

# Electron Transfer from a Solid-State Electrode Assisted by Methyl Viologen Sustains Efficient Microbial Reductive Dechlorination of TCE

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The ability to transfer electrons, via an extracellular path, to solid surfaces is typically exploited by microorganisms which use insoluble electron acceptors, such as iron- or manganese-oxides or inert electrodes in microbial fuel cells. The reverse process, i.e., the use of solid surfaces or electrodes as electron donors in microbial respirations, although largely unexplored, could potentially have important environmental applications, particularly for the removal of oxidized pollutants from contaminated groundwater or waste streams. Here we show, for the first time, that an electrochemical cell with a solid-state electrode polarized at  $-500$  mV (vs standard hydrogen electrode), in combination with a low-potential redox mediator (methyl viologen), can efficiently transfer electrochemical reducing equivalents to microorganisms which respire using chlorinated solvents. By this approach, the reductive transformation of trichloroethene, a toxic yet common groundwater contaminant, to harmless end-products such as ethene and ethane could be performed. Furthermore, using a methyl-viologen-modified electrode we could even demonstrate that dechlorinating bacteria were able to accept reducing equivalents directly from the modified electrode surface. The innovative concept, based on the stimulation of dechlorination reactions through the use of solid-state electrodes (we propose for this process the acronym BEARD: Bio-Electrochemically Assisted Reductive Dechlorination), holds promise for in situ bioremediation of chlorinated-solvent-contaminated groundwater, and has several potential advantages over traditional approaches based on the subsurface injection of organic compounds. The results of this study raise the possibility that immobilization of selected redox mediators may be a general strategy for stimulating and controlling a range of microbial reactions using insoluble electrodes as electron donors.

## Introduction

Trichloroethene (TCE) is a widely used industrial solvent and degreasing agent that has entered and contaminated

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the environment (e.g., soil and groundwater) through leakage from storage tanks and irresponsible disposal practices in the past. TCE is a toxic compound and a suspected human carcinogen, therefore its presence in the environment poses important health risks. At present, the most promising bioremediation approaches for (ground)water polluted by TCE are based on microbial reductive dechlorination, a form of anaerobic respiration in which the chlorinated organic compound is used by bacteria as the terminal electron acceptor in their energy metabolism (1–7). The reductive dechlorination of TCE requires the presence of an electron donor and typically proceeds through the intermediate formation of *cis*-dichloroethylene (*cis*-DCE), vinyl chloride (VC), and finally the nontoxic ethene (8, 9). So far, several organic electron donors, including alcohols, volatile fatty acids, and carbohydrates have been used to stimulate microbial reductive dechlorination reactions both in laboratory-scale and in field-scale studies (10, 11). However, numerous dechlorinating microorganisms use the H<sub>2</sub> produced from the anaerobic fermentation of these organic electron compounds as the actual electron donor for the reduction and dechlorination of TCE in their energy metabolism (12, 13). Interestingly, *Dehalococcoides*, the only microorganism known to fully dechlorinate chloroethenes to ethene, is even restricted to the utilization of H<sub>2</sub> (14–17). At present, current engineered approaches for the bioremediation of chlorinated contaminants (both in situ or in on-site bioreactors) typically involve the addition of H<sub>2</sub> or H<sub>2</sub>-generating organic substrates to stimulate the metabolism of reductive dechlorinating microorganisms (18). Some problems often associated with this approach are the extensive competition for the carbon source and H<sub>2</sub> between dechlorinators and other microorganisms (e.g., sulfate reducers, methanogens, homoacetogens), the accumulation in the subsurface of large amounts of fermentation products with resulting deterioration of groundwater quality, possible aquifer clogging due to excessive biomass growth, and even explosion hazards through excessive methane production (19, 20).

Recently, some authors have proposed the use of bio-electrochemical systems for the reduction of chlorinated compounds or nitrate. As an example, Skadberg and colleagues (21) performed batch studies with a mixed culture in an electrochemical cell and showed that 2,6-dichlorophenol was reductively dechlorinated to 2-chlorophenol when an electric current was passed. Other researchers have employed bio-electrochemical systems for stimulating microbial denitrification (22–24). In all these studies, electrochemically produced H<sub>2</sub> served as the ultimate electron donor in the biological reactions.

Recently, studies by groups led by Zeikus (25, 26), Lovley (27, 28), and others investigated the use of an electrode, and in turn electrical reducing power, as direct electron donor in anaerobic respirations (without intermediate H<sub>2</sub> production). For instance, Park et al. (25, 26) demonstrated that, in the presence of the redox compound neutral red (NR) ( $E^{\circ} = -325$  mV vs standard hydrogen electrode, SHE), electrical reducing power could replace hydrogen as the sole electron donor for growth and metabolism of pure and mixed cultures of H<sub>2</sub>-consuming bacteria in a novel electrochemical bioreactor. More recently, Gregory et al. (28) and Gregory and Lovley (27), demonstrated that members of the *Geobacter* family could directly use electrons from a cathode polarized at  $-300$  mV (vs SHE) to biologically reduce nitrate to nitrite or soluble U(VI) to relatively insoluble U(IV).

Previous studies with *Dehalococcoides ethenogenes* Strain 195 revealed that chemically reduced methyl viologen (MV), could act as an artificial electron donor in the reductive dechlorination of TCE by whole cells (16). Since MV is unable to permeate through lipid bilayers, a periplasmic location for the reductive dehalogenase of this microorganism (or a component feeding electrons to it) was supposed (16).

Based on these previous studies, and taking into consideration the proposed periplasmic location for the reductive dehalogenase of *Dehalococcoides*, in this study we investigated the possibility of using a solid-state electrode to transfer reducing equivalents to dechlorinating microorganisms, thereby stimulating reductive dechlorination reactions, without intermediate H<sub>2</sub> production at the electrode. This approach could potentially overcome some problems associated with the use of fermentable carbon sources and/or molecular hydrogen as electron donors for dechlorination.

## Materials and Methods

**Bio-Electrochemical Cell Setup.** The bio-electrochemical cell used in this study consisted of two gastight borosilicate glass bottles (total volume 300 mL) separated by a 3 cm<sup>2</sup> cross-sectional area, Nafion 117 (DuPont) proton exchange membrane (PEM). The PEM was pretreated by boiling in H<sub>2</sub>O<sub>2</sub> (30%), then in 0.5 M H<sub>2</sub>SO<sub>4</sub>, and finally in DI water, each for 1 h, and then stored in DI water prior to being used. The cathode used was a 20 mm diameter disk of glassy carbon (surface area ~3 cm<sup>2</sup>) and the anode was a 2 cm<sup>2</sup> platinum disk. The electrodes were soaked in DI water prior to being used. The reference electrode (placed in the cathode compartment) was a saturated Ag/AgCl electrode (+200 mV vs SHE) (Amel S.r.l., Milano, Italy). A methyl-viologen-immobilized glassy carbon electrode was prepared as reported in ref 29: an aqueous mediator solution (10 mM MV) and 0.5% (v/v) Nafion ethanol solution were mixed at a ratio of 2:1. The mixed solution (approximately 200 μL) was put on the surface of the glassy-carbon. The solvent was evaporated at room temperature overnight. The so-prepared electrode was soaked in DI water prior to being used.

Electrochemical measurements and monitoring were performed using a Galvanostat/Potentiostat MACCOR 4000 (Kyunggi-Do, Korea). A VMP potentiostat (Bio-Logic, Grenoble, France) was used for cyclic voltammetry experiments which were performed in reduced basal medium (see below) as supporting electrolyte.

**Culture and Medium.** An anaerobic TCE dechlorinating culture (10) was used as the source culture for bio-electrochemical experiments. This mixed culture dechlorinated TCE to predominantly VC and ethene. Fluorescent in situ hybridization analysis (30) indicated that *Dehalococcoides* spp. stably accounted for about one-third of total biomass (27 ± 2.4%, Dhe1259t/DAPI).

The culture was maintained in a 2-L fill-and-draw bioreactor which consisted of a continuously stirred borosilicate glass bottle (liquid volume 1.5 L), sealed by a Teflon-lined gray-butyl stopper (Wheaton, Millville, NJ) and crimped by an aluminum cap. The reactor was fed twice a week with TCE (0.15 mM) and H<sub>2</sub> (1.4 mM) (nominal concentrations, i.e., total mass divided by reactor liquid volume). Before each re-feeding, the reactor was flushed with anaerobic gas (70:30 N<sub>2</sub>/CO<sub>2</sub>) to remove volatile compounds. Once per week, 200 mL of suspended culture was withdrawn (average cell retention time was 52 days) and used in the cathode chamber of the bio-electrochemical cell. Withdrawn liquid volume was replaced by fresh anaerobic basal medium, which contained (g/L): (NH<sub>4</sub>)<sub>2</sub>Cl, 0.5; MgCl<sub>2</sub>·H<sub>2</sub>O, 0.1; K<sub>2</sub>HPO<sub>4</sub>, 0.4; CaCl<sub>2</sub>·H<sub>2</sub>O, 0.05; 10 mL of a trace metal solution (31), and 1 mL of vitamin solution (32). The pH of the medium was ~7.5. The reactor was operated at 25 ± 1 °C.

**Batch Dechlorination Potentiostatic Experiments.** For TCE dechlorination experiments, 200 mL of the source culture was anaerobically transferred into the cathode compartment of the bio-electrochemical cell using a glass syringe, and the anode compartment was filled with 200 mL of reduced medium having the same composition. The two compartments were flushed for 1 h with anaerobic gas (70:30 N<sub>2</sub>/CO<sub>2</sub>). After an equilibration period (1–2 h) the cathode compartment was spiked with the desired volume of TCE and/or a solution of MV, and the cathode was polarized at the desired potential (i.e., –500 mV or –800 mV, vs SHE). At –500 mV vs SHE, MV was electrochemically reduced, according to the one-electron-transfer reaction: MV<sup>2+</sup> + e<sup>–</sup> ⇌ MV<sup>•+</sup> (E<sup>o</sup> = –446 mV vs SHE), but negligible production of H<sub>2</sub> from water electrolysis (E<sup>o</sup> = –442 mV vs SHE, at pH 7.5) occurred. In contrast, at –800 mV vs SHE, significant H<sub>2</sub> production was typically observed. Notably, MV may also undergo a second, one-electron, transfer reaction MV<sup>•+</sup> + e<sup>–</sup> ⇌ MV<sup>0</sup> (E<sup>o</sup> = –760 mV vs SHE). At regular intervals, gaseous samples (100–500 μL) were removed from the headspace of the compartments, using gastight, sample-lock Hamilton (Reno, NV) syringes and analyzed by gas chromatography for volatile compounds. Liquid samples (1 mL) were removed for acetate and volatile fatty acids analysis. During tests, both compartments were maintained at 25 °C in a water bath under magnetic stirring.

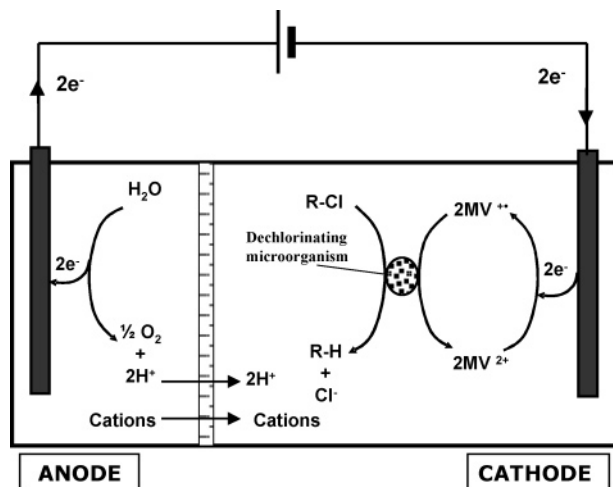
Cumulative electric charge (μeq) that was transferred at the electrodes was calculated by integrating current (A) over the period of electrode polarization. Cumulative reducing equivalents (μeq) that were used for reductive dechlorination were calculated from the measured levels of TCE dechlorination products, considering that 2 μeq/μmol are required for the removal of each chlorine and 2 μeq/μmol are required for the reduction of ethene to ethane. Coulombic efficiency (η<sub>RD</sub> %) for reductive dechlorination was calculated, at any time, as the ratio between the cumulative equivalents used for dechlorination and the cumulative electric charge transferred at the electrode.

**Analytical Methods.** Chlorinated ethenes, ethene, ethane, and methane were analyzed in 250–500 μL gaseous samples, using a gas chromatograph Varian 3400 (Palo Alto, CA) equipped with a flame ionization detector, as described previously (10). Acetate and volatile fatty acids were analyzed, in 1 μL liquid samples, using a gas chromatograph (Perkin-Elmer 8400, Wellesley, MA) equipped with a flame ionization detector and a 2 m × 2 mm glass column packed with Carbograph 1 AL 80/120. Samples were filtered through a 0.2 μm pore diameter membrane and acidified using formic acid (1 M) before analysis. H<sub>2</sub> was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R reduction gas detector (RGD) (Menlo Park, CA) as described elsewhere (33). Headspace concentrations were converted to aqueous-phase concentrations (or total amount of substance per batch) using tabulated Henry's law constants (34, 35). Fluorescent in situ hybridization analysis (FISH) was performed as described in ref 30.

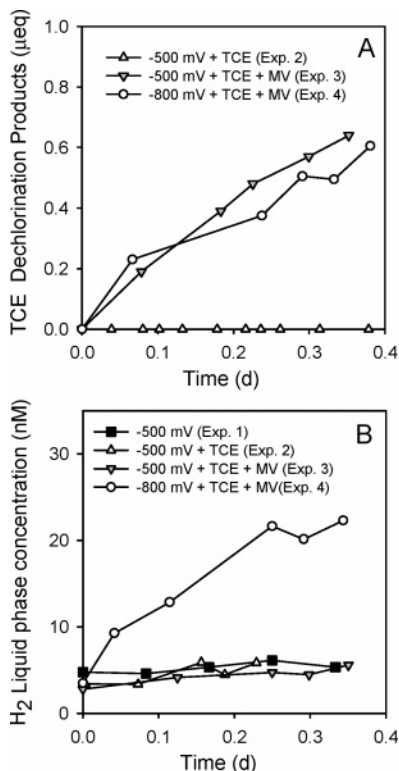
**Chemicals.** Methyl viologen was purchased from Sigma-Aldrich as the dichloride salt and was used as received. Neat TCE (99.5+%) was purchased from Sigma-Aldrich. Vinyl chloride (VC), ethene (ETH), ethane (ETA), hydrogen, and methane gases (99.9+%) were purchased from Scott Specialty Gases (Bellefonte, PA). All the other chemicals used to prepare analytical standards or feed solutions were of analytical grade and were used as received.

## Results

**Microbial Dechlorination of TCE using Electrical Reducing Power and Soluble MV.** Figure 1 shows a schematic of the bio-electrochemical system employed to evaluate the use of



**FIGURE 1. Bio-electrochemically assisted reductive dechlorination. A schematic diagram of the mechanisms by which an electrode polarized at  $-500$  mV vs SHE, in the presence of the redox mediator MV (either in solution or immobilized at the surface of the electrode), can support the microbial reductive dechlorination of TCE.**



**FIGURE 2. Batch dechlorination experiments. (A) Accumulation of TCE reductive dechlorination products in the cathode compartment of the bio-electrochemical cell during batch experiments. (B) H<sub>2</sub> liquid-phase concentration in the batch dechlorination experiments described in panel A, and control experiments. Potentiostatic experiments were carried out on successive days, following the order reported in the legend of the Figure. The cathode was a glassy carbon electrode. All potentials are referred to SHE.**

a glassy carbon electrode, in the presence of a redox mediator, as electron donor for the microbial reductive dechlorination of TCE.

Figure 2A illustrates the cumulative formation of TCE-dechlorination products, in 9-h batch experiments, in the presence of TCE ( $20 \mu\text{mol}$  added) as the electron acceptor, and the glassy carbon electrode as the potential electron donor. When the cathode was maintained at  $-500$  mV (vs

SHE), current flow was as low as  $3\text{--}4 \mu\text{A}$  and negligible formation of TCE dechlorination products was detected, suggesting that almost no direct transfer of electrons to TCE-dechlorinating microorganisms was occurring at the surface of the glassy carbon electrode. In this experiment, H<sub>2</sub> liquid-phase concentration remained nearly constant at  $5.2 \pm 0.6$  nM (mean  $\pm$  standard deviation of data points collected during the 8-h batch experiment) (Figure 2B).

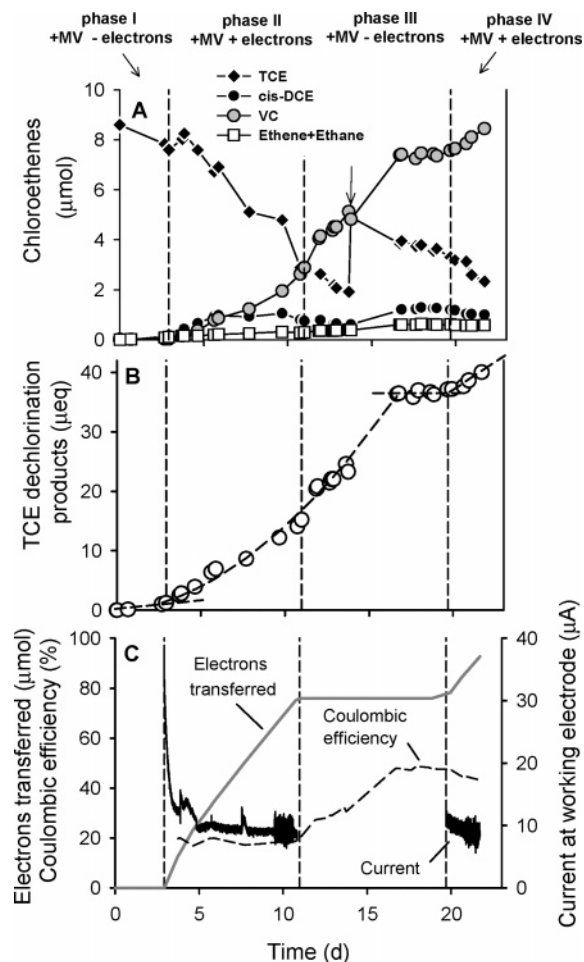
The addition of MV ( $20 \mu\text{mol}$ , corresponding to a concentration of  $100 \mu\text{M}$ ), resulted in an increased current flow ( $15\text{--}20 \mu\text{A}$ ) and TCE dechlorination rate (Figure 2A). Measured TCE dechlorination products in these experiments were typically *cis*-DCE, VC, and, to lesser extent, ETH (Figure S1, Supporting information). Also in this case, the H<sub>2</sub> liquid-phase concentration remained nearly constant at  $4.3 \pm 1.0$  nM (Figure 2B). A similar level of H<sub>2</sub> was also observed in control experiments without TCE and without MV (i.e.,  $4.6 \pm 1.2$  nM) (Figure 2B) and in abiotic tests without microorganisms (data not shown). In abiotic tests, conducted under the same experimental conditions (i.e., cathode polarized at  $-500$  mV vs SHE and  $100 \mu\text{M}$  MV) but without microorganisms, negligible dechlorination occurred (data not shown).

These findings are consistent with MV directly mediating the transfer of electrons from the cathode to dechlorinating microorganisms, without an intermediate H<sub>2</sub> production, according to the mechanism depicted in Figure 1.

The lack of H<sub>2</sub> production at the cathode can be explained by the fact that applied cathode potential was only slightly more reducing ( $-500$  mV vs SHE) than that theoretically needed for H<sub>2</sub> derived from electrolysis of water, i.e.,  $E^\circ = -442$  mV vs SHE, at pH 7.5, and not sufficient to prevail the overpotential at the glassy carbon electrode. Potentiodynamic experiments indicated that water electrolysis required a potential as low as  $-750$  mV vs SHE.

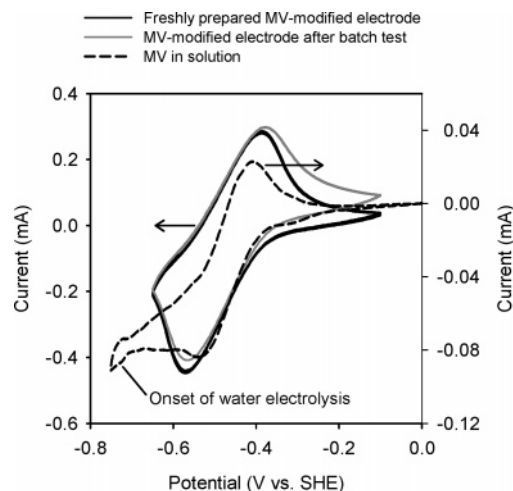
Figure 2B shows the time course of H<sub>2</sub> liquid-phase concentration in a TCE-dechlorination batch experiment with the cathode maintained at  $-800$  mV vs SHE. In spite of the higher H<sub>2</sub> levels, dechlorination proceeded at a rate that was comparable to that observed with MV at  $-500$  mV vs SHE (Figure 2A), indicating that the presence of H<sub>2</sub> had no additional effect in this short-term test. On the other hand, current flow during this test (and accordingly energy consumption) was approximately 5 times higher than that observed without water electrolysis. This finding indicates that the redox mediator allows the reductive dechlorination to occur advantageously at a cathode potential which is significantly lower than that required for H<sub>2</sub> production.

A longer batch TCE-dechlorination experiment (Figure 3) was carried out to verify whether MV could undergo repeated cycles of reduction (at the electrode surface) and oxidation (at microorganism surface) or in other words to carry electrons without irreversibly losing its redox activity. The test was started by spiking the culture with TCE ( $10 \mu\text{mol}$ ) and MV ( $10 \mu\text{mol}$ ) (Figure 3A). At first, no polarization was applied at the cathode. Very little "endogenous" dechlorinating activity was measured during the first 3 days of the experiment (Figure 3A and B, Phase I). On the other hand, as soon as the cathode was polarized at  $-500$  mV vs SHE (Phase II), TCE dechlorination started immediately as indicated by the rapid accumulation of VC and, though at a lesser extent *cis*-DCE, ethene and ethane (Figure 3A). After an initial burst, current flow stabilized at around  $10\text{--}15 \mu\text{A}$ . During phase II Coulombic efficiency for reductive dechlorination remained nearly constant at 20% (Figure 3C). Upon removal of the polarization at the electrode (Phase III), dechlorination continued at a rate that was comparable to or even higher than that observed during previous phase, until day 18, when it suddenly stopped. Such behavior is consistent with the dechlorinating activity being supported, during the period of no polarization (Phase III), by reduced

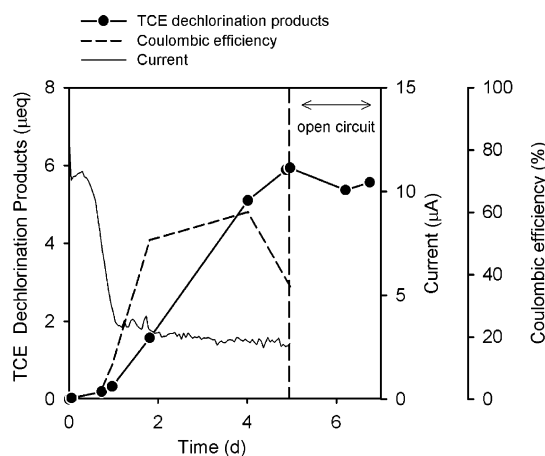


**FIGURE 3. Methyl-viologen-mediated transfer of electrons from the solid-state electrode to dechlorinating microorganisms. (A) Time course of TCE and TCE dechlorination products. (B) Electrons channeled to dechlorination products during different phases of the batch experiment. (C) Current, transferred electric charge, and Coulombic efficiency. At time zero of the test, TCE and MV (10 μmol each) were spiked to the cathode compartment of the bio-electrochemical cell. TCE (10 μmol) was re-spiked on day 13, as indicated by an arrow.**

MV available in solution. As expected, dechlorination ceased when MV was completely reoxidized (on day 18). At that time, around 40 μeq of TCE dechlorination products were formed, divided as follows: *cis*-DCE (14%), VC (81%), sum of ethene and ethane (5%). During phase III no additional electric charge was consumed and, accordingly, Coulombic efficiency for dechlorination (calculated on a cumulative basis) increased (Figure 3C) up to 45%. Interestingly, the reducing equivalents that were channeled to dechlorination during this phase III (~18 μeq) were more than those available from the one-electron oxidation of added MV (i.e., 10 μeq). This fact seems to indicate that, in addition to reduced MV available in solution, other cellular electron carriers or redox enzymes (e.g., NAD<sup>+</sup>, cytochromes) were probably being reduced at -500 mV (vs SHE) (phase II) and could serve as internal electron donors for dechlorination upon removal of the applied potential. Furthermore, it cannot be excluded that during the test a portion of MV<sup>2+</sup> was fully reduced to MV<sup>•</sup>, with the latter being subsequently reoxidized in a two-electron-transfer reaction. Anyway, when on day 20 the potential was applied again (Phase IV), dechlorinating activity immediately resumed, confirming that MV and electric charge were both needed for the reductive dechlorination: the electric charge as the electron donor and MV as the mediator of the electron-transfer process. Interestingly, very little



**FIGURE 4. Cyclic voltammograms of MV and MV-modified electrode. All the voltammograms were measured in reduced basal medium at the scan rate 20 mV/s.**



**FIGURE 5. Microbial reductive dechlorination of TCE using a MV-modified glassy carbon electrode polarized at -500 mV vs SHE as the sole electron donor. On day 5, the electrode was temporarily polarized at -300 mV vs SHE to reoxidize all MV and then the system was stored at open circuit.**

methanogenic activity, accounting for less than 2% of charge transferred, was observed during the test (data not shown).

**Microbial Reductive Dechlorination of TCE using a MV-Modified Electrode.** A modified electrode was prepared by immobilizing MV at the surface of the glassy carbon electrode and cyclic voltammetry experiments were performed to characterize its electrochemical activity. As shown in Figure 4, a pair of redox peaks derived from immobilized MV were observed. At this point, a batch TCE-dechlorination experiment was performed to evaluate the ability of dechlorinating microorganisms to use reducing equivalents potentially available at the surface of the MV-modified glassy carbon electrode (Figure 5). TCE dechlorination started immediately, thereby demonstrating for the first time, heterogeneous-phase, extracellular electron transfer to dechlorinating microorganisms. After the first day of the test, current stabilized at around 2–3 μA, with a corresponding Coulombic efficiency which ranged between 35% and 60% (Figure 5). On day 5, the electrode was temporarily (1 h) polarized at -300 mV (vs SHE) to reoxidize all MV and then the system was stored at open circuit. Thereafter, no further dechlorination was observed. This provided further evidence that MV, in its oxidized state, cannot support reductive dechlorination. The time course of measured dechlorination

intermediates during this experiment is shown in Figure S2 (Supporting Information). Cyclic voltammetry experiments (Figure 4), carried out at the end of this test, indicated an unmodified redox activity of the electrode, indicating that negligible dissolution of immobilized MV occurred during the test.

## Discussion

In this study we demonstrated that a carbon electrode polarized at  $-500$  mV (vs SHE), in combination with the low-potential redox mediator methyl viologen (MV), is a bio-electrochemical system that can be used to transfer electrochemical reducing equivalents to dechlorinating microorganisms. By this approach, the reductive dechlorination of TCE to harmless end-products such as ethene and ethane could be performed. Using a methyl-viologen-modified electrode we could demonstrate that dechlorinating bacteria (probably *Dehalococcoides* spp.) were able to use electrons directly from the electrode surface.

Although the direct involvement of *Dehalococcoides* spp. in the electron-transfer process could not be proved in this mixed-culture study, it is reasonable to suppose that this microorganism was actually responsible for the observed bio-electrochemically assisted dechlorinating activity. This hypothesis is supported by the high percentage of this microorganism in the mixed culture ( $\sim 30\%$ ) and the observed formation (although at low levels) of ethene from TCE, which has been reported, so far, only for members of *Dehalococcoides* group (9, 36). The potential formation of non-chlorinated harmless end-products makes the process attractive for in situ treatment of TCE-contaminated groundwater.

Process efficiency, defined as the ratio between electrons channeled to dechlorination and electrons consumed at the working electrode was quite high and typically in the range 20–60%. In the bio-electrochemical cell used, water oxidation was the main anodic reaction. This reaction produced oxygen which could diffuse into the cathodic chamber and be reduced at the cathode, thereby reducing Coulombic efficiency for dechlorination. This side reaction probably could be minimized through an improved cell design. In addition to dechlorination, no other metabolic processes were apparently stimulated by electric current in our mixed culture study. Interestingly, methane formation was a minimal sink of electrons in spite of the fact that methanogens were present in the  $H_2$ -fed source culture used for bio-electrochemical experiments. Similarly, homoacetogenic activity or biological  $H_2$ -production were not observed. Methanogens are typically regarded as the main competitors of dechlorinators for  $H_2$  utilization. The lack of significant methane formation confirms that negligible  $H_2$  was produced during the experiments, and suggests that methanogens in our mixed-culture were unable to accept reducing equivalents from the electrode, in the presence of MV. In contrast, sustained methane formation from  $CO_2$  was reported by Park and colleagues (26), in a mixed methanogenic culture, in the presence of electrically reduced neutral red (NR) as the redox mediator. This might indicate that different redox mediators are selective toward different microorganisms, thus offering a strategy to control the competition for electric charge and in turn increasing efficiency. At present, little is known on how general is the ability of microorganisms to accept reducing equivalents from an electrode, in the presence of redox mediators. An improved understanding of these aspects will greatly increase the ability to design and control the process, in terms of kinetics, selectivity, and sustainability.

In addition to competitive biological reactions, successful application of the proposed process requires minimization of abiotic reductions of common (competitive) groundwater electron acceptors. The electrochemical reduction of nitrate ( $NO_3^- + 3H_2O + 5e^- \rightarrow 1/2N_2 + 6OH^-$ ;  $E^\circ = 0.26$  V), sulfate

( $SO_4^{2-} + H_2O + 2e^- \rightarrow SO_3^{2-} + 2OH^-$ ;  $E^\circ = -0.92$  V vs SHE), and carbon dioxide ( $CO_2 + 2H^+ + 2e^- \rightarrow HCOOH$ ;  $E^\circ = -0.2$  V vs SHE) typically requires either highly reducing potentials (37) or expensive catalysts (e.g., platinum-group metals) (38, 39). Accordingly these reactions are not likely to occur on carbon electrodes polarized at  $-500$  mV (vs SHE). Differently,  $Fe^{3+}$  will be probably reduced to  $Fe^{2+}$  under such conditions ( $Fe^{3+} + e^- \rightarrow Fe^{2+}$ ;  $E^\circ = 0.77$  V vs SHE). However, the low solubility of  $Fe^{3+}$ , at typical groundwater pH, would probably reduce or exclude this compound from competition in the process.

Long-term assessment of process viability is another major issue that will need to be addressed in future studies. Particularly, it will be of fundamental importance to investigate the effect of long-term operation on composition and activity of microbial communities which enrich at the cathode, as well as the chemical and biological stability of the redox mediator employed.

In this study, the proof-of-concept of an innovative process based on the use of a solid-state electrode, in the presence of a redox mediator, to stimulate the microbial reductive dechlorination of TCE is presented. We propose for this process the acronym BEARD: Bio-Electrochemically Assisted Reductive Dechlorination. This innovative process has the potential to become the “cleanest” and most efficient way to deliver electrons in the subsurface for reductive dechlorination.

## Acknowledgments

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## Supporting Information Available

Additional figures showing the time course of TCE dechlorination products during batch potentiostatic experiments depicted in Figures 2 and 3. This material is available free of charge via the Internet at <http://pubs.acs.org>

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