maximum solubility in water of approximately 8000 mg/L (Klecka et al., 1998). The presence of 1,2-DCA in the environment poses serious environmental concerns, particularly due to the fact that it is a suspected carcinogen and is highly persistent under anaerobic conditions, with an estimated half-life of more than 50 years (Premaratne et al., 1995; Vogel et al., 1987).

Bioremediation, via anaerobic reductive dechlorination, is one of the most promising approaches for the treatment of groundwater contaminated with 1,2-DCA (Arjoon et al., 2013; Baric et al., 2014; De Wildeman et al., 2003, 2002; Grostern and Edwards, 2006; Hirschorn et al., 2007; Klecka et al., 1998; Marzorati et al., 2010). This approach typically requires the subsurface injection of fermentable substrates that serve as electron donors to stimulate autochthonous microbial populations using 1,2-DCA as a respiratory electron acceptor (Duhamel and Edwards, 2007). Typically, the used electron donors are commercial products such as soluble molasses or other fermentable (i.e., H_2-releasing) organic compounds. However, this approach suffers of a number of drawbacks, including the inefficient use of the added electron donors, stimulation of unwanted side reactions, and in general, poor control over reaction conditions (Aulenta et al., 2007). Furthermore, in order to achieve remediation goals, a long period of time and multiple electron donor additions are frequently needed.

In order to overcome some of these limitations, the use of polarized graphite electrodes as the sole electron donors for the microbial reductive dechlorination (RD) processes has been recently proposed (Aulenta et al., 2009; Aulenta et al., 2007; Aulenta et al., 2010; Aulenta et al., 2011; Strycharz et al., 2010, 2008). However, no attempts have been made so far to apply a bioelectrochemical approach to promote the reductive transformation of 1,2-DCA. Hence, in order to further extend the field of applicability of this novel remediation approach, this study explored the possibility to stimulate the microbial RD of 1,2-DCA with a graphite electrode (cathode) serving as the sole electron donor. Attention was given to examining the influence of the set cathode potential on the rate and efficiency of 1,2-DCA dechlorination, as well as on the electron transfer mechanisms possibly involved in the biological process. Molecular analyses of the involved microbial communities (both planktonic and attached onto the electrode surface) were also carried out in order to shed light on the identity of the microorganisms responsible for the electricity-driven dechlorination of 1,2-DCA.

2. Methods

2.1. Bioelectrochemical cell setup

The bioelectrochemical cell setup used in this study consisted of two gastight borosilicate glass bottles (each having a total volume of 270 mL and working volume of 150 mL) separated by a 3-cm^2 cross-sectional area Nafion® 117 proton exchange membrane (PEM). Prior to its use, the PEM was boiled successively in four separate solutions for 2 h each:H_2O_2 (3% v/v), deionized (DI) water, 0.5 M H_2SO_4 and DI water, and then finally stored in DI water until use. The cathode and anode were graphite rods (6 mm diameter, Sigma Aldrich, Milano, Italy). The nominal surface area of the cathode (calculated by taking into account only the part of the electrode that was immersed in the liquid phase) was 9.7 cm^2. The distance between the anode and the cathode was approximately 10 cm. Prior to their use, the graphite electrodes were pretreated as described elsewhere (Gregory et al., 2004). A reference electrode (KCl-saturated Ag/AgCl, +199 mV vs. the standard hydrogen electrode (SHE); Amel S.r.l., Milano, Italy) was placed in the cathodic chamber. The catholyte and anolyte consisted of anaerobic medium as described below. Throughout the manuscript, all potentials are reported with respect to SHE.

At the beginning of the study, the cathode compartment of the cell was inoculated with approximately 100 mL of a dechlorinating culture, previously enriched in the laboratory with 1,2-DCA as electron acceptor and H_2 as electron donor. Briefly, the culture was maintained in an anaerobic serum bottle (total volume 120 mL) that was sealed with a Teflon-faced butyl rubber stopper and aluminum crimp seal, and covered with aluminum foil to prevent growth of phototrophic organisms. The serum bottle was operated in a fill-and-draw mode, receiving a weekly dose of 1,2-DCA (0.5 mmol) and H_2 (3.75 mmol). Before each refeeding, the culture was purged with a N_2/CO_2 gas mixture to remove all volatile compounds. On average, 22.5 mL of suspended culture was removed weekly and replaced with fresh medium. The average cell retention time was 25–30 days. The temperature was maintained at 25 °C and the pH at 7.2–7.5. This 1,2-DCA dechlorinating culture was maintained for over 80 days before being inoculated in the bioelectrochemical cell.

2.2. Bioelectrochemical experiments

The bioelectrochemical system (BES) experiments were carried out over a period of 280 days, during which six different cathode potentials were evaluated, namely open circuit (OCP), −300 mV, −500 mV, −600 mV, −700 mV and −900 mV. Throughout the experimental period, the BES was operated in a fill-and-draw mode. Briefly, every week the cathode and anode compartments of the BES were purged (30 min) with a N_2/CO_2 (70:30 v/v) gas mixture to remove volatile compounds, then a fixed liquid volume was removed from each compartment and replaced with fresh anaerobic medium, in order to maintain an average cell retention time of 68 days. Thereafter, the cathode compartment was spiked with 1,2-DCA at a nominal concentration of 0.5 mmol/L. The pH of the solution was maintained at 7.2–7.5. The BES was incubated at 25 °C (via a water bath), in the dark and received constant magnetic stirring. The bioelectrochemical cell was connected to an IVIUM-n-STAT multichannel electrochemical analyzer (Ivium Technologies, The Netherlands) which allowed setting the cathode potential at the desired value and recording the resulting electric current. Electrochemical data were processed using the Ivium Soft® software package.

The cumulative electric charge (expressed as electron equivalents, $e-eq_i$) that was transferred at the electrodes was calculated by integrating the current (i) over the period of electrode polarization. Cumulative reducing equivalents ($e-eq_{RD}$) that were used for the reductive dechlorination of 1,2-DCA were calculated from the measured amounts of ethene, considering the corresponding molar conversion factor of 2 $e-eq$/mol. The Coulombic efficiency (CE) for the reductive dechlorination process was calculated as follows:

$$CE(\%) = \frac{(e-eq_{RD})}{(e-eq_i)} \times 100$$

To quantify the extent of purely electrolytic hydrogen evolution at the cathode potentials investigated in this work, abiotic BES experiments were also performed. These experiments were carried out under conditions identical to those reported above, with the only exception being the lack of a microbial culture in the cathode compartment.

2.3. Medium

The medium contained the following components: NH_4Cl (0.5 g/L), $MgCl_2{\cdot}6H_2O$ (0.1 g/L), K_2HPO_4 (0.4 g/L), $CaCl_2{\cdot}2H_2O$ (0.05 g/L), trace metal solution (10 mL/L) (Zeikus, 1977), vitamin

solution (10 mL/L) (Balch et al., 1979), and $NaHCO_3$ (15 mL/L, 10% w/v). All solutions were purged for at least 0.5 h with a N_2/CO_2 (70:30% v/v) gas mixture before use. The pH value of the medium was 7.5.

2.4. Microbiological analysis

Liquid samples from the source culture (i.e., the inoculum) and from the cathode compartment of the bioelectrochemical cell, as well as biofilm samples from the graphite cathode, were taken for microbiological analysis. Samples from the bioelectrochemical cell were taken at the end of the study, after the cell had been operated for 3 successive cycles at −300 mV (vs. SHE).

Liquid samples (0.45 mL) were immediately fixed with formaldehyde (2% v/v final concentration), filtered through 0.2 mm polycarbonate filters (Ø 47 mm, Millipore) by gentle vacuum (<0.2 bar) and stored at −20 °C until use.

To fix the biomass from the biofilm formed at the electrode, the surface of the electrode was carefully scraped with a sterile spatula. The detached biomass was initially collected in a solution composed of 45 mL of PBS buffer and 5 mL of 37% (w/v) formaldehyde solution, and then filtered as described above.

Catalyzed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH), carried out following previously published protocols (Di Battista et al., 2012; Fazi et al., 2008), was applied on the filtered samples to characterize their microbial composition. Specifically, CARD-FISH oligonucleotide probes targeting *Bacteria* (EUB338mix probes) and *Archaea* (ARC195 probe), were employed, as well as genus-specific probes targeting known dechlorinating bacteria such as *Dehalococcoides mccartyi* (Dhe1259c and Dhe1259t probes), *Dehalobacter* spp. (DHB643 probe), and *Sulfurospirillum* spp. (SULF220ab probe). The probes, labeled at the 5′-end with horseradish peroxidase (HRP), were purchased from BIOMERS (http://www.biomers.net). Details of the oligonucleotide probes used are available at http://www.microbial-ecology.net/probebase. Oligonucleotide probes were always added together with 4′,6-diamidino-2-phenylindole (DAPI) for the quantification of total cells. Slides were examined by epifluorescence microscopy (Olympus, BX51) and the images were captured with an Olympus F-View CCD camera and processed and analyzed with AnalySIS software (SIS, Germany).

2.5. Analytical methods

Volatile components, namely 1,2-DCA, vinyl chlorine (VC), ethene (ETH), ethane (ETA) and methane (CH_4), were quantified by injecting 50 μL of headspace (taken with a gas-tight syringe) into a Perkin–Elmer GC 8500 gas chromatograph (2 m × 2 mm glass column packed with 60/80 mesh Carbopak B/1% SP-1000 Supelco; N_2 carrier gas 18 mL/min; oven temperature 190 °C; flame ionization detector temperature 250 °C). H_2 was analyzed by injecting 50 μL of headspace with a gas-tight syringe in a PerkinElmer Auto System gas chromatograph (4.6 m × 2.1 mm stainless steel column packed with 60/80 Carboxen-1000 support Supelco; N_2 carrier gas 40 mL/min; oven temperature 225 °C; thermal conductivity detector (TCD) temperature 250 °C). Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants (Chen et al., 2012).

2.6. Chemicals

1,2-DCA (99.8 + %), hydrogen (99.5 + %) and all the other chemicals were purchased from Sigma–Aldrich (Milano, Italy, except where indicated differently). The chemicals used to prepare the mineral medium were of analytical grade and used as received.

3. Results and discussion

3.1. Bioelectrochemical reductive dechlorination of 1,2-DCA

During each feeding cycle, the time course of the 1,2-DCA dechlorination and competing reactions (i.e., methanogenesis) were monitored through daily analyses of headspace samples of the cathode compartment of the bioelectrochemical cell. Regardless the set cathode potential, the removed 1,2-DCA was almost stoichiometrically dechlorinated to ETH via dichloroelimination, with only low amounts of harmful VC (deriving from dehydrochlorination of 1,2-DCA) accumulating over the course of the cycle. Low levels of CH_4 were also occasionally detected.

As an example, Fig. 1 presents the results of a typical feeding cycle, with the cathode potential potentiostatically fixed at −700 mV. The added 1,2-DCA was reductively dechlorinated at a rate of 40 μeq/L d. At the end of the cycle, the removed 1,2-DCA (181 μM, corresponding to 40% of the initial dose) was almost completely converted into ETH (140 μM, corresponding to 77% of the removed 1,2-DCA) and a very low amount of VC (0.11 μM, corresponding to about 0.05% of the removed 1,2-DCA) was detected. Notably, the observed pathway of 1,2-DCA dechlorination and relative distribution of dechlorination products was very similar to the one observed in the source culture which was used as inoculum of the bioelectrochemical cell, where H_2 was supplied as electron donor (data not shown).

3.2. Effect of cathode potential on the rate and yield of 1,2-DCA dechlorination

Throughout an experimental period of 23 weeks, the bioelectrochemical cell was operated at different cathode potentials, randomly changed (and replicated) in the range from −300 mV to −900 mV. Open circuit control experiments were also carried out to quantify the background reductive dechlorination activity of the mixed microbial culture in the bioelectrochemical cell.

The rate of 1,2-DCA dechlorination was found to generally increase (from 10 ± 4 μeq/L d, up to 37 ± 10 μeq/L d) with the decrease of the set cathode potential from −300 mV to −900 mV (Fig. 2). In open circuit tests, the reductive dechlorination proceeded slowly (4 ± 3 μeq/L d) and was most likely driven by electrons deriving from endogenous cell metabolism or from reduced metabolites released into the medium during feeding cycles with the cathode set at more reducing values.

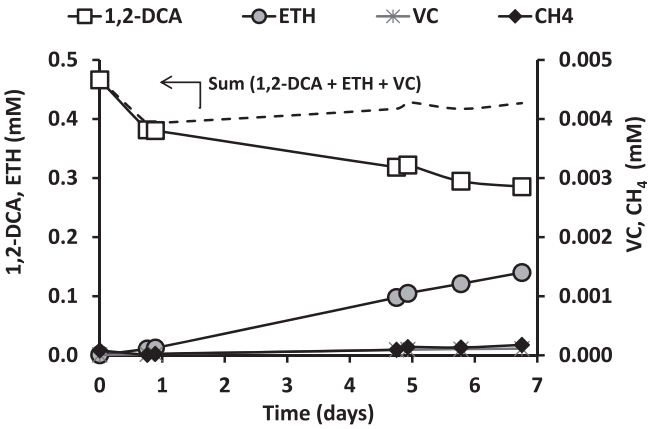

Fig. 1. Time course of bioelectrochemical 1,2-DCA dechlorination and methane formation during a typical 7-day feeding cycle, with the cathode potentiostatically set at −700 mV.

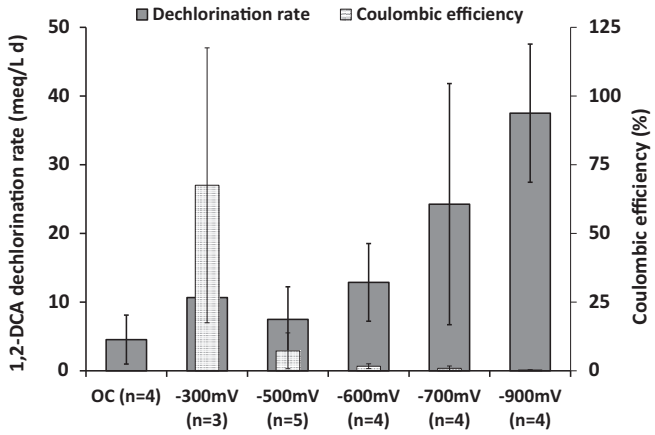

Fig. 2. Effect of the cathode potential on the rate of 1,2-DCA dechlorination and the Coulombic efficiency (data were corrected to account for the reductive dechlorination measured in an Open Circuit Control). For each experimental condition, the number of replicated batch experiments (each consisting of a 7-day feeding cycle) is indicated.

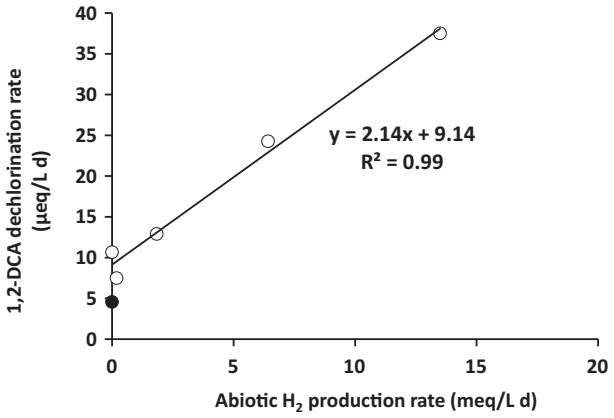

Fig. 3. Correlation between the rate of 1,2-DCA dechlorination and abiotic H_2 production.

Coulombic efficiency (*CE*), i.e. the yield of current utilization for the reductive dechlorination process, was calculated at the end of each feeding cycle (Fig. 2). The highest value (68 ± 50%, corrected to account for the reductive dechlorination observed in open circuit controls) was observed when the cathode was fixed at the less reducing value (−300 mV). Substantially lower and decreasing values were observed as the cathode was set at lower potentials. For example, the CE was 7 ± 6% at −500 mV and lower than 2% for cathode potentials lower than −600 mV.

Since molecular H_2 is known to be a key electron donor in the microbial reductive dechlorination of 1,2-DCA, batch tests were carried out with abiotic (i.e., non-inoculated) cells to quantify the effect of the cathode potential on the purely electrolytic H_2 production.

Consistent with the results of previous studies (Villano et al., 2011), production of H_2 with the graphite electrode was observed only at cathode potentials lower than −500 mV, whereas H_2 remained below instrumental detection limits during the course of the tests carried out at −300 mV. As expected, the rate of abiotic H_2 production increased almost exponentially (from 0.19 ± 0.02 meq/L d to 13.5 ± 3.0 meq/L d) when the cathode potential was decreased from −500 mV to −900 mV. Notably, an almost linear correlation was found between the rate of dechlorination and the rate of abiotic H_2 production, pointing to a direct role of electrolytically-generated H_2 in the electricity-driven and microbially catalyzed reductive dechlorination process (Fig. 3). On the other hand, the fact that the rate of 1,2-DCA dechlorination was substantially higher at −300 mV than that observed in OCP experiments and proceeded with an extremely high CE strongly suggests that it was driven by direct electron transfer, without the intermediate production of H_2.

Overall, the herein reported results compare favorably with those obtained in previous study (Aulenta et al., 2011) whereby the effect of the cathode potential (in the range from −250 mV to −750 mV) was analyzed with reference to TCE dechlorination, using a continuous-flow bioelectrochemical reactor. Also in that case, the rate of dechlorination increased by decreasing the cathode potential (and in turn increasing the H_2 availability), while the highest Coulombic efficiency (almost 100%) was obtained when the cathode was operated at the highest potential (i.e., −250 mV) when the dechlorination was most likely driven by direct electron transfer rather than by H_2. Collectively the results of this and the previous study lead to a trade-off between rate and efficiency, suggesting the existence of an optimal cathode potential range which satisfies both.

3.3. Microbiological characterization

In order to gain insights on the identity of the microorganisms responsible for the bioelectrochemical reductive dechlorination process, at the end of the study the microbial communities in the cathode compartment of the BES (planktonic state and attached to the cathode surface) were analyzed by CARD-FISH. For comparative purposes, the H_2-fed suspended culture that was used as the inoculum for the bioelectrochemical cell was also analyzed.

In the inoculum, *Bacteria* and *Archaea* accounted for 63 ± 14% and 5 ± 3% of DAPI-stained cells, respectively (Fig. 4a). Notably, almost 90% of *Bacteria* could be identified as *Dehalococcoides*, using genus-specific CARD-FISH probes (Fig. 4b). The predominance of *Dehalococcoides* is consistent with its known ability to metabolically dechlorinate 1,2-DCA to ethene (and traces of VC) using H_2 as the primary electron donor (Maymo-Gatell et al., 1999; Schmidt et al., 2014; Tandoi et al., 1994). Other known dechlorinating microorganisms such as *Dehalobacter* and *Sulfurospirillum* together accounted for less than 2% of total Bacteria, suggesting a marginal contribution to the observed dechlorinating activity.

In the BES, both planktonic and attached cells also primarily consisted of *Bacteria*. A slightly higher percentage of *Archaea* (relative to total DAPI-stained cells) was observed in the microbial community attached onto the cathode compared to planktonic cells (16 ± 6% vs. 3 ± 2%). This is possibly due to the fact that H_2 was produced at the electrode or that attached *Archaea* were protected more against the inhibitory effects of chlorinated compounds compared to cells suspended in the bulk liquid.

Remarkably, *Dehalococcoides* accounted for virtually all planktonic bacterial cells in the BES cathode chamber, whereas a substantial portion (≈40%) of bacterial cells collected from the electrode remained unidentified, since they did not bind with any of the tested genus-specific, CARD-FISH probes. A previous study (Di Battista et al., 2012) showed that the presence of *Dehalococcoides* in bioelectrochemical dechlorinating systems is linked to the capacity of this microorganism to thrive on electrolytically produced H_2. This agrees with the observed predominance of this microorganism in the bulk liquid of the BES in the system in this study. On the other hand, the occurrence of other as-yet-unidentified *Bacteria* at the surface of the cathode could be linked to their capacity of direct electron uptake from the surface of the polarized electrode.

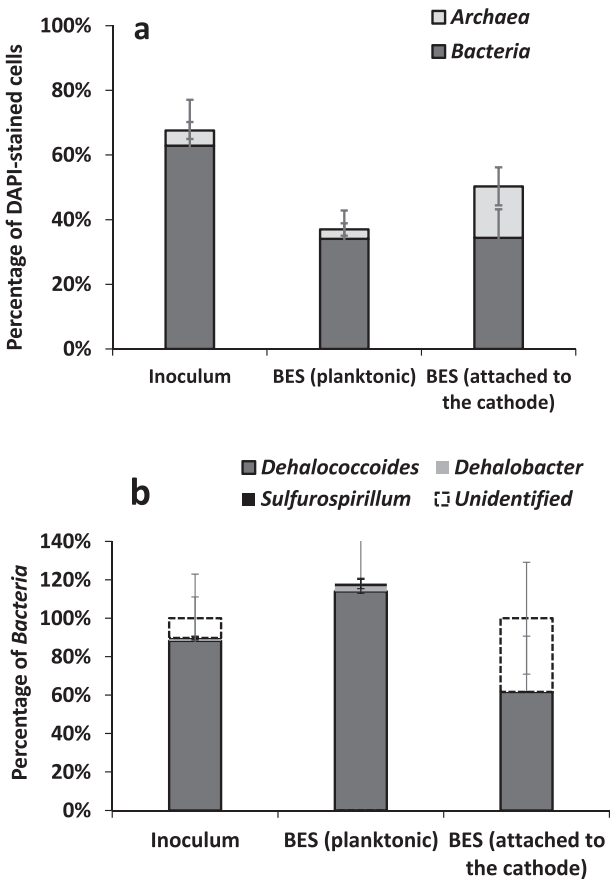

Fig. 4. CARD-FISH analysis of the microbial communities in the inoculum, in the bulk liquid of the BES cathode chamber, and on the surface of the graphite cathode. (a) Percentage of Bacteria and Archaea, relative to the total DAPI-stained cells. (b) Percentage of Dehalococcoides, Sulfurospirillum, and Dehalobacter relative to the total Bacteria.

4. Conclusions

This study demonstrates that graphite electrodes could be used to stimulate the microbially catalyzed reductive dechlorination of 1,2-DCA to ethene, thereby further broadening the range of toxic chlorinated subsurface contaminants which can be treated with BES technology. The set cathode potential turned out to be a key parameter affecting the rate and yield of the reductive dechlorination process.

In conclusion, this study provides further evidence that bioelectrochemical systems represent a versatile and tunable technology to drive the biodegradation of a range of subsurface contaminants.

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