Goldschmidt 2022 Abstract
https://doi.org/10.46427/gold2022.12653

Characterizatin of electroactive microorganisms associated with stibnite mine wastewater

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Arsenic (As) and antimony (Sb) are both naturally occurring toxic metalloids with increasing environmental concerns. Both metalloids mainly exist in two oxidation states, trivalent and pentavalent forms, and are aften strongly adsorbed with Fe(III) oxyhydroxides in natural soils and sediments. Microorganisms play important roles in both iron and metalloids transformations, contributing in geochemical cycling of As and Sb. In this study, we investigated microbe-mineral interactions and characterized electroactive microbial population by using *in situ* electrochemical enrichment and genomic approaches. To enrich cathodic microbial communities under natural environment, polarized electrodes were deployed in stibnite mine wastewater runoffs (As, 292 ppb; Sb, 1.64 ppm), and provided with low current densities (0.1~0.4 mA/m^2). After 6 months of deployment, the linear sweep voltammetry of the polarized cathodes exhibited increase in cathodic current compared to the sodium azide added killed control, suggesting cathodic electron uptake associted with cellular activity. On the cathode surface, the precipitation of calcite and aragonite was observed, and As and Sb were accumulated up to 1000 times (As, 374 ppm; Sb, 99.3 ppm). Scannning electron micrographs showed the development of biofilms containing rod shaped cells with filaments resembling electrically conductive nanowires. The 16S rRNA gene targeted amplicon sequencing of the cathodic microbial community showed the dominance (>80% relative abundance) of novel Gammaproteobacterial phylotypes, distantly related to cultivated strains such as *Acidiferrobacter thiooxydanse* and *Nitrosococcus oceani* (89.1 and 88.8% sequence identity, respectively). The *in situ* electrochemical enrichment was repeated, and the emergence and enrichment of the same dominat phylotypes were observed after 51 days of deployment with electrochemical activity. Metagenome-assembled genome of the dominant phylotype contained genes associated with iron oxidation, CO_2 and nitrogen fixations. These results indicated that the novel electroactive Gammaproteobacteria enriched on the deployed cathode may promote the precipitation of calcite biominerals, which served as natural adsorbent for toxic metalloids. In this study, *in situ* electrochemical enrichment was successfully applied to reveal functional attribute of an uncultivated group of electroactive microorganisms associated with contaminated mine wastewater environment.