Geophysical Research Abstracts, Vol. 8, 04261, 2006 SRef-ID: 1607-7962/gra/EGU06-A-04261 © European Geosciences Union 2006



Genome-wide analysis and *In silico* predictions for *Geobacter sulfurreducens* grown under Electron Acceptor limitation: environmental implications in subsurface metal reduction.

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In uranium bioremediation strategies, acetate is added to the subsurface to promote the growth of *Geobacter* species in order to reduce and precipitate the toxic metal. Under these conditions Geobacter species are likely limited by accessibility to Fe(III) oxides, in contrast to the electron donor-limited growth more typically found in the subsurface. In order to better understand differences in the physiology of Geobacter species under terminal electron acceptor (TEA)-limited growth, the physiology of G. sulfurreducens grown under electron-acceptor limiting and electron-donor limiting conditions was compared in chemostat cultures and modeled with a genome-based in silico model. The results demonstrated that under TEA-limited condition G. sulfurreducens shows a higher respiration rate, thus more acetate is directed to metal reduction, which is the desired reaction. The upregulation of the TCA cycle genes predicted by the In silico model under Fe(III)-limitation conditions explained the higher flux of acetate oxidation found and, consequently, a lower use of acetate as carbon source, both factors desired during in situ uranium bioremediation treatments. Gene expression analysis with a whole-genome DNA microarray demonstrated that this increase in electron transfer activity was correlated with the transcriptional upregulation of c-cytochromes genes known to have a key role in metal. The model also predicted an overproduction of hydrogen as electron sink under the low disponibility of TEA. These results demonstrate that the physiological state of *Geobacter* species during *in*

situ uranium bioremediation is likely to favor electron flow to metal reduction, which should enhance rates of bioremediation. The good agreement between predictions of the model and experimental results suggest that genome-based *in silico* models can be a powerful tool for predicting the outcome of potential bioremediation strategies in the subsurface.