Effect of iron bioavailability on dissolved hydrogen concentrations during microbial iron reduction

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Abstract
Dissolved hydrogen (H₂) concentrations have been shown to correlate with specific terminal electron accepting processes (TEAPs) in aquifers. The research presented herein examined the effect of iron bioavailability on H₂ concentrations during iron reduction in flow-through column experiments filled with soil obtained from the uncontaminated background area of the Field Research Center (FRC), Oak Ridge, TN and amended with acetate as the electron donor. The first column experiment measured H₂ concentrations over 500 days of column operation that fluctuated within a substantial range around an average of 3.9 nM. Iron reduction was determined to be the dominant electron accepting process. AQDS (9,10-anthraquinone-2,6-disulfonic acid) was then used to determine if H₂ concentrations during iron reduction were related to iron bioavailability. For this purpose, a 100-day flow-through column experiment was conducted that compared the effect of AQDS on iron reduction and subsequent H₂ concentrations using two columns in parallel. Both columns were packed with FRC soil and inoculated with Geobacter sulfurreducens but only one was supplied with AQDS. The addition of AQDS increased the rate of iron reduction in the flow-through column and slightly decreased the steady-state H₂ concentrations from an average of 4.0 nM for the column without AQDS to 2.0 nM for the column with AQDS. The results of this study therefore show that H₂ can be used as an indicator to monitor rate and bioavailability changes during microbial iron reduction.

Introduction
Iron reduction, along with denitrification, sulfate reduction and methanogenesis, is a very important anaerobic terminal electron accepting process (TEAP) frequently associated with the remediation of subsurface hazardous wastes ranging from the biodegradation of gasoline contaminated sites to the precipitation and subsequent containment of some trace metals and radionuclides. Many biological transformations that are important to achieve during a remediation scheme are specific to a given TEAP. For example, the reduction and subsequent removal of uranium from groundwater has been observed to occur only after nitrate has been reduced and iron reduction has started (Istok et al. 2004). In addition, uranium precipitation from groundwater decreased when sulfate reduction replaced iron reduction as the dominant TEAP (Anderson et al. 2003). It is therefore important to monitor which TEAP is occurring at a particular time and space in order to understand and maximize the bioremediation/biotransformation processes. Dissolved H₂ has been shown to be an indicator of TEAPs in the subsurface (Chapelle et al. 1996, 2002; Lovley et al. 1994) with specific H₂ concentration ranges measured for different TEAPs; nitrate reduction (<0.1 nM), iron...
reduction (0.2–0.8 nM), sulfate reduction (1–4 nM), methanogenesis (5–15 nM). The TEAP yielding the most energy (if that electron acceptor is present) proceeds before the TEAPs yielding less energy because the microbial population for the higher energy-yielding TEAP is able to utilize the H₂ more efficiently, thus driving the H₂ concentration to levels that cannot be utilized by lower energy-yielding TEAPs (Lovley & Goodwin 1988). The above statement assumes that H₂ is the sole electron donor though additional electron donors may be present during the bioremediation of a contaminated aquifer such as the contaminant itself in the case of petroleum hydrocarbons (Chapelle et al. 1996; Lovley et al. 1994) or an electron donor added to stimulate bioremediation of a trace metal or radionuclide (Anderson et al. 2003). Theoretical analysis has shown that steady state H₂ levels during the simultaneous utilization of H₂ and a carbon source as electron donors will remain relatively constant for a wide range of co-electron donor concentrations (Brown et al., in review), thus providing an explanation as to how H₂ concentrations can be used to determine TEAPs in the presence of additional electron donors.

The more energetically favorable TEAP outcompetes less energetically favorable TEAPs. For example, the presence of sulfate (>6–9 mg/l) has been shown to inhibit methanogenesis (Chapelle et al. 1995) and the presence of amorphous Fe(III) has been documented to inhibit both sulfate reduction and methanogenesis (Lovley & Phillips 1987b). Contrary to this, research has shown that iron reduction can occur simultaneously with sulfate reduction (Watson et al. 2003) even though more energy is gained from iron reduction. One possible explanation for both processes to occur simultaneously is that the limited availability of the iron to the iron reducing microorganisms (referred throughout this manuscript as “bioavailability”) decreases the potential rate of iron reduction, thus allowing other less energetically favorable TEAPs to occur. Iron bioavailability has been shown to be a limiting factor of Fe(III) reduction in soils (Chapelle et al. 2002). The extent of bioavailability is dependent on the chemical structure of the Fe(III) oxide; ranging from a crystalline structure (less bioavailable) to an amorphous structure (more bioavailable). Though the total amount of Fe(III) is an important parameter in characterizing the mineralogy of soil, the bioavailable Fe(III) is key in predicting the extent of microbial iron reduction in soil. Therefore the bioavailability of the iron could limit iron reduction and even though iron is present, it cannot be reduced fast enough to outcompete lower energy yielding TEAPs. Electron shuttling compounds such as AQDS (9,10-anthraquinone-2,6-disulfonic acid) have been shown to increase iron bioavailability in soil (Hacherl et al. 2001; Lovley et al. 1996; Nevin & Lovley 2000) by acting as an electron shuttle between iron surfaces and iron reducing microorganisms that are not in direct contact with the surface bound iron, which significantly increases the rate of iron reduction in soils.

It is unknown to what degree changes in iron bioavailability will affect steady-state H₂ levels during iron reduction. Iron bioavailability may change (i) as the more bioavailable fraction of iron is being reduced and less available iron remains, (ii) as Fe(II) sorbs onto the Fe(III) phase, which may block Fe(III) reaction sites, and (iii) as the bioavailability of iron is increased in the presence of AQDS or other electron shuttling compounds. The objective of this study was to determine via flow-through column experiments how changes in iron bioavailability during long-term iron reduction and/or due to the addition of AQDS affect iron reduction rates and subsequent steady-state H₂ levels. The results of this research will increase existing knowledge pertaining to the use of H₂ as an indicator of redox processes, specifically iron reduction, during bioremediation.

Material and methods

Soil composition

The soil used for all experiments was obtained from the Field Research Center (FRC) which is located within the Y-12 Plant area on the Department of Energy’s (DOE’s) Oak Ridge Reservation in Oak Ridge, Tennessee. The soil was collected from the uncontaminated background area at depths between 36” and 72” and stored at 4°C until its use in microcosm or flow-through column experiments.

Bacterial strain

G. sulfurreducens is an obligate anaerobic bacteria capable of iron reduction with acetate or hydrogen