Biofilm Distribution in a Porous Medium Reactor Simulating Shallow Subsurface Conditions

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

Microorganisms in the terrestrial subsurface play important roles in nutrient cycling and degradation of anthropogenic contaminants, functions essential to the maintenance of healthy aquifers. 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 cycling. Much of the attention given to the shallow terrestrial subsurface has been focused on the effects of contamination and how microorganisms function in these systems, with far less emphasis on understanding how hydraulic variables influence subsurface microbial ecology. To fully understand how environmental factors impact microbial community dynamics, interactions, succession, colonization, and dispersal in the shallow subsurface environment it is essential to understand the link between microbiology and hydrology. An up-flow packed bed reactor (PBR) was designed to simulate select field conditions (*i.e.*, flow rate and particle size) observed at the Oak Ridge National Laboratory-Field Research Center (ORNL-FRC) to observe how environmental factors, including hydraulic variables such as average pore velocity, influences metabolic activity, community establishment, and cell distribution in a micropore environment. The goals were to understand how environmental variables impact distribution and metabolic activity of microbial cells in a pore microenvironment using native sediment bug trap material under hydraulic properties based upon field conditions (flow rate and particle size). 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 distinct pH values (4, 6.3, or 7). The three PBRs were run in parallel with a steady-state flow rate that resulted in an average pore velocity of 0.313 cm/h. 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 (pH 7 and 6.3) had lower biomass concentrations and activity but had microbial communities that were more diverse than pH 4. 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 molecular classes (*i.e.*, sugars, amino acids, nucleotides/nucleosides). Overall, the data and predictions demonstrate that under *ex situ* conditions meant to simulate 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 likely be at sub-micromolar levels between nearest cell/aggregate neighbors. Furthermore, we developed methods to visualize the localization of active and non-active cells within the porous medium and the PBR was able to enrich predominant populations observed in the field. The results have implications for elucidating the impacts of environmental factors on metabolic activity and cell distributions in an impacted, subsurface environment.

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