Accessory publication

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

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Table A1.   Selected properties of Waldenbuch (Wabu) and Schoenbuch forest (Sbu) soils

XRF, X-ray fluorescence analysis; wt% refers to 105°C dried soil

Soil	pH (0.01 M CaCl$_2$)	Water content[A]	Total organic carbon	CaCO$_3$ equivalents[B]	Total Fe by XRF[B]	Total Fe by 6 M HCl extraction[A]	Total Fe by 6 M HCl extraction[A]
		(wt%)	(wt%)	(wt%)	(wt%)	(wt%)	(μmol g^{-1} wet soil)
Wabu	7.1[C]	40	4.1[B]	1.5	2.2	2.0	258.3
Sbu	7.0[B]	49	3.5[A]	12.0	2.6	1.8	214.8

[A]Determined in triplicates or more.

[B]Determined in duplicates.

[C]Determined as single measurement.

Soil analysis

Before soil analysis, large particles (e.g. stones, roots) were removed either by hand with tweezers (Waldenbuch (Wabu) soil) or by sieving (2-mm sieve, Schoenbuch forest (Sbu) soil). The soil was stored in plastic bags at 4°C in the dark until further use. The soil water content was determined and calculated after standard protocols by drying the soil at 105°C until the soil weight was constant.[1] The pH was measured 24 h after addition of 25 mL of 0.01 M CaCl$_2$ solution to 10 g of soil after standard protocols.[1] Total organic carbon content was determined from finely ground 105°C dried soil with a CN analyser (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) after carbonate removal with HCl. The content of CaCO$_3$ equivalents was quantified by mixing finely ground 105°C dried soil with 1 M HCl and determining the consumed HCl by titration with 1-M NaOH. The total Fe (Fe$_{tot}$) content was determined by 6 M HCl extraction and by X-ray fluorescence analysis (XRF). Fe$_{tot}$ was extracted from 1.0 g of field moist soil with 50 mL of 6 M HCl in closed 120-mL serum bottles at 70°C in a water bath for 24 h. The soil–acid mixture was cooled for 15 min at room temperature. After short mixing, 1.8 mL of the suspension was centrifuged (Centrifuge 5417C, Eppendorf AG, Hamburg, Germany) for 15 min at 20817g at 25°C. Fe$_{tot}$ was quantified in the supernatant by the ferrozine

assay[2] as described by Hegler et al.[3] and the concentration was corrected by the water content of the soil. The Fe_{tot} content of finely ground 105°C dried soil was also quantified by XRF (Bruker AXS S4 Pioneer X-ray spectrometer, Bruker AXS GmbH, Karlsruhe, Germany). The two soils were chosen for this experiment because they contained extractable Fe_{tot} concentrations in the wt% range and extractable Fe^{II} (Fig. 1b).

Table A2. **Overview of experiments determining the influence of different factors on the oxidation of Fe^{II} present in soils, $FeCl_2$ solution, and magnetite**

Oxic means that O_2 was present in solution and headspace (from air), anoxic means that no O_2 was present in solution and headspace

| Factor tested	Acid	O_2 conditions	Incubation time	Temperature	Fe^{II} phase	Figures and tables
HCl concentration	1–6 M HCl	oxic	24 h	70°C	Soil $FeCl_2$, magnetite	Fig. 1c, Table 1
Time	6 M HCl	oxic	1–24 h	70°C	Soil	Fig. 1a,b
	1–6 M HCl	oxic	2.5–60 min	70°C	$FeCl_2$, magnetite	Fig. 2a,b
Temperature	1–6 M HCl	oxic	2 weeks	Room temperature	$FeCl_2$, magnetite	Fig. 2c,d
H^+ concentration	3 M H_2SO_4	oxic	24 h	70°C	$FeCl_2$, magnetite	Table 1
Cl^- concentration	1 M HCl + 2 M NaCl	oxic	24 h	70°C	$FeCl_2$, magnetite	Table 1
O_2	6 M HCl	anoxic	24 h	70°C	Soil $FeCl_2$, magnetite	Fig. 1c, Table 1
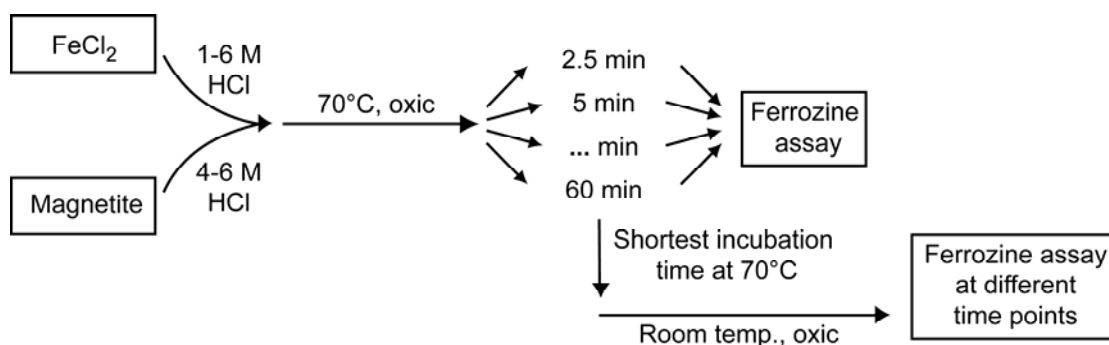
Fig. A1. Experimental set-up to follow Fe^{II} oxidation (i) at 70°C during the first 60 min of incubation (Fig. 2a,b) and (ii) at room temperature for several days in those samples with the shortest incubation time at 70°C (Fig. 2c,d), both under oxic conditions.

Experimental procedure

$FeCl_2$ and magnetite were first incubated in oxic 1–6 M HCl ($FeCl_2$) or oxic 4–6 M HCl (magnetite) for a certain time period at 70°C under oxic conditions (air) (Fig. 2a,b). For each time point and each Fe–HCl mixture, a separate set of two parallels was incubated. After incubation 2 mL of sample were

taken, from which 100 μL were immediately diluted 1:10 with 1 M HCl to stop Fe^{II} oxidation, and Fe^{II} and Fe_{tot} were quantified by the ferrozine assay[2] as described by Hegler et al.[3] The remaining 1.9 mL of the samples with the shortest incubation time at 70°C were further incubated in 2 mL plastic cups at room temperature under oxic conditions (air) in the dark. For $FeCl_2$ in 1–6 M HCl, the shortest incubation time was always 2.5 min. Magnetite needed more time to dissolve in HCl, therefore the shortest incubation time in 4–M HCl was 15 min and in 5–6 M HCl it was 5 min. During the incubation at room temperature, sub-samples of the Fe–HCl mixtures were taken at several time points and Fe^{II} and Fe_{tot} were quantified by the ferrozine assay.

Geochemical equilibrium calculation

The geochemical speciation in HCl–$FeCl_2$ solutions was calculated using the REACT module of *The Geochemist's Workbench 6.0* package (RockWare Inc., Golden, CO, USA) and the 'thermo' database. According to the manual, the calculation of the activity coefficients by this method is not especially accurate at ionic strength > 0.5 mol kg^{-1}.[4,5] We are aware that therefore these calculations cannot be used for an absolute quantitative analysis of the geochemical species at the conditions used in our study (ionic strength > 0.5 mol kg^{-1}). The influence of different HCl concentrations at different temperatures were calculated by using the following input data: 0.01 M $FeCl_2$, 1 M H^+, 1 M Cl^- with water as solvent at 25 and 70°C respectively. The pH was decreased in 10 steps to pH −1. The Cl^- concentration was used as charge balance and therefore increased in the same way as the H^+ concentration. To determine the influence of different NaCl concentrations in 1 M HCl at 70°C, the following input data were used: 0.01 M $FeCl_2$, 1 M H^+, 0.01 M Na^+, 1.01 M Cl^- with water as solvent. The activity of Na^+ was increased in 10 steps to 6 mol kg^{-1} with Cl^- as charge balance.
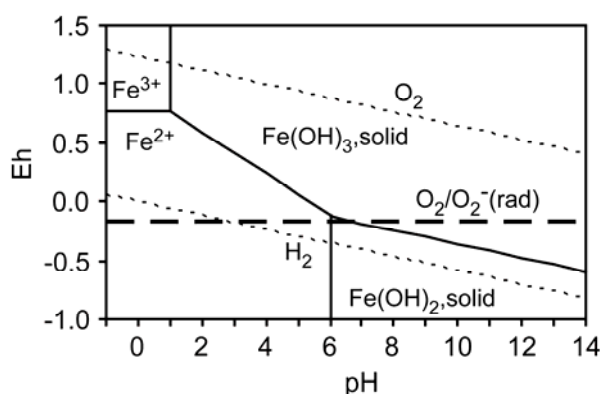

Fig. A2. Eh-pH diagram for Fe^{III}/Fe^{II}, O_2, H_2 and $O_2/O_2^{-\bullet}$ calculated with concentrations of 1 M for all compounds at 25°C. For simplicity, $Fe(OH)_3$ was used as approximation for the Fe^{III} species present.

Free energy calculations

During the oxidation of Fe^{II} by O_2 four electrons are transferred, which is thermodynamically favourable under standard conditions (pH = 0, 25°C):

$$4\ Fe^{2+} + O_{2(aq)} + 4\ H^+ \rightarrow 4\ Fe^{3+} + 2\ H_2O\ (\Delta G^0 = -178\ kJ\ mol^{-1})\ (A1)$$
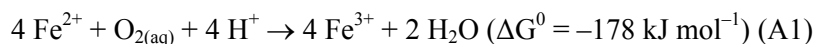
Weiss suggested that the electrons are transferred in four one-electron steps.[6] The first electron transfer step leads to the formation of an $O_2^{-\bullet}$ radical and is thermodynamically unfavourable under standard conditions:

$$Fe^{2+} + O_{2(aq)} \rightarrow Fe^{3+} + O_2^{-\bullet}{}_{(aq)}\ (\Delta G^0 = +90\ kJ\ mol^{-1})\ (A2)$$
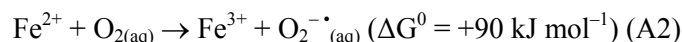
As the free energy depends on the concentration of the compounds involved in a reaction and on temperature, we calculated the free energy of both reactions (A1 and A2) for our experimental conditions to determine if the reactions were thermodynamically favourable in our experimental systems.

The free energy at the beginning of the experiment was calculated by

$$\Delta G = -n \cdot F \cdot \Delta E\ (A3)$$

with n being the numbers of transferred electrons (here $n = 4$ and 1 respectively); F, the Faraday constant; and ΔE, the redox potential of the reaction under experimental conditions.

ΔE was calculated for the four electron transfer reaction according to

$$\Delta E = \Delta E° - (2.303 \cdot R \cdot T/n \cdot F) \cdot \log([Fe^{3+}]^4/[Fe^{2+}]^4 \cdot [O_{2(aq)}] \cdot [H^+]^4)\ (A4)$$

and for the first single electron transfer step according to

$$\Delta E = \Delta E° - (2.303 \cdot R \cdot T/n \cdot F) \cdot \log([Fe^{3+}] \cdot [O_2^{-\bullet}{}_{(aq)}]/[Fe^{2+}] \cdot [O_{2(aq)}])\ (A5)$$

with $\Delta E°$ being the redox potential of the reaction under standard conditions (0.46 V for reaction A4, and –0.93 V for reaction A5 calculated based on the ΔG^0 values given in Stumm and Morgan[7]); R, the universal gas constant; T, temperature (K); and n and F are as given above. According to our experimental conditions, the temperature was set to 298.15 K (25°C) or 343.15 K (70°C), the H^+ concentration to 1 or 6 M, and the Fe^{2+} concentration to 10 mM. The Fe^{3+} concentration was assumed to be very small in the beginning of the reaction and therefore set to 1 μM. At 25°C 8 mg L^{-1} (0.25 mM) O_2 can dissolve in water and at 70°C 4 mg L^{-1} (0.125 mM). The high salt concentration of 6 M HCl, which leads to a smaller dissolved O_2 concentration was not considered. Similarly as for the Fe^{3+} concentration, also the $O_2^{-\bullet}{}_{(aq)}$ concentration was assumed to be very low in the beginning of the experiment and therefore set to be four orders of magnitudes lower than the $O_{2(aq)}$ concentration applied. The results of the free energy calculations for the different experimental set-ups are given in Table A3.

Table A3.   Free energy of the four-electron transfer step (reaction A1) and the first one-electron transfer step (reaction A2) during Fe^{2+} oxidation by dissolved molecular O_2

ΔG was calculated using reactions A3, A4 and A5 for following conditions: 10 mM Fe^{2+}, 1-µM Fe^{3+}, 250-µM $O_{2(aq)}$ and 25-nM $O_2^{-\bullet}{}_{(aq)}$ at 25°C, 125 µM $O_{2(aq)}$ and 12.5-nM $O_2^{-\bullet}{}_{(aq)}$ at 70°C. Please note: the first one-electron transfer step (reaction A2) is independent of the H^+ concentration

	Four-electron transfer step ΔG (kJ mol^{-1})	First one-electron transfer step ΔG (kJ mol^{-1})
25°C, 1 M H^+	−248	+44
25°C, 6 M H^+	−266	+44
70°C, 1 M H^+	−257	+37
70°C, 6 M H^+	−277	+37

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