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Biogeochemistry of humidity cell tests incubated under psychrophilic vs mesophilic conditions

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Humidity cell tests (HCT) are laboratory-scale kinetic tests used to predict the onset of acid mine drainage (AMD) or contaminated neutral drainage (CND) conditions. Vessels containing mine wastes (e.g., tailings) are subjected to repeated wetting and drying cycles to accelerate weathering processes. Weathering products are flushed out of the cells and analysed for key geochemical parameters (e.g., pH, Zn, As, Fe, and sulfate). This data provides quantitative insight on water quality that is used to estimate drainage characteristics over time. Microorganisms (e.g., *Acidothiobacillus*) play an important role in AMD metal(loid) cycling and sulfur oxidation. Rates of weathering and microbial growth in mine wastes are expected to be tied to temperature due to kinetic controls on chemical weathering and differences in microbial community growth patterns with temperature, respectively. We constructed eight HCTs with tailings samples from two sites. Humidity cells were operated for 42 weeks, with four cells incubated at 4°C and four cells at 22°C. We collected physicochemical and geochemical data, high-throughput sequencing (16S rRNA gene, V4 region), XRD, microXRF mapping, and x-ray absorption spectroscopy (XAS) analyses at the Fe, S, and As K-edges at timepoints throughout the HCT experiments to provide us with a basis to examine relationships between microbial communities and geochemistry under psychrophilic and mesophilic conditions. Geochemical analyses demonstrated that (at corresponding timepoints) HCT leachates were lower in key ion loadings at 4°C than 22°C. Microbiological analyses showed changes in microbial community diversity between starting materials and samples from the HCTs at 16 weeks; relatives of typical acidophiles and metal(loid) and sulfur cycling bacteria were identified in all samples, for example *Thiobacillus* (sulfur-oxidizing bacteria), *Acidithrix* (acidophilic heterotrophic iron-oxidizer), *Metallibacterium* (acidophilic heterotroph) and genera known to contain metal(loid) resistant bacteria. Understanding the impact of temperature and microbiology on HCTs can help to improve our understanding of scale effects when translating lab experimental results to site models. The results of this study will help to guide practitioners in interpreting the results of humidity cell tests, with the overall goal of better informed mine site planning and costly decisions on mine waste management and water treatment.