

Report for 2005MD78B: Theoretical and experimental evaluation of acetate thresholds as a monitoring tool for in situ bioremediation

Publications

- There are no reported publications resulting from this project.

Report Follows

Final Report

Theoretical and experimental evaluation of acetate thresholds as a monitoring tool for in situ bioremediation

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The primary objectives of this project were to demonstrate: (1) that characteristic acetate thresholds exist for different terminal electron accepting processes (TEAPs) and increase as the amount of energy released by the electron acceptor reduction decreases; and (2) the usefulness of acetate thresholds as an indicator of dominant TEAPs in contaminated sediments. The thesis research projects of two M.S. students in the Dept. of Civil and Environmental Engineering, Gayle Davis and Supida Piwkhaw, were supported through the Maryland Water Resources Research Center grant. To date, their work has focused primarily on refining methods for conducting the threshold determination experiments and quantifying acetate at micromolar concentrations, characterizing the dominant TEAPs in contaminated sediments, and characterizing acetate thresholds in contaminated sediments and in pure cultures of acetate-oxidizing bacteria.

Example data that were collected using the protocol that was adopted for the pure culture threshold experiments are shown in Fig. 1. The data in Fig. 1 were obtained by growing *Geobacter metallireducens* strain GS-15 under Fe^{3+} (electron acceptor)-limited conditions. Samples were collected at 4-8 h intervals for the analysis of acetate, iron, and biomass concentrations. Acetate was analyzed using an enzymatic/high performance liquid chromatography (HPLC) method. Fe^{3+} and Fe^{2+} were measured using bipyridine colorimetric method. Biomass levels in terms of volatile suspended solids (VSS) were estimated from protein concentrations measured using a bicinchoninic acid assay. The results indicate that within 25 h, strain GS-15 reduced Fe^{3+} from 28 mM down to a steady-state level of 7.8 mM. During this period, biomass and Fe^{2+} concentrations increased, as expected. Biomass and Fe^{2+} are needed to fit a respiration model to the experimental data. Repeated efforts were made to quantify acetate concentrations in the Fe^{3+} -citrate media used to grow strain GS-15. However, constituents in this and other media used to grow pure acetate-oxidizing cultures appeared to be incompatible with the reactants used in the enzymatic method. Therefore, we are in the process of developing a new ^{14}C -based method for quantifying acetate in the pure cultures. In order to implement this method, our HPLC system had to be reconfigured with several new components, including column switching valves, an ion exchange column, and a fraction collector. Briefly, $[\text{U-}^{14}\text{C}]$ acetate will be added along with unlabeled acetate to pure cultures. Aqueous samples obtained from the cultures will be injected onto the HPLC. During the period that acetate elutes from the system, the effluent will be collected in scintillation cocktail, which will be subsequently counted to determine the acetate concentration. There are several advantages to the $[\text{U-}^{14}\text{C}]$ -method compared with the enzymatic method for quantifying acetate concentrations. First, the acetate detection limit will be much lower when $[\text{U-}^{14}\text{C}]$ acetate is used.

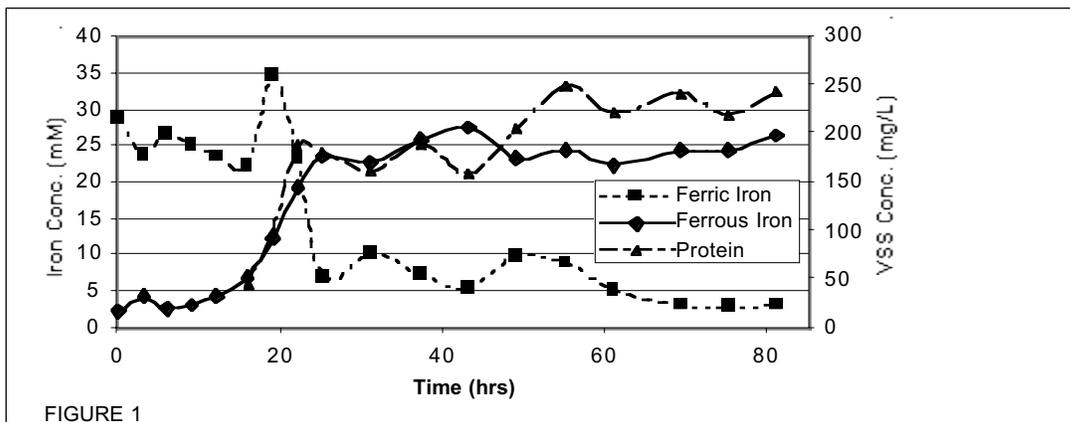


FIGURE 1

Second, $^{14}\text{CO}_2$ and [^{14}C]biomass fractions can also be readily quantified, which will improve our ability to fit model parameters to the experimental data.

The enzymatic method could be used to reliably measure acetate concentrations in natural sediment, provided the pH of the samples and standards was controlled with an organic buffer. It was used to measure acetate thresholds in sediment dominated by different TEAPs. Before threshold experiments involving the sediment could be conducted, the dominant TEAPs in sediments had to be determined. Sediment and groundwater were collected from an Aberdeen Proving Ground (MD) wetland site contaminated with chlorinated volatile organic compounds, including tetrachloroethene (PCE), which can be used as a TEA by some bacteria. After confirming that nitrate, O_2 , and PCE were not present in the groundwater in measurable amounts, the importance of Fe^{3+} -reduction and methanogenesis were evaluated by monitoring $\text{Fe}^{3+}/\text{Fe}^{2+}$ levels and CH_4 production in acetate-amended sediment/groundwater microcosms, respectively. The importance of sulfate reduction was determined by evaluating the effect of molybdate, an inhibitor of sulfate-reducing bacteria, on CH_4 production. CH_4 was produced in the acetate-amended bottles, but greater CH_4 levels were detected in the molybdate-treated bottles. This suggests that sulfate-reduction and methanogenesis were the dominant TEAPs in the sediment. Acetate levels reached threshold concentrations of $10\ \mu\text{M}$ in the molybdate-treated microcosms, but decreased to $4\ \mu\text{M}$ in the microcosms that did not receive molybdate. The amount of free energy released by the reduction of sulfate is greater than that released by the reduction of CO_2 . Thus, the finding that the acetate threshold in the microcosms in which sulfate-reduction was presumably active was lower than in the microcosms dominated by methanogenesis is consistent with theoretical considerations.