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Potential application of (bio)electrochemical systems for organohalide degradation

David Juan Fernández Verdejo

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Universitat Autònoma de Barcelona

Escola d'Enginyeria

Departament d'Enginyeria Química, Biològica i Ambiental

Potential application of (bio)electrochemical systems for organohalide degradation

PhD Thesis

PhD Program in Environmental Science and Technology

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September 2022

Title: Potential application of (bio)electrochemical systems for organohalide degradation

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This work was supported by the Spanish Ministry of Science, Innovation and Universities (projects CTM2017-91879-EXP and PID2019-103989RB-100) and supported by the Generalitat de Catalunya (Consolidated Resarch Group 2014-SGR-476). The author acknowledges a predoctoral grant from Universitat Autònoma de Barcelona (PIF 2017-2018), and the financial support for the research stay performed (ESTPIF2022-04).

This work was done in collaboration with the Research Group on Biological Treatment and Valorization of Liquid and Gaseous Effluents (GENOCOV) of the Universitat Autònoma de Barcelona under the supervision of Dr. Albert Guisasola. Part of this work was done in collaboration with the Group of Microbial Processes, Water Research Institute (IRSA), National Research Council (CNR), Italy (Dr. Federico Aulenta) and the Department of Genetics and Microbiology of the Universitat Autònoma de Barcelona (Dra. Pilar Cortés).

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ERNEST MARCO URREA, Professor Agregat del Departament d'Enginyeria Química, Biològica i Ambiental de la Universitat Autònoma de Barcelona i PAQUI BLÁNQUEZ CANO, Professora Agregada del Departament d'Enginyeria Química, Biològica i Ambiental de la Universitat Autònoma de Barcelona,

CERTIFIQUEM,

Que el Llicenciat en Biotecnologia DAVID JUAN FERNANDEZ VERDEJO ha realitzat sota la nostra direcció, en els laboratoris del Departament d'Enginyeria Química, Biològica i Ambiental de la Universitat Autònoma de Barcelona, el treball que amb el títol "**Potential application of (bio)electrochemical systems for organohalide degradation**", es presenta en aquesta memòria, la qual constitueix la seva tesi per optar al Grau de Doctor per la Universitat Autònoma de Barcelona.

I perquè en prengueu coneixement i consti als efectes oportuns, presentem a l'Escola de Postgrau de la Universitat Autònoma de Barcelona l'esmentada tesi, signant el present certificat a Bellaterra, Setembre de 2022,

Dr. Ernest Marco Dra. Paqui Blánquez

Agraïments

En primer lloc, vull agrair especialment als meus directors, Ernest Marco i Paqui Blánquez, i als meus directors "no oficials", Teresa Vicent i Albert Guisasola, per tot l'ajut i suport d'aquests anys, i fer possible aquesta tesi.

Quiero dar las gracias a todos los que me habéis acompañado en el lab durante estos años, tanto en los del BioremUAB o los del Genocov. Jesi, Dani, Natàlia, Oriol, Guille, Àlex y Maria, gracias por estar siempre en el lab de anaerobis cuando había alguna fuga en la tienda, y gracias Pili, por avisarme cuando había cortes de corriente y había que reactivar el potenciostato. Quiero dar gracias especialmente a Alba, por enseñarme a que no entrara oxígeno a mis botellas y otras tácticas de supervivencia general. Also, thank you Mira, for teaching me which cable is the anode and which one the cathode.

También quiero agradecer al departamento de microbiología de la UAB, y en especial a Pilar, por toda la ayuda y atención que me han prestado para lograr muchos de los resultados de esta tesis. También quiero añadir a los técnicos del DEQBA, Rosi, Pili i Manuel, por hace que todo funcione y facilitar el día a día.

Gracias a todos los otros integrantes presentes y pasados del Biorem: Romina, Edu, Kaidi, Diana, Nacho, Armand, Zewen, Montse i Glòria. Gracias por siempre ayudarme cuando lo he necesitado y por esos eventos de carácter poco académico que hemos hecho de vez en cuando. También a Ana, Edu (otra vez) y Kike por las interminables discusiones (siempre de carácter constructivo) llevadas a cabo en el QC-1117.

Thanks to all the people from IRSA. Federico and Carolina, thank you for welcoming and helping me. Also Matteo, Marco, Pamela, Valentina, Camilla and Paolo,

thanks for your company, constant updates in dropbox and your general positive appreciation regarding Settebagni.

Moltes gràcies als meus pares i germà, Teresa, Luis i Albert, per donar-me sempre suport i estar atents quan us he explicat les últimes notícies del nostre estimat club. Vull agrair als meus oncles, Carme i Ricardo, per totes les quedades que hem fet i donar sempre show quan toca pagar el compte. También a mis tíos del país vecino, Sole y Patrick, y a su buen gusto diseñando menús. Finalmente, quiero añadir al tándem de Luis "le brutal" Santisteban y Adrià Cruells por tantos años de momentos sufridos (y algunos de ellos hasta buenos), entre otros que no me da tiempo a nombrar.

A tots vosaltres, us dedico aquesta tesi.

Abstract

The presence of organohalide compounds contaminating the environment, and more specifically natural groundwater sources, is of important concern due to their adverse effects on environment and human health. Moreover, the relevance of the aquifers due to the exponential increase of needs of freshwater supply highlights the importance related to their decontamination. A possible solution for their treatment is bioremediation, which uses microbial enzymes to degrade organohalide contaminants to less toxic or even innocuous by-products.

In this thesis, electrochemical technologies were employed to degrade organohalides by using two approaches. First, we explored the potential of using electrochemical systems to abiotically transform brominated compounds. Secondly, we exploited the electrochemically generated hydrogen to stimulate the growth and dechlorinating activity of organohalide-respiring bacteria (OHRB) to transform chlorinated compounds into non-toxic products in bioelectrochemical systems (BES). The OHRB and other anaerobic dechlorinating bacteria used in this study were previously enriched in our research group.

During this work, the abiotic electrochemical reduction of two brominated contaminants, dibromomethane (DBM) and 1,2-dibromoetane (DBA), was performed in a two-chamber system operating with a graphite fiber brush as cathode. The complete degradation of 500 μM of both contaminants was achieved between 1 and 7 hours at the three tested cathodic potentials of -0.8, -1.0 and -1.2 V vs standard hydrogen electrode (SHE). Methane and ethene were identified as the main by-products from DBM and DBA dechlorination, respectively. The bromide mass balance further confirmed the complete dehalogenation of both pollutants.

Next, the bioelectrochemical degradation of 1,2-dichloropropane (1,2-DCP) to propene mediated by a *Dehalogenimonas alkenigignens*-containing culture was studied in the cathodic vessel of a two-chamber BES. The usage of a graphite fiber brush as electrode material delivered 5.6-fold higher degradation rates than the ones achieved working with another carbon-based material as carbon cloth. Quantitative PCR confirmed that *Dehalogenimonas* 16S rRNA gene copies increased by two orders of magnitude up to 10^8 16S rRNA gene copies per mL. The application of a pulsed potential operation allowed to obtain high degradation rates coupled to higher coulombic efficiencies (CEs) compared to the operation with a continuous cathodic potential.

The bioelectrochemical degradation of chloroform (CF) was studied in a two-chamber BES by combining the activity of two bacterial consortia containing *Dehalobacter* and *Dehalobacterium*, capable to reduce CF to dichloromethane (DCM) and ferment DCM to acetate, respectively. The application of three sequential cathodic potentials (-0.6, -0.7 and -0.8 V vs SHE) allowed to increase the CF degradation rate and obtain CEs higher than 60% even at the lowest potential. The operation in BES allowed to obtain *Dehalobacter* concentrations up to 10^7 16S rRNA gene copies per mL after four orders of magnitude increase in its initial concentration. Concentration up to 800 μM of CF were successfully degraded without causing an irreversible inhibition in the DCM fermentation by *Dehalobacterium*.

Finally, CF degradation was coupled with the oxidation of toluene in a single-chamber bioelectric well reactor operated in continuous mode. A toluene oxidizing consortium successfully stablished a biofilm in the anodic surface (poised at +0.4 V of potential) and provided a continuous supply of electrons to the cathode, which were used to produce H_2 and drive the reduction of CF by *Dehalobacter*. The effects of different toluene concentrations in the inlet were tested, showing a positive correlation with its

degradation rate by the anodic biofilm. On the other hand, the DCM fermentation by *Dehalobacterium* was inhibited when the concentration of toluene at the inlet was 400 μM, but it recovered its activity when toluene concentration decreased to 160 μM.

Resum

La presència de compostos organohalogenats contaminant el medi ambient i, més específicament, les aigües subterrànies, és un greu problema degut als seus efectes adversos sobre el medi natural i la salut humana. A més a més, la rellevància dels aqüífers degut a l'increment exponencial de la necessitat global d'aigua dolça, fa augmentar la importància de la seva descontaminació. Una possible solució a aquest problema pot ser la bioremediació, que utilitza enzims microbians per tal de degradar els contaminants organohalogenats a productes menys tòxics, o fins i tot innocus.

En aquesta tesi, les tecnologies electroquímiques han sigut utilitzades per tal de degradar organohalogenats mitjançant dos aproximacions. En primer lloc, hem explorat el potencial d'ús dels sistemes electroquímics per tal de transformar abiòticament compostos bromats. En segon lloc, hem aprofitat l'hidrogen generat electroquímicament per tal d'estimular el creixement i l'activitat decloradora de bacteris respiradors d'organohalogenats (OHRB) i de transformar compostos clorats en productes no tòxics en sistemes bioelectroquímics (BES). Els OHRB i altres bacteris decloradors utilitzats en aquest estudi van ser prèviament enriquits al nostre grup de recerca.

Durant aquest treball, la reducció electroquímica abiòtica de dos contaminants bromats, dibromometà (DBM) i 1,2-dibromoetà (DBA), s'ha realitzat en un sistema compost per dos cambres que operava amb un raspall de fibra de grafit com a càtode. La degradació completa de 500 μM d'ambdós contaminants es va aconseguir entre 1 i 7 hores d'operació en els tres potencials de càtode aplicats: -0.8, -1.0 i -1.2 V vs elèctrode estàndard d'hidrogen (SHE). Els principals productes de la reducció del DBM i el DBA han sigut determinats com a metà i etè respectivament. El balanç de matèria del bromur ha permès confirmar la completa dehalogenació dels dos contaminants.

Seguidament, s'ha estudiat la degradació bioelectroquímica del 1,2-dicloropropà (1,2-DCP) a propè mediada per un cultiu que conté *Dehalogenimonas alkenigignens* en el càtode d'un BES de dos cambres. L'ús d'un raspall de fibra de grafit com a material d'elèctrode ha permès obtenir velocitats de degradació 5.6 vegades més elevades que les obtingudes treballant amb un altre material basat en el carboni com és el teixit de carboni. La PCR quantitativa ha confirmat que les còpies del gen 16S rRNA de *Dehalogenimonas* s'han incrementat dos ordres de magnitud fins a 10^8 copies del gen 16S rRNA per mL. L'aplicació d'un potencial polsat ha permès obtenir velocitats de degradació elevades i, alhora, eficiències coulombiques (CEs) més altes que quan s'ha operat a un potencial de càtode continu.

La degradació bioelectroquímica del cloroform (CF) s'ha estudiat en un BES de dos cambres aplicant de manera combinada les activitats de dos consorcis bacterians que contenen *Dehalobacter* i *Dehalobacterium*, capaços de reduir CF a diclorometà (DCM) i de fermentar DCM a acetat, respectivament. L'aplicació de tres potencials de càtode seqüencials (-0.6, -0.7 i -0.8 V vs SHE) ha permès incrementar la velocitat de degradació del CF alhora que s'han obtingut CEs més elevades del 60% inclús en el potencial més negatiu aplicat. L'operació en BES ha permès obtenir concentracions de *Dehalobacter* de fins a 10^7 còpies del gen 16S rRNA per mL després d'haver incrementat quatre ordres de magnitud la seva concentració inicial. Concentracions de CF de fins a 800 μM s'han degradat exitosament sense causar una inhibició irreversible a la fermentació del DCM per part de *Dehalobacterium*.

Finalment, la degradació del CF prèviament descrita es va combinar amb l'oxidació de toluè en un bioreactor d'una sola cambra en forma de pou electroquímic operat en continu. Un consorci oxidador de toluè ha establert satisfactòriament un biofilm adherit a la superfície de l'ànode (operant a +0.4 V de potencial) i ha sigut capaç de

generar un flux continu d'electrons cap al càtode, que s'han utilitzat per tal de produir H_2 i mediar la reducció del CF per part de *Dehalobacter*. S'han estudiat els efectes de diferents concentracions de toluè a l'influent del reactor, demostrant una correlació positiva entre aquestes i les velocitats de degradació per part del biofilm a l'ànode. Per altra banda, la fermentació del DCM per part de *Dehalobacterium* s'ha vist inhibida en presència de concentracions de 400 μM de toluè a l'influent, però s'ha recuperat en quant aquestes concentracions s'han reduït a 160 μM.

Resumen

La presencia de compuestos organohalogenados contaminando el medio ambiente y, más específicamente, las aguas subterráneas, es un grave problema debido a sus efectos adversos sobre el medio natural y la salud humana. Además, la relevancia de los acuíferos debido al incremento exponencial de la necesidad global de agua dulce, hace aumentar la importancia de su descontaminación. Una posible solución a este problema podría ser la biorremediación, que utiliza enzimas microbianas para degradar los contaminantes organohalogenados a productos menos tóxicos, o hasta inocuos.

En esta tesis, las tecnologías electroquímicas han sido utilizadas para degradar organohalogenados mediante dos aproximaciones. En primer lugar, hemos explorado el potencial de uso de los sistemas electroquímicos para transformar abióticamente compuestos bromados. En segundo lugar, hemos aprovechado el hidrógeno generado electroquímicamente para estimular el crecimiento y la actividad decloradora de bacterias respiradoras de organohalogenados (OHRB) y transformar compuestos clorados en productos no tóxicos en sistemas bioelectroquímicos (BES). Las OHRB y otras bacterias decloradoras utilizadas en este estudio fueron previamente enriquecidas en nuestro grupo de investigación.

Durante este trabajo, la reducción electroquímica abiótica de dos contaminantes bromados, dibromometano (DBM) y 1,2-dibromoetano (DBA), se ha realizado en un sistema compuesto por dos cámaras operando con un cepillo de fibra de grafito como cátodo. La degradación de 500 μM de ambos contaminantes se ha conseguido entre 1 y 7 horas de operación en los tres potenciales de cátodo aplicados: -0.8, -1.0 y -1.2 V vs electrodo estándar de hidrógeno (SHE). Los principales productos de la reducción del DBM y el DBA han sido determinados como metano y eteno respectivamente. El balance

de materia del bromuro ha permitido confirmar la completa debromación de ambos contaminantes.

Seguidamente, se ha estudiado la degradación bioelectroquímica del 1,2-dicloropropano (1,2-DCP) a propeno por un consorcio que contiene *Dehalogenimonas alkenigignens* en el cátodo de un sistema de dos cámaras. El uso de un cepillo de fibra de grafito ha permitido obtener velocidades de degradación 5.6 veces más elevadas que las obtenidas trabajando con otro material basado en el carbono como es el tejido de carbono. La PCR cuantitativa ha confirmado que las copias del gen 16S rRNA de *Dehalogenimonas* se han incrementado dos órdenes de magnitud hasta las 10^8 copias del gen 16S rRNA por mL. La aplicación de un potencial pulsado ha permitido obtener velocidades de degradación elevadas y, a la vez, eficiencias coulombicas (CEs) más altas que cuando se opera con un potencial de cátodo continuo.

La degradación bioelectroquímica del cloroformo (CF) se ha estudiado en un BES de dos cámaras aplicando de manera combinada las actividades de dos consorcios bacterianos que contienen *Dehalobacter* y *Dehalobacterium*, capaces de reducir CF a diclorometano (DCM) y de fermentar DCM a acetato, respectivamente. La aplicación de tres potenciales de cátodo secuenciales (-0.6, -0.7 i -0.8 V vs SHE) ha permitido incrementar la velocidad de degradación del CF a la vez que se han obtenido CEs más elevadas del 60% incluso en el potencial aplicado más negativo. La operación en BES ha permitido obtener concentraciones de *Dehalobacter* de hasta 10^7 copias del gen 16S rRNA por mL después de haber incrementado en cuatro órdenes de magnitud su concentración inicial. Concentraciones de CF de hasta 800 μM se han degradado exitosamente sin causar una inhibición irreversible en la fermentación del DCM por parte de *Dehalobacterium*.

Finalmente, la degradación del CF previamente descrita se combinó con la oxidación del tolueno en un biorreactor de una sola cámara en forma de pozo electroquímico operado en continuo. Un consorcio oxidador de tolueno ha establecido satisfactoriamente un biofilm adherido a la superficie del ánodo (operando a +0.4 V de potencial) y ha sido capaz de generar un flujo continuo de electrones hacia el cátodo, que se han utilizado para producir H_2 y mediar la reducción del CF por parte de *Dehalobacter*. Se han estudiado los efectos de diferentes concentraciones de tolueno en el influente del reactor, demostrando una correlación positiva entre estas y las velocidades de degradación por parte del biofilm en el ánodo. Por otro lado, la fermentación del DCM por *Dehalobacterium* se ha visto inhibida en presencia de concentraciones de 400 µM de tolueno en el influente, pero se ha recuperado en cuanto estas concentraciones se han reducido a 160 µM.

Table of contents

List of acronyms

Acronym	Name
1,1-DCA	1,1-dichloroethane
1,2-DCA	1,2-dichloroethane
1,2-DCP	1,2-dichloropropane
ANOVA	Analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
BES	Bioelectrochemical system
BTEX	Benzene, toluene, ethylbenzene and xylene
CE	Coulombic efficiency
CF	Chloroform
cis-DCE	*cis*-dichloroethene
DBA	1,2-dibromoethane
DBM	Dibromomethane
DCM	Dichloromethane
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DNA	Deoxyribonucleic acid
DNAPL	Dense non-aqueous phase liquid
EI	Energetic input
FID	Flame ionization detector
GC	Gas chromatography
HRT	Hydraulic residence time
MCF	Microbial fuel cell
MEC	Microbial electrolysis cell
NMR	Nuclear magnetic resonance
OCP	Open circuit potential
OHRB	Organohalide-respiring bacteria
PCE	Perchloroethene
PCR	Polymerase chain reaction
PVDF	Polyvinylidene fluoride
qPCR	Quantitative polymerase chain reaction
RdhA	Reductive dehalogenase subunit A
RdhB	Reductive dehalogenase subunit B
rRNA	Ribosomal ribonucleic acid
SEM	Scanning electron microscopy
SHE	Standard hydrogen electrode

Acronym	Name
TCE	Trichloroethene
trans-DCE	*trans*-dichloroethene
VC	Vinyl chloride

Thesis overview

This thesis is composed by 8 chapters:

Chapter 1 is the general introduction of this thesis and introduces the environmental impact of the contamination of groundwater by organohalide pollutants. The main remediation technologies for groundwater remediation are described and, more specifically, the bioremediation techniques applying anaerobic bacteria to degrade organohalides. Finally, the feasibility of electrochemical techniques to treat groundwater contaminated with organohalides through abiotic and biotic approaches are introduced.

Chapter 2 enounces the main objective of this thesis followed by the specific objectives which will be treated in the subsequent chapters.

Chapter 3 describes the general materials and methods employed during this thesis.

Chapters 4, 5, 6 and 7 contain the results derived from the experiments carried on during this thesis. These chapters are composed by a brief specific introduction related to the topic of the chapter, materials and methods that were not included in chapter 3, results, discussion, conclusions, and the bibliography.

In **chapter 4**, the abiotic electrochemical degradation of two brominated contaminants, dibromomethane (DBM) and 1,2-dibromoetane (DBA), is studied in a two-chamber system operating with a graphite fiber brush as cathode. The degradation rates for both contaminants were calculated when working at three different cathodic potentials: -0.8, -1.0 and -1.2 V vs standard hydrogen electrode (SHE). The coulombic efficiencies (CE) of the degradation were also assessed.

Chapter 5 presents the bioelectrochemical degradation of 1,2-dichloropropane (1,2-DCP) mediated by a *Dehalogenimonas alkenigignens*-containing consortia grown in the cathodic vessel of a two-chamber system. The degradation performance was assessed when working with two different carbon-based cathode materials: a graphite brush and a carbon cloth. Additionally, the application of a pulsed cathodic potential was compared to a conventional continuous potential operation in terms of degradation rate obtained and CE achieved.

Chapter 6 describes the bioelectrochemical degradation of CF by combining the activity of two bacterial consortia containing respectively *Dehalobacter* and *Dehalobacterium* in a two-chamber system. The effects of three sequential cathodic potentials (-0.6, -0.7 and -0.8 V vs SHE) in the CF degradation rate of *Dehalobacter* are studied, as well as the CEs obtained at each potential. Increasing concentrations of CF were amended to test the inhibiting effect on the fermentation of dichloromethane (DCM) by *Dehalobacterium*.

Chapter 7 couples the degradation of CF described in the previous chapter with the oxidation of toluene in a single-chamber bioelectric well reactor operated in continuous mode. The oxidation of toluene by a bacterial consortium growing in a biofilm attached to the anodic surface (poised at +0.4 V of potential) was used to deliver electrons to the cathode and produce H_2, which was used by the *Dehalobacter* consortia to reduce CF. The effects of different toluene inlet concentrations were studied on the degradation rates and CEs for toluene, CF and its intermediate product DCM.

Chapter 8 presents the general conclusions and achievements of this thesis, based on the results obtained during the previous chapters, as well as several brief thoughts

about the future work that could be performed in the field of the bioelectrochemical degradation of organohalide pollutants in groundwater.

Lastly, the CV of the author, which include his professional achievements and scientific publications, has been included at the end of this document.

Chapter 1

General Introduction

1.1 Organohalide pollutants and their environmental concern

1.1.1 Organohalide pollutants

Organohalide compounds have been used in a wide variety of industrial procedures, ranging from intermediates for the synthesis of chemicals to their use as adhesives, solvents, metal-degreasing agents, pesticides or plasticizers, among others [1–4]. Part of their attractive for the industry resides in their high stability and robustness. Unfortunately, these same characteristics are of environmental concern when these substances are released to the environment due to their long half-lives and persistence [5].

These substances not only affect the environment, but they can potentially have adverse effects on human health. When continuous exposure is produced, many of them have demonstrated to possess toxic, carcinogenic and mutagenic effects, as well as acting as endocrine disruptors [6,7]. These factors caused that the concentrations in drinking water of organohalide compounds such as 1,2-dichloroethane (1,2-DCA), chloroform (CF), bromoform, bromodichloromethane, dibromochloromethane, perchloroethene (PCE), trichloroethene (TCE) and vinyl chloride (VC) were regulated by the Spanish government [8].

1.1.2 Water scarcity and contamination

Despite water covering most of the world surface, taking into account its presence in liquid, solid or gaseous form, only less than 1 % corresponds to liquid freshwater. This volume accounts for 1.4 million of km^3, and 99 % of it corresponds to groundwater sources (Figure 1.1) [9]. The withdrawal of freshwater for anthropogenic purposes has increased exponentially during the last century, ranging from 600 $km^3 \cdot year^{-1}$ in 1900 to 3880 $km^3 \cdot year^{-1}$ in 2017, mainly caused by the growth of global population [9]. Therefore, the correct management of the groundwater, being the main source of

freshwater, is essential in order to sustain the expected increase in population during the next decades.

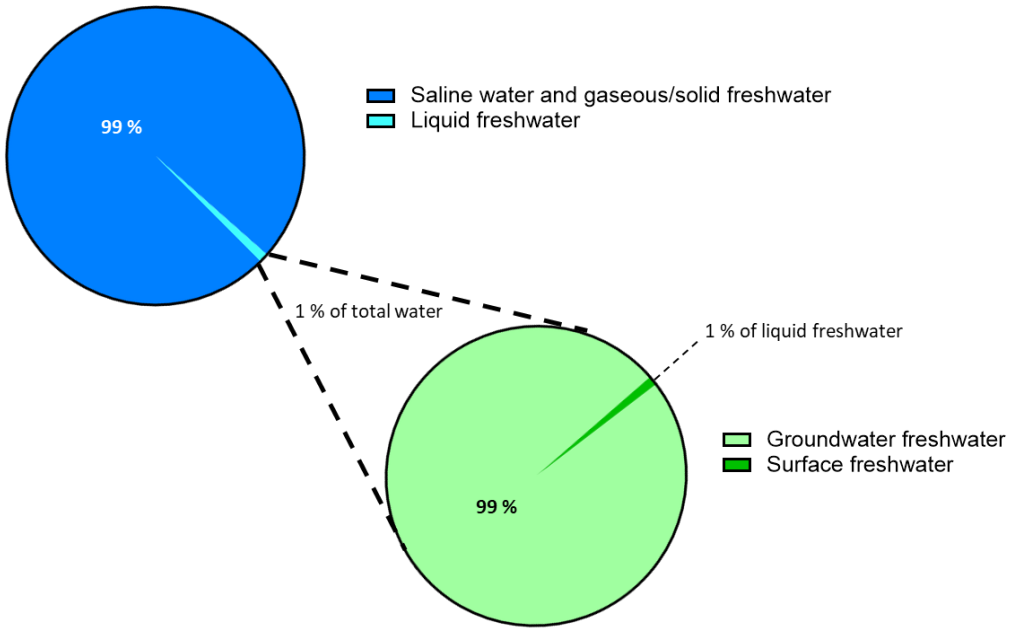

Figure 1.1. Percentages of global freshwater and groundwater.

Unfortunately, not only worldwide needs of water have increased, also the discharge of pollutants to the environment has arisen in the last years [10]. Approximately, 70 % of global water extraction is due to agricultural practices which, in turn, discharge large amounts of pesticides and other contaminants. High percentages of wastewater from settlements, industries and farms are released in the environment without treatment, resulting in tonnes of toxic sludge, heavy metals, chemicals and solvents reaching natural water sources every year [11]. Among these pollutants, a wide variety of organohalides are frequently detected. In Catalonia, the presence of halogenated contaminants was detected in at least 100 groundwater and soil polluted sites between 2000 and 2012 [12].

The current importance of the organohalogenated contaminants was assessed by the Agency for Toxic Substances and Disease Registry (ATSDR) from the United States,

actualizing its Substance Priority List on 2019, which classifies pollutants by a combination of their toxicity, potential of human exposure and frequency of detection [13]. In the list, 128 of the 275 pollutants compounds are organohalides, and 31 of them are ranked in the top 50 spots. Some of the pollutants appearing in the list are CF, dichloromethane (DCM), bromoform or 1,2-dibromoethane (DBA).

1.2 Remediation of organohalide contaminants in groundwater

1.2.1 Fate and behaviour of organohalides in groundwater

One of the main limitations in order to remove the contaminants present in groundwaters is their limited access. Additionally, the hydrogeology of the aquifers can be more heterogeneous than what it can apparently seem, as the contaminant plume, the different flow paths of groundwater or the effects of diffusion are not uniform along the aquifer and can also vary with time [14]. Most chlorinated compounds are classified as dense non-aqueous phase liquids (DNAPLs) because they are only slightly soluble in water and are denser than water. Therefore, once DNAPLs are released into the environment, they tend to move through the soil and accumulate on the non-permeable layer at the bottom of aquifers. The DNAPL stored in these low-permeability zones can act as a source of pollutant to the bulk of liquid by diffusion, slowly dissolving into groundwater and producing plumes of the halogenated pollutant (Figure 1.2) [15,16]. Due to their persistence, potential toxicity and difficulty to access, DNAPLs suppose a major challenge in the bioremediation of aquifers.

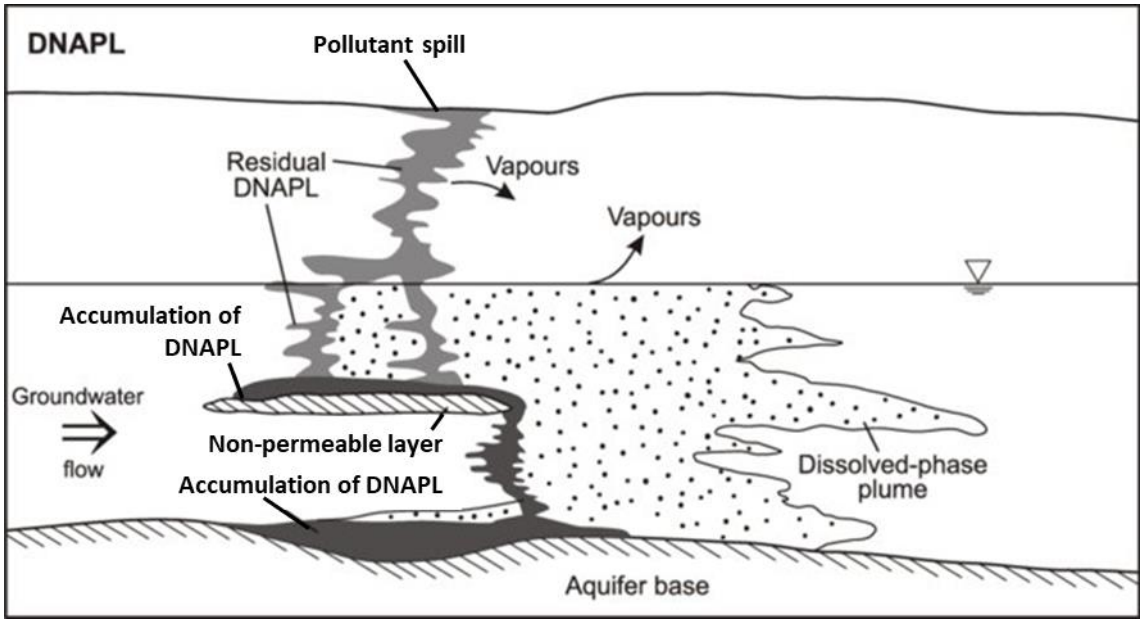

Figure 1.2. Diagram depicting DNAPL distribution in groundwater (modified from Rivett et al, 2006).

Considering all these factors, a deep understanding and characterization of all the hydrogeology and physicochemical characteristics of the aquifer are required in order to predict the behaviour of the pollutants present in groundwater. In recent years, the usage of surfactants has been extensively studied in order to mobilize and/or solubilize DNAPLs by lowering the interfacial tension in the liquid with the goal of increasing the extraction efficiency of contaminated groundwater through sampling wells [17]. Also, the use of computational methods to help in the prediction of the aquifer behaviour has arisen as a major tool for groundwater treatment [17].

1.2.2 Introduction to remediation

The main remediation technologies can be divided in three groups: chemical, physical and biological [18]. In numerous occasions, a single treatment is not enough to reduce the concentration of contaminants to the desired levels, and combinations of different technologies must be employed. Depending on its physical location, the treatment can be in-situ, if performed in the aquifer itself, or ex-situ, if the liquid is

extracted through sampling wells and treated elsewhere (e.g. pump and treat) [19]. If the requirements for the remediation allow for it, it is preferable to decontaminate the polluted site via an in-situ treatment, as it reduces the risk of human exposure to contaminants because it does not require groundwater extraction and it is typically less expensive.

The biological technologies, or bioremediation, deals with the exploitation of the ability of several microorganisms to break down pollutants to innocuous or less toxic products [20]. The degradation of the pollutant is one of their main advantages compared to most physical remediation technologies, as bioventing or adsorption, which only transfer the contaminants from groundwater to another compartment without their transformation to environmentally safer products [18]. Moreover, some physical treatments only intend to avoid the propagation of the contaminant by sealing it in a confined area with the usage of barriers [21]. Also, the mild conditions required for biological reactions are more beneficial for their in-situ application in the environment than most chemical methods, which usually require to produce more extreme reaction conditions, as for example the in-situ chemical oxidation (ISCO) which requires to add strong oxidizing agents [22]. Additionally, among the physical methods, thermal technologies present the same bottlenecks, as they apply high temperatures to degrade or mobilize the contaminant, process that is energetically demanding and negatively affect the properties of the aquifer [23].

Up to date, numerous organohalide remediation studies involving bacteria, fungi and microalgae have been published in the literature [24–26]. Additionally, phytoremediation, the capability of plants to remove contaminants from the environment when assisted by microbial or fungal communities, has also been studied [27].

Occasionally, the polluted sites contain intrinsic microbial populations or natural occurring processes which can degrade the contaminants without external intervention. In these cases, to perform the bioremediation process, it is only required to monitor the concentrations and the evolution of the characteristics in the aquifer in a process known as monitored natural attenuation, avoiding unnecessary human intrusion in the environment [28]. In other occasions, despite detecting in the polluted site endogenous microbial populations which are theoretically able to degrade the contaminant, its degradation is not observed at the necessary rate to conduct the process. In these cases, additional substrates, nutrients or other amendments can be added in the groundwater with the main objective of improving the biological activity, in a process known as biostimulation [29]. Finally, in cases where microorganisms able to perform the required degradation are not present in the polluted site, generally non-native microorganisms, but also enzymes capable to transform the target contaminant, can be introduced in a technology called bioaugmentation [14].

1.3 Degradation of organohalides by bacteria

1.3.1 Organohalide-degrading anaerobic bacteria

One characteristic commonly found in contaminated groundwater sources is their typically low oxygen concentrations. This is a key factor, as it hampers aerobic in-situ bioremediation with strict aerobic microorganisms as fungi or microalgae without the frequent addition of external oxygen to the groundwater, which can severely increase the cost of the process [19]. Nevertheless, this absence of oxygen is beneficial for organohalide-respiring or fermenting bacteria, as they are strictly anaerobic, hence being ideal candidates to perform the bioremediation. The main difference between both groups is that organohalide-respiring bacteria (OHRB) use the halogenated pollutant as terminal electron acceptor during their respiration and typically hydrogen as electron donor,

whereas the anaerobically fermenting bacteria employ the contaminant as an energy-providing carbon source in their catabolic routes [30,31]. Additionally, facultative anaerobic bacteria, which present the capability of surviving in anaerobic environments but do not strictly require them, have been found to degrade organohalides under anaerobic conditions. Facultative methylotrophic bacteria were observed to be able to degrade DCM [32] and several facultative anaerobic bacteria as the genus *Xanthobacter* have been reported to degrade TCE, *cis*-DCE, *trans*-DCE and VC [33].

Several pathways have been described for OHRB during degradation of halogenated compounds. One of the most common is hydrogenolysis, where a chlorine from the organohalogen is substituted by an atom of hydrogen, with a net spent of two electrons (Fig. 1.3 a). A characteristic example of hydrogenolysis is the reduction of PCE performed by *Dehalococcoides mccartyi*, where in each step a chlorine is substituted by a hydrogen atom, going through TCE, *cis*-dichloroethene (*cis*-DCE), vinyl chloride and ethene as final product [29]. Another mechanism is dihaloelimination, where two halogens from adjacent carbon atoms are simultaneously removed in alkanes, resulting in the formation of a double bond between both carbon atoms and with a net spend of two electrons (Fig. 1.3 b). One example of this reaction mechanism can be observed in the reduction of 1,2-dichloropropane (1,2-DCP) to propene in *Dehalogenimonas* genus [34]. A third mechanism, but only observed in abiotic reactions, is dehydrochlorination, where a halogen and a hydrogen atom from two sequential carbon atoms are removed, and a double bond between the carbons is formed (Fig 1.3 c). An example of this mechanism can be observed in studies involving the abiotic conversion of the chlorinated pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) [35].

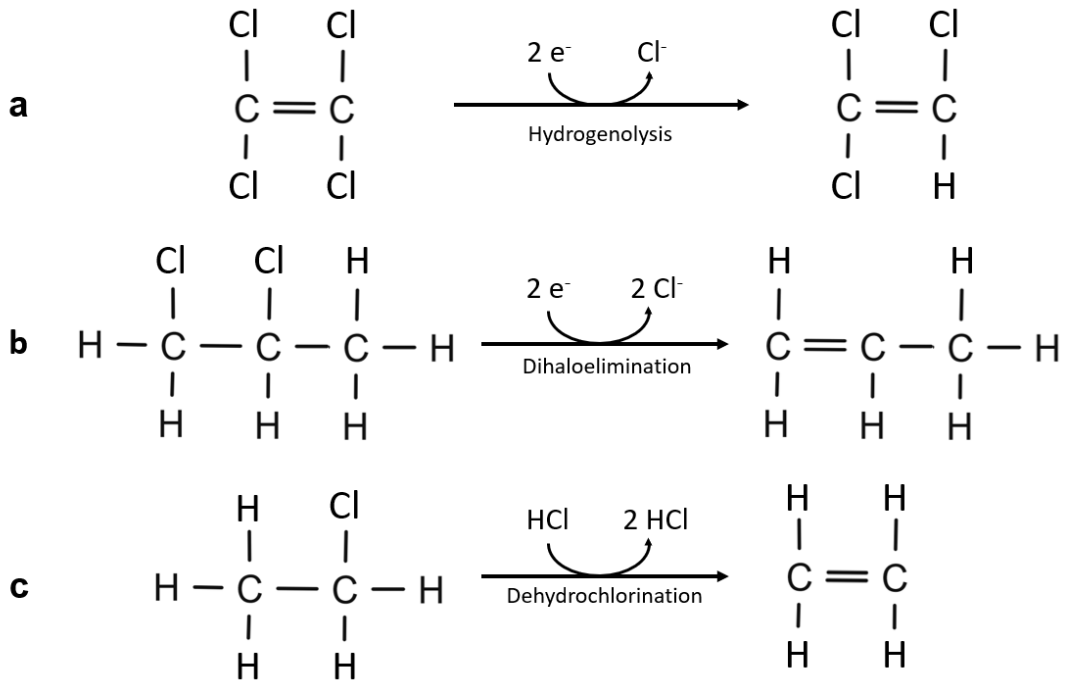

Figure 1.3. Examples of anaerobic degradation pathways of organohalides: hydrogenolysis (a), dihaloelimination (b) and dehydrochlorination (c).

It is important to know which factors and conditions can derive in one reaction pathway over the other in order to avoid the formation of by-products with different physicochemical properties as toxicity, mobility or persistence in the environment. One example is the degradation of tetrachloroethane, which can undergo with all three mechanisms (hydrogenolysis, dihaloelimination and dehydrochlorination), and depending on the degradation pathway can produce VC, which tends to accumulate in the environment and is more toxic than the parental compound [36].

1.3.2 Examples of OHRB and fermenting bacteria

OHRB catalyze the reduction of organohalides by using enzymes known as reductive dehalogenases. Most of the reductive dehalogenases characterized are composed by two subunits: the catalytic subunit A, encoded by gene *rdhA* and located outside of the cell, and subunit B, which acts as a transmembrane anchor for subunit A and is encoded by gene *rdhB* [37]. Depending on how strict is their dependency on the

organohalide respiration for their survivability, OHRB can be divided as obligated or non-obligated [5]. In case of being non-obligated, they have the possibility of using other non-halogenated compounds as electron acceptors, whereas obligated require the presence of the organohalide to grow.

One of the most studied genera of obligate OHRB is *Dehalococcoides*. One of its particularities is that *Dehalococcoides mccartyi* was the first OHRB to catalyze the complete dechlorination of PCE up to ethene by hydrogenolysis, going through TCE, *cis*-DCE and VC [38]. This capability is interesting for bioremediation purposes because the dechlorinating bacteria most commonly found in environmental polluted sites are only capable of performing a partial dechlorination up to cis-DCE, which is more toxic than the parental compound [26]. Resulting from this partial dechlorination, the latter compound is accumulated in a phenomenon called "*cis*-DCE stall" [39].

Another relevant genera of obligate OHRB is *Dehalogenimonas*, which is able to degrade several chlorinated ethanes as 1,2-DCP, 1,2-DCA or DBA by dihaloelimination [40]. The prevalence of this mechanism suggest that *Dehalogenimonas* possess a higher specificity for substrate than other OHRB as *Dehalococcoides*. Nevertheless, in recent years, studies have demonstrated that some *Dehalogenimonas* strains are also capable of degrading chlorinated aromatics (i.e. 1,2,4-trichlorobenzene and dichlorobenzenes) and chlorinated ethenes (i.e. *trans*-DCE and VC) via hydrogenolysis [41–43]. The genus *Dehalobacter* has been reported to degrade an extensive variety of organohalide pollutants as CF, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1-DCA and bromoform [30,44]. As non-obligate OHRB, *Desulfitobacterium* and *Sulfurospirillum* have also been reported to facultatively reduce organohalides [45–47].

Some bacteria degrade organohalides through a fermentation process, where one part of the molecule is oxidized and the other is reduced, avoiding the need of an additional electron acceptor. One of the most prevalent examples is the fermentation of DCM reported by three genera belonging to the *Peptococcaceae* family: *Dehalobacterium formicoaceticum*, '*Candidatus Formimonas warabiya*' (formerly referred as strain DCMF) and '*Candidatus* Dichloromethanomonas elyuquensis' [31,48–52]. The current hypothesis is that these bacteria metabolize DCM via the Wood-Ljungdahl pathway through two distinct routes, where one allows the mineralization of DCM to CO_2 and H_2 in '*Ca*. Dichloromethanomonas elyuquensis', and the other aids in fermentation of DCM to acetate and formate in *D. formicoaceticum*, and solely to acetate in '*Candidatus* Formimonas warabiya' [50,52].

1.4 Electrochemical systems for organohalide remediation

1.4.1 Electrochemistry applied to remediation

In the recent decades, electrochemistry has emerged as a relevant technology for treating organohalogenated pollutants. This technology has opened a wide range of possibilities of catalysing at the same time oxidative and reductive reactions in liquid medium through the application of electric current employing two electrodes. These capabilities have been extensively studied in order to perform the oxidation or reduction of halogenated pollutants through direct or indirect electron transfer between the molecule and the electrode surface [53].

One key factor in the success of the electrochemical degradation is the selection of the electrode material, as its surface is the responsible of delivering or accepting electrons, hence allowing or impeding the performance of the intended degradation. Moreover, the chosen material influences the electric conductivity, the specific surface

area, the required potentials to produce oxygen or hydrogen, the durability, the by-products obtained and the cost of the operation [54]. The selected electrolyte, which can range from aqueous media to organic solvents, also has the potential to define the nature of the reactions performed in the system.

1.4.2 Electrochemical reduction, oxidation and combined processes

The electrochemical reduction of halogenated pollutants is performed in the cathode and allows for the cleavage of the carbon – halogen bond through a direct or indirect mechanism, which will be mainly defined by the electrode material used. In case of a direct reduction, electrons are directly transferred from the cathodic surface to the pollutant molecule, whereas in the indirect mechanism an intermediate compound (i.e. hydrogen adsorbed to the electrode) receives the electrons before delivering them to the target pollutant. Previous studies have treated by direct reduction organohalides as 1,2-DCA and 1,1,2,2-tetrachloroethane with silver, TCE with copper and hexachlorobenzene with graphite electrodes [55–58]. Additionally, Radjenović et al. performed the dehalogenation of a complex mixture of 17 disinfection byproducts by using a graphite electrode, including chlorinated, brominated and iodinated methanes, ethanes and propanes [59]. Indirect reduction has been used to treat contaminants as TCE or 2,4-dichlorophenol by using palladium based electrodes combined with other materials [60–63].

Differently from the reduction, where only the cleavage of the carbon-halogen bond is obtained, the electrochemical oxidation in the anode has the potential to break down the target molecule or, given the required conditions, achieve the complete mineralization of the contaminant to carbon dioxide and water [53]. However, oxidation reactions can produce a wider range of by-products which can cause environmental concern, so an exhaustive analysis of their toxicity is mandatory. This reaction

mechanism has been used to treat chlorophenols and pesticides as diuron and dichloroaniline by using a wide variety of electrode materials [64–66].

Occasionally, the electrochemical oxidation process is unable to fulfil a complete mineralization and still produces halogenated by-products. In order to overcome this environmental concern, the combined application of oxidation and reduction electrochemical processes has been studied. The purpose of this strategy is to obtain the cleavage of the carbon-halogen bond through reduction in the cathode and the subsequent oxidation of the resulting byproducts in the anode. Moreover, exists the possibility of performing simultaneously the anodic and cathodic processes if both electrodes are located in the same vessel. The combined reduction and oxidation reactions in electrochemical processes has been employed to degrade chloroalkanes as 1,2-DCA or 1,1,2,2-tetrachloroethane, aromatics as 2,4-dichlorophenol or pesticides as triclosan, among others [67–70].

1.5 Organohalide degradation with bioelectrochemical systems

1.5.1 Bioelectrochemical remediation of organohalides

Electrochemistry provides the possibility of producing hydrogen in aqueous medium when certain negative potentials are poised in the cathodic electrode. This capability presents a strong synergy with the bioremediation of groundwater sources with OHRB, due to their strict requirements for hydrogen as electron donor, and can avoid the need of adding fermentable substrates as lactate as precursors of the aforementioned gas [71].

The application of bioelectrochemistry methods for bioremediation over abiotic electrochemical catalysis allow the usage of milder reaction conditions regarding the temperature and the electrolyte used to ensure the viability of microorganisms. Some

abiotic degradation processes require high temperatures, extreme pHs, organic solvents or high concentrations of solutes which are conditions not suitable for the growth of living microorganisms [53]. So, milder conditions and the possibility of providing hydrogen directly to the aquifer make the bioelectrochemical processes more suitable for their in-situ application than the electrochemical catalysis, avoiding the additional costs and difficulty of operation associated with the pump and treat methodology [72]. Moreover, electrodes made of carbon-based materials as graphite, especially in brush form, are frequently used to drive biological processes due to their capabilities to adsorb biomass, high ratios of surface area related to the volume and their relatively low economical cost [73].

1.5.2 BES configurations

The two main configurations of bioelectrochemical cells which have been described in the literature are microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). MFCs purpose is to convert the production of chemical energy from the oxidation of substrates in the anode into electric energy, with its potential subsequent conversion to energy-valuable products as H_2. On the other hand, MECs have derived from MFCs and their objective is to catalyze reactions to produce profitable value-added chemicals or to degrade undesired substances in bioremediation processes, while requiring an external supply of energy to drive the reaction [74]. If the MEC only catalyses a biological reaction in the cathode, it is known as a half-biological MEC, whereas if the anode is also involved in a biological degradation it is known as a full-biological MEC.

Usually, half-biological MECs use a dual-chamber system with physical separation between anode and cathode, as a cationic exchange membrane, in order to avoid the loss of the reducing conditions created in the cathodic vessel. This MEC

configuration has been extensively employed in studies involving the reduction of several organohalides as TCE by using bacteria of the genera *Dehalococcoides* under different experimental conditions and testing different electrode materials and coatings [75–79]. Moreover, half-biological MECs have been employed in the degradation of an extended variety of halogenated pollutants including 1,2-DCA with *Dehalococcoides* [80–82], 2-chlorophenol with *Anaeromyxobacter* [83], 2,4,6-trichlorophenol with *Acetobacterium* and *Desulfovibrio* [84], pentachlorophenol with a mixed culture [85] and polychlorinated biphenyls with *Dehalococcoides* [86]. The bioelectrochemical degradation of complex chlorinated antibiotics as chloramphenicol has also been studied [87,88]. Additionally, some studies involving fluorinated pollutants as *p*-fluoronitrobenzene have also been reported [89,90].

Occasionally, the biological reduction obtained in the cathode is not enough to completely dehalogenate the organohalide pollutant, and several by-products are accumulated in the system. In these cases, full-biological MECs can be employed in order to couple sequentially an anodic oxidation driven by bacteria in the anode to the products obtained in the cathodic reduction. A previous study has coupled the bioelectrochemical reduction of TCE to VC in the biocathode of a continuous BES and the subsequent oxidation of the latter in the anode [91]. The use of BES without membrane separating both electrodes has also been studied in order to facilitate the transfer of compounds and enhance the performance of PCE bioelectrochemical degradation [92].

In the recent years, full-biological MECs have been used to study the simultaneous biological oxidation and reduction of mixtures of contaminants [93]. One example of this strategy is the usage of a tubular continuous bioelectrochemical reactor configuration known as “bioelectric well” which has been employed to treat toluene through an anodic oxidation and TCE through a cathodic reduction mediated by *Dehalococcoides* [94].

Despite the theoretical advantages of BESs for the bioremediation of groundwater, their in-situ application in aquifers has not been already reported. Some studies involving the degradation of non-halogenated pollutants by completely submerging a BES in contaminated groundwater under laboratory conditions have been performed, simulating an in-situ operation. This methodology was used by Shu-Hui et al. to simulate the oxidation of benzene in a series of MFC with 3.3 L of total volume [95] and also has been in studies involving denitrification [96,97]. Up to date, the existent pilot-scale BESs have been employed in municipal and industrial wastewater plants for the oxidation of organic matter [98,99] and in the treatment of urine [100]. Further research must be conducted to implement bioelectrochemical systems at pilot scale for the remediation of organohalides in groundwater.

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Chapter 2

Objectives of the thesis

The main objective of this thesis is to provide new insights into the applicability of (bio)electrochemical systems for the remediation of groundwater contaminated with halogenated pollutants, by expanding the number of halogenated pollutants degradable and the anaerobic dehalogenating bacteria capable of growing in bioelectrochemical systems (BES), while studying the main variables affecting the performance of the treatment. In order to fulfil this purpose, the following specific objectives were stablished:

- To study the feasibility of electrochemical systems to abiotically transform brominated pollutants (dibromomethane and 1,2-dibromoethane) to non-toxic products.
- To select a suitable cathodic electrode material for the supply of electrochemically generated hydrogen in a BES to reduce 1,2-dichloropropane to propene by *Dehalogenimonas* sp..
- To study the effect of operating BES with pulsed and continuous cathodic potential on the coulombic efficiency and degradation of 1,2-dichloropropane.
- To study the feasibility of combining the dechlorination capabilities of *Dehalobacter* and *Dehalobacterium* to sequentially transform chloroform to dichloromethane and acetate in BES operated at sequentially decreasing potentials.
- To couple the bioelectrochemical reduction of chloroform by *Dehalobacter* in a cathode with electrons provided by the oxidation of toluene by an anodic biofilm in a continuous flow tubular reactor.

Chapter 3

General materials and methods

3.1 Materials

3.1.1 Chemicals

All the chemicals used had a purity of at least 98% and they are listed with their names, abbreviations, brands, and state of matter in table 3.1.

Table 3.1. Names, abbreviations, brands, and state of matter of the reactants employed during this thesis.

Name	Acronym	Brand	State of matter
1,2-Dibromoethane	DBA	Sigma	Liquid
1,2-Dichlorobenzene	1,2-DCB	Sigma	Liquid
1,2-Dichloropropane	1,2-DCP	Sigma	Liquid
1,3-Dichlorobenzene	1,3-DCB	Sigma	Liquid
1,4-Dichlorobenzene	1,4-DCB	Sigma	Liquid
Acetic acid		Sigma	Liquid
Acetone		Sigma	Liquid
Ammonium chloride		Panreac	Solid
Benzene		Sigma	Liquid
Biotin		Sigma	Solid
Boric acid		Panreac	Solid
Bromoethane		Sigma	Liquid
Calcium chloride		Panreac	Solid
Calcium pantothenate		Sigma	Solid
Chlorobenzene	MCB	Sigma	Liquid
Chloroform	CF	Sigma	Liquid
cis-dichloroethene	*cis*-DCE	Sigma	Liquid
Cobalt chloride 6-hydrate		VWR (Avantor)	Solid
Copper(II) chloride dihydrate		Sigma	Solid
Cyanocobalamin		Sigma	Solid
Dibromomethane	DBM	Sigma	Liquid
Dichloromethane	DCM	Sigma	Liquid
Ethanol		Fisher	Liquid
Ethylene		Sigma	Gas

Name	Acronym	Brand	State of matter
Formic acid		Merck	Liquid
Gas mix CO_2 / N_2 (4:1, v/v)		Carburos Metálicos	Gas
Hydrogen		Carburos Metálicos	Gas
Iron(II) chloride tetrahydrate		Sigma	Solid
L-cysteine		Sigma	Solid
Magnesium chloride hexahydrate		Sigma	Solid
Manganese (II) chloride 4- hydrate		Panreac	Solid
Nickel (II) chloride		Sigma	Solid
Nicotinic acid		Sigma	Solid
Nitrilotriacetic acid		Sigma	Liquid
Nitrogen	N_2	Carburos Metálicos	Gas
Potassium chloride		Panreac	Solid
Potassium dihydrogen phosphate		VWR (Avantor)	Solid
Propene		Chem Service	Gas
Pyridoxine hydrochloride		Sigma	Solid
Resazurin sodium salt		Sigma	Solid
Sodium bicarbonate		Sigma	Solid
Sodium chloride		Panreac	Solid
Sodium hydroxide		Panreac	Solid
Sodium molybdenum oxide dihydrate		VWR (Avantor)	Solid
Sodium sulfide nonahydrate		Sigma	Solid
Thiaminchloride- hydrochloride		Merck	Solid
Toluene		Sigma	Liquid
trans-dichloroethylene	*trans*-DCE	Sigma	Liquid
Vinyl bromide		Sigma	Liquid
Vinyl chloride	VC	Chem Service	Liquid
Vitamin B12		Sigma	Solid
Yeast extract		Scharlau	Solid
Zinc chloride		VWR (Avantor)	Solid

3.1.2 Microorganisms

The bacterial consortia employed during this thesis were enriched previously in our research group and they are outlined below.

3.1.2.1 *Dehalogenimonas alkenigignens* strain BRE15M

This enriched culture was previously obtained from sediments collected at the Besós river estuary (Spain) [1,2]. The bacterial community was dominated by the genera *Dehalogenimonas* (68%), *Desulfovibrio* (31.5%) and others (0.5%) (data not published). The genome of this strain was recently sequenced and annotated, and it was denominated *Dehalogenimonas alkenigignens* strain BRE15M [2]. This strain catalyzes the dichloroelimination of chloroalkanes with two chlorines on vicinal carbon atoms during organohalide respiration.

3.1.2.2 *Dehalobacter* sp. strain 8M

This enriched culture was obtained from groundwater contaminated with CF [3]. This bacterial consortium was composed by members belonging to the genera *Dehalobacter* (20%), *Desulfovibrio* (20%) and *Proteiniphilum* (57%) [3]. This *Dehalobacter* strain used CF and 1,1,2-trichloroethane as electron acceptors during organohalide respiration [3].

3.1.2.3 Dehalobacterium formicoaceticum

This enriched culture was obtained from slurry samples of a membrane bioreactor from a wastewater treatment plant [4]. This bacterial consortium was composed by members belonging to the genera *Acetobacterium* (46.9%), *Dehalobacterium* (24.2%), *Desulfovibrio* (16.2%) and *Wolinella* (12.6%). This *Dehalobacterium* anaerobically ferments dichloromethane to acetate and formate [5].

3.1.3 BES setups

3.1.3.1 Two-chamber electrochemical cells

The electrochemical cells were designed specifically to conduct this thesis and consisted of two 165 mL glass vessels separated by a cation-exchange membrane (CMI-7000, Membranes International INC, USA), with an aperture diameter of 4 cm (Fig. 3.2). A titanium sheet was used as the anode. In the cathodic compartment, depending on the experiment, 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. A power source (Quad Potentiostat, Whistonbrook Software) was used to control the voltage and the potential in the cathode. 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. Teflon-coated 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, as depicted in Figure 3.1.

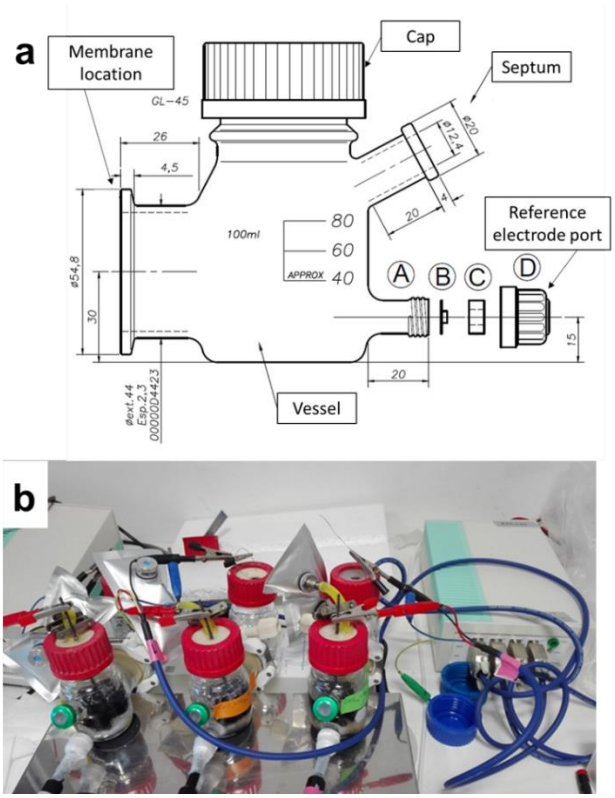

Figure 3.1. Scheme of one chamber of BES (a) and photography of the two-chamber BES used in this thesis (b).

3.1.3.2 Bioelectric well

The "bioelectric well" reactor consisted in a 290 ml glass cylinder containing eight contiguous graphite rods acting as anode (length: 30 cm, diameter: 0.6 cm; Sigma-Aldrich, Italy) and one concentric stainless steel mesh acting as cathode (3 x 30 cm; type 304, Alpha Aesar, USA), as it was previously described (Fig. 3.2) [6]. Both electrodes, anodic and cathodic, were separated physically by a polyethylene mesh (length: 30 cm, diameter: 1 cm), which allowed to maintain a hydraulic connection between the graphite rods and the stainless-steel mesh. The anodic potential was controlled by using an Ag/AgCl reference electrode (+198 mV vs SHE) located at the highest part of the reactor and an IVIUMnSTAT potentiostat (IVIUM Technologies, The Netherlands) and was poised to a value of +0.4 V vs SHE. Titanium wires (diameter: 0.81 mm; Alfa Aesar, USA) were employed as a connection between the electrodes and the power source.

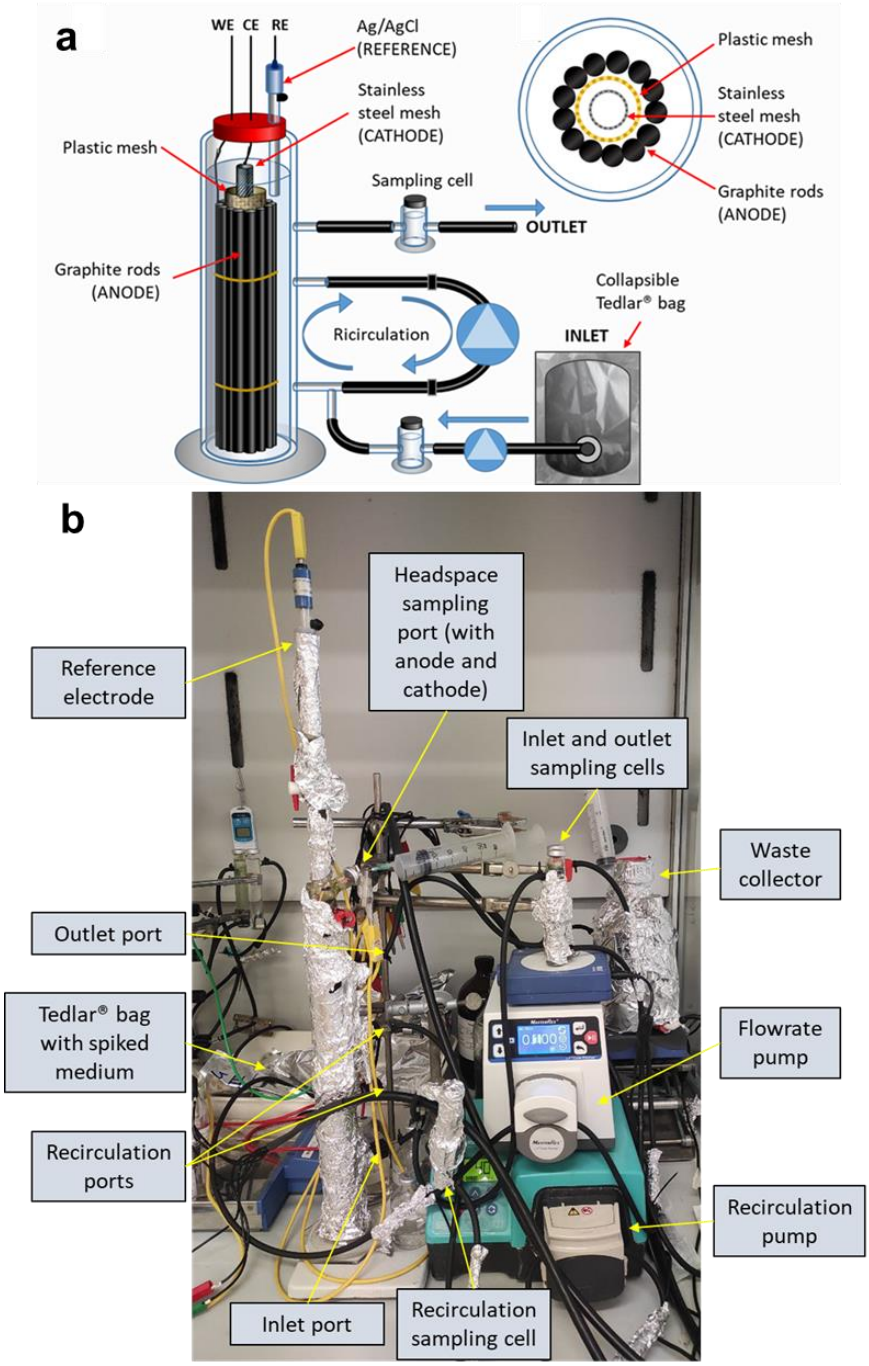

Figure 3.2. Scheme of the bioelectric well and its cross-sectional view representing the position of the electrodes (a) and its photography while operating in continuous mode.

3.2 Methods

3.2.1 Anaerobic medium preparation

Anaerobic basal medium for bacterial microcosms was prepared as described by Adrian et al. (2000) [7]. The solution was prepared under aerobic conditions and its

composition varied depending on the bacterial cultures used. The standard synthetic basal medium used for all cultures contained the following stock solutions: mineral salts, trace metals, sodium acetate, tungsten/selenium and resazurin as redox indicator. The composition and concentrations of each stock solution are listed in Table 3.2.

Table 3.2. Composition of the stock solutions used in the initial mix of the medium.

Name of the Stock Solution	Chemical	Amount per litre of medium
Mineral Salts	KH_2PO_4	0.2 g
	NH_4Cl	0.27 g
	NaCl	1 g
	$MgCl_2 \cdot 6H_2O$	0.41 g
	KCl	0.52 g
	$CaCl_2 \cdot 2H_2O$	0.15 g
Trace Metals	Nitrilotriacetic acid	128 mg
	$FeCl_2 \cdot 4H_2O$	20 mg
	$Na_2MoO4 \cdot 2H_2O$	0.36 mg
	$NiCl_2 \cdot 6H_2O$	0.24 mg
	$CuCl_2 \cdot 2H_2O$	0.02 mg
	$CoCl_2 \cdot 6H_2O$	0.19 mg
	H_3BO_3	0.06 mg
	$MnCl_2.4H_2O$	0.8 mg
	$ZnCl_2$	0.7 mg
Sodium Acetate	$C_2H_3NaO_2$	0.68 g
Tungsten / Selenium	Na_2SeO_3	0.015 mg
	$Na_2WO_4 \cdot 2H_2O$	0.02 mg
Resazurin	Resazurin sodium salt	50 µL

In order to remove oxygen from the basal medium, the solution was connected to a vacuum pump during 45 min and bubbled with nitrogen for 25 min. After that, the basal

medium was introduced to an anaerobic glovebox (Coy) containing a gas mix consisting of nitrogen (97%) and hydrogen (3%) (Fig. 3.3 a). Inside the glovebox, the medium was aliquoted into 100 ml serum bottles (Wheaton) (65 ml per bottle) and sealed with Teflon-coated butyl rubber septums and aluminium crimp caps (Fig. 3.3 b). To ensure sterile conditions, the sealed bottles were autoclaved at 121 ºC for 30 minutes.

Figure 3.3. Anaerobic glovebox used to prepare the anaerobic medium (Panel A) and glass serum bottles with anaerobic medium (Panel B).

Once the bottles recovered room temperature, the following compounds were added from anaerobic sterile stock solutions depending on the bacterial species cultivated: buffering agent, reducing agent 1, reducing agent 2, vitamins and additional nutrients. The composition of each solution is detailed in Table 3.3.

Table 3.3. Composition of the anaerobic stock solutions used in the medium.

Name of the Stock Solution	Chemical	Amount per litre of medium
Buffering agent	$NaHCO_3$	0.7 g
Reducing agent 1	$Na_2S \cdot 9H_2O$	48 mg
	L-cysteine	24 mg
Reducing agent 2	Titanium (III) citrate	0.29 mmol
Vitamins	4-aminobenzoic acid	20 µg
	D(+)-biotin	5 µg
	Nicotinic acid	50 µg
	Calcium D(+)-pantothenate	25 µg
	Pyridoxine hydrochloride	75 µg
	Thiamine chloride hydrochloride	50 µg
	Cyanocobalamin	50 µg
Additional nutrients	Yeast extract	200 mg

Each bottle was inoculated with 3 ml of the corresponding active culture during its exponential degradation phase. Each microcosm was fed with the corresponding halogenated compound from a stock solution with acetone using a glass syringe (Vici) up to the required nominal concentration.

The microcosms were gassed, depending on the culture, with a gas mix (N_2/CO_2, 4:1, v/v) at 0.2 bar of overpressure followed by H_2 at 0.4 bar of overpressure or only with N_2 at 0.4 bar in order to prevent the entrance of oxygen. The inoculated bottles were incubated under dark and static conditions in a thermostatic chamber at 25 ºC. The stock solutions, gases and contaminants employed for each bacterial culture are listed in table 3.4.

Table 3.4. Medium composition, gas amendment and contaminants spiked for each bacterial culture.

Bacterial consortia	*Dehalogenimonas*	*Dehalobacter*	*Dehalobacterium*
Mineral salts	x	x	x
Trace metals	x	x	x
Sodium acetate	x	x	
Tungsten / Selenium		x	x
Resazurin	x	x	x
Buffering agent	x	x	x
Reducing agent 1		x	x
Reducing agent 2	x		
Vitamins	x	x	x
Additional nutrients		x	x
Gases	N_2 + CO_2 , H_2	N_2 + CO_2 , H_2	N_2
Chlorinated compounds used in this study	1,2-Dichloropropane	Chloroform	Dichloromethane
Concentration of the chlorinated compound amended in the microcosms (μM)	500	500	2000
Products obtained	Propene	Dichloromethane	Acetate

3.2.2 Analytical methods

3.2.2.1 Gas analysis in serum bottles

The concentrations of volatile halogenated compounds (CF, DCM, 1,2-DBA, DBM, bromoethane, vinyl bromide, bromomethane, PCE, TCE, *cis*-DCE, VC and 1,2-DCP), methane, ethene, ethane, propene and propane were determined by injecting 0.5 mL headspace samples into an Agilent 6890N 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 previously described method [4]. After the injection of the sample (split ratio 1:2), the initial oven temperature (35°C) was held for 3 min and then ramped at 10 °C·min^{-1} to 240 °C, which was held for 4 min. The inlet and the flame ionization detector were held at 250 and 300 °C during the length of all the analysis. Run time lasted 27 min.

3.2.2.2 Gas analysis in the two-chamber electrochemical cells

The concentration of the compounds mentioned in the previous section contained in the two-chamber electrochemical cells were determined by static headspace gas chromatography. Thus, a 1 ml liquid sample from the cathode was removed through the sampling port and transferred to 10 mL vials sealed with Teflon-coated stoppers containing 5.5 mL deionized water. The vials were placed in a headspace sampler (Agilent 7964) and heated to 85 ºC for 15 min. Automatically, 1 mL headspace gas sample from the vials was injected into the gas chromatograph equipped with the same column and using the same method as described in section 3.2.2.1.

Hydrogen in the cathodic vessel was measured by taking 0.1 mL headspace samples from the sampling port with a pressure-lock syringe (Vici) and using an Agilent 7820A GC equipped with a thermal conductivity detector as previously described [5]. The chromatograph was equipped with a thermal conductivity detector and a MolSieve 5A 60/80 SS (1.82 m x 2 mm, Agilent) and a Porapak Q 60/80 UM (1.82 m x 2 mm, Agilent) columns. The carrier gas was N_2 at 138 kPa. Oven temperature remained at 40 ºC during the analysis and the injector and detector temperatures were held at 200 ºC and 250 ºC respectively. Each analysis lasted 5 min.

3.2.2.3 Gas analysis in the bioelectric well

Oxygen, carbon dioxide, and hydrogen were measured by injecting 50 µL of headspace samples taken with a pressure-lock syringe through the sampling port of the cells into a gas chromatograph (Agilent 8860, GC system, USA) equipped with a thermal conductivity detector (TCD) (2 m x 2 mm stain- less steel column packed with 60/80 Carboxen 1000 Supelco; N_2 was the carrier gas (20 ml min^{-1}), oven temperature was held at 150°C, injector temperature was 200°C and thermal conductivity detector temperature was 200°C [8].

The same chromatograph equipped with a flame ionization detector (FID) and an Agilent 125-1364 column (60 m x 530 µm x 3 µm) was used to quantitate toluene, CF, DCM and methane with a method previously described [9]. The carrier gas was N_2 at 7 $ml \cdot min^{-1}$ and the temperature of oven was held at 40 °C during 3 minutes and then ramped 20 $°C \cdot min^{-1}$ for 7.5 min and was held at 190 °C for 1 min. Injector and detector were held at 250 and 300 °C respectively. Due to the difficulty of using nominal concentrations due to the physical heterogenicity of the different parts of the reactor, the gas phase concentrations measured were converted to liquid phase concentrations by using their respective Henry's law constants [10].

3.2.3 Concentration and degradation rate calculations

3.2.3.1 Calculation of CF, DCM and propene concentrations in the two-chamber cells

Concentration of CF, DCM and propene during the experiments carried out on chapters 5 and 6 were calculated on the basis of the concentration measured by GC in BES and corrected as function of the decrease in their concentration observed in BES

spiked solely with these compounds. The accumulated value of concentration lost during each experiment was estimated in each propene measurement using equation 3.1:

$$[C_{lost}]_n = k_C \cdot \frac{[C_m]_n - [C_m]_{n-1}}{2} \cdot (t_n - t_{n-1}) + [C_{lost}]_{n-1} \quad (3.1)$$

where $[C_{lost}]_n$ is the accumulated amount of concentration of CF, DCM or propene lost during the experiment at the sampling point n (µM), k_C is the CF, DCM or propene leaking constant (d^{-1}), $(([C_m]_n-[C_m]_{n-1})\cdot 2^{-1})$ is the average CF, DCM or propene concentration between the current and previous measurement (µM), (t_n-t_{n-1}) is the time between the current and previous measurement (d) and $[C_{lost}]_{n-1}$ is the previous accumulated CF, DCM or propene loss amount (µM).

k_C was of 0.0396, 0.028 and 0.040 d^{-1} for CF, DCM and propene respectively, and was estimated from sets of triplicate experiments with aqueous standards in the same working conditions than the experimental cells (Fig. 3.4).

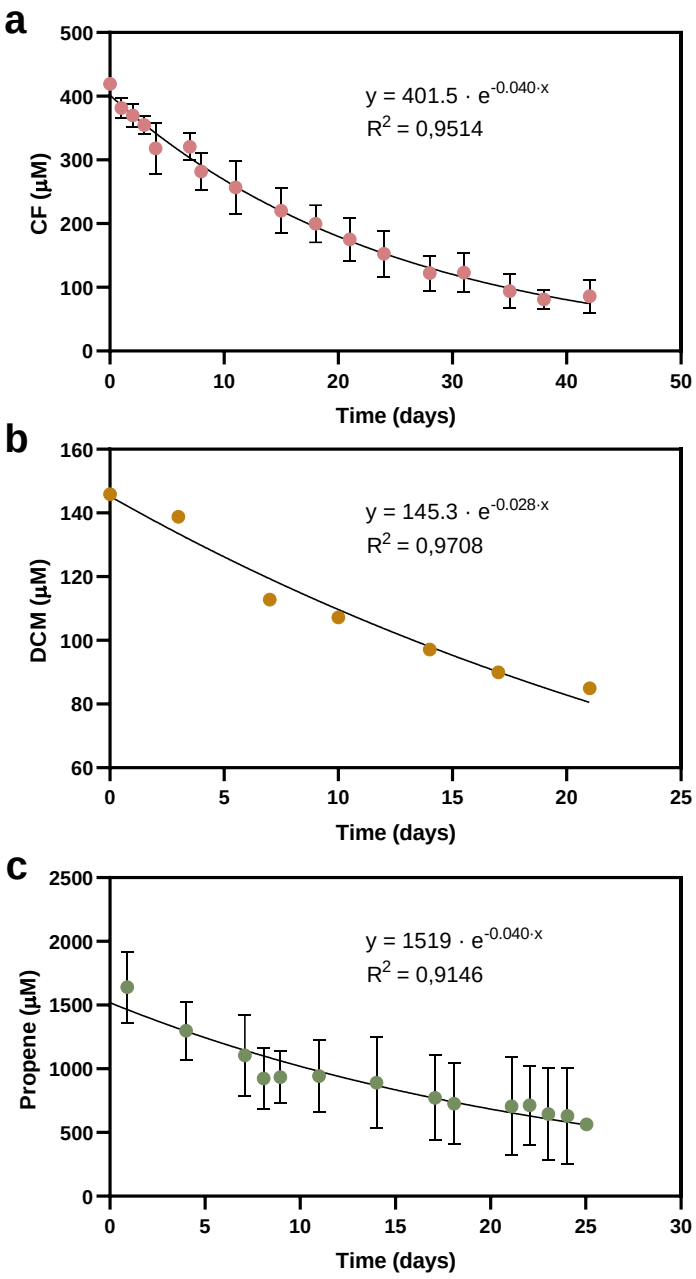

Figure 3.4. Exponential regression of the CF (a), DCM (b) and propene (c) retention in BES triplicates. Bars indicate the deviation for triplicate experiments.

The accumulation of the reaction products (DCM and propene) was calculated as equation 3.2:

$$[C]_n = [C_m]_n + [C_{lost}]_n \quad (3.2)$$

where $[C]_n$ is the corrected value for DCM or propene accumulation at a certain measured point, $[C_m]_n$ is the measured concentration of DCM or propene in the reactor and $[C_{lost}]_n$ is the accumulated amount of DCM or propene loss.

3.2.3.2 Concentration and degradation rates from the bioelectric well

During the continuous phase of the bioelectric well described on chapter 7, the degradation rate of toluene (q_{TOL}) and chloroform (q_{CF}) was calculated using equation 3.3:

$$q = \frac{C_{in} - C_{out}}{V} \cdot Q \quad (3.3)$$

where q is the degradation rate of toluene or CF ($\mu mol \cdot L^{-1} \cdot d^{-1}$), C_{in} and C_{out} are the contaminant concentrations measured in the inlet and the outlet of the reactor respectively ($\mu mol \cdot L^{-1}$), V is the volume of the bioelectric well (L) and Q is the flowrate ($L \cdot d^{-1}$).

The degradation of DCM ($C_{DCM\ deg}$) was calculated under the assumption that each mol of CF degraded was converted exactly to one mol of DCM, as it has been observed in previous studies involving the same *Dehalobacter* bacterial consortia [3]. The concentration of DCM degraded was calculated using equation 3.4:

$$C_{DCM\ deg} = (C_{CF\ in} - C_{CF\ out}) - C_{DCM\ out} \quad (3.4)$$

where C_{DCM} out is the concentration of DCM measured in the outlet.

With the calculated amount of DCM degraded, the degradation rate of DCM (q_{DCM}) was calculated as it follows:

$$q_{DCM} = \frac{C_{DCM\ deg}}{V} \cdot Q \quad (3.5)$$

3.2.4 Coulombic efficiency calculations

3.2.4.1 Coulombic efficiency for the electrochemical degradation experiments of brominated contaminants

The coulombic efficiency (CE) for the electrochemical degradation experiments of brominated contaminants at the cathode from chapter 4 was calculated using equation 3.6:

$$CE = \frac{n_e \cdot V \cdot F \cdot [Br^-]}{\int I(t)dt} \quad (3.6)$$

where n_{e^-} is the number of electrons required to release a bromide ion (corresponding to 2 and 1 in the DBM and DBA reductions respectively, as it can be observed in Fig. 3.5), V is the cathodic liquid volume (L), F is Faraday's constant (96485 $A \cdot s \cdot mol^{-1}$), $[Br^-]$ is the concentration of released bromide ions (μM) and ∫I(t)dt is the integration of the measured intensity throughout the experiment.

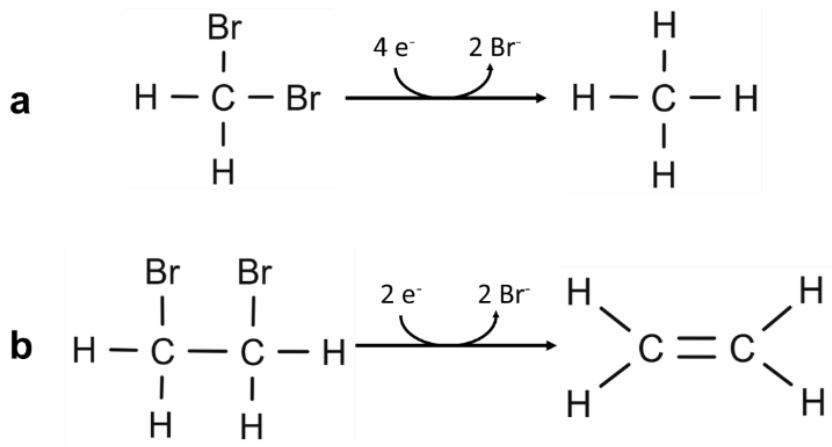

Figure 3.5. Electrochemical reductions observed in the cathodic vessel for DBM (a) and DBA (b).

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

$$EI = \frac{V_A \cdot \int I(t)dt}{V \cdot [Br^-]} \quad (3.7)$$

where V_A is the voltage applied in the system (V).

3.2.4.2 CE for the 1,2-DCP and CF bioelectrochemical degradation experiments

The CE for each degradation experiment of 1,2-DCP and CF at the cathode from chapters 5 and 6 was calculated using equation 3.8:

$$CE = \frac{2 \cdot V \cdot F \cdot [C]_{deg}}{\int I(t)dt} \quad (3.8)$$

where 2 is the number of electrons required to dechlorinate a molecule of 1,2-DCP or CF to propene and DCM respectively, and $[C]_{deg}$ is the degraded concentration of 1,2-DCP or CF (M).

Additionally, the EI required for each experiment was calculated as follows:

$$EI = \frac{V_A \cdot \int I(t)dt}{V \cdot [C]_{deg}} \quad (3.9)$$

3.2.4.3 CE calculation for the bioelectric well experiments

The stoichiometry of complete toluene oxidation and the number of electrons released by it, as published previously in the literature [6], was considered as it follows:

$$C_7H_8 + 14H_2O \rightarrow 7CO_2 + 36H^+ + 36e^- \quad (3.10)$$

Considering the previous value of 36 electrons released per mole of toluene degraded, the CE of toluene degradation during fed-batch phase was calculated by

quantifying the ratio between the charge transferred and the charge corresponding to the amount of toluene degraded:

$$CE = \frac{\int I(t)dt}{[Toluene]_{deg} \cdot 36 \cdot F} \quad (3.11)$$

where $[Toluene]_{deg}$ is the degraded amount of toluene (mol).

Afterwards, during the continuous operation phase, the oxidation of acetate was also included in the CE calculation. Its oxidation stoichiometry was considered as it follows [11]:

$$CH_3COO^- + 2H_2O + 2CO_2 + 7H^+ + 8e^- \quad (3.12)$$

Considering the 8 electrons per mol of acetate oxidized, the equation (3.11) was modified as it follows:

$$CE = \frac{\int I(t)dt}{([Toluene]_{deg} \cdot 36 + [Ac]_{deg} \cdot 8) \cdot F} \quad (3.13)$$

where $[Ac]_{deg}$ is the amount of acetate degraded (mol). The concentration of degraded acetate was considered approximately constant during all the operation, averaging a value of 48 ± 11 mg·L^{-1}.

The cathodic current utilization related to the degradation of CF from the bioelectric well was calculated by using equation 3.8, as it is the same variable than the CE calculated in the two-vessel systems.

3.2.5 Bacterial growth kinetic calculations

During the experiments involving *Dehalogenimonas* and *Dehalobacter* carried out on chapters 5 and 6, the maximum specific growth rates (μ, d^{-1}) were calculated by selecting the exponential growth phase period according to the cell or the 16S rRNA gene

copies concentrations obtained by qPCR of the corresponding bacterial genera and using the following equation:

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \quad (3.14)$$

where X is the cell or the 16S rRNA gene copies concentration in the bioelectrochemical reactor (cells·mL^{-1} or 16S rRNA gene copies·mL^{-1}).

The biomass growth yield during a certain time period (Y, cells generated / µmol contaminant degraded) was calculated as it follows:

$$Y = \frac{X_{gen}}{C_{deg}} \quad (3.15)$$

where X_{gen} is the increase of cells or 16S rRNA gene copies and C_{deg} are the µmol of contaminant degraded during a certain time period.

The maximum specific contaminant utilization rate by *Dehalogenimonas* or *Dehalobacter* (q, µmol contaminant degraded per cell or 16S rRNA gene copy generated per day) can be calculated by applying µ and Y as it is shown in the following equation:

$$q = \frac{\mu}{Y} \quad (3.16)$$

3.2.6 DNA extraction and real-time quantitative PCR analyses

During the experiments carried out on chapters 5 and 6, liquid samples (3 mL) were obtained from the cathode at different time points during the experiments and centrifuged at 9000 g for 20 minutes. The supernatant was removed and the DNA was extracted using a NucleoSpin Tissue kit (Machery Nagel) following the 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* or *Dehalobacter* cells in the BES. The qPCR performed in chapter 5 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 3.5) [12].

Table 3.5. Primer and probe sequences targeting the genus *Dehalogenimonas* used in the quantitative real-time PCR (qPCR) and amplified region obtained.

Primer / Probe / Region	Sequence (5'-3')
Dhgm478F	AGCAGCCGCGGTAATACG
Dhgm536R	CCACTTTACGCCCAATAAATCC
Dhgm500Probe	6FAM-AGGCGAGCGTTAT-TAMRA
Amplified region	**AGCAGCCGCGGTAATACG**TAGG**AGGCGAGC GTTAT**CC**GGATTTATTGGGCGTAAAGTGG**

On the other hand, the qPCR performed in chapter 6 used a set of primers (DhRrespF and DhRrespR) and a TaqMan probe (DhRrespProbe) which specifically targeted *Dehalobacter restrictus* PER-K23 and were previously published in the literature (Table 3.6) [13]. Sequence of TaqMan probe was modified adding one degenerate and one locked nucleic acid (LNA) bases to ensure the hybridization with the 16S sequence of the strain used in this study.

Table 3.6. Primer and probe sequences targeting *Dehalobacter* used in the quantitative real-time PCR (qPCR) and amplified region obtained. The degenerate and the locked nucleic acids added in the TaqMan probe were indicated with underlined and bold characters respectively.

Primer / Probe / Region	Sequence (5'-3')
DhRrespF	CGACGCAACGCGAAGAA
DhRrespR	CGAAGGGCACTCCCATATCTC
DhRrespProbe	6FAM-ACCMAGGCTTGA**C**ATCCAACT -TAMRA
Amplified region	**CGACGCAACGCGAAGA**ACCTT**ACCCAGGCT TGACATCCAACT**AATCCCGTA**GAGATATGGG AGTGCCCTTCG**

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 corresponding primer and TaqMan probe, ultrapure water and 2 µL of the extracted DNA sample. The qPCR cycle conditions for both *Dehalogenimonas* and *Dehalobacter* quantitation 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. Synthetic sequences corresponding to the whole 16S gene of *Dehalogenimonas alkenigignens* (NR_109657.1) and *Dehalobacter restrictus* PER-K23 (CP007033.1) which contained their respective amplified regions were purchased (ATG biosynthetics). The gene sequence of interest was amplified by conventional PCR using the previously described primers and the amplicon (59 and 72 pb for *Dehalogenimonas*

and *Dehalobacter* respectively) 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 the cloned sequence in plasmid DNA was calculated with the formula used by Ritalahti et al. [14]. Also, the 16S gene copies per mL of culture sample corresponding to each bacteria were determined from their respective standard curves (Fig. 3.6) following a previously described equation [15]. All the samples were quantified in triplicate.

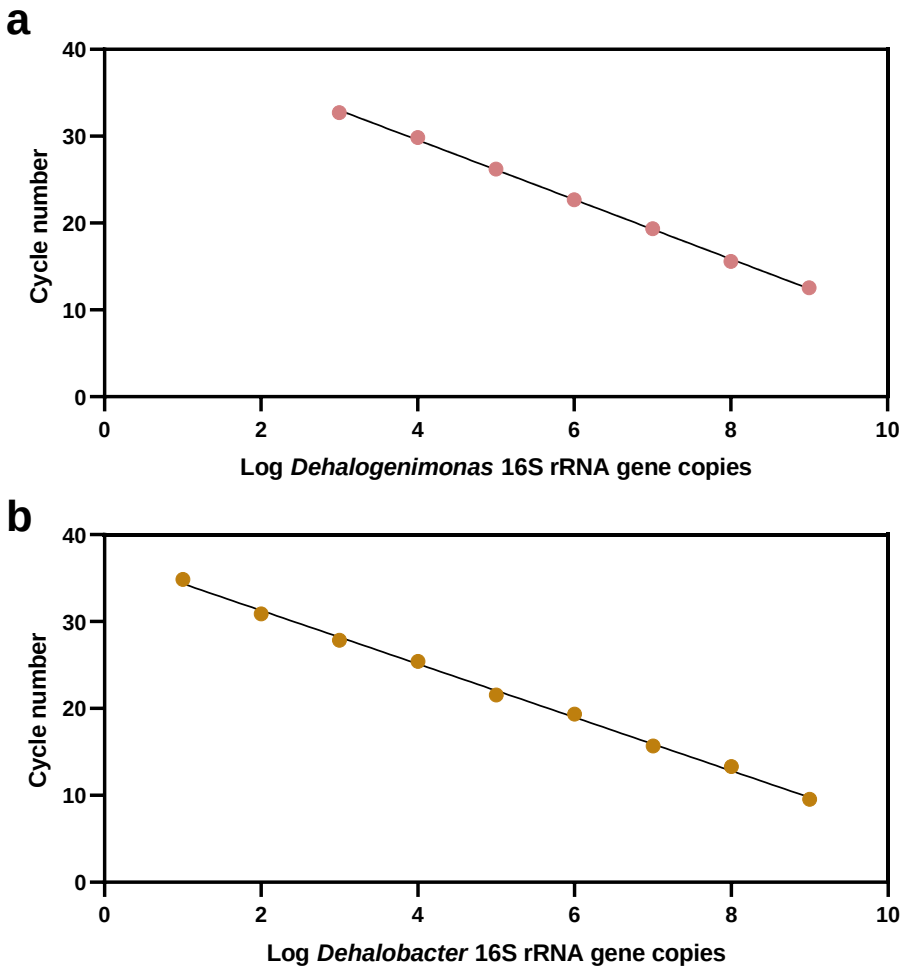

Figure 3.6. Calibration curve for the qPCR of *Dehalogenimonas* (a) and *Dehalobacter* (b) 16S rRNA gene. Points represent the mean value of each dilution and error bars represent the standard deviation of triplicates.

3.2.7 Scanning electron microscopy

Field emission-scanning electron microscopy was performed with liquid samples of the culture contained in the cathode chamber and with small cut fragments of cathodic electrode material at the end of each working period of BES. To provide a solid support, 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 [23]. 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).

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Chapter 4

Electrochemical degradation of dibromomethane and 1,2-dibromoethane to non-toxic products using a carbon fiber brush electrode

Part of this chapter was published as:

D. Fernández-Verdejo, M.L.K. Sulonen, M. Pérez-Trujillo, E. Marco-Urrea, A. Guisasola, P. Blánquez, Electrochemical dehalogenation of dibromomethane and 1,2-dibromoethane to non-toxic products using a carbon fiber brush electrode, J. Chem. Technol. Biotechnol. (2020). doi:10.1002/jctb.6542.

Abstract

Dibromomethane (DBM) and 1,2-dibromoethane (DBA) are two brominated volatile contaminants used in several industrial applications which are often detected in groundwater. The electrochemical degradation of DBM and DBA was studied at different cathode potentials (-0.8, -1 and -1.2 V versus Standard Hydrogen Electrode) in aqueous solution using an inexpensive graphite fiber brush electrode. The degradation followed first-order kinetics with respect to the nominal concentration of the brominated compounds, and the kinetic constant increased concomitantly with the decrease of the cathode potential. During the electrochemical dehalogenation 96.8% and 99.8% of the bromide in DBM and DBA was released as bromine ions, respectively. The main non-brominated compounds detected during the degradation of DBM and DBA were methane and ethene, respectively. In addition, traces of formic acid for DBM and acetic acid for DBA degradation were detected. The non-toxicity of the effluent was confirmed by a Microtox test. The efficient electrochemical degradation of DBM and DBA, needing between 1 and 7 hours to degrade up to 500 μM of contaminant, and the lack of toxic by-products open the door for a simple and non-toxic electrochemical approach for removing aliphatic brominated compounds from aquifers and other water sources. The coulombic efficiencies were not dependent on the cathodic potential and showed values higher than 60% for DBM degradation and 30 % for DBA degradation.

4.1 Introduction

Dibromomethane (DBM) and 1,2-dibromoethane (DBA) are brominated volatile compounds, which are utilized in a wide range of industrial applications, such as petrochemical and pharmaceutical processes, and have been used as agricultural pesticides [1,2]. Due to their extensive usage and accidental spills, their presence has been detected in natural environments, including groundwater sources [2,3]. Both compounds have adverse effects on human health, and are considered probable/potential carcinogens [4]. Based on a combination of the frequency of occurrence, toxicity, and potential human exposure, DBA has been ranked 39th on the 2019 ATSDR Substance Priority List [5].

A wide array of abiotic degradation techniques has been applied to degrade this family of substances in aqueous medium, including aqueous sulfide species (HS^-), iron sulfide (FeS), nano-scale Fe and Ni/Fe particles or Co_3O_4 catalysts [6–9]. Electrochemical techniques have been tested for a broad range of halogenated contaminants, but mostly include chloroalkanes and pesticides [10–14]. Regarding the electrochemical degradation of brominated compounds, Radjenović et al. (2012) [15] reported simultaneous electrochemical reduction of 17 contaminants, including several bromoiodomethanes, bromochloromethanes and bromoform, using resin-impregnated carbon-brushes. Electrocatalysis of halogenated compounds can involve either oxidation or reduction reactions, but electrochemical reduction is a promising approach for the degradation of simple halogenated contaminants because it enables the cleavage of the carbon – halogen bond and further transformation of contaminants to innocuous compounds (i.e. methane and ethane) [16,17]. The main advantages of the electrochemical degradation over biological techniques are i) the short reaction time, ii) the ability to treat streams even with high concentrations of contaminant and iii) the ability to treat complex mixtures of different pollutants in which the growth of microorganisms might be inhibited [18]. On

the other side, the main drawbacks of electrochemical techniques are i) the high cost of the precious metals (i.e. Ag, Al, Au, Cu, Ni, Pd, Ti) required to catalyze the electrochemical reactions [16,19], ii) the need of a high external energy input, and iii) the need of specific medium compositions (as low pH or non-aqueous electrolytes), which hampers its field application [16,20]. Therefore, there is a need to find novel low-cost technologies for the efficient removal of halogenated pollutants from groundwaters.

The main objective of this chapter was to study the abiotic electrochemical reductive debromination of DBM and DBA in electrochemical cells at lab-scale using an inexpensive and untreated graphite brush electrode under non-aggressive electrolytic conditions. The effect of the electrode potential on reaction rates and degradation efficiencies was studied together with the identification of the by-products and the toxicity of the treated effluent.

4.2 Materials and methods

4.2.1 Electrochemical cells description

The two-chamber electrochemical cells used in this chapter have been previously described in section 3.1.3.1 of the general materials and methods chapter.

4.2.2 DBM and DBA electrochemical reduction experiments

Prior the experiments, the anodic and cathodic chambers from the two-vessel electrochemical systems were filled with an anoxic bicarbonate-buffered aqueous solution (50 mM) with a pH value adjusted to 7 and a conductivity of 3.85 $mS \cdot cm^{-1}$. The catholyte was spiked with DBM (Sigma-Aldrich, 99% purity) or DBA (Sigma-Aldrich, ≥98% purity) and vigorously mixed with a magnetic stirrer throughout the experiments. The degradation of both pollutants was tested by triplicate at three different cathode potentials (-0.8, -1 and -1.2 V vs SHE). Open circuit potential (OCP) experiments with

each contaminant in the same experimental conditions were performed to confirm that the compounds were not spontaneously degraded. During the experiments, liquid samples were withdrawn periodically from the catholyte in order to monitor the degradation of the pollutants and the formation of byproducts. Experiments were conducted at room temperature (25±3 °C).

The maximum degradation rate for each contaminant at the different cathode potentials was calculated from the contaminant degradation profile. In addition, a first-order kinetic model (equation 4.1) was fitted to describe the contaminant degradation rate ($\mu M \cdot h^{-1}$) under different applied potentials:

$$\text{Degradation rate} = -k \cdot [\text{Contaminant}] \quad (4.1)$$

where k is the first order rate constant (h^{-1}) and [Contaminant] is the remaining concentration of DBM or DBA in the cathode (µM).

4.2.3 Analysis of volatile compounds

The concentrations of volatile halogenated contaminants, methane and ethene were determined by static headspace gas chromatography as described in section 3.2.2.2 of the general materials and methods chapter.

4.2.4 Analysis of non-volatile compounds

To identify non-volatile compounds produced from the electrochemical degradation of DBA and DBM, 400 mL of aliquot derived from these experiments were mixed with 200 mL of D_2O (99.96 % D, Cortecnet, Voisins-le-Bretonneux, France) in a 5-mm-diameter nuclear magnetic resonance (NMR) tube. A Bruker Avance II 600 NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm TBI probe with Z-gradients, operating at a 1H NMR frequency of 600.13 and at 298.0 K of temperature, was used. 1D 1H NMR spectra were acquired using the noesy1dpr pulse

sequence to suppress the H_2O signal. An acquisition time of 1.70 s and a relaxation delay of 2 s were used. The data were collected into 32 K computer data points, with a spectral width of 9615 Hz and as the sum of 2048 transients. Identification of compounds was done comparing results with those reported in literature and gathered in NMR spectral data bases [21,22].

Bromide ions were quantified from filtered (0.22 μm PVDF filters) liquid samples (1 mL) using ion chromatography with conductivity detection (Dionex ICS-2000 with an Ultimate 3000 Autosampler) as described elsewhere [23]. From each sample, 25 μM were injected in a ion chromatograph with a conductivity detector (Dionex ICS-2000) equipped with a IonPac AS18 REIC anion-exchange column (4 x 250 mm, Dionex). The column was held at 30 °C with a periodical increase in the concentration of potassium hydroxide in the eluent from 25 to 50 mM. Each analysis lasted 10 min. The flowrate was held constant at 1 $mL \cdot min^{-1}$.

4.2.5 Toxicity Analysis

A Microtox bioassay was performed before and after the electrochemical treatment to detect possible toxic intermediates. A Microtox model 500 analyser (Azur Environmental) was used and the 81.9% basic test (adequate for samples with expected low toxicity) was selected. Light emission of the model bacterium *Aliivibrio fischeri* was recorded after 5 and 15 minutes of exposure to the experimental samples and compared to the bioluminescence produced by contaminant-free control samples. The tubes were sealed with a rubber septum to prevent loss of volatile contaminants during the assay.

4.3 Results and discussion

4.3.1 DBM and DBA electrochemical reduction experiments

The electrochemical reduction of DBM and DBA was evaluated at different cathode potentials (-0.8, -1 and -1.2 V vs SHE). The selected potential range was previously scanned with a linear sweep voltammetry without observing any relevant peaks (Fig. 4.1).

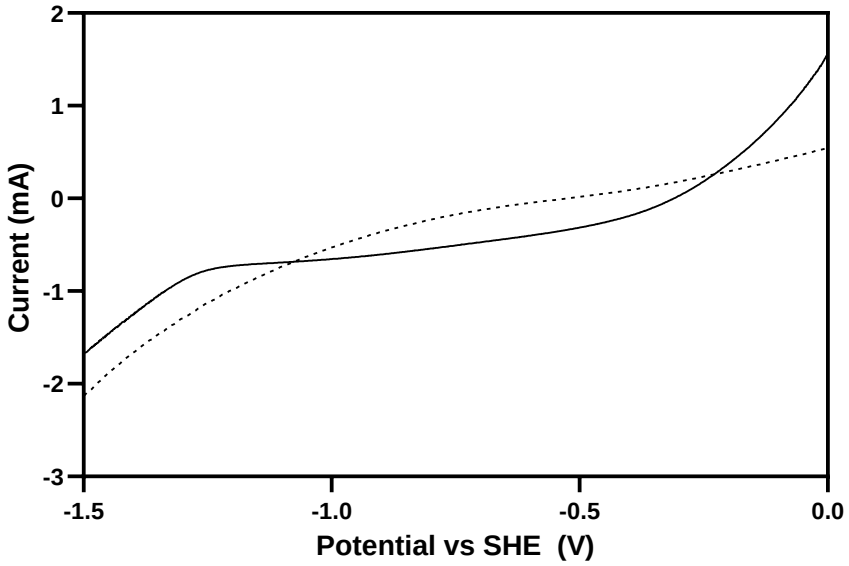

Figure 4.1. Linear sweep voltammetry curves from the cathode for DBM (solid line) or DBA (dashed line) in an aqueous solution. Both contaminants were at a concentration of 500 μM.

The degradation of DBM and DBA was only observed when the cathodes were poised at the desired value and not under OCP conditions (Fig. 4.2 and 4.3), indicating that DBM and DBA degradation was electrochemically mediated.

In the case of DBM, the time needed to obtain a removal higher than 95% was 7, 3 and 1 h for the potentials of -0.8, -1 and -1.2 V, respectively (Fig. 4.2 a). Time-course degradation experiments showed a correlation between the DBM degradation rate and the cathode potential (Fig. 4.2. a), the highest degradation rate (786.7 ± 50.6 $\mu M \cdot h^{-1}$) being

obtained at the lowest cathode potential (-1.2 V) tested. At cathode potentials of -1.0 and -0.8 V, degradation rates of 213.5 ± 31.7 $\mu M \cdot h^{-1}$ and 102.5 ± 26.5 $\mu M \cdot h^{-1}$ were obtained, respectively.

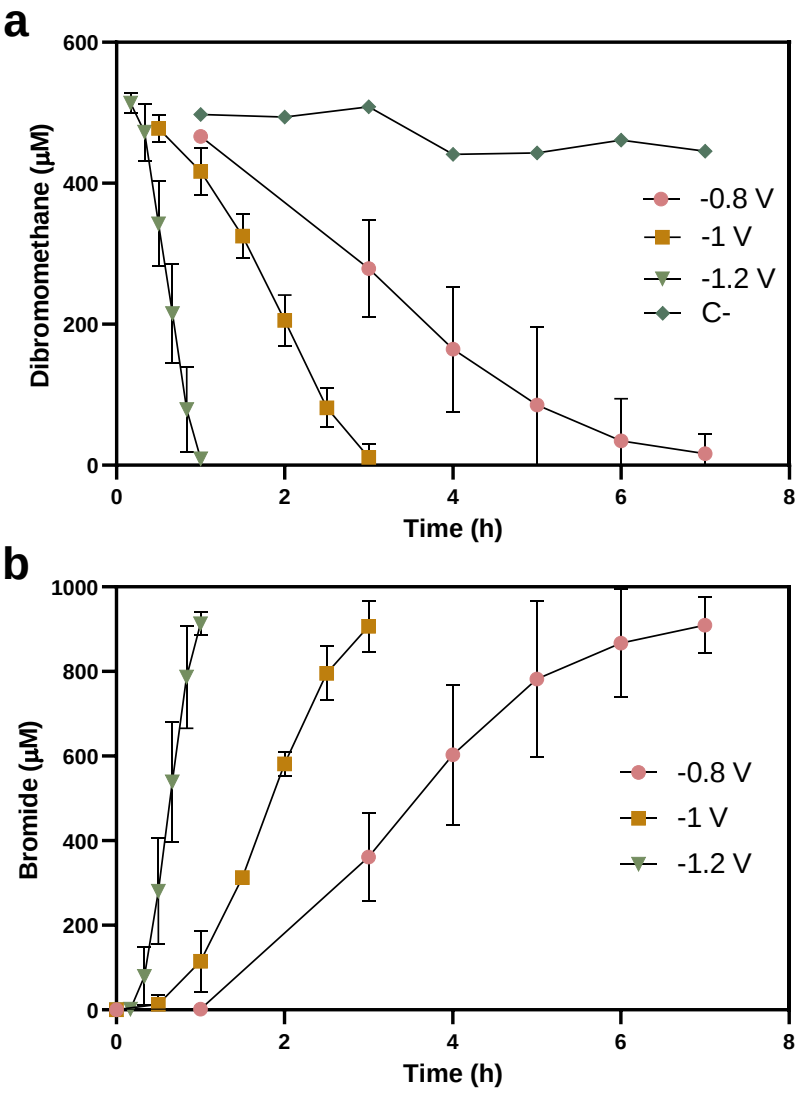

Figure 4.2. Time-course of the electrochemical degradation of DBM (a) and its respective bromide generation (b) in aqueous medium at cathode potentials of -0.8 V, -1.0 V, -1.2 V and OCP. Symbols refer to contaminant and bromide mean concentrations. No bromide generation was detected in OCP experiments. Bars indicate deviation for triplicate experiments.

The degradation of DBA (Fig. 4.3) followed the same trend, the more negative the cathode potential, the higher the maximum degradation rate, but the degradation rates

remained lower. The obtained values were 677.0 ± 107.4, 177.1 ± 18.3 and 76.3 ± 10.9 $\mu M \cdot h^{-1}$ for the potentials of -1.2, -1.0 and -0.8 V, respectively. Although there was a difference among the percentages of degradation between both contaminants, the comparison between the maximum degradation rates obtained for each potential shows a difference lower than 26%. This is the first report describing the electrochemical degradation of DBM. For DBA, two previous studies have addressed the electrochemical degradation with boron-doped diamond and dropping mercury electrodes at cathode potentials around -1.5 V (vs SHE) [17,24]. However, the graphite-brush electrode has a higher specific surface area (800 $m^2 \cdot m^{-3}$) than the boron-doped diamond (0.0007 $m^2 \cdot m^{-3}$) and dropping mercury (0.005 $m^2 \cdot m^{-3}$) electrodes, providing a reacting surface several orders of magnitude higher.

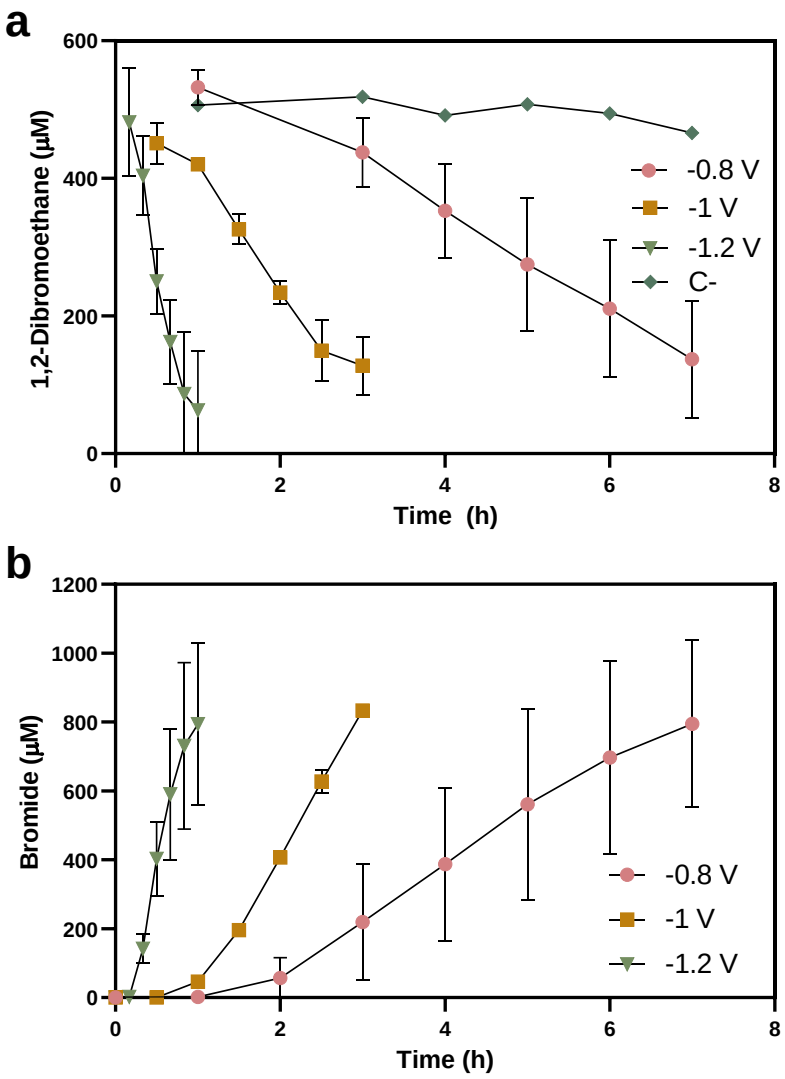

Figure 4.3. Time-course of the electrochemical degradation of DBA (a) and its respective bromide generation (b) in aqueous medium at cathode potentials of -0.8 V, -1.0 V, -1.2 V and OCP. Symbols refer to contaminant and bromide mean concentrations. No bromide generation was detected in OCP experiments. Bars indicate deviation for triplicate experiments.

4.3.2 Identification of by-products from the degradation of DBM and DBA

The chromatographic analyses of the headspace samples indicated that the degraded DBM and DBA were mostly transformed to methane and ethene, respectively (Fig. 4.4). For the average of all three potentials, ethane and ethene accounted respectively for 77 ± 17% of DBM and 155 ± 11% of DBA degraded and were not detected in the

OCP controls, further confirming that degradation of both contaminants was only possible when electric current was applied.

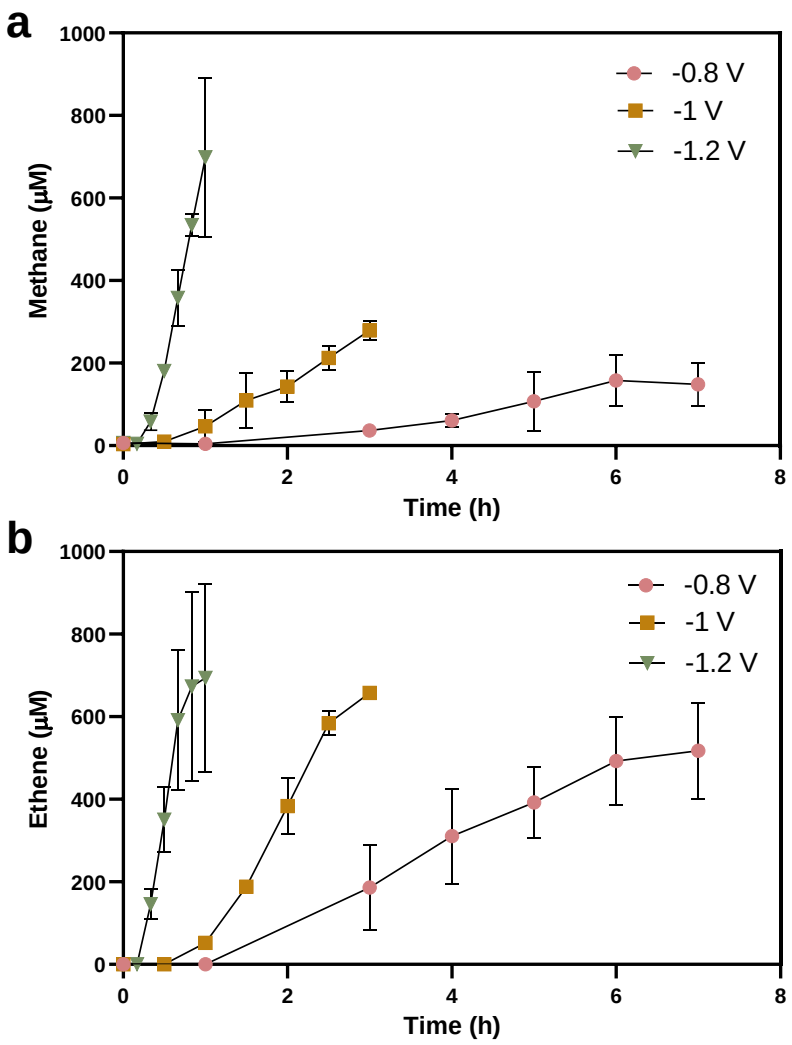

Figure 4.4. Time-course of the electrochemical production of methane (a) and ethene (b) during DBM and DBA degradation respectively in aqueous medium at cathode potentials of -0.8 V, -1.0 V and -1.2 V. No methane or ethene generation was detected in OCP experiments. Bars indicate the deviation for triplicate experiments.

In addition, liquid samples from the degradation experiments of DBM and DBA performed at -1.2 V were removed after 60 min and subsequently analysed by ^{1}H NMR spectroscopy to identify the presence of non-volatile degradation products. Spectra from DBM and DBA presented some very low intense peaks, in form of singlets, which

differed from the blank (Fig. 4.5 a). This blank consisted of a sample derived from the cathodic chamber that was electrochemically operating for 60 min at the same electric potential but without brominated compounds spiked. These signals were difficult to identify due to their singlet nature and to their low intensity (i.e., compounds at very low concentration), which hampered the performance of other NMR experiments like ^{13}C based experiments. However, two compounds were identified due to their typical resonance frequencies at the experimental conditions used. In sample derived from DBM degradation, a small singlet at 8.45 ppm corresponding to formic acid was observed (Fig. 4.5 c). In DBA sample, which presented a cleaner spectrum than the equivalent for DBM, a very low intense singlet at the resonance frequency of acetic acid, 1.92 ppm, was detected (Fig. 4.5 b) [21,22]. No product generation was detected in the OCP experiments, ensuring that the generation of these substances was directly correlated with the contaminant degradation.

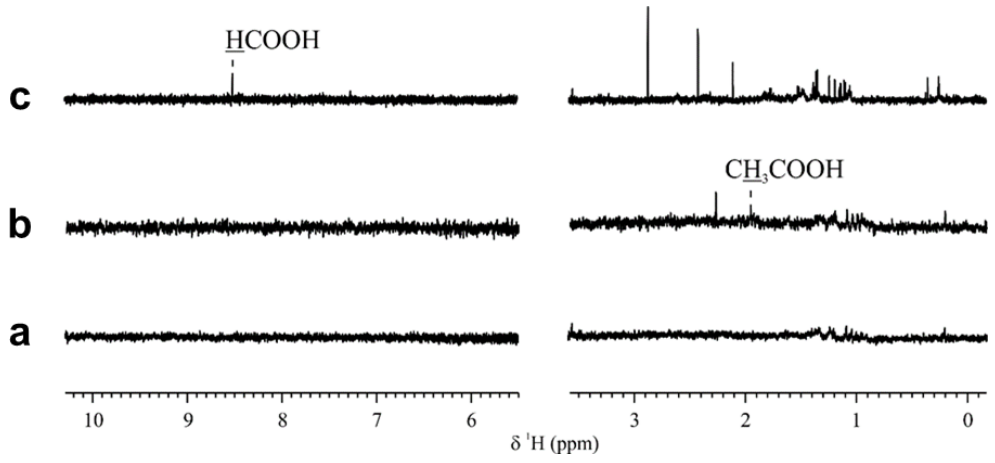

Figure 4.5. 1D 1H NMR spectra of liquid samples taken after 60 min of the electrochemical degradation process of DBA (b) and DBM (c) at -1.2 V (vs SHE). Panel a shows the 1D 1H NMR spectra of a blank that accounted for an electrochemical cell without brominated compounds. The spectra were acquired with suppression of the water signal (noesy1dpr), at 25 ºC and at a magnetic field of 600 MHz.

Increasing amounts of bromide ions were released during the dehalogenation of both brominated compounds (Fig. 4.2 b and 4.3 b). The amount of bromide ion released from DBM degradation was 96.8% of the maximum bromide concentration expected if all the degraded DBM was fully dibrominated (Fig. 4.6 a). Hydrogenolysis of DBM was discarded as a pathway for degradation because no bromomethane was detected by GC analyses during the experiments (detection limit 1 μM). Similarly, DBA degradation yielded a molar ratio of bromide to DBA of 1.995, which means that 99.8% of the expected bromide was released, considering that all the DBA degraded was completely debrominated (Fig. 4.6 b). In contrast, incomplete electrochemical debromination of DBA (ratio between bromide released and DBA degraded of 1.56 and 1.78) was reported at cathode potentials around -1.5 V (vs SHE) in previous studies [17]. Tokoro et al. (1986) hypothesized that carbanion was generated as reaction intermediate and it was further transformed to bromoethane [17]. In this study, neither vinyl bromide nor bromoethane (formed during dehydrohalogenation or hydrogenolysis of DBA, respectively) were detected in the catholyte by GC analyses (detection limit 1 μM). These single-brominated compounds are electrochemically degraded at slower rate than their di-brominated counterparts, and they tend to accumulate during degradation experiments [24].

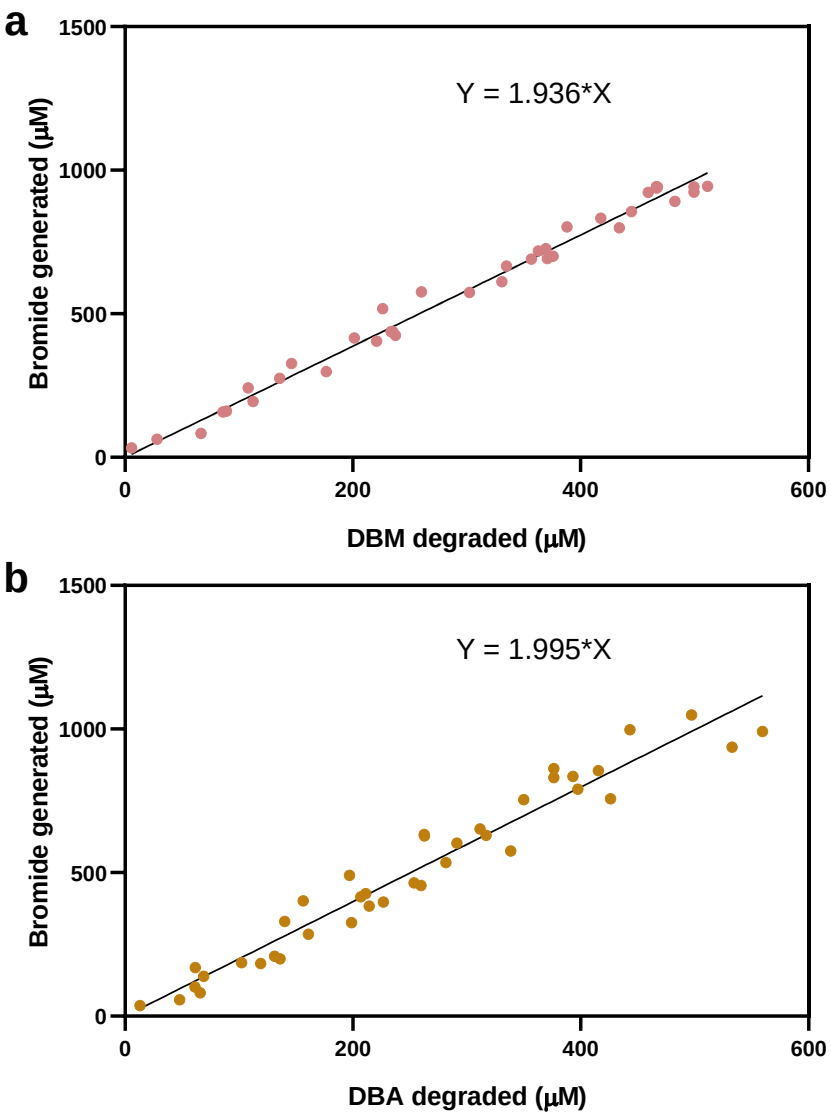

Figure 4.6. Fitted curve of bromide released in the liquid medium per DBM (a) or DBA (b) degraded. Values were obtained from the experiments plotted in Figure 1.

The Microtox toxicity bioassay was used to evaluate the toxicity of the aqueous medium before and after the electrochemical treatment. The results showed that the samples analysed were not sufficiently toxic to produce any effect on the bioluminescence from *A. fischeri*, indicating that electrochemical degradation of DBM and DBA did not result in the accumulation of any toxic products.

4.3.3 Coulombic efficiency and energy consumption

During the operation of the electrochemical cells, the electric current was recorded for each experiment. The intensity was normalized by the graphite brush surface in order to obtain the current density, and its values were depicted in Fig. 4.7. As expected, the intensity flowing across the systems increased with more negative cathodic potentials.

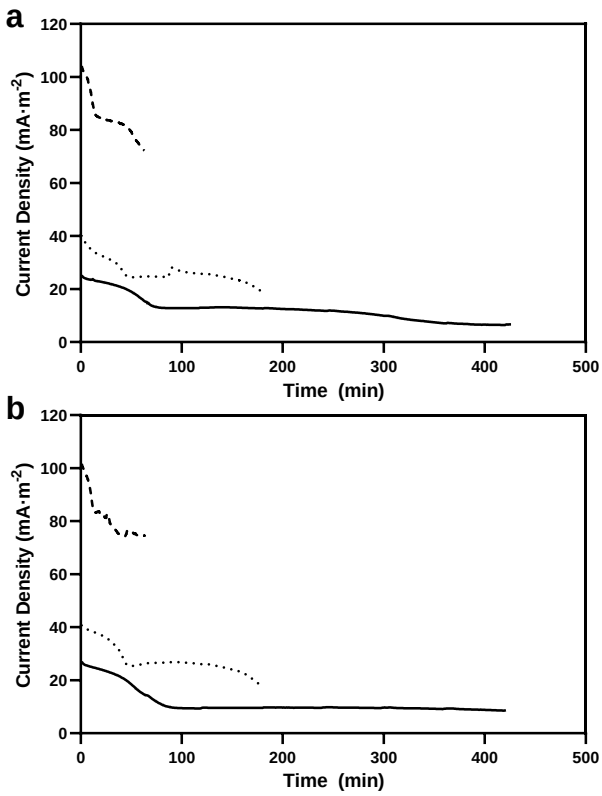

Figure 4.7. Current density profiles in absolute values recorded during DBM (a) and DBA (b) reduction at cathodic potentials of -0.8 V (solid line), -1 V (dotted line) and -1.2 V (dashed line).

Based on the current intensity values recorded, the coulombic efficiency (CE) and the energetic input required to release one mol of bromide were calculated (Table 4.1). The DBM degradation showed high efficiency values (between 68 and 77 %) while the DBA presented values around 30 %, despite presenting similar reaction times and recorded intensities. This difference is explained because DBM reduction to methane required twice the number of electrons than DBA dihaloelimination to ethene (4 and 2

electrons respectively, as it can be observed in Fig. 3.5) while similar intensities were recorded, hence DBA obtaining half of the efficiency obtained for DBM. The difference in applied cathodic potential was not observed to exert any effect in the efficiency values obtained for both contaminants. On the other hand, the energetic input required to release a mol of bromide increased at potential -1.2 V due to the more negative voltages applied. Possible reasons for the decrease of the 100% efficiency could be the reduction of oxygen leaked from the anodic vessel across the cationic exchange membrane and the electrochemical production of cathodic hydrogen.

Table 4.1. Coulombic efficiencies (CE) and Energetic Inputs (EI) per mol of bromide released for DBM and DBA degradation at different potentials.

Contaminant	Applied potential (V vs SHE)	Voltage (V)	Average current density ($mA \cdot m^{-2}$)	CE (%)	EI ($kWh \cdot mol^{-1}$)
DBM	-0.8	-1.9	12.22 ± 0.46	69.8 ± 3.6	0.15 ± 0.01
	-1.0	-2.3	26.52 ± 1.98	76.3 ± 0.8	0.16 ± 0.01
	-1.2	-4.0	84.38 ± 3.80	68.6 ± 1.8	0.31 ± 0.01
DBA	-0.8	-1.9	11.42 ± 2.42	32.6 ± 4.1	0.16 ± 0.02
	-1.0	-2.3	27.65 ± 0.32	33.6 ± 0.3	0.18 ± 0.01
	-1.2	-4.0	81.97 ± 7.61	30.3 ± 7.4	0.37 ± 0.10

The efficiency values obtained in this study differ from the ones previously reported in the literature for DBA dehalogenation [24]. In this previous study, when

working with a boron-doped diamond electrode and in aqueous methanol media, coulombic efficiencies around 60 % were obtained for the cleavage of the first bromine, obtaining an accumulation of bromoethane as intermediate product. On the other hand, extremely low efficiency values of 5 % were obtained in order to debrominate bromoethane to ethane. The usage of the graphite brush applied in this thesis, despite its simplicity, can achieve moderately high efficiency values for the complete DBA debromination while avoiding the accumulation of brominated by-products.

4.3.4 Kinetics of transformation

The degradation of DBM and DBA followed first-order kinetics with respect to the nominal concentration. The rate constants were estimated by fitting the data to a first-order kinetic model (Fig. 4.8 and 4.9), obtaining R^2 values ranging from 0.92 to 0.97.

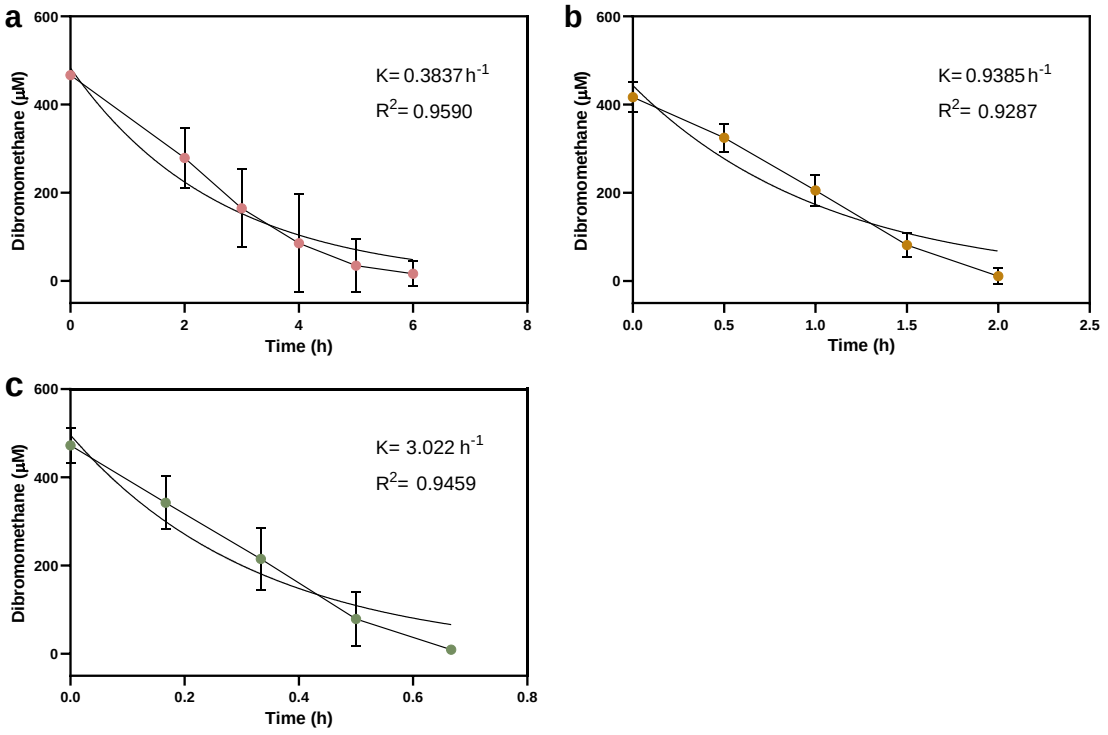

Figure 4.8. First order kinetic models from the electrochemical DBM degradation profiles poised at different cathodic potentials of -0.8 V (a), -1 V (b) and -1.2 V (c). Bars indicate the deviation for triplicate experiments.

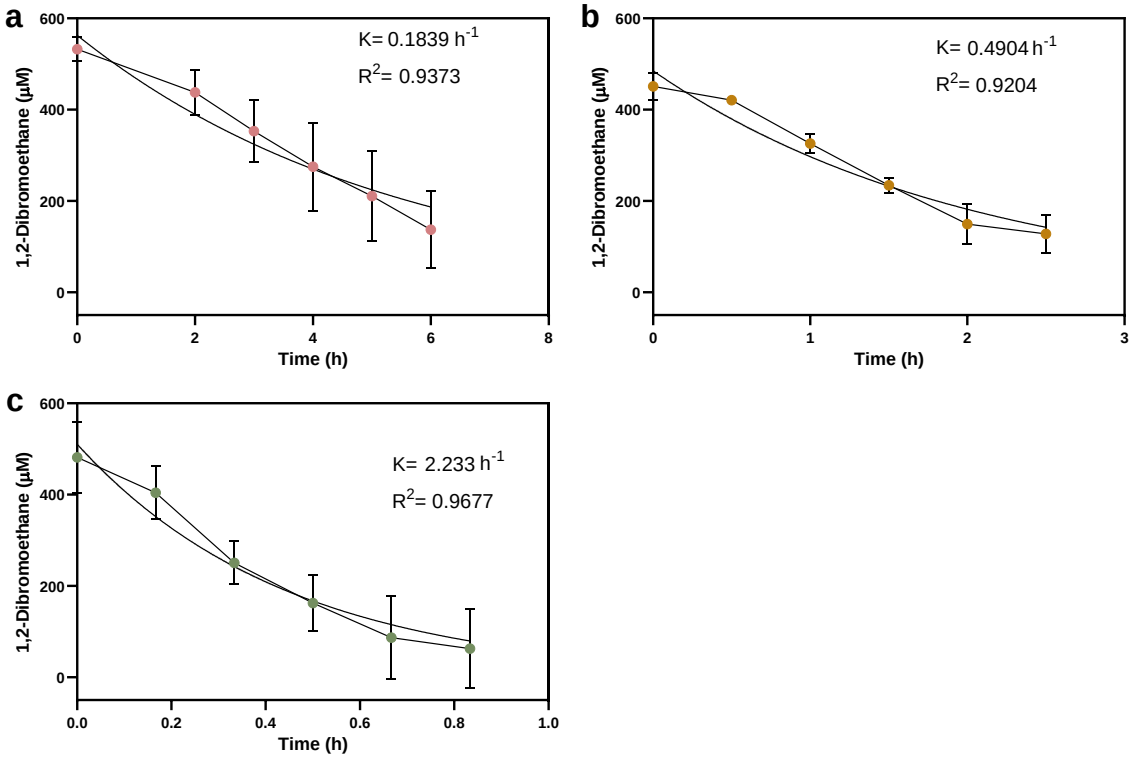

Figure 4.9. First order kinetic models from the electrochemical DBA degradation profiles poised at different cathodic potentials of -0.8 V (a), -1 V (b) and -1.2 V (c). Bars indicate the deviation for triplicate experiments.

The dependence of the rate constants for both brominated contaminants on the cathode potential is illustrated in Fig. 4.10. For both compounds, the first order kinetic constant statistically increased when the electric potential adopted more negative values. These results are in agreement with the maximum degradation rates discussed in the previous section. Slightly higher values were achieved in DBM debromination when compared to the operation at the same potential with DBA.

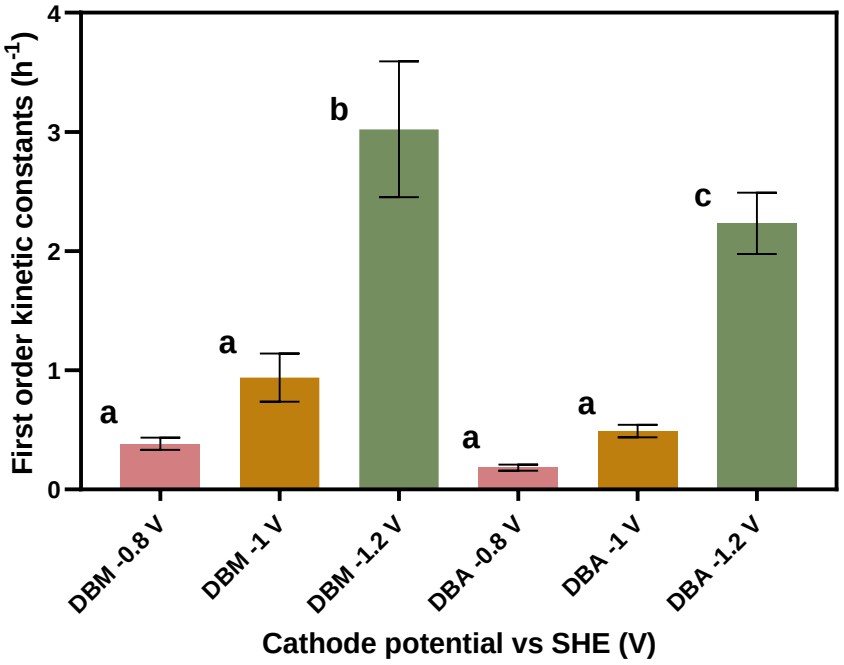

Figure 4.10. Dependence of the first-order kinetic constants for DBM and DBA reductions on cathode potential using a graphite fibre brush cathode (means for the triplicate assays ± standard deviations).

In a previous study reporting the electrochemical reduction of DBA at -1.5 V (vs SHE) with a boron doped diamond electrode and 22 mL of a 30 % methanol in aqueous medium, a kinetic constant value of 0.44 h^{-1} was obtained [24]. This value is 80 % lower than the one obtained in this study at potential of -1.2 V, while the experiments were performed in a 7.5-fold higher reaction volume. This higher degradation rate is mainly due to the high ratio between electrode surface area and catholyte volume provided by the graphite brush, being two orders of magnitude higher. This indicates that an efficient degradation can be reached with cheaper electrode materials and aqueous medium. The kinetic constants obtained in other reports on electrochemical degradation of similar brominated compounds as Radjenović et al. (2012) [15] are difficult to compare due to the complexity of their systems or the use of several simultaneous contaminants.

Further research is needed to assess the optimal conditions and the suitability of the degradation process in a larger scale setup. The key operating parameters to be considered when scaling-up this system are the ratio of cathodic area to volume, the medium electrical conductivity and the contaminants diffusivity to the cathodic surface. Also, the catalytic properties could be enhanced by coating the electrode with platinum or nickel, but this would increase the reactor setup cost. Even considering these parameters, the electrochemical techniques are widely considered to be a low-cost remediation technique due to their simplicity, high degrading efficiency, short required reaction times and soft reacting conditions [16]. Also, when compared to biological remediating treatments, this process is less susceptible to other undesired contaminants in the polluted site. These characteristics coupled with the high coulombic efficiencies observed in this study and the absence of harmful byproducts makes the electrochemical reduction of DBM and DBA a promising remediation strategy.

4.4 Conclusions

This study demonstrates the feasability of electrochemical systems to fully debrominate DBA and DBM using inexpensive and environmentally friendly carbon fibers as electrode materials. The short operation times, requiring less than two hours to degrade arround 500 µM of each contaminant at potentials of -1.2 V, did not lower the coulombic efficiency of the process, maintaining efficiency values higher than 60 and 30 % for DBM and DBA respectively, even in the experiments with higher energetic inputs. The first order kinetic model proposed fits accurately the obtained data and allows to statistically confirm a relationship between the applied cathode potential and the rate of contaminant degradation. The treated effluent was not toxic according to the Microtox bioassay, which is in accordance with the nonhalogenated byproducts identified during the electrochemical degradation of DBM and DBA by gas chromatography and NMR.

Further investigation is required to assess the application of this methodology on environmental contaminated sites.

4.5 References

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Chapter 5

Bioelectrochemically-assisted reduction of 1,2-dichloropropane to propene mediated by *Dehalogenimonas*

Part of this chapter was published as:

D. Fernández-Verdejo, P. Cortés, P. Blánquez, E. Marco-Urrea, A. Guisasola, Enhanced dechlorination of 1,2-dichloropropane to propene in a bioelectrochemical system mediated by *Dehalogenimonas*, J. Hazard. Mater. 416 (2021). doi:10.1016/j.jhazmat.2021.126234.

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.

5.1 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, 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 density 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 chapter 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, as it can be observed in Fig. 5.1. 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.

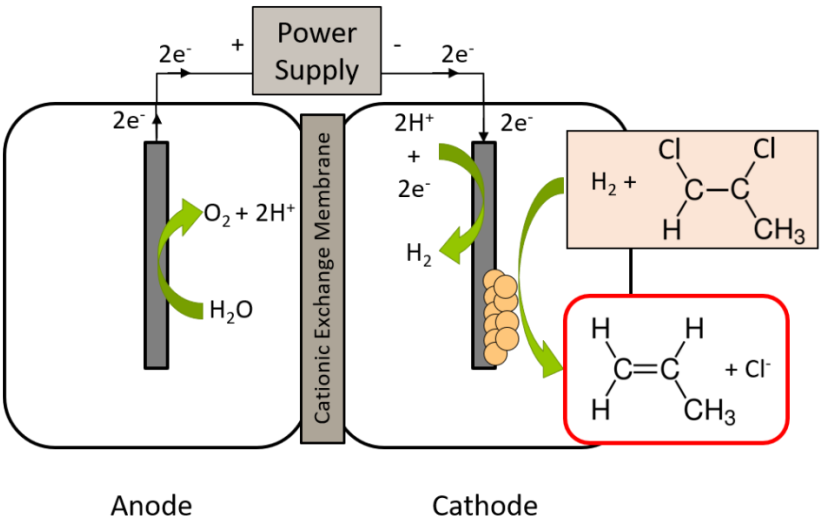

Figure 5.1. Scheme of the reactions occurring in a two-chamber BES inoculated with a 1,2-DCP-degrading culture containing *Dehalogenimonas*.

5.2 Materials and methods

5.2.1 Description of the BES

The two-chamber BES used in this chapter have been previously described in section 3.1.3.1 of the general materials and methods chapter.

5.2.2 Cultivation of the *Dehalogenimonas*-containing culture

The *Dehalogenimonas*-containing culture employed in this chapter is described and cultivated as explained in the general material and methods chapter in sections 3.1.2.1 and 3.2.1 respectively

5.2.3 Cathodic electrode material comparison experiments

130 mL of the *Dehalogenimonas* anaerobic medium was used to fill the cathodic and the anodic chambers of the two-chamber BES containing graphite fiber brush or carbon cloth as cathodic electrodes (Fig. 5.2), providing surface areas of approximately 13.2 dm^2 and 0.213 dm^2 respectively. Cathodic compartments were initially spiked with 500 µM 1,2-DCP and inoculated with 3 mL of an active culture of *Dehalogenimonas* with

a density of ~$9 \cdot 10^7$ cells·mL^{-1}. Biotic BES operated at OCP without electrode were also included as controls. The cathodic potentials were adjusted to -0.7 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 in both graphite brush and carbon cloth-provided BES. The BES were maintained in fed-batch mode by adding 1,2-DCP when exhausted (500 or 1000 µM). A pH probe (Hach) was inserted in the cathodic vessel to adjust pH at a value of 7 by manual additions using an anaerobic stock solution of 1 M of HCl. The liquid samples removed periodically for analysis were refilled with fresh medium and the calculated hydraulic retention time always exceeded the length of the experiments, indicating that BES was virtually operating in fed-batch mode.

Figure 5.2. Carbon cloth (left) and graphite fiber brush (right) electrodes used in the cathode of the experimental BES.

5.2.4 Pulsed and continuous potential comparison

For the experiments carried out with BES containing graphite brush electrodes under pulsed and continuous voltage operation, a cathode potential of -0.6 V and -1.1 V was applied during time-periods of approximately 16 and 8 h respectively from day 0 to

day 46. Afterwards, a cathodic potential of -1.1 V was applied until the end of the experiment at day 136.

5.2.5 Analysis of volatile compounds

The concentrations of volatile halogenated contaminants and propene were determined by static headspace gas chromatography as described in section 3.2.2.2 of the general materials and methods chapter.

5.3 Results and discussion

5.3.1 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 (Fig. 5.3 a). 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. 5.3 a).

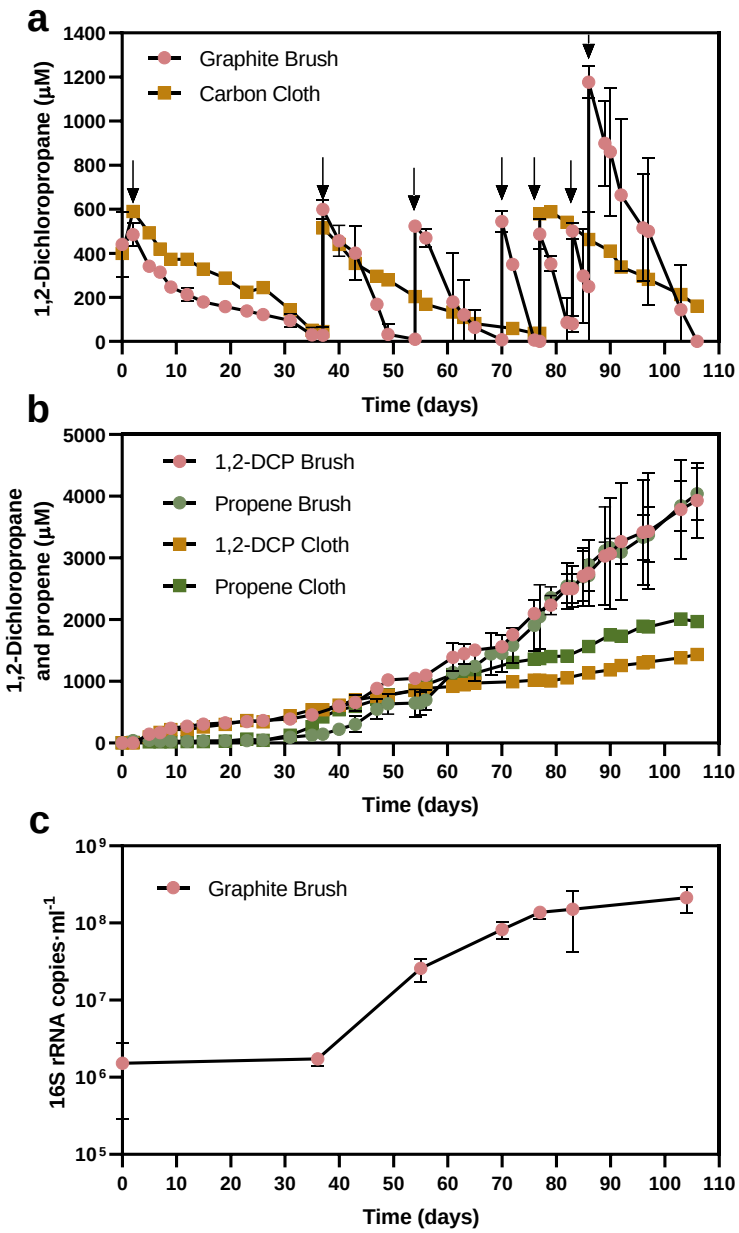

Figure 5.3. Degradation profile of 1,2-DCP in BES (a), accumulated concentration of 1,2-DCP degraded and propene produced (b) and *Dehalogenimonas* 16S rRNA gene copies per mL (c), with graphite brush and carbon cloth as different cathodic electrode materials and cathodic potential set at -0.7 V. Numbers indicate the number of 1,2-DCP amendments added in BES with graphite brush. Values plotted for BES with graphite brush are average of duplicates and error bars indicate the standard deviation. Values plotted for BES with carbon cloth are for an individual BES.

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. 5.3 b). As shown in Fig. 5.3 b, the moles of 1,2-DCP degraded stoichiometrically agree with the propene generated in the BES containing graphite fibre brush. At the end of the experiment (110 days), propene accumulated was 4039 ± 425 µM (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. Propene accumulated in the carbon cloth experiment exceeded the stoichiometric amount expected but accounted for the total 1,2-DCP degradation (Fig. 5.3 b).

Biotic controls that consisted of opened circuit system without electrode required 75 days to degrade 540 µM of 1,2-DCP (Fig. 5.4), showing that 1,2-DCP degradation notably decreased in absence of a continuous supply of electrochemically generated hydrogen from an electrode.

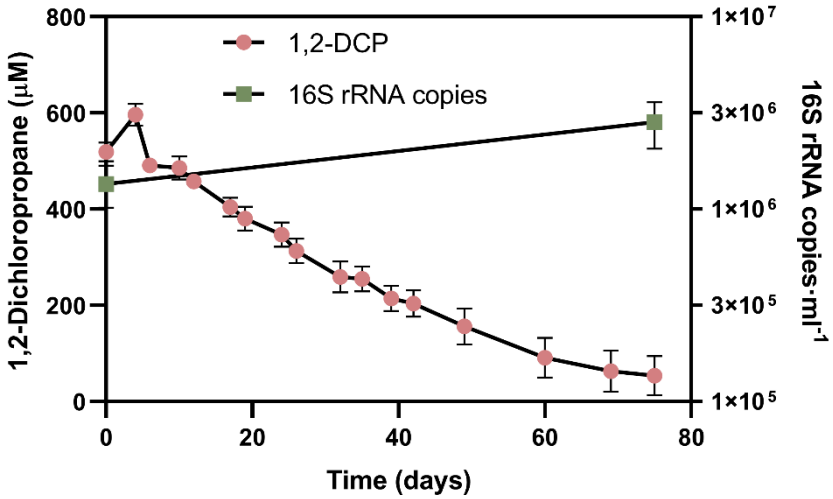

Figure 5.4. Degradation profile of 1,2-DCP and *Dehalogenimonas* growth in biotic OCP BES without electrode. Bars indicate standard deviation for triplicate BES. Symbols: ●, 1,2-DCP; ■: 16S rRNA gene copies.

Abiotic controls conducted for each electrode material with the cathode poised at -0.7 V (Fig. 5.5) showed neither degradation of 1,2-DCP nor propene generation, discarding abiotic electrochemical degradation of 1,2-DCP. This lack of abiotic reduction agrees with the data obtained by cyclic voltammetry when working with a graphite brush electrode, where no reduction peaks were detected when operated in a range of cathodic potentials between +200 and -1100 mV (Fig. 5.6).

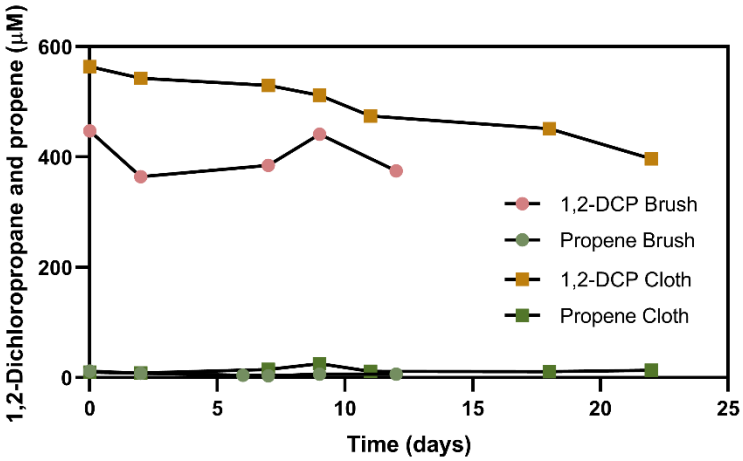

Figure 5.5. Time-course of 1,2-DCP and propene concentrations in an abiotic BES poised at -0.7 V of cathodic potential working with graphite brush or carbon cloth.

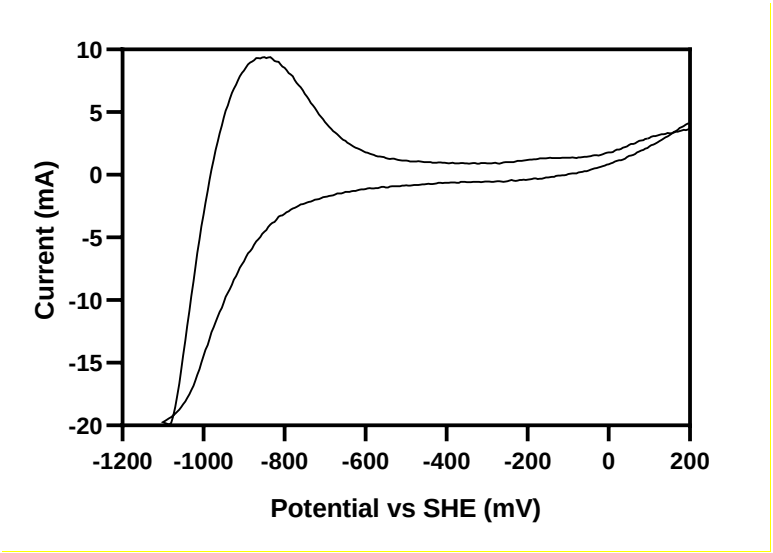

Figure 5.6. Cathodic cyclic voltammetry curve for 1,2-DCP at 500 µM in aqueous medium.

5.3.2 *Dehalogenimonas* growth in BES operated with a graphite brush

Degradation of 1,2-DCP was coupled to an increase in the number of *Dehalogenimonas* 16S rRNA copies in BES containing graphite fibre brush. The consumption of the four first amendments of 1,2-DCP led to an increase of *Dehalogenimonas* 16S rRNA gene copies by over two orders of magnitude (Fig. 5.3 c). The concentration of *Dehalogenimonas* 16S rRNA 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^{-1} *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^{-1} [24]. Relative to the biotic OCP 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 $2.8 \cdot 10^6 \pm 8 \cdot 10^5$ cells of *Dehalogenimonas*·mL^{-1} (Fig. 5.4).

The 1,2-DCP degradation rates obtained in the graphite brush experiment increased during the first four contaminant amendments from 9.84 ± 1.50 µM·d^{-1} to 89.18 ± 6.39 µM·d^{-1} and afterwards exhibited stable values during two consecutive 1,2-DCP amendments (Fig. 5.7 a). The degradation rate reached its maximum at the sixth addition (98.24 ± 82.27 µM·d^{-1}). These degradation rates are in the range of those obtained for this strain degrading 1,2-DCP in H_2-fed serum bottles (50-100 µM·d^{-1}) and similar to that reported for *Dehalococcoides* degrading 1,2-dichloroethane in a graphite rod-containing BES operating at -0.7 V of cathodic potential (50 µM·d^{-1}) [12].The maximum degradation rate in the carbon cloth BES (16 µM·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 OCP controls (10.95 ± 1.38 µM·d^{-1}) indicating that direct transfer of H_2

from electrochemical dehydrogenation promoted the growth of *Dehalogenimonas* and enhanced 1,2-DCP degradation.

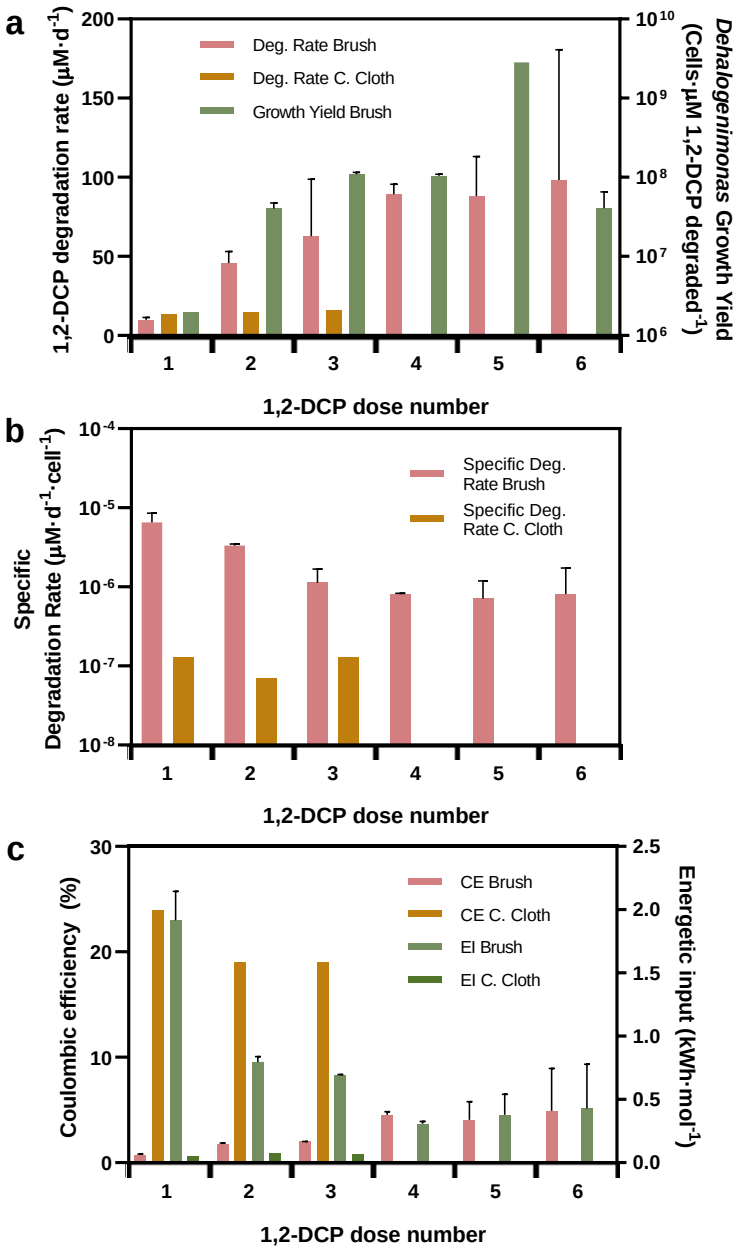

Figure 5.7. Degradation rate, growth yield (a) and specific degradation rate (b) of *Dehalogenimonas* for each dose of 1,2-DCP consumed in BES and coulombic efficiencies and energetic inputs obtained during the degradation (c). Dose number refer to the 1,2-DCP amendments depicted in Fig. 5.3. Values plotted for BES with graphite brush are average of duplicates and error bars indicate the standard deviation. Values plotted for BES with carbon cloth are for an individual BES.

The maximum specific growth rate constant and the growth yield of *Dehalogenimonas* were 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. 3.16, we obtained a specific 1,2-DCP utilization rate of $1.0 \cdot 10^{-9} \pm 5 \cdot 10^{-10}$ µmol of 1,2-DCP degraded per cell generated and day during the whole exponential growth phase of *Dehalogenimonas*. The growth yield of *Dehalogenimonas* in BES obtained in this study of $6 \cdot 10^{7} \pm 2 \cdot 10^{-7}$cells per µmol chloride released was three times higher than that reported for *Dehalogenimonas lykanthroporepellens* strain BL-DC-9 grown with 1,2-DCP [25] but one order of magnitude lower than that obtained by a *Dehalogenimonas*-containing culture growing with 1,1-dichloroethene [27]. Also, the specific utilization rate reported in this study ($2.0 \cdot 10^{-9} \pm 9 \cdot 10^{-10}$ µmol of chloride per cell generated and day) was in the range of those reported in a *Dehalogenimonas*-containing culture ($5.8 \cdot 10^{-8}$ and the $1.9 \cdot 10^{-10}$ µmol of chloride released per cell generated per day) growing with 1,2,3-trichloropropane and *cis*-dichloroethene, respectively [26].

The degradation rates were normalized to the *Dehalogenimonas* cell density for each contaminant dose to obtain the specific 1,2-DCP degradation rates (Fig. 5.7 b). The specific degradation rates ranged between $6.52 \cdot 10^{-6}$ and $7.13 \cdot 10^{-7}$ µM of 1,2-DCP degraded per day and cell for the graphite brush electrode. These values were one or two orders of magnitude higher than those obtained with the carbon cloth electrode likely because of a lower hydrogen bioavailability in the latter.

This hypothesis is supported by the hydrogen measurements at the end of each experiment (Fig. 5.8): hydrogen accumulation in BES with graphite brush was two orders of magnitude higher than that in the carbon cloth. No hydrogen was accumulated in the OCP controls indicating that hydrogen was electrochemically generated.

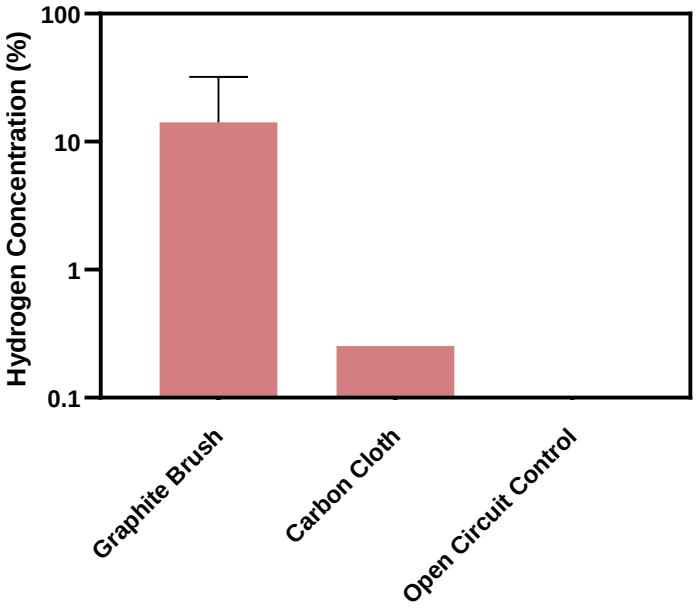

Figure 5.8. Hydrogen accumulated in the cathodic gas phase of each cell at the end of the electrode comparison experiment. The value plotted for BES with graphite brush is the average of a duplicate and the error bar indicate the standard deviation. The value plotted for BES with carbon cloth is for an individual BES.

5.3.3 Coulombic efficiencies and energetic consumptions

The cathodic current intensities recorded during the operation of BES (Fig. 5.9) were used to calculate the coulombic efficiencies (CEs) and the energy inputs for each experiment (Fig. 5.7 c). 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 the fastest. 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·mol^{-1} to 0.31 ± 0.02 kWh·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·mol^{-1}. This increase in the electrical

efficiency and consumption values were caused by the consistently lower intensities recorded during the operation of the cells with carbon cloth as cathode compared to the graphite brush containing ones.

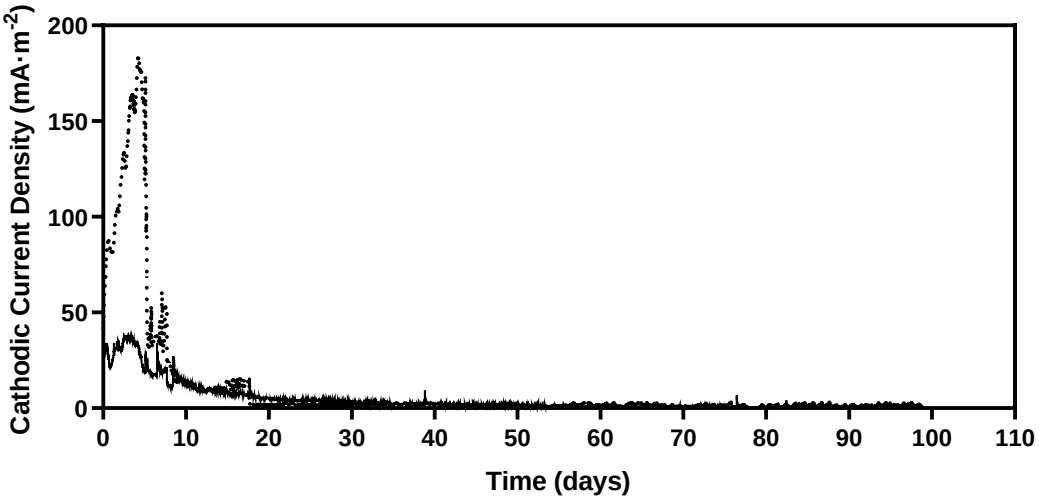

Figure 5.9. Absolute values from the cathodic current density profiles monitored in the graphite fiber brush (solid line) and carbon cloth (dotted line) BES during 1,2-DCP degradation by *Dehalogenimonas*.

The low intensities recorded during the experiments with the carbon cloth electrode could be caused by its low surface area which would negatively affect the hydrogen production and bioavailability by *Dehalogenimonas,* hence drastically decreasing the degradation rate but at the same time benefitting the efficiency values obtained. The concentration of hydrogen measured in the gas phase of each BES supports this hypothesis (Fig. 5.8).

5.3.4 Pulsed and continuous potential operation of BES

The graphite brush was selected for further BES experiments. To increase the low CEs observed in the previous experiments and increase the efficiency of the process while maintaining a high degradation rate, two potential operational modes were tested in a BES for 140 days (Fig. 5.10 a). First, two different cathodic potentials were alternated during

the first 46 days: -0.6 V during 16 h and -1.1 V during 8 h. Then, a continuous cathodic potential of -1.1 V was applied during the next 89 days (Figure 5.10 b).

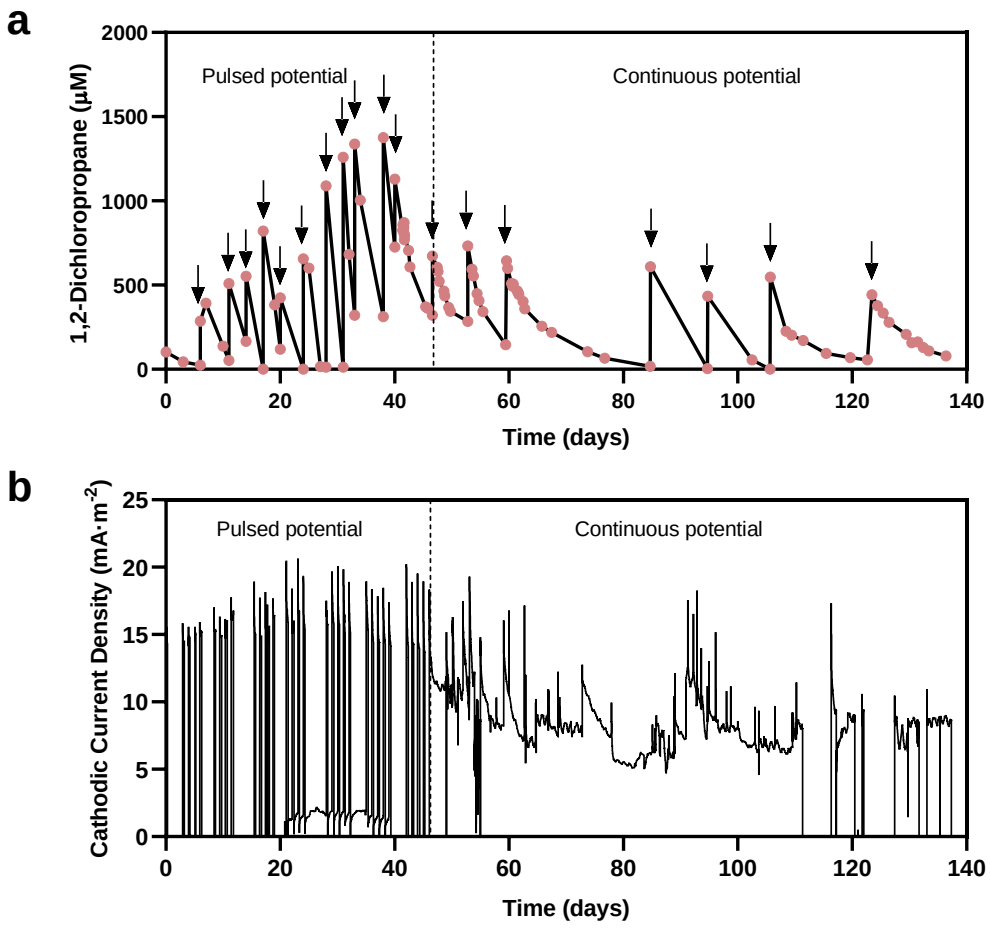

Figure 5.10. Degradation profile of 1,2-DCP in a BES containing a graphite brush as electrode (a) and its measured cathodic current intensities during the operation of pulsed and continuous potential (b). The BES received several additions of 1,2-DCP as indicated by the arrows.

This operation enabled the successful consumption of 10 mM 1,2-DCP in 140 d (Fig. 5.11 a). The maximum degradation rate during the pulsed current period was achieved from day 17 to 43 and it was fitted to a linear regression (Fig. 5.11 b), obtaining a value of 255.9 µM of 1,2-DCP degraded per day. The same methodology was applied to the interval of day 58 to 135, which corresponded to the continuous current application, and an 86.2% lower degradation rate (33.4 $\mu M \cdot d^{-1}$) was obtained. In the abovementioned

periods, the CE was 16.60% and 0.86% for the pulsed and continuous potential operation, respectively.

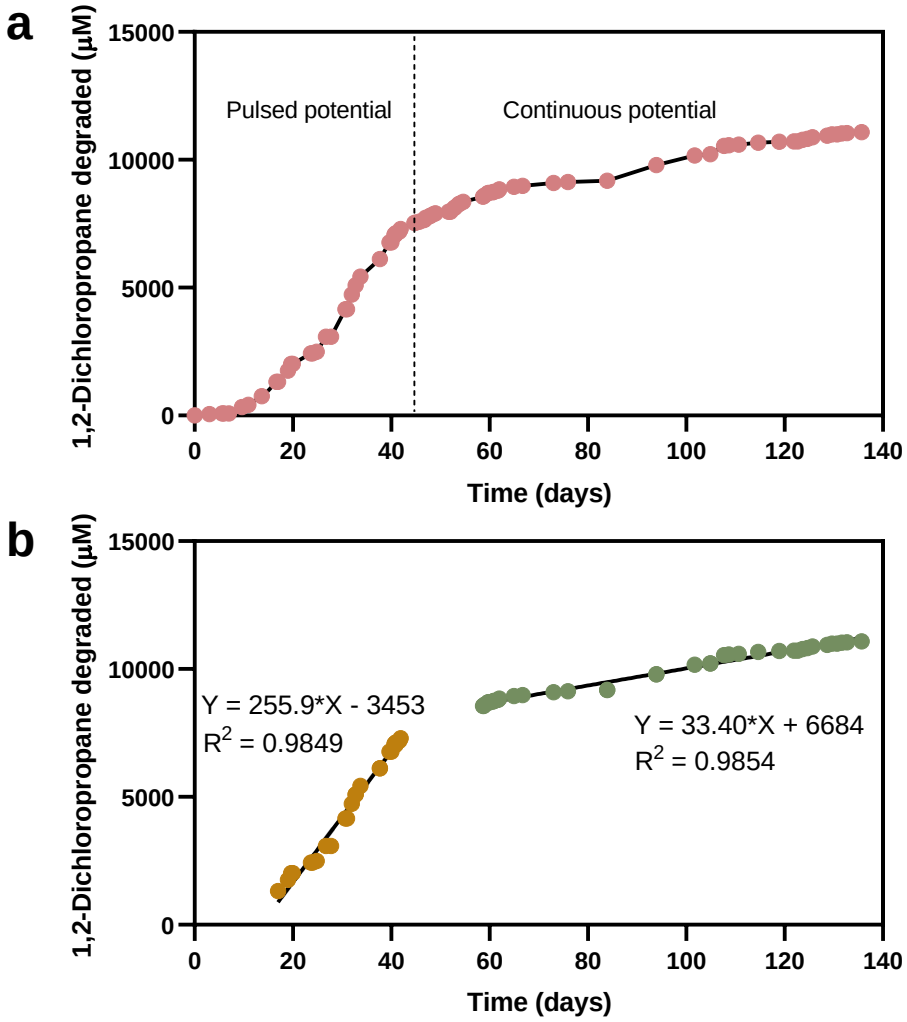

Figure 5.11. 1,2-DCP consumed in a BES containing a graphite brush as electrode (a) and linear regression of the accumulated 1,2-DCP degradation values corresponding to the degradation rate (b) when applying pulsed and continuous potential.

The CE of the pulsed operation was slightly lower than the 19.05% obtained in the carbon cloth containing BES but with a 16-fold degradation rate. This high CE was coupled with a low energetic input of 0.076 $kWh \cdot mol^{-1}$, which was also similar to the values obtained in the carbon cloth cells. The CE value in the pulsed voltage period is

higher than that obtained by *Dehalococcoides* during degradation of 1,2-dichloroethane in a graphite-rod BES working at potentials of -500 mV or lower (i.e. with hydrogen evolution). However, the CE was far lower than that reported for direct electron transfer from the electrode surface to bacteria (CE =68%) [12]. On the other hand, when applying a continuous voltage, the decrease of the degradation rate and the higher intensities applied drastically hindered CE, being a 95% lower than that obtained when applying a pulsed voltage. Also, when applying a continuous voltage to achieve a cathode potential of -1.1 V, the required energetic input increased 33-fold (2.499 $kWh \cdot mol^{-1}$).

Applying a pulsed voltage not only reduced the electric energy input but also stimulated the degrading activity of *Dehalogenimonas*. This increase of degrading activity when working with a pulsed potential could be caused by the high hydrogen generation in a short period of time while avoiding the more aggressive reducing conditions related to a continuous -1.1 V application. A low potential could induce unfavourable conditions for the growth of *Dehalogenimonas* by promoting pH gradients or high local ion concentrations. 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.

5.3.5 Scanning electron microscopy visualization

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 as described in section 3.2.7 of the general materials and methods chapter. Two main cell morphologies comprising irregular-shaped *cocci* and *bacillus* were clearly identified in the filtered suspension cultures (Fig. 5.12 a, 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, as described in section 3.1.2.1 of the general materials and methods [33]. Also, several aggregates were observed on the graphite brush (Fig. 5.12 b and f) and, in less extension, on carbon cloth electrode (Fig. 5.12 d). 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 [34,35]. 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* [36,37]. 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 [38], which are required for a biofilm formation.

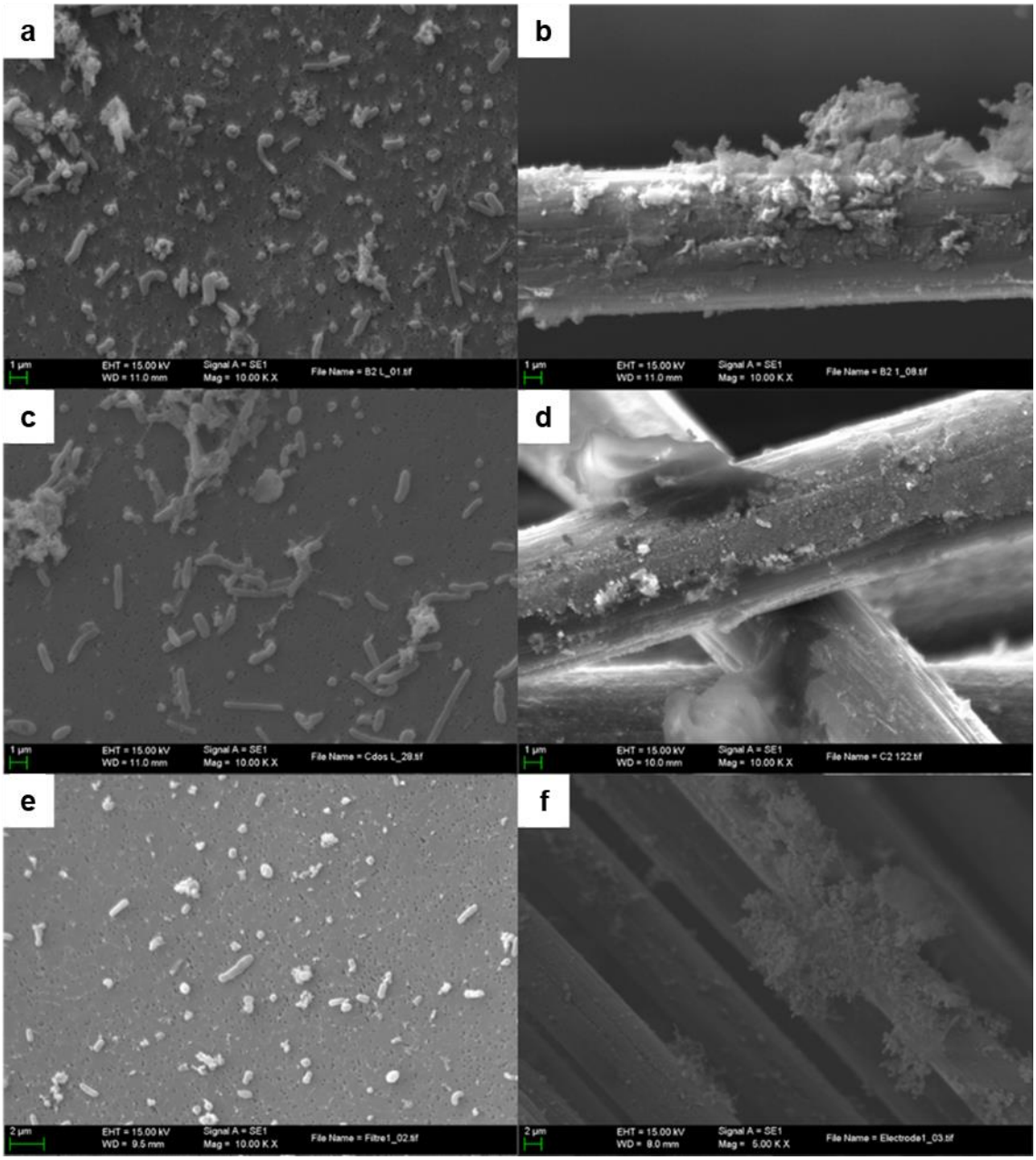

Figure 5.12. Microscopic analysis of the graphite fiber brush (a and b) and carbon cloth experiments (c and d) described in section 5.3.1 and graphite brush experiment described in section 5.3.4 (e and f) at the end of the experiments. For each BES, filtered liquid samples (a, c and e) and solid electrode samples (b, d and f) were analysed by SEM.

5.4 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 rRNA 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 in order to overcome bottlenecks such as the complexity and heterogeneity of a real groundwater environment, the cost of the required energy and materials, or the difficulties related to provide an adequate supply of hydrogen to promote organohalide respiration.

5.5 References

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Chapter 6

Sequential dechlorination of chloroform to dichloromethane and acetate in the cathodic vessel of a bioelectrochemical system

Part of this chapter was published as:

D. Fernández-Verdejo, P. Cortés, A. Guisasola, P. Blánquez, E. Marco-Urrea, Bioelectrochemically-assisted degradation of chloroform by a co-culture of *Dehalobacter* and *Dehalobacterium*, Environ. Sci. Ecotechnology. (2022) 100199. doi:10.1016/j.ese.2022.100199.

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 μM) 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 μM 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.

6.1 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 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 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, 2,3,4,5-tetrachlorobiphenyl [9,13,15–17] or 1,2-dichloropropane (in chapter 5 of this thesis).

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 [18,19]. 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* [20–23]. The fermentation of DCM by *D. formicoaceticum* produces acetate and formate, which are environmentally friendly products [24–26]. Additionally, "*Candidatus* Dichloromethanomonas elyuquensis" was recently found to completely mineralize DCM to H_2 and CO_2 [27,28].

This chapter 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.

6.2 Materials and methods

6.2.1 Description of the BES

The two-chamber BES used in this chapter have been previously described in section 3.1.3.1 of the general materials and methods chapter.

6.2.2 Cultivation of the dehalogenating cultures in serum bottles

The *Dehalobacter*-containing culture employed in this chapter is described in section 3.1.2.2, whereas the *Dehalobacterium*-containing culture is described in section 3.1.2.3. Both cultures are cultivated as detailed in the general material and methods chapter in sections 3.2.1.

6.2.3 Operation of BES

Cathodic and anodic vessels of the two-chamber BES, previously described in the general materials and methods section, were filled with 130 mL of the anaerobic medium of *Dehalobacter*. Cathodic compartments were initially spiked with 500 µM CF and inoculated with 3 mL of the *Dehalobacter*-containing culture (concentration of *Dehalobacter* ~9.38×10^4 ± 2.38×10^4 16S rRNA gene copies per mL). Biotic BESs operated at OCP without electrodes were included as controls to assess biodegradation of CF without electrochemically generated H_2. 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).

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 3.2.2.2 in the general materials and methods chapter) 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.

6.2.4 Analysis of volatile compounds

The concentrations of volatile chlorinated compounds were determined by static headspace gas chromatography as described in section 3.2.2.2 of the general materials and methods chapter.

6.3 Results and discussion

6.3.1 Effect of the cathodic potential on CF dechlorination by *Dehalobacter*

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. 6.1). 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 OCP controls during 20 days of operation (Fig. 6.1). 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.

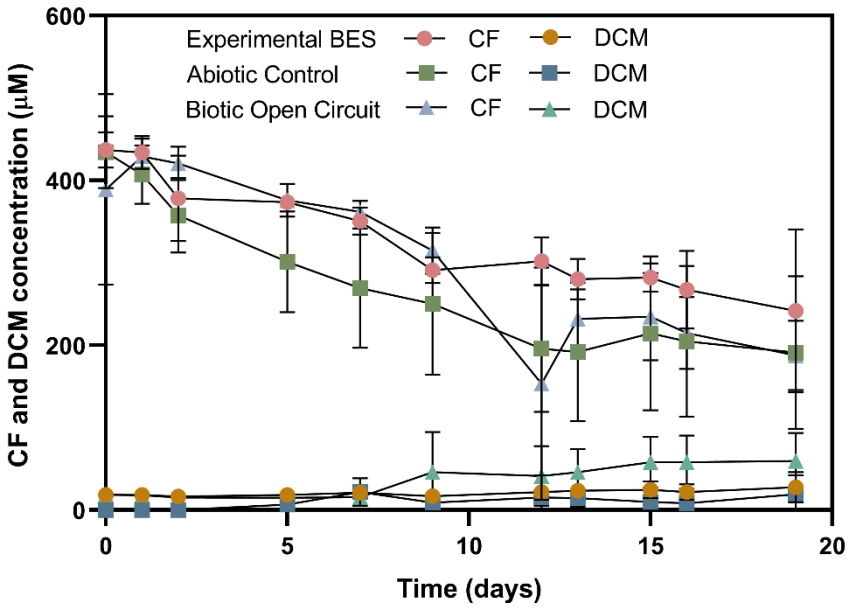

Figure 6.1. Degradation profile of CF and accumulation profile of DCM in BES inoculated with *Dehalobacter* and poised at -0.3 V of cathodic potential, abiotic controls and biotic OCP. Values plotted are average of triplicates and error bars indicate standard deviation.

The dechlorination of CF was almost immediately detected when the cathode was poised at −0.6 V, with the concomitant production of DCM (Fig. 6.2). 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. 6.2).

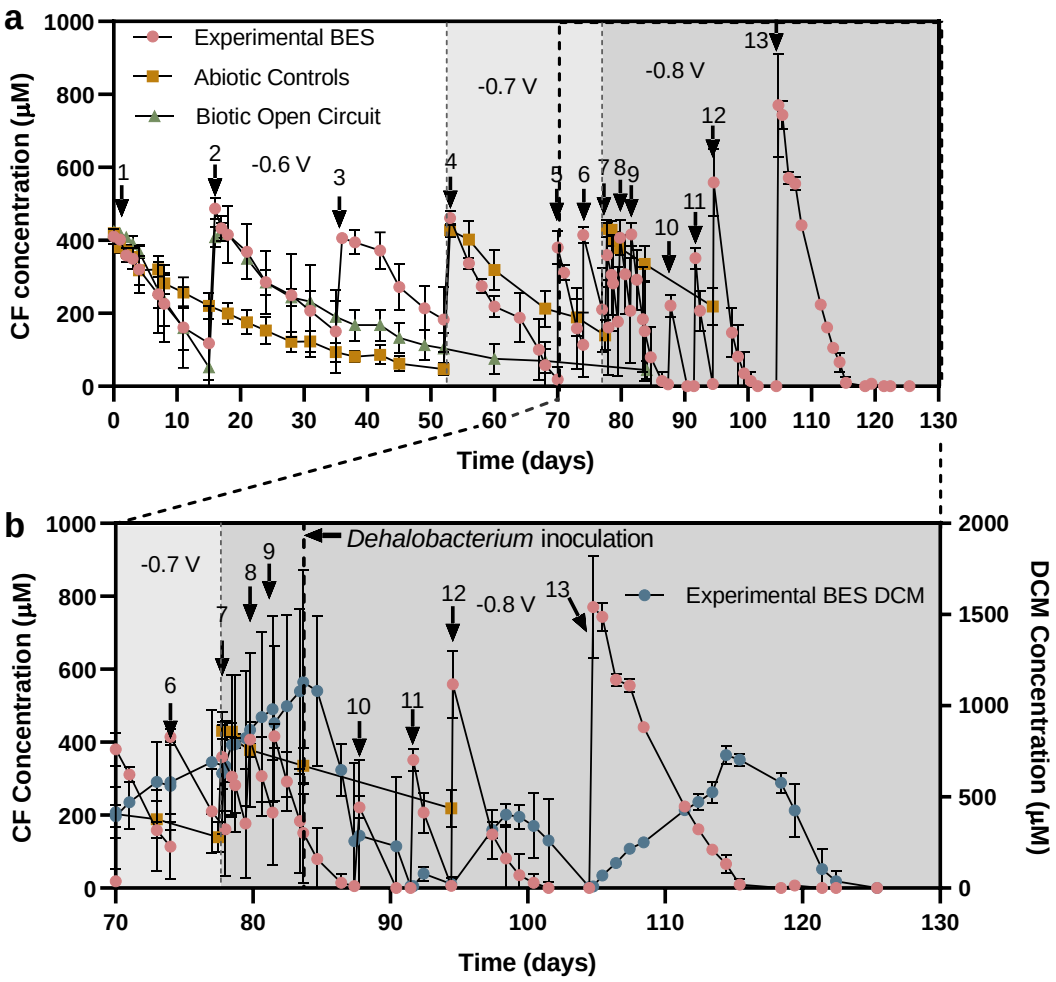

Figure 6.2. Degradation profile of CF in experimental BES inoculated with *Dehalobacter* and poised with a cathodic potential, abiotic controls and biotic OCP. The time lapse between days 70 and 130 was amplified in a subpanel for best readability of measurements. Changes in concentration of DCM was only depicted in the subpanel. Numbers indicate the number of CF amendment in the experimental BES. Values plotted are average of triplicate BES and error bars indicate standard deviation.

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. 6.3 a, 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 ± 7.3 and 10.1 ± 8.5 μM d^{-1} (at −0.6 V cathodic potential) and 131.6 ± 64.9 and 125.2 ± 58.9 μM d^{-1} (at −0.8V cathodic potential), respectively.

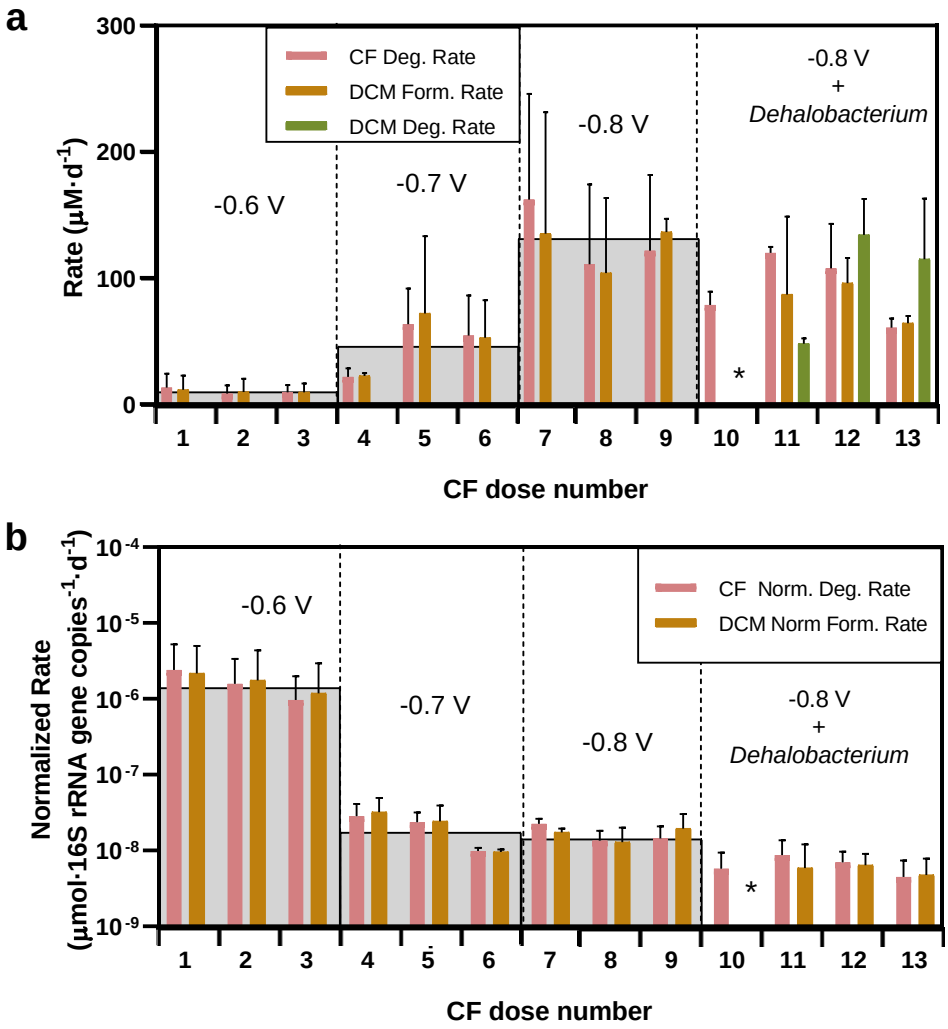

Figure 6.3. Degradation rate of CF and production rate of DCM (a) and CF degradation rate and DCM production rate normalized by the 16S rRNA gene copies (b) for each dose of CF consumed in experimental BES. The numbers refer to the dose number depicted in Fig. 6.2. The grey background represents average 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 its fast consumption by *Dehalobacterium*.

DCM production rates were almost identical to CF degradation rates indicating that molar balance can be considered closed (Fig. 6.3 a). The low basal CF degradation

rate obtained in the abiotic controls was 2.82 ± 0.47, 2.92 ± 1.19, and 2.51 ± 1.90 μM 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 OCP controls (Fig. 6.2), but the second amendment of CF was not completely degraded after ~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 2500 μM DCM for *Dehalobacter* [1].

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 1128 ± 616 μM. DCM was completely degraded in the following seven days at a degradation rate of 481 ± 141 μM d^{-1} (Fig. 6.2). Once DCM was degraded, CF was spiked at increasing concentrations of approximately 200, 400, 600 and 800 μM to couple both *Dehalobacter* and *Dehalobacterium* dechlorination capabilities (Fig. 6.2). At lower concentrations of CF (200 and 400 μM), the degradation of CF and DCM proceeded rapidly (Fig. 6.2, arrow numbers 10 and 11). However, when CF was added at higher concentrations (600 and 800 μM), DCM accumulated (Fig. 6.2, arrow numbers 12 and 13), and degradation only started after CF was almost exhausted. This agrees with a recent study showing that CF at ~800 μM inhibited the DCM degradation activity of *Dehalobacterium*, and the activity could be recovered when the culture was transferred to a CF-free medium [25]. 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 its use as a carbon source by *Dehalobacter* (Fig. 6.4).

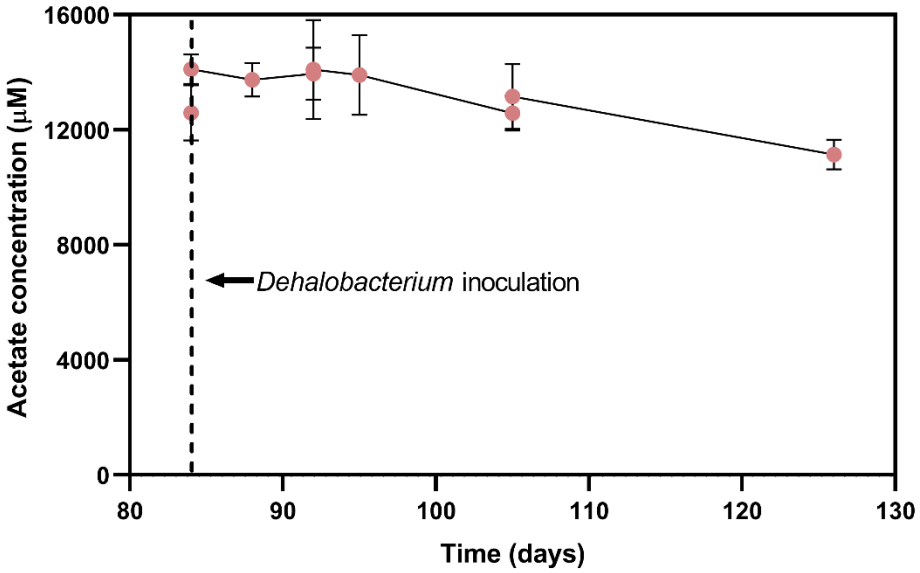

Figure 6.4. Acetate profile in the experimental BES from Fig. 6.2 after the inoculation of the *Dehalobacterium* consortia. Points represent the mean value and error bars represent the standard deviation of triplicates.

6.3.2 *Dehalobacter* growth and degradation kinetics in BES

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. 6.5), as described in the general materials and methods section. *Dehalobacter* 16S rRNA gene copies increased over four orders of magnitude from an initial concentration of $4.33\times10^{3} \pm 1.00\times10^{3}$ to $1.96\times10^{7} \pm 1.01\times10^{7}$ 16S rRNA genes·mL^{-1} after 126 days of operation (Fig. 6.5). Assuming five 16S rRNA gene copies per genome [29], a cell density of $3.92\times10^{6} \pm 2.02\times10^{6}$ *Dehalobacter* cells·mL^{-1} was obtained.

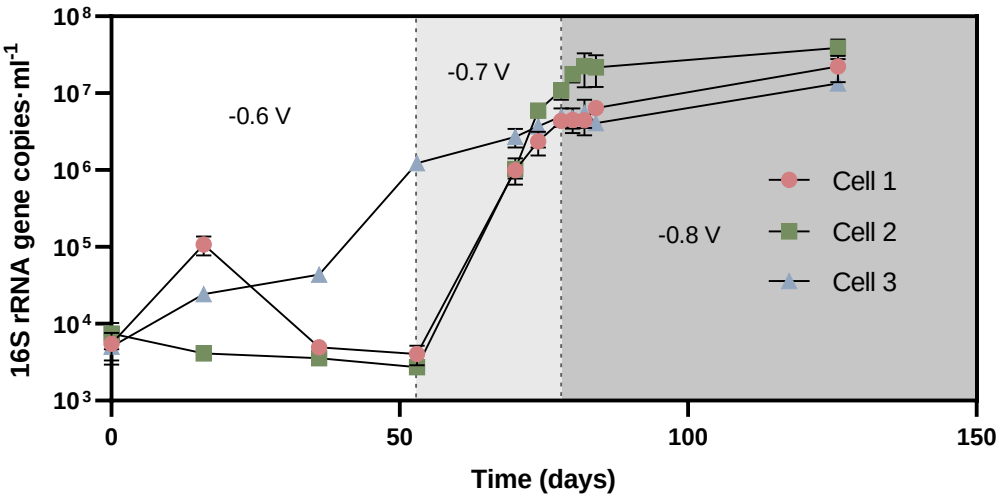

Figure 6.5. Time-course of *Dehalobacter* 16S rRNA gene copies per mL in each replicate of the experimental BES. Bars indicate deviation for duplicate measurements.

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. 6.6). The average growth yield was $7.76\times10^{6} \pm 3.60\times10^{6}$ *Dehalobacter* 16S rRNA gene per µmol of CF degraded (or *Dehalobacter* 16S rRNA gene per µmol 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 ($3.6\times10^{6} \pm 2.6\times10^{6}$ and $2.5\times10^{7} \pm 0.9\times10^{7}$ 16S rRNA gene copies per µmol Cl^{-}) [1,30]. The obtained growth yield is one order of magnitude lower than the one obtained for the CF-respiring *Desulfitobacterium* sp. strain PR ($1.16\times10^{7} \pm 0.16\times10^{7}$ 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 [30–33].

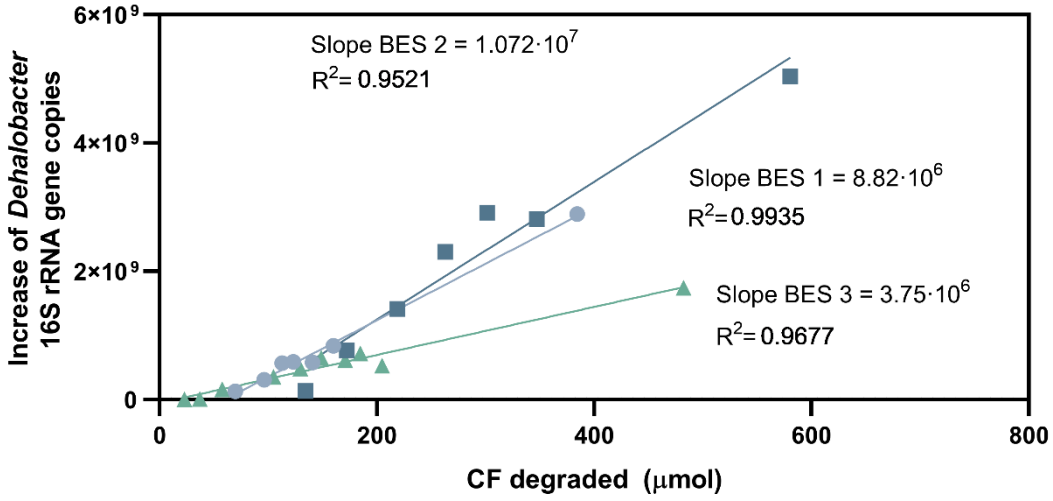

Figure 6.6. Correlation between the moles of CF consumed and changes in the 16S rRNA gene copy numbers for the experimental BES triplicates.

The exponential growth phase from each experimental BES was 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 ± 0.097 d^{-1} and 3.81 ± 2.13 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 ± 1.2 d) [30], suggesting that *Dehalobacter* sp. 8M present in BES grows at a faster rate. A maximum specific CF utilization of 2.83×10^{-8} ± 0.12×10^{-8} μmol of CF degraded per *Dehalobacter* 16S gene copy generated per day was obtained by combining the maximum growth rate and the growth yield.

Fig. 6.3 b 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 [34].

6.3.3 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 ± 0.060, −0.110 ± 0.029, and −0.325 ± 0.043 mA m^{-2} for potentials −0.6, −0.7, and −0.8 V, respectively (Fig. 6.7).

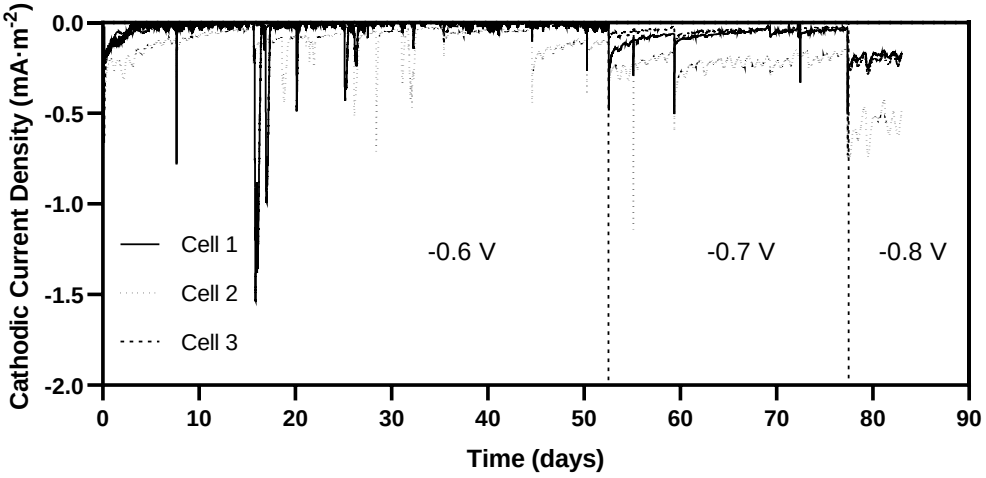

Figure 6.7. Current density profile recorded in the triplicate of experimental systems prior to the inoculation of *Dehalobacterium*.

These current intensity values were used to assess the coulombic efficiency of the CF degradation process, which was 42.33 ± 28.22% when working at a cathodic potential of −0.6 V and increased to values between 60% and 70% when the potential decreased to −0.7 and −0.8 V (Fig. 6.8).

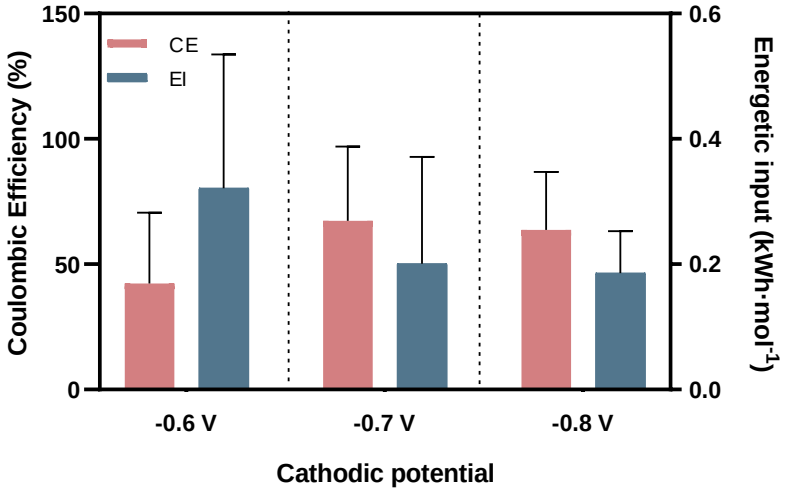

Figure 6.8. Coulombic efficiencies and energetic inputs per mol of CF degraded obtained for each cathodic potential. Bars represent the mean value and error bars represent the standard deviation of triplicates.

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 V and decreased the required value at potentials −0.7 and −0.8 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. 6.9).

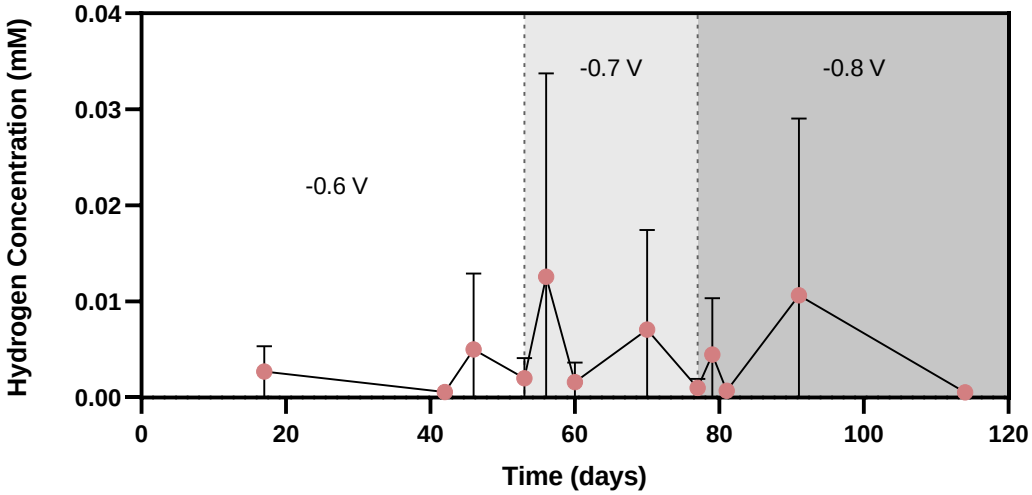

Figure 6.9. Hydrogen concentration profile measured from the gas phase of the triplicate experimental BES depicted in Fig. 6.2. Points represent the mean value and error bars represent the standard deviation of triplicates.

The absolute degradation rates (Fig. 6.3 a) presented similar values during the three CF doses at each poised cathodic potential value despite the 16S rRNA gene concentrations increasing over time (Fig. 6.5). Thus, the observed maximum degradation rate was limited by the hydrogen bioavailability and not cell density. The maximum 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. 6.3 b). 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 [35]. 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 reduced when operating at a higher scale.

6.4 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 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 µM, which would avoid the accumulation of DCM in the environment. At higher concentrations of CF, *Dehalobacterium* was inhibited but recovered the DCM fermenting activity when CF reached 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.

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Chapter 7

Simultaneous degradation of toluene and chloroform in a continuous bioelectrochemical reactor

The work in this chapter was partly performed during a research stay at the Water Research Institute (IRSA) from the National Research Council (CNR) located in Monterotondo (Rome) under the supervision of Dr. Federico Aulenta.

Abstract

The co-contamination of groundwater is a frequent scenario in industrial sites and the simultaneous degradation of the contaminant mixture is often a challenge. In this chapter, we coupled the bioelectrochemical oxidation of toluene by an anodic biofilm with the reduction of CF to acetate in the cathode of a BES operating in a continuous mode after bioaugmentation with *Dehalobacter* and *Dehalobacterium*. The bioelectrochemical system employed, a bioelectric well, consisted of a single-chamber tubular reactor equipped with graphite rods and stainless-steel mesh for anodic and cathodic electrodes respectively. Initially, the system operated in fed-batch mode at +0.4 V vs SHE to promote biofilm formation in the anode using groundwater containing toluene-degrading bacteria and periodical toluene amendments. While operating in fed-batch, the reactor was able to successfully degrade five toluene doses of 270 μM obtaining a stable current of approximately 1 mA and a continuous electrochemical production of H_2 in the cathode. Afterwards, the system was operated in continuous mode, and it was able to simultaneously oxidate toluene and reduce CF to DCM, obtaining rates up to 63 and 49 $\mu M \cdot d^{-1}$ for the oxidative and reductive reactions respectively. Increasing inlet concentrations of toluene were tested to maximize its degradation rate, but DCM fermentation was partially inhibited at a toluene concentration of 400 μM. The degradation of both CF and toluene severely decreased when operating at OCP, demonstrating the requirements of an external source of current to drive the reactions, while the fermentation of DCM, which not required the presence of H_2, remained unaffected. Our findings open the window to the potential application of the bioelectric well as a feasible technology to remediate multi-contaminated sites.

7.1 Introduction

Chlorinated compounds and gasoline aromatics (often referred to as BTEX) are amongst the most widespread groundwater contaminants in urban and industrial areas [1]. Leakages of gasoline are mostly produced from underground storage tanks in gas stations and associated pipes, and chlorinated solvents are mostly released by improper handling and disposal practices [2,3]. The occurrence of both, gasoline and chlorinated solvents, in contaminated groundwater is a common scenario in industrial sites [4] and it is of concern because of their toxicity and adverse effects for human health [5–7]. During the last decades numerous attempts to degrade both families of contaminants through bioremediation have been performed, but the solution often requires the use of several integrated treatment processes to remove them sequentially due to the distinct metabolic needs of the bacterial degraders [8–10]. In order to optimize the bioremediation costs, it would be desirable to conduct the bioremediation of mixtures of both BTEX and chlorinated compounds simultaneously in the same compartment. Environmental pollution by BTEX often leads to a rapid depletion of available oxygen and, therefore, degradation of BTEX and chlorinated compounds rely largely on anaerobic processes. On the one hand, the reductive dechlorination of chlorinated compounds by OHRB is favoured in methanogenic conditions and uses hydrogen as electron donor while using the contaminant as electron acceptor [11]. On the other hand, the oxidative catabolism of BTEX differs in the redox conditions and needs more favourable electron acceptors such as sulphate, ferric iron or nitrate [8,12].

Despite the theoretical possibility of using the oxidation of BTEX as electron donor for the chlorinated compounds reduction, the strict requirements of hydrogen as provider of electrons for OHRB hinders the simultaneous removal of both pollutants in a single-step reaction [13]. This bottleneck can be overcome by operating the

biodegradation process in a BES. The electric current obtained from BTEX oxidation can be transferred to the cathode and electrochemically produce hydrogen which can be used by the OHRB to reduce chlorinated compounds as 1,2-DCP, CF, 1,2-dichloroethane, trichloroethene or perchloroethene, as it has been previously observed in the literature and in chapters 5 and 6 of this thesis [14–20]. Despite these advantages, the application of BES for coupling BTEX oxidation at the anode and the reduction of chlorinated compounds at the anode is still in its infancy [21]. One of the main difficulties facing the filed-scale use BES for aquifer remediation is the low conductivity in the groundwater which is translated to high ohmic resistances. This issue is frequently aggravated by the long distances between the electrodes required when working on the field [22,23].

In this chapter, we selected toluene as compound model of BTEX and intended to couple the CF degradation previously performed in chapter 6 with *Dehalobacter* and *Dehalobacterium* with toluene oxidation in a single reactor. In order to fulfil this objective, a novel BES configuration, the bioelectric well, which possesses both electrodes, anodic and cathodic, in the same vessel, was operated. The concentric position of the anode and cathode allowed to minimize the distance between the electrodes while maintaining hydraulic connection between them, but at the same time avoiding physical contact between their surfaces. This disposition minimizes the ohmic resistance and has been designed in order to be used in-situ in a sampling well without losing the low separation between electrodes. This reactor configuration has been used in previous studies and has been successfully able to treat in continuous-flow mode phenol, toluene, a mix of BTEX and two mixtures of toluene with sulfate and trichloroethene respectively [21,24–27]. In this system, the oxidation of toluene was driven in the anodic electrode and CF was dechlorinated to DCM by *Dehalobacter* using the hydrogen produced at the

cathode. The DCM was further degraded by a *Dehalobacterium*-containing culture, obtaining acetate as final product.

7.2 Materials and methods

7.2.1 Reactor setup and operation

At the start of the operation, the "bioelectric well" reactor (previously described in the section 3.1.3.2 of the general materials and methods chapter) was filled with 0.3 L of groundwater containing a toluene-degrading culture obtained in previous studies in a petrochemical site located in central Italy [21]. The system was operated in fed-batch mode and toluene was spiked at an initial concentration of 130 μM. Further amendments of 270 μM of toluene were done until a stable toluene-oxidizing activity by the anodic bacterial community was observed. Next, the operational system was changed to continuous mode. The mineral medium of *Dehalobacter* described in the general materials and methods chapter (section 3.2.1), excluding yeast extract, and spiked with toluene was pumped into the lower part of the bioelectric well through an entrance port from a collapsible Tedlar® bag (5 L). The inlet flowrate was initially set at 0.2 $ml \cdot min^{-1}$ during 10 days and later was decreased to 0.1 $ml \cdot min^{-1}$, obtaining hydraulic residence times (HRTs) of 24 and 48 hours, respectively. When toluene degradation reached a steady state, after 25 days of continuous operation, 15 ml of the *Dehalobacter* and *Dehalobacterium* cultures, previously described in the general materials and methods chapter in sections 3.1.2.2 and 3.1.2.3, were inoculated respectively in the bioelectric well. Additionally, CF was spiked into the inlet bag in order to support the growth of *Dehalobacter*.

The inlet, outlet and recirculation of the reactor were equipped with sampling cells of 25 mL magnetically stirred containing flow-through. The tubings employed were made

of Viton® (Sigma-Aldrich, Italy) in order to avoid losses of volatile contaminants and minimize adsorption effects. The reactor was operated at room temperature. The final set-up of the reactor can be observed in figure 3.3 in the general materials and methods section.

At the end of the operation of the reactor, the anodic potential control was disconnected and the system operated in OCP in order to determine the effects of the provided electric current in the degradation performances obtained during the previous operation.

All the analytical methods and calculations of this chapter have been previously introduced in general materials and methods chapter.

7.3 Results and discussion

7.3.1 Degradation of toluene in fed-batch mode

The bioelectric well was operated in fed-batch mode and was able to successfully degrade an initial toluene dose of 140 μM and 4 additional sequential doses of approximately 270 μM during 42 days of operation (Fig. 7.1). The averaged toluene degradation rate observed during this period accounted to 25.6 $\mu M \cdot d^{-1}$.

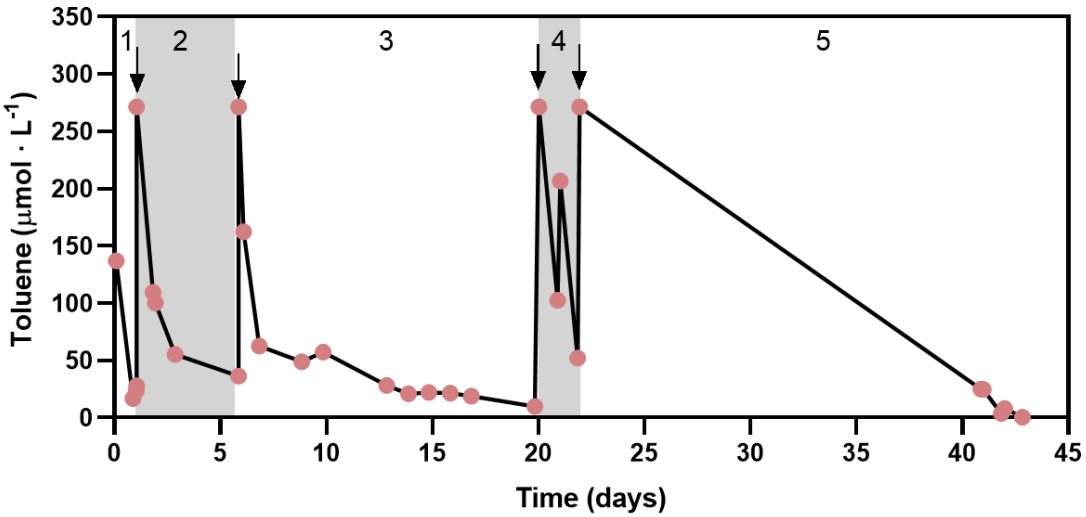

Figure 7.1. Degradation of toluene in the bioelectric well during the fed-batch mode. Arrows represent the amendments of toluene. Numbers and grey/white backgrounds represent the different operational periods.

At the initial steps of the operation, during the two first amendments of toluene, the recorded intensities remained slightly negative, suggesting that the observed decreases in toluene were mostly due to adsorption to surfaces present in the system (e.g. electrodes). This situation changed after 8 - 9 days of operation, when a sudden increase of current was observed in the system (Fig. 7.2), suggesting that the toluene degrading consortia stablished an initial biofilm in the anodic surface and transferred the electrons released from toluene oxidation to the graphite rods. After this initial increase, and excluding peaks corresponding to periods with high toluene degradation rate, as it was observed at the start of dose 5, the current adopted a constant value between 0.5-1 mA, ensuring a continuous electrogenic activity of the formed biofilm. This observation was corroborated by the hydrogen observed in the headspace of the reactor, which was electrochemically produced in the cathode as a result of the toluene oxidation and accumulated from day 9 onwards (Fig. 7.3). Moreover, methane started to accumulate in the reactor following a similar trend than the hydrogen (Fig 7.3), suggesting a

proliferation of methanogenic populations in the medium which benefited from the hydrogen and the reducing conditions produced by the cathode.

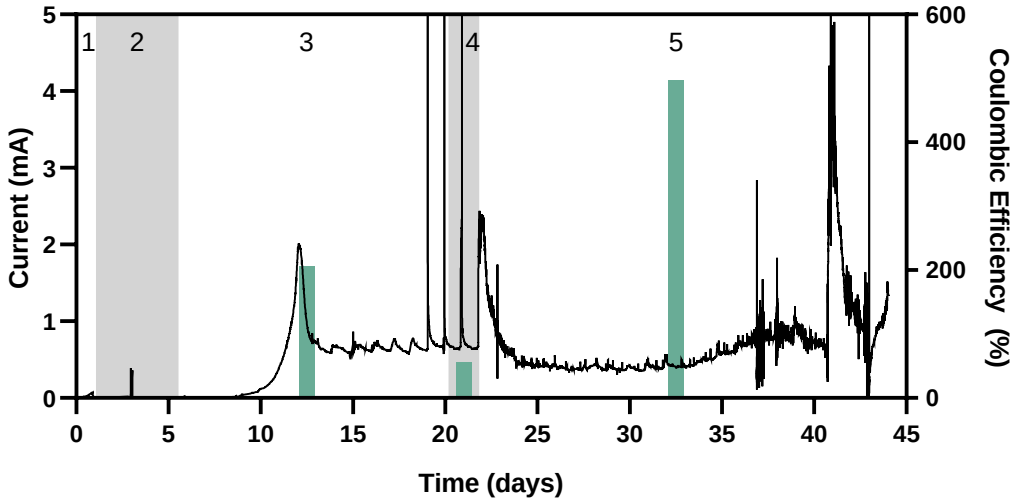

Figure 7.2. Intensities recorded during the batch operation (continuous line) and coulombic efficiency (bars) for each toluene dose of the bioelectric well. Numbers and grey/white backgrounds represent the different operational periods corresponding to the toluene doses amended to the reactor.

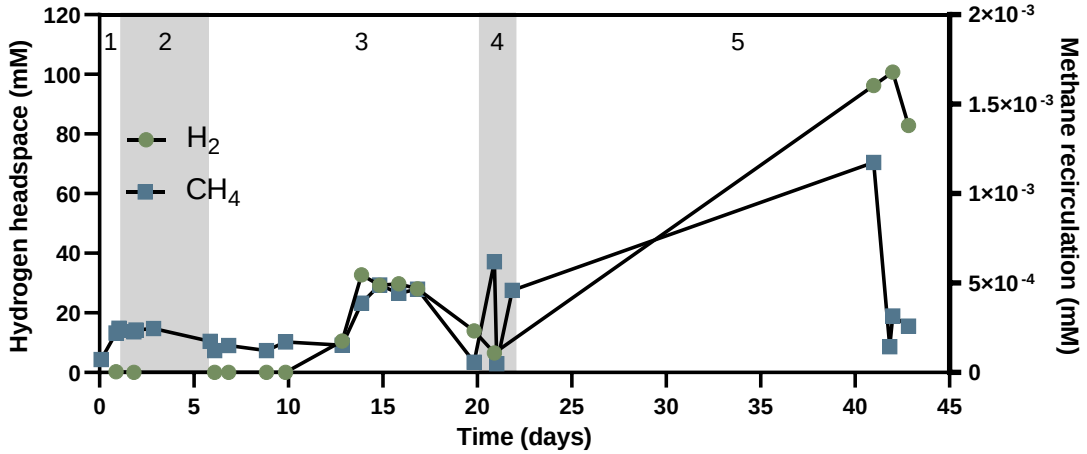

Figure 7.3. Hydrogen accumulated in the headspace and methane measured in the recirculation of the reactor during the batch phase operation. Numbers and grey/white backgrounds represent the different operational periods corresponding to the toluene doses amended to the reactor.

CEs regarding exclusively toluene consumption were calculated from dose 3 onwards and adopted values between 50 and 500% (Fig 7.2). The calculations were neglected in the first two doses because the decreased toluene could not be attributed to biodegradation, as there was no positive intensity flowing through the system. Interestingly, the values of CE higher than 100% observed in the toluene doses 3 and 5 occurred when the highest hydrogen concentrations were observed in the headspace of the reactor. This correlation could point to the oxidation of other substrates at the anode, such as acetate, which could act as an additional electron supply. Another cause could be the hydrogen scavenging, whereby the anode could oxidize the hydrogen produced at the cathode [28]. To calculate CE more accurately, acetate was monitored in the following experimental stages.

7.3.2 Degradation of toluene in continuous mode

After 42 days of operation in fed-batch mode, toluene degradation was considered sustainable and robust enough to switch to a continuous mode operation. Anaerobic mineral medium spiked with ~ 260 μM of toluene was pumped through the inlet port with a flowrate of 0.2 $ml \cdot min^{-1}$ with the main purpose of assuring that the anodic biofilm was able to maintain or enhance its degrading activity in the new operational conditions. After 12 days, the reactor was able to degrade 77 μM of toluene, which was determined by measuring the difference between the inlet and outlet (Fig. 7.4). At that point, in order to increase the hydraulic retention time of the system and improve the degradation values, the flowrate was decreased to 0.1 $ml \cdot min^{-1}$. This change made it possible to increase the amount of toluene degraded by 40 % at the cost of losing 29 % of the degradation rate achieved using the previous flowrate (Fig. 7.5).

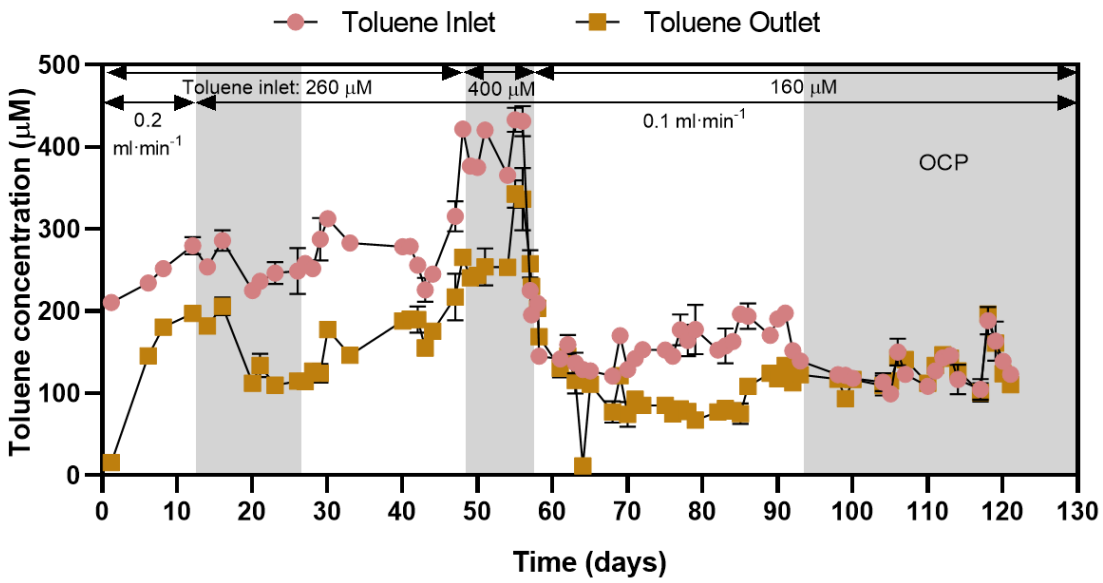

Figure 7.4. Concentration profiles of toluene in the inlet and the outlet of the bioelectric well during its continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of duplicate measurements and error bars indicate standard deviation.

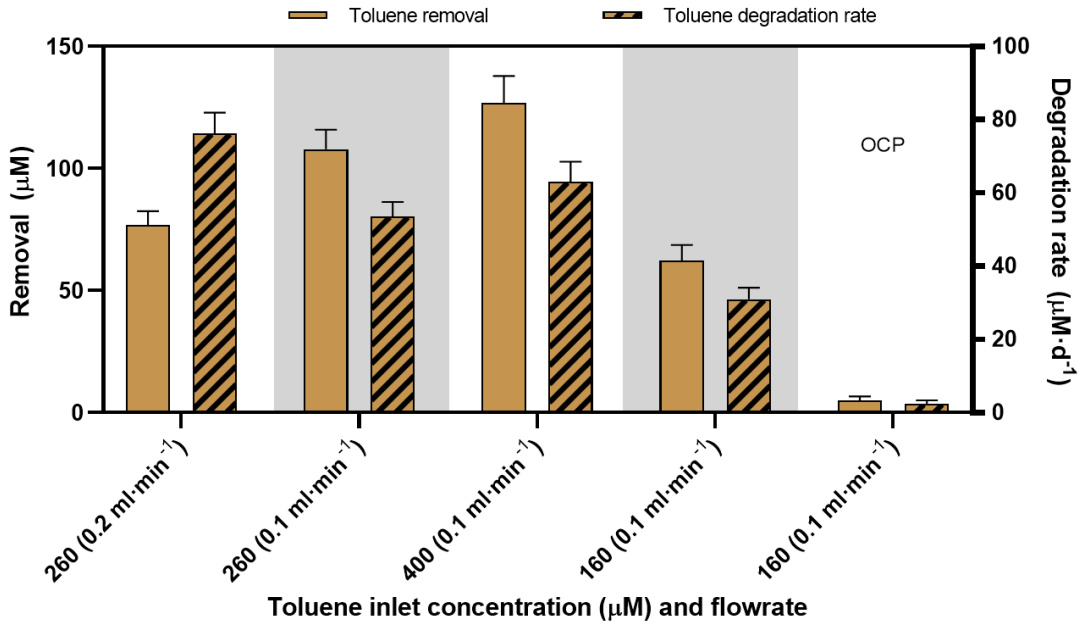

Figure 7.5. Toluene removal between inlet and outlet of the bioelectric well and degradation rates obtained at each stage of the continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of each operational period and error bars indicate standard deviation.

After 14 days of operation at a flowrate of 0.1 mL·min^{-1}, on day 27 of continuous operation, *Dehalobacter* and *Dehalobacterium* were inoculated into the system and 100 µM of CF were spiked to the inlet, in addition to the 260 µM of toluene previously added. The presence of CF, which is a known inhibitor of methanogens [29], and the competition for hydrogen by *Dehalobacter*, had an immediate negative effect on the methanogenic populations present in the reactor, which had previously been proliferating as evidenced by the methane concentration profile (Fig. 7.6). Nevertheless, apparently no adverse effects on toluene degradation were observed after adding CF to the system (Fig. 7.4).

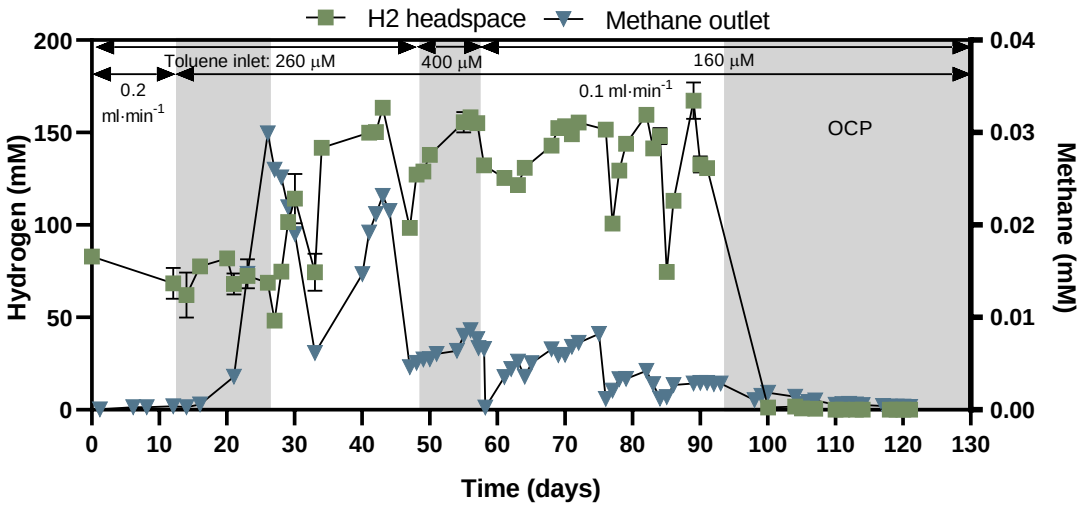

Figure 7.6. Concentration profiles of H_2 and CH_4 in the headspace and the outlet of the bioelectric well respectively during its continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of duplicate measurements and error bars indicate standard deviation.

On day 48 of operation, the toluene concentration in the inlet was increased to 400 µM to study whether a higher concentration of oxidable substrate in the reactor could lead to an increase in its degradation rate (Fig. 7.4). These new conditions, despite providing an immediate increase of 17 % of the average amount of toluene removal, showed a progressively decreasing trend in the degradation rate. This observation suggested that

the prolonged presence of these high toluene concentrations could be detrimental on the long-term for the anodic biofilm. Furthermore, the amounts of toluene present in the bulk of the reactor, as evidenced by the toluene measured in the outlet cell, could potentially lead to a decrease in the activity of *Dehalobacter* and *Dehalobacterium* populations and negatively affect the performance of the CF degradation. Consequently, on day 58, the inlet concentration was reduced to 160 μM with the objective of reducing as much as possible the presence of toluene in the reactor while aiming to maintain a similar performance to that of the previous phases. Unfortunately, this change resulted in a 50 % decrease in the amount of toluene degraded, but allowed a reduction of 63 % in the toluene concentration present in the reactor which potentially could be beneficial for *Dehalobacter* and *Dehalobacterium* activity.

The variations in the degradation rate of toluene at the different amended inlet concentrations are summarized at figure 7.7, obtaining a relatively logarithmic correlation and confirming that the concentration of toluene present at the reactor is a key-factor in its oxidation kinetics by the anodic biofilm. This observation was also supported by the fact that, even at the lowest inlet concentration (160 μM), the toluene degradation rate was 19 % higher than the one obtained during the fed-batch phase. This first phase, by its discontinuous nature, presented extended periods of time with low toluene concentrations in the reactor which could lead to a decrease in the obtained rate. However, the highest toluene degradation rates obtained in this work (63.03 $\mu M \cdot d^{-1}$) are considerably lower than the ones obtained in two previous studies using the bioelectric well reactor, which accounted for 330 and 150 $\mu M \cdot d^{-1}$, when enduring the co-degradation of toluene with sulfate or TCE respectively [21,24]. This rate difference could be due to the presence of acetate in the medium, which was added to support the growth of *Dehalobacter*, but it could be used instead as a preferred substrate for toluene degraders.

During all this continuous operation, the anodic oxidation derived in a continuous presence of 100-150 mM of electrochemically produced H_2 in the headspace of the reactor (Fig. 7.6).

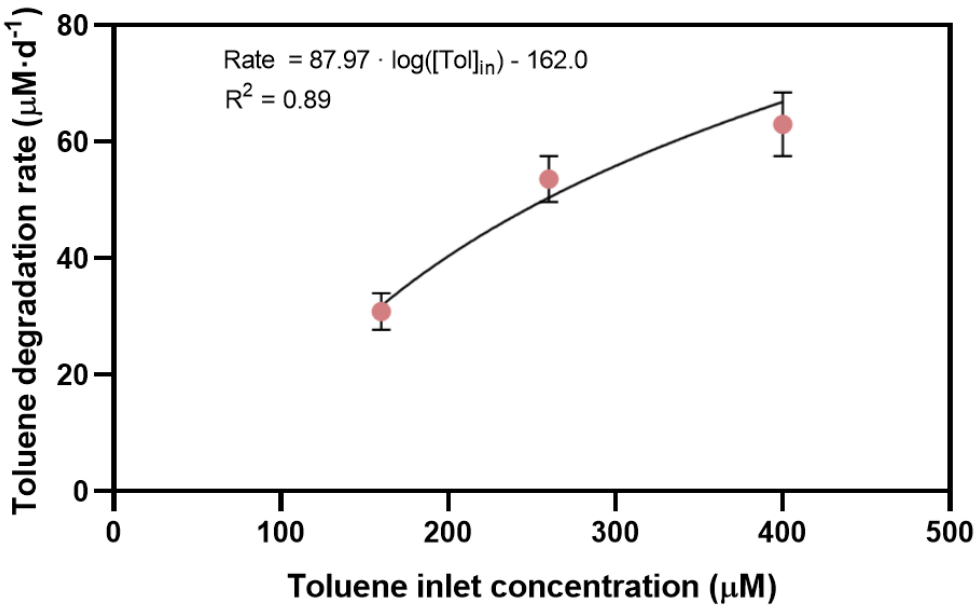

Figure 7.7. Correlation of toluene degradation rate with the amended concentration in the inlet. Values plotted correspond to the average of each toluene inlet concentration and error bars indicate standard deviation.

Finally, on day 93 of operation, the current was disconnected and the system was operated in OCP during the following 28 days. This disconnection was followed by an almost immediate decrease of 98 % in the toluene removal, demonstrating that the oxidation of toluene was mainly driven by the positive potential of +0.4 V poised in the anode.

7.3.3 Degradation of CF in continuous mode

Dehalobacter and *Dehalobacterium* were inoculated to the system on day 27 of the continuous operation and the inlet was spiked with CF at a final concentration of approximately 100 μM. Degradation of CF was detected two days later, and after 14 days the CF outlet concentrations stabilized between 25 and 34 μM (Fig. 7.8). This degradation

was accompanied by a production of DCM, but at 27 % of the stoichiometrically expected concentration, suggesting that *Dehalobacterium* was consuming DCM (Fig. 7.8).

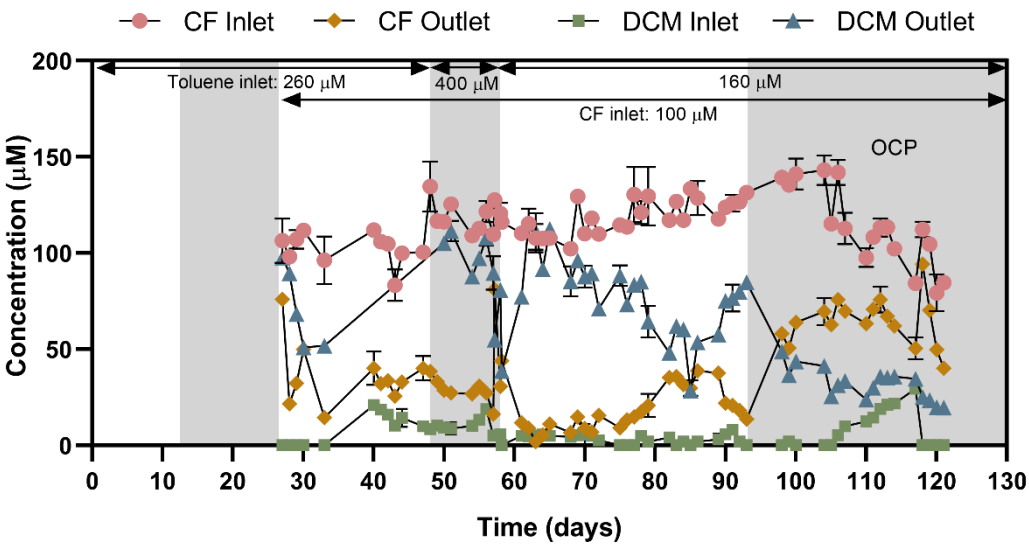

Figure 7.8. Concentration profiles of CF and DCM in the inlet and the outlet of the bioelectric well during its continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of duplicate measurements and error bars indicate standard deviation.

Unfortunately, the measurements in the system showed an almost complete consumption of the acetate that was amended through the inlet (Fig. 7.9), probably due to the oxidation by the anodic microbial consortia. Therefore, the increase of acetate concentration as metabolite of DCM fermentation in the outlet could not be used to confirm the activity of *Dehalobacterium*.

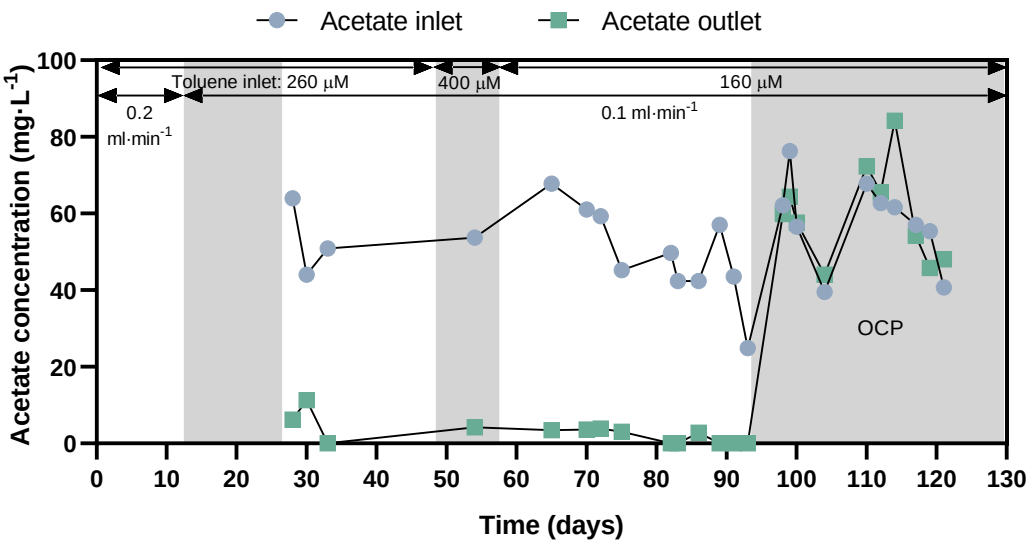

Figure 7.9. Concentration profile of acetate in the inlet and the outlet of the bioelectric well during its continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet.

The DCM degrading activity was lost when, on day 48, the toluene concentration in the inlet was increased to the maximum value of 400 µM (Fig. 7.10). This might be due to an inhibitory effect of toluene on the degrading activity of *Dehalobacterium*, as it has been previously reported in presence of high concentrations of other contaminants [30]. On the other hand, CF degradation not only was unaffected by toluene but increased by 33 %. This improvement could be caused by the growth of the *Dehalobacter* population in the reactor from the consumption of previous doses.

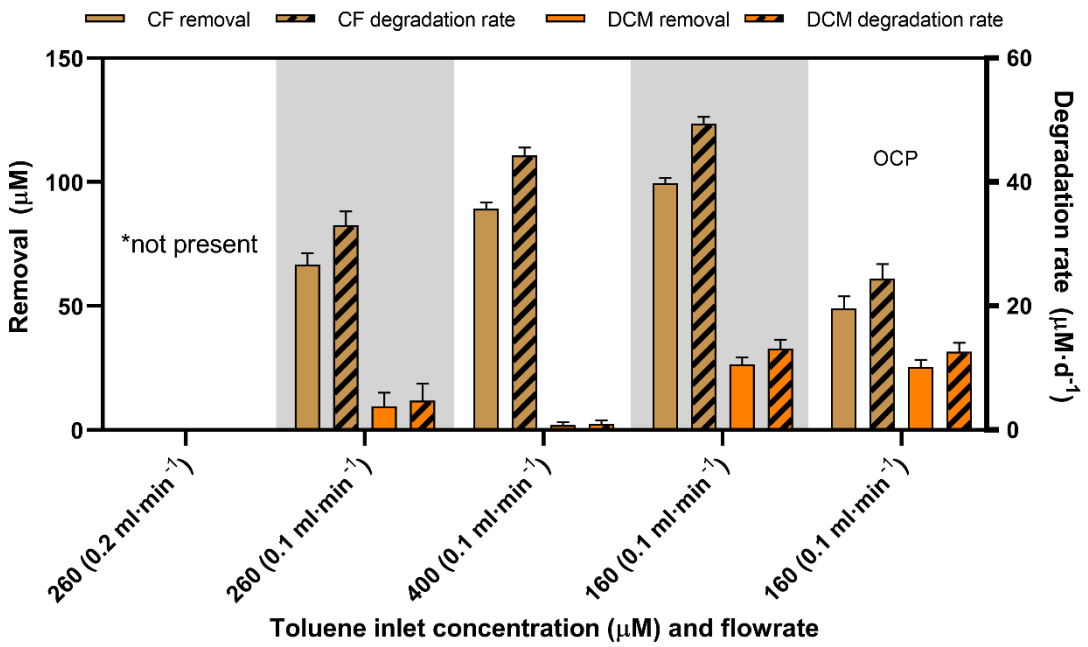

Figure 7.10. CF and DCM removals between inlet and outlet of the bioelectric well and degradation rates obtained at each stage of the continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of each operational period and error bars indicate standard deviation.

In order to recover the degrading activity of DCM, the toluene concentration in the inlet was decreased to 160 μM on day 58, as previously stated. From this day, a steady increase in the degradation of DCM was observed and an average removal of 23.8 ± 3.0 μM between inlet and outlet and an average degradation rate of 11.8 ± 1.47 $\mu M \cdot d^{-1}$ were achieved (Fig 7.10). These degradation rates are considerably lower than the ones observed for *Dehalobacterium* in the Chapter 6 from this thesis. This decrease of activity could be caused by inhibiting effects due to the presence of toluene and CF, or the absence of yeast extract as a supplement of nutrients in the medium. In this experiment, yeast extract, which is used in the enrichment cultures containing the *Dehalobacterium* consortia, which was previously employed in the experiments carried on Chapter 6, was

removed in order to avoid the competence of other carbon sources apart from acetate in the anodic oxidation of toluene.

Additionally, during this period, the reduction of CF increased by 11 % compared to the previous toluene concentration in the inlet, probably caused by a further increase in the *Dehalobacter* concentration, and it was again demonstrated that CF degradation activity was not affected by toluene. The maximum CF consumption rate obtained in this experiment (49 $\mu M \cdot d^{-1}$) was considerably lower than the one obtained in the previous chapter working with a two-vessel BES (132 $\mu M \cdot d^{-1}$) despite of producing an excess of H_2,. The reasons behind this decrease in CF degradation rate could be its lower concentration in the system leading to lower reaction kinetics, as the outlet rarely surpassed 30 μM of CF.

When, on day 93, the system started to operate at OCP, an immediate increase on the CF outlet concentration was observed, resulting in a 50 % decrease in the CF degradation rate. This decrease was correlated with a depletion of the measured hydrogen in the headspace of the reactor, indicating that the electrochemical production of hydrogen was a key factor for *Dehalobacter* activity. Nevertheless, a certain degree of CF reduction was still observed, possibly due to residual H_2 in the reactor. Another explanation could be related to the production of H_2 through the consumption of the sodium acetate present in the medium by bacteria from the consortium (Fig. 7.9) [31]. On the contrary, the fermentation of DCM remained unaffected by the operation at OCP, as *Dehalobacterium* does not need H_2 (Fig. 7.10). Interestingly, the degradation of acetate, which was almost complete when applying current to the system, also stopped in the OCP. This demonstrates that the degradation of acetate, similarly to the consumption of toluene, was

driven by the positive potential and the anodic consortium and possibly caused a competition between both substrates.

7.3.4 Coulombic efficiency in the continuous mode

The degradation of toluene provided a continuous production of electric current with values ranging from 1 to 5 mA approximately (Fig. 7.11). The mean intensity values recorded for each experimental phase increased by 72 % as the inlet concentration of toluene was increased from 260 to 400 μM (Fig. 7.12), concomitant with the increase of toluene degradation rate previously discussed (Fig. 7.7). Also, as observed with the degradation of toluene, a 10% decrease in the average intensity was recorded as the toluene inlet concentration was decreased to 160 μM.

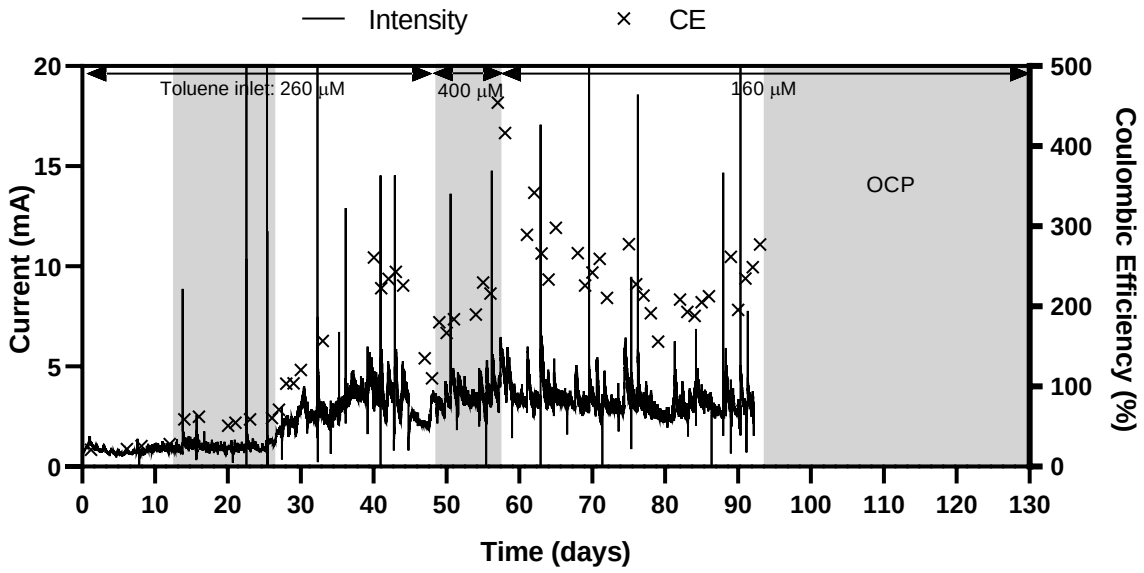

Figure 7.11. Intensity profile and coulombic efficiencies obtained during the continuous operation of the bioelectric well. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet.

The calculated CEs for each experimental phase followed a similar trend than the averaged intensities, increasing from 130 ± 18 to 255 ± 40 % during the first two toluene

concentrations and slightly decreasing to 237 ± 8 % when toluene was reduced to 160 μM. For these calculations, the simultaneous removal of acetate and toluene was included, considering a constant acetate removal of 48 ± 11 $mg \cdot L^{-1}$ during all the continuous operation (excluding the OCP operation) (Fig. 7.9). As observed in the fed-batch phase and even additionally considering the removal of acetate, the CE values vastly exceeded 100 %, indicating that some other source of electrons other than toluene and acetate was present in the reactor. A possible explanation could involve the high excess of hydrogen produced in the cathode, which is likely oxidized in the anode generating this excess of current. Another possible cause could be the presence of other unidentified compounds in the medium, which could be also oxidized by the anodic consortia.

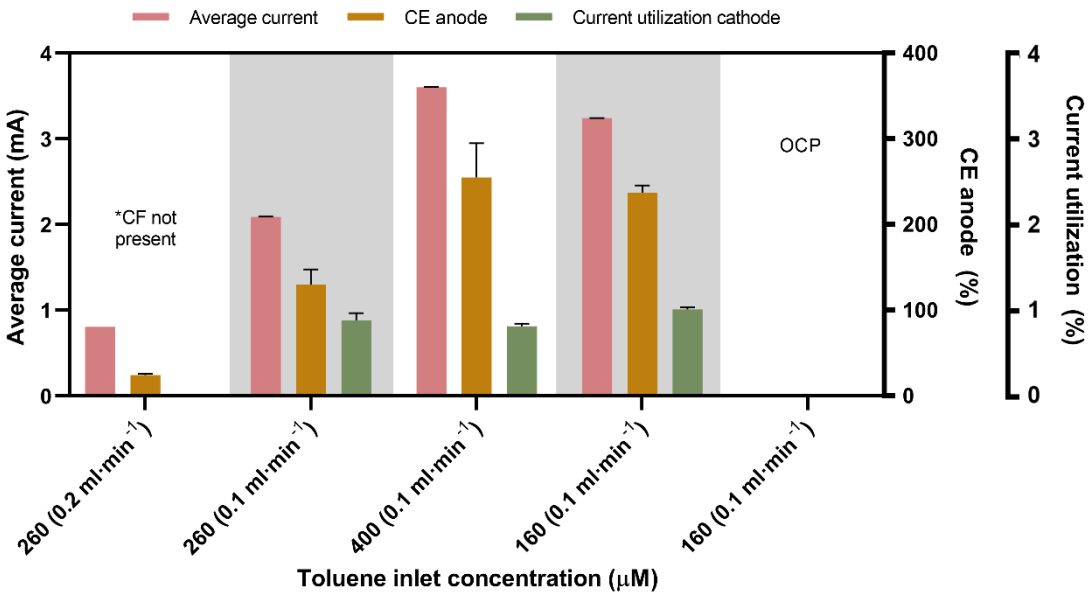

Figure 7.12. Average current, CE and cathodic current utilization at each stage of the continuous operation. Grey/white backgrounds represent the different operational periods corresponding to different flowrates, potentials and concentrations of toluene or CF in the inlet. Values plotted are the average of each operational period and error bars indicate standard deviation.

The hypothesis of hydrogen scavenging at the anode as an explanation for the high observed CEs is supported by the cathodic current utilization calculations, which take

into account the reduction of CF at each operational stage (Fig. 7.12). These measurements in all the toluene inlet concentrations amended did not reach values higher than 1.5 %, meaning that most of the current was not ultimately used in CF degradation. The observed H_2 accumulated in the headspace of the reactor or its scavenging in the anode could be reasons leading to these low utilization values.

The cathodic current utilization of this experiment is a parameter equivalent to the CEs calculated with *Dehalobacter* in Chapter 6, which were higher than 60 % even at the more negative poised potential in the cathode. This difference is caused by the anodic control employed in the bioelectric well and the high number of electrons released per each molecule of toluene or acetate degraded (36 and 8 respectively), which far outweigh the 2 electrons required to reduce each molecule of CF. Moreover, the abovementioned hydrogen scavenging, which did not occur in the previous experiments employing systems composed by two vessels, explains the lower percentages obtained in this experiment.

Further research is needed to assess the optimal parameters to couple the oxidation and reduction of pollutants in a single-vessel system during an in-situ operation. The heterogenicity present in groundwater regarding presence and concentration of toluene, CF or other unexpected co-contaminants and their variability during time can suppose a challenge in order to operate the bioelectric well and maintain the degrading activity of all the bacterial consortia involved, as was observed during this chapter when the concentration of toluene was increased to 400 μM and negatively affected the fermentation of DCM. Moreover, focusing in simultaneous reactions occurring in both, anode and cathode, increases the complexity of the operation and obstructs the system efficiency optimization as compared to the separated-vessel experiments carried on chapters 5 and 6, which presented a higher degree of utilization of the electrons

transferred to the cathode. Overall, these results are promising regarding the decontamination of complex mixtures of contaminants in polluted groundwaters.

7.4 Conclusions

The work performed in this chapter demonstrated the feasibility of coupling the bioelectrochemical degradation of CF with the combined activity of *Dehalobacter* and *Dehalobacterium* with the anodic oxidation of toluene while operating in continuous mode in a single-vessel system. The graphite rod anode poised at potential +0.4 V allowed to obtain the oxidation of toluene at rates up to 63 $\mu M \cdot d^{-1}$. The combined oxidation of toluene and acetate provided electric current exceeding 3 mA, which meant a continuous supply of H_2 in the stainless-steel cathode. The accumulation of H_2 observed in the headspace of the system sustained the reduction of CF to DCM by *Dehalobacter*. The following decrease in the inlet concentration of toluene allowed to avoid the inhibiting effects in *Dehalobacterium* and maximize the DCM-fermenting activity up to 13 $\mu M \cdot d^{-1}$. The operation in OCP demonstrated that the application of an external source of electric current was necessary for sustaining the oxidating and reducing reactions of toluene and CF, respectively. The anodic coulombic efficiencies reached values higher than 100 % due to the excess of hydrogen produced in the cathode, which negatively affected the current utilization related to the CF reduction. This study opens the possibility of treating complex mixtures of heterogeneous contaminants in polluted groundwaters, while further research is needed to optimize the operating conditions leading to increase the removal of the contaminants and maximize the energetic efficiencies of the process.

7.5 References

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Chapter 8

General conclusions

The main objective of this thesis was to study the application of (bio)-electrochemical systems for the remediation of groundwaters contaminated with halogenated pollutants. During its elaboration, the range of halogenated pollutants degraded and anaerobic dechlorinating bacteria grown in (bio)-electrochemical systems was expanded and several electrochemical reactor configurations and operational modes were employed in order to improve the degradation process.

The main conclusions achieved during this thesis are summarized below:

- The complete electrochemical debromination of dibromomethane (DBM) and 1,2-dibromoethane (DBA) to mainly methane and ethene, respectively, was fulfilled at three cathodic potentials (-0.8, -1.0 and -1.2 V) in a two-chamber cell with a graphite brush as cathodic electrode. The non-toxic byproducts generated, the inexpensive and environmentally friendly material used as electrode, and the relatively high coulombic efficiency (CE) achieved open the window to the application of electrochemical systems to treat groundwaters contaminated with brominated alkanes.
- The complete dechlorination of 1,2-dichloropropane (1,2-DCP) to propene mediated by a *Dehalogenimonas*-containing consortia was performed, for the first time, in the cathodic vessel of a two-chamber cell. The effects of two different carbon-based materials, graphite brush and carbon cloth, as cathodic electrodes were tested, obtaining better degradation rates when the former was employed. The operation with a pulsed cathodic potential allowed to maintain the high degradation rates obtained with a continuous potential while maximizing the CE values obtained during the process.
- The degradation of CF to acetate was performed in the cathodic vessel of a two-chamber system by combining the dechlorinating activity of *Dehalobacter* and

Dehalobacterium. The application of a periodically decreasing potential allowed to gradually increase the electrochemical production of hydrogen while the *Dehalobacter* population increased, hence maximizing the CE of the process while also obtaining a high degradation rate of CF.

- The described CF degradation was coupled to the oxidation of toluene in a single-chamber continuous-flow electrochemical reactor known as bioelectric well. A toluene-degrading biofilm was successfully attached to the anodic electrode from groundwater containing toluene-degrading bacteria and was able to consistently deliver electrons to the cathodic electrode, which were used to electrochemically produce H_2 and stimulate the reduction of CF to DCM by *Dehalobacter*. DCM was further fermented by a *Dehalobacterium*-containing culture to acetate, hence obtaining the simultaneous removal of both contaminants in a single system.

The work performed during this thesis has demonstrated the feasibility of bioelectrochemical systems (BESs) as reliable electron donors for the degradation of halogenated pollutants. The ability to catalyze oxidizing reactions in the anode and reducing reactions in the cathode, coupled with their strong synergy with the operation with organohalide-respiring bacteria makes BESs a promising technology to treat complex mixtures of pollutants, which is a frequent scenario in contaminated groundwaters. Despite the theoretical advantages of BESs, which have been discussed during this thesis and also in the literature and have been demonstrated in lab experiments through several reactor configurations, their application to treat real groundwater from polluted sites has been scarcely reported. Consequently, further research is needed to successfully stablish the use of BESs in pilot-plant operations, facing the intrinsic heterogeneity of groundwaters on bioremediation processes.

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List of publications

A. Trueba-Santiso, **D. Fernández-Verdejo**, I. Marco-Rius, J.M. Soder-Walz, O. Casabella, T. Vicent, E. Marco-Urrea, Interspecies interaction and effect of co-contaminants in an anaerobic

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D. Fernández-Verdejo, P. Cortés, P. Blánquez, E. Marco-Urrea, A. Guisasola, Enhanced dechlorination of 1,2-dichloropropane to propene in a bioelectrochemical system mediated by *Dehalogenimonas*, J. Hazard. Mater. 416 (2021). doi:10.1016/j.jhazmat.2021.126234.

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Participation on scientific conferences

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