Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: a comparison of strategies.

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ABSTRACT

The aim of this work is to compare different strategies based on electrokinetic soil flushing and bioremediation for the remediation of diesel-polluted soil. Four options were tested at the laboratory scale: single bioremediation (Bio), performed as a control test; a direct combination of electrokinetic soil flushing and biological technologies (EKSF-Bio); EKSF-Bio with daily polarity reversal of the electric field (PR-EKSF-Bio); and a combination of electrokinetic soil flushing and a permeable reactive biological barrier (EKSF-BioPRB). Four batch experiments of 14 days duration were carried out for comparing technologies at room temperature with an electric field of 1.0 V cm^{-1} (in EKSF). A diesel degrading microbial consortium was used. The

experimental procedure and some specific details, such as the flushing fluids used, varied depending on the strategy. When using the EKSF-Bio option, a high buffer concentration was required to control the pH, causing soil heating, which negatively affected the biological growth and thus the diesel removal. The PR-EKSF-Bio and the EKSF-BioPRB options attained suitable operating conditions and improved the transport processes for biological growth. Polarity reversal was an efficient option for pH, moisture and temperature control. Homogeneous microbial growth was observed, and approximately 20% of the diesel was removed. The BioPRB option was not as efficient as PR-EKSF-Bio in controlling the operating conditions, but the central biobarrier protected the biological activity. Microbial growth was observed not only in the biobarrier but also in a large portion of the soil, and 29% of the diesel was removed in the short remediation test.

Keywords

Electrokinetic soil flushing, electrobioremediation, diesel polluted soil, polarity reversal, permeable reactive barrier.

1. Introduction

Soil pollution is a major environmental problem. Depending on the type of soil and pollutant, there are many possible scenarios whose effective treatment requires the specific research and development of the correct technologies. One common case is the accidental discharge of fuels, which may significantly damage the environment due to the hazardousness of the organic species, affecting not only the ecosystem but also preventing the use of water reservoirs for human supply because of the resultant serious health concerns [1]. Soil

remediation is a solution to these fuel discharges, but despite the large range of possibilities [2], there is not a single technology that can be currently proposed for highly efficient treatment in terms of depollution efficiency and cost.

Biological remediation technologies (bioremediation) use microorganisms to degrade pollution, and they can efficiently remove fuels from soil [3]. Because of the heterogeneity of soil, these biological processes are not as simple as those occurring in the liquid phase, and finding the conditions in which pollutants, microorganisms and nutrients meet in the same treatment region becomes a major concern. Various technological approaches exist to optimize this coexistence using different methodologies [4,5].

To enhance bioremediation efficiency, this technique could be combined with additional technologies [6]. Promising alternatives are electrokinetic processes. The electrokinetic treatment of polluted soils is the application of a low intensity direct electric current through the soil between appropriately distributed electrodes [7,8]. When an electric field is applied between electrodes inserted directly into soil (or inside electrolyte wells), many transport processes may occur (such as electro-osmosis, electromigration and electrophoresis) that could help (or disturb, if not correctly applied) biological processes by collocating pollution, nutrients and microorganisms. Electrical heating, as a consequence of the huge ohmic drops associated with the typically low ionic conductivity of soil, can lead to an increase in the temperature, which may also affect the performance of the microorganisms [9].

There are many possibilities to combining the two technologies, resulting in very different technological approaches that are globally referred to as “soil electro-bioremediation” [10]. One is a combination of electrokinetic soil flushing (EKSF) with bioremediation. EKSF consists of the use of a flushing solution to drag pollutants contained in the soil by the above-mentioned mechanisms. This flushing fluid may contain surfactants, pH regulators, or other species that aid in the efficient removal of pollution [11]. This fluid may also transport other

species such as microorganisms, nutrients and electron acceptors [12,13], thereby addressing the key challenge of biological degradation of combining in the same place all of the species involved in the process. Gill et al. (2014) [14] reviewed the different possibilities, processes and applications of the electrokinetic and biological combined technology for the remediation of organic contaminants.

The present work aims to compare different strategies in a combined technology using electrokinetic soil flushing and bioremediation for a hydrocarbon-polluted soil remediation process. We studied four options in this work: (1) bioremediation alone (Bio) performed as a control test to evaluate the biodegradation process without the application of electric current; (2) a combination of direct electrokinetic soil flushing and the biological technology (EKSF-Bio test) to assess the enhancement of the biological pollutant removal under the influence of electrokinetic phenomena; (3) a combination of direct electrokinetic soil flushing and the biological technology that also includes a daily polarity reversal of the electric field (PR-EKSF-Bio test) to analyse the mixing process that results in the soil when periodic changes are made to the polarity of electric current; and (4) a combination of electrokinetics and a permeable reactive biological barrier (EKSF-BioPRB) located in the soil with microbial consortia attached on the surface of gravel particles. Because diesel fuel is a complex mixture of different hydrocarbons, it was used as a model to study the treatment of this type of pollution in soils. The efficiency and the advantages or disadvantages of the different alternatives have been discussed. Experiments of 14 days duration were carried out to compare the technologies. This period is short enough to allow the complete removal of the pollutant, and hence, it allows us to compare the comparative remediation rates of the technologies. At the same time, it is high enough to be representative of the main processes involved in the remediation of the diesel pollutant.

2. Material and methods

2.1 Bench scale set-up

Fig. 1. Experimental installation

The lab scale set-up is shown in Fig. 1, and it was made of transparent methacrylate and divided into five compartments. The central compartment contained compacted polluted soil. Two graphite electrodes were placed on either side of the polluted soil in compartments separated from the soil by a 0.5 mm nylon mesh. The electrodes were connected to the power supplier device (HQ Power, Gavere, Belgium), constituting the anodic and cathodic compartments. Both electrode compartments were connected to an additional collector compartment at either side to collect the liquid overflowing from the electrode wells. The dimensions of the graphite electrodes, provided by Carbosystem (Madrid, Spain), were $10.0 \times 10.0 \times 1.0 \text{ cm}^3$.

To obtain a homogeneous distribution of current lines throughout the soil, the electrodes were positioned to cover the same cross section as the fraction of the soil to be studied. The

flushing fluids (or electrolytes) were loaded into the anodic and cathodic compartments at the beginning of the tests. The liquid level decrease in the anodic compartment (caused by the electro-osmotic flow) was regulated by a level control loop, and a peristaltic pump was connected to the cathodic compartment to extract the electro-osmotic flow transported towards the cathode.

2.2 Materials

2.2.1 Soil

Electrochemical techniques are especially recommended for treating low permeability soils, such as clays. Therefore, in this study, kaolinite (provided by Manuel Riesgo Chemical Products, Madrid, Spain) was used as a model for clay soil. The properties of this synthetic clay soil are detailed in Table 1. This soil is characterized by its inertness, low hydraulic conductivity, lack of organic content and low cation exchange capacity.

Table 1. Properties of soil.

Mineralogy		Particle size distribution (%)	
Kaolinite	100.00%	Sand	4.0
Fe ₂ O ₃	0.58%	Silt	18.0
TiO ₂	0.27%	Clay	78.0
CaO	0.10%		
K ₂ O	0.75%	Specific gravity	2.6
SiO ₂	52.35%	Hydraulic conductivity (cm s ⁻¹)	1 x 10 ⁻⁸
Al ₂ O ₃	34.50%	Organic content (%)	0.0
Others	11.42%	pH	4.9

2.2.2 Hydrocarbons

Diesel oil, a conventional petroleum-derived fuel, was selected as a model of a hydrocarbon pollutant. Diesel was purchased from a petrol station in Ciudad Real, Spain, and characterized

as previously reported [15]. To artificially pollute the soil, the diesel was diluted tenfold in acetone before evenly distributing the solution drop by drop in a corresponding amount of kaolinite. Simultaneously, the soil was continuously mixed in order to ensure the correct homogenization of the acetone-diesel solution. The solvent (acetone) and the higher volatile diesel fractions (approximately 6% of the initial amount of diesel) were allowed to evaporate at room temperature for at least two days. The concentration of diesel present in the soil at the beginning of the experiment was approximately 10 g kg^{-1} .

2.2.3 Micro-organisms

The diesel-degrading microbial consortium used for the experiments was isolated from diesel oil contaminated soil that was collected from a site in the vicinity of an oil refinery near Ciudad Real, Spain. This consortium of microorganisms was maintained and enriched with Bushnell-Hass Broth (BHB) as the mineral medium (the composition of the medium per litre of deionised water was $0.20 \text{ g l}^{-1} \text{ MgSO}_4$, $0.02 \text{ g l}^{-1} \text{ CaCl}_2$, $1.00 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$, $1.00 \text{ g l}^{-1} (\text{NH}_4)_2\text{HPO}_4$, $0.05 \text{ g l}^{-1} \text{ FeCl}_3$ and $1.00 \text{ g l}^{-1} \text{ KNO}_3$) and diesel hydrocarbon as the sole carbon source (1.0% v/v). After the enrichment process, some of the microorganisms in the consortium were identified, including *Staphylococcus lentus*, *Stenotrophomonas maltophilia* and *Pseudomonas fluorescens*, as reported in a previous work [15]. According to previous studies, these species are commonly found in hydrocarbon-polluted soils.

2.3. Experimental procedure

Four batch experiments of 14 days duration were carried out, all of them at room temperature using an electric field of 20.0 V (1.0 V cm^{-1}), except for the Bio test, which was considered a control experiment (0.0 V cm^{-1}). The operating conditions were successfully

controlled and the fixed variables were identical in all experiments. The experimental procedure and some details such as the flushing fluids used, the removal of the electro-osmotic flow, and the position of the microbial consortium in the soil slightly changed depending on the strategy used, as described below.

Regarding the composition of the flushing fluid, it varied in each experiment according to its expected role in the different strategies used (Table 2): the BHB medium was used as microbial growth medium; the buffer solution ($30.0 \text{ g l}^{-1} \text{ NaHCO}_3$) was used to neutralize pH fronts; the salt solution (30.4 mg l^{-1} of NaNO_3 , 70.0 mg l^{-1} of NaHCO_3 and 88.7 mg l^{-1} of Na_2SO_4) was used as electrolyte medium; and finally the 2.38 g l^{-1} sodium dodecyl sulphate (SDS) solution was used as anionic surfactant to move the diesel by electrokinetic transport across the soil.

Table 2. Procedure details: Microorganism addition and composition of the soil flushing fluid and the electrolytes.

Test	Microorganism addition	Soil liquid medium	Anolyte and catholyte
Bio (control test)	Microbial suspension added to the soil	BHB	salt solution: 30.4 mg l ⁻¹ NaNO ₃ 70.0 mg l ⁻¹ NaHCO ₃ 88.7 mg l ⁻¹ Na ₂ SO ₄
EKSF-Bio	Microbial suspension added to the soil	BHB	buffer solution: 30 g l ⁻¹ NaHCO ₃
PR-EKSF-Bio	Microbial suspension added to the soil	BHB	salt solution: 30.4 mg l ⁻¹ NaNO ₃ 70.0 mg l ⁻¹ NaHCO ₃ 88.7 mg l ⁻¹ Na ₂ SO ₄
EKSF-BioPRB	Microbial biofilm attached to the BioPRB. BioPRB flooded in BHB.	salt solution: 30.4 mg l ⁻¹ NaNO ₃ 70.0 mg l ⁻¹ NaHCO ₃ 88.7 mg l ⁻¹ Na ₂ SO ₄	salt solution (anolyte): 30.4 mg l ⁻¹ NaNO ₃ 70.0 mg l ⁻¹ NaHCO ₃ 88.7 mg l ⁻¹ Na ₂ SO ₄ 2.4 g l ⁻¹ SDS solution (catholyte)

(Bio: Bioremediation; EKSF: Electrokinetic soil flushing; PR: Polarity Reversal; BioPRB: Biological Permeable Reactive Barrier).

2.3.1 Bio test

A 2-ml inoculum of the microbial consortium was grown in a batch reactor for two days using BHB medium, and the obtained culture was concentrated by centrifugation. The concentrated biomass obtained was then suspended again in fresh BHB medium and added to the diesel-polluted soil, which was wet with the biomass suspension up to saturation conditions with a moisture content of approximately 40%. The soil was manually compacted into the central compartment to achieve the highest degree of compaction possible and to avoid the formation of preferential flow paths that could interfere with the results. The electrolyte used in the side compartments consisted of a synthetic aqueous solution composed of 30.4 mg l⁻¹ of NaNO₃, 70.0 mg l⁻¹ of NaHCO₃ and 88.7 mg l⁻¹ of Na₂SO₄, which forestalled the loss of nutrients by diffusion and prevented soil drying. In the control test, no collectors were necessary due to the absence of electro-osmotic flow.

2.3.2 EKSF-Bio test

The diesel-polluted soil was wet with the biomass suspension and then compacted into the central compartment identically to as indicated in the Bio test. In the EKSF-Bio test, a carbonate buffer (30.0 g l⁻¹ NaHCO₃) was used in the side compartments to neutralize the soil pH fronts produced as a consequence of the water electrolysis on the anode and cathode.

2.3.3 PR-EKSF-Bio test

The diesel-polluted soil was wet with the biomass suspension and then compacted into the central compartment identically to as indicated in the Bio test. The electrolyte used in the side compartments consisted of the same synthetic aqueous solution used in the Bio test. Additionally, in this periodic reversal test, the polarity of the electrodes was reversed daily.

2.3.4 EKSF-BioPRB test

The soil was wet to a moisture content of approximately 40% by a synthetic aqueous solution composed of 30.4 mg l⁻¹ of NaNO₃, 70.0 mg l⁻¹ of NaHCO₃ and 88.7 mg l⁻¹ of Na₂SO₄, with no microbial consortium added. The electrolyte used in the anodic well consisted of the same synthetic aqueous solution used in the Bio test, but on the other side, in the cathodic compartment, an anionic surfactant solution was used as a catholyte. A 2.38 g l⁻¹ sodium dodecyl sulphate (SDS) solution was loaded into the cathodic compartment at the beginning of the experiment. The soil was then compacted identically to as in the previous tests. A part of the soil was then removed from the central position in the central compartment, and a 5 cm wide biological permeable reactive barrier (BioPRB) was loaded into this position and flooded with the BHB medium.

The working principle of a BioPRB is the same as that of a conventional biofilm reactor, and it had been previously developed in the laboratory according to the procedure reported by Mena et al. (2014) [16]. The procedure for the BioPRB development has been included as Supplementary Material.

Table 3. Parameters measured in the soil and the electrolyte in both electrode compartments during the experiments.

Test	Bio		EKSF-Bio		PR-EKSF-Bio		EKSF-BioPRB		
	Soil	Electrolyte	Soil	Electrolyte	Soil	Electrolyte	Soil	Electrolyte	BioPRB
Temperature (soil central position)	✓		✓		✓				✓
pH	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓
Conductivity	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓
Moisture	✓ (*)		✓ (*)		✓ (*)		✓ (*)		
Diesel concentration	✓ (*)		✓ (*)		✓ (*)		✓ (*)	✓	✓
Microbial concentration	✓ (*)		✓ (*)		✓ (*)		✓ (*)		✓ (*)
NH ₄ ⁺ , NO ₃ ⁻ , PO ₄ ³⁻ concentration	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓ (*)	✓	✓
SDS concentration							✓ (*)	✓	✓

(*) Parameter measured only at the start (t=0) and the end (t=14d) of the experiment.

2.4. Sampling and analysis

Table 3 shows the parameters that were measured in the soil and in the electrolyte (electrode compartments) in the different experiments. These parameters were periodically measured during the experimentation, and some of them were only measured at the beginning ($t = 0$) and at the end ($t = 14$ d), as indicated in the table footnote.

Apart from the information indicated in Table 3, the following parameters were measured: current density through the soil, using a multimeter able to measure electrical current in the range of milliamps; electro-osmotic water volume collected in the cathodic compartment; and dissolved oxygen (DO) concentration in the liquid medium in the BioPRB (in the EKSFBioPRB test).

At the end of the experiments, soil samples were taken from four longitudinal positions corresponding to four intermediate points between the anode and cathode compartments. In turn, each intermediate longitudinal position also contained four sampling points distributed at different axial positions. The results show the average concentration of the four different samples taken at the same longitudinal position, that is, at the same distance from the electrodes, so it was possible to analyse the axial deviation of the points situated at the same distance from the electrodes.

In the following section, the analytical procedures for the measurement of each parameter are detailed. The moisture in the soil samples was calculated gravimetrically, taking into account the weight loss before and after drying at 105°C for 24h. The pH, conductivity and nitrate, phosphate, and ammonium concentrations were measured from the soil samples as follows: 10 g of dried soil were suspended in 25 ml of Milli-Q water by 20 min of vigorous magnetic agitation. Then, samples were centrifuged at 4000 rpm for 15 min. Measurement of the parameters was made in the supernatant phase. pH was measured using a CRISON pH

meter, and conductivity was measured using a Jenway conductivimeter. Inorganic ionic nutrients concentrations were measured using a Gallery photometric analyser (Thermo Fisher Scientific, Massachusetts, USA).

The diesel concentration was determined using a fractionated serial extraction. Briefly, 10 g of wet soil were mixed with 4 ml of hexane. In every extraction, the soil was mixed with a corresponding volume of dissolvent and agitated vigorously in a vortex agitator for 5 min. After that, the samples were centrifuged at 4000 rpm for 15 min. Samples taken from the organic supernatant phase were analysed using a Trace GC Ultra (Thermo Fisher Scientific, Massachusetts, USA) gas chromatograph equipped with a flame ionization detector (GC-FID). The microbial concentration was expressed as colony-forming units (CFU) per dry gram of soil. It was measured by suspending 10 g of wet soil in 10 ml of saline by 1 min of vortex agitation. After that, an aliquot of 100 μ l of the soil-saline suspension was plated onto Petri dishes. The nutrient solid phase of these dishes were prepared using LB medium (with the following composition per litre of deionised water: NaCl 10.0 g, yeast extract 5.0 g and casein peptone 10.0 g), 15.0 g l⁻¹ of European Bacteriological Agar and 2.0 g l⁻¹ of glucose as a carbon source. The inoculums were evenly spread using Digrafsky handles, and the plates were incubated for 48 h at 26 °C, which is time enough to enable the enumeration of the individual colonies present in each sample. Finally, the concentration of biomass in the biobarrier was also measured as volatile solids before and after the treatment. This value corresponds to the weight difference in the dried samples before and after the calcination at 550 °C for 2 h.

3. Results and Discussion

3.1. Changes in the soil conditions caused by the different technologies

Fig. 2. Main parameters monitored in the experiments daily. (a) Current density across the soil. (b) Soil temperature. (c) Electroosmotic flow. Bio test (▲), EKSF-Bio test (◆), PR-EKSF-Bio test (■) and EKSF-BioPRB test (●).

Fig. 2 shows the time course of the current density (a), soil temperature (b) and electroosmotic flow (c) during the 14 day long tests carried out on the four technologies studied in

this work. Fig. 2 (part a) shows the large differences (pay attention to the different scales) existing between the electric current values monitored in the three electro-bioremediation strategies applied, in spite of the systems being under the same electric field (1.0 V cm^{-1}). Higher values were obtained in the single EKSF-Bio experiment, and this fact can be explained in terms of the increase in the conductivity of the soil due to the necessary addition of the highly concentrated bicarbonate buffer to control the expected pH variations in the anolyte and in the catholyte. As will be discussed afterwards, it could also be explained by the failure of this fluid to control the pH, thereby increasing the proton and hydroxyl ion concentrations near the anode and cathode, respectively. Using the periodic reversal strategy (PR-EKSF-Bio test) or using a biobarrier strategy (EKSF-BioPRB test, which in principle confined and protected the microorganisms in a central position far from the extreme pH changes), the addition of a buffer to control the pH was not considered to be necessary, and the current was maintained at lower values in both situations. This fact has a determining influence on the process, because the rate of electrokinetic processes is known to be related to the current density applied [8]. No important differences between these two options were observed, and a slight current density decrease was observed as the electrokinetic phenomena could remove ionic species from the soil.

One of the most important consequences of the high electrical current registered in the single EKSF-Bio process is the soil heating and the consequent increase in the soil temperature of the system to values of approximately 32°C , as can be observed in Fig. 2 (part b). This soil heating could negatively affect the biological activity because the raw biological culture of diesel-degrading microorganisms was acclimated to a much lower temperature. In comparison to the other processes, only a slight increase in temperature was observed in the biobarrier (that is, the central soil position in the EKSF-BioPRB test), which is not expected to produce any detrimental effect on the biological activity. In the PR-EKSF-Bio test, the polarity reversal

strategy led to a very efficient temperature control, and the temperature values were nearly identical to those obtained in the Bio control experiment without an applied electric field. In general, the use of the different strategies and electrolytes affected the current density, which thereby influenced the temperature profiles.

Another important consequence of the different current densities observed in the experiments is the difference between the values of the electro-osmotic flow rate (Fig. 2, part c). Again, the EKSF-Bio test showed the highest difference, with a clearly higher electro-osmotic flow rate, as expected according to its higher current density. The ratio between the current densities in the EKSF-Bio test and the other two tests (PR-EKSF-Bio and EKSF-BioPRB) at the end of the experiments is over 10, while the ratio between the electro-osmotic flow rates is only approximately 3.

Fig. 3. Soil moisture profile at the start (—) and the end (---) of the experiments: (a) Bio test; (b) EKSF-Bio test; (c) PR-EKSF-Bio test and (d) EKSF-BioPRB test. The line (---) is the average value of the four values in the different axial positions (■▲◆×).

Fig. 3 shows the soil moisture (water content) profile at the start ($t=0$) and the end ($t=14$ d) of the experiments. The horizontal axis indicates the four longitudinal positions that correspond

to four different intermediate points between the anode (left) and cathode (right) compartments. The central BioPRB position is indicated in part d.

In the single bioremediation test (Bio test, part a), there was no electro-osmotic transport of water throughout the soil expected because no electric current was applied to the system. However, at the end of the treatment test, a very low decrease in the moisture of the soil was observed, which can be explained in terms of the losses caused by the evaporation that took place during the two weeks of the experiment.

In the EKSF-Bio experiment, there was a general increase in the moisture of the soil, with a decreasing profile towards the cathode (Fig. 3, part b). In this case, there was significant transport of water throughout the soil because of the electro-osmosis process. This transport can compensate for the loss of water by evaporation. The moisture value is slightly higher at the anode because this is the point at which the flushing fluid solution is added to the electrolyte well to maintain a constant level. A similarly decreasing anode-to-cathode moisture profile is also observed in the EKSF-BioPRB test (Fig. 3, part d), although the water amount transported from the anode well is not so high because of the lower EOF value, and a global moisture reduction is observed. Because of the lower EOF, it is supposed that water loss by evaporation near the cathode zone acquired greater importance. Finally, in the PR-EKSF-Bio test (Fig. 3, part c), there was only a slight decrease in the central position. The polarity reversal caused changes in the EOF direction, and the reversibility of the EOF due to the polarity changes maintained the initial moisture levels near the electrodes. However, the central position is far from the water in the electrode wells and it was supposed that it could produce a slight moisture scarce, and water evaporation could have a slight influence in this zone. It can be concluded that, despite the moisture changes in each of the different strategies, the moisture level was always maintained at an adequate values to support the biological activity.

Fig. 4. Soil conductivity profile at the start (—) and the end (- - -) of the experiments: (a) Bio test; (b) EKSF-Bio test; (c) PR-EKSF-Bio test and (d) EKSF-BioPRB test. The line (- - -) is the average value of the four values at different axial positions (■▲◆×).

Fig. 4 shows the soil ionic conductivity profile at the start ($t=0$) and the end ($t=14$ d) of the experiments. As can be observed, different results were detected depending on the technology used.

In the control Bio experiment (Fig. 4, part a, with no electric field applied), there was a small decrease in the conductivity in the areas near the electrodic wells, which may be due to the diffusion of the ionic species to the electrolyte solutions contained in them. In the EKSF-Bio experiment (applying a 1.0 V cm^{-1} electric field and using the buffer to assist in the control of the pH fronts), there is a great increase in the conductivity in all of the soil sections (Fig. 4, part b). This increase may be explained because of the flushing fluid: with the addition of the buffer to the anolyte and its transport through the soil, the salinity of the soil increases and thereby the soil conductivity. In addition, as will be discussed later, the pH changes were not completely compensated, and hence the local acidification and basification in different parts of

the soil also contribute to the higher conductivity. This increase in the conductivity was more pronounced near the electrodes to which the ions were migrating because of the electric field generated (electromigration) and because of the production of protons or hydroxyl ions (electrolysis).

In the EKSF-BioPRB experiment (Fig. 4, part d), the soil conductivity also increased in the area closest to the anode. The final conductivity values were not as high as those obtained in part b because of the lower current density and lower EOF value. Moreover, the increase in the conductivity in the area near the anode could also be helped by the dissolution and liberation of species under acidic pH [8].

Finally, the results of the PR-EKSF-Bio experiment (part c) are more difficult to explain. The changes in the conductivity profile have a singular shape, with a gradual decrease in all areas of the soil from the zone near the anode to the zone near the cathode, because the processes that affect the ionic species concentrations acted in the two directions for the same period of time (because of the periodic polarity reversal), and it only can be explained as a transient effect caused by the neutralization of the fronts produced the day before when the polarity of the electric field was in the opposite direction.

In general, it is clear that very high conductivity values would adversely affect the biological phenomena, while the other two options maintained adequate conditions.

Fig. 5. Soil pH profile at the start (—) and the end (---) of the experiments: (a) Bio test; (b) EKSF-Bio test; (c) PR-EKSF-Bio test and (d) EKSF-BioPRB test. The line (---) is the average value of the four values at different axial positions (■▲◆×).

Fig. 5 compares the soil pH profile at the start ($t=0$) and the end ($t=14d$) of the experiments. pH variation occurs due to the transport of the protons and hydroxyl ions generated on the surface of the anodes and cathodes, respectively, through the soil.

In the Bio experiment (Fig. 5, part a), there were no changes in the pH of the soil due to the absence of an electric field and hence of electricity-driven changes. In the EKSF-Bio experiment, there was an increase in the pH up to values near 10 across the soil in spite of the presence of the highly concentrated carbonate/bicarbonate buffer (Fig. 5, part b). In the lower sections near the anode, acid pH values were detected, indicating that the buffer was not strong enough to keep the pH value near neutrality, and hence this strategy failed in effectively controlling the pH against the protons produced in the anodic well. Likewise, it also failed in the rest of the soil because the final pH was far from neutrality and became alkaline near the cathode. This would prevent a biological mechanism from the selected consortia. However, the

final value was still far from the extreme values that are typically obtained when no buffer is added, which previous works in the literature [17-19] report can even rise up to 14. In the PR-EKSF experiment, the selected strategy effectively controlled the acidic and basic pH fronts in the treated soil (Fig. 5, part c). Although the electric field was applied in both directions for the same duration, there was a slight decrease in the area closer to the electrode that acted as the anode the day before the end of the test and a slight increase in the area near the electrode that was negatively polarized on this day (cathode). Therefore, although this strategy worked successfully to keep the pH around neutrality, there are transitory changes in the pH of the soil, although a reversal frequency of 1 day^{-1} seems to be suitable to keep them within a practical range. The changes observed in the pH are in agreement with those observed in the conductivity and demonstrate the failure of the EKSF-Bio strategy to coarsely regulate the pH (in spite of the high concentrations of buffering species) and the success of the polarity reversal strategy, which allowed biological processes to occur.

In the EKSF-BioPRB case, significant pH changes were observed near the electrodes according to the expected behaviour (acidic pH near the anode and basic pH near the cathode) because of the lack of buffering. However, the control could be considered successful because the central position (where the biological barrier is located) maintained pH values near neutrality. Thus, it could be assumed that the attached biomass was maintained under adequate pH conditions.

3.2. Microbial growth and hydrocarbon removal under different situations

Fig. 6. Soil microbial concentration at the start (—) and the end (---) of the experiments:

(a) Bio test; (b) EKSF-Bio test; (c) PR-EKSF-Bio test and (d) EKSF-BioPRB test. The line (---) is the average of the values at four axial positions (■▲◆×).

Fig. 6 shows the soil microbial concentration profile at the start ($t=0$) and the end ($t=14$ d) of the experiments. In part d, the initial level corresponds to BioPRB because there was no addition of microorganisms to the soil. As can be seen in part a, the concentration slightly increased uniformly across the soil in the control Bio test, indicating a successful bioaugmentation process with a homogeneous distribution of nutrients across the soil. On the other hand, the unfavourable environmental conditions (such as soil heating, high conductivity, possible lack of nutrients and extreme pH conditions in the EKSF-Bio test) caused the inhibition of the biological activity, and no active biomass was detected in the soil samples (part b), preventing the efficient removal of pollutants with this technology. Thus, the direct combination of bioremediation and electrokinetic soil flushing technology is not possible in such a direct form because the consequences of applying a direct electric field make the

conditions in the treated soil non-viable for the microbes (clear antagonistic effect of the combination of technologies).

On the other hand, the PR-EKSF-Bio and EKSF-BioPRB tests offer promising results according to the microbial growth observed. As discussed before, from the results observed in figures 2 to 5, adequate conditions for microbial life were maintained in these two remediation strategies, and hence there was an increase in the microbial population (parts c and d). In comparing the results of the two strategies, only a low growth was observed in part c, while a significant presence of microorganism across the soil was observed in part d. Because of the relationship between microbial growth and substrate consumption, the microbial concentration profiles will be discussed together with the hydrocarbon removal results.

Fig. 7. Diesel concentration in the soil at the start (—) and the end (- - -) of the experiments: (a) Bio test; (b) EKSF-Bio test; (c) PR-EKSF-Bio test and (d) EKSF-BioPRB test. The line (- - -) is the average of the values at four different axial positions (■▲◆×).

Fig. 7 compares the diesel hydrocarbon concentration profile in the soil before and after the 14 day test. Because the more volatile hydrocarbon fractions were previously removed and the increase in soil temperature was negligible (except in the EKSF-BIO test), hydrocarbon volatilization was not considered, and only the combination of biological and EKSF phenomena was assumed to be responsible for the diesel removal.

In the Bio test (control experiment), a homogeneous removal of diesel from the soil was obtained (Fig. 7, part a). This result is consistent with the uniform increase in the concentration of microorganisms, which clearly indicates that the diesel was biologically degraded by microorganisms in the soil. However, the average diesel removal efficiency was only approximately 12%. In previous studies on diesel biodegradation using the same microbial consortium [15] and using the biobarrier [16], it was found that the biodegradable fraction of the diesel was approximately 70% (expressed as COD). Thus, only a small portion of the biodegradable fraction of the diesel hydrocarbons was removed through the Bio treatment in two weeks, which could be due to the slowness of the static in situ biological process. The transport processes that are responsible for bringing the organic substrate, nutrients and microorganisms into contact are very slow if not aided by EK phenomena.

In the EKSF-Bio test (Fig. 7, part b), as expected according to the microbial death observed, there was a very low removal of diesel, in areas close to the anode. This decrease could be related to thermal desorption caused by the increase in temperature.

The PR-EKSF-Bio test (part c) showed homogeneous diesel removal at approximately 20% efficiency. This is a good result, and an efficiency increase could be expected over a longer experimentation time. The microbial growth was also homogeneous across the soil (Fig. 6, part c), but the increase in the microbial concentration was low compared to the amount of diesel removed according to the previous result observed in the Bio test (part a), and this is a possible contradictory result. It is supposed that significant differences in the microbial growth are

difficult to detect because the differences in diesel removal are low and the microbial process is supposed to be mainly anaerobic, which implies low biomass growth.

It is assumed that the polarity reversal strategy resulted in suitable conditions for the biological process, as it eliminated the soil heating and extreme pH variation and a highly concentrated buffer electrolyte was not necessary. Moreover, this strategy improved the transport processes that brought about the contact between the hydrocarbons, microorganisms and nutrients. The polarity reversal strategy has been previously tested by Kim and Han (2003), Xu et al. (2010), and Huang et al. (2013) [20-22], and all of them concluded that this is a suitable alternative to avoid abrupt changes in the pH values of the soil due to the acid and basic fronts. The same conclusion can also be drawn from the results discussed in this work. The same papers also highlight that the distribution of ionic nutrients in the polluted soils treated with the polarity reversal technique is more homogeneous. This leads to better pollutant biodegradation because the interactions between pollutants, microorganisms and inorganic nutrients occur more frequently. Our polarity reversal frequency was selected according to these previous works.

Finally, the EKSF-BioPRB option (Fig. 7, part d) showed the best results. As previously shown in Fig. 6 (part d), higher microbial concentrations were observed in the central areas near the biobarrier in which the diesel-degrading microorganisms were supported. In this zone, the conditions were optimal for the microbial activity in terms of the pH, and the nutrient concentration was continuously replaced by BHB addition to the biobarrier position. Additionally, high dissolved oxygen concentrations (approximately 6.0 mg l^{-1}) were detected in the BioPRB zone. Although the previous biofilm development was conducted under flooded and non-aerated conditions, and thus high dissolved oxygen concentrations would not be necessary, it is assumed that aerobic conditions in the BioPRB would be more efficient.

Similarly, in the areas near the electrodes, the microbial concentrations were lower. In these zones, the conditions for microbial growth became more extreme as the experiment progressed,

especially with respect to the pH values and the inorganic nutrient availability. The presence of microorganisms in zones outside from the central biobarrier it is supposed to be mainly caused from the biofilm detachment process and the EK transport of the microorganisms [13] rather than the low biomass growth. The biofilm detachment process resulted from biomass growth and the movement of water through the biobarrier.

The results shown in Fig. 7 (part d) indicate that the homogeneous removal of pollutants was achieved in the soil (approximately 29% of the diesel was removed from the soil). The homogeneity of the diesel removal results from two aspects. First, the microorganisms were present nearly throughout the soil portion (not only in the biobarrier) due to the biofilm detachment and the EK transport. In addition, the surfactant mobilized the diesel through the biobarrier, where higher degradation activity would occur. The surfactant was mobilized by electromigration from the cathode to the anode compartment because it was an anionic product. Small amounts of the surfactant were detected in the anodic well, and the surfactant concentration decreased in the cathodic well as the experiment progressed. By the end of the experiment, the surfactant was distributed throughout the soil and contributed to the homogeneous pollutant removal. It is important to note that no diesel oil was detected in the electroodic wells. The current density and EOF were high enough to mix and transport the species involved in the biological process, but also were low enough to avoid negative effects such as soil heating or extreme pH. Taking into account the diesel biodegradability, the result shown in Fig. 7, part d indicates that a significant portion of the biodegradable fraction of the diesel hydrocarbons (41% approximately) was removed through the EKSF-BioPRB treatment over two weeks.

4. Conclusions

The results obtained in this work clearly indicate that the biological treatment could be improved by the use of electrokinetic soil flushing, but only by using the polarity reversal strategy or a central biobarrier. When using the EKSF-Bio option, a high buffer concentration was required that caused a high current density, high conductivity, soil heating and nutrient removal by a high EOF. These conditions negatively affected the biological activity and thereby the diesel removal. The PR-EKSF-Bio and the EKSF-BioPRB options created adequate operating conditions and improved the transport processes for biological activity without the use of a large quantity of buffer. Polarity reversal was shown to be an efficient option for pH, moisture and temperature control. Homogeneous microbial growth was observed, and approximately 20% of the diesel was removed. The BioPRB option was not so efficient in controlling the operating conditions, but the central biobarrier position protected the biological activity. Microbial distribution was observed not only in the biobarrier but also in a large portion of the soil. A surfactant was necessary to improve the contact between microorganisms and diesel, and 29% of the diesel was removed from the soil in only 14 days.

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Appendix A. Supplementary Material

Supplementary material to this article can be found online

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