Hydrogen as an Indicator to Assess Biological Activity during Trace-Metal Bioremediation

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Trace-metal and/or radionuclide bioremediation schemes require that specific redox conditions be achieved at given zones of an aquifer. Tools are therefore needed to identify the terminal electron acceptor processes (TEAPs) that are being achieved during bioremediation in an aquifer. Dissolved hydrogen (H_2) concentrations have been shown to correlate with specific TEAPs during bioremediation in an aquifer. Theoretical analysis has shown that these steady-state H_2 levels are solely dependent upon the physiological parameters of the hydrogen-consuming microorganisms, with H_2 concentrations increasing as each successive TEAP yields less energy for bacterial growth. The objective of this research is to determine if H_2 can still be used as an indicator of TEAPs during a bioremediation scheme when an organic substrate is injected into the subsurface, and organisms may simultaneously consume H_2 and carbon.

Batch experiments were conducted to study the utilization of acetate and H_2 by *Geobacter sulfurreducens* under iron-reducing conditions when either acetate or H_2 is the sole electron donor, and when both are present and utilized simultaneously. The results indicate that though the Monod kinetic coefficients describing the rate of H_2 utilization under iron-reducing conditions correlate energetically with the coefficients found in previous experiments under methanogenic and sulfate-reducing conditions, conventionally measured growth kinetics do not predict the steady-state H_2 levels typical for each TEAP. In addition, with acetate and H_2 as simultaneous electron donors, there is slight inhibition between the two electron donors for *G. sulfurreducens*, and this can be modeled through competitive inhibition terms in the classic Monod formulation, resulting in slightly higher steady-state H_2 concentrations under steady- state conditions in the presence of acetate. This dual-donor model indicates that the steady-state H_2 concentration in the presence of an organic electron donor is not only dependent on the biokinetic coefficients of the TEAP, but also on the concentration of the organic substrate, and that the H_2 concentration does not start to change very dramatically as long as the organic substrate concentration remains below the half-saturation constant.

Column experiments were performed to measure steady-state H_2 concentrations under iron-reducing conditions using NABIR Field Research Center background soil. Steady-state H_2 concentrations in a flow-through column were higher than observed for iron-reducing conditions in the field, even though evidence suggests that iron reduction was the dominant TEAP in the column. Additional column experiments were performed to determine the effect of iron bioavailability on steady-state H_2 concentrations using the humics analogue, AQDS (9,10-anthraquinone-2,6-disulfonic acid). Slightly lower steady-state H_2 levels were observed in the presence of AQDS, indicating that even though iron reduction does occur, a decreased bioavailability of iron may inhibit iron reduction such that H_2 concentrations increase to levels that are more typical for less energetically favorable reactions (sulfate-reduction, methanogenesis).