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 releasing nutrients as they are consumed by the microorganisms. This process is an essentially passive interaction, cells Corundum and the amorphous iron oxide coatings (see below) can dominate the otherwise uncolonized surface.

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 charged oxide surfaces. This model, however, ignores active controls on attachment such as nutrient focusing, nutrient availability, and metal toxicity that might override passive attraction or repulsion due to charge differences or advection. 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 this study area 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(II) is also present at high levels (up to 2000 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 bacterio- and asynchronously distributed methanogens (Bekins et al., 1999).

Research Approach
Microbial attachment to mineral surfaces is often characterized in laboratory settings by rapidly growing monocultures 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 consortia engage in complex, symbiotic interactions. The goal of this study was not to isolate and identify colonizing cells, but to understand the behavior of the entire population. Therefore, field and laboratory techniques were designed to investigate attachment behavior of an entire native consortium, an area where mineral surfaces may be an active component in microbe-mineral interactions.

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 probable number (MPN) determination and examination by scanning electron microscopy (SEM).

Active Controls: Silicate Composition
Quartz and plagioclase are uniformly negatively charged at pH 6.8 (pH 7 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 (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 were near-neutral (pH 7 and ~2.4). 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.

Essential Nutrients
A borosilicate glass containing Fe was moderately colonized by microorganisms in a previous study. The response to Fe glass cannot be attributed to coulombic attraction (pH 7 ~4), but rather is evidence of active preferential colonization of that surface. At the study site Fe 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.

Summary of results from field microcosms

<table>
<thead>
<tr>
<th>Material</th>
<th>Extent of colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corundum</td>
<td>++++</td>
</tr>
<tr>
<td>Hematite</td>
<td>+++</td>
</tr>
<tr>
<td>Fe Glass</td>
<td>+++</td>
</tr>
<tr>
<td>Fe-coated Quartz</td>
<td>+++</td>
</tr>
<tr>
<td>Fe-coated Plagioclase</td>
<td>+++</td>
</tr>
<tr>
<td>Quartz</td>
<td>++</td>
</tr>
<tr>
<td>Plagioclase</td>
<td>--</td>
</tr>
<tr>
<td>Al 0 Glass</td>
<td>++</td>
</tr>
<tr>
<td>Al 20 Glass</td>
<td>--</td>
</tr>
</tbody>
</table>

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

SEM images of iron-coated quartz (left) and plagioclase (right) surfaces after 8 months in the anaerobic Bemidji groundwater. The quartz surface is moderately colonized by a variety of morphotypes and some glycocalyx. The surface is thickly covered by rods and glycocalyx.

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

SEM photomicrograph of Fe glass after 7 months in the anaerobic Bemidji groundwater. The surface is colonized by a variety of cells.

SEM photomicrograph of Al 0 glass (left) and Al 20 glass (right) after 8 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.

SEM photomicrographs of Al 0 glass (left) and Al 20 glass (right) after 8 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.

Community Diversity
The presence of Al in silicates not only impacted the number of surface-colonizing cells but also 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 in iron sequestration, making DIRB less competitive on these surfaces.

Can we implement these experiments into coursework?
The data presented here integrates geology and microbiology and shows that aquifer minerals are 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.