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Inhibition and enhancement of microbial surface colonization: the role of silicate composition

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Abstract

Classical treatment of cell attachment by models of filtration or coulombic attraction assumes that attachment of cells to mineral surfaces would be controlled by factors such as response to predation, collision efficiency, or coulombic attraction between the charged groups at the mineral and cell surfaces. In the study reported here, the passive model of attachment was investigated using a native microbial consortium and a variety of Al- and Fe-bearing silicates and oxides to determine if other controls, such as mineral composition, also influence the interaction between cells and surfaces. Results from in situ colonization studies in an anaerobic groundwater at pH 6.8 combined with most probable number analyses (MPN) of surface-adherent cells demonstrate that electrostatic effects dominate microbial colonization on positively charged oxide surfaces regardless of mineral composition. In contrast, on negatively charged silicate minerals and glasses, the solid phase composition is a factor in determining the extent of microbial colonization, as well as the diversity of the attached community. In particular, silicates containing more than 1.2% Al exhibit less biomass than Al-poor silicates and MPN suggests a shift in community diversity, possibly indicating Al toxicity on these surfaces. When Fe is present in the silicate, however, this trend is reversed and abundant colonization of the surface is observed. Here, microorganisms preferentially colonize those silicate surfaces that offer beneficial nutrients and avoid those that contain potentially toxic elements.

Keywords: Microbial colonization; Nutrients; Toxic metals; Silicate minerals

1. Introduction

The subsurface microbial community can be grossly divided into two distinct but related populations: free-floating or planktonic microorganisms usually assumed to be the smaller fraction, and attached or sessile organisms, which are the more populous fraction in most aquifers (Hazen et al., 1991). Each habitat offers distinct advantages and disadvantages to a population, and there is a rich literature on the mechanisms and tactics for microbial attachment to mineral surfaces (e.g., Fletcher and

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Murphy, 2001), and the transport of pathogenic microorganisms in soils and aquifers (Harvey and Harms 2001, 2002). Microbial attachment and growth can influence flow through small pore spaces, and may alter the nature of the mineral surface or change mineral water reaction equilibria. Microbial attachment to mineral surfaces is a selection mechanism for the microbial community, a component of the porewater geochemistry and can even influence the hydraulic character of the aquifer. The basis for microbial attachment and subsequent colonization of mineral surfaces, therefore, is integral to understanding the biogeochemistry of groundwater habitats.

Models of microbial attachment to and growth on mineral surfaces in aquifers assumes that the initial cell attachment is a passive or random interaction. Often cells are approximated as charged colloids and mineral surfaces as uniform charged surfaces with the primary interaction by passive filtration and columbic attraction (e.g., van Loosdrecht et al., 1989). Most importantly, minerals are treated as surfaces with only the property of charge actively influencing microbial attraction, while passive properties such as hydration forces, hydrophobic or steric interactions, and polymer bridging all influence microbial attachment to some degree but are difficult to separate and quantify (Elimelech et al., 1995). Currently, there are several models used to describe microbial attachment to mineral surfaces, including Derjaguin–Ladau–Verwey–Overbeek (DLVO), extended DLVO (Hermansson, 1999) and surface complexation models (SCM; Fein et al., 1997; Fowle and Fein, 1999; Martinez and Ferris, 2001; Yee et al., 2004). These models are particularly useful in modeling short-term laboratory experiments, as well as microbial transport in porous media where cell attraction and initial attachment occurs over short time periods. Surface complexation models, for example, describe the reactivity of the microbial cell wall by employing acid–base titrations that are used to quantify and assign surface functional groups (e.g., carboxyl, phosphoryl, amino). Equilibrium constants for the deprotonation of these functional groups (pK_a) can then be utilized to model the microbial electric field under specific solution pH and ionic strength conditions. Though often dilute, most groundwaters still contain common major cations (Ca^{2+}, Mg^{2+}, K^{+}, etc.) and therefore both microbial and mineral surface functional groups are likely complexed, potentially leading to a reduction in the electrostatic repulsion between negatively charged bacteria and silicate surfaces such as feldspars, at near-neutral pH (Fowle et al., 2004; Yee et al., 2004).

Significant changes in attachment behavior have been documented as a function of solution pH and ionic strength. Although these parameters have been shown as primary controls on initial surface attachment (Kinoshita et al., 1993; Yee et al., 2000), changes in other matrix properties have profound effects. In particular, the concentration and flux of nutrient and carbon sources has been shown to impact microbial attachment in laboratory settings (Bonet et al., 1993; Knox et al., 1985; McEldowney and Fletcher, 1986; Molin et al., 1982). Field observations suggest that the number of planktonic organisms increases within plumes of carbon contamination (Godsy et al., 1992; Harvey et al., 1984), and Murphy and Ginn (1996) found that initially attached organisms detached to the aqueous phase under non-nutrient-limited conditions, but reattached as one or more nutrients became limiting. Attachment may serve as a means to avoid predation by grazers (protazoan; Harvey, 1997) or may benefit microorganisms because nutrient availability may be greater due to surface-associated organic matter (Davis and McFeters, 1988; Lechevallier and McFeters, 1990; Mueller, 1996) and nutrients. Recent studies have demonstrated that some dissimilatory iron-reducing bacteria use specialized flagella to detect and attach to iron oxide minerals (Caccavo and Das, 2002; Childers et al., 2002; Lower et al., 2001). This type of chemotactic behavior may account for differential colonization of mineral surfaces with similar surface charge but different compositions and may play a role in establishing surface-associated microbial communities.

Over longer time periods, however, other factors must be considered and *colonization* by microorganisms rather than reversible attachment or detachment may occur. Growth of individual cells on a mineral surface may result in the formation of complex interdependent microbial communities, where the cells in contact with the solution are not in contact with the mineral surface, and the surface charge is immaterial to individual cells comprising the layered biofilm. Mineral surfaces can become fouled with exopolysaccarides (EPS) or other organic polymers

changing the surface charge, while exoenzymes may sorb to exposed mineral surfaces. Minerals can dissolve or precipitate due to the presence of viable microorganisms, resulting in a complex and reactive chemical environment around the attached cell that may interfere or potentially enhance basic cell functions associated with metabolism and growth. The longer a cell is attached, the greater the opportunity to directly interact with the mineral surface, and the nature of that interaction will become increasingly important to the success of that population on that habitat (i.e. mineral surface).

The research presented here examines microbial colonization (a steady-state observation of the sum of attachment, detachment, growth and death of surface adherent microbes) of silicate mineral, oxide phases, and glass surfaces in nature over relatively long time periods (months) as a function of both surface charge and detailed composition. The role of silicate-bound metals and nutrients on microbial colonization was investigated and these results were compared to pure silica and simple glass surfaces using both specific coulombic attraction models and broader compositional influences. Microbial colonization on mineral surfaces, as opposed to initial microbial attachment, is strongly influenced by the composition of the mineral, with both negative and positive influences offered by silicate bound metals and nutrients. A compositional model of microbial colonization suggests that all mineral surfaces are not created equally, and a more complete understanding of microbial–mineral surface association requires more than a description of surface charge.

2. Methods

The influence of basic silicate composition on microbial colonization was examined using a variety of iron (Fe)- and aluminum (Al)-bearing minerals and glasses in field colonization experiments. Fe and Al were chosen because both metals have similar electron valence and charge and occur naturally in silicate minerals with silica and charge balancing cations. Microorganisms use Fe as a micronutrient, and in anaerobic systems, as a terminal electron acceptor while Al is known to be inhibitory to some microorganisms. Field in situ microcosm experiments were performed with oxide and silicate minerals and Al- and Fe-doped silicate glasses. Scanning electron microscopy (SEM) was utilized to examine the alteration of mineral and glass surfaces, microbial morphology, and extent of microbial colonization. Microbial diversity was further characterized using a modified most probable number (MPN) technique. This combined approach was used to characterize the influence of mineral composition on microbial colonization.

2.1. Site description

The primary study site is one of the U.S. Geological Survey's Toxic Substances Research Site, a hydrocarbon-contaminated aquifer located in northern Minnesota near the town of Bemidji. On August 20, 1979, a petroleum pipeline ruptured, releasing approximately 1250 m^3 (4.4×10^5 gals) of crude oil, resulting in a floating pool of free-phase petroleum ~1 m thick at the water table. A plume of both dissolved aromatics and secondary metabolites as well as inorganic solutes has formed beneath and downgradient of the source (Baedecker et al., 1993; Hult, 1984). In the anaerobic groundwater, efficient transformation of the dissolved fraction of the petroleum occurs via dissimilatory iron reduction (Anderson, 1998; Baedecker et al., 1993; Bennett et al., 1993; Cozzarelli et al., 1990, 1994; Eganhouse et al., 1993; Lovley et al., 1989; Rooney-Varga et al., 1999) and secondary methanogenesis (Revesz et al., 1995). Bekins et al. (1999b) found that DIRB are the dominant physiologic type in the anaerobic region of the aquifer with fermenting bacteria also present and methanogens occurring only in narrowly distributed zones.

The boreal Lost River peatland is part of a mire deposited on the bed of glacial Lake Agassiz. Peat has accumulated to an average depth of 3 m (Glaser et al., 1991), and the area contains many raised bogs and spring fens, which are hydrologically and geochemically distinct. This study was conducted in a raised bog because its geochemistry was similar to that found in the anaerobic zone of the primary study site. The bog is covered with stunted black spruce and an understory of shrubs and Sphagnum moss. The groundwater chemistry is characterized by oxic, acidic conditions and low TDS (<10 mg l^{-1}) at the surface, with pH and TDS increasing with depth until pH is

circum-neutral and conditions are anoxic. Dissolved Fe(II) and abundant methane suggest active dissimilatory iron-reducing bacteria (DIRB) and methanogenesis at depth. Accelerated weathering of the native silicate grains has been observed and is attributed to complexation by organic ligands produced from metabolism of DOC by indigenous microbes (Bennett et al., 1990). Groundwater geochemistry for both sites, including dissolved carbon, nutrients, anions, cations, and field parameters were determined using standard methods (e.g. Bennett et al., 1993) and are given in Table 1.

2.2. Field microcosms

To investigate the influences of silicate composition and surface charge on microbial colonization, we used in situ microcosms to characterize microbial colonization in field settings. This technique is similar to the standard buried slide technique used by microbiologists to sample native soil microbial populations using glass slides inserted into the soil (Parkinson et al., 1971). The microcosm experiments used in this study were modified to target the planktonic fraction of a groundwater microbial community (e.g., Bennett et al., 1996; Hiebert and Bennett, 1992; Rogers et al., 1998). The microcosms were constructed of sterile polyethylene containers, punctured to permit flow-through of water and suspended material such as colloids and bacteria. At the Bemidji site constructed microcosms were suspended into the screened portion of wells (numbers 9015, 9017 and 532B) in the anaerobic groundwater at Bemidji, while at the Lost River bog microcosms were driven to desired depths (0.3, 0.5, 1.2, 1.7, 2 and 3 m) using a metal rod. Microcosms were left undisturbed for periods of 3–12 months and groundwater samples were taken at the time of placement and removal of the microcosms. Additional microcosms were reserved for reference and control. Biological tissues were fixed using a critical chemical point drying method (Nation, 1983; Vandevivere and Bevaye, 1993). Samples were inspected in the laboratory using conventional scanning electron microscopy (JEOL SEM equipped with electron backscattering spectroscopy, energy-dispersive system). At least 20 different fields on each sample were examined at a variety of magnifications, and then electronic images were collected. The average number of cells per field (~100 μm^2) were calculated for each mineral or glass chip. The patterns of colonization, extent of colonization, presence of attachment features and glycocalyx were noted, as well as changes in the mineral or glass surface.

Table 1
Groundwater chemistry in Bemidji wells and Lost River Bog

Location	Depth	pH	DOC[a] [b]	O_2	DIC[c]	Na	Ca	Mg	Fe[d]	Si	P[e]
BJI[f]	9017	6.64	2.9	0.004	11.3	0.04	4.3	1.5	0.7	0.90	1.0
BJI	9015	6.56	1.83	0.002	11.2	0.05	4.2	0.9	0.7	0.7	1.0
BJI	523B	6.72	**[g]	0.02	8.4	0.04	2.6	0.8	0.4	0.9	1.5
Bog[h]	0.3	4.08	6.7	0.13	3.5	0.1	0.02	0.02	0.02	0.2	b.d.
Bog	0.5	4.06	14.6	**	4.5	0.01	0.07	0.07	0.02	0.4	b.d.
Bog	1.2	4.16	16.1	0.2	4.4	0.03	0.08	0.1	0.02	0.5	b.d.
Bog	1.7	5.15	14.4	0.08	7.4	0.1	0.3	0.3	0.02	0.6	1.7
Bog	2.0	5.62	16.8	0.16	5.9	0.2	0.6	0.5	0.02	0.6	2.3
Bog	3.0	5.94	10.5	0.07	10.3	0.1	1.2	0.7	0.04	0.7	3.8
DL	0.1	0.02	0.01	1×10^{-1}	0.01	4×10^{-3}	3×10^{-4}	2×10^{-4}	9×10^{-5}	7×10^{-4}	0.5
Prec.%			1	5	0.5	6	6	5	5	5	5

[a] Concentrations are in mmol l^{-1}.
[b] DOC is total dissolved organic C, equal to the sum of the nonvolatile and volatile fractions, as mmol l^{-1} C.
[c] DIC is the dissolved inorganic carbon.
[d] Fe is as FeT.
[e] P as μmol l^{-1} PO_4.
[f] Groundwater wells under (9017, 9015), and downgradient of the oil (532b) at Bemidji (BJI).
[g] ** signifies that the constituent was not analyzed.
[h] Sampling depth from the surface in the Lost River Bog (Bog).

2.3. Mineral and glass preparation

Both minerals and silicate glasses were used in field microcosms to assess microbial colonization as a function of silicate composition. Fragments of plagioclase, quartz, hematite, magnetite and corundum were obtained from Wards Scientific. Two silicate float glasses (designated 1830 and 1831) were obtained from the National Institute of Standards and Technology (NIST). Borosilicate glass containing Fe^{3+}, and sodium glasses containing varying amounts of Al were manufactured from stock powder mixtures. The sodium glass powders with 0–20% aluminum (designated Al 0 through Al 20) were obtained from K. Traexler, University of Delaware (Traexler, 1999). Powders were homogenized for 20 min, and then melted in a furnace at 1100 °C for 5 h in platinum crucibles. The resulting one-phase glasses were allowed to cool to room temperature, removed from the crucible, ground to a powder, and remelted to remove bubbles and increase homogeneity.

For field microcosms, minerals and glasses were crushed separately in a tungsten shatterbox (SPEX industries model 8500-115). The pieces were then dry sieved to collect the 5–10 mm size fraction and rinsed with distilled water. To remove fine particles from crystal faces the chips were sonicated 3×15 s in distilled water. The chips were rinsed with distilled water and then dried at 125 °C overnight. Chips of each mineral type and glass were reserved as reference and controls. Surface area of chips was characterized with a Quantachrome Autosorb1 using a seven-point BET with nitrogen or krypton as the adsorbate gas. Major element oxides in the mineral and glass samples were determined by XRF (Activation Laboratories, Ontario, Canada). Elemental composition for the glasses and minerals used in this study are summarized in Table 2.

To examine the role of amorphous iron coatings on silicates as they occur in the aquifer, quartz and plagioclase surfaces were prepared with ferric oxyhydroxide coatings (e.g., Grantham et al., 1997). This was done by titrating a 0.1 M $FeCl_3$ and 0.05 M NaCl solution with 0.5 M NaOH to a pH of ~4, and precipitating the $Fe(OH)_3$ directly onto the clean mineral surfaces to a thickness of 1–5 μm thick based on SEM observations. After coating, minerals were rinsed in distilled water with sonication for 3×15 s and baked in a drying oven as above.

2.4. Diversity of the attached microbial population

Microbial diversity in groundwater and on mineral and glass chips was characterized using a modified most probable number (MPN) method using pre-reduced, anaerobically sterilized (PRAS) (Holdeman and Moore, 1972) prepared media appropriate for DIRB, methanogens, and fermenters. Media formulations were used based on previous site studies by Essaid et al. (1995) and Bekins et al. (1999b) which indicated that these metabolic guilds

Table 2
Compositions of silicates minerals and glasses

Silicate[a]	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	B_2O_3	P_2O_5
Quartz[b]	60.6	19.1	4.4	0.9	3.0	6.6	4.0	0.9	–	0.02
Plagioclase[b]	59.8	20.9	1.1	0.1	2.4	6.7	7.4	–	–	–
Al 0 Gl.[c]	80.0	–	–	–	–	20.0	–	–	–	–
Al 20 Gl.[c]	80.0	20.0	–	–	–	–	–	–	–	–
1830 Gl[d]	73.1	0.1	0.1	3.9	8.6	13.8	–	–	–	–
1831 Gl[d]	73.1	1.2	0.1	3.5	8.2	13.2	0.3	–	–	–
Fe Gl.[e]	80.8	2.2	1.0[d]	–	–	4.3	–	–	12.0	–

[a] Values are expressed as weight percent oxide.
[b] Analysis from Bennett et al. (2001) and Rogers et al. (1998).
[c] Manufactured sodium glasses with no aluminum (Al 0 Gl.) or 20.0 % aluminum (Al 20 Gl.).
[d] NIST glasses 1830 and 1831 (1830 Gl. and 1831 Gl.).
[e] Manufactured borosilicate glass with iron (Fe Gl.).

were dominant in the study zone. All media contained 10 mg l^{-1} Tween-80, a nonionic surfactant used to dislodge cells from mineral surfaces. A volume of 9 ml of media was dispensed into 30-ml glass serum bottles purged with oxygen-free nitrogen. After media was dispensed, bottles were capped with butyl rubber stoppers and aluminum crimp tops, and then sterilized for 20 min at 121 °C.

Mineral and glass chips from field microcosms were transferred to sterilized growth media (one chip in 9 ml of each medium) in a N_2-purged glove bag. The chips and media were incubated at room temperature for 1 h, after which the bottles were shaken vigorously to remove adherent cells. A 10-fold dilution series was made from the supernatant suspension of cells for each mineral or glass type in each medium up to eight bottles, reserving one bottle of each medium as sterile control. Aseptic technique was used throughout the procedure. After inoculation, the methanogen and iron-reducing bottles were pressurized to 140 kPa with a 70:30 mix of H_2:CO_2. All bottles were incubated in the dark at room temperature and scored after 1 week for fermenting bacteria and after 6 weeks for iron-reducing bacteria and methanogens according to the methods of Bekins et al. (1999b). An MPN calculator (VB6 version, Mike Curiale, http://www.totalshareware.com) was used to calculate the final numbers and confidence intervals.

3. Results

3.1. Surface colonization of silicate minerals and glasses

Minerals and glasses were placed into the native groundwater using in situ microcosms and left undisturbed to interact with groundwater, colloids and planktonic organisms for periods up to 1 year. All of the minerals and glasses examined had varying degrees of colonization by microorganisms. The extent of colonization and diversity of organisms differed not only with the composition of the solid phase but also its surface properties.

Previous results of similar experiments using other silicate minerals have been summarized in Rogers and Bennett (2004) and Bennett et al. (2001). Colonization behavior on quartz, plagioclase, and Fe glass was investigated in the anaerobic groundwater at Bemidji and observations were confirmed in the anaerobic groundwater of the Lost River Bog. Subsequent experiments using other Fe and Al bearing phases were performed at the Bemidji site.

The two silicate minerals used in this study, quartz and plagioclase, differed substantially in the extent of surface colonization after 8 months of passive exposure to the anaerobic groundwater. Quartz was colonized by a variety of morphotypes (Fig. 1) and in some extended experiments (>1 year) light etching

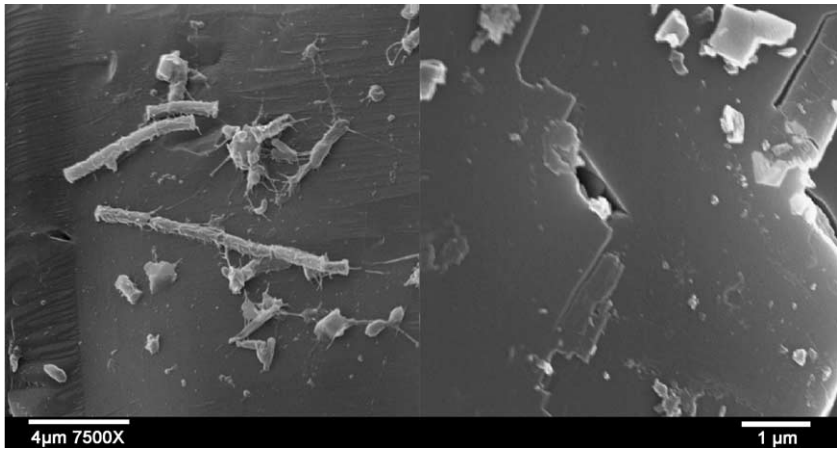

Fig. 1. SEM image of quartz (left) and plagioclase (right) after 8 months in the anaerobic Bemidji groundwater. The quartz surface is lightly colonized by colonies comprised of several morphotypes, while the plagioclase is barren of cells. Quartz scale bar = 4 μm and plagioclase scale bar = 5 μm.

was detected near attached organisms in the petroleum-contaminated aquifer (well #9015, 532B). The cells on quartz were associated with observable glycocalyx and occurred as individuals, as groups or in small colonies. In the bog environment quartz was also heavily colonized but there was no discernable etching associated with attached cells (microcosm depth 1.2 m from surface). Plagioclase, however, had no colonization by microorganisms and no etching was observed. This mineral was investigated in several wells (532B, 9017) using both SEM and environmental SEM and neither cells nor etching were observed. Plagioclase was also barren of cells in the bog environment (0.4 m depth) but exhibited extensive surface etching that was crystallographically oriented along several crystal planes (SEM photomicrographs not shown).

Fig. 2 depicts the effect of varying amounts of Al on microbial colonization. These glasses exhibited different colonization density, with surface-adherent cells only observed on glasses that contained little or no Al. Fig. 2 shows differential colonization of NIST glasses. NIST 1830 glass (0.12% Al_2O_3) was densely colonized by a variety of morphotypes, while no cells were observed on the NIST 1831 glass (1.2% Al_2O_3). No etching was observed on either surface. Colonization of sodium–aluminum glasses was similar to that observed on NIST glasses. The Al 0 (sodium glass) glass was colonized by a variety of morphotypes, occurring primarily in groups with glycocalyx attachment features and extensive surface slime. The other Al glasses (Al 5–20) had no observable colonization (SEM photomicrographs not shown). No colonization was observed on any glass, either NIST or sodium, that contained more than 1.2% Al. An Fe-doped, Al-bearing glass was used to investigate the possibility that the addition of an essential nutrient to an aluminosilicate will counter the inhibitory influence of Al on microbial colonization. After reaction in anaerobic groundwater at both study sites, the Fe/Al glass was colonized moderately by the native microbial communities. Cells occurred in pairs and small groups and were typically associated with glycocalyx (Fig. 3). No etching was observed on the glass surfaces. This mode of colonization was observed in several wells throughout the anaerobic zone of the petroleum-contaminated aquifer (well #9015, 532B) as well as the bog (1.2 m depth).

3.2. Surface colonization of oxide minerals

Iron-oxyhydroxide coatings on quartz and plagioclase were utilized to examine the potentially competing influences of Fe and Al on surface colonization. Coated quartz and plagioclase surfaces were heavily colonized by microorganisms. Cells preferentially attached to the iron coatings but some were also observed on adjacent, clean quartz and plagioclase surfaces. Attached cells on the iron-coated quartz possessed diverse morphologies while rods

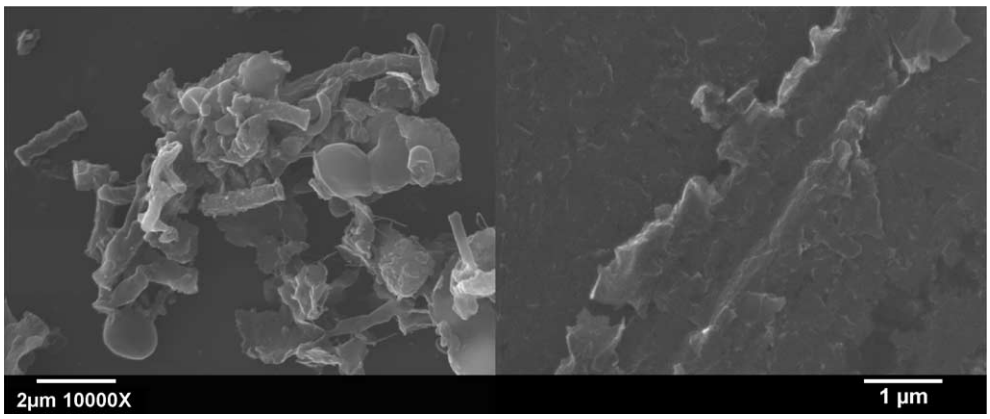

Fig. 2. SEM photomicrograph of NIST 1830 glass (left) and NIST 1831 glass (right) surfaces after 8 months in anaerobic groundwater at Bemidji. The 1830 glass, which lacks aluminum, is moderately colonized by a variety of morphotypes, while the aluminum-bearing 1831 glass is barren of cells. 1830 scale bar=2 μm and 1831 scale bar=1 μm.

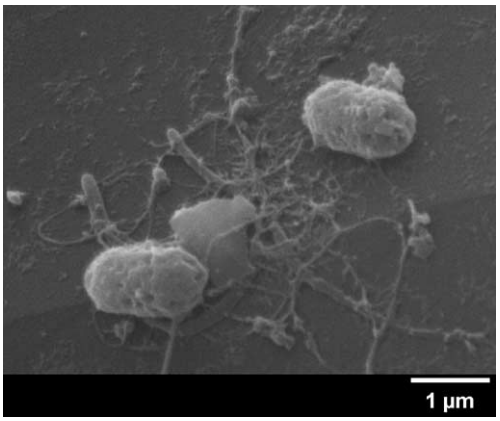

Fig. 3. SEM photomicrograph of Fe glass after 8 months in the anaerobic Bemidji groundwater. The surface is moderately colonized by rods with some glycocalyx. Scale bar=1 μm.

were the predominant morphotype on plagioclase (Fig. 4). While much of the iron was dissolved over the course of the experiment, microorganisms remained even after the iron was removed. There was no etching of the quartz surface and only minor etching of the plagioclase surface was observed in the vicinity of attached cells.

Microbial colonization of both Fe and Al oxide minerals were also examined. Both hematite (Fe_2O_3) and magnetite (Fe_3O_4) were both heavily colonized by a variety of morphotypes. Both rods and cocci occurred as lone cells and in groups. The only striking difference between these two iron oxides was glycocalyx development; hematite had little or no glycocalyx while attached cells on the magnetite surface were associated with extensive glycocalyx (Fig. 5). Although corundum (Al_2O_3) contains Al and not Fe, like the iron oxides, it was very heavily colonized. The surface supported colonies of primarily rods associated with thick glycocalyx (Fig. 6).

3.3. Diversity of the surface adherent microbial population

Measurements of diversity and colonization density of surface-adhering cells by MPN support the hypothesis that silicate composition exerts some control on surface colonization. Because our experimental design selects for cells that are either planktonic or those that can readily attach and detach, we use the composition of the microbial population in the anaerobic groundwater at Bemidji as a point of reference. The planktonic fraction of the microbial population at Bemidji represents approximately 5% of the total microbial population, but accurately reflects the attached population in composition (Bekins, personal communication; Bekins et al., 1999b). The groundwater supported a low titer microbial population of 1.2×10^2 cells ml^{-1} that was dominated by dissimilatory iron-reducing bacteria (DIRB) (~83%) with some methanogens (9%) and fermenters (8%) present (Fig. 7). The colonizing population on quartz was 1.4×10^8 cells m^{-2}, also dominated by DIRB (90%) with 10% methanogens, although there was no significant ferric iron on the quartz surface (quartz

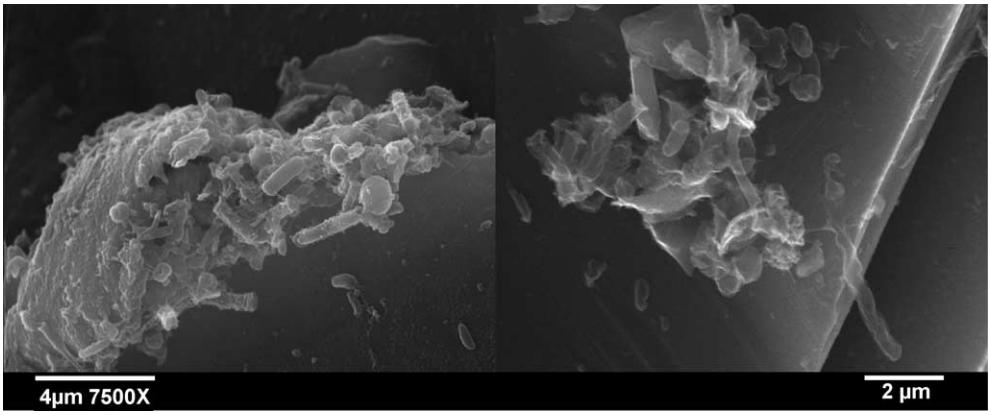

Fig. 4. SEM image of iron-coated quartz (left) and plagioclase (right) surfaces after 8 months in the anaerobic Bemidji groundwater. Both surfaces are covered with a variety of morphotypes and some glycocalyx. Quartz scale bar=4 μm and plagioclase scale bar=2 μm.

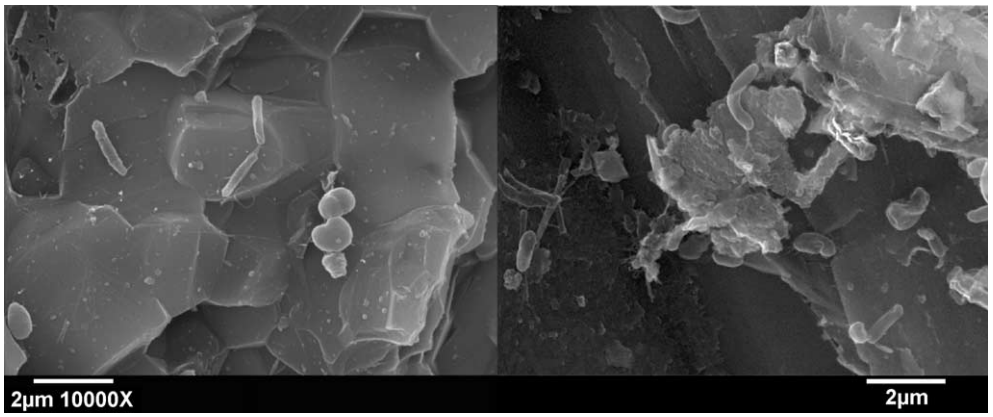

Fig. 5. SEM image of hematite (left) and magnetite (right) surfaces after 7 and 12 months in the anaerobic Bemidji groundwater, respectively. The hematite surface is colonized by a few cocci and rods, with no glycocalyx. The magnetite surface is covered by a variety of morphotypes associated with glycocalyx. Scale bar=2 μm.

was prepared by boiling in aqua regia and inspection of surface with energy-dispersive spectroscopy revealed no Fe peaks.) In contrast, plagioclase supported a smaller population (6.1×10^5 cells m^{-2}) that was dominated by ~75% methanogens and 25% DIRB with less than 1% fermenters (Table 3).

The aluminum glasses exhibited a similar pattern. Al 0 glass supported a small population (9.1×10^4 cells m^{-2}) dominated by DIRB (98% DIRB) with ~1% methanogens and fermenters, while Al 5 supported fewer cells (2.2×10^4 cell m^{-2}) consisting of 93% DIRB, 2% methanogens, and 5% fermenters. Al 20 glass also had scant biomass (2.1×10^2 cells m^{-2}), which consisted of 50% methanogens, 48% DIRB, and 2% fermenters. The Fe glass supported more biomass than the Al glasses with 9.2×10^6 cells

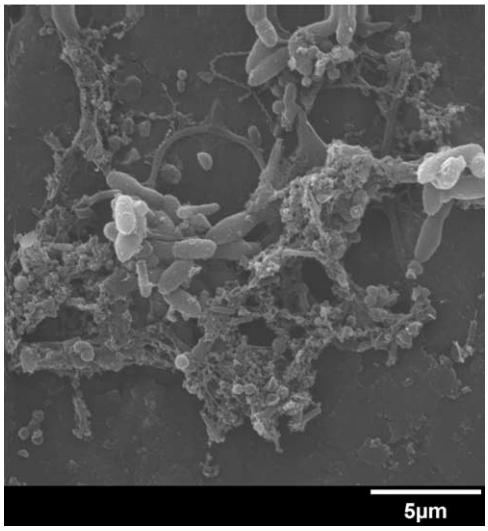

Fig. 6. SEM image of corundum 12 months in the anaerobic Bemidji groundwater. The surface is thickly covered by rods and glycocalyx. Scale bar=5 μm.

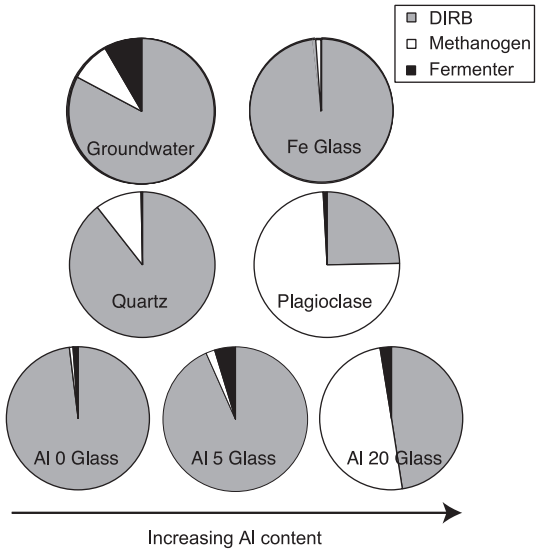

Fig. 7. Fraction of surface colonizing population represented by DIRB, methanogens, and fermenters based on MPN analysis of colonized mineral and glass chips from field microcosms. The fraction of methanogens present increases when Al is present in the solid phase, while DIRB dominate when Al is absent.

Table 3
Summary of results from in situ microcosm oxides, silicates and glasses after exposure to anaerobic, carbon-rich groundwater

	Colonization[a]	Biomass[b]	DIRB	M	FB
Silicates					
Quartz	++	1.4×10^{8}	1.3×10^{8}	1.5×10^{7}	3.0×10^{5}
Plagioclase	–	6.1×10^{5}	1.5×10^{5}	4.6×10^{5}	4.4×10^{3}
1830 glass	++	**[c]	**	**	**
1831 glass	–	**	**	**	**
Al 0 glass	++	9.1×10^{4}	8.9×10^{4}	9.7×10^{2}	8.9×10^{2}
Al 20 glass	–	2.1×10^{2}	1.0×10^{4}	1.1×10^{4}	5.0×10^{2}
Fe glass	+++[d]	9.2×10^{6}	9.1×10^{6}	9.1×10^{4}	4.5×10^{3}
Oxides					
Corundum	+++++				
Hematite	+++				
Magnetite	++++				
Fe-coated quartz	++++				
Fe-coated plagioclase	+++				

[a] Relative amount of colonization (based on average surface cell counts) indicated by + (1–2), ++ (3–5), +++ (6–10), ++++ (11–20), and +++++ (>25) cells per field. – indicates that no cells were observed.

[b] Total biomass based on MPN analysis comprised of dissimilatory iron reducing bacteria (DIRB), methanogens (M), and fermenting bacterial (FB) counts from most probable number determinations of mineral and glass surfaces expressed as cells m^{-2}.

[c] ** signifies that the constituent was not analyzed.

[d] Note that discrepancies in colonization density and biomass based on MPN of mineral and glass chips of the same composition is likely due to differences in sample preparation and methodology. SEM preparation requires extensive washing that may dislodge loosely attached cells, while MPN is a culture-based method that favors only cultureable cells.

m^{-2} that consisted of 98% DIRB and 1% methanogens (Table 3).

4. Discussion

In this study, the influence of major-element composition of silicate minerals on microbial colonization was investigated using silicate minerals and glasses containing these metals. It is proposed here that both mineral surface charge and mineral composition influence microbial colonization. Our results suggest, however, that these two controls dominate attachment and subsequent colonization under different conditions. When the mineral surface is uniformly positively charged, negatively charged cells readily attach and colonize regardless of mineral composition. Conversely, on negatively charged surfaces, though we observe overall less colonization, cell abundance can be correlated to mineral composition. Colonization results are summarized in Table 3.

Silicate minerals, in the absence of Fe, exhibited colonization densities that were inversely correlated to the Al content of the mineral. Quartz was moderately colonized while plagioclase was barren of attached cells. These findings differ from previous laboratory studies, which found little interaction (scant attachment dominated by hydrophobic effects rather than electrostatic interaction) between bacteria and quartz surfaces (Yee et al., 2000). This lack of reactivity is attributed to the predominant negative charge of both quartz and most bacterial cell walls at solution pH above 2 (Harden and Harris, 1953; Marshall, 1980). Plagioclase, however, is also negatively charged in this pH range (pH_{zpc} of 2.4; Stumm and Morgan, 1996) and therefore we would predict that bacteria would exhibit similar attachment behavior with both surfaces. Our observations of attachment and subsequent colonization of mineral surfaces do not adhere to these predictions and while parameters such as surface roughness (e.g., Edwards and Rutenberg, 2001) and solution chemistry (Fontes et al., 1991; Gannon et al., 1991a,b; Gordon and Millero, 1984; Jewett et al., 1995; McEldowney and Fletcher, 1986) may impact surface initial attachment, these are not obvious controls in this environment. Instead, the observed distribution of surface-adhering cells on these minerals cannot be explained by simple passive

coulombic attraction or repulsion, but rather is the result of active colonization and possibly growth on the mineral surface as a function of mineral composition. In this scenario, Al inhibits colonization in the absence of a critical nutrient, such as Fe, which seemingly counters Al as a toxin.

Colonization behavior of microorganisms with manufactured glasses containing stepwise increases in Al concentration (Table 2) supports the assertion that Al is inhibiting colonization on Al-bearing silicates. Microorganisms only colonized NIST glass 1830, which does not contain Al and left the Al-bearing NIST glass 1831 barren. These glasses have very similar compositions except for Al content, but these differences required that we control composition much more precisely in order to isolate Al as a control on colonization. This was done using Al-doped sodium glasses, where only the Al 0 glass was colonized with no observed colonization on any of the other Al glasses (Al 5–20). These results are similar to those obtained using both the minerals quartz and plagioclase as well as the NIST glasses. Silicates with less than 2% Al_2O_3 were colonized likely because they contained neither inhibitory nor beneficial constituents, but provide a surface for cells to attach and form interdependent communities with access to dissolved carbon and nutrients provided by groundwater flow. Silicates containing more than 1.2% Al_2O_3 were uncolonized and the implication is that Al inhibited either attachment or subsequent colonization of the surface. Because these surfaces are charged similarly, it is unlikely that observed differences are attributable to differences in electrostatic interactions between surfaces and cells. Rather, aluminum is likely acting as a toxin (Appanna and Hamel, 1996; Appanna and St. Pierre, 1994; Ilmer and Shinner, 1999) and limiting the ability of adsorbed microorganisms to colonize and grow. Al is known to interfere with microbial cell functions and specifically target DNA, iron proteins and Ca-mediated biochemical reactions (Exley and Birchall, 1992; Nieboer et al., 1995). While many cells use an ATP-dependent pump or the proton motive force to expel toxic metals such as Zn^{2+}, Co^{2+}, and Cd^{2+} (Silver, 1997), Hamel and Appanna (2003) found that *Pseudomonas fluorescens* secreted intracellular Al via its insolubilization as a lipid-containing residue, without the use of the proton motive force nor ATP hydrolysis.

Fe-doped borosilicate glasses were used to determine if this nutrient and TEA could overcome the toxic effects of Al. Fe^{3+} was introduced to the native consortium in a borosilicate glass and the glass surface was moderately colonized in field experiments. The Fe glass supported more biomass (based on SEM) than any of the other silicates although it contained ~2% Al_2O_3 wt.% oxide. The microbial response to Fe glass cannot be attributed to coulombic attraction, but rather is evidence of active preferential colonization of that surface by the native consortium. The valence of iron in the glass is Fe^{3+} and the DIRB in the microbial consortium may find this a viable source of reducible iron. Although there is sediment-extractable Fe(III) in the aquifer sediments, Bekins et al. (1999a) found that DIRB were not using it, possibly because it was less energetically favorable for reduction or because Fe(II) surface coatings inhibited reduction. These limitations may make other sources of Fe, such as silicate-bound Fe, attractive to microorganisms. DIRB are known to extract Fe(III) from aluminosilicate minerals such as glauconite (Kostka et al., 1996, 2002) and previous batch laboratory experiments using Fe glass as the sole source of Fe(III) demonstrated that this iron was available and reducible by a mixed anaerobic consortium (Rogers, 2000; Rogers and Bennett, 2004).

Altering the otherwise uncolonized plagioclase surface with an iron-oxide coating resulted in colonization while coatings enhanced the colonization on quartz. Here, unlike most surfaces examined in this study, distinct colonies and biofilms are apparent. The change in colonization behavior can be attributed to the addition of Fe but unlike the Fe glass is related, in part, to surface charge. Initial reversible attachment to the iron-coated quartz is enhanced by coulombic attraction between the positively charged iron oxyhydroxide surface and the negatively charged microbial cell. Attached cells, however, remain on the surface even when the iron oxide coatings appear to be utilized and energy-dispersive spectroscopy (EDS) confirms that the silicate surface is free of Fe.

Oxide mineral surfaces were also colonized by microorganisms to a greater extent than were silicate surfaces. This is attributed to the fact that these minerals are neutral to positive in surface charge at the

pH of the groundwater, 6.8. Microorganisms are uncharged or slightly positively charged below pH ~2 (the isoelectric point of most cell walls) and become increasingly negatively charged as pH increases (e.g., Harden and Harris, 1953; Marshall, 1980). Microorganisms, therefore, are uniformly negatively charged at neutral pH, while corundum is positively charged (pH_{zpc} 9.1) as are the amorphous iron oxide coatings (pH_{zpc} 8.5; Stumm and Morgan, 1996). Both hematite and magnetite are close to neutrally charged with pH_{zpc} values of 6.7 and 6.5, respectively (Stumm and Morgan, 1996). The observed colonization density on the oxide minerals can, therefore, be explained by the coulombic attraction between the negatively charged cells and positively charged or uncharged surfaces (Fig. 8). Corundum and the amorphous iron oxide coatings exhibit the most colonization as would be predicted from pH_{zpc} values, while the lower pH_{zpc} for hematite and magnetite result in lower colonization density (Table 3). In these experiments, composition does not appear to impact the observed colonization patterns; both aluminum and iron oxide surfaces are heavily colonized, but rather electrostatic interactions between the mineral surface and microbe dominate.

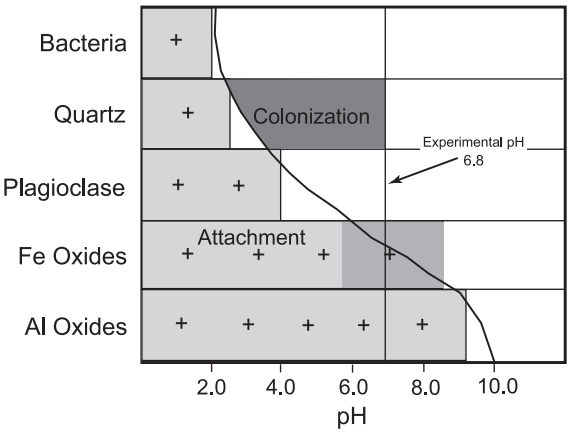

Fig. 8. Schematic of initial attachment and colonization as a function of pH on silicates and microorganisms as observed in this study. The shaded areas and "+" signs represent the pH range of positive charge based on pH_{zpc}. For the Fe oxides, there is a range in pH_{zpc} depending on mineralogy and this range is indicated by darker shading in this region of pH. Initial attachment onto the mineral surface is favored and electrostatically controlled in the region to the left of the curved line. This study observes colonization (the steady-state observation of surface adherent microbial biomass equal to the sum of: attachment−detachment+growth−death) outside of this region, which is shown as a dark grey area between the aquifer pH (shown as a line at pH 6.8) and the curved line. Based on results presented here, it is asserted that in this region the cells are actively colonizing the silicate surfaces and composition of the mineral surface exerts control on the abundance and diversity of cells on the surface.

MPN analysis of the colonizing microbial population reveals that silicate composition is not only controlling the abundance of cells on the surface but also the physiologic types that are present. In groundwater and on silicate surfaces with <5% Al, DIRB were the dominant physiologic type present. On plagioclase and Al 20 glass, however, the methanogens were a significant fraction of the population (Fig. 7; Table 3). When Fe is added to the borosilicate glass, this trend is reversed and DIRB again dominate. Aluminum, therefore, may be specifically toxic to DIRB or alternatively the decrease of DIRB in the presence of Al may relate to the cells' mode of iron sequestration. If DIRB use ligands to chelate and mobilize Fe^{3+}, these ligands may also chelate Al^{3+} decreasing complexation efficiency for iron (e.g., Collinson et al., 1987) and previous studies have demonstrated that this mixed consortium is capable of utilizing chelated Fe(III) (Rogers and Bennett, 2004). DIRB, therefore, may not be as active on these Al-bearing surfaces, making methanogens more competitive in these settings.

5. Summary and conclusions

This study demonstrates two modes of microbial colonization of minerals in circum-neutral pH, anaerobic groundwater. Fig. 8 is a schematic summarizing these findings. On mineral surfaces such as the iron and aluminum oxides investigated in this study, we observe a largely passive interaction dominated by electrostatic interactions between the positively charged mineral surfaces and negatively charged microorganisms shown as the shaded area left of the line in Fig. 8. In contrast, on silicate surfaces that are less electrostatically favorable for microbial attachment, composition controls microbial abundance and diversity on these surfaces. Here, microorganisms preferentially colonize those silicate surfaces that are free of toxic elements or contain beneficial nutrients while avoiding those that contain

toxic elements (shown as the hatched area right of the line in Fig. 8).

The possibility that mineral composition exerts some control over the diversity of the microbial population inhabiting its surface suggests that minerals play a fundamental role in subsurface microbial ecology. Mineral composition, therefore, must be considered when modeling the distribution of microorganisms in the subsurface and may have implications for understanding subsurface microbial transport, biofouling, and biodegradation of contaminants by indigenous microbial communities.

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