Can electro-bioremediation perform as a self-sustainable process?

Silvia Barba[1], José Villaseñor[1,*], Manuel A. Rodrigo[2] and Pablo Cañizares[2]

(1) Chemical Engineering Department. Research Institute for Chemical and Environmental Technology (ITQUIMA). University of Castilla- La Mancha, 13071, Ciudad Real, Spain.

(2) Chemical Engineering Department. Faculty of Chemical Sciences and Technology. University of Castilla- La Mancha, 13071, Ciudad Real, Spain.

*Corresponding author. Tel.: +34 926 29 53 00. E-mail: jose.villasenor@uclm.es

Abstract

In this work, it is evaluated the effect of the treatment period on the results obtained by electro-bioremediation technology, focusing on the efficiency of the removal of oxyfluorfen, viability of the microorganism cultures and reproducibility of experimental results in this complex multiparametric process. To do this, five lab-scale plants were started up simultaneously, operated under an electric field of 1.0 V cm^{-1} with a polarity reversal frequency of 2 d^{-1} over time and they were disconnected at different times ranging from 2 weeks to 6 months (2, 4, 6, 11 and 24 weeks), undergoing a postmortem characterization after their operation period. In addition, during the operation period different parameters were monitored in the electrolyte wells. Results obtained pointed out that despite the low reproducibility of pH and conductivity in the wells (not in soil), main conclusions that can be drawn for the different plants are sound and hence reproducibility can be considered as acceptable. Polarity reversal is a good strategy to keep suitable conditions for life in terms of pH but not in terms of nutrients. There is a depletion of nutrients in the soil, which lead to a decrease in the total population of microorganisms during the treatment. For treatment periods below 10 weeks, there was an appreciable population of microorganism in soil which attain a removal of oxyfluorfen of up to 40%. Longer reaction times were ineffective and this is related to the much lower concentration of microorganisms. In comparing these results with those obtained in single bioremediation, it was found that the application of polarity reversed electric current attains an increase in the average removal of oxyfluorfen from 0.11 to 0.17 mg kg^{-1} d^{-1}, with a much higher decrease in the active microorganisms population from 88.0± 9.0 down to 41.0±6.0% of the initial value seeded.

Keywords

Electro-bioremediation; herbicides; soil; electrokinetic; bioremediation

Highlights

- Acceptable reproducibility of results in the evaluation of soil remediation at lab-scale
- Application of electric field affects negatively to the survival of the microorganisms
- After ten weeks viability of microorganisms population falls down to negligible values
- Electro-bioremediation is able to reach removals of oxyfluorfen of 40% in ten weeks
- Electric fields improves the removal of oxyfluorfen from 0.11 to 0.17 mg kg^{-1} d^{-1} as compared to bioremediation

Introduction

The enhancement of agricultural production, associated to the increase in human population in the last century, has been possible because of an increase in the production and use of agricultural pesticides, especially after the Second World War (Gavrilescu, 2005). However the pollution originated by the use of these pesticides is a serious environmental problem. They can be dispersed through water, soil and air, and they can affect the quality of superficial water reservoirs, aquifers and soil. This problem can affect the ecosystems, and more specifically to the animals through the food chain (Chowdhury et al., 2008), where pesticides may be accumulated. Ususally pesticides are persistent compounds, which remain in the environment for long periods of time and its presence has been detected in food for human consumption, causing different hazardous effects on human's health (Morillo and Villaverde, 2017).

Currently, the Spanish regulation defines the so-called "Reference Pollution Levels" in soil, that is, the maximum pollutant concentration allowed in a soil so that higher concentrations would make it mandatory to perform a risk assessment and, if necessary, the development of remediation works (Spanish Presidential Ministry, 2005). Many organochlorinated compounds are included as soil pollutants in this regulation. Depending on the receptor of the pollutant impact (natural ecosystems or human health) the Reference Pollution Levels are different, but in the case of organochlorinated compounds, they are usually lower than 1.0 mg kg_{soil}^{-1}.

Oxyfluorfen [2-chloro-α, α, α-trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether] is one of the most used organochlorinated pesticides. This product is very sparingly soluble in water (0.116 mg L^{-1}) and it has a low vapour pressure (0.026 mPa at 25 ºC) and a high octanol-water index K_{ow} (log K_{ow} = 4.86). According to these chemical characteristics, oxyfluorfen is strongly adsorbed to most soils, specially soils with high organic matter and clay percentages (United States Environmental Protection Agency, 2002) and thus it has very low mobility in soil. Oxyfluorfen is hardly biodegradable: plants cannot metabolize it and it is only slowly degraded by soil microbial cultures (Sondhia, 2010; Calderón et al., 2015).

In accordance with the above cited environmental and health risks, it is necessary to develop effective soil remediation technologies. There is currently a wide range of remediation options. The so called in-situ technologies try to move or degrade soil pollutants without the need of soil excavation and transport to external remediation facilities. Because of it, the use of in-situ technologies allows a significant reduction of costs, although their main drawback is that the in-situ mass and energy transport processes, which are necessary for the development of physical, chemical or biological decontamination mechanisms inside the soil, are clearly disadvantaged, especially in low permeable soils (Tomei and Daugulis, 2013)

The Electrokinetic (EK) treatment is a recent in situ soil remediation technology that it is achieved by the application of a low voltage direct electric current between electrodes placed in a polluted site. The electric field causes the movement and transport of different substances across the soil by different mechanisms (Virkutyke et al., 2002) such as electromigration (the movement of ions to the opposite electrodes), electrophoresis (the movement of charged particles, to the opposite electrodes), and electroosmosis (the movement of water caused by superficially charged phenomena, usually towards the cathode). EK remediation is usually used for the in-situ treatment of low permeable soils with low hydraulic conductivity values because the conventional forced liquid movement across the soil is not suitable. Despite EK is a recent technology, there are many previous works that reported successful and cost-effective removal of different contaminants from low-permeability soils (Rodrigo et al., 2014).

However, EK is mainly focused on the pollutants movement and transport to an external treatment, rather than the in situ pollutant degradation. Because of it there are some possible combinations between EK and degradation techniques in order to allow the pollutants degradation in situ (Yeung and Gu, 2011). One of these combined technologies is electro-bioremediation (Wick et al., 2007). According to the electro-bioremediation fundamental concepts, EK would cause the movement of pollutants, nutrients, electron acceptors and soil microorganisms by different mechanisms into the soil, and then it would cause the contact between them allowing the biodegradation to occur. However, EK can also produce adverse conditions for the microbial activity, such as extreme pH or high temperatures, and so certain precautions should be taken in the design of the technology to avoid unfavourable conditions (Ramírez et al., 2015).

Previous research works focused on electro-bioremediation of polluted soil with different types of pollutants have been reported by the authors of the present work (Ramírez et al., 2015; Barba et al., 2017). Regarding the electro-bioremediation of oxyfluorfen polluted clay soil, low remediation efficiencies were observed despite that highly acclimated microbial cultures were used (so that the pollutant biodegradability was not the limiting process in this technology). It is well known that oxyfluorfen is a non-polar substance and it has low mobility in soil. Commercial oxyfluorfen is added to the agricultural soil using solvents as xylene, that evaporates quickly and thus oxyfluorfen remains in soil with reduced mobility, especially in clay soil. This situation would cause low pollutant bioavailability for the soil microbial population, and thus the retention times needed for its degradation could drastically increase, causing the EK treatment to increase its operation costs compared to the previous reported values (Cameselle, 2014).

In this context, the objective of the present work is to study the influence of the operation time over the remediation efficiency in the electro-bioremediation of an oxyfluorfen polluted clay soil. Authors want to know the effect of long time electro-bioremediation periods over the operating conditions, the biological activity into the soil and the pollutant removal efficiency, in order to know if long retention time would obtain successful remediation results.

Materials & Methods

2.1. Experimental procedure

The experiments were carried out using the experimental devices described elsewhere (Barba et al., 2017). Basically a rectangular cell (10 x 10 x 20 cm) made in transparent methacrylate with three compartments separated by nylon mesh (0.5 mm of mesh size). Graphite electrodes (10 x 10 x 1 cm) were inserted and connected to a power supply. Each electrodic compartment was filled with electrolyte (80.7 mg L^{-1} Na_2SO_4, 70.0 mg L^{-1} $NaHCO_3$ and 30.4 mg L^{-1} $NaNO_3$)

The soil used in these experiments was a clayey real soil characterized by low permeability, and provided by a quarry of Mora, Toledo (Spain). This soil was contaminated with a solution of Fluoxil24, Cheminova Agro, (Madrid, Spain), a commercial herbicide which active principle is oxyfluorfen (24% p/v). As well as, it contains xylene (<60% p/v), ciclohexanone (<13% p/v) and calcium dodecylbencene sulfonate (<4% p/v) to emulsify oxyfluorfen because of its non-polar nature. Furthermore, the microbial consortium was obtained from an active sludge of wastewater treatment plant of a refinery in Puertollano, Spain. The culture was acclimated previously using Bushnell-Hass broth (BHB) as nutrient solution, which composition per litre of Milli-Q water was the following: 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}. Oxyfluorfen was employed as carbon sole source (200 mg L^{-1}). The microbial culture was checked with several biodegradation tests according to the literature found (Moliterni et al., 2012). When the acclimated period was completed, it was analysed the microbial species by using a

MALDI TOF Mass Spectrometry AXIMA-Assurance supplied by Shimadzy (Germany), in order to characterize our microbial culture. The species found were *achromobacter denitrificans, achromobacter xylosoxidans, pseudomonas putida, pseudomonas oryzihabitans and brevibacterium casei.* The authors consider that due to the species found, the biodegradation of oxyfluorfen is under anoxic mechanism, that is heterotrofic denitrification as it also occurs in similar research works (Ramírez et al., 2015).

2.2. Experimental procedure

The soil was prepared by mixing it with the oxyfluorfen solution to pollute the soil, and microorganisms outside the experimental cell according to a procedure described elsewhere (Barba et al., 2017). Five electro-bioremediation batch test were conducted in potentiostatic mode. The electric field applied was 1.0 V cm^{-1} (20.0 V). Polarity reversal strategy was used in these experiments by applying a polarity reversal frequency (2 d^{-1}) (Barba et al., 2017). The tests were disconnected at 2, 4, 6, 11 and 24 weeks. Additionally, one complementary reference test was conducted. The experiment consists of conventional bioremediation test, which helps to know the removal of oxyfluorfen thanks only to the activity of microorganisms in the system.

2.3. Sampling and analyses

In this electro-bioremediation tests, it was carried out the characterization in operation, it means, it was taken daily liquid samples in anodic and cathodic compartments and in the electroosmotic flow. The variables studied in this case were current intensity, pH, conductivity, nutrients and oxyfluorfen concentration. Moreover, the temperature of the soil was measured when the samples were taken daily. When the experiments finalized it was carried out the post-mortem analysis according to a procedure describe elsewhere (Ruiz et al., 2013). It consists of taking soil samples and analysing the following parameters: pH, conductivity, nutrients, microorganisms concentration and pesticide concentration (Barba et al., 2017).

Oxyfluorfen concentration was measured by using a HPLC with UV detector (Jasco, Japan). The mobile phase was acetonitrile/water 75:25 by volume with an isocratic flow rate of 0.6 ml min^{-1}. The column used was a Kinetex 5 μm Biphenyl 100 Å, 150 x 4.5 mm supplied by Phenomenex (USA), and the UV wavelength was 220 nm. The volume of injection was 20 μL. The moisture of the soil was measured gravimetrically by drying the soil for 24 h at 105 ° C. The measurement of nutrients (phosphate, nitrate and ammonium) were carried out by using a photometer Galery (Thermo Scientific). Microorganisms concentration was determined following the procedure described elsewhere (Mena et al., 2016c). Samples was prepared getting 1 g of wet soil and adding 10 mL of NaCl solution (0.9 %), and it was mixed in an agitator vortex during 3 min. After that, aliquots from the supernatant of 100 μL was taken and put on Petri plates, which contains LB media culture (10.0 g L^{-1} NaCl, 5.0 g L^{-1} yeast extract, 10.0 g L^{-1} caseine peptone, 15.0 g L^{-1} European bacteriological agar and 10.0 g L^{-1} glucose). The dishes were incubated during 24 h at 26.5 ºC.

Results & Discussion

Figure 1 shows the changes underwent in the current intensity and temperature during the five tests carried out in this work. These tests were performed simultaneously and exactly under the same operation conditions by the application of an electric field of 1.0 V cm^{-1} between the anode and the cathode contained in each bench-scale mockup. The only difference is the moment in which the electric field is switched off and the post-mortem characterization of the soil is carried out.

As it can be seen, average current is kept within the range 40-60 mA in all tests and there are not very large deviations between the five tests and also among the current monitored during each of the tests. Thus, standard deviations below 25% of the mean value are obtained. Fluctuation around the average value is not very important and it does not reflect a saw tendency as it has been previously reported in other works in which polarity reversal was applied (Mena et al., 2016b). This means that reversion frequency chosen was high enough to prevent important changes in the soil conformation. These changes (most typically local dryness) are responsible for this kind of saw trend in the intensity produced and their absence here can be initially considered as positive for preserving microbial life. Regarding temperature values are also kept near to the room temperature over the whole tests. This means that the electric ohmic loses were not high and heat produced was dissipated easily. Anyhow, during test 2 and 3 there is an increasing trend in temperature that cannot be explained in terms of the current produced by the electric field and that it can only be related to external factors such as the particular position of the mockups that prevents the dissipation of the small amount of heat passed. At this point, it is worth to say that the amount of energy introduced into the system was small. Thus, a maximum value of 0.20 w kg^{-1} can be estimated by taking into account the average intensity (0.05 A), cell voltage (20 V) and assuming that the mass of soil contained in the mockup is around 5 kg and that the most important contribution of the cell voltage are the ohmic loses (taking into account that the overpotentials for water oxidation and reduction are not very high since the intensity monitored are rather low).

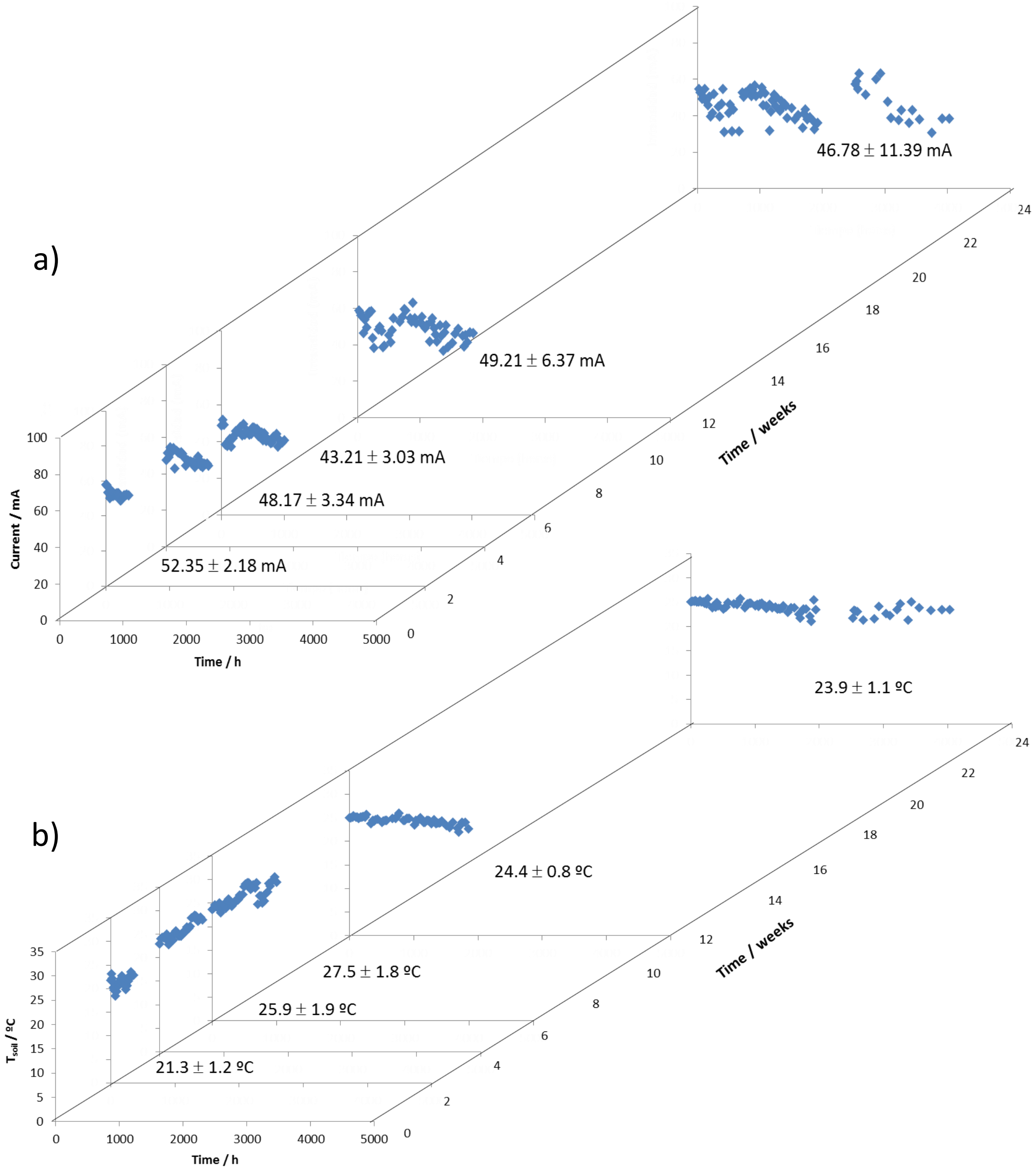

Figure 1. Changes in the current intensity (part a) and temperature (part b) during the five remediation tests

Figure 2 focuses on the changes in the pH in the electrolyte wells and inside the soil (this map is shown only for the final moment of each test because it was obtained from a postmortem analysis). As expected, the reversion in the polarity is a good technique to regulate the pH in the soil and a frequency of 0.5 d^{-1} seems to be suitable to avoid the effects of the acidic and basic fronts in the soil, being them adequate for the combination of the electrochemical and the biological process, as it prevents the main drawback produced by the electrochemical process in the bioprocesses. In fact, these fronts were not detected in the postmortem analysis in any of the tests carried out. Opposite, what it was observed in some of the test was a small difference between the pH measured in the electrolyte well 1 and 2, becoming one of the acidic and the other basic in the moment of the sampling. Initially, this behavior is only obtained in two of the life tests carried out and they indicate that variability in this type of soil remediation technology is important, that is, that the effect of multi-parametricity of the system on results is markedly important. Anyhow, the average pH value in the wells was within the range 7.65-8.45 and the highest standard deviation was 1.66. These ranges of pH were similar in the soil where the average pH was within the range 7.23-8.28 and the maximum standard deviation was 0.32. Differences in the ranges of pH should be explained in terms of the buffering capacity of the soil.

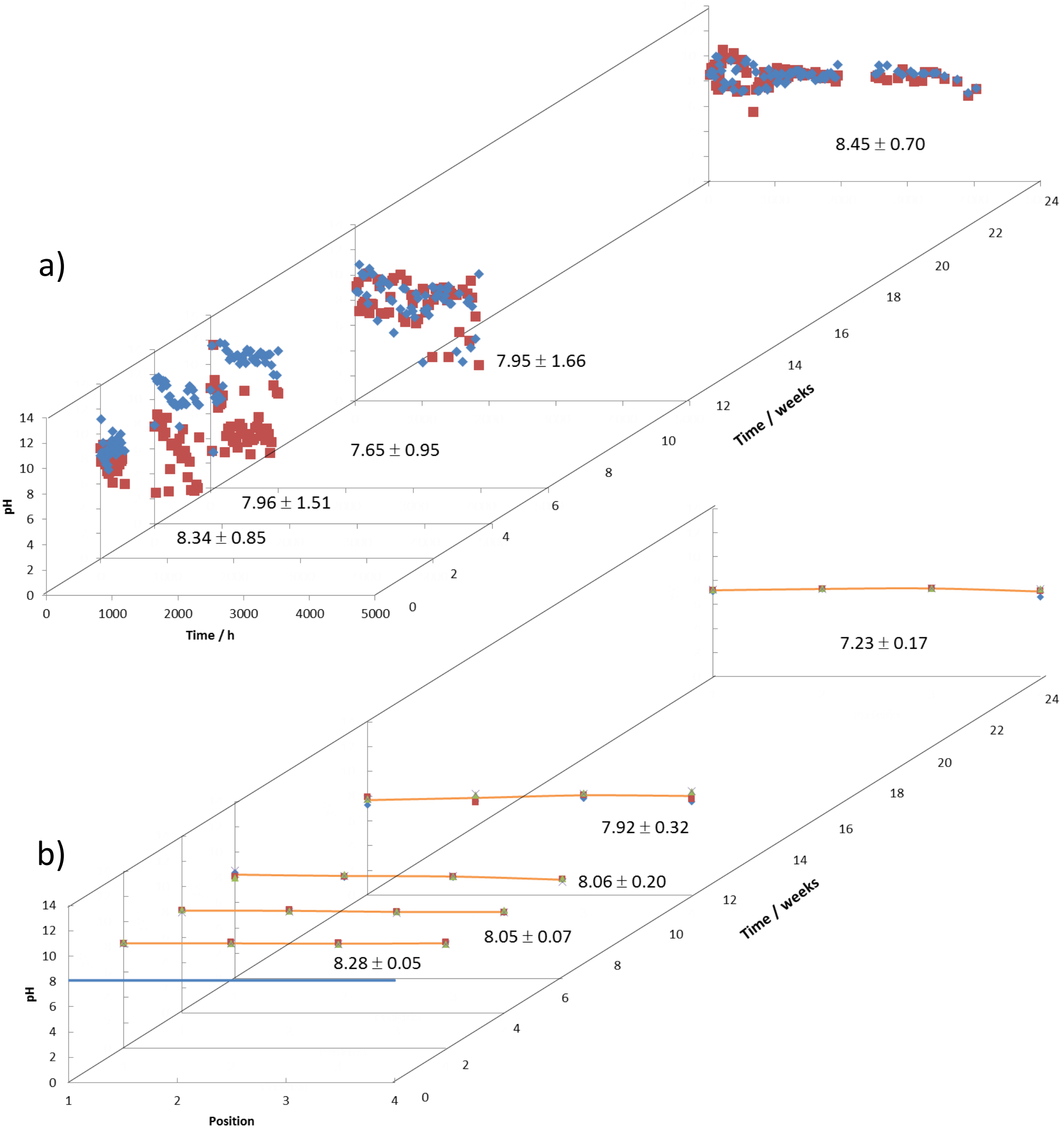

Figure 2. a) Changes in the pH of the liquid contained in the electrode wells during the remediation tests. b) pH profiles in soil after the remediation tests

Opposite to pH changes, the changes in the conductivity are very important (Figure 3), in particular in the central zone of the mockup where the conductivity is clearly below the initial value and it is also below the value reached in the nearness of the electrodes. This informs about partial depletion of ions, which can be dangerous to keep life conditions. As this point, it is worth to take in mind that nutrients are very important to keep life conditions and, for this reason, soil was enriched with nutrients using a BHB solution before the tests. In addition, in the long term tests this medium was added during the tests (in the 11-week test in day 31th and in the 24-week test in days 31th and 80th). Thus, the peaks observed in the two longer tests experiment correspond to the addition of BHB medium to prevent exhaustion of nutrients In addition the flushing fluid added in the electrolyte wells contained 80.75 Na_2SO_4 mg/L, $NaHCO_3$ 70.0 mg/L and 30.36 mg/L of $NaNO_3$.

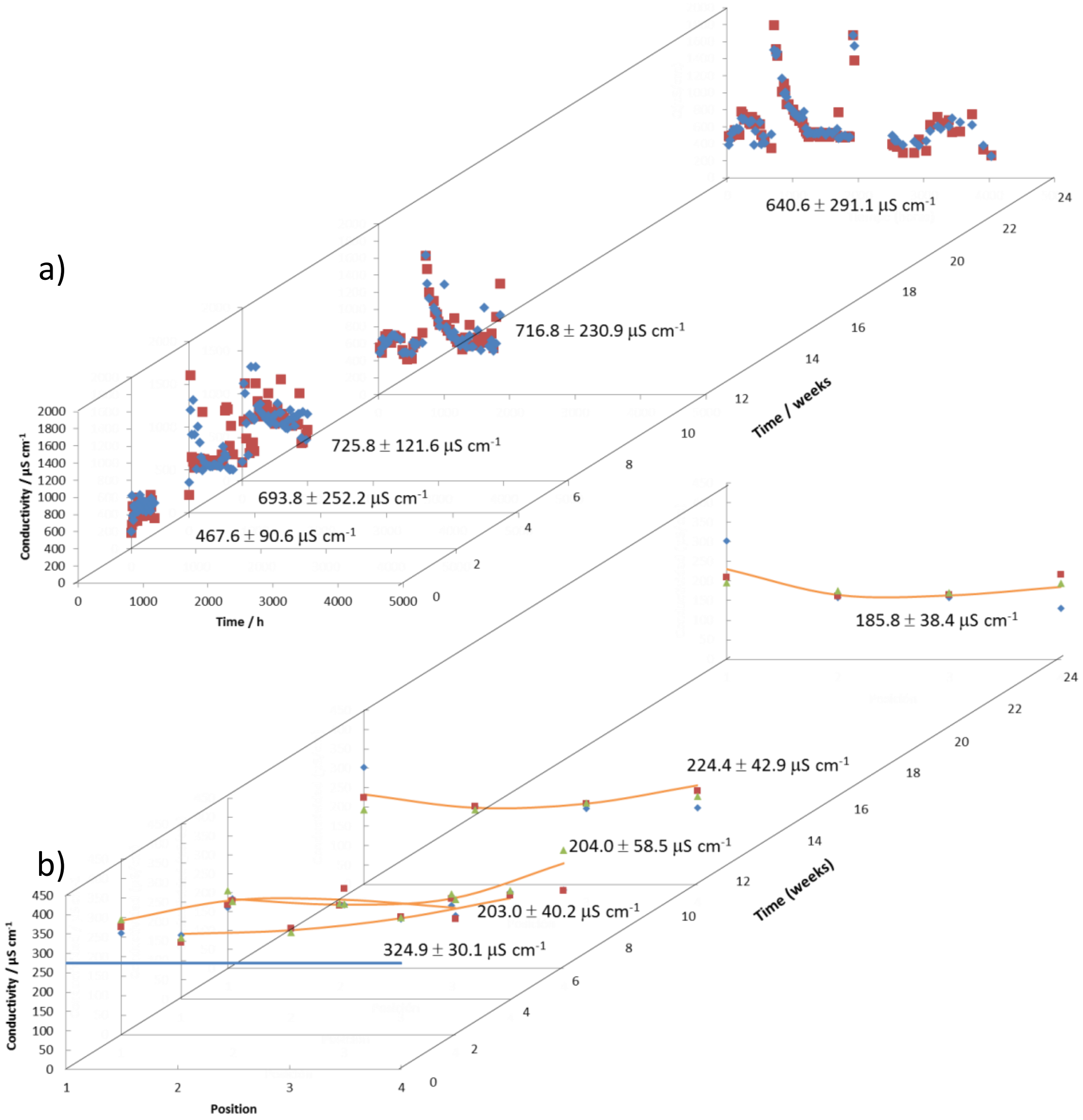

Figure 3. a) Changes in the conductivity of the liquid contained in the electrode wells during the remediation tests. b) Conductivity profiles in soil after the remediation tests

Despite these important income of nutrients, it is important to state that in the postmortem analysis very low concentrations of ammonium and nitrates were found in the soil treated in all the tests (even in the short term ones), meaning the microorganisms may lack these nutrients. This is shown in Figure 4, where the total amount of nitrates and ammonium measured in the postmortem test are compared to the initial amount added.

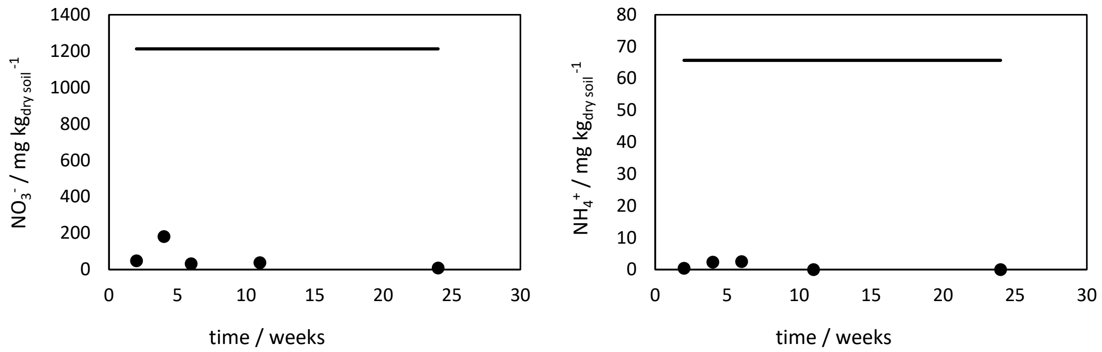

Figure 4. Initial and final values of nitrates and ammonium ions in the five remediation tests performed.

The electro-osmotic flowrates (EOF) are very low and this is the parameter which exhibits the largest discrepancies between the different tests. In fact, each of the five systems behaves in a different fashion and there are test in which the EOF increases up to even 2.5 $cm^3\ h^{-1}$ to finally stabilize in 0.5 $cm^3\ h^{-1}$, while in others the value was kept as low as 0.2 $cm^3\ h^{-1}$. Anyhow, the water content of soil did not undergo very important changes and only for the two longest term tests a minimum value zone is observed in the center of the mockup. As explained in previous works, at least at the bench scale, EK processes increases the water content of the soil and dryness is not a typical problem of operation(Mena Ramirez et al., 2015). Unfortunately, this is not always true at larger scales where it can be found zones in which water is depleted (Mena et al., 2016a).

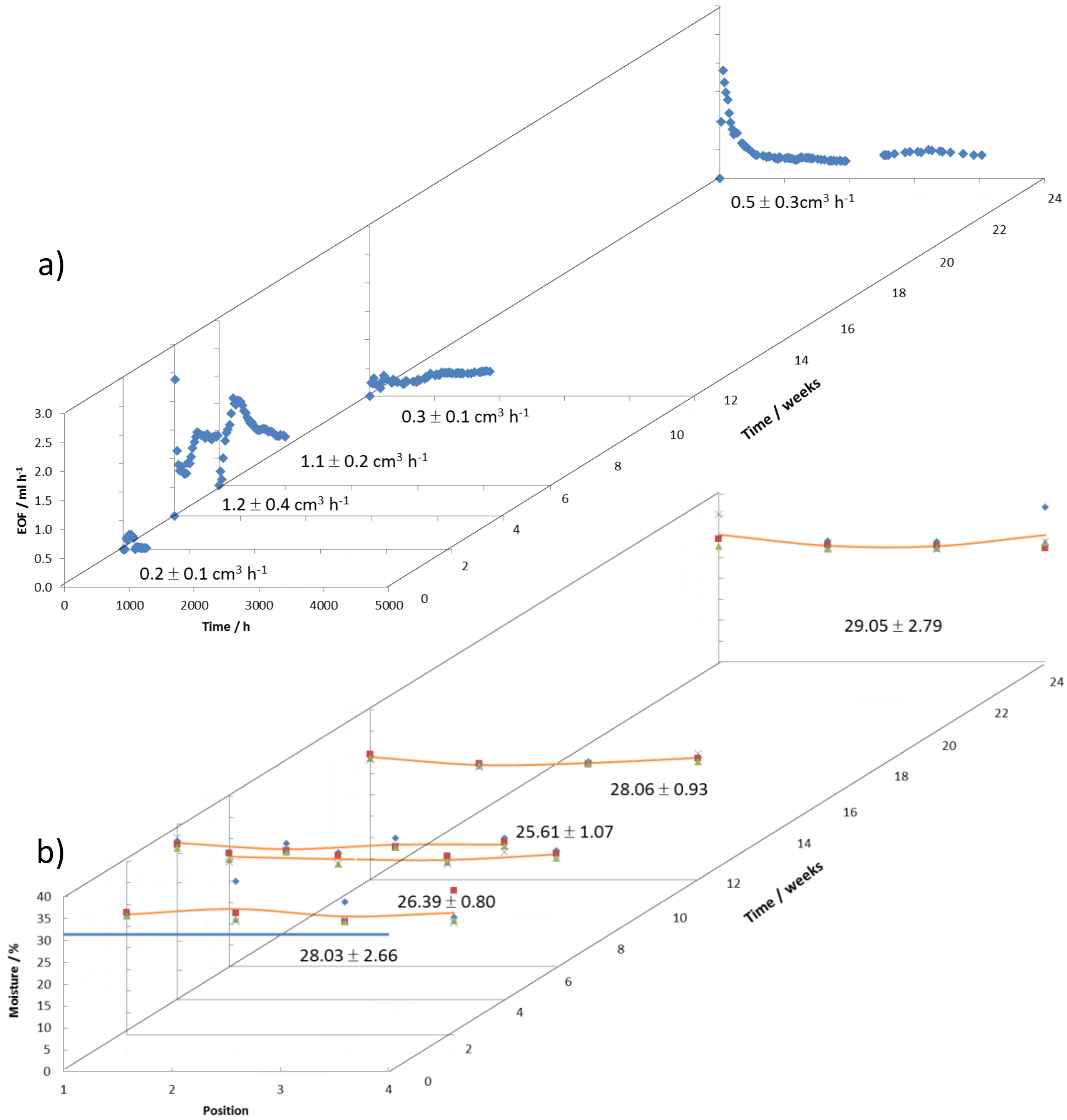

Figure 5. a) Changes in the electro-osmotic flux (EOF) during the remediation tests. b) Water content profiles in soil after the remediation tests

The most important results of this work are plotted in Figure 6, where the changes in the population of microorganisms and in the concentration of herbicide at the end of each of the test are shown. For the sake of clarity, the average values reached are plotted in Figure 7. Clearly it can be seen how the percentage of removal of the pollutant increases with the duration of the test while the concentration of living microorganisms decreases within the same period. Reversion of polarity allows to regulate the pH and avoid potential damages to microorganisms caused by extreme pHs. However, the depletion of nutrients seems to be a major handicap for the technology. Thus, despite important amounts of nutrients that have been added in the electrolyte wells, concentrations in the soil are always very low and, according to results, not enough to keep microorganisms alive. Hence, this process seems to have a very relevant influence on the feasibility of the biological culture and it has to be improved in order to reach a good efficiency.

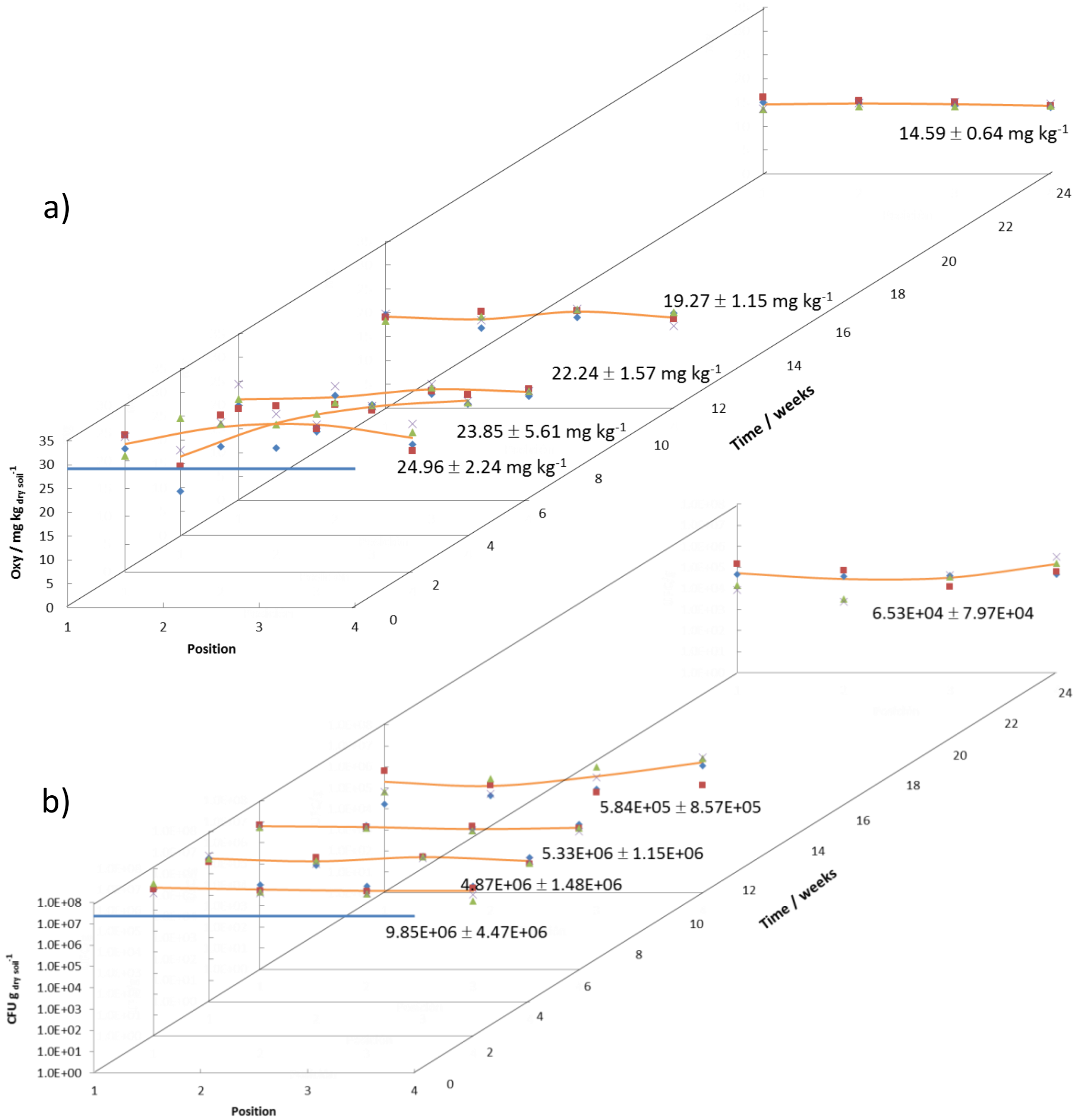

Figure 6. Oxyfluorfen (part a) and microorganisms (part b) profiles in soil after the remediation tests

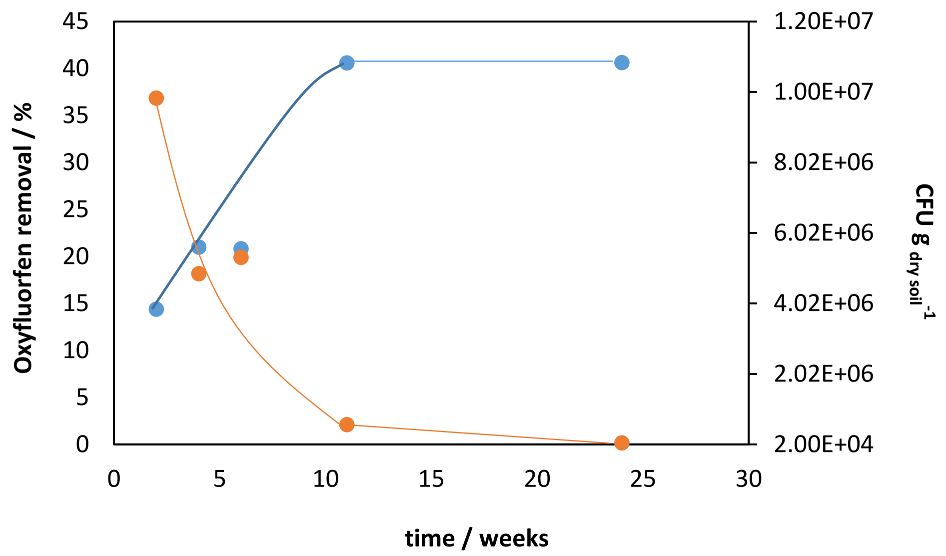

Figure 7. Global results of the test in terms of oxyfluorfen removal and microorganisms contained in soil after the treatment

In order to compare the effect of the electric field, the 2-week test was carried out without the application of an electric field (single bioremediation test). Results of the postmortem analysis carried out in this non-electrochemical test and in the electro bioremediation tests are shown in Figure 8, where it can be observed that the application of the reversible electric field improves the removal of pollutant in a very significant way, while the concentration of microorganisms does not increase but decrease very importantly specially as compared to the single bioremediation test. Thus, the average removal of oxyfluorfen in the single bioremediation was 0.11 mg kg^{-1} d^{-1} and this value increases up to 0.17 mg kg^{-1} d^{-1} with the application of polarity reversed electric current. On the contrary, during the single bioremediation, microorganisms population only decrease slightly being reduced the initial population down to 88.0± 9.0 %. This value compares favorably with the large decay observed when the biological system undergoes the electric field as, in this case, the total population of microorganisms decreases to 41.0±6.0 of the initial value seeded.

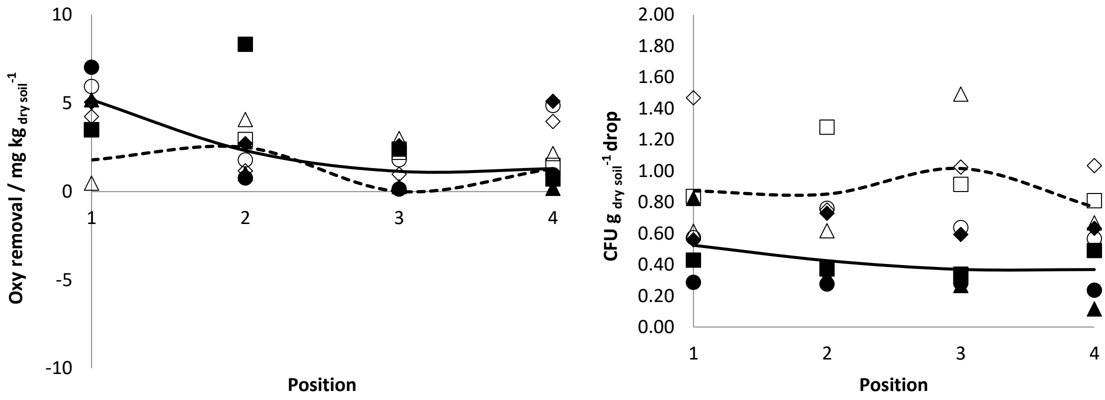

Figure 8. Oxyfluorfen removal and concentration of microorganisms in the soil mockup (△▲ up-right; ●○ up-left; ■□; down-right; ◇◆ down-left). Full points and continuous line: electro-bioremediation test. Empty points and discontinuous line: single bioremediation test)

Conclusions

From this work, the following conclusions can be drawn:

- Electro-bioremediation is a very complex multiparametric process. However, despite the large variability observed in the pH and conductivity of the wells, the key parameters in soil follow a consistent trend and reproducibility of lab-scale tests can be considered acceptable to draw sound conclusions.
- Electro-bioremediation can remove 40% of the oxyfluorfen contained in a polluted soil in less than 10 weeks. During the treatment the microorganisms' population decreases importantly and this decrease can be explained in terms of the depletion of nutrients, because polarity reversal strategy was appropriate to keep the pH of the soil within suitable values.
- Electro-bioremediation attains better performance than single bioremediation and it allows to increase the average removal of oxyfluorfen from 0.11 mg kg^{-1} d^{-1} up

to 0.17 mg kg^{-1} d^{-1}. However, it affects more seriously to microorganisms' viability and the low decrease down to 88.0± 9.0 % of the initial population seeded observed in the bioremediation worsens to 41.0±6.0 with the application of the electric field.

Acknowledgements

Financial support from the Spanish Ministry of Economy, Industry and Competitiveness and European Union through project CTM2016-76197-R (AEI/FEDER, UE) is gratefully acknowledged. The FPI grant BES-2014-069662 is also acknowledged.

References

Barba, S., Villaseñor, J., Rodrigo, M.A., Cañizares, P., 2017. Effect of the polarity reversal frequency in the electrokinetic-biological remediation of oxyfluorfen polluted soil. Chemosphere 177, 120-127.

Calderón, M.J., Real, M., Cabrera, A., Koskinen, W.C., Cornejo, J., Hermosín, M.C., 2015. Influence of olive oil mill waste amendment on fate of oxyfluorfen in southern Spain soils. CLEAN–Soil, Air, Water 43, 1107-1113.

Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N., 2008. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian Journal of Microbiology 48, 114-127.

Mena, E., Barba, S., Saez, C., Navarro, V., Villasenor, J., Rodrigo, M.A., Canizares, P., 2016a. Prescale-Up of Electro-Bioremediation Processes. in: Yesiller, N., Zekkos, D., Farid, A., De, A., Reddy, K.R. (Eds.). Geo-Chicago 2016: Sustainable Waste Management and Remediation, pp. 264-273.

Mena, E., Villasenor, J., Canizares, P., Rodrigo, M.A., 2016b. Effect of electric field on the performance of soil electro-bioremediation with a periodic polarity reversal strategy. Chemosphere 146, 300-307.

Mena, E., Villaseñor, J., Cañizares, P., Rodrigo, M., 2016c. Influence of electric field on the remediation of polluted soil using a biobarrier assisted electro-bioremediation process. Electrochimica Acta 190, 294-304.

Mena Ramirez, E., Villasenor Camacho, J., Rodrigo, M.A., Canizares, P., 2015. Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: A comparison of strategies. Science of the Total Environment 533, 307-316.

Moliterni, E., Jiménez-Tusset, R., Rayo, M.V., Rodriguez, L., Fernández, F., Villasenor, J., 2012. Kinetics of biodegradation of diesel fuel by enriched microbial consortia from polluted soils. International Journal of Environmental Science and Technology 9, 749-758.

Ramírez, E.M., Camacho, J.V., Rodrigo, M.A., Cañizares, P., 2015. Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: a comparison of strategies. Science of the Total Environment 533, 307-316.

Ruiz, C., Mena, E., Cañizares, P., Villaseñor, J., Rodrigo, M.A., 2013. Removal of 2, 4, 6-trichlorophenol from spiked clay soils by electrokinetic soil flushing assisted with granular activated carbon permeable reactive barrier. Industrial & Engineering Chemistry Research 53, 840-846.
Sondhia, S., 2010. Persistence and bioaccumulation of oxyfluorfen residues in onion. Environmental monitoring and assessment 162, 163-168.

References

Calderón, M.J., Real, M., Cabrera, A., Koskinen, W.C., Cornejo, J., Hermosín, M.C., 2015. Influence of olive oil mill waste amendment on fate of oxyfluorfen in southern Spain soils. Clean Soil, Air, Water 43, 1107-1113.

Cameselle, C. 2014, Electrokinetic remediation, costs estimation, in: Kreysa, G., Ota, K., Savinell, R.F. (Eds), Encyclopedia of Applied Electrochemistry, Springer, New York, 2014, 723-725, DOI 10.1007/978-1-4419-6996-5_91.

Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N., 2008. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian Journal of Microbiology 48, 114-127.

Gavrilescu, M., 2005. Fate of pesticides in the environment. Eng. Life Sci. 5, 497–526.

Morillo, E., Villaverde, J., 2017. Advanced technologies for the remediation of pesticide-contaminated soils. Science of the Total Environment 586, 576–597.

Rodrigo, M.A., Oturan, N., Oturan, M.A., 2014. Electrochemically Assisted Remediation of Pesticides in Soils and Water: A Review, Chem. Rev. 114, 8720-8745.

Sondhia, S., 2010. Persistence and bioaccumulation of oxyfluorfen residues in onion. Environ. Monit. Assess. 162, 163-168.

Spanish Presidential Ministry, 2005. Royal Decree 9/2005, of 14 January, which establishes the list of activities that potentially contaminate the soil and criteria and standards for the declaration of polluted soils. Spanish Official Bulletin (BOE), 15, 1833-1843 (in spanish).

Tomei, M.C., Daugulis, A.J., 2013. Ex situ bioremediation of contaminated soils: An overview of conventional and innovative technologies, Crit. Rev. Environ. Sci. Technol. 43, 2107-2139.

USEPA. United States Environmental Protection Agency. Reregistration eligibility decisión (RED), oxyfluorfen; 2002 [EPA: 738-R-02-014].

Virkutyte, J., Sillanpaa, M., Latostenmaa, P., 2002. Electrokinetic soil remediation - critical overview, Sci. Total Environ. 289, 97-121.

Wick, L.Y., Shi, L., Harms, H., 2007. Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions, Electrochim. Acta 52, 3441-3448.

Yeung, A.T., Gu, Y.Y., 2011. A review on techniques to enhance electrochemical remediation of contaminated soils, J. Hazard. Mater. 195, 11-29. Barba, S., Villaseñor, J., Rodrigo, M.A., Cañizares, P., 2017. Effect of the polarity reversal frequency in the electrokinetic-biological remediation of oxyfluorfen polluted soil. Chemosphere 177, 120-127.

Calderón, M.J., Real, M., Cabrera, A., Koskinen, W.C., Cornejo, J., Hermosín, M.C., 2015. Influence of olive oil mill waste amendment on fate of oxyfluorfen in southern Spain soils. CLEAN–Soil, Air, Water 43, 1107-1113.

Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N., 2008. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian Journal of Microbiology 48, 114-127.

Mena, E., Barba, S., Saez, C., Navarro, V., Villasenor, J., Rodrigo, M.A., Canizares, P., 2016a. Prescale-Up of Electro-Bioremediation Processes. in: Yesiller, N., Zekkos, D., Farid, A., De, A., Reddy, K.R. (Eds.). Geo-Chicago 2016: Sustainable Waste Management and Remediation, pp. 264-273.

Mena, E., Villasenor, J., Canizares, P., Rodrigo, M.A., 2016b. Effect of electric field on the performance of soil electro-bioremediation with a periodic polarity reversal strategy. Chemosphere 146, 300-307.

Mena, E., Villaseñor, J., Cañizares, P., Rodrigo, M., 2016c. Influence of electric field on the remediation of polluted soil using a biobarrier assisted electro-bioremediation process. Electrochimica Acta 190, 294-304.

Mena Ramirez, E., Villasenor Camacho, J., Rodrigo, M.A., Canizares, P., 2015. Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: A comparison of strategies. Science of the Total Environment 533, 307-316.

Moliterni, E., Jiménez-Tusset, R., Rayo, M.V., Rodriguez, L., Fernández, F., Villasenor, J., 2012. Kinetics of biodegradation of diesel fuel by enriched microbial consortia from polluted soils. International Journal of Environmental Science and Technology 9, 749-758.

Ramírez, E.M., Camacho, J.V., Rodrigo, M.A., Cañizares, P., 2015. Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: a comparison of strategies. Science of the Total Environment 533, 307-316.
Ruiz, C., Mena, E., Cañizares, P., Villaseñor, J., Rodrigo, M.A., 2013. Removal of 2, 4, 6-trichlorophenol from spiked clay soils by electrokinetic soil flushing assisted with granular activated carbon permeable reactive barrier. Industrial & Engineering Chemistry Research 53, 840-846.
Sondhia, S., 2010. Persistence and bioaccumulation of oxyfluorfen residues in onion. Environmental monitoring and assessment 162, 163-168.