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

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Funding Source: U.S. Department of Energy, NABIR, Award # DE FG02-00ER1263031

(1) Introduction

The design and operation of a trace-metal or radionuclide bioremediation scheme requires that specific redox conditions be achieved at given zones of an aquifer for a pre-determined duration. Tools are therefore needed to identify and quantify the terminal electron accepting processes (TEAPs) that are being achieved during bioremediation in an aquifer, and that this be done at a high spatial resolution. Dissolved hydrogen (H_2) concentrations have been shown to correlate with specific TEAPs during bioremediation in an aquifer (Table 1). Theoretical analysis has shown that these steady-state hydrogen levels are solely dependent upon the physiological parameters of the hydrogen-consuming microorganisms, with hydrogen concentrations increasing as each successive TEAP yields less energy for bacterial growth. The assumptions for this statement may not hold during a bioremediation scheme in which an organic substrate is injected into the subsurface and where organisms may consume hydrogen and carbon simultaneously.

This research examines the effects of simultaneous hydrogen and carbon utilization through obtaining kinetic parameters of both hydrogen and carbon consumption under iron reducing conditions in batch experiments. A dual-donor model was formulated and compared to flow-through column experiments.

Table 1. Typical H_2 concentrations measured for different terminal electron accepting processes (TEAPS).

TEAP	H_2 Concentration (nM)
Methanogenesis	5 - 20
Sulfate Reduction	1 - 4
Iron Reduction	0.1 - 0.8
Nitrate Reduction	< 0.1

Chapelle et al. (1995) Wat. Resour. Res. 31:359-371

(2) Research Objective

In order to better quantify the simultaneous utilization of H_2 and a carbon source and determine the implications on steady-state H_2 concentrations, the following questions were addressed:

- **What are the effects of a carbon source on H_2 consumption?**
- **What are the effects of hydrogen on the carbon source consumption?**
- **Can the dual substrate model predict H_2 concentrations in a continuous flow environment at steady-state?**
- **What is the effect of iron bioavailability on steady state H_2 concentrations?**

(3a) Single and Dual Electron Donor Experiment Model Formulation

Hydrogen consumption by bacteria can be described by equation 1, while growth of hydrogen consuming bacteria can be described by equation 2.

(1) $$\frac{dC_{H_2}}{dt} = q_{max}\frac{C_{H_2}}{K_{SH_2} + C_{H_2}}X$$ (2) $$\frac{dX}{dt} = Y_{cell/H_2}qX - bX$$

q = specific hydrogen uptake rate, q_{max} = maximum rate of hydrogen uptake, K_{SH_2} = hydrogen half-saturation constant, X = biomass conc. of H_2 consuming organisms.
C_{H2} = hydrogen concentration, $Y_{cell/H2}$ = yield coefficient, b = mortality coefficient.

Substituting the growth equation (eq. 2) into the consumption equation (eq. 1) for steady state conditions and solving for C_{H_2} yields equation 3.

(3) $$C_{H_2} = \frac{bK_{SH_2}}{q_{max}Y_{cell/H_2} - b}$$

q_{max} and b are expected to be similar for anaerobic organisms regardless of the TEAP. K_{SH2} and $Y_{cell/H2}$ are dependent upon the amount of energy available to the particular form of respiration. The more energetically favorable the reaction, the lower the K_{SH} value and the higher the $Y_{cell/H2}$. **Therefore, as redox conditions decrease, steady state H_2 concentrations increase (Table 1).** However, the above equation (eq. 3) may not hold during a bioremediation scheme in which an organic substrate is injected into the subsurface and where organisms may consume hydrogen and carbon simultaneously. This dual consumption scenario is described in eqs. 4-6.

(4) $$\frac{dX}{dt} = -Y_{\frac{cell}{H_2}}\frac{dP_{H_2}}{dt} - Y_{\frac{cell}{c}}\frac{dC_c}{dt} - bX$$ **(Biomass Growth)**

(5) $$\frac{dP_{H_2}}{dt} = -\frac{U^{H_2}_{max}}{Y_{\frac{cell}{H_2}}}\frac{P_{H_2}}{K^{H_2}_S + P_{H_2} + K^{C}_{I}C_c}\cdot\frac{C_{Fe^{3+}}}{K^{H_2}_{Fe^{3+}} + C_{Fe^{3+}}}X$$ **(H_2 Consumption)**

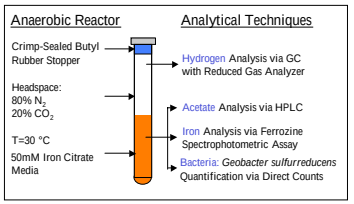
(6) $$\frac{dC_c}{dt} = -\frac{U^{C}_{max}}{Y_{\frac{cell}{c}}}\frac{C_c}{K^{C}_S + C_c + K^{H_2}_{I}P_{H_2}}\cdot\frac{C_{Fe^{3+}}}{K^{C}_{Fe^{3+}} + C_{Fe^{3+}}}X$$ **(Carbon Consumption)**

P_{H_2} = partial pressure of H_2 in headspace
L = Oswalts coefficient for H_2
C_c and C_{Fe3+} = carbon and Fe^{3+} conc., respectively
μ^{H2}_{max} and μ^{C}_{max} = specific H_2 and carbon growth rate, respectively
$K^{H_2}_S$, K^{Fe3+}_{H2} and K^{C}_{S} = half saturation constants for H_2, Fe^{3+} and carbon, respectively
$K^{H_2}_I$ and K^{C}_I = inhibition coefficients

(3b) Single and Dual Electron Donor Experiment – Parameter Estimation

Experimental Design

- **Examined multi-substrate kinetics of simultaneous acetate and H_2 utilization under iron-reducing conditions in anaerobic culture tubes.**
- **Three scenarios were explored:**
  1) **Acetate as sole electron donor**
  2) **Hydrogen as sole electron donor**
  3) **Acetate and hydrogen as dual electron donors.**



Experimental Results

- **Kinetic parameters were determined using Eq. 1-4 and used to model the results of batch experiments (Fig. 1-3).**
- **For these experimental conditions with relatively high acetate concentrations, biomass growth was predicted well using only acetate degradation (biomass produced by H_2 was neglected).**

Iron Reduction Experiments with Acetate

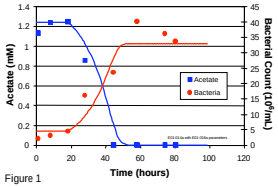


Figure 1

Iron Reduction Experiments with Hydrogen

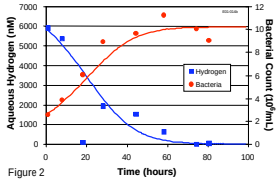


Figure 2

Iron Reduction Experiments with Acetate and Hydrogen

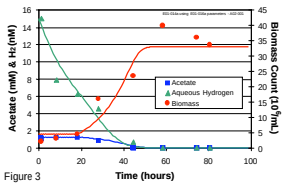


Figure 3

(3c) Single and Dual Electron Donor Experiment Model Results

- **Kinetic parameters for iron reduction agree thermodynamically with previously measured growth kinetics from other TEAPs (Figure 4). Sulfate reduction and methanogenesis kinetics from Robinson and Tiedje *Arch. Microbiol.* 1984, *137*, 26-32.**
- **Results from the model analysis using just the single donor kinetics (H_2 as the sole electron donor) indicate that the calculated steady-state H_2 concentrations are significantly higher than observed in our column experiments as well as field data (Table 2).**
- **Dual donor analysis using eq. 4 (with and without inhibition) indicate that steady-state H_2 concentrations were unaffected by acetate concentrations below acetate's half saturation constant (Figure 5).**

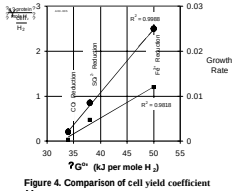


Figure 4. Comparison of cell yield coefficient (•) and first-order growth rate (_) for H_2 degradation as a function of the potential standard free energy for various TEAPs.

Table 2. Steady-state H_2 concentrations with (i) field data from the literature and (ii) H_2 as the sole electron donor (using eq 4 with measured growth kinetics).

	Field Data (Table 1)	H_2 as sole electron donor [b]
Methanogenesis	5 –20 nM	1,305 nM
Sulfate Reduction	1 – 4 nM	90 nM
Iron Reduction	0.1–0.8 nM	52 nM

[b] Using kinetic data for iron reduction (this study),sulfate reduction and methanogensis (Robinson and Tiedje, 1984).

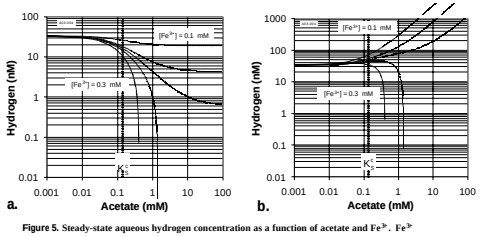


Figure 5. Steady-state aqueous hydrogen concentration as a function of acetate and Fe^{3+}. Fe^{3+} concentrations are 0.3, 0.25, 0.225, 0.2, and 0.1 mM. Curves calculated from Eqn. 4 using coefficients from batch experiments. Figure (a) is with no inhibition between electron donors, and figure (b) uses inhibition coefficients. Dashed line is the Monod half-saturation constant for acetate degradation.

(4) Oak Ridge (FRC-background) Soil Column Experiment

- 30 cm x 5cm column packed with FRC soil
- Phosphate buffered media with 3mM acetate was supplied at 0.5 ml/min to stimulate the indigenous microbial population.

Table 3. Iron conc. (µmol/g soil) after 500 day column operation for different extraction times in 0.5N HCl.

Extraction Time	Fe(II)	Total Iron
1 hour	38.6 ± 8.6	36.1 ± 7.4
22 hour	76.8 ± 12.0	77.2 ± 17.4
21 day	99.5 ± 26.6	230.6 ± 53.1
176 day	96.1 ± 19.7	447.9 ± 88.1

Figure 9. Device used to sample H_2 in column experiments (3.5ml).

Ratio of Iron (II) to Total Iron Over Time

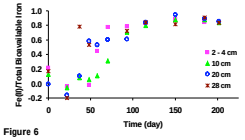


Figure 6

Iron (II) Concentration Over Time

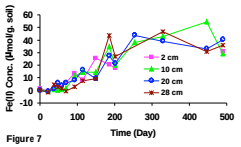


Figure 7

H_2 Concentration Over Time

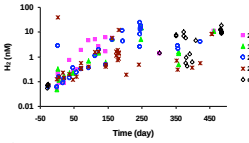


Figure 8

Experimental Summary

- **Bioavailable (1hr 0.5N HCl-extractable) Fe(II)/total bioavailable iron ratios increased over time and leveled off after 100 days (Figure 6), but bioavailable Fe(II) concentrations continue to increase (Figure 7).**
- **Sulfate reduction was observed starting on day 208.**
- **H_2 concentrations typical of iron-reducing processes (0.1-1nM) were observed in the column for the first 75-100 days of the experiment after which the H_2 fluctuated over a substantial range (Figure 8).**

(5) Effect of Iron Bioavailability on H_2 Conc.

Without AQDS (after 90 days) | 50 µM AQDS (after 90 days)

- 15cm x 1cm columns packed with FRC soil.
- Phosphate buffered media with 3mM acetate (Q = 0.5 ml/min).
- Both columns inoculated with *G sulfurreducens*.
- One column supplied 50µM AQDS.

Table 4. Fe(II) concentrations after 96 day column operation for different extraction times in 0.5N HCl.

Extraction Time	Fe(II)[1] in Column without AQDS	Fe(II)[1] in Column with AQDS
1 hour	15.2 ± 1.6	33.8 ± 3.9
6 day	30.8 ± 8.0	66.8 ± 8.0
50 day	42.0 ± 3.8	64.6 ± 4.6

[1] µmol/g soil

Experimental Summary

- **The addition of the electron shuttle AQDS was essential to obtain measurable iron reduction in batch experiments using unsaturated FRC soil over a 35 day period (Figure 10).**
- **In column experiments, the addition of AQDS increased the rate of iron reduction from 0.16 to 0.35 µmol/g soil/d as well as slightly decreased the H_2 concentrations (Figure 11).**

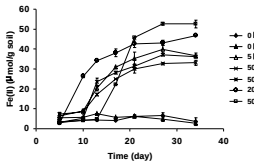


Figure 10. Batch experiments with 1g of unsaturated FRC soil and 1ml of *G. sulfurreducens* growth culture in 9ml basal media with 0 to 500µM of 9,10-Anthraquinone-2,6-Disulfonic Acid (AQDS). Values are average of duplicate samples taken from the same microcosm (+/- std).

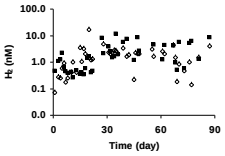


Figure 11. H_2 concentrations over time from the effluent of flow-through column experiments without (_) and with (_) 50 µM AQDS addition.

(6) Summary and Conclusions

- **The presence of acetate did not affect the specific consumption of H_2 and the presence of H_2 did not affect the specific consumption of acetate by *Geobacter sulfurreducens* in batch cultures.**
- **Theoretical analysis shows that at acetate concentrations less than K_S^C, steady-state H_2 levels are not strongly influenced by the presence of acetate.**
- **Steady-state H_2 concentrations calculated using kinetic coefficients from idealized batch conditions differ from H_2 concentrations measured in FRC soil column experiments, as well as in the field.**
- **H_2 concentrations typical of iron-reducing conditions were recorded in a continuous flow column filled with FRC soil during iron reduction by the indigenous microbial population. Sulfate reduction, as well as significant fluctuation in H_2, was measured after the bioavailable Fe(III) was reduced.**
- **Addition of AQDS doubled the rate of iron reduction but only reduced steady-state H_2 concentrations slightly in flow-through FRC soil columns.**

Interesting Side Experiment - Iron Reoxidation

- Column (15cm x 2.5cm) packed with soil from 500 day iron reduction experiment.
- Supplied aerated phosphate buffered media (0.35 ml/min).

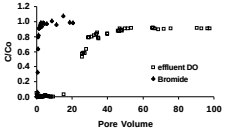


Figure 12. During reoxidation, dissolved oxygen is consumed rapidly at first followed by a slow reaction (long DO trailing, which is not observed for the bromide tracer).

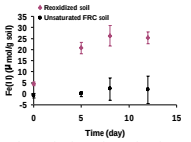


Figure 13. Results from batch experiments show that *G. sulfurreducens* can reduce reoxidized iron much more readily than the iron present in the original FRC soil (ave +- std, n=3)