Fe^{II} oxidation by molecular O$_2$ during HCl extraction

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Environmental context.   In the environment, iron exists mainly as Fe^{II} and Fe^{III} and plays an important role in biogeochemical processes. The Fe^{II} and Fe^{III} content is often quantified by hydrochloric acid extraction and the acid is thought to prevent Fe^{II} oxidation by oxygen. However, we found that with increasing HCl concentration and temperature, oxidation of Fe^{II} by oxygen is accelerated. Therefore, in order to obtain reliable results extractions should be performed with dilute HCl or in the absence of oxygen.

Abstract.   HCl is commonly used to stabilise Fe^{II} under oxic conditions and is often included in Fe extractions. Although Fe^{II} oxidation by molecular O$_2$ in HCl is described in the field of hydrometallurgy, this phenomenon has not been systematically studied in environmentally relevant systems. The extent of Fe^{II} oxidation by O$_2$ during extraction of soils and magnetite by HCl and in HCl/FeCl$_2$ solutions was therefore quantified. Fe^{II} was stable in 1 M HCl at room temperature for several days, whereas in 6 M HCl at 70°C, 90% of the Fe^{II} was oxidised within 24 h. In the absence of O$_2$, no Fe^{II} oxidation occurred. Experiments at low pH with increasing H$^+$ or Cl$^-$ concentration alone and geochemical modelling suggested that the formation of complexes of Fe^{II} and HCl may be responsible for the observed Fe^{II} oxidation. The use of strictly anoxic conditions for Fe extraction by HCl to obtain reliable Fe redox speciation data is therefore recommended.

Additional keywords: abiotic oxidation, biogeochemistry, geomicrobiology, iron minerals, soil extraction.

Introduction

In the environment, Fe mainly exists in the two redox states Fe^{II} and Fe^{III} and undergoes abiotic and microbial redox processes. At neutral pH, Fe^{II} can be oxidised abiotically by MnO$_2$, NO$_2^-$, molecular O$_2$ and H$_2$O$_2$.[1–4] Fe^{III} can be reduced chemically under anoxic conditions e.g. by hydrogen sulfide, the superoxide radical HO$_2^\bullet$ or different organic compounds such as hydroquinones in humic substances.[4–6] Both, Fe^{II} and Fe^{III} can be microbially oxidised and reduced in anoxic and microoxic habitats such as aquifers, sediments and soils.[7,8] The mineralogy of the Fe precipitates formed by abiotic and microbial redox processes strongly depends on geochemical conditions such as pH, the presence of other ions and of humic substances, as well as on Fe oxidation and reduction rate.[9–13]

The mineralogy and crystallinity of the Fe precipitates formed during these reactions affect the fate of organic and inorganic nutrients and pollutants in the environment. Arsenic, for example, co-precipitates during Fe^{III} mineral formation and is released when these minerals are dissolved.[14,15] Furthermore arsenic can undergo redox reactions with Fe phases, such as Fe^{II} sorbed to goethite.[16] The reactivity and therefore the identity of the Fe minerals involved in these redox processes ultimately control the fate of the arsenic. In order to identify and quantify microbial and abiotic Fe mineral (trans)formation and Fe redox processes, the concentrations of the different Fe redox species and Fe minerals present have to be quantified in a reliable and reproducible manner.

Different methods are used to quantify Fe redox species and to characterise Fe mineralogy, e.g. X-ray diffraction (XRD),

Mössbauer spectroscopy or X-ray absorption spectroscopy.[17] As these methods require sophisticated analytical equipment, wet chemical Fe extraction methods are also commonly used. Extraction protocols vary in type of extraction agent, incubation time, temperature, presence or absence of light, and shaking of the sample.[18–20] Different fractions of Fe minerals are dissolved by different extraction agents, e.g. 'ion-exchangeable Fe' by 1 M MgCl$_2$ (pH 7), 'adsorbed Fe' and Fe carbonates by 1 M Na-acetate (pH 5), 'low crystalline Fe minerals' by 0.5–1 M HCl or hydroxylamine-HCl, and 'highly crystalline Fe minerals' by 5–12 M HCl or dithionite.[18–22] If extraction agents with circumneutral pH are applied, the presence of O$_2$ must be excluded to avoid Fe^{II} oxidation by O$_2$. It is generally believed that extraction with HCl circumvents this problem, as Fe^{II} oxidation is reported to be very slow at pH <3.[4] However, extraction of Fe-containing soils with 6 M HCl performed in our laboratory (Fig. 1a,b) revealed that significant Fe^{II} oxidation occurred in the presence of O$_2$ even at acidic pH. Fe^{II} oxidation by molecular O$_2$ in HCl of high concentration has already been described in the field of hydrometallurgy. Several studies determined the kinetics of this process and influencing factors such as O$_2$ concentration, temperature, and effect of catalysts under defined conditions.[23–25] Most of these studies were performed with pure FeCl$_2$ solutions, pure O$_2$ and under constant stirring, conditions which are usually not used for Fe extraction procedures of environmental samples or samples from biogeochemical Fe mineral (trans)formation experiments.

Hence, the aim of our study was (i) to determine the extent of Fe^{II} oxidation during widely used HCl extraction procedures of
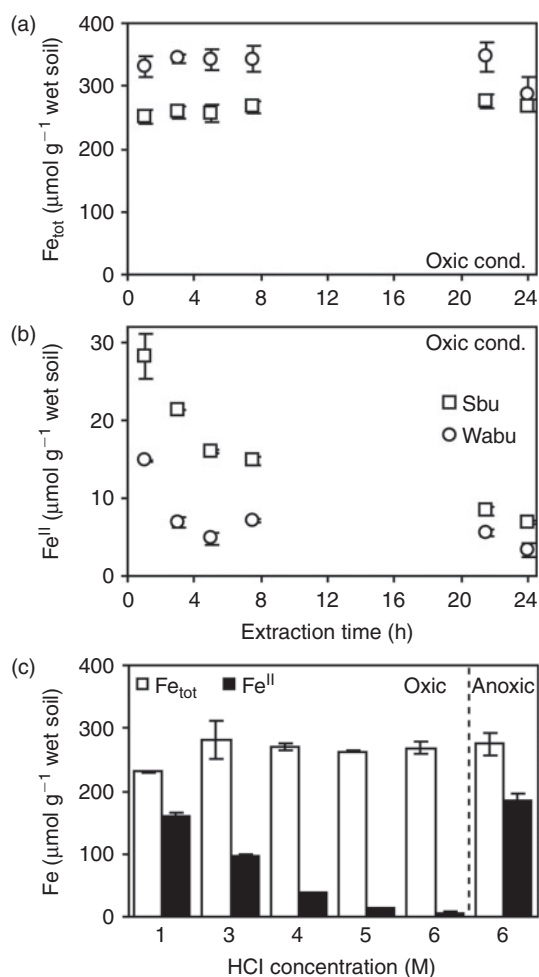
Fig. 1. (a) Fe$_{tot}$ and (b) Fe^{II} extracted from Schoenbuch forest (Sbu) (□) and Waldenbuch (Wabu) (○) soils with oxic 6 M HCl at 70°C under oxic conditions over time. At each time point, a sub-sample was taken from the extraction bottles. (c) Fe$_{tot}$ and Fe^{II} extracted from soil Sbu with oxic and anoxic 1–6 M HCl for 24 h at 70°C under oxic and anoxic conditions. Data for oxic 6 M HCl are the same as in (a) and (b) at 24 h. (a–c) Bars indicate the range of duplicates (oxic conditions) or the standard deviation of triplicates (anoxic conditions).

environmentally relevant samples, (ii) to identify the key factors controlling Fe^{II} oxidation under these conditions, and (iii) to define conditions under which Fe^{II} oxidation is minimised during HCl extraction.

Experimental methods

Fe minerals and chemicals

Magnetite was purchased from Lanxess GmbH, Germany. It was pure according to XRD (Bruker D8 Discover X-ray diffraction instrument, Bruker AXS GmbH, Karlsruhe, Germany) and had a Fe^{II}/total Fe (Fe$_{tot}$) ratio of 0.27 determined by Mössbauer spectroscopy (WissEl – Wissenschaftliche Elektronik GmbH, Starnberg, Germany; for details of Mössbauer spectroscopy see Hohmann et al.[14]). FeCl$_2$ stock solutions of 90 mM (>93% Fe^{II}) were prepared by dissolving FeCl$_2$·4H$_2$O in 1 M HCl and were stored at 4°C in the dark. 1–6 M HCl solutions were obtained by dilution of a 37% HCl solution with de-ionised water. A 95–97% H$_2$SO$_4$ solution was diluted with de-ionised water to 3 M.

Soil sampling and characterisation

Top soil (~20 cm) was sampled from Waldenbuch (Wabu) and the Schoenbuch forest (Sbu) (both located in south-west Germany). The soil was stored in plastic bags at 4°C in the dark until further use. For experiments the soil fraction <2 mm was used. A detailed characterisation of the soils is given in Table A1 of the Accessory publication.

Fe extraction from soils under oxic conditions

Field moist soil (0.5 g) was extracted with 25 mL of 1–6 M HCl in closed 60-mL serum bottles at 70°C in a water bath in the dark for 24 h. After short mixing, 1.8 mL of the suspension was sampled and centrifuged (Centrifuge 5417C, Eppendorf AG, Hamburg, Germany) for 15 min at 20 817g and room temperature (25°C) and Fe^{II} and Fe$_{tot}$ concentrations were quantified in the supernatant. In the experiment with 6 M HCl, Fe^{II} and Fe$_{tot}$ concentrations were followed over time. Before sampling, the bottles were taken from the water bath, mixed and left for 5 min to allow soil particles to sediment. From the liquid, 0.5-mL samples were taken, centrifuged as described above and Fe^{II} and Fe$_{tot}$ were quantified in the supernatant.

Fe^{II} oxidation experiments under oxic conditions

In order to determine the influence of HCl concentration, temperature and proton (H$^+$) and chloride (Cl$^-$) concentrations on Fe^{II} oxidation, an FeCl$_2$ solution (8–9 mM) or magnetite (9–10 mM Fe$_{tot}$) were incubated in closed 23-mL test tubes with a headspace of air at 70°C in a water bath for 24 h in the dark in duplicates or triplicates (Table A2 of the Accessory publication). In order to determine the influence of HCl concentration, 9–10 mL of 1–6 M HCl was added to FeCl$_2$ and magnetite respectively. The initial phase of Fe^{II} oxidation was investigated in short-time experiments at 70°C with maximum incubation times of 15 min (FeCl$_2$ in 1–6 M HCl), 30 min (magnetite in 5–6 M HCl), and 60 min (magnetite in 4 M HCl). For incubation times ≤15 min, the samples were mixed every minute. For incubations times >15 min, the samples were mixed once during incubation. After incubation, 2 mL of each sample were taken and aliquots of 100 µL were immediately diluted 1 : 10 with 1 M HCl. In order to test the effect of storage at room temperature, the remaining 1.9 mL of selected samples (samples taken after 2.5 min for FeCl$_2$ in 1–6 M HCl, after 15 min for magnetite in 4 M HCl and after 5 min for magnetite in 5–6 M HCl) were incubated undiluted at room temperature in the dark and Fe^{II} and Fe$_{tot}$ were followed over time (Fig. A1 of the Accessory publication). In order to differentiate between the effects of H$^+$ and Cl$^-$, FeCl$_2$ and magnetite were incubated at 70°C for 24 h either with 10 mL of 3 M H$_2$SO$_4$ or with 1.17 g of NaCl (end concentration ~2 M) in 10 mL of 1 M HCl. Samples from all experiments were immediately diluted 1 : 10 with 1 M HCl after incubation and analysed for Fe^{II} and Fe$_{tot}$.

Fe oxidation experiments under anoxic conditions

In order to determine if Fe^{II} in HCl is oxidised by molecular O$_2$, soil Sbu, FeCl$_2$ and magnetite were incubated in 6 M HCl for 24 h at 70°C in an anoxic glovebox (M. Braun Inertgas-Systeme GmbH, Garching, Germany, 100% N$_2$). All solutions were made anoxic by purging with N$_2$. As the experiments were performed in a glovebox (100% N$_2$) with anoxic solutions, O$_2$ was present neither in the solutions nor in the headspaces.

Table 1. Recovery of Fe_{tot} and Fe^{II} from 8–9 mM dissolved $FeCl_2$ and 9–10 mM magnetite in different HCl and H_2SO_4 solutions after 24 h at 70°C

The Fe^{II} recovery was calculated based on the measured Fe_{tot} concentration and the stoichiometry of the Fe phases, mean ± s.d. are given, $n = 3$, dash (–) means that these set-ups were not tested. Oxic means that O_2 was present in solution and headspace (air), anoxic means that no O_2 was present in the solution and headspace

Set-up	O_2	$FeCl_2$		Magnetite	
		Fe_{tot} (%)	Fe^{II} (%)	Fe_{tot} (%)	Fe^{II} (%)
1 M HCl	Oxic	101.7 ± 0.1	92.1 ± 1.8	93.3 ± 12.7	101.3 ± 2.0
2 M HCl	Oxic	–	–	103.4 ± 6.4	85.8 ± 0.4
3 M HCl	Oxic	99.3 ± 1.3	59.2 ± 2.4	103.2 ± 6.5	60.5 ± 1.2
4 M HCl	Oxic	–	–	94.4 ± 12.3	34.0 ± 7.0
5 M HCl	Oxic	–	–	95.4 ± 9.7	19.6 ± 8.7
6 M HCl	Oxic	99.9 ± 3.0	9.9 ± 2.3	96.0 ± 2.4	10.7 ± 8.7
6 M HCl	Anoxic	104.4 ± 1.8	100.3 ± 2.0	97.7 ± 36.5	97.5 ± 2.9
3 M H_2SO_4	Oxic	102.5 ± 4.1	90.9 ± 3.2	101.5 ± 1.6	99.4 ± 0.9
1 M HCl + 2 M NaCl	Oxic	99.5 ± 2.3	80.4 ± 0.4	79.9 ± 4.6	88.9 ± 1.6

Analytical methods

Fe^{II} and Fe_{tot} concentrations in the extracts and of the $FeCl_2$ stock solutions were determined by the ferrozine assay (see Hegler et al.[26,27]). The initial Fe^{II} concentration of defined Fe-HCl mixtures before Fe^{II} oxidation was calculated from the measured Fe_{tot} concentration based on the determined initial Fe^{II}/Fe_{tot} ratio of the $FeCl_2$ solutions and magnetite. The Fe^{II} recovery after Fe^{II} oxidation was defined as the ratio between the Fe^{II} measured in the experiments and the calculated initial Fe^{II} concentration.

Geochemical equilibrium calculation

The chemical speciation in anoxic HCl-$FeCl_2$ solutions at 25 and 70°C in the absence and presence of NaCl was calculated using the REACT module of '*The Geochemist's Workbench 6.0*' package (RockWare, Inc., Golden, CO, USA) and the 'thermo' database (for details see the 'Geochemical equilibrium calculation' section of the Accessory publication).

Results

Oxidation of Fe^{II} in soils during Fe extraction with HCl

Two different soils (Table A1 of the Accessory publication) were extracted with oxic 6 M HCl at 70°C for 24 h under oxic conditions (Fig. 1a,b). Fe_{tot} quantification over time showed that after 1 h, more than 90% of the Fe_{tot} was already extracted relative to the maximum extractable Fe_{tot} concentration that was obtained after 21.5 h (275.0 ± 10.5 (Sbu) and 347.1 ± 23.0 (Wabu) μmol Fe per g wet soil). In contrast, the Fe^{II} concentration was highest after 1 h (28.1 ± 2.9 (Sbu) and 14.8 ± 0.1 (Wabu) μmol Fe^{II} per g wet soil) and decreased significantly over time. After 24 h, only 24.5 ± 1.9% (Sbu) and 22.4 ± 6.5% (Wabu) of the Fe^{II} content measured after 1 h were detectable. As the Fe_{tot} concentration did not decrease over time, the observed Fe^{II} decrease suggests that Fe^{II} oxidation occurred in 6 M HCl.

In order to determine the influence of the HCl concentration on Fe^{II} oxidation, soil Sbu was extracted under oxic conditions for 24 h at 70°C with oxic 1–6 M HCl (Fig. 1c). The use of 3–6 M HCl extracted a similar Fe_{tot} concentration, whereas with 1 M HCl the Fe_{tot} concentration was slightly lower. In contrast, the highest Fe^{II} concentration was found in extracts with 1 M HCl (161.3 ± 4.3 μmol Fe per g wet soil). The Fe^{II} concentration decreased with increasing HCl concentration and the measured concentration of Fe^{II} in 6 M HCl was only 4% of that

measured in 1 M HCl. Building on these results with soils, two defined Fe^{II}-containing compounds, an $FeCl_2$ solution and the mixed Fe^{II}–Fe^{III}–mineral magnetite, were used to quantify abiotic Fe^{II} oxidation depending on (i) the presence of molecular O_2, (ii) HCl concentration, (iii) Cl^- v. H^+ concentration, and (iv) temperature (an overview of all experiments is given in Table A2 of the Accessory publication).

Influence of molecular O_2 on Fe^{II} oxidation in HCl

In order to determine if Fe^{II} in HCl is oxidised by molecular O_2 present in air, an $FeCl_2$ solution, magnetite (Table 1) and soil Sbu (Fig. 1c) were incubated in oxic 6 M HCl under oxic conditions and in anoxic 6 M HCl under anoxic conditions, each at 70°C. Fe^{II} present in 8–9 mM $FeCl_2$ and 9–10 mM magnetite was completely recovered under anoxic conditions whereas under oxic conditions 90% of the Fe^{II} was oxidised within 24 h. The Fe^{II}/Fe_{tot} ratio determined after anoxic extraction with 6 M HCl from soil Sbu (67.4 ± 0.3%) was similar to the Fe^{II}/Fe_{tot} ratio obtained with oxic 1 M HCl (69.7 ± 1.6%). In combination with the fact that the total amount of extractable Fe (Fe_{tot}) was only slightly higher for anoxic 6 M HCl compared with oxic 1 M HCl (Fig. 1c), this indicates that Fe^{II} was not oxidised in soil Sbu under oxic conditions in 1 M HCl.

As no Fe^{II} oxidation occurred in 6 M HCl under O_2-free conditions for any of the three Fe^{II}-containing samples, we concluded that (i) Fe^{II} is stable in HCl under anoxic conditions and (ii) Fe^{II} in HCl of high concentration is oxidised by molecular O_2. In order to rule out that during flushing of the HCl with N_2 for deoxygenation, HCl outgassed and a lower HCl concentration was responsible for the absence of Fe^{II} oxidation under anoxic conditions, magnetite was extracted for 24 h at 70°C with deoxygenated 6 M HCl under oxic conditions. For this purpose deoxygenated HCl was added to magnetite in test tubes outside the glovebox, so that O_2 was present in the headspace and diffused back into solution. After the incubation, only 2.8 ± 2.2% of the Fe^{II} was recovered demonstrating that the HCl concentration was high enough to allow significant Fe^{II} oxidation.

Effect of HCl, H^+ and Cl^- concentration on abiotic Fe^{II} oxidation

The effect of HCl concentration on Fe^{II} oxidation was determined by incubation of dissolved $FeCl_2$ and dissolution of magnetite in oxic 1–6 M HCl for 24 h at 70°C under oxic
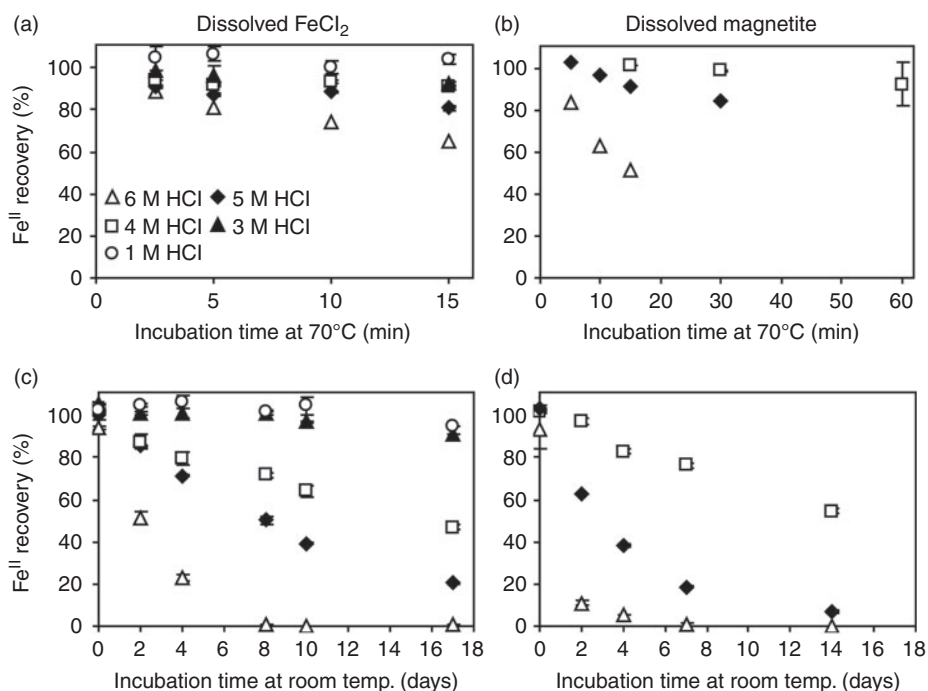
Fig. 2. Fe^{II} recovery after incubation of (a) 8–9 mM FeCl$_2$ and (b) 9–10 mM magnetite in oxic HCl of different concentrations at 70°C under oxic conditions. HCl with magnetite was sampled for the first time after complete dissolution of the mineral phase. For each time point a separate set of two parallel samples was incubated. (c) For FeCl$_2$ in 1–6 M HCl the samples incubated for 2.5 min at 70°C were kept at room temperature under oxic conditions and the Fe^{II} recovery was followed over time. (d) Magnetite samples that were dissolved for 15 min in 4 M HCl at 70°C and magnetite samples that were dissolved for 5 min in 5–6 M HCl at 70°C were kept at room temperature under oxic conditions. The Fe^{II} recovery was followed over time. (a–d) Results are means of duplicates. Bars indicate the range of duplicates.

conditions (Table 1). At all HCl concentrations, Fe$_{tot}$ from magnetite and FeCl$_2$ was recovered to ≥93%. The magnetite used dissolved completely in 1 M HCl and 101.3 ± 2.0% of the expected Fe^{II} was recovered, indicating that no Fe^{II} oxidation occurred. However, in 2 M HCl, a significant fraction of Fe^{II} was oxidised as the Fe^{II} recovery was only 85.8 ± 0.4%. The Fe^{II} concentration decreased further with increasing HCl concentration and with 6 M HCl, only 10.7 ± 8.7% of the Fe^{II} was recovered. For FeCl$_2$ in 1, 3 and 6 M HCl, similar results were obtained. But in contrast to magnetite, some Fe^{II} was oxidised in 1 M HCl after 24 h with a Fe^{II} recovery of 92.1 ± 1.8%.

In order to determine whether high concentrations of H$^+$ or Cl$^-$ cause Fe^{II} oxidation, FeCl$_2$ and magnetite were respectively incubated and dissolved under oxic conditions either with oxic 3 M H$_2$SO$_4$ or with oxic 1 M HCl containing 2 M NaCl for 24 h at 70°C (Table 1). A 3-M solution of H$_2$SO$_4$ has a free H$^+$ concentration of ~3 M, and also dissolved the magnetite. After 24 h, 90.9 ± 3.2% and 99.4 ± 0.9% of the total Fe^{II} concentration of FeCl$_2$ and magnetite were recovered respectively, similar to the results obtained for 1 M HCl. This indicates that no or only minor Fe^{II} oxidation took place. When 1 M HCl with 2 M NaCl was used for extraction, the Fe^{II} recovery for FeCl$_2$ and magnetite lay between the recoveries obtained for 1 and 3 M HCl. These results indicate that the increasing Fe^{II} oxidation observed with increasing HCl concentration is neither triggered by the increasing H$^+$ concentration nor the increasing Cl$^-$ concentration alone.

Kinetics of O$_2$-dependent abiotic Fe^{II} oxidation in HCl

In order to determine the kinetics of Fe^{II} oxidation at 70°C, the recovery of Fe^{II} from FeCl$_2$ and magnetite was quantified over time in short-time experiments with oxic HCl and air as headspace (Fig. 2a,b). Set-ups with FeCl$_2$ were incubated for up to 15 min, and set-ups with magnetite for a maximum of 60 min. For both FeCl$_2$ and magnetite, the Fe^{II} recovery decreased over time with a faster decrease at higher HCl concentrations. For FeCl$_2$ in 1 M HCl, Fe^{II} was completely recovered at each time point, indicating that no Fe^{II} oxidation took place within the first 15 min (Fig. 2a). For FeCl$_2$ in 3 and 4 M HCl a slight decrease in the Fe^{II} concentration was observed, whereas the decrease in 5 and 6 M HCl was already significant within 15 min. In the case of magnetite, only 4–6 M HCl was used, as magnetite dissolved only slowly in 1–3 M HCl and we intended to avoid a mixture of dissolved Fe^{2+}, remaining magnetite and magnetite-sorbed Fe^{II}. In 5 and 6 M HCl, magnetite was dissolved completely within 5 min, and in 4 M HCl within 15 min. As observed for FeCl$_2$, Fe^{II} oxidation was faster with increasing HCl concentration (Fig. 2b).

Influence of sample storage at room temperature on Fe^{II} oxidation in HCl

Fe concentrations in samples from extraction procedures are often not quantified directly after extraction, but are collected and analysed together. To quantify Fe^{II} oxidation during sample storage, selected samples from the short-time experiments at 70°C (samples taken after 2.5 min for FeCl$_2$ in 1–6 M HCl, after 15 min for magnetite in 4 M HCl and after 5 min for magnetite in 5–6 M HCl) were stored undiluted in the dark at room temperature under oxic conditions (Fig. A1 of the Accessory publication). The Fe^{II} recovery during sample storage decreased over time, similar to what had been observed at 70°C, with faster Fe^{II} oxidation at higher HCl concentrations (Fig. 2c,d). However,
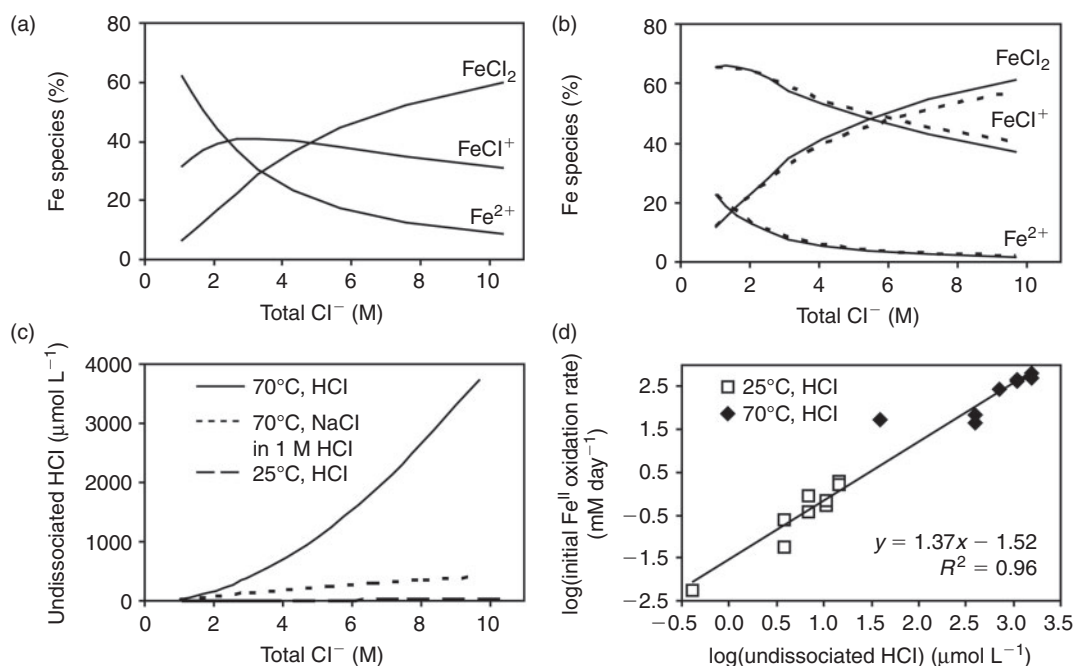
Fig. 3. Concentrations of Fe^{II} species for 10 mM $FeCl_2$ under anoxic conditions calculated using the REACT module of '*The Geochemist's Workbench 6.0*' package (a) at 25°C with increasing HCl concentration and (b) at 70°C with increasing HCl concentration (solid lines) and increasing NaCl concentration in 1 M HCl (dashed lines). (c) Calculated concentration of undissociated HCl for the same conditions as in (a) and (b). (d) Initial Fe^{II} oxidation rate of $FeCl_2$ in 1–6 M HCl at 25°C ($\square$) and 70°C ($\blacklozenge$) versus the concentration of formed undissociated HCl. Initial Fe^{II} oxidation rates were calculated from the Fe^{II} concentrations measured at day 0 and 2 at 25°C (Fig. 2c), and 0 and 2.5 min at 70°C (Fig. 2a) respectively.

Fe^{II} oxidation at room temperature was much slower compared with Fe^{II} oxidation at 70°C. The Fe_{tot} concentration remained constant over time (data not shown).

*Geochemical modelling of Fe^{II} speciation and undissociated HCl*

In order to identify the reactive Fe^{II} species that might be responsible for the observed Fe^{II} oxidation in HCl, the Fe^{II} species present under anoxic conditions and their relative concentrations were estimated by geochemical modelling at different Cl^- concentrations at 25 and 70°C with $FeCl_2$ as a source of Fe^{II}. In all scenarios analysed, the predominant Fe^{II} species were Fe^{2+}, $FeCl^+$ and $FeCl_2$ (Fig. 3a,b). At 25°C, the Fe^{2+} concentration decreased with increasing HCl concentration, while the $FeCl_2$ concentration increased simultaneously (Fig. 3a). The $FeCl^+$ concentration varied between 31 and 41% with a maximum concentration of 3 M total Cl^-. At 70°C, the Fe^{2+} concentration at low Cl^- concentrations was lower than at 25°C but also decreased with increasing HCl concentration accompanied by an increase of $FeCl_2$ (Fig. 3b). In contrast, the $FeCl^+$ concentration was higher at low Cl^- concentrations compared with 25°C and decreased with increasing HCl concentration. When the Cl^- concentration was increased by adding NaCl to $FeCl_2$ in 1 M HCl at 70°C, the change in concentration of the three Fe^{II} species was similar to the changes observed with increasing HCl concentration at 70°C (Fig. 3b). The change in undissociated HCl concentration was also calculated for the three different experimental systems and increased in all scenarios with increasing total Cl^- concentration (Fig. 3c). At 25°C with increasing HCl concentration, the concentration of undissociated HCl did not exceed 50 μM. In contrast, at 70°C the increase of undissociated HCl was up to two orders of magnitude higher and reached the millimolar range. With increasing NaCl

concentration in 1 M HCl at 70°C, less undissociated HCl was formed than with increasing HCl concentration at 70°C.
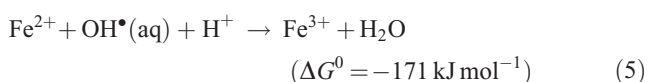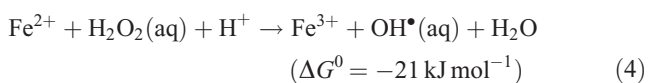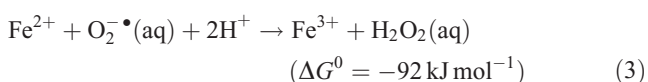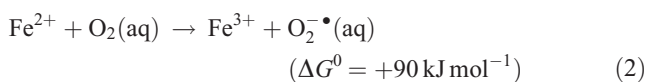
Discussion

Thermodynamics of Fe^{II} oxidation by O_2

During the oxidation of Fe^{II} by O_2 four electrons are transferred:

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$
$$(\Delta G^0 = -178\,kJ\,mol^{-1}) \qquad (1)$$
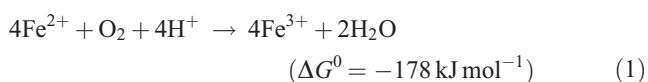
Considering the E_h–pH diagram for Fe^{II}/Fe^{III} and O_2/H_2O at 25°C and a concentration of 1 M of all involved compounds (Fig. A2 of the Accessory publication), from a thermodynamic standpoint Fe^{II} oxidation is expected to occur at all pH values. However, Weiss suggested that the electrons are transferred in four one-electron steps.[28] The free energies of each step were calculated for standard conditions based on the ΔG^0 values given by Stumm and Morgan[4]:

$$Fe^{2+} + O_2(aq) \rightarrow Fe^{3+} + O_2^{-\bullet}(aq)$$
$$(\Delta G^0 = +90\,kJ\,mol^{-1}) \qquad (2)$$

$$Fe^{2+} + O_2^{-\bullet}(aq) + 2H^+ \rightarrow Fe^{3+} + H_2O_2(aq)$$
$$(\Delta G^0 = -92\,kJ\,mol^{-1}) \qquad (3)$$

$$Fe^{2+} + H_2O_2(aq) + H^+ \rightarrow Fe^{3+} + OH^\bullet(aq) + H_2O$$
$$(\Delta G^0 = -21\,kJ\,mol^{-1}) \qquad (4)$$

$$Fe^{2+} + OH^\bullet(aq) + H^+ \rightarrow Fe^{3+} + H_2O$$
$$(\Delta G^0 = -171\,kJ\,mol^{-1}) \qquad (5)$$

Although reaction steps 2–4 are thermodynamically favourable, the first step is thermodynamically unfavourable and is assumed to be the rate limiting step.[4] The free energy of reactions 1 and 2 calculated for our experimental conditions show that the overall reaction (reaction 1) is thermodynamically favourable, whereas the first electron transfer step (reaction 2) is thermodynamically unfavourable both at 25 and 70°C (see 'Free energy calculations' section of the Accessory publication, Table A3). Thus thermodynamic considerations do not explain why Fe[II] oxidation occurs at circumneutral pH and at high HCl concentrations, but not at lower HCl concentrations.

Kinetics of Fe[II] oxidation by O_2

The Fe[II] oxidation rate is strongly influenced by pH. Under circumneutral conditions (pH 6–8), the oxidation of Fe[II] is described by the rate law[3]:

$$-d[Fe^{II}]/dt = k \cdot [Fe^{II}] \cdot p_{O_2} \cdot [OH^-]^2 \qquad (6)$$

where $[Fe^{II}]$ and $[OH^-]$ are the Fe[II] and OH^- concentrations; t, time; k, the reaction constant; and p_{O_2}, the partial pressure of O_2. Accordingly, the oxidation rate increases 100-fold when the pH increases by one unit. OH^- ions enhance Fe[II] oxidation because of the pH dependent formation of Fe[II]–hydroxo complexes. For pH <2 the rate is independent of the OH^- concentration, and therefore does not change with pH.[29] Besides OH^- ions, the kinetics of Fe[II] oxidation was also shown to depend on other anions present. Cl^- and SO_4^{2-} ions, for example, decrease the Fe[II] oxidation rate.[29,30] As slight changes in pH at pH ~0 should have no effect on the Fe[II] oxidation rate and addition of Cl^- is expected to decrease it, one would expect that with increasing HCl concentrations, the Fe[II] oxidation rate decreases. However, we observed the opposite – an increasing Fe[II] oxidation rate with increasing HCl concentration. Our results therefore suggest that in the presence of high HCl concentrations a reactive Fe[II] species is formed that is easily oxidised, and that the concentration of this species increases with increasing HCl concentration. This is in line with other publications that suggested that the Fe[II] oxidation rate depends among other factors on the present Fe[II] species, which are determined by the present anions, but depends not on the formation of a more reactive oxidant species (see e.g. Trapp and Millero[31]).

Role of Fe[II]–Cl species for Fe[II] oxidation

In order to identify this reactive Fe[II] species, the concentrations of different Fe[II] species present in our experimental systems were estimated by geochemical modelling showing that Fe^{2+}, $FeCl^+$ and $FeCl_2$ are the main dissolved Fe[II] species at all HCl concentrations at 25 and 70°C (Fig. 3a,b). Although the calculated concentrations of these three Fe[II] species varies between this and three previous studies,[32–34] all studies exhibit the same trend whereby the concentration of Fe[II]–Cl^- complexes and the number of Cl^- ligands in these complexes increase with increasing Cl^- concentration. Hypothesising that the concentration of an easily oxidisable Fe[II] species increases with increasing HCl concentration, our modelling results suggest that $FeCl_2$ might be this species (Fig. 3a,b). Although the formation of Fe[II]–Cl^- ion pairs slow down Fe[II] oxidation at circumneutral pH,[29,30] the oxidation kinetics of these complexes may be different at acidic pH. If the formation of $FeCl_2$ (or another Fe[II]–Cl^- complex) stimulates Fe[II] oxidation under acidic conditions, one would hypothesise that (i) in the absence of Cl^- at low pH,

no significant Fe[II] oxidation occurs as no Fe[II]–Cl^- complex can form and (ii) at low pH, i.e. at pH ~0, the formation of the Fe[II]–Cl^- species and thus the Fe[II] oxidation are influenced mainly by the Cl^- concentration and not by the H^+ concentration. Hypothesis (i) was tested by incubating Fe[II] in 3 M H_2SO_4 (H^+ concentration ~3 M) and indeed, only minor or no oxidation of Fe[II] occurred compared with the 3 M HCl set-up (Table 1), indicating that Cl^- must be present to enhance Fe[II] oxidation under acidic conditions. This is supported by Awakura et al.[23] who observed no increase in Fe[II] oxidation rates with increasing H_2SO_4 concentration (1–3 M) at 80°C.

Hypothesis (ii) presumes that at low pH, the reactive Fe[II] species forms with increasing Cl^- concentration. And indeed, geochemical modelling yielded almost the same distribution of Fe–Cl^- species with increasing NaCl concentrations as for increasing HCl concentrations (Fig. 3b). Furthermore, Fe[II] oxidation was enhanced in the presence of 2 M NaCl in 1 M HCl in comparison to set-ups with 1 M HCl alone but not as much as by 3 M HCl (Table 1). These results indicate that the Fe[II] oxidation rate is influenced by the Cl^- concentration but that the formation of Fe[II]–Cl^- complexes alone does not explain the experimental data. This suggests that another reactive Fe[II] species besides Fe[II]–Cl^- complexes is formed.

Role of undissociated HCl for Fe[II] oxidation

The formation of a complex of Fe[II] with HCl (Fe–HCl complex) was previously suggested to explain an increase in Fe[II] oxidation rate at room temperature with increasing HCl concentration.[23–25] Fast Fe[II] oxidation in 4 M HCl was also observed by Astanina and Rudenko.[35] They proposed that Fe[II] oxidation occurs by the formation of Fe[II]–(hydroxo)aquo complexes and suggested that at high HCl concentrations, undissociated HCl rather than anions enters these complexes, and thereby changes the oxidation kinetics. In our modelled scenarios, the concentration of undissociated HCl increased with increasing Cl^- concentration but the increase was temperature dependent (Fig. 3c). The higher concentration of undissociated HCl at 70°C than at 25°C may be the reason for the higher Fe[II] oxidation rates observed at 70°C. This is supported by the linear relationship of the initial Fe[II] oxidation rates of $FeCl_2$ in 1–6 M HCl and the concentration of undissociated HCl formed under these conditions (Fig. 3d). As the same relationship can be observed at 25 and 70°C, the formation of more undissociated HCl with increasing temperature is probably the main reason for the increase in Fe[II] oxidation rates rather than temperature alone (which is expected to increase the oxidation rates according to the Arrhenius equation). The potentially important role of undissociated HCl in Fe[II] oxidation is also supported by the fact that an increase in Cl^- by addition of NaCl to 1 M HCl at 70°C resulted in much lower concentrations of undissociated HCl compared with the experiments with increasing HCl concentration (Fig. 3c). Accordingly, we observed lower Fe[II] oxidation in 1 M HCl amended with 2 M NaCl than in 3 M HCl (Table 1). In conclusion, the formation of undissociated HCl and hence the potential formation of a Fe–HCl complex depends on H^+ and Cl^- concentration and on temperature. This formation explains well the observed Fe[II] oxidation in HCl and its increase with increasing HCl concentration and temperature.

Conclusion

The results of this study showed that extraction of Fe from environmental samples with oxic and highly concentrated HCl leads to incorrect quantities of Fe[II] and Fe[III] in the Fe redox

speciation analysis. However, dissolution of Fe minerals in 6 M HCl has been applied in numerous studies to follow microbial changes in Fe mineralogy and Fe redox speciation and no Fe^{II} oxidation was mentioned in these studies.[9,36,37] This may be due to several reasons. First, for extractions with 6 M HCl performed at ambient temperatures we found initial Fe^{II} oxidation rates of $\sim$2 mM day^{-1} (Fig. 3d). Thus, if extraction procedures including Fe quantification take only a few hours, Fe^{II} oxidation is probably too low to be recognised but can still be significant ($\sim$50–100 μM h^{-1}). Second, in some experimental systems, the total concentration of Fe^{II} is unknown, therefore, partial Fe^{II} oxidation will be unrecognised. Third, if Fe is extracted with highly concentrated HCl under anoxic conditions, no Fe^{II} oxidation will occur.

However, there are a few studies in which Fe^{II} oxidation in HCl was indeed observed but not discussed in detail. Matthews et al.[38] for example, quantified stable isotope fractionation between Fe^{II}–Cl$^-$ and Fe^{III}–Cl$^-$ complexes and observed Fe^{II} oxidation in 6 M HCl. Based on our results and previous studies, we recommend that in order to prevent Fe^{II} oxidation during Fe extraction with highly concentrated HCl, the extraction should be performed under anoxic conditions or at least high temperatures should be avoided. If the samples must be stored under oxic conditions, even if it is only for a few days, a dilution of the samples to a HCl concentration of 3 M HCl or lower is suggested.

Accessory publication

Accessory material includes soil properties and experimental methods for their determination (Table A1), an overview of all experiments (Table A2), the experimental set-up for quantification of initial Fe^{II} oxidation at 70°C and Fe^{II} oxidation over time at room temperature (Fig. A1), geochemical modelling of HCl–FeCl$_2$ solutions at 25 and 70°C in the absence and presence of NaCl (see 'Geochemical equilibrium calculation' section of the Accessory publication), E_h–pH diagram for Fe^{III}/Fe^{II}, O$_2$, H$_2$ and O$_2$/O$_2^{-\bullet}$ (Fig. A2), and free energy calculations for our experimental conditions (see 'Free energy calculations' section of the Accessory publication, Table A3). This material is available free of charge online at http://www.publish.csiro.au/?act=view_file&file_id=EN10125_AC.pdf.

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