EFFECT OF THE POLARITY REVERSAL FREQUENCY IN THE ELECTROKINETIC-BIOLOGICAL REMEDIATION OF OXYFLUORFEN POLLUTED SOIL

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Abstract

The aim of this work is the evaluation of the applicability of the periodic polarity reversal strategy (PRS) in a combined electrokinetic-biological process for the remediation of clayey soil polluted with a herbicide. Four two-weeks duration electrobioremediation batch experiments were performed in a bench scale set-up using spiked clay soil polluted with oxyfluorfen (20 mg kg^{-1}) under potentiostatic conditions applying an electric field between the electrodes of 1.0 V cm^{-1} (20.0 V) and using periodic PRS with four frequencies (f) ranging from 1 to 6 d^{-1}. Results are compared with those obtained in remediation test carried out without PRS and also with other in which a single bioremediation test is applied. The microbial consortium used was obtained from an oil refinery wastewater treatment plant and acclimated to oxyfluorfen degradation. Main soil conditions (temperature, pH, moisture and conductivity) were correctly controlled during the tests carried out using PRS. On the contrary, the electroosmotic flow clearly decreased

as f increased. The uniform soil microbial distribution at the end of the experiments indicates that the microbial activity remained in every parts of the soil after two weeks when applying PRS. Despite the adapted microbial culture was capable of degrade 100% of oxyfluorfen in water, the remediation efficiency in soil in a reference test, without the application of electric current, was negligible. However, under the low voltage gradients and polarity reversal, removal efficiencies between 5 and 15% were obtained, and it suggested that oxyfluorfen had difficulties to interact with the microbial culture or nutrients and PRS promotes transport of species which obtain a positive influence on remediation. An optimal f value was observed between 2 and 3 d^{-1}.

1. Introduction

Pesticide pollution is becoming a very serious environmental problem with a highly negative impact on the quality of water reservoirs, which even may prevent their use for human consumption. It may also affect seriously to the surrounding ecosystem in particular to the health of animals through the complete food chain[1], where pesticides may be accumulated.

Persistent pesticides remain in the environment for very long periods and they can produce different harmful effects on humans, some of them even as serious as cancer. Moreover, many of them are known to behave as endocrine disrupters. Oxyfluorfen is framed within this category. This herbicide is frequently used to control weeds. It has low solubility in water (0.116 mg L^{-1}) and low vapour pressure (0.026 mPa at 25 ºC) and its biodegradation is known to be very poor: plants cannot metabolize this compound and it is only slowly assimilated by microorganisms [2,3].

Because of the soil is a non-renewable natural resource, its remediation should be mandatory in countries with a high environmental conciseness and, of course, a topic of the major interest for the environmental authorities. Nowadays, there are different technologies available for the remediation of polluted soils, based on very different biological, chemical, electrochemical, physical or thermal processes. Among them, the electrochemically assisted technologies are becoming more and more studied, because they allow to remediate efficiently soils in situ, and they can be easily combined with other technologies, even as different as the bioremediation, sometimes showing important synergisms.

Thus, the electrokinetic (EK) treatments consist of applying an electric field among electrodes placed the soil. This field mobilizes molecular and ionic species through the soil, including pollutants, nutrients or microorganisms in the case of combination of EK with bioprocesses[4]. Different EK phenomena such as electromigration, electrophoresis and electroosmosis are known to be responsible of this transport of species [5].

Currently, EK treatment is started to be marketed as a cost-effective in-situ technique for removing pesticides from soils with low permeability [4]. However, EK remediation is known to have various important limitations, such as the low mobilization of non-polar pollutants, the important raise in soil temperature or the formation of pH gradients in the treated soil. In addition, EK is a non-destructive technology. It means that the contaminant is not degraded, but it is transported to wells where is accumulated. Then, the pollutant has to be removed and treated off-site [6]. Because of it, as a way to improve the outcomes of this technology, it was recently proposed the combination of EK technology with other conventional technologies [7]. In the recent years, interest has increase in coupling EK treatment to bioremediation, and this combination is known as electro-bioremediation (EBR). The aim of EBR is combining the EK movement with microbiological degradation, which may be an advantage for enabling the in situ pollutant elimination [8] because EK can be efficient in the transport of microorganisms, nutrients or pollutants. At this point, it is worth to remind that there are different ways to promote the biological process while soil undergoes EK treatment, such as

- using autochthonous soil microbial population and adding nutrients in the soil (biostimulation) transported by electromigration,
- adding microorganisms all over the soil previously acclimatized to the pollutant degradation (bioaugmentation) and dragging them with the electro-osmotic flow or

– employing reactive permeable biobarriers, through which the contaminant passes by the different electrokinetic transport phenomena [9].

In all these processes EBR processes have to face several drawbacks being the most important the pH control,which is the most critical parameter in order to keep the microorganisms alive [7,10]. As it is well-known, large pH gradients are caused by the transport of protons and hydroxyl ions generated by water electrolysis on the electrodes surfaces.

Different strategies have been proposed to maintain a suitable pH in the soil during EBR processes [7,10-12]. One of the most interesting is the periodic change of the polarity of the electric field (so-called as periodic polarity reversal) [13]. Periodic polarity reversal has been used in some different treatments such as electroremediation of soils with emerging contaminants [14] and electrobioremediation of diesel polluted soils [13,15,16]. In both cases, successful results were obtained, and pH was properly controlled, while the process has obtained a good treatment efficiency. Based on the experience gained by this research group, it may be guessed that one of the critical factors that can influence this technology is the frequency in the polarity changes, and to the best of our knowledge this factor has not been deeply studied yet. In this context, the aim of this work is to know how the reversal frequency influences on the performance of the periodic polarity reversal strategy in an EK-biological process for the remediation of oxyfluorfen polluted clayey soil. This evaluation of the influence of the frequency represents the main novelty of this work as compared to the previous existing literature.

2. Materials and methods

The experimental devices, and the experimental, sampling and analytical methods have been described elsewhere in previous research performed by the same authors [9,17,18] and related to the same research programme. The following subsections only describe the important details required for a good understanding of this work.

2.1 Electrokinetic installation

The experiments were carried out in a bench scale set-up (Fig. 1). The cell was made is transparent methacrylate and it was divided into five compartments. Pesticide polluted soil was located in central compartment. The anodic and cathodic compartments were placed at both sides of the soil compartment, separated by nylon mesh (0.5 mm. mesh size). They behaved as electrode wells, containing the electrolyte which is transported through the soil. In turn, each electrode compartment was connected to a collection compartment, which the liquid transported by electro-osmosis accumulates. Graphite plates supplied by Carbosystem (Madrid, Spain) (10.0 cm x 10.0 cm x 1.0 cm) are used as anodes and cathodes. They are connected to the power supply (HQ Power, Gavere, Belgium). The section electrodes and soil column is the same.
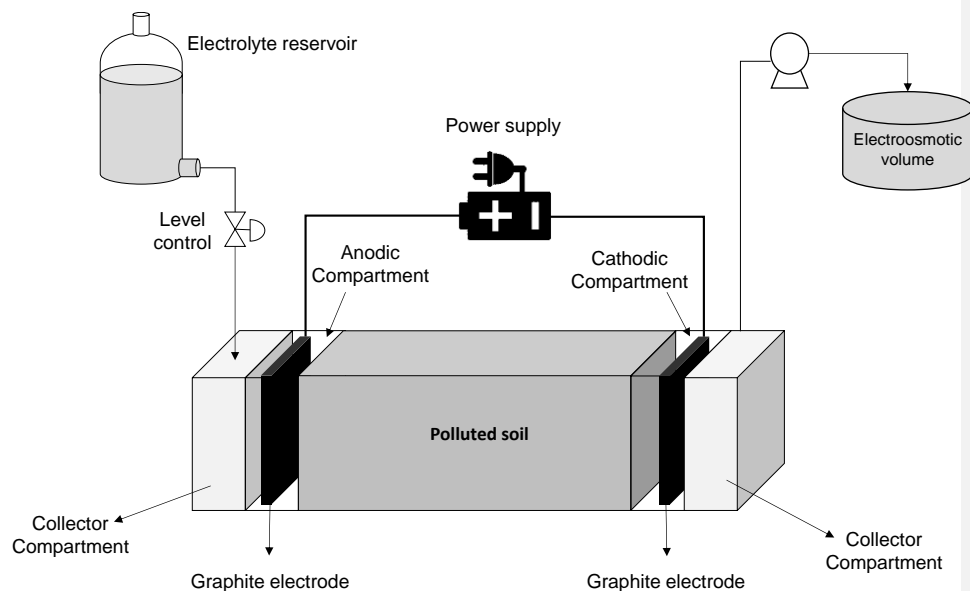
Figure 1. Experimental installation.

2.2 Materials

Clayey soil was provided by Millas Hijos Ceramics (Toledo, Spain). Its main characteristics are shown in Table 1.

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

The oxyfluorfen-degrading microbial consortium was obtained by a standard acclimation procedure described elsewhere [19]. Activated sludge obtained from the biological reactor of an oil-refinery wastewater treatment plant (Puertollano, Spain) was used to seed the

bioreactor. The feeding solution consists of Bushnell-Hass Broth (BHB) culture media (0.20 g Mg SO_4 L^{-1}, 0.02 g $CaCl_2$ L^{-1}, 1.00 g KH_2PO_4 L^{-1}, 1.00 g $(NH_4)_2HPO_4$ L^{-1}, 0.05 g $FeCl_3$ L^{-1} and 1.00 g KNO_3 L^{-1}) and Fluoxil 24 (with a concentration of oxyfluorfen of 200 mg L^{-1}) as organic substrate (sole carbon source). Once the acclimation period was expected to be completed, the microbial culture performance was checked by several batch biodegradation tests. Then, MALDI TOF Mass Spectrometry AXIMA-Assurance equipment (Biotech technology, SHIMADZU, Germany) was used to characterize and identify the microbial species in the acclimated culture.

The electrolyte used was synthetic groundwater (80.75 mg L^{-1} Na_2SO_4, 70.00 mg L^{-1} $NaHCO_3$, 30.36 mg L^{-1} $NaNO_3$). It was introduced into the electrolytic compartments in order to be distributed through the soil by electrokinetic phenomena during the experiments. BHB medium was also used to supply the necessary moisture (25%) and nutrients to the soil. Ammonium, phosphate and nitrate were supplied in excess concentrations in order to avoid nutrient limitations to the biological process as reported in previous works [9].

2.3. Experimental procedure

In order to simulate a degraded soil environment with oxyfluofen pollution and microorganisms, soil was arranged for the experiment by mixing microorganisms and oxyfluorfen outside the electrochemical cell. To do this, microorganisms acclimated for the degradation of oxyfluorfen were centrifuged at 3900 rpm for 10 min and re-suspended with BHB medium. Then, this suspension, Fluoxil 24 and the soil were vigorously mixed up to complete homogenization. Resulting soil had a water content of 25% (near saturation conditions) and a concentration on wet weight basis of oxyfluorfen of 20.0 mg kg^{-1} (26.7 mg kg^{-1} on dry soil basis). After that, this mash was manually compacted in the set-up installation. Synthetic groundwater (electrolyte) was added in both electrode

compartments, and the control level system was connected to the anodic compartment to ensure its replacement during the tests.

Four electro-bioremediation batch experiments were performed under potentiostatic conditions, by applying an electric field between the electrodes of 1.0 V cm^{-1} (20.0 V) using periodic polarity reversal strategy. Duration of tests was 14 days and they were carried out at room temperature. For the polarity reversal, four frequencies were evaluated (1.0, 2.0, 3.0, and 6.0 d^{-1} that is, changing the polarity every 24, 12, 8 and 4 h respectively). Furthermore, two reference experiments were carried out. In the first reference test, no electric field was applied to the soil column (named ad "no EK"). It means that the system is expected to behave as a conventional bioremediation and, hence, this test will help to determine the percentage of oxyfluorfen biodegraded in those conditions and how electrokinetics influenced the process in the four experiments mentioned above. The second reference test was an abiotic EK reference test (named as "no bio") and it was carried out by applying and electric field of 1.0 V cm^{-1} (2.0 d^{-1} polarity reversal frequency) using sterile soil (no microorganisms addition). This tests is expected to shed light on the removal of oxyfluorfen by non-biologically assisted mechanisms, i.e., EK removal to the wells, and evaporation.

2.4. Sampling and analyses

Oxyfluorfen, xylene, and microbial biomass concentrations were measured in the previous batch tests at the end of the acclimation period. Several samples were obtained during 7 days. Oxyfluorfen and xylene concentrations were analyzed by HPLC as described later. The microbial biomass growth was measured by Volatile Suspended Solids concentrations [19].

The pH, conductivity, nutrient concentrations, and organic matter (oxyfluorfen and xylene) concentrations were periodically measured in the electrode wells during the experiments.

To characterize the soil, a post-mortem characterization procedure was followed[20]. Thus, samples of soil were taken only at the start (before the soil was placed in the cell) and at the end of the tests, in order to do not modify the properties of soil. At the end of tests, samples were taken in four longitudinal positions corresponding to intermediate points between anode and cathode compartments. Furthermore, each one of these positions consider four different sample points divided in different axial positions. The average concentration of the four samples taken at the same longitudinal position was represented in results figures, which position 1 is near the anode electrode and position 4 near the cathode electrode. Sample points was chosen according to found literature [21] [18] It is important to remark that anode and cathode change their position owing to the polarity reversal. Because of this, day 0 is taken as reference to label soil portions at end of the remediation for the analytical characterization.

Attending to the analytical methods, the moisture was calculated taking an amount of soil and it was weighed before and after drying at 105 ºC for a day in order to get the loss weight corresponding with water containing in the soil. The pH, conductivity and nutrient concentrations (phosphate, nitrate and ammonium) were measured using the following procedure: 10 g of dried soil adding 25 mL of Milli-Q water and waving by magnetic agitation during 30 minutes; then, it was left to decant a few minutes and supernatant liquid was filtered with 0.2 µm nylon filters; the conductivity and pH were measured by a probe multiparameter SENSLON, HACH; nutrients were measured with a photometer Galery (Thermo Scientific). Oxyfluorfen and xylene concentrations were determined using an HPLC (Jasco, Japan). The mobile phase (acetonitrile/water, 70/30 by volume)

was pumped though a Kinetex 5µm Biphenyl 100Å, 150 x 4.5 mm column (Phenomenex, USA) with an isocratic flow rate of 0.6 ml min^{-1}. UV wavelength detector was set at 220 nm and the injection volume used for all samples was 20 µl. Microorganisms concentration in the soil was measured expressing them as Colony Forming Units (CFU) per gram of dry soil. The preparation of samples was made as follows: 1 g of wet soil with 10 ml of saline solution (0.9%) and it was mixed with the Vortex agitator for 3 min. After that, aliquots of 100 µL of supernatant (soil-saline suspension) were put on Petri dishes. The solid media culture of the dishes was LB medium which composition per liter of Milli-Q water is 10.0 g NaCl, 5.0 yeast extract and 10.0 g casein peptone, 15 g L^{-1} of European Bacteriological Agar and 10.0 g L^{-1} of glucose as carbon source. The plates were incubated for 24h at 26.5 ºC.

Table 1
Properties of the soil used in the experiments shown in this work.

Mineralogy	
Quartz	12%
Feldspar	6%
Calcite	1%
Kaolinite	23%
Glauconite	24%
Muscovite	8%
Montmorillonite	20%
Smectite	-
Illite	6
Other properties	
Dry density / g cm^{-3}	1.65
Hygroscopic moisture	0.115

3. Results and Discussion

3.1. Physico-chemical changes undergone by soil during the electro-bioremediation

Figure 1 shows the average intensity and electro-osmotic flow (EOF) measured during the 15 days-long tests carried out in this work. As it can be seen, reversal of polarity influences both parameters in a different fashion. The higher the frequency, the lower is the resulting EOF and the higher is the current intensity exerted on the electrodes surfaces, for the same electric field applied (1.0 V cm^{-1}). Initially, this is not an expected result, because the rates of the electrochemical and electrokinetic processes is directly related to current. However, there are two important differences that have to be pointed out:

- the reversal of polarity contributes to make less important some of the relevant changes undergone by the soil (pH, depletion of ions) as a consequence of the electrolysis of water, because they are partially balanced in each polarity reversal and

- as the direction of the electroosmotic flux is from cathode to anode, the slight transient draught in the nearness of the cathode may explain that during the polarity reversal some flushing fluid need to be accumulated before an steady state flow is attained, contributing to a decrease in the effective EOF observed.
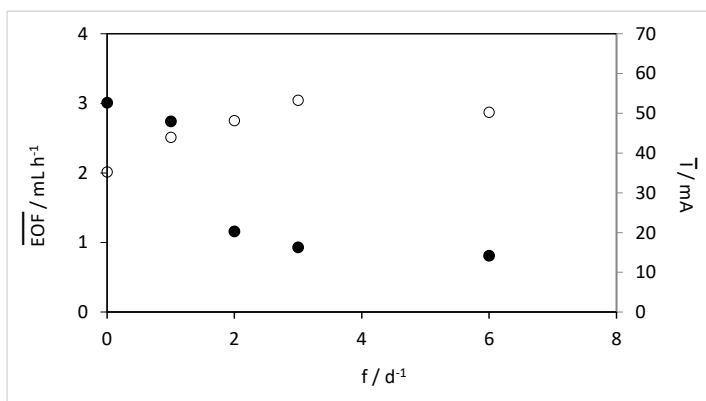

Figure 1. Average exerted intensity (O) and produced electro-osmotic flow (●) in the electro-bioremediation tests carried out in this work

It is important to take in mind that the EOF is obtained alternatively in both electrode compartments because of the PRS applied. Likewise, the EOF values obtained, between 1 and 3 mL h^{-1}, are rather low, and the polarity reversal clearly has a clear negative effect on them [13]. Thus, as compared to single electro-bioremediation treatments (without polarity reversal), a considerably lower value of water volume was moved throughout the soil when applying polarity reveral [17]. It causes a lower transport of water soluble species out of the system but also a lower removal of inorganic nutrients from it, which it is a beneficial effect for the overall process. This is not a negative fact. Thus, under such conditions it has been reported that the contact among the different elements taking part in the biological degradation is increased in low distances (around 100 μm) [8], and no exhaustion of nutrients is obtained. As indicated above, f values from 3 d^{-1} would reduce the need to replenish water, nutrients and electrolytes. At this point, the water removed by EOF contained nitrate, ammonium and phosphate concentrations within the ranges 250-1000 mg L^{-1}, 0.1-1.0 mg L^{-1}, and 1.0-4.0 mg L^{-1} respectively. A single nutrient mass balance indicates that there was enough amount of nutrients available for the complete biological degradation of 20 mg g$^{-1}_{soil}$ of oxyfluorfen. However, it is assumed that higher organic substrate concentrations and retention times would need nutrients replacement [9].

Regarding the reactive processes produced by current, Figure 2 shows the changes in the pH observed in the electrolyte wells and in the soil. As it is well-known, oxidation of water produces protons, while its reduction produces hydroxyl ions. Both ionic species are known to produce the acidic and basic fronts in the soil, which become a real handicap for the combination of this electrochemical technology with any bioremediation process. In addition, they are known to influence many other processes such as ion exchange and precipitation, which also may influence on the viability of the microbial culture.

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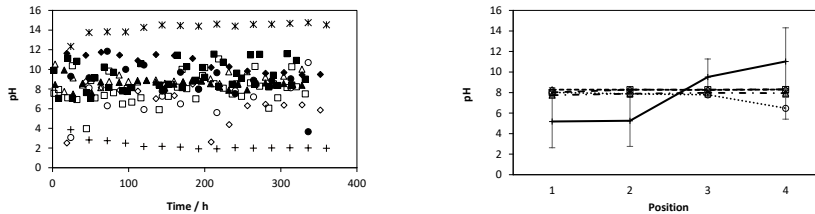
Comentado [MARR2]: Y esto también lo quitaria

Figure 2.Influence of the pH on the electro-bioremediation processes. Part a) Time course of the pH in the electrolyte. Part b) steady state profiles obtained after the post-mortem analysis

The buffering effect of the polarity reversal on the pH is clearly observed in both the pH reached in the electrode wells and in the soil. Changes in the pH of the electrode wells are clearly observed which means that pH is not perfectly neutralized when polarity reversal strategy is applied. However, the acidic and alkaline fronts are not formed. Just a very slight acidification is observed in nearness of one of the electrodes when the soil underwent the lower polarity reversal frequency (as well, with a higher axial variability). On the contrary, the acidic and basic fronts are clearly observed when the soil underwent a non-reversed electric field. This results is very important because it confirms that electrolyte wells and soil are not suitable for microbial life when polarity reversal strategy is not applied.

In addition to the effects caused by the production of protons and hydroxyl radicals on the electrodes, composition of the soil may undergo a significant change in the concentration of ionic species because of the fastest electrokinetic process: the electromigration. This process produces the transport of cations to the cathode and anions to the anode and may cause the depletion of some of them in the soil. As many of the nutrients required for microorganisms to metabolize pollutants are ions (ammonium, phosphates, calcium, iron, etc.), this potential depletion may cause a significant problem

in the combination of biological and electrokinetic processes. Ionic conductivity is a good parameter to follow this problem and Figure 3 shows the changes observed in each of the wells and also in the soil.
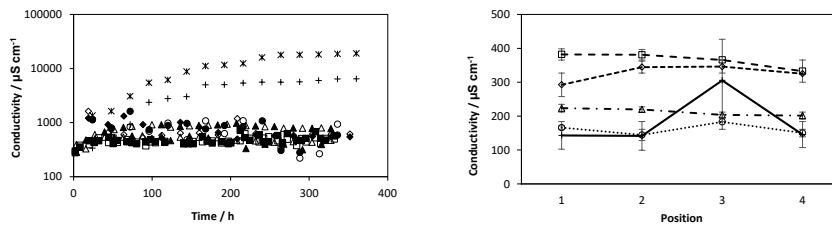

Figure 3. Influence of the conductivity on the electro-bioremediation processes. Part a) Time course of the conductivity in the electrolyte. Part b) steady state profiles obtained after the post-mortem analysis

Regarding the conductivity in the wells, there is a very important increase in the EK test carried out without polarity reversal, which may be associated to the significant increase in the proton and hydroxyl ions concentrations (which in turn, according to Figure 2, is very important in this test). In the tests in which the soil is treated applying polarity reversal strategy, there is a much lower increase in the conductivity, which in this case cannot be associated to changes in the pH but to depletion of ions from soil. Although it seems not to be a direct influence of the frequency on the conductivity of soil, clearly the experiments with the lower polarity reversal (the one with no polarity reversal and that with the lowest frequency) show the lower resulting conductivities in soil, which may suggest a sort of removal of nutrients.

In connection with this observation, a very important point to be discussed is the justification of the choice of the polarity reversal frequencies used in this work. EOF under no periodic polarization is around 72 mL d^{-1}, which means an effective water

transport of around 0.72 cm d^{-1} in the nearness of both electrodes. Distance between anode and cathode is 20 cm and hence the influential EOF zone is around 7% in the nearness of the electrodes wells, which is non negligible. This value is much lower than the expected for the electromigration process, which is known to be at least one log-unit above the EOF (hence covering the complete distance between electrodes) and this means that the reversion frequency chosen is suitable for the distance between electrodes in the lab scale plant because it does not attain the depletion of nutrients, although it clearly has an influence mobilizing water and other important species for the treatment. However, values used in this work are not practical for higher scales because of the well-known complexity of the scale up in soil remediation processes[22-24]. Thus, in order to extrapolate results shown in this paper to higher scale, it has to be taken into account that for a larger scale, reversal time should at least increase in the same ratio that the inter-electrode distance for keeping the same transport properties. Anyhow, this statement needs for further research about the scale-up of polarity reversal electro-remediation processes.

Temperature is also a very important parameter in EK assisted remediation processes. Figure 4 shows the average values of temperature in the whole soil at the start (t = 0) and the end (t = 14 d) in the batch experiments under different polarity reversal frequencies (horizontal axis). The Figure also includes in every case the results obtained in the reference experiment without electrokinetics (labelled as "No EK"). It can be observed that temperature was maintained in values around 20 to 23ºC, and this values were constant during the whole experimental period and every positions in the soil. Compared to the "No EK" experiment, only a slight temperature increase was produced in all cases. This general result is important as the application of the electrical current may produce excessive soil heating which would lead to microbial inactivation. The polarity reversal strategy allowed us to avoid the use of high buffer concentrations for the pH control, and

it also avoided excessive soil heating. Previous works [18] reported soil temperatures around 35ºC, which caused the complete inactivation of the biological process unless polarity reversal and low buffer concentrations were used. Regarding the specific influence of the reversal frequency, a clear effect was not detected.
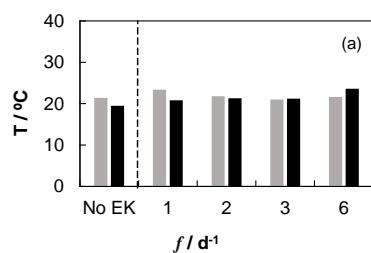

Figure 4. Average temperature in the soil at the start and at the end of the electro-bioremediation tests carried out at 1, 2, 3 and 6 d^{-1} of periodic polarity reversal frequency. Grey bar for the start and black bar for the end of the experiments.

Comentado [MARR5]: Esto lo quitaría

3.2. Oxyfluorfen biodegradation by the acclimated microbial culture

To combine the electrolytic, the electrokinetic and the microbiological processes a microbial suspension was acclimated to the degradation of oxyfluorfen by inoculating sludge coming from an industrial wastewater biological treatment into a bioreactor fed with a synthetic waste containing oxyfluorfen and glucose as co-substrate. This reactor was operated in semicontinuous mode, feeding fresh synthetic wastewater not periodically but only when oxyfluorfen decayed below a set point previously fixed. Results of the acclimation in terms of removal of oxyfluorfen are shown in Figure 5, where it can be seen how the period required for the degradation of oxyfluorfen decreases as time passed by, clearly indicating the generation of a microbiological culture adapted to the degradation of oxyfluorfen.
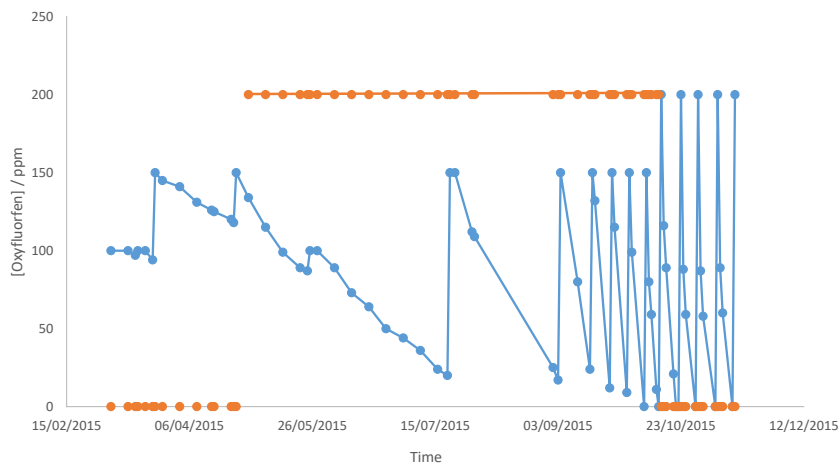
Figure 5. Acclimation of the microbial culture used to remediate the soil.

Biodegradability tests were carried out after this microbial acclimation period of 16 weeks. The tests, performed as reported by [19] proved that the adapted microbial culture was capable to degrade 100% of oxyfluorfen (initial concentration 200 mg L^{-1}) in a period of 5 days. An approximate biomass yield of 1.2 g VSS per g oxyfluorfen removed was observed at 20 ºC and an oxyfluorfen concentration of 200 mg L^{-1}. Also, it was observed a quick xylene removal in these tests, as it was totally evaporated in 3 days. Since the results were repeated over several weeks, it was considered that the culture was acclimated and stationary. According to these successful results, it was considered that the ability of the microbial culture to biodegrade oxyfluorfen would not be the controlling aspect in the oxyfluorfen polluted soil remediation by the in-situ electrobioremediation process. The acclimated microbial culture contained the following species:-----.

It is important to remark that, under our knowledge, there are scarce publications available about biodegradability of oxyfluorfen, or bioremediation of oxyfluorfen polluted soils. [25] studied the biodegradation of oxyfluorfen in soils at 28 ºC and 40ºC, and adding different amounts of mineral fertilizers (NPK) and different concentrations of

oxyfluorfen. The oxyfluorfen removal efficiency at 28 ºC with an initial concentration of 200 mg kg^{-1} for 15 days, was 10.5 %. Furthermore, these authors identified the different species of bacteria founded in their soils, and they concluded that the degradation of oxyfluorfen in soils depended on the temperature of incubation and the microbial species. In this case, [25], performed the experiments using the autochthonous microorganisms in the soil. On the contrary, [26], used a pure microbial culture (*Azotobacter chroococcum*, a typical soil bacteria) after an acclimation process which used a mineral medium and mannitol as carbon source and then, the mannitol was substituted by oxyfluorfen (250 ppm) and incubated at 28 ºC for 7 days. After that, the results shown that at least 60 % of oxyfluorfen was removed.

3.2. Evolution of soil conditions during the batch electrobioremediation tests

The pH results are in strong relationship to the above discussion. pH average values were around 7.7 and 8 in every experiments, during the whole experimentation period and the different soil positions. The pH was constant along the soil positions (from anode to cathode) and the results were similar to the ones observed in the reference "No EK" experiment. The polarity reversal strategy is also considered again as the responsible of such a successful pH control, while again a clear effect of the reversal frequency was not detected.

Soil moisture was also correctly controlled in all cases. The water can be eliminated in soil electrokinetic remediation by the electro-osmotic flow and evaporation. However, the electrolyte level control system continuously supplied water in the anode compartment, and the soil moisture values were kept approximately constant in all experiments and very similar to the values in the "No EK" experiment.

Finally, the average soil electrical conductivity values at the start and end of the experiments showed important variations, but there is not a clear trend in such changes regarding the possible influence of the reversal frequency. Some experiments showed a soil conductivity increase, while other experiments showed the opposite, and no relation with the reversal frequency can be identified. Moreover, these unclear results may be related to the unexpected variation in the initial values during the preparation of the different experiments. However, the final conductivity is, at least, 200 µs cm^{-1}, in every case, and these values ensures the necessary soil conductivity for the EK process. The continuous supply of electrolyte through the water level control system helped to maintain moisture and also the conductivity. At the same time, the conductivity values were low enough to avoid the microbial inactivation by an excess in the salinity of the system. Note again that the microbial culture was acclimated to oxyfluorfen degradation using BHB medium, whose electrical conductivity is 1700 µS cm^{-1}.
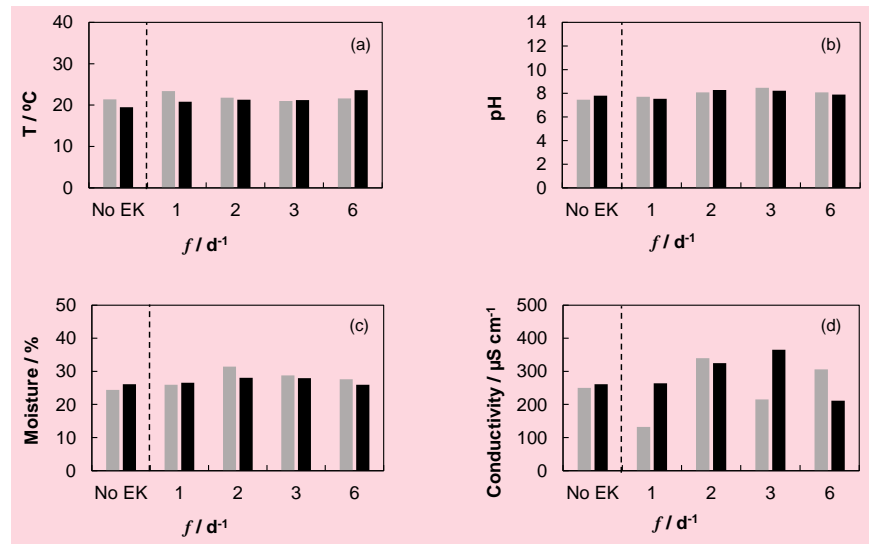
Figure 2. Temperature (a), pH (b), moisture (c) and conductivity (d) average values in the soil at the start and at the end of the electrobioremediation tests carried out at 1, 2, 3 and 6 d^{-1} of periodic polarity reversal frequency. Grey bar for the start and black bar for the end of the experiments.

3.3. Microbial profiles and oxyfluorfen removal results

Figure 6 shows the profiles of the average microbial concentration in the different soil positions at the start and the end of the experiments. The horizontal axis indicates the position from anode (left) to cathode (right) at time t =0. Every experiments shows a homogeneous soil microbial distribution at the end of the treatment and similar concentration values compared to the beginning. This is an important result as it indicates that the microbial activity remains in every parts of the soil after two weeks. Supposing a nutrient scarce or excessive pH gradients, the active microbial concentration would be drastically reduced, as it has been reported in previous works where these aspects were not correctly controlled [18] or higher voltages were applied, even if polarity reversal was used [17] as pH gradients were more difficult to avoid. A negligible microbial growth after the treatment is observed in such logarithmic scales, as the organic concentration for growth is low. Moreover, this biological process cannot be considered as completely aerobic as it has been previously observed [21,27] that the process may be partially anoxic by using another electron acceptors as nitrates, which a lower biomass yield.
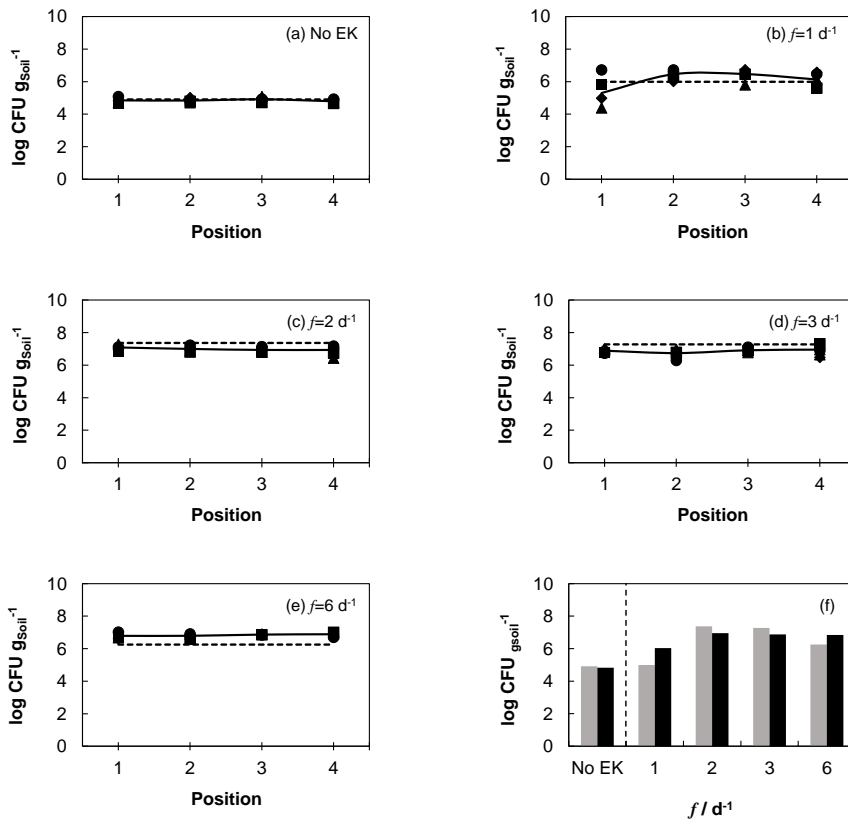
Figure 6. (a – e) Microorganisms concentration profiles in the soil at the start (- -) and at the end (—) of the electrobioremediation tests; (f) average of the values of microorganisms concentration in the whole soil at the start (grey bar) and the end (black bar). The line (—) is the average of the four values in the different axial positions (top right (♦), top left (●), bottom right (■) and bottom left (▲)).

Figure 4 also shows the results in the reference experiment "No EK" where identical microbial profiles were maintained between the start and the end. The organic substrate biodegradation in this experiment was negligible as discussed in the following.

Figure 7 shows the profiles of the average oxyfluorfen concentration in the different soil positions at the start and the end of the experiments. The horizontal axis indicates the position from anode (left) to cathode (right) at time t =0. The results of the reference experiment "No EK", which are not included in the figure, indicated a negligible oxyfluorfen removal efficiency, and these results may be contradictory if compared to the successful removal efficiencies indicated in the biodegradability tests (section 3.1).
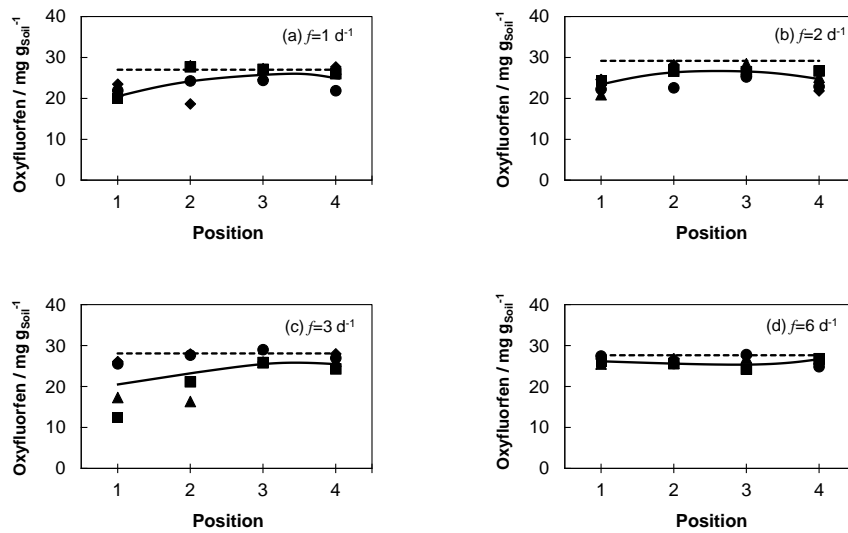
Figure 7. Oxyfluorfen concentration profiles in the soil at the start (- -) and at the end (—) of the electrobioremediation tests. The line (—) is the average of the four values in the different axial positions (top right (♦), top left (●), bottom right (■) and bottom left (▲)).

It is clear that the microbial culture is able to degrade oxyfluorfen in water, but not in the soil matrix in an *in-situ* process without any kind of help to favour the transport and contact between the pollutant, nutrients and microorganisms. Thus, figure 5 shows that under a low voltage gradient, and polarity reversal to control the operating conditions, a

certain removal efficiency is obtained as the oxyfluorfen concentration profile at the end (continuous line) is below the initial concentration (dashed line). There is a homogeneous pollutant removal, as expected because of the homogeneous microbial presence. The fact that there was pollutant removal, although low efficient, seem to suggest that oxyfluorfen has difficulties to interact with the microbial culture or nutrients despite the presence of a low amount of surfactant in the commercial pesticide. It is important to note that xylene, which acts as oxyfluorfen solvent, disappeared in the first 72 hours in the experiments, mainly by evaporation. Thus, important transport limitations may occur.

These results have been compared also to the second reference experiment, that is the abiotic experiment using 1 V cm^{-1} and f=2d^{-1}. In that case, oxyfluorfen removal is also negligible and it does not evaporate while xylene evaporates, and oxyfluorfen is not detected in the electrode compartments. Thus, the pollutant removal from the soil would be related only to the microbial activity and not to any other mechanisms.
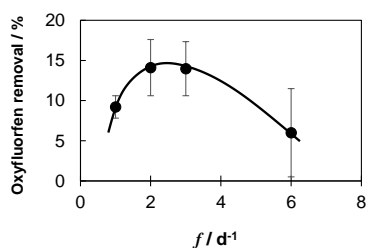

Figure 8. Oxyfluorfen removal percentage profile for all the frequencies of periodic polarity reversal.

Figure 8 shows the oxyfluorfen removal efficiencies after two weeks in the four experiments as a function of the reversal frequency used. Efficiencies are lower compared to the ones obtained in our previous works about electrobioremediation of hydrocarbon

polluted soils [17], and slightly higher than the ones reported by [25] using conventional bioremediation. An optimal frequency value can be observed between 2 and 3 d^{-1}.

There is not any information available in the literature about electrobioremediation of oxyfluorfen polluted soils. In comparison with other works about oil polluted soils [28] and diesel polluted soils [13], it can be concluded that electrobioremediation applying periodic polarity reversal strategy is the technology which offered better results. Additionally, no information is available about the optimization of the reversal frequency. According to our results, we consider that polarity reversal favours the homogenization of the system at microscopic scale as it acted as a mixer which put in contact microorganisms, oxyfluorfen and nutrients. However it is necessary to optimize the reversal frequency as there may be a high reversal frequency (in our work, f values of 3 d^{-1} and higher) which would produce the movement of microorganisms, nutrients and pollutant in very reduced areas, and it would not provide enough time and distance to the desired mixing effect between them. Anyhow, results in this work may be enhanced by improving oxyfluorfen mobility and the retention time.

4. Conclusions

Electrokinetics and polarity reversal allowed the partial biodegradation of oxyfluorfen in the soil. The reversal frequency did not influence the correct control of the operating conditions (T, and pH) but influenced the electroosmotic flow and the oxyfluorfen removal efficiency. It was observed an optimal frequency value between 2 and 3 d^{-1}, which would be directly related to the efficiency in the mixing effect between the pollutant, microorganisms and nutrients in the soil.

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