Linking bacteria-metal interactions to mineral attachment: A role for outer sphere complexation of cations?

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ABSTRACT: In this study, we conducted attachment experiments and electrophoretic mobility titrations to investigate the mechanisms of attachment of *Escherichia coli* K12 onto quartz surfaces. Attachment experiments were performed as a function of pH in 0.01 M KNO₃ and in the presence of 0.01 M Ca²⁺. Scanning electron microscopy and optical density measurements at 600 nm indicate significant pH dependant attachment of *E. coli* onto quartz surfaces. Electrophoretic mobility experiments performed as a function of pH in 0.01 M KNO₃ with and without Ca²⁺ demonstrated that the magnitude of the zeta potential decreased with the introduction of Ca²⁺ for *E. coli*, but had little effect on the surface properties of quartz. These results indicate that the association of hard acid divalent cations with bacterial cells walls may influence the mechanisms and extent of their attachment onto mineral surfaces.

1 INTRODUCTION

The attachment of a bacterium to the surface of a mineral phase marks the initiation of a host of bacterially driven geochemical reactions. Cells attached to minerals can facilitate a series of reactions ranging from the retardation of toxic metals adsorbed to their surfaces polymers, to the accelerated weathering of silicate minerals (e.g. Fowle & Fein, 1999; Liermann et al. 2000a; Bennett et al. 2001). Once attached, microbes may gain a competitive advantage over planktonic or groundwater entrained cells through the preferential acquisition of nutrients from the host mineral phases, protection from predation via biofilm formation, and communalism among various microbial species (Barker & Banfield 1996; Rogers et al. 1998; Ehrlich 2002; Madigan et al. 2002). Attached organisms can enhance or potentially decrease the rate of weathering reactions via the generation of pH gradients at the interface, complexation via exudates, and by enhancing the kinetics of secondary mineral phase formation (e.g. Welch & Ullman 1993; Barker et al. 1998; Welch et al. 1999; Liermann et al. 2000b; Rogers & Bennett 2004). Therefore to accurately predict and quantify bacterial transport in groundwater systems and the role of bacteria in weathering and diagenetic processes we must first quantify the nature and selectivity of the initial attachment of bacteria to mineral surfaces.

In this investigation, we tested whether alteration of bacterial cell wall electrical field via outer sphere complexation of divalent cations influences the attachment of *Escherichia coli* K12 on quartz surfaces. Previous laboratory based studies have found little reactivity between bacteria and quartz surfaces (e.g. Yee et al. 2000), yet field based studies find colonization of quartz grains on clean surfaces free of such impurities as iron oxide coatings (e.g. Rogers et al. 1999; Bennett et al. 2001; Rogers, unpubl.). The objectives of this study are to determine if attachment of *E. coli* onto quartz surfaces is enhanced in the presence of Ca²⁺, reversible, and therefore capable of being incorporated into Donnan and/or DVLO based models of the bacterial electric field to predict attachment in a variety of geological settings.

1.1 Bacteria-Mineral Surface Complexation Models

Significant advances have been made in the development and incorporation of bacteria (e.g. *B. subtilis* and *E. Coli*) into surface complexation models (Fein et al. 1997; Fowle & Fein 1999; Martinez & Ferris 2001; Yee et al. 2003) for metal adsorption. Traditionally these models characterize the reactivity of the bacterial cell wall via acid-base titrations that are utilized to quantify and assign surface functional groups (e.g carboxyl, phosphoryl, amino). The deprotonation of these groups can be then described using equilibria expressions:

$$\text{R-NH}_3^+ \rightleftharpoons \text{R-NH}_2^0 + \text{H}^+ \quad (1)$$
R-COOH ⇔ R-COO⁻ + H⁺ (2)

R-PO₃H⁻ ⇔ R-PO₃⁻ + H⁺ (3)

where R denotes the bacterium to which each functional group is attached (pKa values range from 4-9.4). As the pH of the system of interest increases, these functional groups progressively deprotonate leading to an overall negative charge for the bacterial electrical field under near neutral pH conditions.

The surface of quartz mineral grains can also be modeled using surface functional groups (e.g. >SiO⁻, >SiOH₀, >SiOH₂⁻) approach and deprotonation equilibria expressions:

>SiOH₂⁺ ⇔ SiOH₀ + H⁺ (4)

>SiOH₀ ⇔ SiO⁻ + H⁺ (5)

Deprotonation of these silanol sites at low pH values (pHₛₑ𝑐 ≤ 3.0) insures an increasingly negative surface charge for quartz grains as pH increases.

There are currently several competing models that are utilized to describe the attachment of bacteria to mineral surfaces (e.g. SCM, DVLO, Extended DLVO). There is, however, agreement amongst the models that the attachment behavior is controlled by both electrostatic and hydrophobic interactions. Given the experimental conditions used in this study (pH values of interest 4-8.5) we would predict that electrostatic repulsion limits the approach and attachment of E. coli to quartz surfaces. In most groundwater and soil environments, however, it is improbable that the functional groups of both the mineral phases and bacteria would be uncomplexed with the predominance of common aqueous metal constituents (e.g. Ca²⁺, Mg²⁺, K⁺). Furthermore, gram-negative bacteria rely on many of these divalent cations as important structural components for the stability of the cell wall and cross-linking of the lipopolysaccharide layer (Madigan et al. 2002). Therefore one might question whether the interaction between these cations and cell surfaces suppress the negative electrostatic repulsion effects enough that hydrophobic interaction begins to dominate and attachment occurs. A study by Simoni et al. (2000) revealed that in sand packed column based studies of the attached and unattached bacteria via the injection of 60%wt sucrose solution. Modifications of this protocol included measuring OD density at 600 nm rather then 400 nm, and a dry weight based calibration curve. Control experiments exhibited a loss of 5-10 % of the bacteria during the separation procedure. OD density calibration curves generally possessed R² values ranging from 0.998 – 0.999.

2.3 Bacterial Attachment Experiments

Batch attachment studies were conducted as a function of solution pH, Ca²⁺ concentration, quartz:E. coli ratio and ionic strength at 25°C. The method we utilized closely follows that of Yee et al. (2000) which essentially relies on batch reactions with a set mineral:bacteria ratio, an equilibration time of 2 h with end over end agitation, followed by separation of the attached and unattached bacteria via the injection of 60%wt sucrose solution. Modifications of this protocol included measuring OD density at 600 nm rather then 400 nm, and a dry weight based calibration curve. Control experiments exhibited a loss of 5-10 % of the bacteria during the separation procedure. OD density calibration curves generally possessed R² values ranging from 0.998 – 0.999.

2.4 Electrophoretic Mobility Measurements

The electrophoretic mobilities of E. coli and quartz were measured in 0.01 M KNO₃ at 25°C with or without 0.01 M CaNO₃ utilizing a laser-Doppler ve-
locometric device (Zetasizer 3000, Malvern Instruments, UK). The instrument was tested with a single zeta potential transfer standard (silica colloids) of -50 +/- 5 mV. The mobility of *E. coli* cells or quartz grains was determined with cell/quartz suspensions diluted to a solid-solution concentration of 10 mg solid/L (dry wt.). Velocity measurements were made as a function of pH with an applied field strength of 2500 V/m. The pH of the suspension was adjusted via the addition of small aliquots of standardized HNO₃ or KOH of similar ionic strength. The pH of the cell suspension was measured using an Orion PerpHeCT 3in1 pH/ATC probe to ensure no electrode salt dripped into the solution. The average uncertainty of each measurement was less than 5% RSD.

2.5 Scanning Electron Microscopy

*E. coli*-quartz assemblages were prepared for electron microscopy by utilizing a rapid room temperature chemical fixation technique (Nation 1983; Vandevivere & Bevaye 1993). Individual quartz grains were stub-mounted, gold-sputter coated for 90 seconds, then imaged using a LEO 1550 field emission scanning electron microscope operating at 15 kV. Quartz surfaces from experiments with final pHs of 6.77, 7.53, 8.45, and 8.87 were inspected for the presence of attached cells.

3 RESULTS AND DISCUSSION

Figure 1 displays the pH dependant attachment behaviour of *E. coli* in the presence of quartz. Attachment increases with increasing pH ranging from 0% (by mass) at pH 1.5 to an attachment maxima from pH 5 to 8 of 25%. Analytical error on the OD600 measurements is minimal but preliminary control studies indicate 5-10% experimental error. Notable is the adsorption edge attachment behaviour that has not been previously demonstrated for bacteria-quartz interactions.

![Figure 1](image1.png)

Figure 1. *E. coli* (0.05 g/L) attachment onto quartz (10 g/L) as a function of pH in 0.01 M KNO₃ and 0.01 M Ca²⁺ after 2h equilibration time.

Scanning electron microscopy provided a means to image the quartz-*E. coli* associations at high resolution (Figure 2). Although it seems likely that a significant portion of the attached cells were removed during the fixation of the assemblages (e.g. during rinses with buffer solutions) a number of cells remain attached to the quartz surfaces. Cells are 1 to 2 µm-long rods that generally occur in small groups.

![Figure 2](image2.png)

Figure 2. Representative SEM micrograph of *E. coli* attached to the surface of quartz (final pH 7.45). Scale bar is 1 µm.

The electrophoretic mobility of *E. coli* and quartz in 0.01 M KNO₃ and 0.01 M KNO₃ + 0.01 M Ca²⁺ solutions is displayed in Figure 3. The data indicate that in 0.01 M KNO₃ the *E. coli* cells and quartz grains are dominantly electronegative in the pH range studied. The magnitude of the electronegativity increases with increasing pH. The isoelectric point for quartz as interpreted from this data is at approximately pH 2.1. Similarly the isoelectric point for *E. coli* is approximately pH 2.4. At this pH condition, the concentration of negative charged surface sites is equal to the concentration of positively
charged sites, and the net surface charge is equal to zero. As the pH increases from the isoelectric point, the acidic cell wall functional groups and silanol groups progressively deprotonate, generating a net negative charge within the cell wall and on the surface of the quartz grains. The addition of divalent Ca to the system results in little change to the electrophoretic mobility of quartz. The addition of Ca to the mobility study of *E. coli*, however, seems to shield the electronegativity of the bacterial cell wall at the shear plane. The electrophoretic mobility decreases slightly as a function of pH leveling off at ~ -0.7 [(µV)/(s/cm)] compared to ~ -3.3 [(µV)/(s/cm)] in the mobility studies with only KNO₃ present. The mobility trend mirrors the attachment trend in Figure 1 and adsorption edges for Ca²⁺ determined for other bacterial species (Fowle & Fein 2000). Although this data is preliminary we propose that the screening of the negative charge of the bacterial cell wall by incorporation and adsorption of Ca ions may lead to the enhanced pH dependent attachment of *E. coli* onto the surface of quartz.

4 CONCLUSIONS

The results of this study demonstrate that *E. coli* will attach to the surface of quartz in a pH dependent manner. Attachment appears to be enhanced by the screening of the bacterial electric field by calcium ions associated with the cell wall. This microbe-metal-mineral interaction may account for the observed microbial attachment to quartz in a variety of groundwater environments. Because divalent cations are primary geochemical constituents in natural groundwater they must be incorporated into laboratory studies in order to accurately predict microbial attachment under normal field conditions.

Although the authors first met Dr. Ehrlich recently at ISEB 16, in Oirase, Japan, his body of work in the field of Geomicrobiology and his textbook of the same name were constant companions during the course of their dissertations. For introducing us to this wonderful world of microbe-mineral interactions and his lifelong contributions to our field we are truly grateful.

REFERENCES


