

1 **Effect of electric field on the performance of soil electro-**  
2 **bioremediation with a periodic polarity reversal strategy**

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8 **Abstract**

9 In this work, it is studied the effect of the electric fields (within the range 0.0-1.5  
10 V cm<sup>-1</sup>) on the performance of electrobioremediation with polarity reversal, using a  
11 bench scale plant with diesel-spiked kaolinite with 14-d long tests. Results obtained  
12 show that the periodic changes in the polarity of the electric field results in a more  
13 efficient treatment as compared with the single electro-bioremediation process, and it  
14 does not require the addition of a buffer to keep the pH within a suitable range. The soil  
15 heating was not very important and it did not cause a change in the temperature of the  
16 soil up to values incompatible with the life of microorganisms. Low values of water  
17 transported by the electro-osmosis process were attained with this strategy. After only  
18 14 d of treatment, by using the highest electric field studied in this work (1.5 V cm<sup>-1</sup>),  
19 up to 35.40% of the diesel added at the beginning of the test was removed, value much  
20 higher than the 10.5% obtained by the single bioremediation technology in the same  
21 period.

22 **Keywords**

1           Electrobioremediation, bioremediation, polarity reversal, pH, diesel

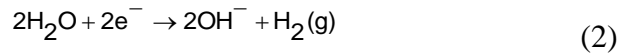
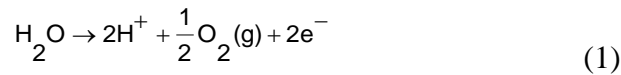
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# 1. Introduction

For the optimal combination of electrokinetics and bioprocesses in the remediation of polluted soils, there is a key point: microorganisms should be kept alive. To attain suitable conditions for life under the application of an electric field, the most critical parameter is pH (Yeung and Gu, 2011; Gill et al., 2014), which is known to be modified very importantly during the electroremediation of soil (Ruiz et al., 2014). These variations are due to the transport by electromigration of the protons and hydroxyl ions generated on the surface of the electrodes, by the well-known water electrolysis reactions (Eq. 1 and 2). (Acar, 1993; Virkutyte et al., 2002)



The protons, produced on the surface of the anode/s, are transported towards the negatively charged electrode/s (cathode/s), forming the so-called acid front. Likewise, the hydroxyl anions, produced on the surface of the cathode/s, are transported towards the positively charged electrode/s (anode/s) forming the basic front. Due to the higher migration constants of the protons (related not only to the size), their transport under an electric field is faster than the transport of the hydroxyl ions. This higher mobility helps to understand that the acid pH front moves along the soil at higher rate than the basic pH front. (Acar et al., 1995)

There are several relevant consequences of the pH fronts in the soil. Thus the dissolution of precipitates is favored by the acid pH front. This front produces the

1 release of metals from hydroxide and carbonate precipitates and those retained in the  
2 soil by ion exchange. This effect is not always important and depends strongly on the  
3 characteristic of the soil: in many types of soil the migration of the protons may be  
4 hindered, in particular if the soil has a relatively high buffering capacity (Reddy and  
5 Cameselle, 2009b). On the other hand, as it could be expected, the basic pH front  
6 produces the opposite processes and it may cause the precipitation of metals (especially  
7 as hydroxide and carbonate salts) and can even prevent the movement of the pollutants  
8 particles by blocking pores of the soil. (Reddy and Cameselle, 2009a)

9 Focusing on the effect of the acid and basic fronts on the biological processes, the  
10 most important consequence is that the zones of the soil with extreme pH values, are  
11 incompatible with the life of the microbial consortia used to degrade pollutants  
12 (Jackman et al., 2001; Page and Page, 2002; Lear et al., 2004; Luo et al., 2006; Lear et  
13 al., 2007; Wick et al., 2010). pH values below 3 and above 9, as well as sudden changes  
14 in the pH of the treatment system matrix, can significantly inhibit microbial growth by  
15 interfering with the microbial metabolism, gas solubility in soil water, nutrients  
16 availability and bioavailability in soil water, and heavy metals solubilities. (Juwarkar et  
17 al., 2010)

18 In this context, diesel-degrading organisms are not characterized for being a type  
19 of biological culture particularly resistant to pH changes. The optimal conditions for the  
20 growth of the microorganisms were evaluated in previous works of this research group  
21 (Moliterni et al., 2012), and they include mild temperature (around 26 °C) and neutral  
22 pH. Taking into account these considerations, it is necessary to look for strategies that  
23 help avoiding the lethal influence of the extreme pH fronts on the microbial  
24 performance.

1           Several authors have suggested different strategies to maintain a proper pH value  
2 for the combined biological and electrokinetic remediation of soil (Pazos et al., 2010;  
3 Yeung and Gu, 2011; Gomes et al., 2012; Gill et al., 2014). Among them, it can be  
4 highlighted the dosing of buffering agents to the flushing fluids, and the recirculation of  
5 electrolyte solutions between the anode and cathode compartments (Lee and Yang,  
6 2000). The main challenge is to find a life-compatible and environmentally-friendly  
7 reagent effective in both the anolyte and the catholyte pH regulation. This strategy is not  
8 easy to be applied and it fails in long treatments because it is not easy to get by  
9 properly. A different strategy is the periodic change of the polarity of the electric field  
10 applied to the soil (periodic polarity reversal). Initially, this is a simple solution for  
11 regulating the changes in the pH and it could also be used for improving the distribution  
12 of ionic inorganic nutrients (Kim and Han, 2003; Luo et al., 2005; Luo et al., 2006; Fan  
13 et al., 2007; Niqui-Arroyo and Ortega-Calvo, 2007; Harbottle et al., 2009; Xu et al.,  
14 2010; Huang et al., 2013) preventing their depletion from the soil by accumulation in  
15 the anolyte or in the catholyte. No information is available in the literature about the  
16 optimization of the electric field applied and the appropriate time intervals for the  
17 change of the polarity of the electric field.

18           Thus, the main objective of this work is the optimization of the polarity reversal  
19 strategy for the synergistic combination of the electrokinetic and the biological  
20 treatments. In order to meet this objective, following are detailed the partial objectives  
21 that are planned to be assessed:

22           - Evaluation of the influence of applying a periodic change in the polarity of the  
23 electric field for the control of the pH value in an electrokinetic-biological treatment of  
24 a diesel polluted clay soil.

1 - Optimization of the value of electric field applied for the optimal performance of  
2 the periodic polarity reversal strategy.

3 - Comparison between results obtained in the direct application of the electro-  
4 bioremediation treatment and results obtained with the polarity reversal strategy.

## 5 6 **2. Material and Methods**

7  
8 The experimental setup used in the tests was described elsewhere (Ramírez et al.,  
9 2015). The soil used was kaolinite clay (provided by Manuel Riesgo Chemical Products,  
10 Madrid, Spain). Diesel oil was selected as pollutant. It was purchased from a petrol  
11 station in Ciudad Real, Spain. To artificially pollute the soil, the diesel was diluted  
12 tenfold in acetone before evenly distributing the solution drop by drop in the  
13 corresponding amount of kaolinite. The solvent (acetone) and the higher volatile diesel  
14 fractions (approximately 6% of the initial amount of added diesel) were allowed to  
15 evaporate at room temperature for at least 2 d. The concentration of diesel present in the  
16 soil at the beginning of the experiment was approximately  $10 \text{ g kg}^{-1}$ . The diesel-  
17 degrading microbial consortium used for the experiments was isolated from a diesel-oil  
18 contaminated soil, which was collected from a site in the vicinity of an oil refinery near  
19 Ciudad Real, Spain. This consortium of microorganisms was maintained and enriched  
20 with Bushnell-Hass Broth (BHB) as the mineral medium (the composition of the  
21 medium per litre of deionised water was  $0.20 \text{ g L}^{-1} \text{ MgSO}_4$ ,  $0.02 \text{ g L}^{-1} \text{ CaCl}_2$ ,  $1.00 \text{ g L}^{-1}$   
22  $\text{KH}_2\text{PO}_4$ ,  $1.00 \text{ g L}^{-1} (\text{NH}_4)_2\text{HPO}_4$ ,  $0.05 \text{ g L}^{-1} \text{ FeCl}_3$  and  $1.00 \text{ g L}^{-1} \text{ KNO}_3$ ) and diesel  
23 hydrocarbon as the sole carbon source (1.0% v/v). The electrolyte used both in the  
24 anolyte and in the catholyte was a synthetic groundwater ( $80.75 \text{ mg L}^{-1} \text{ Na}_2\text{SO}_4$ ,  $70.00$   
25  $\text{NaHCO}_3$ ,  $30.36 \text{ mg L}^{-1} \text{ NaNO}_2$ ).

1

2       The remediation experiments were carried out applying four electric fields (0.0,  
3 0.5, 1.0 and 1.5 V cm<sup>-1</sup>). The duration of the treatment processes was two weeks. A  
4 daily (every 24 h) polarity reversal of the electric field was applied. The periodicity of  
5 the polarity reversal was chosen arbitrarily following some previous tests (not shown)  
6 carried out to check the appropriateness of this choice in order to prevent the exhaustion  
7 of nutrients by their transport to electrolyte wells. At the end of the experiments, the soil  
8 samples were taken in four longitudinal positions, which correspond to four different  
9 intermediate points between anode and cathode compartments. In addition, each  
10 intermediate longitudinal position also considered four different sampling points,  
11 distributed in different axial positions. The results show the average concentration of the  
12 four different samples taken at the same longitudinal position, that is, at the same  
13 distance of the electrodes, and so it was possible to analyze the axial deviation in the  
14 points situated at the same distance of the electrodes. The designation of the sampling  
15 points was done following the same procedure previously discussed in the literature  
16 (Mena Ramírez et al., 2014; Mena et al., 2015). It is relevant to take into account that  
17 electrodes change every day its role as anode or cathode due to the polarity reversal. For  
18 this reason, the function of the electrodes on day 0 was used to label each soil portion in  
19 the *post mortem* characterization.

20       Regarding the analytical procedures, the moisture was calculated in soil samples  
21 gravimetrically, taking into account the weight loss before and after drying at 105 °C for  
22 24h. The pH, conductivity and nitrate, phosphate, and ammonium concentrations were  
23 measured from the soil samples as follows: 10 g of the dried soil samples were  
24 suspended in 25 mL of Milli-Q water by 20 min of vigorous magnetic agitation. After  
25 that, samples were centrifuged at 4000 rpm for 15 min. Measurement of the parameters

1 was made in the supernatant phase. pH was measured using a CRISON pH meter and  
2 conductivity was measured using a Jenway conductivimeter. Diesel concentration was  
3 determined using a fractionated serial extraction: briefly, 10 g of wet soil were mixed  
4 with 4 mL of hexane. In every extraction the soil was mixed with the corresponding  
5 volume of dissolvent and agitated vigorously in a Vortex agitator for 5 min. After that,  
6 samples were centrifuged at 4000 rpm for 15 min. Samples taken from the organic  
7 supernatant phase were analyzed using a Trace GC Ultra (Thermo Fischer Scientific,  
8 Massachusetts, USA) gas chromatograph equipped with a flame ionization detector  
9 (GC-FID). Microbial concentration was expressed as Colony Forming Units (CFU) per  
10 dry gram of soil. It was measured suspending 10 g of wet soil in 10 mL of saline by 1  
11 min of Vortex agitation. After that, an aliquot of 100  $\mu$ L of the soil-saline suspension  
12 was plating on Petri dishes. The nutrient solid phase of these dishes were prepared using  
13 LB medium (with the following composition per litre of deionised water: NaCl 10.0 g,  
14 yeast extract 5.0 g and casein peptone 10.0 g), 15.0 g L<sup>-1</sup> of European Bacteriological  
15 Agar and 2.0 g L<sup>-1</sup> of glucose as carbon source. Inoculums were evenly spread using  
16 Digrafsky handles and the plates were incubated for 48 h at 26 °C, which is time enough  
17 to enumerate the individual colonies present in each sample. Finally, concentration of  
18 biomass in the biobarrier was also measured as volatile solids before and after the  
19 treatment. This value corresponds with the weight difference in the dried samples before  
20 and after the calcination at 550 °C for 2 h.

21

### 22 **3. Results and discussion**

23

24 In a previous work of our group (Ramírez et al., 2015), it was discussed the  
25 reasons that explained why the direct combination (direct electro-bioremediation) of

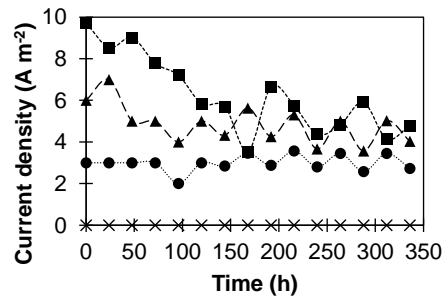


1 E.K.S.F. and bioremediation failed in the remediation of soil polluted with diesel.  
2 Despite the amphoteric behaviour, using a bicarbonate buffer solution to keep regulated  
3 the pH value was not a good strategy and suitable conditions to maintain the viability of  
4 the microbial culture were not met. In this work, a different strategy to keep the pH  
5 under a suitable range is going to be evaluated. It consists of the periodic polarity  
6 reversal. Thus, not a proper flushing of the soil is carried out, because the direction of  
7 the electro-osmotic flow changes periodically. However, mobility of species is  
8 increased and with it, the chances for the increased interaction of pollutant,  
9 microorganisms and nutrients. After some preliminary tests, a frequency of  $1 \text{ d}^{-1}$  was  
10 considered suitable for the polarity reversal periodicity because it avoids depletion of  
11 any nutrient and results in a proper pH distribution. Next, the effect of the applied  
12 electric field on the performance of the electrobioremediation technology is going to be  
13 discussed by evaluating the effect on different key parameters.

14

15 **Current density.** The rate of the mass-transport electrokinetic processes depends  
16 on the value of the resulting current density applied to the system, which, at the same  
17 time, is a consequence of the application of an electric field. In this way, current density  
18 becomes the most important parameter in the monitoring of the electro-bioremediation  
19 processes. Fig. 1 shows the variation in the values of current density in the different  
20 periodic polarity-reversal tests carried out. For the sake of comparison, results obtained  
21 in the single bioremediation test are also included in this Fig. 1.

22



1

2 **Fig. 1.** Time-course of the current density in the electro-bioremediation tests with  
 3 periodic polarity reversal. Experiments at 0.0 V cm<sup>-1</sup> (×), 0.5 V cm<sup>-1</sup> (●), 1.0 V cm<sup>-1</sup>  
 4 (▲) and 1.5 V cm<sup>-1</sup> (■).

5

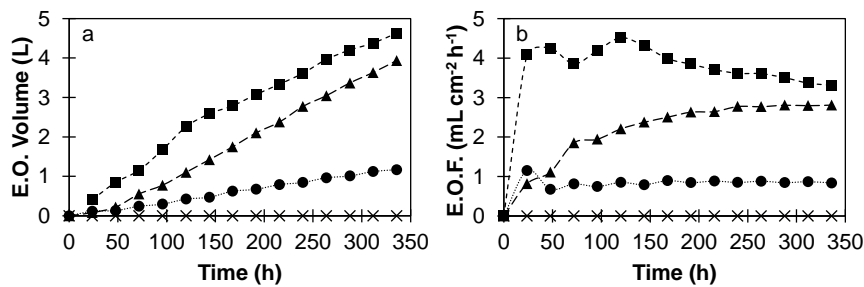
6        There are several relevant aspects to be highlighted related to the current densities  
 7 changes. As expected, the higher the electric field applied, the higher is the resulting  
 8 current density. However, as a major difference, as time passed by in this process, it was  
 9 observed a decrease in the differences between current densities obtained in the three  
 10 tests, reaching very similar current density values for the three different electric field  
 11 experiments. It is also important to note the *zig-zag* shape of the trends, which may be  
 12 explained by the daily change in the polarity of the electric field applied. In general, a  
 13 slightly higher value of current density was obtained when the direction of the electric  
 14 field applied was in the contrary direction to what applied in the first day of the  
 15 experiment. Several authors have suggested that this behavior is due to the change in the  
 16 charge of the double layer that results in a high consumption of the capacitive part of the  
 17 current due the double layer discharging or recharging (Röhrs et al., 2002). These  
 18 changes are more pronounced applying higher values of electric field.

19

20        **Temperature.** One of the most important aspects for the successful application of  
 21 the electro-bioremediation treatment is the control of the temperature. Unsuccessful

1 regulation may prevent the good performance of the microbial culture. As it was  
 2 expected, the maximum heating of the soil was observed in the experiment in which the  
 3 highest value of electric field was applied. However, in this case, the highest values of  
 4 temperature were not above the optimal value for the development of the microbial  
 5 population. In fact, temperature was kept under 30 °C regardless of the electric field  
 6 applied. Hence, the inhibition of the biological process, if occurred, cannot be explained  
 7 in terms of extremely value of temperature due to very accused heating process, because  
 8 in this case mild conditions in terms of temperature are obtained.

10 **Electro-osmotic flow.** The value of the electro-osmotic flux can give an idea of  
 11 the rate at which the other electrokinetic mass transport processes occurs, which have to  
 12 be quantified using more complex procedures. In Fig. 2, the values of the volume and  
 13 the flow rate of the water transported by the electro-osmotic flows are plotted.



15  
 16 **Fig. 2.** Time-course of the volume of water transported by electro-osmosis (a) and time-  
 17 course of the electro-osmotic flow (b). Experiments at 0.0 V cm⁻¹ (×), 0.5 V cm⁻¹ (●),  
 18 1.0 V cm⁻¹ (▲) and 1.5 V cm⁻¹ (■).

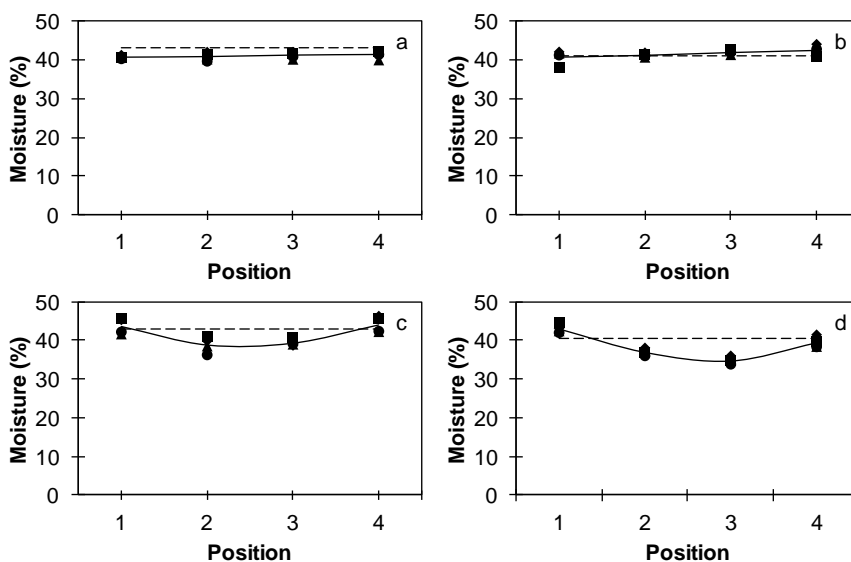
20 In comparing with the direct electro-bioremediation treatment, a considerably  
 21 lower value of water flow was transported throughout the soil. This result had as a  
 22 consequence a lower transport of water soluble species throughout the system but also,

1 and directly related to it, a lower removal of inorganic nutrients from the system. This  
2 can be beneficial for the overall process as the contact among the different elements  
3 taking part in the biological degradation is increased in low distances ( $\approx 100 \mu\text{m}$ ) (Wick  
4 et al., 2007), but no exhaustion of nutrients is obtained.

5

6 **Moisture profiles in the soil.** Moisture is a very important parameter because the  
7 biological degradation develops in the water contained in the soil and a proper operation  
8 of the electrokinetic process needs working with soil near to moisture saturation point.  
9 So, the evaluation of the changes produced in the moisture of the soil after the treatment  
10 is relevant. In the same way, the quantification of the loss of water due to the  
11 evaporation process is also very important for the optimal application of the treatment  
12 technique. Fig. 3 shows the moisture content of the soil before and after the treatment.  
13 Results of the single bioremediation test ( $0.0 \text{ V cm}^{-1}$ ) were also included for the sake of  
14 comparison.

15



16

17 **Fig. 3.** Moisture profile in the soil before (---) and after (—) the periodic reversal  
18 polarity remediation tests carried out at (a)  $0.0 \text{ V cm}^{-1}$ , (b)  $0.5 \text{ V cm}^{-1}$ , (c)  $1.0 \text{ V cm}^{-1}$

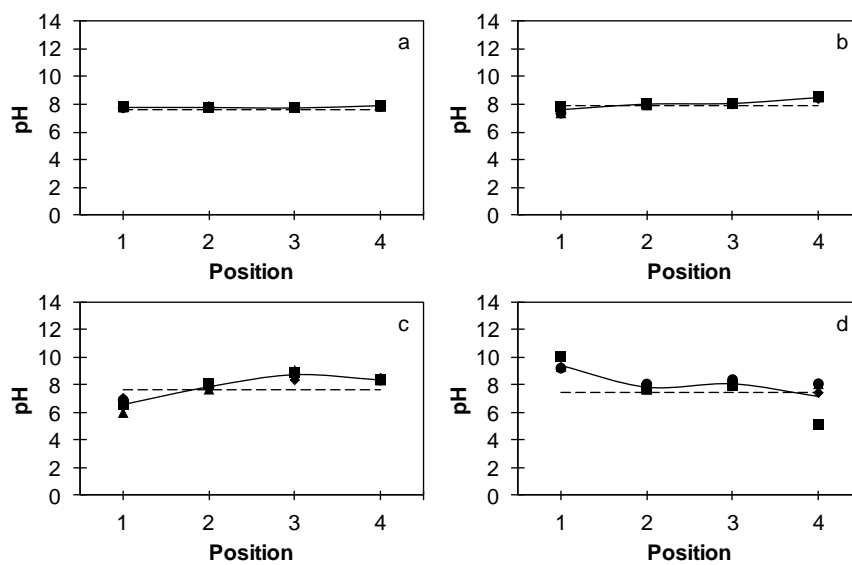
1 and (d)  $1.5 \text{ V cm}^{-1}$ . The line (—) is the average of the four values in the different  
2 axial positions (top right (◆), top left (■), bottom right (▲), bottom left (●)).

3  
4 The most significant differences between the values of moisture in the different  
5 sections of the soil were obtained under the application of the higher values of electric  
6 fields. In these cases, it was also obtained the highest values of water transported by  
7 electro-osmosis. This water was mostly mobilized from the central sections of the soil,  
8 leading to a decrease in the content of water in these areas. As the periodic polarity  
9 reversal strategy consists of applying for the same time the electric field on both  
10 directions, the electro-osmosis transport process also occurs in both directions along the  
11 soil for the same period of time. The volume of water transported was approximately  
12 the same towards both electrodic compartments, obtaining an almost symmetrical  
13 redistribution of the moisture contained in the soil in the highest electric field  
14 experiment. However, this strategy leads to a decrease in the moisture in the central  
15 zones of the soil which becomes more pronounced with the increase in the applied  
16 electric field. In the tests carried out, it does not produces an operating problem but in  
17 longer remediation treatments it should be carefully managed because it could affect the  
18 performance of the system if it decreases down to values in which the electrokinetic or  
19 even the biological processes are prevented.

20  
21 **Changes in the pH.** Due to the absence of a buffer solution used as electrolyte,  
22 pH underwent variation to acid pH in the anolyte and to basic pH in the catholyte which  
23 are corrected daily with the polarity reversal. It was observed a “zig-zag” shape in the  
24 variation of the values corresponding with the daily change of the anodic and cathodic  
25 electrode.

1 In spite of the pronounced changes in the pH observed in the electrolyte, it was  
 2 expected that the daily polarity reversal of the electric field avoided the displacement of  
 3 the extreme pH fronts throughout the soil. As expected, in the bioremediation  
 4 experiment (without applying electric fields) the value of pH in the electrolytes did not  
 5 undergo any significant modification. Fig. 4 compares the pH values of the soil before  
 6 and after the tests.

7



8

9 **Fig. 4.** pH profile in the soil before (---) and after (—) the periodic reversal polarity  
 10 remediation tests carried out at (a) 0.0 V cm<sup>-1</sup>, (b) 0.5 V cm<sup>-1</sup>, (c) 1.0 V cm<sup>-1</sup> and (d) 1.5  
 11 V cm<sup>-1</sup>. The line (—) is the average of the four values in the different axial positions  
 12 (top right (◆), top left (■), bottom right (▲), bottom left (●)).

13

14 One of the most important aspects for the successful implementation of the  
 15 electro-bioremediation treatment is the perfect control of the pH in the soil. The  
 16 displacement of the pH fronts (if no control procedure is used) leads to extremely acid  
 17 pH values in the area near to the anode and, similarly, extreme basic pH in the area near  
 18 to the cathode. The most important objective of the application of the polarity-reversal

1 strategy is the efficient control of the pH to attain values suitable for the biological  
2 process.

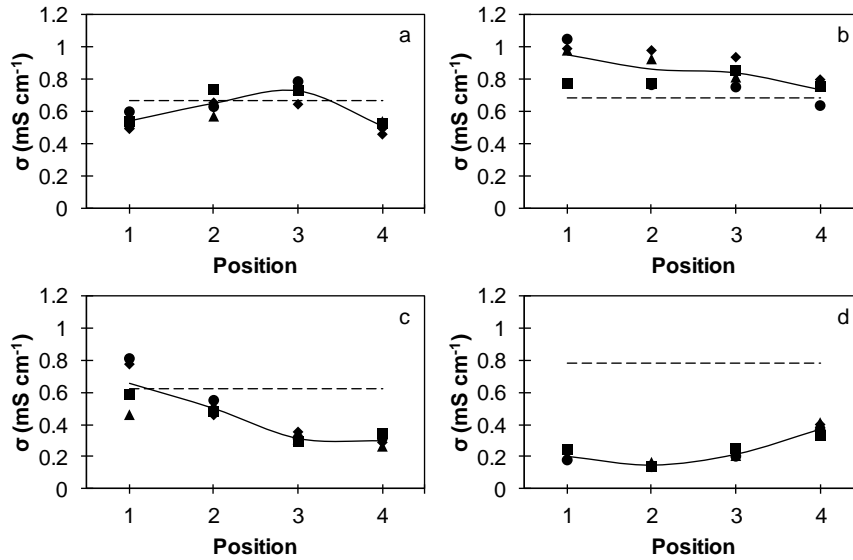
3 The pH in the soil was maintained in suitable values for the development of the  
4 microbial population and the degradation of the pollutant. Only working with the  
5 highest value of voltage, the values of pH reach values slightly above or below the  
6 optimal for the microorganisms, but only in the areas more directly affected by the pH  
7 fronts, near to the electrodes. Consequently, it can be concluded that the periodic  
8 polarity-reversal attained satisfactory results avoiding the influence of the extreme pH  
9 variations as long as the electric field was not too high causing quick pH change in short  
10 periods of time.

11

12 **Conductivity.** It is again relevant the characteristic *zig-zag* shape observed in the  
13 variation of the conductivity values obtained both in the anolyte and also in the  
14 catholyte in the electro-bioremediation tests with periodic polarity reversal,  
15 corresponding with the daily change of the anodic and cathodic electrode. It is worth  
16 noting that, in general, conductivity values in the catholyte were slightly higher than in  
17 the anolyte.

18 The analysis of the changes in the distribution of the conductivity in the soil after  
19 the treatment gives information about the transport of the ionic species. Fig. 5 compares  
20 the values of the conductivity in the soil before and after the treatment. As in the  
21 discussion of previous parameters, results of the bioremediation tests are also included  
22 for the sake of comparison.

23



1

2 **Fig. 5.** Conductivity profile in the soil before (— —) and after (—) the periodic  
 3 reversal polarity remediation tests carried out at (a)  $0.0 \text{ V cm}^{-1}$ , (b)  $0.5 \text{ V cm}^{-1}$ , (c)  $1.0 \text{ V}$   
 4  $\text{cm}^{-1}$  and (d)  $1.5 \text{ V cm}^{-1}$ . The line (—) is the average of the four values in the  
 5 different axial positions (top right (◆), top left (■), bottom right (▲), bottom left (●)).

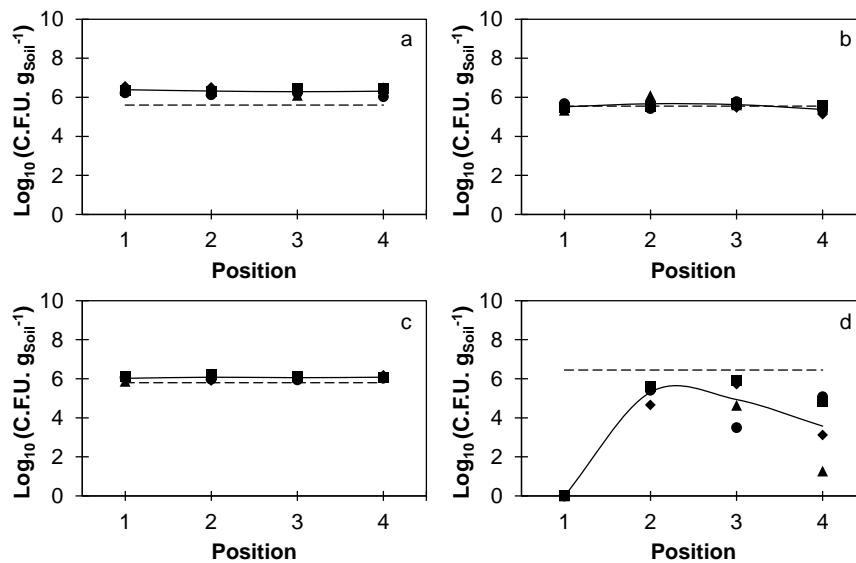
6

7 Conductivity in the soil changed in different ways depending on the value of  
 8 electric field applied. Considering firstly results obtained in the experiment without  
 9 applying electric field, a slight decrease in the conductivity in the areas of the soil near  
 10 to the electrodic wells was obtained. This decrease can be explained in terms of the  
 11 diffusion of the ionic species to the electrolyte contained in the electrodic well. Under  
 12 the application of an electric field of  $0.5 \text{ V cm}^{-1}$ , a general increase in the conductivity  
 13 of the soil was obtained. The electromigration process and in a lesser extension the drag  
 14 with the electro-osmotic flow increase the concentration of ionic species in the soil  
 15 being the influence of this process higher than the removal by accumulation of these  
 16 species in the electrodic wells. However, under application of higher electric fields ( $1.0$   
 17 and  $1.5 \text{ V cm}^{-1}$ ) a general decrease in the conductivity of the soil was obtained, being  
 18 this decrease higher with the increase in the electric field applied. In these cases, the



removal of ionic species from the soil had higher influence than the supply with the electrolyte transported throughout the soil. In the experiment carried out at  $1.0 \text{ V cm}^{-1}$  ionic species are still present at suitable values in the soil.

**Microbial concentration profiles in the soil.** With the periodic polarity reversal strategy, the most important variables affecting the biological process were adequately controlled. The reversal in the polarity of electrodes allows us to keep suitable values of the pH and the temperature in the soil. Consequently, the survival of the microbial population is expected to be higher. Fig. 6 shows the concentrations of microbial population before and after the remediation tests.



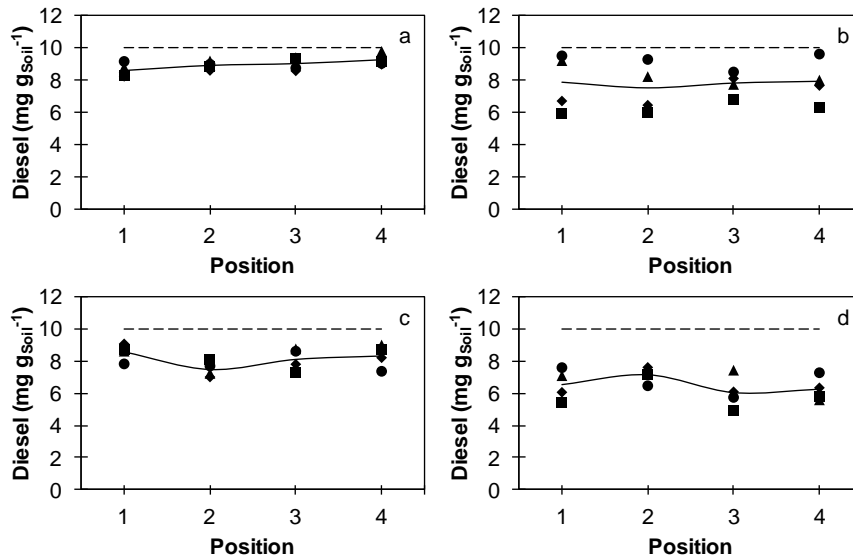
**Fig. 6.** Microorganisms concentration profile in the soil before (— —) and after (—) the periodic reversal polarity remediation tests carried out at (a)  $0.0 \text{ V cm}^{-1}$ , (b)  $0.5 \text{ V cm}^{-1}$ , (c)  $1.0 \text{ V cm}^{-1}$  and (d)  $1.5 \text{ V cm}^{-1}$ . The line (—) is the average of the four values in the different axial positions (top right (◆), top left (■), bottom right (▲), bottom left (●)).

1 As it can be observed, except for the test in which the highest electric field was  
2 applied, there are no significant differences between the bioremediation test and the  
3 electro-bioremediation tests. The microbial cultures survive in the four sections (and  
4 also in the subsections) and even a low increase in the population can be seen. The  
5 worst conditions for the microbial population were under the application of highest  
6 value of electric field and they can be explained if profiles of pH and of microorganisms  
7 concentrations are compared: in the zones near to the electrodic wells the changes of the  
8 pH were higher and these changes affects to the viability of the microorganisms culture.  
9 Anyhow, population in the central positions is maintained and this observation points  
10 out again the extreme importance of keeping well-regulated soil pH in electro-  
11 bioremediation processes, and also that the central position would be the most protected  
12 place regarding possible extreme pH conditions.

13

14 **Diesel concentration profiles in the soil.** Electro-bioremediation with polarity  
15 reversal successes in keeping the pH and temperature under suitable values for life.  
16 These suitable conditions reflect on the viability of the biological culture that is  
17 maintained during the 14 d-long tests (except for zones close to electrodes under the  
18 application of the highest electric field) and this viability maybe reflected in the  
19 performance of the microorganisms and hence in the removal of diesel pollution from  
20 soil. Fig. 7 compares the concentration of diesel oil in the soil at the beginning and at  
21 the end of the different experiments. As it can be observed, removal of diesel obtained  
22 by the electro-bioremediation tests is much higher than that obtained by the single  
23 bioremediation. In comparing the effect of the electric field, it is seen that the higher the  
24 electric field applied, the higher is the removal of pollution. This fact means that

1 periodic polarity reversal is a good alternative for the remediation of soils and that  
 2 electric field has to be carefully managed,  
 3



4  
 5 **Fig. 7.** Diesel concentration profile in the soil before (— —) and after (—) the  
 6 periodic reversal polarity remediation tests carried out at (a) 0.0 V cm<sup>-1</sup>, (b) 0.5 V cm<sup>-1</sup>,  
 7 (c) 1.0 V cm<sup>-1</sup> and (d) 1.5 V cm<sup>-1</sup>. The line (—) is the average of the four values in  
 8 the different axial positions (top right (◆), top left (■), bottom right (▲), bottom left  
 9 (●)).

10

11 The most relevant aspect to highlight in these results is the highest removal of  
 12 diesel oil from the soil obtained in the experiment with the highest value of electric  
 13 field. This is an illustrative result of the complexity of the technology studied in this  
 14 work and it is worth reminding that the most important objective of the combination of  
 15 the biodegradation and the electrokinetic transport processes is the increase in the  
 16 possibilities of interaction among the different elements taking part in the degradation  
 17 process.

18

1 **Efficiency of the treatment.** Table 1 summarizes the most relevant results  
 2 obtained in the experiments for the evaluation of the efficiency of the electro-  
 3 bioremediation with periodic polarity reversal technology. In this way, it can be checked  
 4 that, in general, the application of the electro-bioremediation technology improved  
 5 results obtained about the removal of diesel pollution from the soil, compared with the  
 6 single bioremediation test.

7

8 **Table 1:** Pollutant and inorganic nutrient removal, electrical consumption and soil  
 9 properties.

	Test			
	0.0 V cm <sup>-1</sup>	E.B.R.-P.R. 0.5 V cm <sup>-1</sup>	E.B.R.-P.R. 1.0 V cm <sup>-1</sup>	E.B.R.-P.R. 1.5 V cm <sup>-1</sup>
<b>Soil treated</b> g	2,941	2,848	2,810	2,752
<b>Diesel added</b> mg mg kg <sup>-1</sup> Soil	29,415 10,000	28,480 10,000	28,102 10,000	27,521 10,000
<b>N<sub>Total</sub> added</b> mg mg kg <sup>-1</sup> Soil	513 175	588 206	571 203	541 197
<b>P<sub>Total</sub> added</b> mg mg kg <sup>-1</sup> Soil	346 118	368 129	364 130	349 127
<b>Flushing fluid added</b> mL mL kg <sup>-1</sup> Soil	1,420 483	3,830 1,345	6,560 2,335	6,880 2,500
<b>Moisture</b> mL kg <sup>-1</sup> Soil %	432 43.20	410 40.99	430 42.99	406 40.65
<b>pH</b>	7.64	7.89	7.64	7.41
<b>Electrical conductivity</b> mS/cm	0.673	0.683	0.618	0.781
<b>Diesel removed</b> mg %	3,066 10.42	6,364 22.35	5,389 19.18	9,742 35.40
<b>N<sub>Total</sub> removed</b> Electrode compartments and sampling mg	51	78	190	292
<b>P<sub>Total</sub> removed</b> Electrode compartments and sampling Mg	17	2	6	6
<b>Flushing fluid removed</b> Electro-osmosis Sampling mL	0 280	1,170 280	3,934 280	4,631 280
<b>Moisture</b> mL kg <sup>-1</sup> Soil %	410 41.03	417 41.70	414 41.41	385 38.50
<b>pH</b>	7.80	8.02	7.88	8.10
<b>Electrical conductivity</b> mS/cm	0.606	0.847	0.443	0.233
<b>Electrical consumption</b> Wh kg <sup>-1</sup> Soil	0	42	145	289

10

1           The best results were obtained in the experiment applying  $1.5 \text{ V cm}^{-1}$ , although in  
2 this case also the highest electrical consumption was obtained. In these experiments an  
3 important amount of inorganic nutrients was removed from the system due to the  
4 electrokinetic processes, although in this case the efficiency of the biological process  
5 was not diminished if they are replaced.

6           These results are very promising but they pointed out that this type of technology  
7 needs for further studies in order to be applied in full-scale applications. In particular  
8 the scale-up of the technology is one of the topics that will focus more attention in the  
9 next years as it was recently pointed out in a review about the removal of other organics  
10 with electrochemically assisted technology (Rodrigo et al., 2014).

11

#### 12 **4. Conclusions**

13

14           From this work, the following conclusions can be drawn:

- 15           - The periodic changes in the polarity of the electric field results in a more  
16           efficient treatment as compared with the single electro-bioremediation  
17           process. In addition, it was not necessary the addition of a buffer to keep  
18           the pH within a suitable range. The soil heating was not very important  
19           and it did not cause a change in the temperature of the soil up to values  
20           incompatible with the life of microorganisms. Low values of water  
21           transported by the electro-osmosis process were attained with this strategy.  
22           The removal of nutrients from the soil due to the dragging with the water  
23           transported by the electro-osmosis was lower than in the single electro-  
24           bioremediation technique. However, a nutrient replacement is necessary.

- 1           - The final objective of the periodic polarity reversal was to avoid the  
2           greatly extension of the extreme pH fronts throughout the soil. Results  
3           obtained demonstrated that this objective was successfully achieved. With  
4           the exception of the experiment at the highest electric field, in all the  
5           sections of the soil the pH values were around the neutral point. Hence, the  
6           inhibition of the biological degradation due to the extreme pH was avoided  
7           by the periodic reversion of the electric field. In fact, concentration of  
8           alive microorganisms measured in the soil at the end of the tests was even  
9           increased as compared to the initial concentration.
- 10          - The best pollutant removal results (9,742 mg or, what is the same, 35.40%  
11          of the diesel added at the beginning of the test) were obtained in the  
12          experiment carried out at the highest value of electric field. These  
13          conditions also lead to the highest electrical consumption.

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16

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