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Dissolution of Fe(III)(hydr)oxides by an aerobic bacterium

Although Fe is a necessary metabolic requirement for most plants and microorganisms, Fe oxyhydroxides have very low solubilities (e.g., Lindsay, 1988; Schwertmann, 1991) so that Fe is often a limiting nutrient (Neilands, 1982; Seaman et al., 1992; Hersman et al., 1995; Hersman et al., 1996; Hersman et al., 2001). Our DOE-BES sponsored research (Hersman, Maurice, and collaborator Sposito) focused primarily on the rates and mechanisms whereby aerobic microorganisms obtain Fe from mineral surfaces (Fe (III)(hydr)oxides and kaolinite clay). Initial work used Fe(III)(hydr)oxides and the aerobic bacterium, *Pseudomonas mendocina*. We showed that *P. mendocina* was able to obtain Fe from Fe(III)(hydr)oxides by enhancing mineral dissolution (Hersman et al., 1995, 1996, 2000, 2001; Maurice et al., 1996, 2000; Forsythe et al., 1998). Such dissolution has important implications to pollutant mobility because of the roles that Fe(III)(hydr)oxides play in metal and radionuclide adsorption in the subsurface. Because the bacteria take Fe up into their cells, mineral dissolution could not be monitored based on changes in solution concentrations; rather, we indirectly estimated Fe release using microbial growth rates under Fe-limited conditions.

P. mendocina is a true aerobe that is unable to use Fe(III) as a terminal electron acceptor for oxidative phosphorylation, but that still requires Fe as an essential nutrient for metabolic processes. Therefore, *P. mendocina* only requires μM concentrations of Fe (e.g., Hersman et al., 1996; 2000; Forsythe et al., 1998; Maurice et al., 2000; 2001a,b; Ams et al., 2000), in contrast to dissimilatory Fe-reducing bacteria (DIRB), which require mM concentrations (e.g. Arnold et al., 1988, 90; Lovley, 1991; Lovley and Phillips, 1986, 1988; Nealson and Meyers, 1992; Roden and Zachara, 1996; Zachara et al., 1998; Grantham et al., 1997). Research by our group (Hersman et al., 1995; 1996; 2000; Maurice et al., 1996; 2000; Forsythe et al., 1998) demonstrated that *P. mendocina* enhanced dissolution of Fe(III)(hydr)oxides. Maurice et al. (1999) showed that increasing Al content in the Fe(III)(hydr)oxide mineral goethite, increased the ability of *P. mendocina* to acquire Fe. This was opposed to predictions based on abiotic dissolution of Al-goethites, which tended to decrease with increasing Fe content. Cervini-Silva et al. (in press) showed that fungal siderophores behaved in agreement with the *P. mendocina* bacterium, dissolving more Fe from Al-substituted goethite than from pure goethite.

Hersman et al. (2001) showed that *P. mendocina* dissolved Fe more readily from the well ordered Fe(III)(hydr)oxides hematite and goethite than from more poorly ordered ferrihydrite, and was independent of BET surface area. See Figure 1.

Like many microorganisms (e.g. Nielands, 1981), *P. mendocina* produces Fe(III)-specific ligands known as siderophores when under Fe stress, as a means of acquiring Fe (Hersman et al., 1996; 2000). Hersman et al. (2000) showed that siderophore production by *P. mendocina* increased on a per-cell basis in the order: FeEDTA<hematite <ferrihydrite<no-Fe-added control, as the availability of Fe decreased. This is in agreement with findings of Neilands (1981) suggesting that siderophore production correlates positively with greater Fe deficiencies. Hersman et al. (1995) showed that the siderophore produced by and isolated from *P. mendocina* significantly enhanced hematite dissolution. In addition to producing siderophores, *P. mendocina* produced both extracellular and cell-associated Fe reductants in response to Fe deprivation and Fe

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Description/Abstract
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This project investigated the effects of an aerobic *Pseudomonas mendocina* bacterium on the dissolution of Fe(III)(hydr)oxides. The research is important because metals and radionuclides that adsorb to Fe(III)(hydr)oxides could potentially be remobilized by dissolving bacteria. We showed that *P. mendocina* is capable of dissolving Fe-bearing minerals by a variety of mechanisms, including production of siderophores, pH changes, and formation of reductants. The production of siderophores by *P. mendocina* was quantified under a variety of growth conditions. Finally, we demonstrated that microbial siderophores may adsorb to and enhance dissolution of clay minerals.

source (as FeEDTA or hematite). This reductant response was in the presence of overall aerobic conditions (Hersman et al., 2000). The different responses to Fe source are summarized below:

Table 1. *P. mendocina* responses to Fe source (- no activity, + weak activity, ++strong activity)

Fe source	Siderophore	Reduction activity	
		Cell-associated	Extracellular
no added Fe	+	-	++
FeEDTA	-	+	+(weak)
Hematite	+	+	+(weak)

These results are in agreement with observations of Lee et al. (1999) and Vartivarian and Cowart (1999) who suggested that siderophores are not the primary mobilizers of Fe, as previously thought; i.e., that reductants are also involved in dissolution even in aerobic environments.

Several lines of evidence suggested that Fe acquisition occurred at rates high enough to support growth not only of the microorganisms that were attached to mineral surfaces, but also those growing unattached. At circumneutral pH and oxic conditions, the [Fe] in equilibrium with Fe(III)(hydr)oxides is ~10^{-17}M, at most. In effect, this microorganism increased the equilibrium [Fe] by 10 to 11 orders of magnitude, for each of the Fe(III)(hydr)oxides examined (hematite, goethite, ferrihydrite). This work provided the first description of significantly different growth rates by a strict aerobe on three environmentally relevant, insoluble Fe(III)(hydr)oxides.

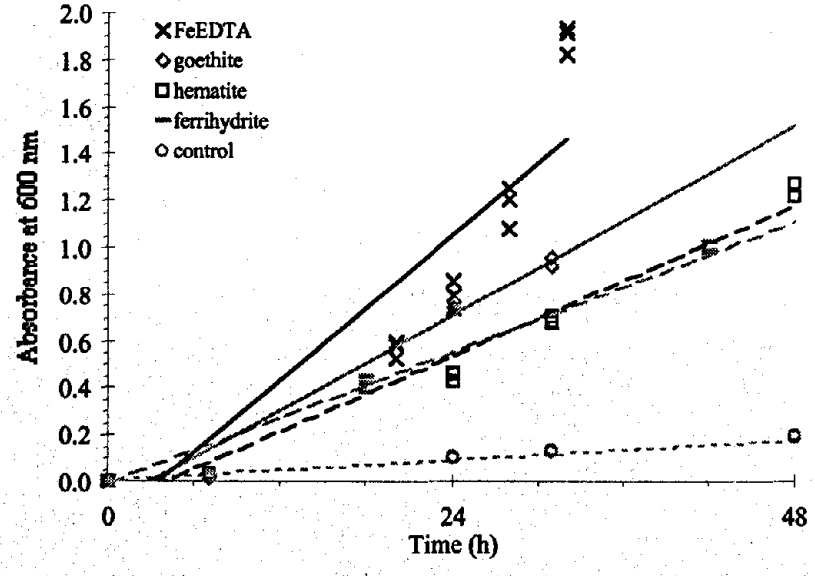

Figure 1. Microbial growth on different sources of Fe (30 uM FeEDTA; minerals approximately 29 m^2 l^{-1}, A_{eff}) and a no Fe added control. [FeEDTA, $y=0.051x - 0.176$, $R^2=0.81$; goethite, $y=0.034x - 0.087$, $R^2=0.97$; hematite, $y=0.027x - 0.102$, $R^2=0.97$; ferrihydrite, $y=0.023x + 0.003$, $R^2=0.99$; control, $y=0.004x + 0.008$, $R^2=0.97$]. Because Fe is limiting, increased growth indicates acquisition of Fe from the mineral sources.

Further analysis using HPLC and Chrome Azural S assay (Schwyn and Neilands 1987) confirmed that siderophore productin varied with different Fe(III)(hydr)oxides as

the Fe source. Interestingly, while *P. mendocina* exhibited greater growth on goethite than on hematite, it produced less siderophore in the process. *P. mendocina* also appeared to use less Kcal/cell to grow on goethite as compared to hematite (Hersman et al., in prep.).

Fe acquisition from kaolinite clay: bacteria and siderophores

Over the course of the bacterial Fe(III)(hydr)oxide dissolution experiments, we observed that *P. mendocina* were efficient at acquiring Fe from mineral sources. This suggested that the bacterium might also be able to access Fe from aluminosilicate clays, even clays such as kaolinite that contain only trace quantities of Fe. Maurice et al. (2001a, b) demonstrated that *P. mendocina* obtained μmol [Fe] from both well and poorly ordered kaolinites, apparently even structural Fe from a poorly ordered sample (KGa-2) with 0.1 weight % Fe. *P. mendocina* enhanced Al dissolution from kaolinite, at least in part by pH changes, but also perhaps by production of Al-binding ligands, including siderophores. The amount of microbial growth under Fe-limited conditions correlated with the concentration of Fe released in 0.001 M oxalate at pH 3, suggesting that growth is controlled by ligand-accessible Fe (Fig. 2a). Ams et al. (2002) showed *that P. mendocina* produced siderophores in the presence of kaolinite and that siderophore production on a per cell basis decreased with decreasing Fe stress, in the order: no-Fe-added controls>*kaolinite*>hematite>Fe-EDTA (Figure 2b and data not shown here). These observations suggested that the bacterially produced siderophore(s) could potentially play a role in Al dissolution and Fe acquisition from kaolinite.

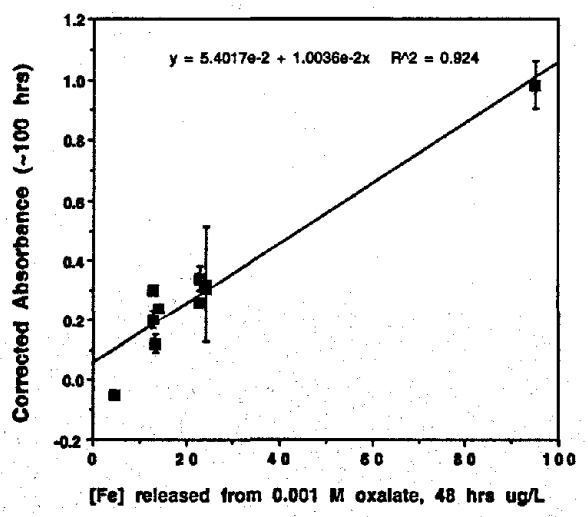

Figure 2a. Microbial growth (measured as absorbance corrected for no-Fe-added control) on a variety of natural kaolinites versus concentration of Fe released in oxalate. This figure demonstrates that microbial growth in Fe-limited experiments is related to the amount of ligand-accessible Fe available in association with kaolinite. (Ams et al., 2002).

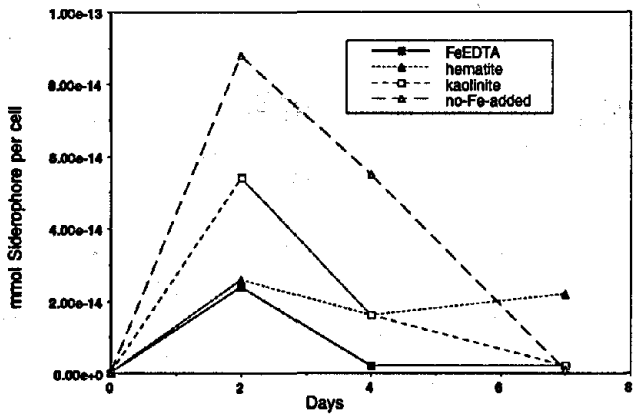

Figure 2b. Comparison of siderophore production on a per cell basis when Fe is supplied to *P. mendocina* as hematite, 30 umol/L FeEDTA, kaolinite, and no-Fe-added control. Siderophore production in the presence of kaolinite is considerably more than when Fe is supplied as FeEDTA, demonstrating that *P. mendocina* is able to obtain Fe from kaolinite, but not as readily as from hematite or FeEDTA.

Rosenberg et al. (2003) showed that the synthetic trihydroxamate siderophore, desferrioxamine mesylate (DFOB), adsorbed to kaolinite and significantly enhanced Al release rates (Table 2). DFOB adsorbed to kaolinite with cation-like adsorption behavior, with adsorption increasing above the pH point of zero net proton condition of kaolinite (pHpznpc), ~5. Adsorption onto kaolinite was considerably greater than onto goethite (Kraemer et al., 1999) (Fig. 3). DFAM released uM concentrations of Fe from kaolinite; i.e., concentrations sufficient for metabolic requirements of aerobic bacteria such as *P. mendocina.*

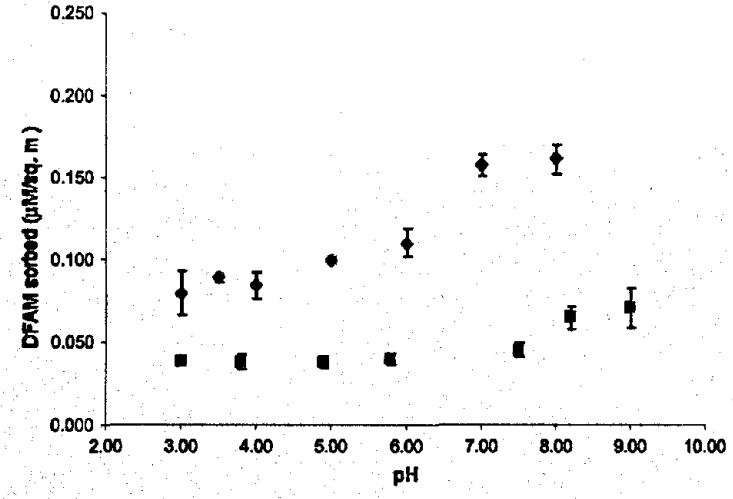

Figure 3. Comparison of adsorption of desferrioxamine B onto goethite (squares; Kraemer et al., 1999) versus kaolinite (diamonds; Rosenberg et al., 2003). Both show cation-like adsorption envelopes, with adsorption increasing with increasing pH. Adsorption onto kaolinite increases above the point of zero net proton condition of kaolinite (pHpznpc = 5.1), and of goethite above the pHpznpc of that mineral (8.1). Overall, adsorption onto kaolinite is greater than onto goethite.

Kraemer et al. (1999) showed that sorption of a trihydroxamate siderophore, desferrioxamine B, by goethite, under oxic conditions also was cation-like, increasing above the point of zero net proton condition of goethite (~8). By comparison with the sorption of acetohydroxamic acid, which has only one hydroxamate group per molecule, it appears that the sorption of the siderophore is hindered by its positively-charged amino group (the goethite surface charge is positive at pH <8). Replacement of this group by a neutral acetyl group (desferrioxamine D1) leads to sorption that is comparable (when suitably normalized for the number of hydroxamate groups) to that of acetohydroxamic acid at the same pH. At pH >6.5, siderophores (desferrioxamine (DFO) B and D1) resulted in desorption of Pb(II) from the surface of goethite. This suggested that Pb-siderophore complexes, which are most stable in this pH range, compete effectively against goethite surface sites for Pb. Adsorbed Pb did not affect goethite dissolution rates in the presence of siderophores at pH 6.5 (Kraemer et al., 1999).

Siderophore characterization.

In collaboration with Prof. Ken Raymond at UC Berkeley. He and a graduate student (Emily Dertz) have made significant progress in characterizing the siderophore produced by *P. mendocina*. Details of this characterization are too lengthy to review, herein.

Table 2. Rates of Al and Si release from KGa-2 in DFAM and HNO_3 at 22°C . Values in parentheses are from Wieland and Stumm (1992) presented for comparison. nm = not measured. bd = below detection. * = steady state not attained.

Sample	R_{Si} nmol m^{-2} h^{-2}	R_{Al} nmol m^{-2} h^{-2}	R_{Al}/R_{Si}
		pH 3	
DFAM	1.20	1.52	1.27
HNO_3	1.60 (2.63, 3.34)	* (1.78, 2.71)	--- (0.30, 1.03)
		pH 5.5	
DFAM	1.06	0.76	0.71
HNO_3	0.92 (1.25)	bd (bd)	--- (---)
		pH 6	
Oxalate	nm (2.30)	nm (bd)	--- (---)
HNO_3	nm (0.90)	nm (bd)	--- (---)
		pH 7	
DFAM	1.69	1.13	0.67
HNO_3	0.53 (0.87 pH 6.5)	bd (bd pH 6.5)	--- (---)

Publications of Hersman and Maurice resulting from this research

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