Immobilization of Heavy Metals and Radionuclides Through Bio-oxidation of Reducing Environments

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Radionuclides and other contaminating heavy metals are readily adsorbed to ferric oxides present in the environment. However, if the environment becomes reducing due to co-contamination with organic compounds, microbial activity will rapidly cause the reduction of the Fe(III)-oxides with a resulting release of the bound metals. Recent studies in our lab have identified several (per)chlorate-reducing bacteria (ClRB) that can couple the anaerobic oxidation of Fe(II) to the reduction of chlorate or nitrate. Oxidized minus reduced difference spectra of whole-cell suspensions of these organisms indicated the presence of *c*-type cytochrome(s). Difference spectra studies on anaerobic H_2-reduced whole-cells demonstrated that the *c*-type cytochrome(s) are involved in the transfer of electrons onto chlorate or perchlorate by these organisms. Furthermore, difference spectra studies of anaerobic whole-cells in the presence of Fe(II) demonstrated that Fe(II) oxidation was an enzymatic process and was not the result of abiotic reactions with highly oxidized intermediates such as chlorite, which are formed as transient intermediates during the reductive metabolism of (per)chlorate.

When anaerobic sediments from contaminated aquifers or aquatic environments were inoculated with an active culture of one of the ClRB isolates, *Dechlorisoma suillus,* and amended with nitrate, the Fe(II) content of the sediment was rapidly oxidized. Interestingly, if acetate was also added, the rate of Fe(II) oxidation decreased. This was probably due to the stimulation of an indigenous acetate-oxidizing, Fe(III)-reducing community which was reducing the bio-oxidized iron as it was formed. In washed whole cell suspensions of *D. suillus* the Fe(III)-oxides formed precipitate out of solution as a green/orange precipitant. XRD analysis of the precipitant yielded a broad absorbance maximum, which is characteristic of an amorphous structure. Similar XRD diffraction patterns were obtained with fresh, abiotic Fe(III)-oxide precipitants. Characteristic peaks indicative of a crystalline structure began to appear in the XRD spectra as the precipitants aged. Interestingly, the biogenically formed Fe(III)-oxides began to crystallize much sooner than the abiotically formed Fe(III)-oxides and significant absorbance peaks were apparent in the biogenic XRD spectra within two weeks.

Although *D. suillus* could not grow with Fe(II) serving as the sole electron donor, it could oxidize Fe(II) while growing in basal media with acetate as a carbon and additional energy source. Growth was rapid and was directly linked to acetate concentration. However, Fe(II) oxidation continued after growth had ceased as long as there was enough electron accepting capacity in the media. Interestingly, concentrations of Fe(II) greater than 1 mM were inhibitory to growth when *D. suillus* was grown with chlorate as the electron acceptor. With nitrate as the sole electron acceptor, *D. suillus* could grow rapidly and oxidize Fe(II) concentrations as high as 25 mM. In addition, the presence of radionuclides/heavymetals such as uranium or cobalt had no significant effect on the growth of *D. suillus* at concentrations of 100 _M. ICP analyses of the soluble uranium and cobalt concentrations indicated that the soluble metal content rapidly decreased as the Fe(III)-oxides were formed and after complete oxidation of the 10 mM $FeCl_2$ added, 80% of the cobalt was removed from solution.

Our results demonstrate that bio-oxidation of the Fe(II) content of reduced environments by ClRB may offer a novel alternative for the immobilization of heavy metals and radionuclides in impacted environments. Previous studies by our group have demonstrated that these organisms are ubiquitous and we have potentially identified the predominant ClRB in the environment. We have now developed specific molecular probes to the predominant ClRB that can be used to monitor their activity during a remediative strategy.