Hydrogen-Based Activity Enhancement in Sediment Cultures and Intact Sediments

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ABSTRACT

The potential for hydrogen gas to stimulate microbial respiratory activity in sediments was investigated. Cell elutions from Passaic River (NJ), San Diego Bay (CA), and Marine Harbor sediments were amended with hydrogen gas to evaluate its impact on microbial activity measured by intracellular reduction of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). The transferability of this approach to sediment slurries and static sediment columns was evaluated based on microbial activity enhancement in Marine Harbor sediments. Results indicate that microbial activity can be increased by a factor of 2–3 at a threshold hydrogen concentration range (0.5 to 1.5 μM). Terminal restriction fragment (T-RF) length polymorphism analysis indicated that the community response to hydrogen resulted in the emergence of previously recessive populations. The causal relationship between hydrogen amendment and an increase in CTC-active cells was most likely due to community structure shifts, as evidenced by the emergence of new T-RFs (19% of total) at hydrogen concentrations above 1.5 μM. No RF was dominant within this emergent group, and no chlororespirers were detected within this group, the latter probably due to the lack of enrichment of halogenated compounds. Nevertheless, the transferability of the observed relationship between hydrogen gas amendment and microbial activity to complex sediment samples suggests a promising remedial strategy for in place contaminated estuarine sediments.

Key words: sediments; dechlorination; hydrogen

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INTRODUCTION

CONTAMINATED SEDIMENT MANAGEMENT represents one of the most challenging contaminated site issues facing freshwater and estuarine coastal environments due to the large volumes to be treated, the wide range of contaminant concentrations, and the need to minimize exposure pathways. Whereas natural processes in anaerobic environments have been demonstrated for many contaminants (Brown *et al.*, 1987; Flanagan and May, 1993; Bedard and Quensen, 1995; Beurskens *et al.*, 1995), the rates are exceedingly slow (e.g., one chlorine removed per 7 to 10 years for both PCBs and dioxins) (Fu *et al.*, 1999). Conventional control technologies for contaminated sediments (e.g., dredging) are limited by high costs and increasingly limited disposal options, such as confined disposal facilities (CDFs). Since natural degradation (e.g., dechlorination) processes are assumed to be slow, enhanced recovery strategies designed to stimulate natural fate pathways may be a promising approach to reduce the human and ecological risks attributed to sediment contamination (Fernandy-van Vlerken, 1998). Whereas in-place sediment management strategies have the potential to provide an alternative to sediment removal, the largely unknown risks associated with leaving contaminants in place and causal relationships have yet to be established (National Research Council, 1997).

Chlorinated aromatic compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDD), chlorobenzenes, and pesticides are persistent, strongly sorbing and bioaccumulative, and their profiles in sediments are reflective of source contributions and reactive transport processes (Adriaens *et al.*, 1999). The hydrophobic nature of these compounds will likely limit their bioavailability (Pignatello, 1998; Schwartz and Scow, 2001). Whereas the success of enhancement strategies have been both compound- and sediment-specific, the scientific understanding of halogenated aromatic dechlorination has evolved from a presumed widespread fortuitous dechlorination activity observed in many reducing environments (Adriaens *et al.*, 1999), to enrichment-dependent regiospecificity in dechlorination patterns (Wu *et al.*, 2000), and even growth-based processes (Bunge *et al.*, 2003). The use of organic electron donors such as aliphatic and aromatic acids is a common technique to supply reducing equivalents for enhanced microbial activity and to stimulate dechlorination of aliphatic and aromatic compounds (Bedard *et al.*, 1995; Adriaens *et al.*, 1999). In reducing environments, this approach results in the production of hydrogen, with reaction stoichiometries dependent on the type of acid electron donor and prevailing microbial community composition (Yang and McCarty, 1998). Hydrogen is central to fermentation processes in anaerobic and reducing lacustrine and marine environments, and plays a central role in the anaerobic metabolism of many physiologies, resulting in discrete equilibrium solution concentrations when different terminal electron accepting processes (TEAPs) are dominant (Chapelle *et al.*, 1996). Moreover, in the last several years, a number of reports have indicated a role for hydrogen during microbially mediated dechlorination of chlorinated aliphatics (Smatlak *et al.*, 1996; Yang and McCarty, 1998; Boyle *et al.*, 1999), and chlorinated aromatic compounds (Kim and Rhee, 1997; Albrecht *et al.*, 1999). For example, Newell *et al.* (1997) reported that hydrogen amendments of aquifer material contaminated with chlorinated solvents resulted in complete dechlorination to ethene, but no mechanistic information or correlations to respiratory activity was inferred. Similarly, hydrogen amendments in freshwater and estuarine sediments contaminated with dioxins resulted in extensive dechlorination of octaCDD to monoCDD at 200-fold accelerated rates, supporting the hypothesis that the beneficial impact from the availability of excess electron equivalents (Albrecht *et al.*, 1999).

Perturbation of complex microbial communities using hydrogen amendments would further be expected to impact community structure and microbial respiratory activity, complicating the causal interpretation of enhanced dechlorination activity (Lendvay *et al.*, 2003). Of the various microbial activity assays available, 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC) has been used most frequently to assess microbiological activity in complex, anaerobic environmental matrices including wastewater, sludge, soil, and sediments (Rodriguez *et al.*, 1992; Winding *et al.*, 1994; Yu *et al.*, 1995; Proctor and Souza, 2001; Gruden *et al.*, 2003b). This dye is favored because it is rapidly reduced by intracellular reductase enzymes to formazan, a fluorescent red precipitate that is easily detected and retains its fluorescence upon storage for up to 2 days (Yu *et al.*, 1995). Effective qualitative monitoring of microbial community structure has been accomplished using terminal restriction fragment length polymorphism (T-RFLP) analysis of amplified bacterial 16S rDNA. This method allows the fingerprinting of a community by analyzing the polymorphism of a certain gene (Liu *et al.*, 1997), therefore overcoming the limitation set by culturing bias when traditional microbiological techniques are applied (Amann *et al.*, 1995). T-RFLP has been successfully applied in the community structure analysis of various environments (Moeseneder *et al.*, 1999; Braker *et al.*, 2001; Avrahami *et al.*, 2002), including contaminated areas and dechlorinating cultures (Flynn *et al.*, 2000; Richardson *et al.*, 2002; Lendvay *et al.*, 2003).

The overarching goal of this work was to demonstrate

activity enhancement of naturally occurring sediment microorganisms in cell elutions, slurries, and sediment columns resulting from hydrogen amendment. The specific objectives were: (1) assessment of sediment microbial activity under mixed conditions as a function of hydrogen fluxes; (2) qualitative comparison of microbial community profiles resulting from varying hydrogen amendment strategies; and (3) demonstration of activity enhancement within a sediment matrix through hydrogen addition in varied conditions from completely mixed slurries to a static sediment column.

EXPERIMENTAL PROTOCOLS

Experimental design

Sediment samples for this research were collected from San Diego Bay (CA), Passaic River (NJ), and Marine Harbor. Table 1 provides descriptive information about each of the sediments. The Marine Harbor site is identified generically at the request of the site owner. The experimental matrices analyzed in this work varied across scale and system complexity. Elution studies separated micro-organisms from sediments prior to studying the effects of hydrogen amendment on microbial activity. This approach simplified the system, and allowed for fast system response with concentrated cells. Activity assays were conducted on elutions from each of the three sediment sites. Dechlorination assays were carried out with cell elutions from Marine Harbor to establish potential applications for this work. Subsequent hydrogen amendments were completed in more complex matrices, slurries, and static columns, to determine the applicability of this technology to field conditions. Slurry studies were performed with diluted Marine Harbor sediments (20%

dry sediment by weight) to allow for complete mixing. Column studies evaluated hydrogen amendment on Marine Harbor sediments in a static column, relying on diffusion to transport dissolved hydrogen through the sediment matrix.

Cell elution experiments

Microbial communities were eluted from historically contaminated sediment cores and suspended in estuarine medium using an established protocol (Barkovskii and Adriaens, 1996). The elution process involved a combination of surfactant addition (1% Tween-80), sonication (5–15 min), and centrifugation ($500 \times g$). Following elution from sediments, micro-organisms were suspended in estuarine medium (40 mM phosphate buffer (KH_2PO_4/Na_2HPO_4; pH = 7.2); 2.0 g/L $NaHCO_3$; 0.9 g/L $(NH_4)_2Cl$; 0.07g/L $CaCl_2 \cdot 6H_2O$; 0.13 g/L $MgSO_4 \cdot 7H_2O$; 0.02 g/L $FeCl_2 \cdot 4H_2O$; Cysteine–HCl; trace minerals; vitamin stock; resazurin). All transfers, enrichments, amendments, and incubations were carried out in an anaerobic chamber. Sediment-derived cultures ($\sim$300 mL) and organic acid cocktail (45 mg/L acetate; 30 mg/L propionate; 15 mg/L butyrate; 10 mg/L benzoate) were dispensed in a SIXFORS fermentation system (ATR Biotech, Maryland). This six-reactor system is equipped with an H_2/N_2 gas delivery system, temperature, mixing (rpm), and pH control. The reactors were amended with varying H_2 fluxes (resulting in 0–4 μM dissolved hydrogen at equilibrium) through a ring sparger to prime cells. For the elution studies, pH remained between 7 and 8, while the temperature was ambient room temperature (22°C), and the reactors were constantly stirred (100 rpm). Preliminary experiments indicated that equilibrium conditions at each concentration, defined by constant dissolved hydrogen and total microbial concen-

Table 1. Sediment characteristics.

Site	Sampling method	Contaminants of interest	Sediment TOC Mean (range)	Site Water salinity (ppt)	References
Passaic River	Vibracore	Dioxins, PCBs, PAHs, DDT/DDE, heavy metals (As, Cd, Cu, Hg, Ni, Pb, Zn)	9% (0.01–40%)	3–14	Barabás Suszkowski
San Diego Bay	Grab sample	PCBs, PAHs, heavy metals (Cr, Cu, Hg Zn)	1.0% (0.2%–2.0%)	33–36	City of San Diego, Port of San Diego
Marine Harbor	Vibracore	Dioxins, PCBs, heavy metals (As, Cr, Zn)	1.2% (0.7–1.7%)	$\sim$35 (estimate)	Limno- Tech, Inc.

trations, were achieved within 3–5 h. Equilibrium hydrogen concentrations were determined using a reduction gas analyzer (Trace Analytical, Sparks, MD).

Activity assays

The metabolic activity of the sediment micro-organisms was determined using the tetrazolium redox dye CTC (5-cyano-2, 3-ditolyl tetrazolium chloride; Polyscience, Inc., Warrington, PA) (ex/em $\lambda = 475/600$ nm) concurrent with a nonspecific DNA stain. The protocol for CTC assays in sediment samples was previously described (Rodriguez *et al.*, 1992; Bhupathiraju *et al.*, 1999a; Proctor and Souza, 2001; Gruden *et al.*, 2003b). The CTC assays were performed using filter-sterilized (0.2 μm) CTC stock solution (20 mM) prepared in ultrapure water and stored at 4°C for a maximum of 2 days (Rodriguez *et al.*, 1992). The appropriate final CTC concentration and incubation time (5 mM and 1 h) for flow cytometric analysis were determined in optimization experiments (Bhupathiraju *et al.*, 1999b; Proctor and Souza, 2001; Gruden *et al.*, 2003a; Gruden *et al.*, 2003b). Total counts of sediment micro-organisms were established using Picogreen (PG) (P-7589; Molecular Probes, Inc., Eugene, OR), a proprietary, high-affinity, green, nucleic acid dye (ex/em $\lambda = 502/523$ nm). Ranges of PG concentrations (5×10^{-4} to 1×10^{-2} of stock) and incubation times (2 min to 4 h) were investigated for determining optimum conditions (5×10^{-3} of stock and 30 min) for enumeration of sediment micro-organisms.

Terminal restriction fragment length polymorphism (T-RFLP)

T-RFLP analysis of cells from Passaic River elution experiments was carried out using a protocol modified from a previously described method (Liu *et al.*, 1997; Flynn *et al.*, 2000). An UltraClean Water DNA Isolation Kit (Cat # 14800-10; MO BIO Laboratories, Inc., Solana Beach, CA) was used for DNA extraction. Bacterial 16S rDNA genes were amplified using PCR primers 8-27F (5′AGAGTTTG-ATCMTGGCTCAG3′) (Giovannoni, 1991) and 1407–1392R (5′ACGGGCGGTGTGTACA3′) (Amann *et al.*, 1995). The forward primer 8–27F was labeled with hexachlorofluorescein (Hex) at the 5′ end (Integrated DNA Technologies, Coralville, IA). The reaction mixture for PCR consisted of 50-ng community DNA, 0.2 μM of each primer, 1× PCR buffer (Promega, Madison, WI), 3 mM MgCl$_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.2 mg/mL bovine serum albumin (New England BioLabs, Beverly, MA), and 2.5 units of Taq DNA polymerase in storage buffer B (Promega). PCR was performed on a Perkin-Elmer 9600 thermocycler (Perkin-Elmer, Norwalk, CT) with the following conditions: 95°C

3 min initial denaturation followed by 30 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 2 min followed by a final extension of 72°C for 7 min. Duplicate PCR reactions for each sample were pooled and concentrated with a Speedvac (Savant Speedvac DNA 120, Thermo Savant, Holbrook, NY) to approximately 40 μL. Gel purification was subsequently performed to remove excess primers, salts, and nonspecific PCR products using a Qiaquick gel extraction kit (Qiagen Inc., Chatsworth, CA) and eluting the band in 50 μL cell culture tested water (Sigma, St. Louis, MO). Aliquots (13 μL) of purified PCR products were incubated for 12 h at 37°C with 10 U of the restriction enzymes *MspI, HaeIII*, and *HhaI* (New England Biolabs) in their corresponding reaction buffers in individual reactions. Restriction enzymes were inactivated by heating to 65°C for 25 min. The inactivated restriction digest was desalted with Microcon Centrifugal Filtration Units (YM-10; Millipore Inc., Bedford, MA). Aliquots (4 μl) were loaded onto an Applied Biosystems PRISM 3100 Genetic Analyzer (Applied Biosystems Instruments, Foster City, CA). The injection time and voltage were 30 s and 3 kV, respectively.

The length of terminal restriction fragments generated was determined by comparison to internal standards using GeneScan® Analysis Software v.2.1 (Applied Biosystems). Peak height was used as measure of abundance of terminal restriction fragments, applying a threshold of 50 fluorescence units. Terminal restriction fragments between 50 and 850 base pairs from different samples were manually aligned using Genotyper® Software v.2.0 (Applied Biosystems). Relative abundance of terminal restriction fragments was determined by dividing individual peak heights by total sample peak height. Similarity between samples was determined by Jaccard's coefficient, which is equal to the ratio of the number of matching terminal restriction fragments to the total number of terminal restriction fragments present in either sample (Blackwood *et al.*, 2003).

Slurry studies

Marine Harbor sediment was mixed with laboratory-prepared estuarine medium containing organic carbon sources in the form of organic acids to form sediment slurry of approximately 20% dry sediment by weight (baseline sample). Approximately 3,300 mL of sediment slurry was created. Sediment slurry (1,650 mL) was divided equally into six reactors and incubated at room temperature on the SIXFORS. The hydrogen-amended slurry and unamended slurry were separately recomposited, thoroughly mixed, and divided into a total of 74 crimptop serumbottles (37 amended and 37 unamended). Each serum bottle, containing approximately 40 mL of slurry

and 30 mL of headspace.The reactors were continuously sparged with 1% hydrogen gas for a period of 4 days to form the hydrogen-amended slurry (enhanced sample). The remaining 1,650 mL of slurry was kept in sealed bottles at room temperature with zero headspace for the 4-day incubation period to form the unamended slurry (unenhanced sample). Microbial activity was measured for each of these three samples.

Column studies

Surface grab samples of Marine Harbor sediments and site water were used for the static column study (6–5/8″ diameter polycarbonate). The column study used a multilayer column set up, with (from bottom to top): 1″ of glass wool saturated with Marine Harbor site water, 4^1/$_2$″ of saturated sediment, ^{1}/$_2$″ layer of site water, and 3″ of headspace. Following column construction, the site water in the lowest layer was replaced with one equal volume of site water bubbled with pure hydrogen gas, to provide the hydrogen supply. This process was repeated after approximately 2 weeks to ensure a sufficient hydrogen supply and a steep hydrogen gradient to drive the diffusion process. Approximately once per week, additional headspace samples were obtained to monitor for hydrogen breakthrough. One month after the initiation of the experiment, the column was opened for sacrificial sampling. The sediment layer was divided vertically into three separate 1^1/$_2$″ thick layers. Triplicate samples of what volume/weight were taken from each layer for ac-

tivity assessment as described earlier in this manuscript. Results from each layer were compared with time-zero results to determine whether the hydrogen, through passive diffusion processes, had impacted microbial activity within the sediment column.

RESULTS AND DISCUSSION

Microbial activity response

Baseline activity assessment of sediment samples indicated that approximately 3–5% of micro-organisms derived from San Diego Bay, Passaic River, and Marine Harbor sediment cores were CTC-active prior to hydrogen amendment. A recent survey (Proctor and Souza, 2001) reported on the percent CTC-active bacteria derived from intact marine sediment core incubations in the range of 9 to 24%. Activity generally increased in anoxic sediments. Van Duyl *et al*. (1999) reported similar results for CTC activity assessment of sediment slurries prepared from surficial (4–20% active) and deeper sediments (up to 30% active) (Van Duyl *et al*., 1999). Dissolved hydrogen amendments (0 to 4 μM) to reactors containing microbial elutions resulted in a statistically significant increase in the percent of CTC active micro-organisms (up to as much as 20%) (Fig. 1). For all three sediments tested, a statistically significant increase in CTC activity was noted above a threshold concentration of approximately 0.5 to 1.5 μM.
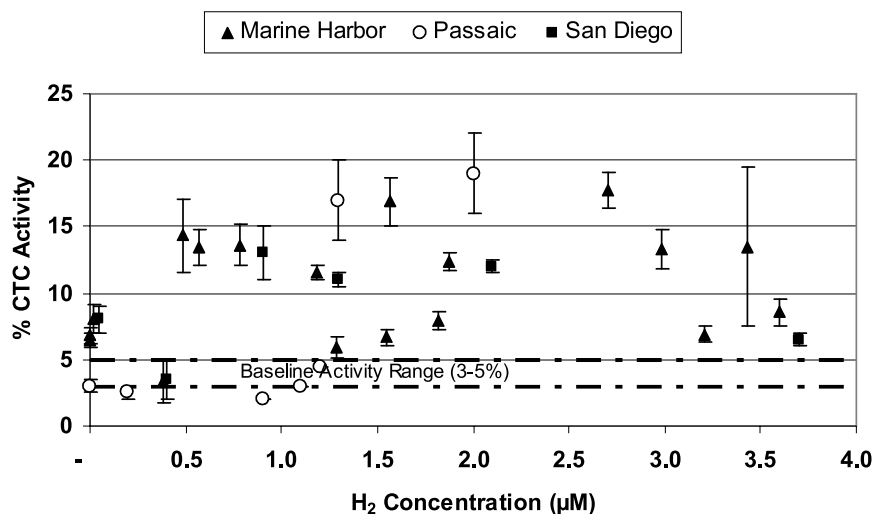

Figure 1. Percent CTC activity in response to dissolved hydrogen concentration for independent experiments using sediments from San Diego Bay (CA), Marine Harbor, and Passaic River (NJ). In each set of experiments, dissolved hydrogen concentrations ranged from 0 to 4 μM. Error bars represent one standard deviation from the mean. In some instances, error bars are too small to be seen.

Since CTC can be reduced by a wide variety of anaerobic micro-organisms (Bhupathiraju *et al.*, 1999b; Proctor and Souza, 2001), CTC activity assessment may serve as an indicator of the increase in bulk activity of the sediment-derived microbial consortia concurrent with an increase in hydrogen flux. Kaprelyants and Kell (1993) reported that CTC was reduced directly by electron transport system-associated dehydrogenase enzymes in *M. luteus*, and Lopez-Amoros *et al.* (1995) demonstrated that CTC could be reduced by NAD(P)H in fixed (formalin) *Escherichia coli* (Kaprelyants and Kell, 1993; Lopez-Amoros *et al.*, 1995). Under anaerobic conditions, CTC was reduced to formazan by a variety of anaerobes during all phases of growth (Bhupathirju *et al.*, 1999b), indicating that intracellular enzymes (e.g., dehydrogenases) present during anaerobic processes (fermentation, nitrate reduction) are capable of reducing CTC. However, further understanding of the anaerobic processes involved in CTC reduction may be complicated, because anaerobic micro-organisms utilize different electron transport system (ETS) enzymes and pathways than their aerobic counterparts.

Our results demonstrated that a two- to threefold increase above baseline sediment microbial activity could be achieved with hydrogen amendment above threshold. In sediments, hydrogen is produced in limited quantities and is subject to competitive metabolic conditions (Chapelle *et al.*, 1996; Hoehler *et al.*, 1998). The hydrogen threshold model describes the syntrophic association between hydrogen-producing and hydrogen-consuming micro-organisms. This concept is based on the assumption that production of hydrogen is limited in sediments, resulting in microbial competition for hydrogen by terminal electron accepting processes, and subsequent redox zonation (Mazur and Jones, 2001). In this work, the microbial competition for hydrogen may have been alleviated through continuous addition and maintenance of a "hydrogen-rich" environment, whereas it is not unusual for concentrations of hydrogen to be between 2 nM and 10 nM in sulfate-rich sediments (Lovley *et al.*, 1994; Yange and McCarty, 1998). However, whereas it is unlikely that hydrogen levels influence TEAPs, they may present a competitive advantage to certain physiologies (Fennell *et al.*, 1997; Fennell and Gosset, 1998).

Microbial community response

The impact of hydrogen amendments on the microbial community profile was evaluated using T-RFLP analysis on samples taken from reactors exposed to no hydrogen, hydrogen concentrations below and above the apparent trigger value of 1.5 μM, as well as the original micro-organisms derived from Passaic River sediments prior to hydrogen amendment. The hypothesis tested is whether hydrogen amendment selectively stimulated archaea, or whether the amendment results in more nonspecific microbial community changes. Three enzymes (*MspI, HhaI, HaeIII*) were used for T-RFLP analysis. Fluorescently labeled PCR amplicons digested with *HhaI* and *HaeIII* resolved fewer ribotypes and were less evenly distributed in size than *MspI*, most likely due to digestion of different bacterial strains resulting in the same T-RF length (data not shown). This is consistent with the results obtained by Liu *et al.* (1997) who, based on simulations, determined that *MspI* and *HhaI* gave the highest number of individual T-RFs when forward primer 8–27F and a different reverse primer (926R) were used. The *MspI* restriction digest produced the greatest number of T-RFs (74) from the original microbial community derived from the Passaic River (NJ) sediments. The samples exposed to no hydrogen resulted in the fewest number of T-RFs (Blackwood *et al.*, 2003), while the sediment micro-organisms exposed to levels of hydrogen <1.5 μM and >1.5 μM resulted in 50 and 56 T-RFs, respectively (Fig. 2A). No microbial species, including archaea, were conclusively identified in the analyzed samples, when the generated T-RFs were compared to the Ribosomal Database Project II 16SrRNA sequences by using TAP T-RFLP (Cole *et al.*, 2003).

Previous reports on the use of T-RFLP to track microbial inocula in groundwater environments indicated the value of this technique to discriminate quantitatively between microbial enhancement strategies by aggregating emergent from previously present T-RFs (Lendvay *et al.*, 2003). However, the sensitivity proved to be inadequate to track the presence of target dechlorinating organisms either in the field, in subcultures derived from dechlorinating enrichments, probably due to their low abundance (Flynn *et al.*, 2000). Further, since multiple bacterial species share the same T-RF and partial restriction products may be formed, this technique is limited to identify specific species (Liu *et al.*, 1997). Here, the T-RFs were plotted as in Lendvay *et al.* (2003) to indicate community shifts as a function of amendment. Figure 2B shows that the storage and manipulation of cores itself or by providing low hydrogen concentrations results in community shifts, resulting in the emergence of T-RFs constituting 20–30% of the total community. However, the application of hydrogen above 1.5 μM led to the appearance of seven additional T-RFs, constituting 19% of the whole microbial community. These organisms were not archaea, but are likely responsible for the increase in bulk microbial activity detected in the
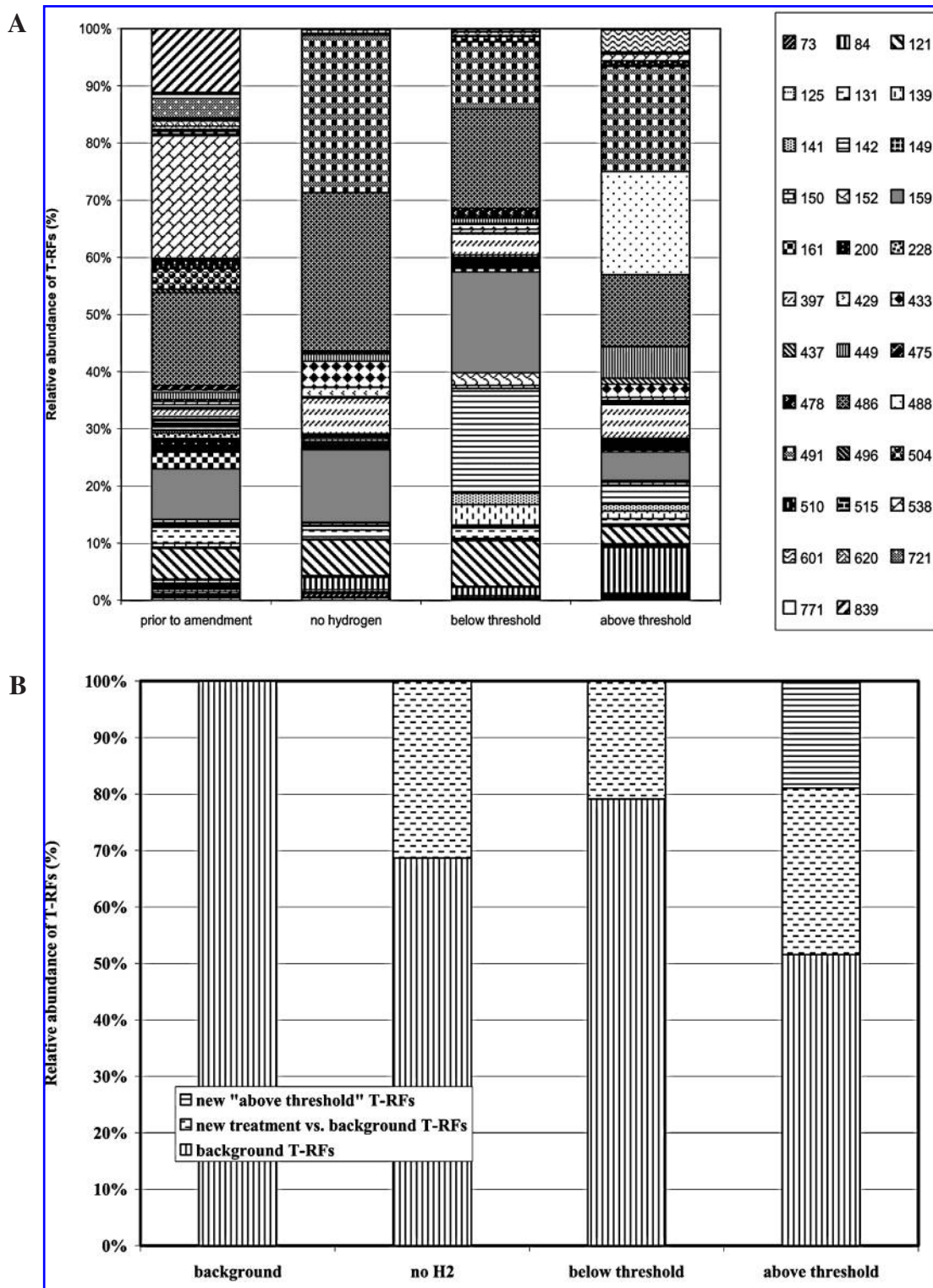
Figure 2. (**A**) Relative abundance of T-RFs (up to 850 bp) generated by *MspI* restriction of amplified 16SrDNAs of *Bacteria* within sediment samples from Passaic River exposed to varying hydrogen amendments. Numbers in the legend indicate length in base pairs of T-RFs with a relative abundance of more than 0.5%. (**B**) Relative abundance of clusters of T-RFs representing T-RFs detected in background sample, new T-RFs detected in samples amended with no or less than 1.5 μM hydrogen, and T-RFs detected exclusively in samples amended with hydrogen concentrations above 1.5 μM.

Table 2. Similarity indices based on T-RFLP community profiles.

Stimulation method:	No hydrogen	Below threshold	Above threshold
Prior to amendment	0.37	0.409	0.461
No hydrogen	—	0.571	0.492
Below threshold	0.571	—	0.606
Above threshold	0.492	0.606	—

sample amended with hydrogen concentrations above 1.5 μM.

Another measure of community comparison is afforded by using similarity indices (Table 2). Based on Jaccard's coefficients, the community profile of the sediment micro-organisms amended with hydrogen greater than the 1.5-μM was not considered similar to the other samples, but demonstrated the highest similarity (Jaccard's coefficient = 0.606) to the sample amended with hydrogen less than 1.5-μM. Similarity indices are statistical methods that are routinely used to compare different microbial communities (as defined by TRFs) among varied treatments (Tiquia *et al*., 2002; Abdo *et al*., 2006).

Transferability to complex matrices

Sediment slurries, using Marine Harbor sediments, were prepared in an effort to increase system complexity while providing complete mixing. Figure 3 shows the percent activity for micro-organisms extracted immediately after slurry preparation (baseline) and after 4-day slurry incubation (enhanced and unenhanced samples). The baseline and unenhanced samples both showed ap-

proximately 5% CTC activity, which is consistent with the baseline activities demonstrated in the cell elution studies (Fig. 1). The enhanced sample showed three times greater activity than the baseline and unenhanced, which is consistent with activity increases observed in the cell elution studies.

In column studies with Marine Harbor sediments, static columns were used to evaluate the applicability of this enhancement approach. During 1 month of incubation, the activity of all column samples increased relative to the time-zero samples (Fig. 4). Activity was highest in samples collected from the bottom 1/3 of the sediment column (nearest the hydrogen source); however, this increase in activity was not statistically significant. Activity in this layer increased to a mean value of 14%. These results are similar to those of both the slurry and the elution studies. Combined results for the top and middle layers show lesser enhancement of activity, consistent with their greater distance from the hydrogen source. The error bars shown in Fig. 4 represent $\pm$ one standard deviation and reflect significant variability among the three samples collected.
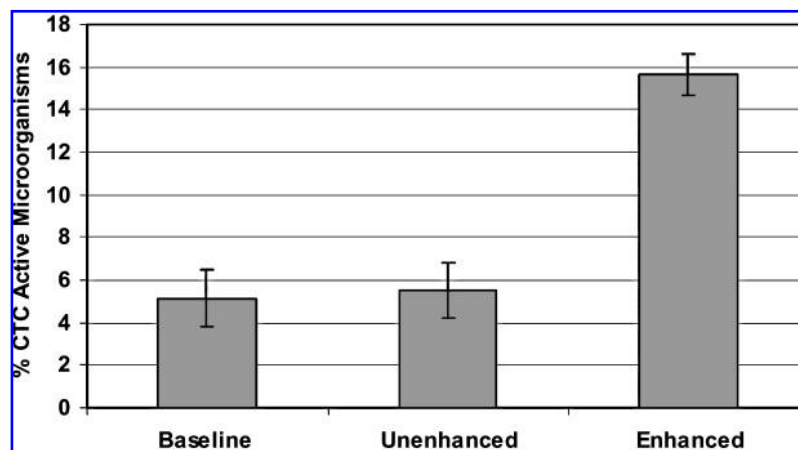
The hydrogen concentrations in the bottom water of

Figure 3. Percent CTC activity in sediment slurries as a function of hydrogen amendment. The background sample was collected prior to incubation. Samples were either unenhanced (no hydrogen) or enhanced (1% hydrogen) for a period of 4 days before this assessment. Error bars represent one standard deviation from the mean.
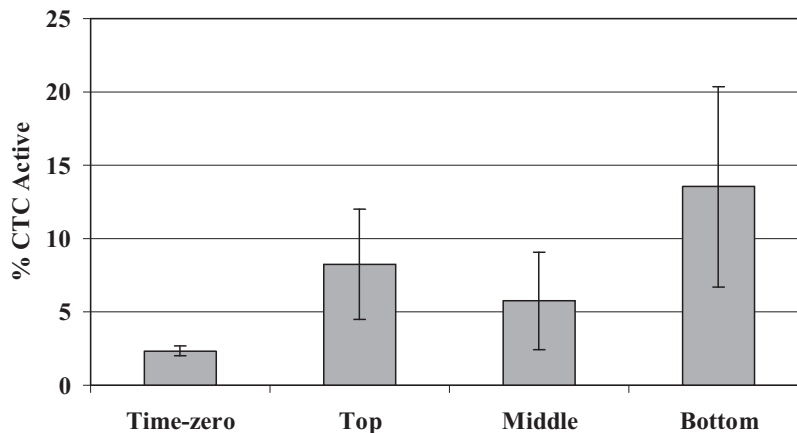
Figure 4. Percent CTC activity in static column studies as a function of location in the column. The bottom 1/3 of the column was proximal to the hydrogen source, whereas the top of the column was furthest from the hydrogen source. These analyses were performed after an incubation of 1 month. Error bars represent one standard deviation from the mean.

the sediment column, ranging from 12 to 20 μM, were roughly 5–10 times higher than those used in the cell elution study. However, the hydrogen concentrations declined quickly, dropping to approximately 100 nM or less within 2 weeks. In addition, the aqueous hydrogen concentration at the top of the sediment column increased over time to a maximum of 16 nM after approximately a month of incubation. This suggests a rate of hydrogen transport within a static sediment matrix of about 0.5 feet per month. However, the maximum concentration of hydrogen observed at the end of the experiment is significantly lower than the potential maximum based on the amount of hydrogen applied to the bottom of the column (12–20 μM hydrogen). The potential maximum is based on the available hydrogen supply. For example, if the initial hydrogen concentration of 12,000 nM in the bottom 1″ layer were spread evenly in porewater throughout the total 6″ column, the concentration would be at least 2,000 nM. In addition, the hydrogen concentration at the top of the column increased only modestly during the 8 days after initial hydrogen breakthrough was observed. These data indicate that hydrogen is used rapidly in these systems as reported in other sediment systems in the literature (Hoehler *et al.*, 2002).

SUMMARY

A screening-level approach aimed at evaluating causal relationships between hydrogen amendments, changes in CTC activity, and concurrent shifts in microbial community profile was presented. Emerging data indicate that amending sediments with dissolved hydrogen may reduce competition between sediment micro-organisms (e.g., sulfate reducers, halorespirers, and methanogens) due to their disparate physiological responses to and affinities for varying hydrogen concentrations. Even though hydrogen amendments influenced community composition (Fig. 2), no mechanistic inferences from the apparent causal relationship between hydrogen levels and the potential for enhanced dechlorination activity can be drawn. Based on the analysis conducted, there was, for example, no evidence of a shift towards chlororespiring populations. Since dechlorination reactions can be cometabolic or linked to respiration (i.e., dehalorespiration), depending upon a variety of factors including prevailing electron-accepting conditions, target compounds, and their relative abundance (Smatlak *et al.*, 1996; Yang and McCarty, 1998; Albrecht *et al.*, 1999; Boyle *et al.*, 1999), the causal relationship would be strengthened if known chlororespiring populations could be detected and tracked in the community. Further studies need to be carried out to evaluate the potential for enhanced dechlorination in complex samples to account for limitations in bioavailability of hydrophobic compounds. Ongoing research endeavors to determine the efficiency of a microbial enhancement technology through hydrogen amendments in sediments with varying geochemistry and contaminant history.

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