Investigating the chemical interactions between *Pseudomonas putida* and hematite using *in situ* flow-cell ATR-FTIR with a hematite-coated Ge crystal

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Hydrophobicity and electrostatic interactions are considered the primary mechanism of bacterial adhesion to mineral surfaces, as they can facilitate a closer approach between the bacteria and the surface so other adhesion interactions can occur. There is new evidence that, although favourable electrostatics contributes to bacterial adhesion, the formation of hydrogen bonds and inner-sphere complexes between a bacterium and a mineral could also play a role. In this work, *in situ* flow-cell ATR-FTIR using a hematite-coated Ge crystal was used to investigate the chemical interactions between *Pseudomonas putida* and hematite. The obtained FTIR spectra were compared to spectra from planktonic cells (not attached to the mineral surface). The symmetric stretching band of carboxylate anions (ν_{sym} COO^-) for the *P. putida* attached to hematite shifted to higher frequencies when compared to the spectra obtained from unattached cells in both H_2O and D_2O suspensions, suggesting that carboxylate groups from macromolecules on the biofilm can be structurally coupled to the atoms on the hematite surface. The spectroscopic data of *P. putida* attached to hematite provided initial evidence that the surface chemistry of bacteria influences cell adhesion at mineral surfaces, and the adhesion of bacteria to solid surfaces may be mediated not only by electrostatic interactions or van der Waals forces, but also by a direct bonding of cell surface macromolecules at the mineral surface functional groups. The use of *in situ* flow cell experiments with a mineral-coated germanium crystal allowed a better description of the bacterial interactions with minerals in real time, as a further step to understand the fundamental mechanisms involved in the relationship between bacteria and mineral surfaces.

Evidence for anaerobic biodegradation of hydrocarbons in the subsurface environment

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Natural attenuation of hydrocarbons in impacted environments is potentially a strategy for site remediation. Biomarkers of anaerobic hydrocarbon degradation can be used to understand the anaerobic biodegradation processes in the subsurface and to evaluate a site for its potential to undergo natural attenuation. The biomarkers include unique metabolites of anaerobic degradation and gene analogues that encode enzymes catalyzing reactions in anaerobic degradation pathways. Groundwater samples from both within and outside the plume of a manufacturing gas plant impacted site were analyzed for the presence of a catabolic gene and metabolic intermediates of anaerobic hydrocarbons degradation. Genes encoding the alpha subunit of benzylsuccinate synthase (*bssA*) were detected in all water samples from within the plume while they were not detected in samples outside the plume. Benzylsuccinate synthase-like enzymes are involved in activation of a suite of hydrocarbons during anaerobic degradation processes, including alkanes, toluene, xylenes, naphthalene and 2-methylnaphthalene. PCR products were also obtained when primers specific for the *bssA* gene in toluene degrading denitrifying or sulfate-reducing bacteria were used suggesting the presence of a diverse community of anaerobic hydrocarbon-utilizing bacteria. GC-MS analysis of solvent extracts of groundwater samples was undertaken to identify the unique metabolic intermediates of anaerobic degradation of PAHs. Metabolites identified from samples from within the plume include 2-naphthoic acid (2-NA), tetrahydro-2-NA, hexahydro-2-NA and methylnaphthoic acid. The highest concentration of these metabolites was detected downstream of the source and within the plume; this was also characterized by low dissolved oxygen and negative oxidation-reduction potential. Collectively, the presence of *bssA* gene along with the specific metabolic intermediates of anaerobic degradation of PAHs within the contaminant plume while generally absent outside the plume, indicate that bacteria capable of anaerobic hydrocarbon degradation are present in the subsurface and are actively degrading the substrates. These data suggest the importance of anaerobic degradation processes *in situ*, at a site, which has been historically impacted with mixed hydrocarbon waste.