Differences Between Heterotrophic and Nitrate-dependent Iron-oxidizing Microbial Communities in Bioreactor Sediment Treating Mine Wastewater

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Abstract

Nitrate-dependent iron oxidation (NDFO) is a novel mechanism for microbial bioremediation of metal and metalloid contaminants. During NDFO, microbes catalyze a redox reaction wherein nitrate is reduced to nitrite and nitrogen gas while Fe(II) is oxidized to solid Fe(III) hydroxide minerals. Metalloid contaminants such as selenium and arsenic have a propensity for adsorption to iron minerals produced during NDFO; some contaminants may also be concurrently bioreduced. A number of bacterial isolates have been shown to be capable of NDFO (e.g., Kappler et al. (2005), Kiskira et al. (2017)), but little work has been done to date characterizing mixed microbial communities performing NDFO. Some autotrophic communities have been characterized, with high relative abundances for strains of *Gallionellaceae* in both a freshwater sediment enrichment culture and an activated sludge culture (Blöthe and Roden 2009, Tian et al. 2020). In mixotrophic activated sludge cultures, the concentration of Fe(II) amendment was found to significantly impact microbial community composition; these cultures were fed with methanol in addition to Fe(II), and the dominant community members were *Methyloversatilis* and other methylotrophic strains (Liu et al. 2018). The work presented here examines microbial communities performing NDFO in the context of remediation, and in particular how differences between NDFO and heterotrophic communities may influence remediation effectiveness.

This research characterizes and compares microbial communities performing NDFO versus heterotrophic denitrification during removal of selenium and nickel from mining wastewater. Sediment and influent water from a subsurface bioreactor treating mining wastewater were used to construct batch bioreactors, which were amended with selenium and nickel as well as either Fe(II) or methanol to investigate contaminant removal and microbial community composition in NDFO versus heterotrophic microbial communities. Both Fe(II) and methanol reactors removed total aqueous selenium to below the quantification limit, but Fe(II) reactors removed it more rapidly, likely due to adsorption of selenite. For nickel, removal to below the detection limit was achieved with

methanol amendment, while Fe(II) amendment resulted in 42-95% removal. This was likely due to precipitation of nickel sulfide during sulfate reduction in methanol-amended reactors.

DNA from the batch bioreactors will be sequenced and the results analyzed for differences among communities. Permutational multivariate analysis of variance and non-metric multidimensional scaling will be used to determine significant correlations of community composition with experimental variables, selenium and nickel removal, and NDFO (Roberts 2023, Kruskal 1964). Indicator species analyses (De Cáceres et al. 2010) will be applied to identify taxa found significantly more often (i.e., at a higher relative abundance) in one group of microbial communities than in any other group. The indicator species analysis may reveal whether there are groups of denitrifiers that predominate in NDFO conditions vs. groups that predominate during heterotrophic denitrification. The results of these microbial community analyses, in combination with the geochemical analyses, will improve our understanding of microbial communities performing NDFO in remediation environments.

Keywords

bioremediation, microbial ecology, denitrification, selenium

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Conflicts of interest

The authors have declared that no competing interests exist.

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