

Effects of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis in wetland sediments

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Iron redox cycling by microorganisms is a significant component of C cycling and energy flux in many aquatic and terrestrial environments. Dissimilatory iron-reducing bacteria (DIRB) are phylogenetically diverse microorganisms that obtain energy by coupling oxidation of organic compounds or H₂ to reduction of Fe(III) to Fe(II). Because of the relative insolubility of most Fe(III)-bearing minerals, many DIRB use soluble electron shuttles (e.g., quinones, flavins, phenazines, and reduced sulfur species) to transfer electrons from the cell to external electron acceptors. Studies investigating effects of electron shuttles on microbial Fe(III) reduction have typically been conducted under axenic conditions. To better understand how electron shuttles influences microbial Fe(III) reduction in the presence of a diverse microbial community, we examined the effects of different electron shuttles (9,10-anthraquinone-2,6-disulfonate acid (AQDS), 9,10-anthraquinone-2-carboxylic acid (AQC), and 5-hydroxy-1,4-naphthoquinone (lawsone, NQL)) on the bioreduction of Fe(III) oxide and methanogenesis in microcosms inoculated with wetland sediment. Our results show no significant enhancement of Fe(III) reduction in the presence of NQL or AQC relative to the no shuttle (NS) control; however both the rate and extent of Fe(II) production were enhanced in the presence of AQDS. The onset of methanogenesis was earlier in the presence of AQDS compared to NQL and NS, but in each case methane production was not evident until Fe(II) production plateaued. Methanogenesis was completely inhibited in the presence of AQC, highlighting the potential for electron shuttles to influence microbial processes not involving microbial respiration using insoluble electron acceptors. Systems amended with AQC were dominated by microorganisms classified in the family *Pelobacteraceae* (avg. 45.4% total abundance), while *Geobacteraceae* dominated in microcosms amended with AQDS (30-48%), NQL (51.9%), or NS (37%). *Geobacteraceae* sequences were of much lower abundance in the AQC enrichments, accounting for only 8% of the total abundance on average and only 3–4% in two of the three replicates. While closely related to the *Geobacteraceae*, organisms in *Pelobacteraceae* lack c-type cytochromes and are unable to transfer electrons directly to ferric iron. This suggests AQC may inhibit direct reduction of ferric iron by organisms such as *Geobacter*, allowing *Pelobacter* spp., which would otherwise be outcompeted, to dominate.