

Article

Abiotic Transient Nitrite Occurrences from Nitrate Reduction through Goethite-Mediated Fe(III)/Fe(II) Cycle with Labile Organic Materials and Ammonia

Hyeoksun Choi ¹  and Sanghwa Oh ^{2,*} 

¹ Department of Civil and Environmental Engineering, Wonkwang University, Iksan 54538, Korea; choihs@wonkwang.ac.kr

² School of Architectural, Civil, Environmental, and Energy Engineering, Kyungpook National University, Daegu 41566, Korea

* Correspondence: shoh@knu.ac.kr; Tel.: +82-53-950-5609

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Abstract: The abiotic reduction of NO_3^- to NO_2^- —coupled with the oxidation of labile organic materials such as citric acid, syringic acid and natural organic matter (NOM) and NH_4^+ through the goethite-mediated Fe(III)/Fe(II) cycle under anaerobic condition—was investigated at pH values of 4 and 7. The concentrations of the produced Fe^{2+} and NO_2^- were monitored. At a pH of 4, concentrations of Fe^{2+} increased, except for citric acid; no NO_2^- was detected. The reason why it was not detected is unclear. A possible reaction was the adsorption of NO_2^- onto goethite at $\text{pH} < \text{point of zero charge (pzc)}$ of goethite (6.42) due to electrical attractive force. The maximum production of NO_2^- at a pH of 7 was in the order of citric acid $\gg$ syringic acid $>$ NOM. However, Fe^{2+} was not detected at this pH even though Fe^{2+} should be required for NO_2^- production. To better understand of these phenomena, the adsorptive removal of Fe^{2+} and NO_2^- onto goethite was experimentally investigated. More than 90% of the produced Fe^{2+} and NO_2^- could be removed rapidly by adsorption onto the surface of goethite at pH 7 and 4, respectively. In addition, the reaction of Fe^{2+} with NO_3^- appeared to determine the overall reaction rate of the Fe(III)/Fe(II) cycle because of its relatively slow reaction rate. Using these results, we conclude that NO_2^- can be produced from NO_3^- reduction through Fe(III)/Fe(II) cycle with labile organic materials and ammonium at a pH of 7; especially, Fe(III)/Fe(II) cycle with citric acid results the maximum NO_2^- production higher than 600 μM for a long time (over 200 h) and then disappeared. But, the reasons for its disappearance were not addressed in this study.

Keywords: ammonia; Fe(III)/Fe(II) cycle; citric acid; syringic acid; natural organic matter (NOM); nitrite; goethite

1. Introduction

Nitrogen has many oxidation states and is important for biomolecules such as enzymes and nucleic acids. Ammonium (NH_4^+) and nitrate (NO_3^-) are two major nitrogen sources in surface and subsurface water systems because they are the final reduced and oxidized nitrogen under anaerobic and aerobic conditions, respectively and nitrite (NO_2^-) is frequently found under specific anaerobic conditions. Nitrogen contamination in surface water and groundwater has mostly been caused by ammonia and nitrate worldwide [1]. Among these nitrogen compounds, NO_3^- contamination is generally derived from the oxidation of NH_4^+ through nitrification by autotrophic bacteria. Ammonia has mainly come from the discharge of agricultural fertilizer and the mineralization of organic nitrogen under aerobic and anaerobic conditions [2–4].

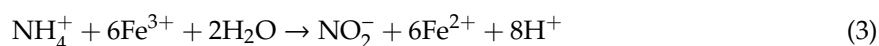
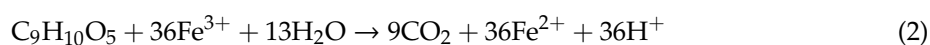
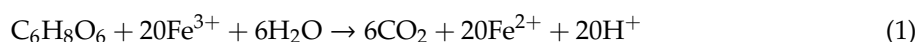
A soil horizon is a layer parallel to the soil surface. The O horizons are dominated by undecomposed or partially decomposed organic materials such as leaves, needles, moss and lichens and, in the A horizons which formed below O horizon, humidification of the organics occurs under anaerobic conditions. In addition, Fe(III) with a high concentration frequently becomes a major electron acceptor in soils and sediments [5,6]. In these conditions, ferric (Fe(III)) and ferrous (Fe(II)) cycle which is the reduction of Fe(III) to Fe(II) and the sequential oxidation of Fe(II) to Fe(III) has been an important way of involving the biotic or abiotic redox of organic or inorganic materials in anaerobic soils and sediments [6]. Abiotic reactions always occur independently regardless of biotic reactions under certain conditions and differ from biotic reactions in important ways for carbon and nitrogen cycles in nature. In nature, abiotic reactions have been an important role for the nutrient cycling and the change of environmental conditions because biologic processes does not correspond all of these abiotic reactions and abiotic reactions can occur anywhere under appropriate conditions [7].

Fe(III)/Fe(II) cycle plays a particularly important role in abiotic nitrogen oxidation or reduction in forest and wetland soils because iron is the most abundant metal in the soil [6,8]. Davidson et al. [8] suggested the iron-mediated rapid abiotic immobilization of NO_3^- and transient generation and sequential disappearance of NO_2^- via Fe(III)/Fe(II) cycle. Zhu-Barker et al. [9] also reported that abiotic reactions between redox-active metals including iron and manganese, organics and reactive intermediates in the nitrogen cycle including nitrite (NO_2^-) can be significant and then reduce NO_2^- to N_2O at low O_2 . As such, NO_2^- is often transiently produced as an intermediate product during abiotic reactions related to N cycle. Factors affecting the concentration of NO_2^- generated from abiotic NO_3^- reduction are pH, low O_2 , NO_3^- concentration and the type of reduced materials.

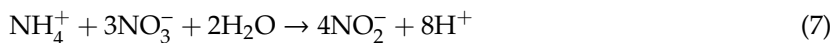
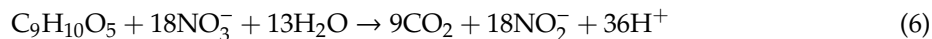
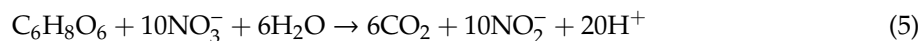
In both biotic and abiotic processes, various reduced organic or inorganic materials are required in order to reduce Fe(III) to Fe(II). Lignin is the most abundant natural compound; syringic acid ($\text{C}_9\text{H}_{10}\text{O}_5$) is a representative lignin monomer in soil that reacts easily with iron [10]. Citric acid ($\text{H}_3\text{C}_6\text{H}_5\text{O}_7$) is also found in a variety of plants, fruits, leaves, roots and in milk, and acts as a chelating agent for heavy metals such as iron, lead and cadmium. Meanwhile goethite (FeOOH), hematite (Fe_2O_3) and magnetite (Fe_3O_4) are typical Fe^{3+} containing soils and the concentration of Fe(III) frequently exceeds that of other electron acceptors in soils. Therefore, an environment suitable for the Fe-mediated redox cycles can be created in anaerobic soils and sediments [11–13]. However, better understanding of the relationship between the NO_2^- transient production during this process and reduced materials is necessary.

This work, therefore, aims to verify the kinetics of the production of NO_2^- from abiotic reduction of NO_3^- in the Fe(III)/Fe(II) cycle under anaerobic conditions with NH_4^+ and several labile organic materials such as citric acid, syringic acid and natural organic matter (NOM) at pH 4 and 7, assuming the conditions as the forest and wetland soils which have generally acidic pH value (about 4) due to anaerobic acidification of organic materials by anaerobic bacteria [14]. With assumption that the pH did not affect the Fe(III)/Fe(II) cycle, relationship between the production of Fe^{2+} and NO_2^- was also investigated. In this study, the Fe^{2+} profile was also observed during the Fe(III)/Fe(II) cycle as well as NO_2^- produced by reacting Fe^{2+} with NO_3^- under anaerobic conditions.

The reaction formula for the oxidation of citric acid, syringic acid and ammonia used in this study and the reduction of Fe^{3+} to Fe^{2+} is as shown in Equations (1)–(3), respectively and the reaction of Fe^{2+} with NO_3^- to produce NO_2^- is presented in Equation (4).



The overall reactions for syringic acid, citric acid and ammonium with goethite and NO_3^- present in Equations (5)–(7).



2. Materials and Methods

2.1. Labile Reduced Materials

Citric acid (Sigma-Aldrich, >99% St. Louis, MO, USA), syringic acid (Sigma-Aldrich, >99% St. Louis, MO, USA), dissolved NOM and ammonium chloride (NH_4Cl , Sigma-Aldrich, >98% St. Louis, MO, USA) were used as labile reduced materials. The dissolved NOM was collected from riparian groundwater in an O-horizon layer near a wetland located in Maryland, USA. The water was collected and stored in polypropylene bottles in the refrigerator. Before use, the water was filtered with 0.2- μm pore size syringe filter. The organic carbon concentrations of NOM measured by TOC analyzer was about 150–240 mg/L (12.5 to 20 mM of TOC). The TOC concentration of NOM was measured for each experiment and the final TOC concentration of NOM was adjusted to be 1 mM.

2.2. Synthesis of Goethite

As an Fe(III) source, goethite was synthesized by a modification of the Atkinson Method [15]. First, 400 mL of a 2.5 M KOH solution was mixed with 1650 mL of prefiltered 0.15 M $\text{Fe}(\text{NO}_3)_3$ solution in a plastic container and stirred vigorously. The mixture was aged for 48 h at 80 °C and then centrifuged to separate the precipitate from the solution. The precipitate was dispersed in 0.01 M HNO_3 solution and centrifuged again. This step was repeated 10 times and rinsed three times with oxygen-free ultrapure water which had been purged with N_2 gas for 24 h to be deoxygenated. The synthesized goethite concentration was 89.4 g/L and the goethite was stored in ultrapure water to minimize oxygen exposure. The mineralogical composition of the synthesized goethite after being freeze-dried by a Freeze Dryer (FDU-2100, Eyela, Japan) was identified by X-ray powder diffraction (D/Max-2500, Rigaku, Japan); the results are shown in Figure 1. The determined d-spacing values of the goethite are compared with those determined by prior researchers in Table 1. The XRD pattern and d-spacing values were very similar to those of goethite synthesized by other researchers [16]. The point of zero charge (pzc) of the synthesized goethite was measured by the point of zero salt effect (PZSE) method [17]; as shown in Figure 2, the resulting pzc was approximately 6.4.

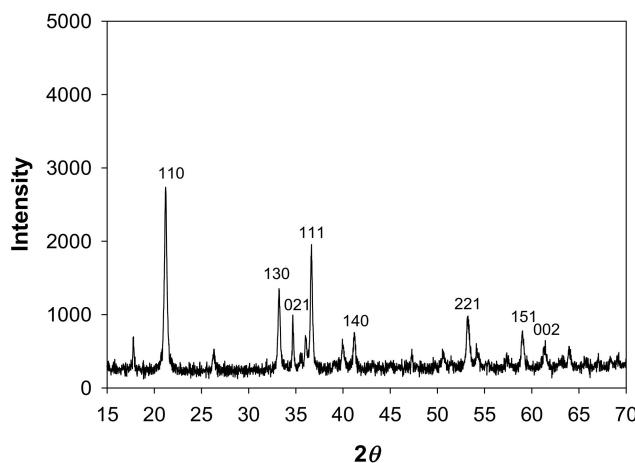
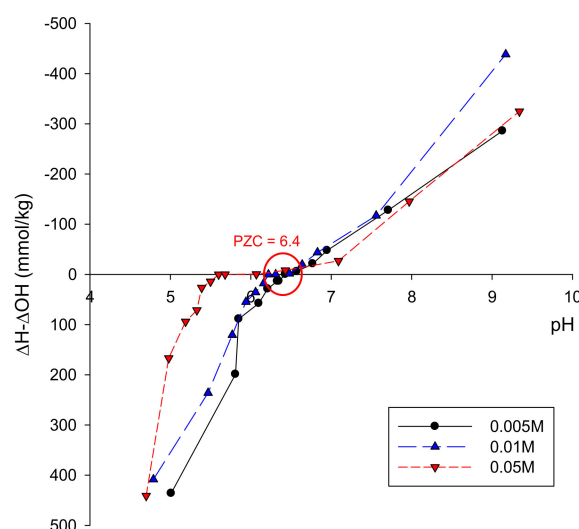


Figure 1. X-ray diffraction (XRD) of synthesized goethite.

Table 1. Comparison of d-spacing of the synthesized goethite in this study with other reference [16].

d_{reported} (Å) [16]	d_{observed} (Å) (This Study)
4.17	4.19
2.69	2.69
2.58	2.58
2.44	2.45
2.19	2.19
1.92	-
1.71	1.72
1.57	1.56
1.51	1.51

**Figure 2.** Point of zero charge (pzc) of the synthesized goethite.

2.3. Fe(III)/Fe(II) Cycling Experiment

2.3.1. Effect of pH and Reduced Material

Next, 11.2 mL of stock suspension containing 89.4 g/L goethite, 20 mL of 100-mM pH buffer solutions, 20 mL of 1-mM reduced carbon stock solutions or 10-mM ammonium stock solutions, 20 mL of 1-mM nitrate stock solution, and 20 mL of 10-mM NaCl stock solution for ionic strength was added into a 250 mL bottle and then milli-Q water was filled to 200 mL of the final volume. For pH buffer, 10-mM MOPs and 10-mM butyric acid was used for pH 7 and 4, respectively. The final concentrations of the reduced carbon, ammonium and nitrate were all about 0.1 mM. After adding goethite and solutions, the bottle was shaken on a magnetic stirrer in an anaerobic glove box (Coy Lab, Grass Lake, MI, USA). Sampling was conducted at a predetermined time schedule in the glove box under strictly anaerobic conditions. The collected samples were filtered with a syringe filter in the glove box and then analyzed for water quality including the concentrations of Fe^{2+} , NO_2^- , NO_3^- and TOC.

To estimate the reaction rate of the second step of the Fe(III)/Fe(II) cycle in which Fe^{2+} and NO_3^- react to produce Fe^{3+} and NO_2^- . The concentration of Fe^{2+} and NO_2^- was monitored at predetermined sampling times. To do so, 20 mL of 2-mM Fe^{2+} and 20 mL of 20-mM NO_3^- stock solutions were added to a 250 mL amber bottle that was then filled to 200 mL with ultrapure water. The final concentrations of Fe^{2+} and NO_3^- in the solution were 0.1 and 1.0 mM, respectively. The solution was mixed well with a magnetic stirrer and sampled by a syringe with a syringe filter with a pore size of 0.2 μm at predetermined times in the anaerobic glove box. Fe^{2+} and NO_2^- concentrations in the filtered samples were analyzed immediately. All experiments were executed in duplicate.

2.3.2. The Fate of Fe^{2+} , NO_2^- and Goethite at pH Values of 4 and 7

As Fe^{2+} and NO_2^- are the target materials, their stability and final concentrations were investigated at pH values of 4 and 7. To do so, 1 g (dry base) of the synthesized goethite solution, pH buffers (butyric acid for a pH of 4 and MOPS for a pH of 7), and 2 mL of 2-mM Fe^{2+} or NO_2^- stock solution was added to a 250 mL bottle that was then filled to 200 mL with ultrapure water. Therefore, the final concentration of Fe^{2+} or NO_2^- was 0.1 mM. The bottle was mixed on a magnetic stirrer and sampled by a syringe with a syringe filter with a pore size of 0.2 μm at predetermined times in the anaerobic glove box.

2.4. Analytic Methods

The pH was measured immediately after sampling and filtration in the glove box. The filtered samples were diluted 25 times with ultrapure water. Then 0.5 mL of the diluted samples were analyzed. The concentration of syringic and citric acids in samples were measured using capillary electrophoresis (CE) (P/ACE MDQ, Beckman Coulter, Brea, CA, USA) with diode-array UV-visible detector with buffer solutions consisting of 25-mM phosphate and 0.5-mM tetradecyltrimethylammonium bromide (TTAB, Sigma-Aldrich, >99%, pH 7) for syringic acid and 25-mM ortho-P plus pyro-P (pH 9.5) and 0.5-mM TTAB for citric acid. The total organic carbon (TOC) content of the NOM was measured using a Dohrmann Phoenix 2000 UV-persulfate oxidation TOC analyzer (Tekmar-Dohrmann, Mason, OH, USA). TOC concentrations of syringic and citric acids were calculated by multiplying the concentrations measured via CE by the number of carbon atoms present in syringic acid or citric acid. The NO_2^- concentration was measured with a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 543 nm by diazotized sulfanilamide with the N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) method [18]. The Fe^{2+} concentration was analyzed with the UV-Vis spectrophotometer at 562 nm by the ferrozine method [19]. The detection limits for NO_2^- and Fe^{2+} were 0.024 μM and 0.046 μM , respectively. The total iron concentration was measured by atomic absorption spectroscopy (Perkin Elmer AAnalyst 100 Waltham, MA, USA).

3. Results

3.1. Effect of Labile Organic Materials on Nitrite and Ferrous Production

The resulting profiles of NO_2^- and Fe^{2+} production from the Fe(III)/Fe(II) reaction cycle with labile organic materials of NOM, syringic acid and citric acid are shown in Figure 3. When using NOM as the organic material and at a pH of 4 (Figure 3a), the concentration of Fe^{2+} increased almost linearly with time whereas that of NO_2^- was almost zero. At a pH of 7 (Figure 3b), a peak NO_2^- concentration of approximately 4 μM was observed at about 120 h of reaction time before the concentration decreased but no Fe^{2+} was detected. A similar pattern was exhibited for syringic acid; Fe^{2+} and not NO_2^- was observed at a pH of 4 (Figure 3c), whereas NO_2^- , but not Fe^{2+} was observed at a pH of 7 (Figure 3d). Moreover, the concentration of syringic acid declined faster than did the concentration of NOM. When citric acid was used, however, a very different pattern was seen. No Fe^{2+} was detected at a pH of 4, even though the concentration of citric acid decreased with time. At a pH of 7, the concentration of NO_2^- increased to approximately 600 μM at 320 h, which was the highest concentration of NO_2^- produced in this experiment. This indicates that citric acid was the best organic carbon source for the Fe(III)/Fe(II) cycle among the compounds studied.

At a pH of 4 (i.e., in Figure 3a,c,e), the kinetics of Fe^{2+} production were nearly linear; the fitted slope, representing the production rate of Fe^{2+} ($\mu\text{M}/\text{h}$) when using NOM, syringic acid and citric acid was 0.0925, 0.5612 and 0.0010 $\mu\text{M}/\text{h}$, respectively, indicating that the syringic acid was the most favorable organic material for Fe^{2+} production. If Fe^{2+} is exposed to oxygen, it rapidly oxidizes to Fe^{3+} [20]; the linear increase in Fe^{2+} concentration seen here thus indicates that this reaction occurred under anaerobic conditions. However, no concentration of Fe^{2+} was detected when citric acid was used as reduced organic carbon compound. Citric acid can be combined easily with Fe^{2+} and Fe^{3+} as

chelating agents to produce iron-citrate, which may have then been filtered out during the sampling step. As a result, no Fe^{2+} was produced when citric acid was used and syringic acid had the highest production rate of Fe^{2+} . Meanwhile, no NO_2^- was detected at this pH. Moreover, even if NO_2^- was produced. This was likely due to the positive surface charge of goethite at $\text{pH} < \text{pzc}$, where the NO_2^- can be combined with the surface of goethite, due to electrical attraction.

At a pH of 7 (i.e., in Figure 3b,d,f), no Fe^{2+} was detected, due to its attachment onto the negative surface charge of goethite at $\text{pH} > \text{pzc}$. The highest peak concentration of NO_2^- was found when using citric acid (600 μM), followed by syringic acid (40 μM) and then NOM (4 μM), indicating that citric acid was the best reactivity compared to other substances due to high affinity of citrate to Fe^{2+} and Fe^{3+} for use as a chelating agent and its relatively simple molecular structure compared to syringic acid. And then the NO_2^- peaks decreased over time.

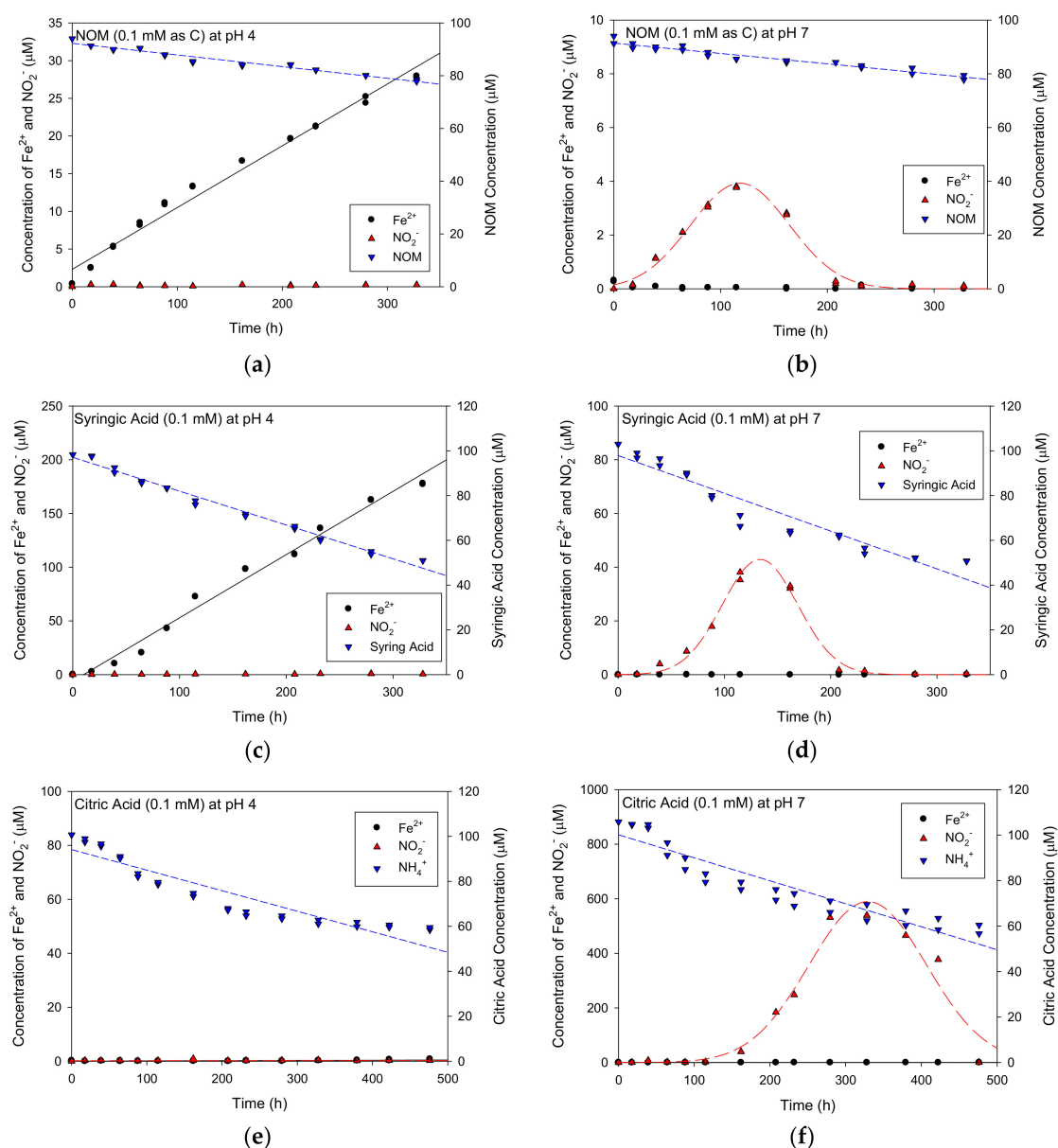


Figure 3. Fe^{2+} and NO_2^- concentrations via time throughout Fe(II)/Fe(III) cycle with natural organic matter (NOM), syringic acid and citric acid at pH 4 and 7: (a) for NOM at pH 4; (b) for NOM at pH 7; (c) for syringic acid at pH 4; (d) for syringic acid at pH 7; (e) for citric acid at pH 4; (f) for citric acid at pH 7.

The TOC was then analyzed as a common organic unit to compare the Fe^{2+} or NO_2^- production kinetics for the varied reduced organic carbon compounds. The TOC concentrations of syringic acid and citric acid were calculated by multiplying their concentrations with the carbon number of the acids (μM). Therefore, the TOC-normalized Fe^{2+} and NO_2^- concentrations or concentration per unit carbon, could be calculated by dividing the observed Fe^{2+} and NO_2^- concentration by the carbon number of the syringic and citric acids. The resulting TOC-normalized Fe^{2+} and NO_2^- production kinetics are shown in Figure 4a,b at pH values of 4 and 7, respectively. At a pH of 4—as in Figure 3a,c,e above—the concentration of Fe^{2+} increased linearly with time and fitted well to a linear model when NOM or syringic acid were used, but not for citric acid. The slope, representing the TOC-based Fe^{2+} production rate in $\mu\text{M}/\text{h}$, was highest when NOM was used, followed by syringic acid, and then citric acid. Thus, the carbon in NOM was utilized most easily. Furthermore, the TOC-based NO_2^- production at a pH of 7 also depended on the carbon type. The maximum TOC-based NO_2^- production (approximately $90 \mu\text{M}$) appeared at about 320 h for citric acid, in spite of no detection of Fe^{2+} at pH 4, followed by syringic acid ($4.2 \mu\text{M}$) and NOM ($3.8 \mu\text{M}$).

The production of NO_2^- at a pH of 4 thus remained unclear. If Fe^{2+} produced by goethite reduction was reacted consecutively with NO_3^- to produce NO_2^- , it would be difficult to increase the Fe^{2+} concentration linearly. Furthermore, NO_2^- is likely to be re-adsorbed onto goethite, as identified in Section 3.3. However, with an initial concentration of NO_3^- of $1000 \mu\text{M}$, it is difficult to detect the minor variation in concentration by reaction. Therefore, NO_3^- is not an indicator to determine whether the concentration decreases due to the reaction.

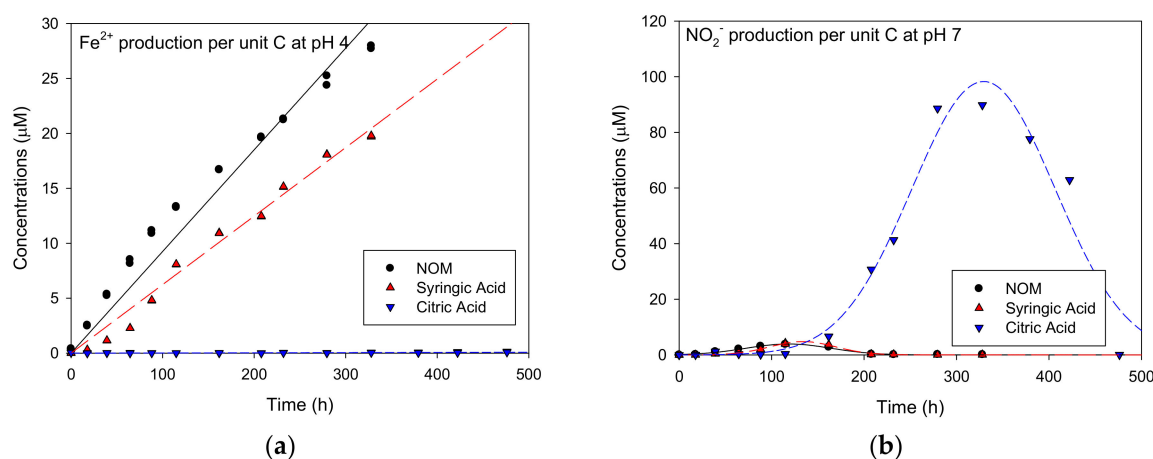


Figure 4. The total organic carbon (TOC)-normalized Fe^{2+} and NO_2^- concentrations via time at (a) pH 4 and (b) pH 7.

3.2. Possibility of Ammonium Used as a Reduced Material for Nitrite and Ferrous Production

The resulting concentration variation of Fe^{2+} and NO_2^- when NH_4^+ was used as a reduced material is shown in Figure 5. Even with an initial NH_4^+ concentration of $1000 \mu\text{M}$, the Fe^{2+} or NO_2^- production for NH_4^+ was very low when compared with other organic materials used in the prior section. The production rate of Fe^{2+} was $0.0281 \mu\text{M}/\text{h}$ at a pH of 4 (Figure 5a) and the maximum production of NO_2^- was approximately $2.2 \mu\text{M}$ and then NO_2^- concentrations decreased at a pH of 7 (Figure 5b). This is in accordance with the hypothesis presented by Clement et al. [5] that coupling the NH_4^+ oxidation to NO_2^- with Fe^{3+} reduction under anaerobic conditions in sediments is thermodynamically feasible. However, from Figure 5, it was difficult to directly analyze whether ammonia influenced nitrite production. To analyze the effect of NH_4^+ on NO_2^- production, it was assumed in this study that it was also generated at pH 7 (Figure 5b) at the same level as the Fe^{2+} concentration generated at pH 4 (Figure 5a). Comparing Figure 5a,b, about $6 \mu\text{M}$ of Fe^{2+} concentration was found at the same time as the observed time (about 210 h) for the highest NO_2^- concentration (approximately $2.3 \mu\text{M}$). At that time, the $\text{NO}_2^-/\text{Fe}^{2+}$ concentration ratio was 0.38 which was lower

than 0.5. The reason for this low ratio was thought to be that the portion in which the generated NO_2^- was converted into another nitrogen compound increased. Therefore, it is necessary to analyze the linear relationship between NO_2^- and Fe^{2+} in the initial time with low NO_2^- conversion rate, which is described in discussion.

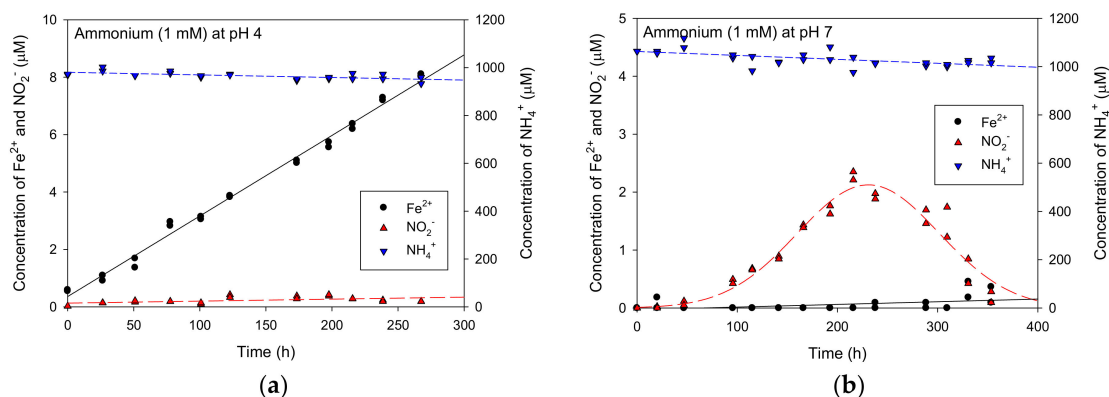


Figure 5. The Fe^{2+} and NO_2^- production kinetics for NH_4^+ used as a reduced material at (a) pH 4 and (b) pH 7.

3.3. Effect of pH on Fe^{2+} and NO_2^- Adsorption onto Goethite

The adsorption of the produced Fe^{2+} onto goethite was then investigated at pH values of 4 and 7; the resulting concentration variation is shown in Figure 6, where the initial concentrations of Fe^{2+} and goethite were 100 μM and 1 g, respectively. At a pH of 4, the concentration of Fe^{2+} produced by Fe^{3+} reduction remained nearly stable, due to the repulsive forces driven by the positive charges of both of them. On the other hand, the Fe^{2+} was almost entirely adsorbed within 1 min at a pH of 7. Thus, even if Fe^{2+} was produced by Fe(III)/Fe(II) cycle, it was not detected in the solution because it was adsorbed onto the goethite quickly, due to the negative charge on the goethite surface at $\text{pH} < \text{pzc}$. This explains why no Fe^{2+} was detected in the results presented in Section 3.1 at a pH of 7 (Figure 3b,d,f).

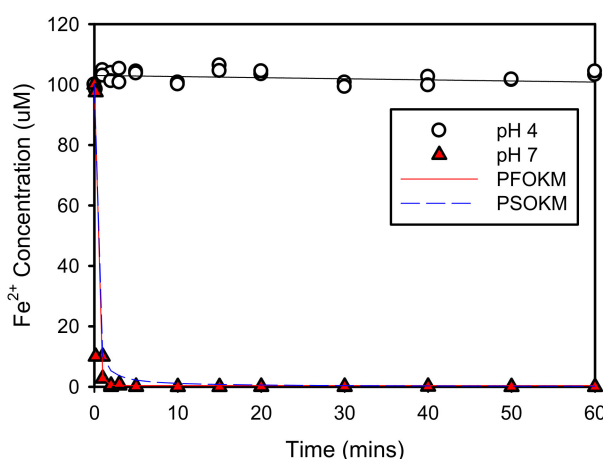


Figure 6. Fe^{2+} adsorption onto goethite at pH 4 and 7.

The resulting adsorption of NO_2^- onto goethite at pH values of 4 and 7 is presented in Figure 7. Unlike Fe^{2+} , the NO_2^- concentration decreased to less than 5 μM within 60 min at a pH value of 4; whereas at a pH value of 7, the NO_2^- concentration remained constant. At a pH value of 7, both NO_2^- and goethite have negative charges at $\text{pH} > \text{pzc}$ of goethite, thus accounting for the lack of adsorption.

To analysis the adsorption kinetics of Fe^{2+} at a pH of 4 and NO_2^- at a pH of 7, the Lagergren pseudo-first-order kinetic model (PFOKM) [21] and the pseudo-second-order kinetic model

(PSOKM) [21,22] were used. The equations for PFOKM and PSOKM are presented at Equations (8) and (9), respectively.

$$\frac{C(t)}{C_0} = 1 - \frac{q_e}{C_0} \frac{W}{V} (1 - e^{-k_1 t}) \quad (8)$$

$$\frac{C(t)}{C_0} = 1 - \frac{1}{C_0} \frac{(W/V)t}{1/(k_2 q_e^2) + t/q_e} \quad (9)$$

where k_1 and k_2 are the rate constant of PFOKM (h^{-1}) and PSOKM (h^{-1}), respectively. C_0 and $C(t)$ denote the solute concentrations in the solution ($\mu\text{mol/L}$) at time 0 and time t (h), respectively. The q_e is the equilibrium concentration in the solid phase ($\mu\text{mol/kg}$), respectively. W and V are the weight of goethite (kg) and solution volume (L). Then, the equilibrium concentration, C_e , of solute in solution can be calculated by Equation (10).

$$C_e = C_0 - q_e \frac{W}{V} \quad (10)$$

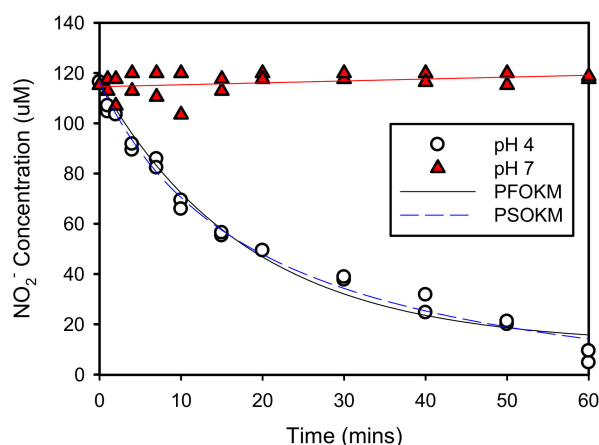


Figure 7. NO_2^- adsorption onto goethite at pH 4 and 7.

Table 2 shows the PFOKM and PSOKM parameters for the adsorption of Fe^{2+} and NO_2^- onto goethite at pH values of 7 and 4, respectively. Of the two models, PFOKM ($R^2 = 0.8491$ and 0.9823 for Fe^{2+} and NO_2^- , respectively) was more accurately interpreted than PSOKM ($R^2 = 0.8147$ and 0.9773 , respectively). Therefore, according to PFOKM analyses, the equilibrium concentrations of Fe^{2+} and NO_2^- were 0.395 and $11.90 \mu\text{mol/L}$, respectively. This means that Fe^{2+} and NO_2^- are removed by 99.6% and 89.8% by adsorption. Moreover, the amount of adsorbed solute on goethite was 19.92 mmol/kg for Fe^{2+} and 20.9 mmol/kg for NO_2^- .

Table 2. Pseudo first order kinetic model (PFOKM) and pseudo second order kinetic model (PSOKM) parameters for the sorption of Fe^{2+} at pH 7 and NO_2^- at pH 4.

Parameter	Fe^{2+}		NO_2^-	
	PFOKM	PSOKM	PFOKM	PSOKM
C_0 ($\mu\text{mol/L}$)	100	100	116.4	116.4
q_e ($\mu\text{mol/kg}$)	$19,920 \pm 551.4$	$20,000 \pm 717.1$	$20,900 \pm 630.7$	$23,280 \pm 793.0$
C_e ($\mu\text{mol/L}$)	0.395	0.000	11.90	0.025
Removal Efficiency (%)	99.6	100.0	89.8	100.0
k_1 (1/d) or k_2 ($\text{kg}/\mu\text{mol/d}$)	3.636 ± 0.976	1.94×10^{-4} $\pm 7.33 \times 10^{-5}$	0.0548 ± 0.0042	3.17×10^{-6} $\pm 5.16 \times 10^{-7}$
R^2	0.8491	0.8147	0.9823	0.9773
Sum of square errors	0.3879	0.4765	0.0381	0.0487

Overall, the adsorption of Fe^{2+} and NO_2^- onto the surface of goethite depends on the surface charge of the goethite, which is dependent on the pH and a very fast adsorption rate at a pH of 4. Therefore, even if NO_2^- is produced, it may not be detected because of its rapid adsorption onto the goethite. Fe^{2+} generated from the Fe(III) reduction was mostly adsorbed onto goethite at a pH of 7 and about 90% of NO_2^- generated from NO_3^- reduction was also adsorbed at a pH of 4.

3.4. The Reaction of Fe^{2+} and NO_3^- to Produce NO_2^-

The kinetics of Fe^{2+} removal and NO_2^- production are shown in Figure 8. Overall, the NO_2^- concentration as the Fe^{2+} concentration decreased, indicating that the reaction occurred successfully, although the Fe^{2+} decreased more rapidly than NO_2^- was produced. Considering the two reaction rates as a first-order reaction ($C = C_0 e^{-kt}$ for Fe^{2+} and $C = C_0 (1 - e^{-kt})$ for NO_2^-), a high coefficient of determination ($R^2 > 0.947$) was obtained (Table 3). Although the resulting reaction rate was relatively slow compared to the adsorption rates of Fe^{2+} and NO_2^- onto goethite, approximately 80% of Fe^{2+} was removed within 140 h, indicating that the overall rate of reaction depends upon the rate of the reaction between Fe^{2+} and NO_3^- .

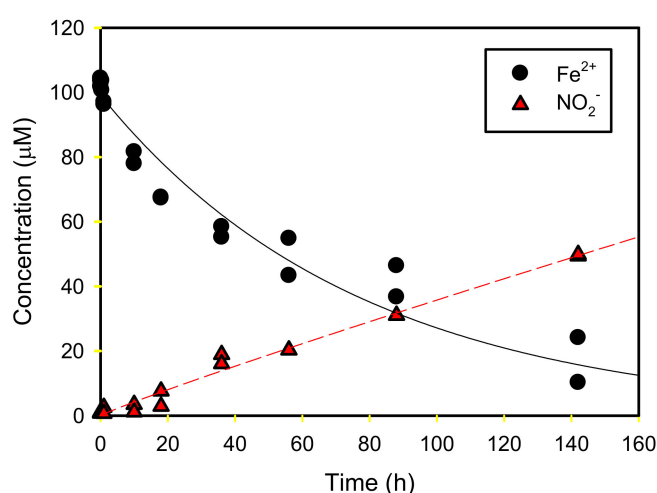


Figure 8. Fe^{2+} and NO_2^- concentration produced from NO_3^- reduction.

Table 3. First-order model parameters for Fe^{2+} removal and NO_2^- production.

Parameter	Fe^{2+}	NO_2^-
Equation	$C = C_0 e^{-kt}$	$C = C_0 (1 - e^{-kt})$
C_0	99.11 ± 2.03	207.20 ± 115.18
k	0.013 ± 0.001	0.0019 ± 0.0012
R^2	0.9477	0.9862
Sum of square errors	6.946	1.904

4. Discussion

From Section 3.3, different situation of the removal of Fe^{2+} and NO_2^- may be involved to the pH values. However, there was no evidence that goethite-mediated Fe(III)/Fe(II) cycle was affected by the pH value in this study. If the pH only controls the adsorptive ability of goethite, the goethite-mediated Fe(III)/Fe(II) cycle can occur regardless of pH changes. The adsorption of NO_2^- and Fe^{2+} onto goethite at pH values of 4 and 7 was the main reason for no detection of them as mentioned in Section 3.3. The concentrations of Fe^{2+} also could not be estimated at a pH of 7 even though NO_2^- was observed from the reaction of Fe^{2+} and NO_3^- . Therefore, it was assumed that the amount of Fe^{2+} produced in a pH of 7 was equal to that in a pH of 4 and the amount of NO_2^- produced through Fe(III)/Fe(II) cycle in a pH of 7 was equal to that in a pH of 4.

The relationships between TOC-normalized Fe^{2+} production at pH 4 and TOC-normalized NO_2^- production at pH 7 for NOM, syringic acid, citric acid and ammonium were shown in Figure 9. For NOM, syringic acid and ammonium, TOC-normalized NO_2^- production increased linearly with TOC-normalized Fe^{2+} production ($0.963 < R^2 < 0.983$) in the relatively low concentration range of TOC-normalized Fe^{2+} production before reaching the peak concentrations. The linearly increase with high R^2 values indicates that it was indirect evidence that Fe(III)/Fe(II) cycle successfully occurred at both pH 4 and 7 with constant rate. The ratios of TOC-normalized Fe^{2+} production and TOC-normalized NO_2^- production for NOM, syringic acid and ammonium were 0.2690, 0.4764 and 0.5585, respectively.

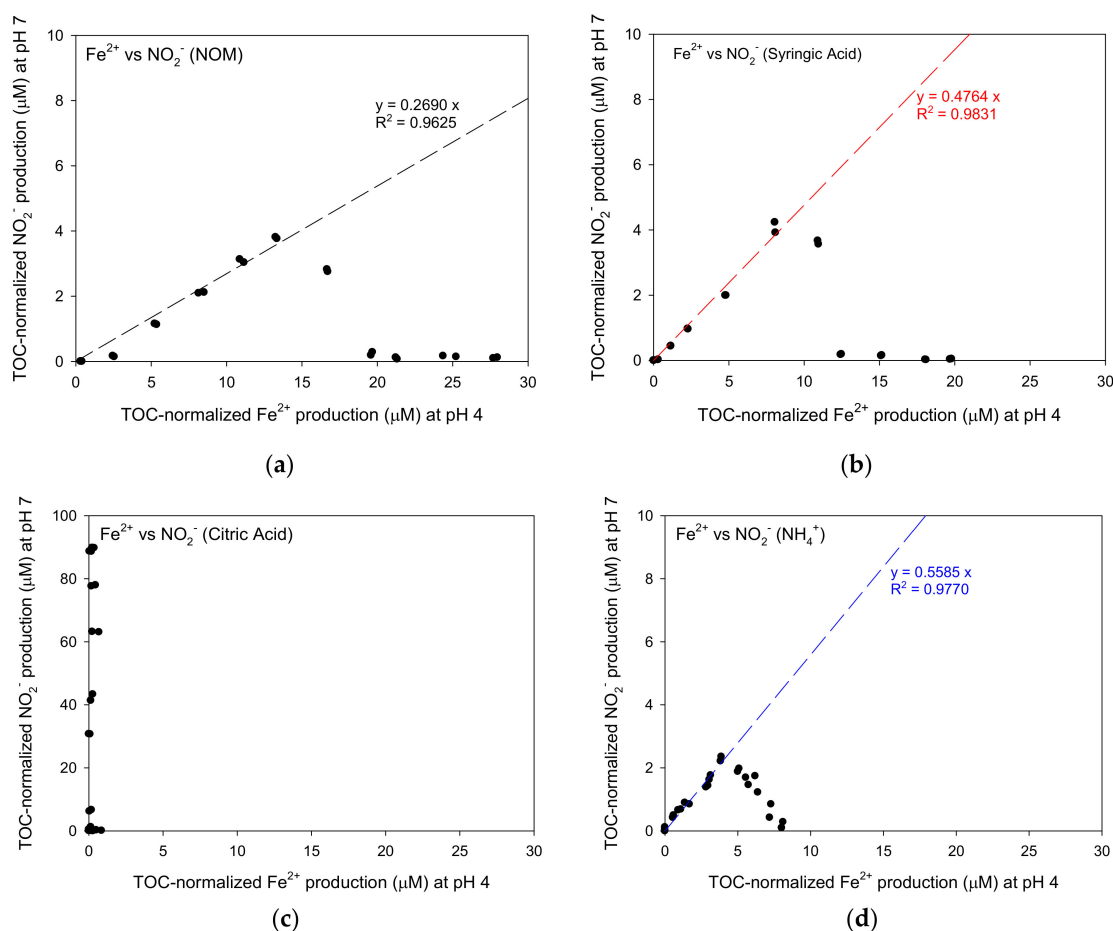


Figure 9. The relationship between TOC-based Fe^{2+} concentrations at a pH of 4 and NO_2^- concentrations at a pH of 7: (a) NOM, (b) syringic acid, (c) citric acid and (d) ammonium.

The theoretical Fe^{2+} productions per mole of syringic acid, citric acid and ammonium are 20, 36 and 8 moles, respectively, as shown in the stoichiometry of the Equations (1)–(3). In addition, the NO_2^- production per mole of syringic acid, citric acid and ammonium through overall reactions are 10, 18 and 4 moles of NO_2^- produced, respectively. Therefore, the theoretical ratio of NO_2^- production per Fe^{2+} production calculated considering overall equations are 0.5, 0.5 and 0.67 mole NO_2^- /mole Fe^{2+} , respectively. If these values were compared to the experimental results presented in Figure 9, the theoretical ratios for syringic acid and ammonium were slightly higher than the experimental ratios: $0.5 > 0.4764$ for syringic acid and $0.67 > 0.5585$ for ammonium. Therefore, the assumption that the Fe(III)/Fe(II) cycle occurred regardless the pH seemed to be right. Even though the Fe^{2+} concentration produced at a pH of 4 was equal to that at a pH of 7, the difference between the experimental Fe^{2+} production and the theoretical Fe^{2+} production was still unknown because some of them can be converted quickly or adsorbed onto the goethite again due to the instability of NO_2^- .

5. Conclusions

In this study, the transient generation of NO_2^- by the abiotic reaction of goethite-mediated Fe(III)/Fe(II) cycle with labile materials and NO_3^- reduction was investigated under anaerobic conditions in the soil. The main reaction was the abiotic Fe(III)/Fe(II) cycle, which was conducted at pH values of 4 (acidic condition) and 7 (neutral condition). The production of NO_2^- through the Fe(III)/Fe(II) cycle was greatly influenced by the relationship between the pH and pzc due to the change in the surface charge of goethite according to pzc. It was confirmed that NO_2^- is produced in a neutral environment and not in an acidic environment. On the TOC basis, citric acid was the best labile organic carbon; using NOM and syringic acid demonstrated similar performances. Using NH_4^+ demonstrated a similar phenomenon, but with a very low production of NO_2^- . The adsorption of the produced Fe^{2+} and NO_2^- onto goethite was also dependent on the relationship between the pH and pzc. The reaction rates of Fe^{2+} and NO_3^- were relatively slow, which had a significant impact on the Fe(III)/Fe(II) cycle reaction rates. According to stoichiometric analyses, the reaction through Fe(III)/Fe(II) cycle was not affected by the pH. Consequently, the best environment to form NO_2^- is likely an anaerobic zone containing a large amount of organic matter at a neutral pH, such as the organic horizon of wetland subsoil.

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