify the microbial community structure during that three operating conditions. They were sent to Shanghai Lingen Biological Technology Co., Ltd to analyze the microbial diversity. After completing the genomic DNA extraction, genes were amplified using the specific primer with the barcode, 16S V4-V5: 515F–907R, 18S V9: 1380F–1510R, ITS1: ITS1F–ITS2R. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina MiSeq platform, and 250 bp/300 bp paired-end reads were generated.

Results and discussion

Parameters with operating conditions and reactor performance

Table 1 summarizes the performance of the UMSR and coupling electric–magnetic field (MF) system concerning the NH_4^+-N, NO_3^--N, NO_2^--N, and TN removal for each stage of operation. For these data, the process continued for 78 ± 2 days until the Fe^0 was corroded under an anaerobic condition with electric–magnetic field effects.

Table 1 Parameters and operating conditions

Operating stages at (HRT) 2.5 h	Effluent NH_4^+-N (mg/L)	Effluent NO_2^--N (mg/L)	Effluent NO_3^--N (mg/L)	Nitrification rate (mg/L day)	Effluent PH	NO_3^--N removal (%)	NH_4^+-N removal (%)	TN removal (%)
Stage (1)	26.2	0.1	9.9	1.2	7.8	58	47	58
Stage (2)	21.9	2.3	9.2	1.3	7.1	64	56	59
Stage (3)	13.6	0.5	8.7	2.3	5.7	76	72	75
Stage (4)	12.4	0.7	11.2	2.5	6.8	55	75	68
Stage (5)	12.9	0.1	8.7	2.4	7.5	76	74	76

NO_3^--N removal efficiency

As shown in Fig. 2, the concentration of NO_3^--N in the effluent dropped quickly for the first few days after Fe^0 was added to the reactor. After a few days, the concentration of NO_3^--N increased again. After two periods of coupling MF with Fe^0 (first coupling with MF and second coupling with MF), the NO_3^--N concentration dropped to a stable level. The NO_3^--N removal efficiencies were 58% before adding Fe^0, 64% after adding Fe^0, 76% at first coupling with MF, 55% without coupling MF, and 76% at second coupling with MF, respectively. The highest and most stable removal was observed during MF coupled with Fe^0. Coupling Fe^0 with electric–magnetic provides stability for nitrate removal by producing H_2 and Fe^{2+}. It is one of the factors that donate to denitrification by Fe^0 corrosion. It can also happen that hydrogen can be used as an electron donor because of the corrosion of Fe^0 in a magnetic field (Liu and Lowry 2006; Němeček et al. 2014). The magnetic field affects electrode kinetics by the accelerated effect on the cathodic reaction of the single Fe^0 corrosion process. It will affect the Fe^{3+} reduction to Fe^{2+} in this Fe^0 recycling. It is possible for nitrate-dependent Fe^{2+} oxidized (NDFO)

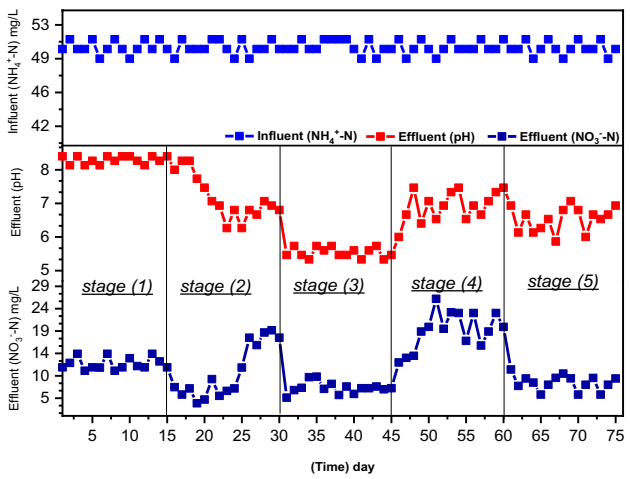

Fig. 2 Effluent pH and NO_3^--N concentration

to occur by producing more Fe^{2+} because the reductive efficiency of nitrate by Fe^0 increased by the increase in dissolved ferrous ion concentration ($Fe^{2+}{}_{aq}$). It may also release cathodic H_2 by reducing water-derived protons (Weathers et al. 1997), as shown in Eqs. (10, 11).

$$Fe^0_{(s)} + H_2O \rightarrow Fe(OH)_{2(aq)} + H_{2(g)} \tag{10}$$

$$Fe^0_{(s)} + 2H^+ \rightarrow Fe^{2+}_{(aq)} + H_{2(g)} \tag{11}$$

As a favored electron donor, hydrogen gas produced by Fe^0 corrosion can be an advantage for several metabolic groups of denitrifying bacteria. The Fe^{2+} oxidation rate could influence various abiotic factors, including a concentration in anionic species; for instance, concentration in anionic species such as Cl^- in water will reduce the Fe^{2+} oxidation rate and laded to a higher accumulation of Fe^{2+} (Adeleye et al., 2013). The oxidation of Fe^{2+} increases the hydrogen ion H^+ concentration in the water, which reflects on the pH, as shown in Eqs. (4, 6). The pH is an important parameter that strongly affects the nitrate reduction by Fe^0 because the nitrate requires proton participation, which affects Fe^0 corrosion rate and Fe corrosion product, and Fe^{2+} occurs at lower pH (Weathers et al. 1997). It was decreased at the first stage of adding Fe^0 to the reactor. The primary way to reduce NO_3^- is by Fe^0; the Fe^0 acts as an electron donor to reduce NO_3^- by Fe^0 surface. The nitrate reduction depends on electron transfer efficiency from the Fe^0 surface $Fe^0 \rightarrow Fe^{2+} + 2e^-$.

The existence of Fe^0 and Fe^{2+} may involve other reactions. They are sources of electrons and protons, as shown in Eqs. (12, 13):

$$3Fe^0 + 4H_2O \rightarrow Fe_3O_4 + 8H^+ + 8e^- \tag{12}$$

$$3Fe^{2+} + 4H_2O \rightarrow Fe_3O_4 + 8H^+ + 2e^- \tag{13}$$

Hematite (Fe_2O_3) and magnetite (Fe_3O_4) were discovered in these probable reactions, as shown in Eqs. (14, 15). It would play a part in the pH decreasing due to Fe^0

corrosion serving as an electron donor from the Fe^0 surface $Fe^0 - 2e \rightarrow Fe^{2+}$.

$$2Fe^0 + 3H_2O - 6e^- \rightarrow Fe_2O_3 + 6H^+ \quad (14)$$

$$3Fe^0 + 4H_2O - 8e^- \rightarrow Fe_3O_4 + 6H^+ \quad (15)$$

Moreover, the nitrate reduction with H^+ will be consumed at neutral pH:

$$NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O \quad (16)$$

When magnetic coupling in all phases, the lowest pH was at the first operating conditions when MF coupling with Fe^0. The pH value decreased to 5.2 at the first stage of coupling MF with Fe^0. The decreasing pH was low and the most stable at all stages of coupling MF with Fe^0, as shown in Figs. 2, 3, and 4.

NH_4^+-N removal efficiency

The effluent NH_4^+-N concentration increased significantly from 21 to 35 mg/L in the first 4 days after Fe^0 was added to the reactor, followed by a decrease in NO_3^--N concentration from 11 to 6 mg/L. Because the reaction between Fe^0 and NO_3^- converts NO_3^- to NH_4^+, as shown in Eq. (1), it increases the NH_4^+ concentration. The nitrogen removal efficiency could not increase during the first few days after Fe^0 was applied to the reactor. It was noticed that the highest NH_4^+-N removal occurred during stage (3) when MF was first coupling with Fe^0. The coupling of NH_4^+ oxidation to NO_2^- with Fe^{3+} reduction is possible, as shown in Eq. (6). After a few days, the NH_4^+-N concentration became lower day by day and stable, as shown in Fig. 3; the NH_4^+-N removal efficiencies were 47% before adding Fe^0, 56% after adding Fe^0, 72% at the first coupled MF with Fe^0, 74% without coupling with MF, and 75% at the second coupled MF with Fe^0, respectively. Thus, the NO_2^- produced from NH_4^+ oxidation with Fe^{3+} will be involved in the ANAMMOX reaction of ammonia, as shown in Eq. (17):

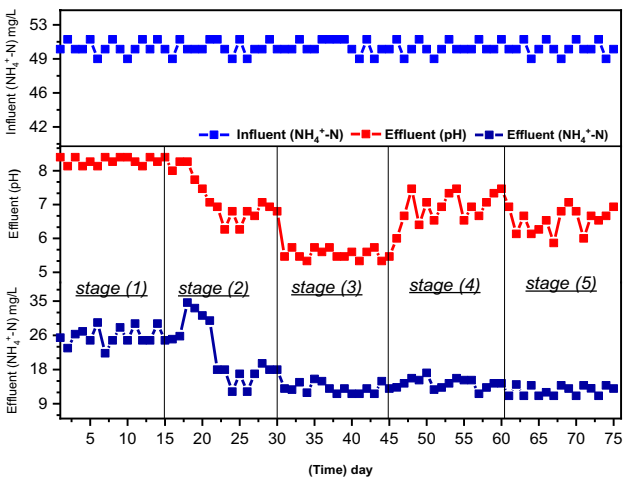

Fig. 3 Effluent pH and NH_4^+-N concentration

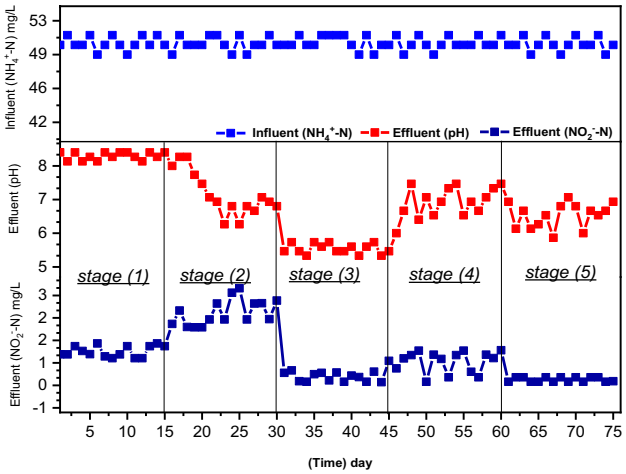

Fig. 4 Effluent pH and NO_2^--N concentration

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (17)$$

NO_2^--N removal efficiency

The NO_2^--N concentration increased to 2.3 mg/L during stage (2) of adding Fe^0 to the reactor due to nitrite formation caused by nitrate reduction, as shown in Eqs. (2, 3) or by NH_4^+ oxidation with Fe^{3+} (Feammox) process, as shown in Eq. (6), whereas the NO_2^--N concentration before adding Fe^0 was 0.1 mg/L, 0.5 mg/L at first coupled MF with Fe^0, 0.7 mg/L without coupled MF, as shown in Fig. 4. Thus, the ANAMMOX reaction that makes increases NO_2^- concentration, as shown in Eq. (18)

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (18)$$

While the Fe^{3+} serves as an electron acceptor, Fe^{3+} oxidized NH_4^+ into NO_2^- and NO_3^-, and the effluent's NO_2^--N concentration was accumulated. On the other hand, by accumulated NO_2^-, the ANAMMOX reactions with NH_4^+ and NO_2^- play a part in the process of reducing NO_2^-, as shown in Eq. (19):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3 + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (19)$$

As well, the electron donated by NO_3^- or which is released by Fe^0 surface with a high concentration of H^+, the NO_2^- will act as an electron donator to produce N_2O, and then N_2O will serve as an electron acceptor to produce N_2, as shown in Eqs. (7–9), which gives removal stability and a decrease in NO_2^- accumulation. Moreover, other research has shown that the adsorption of Fe^{2+} on iron oxides (e.g., Fe_3O_4, FeOH, –FeO(OH)) is crucial in decreasing NO_3^-, NO_2^-, and NH_4^+. Fe^{2+} ions ionize H^+, which may be adsorbed on the surface and reduced to active NO* by hydrogen produced by Fe corrosion after reducing NO_3^- to NO_2^-. This approach was not discussed in this work regarding electric–magnetic field effects.

Structure and function of the microbial community in the reactor

As shown in Fig. 5a, b, microbial community analysis detected that relative abundance changed at the levels of phyla and genus due to the changes in conditions. (The relative domain abundance > 1%). A notable increase and changes in diversity and a decrease in certain bacteria were detected in all samples associated with the three operating conditions. After adding the Fe^0 and coupling it with the magnetic field, there were changes in certain bacteria's richness, abundance, and inhibition.

At the phylum level, the most bacteria abundance of nitrogen removal was identified, including nitrifiers, denitrifiers, and anammox bacteria. Proteobacteria were the most dominant phylum in S1 of which 56% are known for their metabolic diversity and variety including denitrifiers, which are used in crucial environmental cycles such as carbon, nitrogen, sulfur, and phosphorus (Friedrich, Bardischewsky et al. 2006; Meier et al. 2016) and the detected genera in S1 are *Rhodanobacter* of which 16% is capable under acidic and anaerobic conditions of producing N_2 and N_2O by using nitrate, nitrite, and nitrous oxide as electron acceptors (Van Den Heuvel et al. 2010) and *Denitratisoma* of which 14% is anaerobic oxidative that reduces nitrate to N_2O and N_2 and may lead to nitrite accumulation with some N_2 fixation strains (Fahrbach et al. 2006); 45 of *SWB02* and 2% of *Ellin6067* oxidize nitrites to nitrates (NOB) (Fumasoli et al. 2015); 4% of *Thermomonas*, sulfur oxidizers, and carbon fixing convert CO_2 to carbonate, where nitrogen fixation is related (Castelán-Sánchez et al. 2020); 3% of *Limnobacter* is heterotrophic sulfur oxidation and may adapt with anaerobic methane oxidizer bacteria (AOM) (Chen et al. 2016), and 1% of *Thiobacillus* obtains energy from oxidation and reduction of ferrous and sulfide. Moreover, *Thiobacillus* reduces nitrate, nitrite, and other nitrogen compounds to dinitrogen (Kelly and Wood 2000).

However, a sharp decrease in the level of the Proteobacteria was noted after adding the Fe^0 to the reactor, while richness in diversity and diminishing were observed in both samples s2 and s3, at the level of genus *Stenotrophomonas*, which can use nitrate as a terminal electron acceptor for growth with oxygen absence (Crossman et al. 2008) of 4% in s2, *Brevundimonas* which have potential of N_2 fixation (Jiang et al. 2022) of 2% in s2, *Diaphorobacter* under anaerobic conditions reduces nitrate and nitrite to N_2 (Qiu et al. 2015) of 1% in s2, *Qipengyuania* of 1% in s2 has some strains that reported can participate in biogenic nitrogen cycling (Liu et al. 2022), *Pseudoxanthomonas* of 2% in s2 and s3 it is anaerobic methane oxidation bacteria (AOM) it can utilize methane as electron donor to reduce NO_2^-, NO_3^-, Fe^{3+}, and SO_4^{2-}, it typically require additional electron donors such as hydrogen (Fu et al. 2019), *Hydrogenophaga* of 4% in s3 it is capable nitrate denitrification and using the oxidation of H_2 as an energy source and CO_2 as a carbon source (Iannacone et al. 2020; Xu et al. 2021), *Pseudomonas* of 6% in s2, 4% in s3 it has been reported that hydrocarbon-degrading bacteria and may provide a carbon source and energy to other bacteria (Wang et al. 2020a), *Acinetobacter* of 4% in s2, 2% in s3 which use Fe^{2+} as electron donor be capable of denitrifying (Kiskira et al. 2017), the *Limnobacter* of 2% in s2, and of 3% in s3, *SC-I-84_norank* of 1% in s2 and s3, *Steroidobacteraceae_uncultured* was of 1% in s2, 2% in s3, the *Thermomonas* was of 1% in both samples s2 and s3, and *Xanthobacteraceae_uncultured* of 1% in s3, respectively.

Meanwhile, the nitrifiers (AOB) detected in the reactor included Nitrospirota and Bacteroidetes; these nitrifiers bacteria are often present in activated sludge. The

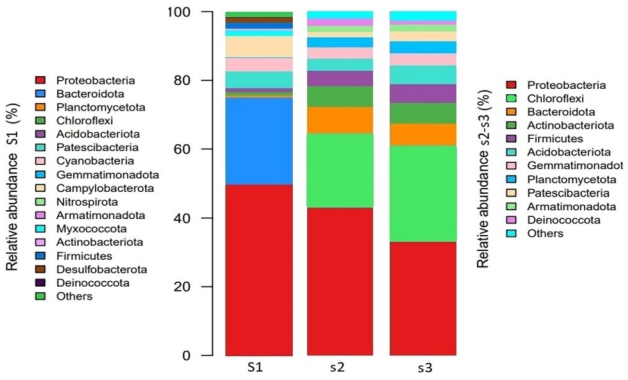

Fig. 5 **a** Relative abundance by phylum. **b** Relative abundance by genus

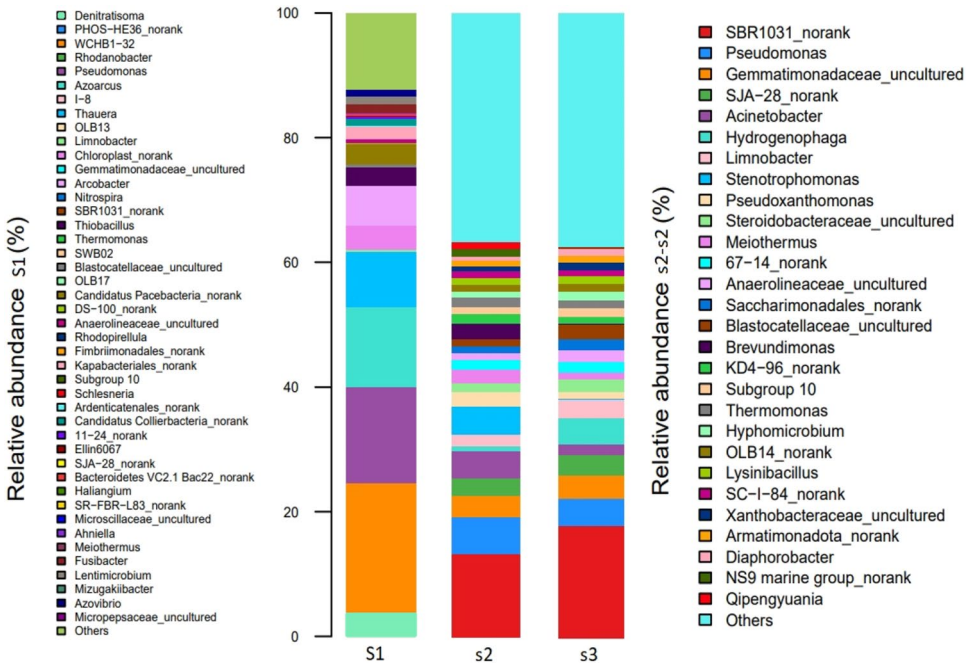

Fig. 5 (continued)

Nitrospirota was 5% in S1 and was shared by the genus *Nitrospira* of 5%. It is a chemolithoautotrophic nitrite-oxidizing bacterium and is essential for nitrification in the completed nitrogen cycle when it oxidizes nitrite to nitrate from ammonia (Yang et al. 2022). However, the *Nitrospira* may also give ammonia oxidizers released from urea or cyanate in interactions known as "reciprocal feeding." Recently found members of the *Nitrospira* can catalyze both nitrification stages independently, earning them the name complete ammonia oxidizers or "Comammox" bacteria (Koch et al. 2019). Certain *Nitrospira* strains may also use hydrogen and formate to supplement their aerobic nitrite oxidation with oxygen or nitrate as the terminal electron acceptor (Palomo et al. 2018; Koch et al. 2019). The *Nitrospira* can adapt to a wide range of oxygen conditions according to its metabolic system (Mehrani et al. 2020) as well as observe that at the level of Chloroflexi, it was not the most dominant bacteria in sample S1. Thus, after the Fe^0 was added or coupled with MF, Chloroflexi became one of the most predominant bacteria at 21% in S2 and 28% in S3, while it was 2% in S1. It was reported that chemoautotrophic desulfurization and denitrification of bacteria and one of the heterotrophic bacteria derive energy from H_2. It was found in low-pH soils with carbon dioxide fixation (Long et al. 2015; Islam et al. 2019). At the genus of desulfurization and denitrification bacteria *SBR1031_norank* of 13% in s2 and 18% in s3, heterotroph *Anaerolineaceae_uncultured* was 1% in s2 and 2% in s3. Chemolithotroph-denitrifying bacteria use Fe^{2+} as electron donor, *OLB14_norank* of 1% in s2 and s3, and *KD4-96_norank* by 1% in both samples s2 and s3, respectively. This can be explained by the effect of the magnetic field on Fe^0 erosion and releasing more hydrogen and ferrous ions that enter into its metabolism process, as well as possibly due to the richness of Firmicutes and hydrogen production in this condition. However, Firmicutes were absent before adding the Fe^0 to the reactor. It has been reported that when coupled with iron, it generates hydrogen (Mohan et al. 2011). Before adding Fe^0, Firmicutes were absent in sample S1; in contrast, after adding Fe^0 and coupling with MF, the abundance was 4% in s2 and 5% in s3, respectively, at the genus *Lysinibacillus* of 1% in both samples s2 and s3. This can be explained by some Firmicutes strains exploiting acids as a source of carbon and energy; the decrease in pH and the existence of Fe^0 provided the richness of the Firmicutes bacteria in the reactor.

Whilst, before adding Fe^0 to the reactor in sample S1, the Gemmatimonadota phylum of 6%, on the level of the genus *Gemmatimonadaceae_uncultured* of 6%. The Gemmatimonadota proportion decreased to 3% after adding Fe^0 in s2, and after coupling with MF, an enrichment of 4% was observed in s3, shared by the same genus *Gemmatimonadaceae_uncultured*. In contrast, the Gemmatimonadota groups have been found in various ecosystems and activated sludge used in wastewater treatment. These groups seem to depend on urea hydrolysis inside cells for energy (Chen et al. 2021; Mujakić et al. 2022). Anammox bacteria Planctomycetota of 4% in S1, 3% in s2 and s3. Planctomycetota

in S1 was shared by genus 2% of *Schlesneria*, which utilizes an organic nitrogen compound as nitrate and ammonium as nitrogen sources; after adding Fe^0 and coupling with MF, that genus vanished in both samples s2 and s3. After adding Fe^0 to the reactor and coupling with MF, the genus *Saccharimonadales_norank* has increased by 1% in s2 and 2% in s3 and noted that the genus *Patescibacteria* increased by 2% in s2 and 3% in s3, while it was 1% in S1, respectively.

In the same context, 1% of Armatimonadota in S1 and 2% in s2 and s3 has been shared by genus *Armatimonadota_norank* by 1% in S1 and 2% in samples s2 and s3, respectively. Furthermore, Actinobacteriota was absent in sample S1 and appeared after Fe^0 was added in s2 and s3 by 6%, with genus *67-14_norank* in both samples s2 and s3 by 2%. Actinobacteriota have some strains that produce organic acids and siderophores which might delay the biogenic Fe^{2+} reoxidation and decrease pH (Zhang et al. 2019). In anoxic conditions, many Actinobacteriota reduces Fe^{3+} in the iron cycle and plays as main important bacterium in the denitrification process (Huang et al. 2022). After adding Fe^0, Deinococcota abundance was increased and returned to the same proportion after coupling with MF at the same genus *Meiothermus* by 1% in S1, 2% in s2, and 1% in s3, which is chemoorganoheterotroph and use nitrate as the terminal electron acceptor (Song et al. 2021).

Conclusions

Adding Fe^0 and coupling it with the electric–magnetic was favorable for large-scale applications due to its simple operation, low cost, and high effectiveness for nitrogen removal. As a result, Fe^0 corrosion under an electric–magnetic field may provide Fe^{2+} and Fe^{3+} and be used as an electron donor to oxidize NO_3^- to NH_4^+. By producing H_2 and decreasing pH and using it as an electron donor donated by NO_3^- to produce N_2O and N_2, the NH_4^+-N, NO_3^--N, and TN removal efficiencies in the UMSR are enhanced under MF with Fe^0. Through coupling electric–magnetic with Fe^0, the energy would be served to electron donors from the Fe^0 surface and increase electron density. The direction of electron transfer from Fe^0 to nitrate would be directly converted into N_2; on the other hand, NO_3^- converted into NH_4^+ and NO_2^- before the coupling MF stage.

Moreover, adding Fe^0 and coupling with electric–magnetic to the reactor decreased the level of Proteobacteria and Nitrospirota. Changing microbial communities became the dominant abundance besides the Proteobacteria, such as Chloroflexi and Firmicutes. After Fe^0 was coupled with electric–magnetic, it gave richness to some anammox such as *Saccharimonadales_norank* and *Patescibacteria*. Denitrification bacteria depend on ferrous Fe^{2+} as the source metabolisms, such as *Anaerolineaceae_uncultured* and *Acinetobacter*, and ferric iron Fe^{3+} such as *Pseudoxanthomonas*, or use hydrogen ion such as *Hydrogenophaga*. However, some bacteria genera have been found with an apparent richness, and the functions have not been determined by researchers yet.

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Declarations

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