Passive and Active Controls on Microbial Colonization of Mineral Surfaces: Aluminum and Iron

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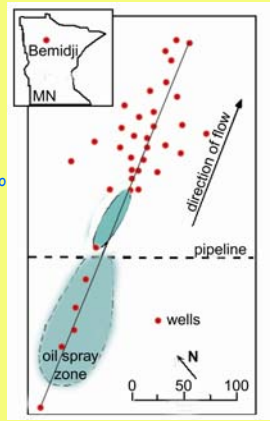
Introduction

Microbial attachment to minerals can impact the subsurface habitat in a variety of ways. In some cases attached cells may alter the mineral surface, in addition to aqueous species, by producing a reactive microenvironment at the point of attachment. Microbial attachment also immobilizes microbes onto mineral surfaces, retarding their transport and serving as a nucleation point for growth and potential clogging of aquifer pores. Classic treatments of cell attachment and surface growth in aquifer systems assumes that this process is an essentially passive interaction; cells attach to mineral surfaces for habitat, by filtration, or by columbic attraction. This model, however, ignores active controls on attachment such as substrate focusing, nutrient availability, and metal toxicity that might override passive attraction or repulsion due to charge differences or advective collision. This study investigates the model of passive attachment using a native microbial consortium and a variety of oxide and silicate surfaces to determine if other, active controls also influence the interaction between cells and surfaces.

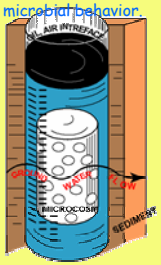
Setting

The study site is a petroleum contaminated aquifer near Bemidji, MN, part of the USGS Toxic Substances Hydrology program. Groundwater in the study zone is completely anaerobic and dissolved organic carbon is >5000 mol l^{-1}, with considerable dissolved methane (up to 1500 mol l^{-1}). pH values are near-neutral (6.9 to 6.5), while dissolved Fe^{2+} is as high as 900 mol l^{-1} where dissimilatory iron-reducing bacteria (DIRB) oxidize carbon and reduce oxidized Fe(III) minerals (Lovley et al., 1989). The microbial biomass is dominated by DIRB with fermenting bacteria and narrowly distributed methanogens (Bekins et al., 1999).



Research Approach

Microbial attachment to mineral surfaces is often characterized in laboratory settings by rapidly growing moncultures in rich media to determine interactions with charged surfaces. While these are appropriate surfaces, the experiments do not represent low abundance, oligotrophic environments, in which native microbial constortia engage in complex, syntrophic interactions. The goal of this study was not to isolate and identify colonizing cells, but to understand the behavior of an entire population. Therefore, field and laboratory techniques were designed to investigate attachment behavior of an entire native consortium *in situ* where mineral surfaces may be an active component in microbial behavior.

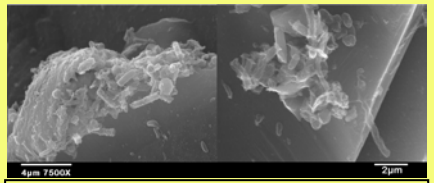


Field Microcosms

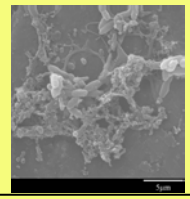
Field microcosms were used to investigate microbial colonization of mineral surfaces *in situ*. Microcosms consisted of sterile mineral and glass chips in a flow-through container suspended in the screened portion of the well for 3-6 months. After reaction in the aquifer the microcosms were recovered and replicate samples were taken and immediately processed for most probably number (MPN) determination and examination by scanning electron microscope (SEM).

Passive Controls: Al and Fe Oxides

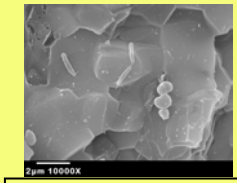
The observed dense colonization on the oxide minerals can be explained by the coulombic attraction between the negatively charged cells and positively- to neutrally-charged oxide surfaces. Corundum and the amorphous iron oxide coatings (see below) exhibit the most colonization as would be predicted from pHzpc values (9.1 and 8.5, respectively), while the lower pHzpc for hematite (6.7) results in lower colonization density.



SEM image of iron-coated quartz (left) and plagioclase (right) surfaces after 8 months in the anaerobic Bemidji groundwater. Both surfaces are covered with a variety of mrophotypes and some glycocalyx. Altering the otherwise uncolonized plagioclase surface (see below) with an iron-oxide coating resulted in colonization, while coatings enhanced the colonization on quartz.

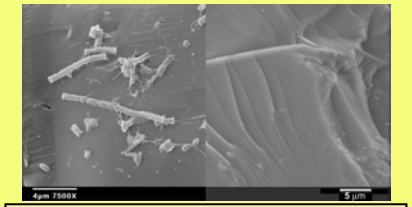


SEM image of corundum 12 months in the anaerobic Bemidji groundwater. The surface is thickly covered by rods and glycocalyx.



SEM image of hematite surface after 7 months in the anaerobic Bemidji groundwater. The hematite surface is colonized by a few cocci and rods, with no discernible glycocalyx.

Active Controls: Silicate Composition

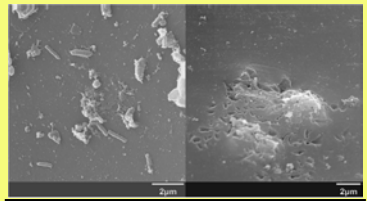


SEM image of quartz (left) and plagioclase (right) after 8 months in the anaerobic Bemidji groundwater. The quartz surface is lightly colonized by colonies comprised of several morphotypes, while the plagioclase is barren of cells.

Quartz and plagioclase are uniformly negatively charged at pH 6.8 (pHzpc 2 and ~2.4; (Stumm and Morgan, 1996) resulting in an overall coulombic repulsion of negatively-charged microorganisms. Microorganisms, however, will overcome this repulsion and attach to silicate surfaces, but to a lesser extent than surfaces with a favorable coulombic attraction. Quartz was moderately colonized while plagioclase was barren of attached cells, although their surface properties were similar.

Inhibitory Metals

Differences in major element chemistry may be responsible for the observed colonization behavior on quartz and plagioclase. Silicate glasses with different concentrations of Al^{3+} (Table right) were used to investigate this theory. Microorganisms colonized sodium-aluminum glasses with less than 5% Al, leaving glasses with 5-20% Al barren, although both glasses had similar surface charge (pHzpc ~4). Aluminum is known to be toxic to some microorganisms or may interfere with iron sequestration by DIRB, by complexing with chelates intended for iron mobilization.



SEM photomicrograph of Al 0 glass (left) and Al 20 glass (right) surfaces after 3 months in anaerobic groundwater at Bemidji. The Al 0 glass, which lacks aluminum, is moderately colonized by a variety of morphotypes, while the Al 20 glass, with 20% Al, is barren of cells.

Essential Nutrients

A borosilicate glass containing Fe^{3+} was moderately colonized by microorganisms in the field. The response to Fe glass cannot be attributed to coulombic attraction (pHzpc ~4), but rather is evidence of active preferential colonization of that surface. At the study site Fe^{3+} is needed as a terminal electron acceptor by DIRB and therefore, sources of Fe, such as silicate-bound Fe, may be attractive to the indigenous microbial population.

SEM photomicrograph of Fe glass after 8 months in the anaerobic Bemidji groundwater. The surface is moderately colonized by rods with some glycocalyx.

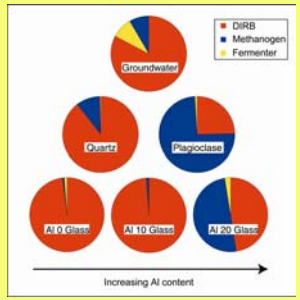
Summary of results from field microcosms

Material	Extent of colonization
Corundum	+++++
Hematite	+++
Fe Glass	+++
Fe-coated Quartz	++++
Fe-coated Plagioclase	+++
Quartz	++
Plagioclase	--
Al 0 Glass	++
Al 20 Glass	--

Increasing density of colonization (C) is indicated by + through +++++. --- indicates that the feature was not observed.

Community Diversity

The presence of Al in silicates not only impacted the number of surface-colonizing cells but also the diversity of physiologic types present. In groundwater and on silicate surfaces without Al, DIRB were the dominant physiologic type. On Al-bearing surfaces (plagioclase and glass with 20% Al), however, the methanogens comprised a significant fraction of the population. The decrease of DIRB in the presence of Al may be due to interference by Al^{3+} in iron sequestration, making DIRB less competitive on those surfaces.



Can we implement these experiments into coursework?

The data presented here integrates geology and microbiology and shows that aquifer mineralogy is a fundamental part of the subsurface microbial ecology. I used an inexpensive and straightforward experimental design aimed at understanding the behavior of an entire microbial population *in situ*. My goal is to implement a similar experiment into my Geomicrobiology class as a class project. The class will design the experiment, collect samples, analyze the data, and write it up a report.