Anaerobic Nitrate-Dependent Metal Bio-Oxidation Associated with Freshwater Surface and Subsurface Sediments

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Our previous studies demonstrated that nitrate-dependent bio-oxidation of Fe(II) by *Azospira suillium* strain PS results in the formation of crystalline mixed Fe(II)/Fe(III) mineral phases that subsequently immobilize heavy metals and radionuclides. Greater than 80% of the added 100 μM U(VI) was sequestered by the most dense, crystalline Fe(II)/Fe(III) mineral phases, which are not readily reduced by Fe(III)-reducing bacteria. Similarly to *A. suillium* strain PS, a novel autotrophic, nitrate-dependent Fe(II)-oxidizing bacterium, *Cosmobacter millennium* strain 2002, yields a mixed phase Fe(II)/Fe(III) mineral phase, identified as green rust by Mössbauer spectroscopy and x-ray diffraction. Green rust (GR), a mixed valence, layered Fe(II)/Fe(III) hydroxide with anion interlayers, is abundant in hydropmorphic soils and sediments. Recent studies suggested that both Fe(II)-oxidizing and Fe(III)-reducing bacteria play a role in GR formation; however, the extent of either process is unknown. In the case of Fe(III)-reduction, GR is transiently produced and subsequently transformed into magnetite. Anaerobic nitrate-dependent oxidation of Fe(II) provides an alternative mechanism of GR formation. In contrast to the GR biogenically formed by Fe(III) reduction, the biogenic GR product formed via nitrate-dependent Fe(II) oxidation by *C. millennium* strain 2002 did not yield transformation products, i.e., magnetite. X-ray fluorescence spectroscopy identified chloride and phosphate in association with the GR product. Furthermore, anion analysis of the GR by ion chromatography indicated that 6 mg SO_4^{2-}, 32 mg Cl^-, and 66 mg PO_4^{3-} was associated with the oxidation of 111 mg Fe(II). These results suggest that GR(Cl^-) was predominantly formed. It is currently unknown whether phosphate is adsorbed to the GR surface or intercalated into the interlayer. Given that nitrate-dependent Fe(II)-oxidizing bacteria are ubiquitous—identified by the most probable number enumeration as being high as 1.47×10^4 cells (g sediment)$^{-1}$ in freshwater lake sediment, 2.04×10^3 cells mL^{-1} in groundwater (NABIR FRC site), and 1.17×10^3 cells (g sediment)$^{-1}$ in subsurface sediment—it is likely that this metabolism is active in soils and sedimentary environments. These results represent the first demonstration of the biogenic formation of green rust in significant quantities, providing strong evidence for the biological mechanism for the production of GR(Cl^-) in soils and sediments.