Mineral defects enhance bioavailability of goethite towards microbial Fe(III) reduction

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1. Methods - Additional details

**Goethite Synthesis.** Natural abundance goethite and $^{56}$Fe goethite were prepared following a modified version of Schwertmann and Cornell.\(^1\) Briefly, zerovalent iron (natural abundance or $^{56}$Fe-enriched) was dissolved in HCl to obtain 15 mL of an Fe(II) stock ($\sim 0.6$ M Fe(II), $\sim 1.8$ M HCl); the solution was oxidized using 2 mL of 30% H$_2$O$_2$ to produce Fe(III). Then, the pH was raised with 16 mL of 5 M KOH, and the resulting precipitate was placed in an oven (70°C, 60 h).

**Mössbauer spectroscopy.** For Mössbauer spectroscopy, solids were collected on a 0.2 $\mu$m nitrocellulose filter, sealed between two pieces of Kapton tape and immediately transferred to the instrument to avoid air oxidation. Mössbauer spectra were collected at 77 K on a spectrometer supplied by Web Research, Inc. (Edina, Minnesota, USA) and equipped with a closed-cycle cryostat (CCS-850 System, Janis Research Co., Wilmington, Massachusetts, USA). We acquired spectra in transmission mode using a constant acceleration drive system and a $^{57}$Co source. The velocity scale was calibrated using a 7-$\mu$m α-Fe(0) foil. Spectra were fitted using the Voigt Based Fitting (VBF) routine available in Recoil software (Ottawa, Canada).\(^2\)

2. Calculation of the percentage of atoms arising from isotope-labeled goethite

To convert the percentage of $^{57}$Fe in the reduced atoms to the percentage of atoms arising from the $^{57}$Fe-labeled goethite we divided the concentration of $^{57}$Fe per 0.0231 (concentration of $^{57}$Fe in our natural abundant samples).
Figure S1. Comparison of $^{57}$Fe isotope released during reduction of goethite by dithionite (yellow and green lines) and Geobacter/AQDS (black line). Reactors containing a labeled mix of goethite$^{(\text{AS})}$ and goethite$^{(\text{HT})}$ samples regular solids or freeze-dried solids. Data corresponds to average of triplicates and the error bars indicate standard deviation. Experimental conditions: 1 g L$^{-1}$ $^{56}$goethite$^{(\text{AS})}$ + 1 g L$^{-1}$ or $^{\text{NA}}$goethite$^{(\text{HT})}$; For biological experiments: $10^8$ cells mL$^{-1}$ Geobacter sulfurreducens; 10 µM AQDS. Data in black corresponds to the average of the biological reduction of the same mix of isotope-labeled goethites (same as in Figure 1).
3. **Calculation of surface area normalized initial rate of microbial Fe(III) reduction**

To calculate the initial rate of reduction in the presence of AQDS, we quantified the Fe(III) reduced for the first time point and divided by the time (960 min) obtaining Fe(III) reduced per minute. We then plotted this value against the goethite surface area contained in the reactor (g x m$^2$ g$^{-1}$) to obtain the surface area normalized initial rate of microbial Fe(III) reduction (Figure S2a).

For reactors without AQDS, the calculation was based on the triplicate of one goethite concentration as we did not run experiments with different loadings without AQDS (Figure S2b).

**Figure S2.** Surface area normalized initial rate of microbial Fe(III) reduction for goethite$^{(AS)}$ and goethite$^{(HT)}$, (a) with and (b) without AQDS. Note that graphs a and b have different y-axis values.
Figure S3. Addition of phenanthroline as an indicator of the presence of Fe(II) sorbed to the inside glass walls of the bottles.
Figure S4. Percentage of Fe(III) reduction for reactors containing different loadings of (a) goethite\textsuperscript{(AS)} and (b) goethite\textsuperscript{(HT)} in the presence of AQDS. Data corresponds to average of biological triplicates and the error bars indicate standard deviation. The shaded areas correspond to the percentage of atoms at the surface for goethite. Experimental conditions: 0.5, 1 or 2 g L\textsuperscript{-1} goethite\textsuperscript{(AS)} or goethite\textsuperscript{(HT)}; 10\textsuperscript{8} cells mL\textsuperscript{-1} \textit{Geobacter sulfurreducens}; 10 µM AQDS.
4. Iron atoms at the surface of goethite

Here we assume that goethite surfaces are dominated by exposure of (101) and (001) crystallographic faces. The surface atom densities were calculated from crystallographic information file models of the goethite crystal structure drawn in CrystalMaker 2.5 in our previous work.

Calculation of surface atom density

(101) surface

\[ 1 \times 8 \text{ Fe atoms} + \frac{1}{2} \times 6 \text{ Fe atoms} + \frac{1}{4} \times 4 \text{ Fe atoms} = 12 \text{ Fe atoms} \]

\[ \rightarrow 12 \text{ Fe atoms} / (9.039 \, \text{Å} \times 10.920 \, \text{Å}) = 0.122 \text{ Fe/Å}^2 = 12.2 \text{ Fe/nm}^2 \]

(001) surface

\[ 1 \times 8 \text{ Fe atoms} + \frac{1}{2} \times 6 \text{ Fe atoms} + \frac{1}{4} \times 4 \text{ Fe atoms} = 12 \text{ Fe atoms} \]

\[ \rightarrow 12 \text{ Fe atoms} / (9.913 \, \text{Å} \times 9.038 \, \text{Å}) = 0.134 \text{ Fe/Å}^2 = 13.4 \text{ Fe/nm}^2 \]

Number of Fe atoms at the surface in a gram of goethite with and without defects

- Goethite\textsuperscript{(AS)}

Specific surface area = 28 m\textsuperscript{2}/g = 2.8 \times 10^{19} \text{ nm}^2/g

\[ 12.2 \text{ Fe/nm}^2 \times 2.8 \times 10^{19} \text{ nm}^2/g = 3.416 \times 10^{20} \text{ Fe surface atoms/g} – (101) surface \]

\[ 13.4 \text{ Fe/nm}^2 \times 2.8 \times 10^{19} \text{ nm}^2/g = 3.752 \times 10^{20} \text{ Fe surface atoms/g} – (001) surface \]

- Goethite\textsuperscript{(HT)}

Specific surface area = 22 m\textsuperscript{2}/g = 2.2 \times 10^{19} \text{ nm}^2/g

\[ 12.2 \text{ Fe/nm}^2 \times 2.2 \times 10^{19} \text{ nm}^2/g = 2.684 \times 10^{20} \text{ Fe surface atoms/g} – (101) surface \]

\[ 13.4 \text{ Fe/nm}^2 \times 2.2 \times 10^{19} \text{ nm}^2/g = 2.948 \times 10^{20} \text{ Fe surface atoms/g} – (001) surface \]

Number of Fe atoms in a gram of goethite

\[
\frac{\text{Number of atoms Fe per mol}}{\text{molar mass goethite}} = \frac{6.022 \times 10^{23}}{88.86} = 6.777 \times 10^{21} \text{ atoms Fe/g}
\]

Fraction of Fe atoms at the surface

- Goethite\textsuperscript{(AS)}

\[
\frac{3.416 \times 10^{20} \text{ Fe surface atoms/g}}{6.777 \times 10^{21} \text{ atoms Fe/g}} = 5.04 \% \text{ of surface atoms} – (101) surface
\]
\[
\frac{3.752 \times 10^{20} \text{ Fe surface atoms/g}}{6.777 \times 10^{21} \text{ atoms Fe/g}} = 5.54 \% \text{ of surface atoms} - (001) \text{ surface}
\]

- **Goethite**(HT)

\[
\frac{2.684 \times 10^{20} \text{ Fe surface atoms/g}}{6.777 \times 10^{21} \text{ atoms Fe/g}} = 3.96\% \text{ of surface atoms} - (101) \text{ surface}
\]

\[
\frac{2.948 \times 10^{20} \text{ Fe surface atoms/g}}{6.777 \times 10^{21} \text{ atoms Fe/g}} = 4.35 \% \text{ of surface atoms} - (001) \text{ surface}
\]

**Calculation of Fe atoms in each reactor**

\[
\frac{2 \text{ g}}{L} \times \frac{1 \text{ mole goethite}}{88.86 \text{ g}} = 0.0225 \text{ M} = 22.5 \text{ mM of goethite} = 22.5 \text{ mM of Fe per reactor}
\]
5. Calculation of surface area normalized instantaneous rate of microbial Fe(III) reduction of goethite\textsuperscript{(AS)} and goethite\textsuperscript{(HT)} using different goethite loadings

To calculate the instantaneous rate of reduction in the presence of AQDS, we calculated the difference between the Fe(II) in the current and previous points divided by the time difference between the points. We used the average of triplicates to calculate the rates.

**Figure S5.** Surface area normalized instantaneous rate of microbial Fe(III) reduction for goethite\textsuperscript{(AS)} and goethite\textsuperscript{(HT)} using (a) 0.5 g L\textsuperscript{-1}, (b) 1 g L\textsuperscript{-1} and (c) 2 g L\textsuperscript{-1} of goethite.
6. Calculation of surface area normalized initial instantaneous rate of microbial Fe(III) reduction of goethite\textsuperscript{(AS)} and goethite\textsuperscript{(HT)} separately or together

To calculate the rate of reduction in the presence of AQDS, we calculated the difference between the Fe(II) in the current and previous points divided by the time difference between the points. We used the average of triplicates to calculate the rates. Note that the data for the reduction separately is derived from the reduction using 1 g L\textsuperscript{-1} goethite (Figure S5b).

![Graph](image)

**Figure S6.** Surface area normalized instantaneous rate of microbial Fe(III) reduction for goethite\textsuperscript{(AS)} and goethite\textsuperscript{(HT)} (a) separately or (b) together.
Figure S7. Fraction of Fe(II) in aqueous phase, acetate extraction and HCl extraction for the microbial Fe(III) reduction of goethite$^{(AS)}$ and goethite$^{(HT)}$ in the presence of AQDS. This data derives from the data in Figure 3. Data corresponds to average of biological triplicates. Note that the shaded areas correspond to the fraction of atoms recovered in the aqueous phase (blue), acetate extraction (yellow) and HCl extraction (red).
Figure S8. X-ray diffractograms of inoculated and abiotic controls of goethite\(^{(AS)}\) and goethite\(^{(HT)}\). Experimental conditions: 2 g L\(^{-1}\) NA goethite or NA goethite\(^{(HT)}\), 0 or 10\(^8\) cells mL\(^{-1}\) Geobacter sulfurreducens; 10 µM AQDS; 20 days of incubation.
Figure S9. Mössbauer spectra of inoculated and abiotic controls of goethite$^{\text{AS}}$ and goethite$^{\text{HT}}$. Experimental conditions: 2 g L$^{-1}$ goethite$^{\text{AS}}$ or goethite$^{\text{HT}}$; 0 or 10$^8$ cells mL$^{-1}$ Geobacter sulfurreducens; 10 μM AQDS; 20 days of incubation.
Table S1. Mössbauer parameters derived from fitting spectra collected at 77 K. Samples of inoculated and abiotic controls for goethite\textsuperscript{(AS)} and goethite\textsuperscript{(HT)}.

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<th>Sample</th>
<th>Component</th>
<th>CS\textsuperscript{a}</th>
<th>QS\textsuperscript{b}</th>
<th>H\textsuperscript{c}</th>
<th>Std(H) or Std(QS)\textsuperscript{d}</th>
<th>Area (%)</th>
<th>Component area (%)</th>
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\textsuperscript{a}Center shift  \\
\textsuperscript{b}Quadrupole splitting for doublets and quadrupole shift parameter for sextets  \\
\textsuperscript{c}Hyperfine Magnetic Field  \\
\textsuperscript{d}Standards deviation of the Voigt profile for the hyperfine field or quadrupole splitting parameters, respectively
Figure S10. XRD diffractograms of goethite\textsuperscript{(AS)} inoculated for 19 days, before and after sodium acetate extraction. Experimental conditions: 2 g L\textsuperscript{-1} Na\textsubscript{2}goethite; 10\textsuperscript{8} cells mL\textsuperscript{-1} \textit{Geobacter sulfurreducens}; 10 µM AQDS; 19 days reduction.
REFERENCES