Microbial chemolithoautotrophic oxidation of pyrite at neutral pH

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The ability of microorganisms to catalyze the oxidation of pyrite (FeS_2) at circumneutral pH is poorly understood, despite the fact that this an energetically favorable process analogous to various well-known chemolithotrophic pathways (e.g. ferrous iron, sulfide, and elemental sulfur oxidation). Neutral pH abiotic oxidation of pyrite by oxygen has been extensively studied; however, there is virtually nothing known about the capacity for microorganisms to compete with abiotic reactions or enhance rates of pyrite oxidation at circumneutral pH. This study examined the potential for microorganisms in Pliocene-age subsurface sediments to oxidize synthetic framboidal pyrite with oxygen as the electron acceptor. Several enrichment cultures capable of chemolithoautotrophic oxidation of synthetic framboidal pyrite linked to sulfate generation were recovered. The extent of sulfate generation was ca. 10-fold higher than in parallel sterile controls. Activity of the cultures was sustained through more than 15 successive transfers over 400 days. TEM analysis showed thin coatings of amorphous Fe(III) oxyhydroxides on oxidized framboids, and linear combination fit analyses of Fe K-edge EXAFS showed increased concentrations ferrihydrite and decreased FeS_2 concentrations in the oxidized pyrite. S K-edge XANES confirmed the absence of S in anything other than the -2 oxidation state, consistent with complete oxidation of FeS_2 to sulfate. DNA-based fluorescence microscopy, cryo-SEM, and Fluoresce In-Situ Hybridization (FISH) showed that microbial cells were intimately associated with the pyrite grains. The composition of the enrichment cultures was assessed using 16S rRNA gene clone libraries. Consistent with FISH results, the clone libraries were dominated by Alphaproteobacteria (*Bradyrhizobiaceae*, *Mesorhizobium*) as well as organisms related to the Betaproteobacterial genus *Ralstonia*. Shotgun metagenomic sequencing revealed the presence of complete sulfur oxidation and CO_2 fixation systems in reconstructed genomes for the dominant organisms in the cultures. Our results have implications for initiation of acid mine drainage in modern environments and weathering-derived sulfate flux over geological time.