Electrocatalytic Dechlorination of 2,4-dichlorophenol in Bioelectrochemical Systems

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24 **Abbreviations**

- 25 Bioelectrochemical systems (BESs)
- 26 Biochemical Oxygen Demand (BOD)
- 27 Chemical oxygen demand (COD)
- 28 Chlorophenols (CPs)
- 29 Electrocatalytic Hydrogenation (ECH)
- 30 Electrochemical reductive dechlorination (ERDC)
- 31 Half-maximal response (EC 50)
- 32 High-performance liquid chromatography (HPLC)
- 33 Joint Committee on Powder Diffraction Standards (JCPDS)
- 34 Linear sweep voltammetries (LSV)
- 35 Microbial Fuel Cell (MFC)
- 36 Reference electrode (RE)
- 37 Working electrode (WE)
- 38 X-Ray diffraction (XRD)
- 39 2,4-dichlorophenol (2,4-DCP)
- 40 4-chlorophenol (4-CP)
- 41 2-chlorophenol (2-CP)

ABSTRACT

In this work, the cathodic electro-dechlorination of 2,4-DCP in a Microbial Fuel Cell (MFC) was studied. To do that, an MFC externally connected with a 120 Ω load was operated by feeding a synthetic wastewater with 300 ppm of 2,4-DCP to the abiotic cathode compartment and sodium acetate to the biotic anode with electroactive bacteria. During the experiments, all the intermediates (2-chlorophenol and 4-chlorophenol) and final products (phenol and Cl^-) from the dechlorination of 2,4-DCP were monitored. Results show that the bio-assisted electroreductive treatment achieved a cathodic dechlorination yield of a 57% after 24 hours, which increases up to 88% after 72 hours. In addition, because of the dechlorination of 2,4-DCP, the biodegradability of the synthetic wastewater, expressed as the ratio BOD/COD, was increased from negative values (corresponding to toxic effluents) up to 0.568. Meanwhile, the toxicity decreased drastically, with EC_{50} values that increased from 0.021 to 0.228 mmol L^{-1} . Operation current densities during the bioelectrodehalogenation processes were significant and they decreased from initial values of 0.14 down to 0.04 mA cm^{-2} . These results indicate that the bioelectrochemical technology can produce small amounts of energy while dechlorinating an extremely hazardous waste.

Keywords: *Bioelectrochemical system; Microbial fuel cell; Organochlorines; 2,4-Dichlorophenol; dechlorination; electro-reductive dehalogenation.*

1. INTRODUCTION

Chlorophenols (CPs) are derivatives of phenol containing one or more covalently bonded chlorine atoms. They are characterised by strong odour, high toxicity, even presenting carcinogenic effects, and low biodegradability, mainly related to the number and position of chlorines in the aromatic ring, which leads to a high persistence in the environment (Igbinosa et al., 2013). Unfortunately, they are found not only in soils but also in superficial and ground water resources (Aristov & Habekost, 2010). One of the most representative members of this family is 2,4-dichlorophenol (2,4-DCP), which is widely used in the chemical industry (Cao et al., 2016), mainly as raw product for the fabrication of a wide spectrum of pesticides such as 2,4-dichlorophenoxyacetic acid (Ya et al., 2017). Despite its industrial production is today strongly restricted, there are still numerous polluted sites and environmental problems related to 2,4-DCP (Zhang et al., 2011).

Conventional methods such as biodegradation, adsorption, ion exchange, liquid–liquid extraction, chemical oxidation or advanced oxidation processes have been widely used for the removal of CPs from waste and natural waters, as well as in soils, presenting all the options advantages but also different drawbacks to be solved (Garba et al., 2019).

One of the most widely used method for CPs removal is the anaerobic biodegradation, also known as halo-respiration (Field & Sierra-Alvarez, 2008; Rodriguez et al., 2013).

Anaerobic biodegradation, as most of biological methods, is cost-effective and environmentally friendly but, unfortunately, it requires long retention times and its efficiency strongly depends on the microbial population performing the treatment.

Instead of removing the CPs, an interesting option could be to transform this molecule into a less harmful one (Arora & Bae, 2014; Farré et al., 2007). Due to the electronegative

characteristics of chlorine substituents, most of the CPs molecules can be transformed via reductive dechlorination. In the reductive dechlorination, CPs accept electrons from an electron donor to break C-Cl bonds. This process can be carried out by using different technologies (Arora & Bae, 2014; Lei et al., 2019; Zhang, 2003). The main advantages of the CPs dechlorination are on the one hand that the products from CPs dechlorination present less toxicity and higher biodegradability than those of the parent molecules (Farré et al., 2007), and on the other hand that they present higher reaction rates than the biotreatments. Because of that, electrochemical reductive dechlorination (ERDC) of CPs stands up as a promising method because of its high reaction rate, low apparatus cost, relatively mild reaction conditions, and absence of secondary toxic contaminants (Sun et al., 2010).

ERDC occurs through both direct and indirect (electrocatalytic hydrogenolysis, ECH) mechanisms. ECH is a process in which chemisorbed hydrogen is generated at the electrode surface by electrolysis of water, described in eq. 1-4 (Chen et al., 2006; He et al., 2016), where R is the aromatic compound and M the metal catalyst.

In the ERDC process, the proton (H^+) in aqueous solution, electrochemically generated from the anodic oxidation step in an electrochemical cell, is reduced to atomic hydrogen (H^* , a strong reducing agent) on the cathode surface, which subsequently attacks and cleaves C-Cl bonds to achieve hydrodechlorination, usually with the aid of a metal catalyst. Unfortunately, as side reactions, H^* may also evolve into molecular hydrogen

(H₂) at a more reductive potential, while finally also H₂ can be directly generated from water electrolysis (Jiang et al., 2017). Several factors such as the applied potentials, electrode materials, and presence of metal catalyst and/or redox mediators are of crucial importance on the ERDC process efficiency, rates and energy consumption (Peters et al., 2014). Pd, Pt and Ni are common catalysts used for the electrodechlorination, but Palladium has shown much more effectiveness in promoting the dechlorination reaction due to its excellent ability to absorb hydrogen into its lattice (Sun et al., 2011). Regarding the energy consumption, in recent years, bioelectrochemical systems (BESs) have emerged focusing on this point. BES are based on the use of exoelectrogenic microorganisms that catalyse the electrochemical reactions taking place on electrode surfaces of an electrochemical cell, because of their ability to participate in the electron transfer mechanisms to/from electrodes surfaces (Rozendal et al., 2008).

BESs can present different configurations, but in most of the cases they are based on a biotic anode, where electrochemically active microorganisms oxidize organic matter releasing electrons to the anode, and a cathode, where electrons are consumed in a reduction reaction such as: the reduction of oxygen to water, protons to hydrogen, or different types of organic pollutants (nitroaromatics, halogenated aromatics, azo dyes, etc) to obtain more reduced and less refractory forms. When the anodic and cathodic reactions coupled leads to a spontaneous reaction, the chemical energy is converted into electrical energy behaving the system as a Microbial Fuel Cell (MFC).

Some works studied the transformation of different types of organochlorines by means of BES, both in bioanodes, degrading them (Hassan et al., 2016; Hassan et al., 2018; Lai

et al., 2017; Wang et al., 2019), or biocathodes, where the target is the dechlorination of the molecule (Chen et al., 2019a; Chen et al., 2019b; Huang et al., 2014).

The coupling of bioanodes with the electrocatalytic hydrodechlorination of CPs is a recent focus of study, showing their feasibility and arising as promising technology, with lower power consumption compared to conventional pure electrochemical technologies (Leon-Fernandez et al., 2019; Wen et al., 2013).

In this context, the present work studies the feasibility of a BES, working under MFC mode, for 2,4-dichlorophenol (24-DCP) bio-electrochemical reductive dechlorination at the cathode, oxidizing acetate at the bio-anode as fuel. Research about electrocatalytic hydrodechlorination of organochlorinated compounds by BES is very scarce and, to the knowledge of the authors, 2,4-DCP electrodechlorination with bioelectrochemical systems has not been studied with this configuration to date.

2. MATERIALS AND METHODS

2.1. Experimental set-up

The set-up used in this work consisted in 3 replicates of a BES operating with biotic anode and abiotic cathode. Additionally, a reference test was carried out in a BES operating with abiotic cathode and abiotic anode, in order to isolate the contribution of the anodic electroactive bacteria. The BES were operated at 25°C as MFCs, with an external resistor of 120 Ω . The cathode and anode potential of the BESs were measured against an Ag/AgCl reference electrode (RE). Each BES consisted of two chambers, with 0.1 L volume each, separated by a proton exchange membrane (Nafion® 117, DuPont). The material used for building the BES was transparent PVC. Silicon plates between membrane and PVC were used to improve the mechanical properties and to avoid liquid leakages (Leon-Fernandez et al., 2019). Carbon felt (KFA10, SGL Carbon Group®) was used as anode material due to its capacity for supporting the biofilm (Mateo et al., 2018). The anode dimensions were $2.5 \times 2.5 \times 0.8 \text{ cm}^3$, specific area of $3.53 \times 10^5 \text{ cm}^2 \text{ g}^{-1}$ and a porosity of 0.95 (Asensio et al., 2017). Carbon cloth was used as cathode, with a Pd load of 0.5 mg cm^{-2} . Its dimensions were $2.5 \times 2.5 \text{ cm}^2$. The catalyst ink for Pd deposition on the electrode consisted of commercial 40% Pd/C Vulcan XC-72R Carbon Black (Fuel Cell Store), Nafion™ ionomener (alcohol-based dispersion at 5 wt. %), and isopropanol as a dispersing solvent. The ratio 40% metal/C – Nafion solution was 1:8. X-Ray diffraction (XRD) measurements were performed on a Philips PW-1700 diffractometer with rotating anode, applying $K\alpha$ corresponding to the transition from copper radiation ($\lambda = 1.5413 \text{ \AA}$) for different samples. Measurements of 2θ angle were located between 20° and 100° with a sweep speed of 0.1° s^{-1} .

A conceptual description of the MFC and more details about the cathodic dechlorination reaction are shown in Figure 1.

Figure 1. (a) Conceptual description of the BES reactor. (b) Reductive dechlorination process at the cathode.

2.2. Anolyte and catholyte composition

A synthetic wastewater containing 300 ppm of 2,4-DCP was prepared to work as catholyte. 100 mmol L^{-1} phosphate buffer was used to keep the cathodic pH at 7. The phosphate buffer was made of 8.66 g L^{-1} of Na_2HPO_4 and 5.31 g L^{-1} of KH_2PO_4 . The conductivity of the synthetic wastewater was 11.21 mS cm^{-1} .

Additionally, a microbial growth medium containing acetate as the sole organic substrate was used as anolyte. The anolyte composition was the following: CH_3COONa 1.00 g L^{-1} , Na_2HPO_4 3.00 g L^{-1} , KH_2PO_4 0.70 g L^{-1} , $(\text{NH}_4)_2\text{SO}_4$ 0.80 g L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.20 g L^{-1} , and $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 0.04 g L^{-1} . The fresh anolyte medium had a pH of about 7.44 and a conductivity of 5.71 mS cm^{-1} .

2.3. Start-up and stationary operation

The seed used for the anodic biofilm development was obtained from the aerobic reactor of a conventional activated sludge facility (volatile suspended solids concentration of approximately 2300 mg L⁻¹, hydraulic retention time of 5 hours and sludge retention time of 7 days), more information about this facility can be found elsewhere (Rodríguez Mayor et al., 2004). At the beginning, the anodic reservoir was filled with activated sludge. After the inoculation stage, and to favor the enrichment of the culture in electrogenic microorganisms, 80% of anolyte was replaced by fresh anodic medium every 2 days. During the start-up stage, to facilitate the anodic biofilm development, the oxygen reduction to water was used as cathodic reaction. Coupling of the acetate oxidation and the oxygen reduction reactions is thermodynamically feasible (Gonzalez del Campo et al., 2014) and ensures the absence of cathodic limitations in the anodic biofilm development, allowing to obtain stationary cycles in every batch reaction. Once the electrogenic biofilm was developed and operated under steady state after 10 days from inoculation, performing reproducible cycles of exerted voltage and achieving a maximum value of around 0.23 V every cycle, the cathodic reduction reaction of oxygen was substituted by the dechlorination of 2,4-DCP. To do that, the aeration was stopped and the catholyte containing the pesticide was added to the cathodic compartment. Additionally, to ensure anaerobic conditions, the MFC's compartments were purged with nitrogen gas at the beginning of each batch cycle.

2.4. Sampling and analytical measurements

Chemical characterization. Every day, in order to avoid significant changes in the total volume of the electrolyte, a sample of 0.75 mL was taken from anolyte and catholyte. The concentration of the 2,4-DCP, as well as the main intermediates of the reduction reaction: 4-chlorophenol (4-CP), 2-chlorophenol (2-CP) and phenol were determined by using HPLC with an UV detector (Jasco, Japan). The mobile phase consisted of 30:70 v/v acetonitrile/0.1% phosphoric acid (flow rate of $1.0 \text{ cm}^3 \text{ min}^{-1}$). The injection volume was 20 μL and the UV detection wavelength as 220 nm. Chloride concentration was determined through ion chromatography using a Metrohm 930 Compact IC Flex coupled to a conductivity detector. A Metrosep A Supp 7 column was used to determine the anions using a mobile phase consisting of 85:15 v/v 3.6 mM Na_2CO_3 /acetone at a flow rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$.

Sodium acetate was determined through high-performance liquid chromatography (HPLC), using an Agilent 1260 Infinity equipment containing a column Hi-plex H (300 $\times$ 7.7 mm, 8 μm). The mobile phase was 5.0 mmol L^{-1} H_2SO_4 , the wavelength was 210 nm and the flow rate 0.4 mL min^{-1} . Chemical oxygen demand (COD) was determined using Spectroquant[®] COD cell tests and a Pharo 100 Merck spectrophotometer. Conductivity and pH were determined with a Crison Cm 35 and a GLP22 Crison devices, respectively.

Electrochemical characterization. Linear sweep voltammetries (LSVs) were performed at 25°C, with magnetic stirring by using an Autolab potentiostat/galvanostat (PGSTAT-302N) at a scan rate of 0.5 mV s^{-1} . The carbon cloth electrode with Pd-C load was used as working electrode (WE), a wire of Pt was the counter electrode and an Ag/AgCl electrode was the RE in a half-cell reactor with magnetic stirring. The dimensions of the WE were 2.5 $\times$ 2.5 cm^2 . Electrical current produced by the BESs was recorded every 5

minutes by means of a Keithley 2000 multimeter. Current density was calculated considering the projected cathodic area. Polarization and power curves were performed by changing the external resistance. Nitrogen gas was sparged into the BES reactor and half-cell reactor prior to the experiment to remove the dissolved oxygen.

Biodegradability/Toxicity characterization. In order to determine the biodegradability, BOD₅ test were performed in an Oxitop IS 6-Var. BOD₅ is determined measuring the oxygen consumed by the microorganisms that oxidize the organic matter contained in the sample for 5 days. According to the literature a trace minerals solution was added (Eaton et al., 2005). The samples were inoculated with 2.0 mL of activated sludge taken from a conventional WWTP described elsewhere (Rodríguez Mayor et al., 2004) to provide an adequate inoculum to degrade the organic matter. In addition, 2 droplets of N-Allylthiourea reagent were added to the samples for nitrification inhibition throughout the tests. The toxicity of the samples was determined by means of a BioTox™ Kit supplied by Aboatox. The inhibitory effect of the sample on the light emission of luminescent bacteria, *Aliivibrio fischeri*, is measured with a luminometer (Junior LB 9509 of Berthold Technologies). The toxicity was evaluated as EC₅₀ by measuring the effective concentration that produces a 50% of inhibition calculated from the loss of luminescence (Rodriguez et al., 2013).

2.5. Faradaic Efficiencies

the Faradaic efficiencies are depending on the reactions considered: anodic (Eq. 5, sodium acetate as reactant and electrons as product) or cathodic (Eq. 6, electrons as reactant and chlorides as final product) (Modin et al., 2017), where n_i is the number of

256 electrons involved in the electrochemical reaction per mol of reactant i, F the Faraday's
257 constant (96485.3 C mol⁻¹); Δm_i is the molar variation of reactant i in mol, t is the time in
258 s and I is the current intensity in A.

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$$\eta_a = \left(\frac{\int_{t_1}^{t_2} I dt}{F \Sigma (n_i \Delta m_i)} \right) \quad (5)$$

$$\eta_c = \left(\frac{\int_{t_1}^{t_2} I dt}{F \Sigma (n_i \Delta m_i)} \right)^{-1} \quad (6)$$

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3. RESULTS AND DISCUSSION

3.1. Fundamental studies with stationary electrodes and electrolyte stirring.

Polarization and Power curves.

Figure 2 reports on X-ray diffraction analysis of the carbon cloth electrode with 0.5 mg cm⁻² catalyst load, corresponding the peaks to Pd (He et al., 2016). The distinct peaks of the X-ray diffractogram were also compared with the data base of the Joint Committee on Powder Diffraction Standards (JCPDS) through software X'pert High Score Plus and they indicated the presence of different faces of palladium in the electrocatalyst.

Figure 2. X-ray diffraction analysis of the carbon cloth electrode, 0.5 mg cm⁻² Pd load

Fig. 3a presents the linear scan voltammetries for the electro-reduction of 2,4-DCP. The carbon cloth electrode with 0.5 mg Pd cm⁻² load used in the BESs was used as working electrode. Same conditions that for the catholyte in the BES were used, with 300 mg L⁻¹ of 2,4-DCP. The OCP value was 0.237 V vs Ag/AgCl and current was increasing by sweeping the potential towards more negative values. The current produced was due

to the electrochemical dechlorination reaction of 2,4-DCP. The dechlorination was also corroborated by HPLC analysis of the electrolyte, after the LSV, where the peaks corresponding to phenol, 4-CP and 2-CP were identified. The limiting current owing to mass transfer was reached at -0.03 V vs Ag/AgCl, and evolution of H₂ occurs at -0.16 V vs Ag/AgCl. Negligible current was generated by using the bare-carbon cloth electrode without Pd load, evidencing the catalytic activity of Pd for the dechlorination of 2,4-DCP. Also, carbon-based materials provide a great overpotential for H₂ evolution, hence, this reaction was not found before -0.3 V vs Ag/AgCl either.

Figure 3. (a) LSV for $2.5 \times 2.5 \text{ cm}^2$ carbon cloth electrode with 0.5 mg cm^{-2} catalyst load, 350 rpm magnetic stirring. (b) LSV for $2.5 \times 2.5 \times 0.8 \text{ cm}^3$ carbon felt with electroactive biofilm, 60 rpm magnetic stirring. (c) Polarization and Power curves performed to the MFCs. (d) Monitoring of anode and cathode potential throughout the performance of the polarization curve test.

Fig. 3b shows the LSV for the carbon felt electrode once the electroactive bacteria were developed on the electrode. The value of OCP was -0.493 V vs Ag/AgCl. This starting potential agrees with the values reported in the literature for a properly developed

biofilm for sodium acetate oxidation (Kato, 2017). The current generated corresponds to the bio-electrochemical oxidation of sodium acetate to HCO_3^- with electron transfer from bacteria to the solid electrodes (Liu et al., 2008). This was also corroborated by HPLC analysis of the electrolyte after the LSV, where the concentration of sodium acetate was lower than at the beginning of the experiment. On the contrary, the concentration of acetate remained constant when the LSV was performed to the blank carbon felt electrode without electroactive bacteria. The value of limiting current was approximately 0.73 mA cm^{-2} , about 5 times higher than in the LSV for the dechlorination of 2,4-DCP. Hence, the cathodic reaction was the limiting stage of the bio-electrochemical process in the MFC.

Since the starting potential for the reduction of 2,4-DCP is over the starting potential for the oxidation of sodium acetate to HCO_3^- , this redox couple is thermodynamically feasible and spontaneous. Therefore, these systems may work with no external energy input required, in MFC mode. Fig. 3c shows the power and polarization of the MFC (average data of the 3 replicates) with the conditions of the batch experiments for 2,4-DCP dechlorination. In addition, anode and cathode potentials were monitored throughout the experiment (Fig. 3d) and show a similar behavior compared to the LSVs described previously. In the operating range of the test, from OCP to maximum current, the reactions that take place at the cathode are the reductive dechlorination of 2,4-DCP through direct mechanisms (Fig. 2b), as well as the competition between promotion of H_2 and indirect dechlorination through electrocatalytic hydrogenolysis (ECH, described in eq. 1-4) at more negative cathode potentials than 0.16 V vs Ag/AgCl. Considering this, different slopes and curves can be seen in Fig. 3c. Regarding the power plot, the first shaded region on the power curve corresponds to the redox couple acetate oxidation /

2,4-DCP reduction through direct electron transfer, where the mass transfer limitations are quite remarkable as previously mentioned from the results of the cathodic operative curve (Fig. 3a). The shaded region on the right on the power curve, which is not affected by mass transfer shows the operating conditions of the MFC at the beginning of the batch experiments. The MFCs worked with an external load of 120 Ω to ensure a maximum dechlorination rate and a higher current density; H_2 evolution is going to occur at the cathode, which also promotes ECH since this mechanism implies water electrolysis. The value of current density and anode and cathode potentials will change throughout the experiment, since the concentration of the reagents will be changing.

3.2. Cathodic dechlorination in MFC systems.

Based on the results shown by the fundamental studies presented in Fig. 3, the cathodic dechlorination of 2,4-DCP is feasible in MFC mode, since the potential of this reaction at OCP conditions is over the anodic potential of the acetate oxidation by electroactive bacteria.

Therefore, a batch experiment in an MFC (3 replicates) was performed and contrasted with a blank reference test with the same operational conditions but abiotic anode.

Fig. 4a shows the evolution of 2,4-DCP and sodium acetate in the MFC and Reference test, and Fig. 4b shows the evolution of the organochlorinated species and chlorides in the catholyte of the MFC, in molar concentration. As can be seen in Fig. 4a, the concentration of 2,4-DCP drops a 72% in the first 24 hours, being negligible after 3 days. The final products of the dechlorination are phenol and chlorides, whose concentration increases over time until the end of the batch experiment. The removal of the chlorines bonded to the aromatic ring of the 2,4-DCP involves the generation of 2-CP and 4-CP as

intermediates. These intermediates were found in the catholyte, increasing their concentration until 0.234 mmol L⁻¹ and 0.057 mmol L⁻¹ for 2-CP and 4-CP, respectively. Regarding the chemistry of the molecule, the chlorine bonded in ortho position is more electropositive than the chlorine in para position due to the inductive -I effect of the OH group, which pulls the electrons towards itself, since the conjugative +K effect of the oxygen atom, which involves the electronic delocalization along the aromatic ring, affects equally to both chlorines bonded to the aromatic ring. Assuming this difference in the electron density on chlorine atoms, chlorine in ortho position should be more electrophile, and hence it should be more likely to leave the molecule in the reductive dechlorination process. However, results show a higher accumulation of 2-CP rather than 4-CP, contrarily. These results suggest that the steric effect of the attached –OH group would hinder the cleavage of C–Cl bond in ortho position. This phenomenon has been reported in other studies with similar chlorophenols (Sun et al., 2014; Tsyganok & Otsuka, 1999). The final concentration of phenol (averaged data of the three replicates) was 1.501 mmol L⁻¹ and the final concentration of chlorides was 2.905 mmol L⁻¹.

Figure 4. Evolution of the species in the MFC. (a) Evolution of 2,4-DCP and sodium acetate in the MFC and blank reference test. (b) Evolution of phenolic products and chlorides generated in the dechlorination process.

The reconciliation of the mass balance was verified along the experiment: considering the total dechlorination of 2,4-DCP, the final molar concentration of phenol would be the same that the initial concentration of 2,4-DCP (assuming the absence of losses of the CPs by volatilization or adsorption), and, consequently, twice of chlorides (see Fig. 4b). In other terms:

$$\sum [\text{Organochlorines}] = [2,4\text{-DCP}]_t + [4\text{-CP}]_t + [2\text{-CP}]_t + [\text{phenol}]_t$$

$$= \sum [2,4\text{-DCP}]_0$$

$$[\text{Cl}^-]_t = [4\text{-CP}]_t + [2\text{-CP}]_t + 2 \cdot [\text{phenol}]_t$$

The average % of dechlorination in the MFCs was 88%, calculated as follows (concentration in mol L⁻¹):

$$\% \text{ dechlorination} = \frac{[\text{Cl}^-]_{t=3d}}{2 \cdot [2,4\text{-DCP}]_{t_0}} \cdot 100$$

The concentration of 2,4-DCP remained constant in the blank reference test, and no other intermediates were found through chromatography. This agrees with the cathode potential in the MFC and reference test, shown in Fig. 5b. The cathode potential in the MFC reached the negative values expected according to the polarization curve, whereas in the reference test this value is close to the starting potential for the dechlorination of 2,4-DCP. In consequence, this reaction did not take place in the reference test and the current exerted was negligible (Fig. 5a).

The current decreased gradually due the abatement of chlorophenols, however it was not nil as in the blank reference test (Fig. 5a). This surplus current can be explained in terms of the H_2 promotion reaction at the cathode.

Sodium acetate concentration decreased steadily in the analyte during the first 2 days and more dramatically during the last 24 hours (Fig. 4a); however, the exerted current during the last 24 hours was lower as previously mentioned. This is due to the more significant biodegradation by means of non-electrochemical pathways. As expected, sodium acetate concentration in the reference test, with abiotic anode, remained constant (Fig. 4a).

Figure 5. (a) Current density; (b) Cathode and anode potential.

3.3. Electrical performance of the BES

To assess the performance of the systems throughout the experiments, the Faradaic efficiencies over time were determined (Fig. 6).

Cathodic faradaic efficiencies were lower than 70% at the beginning of the batch cycle, decreasing over time until reaching a value of 11%. These low efficiencies are due to the parallel H_2 promotion at the cathode, as previously justify. The efficiencies decreased owing to the depletion of chlorophenols to be reduced at the cathode. Anodic faradaic efficiencies are low too, in agreement with the literature for bioanodes inoculated with a mixed culture, since not all the sodium acetate is oxidized through (bio)electrochemical pathways, giving electrons (Das et al., 2020). The efficiency drops more dramatically in the last day of operation, due to the growth of non-electroactive bacteria.

Figure 6. Evolution over time of the faradic efficiencies in the MFC.

3.4. Toxicity and biodegradability

Table 1 reports on biodegradability and toxicity data of the initial wastewater and once treated. The Biochemical Oxygen Demand (BOD) at $t = \infty$ was calculated according to Eq.7, where Y is the BOD at certain time t , t is the time in days, BOD_f is the BOD reached at $t = \infty$ in $mg L^{-1}$ and k is the biodegradation constant in d^{-1} . The constant k gives an

idea of the kinetics of the biodegradation test. BOD_f and k parameters were estimated though a fitting of the experimental data obtained in the BOD_5 tests performed to the wastewater at the beginning and at the end of the (bio)electrochemical dechlorination treatment.

$$Y = BOD_f \cdot (1 - e^{-kt}) \quad (7)$$

In order to fit the equations to the data set the Simplex algorithm was used according to the protocol defined in the literature (Carboneras et al., 2017; Fernández-Morales et al., 2010). The fitting aim was to achieve the minimum value of the objective error function, defined as shown in eq. 8, where n is the number of data points, X_i is the predicted values of the variable at the i^{th} measurement, and $X_{exp,i}$ is the actual values at the i^{th} measurement.

$$\varphi = \sum_{i=1}^n (X_i - X_{exp})^2 \quad (8)$$

Table 1. Parameters of biodegradability and toxicity before and after the treatment.

	BOD_f (mg L ⁻¹)	K (d ⁻¹)	COD (mg L ⁻¹)	BOD/COD	EC 50 _{5 min} (mmol L ⁻¹)
Before	-5	0.2	372	-0.013	0.021
After	240	0.9	418	0.568	0.228

Regarding the table, the negative initial value of the BOD_f indicates its toxicity. Additionally, the increase in the value of COD, once the wastewater was treated, was in

agreement with the reductive treatment carried out, since the dechlorination process implies a reduction of the molecule. In addition, the treated wastewater is far more biodegradable than before the reductive treatment, as expected. Chlorines bonded to the aromatic ring provide the molecule with toxicity and biorefractoriness, therefore once 2,4-DCP is dechlorinated, the biodegradability is enhanced resulting in a $BOD_f=240$ $mg\ L^{-1}$ and $BOD/COD=0.568$. The negative value of BOD of the wastewater with 300 $mg\ L^{-1}$ of 2,4-DCP gives not only idea of how unbiodegradable it is, but also about its toxicity (although this BOD tests are not destined to toxicity determinations). A negative BOD implies inhibition of the endogenous respiration, in consequence BOD of the tests were lower than BOD of the blank reference test.

Toxicity of the wastewater before and after the cathodic dechlorination treatment was determined through the light emission of luminescent bacteria, *Aliivibrio fischeri*, as detailed in Materials and Methods section. The toxicity was evaluated as EC_{50} by measuring the effective concentration that produces a 50% of inhibition, in molar concentration of phenolic compound, since the molar concentration of phenolic compounds is constant over the treatment, but not the mass concentration (the aromatic ring is losing chlorine atoms). Values of EC_{50} at 5 min and 15 min were very similar, therefore only EC_{50} at 5 min is reported on the graph. The toxicity after the treatment was reduced in one order of magnitude.

4. CONCLUSIONS

From this work, the following conclusions can be drawn:

- Bioelectrochemical technology under the conditions used in this work proved to be viable for the electrochemical dechlorination of 2,4-DCP. The MFCs, which worked with an external load of 120 Ω , operated in batch mode achieving a dechlorination of a 57% after 24 hours and an 88% after 72 hours in the batch experiment.
- The current density generated by the systems ranged from 0.14 to 0.04 mA cm^{-2} , because of the depletion of the 2,4-DCP throughout the dechlorination batch process. On the contrary, the current generated in the reference test with abiotic anode was negligible, what proves the bio-electro-catalytic effect of the electroactive bacteria in the MFCs. Moreover, the concentration of sodium acetate and 2,4-DCP remained constant throughout the process, indicating that the dechlorination reaction did not take place in the abiotic reference test.
- After the dechlorination treatment, the biodegradability (BOD/COD) was enhanced from a -0.013 to a 0.568 and the toxicity decreased as indicates the increase of the EC_{50} from 0.021 to 0.228 mmol L^{-1} .

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