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ABSTRACT TITLE: Molecular mechanisms and kinetics of microbial anaerobic nitrate-dependent U(IV) and Fe(II) oxidation

ABSTRACT: The stability and dissolution rate of solid U(IV)-oxide phases in aquifers are important for transport of dissolved U as the mobile uranyl cation (UO\textsubscript{2}\textsuperscript{2+}). Recent studies have suggested that microbially mediated, nitrate-dependent U(IV) oxidation under anaerobic conditions could complicate efforts at long-term reductive immobilization. We investigated the anaerobic, oxidative dissolution of biogenically produced U(IV)-oxide (nominally UO\textsubscript{2}(s)) by either chemical oxidants (nitrate or nitrite) or by Thiobacillus denitrificans, a chemolitho-autotrophic bacterium that catalyzes anaerobic, nitrate-dependent U(IV) and Fe(II) oxidation. Mixtures of biogenic UO\textsubscript{2}(s) and quartz with and without T. denitrificans were used in flow-through column experiments to examine coupled and competing oxidation-reduction processes. Abiotic oxidation of UO\textsubscript{2}(s) in the presence of nitrate under anaerobic conditions was slow but faster than control experiments of non-oxidative dissolution. Abiotic UO\textsubscript{2}(s) oxidation by nitrite was more rapid by several orders of magnitude. In the presence of T. denitrificans and dissolved nitrate, higher rates of dissolved U release were observed compared with abiotic controls, suggesting that T. denitrificans catalyzed the oxidative dissolution of UO\textsubscript{2}(s) in addition to the abiotic oxidation pathways. X-ray spectroscopic characterization of reaction products indicated a fraction of solid-associated oxidized U(VI) that is retained in the column. Analysis of local atomic structures showed formation of U-oxo molecular moieties within or on particle surfaces that are similar but not identical to aqueous or sorbed uranyl species, suggesting mostly surface particle oxidation rather than detachment and re-adsorption of uranyl in the column. Reactive transport modeling (using Crunchflow) of column experiments incorporating thermodynamic solubility, irreversible overall abiotic and biotic kinetic reactions, and uranyl sorption on quartz was used to derive rates for overall kinetic reactions from column effluent U concentrations. Steady-state U concentrations were well simulated for a small amount of UO\textsubscript{2}(s) oxidation relative to total UO\textsubscript{2}(s) mass, but calculations were sensitive to particle surface area.

We investigated the enzymes involved in anaerobic, nitrate-dependent Fe(II) and U(IV) oxidation in T. denitrificans, as these enzymes have not yet been described for any other microorganisms that catalyze these processes. We previously reported that two c-type cytochromes, Tbd_0187 and Tbd_0146, were involved in anaerobic nitrate-dependent U(IV) oxidation in T. denitrificans and we hypothesized that c-type cytochromes would also catalyze nitrate-dependent Fe(II) oxidation. Studies to identify genes associated with nitrate-dependent Fe(II) oxidation included whole-genome transcriptional assays (including the use of FeCO\textsubscript{3}, Fe\textsuperscript{2+}(aq), and U(IV) oxides as electron donors under denitrifying conditions), targeted insertion mutations of 26 genes of interest, and random transposon-mutagenesis studies with screening for Fe(II) oxidation. Non-defective mutants included the c\textsubscript{1}-cytochrome subunit of the cytochrome bc\textsubscript{1} complex, which has relevance to a previously proposed role for this complex in nitrate-dependent Fe(II) oxidation. Of the transposon mutants defective in Fe(II) oxidation, one mutant with a disrupted gene associated with NADH:ubiquinone oxidoreductase (complex I) was >30% defective relative to the wild-type strain. Overall, our results indicate that nitrate-dependent Fe(II) oxidation in T. denitrificans is not catalyzed by the same c-type cytochromes involved in U(IV) oxidation, nor have other c-type cytochromes yet been implicated in the process.