

# Trichloroethene Dechlorination and H<sub>2</sub> Evolution Are Alternative Biological Pathways of Electric Charge Utilization by a Dechlorinating Culture in a Bioelectrochemical System

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Recently, the proof-of-principle of an innovative bioelectrochemical process for trichloroethene (TCE) bioremediation was presented. In this newly developed process, a solid-state electrode polarized to  $-450$  mV (vs SHE), in the presence of a low-potential redox mediator (methyl viologen), is employed as an electron donor for the microbial reductive dechlorination of TCE to lower or nonchlorinated end products. In the present study, we investigated the influence of methyl viologen and TCE concentrations on process performance. Using a highly enriched hydrogenotrophic dechlorinating culture, as a source culture in batch experiments, we found that TCE dechlorination and H<sub>2</sub> evolution were the two main biological reactions which were stimulated. The relative contribution of the two reactions appeared to be strongly dependent on the mediator concentration. At the lowest methyl viologen (MV) concentrations (25–750  $\mu$ M), only TCE dechlorination was stimulated, and no H<sub>2</sub> was produced; at higher MV concentrations, both reactions occurred simultaneously, although they showed distinct kinetic features. In batch experiments in which TCE was omitted from the system, the rate of H<sub>2</sub> production was remarkably increased (up to 80 times), suggesting that protons represented an alternative electron sink in the absence of the more energetically favorable TCE. Clearly, optimization of the process for TCE dechlorination requires H<sub>2</sub> evolution to be minimized by, for instance, operating the system at low mediator concentrations, and this can be possibly achieved through proper physical immobilization of the mediator at the electrode surface. On the other hand, the observed bioelectrocatalytic H<sub>2</sub> production occurred at virtually no overpotentials with respect to the thermodynamic 2H<sup>+</sup>/H<sub>2</sub> potential. This finding revealed that the dechlorinating culture employed represented quite an exceptional and previously unrecognized biocatalytic system for H<sub>2</sub> production.

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## Introduction

The ability of microorganisms to “communicate” with solid-state electrodes by exchanging electrons with them, either directly or via redox mediators (1), has recently received considerable attention by the scientific community. This interesting feature is currently being explored as a strategy to produce sustainable electricity (2–6) or H<sub>2</sub> (7–10) from the bacterial oxidation of organic waste materials in microbial fuel cells or to biologically reduce oxidized pollutants in bioremediation systems (11–14). As for the application of bioelectrochemical processes in the field of bioremediation, it was shown that members of the *Geobacter* family biologically reduce nitrate to nitrite or U(VI) to relatively insoluble U(IV) (13, 14) using a graphite cathode polarized to  $-500$  mV (vs Ag/AgCl) as the sole electron donor. In *Geobacter*, the extracellular electron-transfer process is mediated by membrane-bound proteins such as cytochromes or highly conductive pili or nanowires (15, 16). Some other microorganisms cannot directly transfer electrons to solid electrodes but, rather, require a soluble redox mediator, which serves as an electron shuttle between the cells and the electrodes (17, 18). Humic substances, quinones, phenazines, and riboflavin, have been proved to function as exocellular electron mediators (19, 20). As an example, Thrash and colleagues showed that washed cells of *Dechloromonas* and *Azospira* species readily reduced 90 mg/L perchlorate, with a solid-state electrode as an electron donor, with 2,6-anthraquinone disulfonate (AQDS) as a mediator (12); no perchlorate was reduced in the absence of cells or AQDS, or in an open-circuit control.

In a recent paper, we described the proof-of-principle of a novel process named BEARD (i.e., Bio-Electrochemically Assisted Reductive Dechlorination) in which a polarized glassy carbon electrode (i.e., cathode), in the presence of methyl viologen (MV), as a redox mediator, is used as an electron donor for the microbial reductive dechlorination of trichloroethene (TCE), a common groundwater pollutant, to harmless ethene (21). In the BEARD process, the mediator, either dissolved in the bulk liquid or physically “anchored” to the electrode surface, facilitated the shuttling of electrons from the carbon electrode to the dechlorinating bacteria. The proposed process potentially carries several advantages over traditional bioremediation processes for chlorinated solvents which rely on the use of chemicals as electron donors in the bacterial metabolism. Among them are the possibility of continuously monitoring the microbial activity and controlling the supply of electron donors both in terms of current and cathode potential; no chemicals need to be added for electron supply, which omits the need for transport, storage, dosing, post-treatment, and secondary groundwater contamination phenomena (22, 23).

The aim of the present study was to gain a deeper insight into the factors affecting the performance of the BEARD process and the mechanisms of electron transfer in a mixed dechlorinating culture. By investigating the influence of redox mediators and TCE concentration, we discovered that proton reduction to molecular H<sub>2</sub> represents an alternative route of electron utilization.

## Experimental Section

**Source Culture.** An anaerobic TCE dechlorinating culture was used as the source culture in bioelectrochemical batch experiments. The culture was originally enriched from brackish sediments originating from the Venice Lagoon (24). 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-faced gray-butyl stopper (Wheaton, Millville, NJ) and crimped by an aluminum cap. The reactor was fed once per week with TCE (0.75 mmoles) and H<sub>2</sub> (1.64 mmoles). Before each refeeding, the reactor was flushed with anaerobic gas (70:30 N<sub>2</sub>/CO<sub>2</sub>) to remove volatile compounds. On average, 450 mL of suspended culture was weekly removed from the reactor and used in bioelectrochemical batch experiments (the resulting average cell retention time was 20–25 days). The removed volume was weekly replaced by a 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 (25); 1 mL of a vitamin solution (26); and 15 mL of NaHCO<sub>3</sub> (10% w/v). The pH of the medium was 7.5. The reactor was maintained in a water bath at 25 ± 1 °C. After several feeding cycles, the culture could steadily dechlorinate TCE at a maximum rate of 255 ± 19 μeq/day. The spiked TCE was dechlorinated to predominantly *cis*-dichloroethene (cDCE), although lower amounts of vinyl chloride (VC) and ethene (ETH) were also formed. No methane was ever produced in the culture. The steady-state biomass concentration was around 20–25 mg VSS/L. A mass balance of reducing equivalents in the reactor indicated that typically over 90% of the added hydrogen was typically recovered as TCE dechlorination products. A molecular analysis of the culture by fluorescent *in situ* hybridization (FISH) carried out using previously developed probes and protocols (27, 28) indicated that *Desulfitobacterium* sp. (i.e., a known H<sub>2</sub>-utilizing, TCE-to-cDCE dechlorinating bacterium) (29, 30) was the predominant (58.4 ± 5.7% Dsf440/EUB338mix) member of the microbial community. Although in lower percentages (11.8 ± 2.8% Dhe1259t-c/EUB338mix), *Dehalococcoides* spp. was also detected in the reactor. FISH analysis also confirmed the absence of methanogenic populations. Epifluorescence micrographs and additional information on the characterization of the microbial culture by FISH can be found in the Supporting Information. It is worth mentioning that, when the same source culture was operated at a higher cell retention time (52 days), the composition of the community was remarkably different, with *Dehalococcoides* spp. being the dominant member (21).

**Bioelectrochemical Cell Setup.** The bioelectrochemical cell used in this study consisted of two gastight borosilicate glass bottles (total volume 250 mL) separated by a 3 cm<sup>2</sup> cross-sectional area, a 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. Each bottle was equipped with side arms for the sampling of the headspace (total volume 100 mL) and the liquid phase (total volume 150 mL), which ended with Teflon-faced butyl rubber stoppers and aluminum crimps. The cathode we used was a glassy carbon rod (HTW GmbH, Germany; 5 mm diameter, 150 mm length, working surface area ~7 cm<sup>2</sup>), and the anode was a 2 cm<sup>2</sup> platinum disk. The electrodes were repeatedly soaked in DI water prior to being used. The reference electrode (placed in the cathode compartment) was a saturated Ag/AgCl electrode (+199 mV vs SHE; Amel S.r.l., Milano, Italy). Electrochemical measurements and monitoring were performed using a Galvanostat/Potentiostat MACCOR 4000 (Kyunggi-Do, Korea) interfaced to a PC. A VMP potentiostat (Bio-Logic, Grenoble, France) was used for cyclic voltammetry experiments, which were performed in a reduced basal medium (see below) as the supporting electrolyte.

**Batch Dechlorination Experiments.** For bioelectrochemical batch experiments, 150 mL of the source culture was anaerobically transferred into the cathode compartment of the bioelectrochemical cell using a glass syringe. In parallel,

the anode compartment was filled with an identical volume of a mineral medium. Thereafter, the two compartments were flushed with anaerobic gas (70:30 N<sub>2</sub>/CO<sub>2</sub>). After an equilibration period (~1 h), the cathode compartment was spiked with the desired volume of TCE and/or a solution of MV; cyclic voltammograms were recorded to characterize the electrochemical activity of the redox mediator, and then the cathode potential was set to -450 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. Control experiments were also performed in the absence of TCE, or in the absence of the microbial culture, or in the absence of cathode polarization.

In all tests, the bioelectrochemical cell was maintained at 25 °C in a water bath under magnetic stirring. The cumulative electric charge (μeq) that was transferred at the electrodes was calculated by integrating the current (A) over the period of electrode polarization. Cumulative reducing equivalents (μeq) that were used for reductive dechlorination were calculated from the measured amounts of TCE dechlorination products formed, considering that 2 μeq/μmol are required for the removal of each chlorine. Hydrogen formed was also expressed as reducing equivalents (μeq) using 2 μeq/μmol as molar conversion factor.

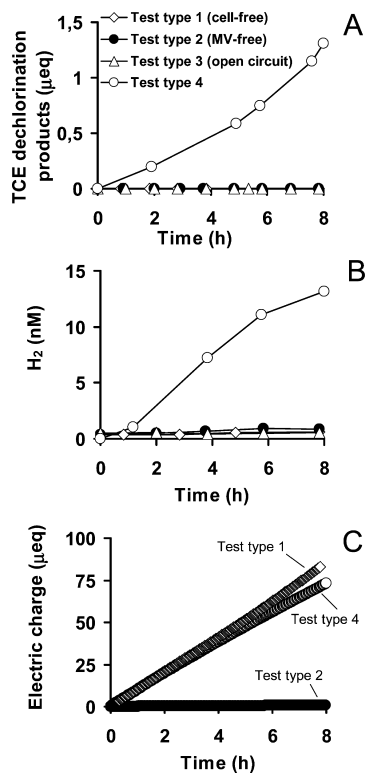
**Analytical Methods.** Volatile compounds (TCE, cDCE, VC, ETH, and methane) were analyzed in 100 μL gaseous samples, using a gas chromatograph Varian 3400 (Palo Alto, CA) equipped with a flame ionization detector, as described previously (31, 32). H<sub>2</sub> was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R reduction gas detector (RGD; Menlo Park, CA). When the H<sub>2</sub> level was above the range of the RGD (i.e., >0.5 μM), it was quantified using a Varian 3400 gas chromatograph and a thermoconductivity detector (32). Headspace, concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants (33, 34).

**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, ethene, ethane, hydrogen, and methane gases (99.9+ %) were purchased from Scott Specialty Gases (Bellefonte, PA). All of the other chemicals used to prepare analytical standards or feed solutions were of analytical grade and were used as received.

## Results

**BEARD at -450 mV (vs SHE) with Dissolved MV.** In order to achieve significant rates of electron transfer between the glassy carbon electrode and the dechlorinating bacteria, the BEARD process required the presence of a low-potential ( $E^{\circ} = -446$  mV vs SHE) redox mediator (i.e., MV). Indeed, as shown in Figure 1, negligible dechlorination of the spiked TCE (15 μmol) occurred, in batch experiments, when either the dechlorinating culture or MV was omitted from the system or when no polarization was applied to the cathode (test types 1–3; Figure 1A). Conversely, an almost linear accumulation of TCE dechlorination products (i.e., *cis*-DCE and lower levels of VC) occurred under typical BEARD conditions (test type 4), that is, in the presence of the dechlorinating culture, MV (500 μM), and cathode polarization to -450 mV (vs SHE). At the end of the test, the amount of produced *cis*-DCE, on a molar basis, was approximately 10 times more than the amount of produced VC.

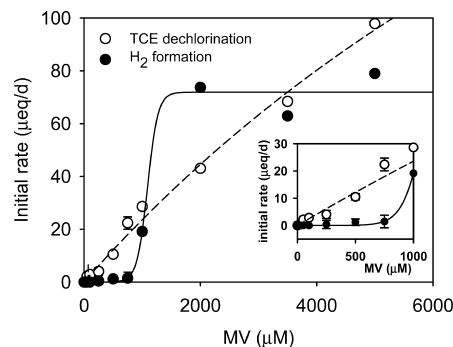
Since H<sub>2</sub> is an important electron donor in the microbial reductive dechlorination of TCE, the H<sub>2</sub> liquid-phase concentration during the above-described tests was monitored and is shown in Figure 1B. In control experiments (test types 1–3), the H<sub>2</sub> concentration remained nearly constant at



**FIGURE 1.** Reducing equivalents channeled to TCE dechlorination (A) and liquid-phase H<sub>2</sub> concentration (B), in batch experiments, and cumulative electric charge transferred (C). Test type 1 (cell-free): abiotic batch experiment with TCE (15 µmoles), MV (500 µmol/L), cathode polarization (−450 mV vs SHE), but without the microbial culture. Test type 2 (MV-free): biotic batch experiment with TCE (15 µmol), the microbial culture; cathode polarization (−450 mV vs SHE), but without MV. Test type 3 (open circuit): biotic batch experiment with TCE (15 µmoles), MV (500 µmol/L), the microbial culture, but without cathode polarization. Test type 4: biotic batch experiment with TCE (15 µmoles), MV (500 µmol/L), cathode polarization (−450 mV vs SHE), and the microbial culture.

around 0.5 nM, confirming that H<sub>2</sub> could not be produced from the abiotic electrochemical proton reduction. Analogously, the endogenous metabolism of the microbial suspension also resulted in a negligible accumulation of H<sub>2</sub>. By contrast, H<sub>2</sub> accumulated at −450 mV (vs SHE), in the presence of the microbial culture and MV (500 µM), even though at a very low rate (0.7 µeq/day) and concentration (~15 nM). This finding proved that electrically reduced methyl viologen could act as a reductant for a biologically mediated reduction of protons to H<sub>2</sub>.

During type 1 and type 4 batch experiments (i.e., in the presence of MV and cathode polarization), the current flowing in the circuit was nearly the same (~250 µA) and remained constant throughout the test, independently of the presence of the microbial culture. Conversely, in the absence of MV (test type 2), the current remained always below 5 µA. Accordingly, the cumulative electric charge transferred during the 8 h of the test was nearly the same for type 1 and type 4 batch experiments, and almost negligible for type 2 batch experiments (Figure 1C). Therefore, under the experimental conditions adopted for test type 4, only a low percentage of the electric charge could be channeled to the slow dechlorination and H<sub>2</sub> production. The steady-state current reached in the presence of MV (regardless of the presence of the microbial culture and dechlorinating activity) was presumably controlled by the rate of the reaction between electrochemically reduced MV with the oxygen generated at the anode (from water oxidation) and diffusing into the cathode



**FIGURE 2.** Influence of MV concentration on the initial rate of TCE dechlorination (open circles) and H<sub>2</sub> formation (filled circles) by the microbial culture in batch experiments at −450 mV (vs SHE). Error bars represent the standard deviation of replicated experiments.

chamber through the Nafion membrane. Under such experimental conditions, we could reasonably assume that the concentration of the reduced form of MV remained nearly constant throughout each test. Thus, since the maximization of current efficiency was not, at this stage, the final aim of these tests, it was considered that the latter condition (constant concentration of the reduced form of MV at steady-state current) was favorable to investigate the role of MV on the different biological reactions.

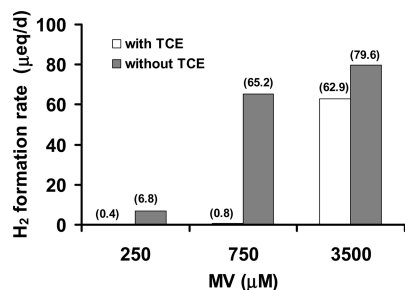
**Influence of MV Concentration on TCE Dechlorination and H<sub>2</sub> Formation.** Figure 2 shows the influence of MV concentration on the initial rate of TCE-dechlorination and H<sub>2</sub> formation by the mixed culture, with the cathode polarized at −450 mV (vs SHE). Each data point in the graph shown in Figure 2 resulted from an independent batch experiment. In the range of MV concentration 25–5000 µM, the initial TCE dechlorination rate increased in nearly direct proportion to MV concentration (Figure 2). In contrast, at MV concentrations of 7500 µM (data not shown), the initial rate of TCE dechlorination (and also H<sub>2</sub> formation) was significantly lower than at 5000 µM, indicating that such a high MV concentration proved toxic to microorganisms or that dimerization reactions of the radical MV<sup>•+</sup> (i.e., the reduced form of MV) resulted in a decreased bulk concentration of the biologically active form of the redox mediator.

Compared to TCE dechlorination, the rate of H<sub>2</sub> formation showed a remarkably different dependency on MV concentration. More specifically, no (or very slow) H<sub>2</sub> production was observed in the range of MV concentrations from 25 to 750 µM; a sharp, 100-fold, increase in the H<sub>2</sub> formation rate was observed in the range of MV concentrations from 750 to 2000 µM; then, at higher MV concentrations, the H<sub>2</sub> formation rate did not increase further (Figure 2).

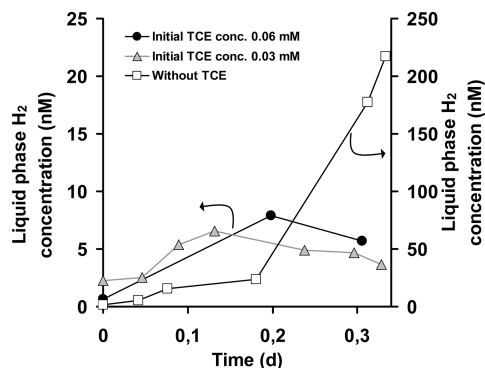
It is worth mentioning that control experiments carried out over the same range of MV concentrations (i.e., 25–5000 µM) confirmed that neither was H<sub>2</sub> produced nor was TCE dechlorinated unless microorganisms, MV, and cathode polarization (at −450 mV vs SHE) were simultaneously present in the system (data not shown).

**Influence of TCE on H<sub>2</sub> Formation.** Batch experiments at three different MV concentrations (i.e., 250, 750, and 3500 µM) were conducted also in the absence of TCE. As shown in Figure 3, also in the absence of TCE, the rate of H<sub>2</sub> formation showed an apparent saturation dependency on the MV concentration: it sharply increased (from 6.8 µeq/day to 65.2 µeq/day) when the added MV increased from 250 to 750 µM, then almost leveled off (at a value of around 80 µeq/day) when the MV concentration was further increased to 3500 µM.

The most striking finding that emerged from the couples of batch experiments with and without TCE, at different MV concentrations, is that the initial rate of H<sub>2</sub> formation in the



**FIGURE 3.** Influence of MV concentration on the initial H<sub>2</sub> formation rate (values are indicated in parentheses) by the microbial culture with or without TCE (15 µmol). All of the experiments were performed with the cathode polarized to -450 mV (vs SHE).



**FIGURE 4.** Time course of H<sub>2</sub> liquid-phase concentration during batch experiments in the presence of different initial TCE concentrations.

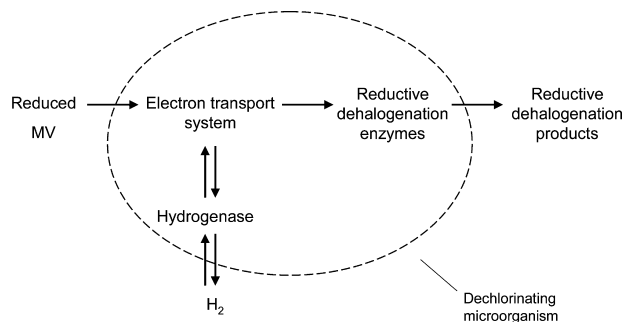
absence of TCE was much greater than in the presence of TCE. Such an enhancement was observed at all of the tested MV concentrations, although it was maximum (~80 times) when the MV concentration was 750 µM (Figure 3).

To verify whether the higher H<sub>2</sub> formation rate was due to the absence of inhibitory effects of TCE on H<sub>2</sub>-producing microorganisms, additional tests with lower amounts of spiked TCE were conducted. In these tests, the initial liquid phase concentration of TCE was decreased from 60 µM down to 30 µM (Figure 4), while the MV concentration was maintained at 250 µM. In spite of the diminished initial TCE concentration, no differences were observed among the observed H<sub>2</sub> profiles, suggesting that toxicity effects were unlikely the reason for the reduced H<sub>2</sub> formation in the presence of TCE (Figure 4). Figure 4 also shows the time course of H<sub>2</sub> concentration when TCE was completely omitted from the system: at the end of the 8 h experiment, the H<sub>2</sub> concentration was over 1 order of magnitude higher than in the experiments with various initial amounts of TCE.

Interestingly, at the highest MV concentrations, both in the presence and in the absence of TCE, the H<sub>2</sub>-formation rate reached almost the same value of around 80 µeq/day, thereby suggesting that this value represented a maximum rate of this reaction, for the microbial culture under consideration. In support of this consideration, thermodynamic calculations indicated that, at the highest H<sub>2</sub> partial pressures reached (e.g., 0.0025 atm), for the reaction to be energetically feasible, it was sufficient that only 1/30 of added MV was in its reduced state. Accordingly, at the highest MV concentrations, H<sub>2</sub> production was most probably limited by the intrinsic kinetic activity of the culture rather than by thermodynamic constraints.

## Discussion

**Relevance of H<sub>2</sub> Evolution on BEARD Efficiency.** This study revealed that TCE dechlorination and H<sub>2</sub> evolution are the



**FIGURE 5.** Proposed scheme of reduced MV utilization, TCE dechlorination, and H<sub>2</sub> production by dechlorinating bacteria (modified from ref 35).

two main biological reactions which are stimulated in the BEARD process when a mixed, hydrogenotrophic, TCE-dechlorinating culture is employed. The relative importance of the two reactions appeared to be strongly dependent on the mediator concentration. At the lowest MV concentrations (25–750 µM), only TCE dechlorination was stimulated, whereas at higher MV concentrations, both reactions occurred simultaneously, although they showed distinct kinetic features. Although in principle, H<sub>2</sub> is also an electron donor for TCE dechlorination, its accumulation in the system actually represents a source of current and process inefficiency (e.g., other metabolisms can be stimulated causing the formation of unwanted end products such as methane), which thereby needs to be minimized by operating the system at low mediator concentrations. Clearly, this would also result in lower degradation rates. Possibly, a combination of high degradation rates and high current efficiency could be obtained by anchoring the mediator at the electrode surface and employing high specific surface electrodes. Indeed, in a previous study (21), we found that, using a glassy carbon electrode with the mediator immobilized in a Nafion film on its surface, high current efficiencies were obtained without hydrogen accumulation. At the same time, the use of high specific surface area electrodes should, in principle, also allow the achievement of high bacterial densities in the system and accordingly high dechlorination rates.

**Putative Microbial Populations Responsible of H<sub>2</sub> Formation and Reductive Dechlorination.** The results obtained in this study suggest two alternative hypotheses concerning the microbial populations responsible for H<sub>2</sub> formation and TCE dechlorination. In a first hypothesis, H<sub>2</sub> production and TCE dechlorination are catalyzed by distinct microbial populations which both use electrochemically reduced MV as an electron donor in their metabolism. The dechlorinating bacteria, in addition to MV, also use the H<sub>2</sub> produced by other community members as an electron donor for TCE dechlorination. According to this hypothesis, the apparent lack of H<sub>2</sub> formation at the lowest MV concentrations (25–750 µM) could actually be explained by a rapid scavenging of the slowly produced H<sub>2</sub> by dechlorinators. In a second, and we believe more convincing, hypothesis, the observed H<sub>2</sub> production would result from a branched metabolism of the dechlorinating bacteria themselves (Figure 5): when electron donation by reduced MV exceeds electron utilization by TCE reductive dehalogenation enzymes (this occurs at increasing MV concentrations), the electrons are diverted, most likely via a hydrogenase, to an alternative electron sink, that is, H<sup>+</sup>. However, at the lowest MV concentrations and in the presence of TCE, the H<sub>2</sub>-producing pathway is not active because the electrons are channeled to the most energetically favorable electron acceptor (i.e., TCE). Alternatively, in the absence of TCE, all of the electron flow is diverted toward protons' reduction, and the H<sub>2</sub> formation is greatly enhanced. It is worth noting that the latter strategy has been previously

described for a sulfate-reducing microorganism using metallic iron as a direct source of electrons via an extracellular pathway (35). Similarly to sulfate-reducing bacteria, also dechlorinators, including those in our mixed culture, can use H<sub>2</sub> as an electron donor in their metabolism. In these bacteria, the H<sub>2</sub> metabolism involves key-enzymes, hydrogenases, which are known to catalyze the reversible reaction  $H_2 \rightleftharpoons 2H^+ + 2e^-$  (36). In support of this hypothesis is also the fact that TCE dechlorination was apparently the only metabolism responsible for H<sub>2</sub> consumption in the present mixed culture used as inoculum in the batch experiments. Indeed, no methane was ever detected, and regularly, nearly all of the weekly fed H<sub>2</sub> was recovered as TCE dechlorination products (data not shown). Moreover, the microbiological characterization of the mixed culture by FISH revealed that known hydrogenotrophic dechlorinating microorganisms (*Desulfotobacterium* spp. and *Dehalococcoides* spp.) accounted for over 70% of the total bacteria.

**Relevance of Biocathodic H<sub>2</sub> Production.** The interest in hydrogen evolution catalysts is currently increasing, as molecular hydrogen is being considered as an optimal energy carrier. Hydrogenases are very effective catalysts for the hydrogen evolution process (37, 38); therefore, hydrogenase-anchored electrodes aimed at exploiting the interesting catalytic features of these enzymes are currently being developed (39, 40). However, these systems suffer from the fact that enzymes are typically instable and are not self-regenerating. Conversely, the bioelectrocatalytic H<sub>2</sub> production by self-regenerating whole cells (instead of enzymes) would be more appealing. So far, bioelectrocatalytic H<sub>2</sub> production has been reported only for an immobilized pure culture of *Desulfovibrio vulgaris* (which contains periplasmic hydrogenases) with MV as a redox mediator (38, 41). Only very recently, Rozendal and colleagues (42) developed, for the first time, a H<sub>2</sub>-producing biocathode based on a mixed culture of "electroactive" bacteria able to directly accept electrons from a polarized electrode, via an extracellular electron transfer pathway. However, the composition of the microbial culture described in that study was unknown. A preliminary comparison of the maximum volumetric H<sub>2</sub> production rate obtained in this study with those reported by Rozendal and colleagues (42) indicates that rates here obtained are about 100 times lower. However, it needs to be considered that the biomass concentration in batch experiments was extremely low and typically around 20 mg VSS/L. Accordingly, it is anticipated that H<sub>2</sub> production rates comparable to those reported by Rozendal and colleagues (42) should be potentially achieved by employing high surface area electrodes, which, in the end, should allow an increase of the bacterial density by orders of magnitude. Overall, our findings revealed that a dechlorinating culture (and most likely hydrogenotrophic dechlorinating bacteria) is quite an exceptional biocatalyst for H<sub>2</sub> production, exhibiting almost no overpotentials with respect to the thermodynamic  $2H^+ / H_2$  potential (-440 mV vs SHE, pH = 7.5). Although promising, the practical applicability of such microorganisms for sustainable bioelectrocatalytic H<sub>2</sub> production will surely require further investigations, including a long-term assessment of culture viability and stability of the redox mediator.

## Acknowledgments

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## Supporting Information Available

Epifluorescent micrographs and information on the molecular characterization of the dechlorinating culture by FISH. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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