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COUPLING A PERMEABLE FIXED-BED BIOLOGICAL BARRIER AND ELECTROKINETICS FOR THE IN-SITU ELECTROBIOREMEDIATION OF OXYFLUORFEN POLLUTED SOIL

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Abstract:	<p>This work studies the in-situ electrobioremediation of an oxyfluorfen polluted clay soil in a two-stage method. First, a fixed bed biofilm reactor for oxyfluorfen biodegradation in wastewater was developed; It treated wastewater with 200 mg L⁻¹ of oxyfluorfen and reached 100% of oxyfluorfen degradation in 30 h. Second, a portion of the biofilm-covered bed was included into the polluted soil and it was used as a biological permeable reactive barrier (BioPRB), whereas electrokinetics were applied to promote the contact between the pollutant and microorganisms into the soil. The electrobioremediation study was performed in a bench scale setup under 1.0 V cm⁻¹ at room temperature and under periodic polarity reversal (2 d⁻¹) in a two-week batch experiment. Two reference tests were done: (i) a conventional in-situ biological test without electrokinetics and (ii) a conventional in-situ electrokinetic test without using microorganisms. The experimental conditions (temperature, pH, moisture) were correctly controlled in the soil and enabled the microbial activity during the process. A low oxyfluorfen removal efficiency was obtained after 2 weeks (11%) because of the low electrokinetic mobility of such non-polar pollutant into the soil. Despite this low efficiency value, it was considered that the combined biological-electrokinetic technology could be used as a bioaugmentation procedure to perform electrobioremediation processes because the results of both reference tests showed negligible removal efficiencies when using only biological or only electrochemical methods. According to these results, electrobioremediation could be considered as a feasible technology although more retention time would be required to achieve successful remediation results.</p>	
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Dear Editor:

Attached you will find the submitted manuscript “*Coupling a permeable fixed-bed biological barrier and electrokinetics for the in-situ electrobioremediation of oxyfluorfen polluted soil*”, by Silvia Barba, Helena Ocaña, José Villaseñor, Manuel Andrés Rodrigo and Pablo Cañizares (corresponding author: jose.villasenor@uclm.es), in order to be reviewed for a possible publication as original research paper in *Water, Air and Soil Pollution*.

The work described has not been published previously, and it is not under consideration for publication elsewhere. The authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations, and all authors mutually agree that it should be submitted to *Water, Air and Soil Pollution*. This is an original work reached out by the authors in the Chemical Engineering Department of the University of Castilla- La Mancha, Spain. The manuscript was not previously submitted to *Water, Air and Soil Pollution*. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Statement of novelty: This work describes a procedure for the *in-situ* electro-bioremediation of polluted soils. An external biofilm reactor able to degrade a pesticide (oxyfluorfen) was developed and then it was included as a permeable biological barrier into a clay soil polluted with this pesticide. Then, the electrokinetic mechanisms into the soil moved pollutants, nutrients and microorganisms and allowed the bioremediation process. This technology could be considered as a method to introduce microorganisms and activate bioremediation processes into the soil because of the positive contribution of the electrokinetic phenomena in low permeable polluted soils. Moreover, it is focused in the removal of a very common type of pollutants, such as agricultural pesticides.

Yours sincerely

Dr. José Villaseñor

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COUPLING A PERMEABLE FIXED-BED BIOLOGICAL BARRIER AND ELECTROKINETICS FOR THE IN-SITU ELECTROBIOREMEDIATION OF OXYFLUORFEN POLLUTED SOIL

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Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract

This work studies the in-situ electrobioremediation of an oxyfluorfen polluted clay soil in a two-stage method. First, a fixed bed biofilm reactor for oxyfluorfen biodegradation in wastewater was developed; It treated wastewater with 200 mg L⁻¹ of oxyfluorfen and reached 100% of oxyfluorfen degradation in 30 h. Second, a portion of the biofilm-covered bed was included into the polluted soil and it was used as a biological permeable reactive barrier (BioPRB), whereas electrokinetics were applied to promote the contact between the pollutant and microorganisms into the soil. The electrobioremediation study was performed in a bench scale setup under 1.0 V cm⁻¹ at room temperature and under periodic polarity reversal (2 d⁻¹) in a two-week batch experiment. Two reference tests were done: (i) a conventional in-situ biological test without electrokinetics and (ii) a conventional in-situ electrokinetic test without using microorganisms. The experimental conditions (temperature, pH, moisture) were correctly controlled in the soil and enabled the microbial activity during the process. A low oxyfluorfen removal efficiency was obtained after 2 weeks (11%) because of the low electrokinetic mobility of such non-polar pollutant into the soil. Despite this low efficiency value, it was considered that the combined biological-electrokinetic technology could be used as a bioaugmentation procedure to perform electrobioremediation processes because the results of both reference tests showed negligible removal efficiencies when using only biological or only electrochemical methods. According to these results, electrobioremediation could be considered as a feasible technology although more retention time would be required to achieve successful remediation results.

Keywords

Oxyfluorfen, polluted soil, electrobioremediation, biological permeable barrier, biofilm reactor.

1. Introduction

Currently, the pollution originated because of the use of pesticides is becoming a serious environmental problem. Because of this type of pollutants, the quality of several water reservoirs is negatively affected, and their use for human consumption is sometimes not advised. This problem can affect the ecosystems and specifically the animals in the food chain (Chowdhury et al., 2008), where pesticides may accumulate. Moreover, because of the persistence of pesticides, these compounds remain in the environment for a long time, and their presence has been recognized as able to produce different hazardous effects on humans (Morillo and Villaverde, 2017). Oxyfluorfen is a frequently used herbicide to control weeds. It has very low solubility in water (0.116 mg L^{-1}), low vapour pressure (0.026 mPa at $25 \text{ }^{\circ}\text{C}$), and low biodegradability because the metabolism of this compounds by the plants is not possible, and it is only slowly assimilated by microorganisms (Sondhia, 2010; Calderón et al., 2015).

Soil is a non-renewable natural resource that is affected by pesticide pollution. Thus, its remediation should be performed in countries with high environmental awareness and is a topic of interest for environmental authorities. Currently, different technologies are available to remediate polluted soils based on notably different biological, chemical, electrochemical, physical or thermal techniques. Among them, the study of electrochemically assisted technologies is increasing because the remediation of soil is enabled *in situ*, i.e., it is not necessary to excavate and transport the polluted soil to the remediation facilities (Reddy and Cameselle, 2009).

The electrokinetic (EK) treatment consists of the application of an electric field between electrodes, which are inserted in the soil. The direct electric current mobilizes ionic and molecular species through the soil, including nutrients, pollutants or even microorganisms in the case of combining with bioprocesses (Reddy and Cameselle, 2009).

Electromigration, electrophoresis and electroosmosis are different EK phenomena that are responsible for the transport of the species in the soil (Paillat et al., 2000), so the main objective of the EK technology is to mobilize pollutants to external treatment systems. Previous works report that EK is a cost-effective (Cameselle, 2014) and successful *in situ* technique to remove pesticides from low-permeability soils, where conventional pumping methods are ineffective (Reddy and Cameselle, 2009). Nonetheless, the EK remediation presents some limitations as to the low mobilization of non-polar pollutants, the temperature increase in soil because of ohmic resistance or the formation of strong pH gradients near the electrodes, etc. To improve the EK technology, it can be combined with other technologies (Yeung and Gu, 2011). In recent years, the interest in combining EK treatment with bioremediation processes has increased. This technology is known as electrobioremediation (EBR). The target of EBR is combining the EK phenomena with microbiological degradation, which may be an advantage to enable the *in situ* pollutant degradation (Wick et al., 2007). Particularly, one EBR option is using a permeable reactive biological barrier (BioPRB or biobarrier) as a biological reactor in the soil and coupled with the EK processes (Mena et al., 2015). The biobarrier is commonly a porous supporting material, where the pollutant-degrading microorganisms are adhered on its surface to form a biofilm, and it works as a fixed-film bioreactor. The use of a biobarrier can achieve *bioaugmentation* (Gill et al., 2014), which is the inoculation in the soil of an external microbial culture adapted to the biodegradation of the soil pollutant. This biobarrier can be located in the centre of the soil to be remediated to avoid the extreme pH fronts near the electrodes and in the direction of the groundwater flow. The working principle of such system (EK-BioPRB) is that the pollution plume must be moved through the biobarrier inserted in the soil by EK processes; thus, it can be eliminated there (Mena et al., 2016a).

A BioPRB for *in situ* soil remediation purposes must be developed using a conventional method with two stages. First, a microbial acclimation process is necessary because the microbial culture must be adapted to use the soil pollutant as substrate. The second stage is the microbial inoculation, biofilm formation and development until a stationary biofilm reactor has been obtained. These two stages can last several weeks. The treatment of wastewater polluted with different types of pesticides using biofilm reactors or biobarriers has been achieved (Ramos-Monroy et al., 2013; Sandoval-Carrasco et al., 2013). However, to the author's knowledge, oxyfluorfen biodegradation in water has hardly been studied (Chakraborty et al., 2002) because it is a low biodegradable pollutant, and no previous studies about oxyfluorfen biodegradation in biofilm reactors are known.

The present work aims to study the feasibility of a combined technology for the *in-situ* EBR of an oxyfluorfen polluted clay soil. The proposed technology has two stages. First, a fixed-bed biofilm reactor that can biodegrade oxyfluorfen in wastewater is developed, and its stationary performance is monitored. Second, a portion of the biofilm reactor is included into the soil and used as a BioPRB, whereas electrokinetics are used to promote contact between the pollutant and microorganisms. The feasibility of the proposed technology is checked by the comparison with two reference tests: (i) a conventional *in-situ* biological test without the application of electrokinetics (Bio test) and (ii) a conventional *in-situ* EK test without the application of microorganisms to the soil (EK test). As previously stated, despite the serious environmental problem related to this type of herbicides, studies about the biodegradation of oxyfluorfen are notably scarce, and no previous research about the EBR of oxyfluorfen polluted soils by other authors has been found.

2. Materials and methods

The description of experimental devices and the experimental, sampling and analytical methods have been carefully described in previous works by the same authors and related to the same research programme (Ramírez et al., 2015a; Mena et al., 2016b,c). In the following subsections of this work, only the important details are described.

2.1. Materials

The soil in this work is a natural clayey soil provided by Millas Hijos Ceramics (Toledo, Spain), whose characteristics are shown in Table 1.

A commercial herbicide, whose composition contains oxyfluorfen (Fluoxil 24 EC, from Cheminova Agro, Madrid), was used as a model of chlorinated pesticide to artificially pollute the soil. Its composition is 24% oxyfluorfen, solvents such as xylene (<60%) and cyclohexanone (<13%) and a low amount of surfactant (calcium dodecylbencene sulfonate) (<4%) which is added to the formulation to promote the correct spreading of oxyfluorfen in soils.

The soil was artificially polluted. It was vigorously mixed with a solution of Fluoxil 24 to obtain the complete homogenization to provide a moisture of 25% (saturation conditions of this type of soil). The oxyfluorfen concentration on the wet weight basis was 20.0 mg kg⁻¹ (26.7 mg kg⁻¹ on the dry soil basis).

The oxyfluorfen-degrading microbial consortium was acclimated by a procedure described in previous works (Moliterni et al., 2012). Activated sludge, which was obtained from the biological reactor of an oil-refinery wastewater treatment plant (Puertollano, Spain), was used as the starting seed for this process. The culture medium was Bushnell-Hass Broth (BHB), including inorganic nutrients (0.20 g Mg SO₄ L⁻¹, 0.02 g CaCl₂ L⁻¹, 1.00 g KH₂PO₄ L⁻¹, 1.00 g (NH₄)₂HPO₄ L⁻¹, 0.05 g FeCl₃ L⁻¹ and 1.00 g KNO₃ L⁻¹) and Fluoxil 24 (reaching 200 mg L⁻¹ of oxyfluorfen as the final concentration

in the medium) was used as the organic substrate source. Glucose was also used as the co-substrate during the first weeks. Then, MALDI TOF Mass Spectrometry AXIMA-Assurance equipment (Biotech technology, SHIMADZU, Germany) was used to identify the microbial species in the acclimated culture. Several batch biodegradation tests were performed using the acclimated culture to check the feasibility of the oxyfluorfen biodegradation.

2.2. Biobarrier development and performance

A fixed-bed biofilm bioreactor for the oxyfluorfen-polluted wastewater treatment was developed using the acclimated culture, feeding BHB as the culture media and 200 mg L⁻¹ of oxyfluorfen as the carbon source (Fig. 1a).

The biofilm reactor worked in a weekly sequencing batch reactor operation mode using pumice stone as the supporting material (average diameter 3 mm), and the hydraulic retention time was 150 h. The reactor performance was checked under stationary conditions; subsequently, to check its robustness under increasing organic loadings, five batch experiments with different oxyfluorfen concentrations (100, 200, 300, 500 and 800 mg L⁻¹) were performed. A portion of the stationary biofilm will be later used as BioPRB in the polluted soil (section 2.4).

2.3 Electrokinetic installation

The soil remediation process was performed in a bench scale setup (Fig. 1b). The facility is a transparent methacrylate EK cell divided into five compartments. The polluted soil is located in the central compartment (20 cm), and the biobarrier (5 cm wide) is introduced in the central position of this compartment and separated by a nylon mesh (0.5 mm mesh size). At both sides of the soil are the anodic and cathodic compartments, which are also separated by nylon mesh. They behave as electrode wells that contain the electrolyte, which is transported through the soil. In turn, each electrode compartment is connected

to an external collection compartment, which accumulates the liquid transported via electro-osmosis. Graphite electrodes were used, which were supplied by Carbosystem (Madrid, Spain), whose dimensions were 10.0 cm x 10.0 cm x 1.0 cm (same section as the soil). They were connected to the power supply (HQ Power, Gavere, Belgium). Electrolyte (as groundwater) was used (80.75 mg L⁻¹ Na₂SO₄, 70.00 mg L⁻¹ NaHCO₃, 30.36 mg L⁻¹ NaNO₃) and introduced into the electrolytic compartments to homogeneously distribute in the soil by the EK phenomena during the experimental process. The initial soil moisture was 25%. As reported in previous works (Mena et al., 2016c), nutrient limitations to the biological process can occur. To avoid this issue, the concentrations of ammonium, phosphate and nitrate were provided in excess by supplying BHB medium in the biobarrier position (Fig. 1). In addition, the water removed by electro-osmosis was replaced by the electrolyte control level system.

2.4. Experimental procedure of soil electrobioremediation

The biobarrier was located in the compartment at the centre of the setup. The polluted soil was compacted on both sides of the biobarrier. An electrolyte solution (synthetic groundwater) was added in the anodic and cathodic compartments. The anodic compartment was connected to the control level system to ensure the moisture and electrolyte levels in the soil during the tests.

An EBR study using the active and stationary permeable reactive biobarrier was conducted in a two-week batch experiment. The experiment was performed using potentiostatic conditions at 1.0 V cm⁻¹ (20.0 V) at room temperature, and the frequency in the periodic polarity reversal was 2 d⁻¹ (changed every 12 hours).

Additionally, two experiments were performed to act as the reference tests: (i) a test without the application of electrokinetics (*Bio* test), where the microorganism and nutrients were homogeneously distributed into the soil, but no electric current was

applied. The *Bio* test helps to know the possible oxyfluorfen biodegradation in a soil that contained acclimated microorganisms without the contribution of mobility caused by the EK phenomena. The second reference test (ii) was a conventional EK test without the biobarrier, i.e., without the application of microorganisms to the soil (*EK* test). The *EK* test is abiotic and will help to know the possible removal of oxyfluorfen by non-biologically assisted mechanisms, i.e., EK removal to the wells, or evaporation.

2.5. Sampling and analyses

Biofilm reactor stationary performance and batch experiments

Oxyfluorfen, xylene, inorganic nutrients and microbial biomass concentrations were measured during the biofilm reactor stationary operation and batch experiments. Oxyfluorfen and xylene concentrations were analysed by HPLC as later described. The Volatile Suspended Solid concentration method was used to measure the microbial biomass growth (Moliterni et al., 2012). FESEM (Field Emission Scanning Electron Microscopy) images were taken by the specialized microscopy services of University of Castilla La Mancha.

Electro-bioremediation study

The conductivity, pH, nutrient concentrations, and oxyfluorfen concentrations in the electrolytic compartments and electroosmotic flow (EOF) were monitored daily. To characterize the soil, samples were taken only at the beginning (before placing it in the cell) and the end of the tests, not to modify the permeability properties of the soil (*post-mortem* analysis) (Ruiz et al., 2014). At the end of the experiment, soil samples were taken in four longitudinal positions that correspond to intermediate points between anode and cathode compartments. In addition, each position considers four different sample points divided in different axial positions. The experimental points in Figs. 6 and 7 indicate the concentrations from the four samples at each longitudinal position (position

1 is near the anode, and position 4 is near the cathode at time $t = 0$). Sampling points were selected according to previous studies (Ramírez et al., 2014). It is important to note that the anode and cathode electrodes changed their position because of the polarity reversal. Thus, day 0 ($t=0$) is used as the reference to label soil portions that were taken at end of the remediation study.

Oxyfluorfen and xylene soil concentrations were measured by HPLC (Jasco, Japan). The mobile phase was acetonitrile/water, 70/30 by volume, and the column was a Kinetex 5 μm Biphenyl 100 Å, 150 x 4.5 mm column (Phenomenex, USA) with an isocratic flow rate of 0.6 ml min^{-1} . The wavelength of the UV detector was 220 nm and 20 μl of the injection volume.

To calculate the soil moisture, an amount of soil was dried at 105°C in 24 hours, and the weight loss was measured, which corresponds with the evaporated water. To measure the pH, conductivity and nutrient concentrations (phosphate, nitrate and ammonium), the following procedure was used: 25 ml of Milli-Q water was added to 10 g of dried soil with vigorous agitation for 30 minutes. Then, it was left to decant for a few minutes, and the supernatant liquid was filtered using 0.2- μm nylon filters. A probe multiparameter (SENSLON, HACH) was used to measure the conductivity and pH, and the nutrients were measured using a photometer Galery (Thermo Scientific). The concentration of microorganisms in the soil was expressed as Colony Forming Units (CFU) per gram of dry soil (Ramírez et al., 2015b). The samples were prepared as follows: 1 g of wet soil was added to 10 ml of saline solution (0.9%) and mixed for 3 min using a vortex agitator. Then, aliquots of supernatant (100 μl) were obtained and put on Petri dishes that contained LB as the solid media culture (composition per litre of Milli-Q water: 10.0 g NaCl, 5.0 g yeast extract and 10.0 g casein peptone, 15 g of European Bacteriological Agar and 10.0 g of glucose as carbon source). The incubation time of the plates was 24 h

at 26.5 °C. Colonies were counted after incubation.

3. Results and discussion

3.1 Biofilm reactor performance

Prior to the biobarrier development, the acclimation step in Section 2.1 enabled one to obtain an acclimated mixed microbial culture to degrade oxyfluorfen. The time required to completely degrade 200 mg L⁻¹ oxyfluorfen gradually decreased by consecutive weekly batch biodegradation cycles, which clearly indicates the generation of a microbiological culture adapted to the degradation of this pesticide. The final acclimated microbial culture contained the following species: *Achromobacter denitrificans*, *Achromobacter xylosoxidans*, *Pseudomonas putida*, *Pseudomonas oryzae* and *Brevibacterium casei*. A quick xylene removal was observed in these batch tests: it was totally evaporated in less than 3 days.

Then, the acclimated culture was inoculated into the fixed-bed reactor, and a stationary biofilm reactor was obtained after approximately two months of weekly sequential batch cycles as indicated in Section 2.2. The final stationary biofilm reactor contained approximately 15.3 g of attached volatile solids (VS) per kg of solid carrier (i.e., 12.1 gVS L⁻¹), and a low amount of suspended biomass, which was detached from the biofilm as a result of excess biomass growth, was settled to the bottom and removed from the reactor after every batch cycle. The temperature and electrical conductivity were maintained approximately constant (22°C and 2500 µS cm⁻¹), and the pH was controlled at 7.5 using the buffer capacity of the BHB medium.

Fig. 2a shows the average oxyfluorfen and soluble Chemical Oxygen Demand (COD) concentrations in water in a stationary batch cycle in the biofilm reactor. Oxyfluorfen and the readily biodegradable fraction of the total soluble COD in water were completely

removed after approximately 30 h, whereas approximately 100 h were necessary to eliminate the slowly biodegradable fraction of soluble COD, which contained the remaining compounds in the commercial herbicide and possible organic material from the cell lysis.

Fig. 2b shows the consumption of electron acceptors (dissolved oxygen DO, and nitrate) during a stationary batch cycle in the biofilm reactor. DO was consumed during approximately the first 10 h of the batch operation, which corresponded to most of the removal of oxyfluorfen. Then, DO was nearly depleted, and nitrate was used as the electron acceptor to remove the remaining oxyfluorfen and the remaining easily biodegradable dissolved organic material up to approximately 40 h. After this period, the nitrate concentration slowly decreased, and the initial DO level was attained again because of the slow oxygen transfer from the atmosphere. This result confirms that although oxygen is the most efficient electron acceptor, the microbial culture can degrade oxyfluorfen in the biobarrier under both aerobic and anoxic conditions using oxygen or nitrate depending on the availability of the electron acceptors. This result is a successful result because the biobarrier was designed as a flooded reactor to avoid turbulence as much as possible and the oxygen transfer from the atmosphere at the top of it.

One of the objectives of the biobarrier development is to ensure the capacity of the microbial culture to degrade oxyfluorfen without dissolved oxygen. A portion of this biofilm reactor is later used as BioPRB in the in-situ EBR process, and the oxygen availability is notably low into the soil as observed in previous works (Ramírez et al., 2014). The ammonium and phosphate concentrations during the batch cycles were always sufficiently high to ensure that there were no nutrient limitations, and only a slight decrease from their initial values (130 mgL^{-1} of ammonium and 560 mgL^{-1} of phosphate) was observed.

Fig. 3 shows two FESEM images of the stationary biofilm reactor using different zoom levels: (left) 400 μm and (right) 100 μm . A homogenous biofilm that covered the solid carrier was not observed because biomass particles were distributed in different locations on the surface. We can distinguish: (i) the clean surface of the solid carrier in different zones, (ii) a great amount of inorganic solid particles from the inorganic growth medium, and (iii) microbial biofilm particles (indicated with circles) at different points of the surface.

Fig. 4a shows the results of the batch experiments under different oxyfluorfen initial concentrations. There is a successful biodegradation, although a higher pollutant concentration requires more retention time. No substrate inhibition effects seemed to appear when using such high oxyfluorfen levels, and no bioadsorption (instead of biodegradation) was detected as the removal mechanism.

Fig. 4b shows the suspended biomass concentration (data points) generated during the batch experiments as a result of the microbial growth and detachment. The biomass growth was notably low compared to the total amount of biomass attached as a biofilm in the stationary process (marked as dashed horizontal line), as expected in a biofilm reactor with such a high biomass concentration. There was no appreciable difference when different substrate concentrations were used.

According to the results of the performance of the biofilm reactor, the biodegradation mechanism was not considered a limiting step in the possible removal of oxyfluorfen in soil when this culture was used. Then, a portion was located as BioPRB in the soil EBR experiment.

3.2 Electro-bioremediation results

Fig. 5 shows the experimental conditions monitored in the polluted soil and electrolyte wells during the EBR experiment using the BioPRB. The behaviour and evolution of such

parameters help to explain the results of the oxyfluorfen removal from the soil. Moreover, most of these results have been compared to reported results in a previous work (Barba et al., 2017) which showed the experimental conditions in an EBR process without the BioPRB, i.e., using a biostimulation option with the distributed microbial culture in the polluted soil. Fig. 5a shows that the electric current through the soil is approximately constant. Although the electro-osmotic flow (EOF) removed water and electrolytes from the soil (Fig. 5d), the water level control system in the electrode wells enabled one to replace electrolytes and maintained the electric current density. Previous works in our research group showed a gradual current decrease because of the removal of electrolytes in soil under potentiostatic conditions (Ramírez et al., 2015a). The pH values were maintained approximately constant (8.0 and 10.5) in the anode and cathode compartments (Fig. 5b). The soil temperature (Fig. 5c) was maintained at 22-25°C, i.e., a slight soil heating occurred because of the ohmic losses, but the soil temperature was always adequate for the expected microbial activity. Finally, the EOF (Fig. 5d) gradually decreased, and it was clearly lower than the previously reported value (Barba et al., 2017) under identical experimental conditions but without the BioPRB. Thus, the inclusion of a porous material such as the biofilm-covered carrier as BioPRB caused a clear disturbance in the low permeability of the clay soil. It is well known that the EOF is inversely proportional to the soil porosity (Reddy and Cameselle, 2009), and the EOF through low-permeability regions is significantly greater than the EOF in higher-porosity regions. Fig. 6 shows the concentration values of different parameters in the soil at the beginning and the end (two weeks) of the experiment. The left and right vertical axes in Fig. 6 indicate the position of the anode and cathode wells respectively at the beginning, and the horizontal axis indicates different soil positions between the electrodes. The grey vertical bar indicates the BioPRB position. The soil moisture and soil pH were successfully

controlled (Figs. 6a and b). As indicated, the water level control system enabled one to replace the water removal because of the EOF. The pH was correctly controlled using the polarity reversal strategy as proven in several previous works about soil electroremediation by different authors (Mena et al., 2016c). Despite the water and electrolyte replacement and the constant electric current, a clear decrease in soil electrical conductivity was observed (Fig. 6c). However, the soil conductivity values were always included in the range suitable for microbial activity.

Fig. 6d shows the microbial concentration profile at the end of the experiment. The initial microbial concentration (horizontal dashed line) only refers to the initial autochthonous heterotrophic microbial population in the soil. This population was not acclimated to oxyfluorfen consumption, and no biodegradation activity was considered. The solid line across Fig. 6d indicates a homogeneous microbial distribution from the BioPRB to the soil because of the biofilm detachment and EK transport through the soil in both directions because of the changes in polarity (electrophoresis) and EOF. This behaviour has been reported in previous works using different microbial cultures and types of barriers (Mena et al., 2016c). According to these results, we can assume that the microbial activity has been present in the soil and not only in the central Bio-PRB zone. Thus, the BioPRB functions as a central permeable barrier and a method to introduce the acclimated culture (bioaugmentation) to the entire soil sample. Alternatively, the bioaugmentation through the electrode wells is not advisable because of the expected harmful effects of extreme pH values on the microbial culture.

Fig. 7 shows the results of the remediation experiment. Fig. 7a focuses on the oxyfluorfen concentration profile across the soil at the beginning (horizontal dashed line) and at the end of the experiment (the solid line indicates the average value from different axial measurements). Two important results are observed: the oxyfluorfen concentration

decreased, so a partial removal was achieved, and the oxyfluorfen removal was extended to the entire soil instead of locating in the central soil zone near the BioPRB. These results are consistent with the detected microbial activity across the soil.

Fig. 7b shows the oxyfluorfen removal efficiency after two weeks in the EBR experiment using BioPRB and in the two reference experiments (Bio and EK). The removal efficiency of both reference tests was negligible, i.e., both contributions (biological and EK) are necessary to remediate the soil. On one hand, although the pollutant can be easily degraded by the microbial culture (as shown in Section 3.1) and the microbial culture was homogeneously distributed across the soil in the Bio test, the pollutant was not degraded in the soil because of the transport limitations in this low-permeability soil. Oxyfluorfen is a non-soluble compound, and xylene (used as the surfactant) was quickly evaporated. Thus, it was assumed that oxyfluorfen was not accessible to the microbial population. On the other hand, the EK test moved water alternatively in both directions, but oxyfluorfen was not removed, and it was not detected in the electrolyte wells. Thus, it can be assumed that the oxyfluorfen removal in the BioPRB experiment was the consequence of coupling the biological and EK mechanisms.

However, the removal efficiency was low: only 11% after two weeks. This result is low compared to those in previous works using oxyfluorfen-polluted soil (Barba et al., 2017) and diesel-hydrocarbon-polluted soil (Mena et al., 2016b). The reason of these differences is mainly related to the low mobility of oxyfluorfen, and the inclusion of a BioPRB decreases the EOF as previously stated. According to these results, higher retention time is necessary to make this technology effective.

4. Conclusions

A microbial culture that can biodegrade oxyfluorfen was grown as a biofilm in a fixed-bed biofilm reactor. The biofilm reactor works under stationary conditions and can

degrade 200 mgL⁻¹ of oxyfluorfen in approximately 30 h. The culture uses both oxygen and nitrate as electron acceptors and can degrade oxyfluorfen concentrations up to 800 mgL⁻¹. A portion of the biofilm reactor was used as a central permeable biological barrier for the *in-situ* EBR of oxyfluorfen-polluted clay soil. The microbial activity was detected in the soil after a two-week batch period probably because of the biofilm detachment and microbial EK transport. The reference tests prove that the combination of the biological and EK mechanisms is necessary. This technology can be used as a bioaugmentation method for the EBR technology, although the low removal efficiency suggests that oxyfluorfen has low mobility, and higher retention time is necessary to obtain successful results.

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Fig. 1 (a) Biofilm reactor scheme. (b) Experimental installation for soil EBR: scheme and photograph

Fig. 2 Biofilm reactor performance: (a) oxyfluorfen and soluble COD degradation and (b) dissolved oxygen and nitrate consumption in a stationary batch cycle

Fig. 3 FESEM images of the stationary biofilm

Fig. 4 Biofilm reactor batch experiments using different oxyfluorfen initial concentrations: (a) Oxyfluorfen concentration; (b) Suspended biomass growth: legend indicates the initial oxyfluorfen concentrations, and the dashed horizontal line indicates de fixed biomass concentration in the biofilm reactor

Fig. 5 Experimental conditions monitored during the EBR process: (a) electric current through the soil; (b) pH in the electrode wells ($\diamond$ cathode, $\blacklozenge$ anode); (c) average soil temperature; (d) EOF through the soil

Fig. 6 Concentration profiles in the soil: (a) moisture; (b) pH; (c) electrical conductivity; (d) microorganisms. Horizontal dashed lines: initial value; Solid lines: final values obtained as the average of different measurements at different axial positions in the soil (data points). The grey vertical bar indicates the central BioPRB position

Fig. 7 Oxyfluorfen removal results. (a) Oxyfluorfen concentration profiles in the soil (dashed line: initial; continuous line: average final; the grey vertical bar indicates the central BioPRB position). (b) Oxyfluorfen removal efficiency compared to the two reference tests

Table 1. Properties of the soil used in the experiments.

Mineralogy (%)	
Quartz	12
Feldspar	6
Calcite	1
Kaolinite	23
Glauconite	24
Muscovite	8
Montmorillonite	20
Illite	6
Other properties	
Dry density / g cm ⁻³	1.65
Hygroscopic moisture	0.115

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Fig.7