



## Bioelectrochemically-assisted degradation of chloroform by a co-culture of *Dehalobacter* and *Dehalobacterium*

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### ABSTRACT

Using bioelectrochemical systems (BESs) to provide electrochemically generated hydrogen is a promising technology to provide electron donors for reductive dechlorination by organohalide-respiring bacteria. In this study, we inoculated two syntrophic dechlorinating cultures containing *Dehalobacter* and *Dehalobacterium* to sequentially transform chloroform (CF) to acetate in a BES using a graphite fiber brush as the electrode. In this co-culture, *Dehalobacter* transformed CF to stoichiometric amounts of dichloromethane (DCM) via organohalide respiration, whereas the *Dehalobacterium*-containing culture converted DCM to acetate via fermentation. BES were initially inoculated with *Dehalobacter*, and sequential cathodic potentials of  $-0.6$ ,  $-0.7$ , and  $-0.8$  V were poised after consuming three CF doses ( $500 \mu\text{M}$ ) per each potential during a time-span of 83 days. At the end of this period, the accumulated DCM was degraded in the following seven days after the inoculation of *Dehalobacterium*. At this point, four consecutive amendments of CF at increasing concentrations of 200, 400, 600, and  $800 \mu\text{M}$  were sequentially transformed by the combined degradation activity of *Dehalobacter* and *Dehalobacterium*. The *Dehalobacter* 16S rRNA gene copies increased four orders of magnitude during the whole period. The coulombic efficiencies associated with the degradation of CF reached values  $> 60\%$  at a cathodic potential of  $-0.8$  V when the degradation rate of CF achieved the highest values. This study shows the advantages of combining syntrophic bacteria to fully detoxify chlorinated compounds in BESs and further expands the use of this technology for treating water bodies impacted with pollutants.

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### 1. Introduction

Chloroform (CF) is a chlorinated compound usually employed as a solvent and in the chemical synthesis of fluorocarbons [1]. Due to improper handling and disposal practices, CF can be discharged into the subsurface environment, which constitutes a health risk to humans. According to the 2019 ATSDR Substance Priority List, CF is ranked 11th (out of 275) based on a combination of frequency, toxicity and potential for human exposure [2].

Due to the typically anoxic conditions of contaminated groundwater, applying anaerobic bioremediation treatments catalyzed by organohalide-respiring bacteria (OHRB) is a potential solution to remediate sites impacted with chlorinated pollutants [3,4]. To date, bacteria belonging to the genera *Dehalobacter* and *Desulfitobacterium* are the only OHRB described using CF as a terminal electron acceptor, dechlorinating CF to predominantly dichloromethane (DCM) [5,6]. *Dehalobacter* is a strict hydrogenotroph; therefore, an external and continuous supply of

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hydrogen in contaminated groundwaters is required to promote the dechlorination of CF by this genus [7]. The direct injection of fermentable organic substrates, such as lactate, ethanol and molasses, is the common procedure to deliver hydrogen for enhanced anaerobic bioremediation. Still, some disadvantages of this procedure include the rapid degradation of the soluble substrates and the subsequent need for repetitive reinjections that could lower the pH in groundwater [8].

In the last years, intensive research with bioelectrochemical systems (BESs) has been performed as a novel strategy to supply hydrogen as the electron donor to promote anaerobic reductive dechlorination of chlorinated pollutants in groundwaters [9–14]. BESs consist of two-chamber devices where an oxidation reaction occurs in the anode, and a reduction reaction occurs in the cathode, with one or both reactions catalyzed by bacteria. BESs aiming to produce hydrogen in cathodes are not thermodynamically spontaneous; thus, an additional voltage is required to drive the process. In previous studies, a correlation has been observed between more negative cathodic potentials and hydrogen production, followed by a noticeable increase in the degradation rates of dechlorinating bacteria [9,11]. However, this increase has been at the cost of an excess of hydrogen production and a concomitant decrease in the coulombic efficiency of the process. To date, the OHRB studied in BESs belonged to the genera *Dehalococcoides*, *Geobacter*, and *Dehalogenimonas* and degraded contaminants as 1,2-dichloroethane, tetrachloroethene, trichloroethene, *cis*-dichloroethene, or 2,3,4,5-tetrachlorobiphenyl [9,13,15–18].

To detoxify CF-impacted sites, the next step after the dechlorination of CF by *Dehalobacter* is the remediation of DCM, which is still a toxic compound of environmental concern [19,20]. To date, only two DCM fermentative bacteria, all belonging to the Peptococcaceae family, have been identified to ferment DCM: “*Candidatus Formimonas warabiya*” (formerly referred to as strain DCMF) and *Dehalobacterium formicoaceticum* [21–24]. The fermentation of DCM by *D. formicoaceticum* produces acetate and formate, which are environmentally friendly products [25–27]. Additionally, “*Candidatus Dichloromethanomonas elyuquensis*” was recently found to completely mineralize DCM to H<sub>2</sub> and CO<sub>2</sub> [28,29].

The study aims to assess the feasibility of coupling the dechlorination potential of two mixed cultures, with one containing *Dehalobacter* and the other *Dehalobacterium*, in the cathodic chamber of a BES to transform CF into non-toxic final products for the first time. The sequential dechlorination proceeds through the organohalide respiration of CF to DCM via *Dehalobacter*, followed by the fermentation of DCM to acetate and formate via *Dehalobacterium*. The application of sequentially decreasing cathodic potentials was tested to balance the electrochemical production of hydrogen to the growth of the *Dehalobacter* population to maximize at the same time the degradation rates and the coulombic efficiency of the process. This work expands the number of bacteria and contaminants degraded utilizing BES-based technologies while combining for the first time the degradation of CF in a two-step dechlorination process in a single BES.

## 2. Materials and methods

### 2.1. Bioelectrochemical cells and operation

The bioelectrochemical system used in this study was described previously [18]. In brief, it consisted of two glass vessels with 165 mL of total volume separated by a cation-exchange membrane (CMI-7000, Membranes International INC, USA) with an aperture diameter of 4 cm. A titanium sheet and a graphite brush were used as the anodic and cathodic electrodes, respectively. Mixing of the liquid medium of each vessel was performed by a magnetic stirrer.

The system was sealed with butyl rubber septa and aluminum crimp caps, allowing liquid samples to be added and withdrawn from the anodic and cathodic vessels.

### 2.2. Cultivation of the *Dehalobacter* and *Dehalobacterium*-containing cultures in serum bottles

The enriched cultures containing *Dehalobacter* used in this study were derived from groundwater contaminated with CF [30]. The inoculum for the *Dehalobacterium*-containing consortia was obtained from slurry samples of a membrane bioreactor from a wastewater plant and subsequently enriched in the laboratory [25]. The *Dehalobacter* sp. contained in the culture, denominated *Dehalobacter* sp. strain 8 M, transformed CF to DCM via organohalide respiration [30], whereas the *Dehalobacterium*-containing culture transformed DCM to acetate in a three-step process [26]. Both cultures were maintained in 100 mL glass bottles containing 65 mL of reduced bicarbonate-buffered medium (pH = 7) as described elsewhere [31]. In brief, the growth medium of *Dehalobacterium* contained trace elements, tungsten (22.8 μM), selenium (24.2 μM), vitamins, yeast extract (200 mg L<sup>-1</sup>), Na<sub>2</sub>S·9H<sub>2</sub>O, and L-cysteine (0.2 mM each) as reducing agents and sodium bicarbonate (12 mM) as a buffering agent. This same medium was used for the *Dehalobacter* microcosms but included sodium acetate (5 mM) as an additional carbon source. Butyl rubber septa and aluminum crimp caps were used to seal the bottles. *Dehalobacter* cultures were gassed with N<sub>2</sub>/CO<sub>2</sub> (4:1 at 0.2 bar of overpressure) and H<sub>2</sub> (0.4 bar of overpressure), while *Dehalobacterium* cultures were gassed with N<sub>2</sub> (0.4 bar of overpressure).

### 2.3. Operation of BESs

Cathodic and anodic vessels were filled with 130 mL of the anaerobic medium of *Dehalobacter* described above. Cathodic compartments were initially spiked with 500 μM CF and inoculated with 3 mL of the *Dehalobacter*-containing culture (concentration of *Dehalobacter*  $\sim 9.38 \times 10^4 \pm 2.38 \times 10^4$  16S rRNA gene copies per mL). Biotic open circuit BESs without electrodes were included as controls to assess biodegradation of CF without electrochemically generated H<sub>2</sub>. In addition, abiotic BESs with the cathodes poised at certain potentials were included to assess that CF and DCM were not transformed electrochemically. The cathodic potentials were initially adjusted to -0.6 V vs. Standard Hydrogen Electrode (SHE) against an Ag/AgCl reference electrode (RE-1B, BAS Inc., +197 mV vs. SHE) by applying a potentiostatic control using a power source (Quad Potentiostat, Whistonbrook Software). All the potential values mentioned are V vs. Standard Hydrogen Electrode (SHE) unless otherwise stated.

Each cell configuration was operated with three parallel replicates. CF was re-spiked with 500 μM whenever depleted. After consuming three CF amendments at -0.6 V, the cathodic potential was sequentially reduced to -0.7 and -0.8 V after degrading three CF amendments at each potential. Afterward, the systems were inoculated with 6 mL of the *Dehalobacterium*-containing culture to proceed with the consumption of the DCM produced. At this point, several CF amendments of increasing concentration were periodically added to degrade both CF and DCM sequentially. During the whole BES operation, 1-mL liquid samples were periodically taken to monitor pH, and it was adjusted to pH 7 by adding HCl from an anaerobic stock solution (1 M). The liquid medium removed for analytical measurements (see section 2.5) was replaced with the addition of a fresh medium. The hydraulic retention time was longer than the length of the experiments, so the BESs were considered to operate in fed-batch mode.

#### 2.4. Coulombic efficiency and energy consumption

The coulombic efficiency (CE) after the consumption of each CF amendment was calculated using equation (1):

$$CE = \frac{2 \cdot V \cdot F \cdot [CF]_{deg}}{\int I(t) dt} \quad (1)$$

where 2 is the number of electrons required to dechlorinate a molecule of CF to DCM, V is the liquid volume at the cathodic vessel (L), F is Faraday's constant (96,485 A s mol<sup>-1</sup>), [CF]<sub>deg</sub> is the concentration of CF degraded (M) and ∫I(t)dt is the integration of the monitored intensity throughout the experimental time (A s).

Also, the energetic input (EI) required for each mol of CF degraded at each experiment was calculated as follows:

$$EI = \frac{V_A \cdot \int I(t) dt}{V \cdot [CF]_{deg}} \quad (2)$$

where V<sub>A</sub> is the applied voltage in the system (V).

#### 2.5. Analytical methods

The concentrations of CF and DCM were determined by transferring 1 mL of the liquid medium from the cathode to a 10 mL vial sealed with Teflon-coated stoppers that contained 5.5 mL of deionized water. The vials were placed in a headspace sampler (Agilent 7964) and heated for 15 min to 85 °C. Automatically, 1 mL headspace gas sample from the vials was injected into an Agilent 6890 N gas chromatograph provided with an Agilent DB-624 column (30 m × 0.32 mm with 0.25 μm film thickness) and a flame ionization detector following a method previously described [25]. The calibration of both compounds was based on aqueous external standards with the same liquid and gas volumes as to the experimental BES. Details for the calculation of CF and DCM concentrations are provided in the Supplementary Information (SMM1). Hydrogen concentration was measured on 0.1-mL headspace samples from the cathode using an Agilent 7820A GC equipped with a thermal conductivity detector as previously described [26].

#### 2.6. Real-time PCR (qPCR)

Liquid samples (3 mL) were obtained from the cathode vessel at different time points, centrifuged at 9000 g for 20 min and extracted as previously described [18]. An experimental procedure quantifying the 16S rRNA gene copies of *Dehalobacter* by qPCR previously used in another study was employed [30]. The employed calibration curve was depicted in Fig. S1.

#### 2.7. Cell growth kinetic calculations

The maximum specific growth rates (μ, d<sup>-1</sup>) were calculated by selecting the exponential growth phase period according to the 16S rRNA gene concentrations of *Dehalobacter* obtained by qPCR and using the following equation:

$$\mu = \frac{1}{X} \frac{dX}{dt} \quad (3)$$

where X is the 16S rRNA gene copies concentration in the BESs (16S rRNA gene copies per mL).

The biomass growth yield (Y, 16S rRNA gene copies generated per μmol CF degraded) was calculated as follows:

$$Y = \frac{X_{gen}}{CF_{deg}} \quad (4)$$

where X<sub>gen</sub> is the increase of *Dehalobacter* 16S rRNA gene copies and CF<sub>deg</sub> is the μmol of CF degraded during a certain time period.

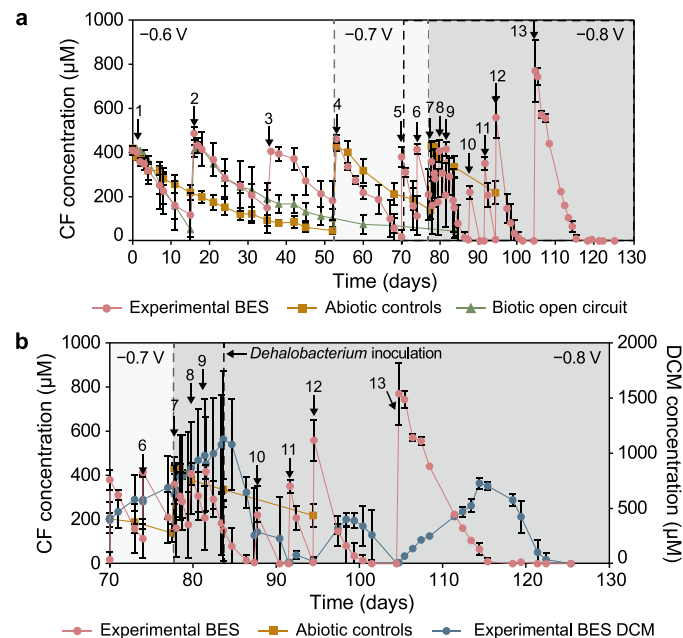
The maximum specific CF utilization rate by *Dehalobacter* (q, μmol CF degraded per 16S rRNA gene copy generated per day) can be calculated as follows:

$$q = \frac{\mu}{Y} \quad (5)$$

### 3. Results and discussion

#### 3.1. Effect of the cathodic potential on CF dechlorination by *Dehalobacter*

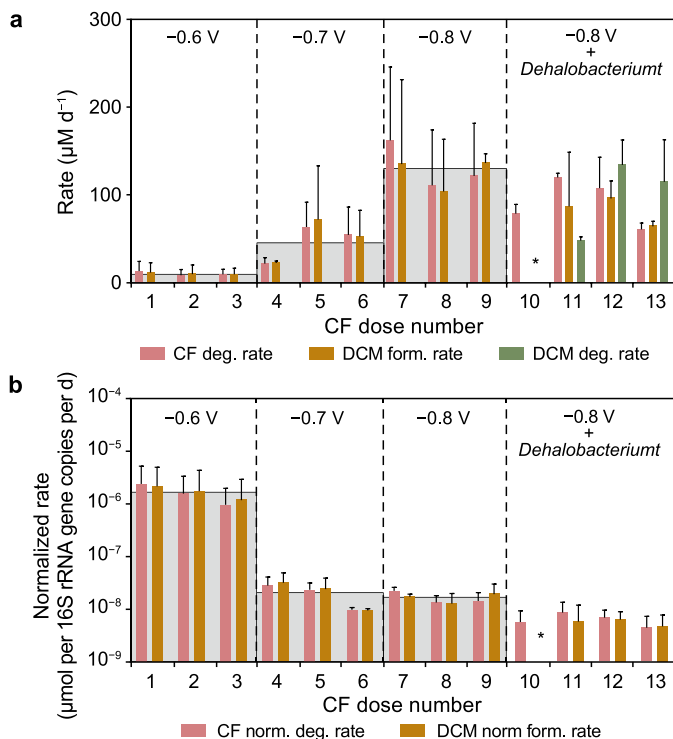
BESs inoculated with *Dehalobacter* successfully dechlorinate several doses of CF operating at sequential cathodic potentials of -0.6, -0.7, and -0.8 V (Fig. 1). CF was not bioelectrochemically transformed in BESs operating at the non-hydrogen producing potential of -0.3 V, discarding direct electron transfer from the graphite brush electrode surface to *Dehalobacter* (Fig. S2). In this set of experiments, neither the dechlorination of CF nor the continuous production of DCM was observed in three different set-ups: a conventional BES system, abiotic controls and biotic open circuit controls during 20 days of operation (Fig. S2). The CF concentration steadily decreased for all the experimental replicates at a similar rate. The measured residual concentrations of DCM (less than 60 μM) did not amount to the total CF lost and were probably caused by traces of hydrogen. Therefore, CF decrease could only be explained by its diffusion through the cationic exchange membrane and not any kind of electrochemical or biological degradation.



**Fig. 1.** a, Degradation profile of CF in experimental BES inoculated with *Dehalobacter* and poised with a cathodic potential, abiotic controls, and biotic open circuits. The time lapse between days 70 and 130 was amplified in subpanel b for best readability of measurements. Changes in the concentration of DCM were only depicted in the sub-panel b with blue circles. Numbers indicate the number of CF amendments in the experimental BES. Values plotted are the average of triplicate BES, and error bars indicate standard deviation.

The dechlorination of CF was almost immediately detected when the cathode was poised at  $-0.6$  V, with the concomitant production of DCM (Fig. 1). The BESs were sequentially operated at  $-0.6$ ,  $-0.7$ , and  $-0.8$  V after the consumption of three doses of CF per cathodic potential (Fig. 1). The decrease of the poised cathodic potential showed a significant increase in both the CF degradation rate and the DCM production rate. There were statistical differences ( $p$ -value  $< 0.02$ ) for all three potentials, as shown with a Brown-Forsythe and Welch ANOVA test for multiple comparisons. As observed in Fig. 2a, CF degradation and DCM production rates increased one order of magnitude when moving from  $-0.6$  V to  $-0.8$  V, reaching values of  $10.3 \pm 7.3$  and  $10.1 \pm 8.5$   $\mu\text{M d}^{-1}$  (at  $-0.6$  V cathodic potential) and  $131.6 \pm 64.9$  and  $125.2 \pm 58.9$   $\mu\text{M d}^{-1}$  (at  $-0.8$  V cathodic potential), respectively (Fig. 2a).

DCM production rates were almost identical to CF degradation rates indicating that molar balance can be considered closed (Fig. 2a). The low basal CF degradation rate obtained in the abiotic controls was  $2.82 \pm 0.47$ ,  $2.92 \pm 1.19$ , and  $2.51 \pm 1.90$   $\mu\text{M d}^{-1}$  for cathodic potentials of  $-0.6$ ,  $-0.7$  and  $-0.8$  V, respectively, and confirmed that CF degradation was biologically-mediated in BESs. On the other hand, the first dose of CF was rapidly depleted in the biotic open circuit controls (Fig. 1), but the second amendment of CF was not completely degraded after  $\sim 70$  days, indicating that the first amendment was probably consumed linked to the residual hydrogen dissolved in the inoculum. Dechlorination of CF was not inhibited by the DCM accumulated in the cathode, and this is in accordance with previous studies showing an inhibitory threshold of  $2,500$   $\mu\text{M}$  DCM for *Dehalobacter* [1].



**Fig. 2.** Degradation rate of CF and production rate of DCM (a) and CF degradation rate and DCM production rate normalized by the 16S rRNA gene copies (b) for each dose of CF consumed in experimental BESs. The numbers refer to the dose number depicted in Fig. 1. The grey background represents the average CF degradation rate (a) and average CF normalized degradation rate (b) for each applied potential. Asterisks indicate CF doses where the formation of DCM accumulation was not detected due to a fast degradation by *Dehalobacterium*.

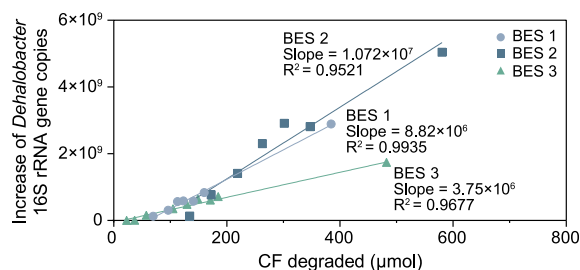
After the degradation of nine CF doses, the experimental BESs were inoculated with an enriched culture containing *Dehalobacterium* on day 83 of operation. At this point, the DCM accumulated in the BES was  $1,128 \pm 616$   $\mu\text{M}$ . DCM was completely degraded in the following seven days at a degradation rate of  $481 \pm 141$   $\mu\text{M d}^{-1}$  (Fig. 1). Once DCM was degraded, CF was spiked at increasing concentrations of approximately 200, 400, 600 and 800  $\mu\text{M}$  to couple both *Dehalobacter* and *Dehalobacterium* dechlorination capabilities (Fig. 1). At lower concentrations of CF (200 and 400  $\mu\text{M}$ ), the degradation of CF and DCM proceeded rapidly (Fig. 1, arrow numbers 10 and 11). However, when CF was added at higher concentrations (600 and 800  $\mu\text{M}$ ), DCM accumulated (Fig. 1, arrow numbers 12 and 13), and degradation only started after CF was almost exhausted. This agrees with a recent study showing that CF at  $\sim 800$   $\mu\text{M}$  inhibited the DCM degradation activity of *Dehalobacterium*, and the activity could be recovered when the culture was transferred to a CF-free medium [26]. The production of acetate derived from DCM fermentation was not quantified because it was masked by the high concentrations of acetate in the medium and used as a carbon source by *Dehalobacter* (Fig. S3).

### 3.2. *Dehalobacter* growth and degradation kinetics in BESs

Periodic samples from the cathodic vessel were withdrawn, and the DNA was extracted to quantify the increase of 16S rDNA gene copies during the BES operation period (Fig. S4). *Dehalobacter* 16S rRNA gene copies increased over four orders of magnitude from an initial concentration of  $4.33 \times 10^3 \pm 1.00 \times 10^3$  to  $1.96 \times 10^7 \pm 1.01 \times 10^7$  16S rRNA genes per mL after 126 days of operation (Fig. S4). Assuming five 16S rRNA gene copies per genome [32], a cell density of  $3.92 \times 10^6 \pm 2.02 \times 10^6$  *Dehalobacter* cells per mL was obtained.

The growth yield was calculated for each BES set-up by determining the changes in *Dehalobacter* 16S rDNA gene copies produced during the consumption of each CF dose (Fig. 3). The average growth yield was  $7.76 \times 10^5 \pm 3.60 \times 10^6$  *Dehalobacter* 16S rRNA gene per  $\mu\text{mol}$  of CF degraded (or *Dehalobacter* 16S rRNA gene per  $\mu\text{mol Cl}^-$ ). The average yield values reported in this study are in the same order as the magnitude of those previously reported for other *Dehalobacter* sp. respiring CF ( $3.6 \times 10^6 \pm 2.6 \times 10^6$  and  $2.5 \times 10^7 \pm 0.9 \times 10^7$  16S rRNA gene copies per  $\mu\text{mol Cl}^-$ ) [1,33]. The obtained growth yield is one order of magnitude lower than the one obtained for the CF-respiring *Desulfitobacterium* sp. strain PR ( $1.16 \times 10^7 \pm 0.16 \times 10^7$  cells per mol of chlorine released) [6]. The growth yield values obtained for *Dehalobacter* respiring CF are between one or two orders of magnitude lower compared with other chlorinated compounds as 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, or 1,2,4-trichlorobenzene [33–36].

The exponential growth phase from each experimental BES was



**Fig. 3.** Correlation between the moles of CF consumed and changes in the 16S rRNA gene copy numbers for the experimental BES triplicates.

selected from the qPCR results and linearized. From their slope, the maximum specific growth rates and their corresponding doubling times were calculated, reaching a value of  $0.218 \pm 0.097 \text{ d}^{-1}$  and  $3.81 \pm 2.13 \text{ d}$ , respectively. The doubling times observed in this study are approximately 60% lower than those obtained in previous enriched cultures of *Dehalobacter* growing with CF ( $9.9 \pm 1.2 \text{ d}$ ) [33], suggesting that *Dehalobacter* sp. 8 M present in BES grows at a faster rate. A maximum specific CF utilization of  $2.83 \times 10^{-8} \pm 0.12 \times 10^{-8} \mu\text{mol}$  of CF degraded per *Dehalobacter* 16S gene copy generated per day was obtained by combining the maximum growth rate and the growth yield.

Fig. 2b displays the normalized degradation rates, referring to the concentration of *Dehalobacter* 16S rDNA gene copies. The highest normalized degradation rates were obtained for the highest cathodic potentials, as also observed in a previous study with BES inoculated with a *Dehalogenimonas*-containing culture respiring 1,2-dichloropropane [18].

### 3.3. Coulombic efficiency and energetic input of the process

The current intensities decreased when the cathodic potential was lowered, obtaining average current density values of  $-0.050 \pm 0.060$ ,  $-0.110 \pm 0.029$ , and  $-0.325 \pm 0.043 \text{ mA m}^{-2}$  for potentials  $-0.6$ ,  $-0.7$ , and  $-0.8 \text{ V}$ , respectively (Fig. S5). These values were used to assess the coulombic efficiency of the CF degradation process, which was  $42.33 \pm 28.22\%$  when working at a cathodic potential of  $-0.6 \text{ V}$  and increased to values between 60% and 70% when the potential decreased to  $-0.7$  and  $-0.8 \text{ V}$  (Fig. 4). The values of coulombic efficiency and required energetic input to degrade one mol of CF were similar between the two most negative potentials poised in this study. As expected, the energetic input required for degrading a certain amount of contaminant was inversely correlated to the coulombic efficiency. In this study, a higher amount of energy to degrade a mol of contaminant was required when working at potential  $-0.6 \text{ V}$  and decreased the required value at potentials  $-0.7$  and  $-0.8 \text{ V}$ . This observation does not correlate with the results of previous studies working with OHRB in BESs which showed that the decrease of cathodic potential produced faster degradation rates at the cost of severely decreasing the coulombic efficiency of the process [9]. This could be explained by the sequential decrease of the cathodic potential applied in this study that is accompanied by a continuous growth of *Dehalobacter* that would potentially consume the hydrogen supplied as it is produced. This observation is supported by gas measurements from the headspace of the BESs, which showed no hydrogen accumulation (Fig. S6).

The absolute degradation rates (Fig. 2a) presented similar values during the three CF doses at each poised cathodic potential value despite the 16S rRNA gene concentrations increasing over time (Fig. S4). Thus, the observed maximum degradation rate was limited by the hydrogen bioavailability and not cell density. The maximum

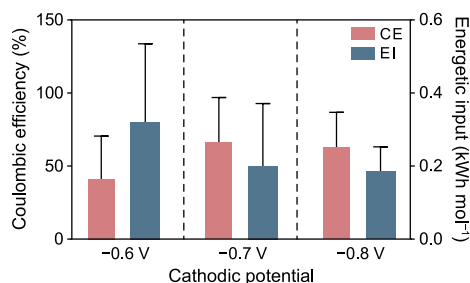


Fig. 4. Coulombic efficiencies and energetic inputs per mol of CF degraded obtained for each cathodic potential.

degradation potential of the culture was not used, which would explain the decrease of the specific dechlorination activity of CF at lower cathodic potentials (Fig. 2b). Overall, operating BESs with a sequential increase in the cathodic potential is a feasible strategy to control the maximum degradation rate values attained and reduce energetic costs during bioremediation processes assisted with electrochemically generated hydrogen. Several parameters other than the cathodic potential should be studied in view of upscaling BES reactors. The reactor configuration should aim at reducing the overpotentials; therefore, anodic and cathodic areas should be maximized and placed as close as possible. A possible alternative to reduce the overall energy requirements is to replace water oxidation as an anodic reaction with another more favorable reaction, such as the (bio)electrochemical oxidation of another pollutant. The coupled oxidation of toluene and the reduction of trichloroethene have recently been reported [37]. Moreover, this high-scale configuration should be able to operate under continuous mode. From an economic point of view, the cost of the electrode materials to drive hydrogen reduction should also be decreased.

## 4. Conclusions

This study demonstrates the full dechlorination of CF by combining *Dehalobacter* and *Dehalobacterium* in a BES for the first time. The application of a steadily decreasing cathodic potential instead of a fixed one allowed to adjust the supply of hydrogen and maximize the coulombic efficiency of the process and the degradation rates obtained even at the most negative cathodic potential poised ( $-0.8 \text{ V}$ ). We operated the BESs in a fed-batch mode for 126 days, obtaining *Dehalobacter* concentrations up to  $10^7$  16S rRNA gene copies per mL after increasing in four orders of magnitude the initial *Dehalobacter* concentration. This study provides a basis to use BESs as *on-site* bioreactors to deliver enriched OHRB and hydrogen in contaminated groundwater requiring biostimulation and bioaugmentation. The organohalide respiration of CF and the fermentation of DCM proceeded almost simultaneously at concentrations of CF up to  $400 \mu\text{M}$ , which would avoid the accumulation of DCM in the environment. At higher concentrations of CF, *Dehalobacterium* is inhibited but recovers the DCM fermenting activity when CF reaches lower concentrations. In all, these results show the potential of the constructed co-culture and BESs to completely dechlorinate CF, which cannot be fully dechlorinated by a single anaerobic bacterium to date.

### Conflict of interest

The authors declare no conflict of interest.

### 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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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.es.2022.100199>.

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