Coupling of Field- and Lab-based Experiments to resolve controls of Nitrate Respiration Pathway Partitioning at the Oak Ridge Shallow Aquifer

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Project Goals: Surveying the fate of nitrate at the Oak Ridge site through field assays and experimental lab systems for understanding the drivers of nitrate respiration pathway partitioning

The Oak Ridge site has a long history of research on biogeochemical impacts of contamination. Past studies largely focused on the fate and transport of radioisotopes, heavy metals, halogenated organic compounds, and mercury. Less studied is the consequence of the disposal of heavy metal laden nitric acid reaching nitrate concentrations of up to 2 g/l, acidifying large regions of this shallow aquifer. Thus, the combined impact of nitrate and lowered pH on microbial respiration and associated processes in this oxygen-limited environment is not well constrained in the literature. Microbially-driven nitrate respiration can operate through different pathways, resulting in either removal of nitrate through gaseous metabolites or retention through dissimilatory nitrate reduction to ammonium (DNRA). In this project, we focus on biotic and abiotic controls of nitrate transformation through field assays and experiments in lab systems.

A grand challenge is to understand the function of microbial communities in the field within the limitations of available sampling methods. Therefore, we use a two-tier approach by combining field data and model laboratory systems. In the field, we survey the potential for nitrate respiration through the acetylene block method and nitrate isotope fractionation. For experiments with model isolates and communities in the lab, we are characterizing different reactor system formats but since biology in the subsurface is a combination of sediment-associated biofilms and planktonic organisms in pore water, we here focus on fluidized bed reactor (FBR) technology. Fluidization of sediment selects for both planktonic and attached populations, which can be challeng-

ing in planktonic chemostats, thus better emulating subsurface conditions while avoiding physical heterogeneities, such as channeling, that develop in packed-bed reactors.

Abiotic characterization of FBR fluidization has shown that particles of less than 100 μm in diameter have impractically low fluidization velocities while particles larger than 300 μm in diameter require excessive flow rates to fluidize. We have established controlled fluidization under both oxic and anoxic conditions, and are evaluating the influence of medium composition, substratum size, composition as well as mass per reactor volume on biomass accumulation and activity. FBR operating conditions are initially refined with monocultures of *Desulfovibrio vulgaris* to select for both attached and planktonic populations or primarily attached populations. These studies are also evaluate the influence of physical parameters (particle size/composition, shear stress, and surface area) on colonization and factors controlling the partitioning between planktonic and attached organisms, a common issue in the field. These data inform the range of operating conditions needed to develop more complex reactor communities, now being evaluated by reactor colonization by *Desulfovibrio* spp. syntrophically coupled with different hydrogenotrophic methanogens.

A simple acetylene block test serves to measure active regions of nitrate respiration in the field, identifying active source material for the FBR experiments. Using a model mixture of carbon sources to stimulate nitrate respiration with either biomass from filtered groundwater or sediment in native sterile groundwater, we trace the formation of nitrous oxide and ammonium over relatively short incubation periods in different incubations setups. Those studies are complemented by stable isotope fractionation data to constrain abiotic and biotic sinks/sources of nitrate and its transformation products, including nitrous oxide. Initial acetylene block and nitrate isotope fractionation data are consistent with a significant abiotic source of nitrous oxide in the highly contaminated area EB106 while biotic processes dominate at lower nitrate concentrations in both groundwater and sediment fractions in a region of lower contamination (EB271). DNRA and denitrification were significant processes at EB271, with DNRA more prominent in the vadose zone and denitrification in the transition zone between capillary fringe and saturated zone.

The field data will guide FBR design and operation, as needed to identify and quantify variables governing microbial community dynamics, such as activity, resilience, and persistence as they relate to different respiratory processes at the Oak Ridge site. While the FBR reactor configuration may better emulate the subsurface environment through retention of both planktonic and attached microbial populations than standard liquid cultures, we anticipate that a more fully predictive understanding of variables controlling field site processes will derive from comparative studies of different reactor formats, including ongoing complementary studies of both field chemostats and packed-bed columns. Another connection to the field is the use isotopic fractionation signatures, as well as the metabolic and thermodynamic modeling of reactors operated under field relevant conditions.

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