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Enhanced dechlorination of 1,2-dichloropropane to propene in a bioelectrochemical system mediated by *Dehalogenimonas*

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Abstract

Bioelectrochemical systems (BES) are promising technologies to enhance the growth of organohalide-respiring bacteria and to treat chlorinated aliphatic hydrocarbons. In this study, two carbon-based cathodic electrode materials, a graphite brush and a carbon cloth, were used as hydrogen suppliers to couple growth of *Dehalogenimonas* and dechlorination of 1,2-DCP to nontoxic propene in the cathode vessel. The BES with graphite brush electrode consumed ~4000 µM 1,2-DCP during 110 days and exhibited a degradation rate 5.6-fold higher than the maximum value obtained with the carbon cloth electrode, with a cathode potential set at -0.7 V. Quantitative PCR confirmed that *Dehalogenimonas* gene copies increased by two orders of magnitude in the graphite brush BES, with an average yield of $1.2 \cdot 10^8 \pm 5 \cdot 10^7$ cells per µmol of 1,2-DCP degraded. The use of a pulsed voltage operation (cathode potential set at -0.6 V for 16h and -1.1 V for 8h) increased the coulombic efficiency and degradation of 1,2-DCP when compared with a continuous voltage operation of -1.1 V. Bacterial cell aggregates were observed in the surface of the graphite brush electrodes by electron scanning microscopy, suggesting biofilm formation. This study expands the range of chlorinated compounds degradable and organohalide-respiring bacteria capable of growing in BES.

Keywords: Dehalogenimonas, 1,2-Dichloropropane, Bioelectrochemistry, Remediation, Organohalide respiring bacteria.

1. Introduction

1,2-dichloropropane (1,2-DCP) is an halogenated organic compound used in industry either as a chemical intermediate in the production of other valuable organic substances, such as tetrachloroethene and tetrachloromethane or as a solvent [1]. Due to its toxicity and carcinogenic effects, it has been designated as a high priority chemical substance for risk evaluation by the United States Environmental Protection Agency [2].

Due to accidental spills, 1,2-DCP has been frequently detected in groundwater sources. The anoxic conditions typically found in contaminated groundwater limit the aerobic biodegradation of 1,2-DCP [3–6]. Under anaerobic conditions, 1,2-DCP can serve as terminal electron acceptor for organohalide-respiring bacteria (OHRB) belonging to the genera *Dehalogenimonas* and *Dehalococcoides mccartyi*, among others [1,7–9]. The reductive dechlorination reaction of 1,2-DCP for both genera involve a dichloroelimination mechanism producing propene as final non-toxic product [1,7–9].

Enhanced *in situ* anaerobic bioremediation can be an effective method of degrading chlorinated compounds dissolved in groundwater [10,11], but the injection of electron donors in groundwaters to stimulate OHRB growth presents some limitations (i.e. the uneven distribution of the substrates, possible induction of secondary contamination or competition with non-dechlorinating hydrogenotrophic bacteria). In the recent years, bioelectrochemical systems (BES) have been proposed as an efficient technology to promote anaerobic reductive dechlorination processes [12–18]. BES catalyse reductive dechlorination reactions in the cathode by supplying electrons either by direct electron transfer from the electrode surface or by indirect electron transfer via soluble redox mediators or H_2 generation through electrochemical dehydrogenation [19]. One of the advantages of using BES over conventional enhanced *in situ* anaerobic bioremediation is

that the supply of electron donor, i.e. hydrogen, can be easily monitored and fine-tuned. In addition, the utilisation of BES could increase the low cell densities obtained for OHRB using conventional suspended lab culture techniques, which is one of the bottlenecks for the obtention of commercial bioaugmentation cultures [20]. This could be achieved by the formation of biofilms attached to high-surface carbon electrodes, that would allow a higher OHRB concentration in the reactor [17].

To date, the application of biocathodes for the treatment of chlorinated aliphatic hydrocarbons by OHRB has been limited to the degradation of chlorinated ethenes (tetrachloroethylene, trichlorethylene and 1,2-dichloroethylene) and the chlorinated ethane 1,2-dichloroethane [21]. The OHRB identified playing a role in these BES systems belonged to the genera *Dehalococcoides mccartyi* or *Geobacter* [12,15,21,22].

The aim of this study is to explore the feasibility of performing the dechlorination of 1,2-DCP to non-toxic propene by a *Dehalogenimonas*-containing culture in a two-chamber BES separated by a cationic exchange membrane, where the growth of *Dehalogenimonas* is driven by abiotically generated H_2 in the cathode. Graphite fibre and carbon cloth were selected as the cathodic electrode materials in view of its biocompatibility with *Dehalogenimonas* sp. Both cathodes were compared in terms of energetic consumption and degradation rate at the same cathode potential. This work further expands the range of chlorinated compounds and OHRB that can be stimulated by using BES.

2. Materials and methods

2.1. Bioelectrochemical cells description

The electrochemical cells consisted of two 165 mL glass vessels separated by a cation-exchange membrane (CMI-7000, Membranes International INC, USA) with 4 cm of aperture diameter. In the anodic compartment, a titanium sheet was used as the electrode.

In the cathodic compartment, a graphite fiber brush (35 mm length x 30 mm diameter, 7.2 µm fiber diameter, Millrose Co., USA) or a carbon cloth fragment (85 mm length x 25 mm width x 0.673 mm thickness, Zoltek Co., USA), provided with a surface area of approximately 13.2 dm^2 and 0.213 dm^2 respectively, were used as working electrodes. Both vessels were mixed with a magnetic stirrer. A power source (Quad Potentiostat, Whistonbrook Software) was used to control the voltage and the potential of the system. The applied cathode potential was measured against an Ag/AgCl reference electrode placed in the cathodic vessel. When controlling the voltage, a digital multimeter (Hayoue DT830B) was used to ensure the cathodic potential. All the potential values mentioned are V vs Standard Hydrogen Electrode (SHE) unless otherwise stated. Butyl rubber septums and aluminium crimp caps were used to seal the system while providing a sampling port to allow liquid extraction or addition from both cell compartments.

2.2. Cultivation of the *Dehalogenimonas*-containing culture in serum bottles

A 1,2-DCP-degrading *Dehalogenimonas*-containing culture was previously enriched from sediments obtained in the Besós river (Spain) [1]. The genome of the strain contained in this culture was recently sequenced and annotated, and it was denominated *Dehalogenimonas alkenigignens* strain BRE15M [23]. The culture was maintained in 100 mL glass bottles containing 65 mL of anaerobic medium previously described [24]. In brief, it contained minerals, trace elements, vitamins, sodium acetate (5 mM) as a carbon source, titanium (III) citrate (0.29 mM) as reducing agent and sodium bicarbonate (12 mM) as buffering agent. Bottles were sealed with butyl rubber septums and aluminium crimp caps and were gassed with N_2/CO_2 (4:1 at 0.2 bar of overpressure) and H_2 (0.4 bar of overpressure). Cultures were periodically spiked with 1,2-DCP (500 µM, nominal concentration) and transferred into fresh medium after the consumption of ~3000 µM.

117 2.3. Operation of BES

118 Cathode and anode chambers contained 130 mL of the anaerobic medium described
119 above. Cathodic compartments were initially spiked with 500 µM 1,2-DCP and
120 inoculated with 3 mL of an active culture of *Dehalogenimonas* with a density of ~$9 \cdot 10^7$
121 cells·mL^{-1}. Biotic open circuit BES without electrode were also included as controls. The
122 cathodic potentials were adjusted to -0.7 V vs Standard Hydrogen Electrode (SHE)
123 against an Ag/AgCl reference electrode (RE-1B, BAS Inc., +197 mV vs SHE) by
124 applying a potentiostatic control in both graphite brush and carbon cloth-provided
125 reactors. The BES were maintained in fed-batch mode by adding 1,2-DCP when
126 exhausted (500 or 1000 µM).

127 For the experiments carried out with BES containing graphite brush electrodes under
128 pulsed and continuous voltage operation, a cathode potential of -0.6 V and -1.1 V was
129 applied during time-periods of approximately 16 and 8 h respectively from day 0 to day
130 46. Afterwards, a cathodic potential of -1.1 V was applied until the end of the experiment
131 at day 136. In this case, a pH probe (Hach) was employed to ensure the acidity of the
132 cathodic vessel and manual additions of an anaerobic stock solution of 1 M of HCl were
133 employed to adjust pH at a value of 7.

134 2.4. Coulombic efficiency and energy consumption

135 The coulombic efficiency (CE) for each degradation experiment at the cathode was
136 calculated using equation 1:

137 $$\mathrm{CE} = \frac{2 \cdot \mathrm{V} \cdot \mathrm{F} \cdot [1,2\mathrm{-DCP}]_{\mathrm{deg}}}{\int \mathrm{I(t)dt}} \quad (1)$$

138 where 2 is the number of electrons required to dechlorinate a molecule of 1,2-DCP to
139 propene, V is the cathodic liquid volume (L), F is Faraday's constant (96485 A·s·mol^{-1}),

140 $[1,2\text{-DCP}]_{deg}$ is the degraded concentration of 1,2-DCP (M) and $\int I(t)dt$ is the integration
141 of the measured intensity throughout the selected experimental time (A·s).

142 Additionally, the energetic input (EI) required for each experiment was calculated as
143 follows:

144 $$EI = \frac{V_A \cdot \int I(t)dt}{V \cdot [1,2-DCP]_{deg}} \quad (2)$$

145 Where V_A is the voltage applied in the system (V).

146 2.5. Analytical methods

147 1,2-DCP and propene were measured by static headspace gas chromatography. Liquid
148 samples (1 mL) were taken from the cathode and were transferred to 10 mL sealed vials
149 containing 5.5 mL deionized water. The vials were placed in a headspace sampler
150 (Agilent 7964) and heated to 85 ºC for 15 min. Automatically, 1 mL headspace gas sample
151 from the vials was injected into an Agilent 6890N gas chromatograph provided with an
152 Agilent DB-624 column (30 m × 0.32 mm with 0.25 µm film thickness) and a flame
153 ionization detector following a method described elsewhere [25]. Calibration of both
154 compounds was based on aqueous standards with the same liquid and gas volumes than
155 the experimental BES. Details for the calculation of propene concentration is provided in
156 the Supplementary Information Materials and Methods section.

157 2.6. Microbiological analysis

158 Liquid samples (3 mL) were obtained from the cathode at different time points during the
159 experiments and centrifuged at 9000 g for 20 minutes. The supernatant was removed and
160 the DNA was extracted using a NucleoSpin Tissue kit (Machery Nagel) following the
161 manufacturer instructions and eluted in a final volume of 50 µL.

Quantitative hydrolysis probes based real-time PCR (qPCR) was performed with a Lightcycler 480 instrument (LC480; Roche) in order to monitor the increase of *Dehalogenimonas* cells in the BES cultures. The qPCR used a set of primers (Dhgm478F and Dhgm536R) and a TaqMan probe (Dhgm500Probe) which were used in a previous study and specifically targeted the 16S gene from the genus *Dehalogenimonas* (Table S1) [26]. Each qPCR assay was performed in a total volume of 20 µL and containing 1x LightCycler 480 Probes Master Mix (Roche Diagnostics), 0.4 µM final concentration of each primer and TaqMan probe, ultrapure water and 2 µL of the extracted DNA sample. The qPCR cycle conditions were: pre-incubation at 95ºC for 10 min, followed by 50 cycles of 15 s at 95 °C, 1 min at 56 °C and 1 second at 72 ºC (fluorescence detection).

Standard curve for quantifying the gene copies obtained in the assays was created as following. A synthetic sequence corresponding to the whole 16S gene of *Dehalogenimonas alkenigignens* (NR_109657.1) and containing the amplified region was purchased (ATG biosynthetics). The gene sequence of interest was amplified by conventional PCR using the previously described primers and the amplicon (59 pb) presence was checked with a 2% agarose electrophoresis gel. The PCR product was purified using an NZYGelpure kit (Nzytech) following the manufacturer instructions, and was ligated to a pGEMT vector (Promega) and transformed into *Escherichia coli* DH5α following standard procedures. The presence of the cloned sequence in the transformed vector was assessed by sequencing (Macrogen).

Plasmid DNA was prepared using an NZY Miniprep kit (Nzytech) according to the manufacturer instructions and it was diluted in 10-fold dilutions ranging from 10^3 to 10^9 gene copies per reaction mix and used as template for qPCR. The quantification cycle (Cq) obtained for each dilution was plotted against the logarithm of the gene copy numbers in the template. Each dilution was tested by triplicate. The number of copies of

187 the cloned sequence in plasmid DNA was calculated with the formula used by Ritalahti
188 et al. [27]. Also, the 16S gene copies per mL of culture sample were determined from the
189 standard curve (Fig. S1) following a previously described equation [28]. All the samples
190 were quantified in triplicate.

191 Due to the significant similarities in the amplified region between the genus
192 *Dehalococcoides* and *Dehalogenimonas*, two negative controls using a DNA extraction
193 from a *Dehalococcoides mccartyi*-containing culture and water instead of experimental
194 sample were used to assess the specificity of the amplification.

195 2.7. Cell growth kinetic calculations

196 The maximum specific growth rates (μ, d^{-1}) were calculated by selecting the exponential
197 growth phase period according to the cell concentrations of *Dehalogenimonas* obtained
198 by qPCR and using the following equation:

199 $$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \quad (3)$$

200 where X is the cell concentration in the bioelectrochemical reactor (*Dehalogenimonas*
201 cells·mL^{-1}).

202 The biomass growth yield (Y, cells generated / μmol 1,2-DCP degraded) was calculated
203 as it follows:

204 $$Y = \frac{X_{gen}}{1,2\text{-}DCP_{deg}} \quad (4)$$

205 Where X_{gen} is the increase of *Dehalogenimonas* cells and 1,2-DCP_{deg} are the μmol of 1,2-
206 DCP degraded during a certain time period.

207 The maximum specific 1,2-DCP degradation rate by *Dehalogenimonas* (q) can be
208 calculated by applying μ and Y as it is shown in the following equation:

209 $$q = \frac{\mu}{Y} \quad (5)$$

2.8. Scanning electron microscopy

Field emission-scanning electron microscopy was performed with liquid samples of the culture contained in the cathode chamber and with cathodic electrode material at the end of each working period of BES. Liquid samples (5 mL) were filtered through 0.2 µm pore-size polycarbonate filters to collect the cells in suspension. Both kind of samples were fixed with a 2.5 % glutaraldehyde solution and treated as previously described [29]. Imaging of the samples was done with a Zeiss EVO-MA10 field emission scanning electrode microscope at the Servei de Microscopia (Universitat Autònoma de Barcelona).

3. Results and discussion

3.1. Effect of electrode material on 1,2-DCP dechlorination

BES containing graphite fibre brush and carbon cloth cathodes were poised at -0.7 V of cathode potential and operated during 110 days with periodical amendments of 1,2-DCP when consumed (Figure 1A). The first dose of 1,2-DCP was completely dechlorinated to propene in 37 days regardless of the electrode. However, the subsequent amendments of 1,2-DCP were consumed at increasing rates over time in BES containing graphite fibre brush whereas dechlorination of 1,2-DCP in BES containing carbon cloth proceeded slower (Fig. 1A).

During this time-span, graphite fiber brush-containing cells were capable of depleting seven 1,2-DCP doses, while the carbon cloth-containing cell was only capable of degrading three, which resulted in a final accumulated degradation of 3930 ± 605 and 1442 µM of 1,2-DCP, respectively (Fig. 1B). The production of propene was monitored in the BES containing graphite fibre brush because exhibited an enhanced 1,2-DCP dechlorination performance. As shown in Fig. 1B, the moles of 1,2-DCP degraded

stoichiometrically agree with the propene generated. At the end of the experiment (110 days), propene accumulated was 4039 ± 425 (102.7% of the 1,2-DCP consumed), demonstrating that full dechlorination of 1,2-DCP was achieved during the process and discarding the presence of other undesired and potentially harmful by-products. Biotic controls that consisted of opened circuit system without electrode required 75 days to degrade 540 µM of 1,2-DCP (Figure S2), showing that 1,2-DCP degradation notably decreased in absence of a continuous supply of electrochemically generated hydrogen from an electrode.

Degradation of 1,2-DCP was coupled to an increase in the number of *Dehalogenimonas* 16SrDNA copies in BES containing graphite fibre brush. The consumption of the four first amendments of 1,2-DCP led to an increase of *Dehalogenimonas* 16SrDNA gene copies by over two orders of magnitude (Figure 1C). The concentration of *Dehalogenimonas* 16S rDNA genes during the conversion of the following two 1,2-DCP doses continued increasing but at lesser extent, obtaining a final cell density of $2.1 \cdot 10^8 \pm 8 \cdot 10^7$ cells/mL *Dehalogenimonas* (assuming one 16S rRNA gene per genome). This value fits well with cell densities reported for organohalide-respiring bacteria, which rarely grow above 10^8 cells/mL [30]. Relative to the biotic controls, the consumption of a single dose of 540 µM 1,2-DCP in 75 days was consistently accompanied by a modest growth from $1.3 \cdot 10^6 \pm 3 \cdot 10^5$ to $2.8 \cdot 10^6 \pm 8 \cdot 10^5$ (Figure S2).

The 1,2-DCP degradation rates obtained in the graphite brush experiment increased during the first four contaminant amendments from 9.84 ± 1.50 $\mu M \cdot d^{-1}$ to 89.18 ± 6.39 $\mu M \cdot d^{-1}$ and afterwards exhibited stable values during three consecutive 1,2-DCP amendments (Figure 2A). The degradation rate reached its maximum at the sixth addition (98.24 ± 82.27 $\mu M \cdot d^{-1}$). The maximum degradation rate in the carbon cloth BES (16 $\mu M \cdot d^{-1}$) was 5.6-fold lower than that obtained in the graphite brush BES. Both graphite

and carbon cloth electrodes showed higher degradation rates than biotic controls (10.95 ± 1.38 $\mu M \cdot d^{-1}$) indicating that direct transfer of H_2 from electrochemical dehydrogenation promoted the growth of *Dehalogenimonas* and enhanced 1,2-DCP degradation.

The maximum specific growth rate constant and the growth yield of *Dehalogenimonas* was 0.105 ± 0.005 d^{-1} and $1.2 \cdot 10^8 \pm 5 \cdot 10^7$ cells per µmol of 1,2-DCP degraded, respectively, calculated from values obtained from the exponential growth phase of BES with graphite brush in the 1,2-DCP amendments 2 to 5. By applying Eq. (5), we obtained a specific 1,2-DCP degradation rate of 1.0·10-9 ± $5 \cdot 10^{-10}$ µmol of 1,2-DCP degraded per cell and day during the whole exponential growth phase of *Dehalogenimonas*. The growth yield of *Dehalogenimonas* in BES was one order of magnitude higher than those reported for *Dehalogenimonas lykanthroporepellens* strain BL-DC-9 grown with 1,2-DCP [31] but one order of magnitude lower than growth yields of *Dehalogenimonas* grown with chlorinated ethenes [26].

The current intensities recorded during the operation of BES (Figure S3) were used to calculate the coulombic efficiencies (CEs) and the energy inputs for each experiment (Figure 2B). The graphite fibre brush cells, which reached higher degradation rates than those with carbon cloth, presented low CEs, ranging from 0.73 ± 0.9% in the first amendment, to 4.91 ± 4.02% in the amendment that was consumed faster. At the same time, the energy input per mole of contaminant degraded was inversely correlated to the CEs, obtaining lower values when the degradation rate was higher, and ranging from 1.91 ± 0.23 $kWh \cdot mol^{-1}$ to 0.31 ± 0.02 $kWh \cdot mol^{-1}$. On the other hand, the carbon cloth BES, despite presenting lower degradation rates, showed higher CEs and lower energetic consumptions per mole of contaminant degraded, obtaining values at its final 1,2-DCP amendment of 19.05% and 0.069 $kWh \cdot mol^{-1}$. This increase in the electrical efficiency and consumption values were caused by the consistently lower intensities recorded during

284 the operation of the cells with carbon cloth as cathode compared to the graphite brush
285 containing ones.

286 3.2. Pulsed and continuous voltage operation of BES

287 The graphite brush was selected for further BES experiments. To increase the low CEs
288 observed in the previous experiments and increase the efficiency of the process while
289 maintaining a high degradation rate, two voltage operational modes were tested in a BES
290 for 140 days (Figure 3A). First, two different cathodic potentials were alternated during
291 the first 46 days: -0.6 V during 16 h and -1.1 V during 8 h. Then, a continuous cathodic
292 voltage of -1.1 V was applied during the next 89 days (Figure 3B). This operation enabled
293 the successful consumption of 10 mM 1,2-DCP in 140 d (Figure 4A). The maximum
294 degradation rate during the pulsed current period was achieved from day 17 to 43 and it
295 was fitted to a linear regression (Figure 4B), obtaining a value of 255.9 µM of 1,2-DCP
296 degraded per day. The same methodology was applied to the interval of day 58 to 135,
297 which corresponded to the continuous current application, and an 86.2% lower
298 degradation rate (33.4 $\mu M \cdot d^{-1}$) was obtained. In the abovementioned periods, the CE was
299 16.60% and 0.86% for the pulsed and continuous voltage operation, respectively. The CE
300 of the pulsed operation was slightly lower than the 19.05% obtained in the carbon cloth
301 containing BES but with a 16-fold degradation rate. This high CE is coupled with a low
302 energetic input of 0.076 $kWh \cdot mol^{-1}$, which is also similar to the values obtained in the
303 carbon cloth cells. On the other hand, when applying a continuous voltage, the decrease
304 of the degradation rate and the higher intensities applied drastically hindered CE, being a
305 95% lower than that obtained when applying a pulsed voltage. Also, when applying a
306 continuous voltage to achieve a cathode potential of -1.1 V, the required energetic input
307 increased 33-fold (2.499 $kWh \cdot mol^{-1}$). Applying a pulsed voltage not only reduced the
308 electric energy input but also stimulated the degrading activity of *Dehalogenimonas*.

Therefore, the pulsed voltage operation can be a suitable strategy in a hypothetical scale-up of the process to reduce the energy input in a large-scale process while maintaining the high degradation rates required for the sustainability of the process.

3.3. Scanning electron microscopy

Scanning electron microscope (SEM) was applied to uncover cell distribution and cell morphology in the cathodic electrodes and liquid samples at the end of each operation. Two main cell morphologies comprising irregular-shaped *cocci* and *bacillus* were clearly identified in the filtered suspension cultures (Figure 5A, C and E). These *cocci* and *bacillus* morphologies are in agreement with the presence of *Dehalogenimonas* and *Desulfovibrio*, the two predominant bacterial *genus* in this culture [32]. Also, several aggregates were observed on the graphite brush (Figure 5B and F) and, in less extension, on carbon cloth electrode (Figure 5D). The formation of cell aggregates is common in co-cultures with obligate syntrophic interactions, which facilitates the cell-to-cell contacts and enhance metabolite fluxes between species. *Desulfovibrio* has been frequently detected in co-cultures and tri-cultures with organohalide-respiring bacteria, and in some cases its presence is required to proceed with the dechlorination [33,34]. The role of *Desulfovibrio* is not fully understood but some studies suggest that might provide organic cofactors (i.e. corrinoids) to organohalide-respiring bacteria, which are essential for the growth of *Dehalogenimonas* [35,36]. The micrographs obtained in our study suggest that the surface of the graphite brush electrode can provide suitable characteristics to attach aggregates of *Dehalogenimonas* and *Desulfovibrio*, especially considering that it has been previously reported the capability of *Desulfovibrio* populations of synthetizing extracellular polymeric substances (EPS) [37], which are required for a biofilm formation.

4. Conclusions

This study demonstrates for the first time the capability of growing *Dehalogenimonas* in a BES to degrade 1,2-DCP into the non-toxic product propene. The graphite brush electrodes resulted in a 5.6-fold higher degradation rates than those with carbon cloth. The application of periodic pulses of voltage allowed to maintain high CE and degradation rates while decreasing the energetic input required with the graphite brush electrode. The use of BES allowed to obtain final *Dehalogenimonas* concentrations up to 10^8 16S gene copies per mL of liquid culture, making these systems a promising technology to produce high density *Dehalogenimonas* cultures for bioaugmentation purposes. Further research is required to assess the optimal operational conditions for the application of this technology in a scaled-up process.

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497 **Figure captions**

498 **Figure 1.** Degradation profile of 1,2-DCP in BES (Panel A), accumulated concentration
499 of 1,2-DCP degraded and propene produced (Panel B) and *Dehalogenimonas* 16S rDNA
500 gene copies per mL (Panel C), with different cathodic electrode materials (●: Graphite
501 brush, ■: Carbon cloth) and cathodic potential set at -0.7 V. Solid symbols and open
502 symbols refer to 1,2-DCP and propene, respectively. Numbers indicate the number of 1,2-
503 DCP amendments added in BES with graphite brush. Values plotted for BES with
504 graphite brush are average of duplicates and error bars indicate 1 standard deviation.
505 Values plotted for BES with carbon cloth are for an individual BES.

506 **Figure 2.** Degradation rate and growth yield of *Dehalogenimonas* obtained for each dose
507 of 1,2-DCP consumed in BES (Panel A) and coulombic efficiencies and energetic inputs
508 obtained during the degradation (Panel B). Dose number refer to the 1,2-DCP
509 amendments depicted in Fig. 1.

510 **Figure 3.** Degradation profile of 1,2-DCP in a BES containing a graphite brush as
511 electrode (Panel A) and its measured current intensities during the operation of pulsed
512 and continuous voltage (Panel B). The BES received several additions of 1,2-DCP as
513 indicated by the arrows.

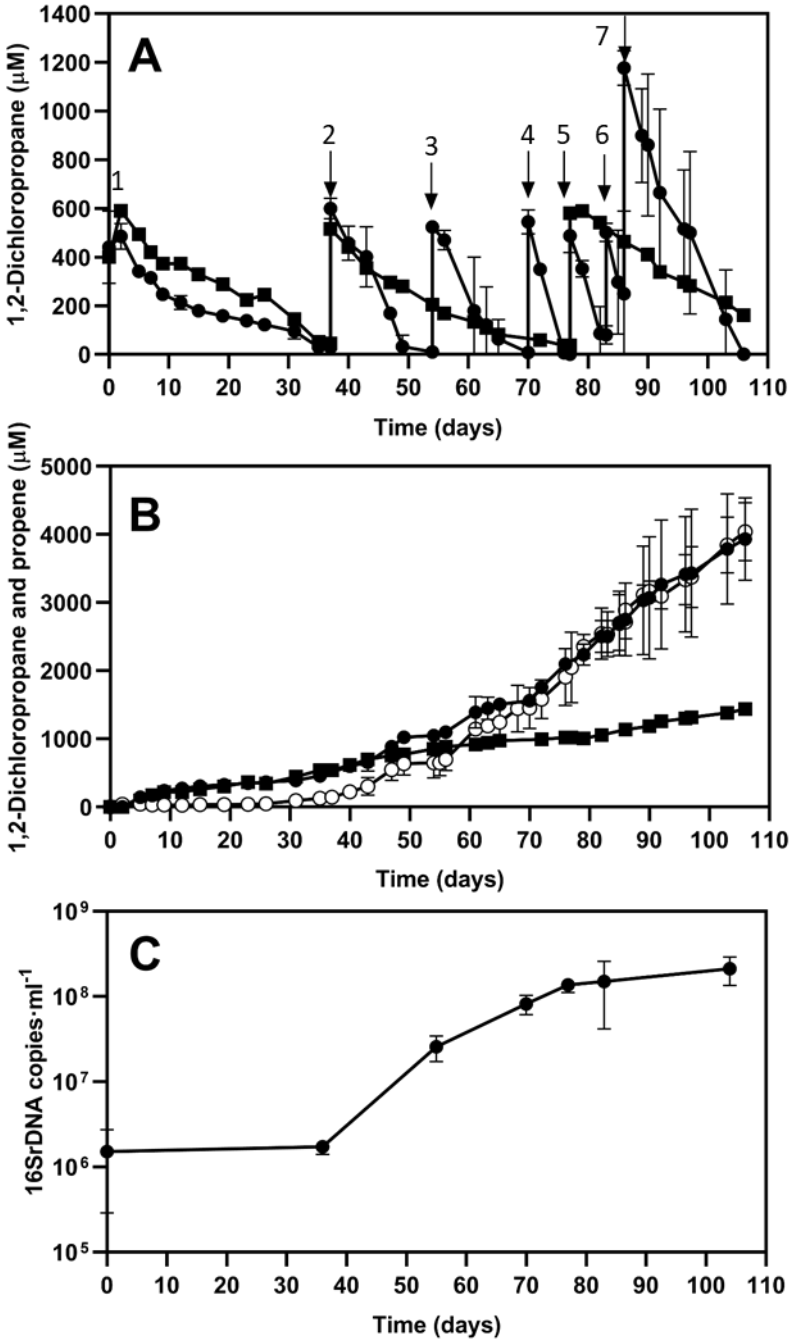
514 **Figure 4.** 1,2-DCP consumed in a BES containing a graphite brush as electrode (panel
515 A) and linear regression of the accumulated 1,2-DCP degradation values corresponding
516 to the degradation rate when applying pulsed (solid symbols) and continuous (open
517 symbols) voltage.

518 **Figure 5.** Microscopic analysis of the carbon fiber brush (A and B) and carbon cloth
519 experiments (C and D) described in section 3.1 and graphite brush experiment described

520 in section 3.2 (E and F) at the end of the experiments. For each BES, filtered liquid
521 samples (A, C and E) and solid electrode samples (B, D and F) were analysed by SEM.

522

523 **Figure 1**



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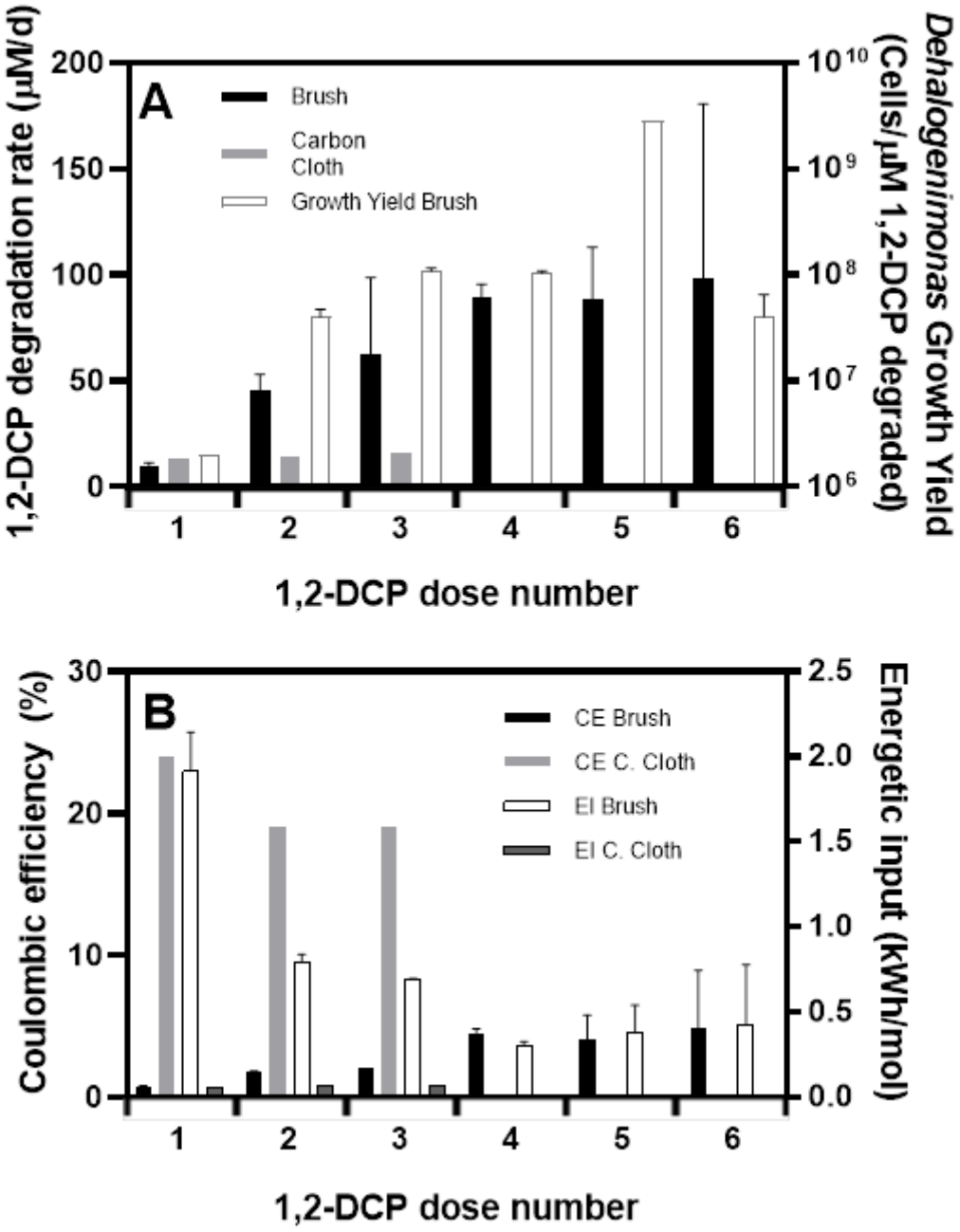
528 **Figure 2**



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533 **Figure 3**

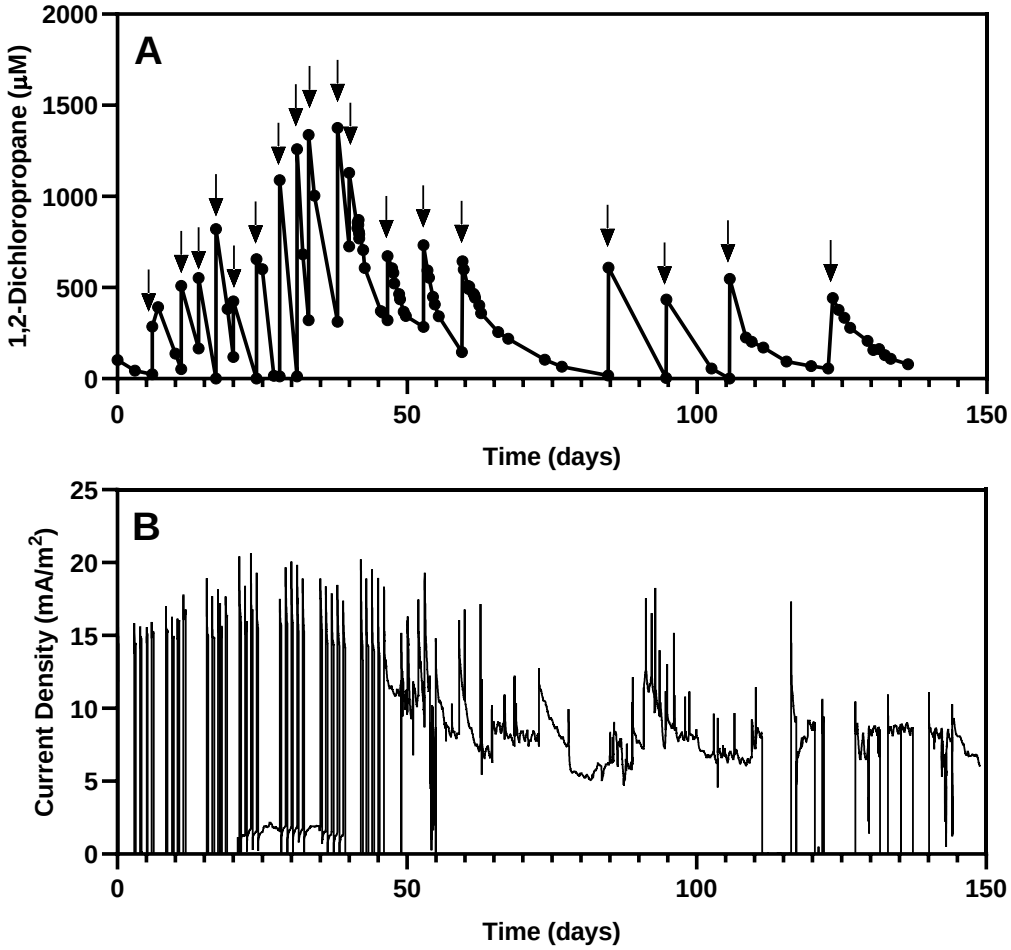

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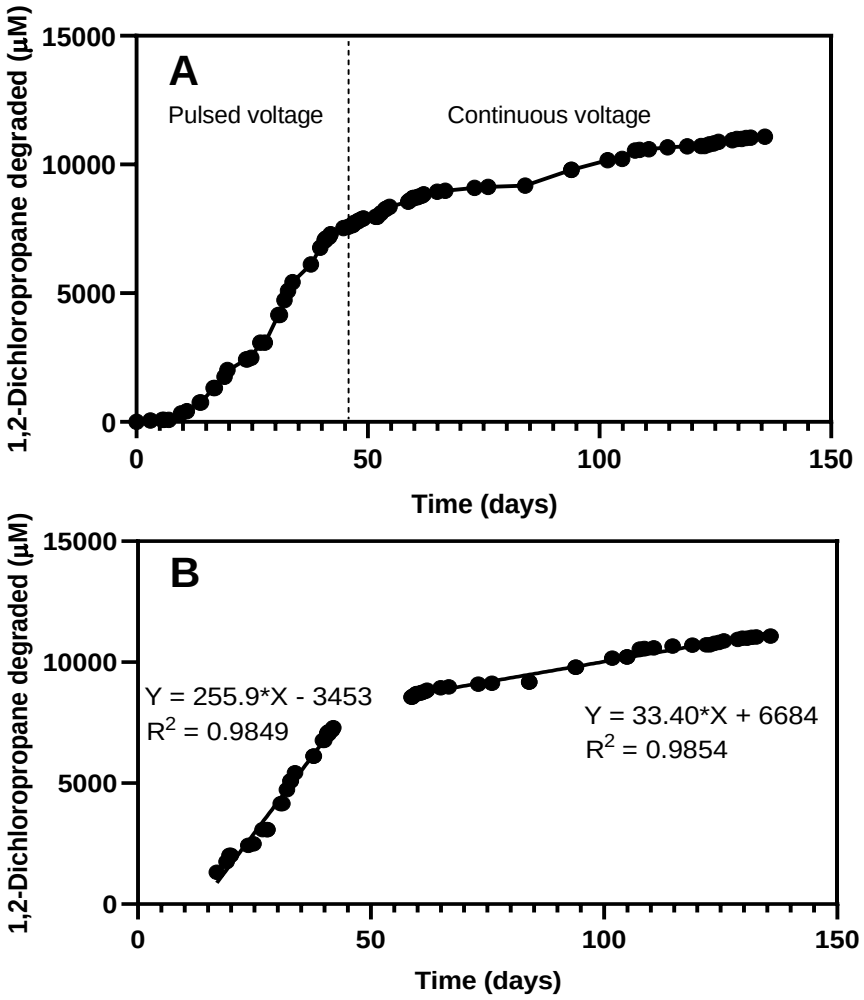
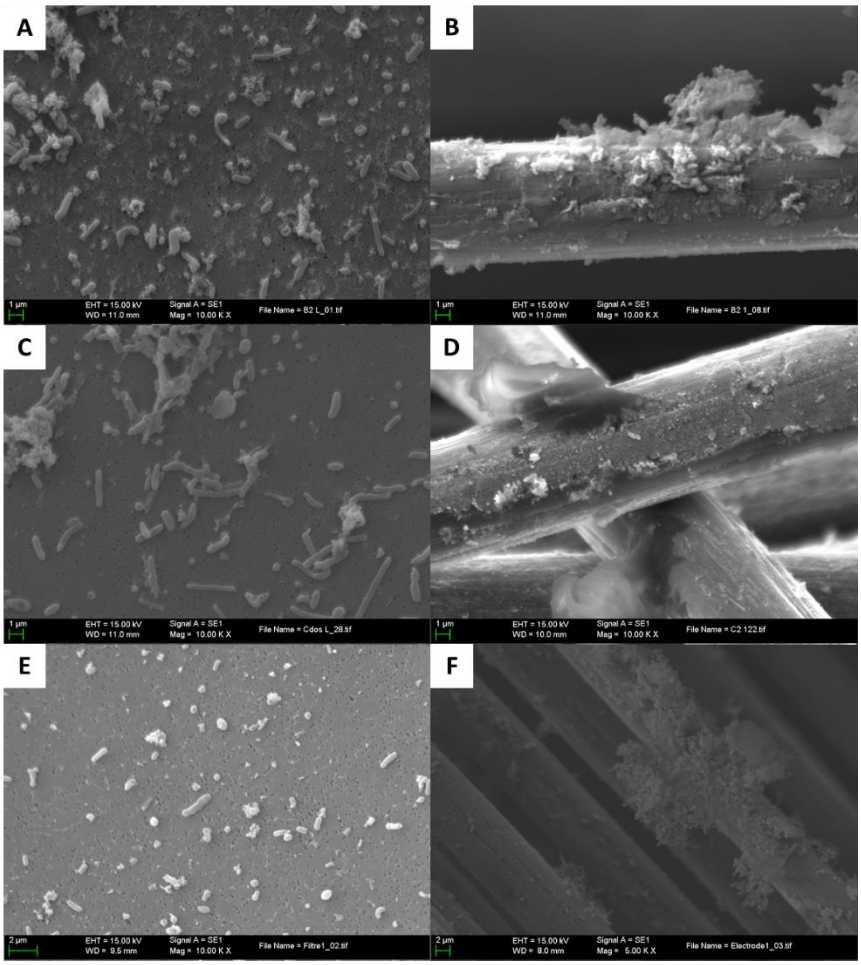
Figure 4


A
Pulsed voltage
Continuous voltage
1,2-Dichloropropane degraded (µM)
15000
10000
5000
0
0
50
100
150
Time (days)
B
Y = 255.9*X - 3453
R2 = 0.9849
Y = 33.40*X + 6684
R2 = 0.9854
1,2-Dichloropropane degraded (µM)
15000
10000
5000
0
0
50
100
150
Time (days)

549

Figure 5



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