2 **Effect of electrokinetics on the bioaccessibility of polycyclic**
3 **aromatic hydrocarbons in polluted soils**

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ABSTRACT

Bioaccessibility is one of the most relevant aspects to be considered in the restoration of soils using biological technologies. Polycyclic aromatic hydrocarbons (PAH) usually have residual fractions, resistant to biodegradation, at the end of the biological treatment. In some situations, these residual concentrations could be still above legal standards. Here, we propose that the available knowledge about electroremediation technologies could be applied to enhance bioremediation of soils polluted with PAH. The main objective of this study was to show that a previous electrokinetic treatment could reduce the PAH residual fractions when the soil is subsequently treated by means of a bioremediation process. The approach involved the electrokinetic treatment of PAH-polluted soils at a potential drop of 0.9-1.1 V/cm and the subsequent estimations of bioaccessibility of residual PAHs after slurry-phase biodegradation. Bioaccessibility of PAH in two creosote-polluted soils (clay and loamy sand, total PAH content averaging 300 mg/Kg) previously treated with an electric field in the presence of the nonionic surfactant Brij 35 was often higher than in untreated controls. For example, total PAH content remaining in clay soil after bioremediation only was 62.65 ± 4.26 mg/Kg, whereas a 7-days electrokinetic pre-treatment lead, under the same conditions, to a residual concentration of 29.24 ± 1.88 mg/Kg after bioremediation. Control treatments without surfactant indicated that the electrokinetic treatment increased bioaccessibility of PAHs. A different manner of electric field implementation (continuous current vs. current reversals) did not induce changes in PAH bioaccessibility. We suggest that this hybrid technology may be useful in certain bioremediation scenarios, such as soils rich in clay and black carbon, which show

1 limited success due to bioavailability restrictions, as well as in highly
2 heterogeneous soils.

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1 Polycyclic aromatic hydrocarbons (PAHs) represent a risk to public health
2 due their carcinogenic and/or mutagenic properties. In addition, the hydrophobicity
3 of these chemicals causes their tendency to persist in environmental systems for a
4 long time. Despite bioremediation is considered as a reasonable alternative to restore
5 soils and sediments polluted with this group of organic compounds (Breedveld and
6 Karlsen, 2000; Carriere and Mesania, 1995; Eriksson, 2000; Mueller et al., 1989), the
7 success of this technology is often limited by the low bioaccessibility of these
8 chemicals to the microbial populations active in the bioremediation process (Semple
9 et al., 2007). As result of its particular kinetics of biodegradation, known as “hockey
10 stick” kinetics, relevant residual fractions – in some cases, above legal standards – of
11 these compounds can be found at the end of soil restoration by means of biological
12 processes.

13 The combined use of bioremediation with other soil remediation technologies
14 may help to overcome this limitation, thus increasing the possibilities of
15 bioremediation as a treatment option. This may be the case with electroremediation.
16 This emerging technology has been successfully used to restore sites polluted with
17 heavy metals (Lageman et al., 2005) and organic compounds (Ho et al., 1995; Ho et
18 al., 1999; Lageman and Pool, 2001; Lageman et al., 2005; Sonja et al., 2007), and
19 recent research has evidenced the usefulness in integrating bioremediation and
20 electrokinetics – in the so-called electrobioremediation – for an optimized PAH
21 removal from polluted soils (Niqui-Arroyo and Ortega-Calvo, 2007; Niqui Arroyo et
22 al., 2006). The processes involved in the enhanced bioremediation performance
23 caused by electrokinetics may include an optimized biological activity due to heating
24 (Sun et al., 2007), an increased dispersal of degrading cells through the soil matrix

(Wick et al., 2004), and the promotion of pollutant release into porewater caused by electroosmotic flow (Shi et al., 2008).

In spite of these advancements in the field, there is still a limited knowledge of the exact effect of electrokinetic processes on the bioaccessibility of these pollutants and their residual fractions, which, for reasons given above, is a key limitation in the bioremediation of PAHs. The main objective of the present paper was to test whether an electrokinetic treatment of polluted soil is able to enhance PAH bioaccessibility, leading to significant pollutant removal in soil after slurry-phase bioremediation. In order to assess the eventual changes in bioaccessibility after electrokinetic treatment in the two soils considered, a dual ^{14}C /residue analysis method was employed (Posada-Baquero et al., 2008), consisting of the inoculation of the soil with a sufficient number of microorganisms, monitoring of biodegradation activity by means of labelled substrates (^{14}C) and single-step chemical analysis (HPLC) of the native compound at the end of biodegradation process. In this study, the nonionic surfactant Brij 35 was used in all cases, except for control assays, to promote PAH desorption from soil, and therefore to improve bioremediation performance. Based on an extensive literature review that focused on the desorption, solubilization, and/or biodegradation performance of various nonionic surfactants (Liu et al., 1991; Liu et al., 1992; Liu et al., 1995; Yeom et al., 1996; Garcia et al., 2001; Kim et al., 2001), we selected Brij 35 [$\text{C}_{12}\text{H}_{25}\text{O}(\text{CH}_2\text{CH}_2\text{O})_{23}\text{H}$], a biodegradable, nonionic alkylpoly (ethylene glycol) ether surfactant. Moreover, Brij 35 was chosen because it had been found to be nontoxic to bacteria (Tiehm, 1994) and it did not serve as carbon source for growth of the bacterial strain used in this study. The capability of Brij 35 and other non-ionic surfactants to increase, at similar concentrations as those used in this study, PAH desorption from soil during

electrokinetic treatment has also been previously reported (Reddy and Saichek, 2003; Saichek and Reddy, 2003).

MATERIALS AND METHODS

Chemicals

Radiolabelled PAHs, [9-¹⁴C]-phenanthrene (13.1 mCi mmol⁻¹), [3-¹⁴C]-fluoranthene (45.0 mCi mmol⁻¹) and [4,5,9,10-¹⁴C]-pyrene (58.7 mCi mmol⁻¹), all of them with a radiochemical purity >98%, were provided by Sigma Chemical Co., St. Louis. Brij 35, nonionic alkylpoly (ethylene glycol) ether surfactant [C₁₂H₂₅O(CH₂CH₂)H] was also obtained from Sigma.

Soils

Three soils were used in this study: two creosote-polluted clay soils and an agricultural soil. Clay soils (samples I and E2), both of them classified as calcareous fluvisols, were provided by EMGRISA (Madrid, Spain) from a wood-treating facility located in Southern Spain, with a record of pollution by creosote exceeding 100 years. These soils were sampled from different locations of this site, but both samples had the same organic matter content and particle size distribution, namely 3 % organic matter, 1 % coarse-grained sand (2.0-0.2 mm), 2 % fine-grained sand (0.20-0.05 mm), 37 % silt (0.050-0.002 mm), and 60 % clay (< 0.002 mm). However, the PAH content was different for these soils: sample I showed, according to EMGRISA data, heavy pollution by PAHs (4,501 mg/Kg, sum of 16 EPA PAH), while total PAH concentration in

sample E2 was 50 mg/Kg. A more detailed description of clay soil characteristics is given elsewhere (Niqui Arroyo et al., 2006). The agricultural soil, non polluted soil, was a loamy sand from Coria del Río, Seville, Spain (Typic Xerochrepts, 0.8 % organic matter, pH 6.5). Its particle size distribution was 73.3 % coarse-grained sand (2.0-0.2 mm), 6.0 % fine-grained sand (0.20-0.05 mm), 14.0 % silt (0.050-0.002 mm), and 6.7 % clay (< 0.002 mm), and had no detectable PAH pollution (Niqui-Arroyo and Ortega-Calvo, 2007). The soils were air-dried and sieved (2 mm mesh). Clay soil I was used in 1:10 mixtures with sample E2 and loamy sand. These PAH-containing soil mixtures, obtained after mild homogenization in a glass vessel with a rotary shaker for 6 h, will be referred from now on as “clay soil” and “loamy sand” respectively. This homogenization period was necessary, to allow reproducible results – as related to soil PAH contents – due to the high consistency of the clay-rich soil aggregates present in the field-polluted soil (sample I). However, to account for possible variability of PAH concentrations among the batches prepared for each electrokinetic experiment, analysis of each batch was carried out and initial concentration is reported for every case.

Bacteria, media and cultivation

The bacterium used in this study, *Mycobacterium gilvum* VM552, was able to grow with phenanthrene, fluoranthene and pyrene as carbon and energy sources, and was also able to degrade anthracene and benzo[a]pyrene, probably through growth-linked or cometabolic reactions. The strain was cultured with phenanthrene as sole carbon source and prepared for bioaccessibility experiments as previously described (Niqui-Arroyo and Ortega-Calvo, 2007).

Electrokinetic treatment

Soil samples used in this study were subjected to different electrokinetic treatments. The treatments were performed within the framework of a separate study on the simultaneous integration of bioremediation and electrokinetics (Niqui-Arroyo and Ortega-Calvo, 2007). The samples originated from electrokinetic treatments in the presence of surfactant, and were studied in relation to the bioaccessibility of residual PAHs, what was not the subject of that previous research. The electrokinetic cells consisted of a polyethylene body protected with an inner layer of glass, to prevent PAH loss due to sorption. Two rod electrodes, made of a titanium base and coated with a mix of platinum metal group oxides (METAKEM GmbH, Usingen, Germany), were inserted in the soil inside cylindrical glass filtercandles (Robuglasfilter-Geräte GMBH, Hattert, Germany) which served as electrode reservoirs. The filtercandles had porous walls (160-250 μm pore size), allowing the exchange of electrode solution into and out of the reservoirs. The separation distance between the electrodes was 16 cm. The test soil (200 g) was packed in the cell as a 5 cm-wide zone perpendicular to the line between the electrodes. The PAH-polluted soil was between two zones containing each 300 g of uncontaminated, agricultural soil, which was in contact with electrode reservoirs. Potential drop applied to electrokinetic cells ranged from 0.9 to 1.1 V/cm. This potential, being somewhat higher than that used in the field (0.3-0.4 V/cm), is normally used to shorten experimental periods in electrokinetic studies under laboratory conditions. The electrokinetic treatment conditions did not cause significant increases in temperature. Clay soil received two different treatments, with and without the surfactant Brij 35. A first soil batch was treated during 7 days in the presence of Brij 35 at 35 mg/g dry soil. This concentration was well above the critical micelle concentration (CMC) of

1 this surfactant in soil suspensions, 0.078 mg/mL (Bueno-Montes, personal
2 communication). A second electrokinetic treatment was carried out during 26 days.
3 Continuous application of current was employed along both clay soil assays. Treatments
4 of loamy sand were carried out under continuous current and no changes in polarity
5 during 7 or 18 days, respectively, and with 24-h inversions of polarity during 18 days.
6 All treatments of loamy sand were performed in the presence of Brij 35 at 35 mg/g. A
7 more detailed description of the experimental conditions for electrokinetic treatments is
8 given elsewhere (Niqui-Arroyo and Ortega-Calvo, 2007).

9 10 **Bioaccessibility experiments**

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12 PAH bioaccessibility in soil samples previously treated by electrokinetics was
13 estimated in slurries through a dual ^{14}C /residue analysis method (Posada-Baquero et al.,
14 2008). Experiments were performed in duplicate in 250 mL Erlenmeyer flasks. Fifty
15 microliters of an acetone solution, with approximately 100,000 dpm of respective ^{14}C -
16 radiolabeled substrate (phenanthrene, fluoranthene or pyrene) was added to 2 g of soil.
17 Immediately, 100 mL of inorganic salts solution (pH 5.8) was added to every flask and
18 soil slurries were homogenized in an orbital shaker for 48 h. No $^{14}\text{CO}_2$ production was
19 detected during this homogenization period. Soil slurries were then inoculated with the
20 bacterium *Mycobacterium gilvum* VM 552. Every flask received 1 mL of inoculum
21 containing between 10^7 - 10^9 cells/mL. The flasks were closed with Teflon-lined
22 stoppers, from which an 8-mL vial containing 1 mL of 0.5M NaOH was suspended to
23 trap $^{14}\text{CO}_2$. The flasks were incubated at $23 \pm 2^\circ\text{C}$ on an orbital shaker operating at 150
24 rpm and production of $^{14}\text{CO}_2$ was measured as radioactivity appearing in the alkali trap.
25 The NaOH solution was mixed with 5 mL of scintillation cocktail, kept in darkness for
26 at least 8 h to dissipate chemiluminescence. Finally, radioactivity was measured with a

liquid scintillation counter (Ready Safe, Beckman Instruments, Inc., Fullerton, Calif.; model LS5000TD). Maximum mineralization rates were calculated and compared as the slope of the regression lines drawn with the points belonging to the phase of maximum mineralization and initial concentrations of the native PAHs (Ortega-Calvo et al., 1995). Simultaneously, PAH residual content at the end of biodegradation process was measured in soil samples incubated under the same conditions described above but contained no ^{14}C -labeled compound. Once mineralization reached the plateau phase, duplicated flasks were sacrificed and kept frozen at -80°C to prevent biodegradation and PAH concentration was determined by high performance liquid chromatography as described below. Additionally, three times autoclaved soil samples, all of them not previously subjected to electrokinetic treatment, with and without surfactant Brij 35 at 35 mg/g, were incubated under the same conditions and worked as controls of the assays described above. Soil samples were sterilized three times by means of autoclave (1 atm, 120°C) before electrokinetic treatment. Although this step could modify the desorption pattern of PAHs, all samples, including the controls, were subjected to same treatment.

PAH analysis

Soil samples were defrosted, subsequently subjected to an initial drying stage at $35 - 40^{\circ}\text{C}$, and finally dried completely using a similar weight of anhydrous sodium sulphate to ground the mixture in a mortar. Samples were extracted in a Soxhlet with 100 mL acetone for 8 hours. Preliminary experiences showed no significant effect of Brij 35 on the recovery of PAH with this extraction method. Then, the organic solvent was evaporated to near dryness, and the residue was re-dissolved in 4-5 mL of dichloromethane. The clean-up of the samples was then carried out using Sep-Pak Plus

1 Florisil cartridges (WATERS Corp., Milford, MASS, USA) to remove polar
2 compounds. Once this clean-up was performed, organic phase was evaporated in a
3 gentle stream of nitrogen and the residue was dissolved in a known volume of
4 acetonitrile (between 8 and 10 mL). Finally, samples were filtered through a syringe
5 filter of nylon (0.45 μm , 13 mm $\varnothing$, Teknokroma, Barcelona, Spain), and analysis of
6 PAH was carried out using a Waters HPLC system (2690 separations module, 996
7 photodiode array and 474 scanning fluorescence detectors. Column: Inertsil ODS-P C₁₈
8 Teknokroma, 5 μm , 4.6 I. D. x 250 mm; flow: 1 mL min⁻¹; mobile phase: 45%
9 acetonitrile 55% water). The mobile phase used was an acetonitrile-milli-Q water
10 gradient comprising 45% (v/v) acetonitrile from 0 to 5 min, 95% (v/v) acetonitrile from
11 5 to 25 min programmed to 100 % acetonitrile between 25 to 38 min. The initial solvent
12 composition was then maintained for further 13 min. Analysis of phenanthrene content
13 of a reference material was in agreement with the certified value, with deviations not
14 exceeding 5% for this PAH (Niqui Arroyo et al., 2006). When the surfactant Brij 35
15 was not present in soil samples, PAH extraction was carried out with dichloromethane
16 as organic solvent, instead acetone. Concentrations of PAHs are reported as mg/Kg of
17 dry soil.

18 19 **RESULTS**

20 21 **Effect of electrokinetic treatment on bioaccessibility of PAHs**

22
23 The maximum rates and extents of ¹⁴CO₂ production observed in bioaccessibility
24 assays of electrokinetically-treated soils were similar to those observed in controls (data
25 not shown). This indicates that the possible effect of electrokinetic treatment on

bioaccessibility was restricted to the second, slow phase of biodegradation, what cannot be estimated through radiorespirometry only due to inherent uncertainties related to the direct translation of final mineralization extents into percentages of chemical loss (Posada-Baquero et al., 2008). Nevertheless, the use of ^{14}C -labeling allowed the determination of the time period needed for biodegradation of the bioaccessible fraction of the native chemicals.

Residual contents of native PAH at the end of the bioaccessibility assays indicated an increase of compound bioaccessibility as a result of 7 days of electrokinetic treatment (Tables 1 and 2). In these tables, PAH concentrations before and after treatment are indicated as C_o and C_e , respectively, whereas C_f refers to the PAH concentration at the end of the bioaccessibility assay. According to previous results (Niqui-Arroyo and Ortega-Calvo, 2007), the surfactant Brij 35 was used in these experiments in order to optimize and shorten the electrokinetic treatment. The 7-day period was short enough to avoid significant losses of PAH during electrokinetic treatment (as indicated by C_e values in Tables 1 and 2). The increased bioaccessibility was evidenced by the significantly lower ($P=0.05$) C_f values as compared with the untreated controls, and resulted in a 50% decrease of total PAH content at the end of the bioaccessibility assays with both types of soil.

To assess the relative contribution of the surfactant and the electrokinetic treatment to these losses, control bioaccessibility assays were carried out in the absence of the surfactant. The resulting C_f values for total PAH concentrations in these controls were 57.01 ± 0.42 mg/Kg and 23.40 ± 9.16 mg/Kg for clay soil and loamy sand, respectively. These values were not significantly different ($P=0.05$) to the residual contents resulting in bioaccessibility controls with surfactant (Tables 1 and 2), thus indicating that the surfactant alone had no apparent effect, as evidenced by these control

assays. A separate experiment was also performed with clay soil that was treated electrokinetically during 26 days under the same conditions but without surfactant. The corresponding C_o , C_e and C_f values for total PAH concentrations in bioaccessibility assays with this soil were, respectively, 282.04 ± 14.30 mg/Kg, 259.07 ± 7.18 mg/Kg and 47.45 ± 10.84 mg/Kg. The differences with C_f values from Table 1 further indicate that the surfactant was needed for a significant effect of electrokinetic treatment on bioaccessibility.

Extending to 18 d the period of electrokinetic treatment did not result in significantly lower C_f values (Table 3), as compared with 7-d treatment (Table 2), although compound losses were significantly lower than the control (Table 1). This occurred both when the current was applied in the continuous mode or with 24-h inversions, what further indicates an effect of electrokinetics on bioaccessibility. Interestingly, in spite of the differences observed in the losses after electrokinetic treatment (as revealed by different C_e values indicating losses of 47 % and 63 % of total PAH with continuous current and current reversal, respectively), the C_f values were very similar with both current application regimes (7.20 ± 1.70 mg/Kg and 7.11 ± 1.75 mg/Kg, respectively- Table 3).

Kinetics of biodegradation of slowly-degrading PAH fractions present in treated soils

In this study, the electrokinetic treatments and bioaccessibility assays were performed using different soil batches and, therefore, initial PAH concentrations (C_o and C_e values in controls and electrokinetic treatments, respectively, Tables 1-3) were

slightly different. However, the experimental data obtained on residual fractions after different treatments (C_f values) can be compared. To illustrate this, Fig. 1 shows the simulation of biodegradation of native phenanthrene in clay soil slurries. The different plots compare the biodegradation process under the optimal conditions set in the bioaccessibility assays, assuming that biodegradation follows the next equation:

$$S_t/S_o = F_{rap} \exp(-k_{rap}t) + F_{slow} \exp(-k_{slow}t)$$

where S_t and S_o (g) are the amounts of phenanthrene at time t (h) and at the start of the experiment, respectively. F_{rap} and F_{slow} are the rapidly and slowly-biodegrading fractions while k_{rap} and k_{slow} (h^{-1}) are the rate constants of rapid and slow biodegradation. The parameter values employed for this simulation, already published (Niqui Arroyo et al., 2006), were, for untreated soil, 0.046 h^{-1} and 0.001 h^{-1} for k_{rap} and k_{slow} , respectively, and 0.95 for F_{rap} (solid line in Fig. 1). The short-dash line corresponds to the biodegradation of phenanthrene in electrokinetically-treated soil. If we assume that the rate constants (k_{rap} and k_{slow}) do not change upon treatment and that the initial phenanthrene concentrations (158.55 mg/Kg vs 110.17 mg/Kg in control and treated soils, respectively – C_0 and C_e values in Table 1) can be attributed only to differences in the rapidly biodegrading fractions (resulting in a decrease of F_{rap} from 0.95 to 0.93 after treatment), it can be observed that the residual phenanthrene concentrations are very similar (5.87 mg/Kg and 5.71 mg/Kg, respectively). However, the long-dash plot in Fig. 1 represents a different situation, where the same decrease in F_{rap} is produced as a result of electrokinetic treatment but also a change in the rate constant of slow biodegradation (from 0.001 to 0.004 h^{-1}). The resulting concentration of residual phenanthrene (2.32 mg/Kg dry soil) is appreciably lower than that observed

1 in the previous situations, thus reproducing the relative differences observed
2 experimentally in the bioaccessibility assays between the control and the treated soils
3 (Table 1). Accordingly, the residual PAH fractions after biodegradation are not
4 significantly affected by slight differences in the initial concentrations, but a change of
5 k_{slow} is needed to produce a fall of this residual fraction. These calculations suggest,
6 therefore, that the electrokinetic treatment could have increased the constant rate of
7 slow biodegradation.

8 9 **DISCUSSION**

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11 Our previous assessments of bioremediation potential in PAH-polluted soils
12 showed the common occurrence of hydrocarbon residual fractions, that resisted
13 biodegradation even when optimal conditions for microbial activity were used (Niqui-
14 Arroyo and Ortega-Calvo, 2007; Niqui-Arroyo et al., 2006). The desorption kinetics of
15 these compounds seemed to be responsible of those resistant fractions. In this study, the
16 slowly-degrading compounds remaining at the end of the bioaccessibility assays very
17 likely exhibited slow desorption, what usually limits their biodegradation by
18 microorganisms (Gómez-Lahoz and Ortega-Calvo, 2005). Therefore, we suggest that
19 the application of an electric field to soils (and the electroosmotic flow generated
20 consequently) in the presence of the non ionic surfactant Brij 35 modified the
21 bioaccessibility of PAHs by changing their desorption kinetics. Probably, the
22 development of an electroosmotic flow through soil aggregates caused the conversion of
23 the fraction of slowest desorption into a faster fraction, with a higher bioaccessibility
24 (Gómez-Lahoz and Ortega-Calvo, 2005; Shi et al., 2008). The induced physical and
25 chemical changes could have mobilized the pollutant fraction present in soil nanopores

1 and/or strongly sorbed to black carbon, situations which usually result in a diminished
2 bioaccessibility to soil microorganisms (Nam et al., 1998; Rhodes et al., 2008).
3 Experimental approaches focused on changes on the desorption kinetics of these
4 chemicals (e.g. using Tenax as sorbent polymer (Braida et al., 2004; Cornelissen et al.,
5 2001)) as result of electrokinetic treatment will be part of future investigations.

6 The results of bioaccessibility tests on samples of clay soil, both the absence and
7 presence of Brij 35, suggest that the surfactant plays an important role in the
8 effectiveness of the electrokinetic treatment on the bioavailability of PAHs. In this
9 sense, some studies have shown the capability of several agents, mainly surfactants, to
10 enhance the solubilization or desorption of organic pollutants in soils in combination
11 with electroosmotic flow for soil washing (Jiradecha et al., 2006; Ko et al., 2000; Reddy
12 and Saichek, 2003; Reddy and Saichek, 2004; Saichek and Reddy, 2003; Yuan and
13 Weng, 2004). Our study extends those findings by showing that this approach can also
14 be used in certain bioremediation scenarios to promote bioaccessibility and hence
15 biodegradation by soil microbial populations. The benefits from this process will
16 depend of the type of soil, i.e., clay-rich soils exhibiting high consistency, nanoporosity
17 and surface area available for sorption may be more difficult to treat biologically than
18 sandy soils but their treatment affordable by this combined
19 electrokinetic/bioremediation approach. Also, successful bioremediation of PAH-
20 polluted soils rich in black carbon, such as manufactured gas plant soils, may also be
21 limited by bioaccessibility restrictions (Reichenberg et al. 2010), that could be also
22 overcome through this hybrid technology. Treatment of highly heterogeneous soils,
23 where different materials may have highly dissimilar hydraulic conductivities, may also
24 benefit from the electroosmotic flow, which is not affected by the distribution of pore
25 size and the presence of macropores in soil.

The results from this study also indicated that a different manner of electric field implementation did not induce changes on PAH bioaccessibility. Nevertheless, it should be noted that, according to previous results (Maturi and Reddy, 2006; Reddy and Saichek, 2004), changes in polarity or in the period of application of the current (i.e. pulses) could be very useful to optimize the electrobiorremediation process *in-situ*, specially for clay-rich soils. Using surfactants for environmental remediation has potential risks due to their possible toxicity and effects on soil quality, and has also economical implications. Therefore, the effect of minimization of surfactant dose on electrobioremediation performance is also of interest to reduce the environmental impact of this technology and the costs of treatment, and will be the subject of future investigations.

CONCLUSIONS

The results presented in this study suggest that the electrokinetic treatment of PAH polluted soils could increase the bioaccessibility of these chemicals to PAH-degrading bacteria. This stimulating effect seems to be more efficient when a nonionic surfactant (Brij 35) is applied to the soil. We propose therefore the electrokinetic treatment as an innovative step to improve the effectiveness of PAH removal from soil through bioremediation.

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Table 1. Effect of electrokinetic treatment on the bioaccessibility of polycyclic aromatic hydrocarbons (PAH) in slurries of clay soil, as compared with an untreated control. The electrokinetic and the control treatments were carried out in presence of the nonionic surfactant Brij 35 at 35 mg/g.^{†, ††}

PAH	Control		Electrokinetic treatment		
	C_o	C_f	C_o	C_e	C_f
Phenanthrene	158.55 ± 4.58	13.92 ± 0.98A	161.73 ± 3.74	110.17 ± 7.40	4.36 ± 0.17B
Anthracene	31.04 ± 0.23	3.68 ± 1.29A	31.10 ± 0.28	26.88 ± 0.37	1.42 ± 0.17A
Fluoranthene	48.76 ± 2.77	33.96 ± 1.64A	101.18 ± 0.94	95.15 ± 4.45	17.64 ± 1.80B
Pyrene	47.37 ± 5.22	9.54 ± 0.60A	63.70 ± 3.61	52.21 ± 2.40	3.72 ± 0.16B
Benzo(a)pyrene	1.62 ± 0.04	1.55 ± 0.14A	5.11 ± 0.25	5.68 ± 1.65	2.12 ± 0.02B
Σ PAH ^{†††}	287.34 ± 8.11	62.65 ± 4.26A	362.83 ± 7.77	290.08 ± 16.29	29.24 ± 1.88B

[†] Values are reported as PAH concentration in mg/Kg. For each PAH, C_f values followed by the capital letter are not significantly different (t -student, $P = 0.05$).

^{††} C_o (Initial PAH concentration); C_e (PAH concentration after 7 days of electrokinetic treatment); C_f (PAH concentration after bioaccessibility assay).

^{†††} Sum of PAH degraded by *M. gilvum* VM552 (phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene).

Table 2. Effect of electrokinetic treatment on the bioaccessibility of polycyclic aromatic hydrocarbons (PAH) in slurries of loamy sand, as compared with an untreated control. The electrokinetic and the control treatments were carried out in presence of the nonionic surfactant Brij 35 at 35 mg/g.^{†, ††}

PAH	Control		Electrokinetic treatment		
	C_o	C_f	C_o	C_e	C_f
Phenanthrene	105.81 ± 19.00	2.77 ± 1.14A	164.24 ± 19.52	73.03 ± 0.28	1.81 ± 1.40A
Anthracene	14.69 ± 3.05	1.77 ± 0.26A	32.06 ± 3.63	13.68 ± 0.53	1.25 ± 0.06AB
Fluoranthene	57.90 ± 4.20	9.25 ± 0.31A	114.45 ± 8.29	58.59 ± 0.78	5.70 ± 0.34B
Pyrene	29.77 ± 5.11	3.93 ± 0.21A	54.34 ± 4.70	25.77 ± 0.27	1.29 ± 0.59B
Benzo(a)pyrene	1.59 ± 0.09	0.69 ± 0.01A	3.55 ± 0.01	1.80 ± 0.16	0.85 ± 0.08B
ΣPAH^{†††}	209.75 ± 31.45	18.40 ± 0.78A	368.63 ± 23.75	173.16 ± 2.02	10.89 ± 1.80B

[†] Values are reported as PAH concentration in mg/Kg. For each PAH, C_f values followed by the capital letter are not significantly different (analysis of variance, $P = 0.05$). Statistical comparisons were performed together with C_f values in Table 3.

^{††} C_o (Initial PAH concentration); C_e (PAH concentration after 7 days of electrokinetic treatment); C_f (PAH concentration after bioaccessibility assay).

^{†††} Sum of PAH degraded by *M. gilvum* VM552 (phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene).

Table 3. Effect of current reversal every 24 h on the bioaccessibility of polycyclic aromatic hydrocarbons (PAH) after electrokinetic treatment in slurries of loamy sand, as compared with continuous current application. The electrokinetic treatments were carried out in presence of the nonionic surfactant Brij 35 at 35 mg/g.^{†, ††}

PAH	Continuous current			24 h inversion		
	C_o	C_e	C_f	C_o	C_e	C_f
Phenanthrene	169.37 ± 1.42	81.75 ± 7.23	1.38 ± 0.25A	105.81 ± 19.00	30.35 ± 2.77	1.52 ± 0.40A
Anthracene	26.95 ± 1.52	16.19 ± 0.77	0.73 ± 0.37BC	14.69 ± 3.05	5.67 ± 0.07	0.51 ± 0.10C
Fluoranthene	118.63 ± 0.10	68.75 ± 5.14	3.92 ± 1.48B	57.90 ± 4.20	24.25 ± 1.85	3.35 ± 0.83B
Pyrene	94.55 ± 0.04	51.19 ± 0.00	0.56 ± 0.14B	29.77 ± 5.11	17.40 ± 0.05	1.21 ± 0.45B
Benzo(a)pyrene	1.65 ± 0.42	1.12 ± 0.13	0.62 ± 0.04AC	1.59 ± 0.09	0.63 ± 0.01	0.54 ± 0.02C
ΣPAH^{†††}	411.14 ± 0.59	218.99 ± 13.27	7.20 ± 1.70B	209.75 ± 31.45	78.30 ± 0.91	7.11 ± 1.75B

[†] Values are reported as PAH concentration in mg/Kg. For each PAH, C_f values followed by the capital letter are not significantly different (analysis of variance, $P = 0.05$). Statistical comparisons were performed together with C_f values in Table 2.

^{††} C_o (Initial PAH concentration); C_e (PAH concentration after 18 days of electrokinetic treatment); C_f (PAH concentration after bioaccessibility assay). See Table 2 for bioaccessibility control.

^{†††} Sum of PAH degraded by *M. gilvum* VM552 (phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene).

Figure legend

Fig. 1. Simulated biodegradation of native phenanthrene in clay soil slurries under different conditions: (a) no previous electrokinetic treatment (solid line), (b) soil treated electrokinetically, resulting in a lower F_{rap}/F_{slow} ratio (short dash plot) and (c) soil treated electrokinetically, with a lower F_{rap}/F_{slow} ratio and a four times higher k_{slow} (long dash plot).

Fig. 1