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Validating biological arsenic attenuation mechanisms for aquifers influenced by thermal bitumen extraction

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Arsenic is a metal(loid) of environmental and human health concern naturally present in aquifers that can be mobilized as inorganic As(III) or As(V) during resource extraction (*e.g.,* mining and petroleum production). Microbes regulate arsenic bioavailability through direct redox transformations and indirect processes linked to iron and sulphur cycling. While these pathways are well studied in non-engineered systems, their roles in engineered systems where arsenic is thermally mobilized remain poorly characterized. Addressing this gap is essential for predicting arsenic's fate in aquifers affected by thermal mobilization and developing attenuation strategies that rely on microbes naturally occurring in aquifer habitats.

Our previous multi-year survey established a conceptual model for microbial arsenic cycling in aquifers linked to thermal bitumen extraction. We detected abundant sulphate-reducing bacteria (SRB) in groundwater arsenic plumes, suggesting As(III) could be immobilized through sulphide mineral precipitation. The current study tested whether enrichment cultures established using biomass from these plumes could decrease total dissolved arsenic through such pathways under environmentally representative conditions.

Biomass from three sites spanning an arsenic gradient was used to inoculate enrichment cultures grown in synthetic groundwater amended with 5 µM As(III), As(V), or no arsenic (control). Dissolved arsenic concentrations and speciation were monitored in cultures and sterile controls using LC-ICP-MS and microscopy. Microbial community composition and metabolic capacity were assessed using 16S rRNA amplicon and whole-community DNA sequencing to identify taxa and functional genes involved in arsenic cycling.

All cultures decreased total dissolved arsenic by ~50% within one month and up to 80% after one year, despite As(III) dominating speciation (Fig1). Genes associated with As(III) methylation (*arsM*) and As(V) reduction (*arrA*) were abundant, independent of arsenic treatment, whereas genes for As(III) oxidation were rare, consistent with our previous field observations (Fig2). Sequencing revealed strong enrichment of SRB families and dissimilatory sulphur reduction pathways, suggesting sulphate reduction was involved in arsenic removal. SEM-EDX supported this explanation by confirming that arsenic was incorporated heterogeneously into precipitates alongside sulphur and silicon.

These findings validate our model for arsenic cycling in these systems and demonstrate that naturally occurring SRB can promote long-term arsenic immobilization in aquifers where arsenic is thermally mobilized.

Figure 1: Mean total dissolved As over time

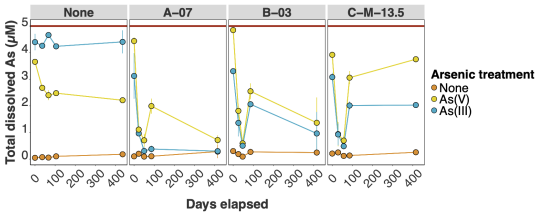

Figure 2: Arsenic gene counts at the contig level

