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Dechlorination of Trichloroethene in a Continuous-Flow Bioelectrochemical Reactor: Effect of Cathode Potential on Rate, Selectivity, and Electron Transfer Mechanisms

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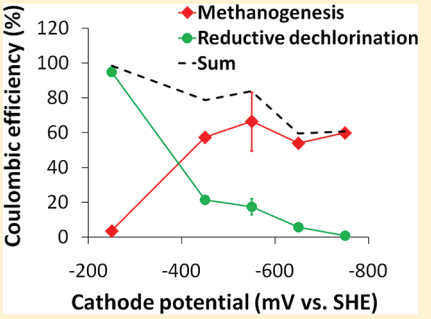
Supporting Information

ABSTRACT: The exciting discovery that dechlorinating bacteria can use polarized graphite cathodes as direct electron donors in the reductive dechlorination has prompted investigations on the development of novel bioelectrochemical remediation approaches. In this work, we investigated the performance of a bioelectrochemical reactor for the treatment of trichloroethene (TCE). The reactor was continuously operated for about 570 days, at different potentiostatically controlled cathode potentials, ranging from −250 mV to −750 mV vs standard hydrogen electrode. The rate and extent of TCE dechlorination, as well as the competition for the available electrons, were highly dependent on the set cathode potential. When the cathode was controlled at −250 mV, no abiotic hydrogen production occurred and TCE dechlorination (predominantly to *cis*-DCE and VC), most probably sustained via direct extracellular electron transfer, proceeded at an average rate of 15.5 ± 1.2 μmol e^-/L d. At this cathode, potential methanogenesis was almost completely suppressed and dechlorination accounted for 94.7 ± 0.1% of the electric current (15.0 ± 0.8 μA) flowing in the system. A higher rate of TCE dechlorination (up to 64 ± 2 μmol e^-/L d) was achieved at cathode potentials lower than −450 mV, though in the presence of a very active methanogenesis which accounted for over 60% of the electric current. Remarkably, the bioelectrochemical reactor displayed a stable and reproducible performance even without the supply of organic carbon sources with the feed, confirming long-term viability.



1. INTRODUCTION

Chlorinated aliphatic hydrocarbons (CAHs), such as perchloroethene (PCE) and trichloroethene (TCE) are among the most common and harmful subsurface contaminants due to their extensive industrial use, persistence in the environment, and toxicity.[1] Anaerobic bioremediation via reductive dechlorination is regarded as an efficient and cost-effective remediation technology for CAHs in groundwater. The technology relies on the capacity of anaerobic dechlorinating bacteria to "respire" chlorinated contaminants, using them as terminal electron acceptors in their energy metabolism.[2] During microbial reductive dechlorination, the contaminant is sequentially dechlorinated to less-chlorinated (more amenable to further aerobic biodegradation) or even nonchlorinated end-products. As an example, the microbial reductive dechlorination of TCE proceeds via the formation of *cis*-dichloroethene (*cis*-DCE), vinyl chloride (VC), and ultimately the nontoxic ethene and ethane.[3,4] Hydrogen gas has been identified as a key electron donor for most dechlorinating bacteria, including *Dehalococcoides* spp., which is the only known microorganism capable of anaerobically dechlorinating chloroethenes all the way to ethene.[5] Different engineered approaches have been proposed to deliver H_2 to dechlorinating bacteria (either in situ or in ex situ bioreactors), such as the use of organic substrates which release H_2 upon fermentation,[6] passive dissolution using hollow-fiber membranes,[7] and more recently bioelectrochemical systems based on water electrolysis.[8−10] In principle, these latter (bioelectrochemical) methods hold the advantage that the rate of hydrogen (and oxygen) supply can be easily controlled by adjusting the electric current. In spite of that, none of the above-mentioned approaches allow directly controlling the rate and extent of microbial dechlorination as well as the competition for the produced hydrogen between dechlorination and competing metabolisms.

The recent and exciting discovery that certain dechlorinating bacteria can accept electrons from the surface of polarized graphite electrodes has drastically changed the paradigm that hydrogen gas is necessary to sustain the reductive dechlorination process.[11−14] These findings have prompted investigations on the development of novel bioelectrochemical systems for groundwater remediation, based on direct extracellular electron transfer to dechlorinating bacteria. To date, this concept has been demonstrated only in short-term (<3 days) batch experiments and under a narrow range of operating conditions (e.g., cathode potentials). In this work, we investigated the long-term (570 days) dechlorination performance of a continuous-flow bioelectrochemical reactor operated in a

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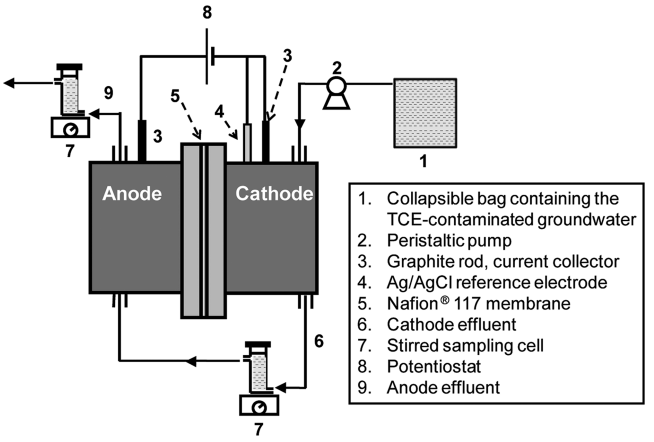

Figure 1. Schematic overview of the bioelectrochemical reactor employed in this work. The drawing reflects the configuration adopted in Runs 5 to 8, in which the cathode effluent is treated in the anode chamber. Conversely, during Runs 1 to 4, the cathode effluent was discharged, after being analyzed, and the anode was continuously fed with anaerobic mineral medium.

range (−250 mV to −750 mV vs SHE) of potentiostatically controlled cathode potentials. Great attention was paid to the rate and extent of reductive TCE dechlorination, long-term viability, involved electron transfer mechanisms (i.e., direct vs H_2-mediated), as well as on competing electrons and hydrogen consuming processes, such as methane formation.

2. EXPERIMENTAL SECTION

2.1. Bioelectrochemical Reactor Setup. The bioelectrochemical reactor employed in this work consisted of two nearly identical cylindrical borosilicate glass chambers, separated by a Nafion 117 proton exchange membrane (PEM) (Figure 1). The two chambers were held in place by an aluminum clamp and a Viton seal, which also ensured air-tightness. Prior to being used, the PEM was pretreated by boiling successively in H_2O_2 (3% v/v), DI water, 0.5 M H_2SO_4, and finally in DI water again, each for 2 h. The anodic and cathodic chambers were filled with conductive graphite granules with diameter between 2 and 6 mm (El Carb 100, Graphite sales, Inc., U.S.). The specific electrodic surface area of each chamber was about 1290 m^2/m^3. Prior to use, the graphite granules were submerged for 24 h in 37% HCl solution, then thoroughly rinsed in DI water and finally dried at 100 °C overnight. The total empty volume of the cathode and anode chambers were 0.82 and 0.95 L, respectively. After filling with graphite granules, the liquid phase volume at the cathode and at the anode reduced to 0.52 and 0.54 L, respectively. External electrical connections were guaranteed by inserting graphite rod current collectors (5 mm diameter, Sigma-Aldrich, Italy) in each compartment. An Ag/AgCl reference electrode (+0.199 vs standard hydrogen electrode, SHE) (Amel, Milan, Italy) was placed in the cathode chamber. Throughout the manuscript, all voltages are reported with respect to SHE. Cathode (working electrode), anode (counter electrode), and reference electrode were connected to a potentiostat Amel Model 549 (Milan, Italy) which was used to set the cathode potential at the desired value.

The system was also equipped with two flow-through sampling cells (placed at the outlet of the cathode and the anode chambers, respectively) that were continuously and vigorously mixed with magnetic stirrers, to ensure that the liquid phase (approximately 40 mL) and the headspace (approximately 10 mL) of the sampling cell were in equilibrium. Volatile compounds were analyzed in the headspace of the sampling cells. The cells also allowed taking liquid samples that were routinely analyzed for pH, and occasionally for volatile fatty acids. All tubings were made of Viton in order to minimize the adsorption of chlorinated compounds and volatilization losses.

2.2. Bioelectrochemical Reactor Operating Conditions. For the purpose of this work, the bioelectrochemical reactor was operated at ambient temperature (22 ± 3 °C) for a period of 570 days. Prior to the start-up, the cathode chamber was inoculated with 0.15 L of a TCE-to-ethene dechlorinating culture,[8] previously enriched on hydrogen and TCE as electron donor and acceptor, respectively. Throughout the entire operational period, the cathode chamber of the bioelectrochemical reactor was continuously fed, at a flow-rate of 0.58 L/d, with anaerobic medium spiked with TCE to a final concentration of approximately 35 μmol/L. The hydraulic retention time in the cathode chamber was 0.9 days, and the organic loading rate (referred to the empty volume of the cathode chamber) was 24.5 μmol/L d. The medium contained (in g/L): NH_4Cl, 0.5; $MgCl_2 \cdot 6H_2O$, 0.1; K_2HPO_4, 0.4; $MgCl_2 \cdot 2H_2O$, 0.05; 2 mL/L of a trace metal solution,[15] 2 mL/L of vitamin solution.[16] The electrical conductivity of the medium was 4.8 mS/cm, hence within the range of values typically reported for highly contaminated groundwater (i.e., 0.67 to 7.98 mS/cm).[17] The pH of the medium was maintained at values between 7 and 7.5 with a $NaHCO_3$ solution (10% w/v). The medium was maintained in a collapsible Tedlar bag that contained no headspace, thus eliminating TCE partitioning into the gas phase. Twice a week, a sample of medium was removed from the Tedlar bag and analyzed to determine the influent TCE concentration.

Eight experimental runs were conducted, each characterized by a different potential applied to the cathode (Table 1) with the potentiostat. The Runs at −550 mV and −450 mV were randomly replicated (three and two times, respectively) in order to check the reproducibility of the system. At the end of the study, the reactor was disconnected from the potentiostat and operated for approximately 50 days under open circuit conditions in order to determine the extent of TCE dechlorination in the absence of electric current. In parallel, abiotic experiments were also performed under batch conditions, as described elsewhere.[18] For these experiments, the cathode and anode compartments were each filled with approximately 50 g of granular graphite. In order to conservatively estimate the extent of electrochemical TCE dechlorination, these abiotic experiments were carried out by controlling the cathode at −750 mV (i.e., the most reducing potential tested in the biotic Runs), by spiking the cathode with TCE at initial concentration of 0.1 mM.

During Runs 1−4, the groundwater was discharged after the passage through the cathode chamber, and the (abiotic) anode was separately and continuously (at a flow rate of 0.2 L/d) fed with anaerobic basal medium (not containing TCE). Hence, only the cathodic (reductive) TCE dechlorination process was assessed during the aforementioned Runs.

Successively (Runs 5−8), the anode was inoculated with 0.1 L of an aerobic culture, capable of degrading *cis*-DCE and VC in the presence of ethene as a growth substrate, and the cathode effluent was used as the feed of the anode chamber. Here, the anodic

Table 1. Operational Conditions of the Bioelectrochemical Reactor

BES run no.	days of operation	set cathode potential (mV vs SHE)	treatment
1	0−147	−550	cathodic only
2	148−163	−450	cathodic only
3	164−174	−550	cathodic only
4	175−266	−650	cathodic only
5	267−349	−550	cathodic/anodic
6	350−399	−750	cathodic/anodic
7	400−436	−450	cathodic/anodic
8	437−570	−250	cathodic/anodic

(oxidative) biodegradation of TCE dechlorination products (formed at the cathode) was assessed. The hydraulic retention time in the anode chamber was also around 0.9 days.

Throughout the work, the anode potential was not controlled, instead it varied as a function of the current generated at the cathode. As expected, the highest value of the anode potential (i.e., +1.6 V vs SHE) was reached when the cathodic current was the highest (i.e., when the cathode was set at −0.75 V vs SHE); whereas the lowest value (i.e., +0.44 V vs SHE) was reached when the cathode was polarized to the less reducing value (i.e., −0.25 V, vs SHE).

2.3. Analytical Methods. Volatile compounds (TCE, *cis*-DCE, VC, ethene, ethane, and methane) were analyzed, in a 100 μL gaseous sample (removed from the headspace of the sampling cells) by a Varian 3400 gas-chromatograph equipped with a glass column packed with 60/80 mesh Carbopack B/1% SP-1000 (Sigma-Aldrich, Milano, Italy) and a flame ionization detector (FID).[8] Hydrogen was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R gas-chromatograph (Menlo Park, CA, U.S.) equipped with a molecular sieve packed column and a reduction gas detector (RGD). The H_2 detection limit of the instrument is 0.02 ppmv. When the hydrogen level was above the range of the RGD (i.e., over 100 ppmv), it was quantified with the Varian 3400 gas-chromatograph using a stainless-steel column packed with molecular sieve and a thermal-conductivity detector (TCD). Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants.[19,20]

The total biomass concentration in the cathode effluent was analyzed using DAPI staining, following a protocol described elsewhere.[21]

2.4. Calculations. The rate of TCE reductive dechlorination was calculated from the measured concentrations of TCE dechlorination intermediates in the cathode effluent as follows:

$$r_{RD}\left(\frac{\mu mole^-}{Ld}\right) = \frac{(2 \times [cis\text{-DCE}] + 4 \times [VC] + 6 \times [ethene] + 8 \times [ethane]) \times Q}{V_C}$$

where 2, 4, 6, or 8 are the number of moles of electrons required for the formation of 1 mol of *cis*-DCE, VC, ethene, or ethane from TCE, respectively; [*cis*-DCE], [VC], [ethene], and [ethane] are the concentrations (μmol/L) of TCE dechlorination products in the cathode effluent; Q is the flow rate (L/d); V_C is the empty volume of the cathode chamber (L); analogously, the rate of methane production was calculated as follows:

$$r_{CH_4}\left(\frac{\mu mole^-}{Ld}\right) = \frac{8 \times [CH_4] \times Q}{V_C}$$

where 8 is the number of mol of electrons required for the formation of 1 mol of methane, and $[CH_4]$ is the effluent methane concentration (μmol/L).

The Coulombic efficiency (%) for TCE dechlorination was calculated as the ratio of the theoretical electric current due to the formation of dechlorination products and the current flowing across the system according to the following equation:

$$\varepsilon_{RD}(\%) = \frac{\frac{r_{RD} \times V_C}{24 \times 3600} \times F}{I} \times 100$$

where F is the Faraday's constant (96485 C/mol electrons); I is the current (μA). Similarly, the Coulombic efficiency for methane formation was calculated as follows:

$$\varepsilon_{CH_4}(\%) = \frac{\frac{r_{CH_4} \times V_C}{24 \times 3600} \times F}{I} \times 100$$

Every time the cathode potential was changed, we waited (at least for 5 hydraulic retention times) for the reactor to reach a new stable performance before calculating reaction rates and yields.

2.5. Chemicals. TCE and *cis*-DCE (99.5+ %) were purchased from Sigma-Aldrich (Milano, Italy). Vinyl chloride, ethene, ethane, methane, and hydrogen gases were purchased from Sigma-Aldrich (Milano, Italy). All of the other chemicals used to prepare analytical standard for feed solutions were of analytical grade and were used as received.

3. RESULTS AND DISCUSSION

3.1. Reductive Dechlorination Performance at −550 mV. Figure 2 shows the bioelectrochemical reactor performance during Run 1, with the cathode polarized to −550 mV. During most of the Run, the concentration of TCE in the cathode effluent was lower than 3 μmol/L, and accordingly the average TCE removal efficiency was higher than 90% (Figure 2A). However, during the start-up (initial 20−30 days of reactor operation), the concentration of TCE dechlorination products in the cathode effluent was quite low (Figure 2B), suggesting that adsorption onto the granular graphite was the main mechanism responsible for TCE removal. Thereafter, the concentrations of VC, ethene, and ethane gradually increased prior to reaching a nearly stable value of 19 ± 1 μmol/L for VC and 1.7 ± 0.1 μmol/L for ethene + ethane, respectively (mean value ±1 standard deviation), on day 60. Although its concentration did not show a clear trend, *cis*-DCE was also occasionally detected in the cathode effluent, at an average concentration (calculated from day 60 until the end of the Run) of around 2.6 ± 0.4 μmol/L (Figure 2B). During this run, the average cumulative reductive dechlorination rate (calculated over a time period ranging from day 60 to the end of the run) was 62 ± 2 μmol e^-/ L d (referred to the total empty volume of the cathode chamber).

A mass balance of TCE and its dechlorination products for the continuous-flow bioelectrochemical reactor is presented in Figure 2C. Notably, to account for the different volumetric flow rates applied to the cathode and to the anode, the mass balance

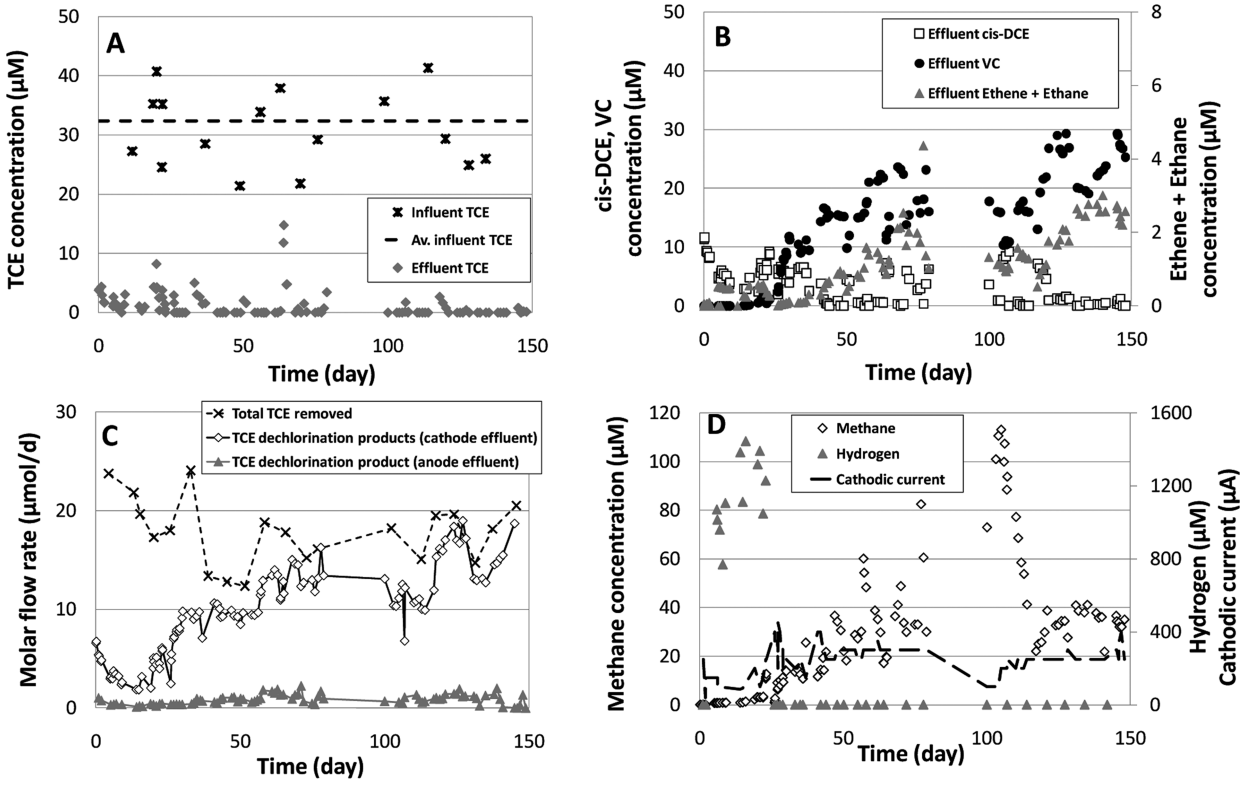

Figure 2. Performance of the bioelectrochemical reactor during Run 1 with the cathode potentiostatically controlled at −550 mV vs SHE: (A) TCE concentration in the influent and effluent of the cathodic chamber; (B) Concentration of dechlorination products in the effluent of the cathodic chamber; (C) Mass balance for the reductive dechlorination process in the continuous-flow bioelectrochemical reactor, based on molar flow rates; and (D) Hydrogen and methane concentrations in the effluent of the cathodic chamber, and cathodic current.

was based on molar flow rates (rather than simply on cathode and anode effluent concentrations), the latter calculated by multiplying the measured concentrations for the volumetric flow rates. From day 60, around 75% of the removed TCE was recovered as dechlorination products (i.e., *cis*-DCE, VC, ethene, and ethane) in the cathode effluent. However, around 6% was recovered in the anode effluent, indicating the occurrence of slow molecular diffusion of TCE and its dechlorination products from the cathode to the anode through the Nafion membrane.

Figure 2D shows the time course of hydrogen and methane (liquid phase) concentrations during Run 1. Until day 20, the hydrogen concentration was high (around 1400 μmol/L), thereby suggesting that the cathode potential (−550 mV) was reducing enough to sustain the abiotic reduction of H^+ to H_2 (standard reduction potential of −414 mV at pH 7). From day 25 onward, the hydrogen concentration remained stably below 20 nmol/L. Differently, methane concentration steadily increased (up to 120 μmol/L) during the initial 100 days of operation, then remained nearly constant at a substantially lower value (40 μmol/L) for the remainder of the Run. From day 60, the average electric current flowing in the circuit was 266 ± 5 μA.

3.2. Effect of the Cathode Potential on the Reductive Dechlorination Performance. An overview of the effect of set cathode potentials, on the reductive dechlorination performance is shown in Figure 3. The cathodic TCE removal efficiency gradually decreased, from 100% to around 60%, when the cathode potential was increased from −750 to −250 mV (Figure 3A). VC was the main end-product of TCE dechlorination when the cathode was controlled in the range −450 to −750 mV. More specifically, its concentration was the highest (16−18 μmol/L) when the cathode was set in the range −450 to −650 mV, whereas a slightly lower value (11 μmol/L) was obtained at −750 mV, where ethene (and ethane) concentration was higher (Figure 3A). The observed increase of ethene and ethane concentration when the cathode potential was set to gradually decreasing values is consistent with a higher abiotic (purely electrolytic) hydrogen production rate and, accordingly, with a higher hydrogen availability to the reductive dechlorination process.

The run carried out at a cathode potential of −250 mV deserves ad hoc considerations. At −250 mV, the occurrence of abiotic hydrogen production is extremely unlikely.

In principle, at a pH of 7.1 (i.e., the average measured value during Run 8, at −250 mV), the abiotic hydrogen production would become exergonic at a hydrogen partial pressure of 10^{-6} atm, which corresponds to a liquid phase hydrogen concentration lower than 0.8 nM, a value that is pretty close to the reported hydrogen threshold concentration of dechlorination (i.e., the lowest hydrogen concentration exploitable by dechlorinating bacteria).[22−25] It is also worth mentioning that for this calculation it was assumed that hydrogen evolution is not affected by overpotentials; this assumption may be reasonable for Pt or a few other noble metals, but certainly not for graphite electrodes. As an example in previous studies, we found that hydrogen evolution at carbon-based electrodes starts for cathode potentials more negative that −550 mV.[13,26] Similar results have been reported in many other studies.[12,27] Throughout Run 8, the measured liquid phase H_2 concentration remained nearly constant at 0.7 ± 0.1 nM, thereby supporting the absence of abiotic

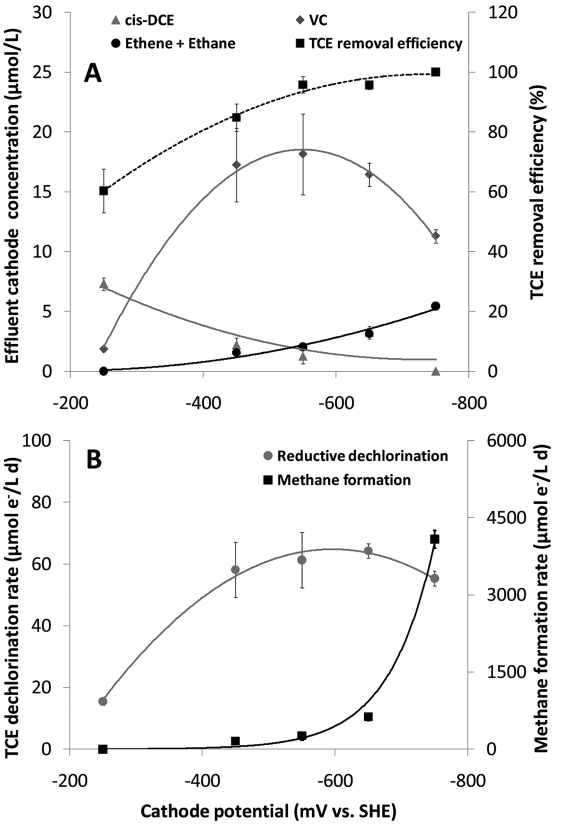

Figure 3. Performance of the bioelectrochemical reactor as a function of the set cathode potential: (A) Average concentration (±1 standard deviation) of dechlorination products in the effluent of the cathodic chamber, and TCE removal efficiency; (B) Average rates (±1 standard deviation) of reductive dechlorination and methane formation.

H_2 production at −250 mV and providing convincing indications that the observed reductive dechlorination activity was sustained by a direct extracellular transfer of electrons from the cathode surface to the dechlorinating bacteria. This mechanism was previously demonstrated in batch experiments conducted on the same dechlorinating culture hereby used, but only for cathode potentials more reducing than −450 mV.[13,14] Here, the occurrence of reductive dechlorination at less reducing potentials (i.e., −250 mV) suggests that the long-term operation of the bioreactor, which may have resulted in the formation of a biofilm on the surface of the graphite cathode, allows decreasing the activation overpotentials, thereby obtaining a more effective extracellular electron transfer process.

However, during the Run at −250 mV, the TCE removal efficiency was lower (60.3 ± 7.3%) and, differently from the other Runs, *cis*-DCE (7.3 ± 0.5 μmol/L) was the dominant TCE dechlorination intermediate in the cathode effluent, although VC was also present (1.9 ± 0.2 μmol/L). Ethene and ethane were only occasionally detected and only at very low concentrations (<0.5 μmol/L) (Figure 3A).

Figure 3B shows the effect of the set cathode potential on the overall rate of TCE dechlorination (referred to the total empty volume of the cathode compartment). A sharp increase (from 15.5 ± 1.2 to 58 ± 1 μmol e^-/L d) was observed when the cathode potential was decreased from −250 to −450 mV, consistent with the probable onset of (abiotic) hydrogen production for cathode potentials lower than −250 mV. Notably,

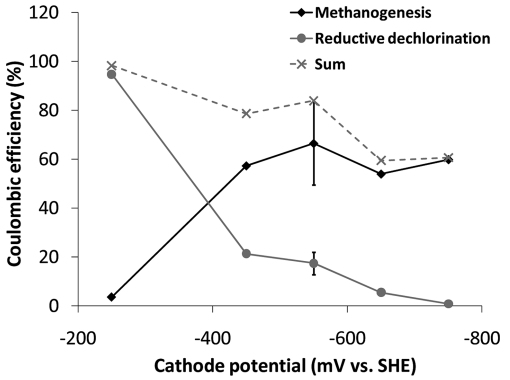

Figure 4. Average Coulombic efficiency (±1 standard deviation) of the reductive dechlorination and methanogenesis (i.e., percentage of electric current recovered as dechlorination products or methane) as a function of the set cathode potential.

the rate of TCE dechlorination did not substantially increase when the cathode was operated at more reducing values, instead it slightly decreased when the cathode was set at −750 mV (Figure 3B). Under open circuit conditions, TCE was slowly removed and only *cis*-DCE was formed, at a rate of 8.9 ± 0.8 μmol e^-/L d. Notably, the removed TCE was nearly stoichiometrically recovered as *cis*-DCE, pointing to a biotic reductive dechlorination sustained by the endogenous metabolism of the biomass present in the reactor. The dechlorination rate observed under open circuit conditions is approximately one-half of that observed at −250 mV and almost 7-times lower than that observed at more negative cathode potentials, hence providing an additional confirmation of the key stimulatory effect of the electric current on the metabolism of dechlorinating bacteria. Moreover, negligible TCE dechlorination was observed in abiotic control tests.

The most striking effect of the cathode potential was on methanogenesis. The effluent methane concentration and accordingly the rate of methane production increased exponentially by decreasing the cathode potential (Figure 3B). This is consistent with the rate of abiotic hydrogen production increasing exponentially with a decrease in the cathode potential, as predicted by the Butler−Volmer equation.[28] Remarkably, the rate of methane production rate increased much more than the reductive dechlorination rate (up to 4100 μmol e^-/L d, at −750 mV). Consequently, the Coulombic efficiency for the reductive dechlorination steadily decreased from 23% at −450 mV down to less than 1% at −750 mV (Figure 4). Overall, in the range −450 to −750 mV, methanogenesis (most likely supported by abiotically produced hydrogen gas) was the main electron sink and the predominant metabolic activity in competition with the reductive dechlorination for current "consumption".

Again, the run conducted at a cathode potential of −250 mV deserves ad hoc considerations. Indeed, after an initial transient phase of around 15 days, the methane concentration in the effluent dropped down to low values (data not shown), clearly demonstrating the poor ability of methanogenic microorganisms to thrive at this cathode potential and providing additional lines of evidence of the lack of abiotic hydrogen production during this run. The inability of the cathode polarized to −250 mV to serve as a direct electron donor for methane production is also in agreement with previous studies demonstrating that "electromethanogenesis" requires cathode potentials more negative

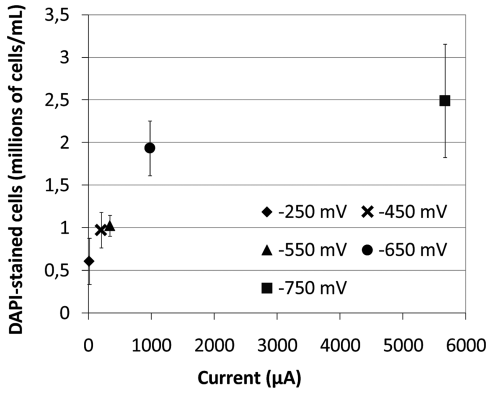

Figure 5. DAPI-stained cells in the cathode effluent as a function of the average electric current, for each set cathode potential.

than −650 mV.[29,30] As a major outcome of the very low methane production, the average Coulombic efficiency for the reductive dechlorination was 94.7 ± 0.1% (Figure 4). This remarkable experimental evidence demonstrates for the first time that the competition for electrons between dechlorinators and methanogens can be fully addressed by tuning the cathode potential. This electrochemical approach is perhaps more straightforward than conventional ones, commonly based on a controlled dosage of selected fermentable substrates to release hydrogen gas at concentrations that favor dechlorinators over methanogens.[31]

Biomass was present (as detected by DAPI-staining) in the cathode effluent throughout the entire experimental period, clearly indicating the occurrence of growth within the bioelectrochemical reactor. Due to the presence of the graphite support and its active participation into the microbial reactions, a biofilm was most likely formed (even if it was not specifically visualized in the present study). Figure 5 clearly shows that the concentration of DAPI-stained cells increases with the current, although not in a linear way. This finding suggests that biomass density in the cathode compartment increased as the cathode potential was controlled at decreasing values. However, a direct effect on detachment rates of the biofilm cannot be excluded.

3.3. Long-Term (570 Days) Reductive Dechlorination of TCE in the Absence of Organic Carbon Sources. The reductive dechlorination of TCE could be sustained for over 570 days without the addition of organic carbon sources (the only exception being the vitamins contained in the basal medium). Although, acetate is typically regarded as a key carbon source in the anabolism of *Dehalococcoides* spp. (the only known microorganism capable of dechlorinating past *cis*-DCE),[32] it was never found at measurable concentrations (as well as all other volatile fatty acids) in the cathode effluent, regardless the set cathode potential. Clearly, it is still possible that low amounts of acetate were produced from hydrogen and carbon dioxide via homoacetogenesis [9] and then rapidly consumed by the dechlorinators and methanogens. However, this hypothesis is not supported by the stable performance observed, for over 130 days, at −250 mV during which hydrogen formation did not occur. Analogously, it is possible that low levels of organic carbon were excreted by methanogens or released upon their death and subsequent lysis.

Clearly, further investigations on this issue, specifically addressing the organic carbon requirements of dechlorinating bacteria in bioelectrochemical systems, are necessary. It also needs to be ascertained if ATP can be generated during the electricity-driven dechlorination, particularly when the reaction proceeds via direct extracellular electron transfer.[33]

In any case, the possibility of sustaining for long-periods the reductive dechlorination of chlorinated contaminants by solely providing bacteria with a solid electrode and without supplying organic substrates is a major finding of this study and it is extremely relevant from a practical point of view.

3.4. Oxidative (Anodic) Treatment of the Cathode Effluent. Starting from Run 5, the effluent of the cathode was used as a feed of the anode chamber (previously inoculated with a mixed culture capable of aerobically degrading *cis*-DCE, and VC in the presence of ethene as the growth substrate) in order to promote the oxidative degradation of (lesser chlorinated) TCE intermediates contained therein. In spite of the presence of favorable conditions (i.e., the presence of oxygen at concentrations higher than 1 mg/L and ethene), during most of the Runs, the extent of microbial VC (and also ethene) degradation at the anode never exceeded 40% (Supporting Information, Figure S1). A lower degradation efficiency was observed during Run 8 (with the cathode polarized at −250 mV), where *cis*-DCE, which is much more recalcitrant than VC to aerobic oxidation,[34,35] accumulated as the main TCE dechlorination intermediate (Supporting Information, Figure S1).

One possible explanation for the poor degradation efficiencies is the presence at the anode of microbiologically hostile conditions, possibly induced by the presence of electrochemically generated oxidants, such as chlorine, halogenated organic compounds or trihalomethanes.[36,37] Differently, in another study,[38] it was the electric field generated by electrode reactions to inhibit the activity of aerobic VC degrading microorganisms; in that work, however, the current densities were much higher (higher than 8 mA/cm^2) than those applied in the present work.

Clearly, the anodic oxidation was not the main target of this research and additional efforts are needed to identify which operating conditions allow establishing an effective anodic degradation of TCE reductive dechlorination intermediates, as for instance obtained by other researchers with 1,2-dichloroethane.[39]

3.5. Perspectives of Bioelectrochemical Approach for Groundwater Remediation. The present work demonstrates that a fine-tuning of the cathode potential allows gaining a good control over the rate and extent of microbial reductive dechlorination processes, as well as over the competition for the supplied electrons between dechlorinators and methanogens. Specifically, when the cathode was controlled at −250 mV, no hydrogen was (abiotically) produced, methanogenesis was fully suppressed and TCE dechlorination, although slower that at more negative potentials, was the only electron-consuming process, resulting in a Coulombic efficiency of approximately 100%. Apparently, the bioelectrochemical strategy adopted in this work (i.e., based on the individual control of the cathode potential) turned out to be more selective in stimulating the reductive dechlorination process than that recently proposed by Lohner and Tiehm,[9] consisting in the application of a constant electric current (in the range 0−0.05 mA/cm^2), whereby the reductive dechlorination never accounted for more than 13% of the consumed electric current. On the contrary, the extent of anodic biodegradation achieved by Lohner and Tiehm was substantially greater than that obtained here, with the difference being possibly related to the different anodic material (stainless steel vs graphite) and microbial inoculum employed in that study.[9]

In this work, a clear trend for an increased ethene formation yield was observed by operating the cathode at more reducing values. Along this line, a complete conversion of the influent TCE into ethene would eliminate the need for an anodic (post) treatment; however, on the basis of the results obtained in this work, this strategy seems impractical (unless the TCE load is drastically reduced) due to the requirement for very negative cathode potentials and the resulting very low Coulombic efficiencies (due to the extensive competition with methanogens).

With respect to long-term viability, the bioelectrochemical reactor was operated for over 470 days, displaying a stable and reproducible (all replicated Runs behaved very similarly) performance even without the supply of organic carbon sources with the feed.

An additional interesting feature of the bioelectrochemical remediation process described in this study is that, at least in principle, it can be implemented into a variety of remediation schemes either in situ (e.g., in biological permeable barrier) or ex situ (e.g., in a above ground bioreactor), provided that suitable strategies are implemented to control at large scale individual electrode potentials.

In conclusion, this work confirmed the sustainability, robustness, and versatility of the bioelectrochemical approach: by fine-tuning the cathode potential, it is possible to directly control the microbiological activity occurring at the electrode surface and in turn gaining a remarkable control over the reductive dechlorination process.

■ ASSOCIATED CONTENT

Supporting Information. Effect of the anodic treatment on the concentration of TCE dechlorination products and methane. This material is available free of charge via the Internet at http://pubs.acs.org.

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