Depth profiling adsorption of extracellular polymeric substances (EPS) from *Pseudomonas aeruginosa* (PAO1) onto α-Fe_2O_3 using variable angle ATR-FTIR

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Identification of bacteria or EPS–mineral substrate surface bonds as well as adsorptive fractionation processes during conditioning film formation is crucial to understanding and controlling bacterial adhesion. In this study, we extracted and characterized EPS from the growth media of *P. aeruginosa* (PAO1) cultured to the early stationary growth phase. We have then employed a novel use of depth profiling ATR-FTIR spectroscopy to monitor the adsorption of EPS onto a hematite-coated Ge-IRE in situ and in real time. All the adsorption experiments were conducted in 10 mM NaCl at pH 6.0.

FTIR and C-13 NMR spectra of the isolated PAO1 EPS show bands of macromolecules containing heterogeneous functional groups. Depth FTIR profiles for EPS (hematite-free, Fig. 1a) on Ge show no significant spectral change with increasing penetration depth (d_p); however, profiles for EPS contacted with hematite (Fig. 1b) show increase in amide II band intensity (protein) as well as variation in amide I/amide II intensity ratios with d_p. Variation in amide I/amide II intensity ratio is consistent with conformational changes resulting from protein adsorption and/or fractional adsorption of different protein types. Our data also show an increasing time dependent preferential adsorption of proteins relative to polysaccharide (Fig. 1c). Furthermore, spectral characteristics in the region 1250-950 cm^{-1} suggest chemical interaction between Fe centers and phosphate groups in DNA.

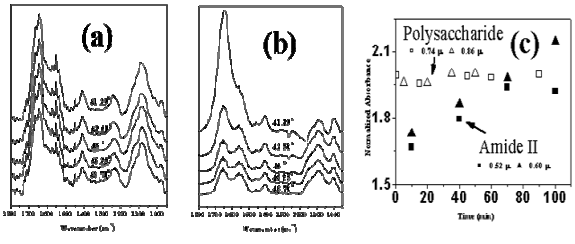

Figure 1. (a) EPS solution on Ge IRE and (b) EPS contacted with α-Fe_2O_3 (region 1800-950 cm^{-1}, 4h contact period) as a function of incident beam angle; and (c) Normalized (using ν_s COO^-) amide II (protein) and polysaccharide absorbances at different d_p as a function of reaction time for EPS adsorption on α- Fe_2O_3.

Bacterial surface charge heterogeneity: Implications for cell-metal/mineral interaction

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Important biogeochemical processes such as bacterial metal adsorption and mineral adhesion are underpinned by the deprotonation of cell wall ligands which impart a negative charge to the cell. Correlating how surface charge mediates these processes often assumes the cell surface charge is evenly distributed. However, the cell surface can be quite heterogeneous, resulting in an uneven distribution of charge which may significantly impact cell surface interactions with dissolved metals and minerals.

Polycationized ferritin (PCF), was used to label the negative sites on the surface of gram positive *Bacillus subtilus*, and gram negative *Shewanella putrefaciens* CN32 bacteria and labelling detected by transmission electron microscopy.

PCF labelling density increased with pH, reflecting an increase in negative charge of the cell surface. However, at undersaturating concentrations, PCF labelling was patchy and heterogeneous, indicating some areas of the cell surface were more electronegative than others. Both organisms also displayed intercellular heterogeneity, indicating some cells were more electronegative than others. This intercellular heterogeneity was found to increase with pH. Interestingly, the polar ends of *B. subtillus* were more electronegative than the lateral walls; a characteristic not displayed by *S. putrefaciens.* This is possibly due to the fluidity of the outer membrane of *S. putrefaciens,* preventing localization of charged ligands. Overall, these results suggest that different regions of the cell surface will interact differently with charged metals and minerals.