Impact of Iron-Reducing Bacteria on Metals and Radionuclides Adsorbed to Humic-Coated Iron(III) Oxides

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Studies were conducted to investigate the role of natural organic matter (NOM) on bacterial dissimilatory reduction of ferric iron oxides. Natural organic matter has been proposed to enhance dissimilatory iron reduction by two mechanisms: (1) shuttling of electrons from the bacterium to the ferric iron surface, and (2) complexation of ferrous iron. Electron shuttling by soluble quinone components of NOM may enhance both the rate and extent of ferric oxide bioreduction by relieving the requirement for direct bacterial attachment to the oxide surface. The adsorption of biogenic ferrous iron to oxide surfaces may limit further reduction due to oxide surface passivation, while ferrous iron adsorption to cell surfaces may decrease cell viability or activity. The complexation of ferrous iron by NOM may reduce these effects and enhance bioreduction.

NOM collected from a wetland pond (Georgetown NOM, GNOM), a known electron shuttling compound (anthraquinone-2,6-disulfonate, AQDS), and a strong ferrous complexing agent (Ferrozine) were employed to quantify the effects of NOM on iron reduction and to comparatively evaluate the two proposed mechanisms. Test systems consisted of serum bottles sealed with Teflon-faced butyl rubber stoppers and aluminum crimp seals containing 10 mL of medium inoculated to produce a known density of the dissimilatory iron reducing bacterium *Shewanella putrefaciens*, strain CN32. Tests were conducted under non-growth conditions with H_2 as the sole electron donor. Test medium contained 10 mM PIPES, pH = 6.8, 30 µM PO_4^{3-}, and 2.0 g_L $^{-1}$ of commercial hematite. All test vessels were incubated at 20°C on gyrarotory shakers outside of an anaerobic chamber, while all test preparation was performed in an anaerobic chamber under an N_2:H_2 (ca. 97.5:2.5) atmosphere. Specific amendments to the test medium were (final concentrations): 100 µM AQDS, 1.0 g_L $^{-1}$ Ferrozine and 250 mg_L $^{-1}$ GNOM.

Test conditions were replicated in parallel with all treatments and included uninoculated controls. Ferrous iron production and pH were measured in sacrificed samples at various incubation times. Soluble ferrous iron was determined by filtering (0.1 _m) the medium and analyzing the filtrate with Ferrozine. Acid-extractable ferrous iron was determined by allowing a sample of the medium to react with HCl (final normality 0.5 N) overnight, filtering (0.1 _m) the extracted sample and analyzing the filtrate by Ferrozine. The pH of the unacidified filtrate was measured under anaerobic conditions using a combination electrode. Student t-tests were used to determine if significant differences existed in ferrous iron production between treatments. Preliminary results revealed statistically significant differences between both AQDS and Ferrozine treatments compared to unamended inoculated controls and AQDS compared to Ferrozine.