# Biomolecular complexation affects microbial adhesion to iron (oxyhydr)oxides

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## Abstract

Interfacial reactions in soil biogeochemical systems are often mediated by microorganisms that become directly attached or closely associated with mineral and organic surfaces. Elucidation of mechanisms of microbe-mineral interactions at the molecular-scale requires the use of *in situ* molecular spectroscopy. We have employed attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy to identify the types of molecules and the nature of bonds that mediate interaction between surficial biomolecules and mineral surface functional groups for a variety of systems of interest. This paper reviews our recent research on biomolecule-iron oxide interactions, with a particular emphasis on adhesion of extracellular polymeric substances (EPS), bacterial cells (*Pseudomonas aeruginosa*, *Bacillus subtilis*), and pathogenic protozoa (*Cryptosporidium parvum*). Evidence for direct, inner-sphere complexation of biomolecular functional groups is discussed, and linkages are made to macro-scale transport phenomena.

## Key Words

Biogeochemical interfaces, mineral-microbe interaction, ATR-FTIR spectroscopy, biomolecule adsorption

## Introduction

Microbial cells, especially bacteria and protozoa, can be transported over relatively long distances in porous media prior to attaching to mineral or organic surfaces (Hornberger *et al.* 1992; Schafer *et al.* 1998; Redman *et al.* 2004). Bacterial adhesion often involves the coating of substrata and eventually of growing cells with extracellular polymeric substances (EPS), i.e., an incipient biofilm that evolves into a complex micro-environment at the solid-water interface. Because of the diversity of biomolecular and mineral surface structures and functionalities, their mutual interactions have the potential to involve a multitude of biomolecule-surface interactions that can contribute to or diminish cell-surface adhesion. Steric and dispersion forces similar to those that act on bare and polymer-coated surfaces, are superimposed on wide range of localized molecular bonding possibilities (hydrophobic, van der Waals, ion bridging, covalent).

As a result of the predominance of weakly acidic carboxyl, phosphoryl, and hydroxyl functional groups, microbial surfaces tend to exhibit net negative charge over a wide pH range representative of natural waters. Thus, individual microbial cells may be transported over long distances in porous media. This can be beneficial when contaminant remediation requires microbiological augmentation (Scow and Hicks, 2005). Or, it can be detrimental when it involves the transport of pathogenic microbes (e.g., *Cryptosporidium sp.*, *Giardia sp.*, or *Escherichia coli*). Recent history contains several cases where pathogens have been transported in aqueous suspension through porous geomedia and into community water supplies (Curriero *et al.* 2001). Hence, an understanding of adhesion processes is essential. Mean field models, based on Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of reactive particle transport and adhesion to surfaces, do not closely match experimental adhesion measurements because key interaction mechanisms of bio-particles and mineral surfaces are not included among the classical DLVO (van der Waals and electrostatic) forces (Ginn *et al.* 2002). More effective data fits for *E. coli* adhesion to silica, for example, were made when models included a steric term to account for the “polymer brush” surface of microbial cells (Camesano and Logan, 2000). Toward the goal of better predicting adhesion processes, our work has focused on developing an understanding of molecular-scale bonding interactions using *in situ* spectroscopy of these biogeochemical interfaces.

## Biomolecular Bonding to Mineral Surfaces

In addition to providing the potential for steric effects and/mixing entropy of the polymer brush, surface biomolecules also can enter into direct (e.g., covalent) bonding interactions with hydroxylated mineral surfaces (Parikh and Chorover, 2006). Laboratory and field experiments with microorganisms have shown that ferric oxyhydroxide coatings are important in the removal of microorganisms in saturated porous media, even in the presence of organic matter and phosphate, which can block microorganism attachment (Harvey and Ryan, 2004). As a sorbent for biomolecules, the hydroxylated surfaces of Fe and Al (oxyhydr)oxides differ significantly from the siloxane surfaces of primary silicates and clay minerals. Whereas the basal surfaces of clay minerals tend to effectively sequester aliphatic molecules such as lipids, the hydroxylated surfaces of hydrous oxides exhibit affinity for phosphorylated and carboxylated compounds.

Early adhesion processes likely involve some degree of mineral-surface conditioning film formation by free or cell-bound EPS. Microbial exudates and lysates comprise a mixture of polysaccharides, proteins, nucleic acids and lipids whose relative affinities for mineral surfaces depend on the structure and functional group chemistry of the substrate surface, as well as solution chemistry conditions. Data on the adsorption of EPS from the bacterial strains *Bacillus subtilis* and *Pseudomonas aeruginosa* to the goethite (α-FeOOH) surface are shown in Figure 1. Macroscopic sorption data (depicted as $K_d$ values in left side of Figure 1) indicate that reaction of EPS with goethite favors preferential uptake of P-containing biomolecules relative to bulk C or N (Omoike and Chorover, 2006). One explanation for this organic P-selectivity comes from infrared spectroscopic data (right side of Figure 1) of goethite-EPS complexes indicating the ligand exchange of biomolecular phosphoryl groups at goethite surface hydroxyls (i.e., ≡Fe-OP-R bonding). These infrared spectroscopic results are consistent with ab initio quantum chemical calculations of complexation of phosphodiester groups, such as those associated with nucleic acids, bonded to Fe metal centers (Omoike *et al*. 2004). ATR-FTIR spectroscopic studies of live bacterial-Fe oxide systems also show the formation of ≡Fe-OP-R(s) bonds, suggesting that cell-bound phosphorylated molecules also play an important role in whole cell adhesion to unconditioned oxide surfaces (Parikh and Chorover, 2006).

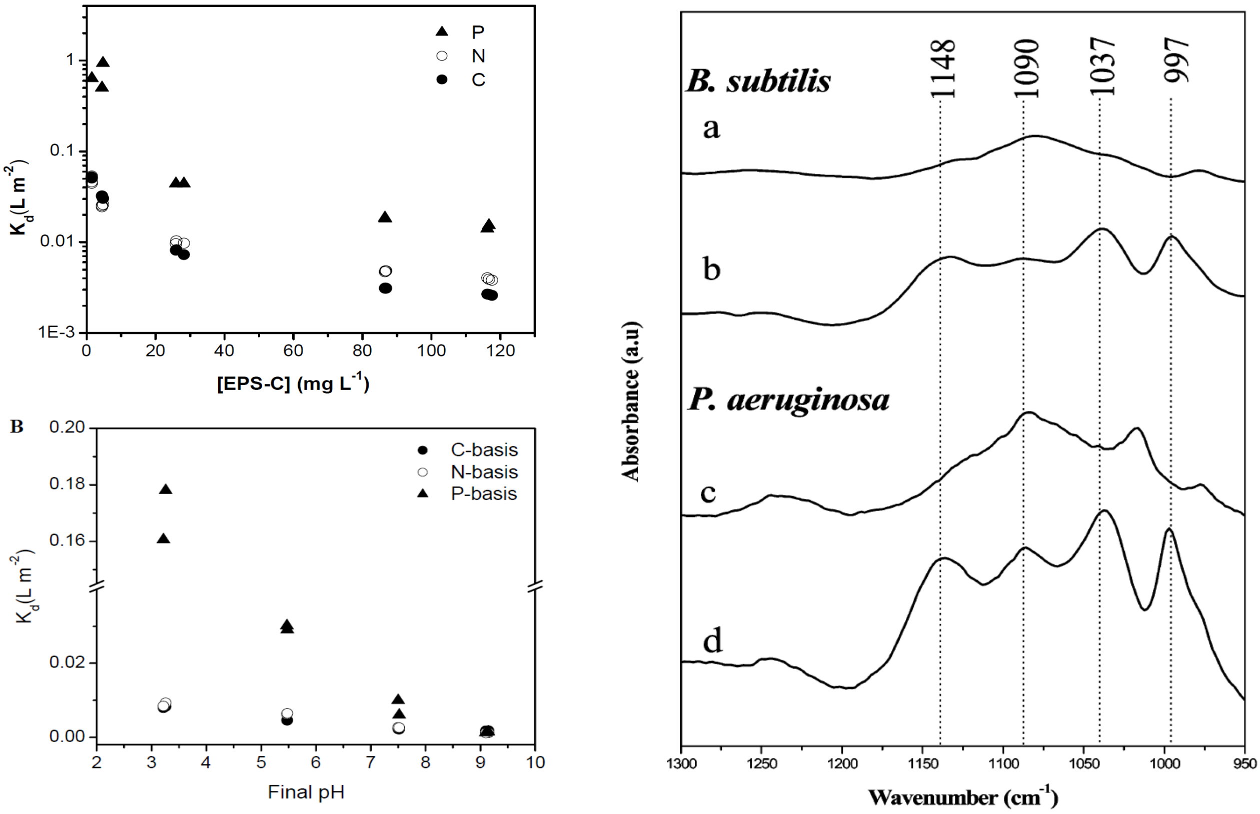

**Figure 1. Left side shows macroscopic data on adsorption of EPS from *Bacillus subtilis* to the surface of α-FeOOH as a function of EPS concentration (carbon basis, top left) and pH (bottom left). Data indicate preferential adsorption of P-containing moieties (Omoike and Chorover, 2006). Right side shows attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of EPS from *B. subtilis* and *Pseudomonas aeruginosa* either free in solution (a, c) or adsorbed to the surface of goethite (b, d). Emergence of new peaks for adsorbed EPS is indicative of formation of inner-sphere PO-Fe bonds as a result of ligand exchange (Omoike et al., 2004).**

The transport of pathogenic microbial contaminants is likewise affected by mineral-microbe bonding interaction. As shown in the left side of Figure 2, the progressive coating of quartz sand surfaces with iron oxyhydroxide results in a significant decrease in *Cryptosporidium parvum* öocyst breakthrough from porous media columns, and an increase in adhesion, as reflected in the collision efficiency parameter, α (Abudalo *et al*. 2005). The right side of Figure 2 shows ATR-FTIR studies of öocyst surface functional group chemistry upon adhesion to uncoated (top) and iron-oxide-coated (bottom) surfaces. The data show that at the pH of the transport experiments, öocysts are adhered to Fe oxide surfaces through inner-sphere complexation of carboxylate groups at Fe(III) metal centers (Gao *et al.* 2009). Hence, in addition to the influence of electrostatic attraction for microbial adhesion to Fe oxide at this pH, direct bonding effects likely also play a role in retarding pathogen transport.

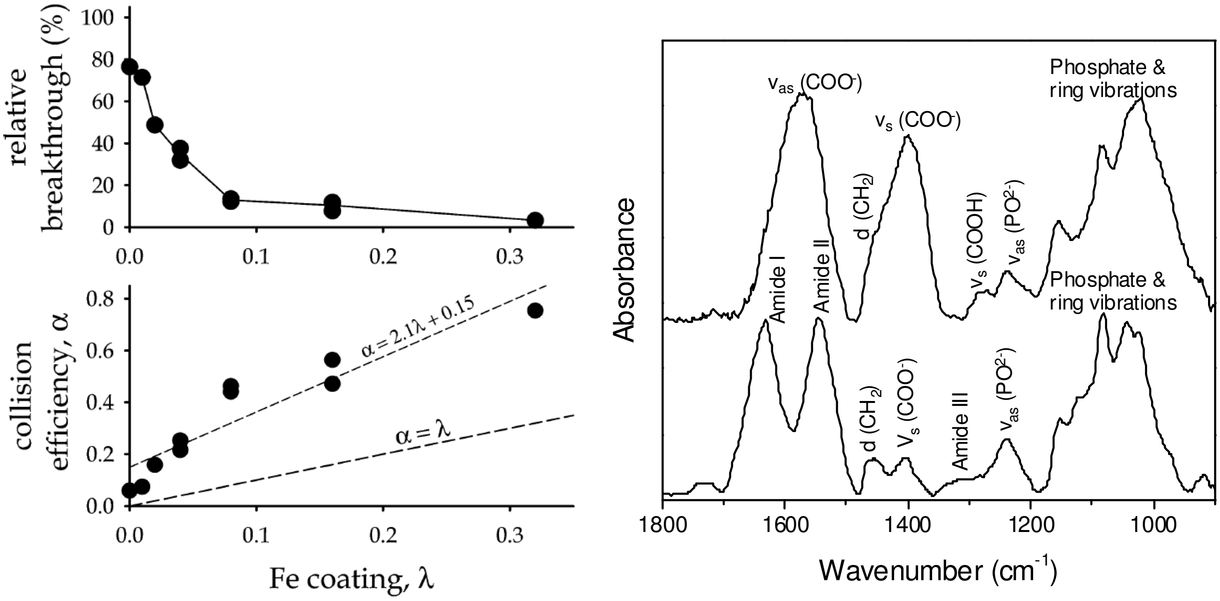

**Figure 2. Macroscopic transport data left side shows that relative breakthrough decreases and collision efficiency increases for *Cryptosporidium parvum* öocysts as a function of the fraction of the Fe(III) oxide surface coating of a quartz sand at pH 5.6-5.8 and 0.1 mM NaCl (Abudalo *et al.* 2005). (Zeta potential of öocysts under these conditions was -10 mV.) Right side uses molecular spectroscopy to depict the mechanism. ATR-FTIR spectra of öocysts adhering to an uncoated (bottom spectrum) and α-$Fe_2O_3$-coated (top spectrum) internal reflection element surface indicates the inner-sphere complexation of öocyst carboxylate groups at hematite Fe(III) metal centers (Gao *et al.* 2009).**

Following initial adhesion to mineral surfaces, further accumulation of cells and EPS can result in the formation of incipient biofilm, where the nature of biopolymeric exudates are driven partly by cell physiological response to the local interfacial environment. Indeed, Geoghegan *et al.* (2008) proposed that "*cell attachment and related cell growth behaviour is mediated by macromolecular physics and chemistry in the interfacial environment. Ecological success depends on the genetic potential to favourably influence the interface through adaptation of the macromolecular structure.*"

## Conclusions

In-situ ATR-FTIR spectroscopy studies indicate that adhesion of microbial cells to iron (oxyhydr)oxide surfaces involves not only mean field interactions, such as electrostatic and van der Waals associations that are incorporated into traditional DLVO theory, but also biomolecule-surface metal bonding interactions. We have observed inner-sphere complexation of phosphoryl and carboxyl groups during adhesion of bacterial cells and *C. parvum* öocysts, respectively, and contend that such interactions play an important role in the formation of the microbe-mineral interface.

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