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Reductive/oxidative sequential bioelectrochemical process for Perchloroethylene (PCE) removal: effect of the applied reductive potential and microbial community characterization

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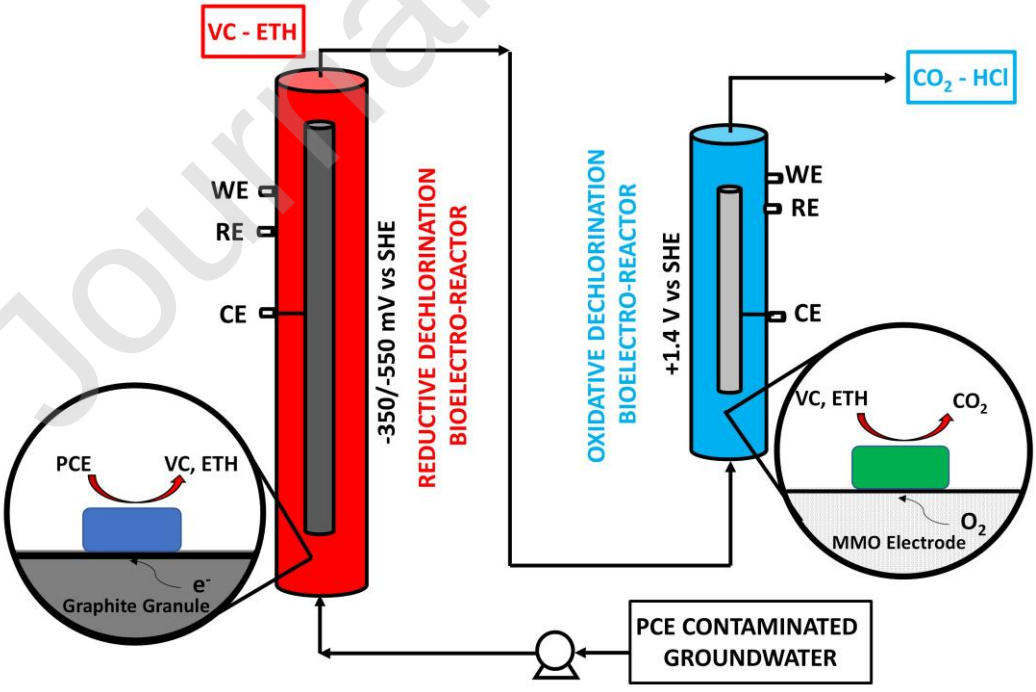


Highlights

- Perchloroethylene was completely reduced into VC and Eth in the reductive reactor
- The oxidative reactor oxidized all the VC and Eth coming from the reductive reactor
- The -350 mV potential increased the coulombic efficiency of the reductive step
- *Dehalococcoides mccartyi* was found at high abundance in the reductive reactor
- The MEC configuration allowed the existence of different redox niches in each reactor

Abstract

In this paper, a bioelectrochemical process has been developed by the combination of two membrane-less reactors equipped with an internal graphite granules counterelectrode for the perchloroethylene (PCE) removal through a reductive/oxidative sequence. In the reductive reactor, the cathodic chamber supplied the reducing power to PCE dechlorinating biomass while a rutile electrode promoted the aerobic dechlorination of the less chlorinated PCE byproducts by oxygen *in situ* evolution. Two potentiostatic conditions, -350 and -550 mV vs SHE, were tested on the reductive reactor, which showed the capability to completely reduce the PCE into vinyl chloride (VC) and ethylene (Eth). These compounds were completely removed by the oxidative reactor with an average VC and Eth removal efficiency of 94 ± 1% and 98 ± 1%. The -350 mV vs SHE condition resulted in the higher coulombic efficiency for the reductive dechlorination which reached 22 ± 7 % while by increasing the reductive potential to -550 mV the coulombic efficiency drop down to 6 ± 1 % in favor of the methanogenesis reaction. *Dehalococcoides mccartyi* was found at high abundance in the reducing reactor while a heterogeneous bacterial consortium was observed in the oxidative reactor. Microbiome characterization of the reductive and oxidative reactors showed the concomitant presence of different redox niches in each compartment suggesting that the exchange of ionic species between the electrode and the counterelectrode allowed the co-existence of both reducing and oxidative reactions.

Keywords: Reductive dechlorination; Oxidative dechlorination; Bioremediation; Bioelectrochemical systems; Chlorinated Aliphatic Hydrocarbons; Groundwater remediation

1.Introduction

Perchloroethylene and in general Chlorinated aliphatic hydrocarbons (CAHs) groundwater contaminations are worldwide presents in relation with their intensive industrial use followed by inappropriate disposal procedures of the past [1]. Perchloroethylene (PCE) and trichloroethylene (TCE), widely utilized for their good degreasing properties, are classified as suspected human carcinogen, moreover, the PCE and TCE usually release Vinyl Chloride (VC) from their partial degradation which represents the most harmful chlorinated compound [2]. Engineered bioremediation processes represents innovative remediation technologies that permit the CAHs removal from contaminated groundwater directly *in situ* resulting in a more environmental and cost-effective remediation [3-5]. The engineered bioremediation consists in the stimulation of microorganisms already present in the contaminated matrix by the addition of electron donors or nutrients directly to the subsurface to boost biological removal of pollutants [6]. The highly chlorinated aliphatic compounds can be transformed to less toxic compounds by the reductive dechlorination (RD), a biological reaction where the chlorinated compounds are reduced through sequential reactions in which a chlorine atom is loss at each step [7, 8]. Specialized microorganisms, primarily *Dehalococcoides mccartyi*, couple the reductive dechlorination of chlorinated ethenes with growth in a process called organohalide respiration [9]. *D. mccartyi* strains differ from one another for the presence in the genome of reductive dehalogenase genes (RDases) coding for proteins involved in different steps of the RD process. Among them, the most representative RDases are *tceA, vcrA, bvcA* genes catalyzing the conversion of PCE/TCE to vinyl chloride (VC), cis-1,2-Dichloroethylene (cis-DCE) to VC and VC to ethylene (Eth) [10]. Being *D. mccartyi* and the RDases unequivocally associated with the RD process, they are considered as elective "biomarkers" and routinely screened and quantified in research studies and in field applications [11]. Indeed, VC dechlorination into Eth is the rate-limiting step during reductive dechlorination of chlorinated ethenes and can be affected by the lack of electron donor and/or the

absence of specialized *D. mccartyi* strains (e.g. *vcrA* gene-carrying strains) able to mediate this reductive step. The highly chlorinated compounds are more easily degradable by RD while, the progressive loss of chlorine atoms, which present high electronegativity character, increase the tendency of the less chlorinated compounds (e.g. cis-DCE, VC) to oxidative dechlorination pathways. The cis-DCE and VC oxidation via co-metabolic or metabolic pathways involves dioxygenase or monooxygenase enzymes, which are involved in the hydrocarbon's degradation by phenols or epoxides formation [12, 13]. Previous studies showed an ethene-assimilating mixed culture performed the co-metabolic oxidation of the cis-DCE through the formation of the oxyrane (epoxyethane) under aerobic condition [14]. An efficient integration of anaerobic and aerobic dechlorinating mechanisms can be adopted to control the accumulation of more toxic intermediates, produced by the incomplete RD, leading to the complete degradation of the contaminants into CO_2. More in details, the adoption of a sequential reductive/oxidative environment can promote both anaerobic reduction of high chlorinated compounds and aerobic oxidation of low chlorinated intermediates, resulted in an effective strategy to prevent secondary contamination by more toxic (e.g. VC) or mobile compounds. The stimulation of the RD by injecting fermentable electron donors in the groundwater, followed by the application of in situ biosparging has been already tested leading to a considerable VC degradation [15, 16]. Despite the good removal rates, this remediation approach showed some limitations and disadvantages, such as the decrease of the aquifer quality and the clogging effects due to ferric iron precipitation [17]. Bioelectrochemical remediation strategies are good candidates to overcome these limitations [18, 19]. In a bioelectrochemical system, the application of electric potential creates a reductive/oxidative environment to sustain the biodegradation of contaminants and stimulates the metabolic activity of electroactive microorganisms involved [20-22]. In a microbial electrolysis cells (MEC) the cathode is used as electron donor for the anaerobic RD of chlorinated ethenes [23-25] while the anode promotes the oxidative dechlorination of low chlorinated products. Several studies already tested the bioelectrochemical stimulation of RD with different two-chamber/bench-scale reactors for the

TCE and PCE degradation under continuous flow condition [26-28]. These studies showed the effect of both applied potential in the cathode compartment and TCE loading rate, demonstrating the effectiveness of the bioelectrochemical approach in the dechlorinating activity stimulation. Moreover, an aerobic mixed culture capable to oxidize cisDCE and Eth, which usually are produced together with VC during the RD reaction, by using a graphite bioanode has been reported [29], the graphite bioanode allowed for the cometabolic degradation of cisDCE and Eth by prodcuing an oxidative environment. Even if under batch tests the graphite bioanode promoted the aerobic degradation of less chlorinated ethenes, in a continuous flow bench scale reactor the use of graphite granules as anodic material [30, 31] resulted in a low oxidation activity deriving from the low efficiency of the graphite for the oxygen evolution, which is affected by parasitic reactions [32]. On the other hand, the adoption of an efficient rutile electrode for the oxygen evolution allowed for the complete degradation in the bioanode of the VC produced by the incomplete dechlorination of the TCE.

In the present study, an innovative configuration of membrane-less bioelectrochemical reactor has been used for a sequential reductive/oxidative dichlorination of high chlorinated compounds. Reductive dechlorination rate, coulombic efficiency and competitive mechanisms have been evaluated at two different cathodic potentials (-350 vs SHE and -550 vs SHE), while the oxidative reactor was maintained at +1.4 V vs SHE to ensure the in situ oxygen evolution necessary for the oxidation of the low chlorinated RD by-products.

The sequential bioelectrochemical process involved a reductive reactor to stimulate the RD and an oxidative reactor for the stimulation of the dechlorination of low chlorinated by-products. While the reductive reactor adopted graphite granules as electron donor, the oxidative reactor utilized a rutile electrode anode for in situ oxygen evolution. In both bioelectrochemical reactors a membrane-less configuration was used, in which the presence of an internal graphite counterelectrode guaranteed the cost's reduction and the simplification of the construction. Moreover, in the reductive reactor,

the scavenging effect of the graphite granules, avoided electrolytic oxygen evolution preventing poisoning effects on the dechlorinating bacteria [32]. Preliminary investigations already confirmed the capability of the sequential reductive oxidative process to completely remove the PCE into non harmful by-products through the bioelectrochemical stimulation of both anaerobic and aerobic dechlorinating microorganisms [33].

At the end of the sequential process operation here reported, the microbial community composition of the working electrodes of the reductive and oxidative bioelectrochemical reactors has been compared with the anaerobic and aerobic dechlorinating consortia used as inocula for the start up. Moreover, the abundance of specific biomarkers involved in PCE reductive dechlorination were analysed on both biofilm and planktonic populations.

2.Material and Methods

2.1 Anaerobic and aerobic dechlorinating inoculums

The anaerobic dechlorinating consortium used for the start-up of the reductive reactor derived from a 150 mL PCE-fed enriched culture previously acclimated on lactate as electron donor and maintained under fill-and-draw conditions with an Hydraulic Retention Time (HRT) of 30 days. The culture maintenance has been performed by the periodic feeding of PCE (0.8 mM) and a lactate 5 mM solution as electron donor. Each 10 days 50 mL of liquid phase was withdrawn from the enriched culture and replaced by fresh mineral medium. Before each new feeding, any organic volatile metabolites (i.e. mainly VC and Eth) were removed by flushing the headspace and liquid phase with a mixture of N_2/CO_2 (70:30) which ensured the anaerobic environment necessary to the dechlorinating microorganisms. Mineral medium composition has been reported elsewhere [34, 35]

The 1.2 L of aerobic dechlorinating consortium, which resulted capable to oxidize cis-DCE in presence of Eth, has been maintained by the withdrawal of 400 mL of liquid phase each 7 days, corresponding to a HRT of 21 days. The mineral medium refreshment was followed by the aeration of the culture by an air pump to ensure the oxygen dissolution in the liquid media [29]. After the liquid phase aeration, the cis-DCE (3.5 mM) and Eth (60 mM) were dosed.

2.2 Sequential process set up

The Sequential Reductive/Oxidative system presents two tubular microbial electrolysis cell (MEC) (Figure 1). Each borosilicate glass MEC is equipped with a graphite internal counter electrode. The internal counter electrode adopted in the two reactors is concentric and is constituted by a double layer of a plastic grid in where inside is set an HDPE geotextile membrane that allows the ion diffusion by avoiding electrical shortcuts. The first MEC, also named reductive reactor, with an empty volume of 8.24 L, is devoted to the reductive dechlorination using a graphite cathode chamber as working electrode. The second MEC; named oxidative reactor, has an empty volume of

3.14 L. The working electrode of the oxidative reactor was a commercial rutile electrode consisting in a titanium mesh which supported Iridium and Ruthenium oxides, usually indicated as mixed metal oxides. Rutile electrodes coated with mixed metal oxides (MMO) ensures an efficient oxygen evolution by water electrolysis. The working electrode of the oxidative reactor is in a silica granules bed. The two bioelectrochemical reactors, were polarized by using two single channel AMEL 509 potentiostat (AMEL, Italy) that enabled the utilization of a three-electrode configuration by the use of a Ag/AgCl (3 M KCl, +0.199 V vs SHE) reference electrode. The sequential process was fed with the same peristaltic pump by using a synthetic mineral medium contaminated with PCE 100 μM. The composition of the contaminated mineral medium was: 1 g/L NaCl, 0.5 g/L MgCl2 *6H2O, 0.2 g/L KH2PO 4 , 0.3 g/L NH4Cl, 0.3 g/L KCl, 0.015 g/L CaCl2 *2H2O, 0.05 g/L Na2S, 2.5 g/L NaHCO3 [36], 1 mL/L metal solution[37], 10 mL/L vitamin solution [38]. Both the influent feeding solution and the effluent were collected into Tedlar bag®, a self-collapsing bag that permits the quantification of liquid and gaseous flow rates. Analytical procedures already described [33], are reported in the supplementary material section S1.

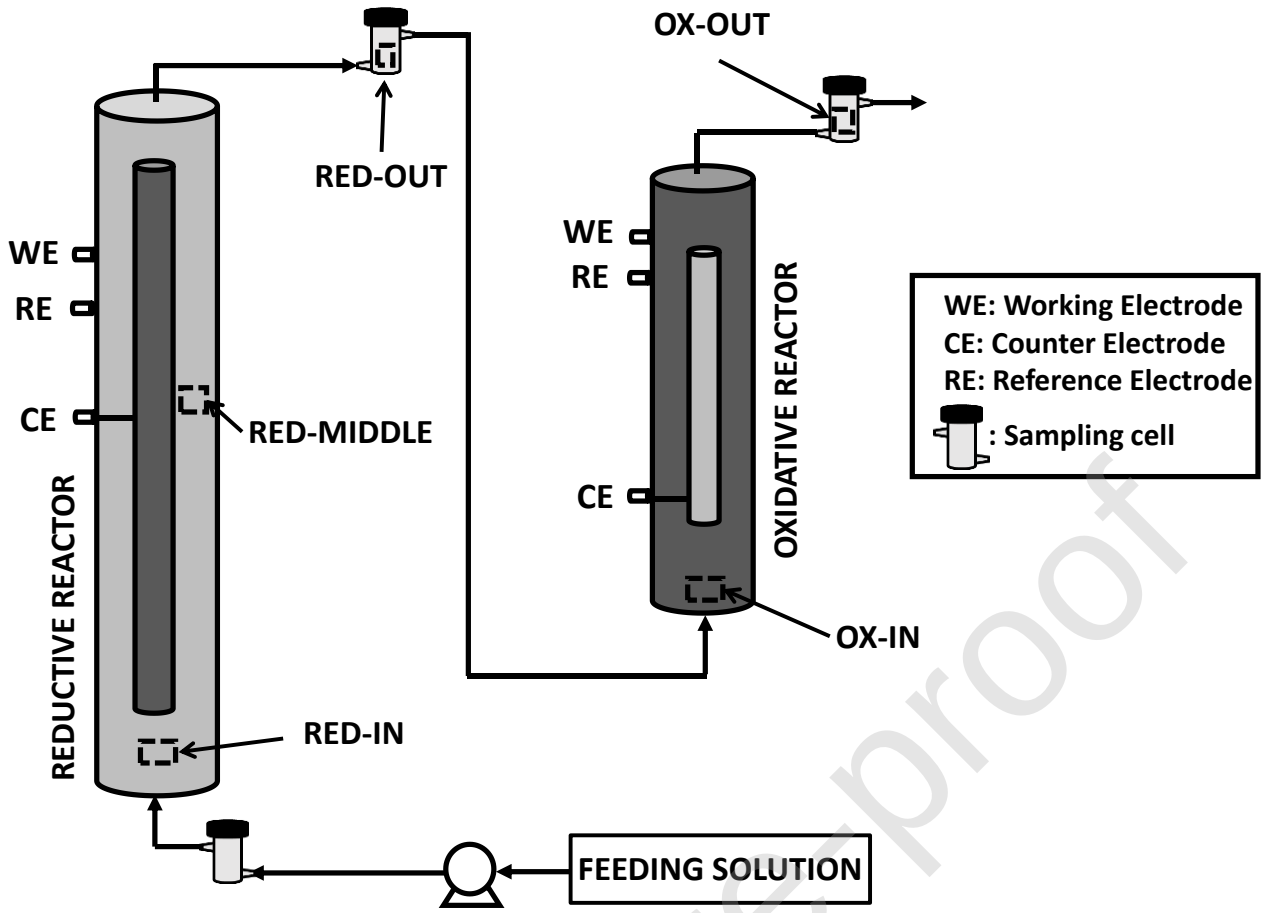


Figure 1. Schematic view of the reductive/oxidative bioelectrochemical reactor. Sampling points for the biomolecular analysis are indicated. RED-IN: Liquid phase on the bottom of the reductive reactor; RED-MIDDLE: Liquid phase from the middle part of the reductive reactor; RED-OUT: Liquid phase in the outlet sampling cell of the reductive reactor; OX-IN: Liquid phase on the bottom of the oxidative reactor; OX-OUT: Liquid phase in the outlet sampling cell of the oxidative reactor.

2.3 Calculation

For the enriched cultures it has been possible to evaluate the rate for the dichlorination reaction, both reductive and oxidative with the follow equation:

$$r_{Dr} = \frac{C_{in} - C_{fin}}{T_{in} - T_{fin}}$$

In which Dr means a generical dechlorinating reaction, C_{in} is the initial concentration of the target species, C_{fin} is the final concentration of the target species, Tin is the initial time and is equal to the analytical determination of the C_{in} and T_{fin} the final concentration.

The RD rate, which represent the amount of equivalents utilized by the microorganism for the PCE dechlorination in the reductive reactor has been evaluated by equation (1)

$$RD\ \left(\frac{\mu eq}{L\ d}\right) = \frac{Q}{V_{reductive}}\left(\frac{L}{d}\right) * \{2[TCE] + 4[cisDCE] + 6[VC] + 8[Eth]\}\ (1)$$

In which Q is the liquid flow rate adopted in the experiment, $V_{reductive}$ is the empty volume of the reductive reactor, [TCE], [cisDCE], [VC], [Eth] are the nominal concentrations expressed as mM while 2, 4, 6 or 8 are the number of moles of electrons required for RD intermediates [27]. The RD rate is converted into current by the equation 2

$$RD\ (mA) = RD\ \left(\frac{\mu eq}{L\ d}\right) * V_{reductive} * \frac{F}{\frac{s}{d}}\ (2)$$

In which F is the Faraday constant (96485 C/mole-) and s/d are the 86400 s in a day. The amount of electricity flowing in the reductive reactor utilized for the RD reaction, also named coulombic efficiency of the reductive dechlorination ($CE_{(RD)}$) has been calculated by equation 3:

$$CE_{RD}\ (\%) = \frac{RD_{(mA)}}{Current\ (mA)} * 100\ (3)$$

As a competitive reduced product, as also described in the literature [39, 40], the coulombic efficiency for methane production [41], described by the equation (4) [42], has been evaluated according to the equation (5) and (6) [43]:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O\ (4)$$

$$CH_4\ (mA) = F\left(\frac{L}{d}\right) * 8[CH_4] * \frac{F}{\frac{s}{d}}\ (5)$$

$$CE_{CH4}\ (\%) = \frac{CH_{4(mA)}}{Current\ (mA)} * 100\ (6)$$

In the oxidative reactor the current is mainly devoted to the oxygen evolution, i.e. the oxidative coulombic efficiency was evaluated by considering the stoichiometry of the complete VC and Eth oxidation, reported in equation (7) and (8), which required 2.5 and 3 molO_2, respectively.

$$C_2H_3Cl + 2.5\ O_2 \rightarrow 2CO_2 + H_2O + HCl \quad (7)$$

$$C_2H_4 + 3O_2 \rightarrow 2CO_2 + 2H_2O \quad (8)$$

Moreover, considering a complete conversion of the current into oxygen in the oxidative reactor, which release 4 electrons for each mole of oxygen produced (equation 9), the coulombic efficiency for the oxidative dechlorination has been calculated according to equation (10).

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (9)$$

$$CE\ (ox) = \frac{(VC\ removal * 2.5 + Eth\ removal * 3\ )}{Oxidatiove\ Curent\ (O2)} * 100 \quad (10)$$

2.4 Sampling for Biomolecular Analysis and Nucleic Acids Extraction

The analysis was conducted on the *inocula* used for the start-up of the BES (1 mL of each aerobic and anaerobic liquid culture) and on samples taken from the reductive and oxidative reactors at the end of the operation at -550 mV vs SHE. In detail, 5 mL of liquid medium and 1 g of graphite or silica granules were collected at different sampling points of the reductive (Red-In, Red-Middle, Red-Out) and the oxidative (Ox-Out) reactors (Fig. 1).

Liquid samples were filtered on polycarbonate filters (pore size 0.22 μm, 47 mm diameter, Nuclepore) to harvest the biomass. DNA was extracted directly from the filters and from graphite or silica particles by PowerSoil DNA extraction kit (Qiagen, Italy) following the manufacturer's instructions.

Purified DNA from each sample was eluted in 100 μL sterile Milli-Q and stored at -20°C until further biomolecular analysis.

2.5 Next Generation Sequencing (NGS)

4 ng of DNA extracted from each sample was used for NGS analysis. 16S rRNA amplicon library targeting the V1–3 variable region was constructed as previously reported [44]. PCR reactions were performed in 25 μL reaction volume containing Phusion Master Mix High Fidelity (Thermo Fisher Scientific, United States) and 0.5 μM final concentration of the library adaptors with V1–3 primers (27F and 534R). All PCR reactions were run in duplicate and pooled afterward. The amplicon libraries were purified using the Agencourt® AMpureXP-beads protocol (Beckmann Coulter, United States) and the concentration was measured with Qubit 3.0 fluorometer (Thermo Fisher Scientific, United States). Purified libraries were pooled in equimolar concentrations and diluted to 4 nM. Phix control was added at 10% in the pooled libraries to overcome issues often observed in 16S rRNA sequencing. Samples were paired-end sequenced (2 × 301 bp) on a MiSeq (Illumina, United States) instrument using a MiSeq Reagent kit v3, 600 cycles (Illumina, United States) following the standard guidelines. Bioinformatic analyses were performed using QIIME2 software tools 2018.2 release [45] and reads were managed DADA2 algorithm [46]. The taxonomic analysis was performed on 16S rRNA gene sequences clustered at 99% similarities within the Silva 128 database [47] and a dataset of amplicon sequence variants (ASVs) was obtained. Shannon index (H) was calculated by PAST version 4.0 [48]. Data are reported as relative percentage of single or grouped ASVs. Corresponding ASV identifiers are summarized in Supplementary Material (S.2, Tab S1)[49].

2.7 Real time PCR (qPCR)

D. mccartyi 16S rRNA gene and RDase genes *tceA, bvcA, vcrA* were quantified by Real time PCR (qPCR). Absolute quantification method with TaqMan® chemistry, composed by 6-carboxyfluorescein (FAM) as the 5′ end reporter fluorophore and N,N,N,N,-tetramethyl-6-carboxyrhodamine (TAMRA) as the 3′ end quencher, was employed. Reactions were conducted in

20 µL total volume including 3 µL of DNA as template, 300 nM of each primer and TaqMan® probe and SsoAdvancedTM Universal Probes Supermix (Bio-Rad, Italy). Primers and probes used for each reaction were previously reported in [50]. Standard curves for the absolute quantification were constructed by using the long amplicons method previously reported in [51]. Each reaction for each sample was performed in triplicate with CFX96 TouchTM Real-Time PCR Detection System (Bio-Rad, Italy). Quantitative data were reported as gene copy numbers L^{-1} of liquid culture or g^{-1} of graphite (from the reductive reactor) or sand (from the oxidative reactor). Error bars were calculated with Microsoft Excel® on triplicate reactions for each sample.

3. Results and Discussions

3.1 PCE removal and daughter products oxidation under continuous flow experiments

The anaerobic and the aerobic dechlorinating cultures were utilized as *inocula* for the two different membrane-less bioelectrochemical reactors in series, the first one reductive and the second one oxidative, which adopted an internal counterelectrode configuration. A reductive potential stimulated the dechlorination of high chlorinated compounds in the reductive reactor, while an oxidative potential of +1.4 V vs SHE allowed oxygen *in situ* production in the oxidative reactor. After the first potentiostatic condition in which the reductive reactor was operated at -450 mV vs SHE and the oxidative reactor at +1.4 V vs SHE, already described in [33], a less reductive condition was adopted in the reductive reactor by applying a -350 mV vs SHE potential. This condition was selected to minimize the energy consumption of the reductive step. The sequential reductive/oxidative process was operated with an average flow rate of 1.52 ± 0.04 L/d, that allowed an HRT of 5.5 days for the reductive reactor while a 2.6 days HRT for the oxidative reactor. The -350 mV vs SHE potential was also tested to evaluate a possible direct electron transfer between graphite granules and the dechlorinating microorganisms. Even if the -350 mV allowed for a theoretical hydrogen partial pressure of $8*10^{-3}$ atm sufficient to sustain the dechlorinating activity

(hydrogen partial pressure lower limit of 10^{-7} atm [9]), the proton reduction on the graphite results in a considerable overpotential. As expected, during the - 350 mV vs SHE potentiostatic condition, the current drops down to an average value of -3.1 ± 0.3 mA. Therefore, the average applied cell voltage was equal to -1.37 ± 0.02 V while the counter electrode potential was +0.77 ± 0.02 V vs SHE. During the -350 mV vs SHE condition, no PCE has been detected in the effluent of the reductive reactor being completely removed with an average rate of 16 ± 3 μmol/Ld corresponding to a removal efficiency of 100 ± 1% (Fig. 2a). Similarly, at the potentiostatic condition of - 450 mV vs SHE condition, neither TCE nor cis-DCE were detected in the effluent of the reductive reactor; only VC and Eth, with an average concentration of 41 ± 7 and 19 ± 4 μmol/L, respectively (Fig. 2b) were detected. At -350 mV vs SHE potentiostatic condition, reductive dechlorination allowed for an average coulombic efficiency of 22 ± 7 %, higher than the value obtained at -450 mV vs SHE.

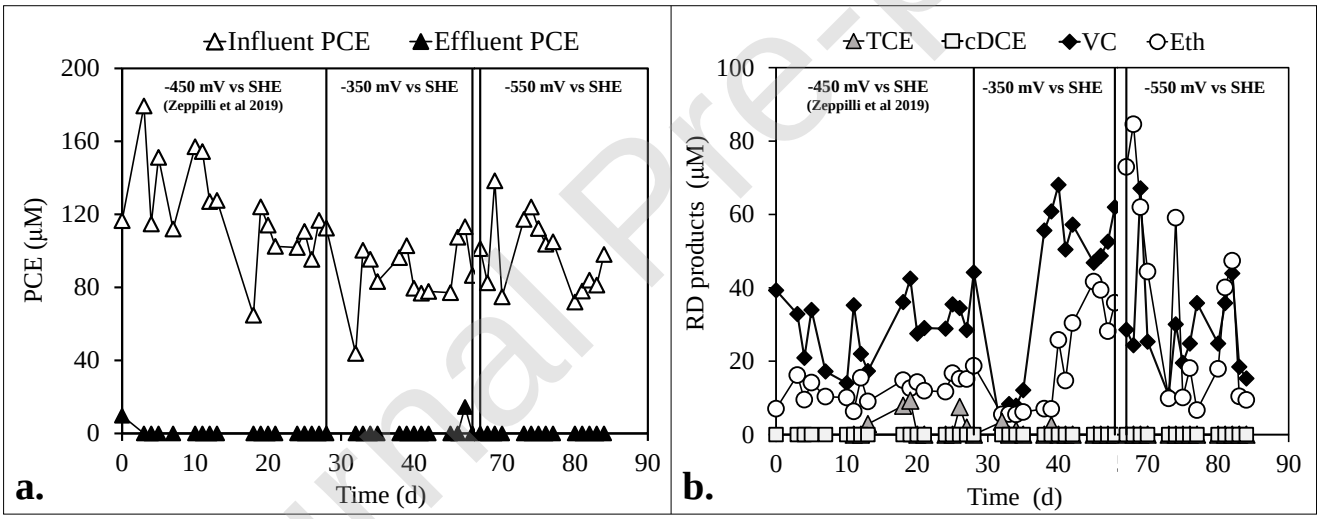


Figure 2. PCE concentration in the influent and effluent of the reductive reactor (a) and reductive dechlorination products in the effluent of the reductive reactor (b) during the different potentiostatic conditions.

In the reductive reactor, the main competitive mechanism for the reducing power consumption, was the methane production through the bioelectromethanogenesis. This process was considerably lowered at -350 mV vs SHE, which shown an average methane production rate of 16 ± 2 μmol/Ld.

Indeed, under the -350 mV vs SHE potentiostatic condition, methane production accounted for the consumption of the 8 ± 4 % of the current flowed in the circuit (methane coulombic efficiency), giving on the other hand an overall current recovery of 30 ± 4 % in the reductive reactor. Thus, a considerable amount of electric current resulted unrecovered and no other reduction products were detected to justify the current flowed in the circuit. The large unrecovered current can be reasonable justified by the utilization of the internal counter electrode that promoted electron loops, in which some species contained in the mineral medium (i.e. sulphide) or the molecular hydrogen, are oxidized in the oxidative part of the reactor (the internal counter electrode) and reduced in the external cathodic part of the reactor (external working electrode).

The oxidative reactor was fed directly by the reductive reactor effluent, the anode of the oxidative reactor was polarized at +1.4 V vs SHE, a potential that permits the oxygen evolution necessary for the oxidative dechlorination of PCE reduction daughter products [24]. As reported in figure 3, the oxidative reactor performed the almost complete removal of VC (Fig. 3a) and Eth (Fig. 3b) with average removal efficiency of 94 ± 2 % and 98 ± 5%, respectively. The current flowing in the reactor was 7.9 ± 0.3 mA and the oxidative coulombic efficiency for the VC and Eth resulted 8 ± 1 and 5 ± 1 %, in the respective cases. The methane produced in the reductive rector under all the potentiostatic conditions explored, were removed with an average removal efficiency of 51 ± 4 % in the oxidative reactor and a corresponding oxidative coulombic efficiency of 7 ± 2 %.

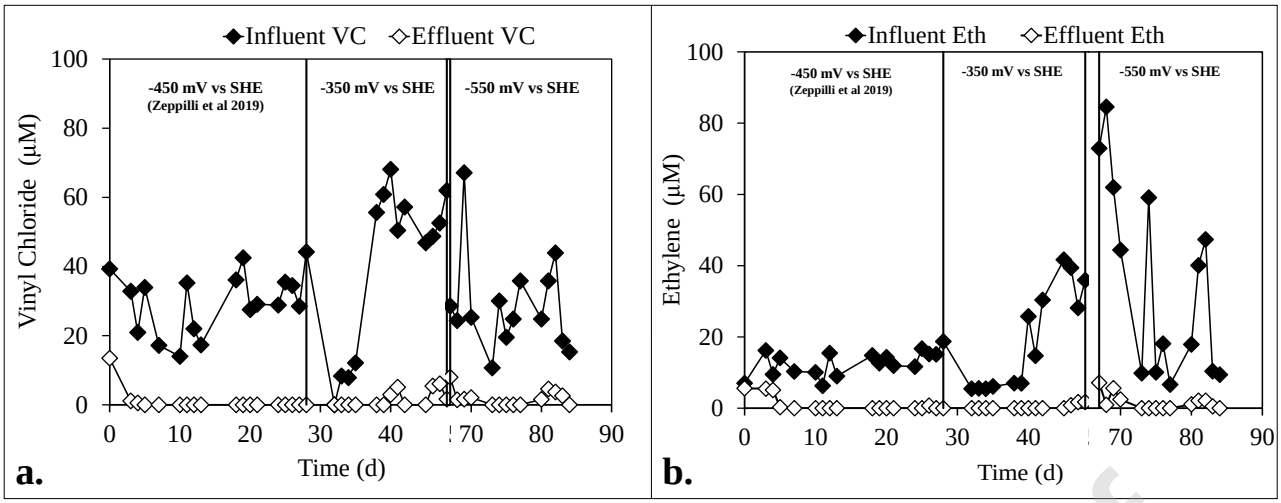


Figure 3. Removal of the VC (A) and Eth(B) in the oxidative reactor during the two potentiostatic condition explored.

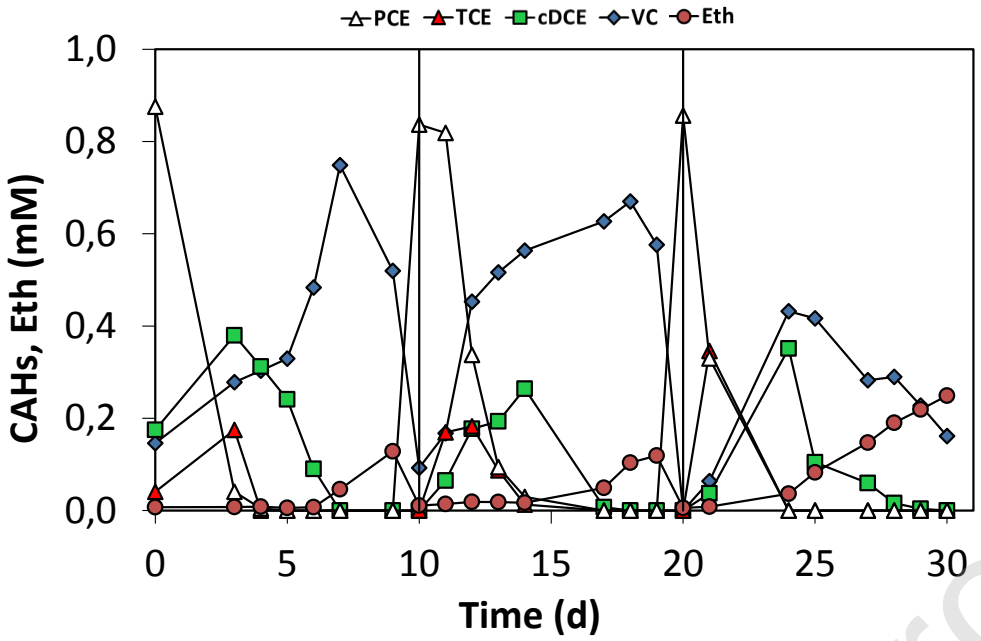
After the mild reductive potentiostatic condition, the cathodic potential of the reductive reactor was shifted to a potential of -550 mV vs SHE. This condition was adopted to test the possibility to increase the dechlorination rate boosting VC dechlorination to Eth. The flow rate utilized during the -550 mV vs SHE condition was 1.62 ± 0.06 L/d, resulting in a HRT of 5.1 and 1.9 days for the oxidative and reductive step, respectively. The shift of the reductive reactor potential to -550 mV vs SHE promoted a current increase which quickly stabilized at -15.1 ± 0.1 mA, moreover, the cell voltage and the anodic potential increased to -1.71 ± 0.07 V and + 0.99 ± 0.03V vs SHE, respectively. Under the -550 mV vs SHE condition, PCE was completely removed with a removal rate of 19 ± 1 µmol/Ld, corresponding to a removal efficiency of 100 ± 0.1 %. Moreover, VC and Eth were the RD products observed with an average concentration of 29 ± 4 µmol/L and 35 ± 7 µmol/L, respectively. Even if Eth concentration increase has been observed during the -550 mV vs SHE condition, the coulombic efficiency to sustain the RD drops down to an average value of 6 ± 1 %: this values is comparable to a coulombic efficiency of 5 ± 1 %, already reported in the first potentiostatic condition at -450 mV vs SHE. On the other hand, the higher reductive equivalent availability in the reductive reactor obtained by the utilization of the -550 mV vs SHE potential, promoted the methane production increase to 42 ± 5 µmol/Ld, accounting for the consumption of

the 21 ± 4 % of the overall current (i.e. methane coulombic efficiency). An overall coulombic efficiency of 26 ± 5 % has been reached in the reductive reactor at -550 mV vs SHE, which resulted in a similar global coulombic efficiency value obtained in the -350 mV vs SHE condition where the 30 % of the current was utilized for the reductive dechlorination and the methanogenesis reaction. As reported in table 1, under -550 mV vs SHE, the predominant electron-consuming mechanism resulted the methanogenesis with respect the reductive dechlorination, however, the lower coulombic efficiency reached by the reductive dechlorination didn't resulted in a loss of performance of the dechlorination rate, which remained almost constant during the two conditions with average values of 83 ± 4 and 99 ± 5 μeq/Ld, for the -350 and -550 mV vs SHE, respectively. Also, during the -550 mV vs SHE condition a large amount of unrecovered current was obtained in the reductive reactor. Indeed, due to the use of a permeable membrane, some parasitic reductive/oxidative reactions can occur between the working electrode and the counter electrode which shares the same electrolyte. The oxidative reactor, which received the reductive reactor effluent as feeding solution, was maintained polarized at +1.4 V vs SHE, thus, a similar performance was obtained with the previous explored run with the reductive reactor polarized at -350 mV vs SHE, obtaining an almost complete removal of the influent VC and Eth with removal efficiencies of 93 ± 2 % and 96 ± 1 %, respectively. Finally, also the methane produced in the reductive reactor was oxidized in the oxidative reactor with an average removal efficiency of 51 ± 5 %, which corresponded to a daily removal of 57 ± 11 μmol/Ld, a three times higher amount with respect to the previous -350 mV vs SHE condition in which 22 ± 3 μmol/Ld of methane were oxidized. Considering the average current flowing in the reactor, that was 7.5 ± 0.3 mA, the oxidative coulombic efficiencies for the VC, the Eth and methane resulted on average, 7 ± 2 %, 13 ± 1 %, and 16 ± 2 %, respectively. All the main parameters about the reductive and the oxidative reactor are reported in table 2.

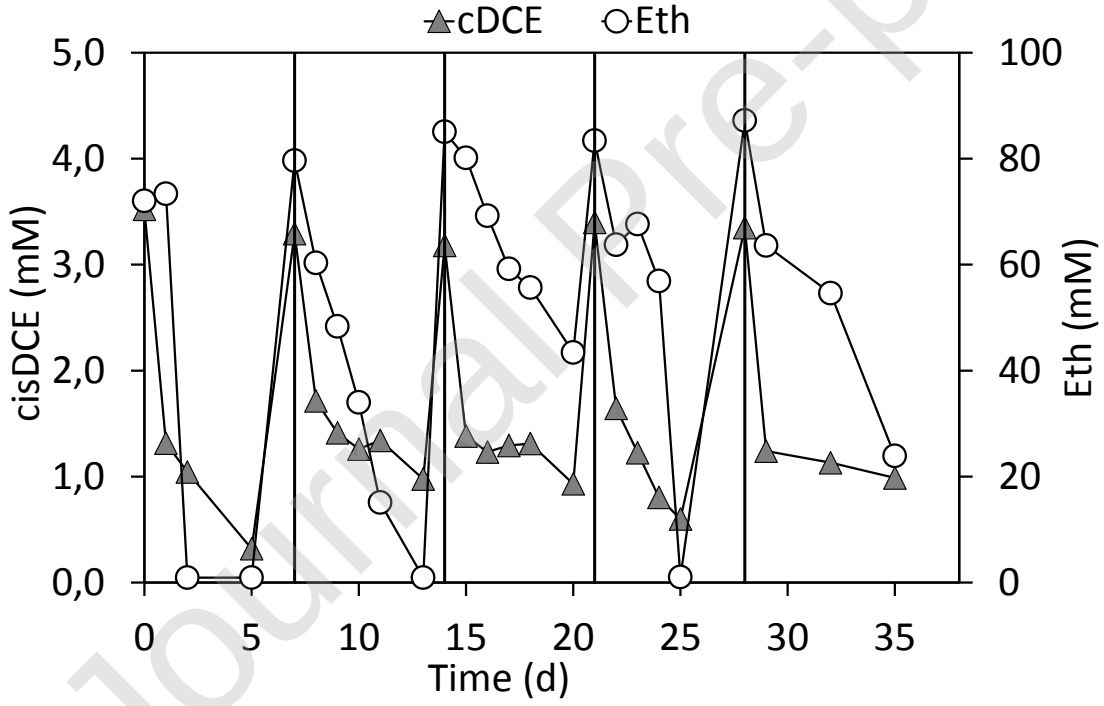
3.2 Microbial community characterization

3.2.1 Dechlorinating cultures characterization

An anaerobic PCE-dechlorinating mixed culture (hereafter cited as PCEed) was maintained under fill-and-draw conditions by 10-days cycles (HRT= 30 days) and was able to completely convert PCE to VC and Eth (Fig. 4a).



a.



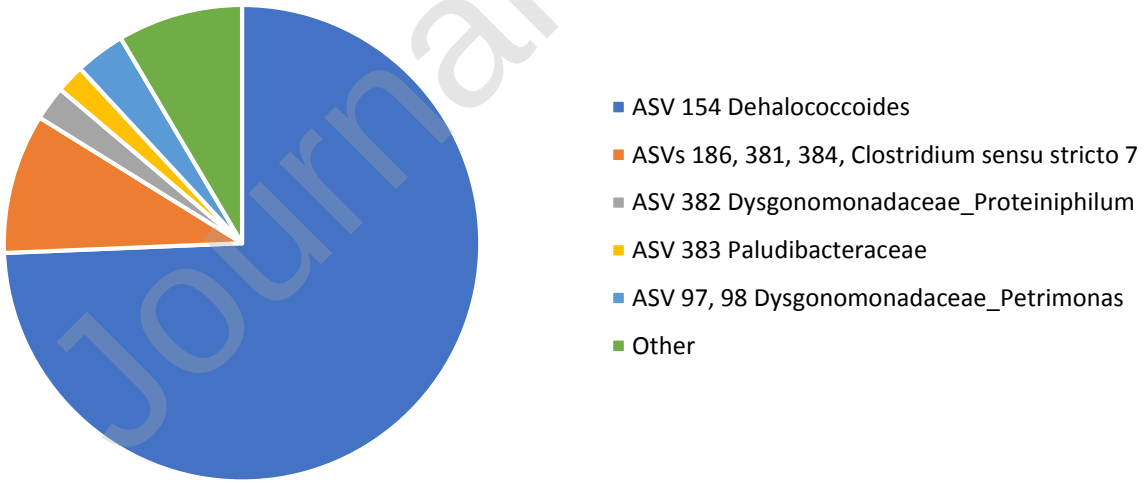
b.

Figure 4. Reductive dechlorination cycles of the PCE-dechlorinating enrichment culture used as inoculum for the reductive reactor (a). In the panel b, cycles of the aerobic dechlorinating culture utilized as inoculum of the oxidative reactor are reported (b).

In line with the RD performances, the anaerobic dechlorinating culture was highly selected and mostly composed by *D. mccartyi* (ASV 154) representing 76% of total reads (Fig. 5a). A total of 4.24E+10 *D. mccartyi* 16S rRNA gene copy number L^{-1} was estimated by qPCR. *D. mccartyi* strains carrying *tceA*, *bvcA* and *vcrA* RDase genes were found at concentration of 4.3E+08, 1.14E+10 and 1.06E+10 gene copy number L^{-1} respectively.

The co-metabolic oxidation of cis-DCE in presence of Eth is shown in Fig.4b. The aerobic dechlorinating culture was maintained in a fill-and-draw mode by 7-days cycles with an average HRT of 21. The aerobic dechlorinating culture reached dechlorinating capacity of 0.49 ± 0.08 and 12.79 ± 2.36 mmol/Ld for cis-DCE and Eth, respectively.

The aerobic culture used as inoculum for the oxidative reactor, was mostly composed by *Ignavibacteriales* (35%), *Anaerolineaceae* (13.5%) and *Saccharimonadaceae* (11.8%) members (Fig. 5b). Sequences affiliated to *Mycobacterium* were also found (ASVs 8, 10, 13, 15, 55 for a total of 4.4%). Members of this genus were previously shown to be able to metabolically or co-metabolically degrade cis-DCE [52].



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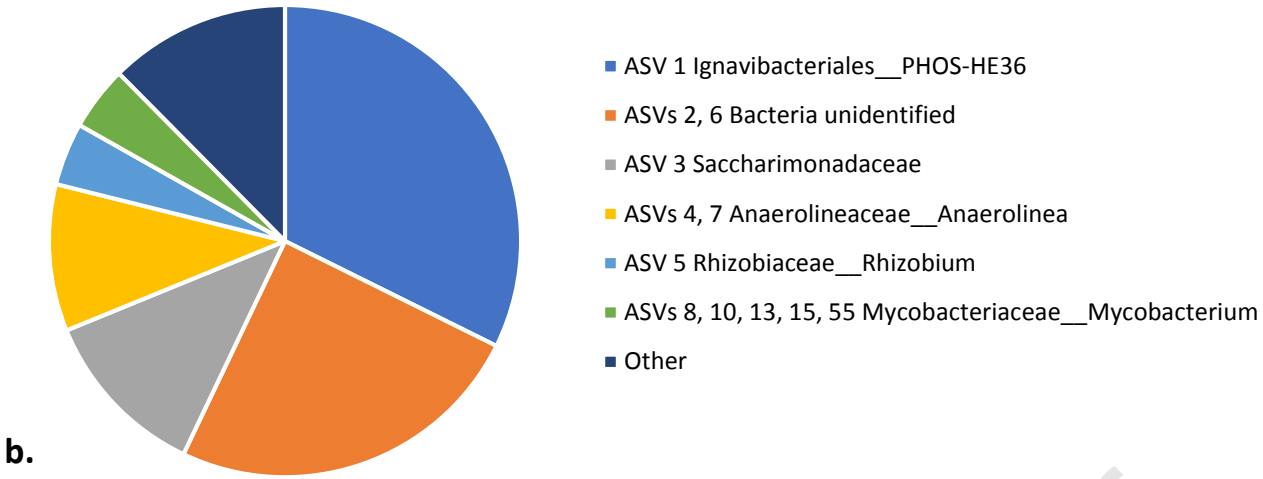


Figure 5. Microbiome composition of the enrichment cultures used as inocula for the reductive (PCEed culture, panel *a*) and oxidative (cis-DCE/Ethylene culture, panel *b*) reactors.

3.2.2 Microbial characterization of the MEC biofilm and planktonic communities

The overall bacterial composition and the abundance of the main biomarkers of the RD process, including *D. mccartyi* 16S rRNA and reductive dehalogenase genes *tceA*, *bvcA*, *vcrA* were analyzed at the end of the MEC operation (-550 mV vs SHE) on both biofilm and liquid samples taken from reductive and oxidative compartments.

3.2.2.1 *D. mccartyi* abundance and distribution in the bioelectrochemical reactors

Consistent with the observed PCE dechlorination, *D. mccartyi* was detected in all sampling points along the reductive reactor up to 5.61E+08±7.01E+07 gene copies L^{-1} of liquid medium (Fig. 6). RDase genes found were mostly associated to *tceA*-carrying strains. Although the main biomarkers of the RD process were found on the graphite biofilm, quantitative data also showed high abundances of planktonic *D. mccartyi* cells suggesting the occurrence at this potential (-550 mV vs SHE) of PCE reductive dechlorination probably sustained also by hydrogen generated at the cathode from water electrolysis.

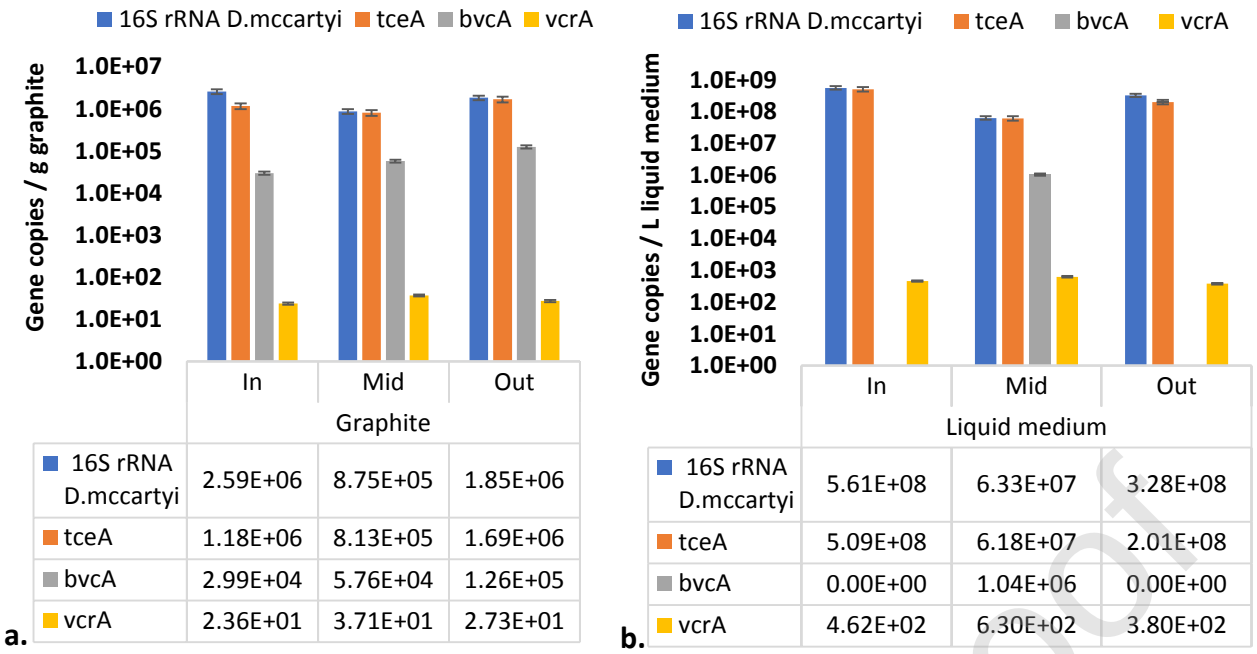


Figure 6. Quantification of D. mccartyi 16S rRNA and functional genes in the graphite biofilm and the liquid medium collected at different sampling points of the reductive reactor operating at -550 mV. Data are reported in Log scale.

3.2.2.2 Cathodic and anodic microbiome composition.

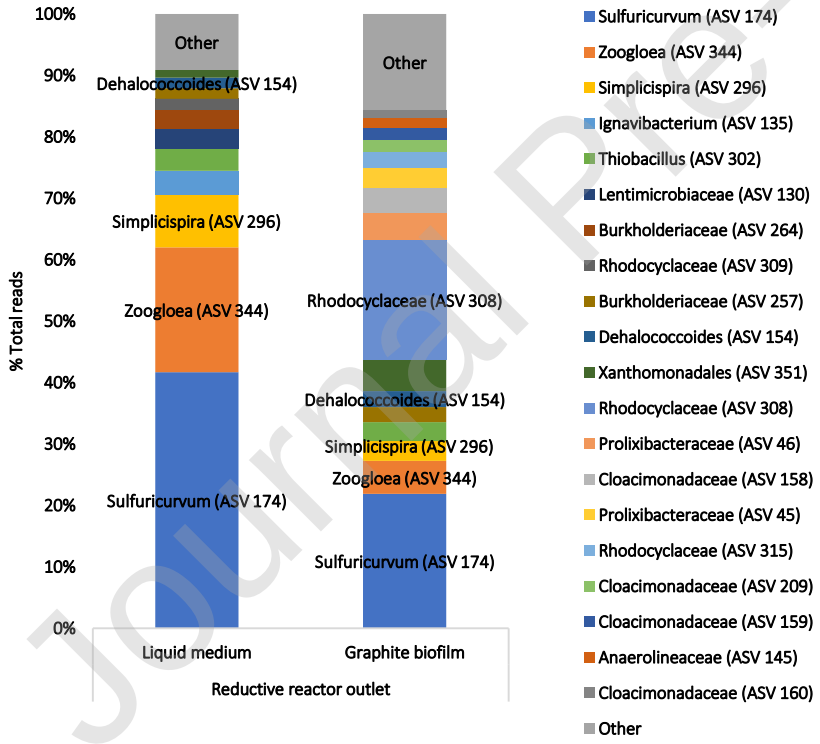
High-throughput sequencing showed the dominance of 11 ASVs in the liquid medium and 16 ASVs in the cathodic biofilm accounting for 90.9% and 84.34% of total reads, respectively (Fig. 7a). In terms of taxonomic composition, ASV 174 affiliated with *Sulfuricurvum kujiense* was found at high relative abundance both in the liquid and in the graphite biofilm (41.7% and 21.9% of total reads, respectively). *Sulfuricurvum kujiense* is a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium widely found in different groundwater environments (Kodama and Watanabe, 2014). It can utilize sulfide, elemental sulfur, thiosulfate and hydrogen as the electron donors and nitrate as the electron acceptor under anaerobic conditions. *S. kujiense* was shown in groundwater heavily polluted by 1,2-DCA during a treatability study [53] and associated with nitrate reduction in a membrane biofilm reactor treating perchlorate [54].

A sequence affiliated with *Rhodocyclaceae* family (ASV 308) was abundant (19.5% of total reads) in the graphite biofilm. Members of this family are commonly found in bioelectrochemical systems

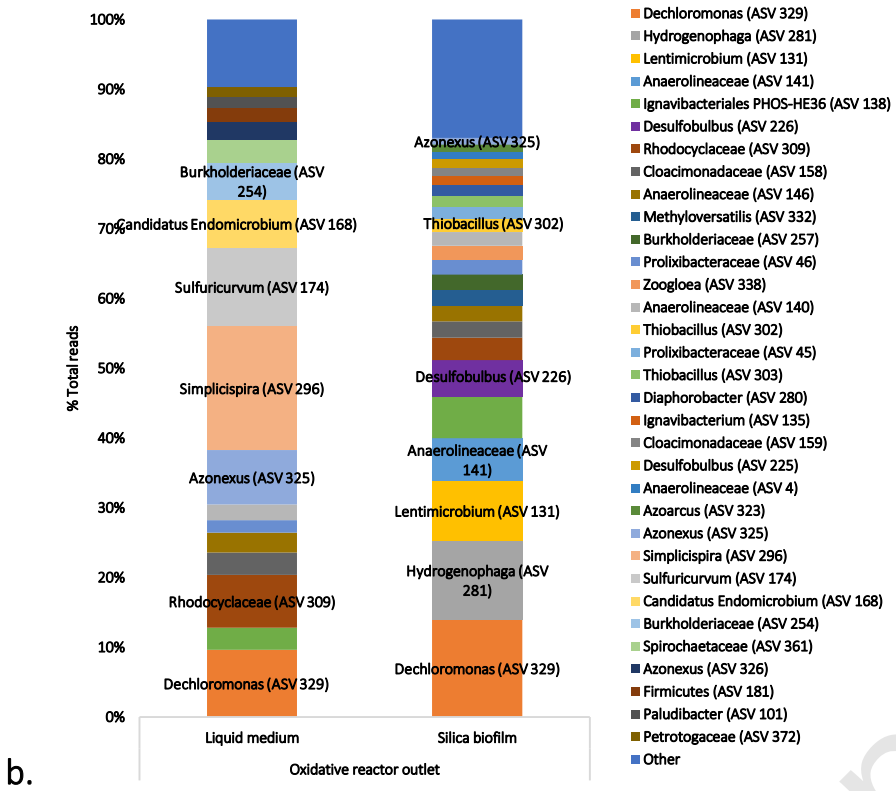
and many of them possess the ability to degrade halogenated compounds and behave as sulfate-, perchlorate- and iron-reducing bacteria with high tolerance to oxygen [55, 56].

Additionally, consistently with qPCR data, the sequence belonging to *D. maccartyi* (ASV 154) was found in the liquid at higher relative abundance (up to 11%) compared to graphite biofilm (up to 3.2%) suggesting the concomitant occurrence of an indirect bioelectrochemically-assisted reductive dechlorination.

The co-existence of multiple hydrogen oxidizing bacteria in addition to dechlorinators (e.g. nitrate-, perchlorate-and sulphate- reducing microorganisms) might explain the large amount of unrecovered current likely associated with parasitic reductive/oxidative reactions occurring between the working and the counter electrode.



a.



b.

Figure 7. Microbial composition of the reductive (a) and oxidative (b) reactor at the outlet sampling points, respectively. Samples analyzed are liquid medium and graphite (reductive reactor) or silica (oxidative reactor) biofilm.

The oxidative reactor showed higher biodiversity compared to the reductive unit (liquid H=2.8; biofilm H=3.4 compared to H=2.1 and H=2.9 of the liquid and biofilm of the reducing reactor respectively) (Fig 7b).

Planktonic microbial components were mostly represented by sequences affiliated with *Simplicispira* (ASV 296, 18%), *Sulfuricurvum* (ASV 174, 11.27%), *Dechloromonas* (ASV 329, 9.7%) and *Azonexus* (8%) while silica biofilm was mostly characterized by sequences affiliated with *Dechloromonas* (ASV 329, 14%) and *Hydrogenophaga* (ASV 281, 11.3%).

Previously described bacteria able to degrade low chlorinated ethenes (e.g. *Polaromonas;*[52]) were not found in the oxidative reactor at the end of operation, including those microorganisms (*Mycobacterium* ASVs 8, 10, 13, 15, 55), present in the aerobic cis-DCE/ethene oxidizing inoculum.

Dechloromonas ASV 329 was one of the main planktonic and biofilm components (Fig. 7b). Members of this genus were shown to perform bioelectrochemical perchlorate reduction [57], and often associated to other functional groups such as denitrifiers [58-60]. ASVs affiliated with *Azonexus* (ASV 325) and *Thiobacillus* (ASV 302) were also found in the oxidative reactor (Fig. 7b): *Azonexus* is an aerobic denitrifying microorganism that uses oxygen and nitrate as oxidizing agents [61], while *Thiobacillus* is an autotrophic denitrifier that uses hydrogen or reduced sulfur as electron donor [62]. *Hydrogenophaga* (ASV 281, 11.3%) was also found as a component of the biofilm of the oxidative reactor. Even though members of this genus behave mainly as hydrogenotrophs and are not usually associated with dehalogenation, they were often found in chlorinated solvents contaminated groundwaters [63-65].

3.3 Reductive reactor mass balance

The daily amount of removed PCE in the reductive reactor has been evaluated also by the mass balance of the chlorinated species. The sum of the less chlorinated products has been compared with the daily PCE removal in the reductive reactor. During the -350 mv vs SHE potentiostatic condition, a mass recovery of 70 ± 20 % has been obtained, which indicates that the main mechanism that occurs in the reactor can be quantified. During the -550 mV vs SHE condition, the mass balance recovery resulted similar to the -350 mV vs SHE condition, with an average value of 65 ± 10 %, similar to the one reported for the -350 mV vs SHE condition. The mass balance recovery indicates that a consistent fraction of the removed PCE is not recovered as a dechlorination product, thus, other possible removal mechanisms can be assumed. More in details, the presence of the internal counter electrode in the reductive reactor can promote aerobic/oxidative pathways on RD low chlorinated byprodicts such as cis DCE and VC. Moreover, as reported in the literature possible PCE co-metabolic oxidative pathways have been identified in presence of several hydrocarbons including methane [66].

3.4 Energetic evaluation of the process

The energy consumption of each potentiostatic run of the sequential bioelectrochemical process has been evaluated from the electrical energy consumed by the two reactors, the following energy consumption did not consider any pumping or other facilities energy consumption. The energy consumption of each reactor for the different runs have been evaluated with respect to the volumetric daily treated water, expressed as kWh/m^3. As reported in figure 8 a lower energy consumption has been reported during the -350mV vs SHE thanks to the mild reductive potential adopted which significantly reduced the current flowed in the circuit. The reductive reactor energy consumption contributes for the 19 and 63 % of the overall energy consumption of the process in the -350 mV and - 550 mV vs SHE condition, while the oxidative reactor maintained a similar energy consumption throughout the two potentiostatic conditions.

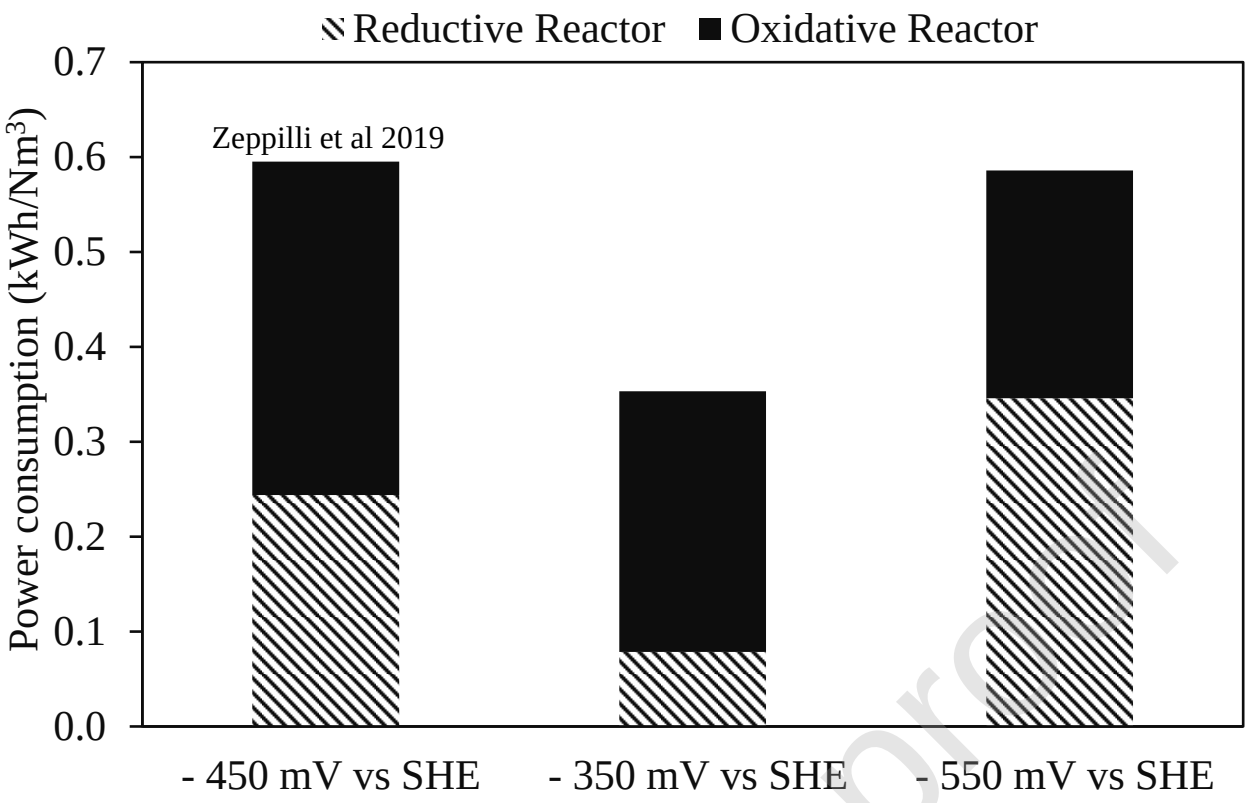


Figure 8. Energy consumption of the sequential process expressed as kWh/m^3 of treated contaminated synthetic solution

4. Conclusions

The sequential reductive/oxidative bioelectrochemical process allowed for the complete removal of PCE from the synthetic feeding solution, with average PCE removal efficiencies of 100 ± 1% and 97 ± 1 % during the operation at -350 and -550 mV vs SHE, respectively. The reductive reactor resulted capable of the complete transformation of PCE into VC and Eth as by-products of the reductive dechlorination, moreover, through the presence of the oxidative reactor more than 94 ± 1% and 92 ± 2% of the VC produced by the reductive step was removed during the -350 and -550 mV vs SHE runs through the stimulation of the aerobic dechlorination of low chlorinated intermediate. The adoption of a mild reductive potential of -350 mV vs SHE allowed for the increase of the coulombic efficiency for the reductive dechlorination which accounted for the 22 ± 7

%, while, by using the -550 mV vs SHE potential, the coulombic efficiency for the reductive dechlorination drops down to 6 ± 1 %. It is interesting to underline that even if the coulombic efficiency of the RD resulted reduced by the reductive potential increase, the PCE removal performances and the RD rate were not affected: PCE removal efficiency remained almost complete while the RD rate increased from 83 ± 4 to 99 ± 5 μeq/Ld. The reductive potential increase in the reductive reactor resulted in a higher average current which stimulated the competitive mechanisms like the methanogenesis as well as the energy consumption of the whole process.

High throughput sequencing highlighted multiple microbial functional traits likely associated to the concomitant presence of different redox niches in each MEC compartment. The system configuration promotes the exchange of ionic species between the electrode and the counter electrode of each reactor thus allowing the co-existence of both reducing and oxidative reactions. This peculiarity may explain the observed establishment of a community of hydrogen oxidizing bacteria capable of nitrate-, perchlorate-and sulphate- reduction. PCE reductive dechlorination was led by *D. mccartyi* found at high concentration in the reducing reactor. Even more heterogeneous bacterial consortium was observed in the oxidative reactor at the end of the MEC operation. Interestingly, no microorganisms previously found associated to ethylene/vinyl chloride oxidation processes were found. This finding might be also in this case linked to the peculiar system configuration supporting microorganisms able to grow at low dissolved oxygen concentration and to use multiple oxidizing agents.

Author Contributions

Marco Zeppilli: Preparation of the original Draft, Validation, Investigation

Bruna Matturro: Preparation of the original Draft; Investigation, Validation

Edoardo Dell'Armi: Investigation, Validation

Lorenzo Cristiani: Investigation, Validation

Simona Rossetti: Supervision

Marco Petrangeli Papini: Supervision

Mauro Majone: Supervision, Funding Acquisition.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Main performance of the sequential Reductive /Oxidative process in the two different potentiostatic condition explored

Reductive reactor potential	**-450 mV vs SHE [33]**	**-350 mV vs SHE**	**-550 mV vs SHE**
PCE removal rate (µmol/Ld)	23 ± 2	16 ± 3	19 ± 1
PCE removal efficiency (%)	100 ± 1	100 ± 1	97 ± 1
RD rate (µeq/Ld)	60 ± 3	83 ± 4	99 ± 5
Coulombic Efficiency RD (%)	5 ± 1	22 ± 7	6 ± 1
Coulombic Efficiency CH_4 (%)	22 ± 4	8 ± 4	20 ± 5
Oxidative reactor potential	+1.4 V vs SHE		
VC removal efficiency (%)	100 ± 2	94 ± 1	92 ± 2
Ethylene removal efficiency (%)	92 ± 5	98 ± 1	96 ± 1
Coulombic efficiency Oxi VC-Eth(%)	7 ± 2	13 ± 1	16 ± 2
Coulombic efficiency Oxi CH_4(%)	8 ± 1	7 ± 2	21 ± 1