Genome-enabled studies of anaerobic, nitrate-dependent U(IV) oxidation

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Anaerobic, nitrate-dependent U(IV) oxidation has considerable relevance to the bioremediation of uranium-contaminated aquifers and also represents a novel bacterial metabolic capability of fundamental scientific interest. A favored process for U bioremediation is *in situ* reductive immobilization, a process by which anaerobic bacteria reduce water-soluble U(VI) complexes to poorly soluble U(IV) phases. The discovery that *Thiobacillus denitrificans* [1] and other bacteria can anaerobically re-oxidize, and thus, re-mobilize, uranium in groundwater highlights a process that could compromise the efficiency of this bioremediation approach. While microbial U(VI) reduction has been the subject of extensive research, far less is known about anaerobic U(IV) re-oxidation.

We will discuss our efforts to identify the genes/proteins that are key to nitrate-dependent U(IV) oxidation in *T. denitrificans*. These efforts included: (a) detailed analysis of the *T. denitrificans* genome [2], (b) whole-genome transcriptional analyses of *T. denitrificans* with high-density, oligonucleotide microarrays [3], (c) proteomic studies of membrane-associated, *c*-type cytochromes in *T. denitrificans* [4], and (d) development of a genetic system in *T. denitrificans* [5].

We identified two diheme, *c*-type cytochromes critical to anaerobic U(IV) oxidation in *T. denitrificans* (putatively c_4 and c_5 cytochromes, Tbd_0187 and Tbd_0146, respectively). Insertion mutations in each of the two genes encoding these cytochromes resulted in a greater than 50% decrease in nitrate-dependent U(IV) oxidation activity, and complementation *in trans* restored activity to wild-type levels. Sucrose-density-gradient ultracentrifugation confirmed that both cytochromes are membrane associated. Sequence-based evidence links the Tbd_0187 protein to the high midpoint reduction potentials that would be required to catalyze U(IV) oxidation. Insertion mutations in other membrane-associated *c*-type cytochromes in *T. denitrificans* did not diminish U(IV) oxidation. To date, Tbd_0146 and Tbd_0187 are the only genes identified as being associated with anaerobic U(IV) oxidation.

We are also investigating nitrate-dependent Fe(II) oxidation in *T. denitrificans*, a process that we observed to accelerate U(IV) oxidation. Notably, the two cytochromes involved in U(IV) oxidation in *T. denitrificans* do not appear to be involved in Fe(II) oxidation. Random transposon mutagenesis studies to further investigate Fe(II) oxidation in *T. denitrificans* are ongoing.

[1] Beller (2005) *Applied and Environmental Microbiology* **71**, 2170-2174. [2] Beller *et al.* (2006) *Journal of Bacteriology* **188**, 1473-1488. [3] Beller *et al.* (2006) *Journal of Bacteriology* **188**, 7005-7015. [4] Beller *et al.* (2009) *Biodegradation* **20**, 45-53. [5] Letain *et al.* (2007) *Applied and Environmental Microbiology* **73**, 3265-3271.

Stable Isotope and Isotopomeric Constraints on N_2O Production in Wastewater Treatment Plants

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Wastewater treatment plants (WWTPs) constitute a substantial source of N_2O to the atmosphere, with a wide range of estimated emission factors, varying from 0.3 to 140 g N_2O/person/yr [1]. The majority of N_2O emissions occur in the aerobic reactors, where both incomplete nitrification and denitrification might contribute to the overall N_2O emissions. To better constrain production mechanisms and overall N_2O fluxes, we measured N and O isotope ratios of NH_4^+, NO_2^-/NO_3^-, and N_2O, and isotopomer ratios (N isotope site preference) of N_2O at two large-scale activated-sludge WWTPs in Chicago.

The average N2O concentration in the off-gas from the aerobic reactors was 34 and 56 ppmv for plants 1 and 2, respectively. At both plants the isotopic distribution of the major aqueous N-species (NO_3^- and NH_4^+) within the aerobic reactor can be approximated with a Rayleigh-type fractionation model determined by nitrification of ammonium along the wastewater flow path, accompanied by denitrification of about 5 % of the NO_3^- produced, with constant fractionation factors of about -15 ‰ and -20 ‰ for NH_4^+ nitrification and NO_3^- denitrification, respectively. The N isotope site preference in N_2O, averaging +2.7 ‰ and +3.9 ‰ at the two plants, suggests that N_2O was mainly produced by incomplete denitrification [2] in the portions of the tanks where dissolved oxygen was between 0.2 and 2.5 mg/L (fig. 1).

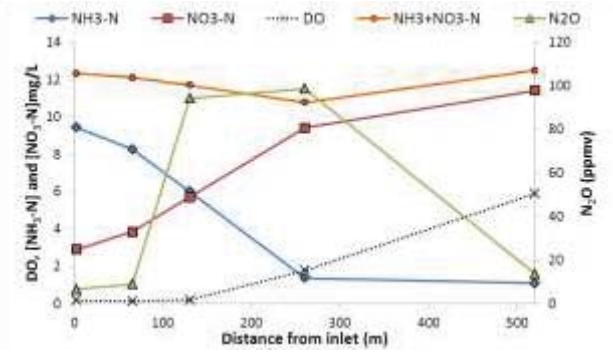

Figure 1. Average concentrations of dissolved oxygen (DO), N aqueous species, and off-gas N_2O along the wastewater flow path in a single tank of the aerobic reactor at plant 1.

[1] Ahn *et al.* (2010) *Environ. Sci. Technol.* **44**, 4505-4511.
[2] Sutka *et al.* (2006) *Appl. Environ. Microbiol.* **72**, 638-644.