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Coupling electrokinetic soil flushing with bioremediation for the removal of chlorinated benzenes and hexachlorocyclohexane

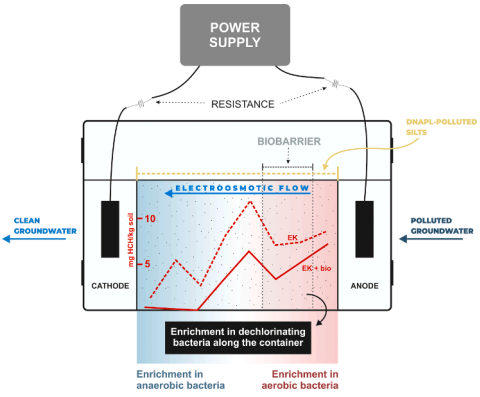
Dani Salom [a], David Fernández-Verdejo [a], Julia Isidro [b,c], Teresa Vicent [a], Paqui Blánquez [a,*]

[a] Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona 08193, Spain
[b] Chemical Engineering Department, Faculty of Chemical Sciences and Technology, University of Castilla – La Mancha, Ciudad Real 13071, Spain
[c] Centro Nacional del Hidrógeno, Prolongación Fernando El Santo s/n, Puertollano, Ciudad Real 13500, Spain

HIGHLIGHTS

- Direct electric current does not hinder but promotes dichlorination activity.
- Aerobic conditions allowed a higher dichlorination percentage.
- The biobarrier is needed to further remove pollutants from the silts.
- Direct electric current determines the distribution of the dechlorinating bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

The improper deposit of the lindane production wastes in the Sardas landfill (Sabiñánigo, Huesca) during the last century led to the pollution of this site, mainly with hexachlorocyclohexane (HCH) isomers and chlorobenzenes. The pollution affected different soil layers, including the silts and groundwater. Previous works have used electrokinetic (EK) remediation techniques to address this problem in fine-grained soil layers. However, EK often needs to be coupled with other remediation techniques. In this work, the impact of establishing a biobarrier during the application of the electroremediation (electro-bioremediation system, EBS) is studied to compare with the case in which no biological treatment is conducted. Experiments were conducted at bench scale using 1.5 dm^3-containers filled with polluted silts and groundwater from Sardas, and biobarriers with bacterial cultures enriched from the polluted groundwater of Sardas. Applying 1 V/cm did not prevent the biological activity, and the EBS improved by 54 % and 36 % the remediation of HCHs and chlorinated benzenes, respectively, in the silts under aerobic conditions, in contrast to the case in which only electroremediation was performed. Microbial relative population analysed by sequencing 16S genes from silts pointed out that the direct electric current

* Corresponding author.
E-mail address: paqui.blanquez@uab.cat (P. Blánquez).

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affected the microorganisms' distribution along the containers due to the oxidative or reductive conditions promoted by the anode and the cathode, respectively.

1. Introduction

1,2,3,4,5,6-hexachlorocyclohexane (HCH) is an organochlorine pesticide widely used in the twentieth century for pest control. It is produced from the photochlorination of benzene using UV radiation [25], yielding a mixture of α- (55 – 80 %), β- (5 – 14 %), γ- (8 – 15 %), δ- (2 – 16 %) and ε- (3 – 5 %) HCH as the main isomers. This mixture is known as technical HCH, but only the γ-isomer has insecticidal properties. For this reason, companies purified the technical HCH to obtain almost pure (> 99 %) γ-HCH [29]. This purified γ-HCH is called lindane, and its production is very inefficient, as 8–12 tonnes of hazardous wastes are produced per tonne of purified lindane [29]. It is estimated that between 4 and 7 million tonnes of this hazardous waste have been dumped worldwide, being often improperly deposited in open piles [29]. HCHs have a great migration potential as they are sufficiently soluble and volatile for, when dumped into the field, leading to the pollution of nearby areas spreading through the air or leaching into the soil and the groundwater. HCHs are bioaccumulative in living tissues, and toxic effects have been reported (*e.g.*, affections to the nervous, reproductive, and immunologic systems, convulsions, endocrine disrupting, carcinogenic, and even death) [22,32]. Consequently, lindane and technical HCH have been banned in the European Union since 2008. In 2009, α-, β-, and γ-HCH were included in the Stockholm Convention persistent organic pollutants (POPs) list [29], and α-, β-, γ-, δ- and technical-HCH were listed in the ATSDR's Substance Priority List. However, due to their persistence, HCHs are still found in the environment and their remediation works are still ongoing [30].

One of these HCH-polluted sites is found in Sabiñánigo (Aragón, Spain), where INQUINOSA company produced lindane from 1975 to 1988 and improperly deposited around 160000 tonnes of waste in the nearby landfills (Sardas and Bailín), making it the largest HCH deposit worldwide [10]. The landfills were not prepared to confine the wastes, resulting in the migration of these substances through the subsurface. A dense non-aqueous phase liquid (DNAPL), mainly composed of HCH isomers, benzene, chlorinated benzenes, and non-aromatic chlorinated cyclic compounds [14,25], was detected downstream of the Sardas landfill, with a plume of contamination polluting the soil and the groundwater, and threatening the Gállego River. Therefore, it is crucial to remove the DNAPL.

The silts are one of the affected subsurface layers by the contamination in Sardas. Several remediation approaches can be applied in this area, including soil vapor extraction, that can be coupled with thermal or electrical resistance heating [13,34], pump-and-treat systems [33] or chemical treatments, such as *in-situ* chemical oxidation (ISCO) [26]. These technologies are frequently enhanced by the injection of surfactants to facilitate the solubilization of DNAPLs [27,31]. However, the low hydraulic conductivity of the silt layer poses significant challenges for the effective implementation of these technologies [14]. A proper strategy to remove the contamination from the silts is the electrokinetic (EK) remediation technique [11,21]. It consists of the application of a direct electric current (DEC) between an anode and a cathode in the polluted zone, promoting transport processes (electroosmosis, electrophoresis, and electromigration). The applied current promotes the transport of charged substances, whereas non-charged organic molecules (*e.g.*, lindane, chlorobenzenes) are transported by electroosmosis, as they dissolve in the pore water and flow within it from the anode to the cathode [3].

However, the remediation efficiency of the EK technique can be improved, so it often needs to be coupled with further remediation treatments to degrade the pollutants that the electric current moves [20]. Bioremediation, as a sustainable, low-cost technology that also allows *in situ* treatment, possesses a good synergy with the application of EK treatments. HCHs and chlorobenzenes have been reported to be biodegraded in both aerobic and anaerobic conditions [12,23]. However, in low porosity soils (*i.e.*, silts) and when the pollutants are adsorbed to the soil particles, the bioavailability of the pollutants and nutrients for the microorganisms is limited. The application of a DEC improves the bioavailability of the pollutants through the EK transport processes promoted [20,21], in which the movement of microorganisms can be also promoted by electrophoresis or electroosmosis. Nevertheless, a high DEC may come with harmful effects for the bacterial cultures. One such effect is electrical heating of the soil, which can occur due to its inherent resistance to the applied voltage, resulting in high soil temperatures that are detrimental to microbial activity. Additionally, the DEC induces electrolysis reactions in the liquid medium within the electrolytic wells. At the anode, water is oxidized to O_2 and H^+, leading to acidification, whereas at the cathode, water is reduced to H_2 and OH^-, resulting in alkalinization. This phenomenon creates an acidic and a basic front on their respective sides of the container, which progressively propagate because of the electromigration of H^+ and OH^- ions. These pH gradients can potentially cause a threat to microbial viability. Also, other side reactions can occur at the electrodes: in the cathode, the presence of oxygen or nitrate can yield hydrogen peroxide and nitrite, respectively; in the anode, elevated chloride concentrations can produce free chlorine. These toxic compounds, together with the production of other radical species, contribute to oxidative stress in microbial cells and may cause irreversible permeabilization of their external membranes (electroporation), leading to cell death [20,21]. However, it has been reported that low DECs can biostimulate the microorganisms, resulting in higher growth and an increase in ATP production [3]. Therefore, the combination of both EK and bioremediation strategies (electro--bioremediation) at low DEC (1 V/cm) [20] could result in a good remediation strategy. Some attempts to combine EK and bioremediation have been previously tested, such as the use of a biobarrier where the pollutants move through [20].

Thus, this work aims to assess the remediation of the DNAPL-polluted silts and groundwater of Sardas in an electro-bioremediation system (EBS) at a bench scale, in which a DNAPL-acclimated bacterial culture has been inoculated in a vertical layer of the silts, forming a biobarrier between two electrodes acting as anode and cathode. The biobarrier is a simple approach to inject the enriched culture in the silts that allows low disturbance and highly localized implementation in the site.

In this EBS, pollutants from the real silts and groundwater are forced to flow through the biobarrier by electroosmosis, where they are biodegraded. The possible extreme conditions promoted by the DEC (*i.e.*, temperature, moisture, pH) have been studied and the redox conditions (*i.e.*, aerobic and anaerobic) have been tested to optimize biological activity. Biodegradation results of the EBS have been compared with the scenario in which only the EK remediation is applied to study whether there is an improvement concerning the EBS. In addition to the pollutants removal, the microorganisms' relative population has been addressed to study the effect of the DEC on their distribution in the presence of chlorinated benzenes and HCH isomers and assess the contribution of the bioremediation in an EBS. The findings of this work can be potentially extrapolated to other studies involving the degradation of complex mixtures of chlorinated pollutants.

2. Methodology

2.1. Materials

2.1.1. Containers

The containers used for the bench-scale experiments were made of transparent methacrylate (26.0 × 3.5 × 16.0 cm) and divided into three compartments, separated by a 0.5 mm nylon mesh. The central compartment (17 cm width) contained the silts up to a height of 11 cm. The silts were compacted by applying physical pressure to avoid preferential pathways of the flushing fluid. The two side compartments (4.5 cm width each) corresponded to the electrolytic wells (anode and cathode), filled with the flushing fluid (up to 11 cm of height) and the graphite rod electrodes, each one with 37.7 cm^2 of surface area. Both electrodes were connected to a power source (HQ POWER) which allowed the application of the selected potential into the system. The containers were completely sealed and included four boreholes (two in the anodic compartment and two in the cathodic compartment) sealed with Teflon-coated butyl rubber to allow electrolyte sampling and addition.

2.1.2. Soil and groundwater

The non-polluted, silty soil was collected from the Sardas site, located downstream of the landfill. Depending on the experiment, additional amendments of DNAPL extracted from the site were added to the silts. Artificial contamination was applied to avoid analytical limitations associated with the use of the real polluted soil, which contained very low pollutant concentrations and would have required larger sample volumes for reliable analysis. To pollute the silts, the DNAPL was previously diluted in acetone (10^{-3} mL DNAPL/mL acetone). This solution was homogenized with the silts, which were immediately added to the containers. The DNAPL-polluted groundwater was collected from a piezometer located in the contaminated plume (PS16E) (Fig. S1). Table S1 shows the pollutant composition of the groundwater.

2.1.3. Bacterial cultures

2.1.3.1. Physicochemical parameters and biological activity. For the first experiment (Section 3.1), an anaerobic *Dehalogenimonas*-containing culture was used. It is an anaerobic culture derived from sediments of the Besòs river estuary (Barcelona, Spain). The culture was maintained for more than seven years under anaerobic conditions in microcosms consisting of serum bottles sealed with Teflon-coated butyl rubber aluminium crimp caps, containing 70 mL of a defined culture medium described elsewhere [17]. Briefly, it consisted of vitamins, acetate (5 mM), trace elements (NH_2PO_4, NH_4Cl, $CaCl_2$, $MgCl_2{\cdot}6\ H_2O$, KCl, $CaCl_2{\cdot}2\ H_2O$, nitriloacetic acid (NTA), $FeCl_2{\cdot}4\ H_2O$, $ZnCl_2$, $MnCl_2{\cdot}2\ H_2O$, $CoCl_2{\cdot}6\ H_2O$, H_3BO_3, $NiCl_2{\cdot}6\ H_2O$, and $NaMoO_4{\cdot}H_2O$), $Na_2S{\cdot}9\ H_2O$ and L-cysteine (0.2 mM each) as reducing agents and bicarbonate solution (0.01 M) as pH buffer. Cultures were gassed with N_2/CO_2 (4:1, v/v, 0.2 bar) and H_2 (0.4 bar).

2.1.3.2. Aerobic and anaerobic EBS using non-polluted silts and polluted groundwater. For the second experiment (Section 3.2), two bacterial cultures previously enriched from the PS16E piezometer from Sardas were used to inoculate a biobarrier. One bacterial culture was enriched in aerobic conditions by filling serum bottles with 100 mL of the groundwater and adding NaH_2PO_4 20 mg/L and 10 – 100 µM 1,4-dichlorobenzene (1,4-DCB) as model pollutant purchased from Sigma-Aldrich. 1,4-DCB came from a stock solution diluted in acetone. O_2 overpressure was added (0.5 bar). For the anaerobic culture, microcosms were set up by filling with 100 mL of the groundwater into an anaerobic glovebox and adding NaH_2PO_4 20 mg/L, sodium lactate (3 mM), and 1.5–5 µM of lindane purchased from Sigma-Aldrich. HCH came from a stock solution diluted in acetone. N_2 overpressure (0.4 bar) was added. Microcosms were sealed using Teflon-coated butyl rubber aluminium crimp caps. When the bacterial cultures consumed the pollutant (1,4-DCB or HCH), it was reamended.

2.1.3.3. Aerobic treatment of polluted silts and groundwater in EBS. For the third experiment (Section 3.3), an aerobic, 1,4-DCB-degrading bacterial culture was used. It came from groundwater samples from piezometer PS5E, which were cultured in an agar plate with a selective medium, spiked with DNAPL extracted from Sardas. Three dilutions were performed (1 mL/10 mL, 1 mL/100 mL, 1 mL/1000 mL). Separated colonies were obtained in the second dilution, and each of the different colonies was used separately to set microcosms in 70 mL of aerobic culture media consisting of KNO_3, NH_4Cl, KH_2PO_4, $Na_2HPO_4{\cdot}2\ H_2O$, $MgCl_2{\cdot}6\ H_2O$, $ZnCl_2$, $CuSO_4{\cdot}5\ H_2O$, $FeSO_4{\cdot}7\ H_2O$, $CaCl_2{\cdot}2\ H_2O$, $MnSO_4{\cdot}H_2O$, $NaMoO_4{\cdot}H_2O$, H_3BO_3, NTA. Overpressure of O_2 (0.5 bar) and 1,4-DCB 15 µM from a stock solution diluted in acetone were added to the microcosms. A microcosm inoculated with a white, transparent, wrinkled, and flat colony fully depleted the 1,4-DCB after several reamendments and was used as inoculum for the biobarrier.

2.2. Experimental set-up

Batch experiments lasted 14 days except for experiment 1 (Section 3.1), which lasted 10 days. This duration was selected as it was sufficient to observe changes in pollutant concentrations and still short enough to prevent complete pollutants removal, thereby allowing comparative assessment of the different treatments, as previously determined [11]. Each treatment was performed once.

All the experiments were performed at room temperature and an electric field of 17 V was applied (1 V/cm).

The cathodic compartment was always filled with a salinity solution formulated to simulate a synthetic tap water (deionized water Millipore Milli-Q System containing 30 mg/L KNO_3, 70 mg/L $NaHCO_3$, 90 mg/L Na_2BO_4), while the flushing fluid in the anodic compartment was synthetic tap water or polluted groundwater, depending on the experiment. The synthetic tap water was aerobic or anaerobic when indicated. To turn it into anaerobic conditions, 500 mL were vacuumed for 45 min and bubbled with N_2 for 20 min.

The biobarrier (160 cm^3) was composed by mixing the silts (polluted or not polluted, depending on the experiment) and the culture media at a 70/30 ratio (v/v) respectively.

If the experiment operated in anaerobic conditions, the biobarrier and the containers were set up in an anaerobic glovebox filled with an N_2 and H_2 atmosphere.

Microcosms inoculated with 1 – 2 g of the silts (experiment 1, Section 3.1) were also set up inside an anaerobic glovebox. The culture medium used (70 mL) was the one described in Section 2.1.3 for a *Dehalogenimonas*-containing culture [17]. It was spiked with 1,2-dichloropropane (1,2-DCP) at a final concentration of 1000 µM and sealed with Teflon-coated butyl rubber aluminium crimp caps.

2.3. Analysis

During the experiments, temperature (only for the first experiment) in the central section of the container and pH, conductivity, and pollutants concentration in both electrolyte wells were measured. In experiment 3 (Section 3.3), microbial relative populations were also analysed in the silts. The water volume collected in the cathodic well and the loss of water volume in the anodic well were measured daily to estimate the electroosmotic flow. The intensity was calculated through the measure of the potential at both sides of a 70 Ω resistor located in the anodic connection.

2.3.1. Physicochemical parameters

The central compartment of the container (silts) was divided into 16

sections (two horizontal and eight vertical divisions) (Fig. S2) for analysis purposes. In each section, post-mortem analyses of moisture, pH, and pollutants concentration were conducted. pH and moisture measurements are described by Ramírez, et al. [20]. Silts moisture was calculated by the difference between the wet and dried (at 105 ºC for 24 h) weight. pH of the silts was measured by vortexing 10 g of silts in 25 mL of Mili-Q water. When the soil was sedimented, pH was measured with a pH probe 50 + DHS®. Initial analysis of moisture and pH were carried out too from a sample of the filling silts. The pH at the point of zero charge of silts (pH_{zpc}) was determined using the salt addition method described by Bakatula et al. [4]. In brief, 40 mL of 0.1 M $NaNO_3$ solutions were prepared and adjusted to a range of initial pH values (2 – 11) by triplicate. Subsequently, 0.2 g of silts were added to each solution, and suspensions were shaken for 24 h at 200 rpm. When soil particles settled, final pH values were measured, and ΔpH (final pH – initial pH) was plotted against the final pH to determine the pH_{zpc} as the pH at which ΔpH is zero.

2.3.2. Pollutant concentrations

For the solid samples, an extraction with methanol was performed to analyse the pollutants. Ten g of solid sample were suspended in 50 mL of methanol and sonicated at 40 kHz for 24 h in an Ultrasonic Cleaner CD-4800. Later, the sample was dried with a nitrogen stream and the extract was recovered with a mixture of hexane/ethyl acetate (70:30). For the liquid samples, HCHs were extracted with hexane, and for the analysis of chlorobenzenes and benzene, 1 – 10 mL of the sample was transferred to a vial and sealed immediately with a PTFE stopper, and the headspace was analysed. All the samples were analysed by GC-MS (Agilent 6890 N) equipped with an HP-5MS column (30 m × 250 μm × 0.25 μm). The injection was automatic, in splitless mode and the front inlet was set at 250 ºC. The oven started at 60 ºC and increased to reach 350 ºC in 35 min. The injection volume was 2 μL. Helium was used as the carrier gas at 0.5 mL/min flow rate.

1,2-DCP and propene were analysed in a gas chromatograph (GC) (6890 N, Agilent Technologies) taking 0.5 mL of the headspace from the microcosms. The GC was equipped with a DB-624 column (30 m × 0.32 × 0.25 μm) and coupled to a flame ionization detector (FID). The method for the analysis was described by [17].

Internal standards were used for all chromatographic analysis, and a verification control using calibration standards was performed daily. Instrumental calibrations were also carried out daily, and chromatography calibrations were done, when necessary, based on the verification checks. Additionally, a maintenance schedule was followed for all the analytical equipment, covering internal and external servicing, and the laboratory is involved in interlaboratory comparison exercises.

2.3.3. Microbial population analysis

To analyse the microbial relative populations in each section of the silts, the DNA was extracted from 2 g of each section using the DNeasy PowerSoil Pro Kit (QIAGEN) following the manufacturer's instructions. DNA samples were analysed at Serveis de Genòmica i Bioinformàtica from the Universitat Autònoma de Barcelona by Illumina MiSeq, as described in Soder-Walz et al. [28].

2.4. Calculations

The amount of pollutants remaining in the system was calculated as the sum of the pollutants contained in all silt sections, considering the mass of silts and each pollutant concentration. The pollutants mass remaining in the electrolyte wells was also considered, accounting for both water volume (96 mL in each well) and the volume dragged by electroosmotic flow.

The cost associated with the energy consumption was estimated by multiplying the average intensity measured, the average electric potential, and the operation time. An electricity prize of 0.2 €/kWh was considered, based on Eurostat data from the second semester of 2024 for non-household consumers in the European Union [9].

3. Results and discussion

3.1. Physicochemical parameters and biological activity

A first experiment was set up to determine i) how the hypothetical extreme conditions (*e.g.*, temperature, pH, moisture) promoted by the DEC applied to the silts could affect the biological activity and ii) the migration of cells through the silts due to transport processes promoted by the DEC [20,21]. The experiment was performed under anaerobic conditions, inoculating an anaerobic *Dehalogenimonas* culture in the biobarrier, which was in the middle of the container (Sections 4, 5, 12, and 13). The electrolytic wells were filled with an anaerobic salinity solution. Fig. 1A shows the temperature in the biobarrier during the 10 days that lasted the experiment, which ranged from 22 to 26 °C (room temperature), being suitable values for the biological activity of *Dehalogenimonas*. Moisture was also analysed at the initial and final times (Fig. 1B). Silts were initially dry when introduced into the container and then were flooded with the flushing salinity solution from the electrolytic wells during the ten days of the experiment, producing a moisture increase from ~ 8 % at the initial time to ~ 25 % at the final time. As the biobarrier was composed of 30 % liquid culture, it started with a high percentage of moisture (~ 33 %). At the final time, it slightly decreased to ~ 27 %, presenting a homogenization in the water content with the other sections of the silts. The areas closest to the anode (Section 1 and 9) and the cathode (sections 8 and 16) showed slightly higher moisture levels due to their proximity to the anodic and cathodic wells.

pH values in the electrolyte wells were periodically monitored during the operation of the system (Fig. 1C). In both the anodic and cathodic wells, pH reached extreme values since the first day of operation (pH of ~ 2 in the anode and ~ 11.5 in the cathode). This is due to the electrolysis reactions occurring in the liquid medium when the DEC is applied, resulting in the generation of H^+ and OH^- ions due to water oxidation and reduction at the anode and cathode, respectively. Specifically, OH^- ions produced at the cathode migrate through the silts toward the anodic region, driven by their negative charge, leading to an increase in alkalinity near the cathode. This phenomenon is referred to as the advancement of the basic front. Conversely, H^+ ions generated at the anode migrate toward the cathodic region, forming the so-called acid front, which is gradually neutralized upon encountering the basic front [21]. Fig. 1D shows pH values at the initial and final time in the silts. As expected, the transport of OH^- ions through the container increased the basicity of the silts near the cathode. However, the silts close to the anode kept the neutral pH observed in the initial conditions, indicating that they possess enough acid buffering capacity, which is optimal for biological activity. Santos et al. [26] reported a high carbonate content in Sarda's soil, which could be responsible for this strong buffering capacity. Soil pH is one of the parameters affecting the velocity of the electroosmotic flow (v_{EO}), which is directly related to the zeta potential of the soil (ζ). ζ is the electric potential between the soil particles and the pore fluid. When the more negative is the ζ, the higher values adopts v_{EO} (flowing from anode to cathode) in a phenomenon dependent, among other parameters, on pH. When the more basic are the conditions, the higher amount of negative charges accumulate in the soil, resulting in a more negative ζ value and a higher v_{EO} (from anode to cathode) [21,35]. Thus, the increase in the soil pH along the length of the silts improved the electroosmotic flow, especially near the cathode. Electroosmotic flow increased during the initial temporary phase of the setup (Fig. S3), likely due to the rise in the soil pH. The maximum electroosmotic flow was 0.7 cm/d during this initial period. Subsequently, it decreased following the same trend as the intensity values (Fig. S3), probably due to depletion of the ions contained in the silts, as it was observed in previous studies [11]. Despite the basic pH conditions, the electroosmotic flow remained relatively low, although it should be enough to drag the pollutants over the 14-day experimental period [11].

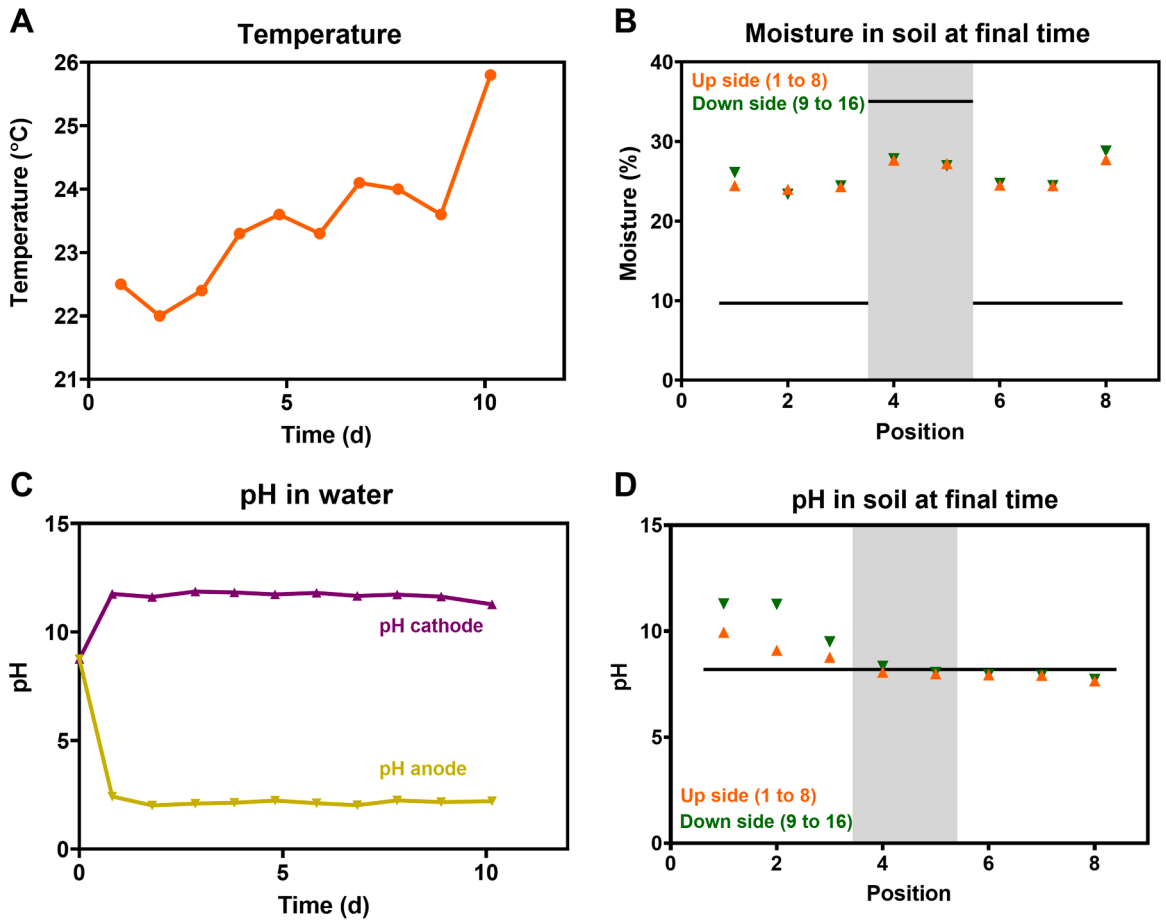

Fig. 1. Physicochemical parameters of experiment 1. (A) Time course of the temperature in the biological barrier. (B) Moisture profile in solid samples analysed post-mortem. (C) Time-course of the pH in both anodic and cathodic wells. (D) pH profiles in solid samples analysed post-mortem. Grey areas indicate the location of the biological barrier. Solid horizontal lines indicate the initial values of the silts and the biobarrier at the start of the experiment.

This low value is caused by the high pH_{zpc} of the silts, which is 9.2 ± 0.3, which is a typical value for soils with a high carbonate content [5].

Once the experiment 1 finished, the survivability of the *Dehalogenimonas* consortium under DEC treatment and its potential migration through the silts –driven by transport processes induced by the DEC– were investigated. This culture was selected for this experiment because it had previously been characterized and reported to use 1,2-DCP as growth substrate, producing propene in stoichiometric concentrations as the final dichlorination product [17]. Thus, this dichlorination activity was used as a proxy to monitor the *Dehalogenimonas* response to DEC. One to two grams from each of the 8 vertical layers of the silts (each one comprising 2 sections) were used as inoculum for microcosms containing anaerobic medium (70 mL) spiked with 1,2-DCP at 1000 μM. All microcosms consumed 1,2-DCP (Fig. 2A) and produced propene (Fig. 2B), except for Section 3, 11. Part of the decrease in 1,2-DCP can be attributed to absorption to the soil matrix; thus, propene production provides a more confident insight of the dichlorination activity. Metabolization of 1,2-DCP observed along the silts confirmed that cells migrated from the biobarrier, likely due to the electroosmotic flow.

Areas close to the cathode showed a lower dichlorination activity, likely due to the basic front. Consequently, to achieve more suitable conditions for the grow of the bacteria employed in the electro-bioremediation process, in the following experiments, the biobarrier was located closer to the anode, more precisely in Section 6, 7, 14, and 15, as it was determined that this area keeps the original pH due to the natural buffering capacity of the silts. Later, the electroosmotic flow will provide of these grown cells from the biobarrier to the other parts of the silts.

3.2. *Aerobic and anaerobic EBS*

Two batch experiments were performed (experiment 2), one under aerobic conditions and the other under anaerobic conditions, to assess which of these two redox states optimizes the removal of the pollutants present in the DNAPL. The biobarriers were inoculated with aerobically- and anaerobically-grown cultures, respectively, which were obtained from the polluted groundwater of Sardas (PS16E) and were grown as described in Section 2. The flushing solution used in these experiments was the polluted groundwater sampled from Sardas, aiming to simulate the real conditions and to assess the treatability of this groundwater by the biobarriers as well. Due to the electroosmotic flow, the flushing fluid flowed from the anode towards the cathode through the silts and the biobarrier. The cathodic well was filled with an aerobic or anaerobic salinity solution, depending on the experiment.

Fig. S4 shows the electrokinetic parameters resulting from this experiment, showing similar trends in electroosmotic flow and intensity than experiment 1. Fig. 3 shows the initial and final pH values in the silts. As observed in the first experiment, the pH rose to alkaline values near the cathode, while it remained closer to the initial values near the anode in both experiments.

Fig. 4 shows the degradation percentages of the groundwater pollutants by comparing the inputs (sections 8 and 16) and the outputs (Section 5 and 13) of the biobarrier. A high removal percentage was observed in the biobarrier segments, indicating good biological activity performance in both aerobic and anaerobic experiments.

The aerobic experiment (Fig. 4A) showed a degradation percentage higher than 80 % for most of the pollutants except for 1,4-DCB and

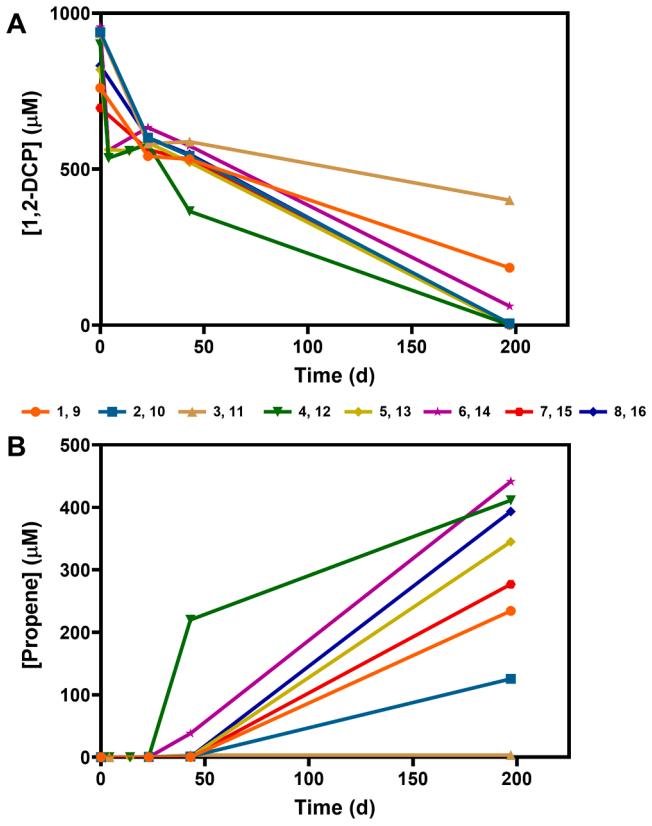

Fig. 2. Time-course of 1,2-DCP (A) and propene (B) concentrations in vials containing 1 – 2 g from each of the 8 vertical sections of the silts.

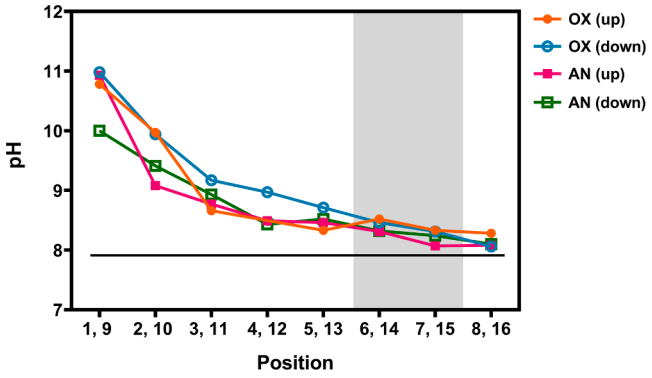

Fig. 3. Initial and final pH values in each section of the aerobic and anaerobic EBS. The black, horizontal line points at the initial pH value of the silts. The grey zone indicates the location of the biobarrier. OX: aerobic experiment; AN: anaerobic experiment. Up: Section 1–8; down: sections 9–16.

tetrachlorobenzenes. The 1,4-DCB was further accumulated because it was used as the carbon source during the enrichment of the culture.

In a general trend, degradation percentages of pollutants were similar or slightly lower under anaerobic conditions than under aerobic conditions, except for the MCB, that was accumulated under anaerobic conditions, and the TeCBs, which showed a higher degradation rate in absence of oxygen (Fig. 4).

Under anaerobic conditions, the monochlorobenzene (MCB) concentration was shown to be higher at the output of the biobarrier compared to the inlet (Figs. 4B and 5), and it is because, in anaerobic conditions, higher chlorinated pollutants were being reductively dechlorinated, yielding lower chlorinated benzenes (*i.e.*, MCB) [1,12]. Anaerobic degradation pathways of MCB to CO_2 and methane, with benzene as an intermediate, have been described in methanogenic conditions at low reaction rates [1,19]. Therefore, MCB was being accumulated at the end of the biobarrier in the anaerobic EBS. However, this pollutant did not accumulate along the EBS, as it was removed in sections near the cathode mainly due to the drag caused by the electroosmotic flow (Fig. 5), which is the main transport mechanism of the chlorinated benzenes [21,35,3]. As the electroosmotic flow increases towards the cathode when the conditions are more alkaline, the drag of the MCB is higher close to that electrode. This phenomenon explains the MCB profile shown in Fig. 5 from Sections 1–9–5–13, observing the lowest MCB concentrations in the cathodic Section 1–9.

The other pollutants were mainly removed once passed through the biobarrier (Fig. 4); however, trichlorobenzenes showed punctual rises in their concentrations near the cathode, in both aerobic and anaerobic conditions (Fig. S1). It is probably due to the alkaline dehydrochlorination of the lindane, which has been reported to yield trichlorobenzenes [18,25].

A good performance was observed for the aerobic EBS; besides, it avoids the operational complexities involved in an operation strictly in anaerobic conditions. Therefore, the following experiments were performed under aerobic conditions.

3.3. Aerobic treatment of polluted silts and groundwater in EBS

The next step was to apply the electrobioremediation technique to simultaneously treat DNAPL-polluted silts and groundwater (experiment 3). An aerobic biobarrier was located, as in the previous experiment, in Section 6, 7, 14, and 15, and a control treatment in which a DEC is applied without an inoculated biobarrier was also established in order to assess its effects in the observed depletion of the pollutants.

After 14 days of operation, pH was measured in the soil samples of both, experimental and control experiments, observing neutral values except for the closest areas to the cathode (Sections 1–9, 2–10 and 3–11), where pH was alkaline (Fig. 6). Electrokinetic parameters of this experiment are shown in Fig. S6 (pH in electrolytic wells, electroosmotic flow, and current intensity). As observed in previous experiments conducted in this work, and also previous studies [11], these silts showed the maximum electroosmotic flow during the initial time (0.5 cm/d) and later decreased, in line with the current intensity. From day 5 onwards, the average electroosmotic flow in the EBS treatment was 0.13 $\pm$ 0.02 cm/d, whereas it was 0.24 $\pm$ 0.02 cm/d in the control treatment.

3.3.1. Pollutants removal

Volatilization of pollutants in the closed containers and variations in the concentrations of the feeding groundwater hindered the closure of the mass balance. This was previously assessed by Fernández-Cascán et al. [11], together with the effects of the DEC on the pollutants in silts from Sardas. Building on that work, the present study focuses on evaluating the additional contribution of the biobarrier in the EBS, in comparison to a conventional abiotic electroremediation. Fig. 7 shows the pollutants concentrations in silts at the different positions of the EBS and the control treatment at the end of the experiment.

The total amount of HCHs (silts and electrolytic wells) detected in the control treatment after 14 days was 7.1 mg HCHs, while it accounted for 3.3 mg HCHs in the EBS. Hence, the effect of the biobarrier improved the removal of HCHs in a 54 %. HCHs from the control treatment (Fig. 7B) were accumulated at higher concentrations in the central sections and the ones near the anode compared to the EBS (Fig. 7A).

In the area close to the cathode, HCHs concentrations in both, EBS and control treatments, were completely removed. It is due to the reductive conditions of the basic front, which promote HCHs dechlorination [18,25]. The HCHs abiotic removal was especially notable in Sections 1, 2 and 3 upside and 9, 10 and 11 downside, aligning with the pH (Fig. 6), as pH was 10–11 in Section 1 and 9 and decreased to the initial pH (7.7) in Section 3 and 11. On the other hand, the other sections of the container showed neutral pH values, so the basic front had no

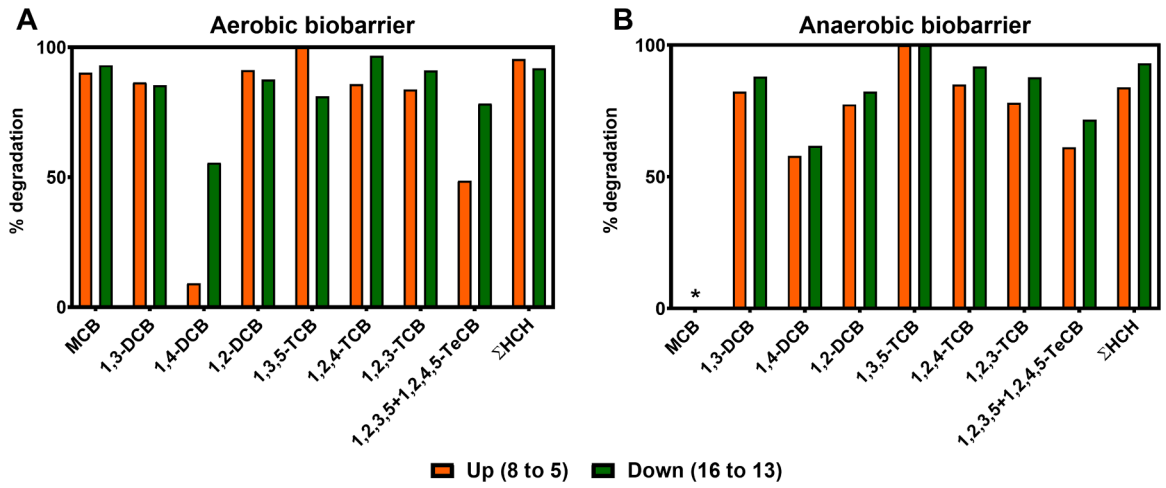

Fig. 4. Percentage removal of the pollutants performed by the aerobic (A) and anaerobic (B) biobarrier by comparing the inputs (sections 8 and 16) and the outputs (Section 5 and 13). * indicates negative degradation percentage for the MCB in anaerobic conditions (-114 % up; −62 % down).

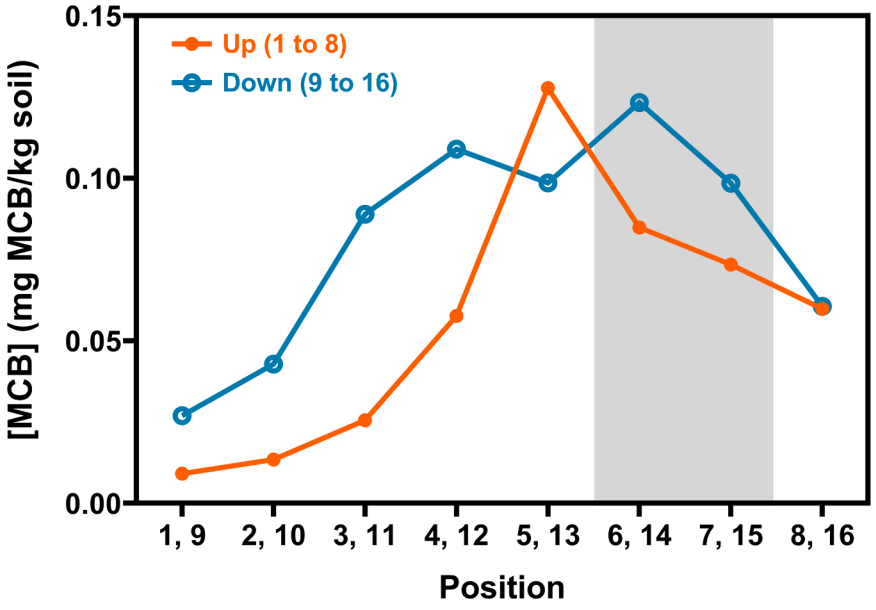

Fig. 5. Monochlorobenzene concentration in the silts of the anaerobic experiment along the container. The grey area indicates the location of the biobarrier. Up: Section 1–8; down: sections 9–16.

influence and therefore alkaline hydrodechlorination reactions were not observed. Concomitantly to HCHs abiotic degradation, 1,3-DCB, 1,2,4-TCB, and 1,2,3-TCB were accumulated in Sections 1, 2 and 3 upside and 9, 10 and downside as the main products (Fig. 7 I, J, K, L, O, P) (Santos et al., 2018; [11]).

The amount of chlorinated benzenes remaining in the control treatment after 14 days was 11.23 mg, whereas it was 7.23 mg in the EBS. Thus, the biobarrier contributed to a global 36 % reduction in these pollutants. However, considering that chlorinated benzenes are byproducts of the dichlorination of HCH isomers, the extent of their removal in the EBS can be even higher. In the control treatment, MCB was accumulated as a degradation product in most of the sections of the container (Fig. 7D), while the experimental one could minimize its accumulation due to the biological activity of the biobarrier in a 76 % (Fig. 7C). However, as it was seen in the previous experiment (Section 3.2), independently of the treatment, MCB was removed in the nearest sections to the cathode (1 and 9). It has been demonstrated that chlorobenzenes are not removed in alkaline conditions, as proved for HCHs [25]. Hence, the decrease of MCB in the cathode immediacies was caused by the higher electroosmotic flow occurring in these areas, as commented in Section 3.2 [35]. The same pattern can also be seen for the other chlorinated benzenes, although this effect is less noticeable when the more chlorinated and the less insoluble are the benzene molecules. Lastly, the more chlorinated benzenes as the TCBs, the TeCBs, and pentachlorobenzene (PentaCB), were not affected by the electroosmotic flow and even accumulated in the cathodic areas (Fig. 7 K-T), especially in the control treatment (Fig. 7 M, N, O). The dichlorobenzenes decrease due to the biobarrier compared to the control treatment was 21 %. In the EBS experiment, 1,4-DCB presented a maximum concentration in the biobarrier (Fig. 7G) because it was amended together with the inoculum source, as it was employed as its carbon source. However, the reduction of trichlorobenzenes in EBS with respect to the control treatment was 38 % (Fig. 7 K, L, M, N, O, P). 1,2,3-TCB and 1,2,4-TCB were produced from the reductive hydrodechlorination of lindane in this section [25], whereas the increase of 1,3,5-TCB in section 9 (Fig. 7M) could be explained by the biologically-mediated anaerobic removal of higher chlorinated benzenes, such as TeCBs or PentaCB. The anaerobic biodegradation

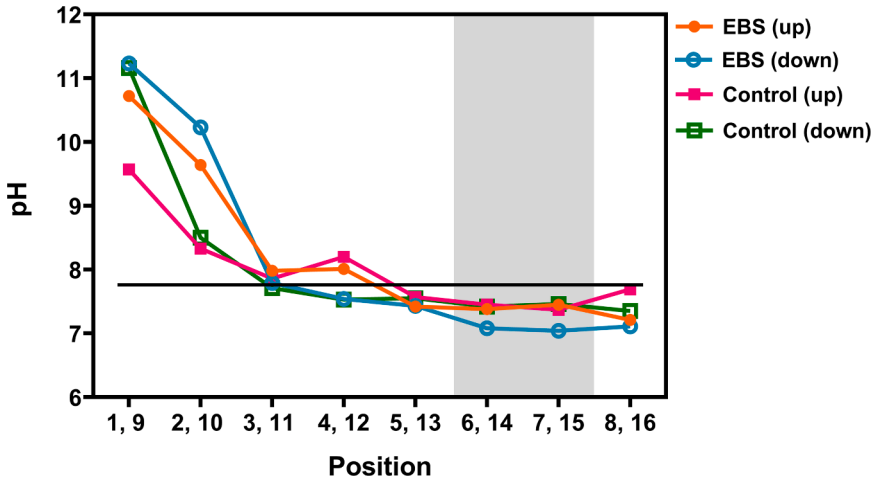

Fig. 6. Initial and final pH values in each section of the EBS and control treatment. The black, horizontal line points at the initial pH value of the silts. The grey zone indicates the location of the biobarrier in the EBS. Up: Section 1–8; down: sections 9–16.

pathway yielding 1,3,5-TCB (from TeCBs or PentaCB) has been reported to be the most thermodynamically favourable as it comes from the doubly flanked chlorine removal, giving a more negative ΔG°' value [1]. Tetrachlorobenzenes amount in EBS was a 35 % lower than in the control treatment, and a 37 % for PentaCB, which was detected at very low concentrations, as seen in Fig. 7 **S** and **T**.

Pollutant concentrations (HCHs, MCB and 1,3-DCB) in the electrolyte wells during the experiment are represented in Fig. 8. HCHs inlet concentrations ranged mostly from 200 to 400 μM during the experiment. The anode concentrations of HCHs, despite being similar, are slightly higher for the control system, probably because the silts in this treatment show higher HCHs concentrations (Fig. 7B). However, in the EBS, there were no HCHs detected in the cathode well due to the hydrodechlorination reactions which take place with the OH^- ions, yielding 1,2,3-TCB and 1,2,4-TCB [25]. A similar behaviour was observed in the control treatment during most of the operation until long operational times ($t = 14$ d) when an accumulation of HCHs at similar concentrations than in the anode was observed. This cathodic accumulation can be explained because, in the absence of bioremediation, higher levels of HCHs accumulate in the silts, being dragged by electroosmosis to the cathode and surpassing the capability of the alkaline hydrochlorination kinetics to completely degrade them (Fig. 7B).

MCB concentrations measured in the water introduced in the anodic well were clearly lower than the concentrations analysed in the experimental samples (Fig. 8B), suggesting that MCB was being produced. Its production in the anodic well was likely attributed to the lindane electrooxidation reactions that can take place on the anode surface. Water electrolysis allows the formation of reactive intermediates, such as hydroxyl radicals, which can be physically or chemically adsorbed to the anode surface [24]. Dominguez et al. [8] found that lindane electrooxidation entails the formation of MCB, as such as other chlorinated benzenes (*i.e.*, 1,2-DCB, 1,4-DCB, 1,3,5-TCB, 1,2,4-TCB, TeCBs). On the cathodic well, it can be seen how MCB was being accumulated in the control treatment, while its concentrations were lower in the experimental treatment. As previously discussed, MCB from the container was transported to the cathode by electroosmosis, where it is further accumulated, as its alkaline removal has been reported to not occur [25]. Consequently, MCB in the EBS is biologically depleted, thus it is further accumulated in the control cathodic well than in the experimental treatment. Consequently, the cathodic water from the control treatment would need further remediation treatments to remove MCB, HCHs, and other pollutants that were not biodegraded and accumulated there (Fig. S3).

The decrease through time observed in MCB concentrations may be attributable to volatilization. Similarly, it could be also attributable to 1,3-DCB and 1,2-DCB (Fig. 8C, Fig. S3), where their concentrations at the cathode are almost zero in both treatments. 1,4-DCB (Fig. S3) showed a higher concentration in the cathode because it was present in higher amounts in the silts (it was used as the carbon source for the inoculum in the experimental treatment).

No PentaCB was detected in the filling groundwater, but small concentrations were detected in the electrolyte wells, likely coming by diffusion from the silts, where it was initially detected (Fig. 7).

3.3.2. *Microbial population*

The microbial population at the initial and final time of experiment 3 in the EBS, as well as in the control treatment at the final time, is shown in Fig. S7. Phyla and genera pointed in this Fig. S7 are those which include strains able to remove benzene, chlorinated benzenes or lindane under aerobic or anaerobic conditions (see Table S2).

From the initial time, silts presented a great predominance of the Actinobacteria phylum (42.3 %); these are very frequent in soils [2]. Actinobacteria are also present in the initial biobarrier at a high proportion (54.5 %), with *Arthrobacter* as the main genus (36.8 %). The prevalence of this genus could be attributed to the biostimulation and enrichment of the groundwater culture by amending an external source of nutrients (N and P), O_2, and 1,4-DCB (as mentioned in Section 2.1.3). Then, these results suggest that *Arthrobacter* could have an active role in the removal of the compounds present in the DNAPL in aerobic conditions. Other phyla detected in a lower proportion in the initial silts and biobarrier are Acidobacteria (14.0 and 6.4 %, respectively), Proteobacteria (11.9 and 14.2 %, respectively), and Gemmatimonadetes (6.3 and 2.3 %, respectively). Acidobacteria [7] and Gemmatimonadetes [6] can be also typically found in soils.

At the final time of the EBS operation, the initial distribution in silts was mostly maintained in the closest area to the anode, except for an increase in *Arthrobacter* (from 1.6 % in initial silts to 11.1 % at the final time in section 8). This increase was also seen in the control treatment, where *Arthrobacter* accounted for 12.1 % of the total bacterial genera reads. A similar increase was also observed by Lear et al. [15], pointing the survival of this genus to the extreme oxidating conditions promoted by the anode. However, in the biobarrier, *Arthrobacter* lowered their proportion from 36.8 % at the initial time to 14.8 % as the maximum value in Section 6, while Proteobacteria increased from 14.1 % to 23.1 %, presenting a greater proportion of *Xanthobacter* (9.4 % in section 14), *Sphingobium* (21.2 % in section 15), and *Sphingomonas* (6.7 % in section 15). These two last genera have been described to be capable of aerobically removing lindane, yielding 1,2,4-TCB [2,18]. It could explain the increase of this pollutant in this section (Fig. 7**K**). The control treatment also showed an increase in 1,2,4-TCB in Section 5 (Fig. 7**L**). As

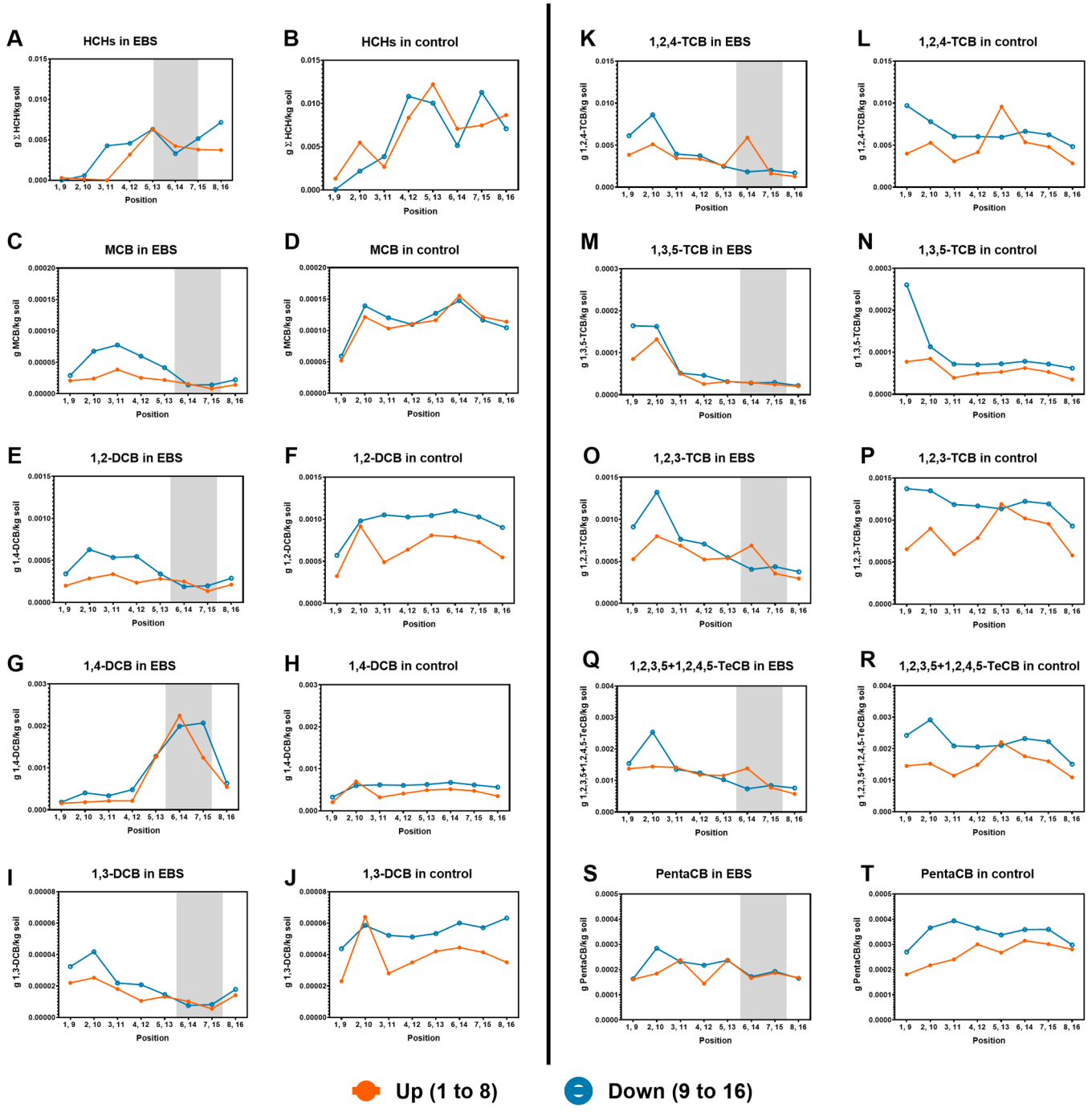

Fig. 7. Pollutant concentrations in silts at final time (14 days). Grey area indicates the location of the biobarrier.

previously commented, the DEC can biostimulate the autochthonous microorganisms in the silts and could be responsible for the detection of *Sphingomonas* in the control treatment (1.0 % in section 12 and 0.8 % in section 15). Firmicutes and Gemmatimonadetes also increased close to the anode and in the middle positions at the final time. Most of the genera included in these phyla, which have been detected in higher proportions in the biobarrier, have been described as dechlorinating bacteria. Thus, this increase could be due to their growth from the consumption of lindane, chlorobenzenes, or their chlorinated byproducts in the biobarrier.

As we move closer to the cathode, the proportion of Firmicutes increased at the expense of Actinobacteria as it is benefited by the reducing conditions promoted by this electrode [21], which can also stimulate the growth of other anaerobic bacteria. In this section, *Arthrobacter* still had a high representation (8.8 % in section EBS3, 1.5 % in EBS1) likely because it was enriched in the biobarrier and dragged by electroosmotic flow [3,21]. In comparison, the control treatment only possessed an *Arthrobacter*'s proportion (section CONT12) of 0.9 %. The main Firmicutes genus observed was *Bacillus*, which achieved high proportions (6.4 % in section EBS3, 30.0 % in section EBS1). Lear et al. [15] also reported a great development of *Bacillus* close to the cathode because of the ability of the genus to grow under the extreme conditions promoted in these areas. Other genera belonging to the Firmicutes phylum which also showed a greater proportion close to the cathode were *Exiguobacterium* (10.7 % in EBS2, 5.1 % in EBS1), *Clostridium sensu stricto* (3.9 % in EBS3, 3.7 % in EBS1) and *Sedimentibacter* (6.8 % in

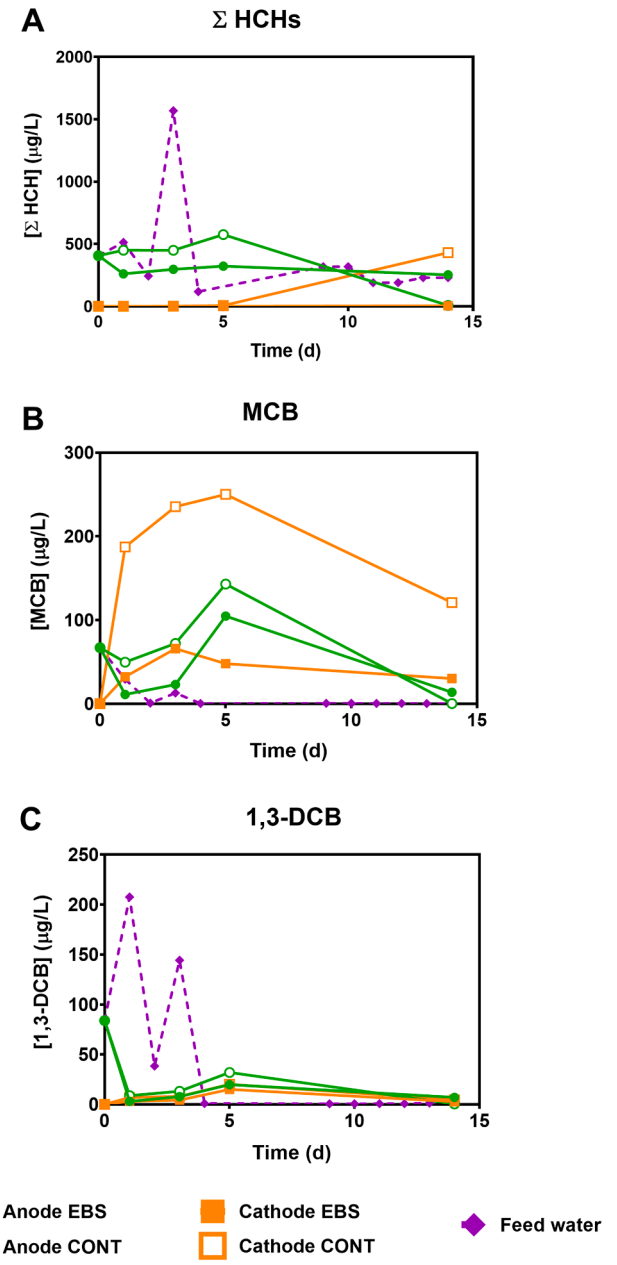

Fig. 8. Pollutants concentration along time in the electrolyte wells. EBS: electrobioremediation system; CONT: control treatment (electroremediation, without an inoculated biobarrier).

section EBS3, 3.1 % in section EBS1). In the control treatment (CONT12), similar distributions were seen in the closest area to the cathode.

Then, Fig. S7 demonstrates that the DEC applied affects the axial microbial distribution along silts in the containers. Basically, aerobic microorganisms are stimulated in the area near the anode (mainly *Arthrobacter*), as oxygen is provided, while anaerobic bacteria have an advantage in the areas near the cathode (mainly *Bacillus*), as hydrogen is provided [21]. Between all the microorganisms present, there are many dechlorinating groups able to degrade both lindane and chlorobenzenes, which may have been previously enriched in these silts coming from a polluted site in Sardas. Great similitudes are seen in the microbial composition of the experimental and control treatment, although higher removals were obtained in the experimental treatment (Figs. 7, 8).

In this experiment, two bioremediation treatments have been compared: a biostimulation (the control treatment) and a bioaugmentation (the EBS treatment). Fig. S7 proves that microorganisms able to remove the pollutants present in the DNAPL were detected in both control and EBS treatments. However, results from Fig. 7 indicate that the bioaugmentation performed better than the biostimulation. Bioremediation in soils or groundwaters with low hydraulic conductivity has some transport constraints as microorganisms, pollutants, and nutrients cannot find each other. When the DEC is applied, the improved mobility in the soil allows for the stimulation of the bioremediaiton process by increasing the opportunities for interaction among these three elements. In addition, the anode provides O_2, stimulating the oxidative remediation, while the cathode promotes reductive degradation by providing H_2, which is a suitable electron donor [20,21]. However, as reported in this section, biostimulation did not perform as well as the bioaugmentation. A reason could be that autochthonous microorganisms needed an adaptation period to the DNAPL pollutants, while those microorganisms bioaugmented were already acclimated to them. Another reason could also be that, although microorganisms could be provided with electron donors and acceptors, they would need other requirements, such as the provision of some nutrients lacking in silts and groundwater. However, there is the possibility that the control treatment could present biological activity, but showed less degradation rates than the experimental treatment, or just biological activity in specific reactions; for instance, in the production of 1,3,5-TCB from the biodegradation of higher chlorinated benzenes.

Another evidence for the better performance of the EBS was the higher accumulated electroosmotic flow of the control treatment (138 mL) compared to the experimental treatment (95 mL). Thus, although the control treatment showed a higher electroosmotic flow able to remove more pollutants from the containers by dragging them to the cathode, the results showed lower concentrations in the EBS treatment.

3.3.3. *Cost-effectivness comparison to other remediation technologies*

As seen, the combination of electroremediation and bioremediation techniques improved the degradation efficacy of HCH isomers and chlorobenzenes compared to the application of an abiotic electroremediation. Moreover, the application of DEC enhances bioremediation by stimulating the activity of the injected microbial consortia, thereby shortening the operational time. This results in reduced operational costs compared not only to each technology applied independently, but also to other conventional remediation strategies. Derived operational costs specific associated with EK-remediation include energy consumption and waste management [16]. In this study, the establishment of a biobarrier achieved a decrease in the concentration of the pollutants along the silts. Therefore, in an EBS, waste management costs can be reduced or even eliminated, as the remaining pollution could be further treated using less intensive procedures, such as bioreactors. The operational costs due to energy consumption achieved in this study were 0.16 €/m^3/d. However, it should be noted that the electrical resistance of the soil increases with the treated volume, potentially raising energy requirements in the field.

This remediation strategy also offers cost savings compared to other remediation technologies. In the first place, the *in-situ* treatment avoids operational costs related to soil excavation, transportation and possible containment [29]. Secondly, it does not require the consumption of chemical reagents such as oxidants used in *in-situ* chemical oxidation (ISCO). Moreover, since pollutant transport was enhanced by electroosmotic flow, the use of surfactants was considered unnecessary. The DEC also eliminates the need for biostimulation substrates injection or aeration, as it generates oxygen at the anode and hydrogen at the cathode. Besides, it promotes the homogeneization of the soil, thus increasing the bioavailability of nutrients.

Overall, the EBS approach is a cost-effective and promising strategy for remediating polluted soils, particularly those presenting low permeability.

4. Conclusions

Pollution by HCH isomers and chlorinated benzenes still represents a significant global concern. The remediation of low-permeable soils contaminated with these compounds remains a major challenge due to their limited accessibility. The application of a DEC acts by destroying part of these compounds and promoting mobilization of the pollutants to be recovered and subsequently treated *ex-situ*. In this study, the addition of a biobarrier was investigated to assess the contribution of bio-augmentation during electrokinetic treatments. The combined biological activity of the biobarrier with the application of a DEC was tested under different conditions to maximize the performance of microorganisms (*i.e.*, avoiding extreme pH). Their combined synergic effects improved the degradation efficiency of most pollutants present in the silts compared to the single application of DEC, decreasing the need for further *ex-situ* treatments. Microbial analysis revealed a high diversity of dechlorinating bacteria in the silts and groundwater coming from the site, including genera such as *Bacillus*, *Sphingobium*, *Arthrobacter*, *Pseudomonas* and *Clostridium*. Their distribution within the silts was influenced by the DEC, which biostimulated the growth of aerobic bacteria in areas influenced by the anode and anaerobic bacteria in the areas influenced by the cathode.

The combination of electrokinetic and bioremediation techniques integrated the advantages of both approaches by enhancing the clean-up efficiency and reducing required time, thereby resulting in a cost-effective remediation strategy. This study assessed the effect of the biobarrier located close to the anode to prevent potential harmful effects to biological activity due to alkaline conditions in areas near the cathode. However, to further enhance the pollutant removal efficiency, different relative positions of the biobarrier to the electrolytes or the application of recirculations of the water flow should be investigated. In another hand, with a view to field-scale application, further research is required to assess the limitations associated with scale-up, in which electrical heating effects will increase and may pose a threat to the biological activity by causing extreme temperatures and potential soil desiccation.

Environmental implication

Lindane production was an inefficient process that resulted in the generation of large quantities of toxic, persistent waste compounds, which were often disposed of in landfills. Remediating these sites is difficult due to the recalcitrant nature of these substances, their low solubility, and the complex mixture of pollutants. The combined use of electrokinetic soil flushing and bioremediation proposed in this work offers a sustainable, scalable solution for remediating soils contaminated with lindane. By enhancing contaminant mobility and microbial degradation, this approach reduces long-term environmental risks and promotes in-situ cleanup of complex sites, supporting healthier ecosystems and safer land use.

CRediT authorship contribution statement

Paqui Blánquez: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Teresa Vicent:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **David Fernández-Verdejo:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julia Isidro:** Methodology, Investigation, Conceptualization. **Dani Salom:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.139397.

Data availability

Data will be made available on request.

References

[1] Adrian, L., Görisch, H., 2002. Microbial transformation of chlorinated benzenes under anaerobic conditions. Res Microbiol 153 (3), 131–137.
[2] Alvarez, A., Benimeli, C.S., Saez, J.M., Fuentes, M.S., Cuozzo, S.A., Polti, M.A., Amoroso, M.J., 2012. Bacterial bio-resources for remediation of hexachlorocyclohexane. Int J Mol Sci 13 (11), 15086–15106.
[3] Annamalai, S., Sundaram, M., 2020. Electro-bioremediation: an advanced remediation technology for the treatment and management of contaminated soil. Bioremediation Ind Waste Environ Saf Vol II Biol Agents Methods Ind Waste Manag 183–214.
[4] Bakatula, E.N., Richard, D., Neculita, C.M., Zagury, G.J., 2018. Determination of point of zero charge of natural organic materials. Environ Sci Pollut Res 25 (8), 7823–7833.
[5] Davranche, M., Beaufreton, S., Bollinger, J.C., 2002. Influence of carbonates on the surface charge of a natural solid. J Colloid Interface Sci 249 (1), 113–118.
[6] DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., Radosevich, M., 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Appl Environ Microbiol 77 (17), 6295–6300.
[7] Dedysh, S.N., Damsté, J.S., 2018. Acidobacteria. eLS., 10. John Wiley & Sons, Ltd, *Chichester, UK*.
[8] Dominguez, C.M., Oturan, N., Romero, A., Santos, A., Oturan, M.A., 2018. Lindane degradation by electrooxidation process: effect of electrode materials on oxidation and mineralization kinetics. Water Res 135, 220–230.
[9] Eurostat. (2025, April). *Electricity price statistics*. In *Statistics Explained*. Retrieved June 6, 2025, from https://ec.europa.eu/eurostat/.
[10] Fernández, J., Arjol, M.A., Cacho, C., 2013. POP-contaminated sites from HCH production in Sabiñánigo, Spain. Environ Sci Pollut Res 20, 1937–1950.
[11] Fernández-Cascán, J., Isidro, J., Guadaño, J., Sáez, C., Rodrigo, M.A., 2024. Electrokinetic remediation of real soils polluted with chlorinated hydrocarbons: relevance of dichlorination and volatilization processes. J Solid State Electrochem 1–10.
[12] Field, J.A., Sierra-Alvarez, R., 2008. Microbial degradation of chlorinated benzenes. Biodegradation 19, 463–480.
[13] Geng, Z., Liu, B., Li, G., Zhang, F., 2021. Enhancing DNAPL removal from low permeability zone using electrical resistance heating with pulsed direct current. J Hazard Mater 413, 125455.
[14] Guadaño, J., Gómez, J., Fernández, J., Lorenzo, D., Domínguez, C.M., Cotillas, S., García-Cervilla, R., Santos, A., 2022. Remediation of the Alluvial Aquifer of the Sardas Landfill (Sabiñánigo, Huesca) by Surfactant Application. Sustainability 14 (24), 16576.
[15] Lear, G., Harbottle, M.J., Van Der Gast, C.J., Jackman, S.A., Knowles, C.J., Sills, G., Thompson, I.P., 2004. The effect of electrokinetics on soil microbial communities. Soil Biol Biochem 36 (11), 1751–1760.
[16] López-Vizcaíno, R., Yustres, A., Sáez, C., Cañizares, P., Asensio, L., Navarro, V., Rodrigo, M.A., 2019. Techno-economic analysis of the scale-up process of electrochemically-assisted soil remediation. J Environ Manag 231, 570–575.
[17] Martín-González, L., Hatijah Mortan, S., Rosell, M., Parladé, E., Martínez-Alonso, M., Gaju, N., Caminal, G., Adrian, L., Marco-Urrea, E., 2015. Stable carbon isotope fractionation during 1, 2-dichloropropane-to-propene transformation by an enrichment culture containing Dehalogenimonas strains and a dcpA gene. Environ Sci Technol 49 (14), 8666–8674.
[18] Phillips, T.M., Seech, A.G., Lee, H., Trevors, J.T., 2005. Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. Biodegradation 16, 363–392.
[19] Puentes Jácome, L.A., Lomheim, L., Gaspard, S., Edwards, E.A., 2021. Biodegradation of lindane (γ-hexachlorocyclohexane) to nontoxic end products by sequential treatment with three mixed anaerobic microbial cultures. Environ Sci Technol 55 (5), 2968–2979.
[20] Ramírez, E.M., Camacho, J.V., Rodrigo, M.A., Cañizares, P., 2015. Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: a comparison of strategies. Sci Total Environ 533, 307–316.
[21] Reddy, K.R., Cameselle, C., 2009. Electrochemical remediation technologies for polluted soils, sediments and groundwater. John Wiley & Sons.
[22] *Resumen de Salud Pública: Hexaclorociclohexano [Hexachlorocyclohexane] | PHS | ATSDR*. (2021, January 26). ⟨https://www.atsdr.cdc.gov/es/phs/es_phs43.html⟩.

[23] Roy, A., Vajpayee, P., Srivastava, S., Srivastava, P.K., 2023. Revelation of bioremediation approaches for hexachlorocyclohexane degradation in soil. World J Microbiol Biotechnol 39 (9), 243.
[24] Rueffer, M., Bejan, D., Bunce, N.J., 2011. Graphite: An active or an inactive anode? Electrochim Acta 56 (5), 2246–2253.
[25] Santos, A., Fernandez, J., Guadano, J., Lorenzo, D., Romero, A., 2018. Chlorinated organic compounds in liquid wastes (DNAPL) from lindane production dumped in landfills in Sabinanigo (Spain). Environ Pollut 242, 1616–1624.
[26] Santos, A., Fernandez, J., Rodriguez, S., Dominguez, C.M., Lominchar, M.A., Lorenzo, D., Romero, A., 2018. Abatement of chlorinated compounds in groundwater contaminated by HCH wastes using ISCO with alkali activated persulfate. Sci Total Environ 615, 1070–1077.
[27] Santos, A., García-Cervilla, R., Checa-Fernández, A., Domínguez, C.M., Lorenzo, D., 2022. Acute toxicity evaluation of lindane-waste contaminated soils treated by surfactant-enhanced ISCO. Molecules 27 (24), 8965.
[28] Soder-Walz, J.M., Salom, D., Granados-Rigol, E., Fernández-Verdejo, D., Vicent, T., Marco-Urrea, E., Blánquez, P., 2025. Enhanced aerobic bioremediation of an aquifer heavily contaminated with a mixture of chlorobenzenes and hexachlorocyclohexanes at the Sardas landfill (Spain). J Hazard Mater 484, 136717. https://doi.org/10.1016/j.jhazmat.2024.136717.
[29] Vijgen, J., de Borst, B., Weber, R., Stobiecki, T., Forter, M., 2019. HCH and lindane contaminated sites: European and global need for a permanent solution for a long-time neglected issue. Environ Pollut 248, 696–705.
[30] Vijgen, J., Fokke, B., van de Coterlet, G., Amstaetter, K., Sancho, J., Bensaïah, C., Weber, R., 2022. European cooperation to tackle the legacies of hexachlorocyclohexane (HCH) and lindane. Emerg Contam 8, 97–112.
[31] Wang, Z., Yang, Z., Chen, Y.F., 2023. Pore-scale investigation of surfactant-enhanced DNAPL mobilization and solubilization. Chemosphere 341, 140071.
[32] Williams, M., Carlson-Lynch, H., Buser, M., Heit, C., Casillas, G., Melia, J., Rhoades, J. & Sierco, S. (2024). Toxicological Profile for Hexachlorocyclohexane (HCH).
[33] Xie, T., Dang, Z., Zhang, J., Zhang, Q., Zhang, R.H., Liao, C.J., Lu, G.N., 2021. Decontamination of dense nonaqueous-phase liquids in groundwater using pump-and-treat and in situ chemical oxidation processes: a field test. RSC Adv 11 (7), 4237–4246.
[34] Yu, Y., Liu, L., Yang, C., Kang, W., Yan, Z., Zhu, Y., Wang, J., Zhang, H., 2019. Removal kinetics of petroleum hydrocarbons from low-permeable soil by sand mixing and thermal enhancement of soil vapor extraction. Chemosphere 236, 124319.
[35] Yuan, S.H., Wan, J.Z., Lu, X.H., 2007. Electrokinetic movement of multiple chlorobenzenes in contaminated soils in the presence of β-cyclodextrin. J Environ Sci 19 (8), 968–976.