MONTANA STATE UNIVERSITY
NORM ASBJORNSON College of ENGINEERING

Master's Thesis Defense by KaeLee Massey
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Via WebEx

"Biofilm Distribution in a Porous Medium Reactor Emulating Shallow Subsurface Conditions"

Abstract:
Microorganisms have the potential to change the geochemical properties of the shallow terrestrial subsurface, and previous studies have uncovered significant roles microorganisms can play in groundwater processes, such as biogeochemical processes. While much attention given to the shallow terrestrial subsurface has been focused on the effects of contamination and how microorganisms function in these systems, knowledge of the distribution of microbial biomass and activity related to hydraulic properties is less understood. In this study, an up-flow packed bed reactor (PBR) was designed to emulate select field conditions (i.e., flow rate and particle size) observed at the Oak Ridge National Laboratory-Field Research Center (ORNL-FRC) to observe microbial biomass and activity distribution in a micropore environment. The PBR contained a porous medium of silica oxide particles (74-300 μm), and the size range was based upon particle size assessment of sediment material from the ORNL-FRC. The water phase of the system was a basal groundwater medium that contained low levels of sugars, amino acids, and nucleosides/nucleotides as the C and N sources that were based upon metabolomic characterization of sediment extracts from the ORNL-FRC. The inocula for the PBRs consisted of sediment material in samplers that were incubated down-well and retrieved from three FRC wells each at a distinct pH (4, 6.3, or 7). Following 4 months of incubation, biomass, cell concentrations, cell distribution, and microbial community analysis for each reactor were evaluated. The pH 4 reactor had the largest biomass and highest activity but had the lowest diversity amongst the pH conditions. The two circumneutral reactors (1 at pH 7, 2 at pH 6.3) had lower biomass concentrations and activity but had microbial communities that were more diverse than pH 4.

Methods were also developed to allow the embedding and sectioning of an intact core from the PBRs, and this allowed visualization of cell localization within the porous medium. The reactors showed different trends in how microbial biomass was distributed through the porous medium as well as distances to other cells and/or cell aggregates. The measured distances were also compared to substrate concentrations over distances predicted by a model based upon diffusion coefficients for compound classes (i.e., sugars, amino acids, nucleotides/nucleosides). Overall, the data and predictions demonstrate that under ex situ conditions meant to emulate porous media flow (e.g., porosity, flow, particle size) at the ORNL-FRC, cells that are part of a diverse microbial community can be on average 20 to 80 μm apart with an average of 2 to 9 cells/particle. Based on diffusivity of potential substrates and measured distance ranges between cells, sugar levels could be approximately 5 to 20 μM whereas amino acids and nucleotides/nucleosides would be sub-micromolar between nearest cell/aggregate neighbors.

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